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Quick Review Cards *for* Medical Laboratory Science

Second Edition

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Dedicated to my husband, Gary, for his support and encouragement through another long project, and in loving memory of my parents, Bill and Lee Dietz, who provided me with the education that was the foundation of my career.

Preface

Quick Review Cards for Medical Laboratory Science were developed as a study aid to improve student performance on Board examinations in medical laboratory science (medical technology) at both the technician and technologist levels. (Technician candidates may skip the section on management and education.)

This card deck is the product of more than 30 years of experience teaching hundreds of students who have successfully passed Board examinations at both levels. The card format allows for easy sorting and portability, making them ideal for quick reviews and last-minute studying. Use of these cards alone, however, does not guarantee a passing score; they are intended to be used as an adjunct to traditional textbooks. Students are encouraged to highlight unfamiliar information and to refer to textbooks and class notes to supplement their study of those topics. The use of a multiple-choice review book and practice exams also will help to round out a student's preparation for the Board exam.

The review cards will also be beneficial to MSL and MLT students before graduation as they prepare for course examinations. Professionals who are cross-training or reentering the workplace will find these cards useful as well.

No review of this type can include all topics. This review focuses on common procedures and disorders, other knowledge that entry-level laboratory professionals are expected to have, and topics that are frequently included on Board exams. The review cards are written in an informal note-taking style, using abbreviations, symbols, and short phrases to maximize the amount of information included. A list of abbreviations is found in the frontmatter.

New to the second edition are a chapter on molecular diagnostics and graphics for select topics. Space did not allow for inclusion of drawings of all cells/organisms. Students are encouraged to refer to textbooks to supplement their review with additional pictures and diagrams. Further benefit could be derived from making their own drawings, diagrams, and flow charts. Active and frequent review will lead to higher scores.

Every effort was made to ensure the accuracy of the content. In some cases, discrepancies were found within and among references; then information was either selected from the most recent publication or confirmed in another source. Please let the publisher know if you have suggestions for improving future editions.

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Partial List of Abbreviations Used

Ab	Antibody
Ag	Antigen
AHG	Antihuman globulin
AIDS	Acquired immunodeficiency syndrome
ASAP	As soon as possible
AT	Antithrombin
BBP	Bloodborne pathogens
BP	Blood pressure
CAP	College of American Pathologists
CDC	Centers for Disease Control and Prevention
CIA	Chemiluminescent immunoassay
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLSI	Clinical Laboratory and Standards Institute
CMS	Centers for Medicare and Medicaid Services
CMV	Cytomegalovirus
CNS	Coagulase-negative staphylococci
CNS	Central nervous system
Ck	Check
CSF	Cerebrospinal fluid
CV	Coefficient of variation
DAT	Direct antiglobulin test
Diff	Differential
DTaP	Diphtheria, tetanus, pertussis vaccine
Dx	Diagnosis
EBV	Epstein-Barr virus
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
FFP	Fresh frozen plasma
GPC	Gram-positive cocci
GAS	Group A *Sreptococcus*
GBS	Group B *Sreptoccus*
GI	Gastrointestinal
GN	Gram negative
GNCB	Gram-negative coccobacilli
GNDC	Gram-negative diplococci
GNR	Gram-negative rods
GP	Gram positive
GPC	Gram-positive cocci
GPR	Gram-positive rods
GU	Genitourinary
HAV	Hepatitis A virus
HBIG	Hepatitis B immune globulin
HBV	Hepatitis B virus
HCB	Hepatitis C virus
HCT	Hematocrit
HDFN	Hemolytic disease of the fetus & newborn
Hgb	Hemoglobin

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Partial List of Abbreviations Used *continued*

Hib	*Haemophilus influenzae* type b
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPF	High power field
Hr	Hour(s)
IAT	Indirect antiglobulin test
ID	Identify, identification
IFA	Indirect fluorescent antibody
Ig	Immunoglobulin
IM	Infectious mononucleosis
IS	Immediate spin
LPF	Low power field
LF	Lactose fermenter
Min	Minute(s)
MMR	Measles, mumps, rubella vaccine
Mo	Month(s)
MRSA	Methicillin-resistant *Stapylococcus aureus*
MW	Molecular weight
N	Normal
NAT	Nucleic acid testing
N:C	Nucleus to cytoplasm
Neg	Negative
NLF	Nonlactose fermenter
NRBC	Nucleated red blood cell
OIF	Oil immersion field
OSHA	Occupational Safety and Health Administration
PCR	Polymerase chain reaction
PHI	Protected health information
Plt	Platelet(s)
Poly	Polymorphonuclear leukocyte, granulocyte
Pos	Positive
PPD	Purified protein derivative
Pt	Patient
QA	Quality assurance or assessment
QC	Quality control
RBC	Red blood cells
RE	Reticuloendothelial
RhIG	Rh immune globulin
RT	Room temperature (20°–24°C)
RTI	Respiratory tract infection
Rxn	Reaction
SD	Standard deviation
Sec	Second(s)
SG	Specific gravity
SOP	Standard operating procedures
Temp	Temperature

continued...

Partial List of Abbreviations Used *continued*

Tf	Transfuse, transfusion
UTI	Urinary tract infection
VRE	Vancomycin-resistant enterococci
WB	Whole blood
WBC	White blood cells
Wk	Week(s)
Xmatch	Crossmatch
Yr	Year(s)
#	Number
↑	Increase(s), increased
↓	Decrease(s), decreased
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
=	Equals

Other abbreviations are defined in the text.

SECTION 1

Laboratory Operations Review

Credentialing

PROCESS	DEFINITION	EXAMPLES
Accreditation	Recognition granted by nongovernmental agency to institutions that meet certain standards. ***Voluntary.***	AABB (formerly American Association of Blood Banks) College of American Pathologists (CAP) The Joint Commission (formerly JCAHO) National Accrediting Agency for Clinical Laboratory Sciences (NAACLS)
Certification	Recognition granted by nongovernmental agency to individuals who meet education requirements & demonstrate entry-level competency by passing exam. ***Voluntary.***	American Society for Clinical Pathology (ASCP) American Association of Bioanalysts (AAB) American Medical Technologists (AMT)
Licensure	Permission granted by state to individuals/organizations to engage in certain professions/businesses. ***Mandatory.*** Illegal to practice/operate in that state without license.	Licensure of laboratory personnel is required in CA, FL, HI, LA, MT, NV, NY, ND, RI, TN, WV. Many states require licensure of clinical labs.

Agencies That Issue Guidelines/Standards

AGENCY	GUIDELINES/STANDARDS
AABB (formerly American Association of Blood Banks)	Technical standards & accreditation of blood banks.
Centers for Disease Control & Prevention (CDC)	Standards & guidelines primarily related to infection control & safe work practices.
Clinical Laboratory & Standards Institute (CLSI, formerly NCCLS)	Standards on all aspects of lab practice developed through voluntary consensus.
International Organization for Standardization (ISO)	Standards to facilitate international exchange of goods & services. Developed through voluntary worldwide consensus. ISO 15189 defines standards for quality management in medical labs.

Federal Regulatory Agencies

AGENCY	AUTHORITY
Centers for Medicare & Medicaid Services (CMS)	Writes regulations for & enforces Clinical Laboratory Improvement Amendments of 1988 (CLIA '88).
Department of Health & Human Services (HHS)	Interprets & implements federal regulations related to health care. Oversees CDC, CMS, FDA, SAMSHA.
Department of Transportation (DOT)	Regulates packaging, labeling, & transportation of biological products.
Environmental Protection Agency (EPA)	Regulates disposal of toxic chemical & biohazardous wastes.
Food & Drug Administration (FDA)	Regulates market entry of instruments/reagents & production of donor blood & components. Licenses blood banks.
Nuclear Regulatory Commission (NRC)	Licenses labs that use radionucleotides.
Occupational Safety & Health Administration (OSHA)	Regulates employee safety in the workplace.
Substance Abuse and Mental Health Services Administration (SAMHSA)	Certifies laboratories to conduct forensic drug testing for federal agencies.

Federal Regulations

STANDARD	SUMMARY
Hazard Communication Standard (OSHA 1983) "Right-to-Know Law"	Requires employers to inform employees about hazardous substances in workplace & educate them in safe handling.
Clinical Laboratory Improvement Amendments of 1988 "CLIA '88"	Regulates all lab testing (except research) performed on humans in U.S. Requirements for personnel & quality assurance determined by test complexity. Administered by CMS.
Occupational Exposure to Hazardous Chemicals in Laboratories (OSHA 1990) "Laboratory Standard"	Requires chemical hygiene plan to minimize personnel exposure to hazardous chemicals in labs.
Bloodborne Pathogens Standard (OSHA 1991)	Mandates work practices & procedures to minimize worker exposure to bloodborne pathogens.
Formaldehyde Standard (OSHA 1992)	Requires monitoring of formaldehyde exposure.
Health Insurance Portability and Accountability Act of 1996 "HIPAA"	Regulates use & disclosure of protected health information (PHI).

CLIA '88 Test Complexities

COMPLEXITY	CRITERIA	QUALITY CONTROL	PROFICIENCY TESTING (PT)	TESTING PERSONNEL (MINIMUM QUALIFICATIONS)
Waived	Tests cleared by FDA for home use, negligible likelihood of erroneous results, or no reasonable risk of harm to patient if performed incorrectly	None required other than to follow manufacturers' directions	Not required	None specified
Provider-Performed Microscopy (PPM)*	Certain microscopic exams performed by provider during patient's visit, e.g., direct wet mount, KOH prep, urine sediment	Required when controls are available; otherwise, reference materials (e.g., photomicrographs) fulfill requirement	PT not specifically required, but labs must verify accuracy of testing twice annually. Can be through PT, split sampling, or blind testing.	Physician, midlevel practitioner, or dentist
Moderate Complexity	Score ≤ 12 on 7 criteria**	2 levels of external controls each day of testing	Required	High school diploma or equivalent & training for testing performed
High Complexity	Score > 12 on 7 criteria**	2 levels of external controls each day of testing	Required	Associate degree in medical laboratory technology or equivalent

*PPM is a subcategory of moderate complexity.

**Criteria used to evaluate text complexity: knowledge, training/experience, reagent/material preparation, characteristics of operational steps, calibration/quality control/proficiency testing materials, test system troubleshooting, interpretation/judgment. Each of the 7 criteria is rated 1–3 (lowest to highest), & scores are totaled.

Bloodborne Pathogens Standard

History	Published in 1991. Revised in 2001 following passage of Needlestick Safety & Prevention Act to include stronger requirements for employers to evaluate & adopt safer medical devices.
Purpose	To protect health-care workers from occupational exposure to bloodborne pathogens (BBP; e.g., HIV, HBV, HCV)
Primary Requirements	**Exposure control plan**: Determination of employees' risk of exposure & implementation of methods to control exposure. Plan must be reviewed & updated annually to reflect new technologies. Documentation of evaluation & adoption of safer devices is required. Nonmanagerial employees must be involved in evaluation & selection of devices. **Universal precautions**: All blood & certain body fluids are to be handled as if known to be infectious for bloodborne pathogens. **Engineering controls**: Control measures that isolate or remove a hazard from workplace, e.g., sharps containers, self-sheathing needles, plastic capillary tubes, Plexiglas shields. **Work practice controls**: e.g., hand washing, disposal of needles with safety device activated & holder attached, ban on eating/drinking/smoking in lab. **Personal protective clothing & equipment**: e.g., lab coats, gloves, face shields. Employer must provide & must launder lab coats. **Housekeeping**: e.g., proper disposal of biohazardous waste, decontamination of work surfaces. **Training**: On assignment & annually thereafter. **Medical surveillance**: Postexposure evaluation & follow-up at no cost to employee. **Hepatitis B vaccine**: Provided by employer within 10 days of assignment at no cost to employee. **Hazard communication**: e.g., biohazard labels, red bags. **Sharps injury log**: Must include description & location of incident, device involved. Employee privacy must be protected.

Specimen Infectivity

POTENTIALLY INFECTIOUS	USUALLY NOT INFECTIOUS (UNLESS VISIBLY BLOODY)
Blood	Feces
Tissues	Nasal secretions
Semen	Sputum
Vaginal secretions	Sweat
Cerebrospinal fluid	Tears
Synovial fluid	Urine
Pleural fluid	Vomitus
Peritoneal fluid	
Pericardial fluid	
Amniotic fluid	
Saliva in dental procedures	

Packaging of Biologics for Shipping

REQUIREMENT	EXPLANATION
Primary container	Test tube, vial, etc. containing etiologic agent. Must be securely closed, watertight, surrounded by absorbent material, & placed in secondary container.
Secondary container	Must be watertight, sealed, & placed in approved mailing container.
Mailing container	Must be made of fiberboard.
Labeling	Biohazard label required on primary & mailing containers.
Training	Employees must be trained & retrained every 2–3 yr or when regulations change.

Hazard Communication Standard (HCS)

History	Issued by OSHA in 1983. Written for manufacturing industry, but courts expanded jurisdiction to clinical labs.
Also Known As	"Right-to-Know Law"; "HAZCOM"
Purpose	To inform employees about chemical hazards in workplace & protective measures
Primary Requirements	Written hazard communication plan Inventory of hazardous chemicals on site Hazard labeling Material safety data sheet (MSDS) for each chemical readily accessible to employees on each shift. Training on initial assignment & when new hazard introduced.

Occupational Exposures to Hazardous Chemicals in Laboratories Standard

History	Issued by OSHA in 1990. Extension of HCS written specifically for labs.
Also Known As	“Laboratory Standard”; “Chemical Hygiene Standard”
Purpose	To limit employee exposure to hazardous chemicals to levels at or below permissible exposure levels (PELs).
Primary Requirements	Written chemical hygiene plan outlining standard operating procedures for use, storage, exposure control, & disposal of hazardous chemicals. Designation of chemical hygiene officer. Hazard identification & labeling. Material safety data sheet (MSDS) for each chemical readily accessible to employees on each shift. Use of personal protective equipment. Proper maintenance of fume hoods & other protective equipment. Monitoring of employee exposure to hazardous chemicals. Medical exams at no cost in cases of suspected overexposure. Training on initial assignment & before assignments involving new exposures.

Hazard Categories of Chemicals

CLASSIFICATION	EXAMPLE	EFFECT	COMMENTS
Corrosives	Glacial acetic acid, hydrochloric acid, sodium hydroxide	Visible destruction of human tissue on contact. Can cause injury on inhalation or contact.	Chemicals with pH <2 or >12. Separate inorganic acids from organic acids. Concentrated acids & bases can generate large amounts of heat when mixed with water.
Toxic substances	Cyanides, sulfides	Interfere with metabolic processes when ingested, inhaled, or absorbed through skin.	Threshold limit values (TLVs) = safe level of exposure.
Carcinogens	Benzidine, formaldehyde	Capable of causing cancer.	OSHA requires monitoring of formaldehyde exposure.
Mutagens & teratogens	Benzene, lead, mercury, radioactive material, toluene	Mutagens induce genetic mutations; teratogens cause defects in embryo.	Special precautions during pregnancy.
Ignitables	Acetone, alcohols, ether, xylene	Fire	Flashpoint = lowest temp that produces ignitable vapor. Flammables <100°F; combustibles ≥100°F.

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Hazard Categories of Chemicals *continued*

CLASSIFICATION	EXAMPLE	EFFECT	COMMENTS
Reactives	Ether, perchloric acid, picric acid, sodium azide	Explosion	Ether forms explosive peroxides on exposure to air or light; store in explosion-proof refrigerator. Perchloric acid may react explosively with organic compounds; separate from other acids. Picric acid is shock sensitive when dehydrated; more powerful than TNT. Sodium azide solutions can form explosive lead or copper azides in drains.

National Fire Protection Association (NFPA) Hazmat Diamond

HAZARD	SYMBOL	0	1	2	3	4
Health	Blue diamond (left)	No hazard	Can cause significant irritation	Can cause temporary incapacitation or residual injury	Can cause serious or permanent injury	Can be lethal
Flammability	Red diamond (top)	Will not burn	Must be preheated for ignition to occur	Must be heated or in ↑ ambient temp to burn	Can be ignited under almost all ambient temps	Will vaporize & burn at normal temp
Instability	Yellow diamond (right)	Stable	↑ temp makes unstable	Violent chemical change at ↑ temp or pressures	May explode from ↑ temp or shock	May explode at normal temp & pressures
Special hazards	White diamond (bottom)	W = unusual reactivity with water OX = oxidizer				

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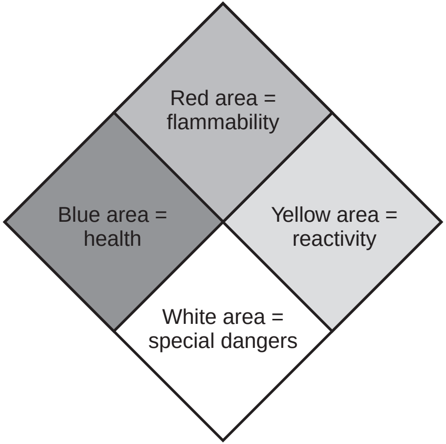
Hazard Identification System *continued*



Safety diamond. Colored areas within the diamond indicate types of danger: red area (top) = flammability; blue area (left) = health; yellow area (right) = reactivity; and white area (bottom) = special dangers. *(From Arneson W, Brickell J. Clinical Chemistry: A Laboratory Perspective. Philadelphia: FA Davis; 2007:5.)*

Storage of Chemicals

CHEMICAL CATEGORY	EXAMPLES	STORAGE GUIDELINES
Acids	Organic: formic, glacial acetic, citric Inorganic: hydrochloric, nitric, sulfuric Oxidizing: chromic, nitric, perchloric, sulfuric	Store below counter level or in acid cabinets. Separate from flammable & combustible material, bases, & active metals (e.g., sodium, potassium, magnesium). Separate organic acids from inorganic acids. Separate oxidizing acids from organic acids.
Bases	Ammonium hydroxide, potassium hydroxide, sodium hydroxide	Separate from acids. Store inorganic hydroxides in polyethylene containers.
Flammables	Acetone, alcohols, xylene	Limit amount in work area. Store in approved safety cans or cabinets. Separate from oxidizing acids & oxidizers.
Oxidizers	Nitric acid, perchloric acid, sulfuric acid, acetic acid, potassium chloride, hydrogen peroxide	Separate from reducing agents (e.g., zinc, alkaline metals, formic acid), flammable & combustible materials.
Water-reactive chemicals	Sodium, potassium	Keep away from water. Store in a dry, cool place.

Fire Safety

CLASS OF FIRE	COMBUSTIBLE MATERIAL	EXTINGUISHERS TO USE	COMMENTS
A	Cloth, wood, paper	Pressurized water (A) Dry chemical (ABC)	Don't use water on electrical fires or burning liquids.
B	Flammable or combustible liquids	Dry chemical (ABC) CO_2 (BC)	
C	Electrical equipment	Dry chemical (ABC) CO_2 (BC)	Never use water. Dry chemical may damage electrical equipment. CO_2 leaves no residue; good choice for computers, analyzers.
D	Combustible metals	Leave to professional firefighters.	

Commonly Used Anticoagulants/Additives

ANTICOAGULANT/ ADDITIVE	STOPPER COLOR	MODE OF ACTION	EXAMPLES OF USE	COMMENTS
EDTA	Lavender	Prevents clotting by chelating Ca^{2+}	CBC, diff, sed rate	Prevents platelets from clumping. Minimal morphologic changes to WBCs. Tube should be at least ½ full.
Heparin	Green	Prevents clotting by neutralizing thrombin	Many chemistries, osmotic fragility, plasma hgb, blood gases	Best anticoagulant for prevention of hemolysis. Don't use for diffs (blue background).
Sodium citrate	Light blue	Prevents clotting by binding Ca^{2+}	Most coagulation tests	Preserves labile clotting factors. Tube must be full for 9:1 blood-to-anticoagulant ratio or coag results falsely ↑. To ensure proper ratio when drawing with butterfly, use discard tube to clear air from tubing. Discard tube not required in other situations. Reduce anticoagulant when HCT >55%.
Sodium fluoride	Gray	Inhibits glycolysis (not an anticoagulant)	Glucose, lactic acid, blood alcohol	Preserves glucose for 24 hr. Combined with K oxalate if anticoagulation needed. Oxalate binds Ca^{2+}.

Recommended Order for Drawing Evacuated Tubes & Filling Tubes From a Syringe

(CLSI H3-A6, 2007)

TUBE	STOPPER OR CLOSURE	COMMENTS
Blood culture	Yellow (SPS) or blood culture bottle	Drawing 1st avoids bacterial contamination from needle that has pierced other stoppers.
Coagulation (citrate)	Light blue	Drawing before other anticoagulant & clot activator tubes avoids contamination with additives that can affect coag results.
Serum (with/without clot activator; with/without gel)	Red, gold, speckled	Drawing before green avoids contamination with sodium heparin (↑ Na^+) or lithium heparin (↑ Li^+). Drawing before lavender avoids contamination from K_2EDTA (↓ Ca^{2+}, Mg^{2+}; ↑ K^+). Drawing before gray avoids contamination with sodium fluoride/potassium oxalate (↓ Ca^{2+}, ↑ Na^+, ↑ K^+, interference with some enzyme assays).
Heparin (with/without gel)	Green	Drawing before lavender avoids contamination from K_2EDTA (↓ Ca^{2+}, Mg^{2+}; ↑ K^+). Drawing before gray avoids contamination with sodium fluoride/potassium oxalate (↓ Ca^{2+}, ↑ Na^+, ↑ K^+).
EDTA	Lavender, pink, white	Drawing before gray avoids contamination with oxalate, which alters cellular morphology
Glycolytic inhibitor (Na fluoride/K oxalate)	Gray	

Recommended Order for Filling Microcollection Tubes From Capillary Punctures

(CLSI H4-A6, 2008)

TEST/TUBE	RATIONALE FOR ORDER
Blood gases	Minimizes exposure to air
EDTA	Minimizes clumping of platelets
Other additive tubes	Minimizes clotting
Serum tubes	Clotting is not a concern

Special Situations in Phlebotomy

SITUATION	APPROPRIATE COURSE OF ACTION
IV	Use opposite arm or perform fingerstick, if possible; otherwise, have nurse turn off IV for 2 min, apply tourniquet below IV, use different vein (if possible). Document location of IV & venipuncture, type of fluid.
Fistula	Draw from opposite arm.
Indwelling lines & catheters, heparin locks, cannulas	Usually not drawn by lab. First 5 mL drawn should be discarded. Lab may draw below heparin lock if nothing is being infused.
Sclerosed veins	Select another site.
Hematoma	Draw below.
Streptokinase/tissue plasminogen activator (TPA)	Minimize venipunctures. Hold pressure until bleeding has stopped.
Edema	Select another site.
Scars, burns, tattoos	Select another site.
Mastectomy	Draw from opposite arm.
Patient refuses	Try to persuade. If unsuccessful, notify nurse. Never draw without consent; could lead to charges of assault & battery.
Unidentified patient	Ask nurse to ID before drawing.

Special Test Requirements

REQUIREMENT	EXAMPLES*	COMMENTS
Fasting	Fasting blood sugar, triglycerides, lipid panel, gastrin, insulin	Nothing to eat or drink (except water) for at least 8 hr
Chilling	ACTH, acetone, ammonia, gastrin, glucagon, lactic acid, pyruvate, PTH, renin	Place in slurry of crushed ice & water. Don't use ice cubes alone because RBCs may lyse.
Warming	Cold agglutinins, cryoglobulins	Use 37°C heat block, heel warmer, or hold in hand.
Protection from light	Bilirubin, carotene, erythrocyte protoporphyrin, vitamin A, vitamin B_{12}	Wrap in aluminum foil.
Chain of custody	Any test used as evidence in legal proceedings; e.g., blood alcohol, drug screens, DNA analysis	Chain of custody form. Lock box may be required.

*Follow laboratory's established procedures.

Phlebotomy Sources of Error

ERROR	POSSIBLE EFFECT
Misidentification of patient	Treatment errors, possibility of transfusion fatality
Drawing at incorrect time	Treatment errors if samples for certain tests aren't drawn at appropriate time, e.g., therapeutic drug monitoring, analytes that exhibit diurnal variation, analytes that are affected by recent eating/drinking
Improper skin disinfection	Infection at site of puncture. Contamination of blood cultures & blood components. Isopropyl alcohol wipes can contaminate samples for blood alcohol.
Drawing from edematous site	Dilution of sample with tissue fluid
Fist pumping during venipuncture	↑ K^+, lactic acid, Ca^{2+}, phosphorus; ↓ pH
Tourniquet >1 min	↑ K^+, total protein, lactic acid
IV fluid contamination	↑ glucose, electrolytes (depending on IV)
Expired collection tubes	↓ vacuum, failure to obtain specimen
Incorrect anticoagulant or contamination from incorrect order of draw	K_2EDTA before serum or heparin tube: ↓ Ca^{2+}, Mg^{2+}, ↑ K^+ Contamination of citrate tube with clot activator: erroneous coag results.
Failure to hold bottom of tube lower than top during collection	Carryover from one tube to another. Possible additive contamination.

continued...

Phlebotomy Sources of Error *continued*

ERROR	POSSIBLE EFFECT
Short draws	Incorrect blood: anticoagulant ratio affects some results, e.g., coag tests.
Inadequate mixing of anticoagulant tube	Micro-clots, fibrin, platelet clumping can lead to erroneous results.
Hemolysis from alcohol contamination, "milking" site of capillary puncture, probing with needle, vigorous shaking of tubes, exposure of samples to extremes of temperature	↑ K^+, Mg^{2+}, LD, iron

Guidelines for Specimen Handling & Processing*

- Transport blood specimens carefully to avoid hemolysis.
- Protect tubes for bili, carotene from light.
- Transport samples for ACTH, lactic acid, ammonia, blood gases in ice slurry.
- Maintain tubes in vertical position to promote complete clotting.
- Allow serum & gel separator tubes to clot for 30–60 min before centrifugation to avoid fibrin strands.
- Centrifuge within 2 hr of collection.
- Spin most tubes at 1,000–1,300 RCF for 10–15 min.
- Spin citrate tubes at 1,500 RCF for 15 min to produce platelet-poor plasma.
- Keep tubes capped during centrifugation to avoid loss of CO_2, change of pH, evaporation, or aerosol formation.
- Don't re-spin primary tubes. Can cause hemolysis. If recentrifuging is necessary, transfer serum/plasma to another tube.
- Don't re-spin serum separator tubes. Serum in contact with RBCs under gel can be expressed & ↑ K^+.
- Separate serum or plasma from cells within 2 hr of collection (exception: centrifuged gel tubes).
- When transferring samples to secondary containers, aspirate to avoid cellular contamination. Don't pour.
- Lipemic specimens can be ultracentrifuged at 10^5 x g to remove chylomicrons (triglycerides).
- Separated serum/plasma may be kept at RT for 8 hr or at 2–8°C for 48 hr. For longer storage, freeze at –20°C. Avoid repeated freezing & thawing.
- Don't freeze whole blood.

*Always follow laboratory's established procedures.

Centrifuges

TERM	EXPLANATION
Relative centrifugal force (RCF)	Force acting on sample being centrifuged. Gravities (g). Function of rpm and radius. $RCF = 1.12 \times 10^{-5} \times r \times rpm^2$.
rpm	Revolutions per minute. Speed of centrifugation. Determined by tachometer.
Radius (r)	Distance in cm from center of rotation to bottom of tube when rotating.
Horizontal-head centrifuge (swinging-bucket)	Tubes are in horizontal position when rotating. Produces a tightly packed, flat sediment surface. Recommended for serum separator tubes.
Angle-head centrifuge	Tubes are at fixed angle (25°–40°) when rotating. Capable of higher speeds. Produces a slanted sediment surface that isn't tightly packed. Decantation is not recommended.
Ultra centrifuge	High-speed. Capable of 100,000 rpm. Refrigerated to reduce heat.

Always make sure centrifuge is balanced. Don't open while spinning. Keep tubes capped.

Examples of Criteria for Specimen Rejection*

- Missing or inadequate label
- Collected at wrong time
- Collected in wrong tube
- Insufficient specimen
- Inadequate volume of blood in anticoagulant tube
- Exposure to temperature extremes
- Prolonged transit
- Clots in CBC tube
- Hemolysis (depending on test ordered)
- Lipemia (depending on test ordered)

*Follow lab's written policies.

Types of Glass

TYPE	CHARACTERISTICS
Borosilicate glass (Kimax, Pyrex)	High resistance to thermal shock & chemical attack. Heavy walls to minimize breakage. Used for most beakers, flasks, & pipets. Minimal contamination of liquids by elements in glass. Can be heated & autoclaved.
Aluminosilicate glass (Corex)	6 times stronger than borosilicate. Better able to resist clouding due to alkali & scratching.
Boron free	Used for highly alkaline solutions. Alkali resistant. Poor heat resistance.
High silica	Heat, chemical, & electrical tolerance. Excellent optical properties. Used for high-precision analytic work, optical reflectors, mirrors.
Flint glass	Soda-lime glass containing oxides of sodium, silicon, & calcium. Least expensive but poor resistance to high temp & sudden changes of temp. Only fair resistance to chemicals. Can release alkali & affect some determinations. Used for some disposable glassware.
Low actinic	Amber or red. Used to ↓ exposure to light, e.g., bilirubin standards.

Types of Plastic

TYPE	CHARACTERISTICS
Polypropylene	Relatively inert chemically. Resistant to most acids, alkalis, & salts. Can be autoclaved. Used for pipet tips, test tubes.
Polyethylene	Relatively inert chemically. Resistant to most acids (except concentrated H_2SO_4), alkalis, & salts. Used for test tubes, bottles, disposable transfer pipets, test tube racks. Can't be autoclaved.
Polycarbonate	Stronger than polypropylene & better temp tolerance, but chemical resistance not as good. Clear. Resistant to shattering. Used for centrifuge tubes, graduated cylinders.
Polystyrene	Rigid, clear. Shouldn't be autoclaved. Will crack & splinter. Used for test tubes, graduated tubes.
Polyvinyl chloride	Soft & flexible but porous. Frequently used as tubing.
Teflon	Extremely inert. Excellent temp tolerance & chemical resistance. Used for stir bars, stopcocks, tubing.

Glassware Inscriptions

INSCRIPTION	EXPLANATION
A	Class A. Meets high standards for accuracy.
20°C	Temp of calibration. Temp glassware & solutions should be for maximum accuracy.
TC	To contain. Vessel calibrated to hold specified volume (e.g., volumetric flask).
TD	To deliver. Vessel calibrated to deliver specified volume (e.g., graduated cylinder).

Volumetric Glassware

GLASSWARE	DESCRIPTION/USE
Beaker	Wide-mouthed, straight-sided jar with pouring spout. Not accurate enough for critical measurements.
Erlenmeyer flask	Sloping sides. Graduated markings. Used to hold liquids, mix solutions, measure noncritical volumes.
Florence flask	Spherical base with long cylindrical neck. Single calibration mark. Only for noncritical measurements.
Volumetric flask	Pear shaped. Long neck with single calibration mark. Manufactured to strict standards. Glassware & solutions should be at RT. Used to prepare standards & reagents. Shouldn't be used to store solutions.
Graduated cylinder (graduates)	Upright, straight-sided tube with flared base. Used for noncritical measurements. Most are TD. Shouldn't be used to measure <5 mL or <10% of capacity. Use graduate closest in size to volume to be measured.

Glass Pipets

PIPET	DESCRIPTION/USE
Volumetric	Transfer pipet. Single calibration mark. Calibrated to accurately deliver fixed volume of nonviscous samples & standards. Touch off last drop against wall of receiving vessel.
Ostwald-Folin	Transfer pipet. Similar to volumetric pipet, but bulb closer to tip. Etched ring means blowout. Used for accurate measurement of viscous fluids, e.g., whole blood. Not widely used.
Serological	Graduated or measuring pipet. Graduation marks down to tip. Etched ring means blowout. Can use for serial dilutions & measuring reagents. Not accurate enough for measuring samples or standards.
Mohr	Graduated or measuring pipet. Doesn’t have graduation marks all the way to tip or frosted band near upper end. Delivery is made “point to point.” Not widely used.
Micropipet	Disposable pipet for volumes ranging from 1–1,000 µL. Single calibration mark. Filled by capillary action. TC. Must be rinsed out with diluent to deliver exact amount. Small pipetting bulb is used.

Mechanical Micropipets

Types	
Air displacement	Uses suction to aspirate & dispense sample through polypropylene tip. 1- or 2-stop. With 2-stop, button is depressed to 2nd stop to "blow out." Tips can only be used once. Seals require periodic lubrication. Follow manufacturer's instructions for use.
Positive displacement	Uses a glass capillary tip fitted with Teflon-tipped plunger. No carryover. Tips are reusable. Plunger setting must be checked & Teflon tip replaced periodically. Follow manufacturer's instructions for use.
Calibration	Verify accuracy & precision on receipt, after service or repair, & on regular schedule. Most accurate method for calibration is gravimetric method (weight of distilled water delivered). Secondary method is spectrophotometric (absorbance of potassium dichromate or *p*-nitrophenol delivered).

Grades of Chemicals

GRADE	CHARACTERISTICS/USE
Analytic reagent grade	Very high purity. Meets specifications of American Chemical Society. Recommended for qualitative & quantitative analysis.
Ultra pure	Spectrograde, nanograde, or HPLC grade. Used for gas chromatography, HPLC, fluorometry, & trace metal determinations.
Chemically pure	Limits of impurities not specified. May be acceptable for some lab applications when higher purity chemicals aren't available.
Practical, technical, or commercial grade	For industrial use. Not of sufficient purity to use as analytic reagents.
USP or NF grade	Meet specifications of U.S. Pharmacopeia or National Formulary. Not injurious to health. Not necessarily of sufficient purity to use as analytic reagents.

Purified Water

CLSI C3-A4, 2006

TYPE	USE
Clinical laboratory reagent grade (CLRW)	Meets CLSI specifications for ionic, microbiological, & organic impurities, particulate & colloid content. Pure enough for most routine testing. Replaces previously designated type I and type II water. No single purification method can produce water of this quality. Purification systems use various combinations of distillation, deionization, reverse osmosis, & filtration. Lab must test at regular intervals for resistivity (the ↑ the resistivity, the ↓ the ion concentration), microbial content, & total organic carbon (TOC).
Special reagent water (SRW)	For applications that require water of different purity than CLRW, e.g., DNA/RNA analysis, trace metals. Must meet specifications of assay.
Instrument feed water	Used in automated analyzers for rinsing, dilutions, water baths. Specifications set by manufacturer.
Water supplied by a method manufacturer	Water provided by manufacturer for use in particular test system. Not for use in other applications.
Autoclave & wash water	Feed water for autoclaves & dishwashers. Impurities that could contaminate washed labware or solutions in autoclave are removed. Replacement for previously designated type III water.
Commercially bottled, purified water	Must meet specifications for intended use & be packaged to protect from degradation & contamination.

CAP Reagent Labeling Requirements

REQUIRED	NOT REQUIRED
Content	Date received
Concentration	Date opened
Storage requirements	
Date prepared or reconstituted	
Expiration date	
Lot #, if applicable	

Information may be recorded in paper or electronic log, rather than on container.

Brightfield Microscopy

TERM	EXPLANATION
Achromatic objective	Least expensive objective. Partially corrects for chromatic & spherical aberrations.
Aperture diaphragm	Controls angle & amount of light sent to objective.
Binocular microscope	One with 2 oculars.
Blue filter	Used to eliminate yellow color emitted by tungsten.
Brightfield microscope	Uses transmitted light & lenses. Objects appear dark against white background. Used for most routine clinical work.
Compound microscope	One with 2 lens systems—objectives & oculars.
Condenser	Focuses light on specimen.
Depth of focus	Distance throughout which all parts of specimen are in focus simultaneously.
Field diaphragm	Limits area of illumination to image field.
Field of view	Area of specimen that can be seen.
Immersion oil	Used to help objective gather light from a wide numerical aperture. Provides high resolution. Type B (high viscosity) is commonly used.
Kohler illumination	Method of focusing & centering light path & spreading light uniformly. Ensures optimum contrast & resolution.

continued...

Brightfield Microscopy *continued*

TERM	EXPLANATION
Magnification, total	Magnification of ocular × magnification of objective. 1,000× is highest magnification achievable with brightfield microscope.
Numerical aperture (NA)	Mathematical expression of light admitted by lens. The higher the NA, the greater the resolution.
Objectives	Lenses attached to revolving nosepiece. Most commonly used are low power (10×), high power (40×), & oil immersion (50× or 100×).
Ocular	Eye piece. Usually 10×.
Parcentric	Object in center of field at 1 magnification will be in center of field at other magnifications
Parfocal	Object remains in focus from 1 magnification to another
Planachromatic objective	More expensive objective that corrects for curvature of field. Results in flat field with uniform focus.
Resolution	Ability to reveal fine detail & distinguish between 2 close points.
Rheostat	Light control knob. Light intensity shouldn't be regulated by condenser or diaphragms.
Tungsten-halogen bulb	Type of bulb used for brightfield microscopy.
Virtual image	Image seen through microscope. Upside down & reversed.
Working distance	Distance between slide & objective. Decreases with higher magnification objectives.

Other Types of Microscopy

	EXPLANATION	APPLICATION IN CLINICAL LABORATORY
Light Microscopes		
Darkfield	Brightfield microscope with special condenser. Objects appear white against black background.	Identification of live *Treponema pallidum* & other microorganisms
Fluorescent	Brightfield microscope with 2 special filters. Fluorescent dyes absorb light of 1 wavelength & emit light of longer wavelength. Objects appear green, yellow, or orange against black background.	Direct & indirect fluorescent antibody stains in microbiology & immunology
Interference contrast	Brightfield microscope with special slit aperture below condenser, polarizer, & special amplitude filter (modulator) in back of each objective. Gives 3-D effect to unstained specimens.	Wet mounts
Phase contrast	Brightfield microscope with phase condenser & phase objectives. Subtle differences in refractive index converted to clear-cut variations of light intensity & contrast. Good for living cells, unstained specimens.	Manual platelet counts, urine sediments (good for hyaline casts)
Polarizing	Brightfield microscope with 2 crossing filters—polarizing filter below condenser, analyzer between objective & eyepiece. Objects that can refract light (birefringent) appear white against black background.	Identification of crystals in urine & synovial fluid. Confirmation of fat or oval fat bodies in urine sediment.

continued...

Other Types of Microscopy *continued*

	EXPLANATION	APPLICATION IN CLINICAL LABORATORY
Electron Microscopes		
Transmission	Beam of electrons passes through specimen, focused onto fluorescent screen or photographic plate. Magnification >100,000×.	Virology, cells (organelles)
Scanning	Beam of electrons strikes surface of specimen, focused onto photographic film or cathode ray tube. 3-D image. Magnification >1,000×.	Virology, cells (surface)

Informatics

TERM	EXPLANATION
Informatics	Information science. Science of processing data for storage, retrieval, & use.
Laboratory informatics	Use of computers & information systems to process & communicate information generated in clinical lab
Electronic medical record (EMR)	Computerized medical record. American Recovery & Reinvestment Act of 2009 (ARRA) calls for EMRs for all patients by 2014 to help ↓ cost & ↑ efficiency of health-care delivery.

Computer Hardware

TERM	EXPLANATION
Hardware	Physical parts of computer
CPU	Central processing unit. Executes software instructions.
RAM	Random access memory. Working memory used for temporary storage of programs & data. Content is lost each time computer is turned off.
ROM	Read-only memory. Part of memory that is permanently protected from being modified, erased, or written over. Not affected by power loss. Used for boot-level & other system instructions.
Hard drive	Magnetic-coated metal plate inside CPU for storing data
Disk drive	Device that reads data stored on magnetic or optical disk & writes data onto disk for storage
Optical disks	CDs, DVDs. Store data.
Peripheral devices	Input/output & information storage components
Input devices	Devices that deliver data to computer, e.g., keyboards, barcode readers, computer links (interfaces)
Output devices	Devices by which computer delivers data, e.g., printers, monitors
Modem	Input/output device that allows computers to communicate over telephone lines

Computer Software

TERM	EXPLANATION
Software	Programs that tell computer what to do
Operating system software	Program that controls basic functions of computer, e.g., Microsoft Windows
Application software	Programs designed to meet specific needs of users, e.g., word processing
Word processor	Application program that allows for manipulation of text. Used to write letters, reports, etc.
Spreadsheet	Application program to manipulate numbers & perform mathematical calculations. Used to prepare financial statements, budgets, etc.
Database	Application program to organize, store, sort, & retrieve data (words or numbers).
Browser	Program that provides access to Internet, e.g., Microsoft Internet Explorer

Information Systems

TERM	EXPLANATION
Laboratory information system (LIS)	System of hardware, software, connections, & communication protocols to handle all informational needs of lab, from intake of requests to delivery of results. Can provide patient information, test information, collection lists, work lists, test results, financial functions, productivity/workload monitoring, quality management, & interface with other computer systems.
Hospital information system (HIS)	Information system to handle all informational needs of hospital, both clinical & administrative.
Interface	Hardware & software that allow for electronic communication between 2 computer systems, even if they use different programming languages. The LIS is typically interfaced to HIS & automated analyzers.
Unidirectional interface	Interface that transmits electronic information in 1 direction, e.g., a point-of-care analyzer downloads test results to LIS.
Bidirectional interface	Interface that transmits electronic information in 2 directions, e.g., the LIS downloads orders from the HIS & uploads results to the HIS.
Middleware	Interface between an analyzer and LIS. Can apply rules to automate processes, e.g., autoverification (automatic release of results without tech review when certain criteria are met).
System validation	Documentation that LIS functions as expected. Required by regulatory agencies.

Computer Networks

TERM	EXPLANATION
Local area network (LAN)	Computer network that connects computers in close geographic proximity (e.g., building, campus)
Wide area network (WAN)	Computer network that connects computers over larger geographic area (e.g., multisite health-care facility, Internet)
Internet	Global system of interconnected computer networks
Intranet	Computer network within an organization. Access is usually restricted to employees.
Extranet	Extension of a private network onto the Internet where it can be accessed by authorized clients, suppliers, etc.
Protocol	Common set of signals & rules that network uses for communication
Ethernet	One of the 1st protocols developed for connecting computers
TCP/IP	Transmission Control Protocol/Internet Protocol. Originally developed as transfer protocol for Internet; adapted for transmission in LANs.
Health Level 7 standard (HL7)	Standardized message protocol that facilitates exchange of medical data among computer systems

Quality Assessment

TERM	EXPLANATION
Quality assessment or quality assurance (QA)	Process by which lab ensures quality results by closely monitoring preanalytical, analytical, & postanalytical stages of testing.
Preanalytical QA	Everything that precedes test performance, e.g., test ordering, patient preparation, patient ID, specimen collection, specimen transport, specimen processing.
Analytical QA	Everything related to assay, e.g., test analysis, QC, reagents, calibration, preventive maintenance.
Postanalytical QA	Everything that comes after test analysis, e.g., verification of calculations & reference ranges, review of results, notification of critical values, result reporting, test interpretation by physician, follow-up patient care.
Quality system	All of the lab's policies, processes, procedures, & resources needed to achieve quality testing.

Quality Control

TERM	EXPLANATION
Quality control (QC)	Part of analytical phase of quality assurance; process of monitoring results from control samples to verify accuracy of patient results.
Control	Sample that is chemically & physically similar to unknown specimen & is tested in exactly the same manner. Monitors precision of test system. For nonwaived quantitative tests, CLIA requires at least 2 levels of controls each day test is performed. (See "Equivalent QC" below.) For qualitative tests, pos & neg controls must be included with each run.
External QC	Testing control material not built into test system. Term also used for QC that extends beyond lab, e.g., participation in proficiency testing program.
Internal monitoring systems	Electronic, internal, or procedural controls that are built into test system.
Equivalent QC	Labs may reduce frequency of testing external QC materials for certain test systems if they can demonstrate through a CMS-approved QC evaluation process that test system is stable.

TERM	EXPLANATION
Measures of dispersion	Statistical parameters describing spread of data about mean, e.g., standard deviation, coefficient of variation, range. Measurements of precision.
Range	Difference between highest & lowest values in data set.
Mean	Sum of all observations divided by number of observations. Average of all observations.
Standard deviation (SD)	Statistical expression of dispersion of values around mean. Requires a minimum of 20 values. $SD = \sqrt{\frac{\Sigma(\bar{x} - x)^2}{n - 1}}$ where Σ = sum, x = individual value, $\bar{x}$ = mean, n = number of values
Coefficient of variation (CV)	Expresses standard deviation as percentage. CV % = (SD ÷ mean) × 100. The ↓ the CV, the ↑ the precision.

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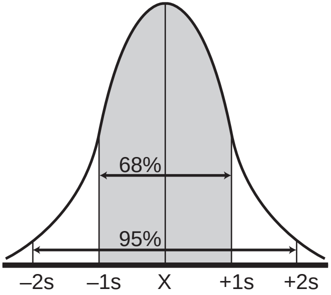
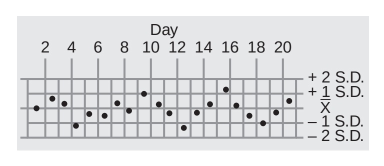
TERM	EXPLANATION
Normal distribution	The Gaussian bell-shaped curve: 68% of values fall within ±1 SD of mean 95% of values fall within ±2 SD of mean (95% confidence interval) 99.7% of values fall within ±3 SD of mean



Gaussian frequency distribution with 95% limits indicated. *(From Arneson W, Brickell J. Clinical Chemistry: A Laboratory Perspective. Philadelphia: FA Davis; 2007:54.)*

TERM	EXPLANATION
Control limits	Range within which control values must fall for assay to be considered valid. Many labs use mean ±2 SD. 1 determination in 20 will fall outside ±2 SD. This is anticipated part of normal variation.  Levey-Jennings chart showing in-control results. *(From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:133.)*
Levey-Jennings chart	Normal distribution curve lying on its side, marked with mean, ±1, ±2, ±3 SD.

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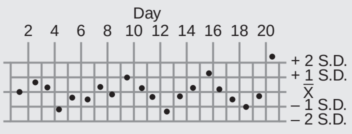
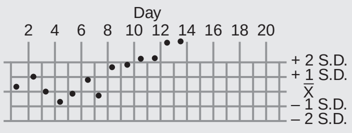
Interpretation of Quality Control Data *continued*

TERM	EXPLANATION
Outlier	A control result outside established limits. May be due to chance or may indicate problem in test system. If it occurs more than once in 20 successive runs, investigation must be carried out.  Levey-Jennings chart showing outlier. *(Modified from Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:133.)*
Shift	6 consecutive control values on same side of mean.  Levey-Jennings chart showing shift. *(From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:133.)*

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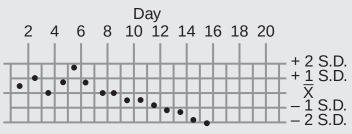
Interpretation of Quality Control Data *continued*

TERM	EXPLANATION
Trend	Control values increasing or decreasing for 6 consecutive runs.  Levey-Jennings chart showing trend. *(From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:133.)*
Error, random	Error that doesn't recur in regular pattern, e.g., error due to dirty glassware, use of wrong pipet, voltage fluctuation, sampling error, anticoagulant or drug interference. Indicated by control value significantly different from others on Levey-Jennings chart, or violation of the 1_{3S} or R_{4S} Westgard rules. Usually a 1-time error, & controls & samples can be rerun with success.
Error, systematic	Recurring error inherent in test procedure, e.g., dirty photometer, faulty ISE, evaporation or contamination of standards or reagents. Affects all results. Indicated by trend or shift on Levey-Jennings chart, or violation of 2_{2S}, 4_{1S}, or $10_{\times}$ Westgard rules (see following). Requires investigation to determine cause.
False rejection	Rejection of run because QC results indicate problem when none is present. Use of Westgard rules minimizes false rejections.

Westgard Multirules

RULE	EXPLANATION	TYPE OF ERROR	COMMENTS
1_{2S}	1 control >±2s from mean. Warning flag of possible change in accuracy or precision.		Initiates testing of other rules. If no violation of other rules, run is considered in control.
1_{3S}	1 control >±3s from mean	Random	Rejection rule
2_{2S}	2 consecutive controls >2s from mean on same side	Systematic	Rejection rule
R_{4S}	2 consecutive controls differ by >4s	Random	Rejection rule
4_{1S}	4 consecutive controls >1s from mean on same side	Systematic	Rejection rule
10_x	10 consecutive controls on same side of mean	Systematic	Rejection rule

Typical Steps Taken When a Control Is Outside Acceptable Range*

STEP	RATIONALE
Hold patient results until problem is resolved.	If a control value is inaccurate, patient values might be inaccurate.
Rerun control (1 time only).	Value might have been due to expected random error. (1 in 20 results will be outside ±2 SD.)
If control is still out, run a new vial of control or another lot #.	Control might have been outdated, improperly stored, contaminated.
If control is still out, look for & correct any problems, then run control.	Consider reagents (low, outdated, improperly stored, contaminated, change in lot #), preventive maintenance (overdue), mechanical problems, clots, etc.
If control is still out, recalibrate, then run control.	Calibration may have shifted.
If control is still out, get assistance.	Supervisor or service rep may be able to determine problem.
Once resolved, document corrective action.	Provides a record for future reference, points out repetitive problems.
Evaluate all patient results in rejected run & since last run with acceptable QC. Repeat tests & issue corrected reports, as needed.	Ensure accuracy of reported results.

*Follow laboratory's written protocol.

Calibration

TERM	EXPLANATION
Calibration	Process of testing & adjusting analyzer's readout to establish correlation between measured & actual concentrations.
Calibrator	Reference material with known concentration of analyte. Programmed into analyzer's computer for use in calculating concentration of unknowns. Formerly called standard.
Calibration verification	Testing materials of known concentrations (calibrators, controls, proficiency testing samples, patient specimens with known values) to ensure accuracy of results throughout reportable range. Test 3 levels—high, midpoint, & low. Required every 6 months, when lot # of reagents changes, following preventive maintenance or repair, & when controls are out of range.

Test Performance Specifications and Verification

CLIA requires labs to verify manufacturer's analytical performance claims for # 1–4 below on new methods introduced after April 24, 2003.

PARAMETER	EXPLANATION	VERIFICATION
1. Accuracy	How close measurement is to true value.	Lab tests samples of known values (controls, calibrators, proficiency samples, previously tested patient specimens) to see how close results are to known value.
2. Precision	Reproducibility. How close results are when same sample is tested multiple times. (Note: A procedure can be precise but not accurate.)	Lab repeatedly tests same samples (on same day & different days) to see how close the results are.
3. Reportable range	Range of values over which lab can verify accuracy of test system. Also known as linearity.	Lab tests samples with known values at highest & lowest levels claimed to be accurate by manufacturer.
4. Reference interval	Formerly called normal value. Can vary for different patient populations (age, gender, race). Established by testing minimum of 120 healthy subjects & determining range in which 95% fall. (Note: 5% of healthy population falls outside of reference range.) Labs may use manufacturer's reference ranges or published reference ranges, if appropriate for their patient population.	If manufacturer's or published reference ranges are used, lab must test specimens from normal subjects to verify ranges. Ranges may need to be adjusted to fit lab's patient population.

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Test Performance Specifications and Verification *continued*

PARAMETER	EXPLANATION	VERIFICATION
5. Analytical sensitivity	Same as detection limit. Lowest concentration of substance that can be detected by test method. ↑ sensitivity means ↓ false negs. Desirable in screening tests.	Determined by manufacturer. For unmodified FDA-approved tests, verification isn't required.
6. Analytical specificity	Ability of method to measure only analyte it's supposed to measure & not other related substances. ↑ specificity means ↓ false pos, ↓ cross-reactivity. Desirable in confirmatory tests.	Determined by manufacturer. For unmodified FDA-approved tests, verification isn't required.

Diagnostic Value of a Test

CHARACTERISTIC	EXPLANATION
True positive (TP)	Pos result in patient who has the disease
False positive (FP)	Pos result in patient who doesn't have the disease
True negative (TN)	Neg result in patient who doesn't have the disease
False negative (FN)	Neg result in patient who does have the disease
Diagnostic sensitivity *	% of population with the disease that test pos $TP / (TP + FN) \times 100$
Diagnostic specificity *	% of population without the disease that test neg $TN / (TN + FP) \times 100$
Positive predictive value * (PPV)	% of time that a pos result is correct $TP / (TP + FP) \times 100$
Negative predictive value * (NPV)	% of time that a neg result is correct $TN / (TN + FN) \times 100$

*Values are determined by manufacturer. Information helps physicians interpret results.

Other Components of a QA Program

ACTIVITY	EXPLANATION
Correlation study	Study to verify accuracy of new method. Split patient samples are analyzed by existing method & new method. Requires a minimum of 40 patient samples representing wide range of concentrations. Reference values (existing method) are plotted on *x* axis, values from new method on *y* axis. Perfect correlation is straight line passing through zero at 45° angle. The correlation coefficient (*r*) can be derived mathematically. Values range from -1 to $+1$. $0 =$ no correlation between methods. $+1 =$ perfect direct correlation. $\geq 0.95 =$ excellent correlation.
Preventive maintenance	Schedule of maintenance to keep equipment in peak operating condition. Maintenance must be documented & must follow manufacturer's specifications & frequencies.
Function checks	Procedures specified by manufacturer to evaluate critical operating characteristics of test system, e.g., stray light, background counts. Must be within manufacturer's established limits before patient testing is conducted. Documentation required.
Delta checks	Comparison of patient data with previous results. Detects specimen mix-up & other errors. When limit is exceeded, must determine if due to medical change in patient or lab error.
Critical values	Test results that indicate a potentially life-threatening situation. List typically includes glucose, Na^+, K^+, total CO_2, Ca^{2+}, Mg^{2+}, phosphorus, total bili (neonates), blood gases. Patient care personnel must be notified immediately. The Joint Commission requires "read-back" policy. Person receiving critical values must record & read back patient's name & critical values. Lab must document person who received information & time of notification.

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Other Components of a QA Program *continued*

ACTIVITY	EXPLANATION
Personnel competency assessment	CLIA requires documentation of competency assessment on hire, at 6 months, & then annually.
Proficiency testing (PT)	Testing of unknowns submitted by outside agency, e.g., CAP. Unknowns must not receive preferential treatment. Results reported to agency, which compares them to results from other labs. CLIA requires all labs performing nonwaived tests (moderate or high complexity) to participate in PT.
Standard operating procedure (SOP)	Set of instructions for methods used in the laboratory. Also known as procedure manual.

CLIA Requirements for Procedure Manuals

A written procedure manual must be available to testing personnel & must include:

1. Requirements for patient preparation, specimen collection, labeling, storage, preservation, transportation, processing, referral & criteria for specimen acceptability & rejection
2. Procedures for microscopic examinations, including detection of inadequately prepared slides
3. Step-by-step performance of the procedure, including test calculations & interpretation of results
4. Preparation of slides, solutions, calibrators, controls, reagents, stains, & other materials used in testing
5. Calibration & calibration verification procedures
6. Reportable range for patient test results
7. Control procedures
8. Corrective action when calibration or control results fail to meet lab's criteria for acceptability
9. Limitations in methodology, including interfering substances
10. Reference intervals
11. Imminently life-threatening laboratory results (critical values)
12. Pertinent literature references
13. System for entering results in patient record & reporting (including protocol for critical values)
14. Action to take if test system becomes inoperable

Manufacturer's instructions may be used for #1–12. Procedures & changes must be approved, signed, & dated by director before use. Copies of procedures must be retained for 2 yr after discontinuance & must include dates of initial use & discontinuance.

TERM	EXPLANATION
Confidentiality	The right of a patient to have his/her medical information kept private. Restriction of access to information to those who have authorization and a need to know.
Protected health information (PHI)	All individually identifiable health information, including lab results. HIPAA requires health-care providers to establish extensive security measures to ensure privacy. Unauthorized disclosure of medical information could lead to charges of breach of confidentiality or invasion of privacy.
Invasion of privacy	The wrongful intrusion into a person's private affairs, e.g., unauthorized disclosure of confidential medical information.
Informed consent	Consent for a medical procedure given by patient after procedure & possible risks have been explained. May be expressed or implied.
Expressed consent	Written or verbal consent.
Implied consent	Consent that is inferred from action or signs; e.g., a patient sits in phlebotomy chair & extends arm. Action implies consent for venipuncture.
Informed refusal	Patients have right to refuse medical treatment/procedures.
Negligence	Violation of duty to exercise reasonable skill & care.
Malpractice	Misconduct or negligence that results in injury to patient.

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Ethical and Legal Issues *continued*

TERM	EXPLANATION
Assault & battery	Touching another person without his/her consent. Drawing blood against a patient's wishes could lead to charges of assault & battery.
Chain of custody	Procedure to guarantee integrity of specimen to court, e.g., legal blood alcohol, drug test. Each person handling specimen must sign chain-of-custody form that accompanies specimen & documents custody of specimen at all times. Specimen may be transported in locked box to prevent tampering.

Commonly Used Prefixes in the Metric System

PREFIX	DEFINITION
Deci-	10^{-1}
Centi-	10^{-2}
Milli-	10^{-3}
Micro-	10^{-6}
Nano-	10^{-9}
Pico-	10^{-12}
Femto-	10^{-15}

CALCULATION	EXAMPLE
$F = 1.8°C + 32$	If the temperature of a refrigerator is 4°C, what is the temperature in °F? $°F = (1.8 \times °C) + 32 = 39.2$ If the room temperature is 73°F, what is the temperature in °C? $73 = 1.8°C + 32$ $°C = \dfrac{73 - 32}{1.8} = 22.8$
$\text{Mean} = \Sigma x / n$	Σ = sum, x = individual values, n = number of values
Standard Deviation (SD) $= \sqrt{\dfrac{\Sigma(\bar{x} - x)^2}{n - 1}}$	Σ = sum, x = individual value, $\bar{x}$ = mean, n = number of values
Coefficient of variation (%) $= \dfrac{SD}{\text{Mean}} \times 100$	What is the CV for a procedure whose mean is 100 and whose standard deviation is 3? $CV = \dfrac{3}{100} \times 100 = 3\%$

continued...

CALCULATION	EXAMPLE
$\text{Dilution} = \dfrac{\text{Vol. of specimen}}{\text{Vol. of specimen} + \text{vol. of diluent}}$	What is the dilution if 0.1 mL of serum is diluted with 0.4 mL of saline? $\dfrac{0.1}{0.1 + 0.4} = \dfrac{0.1}{0.5} = \dfrac{1}{5}$ How would you prepare a 1:10 dilution of urine? 1 part of urine + 9 parts of diluent
Correcting for a dilution: Value obtained for diluted specimen $\times$ reciprocal of dilution	A specimen for glucose is diluted 1:5. The value of the diluted specimen is 100 mg/dL. What value should be reported? $100\ \text{mg/dL} \times 5 = 500\ \text{mg/dL}$

SECTION 2

Clinical Chemistry Review

Comparison of Conventional and SI Units for Selected Reference Ranges

ANALYTE	CONVENTIONAL UNITS	SI UNITS
Bilirubin, total	0.2–1 mg/dL	3.4–17.1 μmol/L
BUN	6–20 mg/dL	2.1–7.1 mmol/L
Calcium, total	8.6–10 mg/dL	2.15–2.5 mmol/L
Chloride	98–107 mEq/L	98–107 mmol/L
Creatinine	M: 0.9–1.2 mg/dL, F: 0.6–1.1 mg/dL	M: 80–106 mmol/L, F: 53–97 mmol/L
Glucose, fasting	70–99 mg/dL	3.9–5.5 mmol/L
Potassium	3.5–5.1 mEq/L	3.5–5.1 mmol/L
Sodium	136–145 mEq/L	136–145 mmol/L
Total protein	6.4–8.3 g/dL	64–83 g/L
Uric acid	M: 3.5–7.2 mg/dL, F: 2.6–6 mg/dL	M: 208–428 μmol/L, F: 155–357 μmol/L

SI = Système International d'Unités (international system of units).

Examples of Patient Variables That May Affect Chemistry Values

VARIABLE	ANALYTES AFFECTED
Diurnal variation	↑ in am: ACTH, cortisol, iron ↑ in pm: growth hormone, PTH, TSH
Day-to-day variation	≥20% for ALT, bili, CK, steroid hormones, triglycerides
Recent food ingestion	↑ glucose, insulin, gastrin, triglycerides, Na^+, uric acid, iron, LD, Ca^{2+}; ↓ chloride, phosphate, K^+ Fasting required: fasting glucose, triglycerides, lipid panel
Alcohol	↓ glucose; ↑ triglycerides, GGT
Posture	↑ albumin, cholesterol, Ca^{2+} when standing
Activity	↑ in ambulatory patients: creatinine kinase (CK) ↑ with exercise: K^+, phosphate, lactic acid, creatinine, protein, CK, AST, LD
Stress	↑ ACTH, cortisol, catecholamines
Age, gender, race, drugs	Various

Examples of Preanalytical Factors That May Affect Chemistry Results

FACTOR	EFFECT
Use of isopropyl alcohol wipes to disinfect venipuncture site	Can compromise blood alcohol determination
Squeezing site of capillary puncture	↑ K^+
Pumping fist during venipuncture	↑ K^+, lactic acid, Ca^{2+}, phosphorus; ↓ pH
Tourniquet >1 min	↑ K^+, total protein, lactic acid
IV fluid contamination	↑ glucose, K^+, Na^+, Cl^- (depending on IV). Possible dilution of other analytes.
Incorrect anticoagulant or contamination from incorrect order of draw	K_2EDTA: ↓ Ca^{2+}, Mg^{2+}; ↑ K^+ Sodium heparin: ↑ Na^+ if tube not completely filled Lithium heparin: ↑ lithium Gels: Some interfere with trace metals & certain drugs
Hemolysis	↑ K^+, Mg^{2+}, phosphorus, LD, AST, iron, ammonia (May be method dependent. Refer to reagent package inserts.)
Exposure to light	↓ bilirubin, carotene
Temperature between collection & testing	Chilling required for lactic acid, ammonia, blood gases

continued...

Examples of Preanalytical Factors That May Affect Chemistry Results *continued*

FACTOR	EFFECT
Inadequate centrifugation	Poor barrier formation in gel tubes can result in ↑ K^+, LD, AST, iron, phosphorus
Recentrifugation of primary tubes	Hemolysis, ↑ K^+
Delay in separating serum/plasma (unless gel tube is used)	↑ ammonia, lactic acid, K^+, Mg^{2+}, LD ↓ glucose (unless collected in fluoride)
Storage temperature	↓ at RT: glucose (unless collected in fluoride) ↑ at RT: lactic acid, ammonia ↓ at 4°C: LD ↑ at 4°C: ALP

Differences in Analyte Concentrations

DIFFERENCE	ANALYTE(S)
Higher in plasma than serum	Total protein, LD, Ca^{2+}
Higher in serum than plasma	K^{+}, phosphate, glucose, CK, bicarbonate, ALP, albumin, AST, triglycerides
Higher in plasma than whole blood	Glucose
Higher in capillary blood than venous blood	Glucose (in postprandial specimen), K^{+}
Higher in venous blood than capillary blood	Ca^{2+}, total protein
Higher in RBCs than plasma	K^{+}, phosphate, Mg^{2+}
Higher in plasma than RBCs	Na^{+}, chloride

Photometric Methods

METHOD	PRINCIPLE	COMPONENT PARTS	OTHER
Spectrophotometry	Chemical rxn produces colored substance that absorbs light of a specific wavelength. Amount of light absorbed is directly proportional to concentration of analyte.	Light source (tungsten lamp for visible range, deuterium lamp for UV), monochromator (diffraction grating), cuvette, photodetector, readout device	One of most common methods. Used for many routine chemistry assays. A = 2-log % T.
Atomic absorption spectrophotometry	Measures light absorbed by ground-state atoms.	Hollow cathode lamp, atomizer, flame, mixing chamber, chopper, monochromator, detector, readout device	Hollow cathode lamp with cathode made of analyte produces wavelength specific for analyte. Sensitive. Used to measure trace metals.
Fluorometry	Atoms absorb light of specific wavelength & emit light of longer wavelength (lower energy).	Light source (mercury or xenon arc lamp), primary monochromator, sample holder (quartz cuvettes), secondary monochromator, detector, readout device	Detector at 90° to light source so that only light emitted by sample is measured. More sensitive than colorimetry. Used to measure drugs, hormones.

continued...

Photometric Methods *continued*

METHOD	PRINCIPLE	COMPONENT PARTS	OTHER
Chemiluminescence	Chemical rxn that produces light. Usually involves oxidation of luminol, acridinium esters, or dioxetanes.	Reagent probes, sample & reagent cuvette, photomultiplier tube, readout device	Doesn't require excitation radiation or monochromators like fluorometry. Extremely sensitive. Used for immunoassays.
Turbidimetry	Measures reduction in light transmission by particles in suspension.	Light source, lens, cuvette, photodetector, readout device	Used to measure proteins in urine & CSF.
Nephelometry	Similar to turbidity, but light is measured at angle from light source.	Light source, collimator, monochromator, cuvette, photodetector, readout device	Used to measure ag-ab rxn.

Visible Light

WAVELENGTH (nm)	COLOR ABSORBED	COLOR TRANSMITTED (COLOR SEEN)
350–430	Violet	Yellow
430–475	Blue	Orange
475–495	Blue-green	Red-orange
495–505	Green-blue	Orange-red
505–555	Green	Red
555–575	Yellow-green	Violet-red
575–600	Yellow	Violet
600–650	Orange	Blue
670–700	Red	Green

Wavelengths Used in Spectrophotometry

WAVELENGTH (nm)	RANGE	COMMON LIGHT SOURCE	CUVETTE
220–380	Near-ultraviolet	Deuterium or mercury arc	Quartz (silica)
380–750	Visible	Incandescent tungsten or tungsten-iodide	Borosilicate
750–2,000	Near-infrared	Incandescent tungsten or tungsten-iodide	Quartz (silica)

Chromatography*

TYPE	COMPONENTS	USE	OTHER
Thin-layer chromatography (TLC)	Sorbent-coated glass or plastic plate, closed container, solvent	Screening test for drugs of abuse in urine	Substances identified by R_f value (distance traveled by compound/ distance traveled by solvent).
High-performance liquid chromatography (HPLC)	Solvent, pump, injection port, column, detector, recorder	Separation of thermolabile compounds	Concentration determined by peak height ratio (height of analyte peak/height of internal standard peak). Mass spectrometry (MS) can be used as detector for definitive ID (LC/MS).
Gas chromatography (GC)	Gas, injection port, column, oven, detector, recorder	Separation of volatile compounds or compounds that can be made volatile, e.g., therapeutic & toxic drugs	Compounds identified by retention time. Area of peak is proportional to concentration. MS can be used as detector for definitive ID (GC/MS).

*Separation of compounds based on differential distribution between mobile phase & stationary phase.

Other Analytic Techniques

METHOD	PRINCIPLE	COMPONENT PARTS	USE
Ion-selective electrodes	Potential difference between 2 electrodes directly related to concentration of analyte.	Reference electrode, indicator electrode, liquid junction, measuring device	pH, Pco_2, Po_2, Na^+, K^+, Ca^{2+}, Li^+, Cl^-
Osmometry	Determines osmolality (measurement of # of dissolved particles in solution, irrespective of molecular weight, size, density, or type) based on freezing-point depression. (Vapor depression osmometers not widely used in clinical labs. Don't measure volatile solutes.)	Cooling bath, thermistor probe, stirring wire, galvanometer	Serum & urine osmolality
Electrophoresis	Separation of charged particles in electrical field. Anions move to positively charged pole (anode); cations to negatively charged pole (cathode). The greater the charge, the faster the migration.	Power supply, support medium, buffer, stain, densitometer	Serum protein electrophoresis, hemoglobin electrophoresis

Steps in Automated Analysis

STEP	COMMENTS
Sample ID	Usually by bar code reader
Test selection	Usually communicated by LIS
Sampling	Usually closed-tube sampling from primary collection tubes. Some analyzers have short sample & clot detection
Reagent delivery	Usually by syringes, pumps, or pressurized reagent bottles. Vitros uses dry slides. Some offer reagent inventory
Chemical reaction	Mixing & incubation
Measurements	Visible & UV spectrophotometry, ion selective electrodes, fluorescence polarization, chemiluminescence, bioluminescence. Most offer automatic dilution & retesting when linearity is exceeded
Data handling	Concentration derived from calibration curve stored in analyzer
Reporting	Usually reported to LIS through interface
Troubleshooting	Can be done remotely by modem on many analyzers

Chemistry Panels

PANEL	TESTS
Basic metabolic panel	Na^+, K^+, chloride, CO_2, glucose, creatinine, BUN, Ca^{2+}
Comprehensive metabolic panel	Na^+, K^+, chloride, CO_2, glucose, creatinine, BUN, albumin, total protein, ALP, AST, bilirubin, Ca^{2+}
Electrolyte panel	Na^+, K^+, Cl^-, CO_2
Hepatic function panel	Albumin, ALT, AST, ALP, bilirubin (total & direct), total protein
Lipid panel	Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides
Renal function panel	Na^+, K^+, CO_2, glucose, creatinine, BUN, Ca^{2+}, albumin, phosphate

ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Glucose, fasting	70–99 mg/dL	↑ (hyperglycemia): diabetes mellitus, other endocrine disorders, acute stress, pancreatitis ↓ (hypoglycemia): insulinoma, insulin-induced hypoglycemia, hypopituitarism	Major source of cellular energy. Levels ↓ at RT. Use sodium fluoride to prevent glycolysis. Glucose oxidase & hexokinase are most common methods. Hexokinase considered more accurate, fewer interfering substances.
Cholesterol, total	Desirable: <200 mg/dL	Limited value for predicting risk of coronary artery disease (CAD) by itself. Used in conjunction with HDL & LDL cholesterol	Enzymatic methods most common.
HDL cholesterol	Desirable: ≥60 mg/dL	Appears to be inversely related to CAD	Homogeneous assays don't require pretreatment to remove non-HDL. 1st reagent blocks non-HDL, 2nd reacts with HDL.
LDL cholesterol	Optimal: <100 mg/dL	Risk factor for CAD	May be calculated from Friedewald formula (if triglycerides not >400 mg/dL) or measured by direct homogeneous assays.
Triglycerides	Desirable: <150 mg/dL	Risk factor for CAD	Main form of lipid storage. Enzymatic methods using lipase. Requires fasting specimen.

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ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Total protein	6.4–8.3 g/dL	↑ dehydration, chronic inflammation, multiple myeloma ↓ nephrotic syndrome, malabsorption, overhydration, hepatic insufficiency, malnutrition, agammaglobulinemia	<4.5 g/dL associated with peripheral edema. Biuret method. Alkaline copper reagent reacts with peptide bonds.
Albumin	3.5–5 g/dL	↑ dehydration ↓ malnutrition, liver disease, nephrotic syndrome, chronic inflammation	Largest fraction of plasma proteins. Synthesized by liver. Regulates osmotic pressure. Measure by dye binding, e.g., bromocresol green (BCG), bromocresol purple (BCP).
Microalbumin (on urine)	50–200 mg/24 hr predictive of diabetic nephropathy	↑ in diabetics at risk of nephropathy	Detects albumin in urine earlier than dipstick protein. Strict control of glucose & blood pressure can prevent progression to end-stage renal disease. Immunoassays on 24-hr urine. Alternative is albumin-to-creatinine ratio on random sample. 30–300 mg albumin/g creatinine = microalbuminuria. Urine dipsticks available for albumin & albumin-to creatinine ratio.

Regulation of Glucose

HORMONE	ACTION	RELATIVE IMPORTANCE
Decreases glucose levels		
Insulin	Responsible for entry of glucose into cells. Increases glycogenesis.	Primary
Increases glucose levels		
Glucagon	Stimulates glycogenolysis & gluconeogenesis. Inhibits glycolysis.	Primary
Cortisol	Insulin antagonist. Increases gluconeogenesis.	Secondary
Epinephrine	Promotes glycogenolysis & gluconeogenesis.	Secondary
Growth hormone	Insulin antagonist.	Secondary
Thyroxine	Increases glucose absorption from GI tract. Stimulates glycogenolysis.	Negligible

TYPE	CAUSE	CHARACTERISTICS
Type 1 (formerly type I juvenile-onset diabetes, insulin-dependent diabetes mellitus)	Autoimmune destruction of beta cells. Absolute insulin deficiency. Genetic predisposition (HLA-DR 3/4).	Acute onset. Most develop before age 25 yr. Dependency on injected insulin. Prone to ketoacidosis & diabetic complications.
Type 2 (formerly type II, adult-onset diabetes, non–insulin-dependent diabetes mellitus)	Insulin resistance in peripheral tissue. Insulin secretory defect of beta cells. Associated with obesity.	Most common type. Usual onset was after age 40 yr but being seen in obese youth. Not dependent on exogenous insulin. Not prone to ketoacidosis or diabetic complications.
Gestational diabetes mellitus (GDM)	Placental lactogen inhibits action of insulin.	Usually Dx during latter half of pregnancy. Some develop type 2 diabetes years later. Risk of intrauterine death or neonatal complications (macrosomia, hypoglycemia, hypocalcemia, polycythemia, hyperbilirubinemia).

Tests for Diabetes Mellitus

TEST	PATIENT PREPARATION	DIABETES MELLITUS	COMMENTS
Random plasma glucose	None	≥200 mg/dL	Collected any time of day without regard to time since last meal. Only for use in patients with symptoms of hyperglycemia.
Fasting plasma glucose (FPG)	Fast of at least 8 hr	≥126 mg/dL on 2 occasions	
2-hr plasma glucose	75-g glucose load	≥200 mg/dL on 2 occasions	
Oral glucose tolerance test (OGTT)	Fast of at least 8 hr; 75-g glucose load	Fasting ≥92 mg/dL, or 1 hr ≥180, or 2 hr ≥153	Only for Dx of gestational diabetes mellitus. Performed at 24–28 wk of gestation.
Hemoglobin A_{1c}	None; fasting not required	≥6.5%	Gives estimate of glucose control over previous 2–3 months. Originally only used to monitor therapy. Now accepted for Dx except in patients with hemoglobinopathies or abnormal RBC turnover. Should be performed using method certified by National Glycohemoglobin Standardization Program. Point-of-care assays currently not accurate enough for diagnosis.

Typical Laboratory Findings in Uncontrolled Diabetes Mellitus

INCREASED	DECREASED
Blood glucose	Bicarbonate
Urine glucose	Blood pH
Urine specific gravity	
Glycohemoglobin	
Ketones (blood & urine)	
Anion gap	
BUN	
Osmolality (serum & urine)	
Cholesterol	
Triglycerides	

Metabolic Syndrome

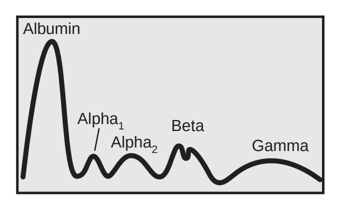
Definition	Group of risk factors that seem to promote development of atherosclerotic cardiovascular disease & type 2 diabetes mellitus
Risk factors	↓ HDL-C ↑ LDL-C ↑ triglycerides ↑ blood pressure ↑ blood glucose

DISEASE	CAUSE	EFFECT	DIAGNOSIS
Phenylketonuria	Deficiency of enzyme that converts phenylalanine to tyrosine. Phenylpyruvic acid in blood & urine.	Mental retardation. Urine has “mousy” odor.	Guthrie bacterial inhibition assay, HPLC, tandem mass spectrometry (MS/MS), fluorometric & enzymatic methods. All newborns are screened
Tyrosinemia	Disorder of tyrosine catabolism. Tyrosine & its metabolites are excreted in urine.	Liver & kidney disease, death.	MS/MS
Alkaptonuria	Deficiency of enzyme needed in metabolism of tyrosine & phenylalanine. Buildup of homogentisic acid.	Diapers stain black due to homogentisic acid in urine. Later in life, darkening of tissues, hip & back pain.	Gas chromatography & mass spectroscopy
Maple syrup urine disease (MSUD)	Enzyme deficiency leading to buildup of leucine, isoleucine, valine.	Burnt-sugar odor to urine, breath, skin. Failure to thrive, mental retardation, acidosis, seizures, coma, death.	Modified Guthrie test, MS/MS
Homocystinuria	Deficiency in enzyme needed for metabolism of methionine. Methionine & homocysteine build up in plasma & urine.	Osteoporosis, dislocated lenses in eye, mental retardation, thromboembolic events.	Guthrie test, MS/MS, LC-MS/MS
Cystinuria	Increased excretion of cystine due to defect in renal reabsorption.	Recurring kidney stones.	Test urine with cyanide nitroprusside. Pos = red-purple color

Protein Electrophoresis

Rate of migration	Depends on size, shape, & charge of molecule
Support medium	Cellulose acetate or agarose
Buffer	Barbital buffer, pH 8.6
Stains	Ponceau S, amido blue, bromphenol blue, Coomassie brilliant blue
Charge	At pH 8.6, proteins are negatively charged & move toward anode
Order of migration (fastest to slowest)	Albumin, alpha-1 globulin, alpha-2 globulin, beta globulin, gamma globulin
Largest fraction	Albumin
Electroendosmosis	Buffer flow toward cathode. Causes gamma region to be cathodic to point of application
Urine	Must be concentrated first because of low protein concentration. Bence Jones proteins migrate to gamma region in urine electrophoresis
CSF	Must be concentrated first because of low protein concentration. CSF has a prealbumin band

Common Serum Protein Electrophoresis Patterns

CONDITION	PATTERN
Normal	 Serum protein electrophoresis showing patterns of normal serum. *(From Ciesla B. Hematology in Practice, 2nd ed. Philadelphia: FA Davis; 2012:212.)*
Acute inflammation	↑ alpha-1 & alpha-2
Chronic infection	↑ alpha-1, alpha-2, & gamma
Cirrhosis	Polyclonal ↑ (all fractions) in gamma with beta-gamma bridging
Monoclonal gammopathy	Sharp ↑ in 1 immunoglobulin (“M spike”). ↓ in other fractions

continued…

Common Serum Protein Electrophoresis Patterns *continued*

CONDITION	PATTERN
Polyclonal gammopathy	Diffuse ↑ in gamma
Hypogammaglobulinemia	↓ gamma
Nephrotic syndrome	↓ albumin, ↑ alpha-2
Alpha-1-antitrypsin deficiency	↓ alpha-1
Hemolyzed specimen	↑ beta or unusual band between alpha-2 & beta
Plasma	Extra band (fibrinogen) between beta & gamma

ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
BUN	8–26 mg/dL	↑ kidney disease ↓ overhydration or liver disease	Synthesized by liver from ammonia. Excreted by kidneys. Urease reagent. Don't use sodium fluoride, EDTA, citrate, or ammonium heparin. Test isn't sensitive. Dilute urine 1:20 or 1:50 & refrigerate or acidify.
Creatinine	0.7–1.5 mg/dL	↑ kidney disease	Waste product from dehydration of creatine (mainly in muscles). Jaffe's reaction (alkaline picrate) is nonspecific. Enzymatic methods are more specific. Tests aren't sensitive. Normal BUN: creatinine ratio = 12–20. Dilute urine 1:100.
Uric acid	M: 3.5–7.2 F: 2.6-6 mg/dL	↑ gout, renal failure, ketoacidosis, lactate excess, high nucleoprotein diet, leukemia, lymphoma, polycythemia ↓ administration of ACTH, renal tubular defects	Increased = risk of renal calculi & joint tophi. Uricase method. EDTA & fluoride interfere. Adjust urine pH to 7.5–8 to prevent precipitation.
Ammonia	19–60 µg/dL	↑ liver disease, hepatic coma, renal failure, Reye's syndrome	Produced in GI tract. High levels are neurotoxic. Collect in EDTA or heparin. Serum may cause ↑ levels as NH_3 is generated during clotting. Chill immediately. Analyze ASAP. Avoid contamination from ammonia in detergents or water.

Major Electrolytes

ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Sodium (Na^+)	136–145 mmol/L	↑ (hypernatremia): Due to ↑ intake or IV administration, hyperaldosteronism, excessive sweating, burns, diabetes insipidus. Causes tremors, irritability, confusion, coma. ↓ (hyponatremia): Due to renal or extrarenal loss (vomiting, diarrhea, sweating, burns) or ↑ extracellular fluid volume. Causes weakness, nausea, altered mental status.	Major extracellular cation. Contributes almost half to plasma osmolality. Maintains normal distribution of water & osmotic pressure. Levels regulated by aldosterone. Ion-selective electrode (ISE) is most common method. Normal Na^+/K^+ ratio in serum approximately 30:1.
Potassium (K^+)	3.5–5.1 mmol/L	↑ (hyperkalemia): Due to ↑ intake, ↓ excretion, crush injuries, metabolic acidosis. Can cause muscle weakness, confusion, cardiac arrhythmia, cardiac arrest. ↓ (hypokalemia): Due to ↑ GI or urinary loss, use of diuretics, metabolic alkalosis. Can cause muscle weakness, paralysis, breathing problems, cardiac arrhythmia, death.	Major intracellular cation. Artifactual ↑ due to squeezing site of capillary puncture, prolonged tourniquet, pumping fist during venipuncture, contamination with IV fluid, hemolysis, prolonged contact with RBCs, leukocytosis, thrombocytosis. Serum values 0.1–0.2 mmol/L higher than plasma due to release from platelets during clotting. Most common method is ISE with valinomycin membrane.

continued...

Major Electrolytes *continued*

ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Chloride (Cl^-)	98–107 mmol/L	↑ (hyperchloremia): Due to same conditions as ↑ Na^+ & excess loss of HCO_3^-. ↓ (hypochloremia): From prolonged vomiting, diabetic ketoacidosis, aldosterone deficiency, salt-losing renal diseases, metabolic alkalosis, compensated respiratory acidosis	Major extracellular ion. Helps maintain osmolality, blood volume, electric neutrality. Passively follows Na^+. Most common method is ISE. Sweat chloride test for Dx of cystic fibrosis.
CO_2, total	23–29 mmol/L	↑ in metabolic alkalosis, compensated respiratory acidosis ↓ in metabolic acidosis, compensated respiratory alkalosis	>90% is bicarbonate (HCO_3^-); remainder is carbonic acid (H_2CO_3) & dissolved CO_2. HCO_3^- important in maintaining acid-base balance. Keep sample capped to prevent loss of CO_2. Measured by ISE or enzymatic method.

Other Electrolytes

ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Magnesium (Mg^{2+})	1.6–2.6 mg/dL	↑ due to renal failure, ↑ intake (e.g., antacids), dehydration, bone cancer, endocrine disorders. Can cause cardiac abnormalities, paralysis, respiratory arrest, coma. ↓ due to severe illness, GI disorders, endocrine disorders, renal loss. Can lead to cardiac arrhythmias, tremors, tetany, paralysis, psychosis, coma. Rare in non-hospitalized patients.	Essential cofactor for many enzymes. 10× more concentrated in RBCs. Avoid hemolysis. EDTA, citrate, oxalate bind Mg^{2+}. Colorimetric methods are most common.
Calcium (Ca^{2+})	Total: 8.6–10 mg/dL Ionized: 4.60–5.08 mg/dL	↑ with primary hyperparathyroidism, cancer, multiple myeloma. Can cause weakness, coma, GI symptoms, renal calculi. ↓ with hypoparathyroidism, malabsorption, vitamin D deficiency, renal tubular acidosis. Leads to tetany (muscle spasms), seizures, cardiac arrhythmias.	Most abundant mineral in body. 99% in bones. Regulated by parathyroid hormone (PTH), vitamin D, calcitonin. Anticoagulants other than heparin bind Ca^{2+}. Colorimetric methods for total Ca^{2+}. Ionized (free) Ca^{2+} is biologically active form, better indicator of Ca^{2+} status. Measured by ISE. Affected by pH & temp.

continued...

ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Phosphorus, inorganic (phosphate)	2.5–4.5 mg/dL	↑ with renal disease, hypoparathyroidism. ↓ with hyperparathyroidism, vitamin D deficiency, renal tubular acidosis.	Major intracellular anion. Mostly in bones. Component of nucleic acids, many coenzymes. Important reservoir of energy (ATP). Limited value alone. Should be correlated with Ca^{2+} (normally reciprocal relationship). Higher in children. Citrate, oxalate, EDTA interfere. More in RBCs than plasma. Avoid hemolysis. Separate promptly.
Lactate (lactic acid)	4.5–19.8 mg/dL	Sign of ↓ 0_2 to tissues.	By product of anaerobic metabolism. Best not to use tourniquet. Patient shouldn't make fist. Collect in heparin & put on ice or use fluoride to inhibit glycolysis. Enzymatic methods.

Iron and Related Tests

ANALYTE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Iron	M: 65–175 F: 50–170 µg/dL	↑ iron overdose, hemochromatosis, sideroblastic anemia, hemolytic anemia, liver disease ↓ iron deficiency anemia	Necessary for hgb synthesis. Transported by transferrin. Hemolysis interferes. Oxalate, citrate, & EDTA bind iron. Early-morning specimen preferred because of diurnal variation. Colorimetric methods.
Total iron binding capacity (TIBC)	250–425 µg/dL	↑ iron deficiency anemia ↓ iron overdose, hemochromatosis	Iron added to saturate transferrin. Excess removed. Iron content determined.
% saturation or transferrin saturation	20%–50%	↑ iron overdose, hemochromatosis, sideroblastic anemia ↓ iron deficiency anemia	Calculated value. 100× serum iron/TIBC.
Transferrin	200–360 mg/dL	↑ iron deficiency anemia ↓ iron overdose, hemochromatosis, chronic infections, malignancies	Complex of apotransferrin (protein that transports iron) & iron. Immunoassay.
Ferritin	M: 20–250 F: 10–120 µg/L	↑ iron overload, hemochromatosis, chronic infections, malignancies ↓ iron deficiency anemia	Storage form of iron. Rough estimate of body iron content. Immunoassay.

Factors That Influence Enzymatic Reactions

FACTOR	EXPLANATION	OTHER
Substrate concentration	First-order kinetics: [enzyme] > [substrate]. Reaction rate proportional to [substrate]. Zero-order kinetics: [substrate] > [enzyme]. Reaction rate proportional to [enzyme].	Assays are zero-order (excess substrate).
Enzyme concentration	Velocity of rxn proportional to [enzyme] as long as [substrate] > [enzyme].	Unit of measure is international unit (IU). Amount of enzyme that will catalyze 1 μmol of substrate per min under standardized conditions.
pH	Extremes of pH may denature enzymes.	Most rxn occur at pH 7–8. Use buffers to maintain optimal pH.
Temperature	Increase of 10°C doubles rate of rxn until around 40°–50°C; then denaturation of enzyme may occur.	37°C is most commonly used in U.S.
Cofactors	Nonprotein molecules that participate in rxn. Must be present in excess. Inorganic cofactors (e.g., Cl^-, Mg^{2+}) called activators. Either required for or enhance rxn. Organic cofactors (e.g., nicotinamide adenine dinucleotide) called coenzymes. May serve as 2nd substrate in rxn.	Rxn commonly used in enzyme determinations: Nicotinamide adenine dinucleotide (NAD) ⇔ nicotinamide adenine dinucleotide, reduced form (NADH). NADH has absorbance at 340 nm; NAD does not.
Inhibitors	Interfere with rxn.	

Enzymes of Clinical Significance

ENZYME	TISSUE(S)	CLINICAL SIGNIFICANCE	OTHER
Acid phosphatase (ACP)	Prostate	↑ in prostate cancer	Limited value today. PSA is more specific.
Alkaline phosphatase (ALP)	Almost all	↑ liver & bone disease. Levels higher in biliary tract obstruction than in hepatocellular disorders (hepatitis, cirrhosis)	↑ in children, adolescents, pregnant women, & with healing bone fractures. Optimum pH = 9–10.
Aspartate aminotransferase (AST)	Many. Highest in liver, heart, skeletal muscle.	↑ with liver disease (marked ↑ with viral hepatitis), acute myocardial infarction (AMI), muscular dystrophy	Avoid hemolysis.
Alanine aminotransferase (ALT)	Liver, RBCs	↑ with liver disease	More specific for liver disease than AST. Marked ↑ with viral hepatitis.
Gamma glutamyl transferase (GGT)	Liver, kidneys, pancreas	↑ in all hepatobiliary disorders, chronic alcoholism	Most sensitive enzyme for all types of liver disease. Highest levels with obstructive disorders. Treatment centers use to monitor abstention from alcohol.

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ENZYME	TISSUE(S)	CLINICAL SIGNIFICANCE	OTHER
Lactate dehydrogenase (LD)	All. Highest in liver, heart, skeletal muscle, RBCs	↑ with AMI, liver disease, pernicious anemia	Catalyzes lactic acid ⇔ pyruvic acid. Avoid hemolysis. Unstable. Store at 25°C, not 4°C. Highest levels with pernicious anemia. Some anticoagulants interfere.
Creatine kinase (CK)	Cardiac muscle, skeletal muscle, brain	↑ with AMI, muscular dystrophy	Catalyzes phosphocreatine + ADP ⇔ creatine + ATP. Most sensitive enzyme for skeletal muscle disease. Highest levels with muscular dystrophy. Inhibited by all anticoagulants except heparin. ↑ with physical activity, IM injections. CK-MB isoenzyme used in Dx of AMI.
Amylase (AMS)	Salivary glands, pancreas	↑ in acute pancreatitis, other abdominal diseases, mumps	Breaks down starch to simple sugars. In acute pancreatitis, levels ↑ 2–12 hr after attack, peak at 24 hr, return to normal in 3–5 days.
Lipase (LPS)	Pancreas	↑ in acute pancreatitis	Breaks down triglycerides into fatty acids & glycerol. Levels usually parallel amylase, but may stay ↑ longer. More specific than amylase for pancreatic disease.
Glucose-6-phosphate dehydrogenase (G6PD)	RBCs	Inherited deficiency can lead to drug-induced hemolytic anemia	Measured in hemolysate of whole blood.

Summary of Diagnostic Enzymology

CARDIAC DISORDERS	HEPATIC DISORDERS	SKELETAL MUSCLE DISORDERS	BONE DISORDERS	ACUTE PANCREATITIS
CK-MB	Hepatocellular disorders: AST, ALT, LD Biliary tract obstruction: ALP, GGT	CK, AST, LD, aldolase	ALP	Amylase, lipase

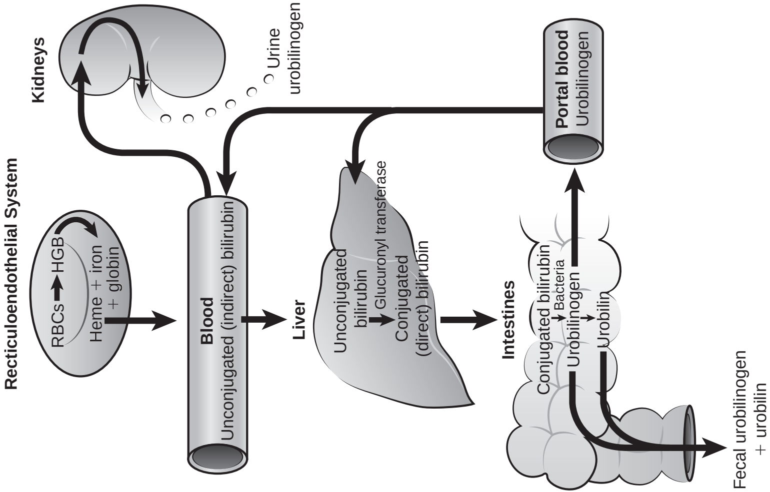
Cardiac Markers for Diagnosis of Acute Myocardial Infarction

	CK-MB	MYOGLOBIN	CARDIAC TROPONINS (cTn)
Elevation after chest pain	4–6 hr	1–4 hr	4–10 hr
Duration of elevation	2–3 days	18–24 hr	4–10 days
Sensitivity/specificity	Not entirely specific for AMI	Sensitive but not specific	High sensitivity & specificity
Methods	Immunoassay	Immunoassay	Immunoassay
Comments	Used to be "gold standard." Use declining because of newer tests	Negative predictive marker. If not ↑ within 8 hr of chest pain, AMI ruled out	Considered definitive marker for AMI
Testing recommendations	Use 2 biomarkers—1 that is ↑ within 6 hr (CK-MB or myoglobin) & 1 with high sensitivity & specificity that is ↑ within 6–9 hr & remains elevated for several days (troponin). Draw blood at admission, at 6–9 hr, & at 12–24 hr, if previous results were not ↑		

Other Cardiac Tests

TEST	CLINICAL SIGNIFICANCE
Tests for Heart Failure	
B-type natriuretic peptide (BNP)	Released from heart muscle of left ventricle when fluid builds from heart failure. Acts on kidneys to ↑ excretion of fluid.
Tests to Assess Risk of Coronary Artery Disease (CAD)	
Cardiac C-reactive protein (cCRP)	High-sensitivity CRP (hs-CRP) to ID individuals at risk of cardiovascular disease. Nonspecific marker of inflammation. Best single biomarker for predicting cardiovascular events. Test on 2 occasions because of individual variability. Methods: nephelometry, immunoassay.
Total cholesterol	Limited value for predicting risk of CAD by itself. Used in conjunction with HDL & LDL cholesterol. Desirable: $<$200 mg/dL.*
HDL cholesterol	Inversely related to risk of CAD. Low levels are risk factor. Desirable: $\geq$60 mg/dL.*
LDL cholesterol	Major cause of CAD. Primary target of therapy. Optimal: $<$100 mg/dL.*
Triglycerides	Independent risk factor for CAD. Desirable level: $<$150 mg/dL.*

* National Cholesterol Education Program Adult Treatment Panel III.
Coronary artery disease (CAD, atherosclerosis) = coronary heart disease (CHD).



Normal metabolism of bilirubin.

Types of Bilirubin

TYPE	REFERENCE RANGE	CLINICAL SIGNIFICANCE	OTHER
Total bilirubin	0.2–1 mg/dL	↑ liver disease, hemolysis, hemolytic disease of newborn. In infants, >20 mg/dL associated with brain damage (kernicterus)	Sum of conjugated, unconjugated, & delta bilirubin. Avoid hemolysis. Protect sample from light. Jendrassik-Grof method. Diazo reagent. Accelerator added so conjugated bilirubin reacts. Bilirubinometry for neonates only. Measures reflected light from skin using 2 wavelengths.
Conjugated bilirubin (direct bilirubin)	<0.2 mg/dL	↑ liver disease, obstructive jaundice	Bilirubin monoglucuronide, bilirubin diglucuronide, & delta bilirubin (bound to albumin; only seen with significant hepatic obstruction). Avoid hemolysis. Protect from light. Jendrassik-Grof method. Diazo reagent. No accelerator required.
Unconjugated bilirubin (indirect bilirubin)	<0.8 mg/dL	↑ prehepatic, posthepatic, & some types of hepatic jaundice	Calculated value. Total bili – direct bili.

Unconjugated Versus Conjugated Bilirubin

	UNCONJUGATED BILIRUBIN	CONJUGATED BILIRUBIN
Structure	Bilirubin	Bilirubin monoglucuronide, bilirubin diglucuronide, & delta bilirubin
Bound to protein	Yes (albumin)	No (except delta bilirubin)
Type of compound	Nonpolar	Polar
Soluble in water?	No	Yes
Present in urine?	No	Yes
Reaction with diazotized sulfanilic acid	Indirect (only reacts in presence of accelerator)	Direct (reacts without accelerator)
Affinity for brain tissue	High	Low

Differential Diagnosis of Jaundice

TEST	PREHEPATIC JAUNDICE	HEPATIC JAUNDICE	POSTHEPATIC JAUNDICE
Total bilirubin	↑	↑	↑
Direct bilirubin	N	V	↑
Urine bilirubin	Neg	V	Pos
Urine urobilinogen	↑	↓	↓

N = normal, V = variable.

HORMONE	REGULATES	COMMENTS
Anterior pituitary ACTH	Production of adrenocortical hormones by adrenal cortex	Regulated by corticotropin-releasing hormone (CRH) from hypothalamus. Diurnal variation: highest levels in early am, lowest in late afternoon. ↑ Cushing's disease. Collect on ice. Store frozen.
FSH	Sperm & egg production	Regulated by gonadotropin-releasing hormone (GnRH) from hypothalamus. Sharp ↑ just before ovulation.
Growth hormone (GH)	Protein synthesis, cell growth, & division	Regulated by growth-hormone releasing hormone (GHRH) & somatostatin from hypothalamus. ↑ gigantism, acromegaly. ↓ dwarfism.
LH	Maturation of follicles, ovulation, production of estrogen, progesterone, testosterone	Regulated by GnRH from hypothalamus. Sharp ↑ just before ovulation. Home ELISA kits to detect ovulation.
Prolactin (PRL)	Lactation	Regulated by prolactin-releasing factor (PRF) & prolactin-inhibiting factor (PIF) from hypothalamus.
TSH	Production of T_3 & T_4 by thyroid	Regulated by thyrotropin-releasing hormone (TRH) from hypothalamus. ↑ hypothyroidism. ↓ hyperthyroidism.

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Pituitary Hormones *continued*

HORMONE	REGULATES	COMMENTS
Posterior pituitary		
ADH	Reabsorption of water in distal renal tubules	Produced in hypothalamus. Stored in posterior pituitary. Release stimulated by ↑ osmolality, ↓ blood volume or blood pressure. ↓ in diabetes insipidus.
Oxytocin	Uterine contractions during childbirth, lactation	Produced in hypothalamus. Stored in posterior pituitary. Not clinically useful.

HORMONE	REGULATES	COMMENTS
Thyroid Thyroxine (T_4)	Metabolism, growth, & development	Principle thyroid hormone. 50 × more concentrated than T_3. Contains 4 atoms of I. Regulated by TSH. Most bound to thyroxine-binding globulin (TBG). ↑ in hyperthyroidism, ↓ in hypothyroidism
Triiodothyronine (T_3)	Metabolism, growth, & development	Most formed from deiodination of T_4 by tissues. Contains 3 atoms of I. 4×–5× more potent than T_4. Regulated by TSH. ↑ in hyperthyroidism, ↓ in hypothyroidism
Calcitonin	Inhibition of Ca^{2+} resorption	Important in diagnosis of thyroid cancer
Parathyroid Parathyroid hormone (PTH)	Regulation of Ca^{2+} & phosphate	Primary hyperthyroidism = ↑ PTH, ↑ Ca^{2+}, ↓ phosphate Hypoparathyroidism = ↓ PTH, ↓ Ca^{2+}, ↑ phosphate

TEST	PRIMARY HYPOTHYROIDISM	SECONDARY HYPOTHYROIDISM	HYPERTHYROIDISM	T_3 THYROTOXICOSIS	COMMENTS
Thyroid-stimulating hormone (TSH)	↑	↓	↓	↓	1st test for screening. If normal, no further testing.
Free T_4 (FT_4)	↓	↓	↑	N	Biologically active form of T_4. 2nd step in screening if TSH abnormal.
Free T_3 (FT_3)	↓	↓	↑	↑	Biologically active form of T_3. Usually not helpful in Dx of hypothyroidism because last test to become abnormal. Usually only tested when TSH is ↓ & FT_4 isn't ↑.

Primary hypothyroidism = thyroid insufficiency. Secondary hypothyroidism = pituitary insufficiency. Graves' disease (type of autoimmune disease) is most common cause of hyperthyroidism. Thyroid tests are immunoassays.

Adrenal Hormones

HORMONE	REGULATES	COMMENTS
Adrenal cortex Aldosterone	Reabsorption of Na^+ in renal tubules	↑ causes hypertension due to water & Na^+ retention. ↓ leads to severe water & electrolyte abnormalities.
Cortisol	Carbohydrate, fat, & protein metabolism. Water & electrolyte balance. Suppresses inflammatory & allergic reactions	Regulated by ACTH. Diurnal variation. Highest in am. ↑ and loss of diurnal variation in Cushing's syndrome, ↓ in Addison's disease.
Adrenal medulla Epinephrine, norepinephrine (adrenaline, noradrenaline)	"Fight or flight syndrome." Stimulation of sympathetic nervous system	Epinephrine is primary hormone of adrenal medulla. Epinephrine & norepinephrine = catecholamines. Metabolites are metanephrines & VMA. ↑ with pheochromocytoma (rare catecholamine producing tumor). Tests: plasma & urine catecholamines & metanephrines, urine VMA.

Reproductive Hormones

HORMONE	REGULATES	COMMENTS
Ovaries		
Estrogens	Development of female reproductive organs & secondary sex characteristics. Regulation of menstrual cycle. Maintenance of pregnancy	Estradiol (E_2) is major estrogen produced by ovaries; most potent estrogen. Also produced in adrenal cortex.
Progesterone	Preparation of uterus for ovum implantation, maintenance of pregnancy	Also produced by placenta. Metabolite is pregnanediol. Useful in infertility studies & to assess placental function.
Placenta		
Estrogen (estriol)	No hormonal activity	Used to monitor fetal growth & development.
Progesterone	See above	
HCG	Progesterone production by corpus luteum during early pregnancy. Development of fetal gonads	Used to detect pregnancy, gestational trophoblastic disease (e.g., hydatidiform mole), testicular tumor, & other HCG-producing tumors.
Human placental lactogen (HPL)	Estrogen & progesterone production by corpus luteum. Development of mammary glands	Used to assess placental function.
Testes		
Testosterone	Development of male reproductive organs & secondary sex characteristics	Also produced in adrenal cortex.

Pancreatic Hormones

HORMONE	REGULATES	COMMENTS
Insulin	Carbohydrate metabolism	Produced in beta cells of islets of Langerhans. Causes ↑ movement of glucose into cells for metabolism. Decreases plasma glucose levels. ↓ in diabetes mellitus, ↑ with insulinoma, hypoglycemia.
Glucagon	Glycogenolysis, gluconeogenesis, lipolysis	Produced in alpha cells of islets of Langerhans. Increases plasma glucose levels. ↑ with glucagonoma, diabetes mellitus, pancreatitis, trauma.

Therapeutic Drug Monitoring (TDM)

TERM	EXPLANATION
Minimum effective concentration (MEC)	Lowest concentration of drug in blood that will produce desired effect
Minimum toxic concentration (MTC)	Lowest concentration of drug in blood that will produce adverse response
Therapeutic index	Ratio of MTC to MEC
Trough	Lowest concentration of drug measured in blood. Reached just before next scheduled dose. Shouldn't fall below MEC
Peak	Highest concentration of drug measured in blood. Drawn immediately on achievement of steady state. Should not exceed MTC
Steady state	Amount of drug absorbed & distributed = amount of drug metabolized & excreted. Usually reached after 5–7 half-lives
Half-life	Time required for concentration of drug to be ↓ by half.
Pharmacokinetics	Rates of absorption, distribution, biotransformation, & excretion

Most common methods: immunoassay, chromatography.

Therapeutic Drug Groups

GROUP	REPRESENTATIVE DRUGS
Analgesics	Salicylates, acetaminophen
Antiepileptics	Phenobarbital, phenytoin, valproic acid, carbamazepine, ethosuximide, felbamate, gabapentin, lamotrigine
Antineoplastics	Methotrexate
Antibiotics	Aminoglycosides (amikacin, gentamicin, kanamycin, tobramycin), vancomycin
Cardioactives	Digoxin, disopyramide, procainamide, quinidine
Psychoactives	Tricyclic antidepressants, lithium
Immunosuppressants	Cyclosporine, tacrolimus (FK-506)

Toxic Agents

SUBSTANCE	ANALYTIC METHOD
Ethanol	Gas chromatography, enzymatic methods
Carbon monoxide	Differential spectrophotometry (co-oximeter), gas chromatography
Arsenic	Atomic absorption
Lead	Atomic absorption
Pesticides	Measurement of serum pseudocholinesterase

Drugs of Abuse Urine Screen

Drugs routinely tested	Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, phencyclidine, tricyclic antidepressants
Adulterated urine*	Value outside physiological range or presence of substance that isn't found in human urine, e.g., pH <3 or ≥ 11; nitrite ≥ 500 mg/dL; presence of chromium, halogens (bleach, iodine, fluoride), glutaraldehyde, pyridine, or surfactant
Substituted urine	Values that aren't consistent with normal human urine, e.g., creatinine <2 mg/dL & specific gravity ≤ 1.0010 or ≥ 1.0200
Diluted urine	Creatinine & specific gravity lower than expected for normal human urine, e.g., creatinine ≥ 2 mg/dL but ≤ 20 mg/dL & specific gravity ≥ 1.0010 but ≤ 1.0030
Method	Immunoassay
Confirmation	Method using different measurement principle

*Urine adulteration test strips are available.

Common Tumor Markers*

TUMOR MARKER	TYPE OF CANCER FOR WHICH MARKER IS MOST OFTEN USED	CLINICAL USE	COMMENTS
α-Fetoprotein (AFP)	Liver	Aid Dx, monitor therapy, detect recurrence	Produced by fetal liver; re-expressed in certain tumors. Also ↑ in hepatitis, pregnancy.
Cancer antigen 15-3 (CA 15-3) & cancer antigen 27.29 (CA 27.29)	Breast	Stage disease, monitor therapy, detect recurrence	Two different assays for same marker. Can be ↑ with other cancers & non-cancerous conditions.
Cancer antigen 19-9 (CA 19-9)	Pancreatic	Stage disease, monitor therapy, detect recurrence	Can be ↑ with other cancers & non-cancerous conditions.
Cancer antigen 125 (CA 125)	Ovarian	Aid Dx, monitor therapy, detect recurrence	Can be ↑ with other cancers & gyneco-logical conditions.
Carcinoembryonic antigen (CEA)	Colorectal	Monitor therapy, detect recurrence	Fetal antigen re-expressed in tumors. Can be ↑ with other cancers, non-cancerous conditions, & in smokers.
Human chorionic gonadotropin (hCG)	Ovarian & testicular. Also gesta-tional trophoblastic diseases	Aid Dx, monitor therapy, detect recurrence	↑ in pregnancy.

continued...

Common Tumor Markers* *continued*

	TYPE OF CANCER FOR WHICH MARKER IS MOST OFTEN USED	CLINICAL USE	COMMENTS
Prostate-specific antigen (PSA)	Prostate	Screening, aid Dx, monitor therapy, detect recurrence	Currently most widely used tumor marker. Screening asymptomatic men is controversial. Some men with prostate cancer don't have ↑ PSA. PSA can be ↑ in other conditions. Measurement of free PSA may be helpful when PSA is borderline.
Thyroglobulin	Thyroid	Monitor therapy, detect recurrence	↑ in other thyroid diseases. Antithyroglobulin antibodies should be measured at same time. Can interfere with assays.

* Tumor markers alone cannot Dx cancer. Most are not useful for screening. Tumor markers are nonspecific & can be elevated in noncancerous conditions. Some patients with cancer do not have elevated tumor markers. Serial testing is more useful than a single test. With successful treatment, tumor marker levels should decline & return to normal. Increasing levels following treatment might indicate a recurrence. All of the tumor markers above are measured by immunoassay.

Acid-Base Balance Terminology

TERM	EXPLANATION
pH	$-\text{Log}\,[H^+]$ or $\log \frac{1}{[H^+]}$
Acid	Chemical that can yield H^+. Proton donor. pH <7
Base	Chemical that can accept H^+ or yield OH^-. pH >7
Buffer	Weak acid & its salt or conjugate base. Minimizes changes in pH. Most important 1 for maintaining blood pH is bicarbonate/carbonic acid. ($H^+ + HCO_3^- \Leftrightarrow H_2CO_3 \Leftrightarrow H_2O + CO_2$). Others: phosphates, proteins, hemoglobin
Bicarbonate	HCO_3^-. Second largest fraction of anions. Proton acceptor or base. Equal to total $CO_2 - 1$. Regulated by kidneys
Carbonic acid	H_2CO_3. Proton donor or weak acid. Equal to $PCO_2 \times 0.03$. Regulated by lungs
Total CO_2	All forms of CO_2. ($HCO_3^- + H_2CO_3 +$ dissolved CO_2)
Pco_2	Partial pressure of CO_2. Directly related to amount of dissolved CO_2.
Henderson-Hasselbalch equation	$pH = 6.1 + \log \frac{[HCO_3^-]}{[H_2CO_3]}$ or $6.1 + \log \frac{HCO_3^-}{PCO_2 \times 0.03}$

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Acid-Base Balance Terminology *continued*

TERM	EXPLANATION
Acidosis (acidemia)	Blood pH <7.38. ↓ HCO_3^-: H_2CO_3 ratio. (Normal 20:1). May be due to ↓ in HCO_3^- (metabolic acidosis) or ↑ in H_2CO_3 (respiratory acidosis)
Alkalosis (alkalemia)	Blood pH >7.42. ↑ HCO_3^-: H_2CO_3 ratio. May be due to ↑ in HCO_3^- (metabolic alkalosis) or ↓ in H_2CO_3 (respiratory alkalosis)
Compensated acidosis or alkalosis	When compensatory mechanisms have succeeded in restoring the 20:1 ratio & pH returns to normal. Kidneys compensate for respiratory problem; lungs compensate for metabolic problem

Acid-Base Imbalances

CONDITION	pH	P_{CO_2}	HCO_3^-	COMPENSATION TO RE-ESTABLISH 20:1 RATIO
Respiratory acidosis	↓	↑	N	Kidneys retain HCO_3^-, excrete H^+
Metabolic acidosis	↓	N	↓	Hyperventilation (blow off CO_2)
Respiratory alkalosis	↑	↓	N	Kidneys excrete HCO_3^-, retain H^+
Metabolic alkalosis	↑	N	↑	Hypoventilation (retain CO_2)

Arterial Blood Gases Terminology

TERM	EXPLANATION
Hypoxemia	Low O_2 content in arterial blood
Hypoxia	Lack of O_2 at cellular level
Partial pressure	Barometric pressure × % gas concentration
Pco_2	Partial pressure of CO_2 expressed in mm of Hg. Directly related to amount of dissolved CO_2. Measure of respiratory component (inversely proportional to respiration)
Po_2	Partial pressure of O_2. Assesses pulmonary function
Oxygen dissociation curve	Graph showing relationship between oxygen saturation & Po_2. Provides information about hemoglobin's affinity for O_2
2,3-Diphosphoglycerate (2,3-DPG)	Phosphate compound in RBCs that affects O_2 dissociation curve. Low levels inhibit release of O_2 to tissues
Oxygen saturation	Amount of O_2 that *is* combined with hemoglobin, expressed as % of amount of O_2 that *can be* combined with hemoglobin. 1 g of hemoglobin can combine with 1.34 mL of O_2
P_{50}	Partial pressure of O_2 at which hemoglobin oxygen saturation is 50%. Low value = ↑ oxygen affinity (shift to the left in O_2 dissociation curve). High value = ↓ oxygen affinity (shift to right)

Blood Gas Parameters

PARAMETER	MEASUREMENT OF:	DERIVATION	REFERENCE RANGE (ARTERIAL BLOOD)
pH	$[H^+]$	pH electrode on blood gas analyzer	7.35–7.45
P_{CO_2}	Partial pressure of CO_2	P_{CO_2} electrode on blood gas analyzer	35–45 mm Hg
P_{O_2}	Partial pressure of O_2	P_{O_2} electrode on blood gas analyzer	80–100 mm Hg
HCO_3^-	Bicarbonate	Calculated value on blood gas analyzer	22–26 mmol/L
Total CO_2	Bicarbonate + carbonic acid	Calculated value on blood gas analyzer	23–27 mmol/L
Base excess*	Metabolic component of acid-base status. Difference between titratable bicarbonate of sample & that of normal blood sample	Calculated value on blood gas analyzer	−2 to +2 mEq/L
Oxygen saturation	Amount of oxygenated hemoglobin	Measured by oximeter	94%–100%

*Negative values indicate base deficit.

DEVICE	DESCRIPTION	MEASURES	CALIBRATION
pH electrode	H^+-sensitive glass electrode containing Ag/AgCl wire in electrolyte of known pH & reference (calomel) electrode (Hg/Hg_2Cl_2). Measurement is potentiometric (change in voltage indicates activity of analyte).	$[H^+]$	2 phosphate buffers of known pH. (Store at RT; don't expose to air)
Pco_2 electrode (Severinghaus electrode)	pH electrode covered with membrane permeable to CO_2, with bicarbonate buffer between membrane & electrode. Measurement is potentiometric.	Dissolved CO_2	2 gases of known Pco_2
Po_2 electrode (Clark electrode)	Platinum cathode & Ag/AgCl anode covered with semipermeable membrane. Measurement is amperometric (amount of current flow is indication of O_2 present).	Dissolved O_2	2 gases of known Po_2
Co-oximeter	Spectrophotometer that reads absorbance or reflectance at isobestic point (wavelength where reduced & oxyhemoglobin have same absorbance or reflectance, e.g., 805 nm) & differential point (wavelength where reduced & oxyhemoglobin have different absorbance or reflectance, e.g., 650 nm).	Oxygen saturation. Some also measure carboxyhemoglobin, methemoglobin, & sulfhemoglobin by using additional wavelengths.	Calibration curve prepared from specimens with 0% & 100% O_2 saturation

Sources of Error in Arterial Blood Gases

ERROR	EFFECT
Hyperventilation	↓ Pco_2, ↑ pH, ↑ Po_2
Specimen exposed to air	↓ Pco_2, ↑ pH, ↑ Po_2
Specimen at RT >30 minutes	↓ Po_2, ↓ pH, ↑ Pco_2

VALUE	CALCULATION	NORMAL RANGE	CLINICAL SIGNIFICANCE
A/G ratio	$\frac{\text{Albumin}}{\text{Total protein} - \text{albumin}}$	1–2.5	Reversed A/G ratio with multiple myeloma, liver disease.
Amylase: creatinine clearance ratio	$\frac{\text{Urine amylase (U/L)} \times \text{serum creatinine (mg/L)}}{\text{Serum amylase (U/L)} \times \text{urine creatinine (mg/L)}}$	2%–5%	↑ acute pancreatitis. ↓ macroamylasemia.
Anion gap	$(Na^+ + K^+) - (Cl^- + HCO_3^-)$ or $Na^+ - (Cl^- + HCO_3^-)$	10–20 or 7–16	Difference between unmeasured anions & unmeasured cations. ↑ in renal failure; diabetic acidosis; lactic acidosis; methanol, ethanol, ethylene glycol, or salicylate poisoning; laboratory error. Useful QC check. Can't be a negative number. If all determinations are ↑ or ↓, possible instrument error in 1 of the determinations.

continued...

Calculated Chemistry Values *continued*

VALUE	CALCULATION	NORMAL RANGE	CLINICAL SIGNIFICANCE
BUN-to-creatinine ratio	$\frac{\text{BUN}}{\text{Creatinine}}$	10–20	Normal ratio with renal disease. Prerenal conditions: ↑ ratio with ↑ BUN & normal CR. Postrenal conditions: ↑ ratio with ↑ CR. Ratio ↓ with ↓ urea production (e.g., severe liver disease, ↓ protein intake).
Creatinine clearance	$\frac{\text{urine creatinine (mg/dL)} \times \text{urine mL per 24hr/1,440}}{\text{plasma creatinine (mg/dL)}} \times \frac{1.73}{\text{body surface area}}$	M: 97–137 mL/min F: 88–128 mL/min	↓ renal disease (early indicator).
Indirect (unconjugated) bilirubin	Total bilirubin – direct (conjugated) bilirubin	<0.2 mg/dL	↑ prehepatic, posthepatic, & some types of hepatic jaundice.
LDL cholesterol	Friedewald formula: LDL-C = total cholesterol – [HDL-C + (triglycerides/5)]	Desirable level <130 mg/dL	↑ LDL cholesterol associated with ↑ risk of CAD. Not valid if triglycerides >400 mg/dL. Direct measurements now available.

continued...

Calculated Chemistry Values *continued*

VALUE	CALCULATION	NORMAL RANGE	CLINICAL SIGNIFICANCE
Calculated osmolality	Formula 1: $1.86\ Na^{+}\ mEq/L + \frac{glucose\ mg/dL}{18} + \frac{BUN\ mg/dL}{2.8} + 9$ Formula 2: $2\ Na^{+}\ mEq/L + \frac{glucose\ mg/dL}{20} + \frac{BUN\ mg/dL}{3}$	275–295 Osm/kg	Concentration of solute. Electrolytes contribute most. One of colligative properties. ↑ dehydration, uremia, uncontrolled diabetes, alcohol or salicylate intoxication, excessive electrolyte IVs. ↓ excessive water intake.
Osmolal gap	Measured osmolality – calculated osmolality	0–10 mOsm/kg	Similar to anion gap but based on osmotically active solute concentration rather than concentration of ions. >10 indicates abnormal concentration of unmeasured substance (ex: isopropanol, methanol, acetone, ethylene glycol). Used to Dx poisonings.
Urine-to-serum osmolality	$\frac{\text{Urine osmolality}}{\text{Serum osmolality}}$	1–3	↓ renal tubular deficiency, diabetes insipidus.

Chemistry Calculations

FORMULA	EXAMPLE
Beer's law: Concentration of unknown = $\dfrac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times$ concentration of standard	A manual glucose assay gave the following results: Absorbance of 100 mg/dL standard = 0.3. Absorbance of patient = 0.4. What is the glucose concentration of the patient? $$\text{Concentration} = \frac{0.4}{0.3} \times 100 = 133 \text{ mg/dL}$$ (Note: If a dilution is run, multiply answer by reciprocal of dilution.)
$\text{mEq/L} = \dfrac{\text{mg/dL}}{\text{GEW}^*} \times 10$	A calcium is reported as 10 mg/dL. What is the concentration in mEq/L? (Atomic weight of calcium = 40. Valence of calcium = 2^+.) $$\text{mEq/L} = \frac{10 \times 10}{20} = 5$$
$\text{mmol/L} = \dfrac{\text{mg/dL}}{\text{GMW}^\dagger} \times 10$	A calcium is reported as 10 mg/dL. What is the concentration in mmol/L? (Atomic weight of calcium = 40. Valence of calcium = 2^+.) $$\text{mmol/L} = \frac{10 \times 10}{40} = 2.5$$

*GEW = gram equivalent weight (gram molecular weight ÷ valence).
†GMW = gram molecular weight

continued...

FORMULA	EXAMPLE
$\text{mmol/L} = \dfrac{\text{mEq/L}}{\text{valence}}$	A calcium is reported as 5 mEq/L. What is the concentration in mmol/L? (Atomic weight of calcium = 40. Valence of calcium = 2^{+}.) $$\text{mmol/L} = \frac{5}{2} = 2.5$$
$\text{Molarity (M)} = \dfrac{\text{grams per liter}}{\text{GMW}}$	What is the molarity of a solution that contains 45 grams of NaCl per liter? (Atomic weights: Na = 23, Cl = 35.5.) $$M = \frac{45}{58.5} = 0.77$$
$\text{Normality (N)} = \dfrac{\text{grams per liter}}{\text{GEW}}$	What is the normality of a solution that contains 98 grams of H_2SO_4 per 500 mL? (Atomic weights: H = 1, S = 32, 0 = 16.) $$N = \frac{196}{49} = 4$$

continued...

FORMULA	EXAMPLE
% concentration = grams or milliliters per 100 mL	What is the concentration in % of a solution that contains 8.5 grams of NaCl per liter? $\frac{8.5\text{ g}}{1{,}000\text{ mL}} = \frac{x}{100\text{ mL}}$ $1{,}000\,x = (8.5) \times 100$ $x = 0.85\%$
$N = M \times \text{valence}$	What is the normality of a 3 M H_2SO_4 solution? $N = 3 \times 2 = 6$ What is the molarity of a 0.3 N H_2SO_4 solution? $M = \frac{0.3}{2} = 0.15$
$V_1C_1 = V_2C_2$	How many mL of 95% alcohol are needed to prepare 100 mL of 70% alcohol? $(x)(95) = (100)(70)$ $x = 73.7\text{ mL}$

V = volume, C = concentration.

SECTION

3

Clinical Microbiology Review

Biosafety Levels

LEVEL	RISK	TYPES OF AGENTS	EXAMPLES OF AGENTS	PRECAUTIONS
I	Minimal	Those not known to cause disease in healthy adults	*Bacillus subtilis, Mycobacterium gordonae*, soil microbes	Standard microbiological practices. No special equipment.
II	Moderate	Common human pathogens	*E. coli, Salmonella*, HIV, HBV, influenza	Biological safety cabinet (BSC) I or II. Personal protective equipment (PPE). Autoclave must be available. Limited access. Most micro labs fall in this category.
III	High	Those that may cause serious or lethal disease via inhalation. Effective treatment available	*Bacillus anthracis, Francisella, Brucella, Mycobacterium tuberculosis, Rickettsia rickettsii, Coxiella burnetii*, mold stages of systemic fungi	Same as above plus negative air flow, sealed windows.
IV	Extreme	Those that pose high risk of life-threatening disease. May be transmitted by aerosols. No vaccine or therapy	Ebola virus, Lassa virus, others that cause hemorrhagic fevers	Requires use of class III BSC; full-body, air-supplied positive pressure suit; independent unit with specialized ventilation & waste management to prevent release into environment.

Centers for Disease Control and Prevention Classification of Biological Agents

CATEGORY	PRIORITY	SPREAD	IMPACT	EXAMPLES
A	Highest	Easily disseminated or transmitted from person to person	High mortality, potential for major public health impact	*Bacillus anthracis, Yersinia pestis, Francisella tularensis, Clostridium botulinum* toxin, smallpox, hemorrhagic fever viruses (Ebola, Marburg, Lassa, Machupo)
B	2nd highest	Moderately easy to disseminate	Moderate illness, low death rate	*Brucella, Salmonella, Shigella, E. coli* 0157:H7, *Burkholderia mallei, Burkholderia pseudomallei, Chlamydia psittaci, Coxiella burnetii, Clostridium perfringens* toxin, ricin toxin, staphylococcal enterotoxin B, *Rickettsia prowazekii*, viral encephalitis viruses, *Vibrio cholerae, Cryptosporidium parvum*
C	3rd highest	Could be engineered for mass dissemination	High morbidity/mortality, major public health impact	Nipah virus, hantavirus

CLASS	DESCRIPTION	USE
I	Open front. Unsterilized room air enters. Air passes through high-efficiency particulate air (HEPA) filter before being exhausted.	Provides minimal personnel protection. Doesn't protect work surface.
II	Laminar flow cabinets with variable sash opening. Air passes through 1 HEPA filter before reaching work surface & 2nd one before being exhausted.	Type most commonly used in hospital micro labs. Provides protection for worker & work.
III	Completely enclosed. Negative pressure. Air is filter sterilized coming in & going out. Gloves are attached to front.	Provides maximum protection. Used in labs that work with extremely hazardous organisms.

Temperature	Most reliable
Steam under pressure	Autoclave. Most practical & dependable method. 15 lb of pressure for 15 min. 121°C. Kills spores.
Boiling	Not reliable. Spores may not be killed.
Pasteurization	Used in food industry to kill foodborne pathogens. Doesn't sterilize. Liquid is heated to 71.7°C for 15 sec.
Hot air sterilization	Used when steam may damage or fail to penetrate. 2 hours at 170°C. Kills spores.
Incineration	Used to sterilize inoculating loops & biomedical wastes.
Filtration	Used to sterilize liquids that are thermolabile (e.g., urea broth). HEPA air filters are used in BSC.
Radiation	UV light. Used in BSC.
Chemicals	
Alcohols	Ethyl & isopropyl (70%–80%) frequently used as antiseptics & disinfectants. Don't kill spores.
Chlorine	Sodium hypochlorite (household bleach). One of most effective agents against HIV & HBV. 10% solution. Prepare daily.
Formalin	Aqueous solution of formaldehyde. 5%–10% formalin used to preserve & fix specimens. Exposure must be monitored.

continued...

Glutaraldehyde	Effective against most vegetative cells other than mycobacteria. Used for cold sterilization of items damaged by heat, e.g., inhalation therapy equipment, equipment with optical lenses such as endoscopes.
Hydrogen peroxide	3% solution used as antiseptic
Iodine & iodophors	Iodophors (e.g., povidone-iodine) release iodine slowly & are less irritating & nonstaining. Effective skin antiseptics.
Phenolics	Don't kill spores. Other disinfectants are evaluated by comparing effectiveness to phenol. "Phenol coefficient."
Quaternary ammonium compounds	Effective against wide range of vegetative bacteria. Not effective against spores, mycobacteria, or nonenveloped viruses. Used to disinfect floors, walls, furniture.
Gases	
Ethylene oxide	Widely used in hospitals to sterilize materials that can't withstand steam.

Bacterial Toxins

	EXOTOXIN	ENDOTOXIN
Produced by	Gram positives	Gram negatives
Location in cell	Within & without	Within
Composition	Protein	Lipopolysaccharide
Effect	Systemic	Local
Toxicity	High	Low
Stability to heating	Unstable	Stable
Antigenicity	High	Low
Stimulates antitoxin productions?	Yes	No
Converted to toxoid?	Yes	No

Specimen Collection Guidelines

- Obtain during acute phase of infection (within 2–3 days for viruses).
- Collect before antibiotics are administered.
- Sample appropriate site.
- Aspirates or tissues are preferred to swabs.
- Use swabs with Dacron or polyester tips & plastic shafts. Wood, cotton, & calcium alginate may be toxic.
- For anaerobes, aspirates preferred to swabs.
- Avoid contamination with environmental or normal flora.
- Obtain sufficient quantity.
- Use sterile, leak-proof container.
- Properly label container, not lid.
- Transport in secondary container (plastic bag) marked with biohazard symbol.
- Protect requisition from contamination.
- Syringes with needles attached should never be transported.
- Deliver to lab within 30 min of collection.
- For prolonged transit, use special preservatives or holding media.

Specimen Preservation and Storage

SPECIMEN	PRESERVATIVE OR TRANSPORT DEVICE	STORAGE TEMPERATURE (UNPRESERVED)
Anaerobes	Anaerobic transport systems to eliminate O_2	25°C. Don't refrigerate
CSF	None	25°C or 35°C. Don't refrigerate
Neisseria gonorrhoeae	Amies transport medium with charcoal, Transgrow, JEMBEC plates, Gono-Pak, BioBag (best to inoculate medium directly)	25°C. Don't refrigerate
Sputum	None	4°C
Stool for culture	Transport media, e.g., Cary-Blair transport medium if specimen can't be cultured within 2 hr of collection	4°C. Best to process without delay. Refrigeration may kill *Shigella*
Stool for ova & parasites	Depending on procedures to be performed: polyvinyl alcohol (PVA), 10% formalin, sodium acetate–acetic acid–formalin (SAF), merthiolate-iodine-formalin (MIF), others without formaldehyde or mercury (e.g., Ecofix, Parasafe)	Can hold formed specimens at 4°C. Soft or liquid specimens should be examined immediately or preserved
Urine	Transport tubes with boric acid–glycerol if specimen can't be processed within 2 hr of collection	4°C for not more than 24 hr
Viruses	Viral transport medium	4°C. For delay >24 hr, freeze at −70°C

Fragile Organisms

- Anaerobes
- *Chlamydia*
- *Haemophilus influenzae*
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Salmonella*
- *Shigella*
- *Streptococcus pneumoniae*
- Viruses
- Parasites

Criteria for Rejection of Specimens in Microbiology

- Unlabeled or improperly labeled specimen
- Improper collection site
- Prolonged transit (over 2 hr without preservation)
- Improper temperature during transport or storage
- Leaking specimens
- Specimens in nonsterile containers
- Improper transport medium
- Culturette ampule not broken, swab dried out
- Improper swab, e.g., wood or calcium alginate for viruses or *Chlamydia*
- Syringes with needles attached
- Culture for anaerobes requested on inappropriate sources
- Specimen received in formalin (other than stool for ova & parasites)
- Saliva instead of sputum
- Foley catheter tip
- Insufficient quantity

Follow lab's written policies.

Gram Stain

REAGENT	FUNCTION	OTHER
Crystal violet	Basic dye	Stains all bacteria purple
Iodine	Mordant	Fixes crystal violet to cells
95% ethyl alcohol or acetone or combination	Decolorizer	Removes crystal violet from gram neg
Safranin	Counterstain	Stains gram neg pink

Staining Properties of Gram-Positive and Gram-Negative Bacteria

GRAM REACTION	CELL WALL	STAINED BY	COLOR IN GRAM STAIN
Gram positive	Thick peptidoglycan layer with teichoic acid & lipoteichoic acid. Teichoic acid cross-links prevent decolorization in Gram stain.	Crystal violet	Purple
Gram negative	Thin peptidoglycan layer covered with proteins, phospholipids, & lipopolysaccharides. Decolorizer causes ↑ permeability of lipid-rich cell wall. Primary stain (crystal violet) washes out.	Safranin	Pink

Types of Media

TYPE	EXPLANATION	EXAMPLES
Supportive	Supports growth of most nonfastidious bacteria	Nutrient agar, trypticase soy agar
Enrichment	Contains added growth factors, e.g., blood, vitamins, yeast extract	Sheep blood agar, chocolate agar, brain-heart infusion, buffered charcoal-yeast extract agar
Selective	Contains additives such as dyes, bile salts, alcohols, acids, or antibiotics to inhibit growth of certain bacteria (e.g., gram pos)	Columbia colistin–nalidixic acid (CNA) agar, eosin methylene blue (EMB), MacConkey, Hektoen enteric (HE), xylose lysine deoxycholate (XLD), Thayer-Martin
Differential	Formulated to provide distinct colonial appearances based on certain biochemical rxn (e.g., lactose fermentation, hydrogen sulfide [H_2S] production)	EMB, MacConkey, HE, XLD

Note: Media may be of more than 1 type, e.g., selective & differential.

Routine Media for Aerobes and Facultative Anaerobes

MEDIUM	TYPE	FOR ISOLATION OF	OTHER
Sheep blood agar (SBA)	E, D	Most nonfastidious bacteria	Tryptic soy agar with 5% sheep blood. Allows differentiation of hemolysis.
Chocolate agar (CHOC)	E	*Haemophilus* & *Neisseria*	Supplies X & V factors. Incubate in ↑ CO_2.
Columbia colistin–nalidixic acid agar (CNA)	S	GP	Colistin & nalidixic acid suppress most GN. Contain 5% sheep blood.
Phenylethyl alcohol agar (PEA)	S	GPC & anaerobic GNR	Phenylethyl alcohol inhibits enteric GNR. Contains 5% sheep blood.
Group A–selective strep agar with 5% sheep blood (SSA)	S	Group A strep from respiratory sources	Contains antibiotics to suppress normal throat flora. Group B strep will also grow.
Eosin methylene blue (EMB)	S, D	Enteric GNR	Eosin & methylene blue inhibit GP. Lactose fermenters (LF) green-black or purple. *E. coli* produces green metallic sheen. Non–lactose fermenters (NLF) colorless.
MacConkey (MAC) agar	S, D	Enteric GNR	Bile salts & crystal violet inhibit most GP. LF pink. NLF colorless.
Sorbitol MacConkey (SMAC) agar	S	*E. coli 0157:H7*	*E. coli 0157:H7* doesn't ferment sorbitol. Colorless colonies. Some labs have stopped using because non-0157 serotypes can be pathogens.

continued...

Routine Media for Aerobes and Facultative Anaerobes *continued*

MEDIUM	TYPE	FOR ISOLATION OF	OTHER
Hektoen enteric (HE) agar	S, D	*Salmonella* & *Shigella* in stool	Bile salts, bromothymol blue, & acid fuchsin inhibit normal GI flora. Nonpathogens orange to salmon pink. NLF green to blue-green. H_2S-pos colonies have black precipitate.
Xylose lysine deoxycholate (XLD)	S, D	*Salmonella* & *Shigella* in stool	Deoxycholate inhibits many GNR & GP. 4 types of colonies: yellow (e.g., *E. coli*), yellow with black centers (e.g., some *Proteus* species), colorless or red colonies (e.g., *Shigella*), red colonies with black centers (e.g., *Salmonella*). (Some shigellae may be inhibited. Some salmonellae may not produce H_2S.)
Salmonella-Shigella (SS) agar	S	*Salmonella* & *Shigella* in stool	Brilliant green & bile salts inhibit other enterics. *Salmonella* & *Shigella* don't ferment lactose (colorless colonies). *Salmonella* produces H_2S (black center).
Gram-negative broth (GN)	E, S	*Salmonella* & *Shigella* from stools & rectal swabs	Deoxycholate & citrate salts retard growth of GP. Subculture onto selective differential agar after 6–8 hr & 18–24 hr incubation. Use of enrichment broths for stool cultures is decreasing.

continued...

Routine Media for Aerobes and Facultative Anaerobes *continued*

MEDIUM	TYPE	FOR ISOLATION OF	OTHER
Deoxycholate-citrate agar	S	*Salmonella* & *Shigella*	Other enterics inhibited.
Campylobacter broth	E	*Campylobacter* from stool	Subculture to Campy-selective agar after overnight incubation at 4°C.
Campylobacter blood agar (Campy BAP)	E, S	*Campylobacter* from stool	Incubate plates in ↑ CO_2 at 42°C.

E = enriched; S = selective; D = differential; GP = gram positive; GN = gram negative; C = cocci; R = rods.

Selective Media for Isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*

MEDIUM	COMMENTS
Modified Thayer-Martin (TM)	Vancomycin, colistin, nystatin, & trimethoprim inhibit growth of other bacteria & fungi. Incubate in ↑ CO_2. Some *N. gonorrhoeae* may be inhibited.
Martin-Lewis	Similar to Thayer-Martin, but different antibiotics. Inhibits yeast better. Incubate in ↑ CO_2.
New York City medium (NYC)	Incubate in ↑ CO_2. Some *N. gonorrhoeae* are inhibited by antibiotics. Genital mycoplasmas will grow.
GC-LECT	Antibiotics to inhibit GN & GP bacteria & yeast.
JEMBEC plates	For transportation & growth of *N. gonorrhoeae*. Plates contain *Neisseria*-selective medium & come with resealable polyethylene bag & CO_2-generating tablet. No need to transfer to culture plate.

Used for specimens from sites with normal flora.

Special Bacteriologic Media

MEDIUM	USE	OTHER
Cystine-tellurite blood agar	Differential medium for isolation of *Corynebacterium diphtheriae*	*C. diphtheriae* produces black colonies.
Loeffler medium	Enrichment medium for *C. diphtheriae*	Promotes development of metachromatic granules.
Tindale agar	Selective differential medium for *C. diphtheriae*	*Corynebacterium* spp produce gray to black colonies due to reduction of tellurite. *C. diphtheriae* colonies are surrounded by a brown halo.
Bismuth sulfite agar	Selective for *Salmonella*	Bismuth sulfite & brilliant green inhibit most others. *S. typhi* colonies are black, surrounded by metallic sheen. Others are light green. (Some salmonellae may be inhibited.)
Cefsulodin-irgasan-novobiocin (CIN) agar	Selective medium for *Yersinia enterocolitica, Aeromonas,* & *Plesiomonas shigelloides*	Crystal violet inhibits most GN. Novobiocin inhibits GPC. Cefsulodin inhibits most GP & GN. *Y. enterocolitica* ferments mannitol, appears as red "bull's-eye" colonies surrounded by colorless halo.
Alkaline peptone water (APW)	Enrichment medium for recovery of *Vibrio* from stool	Alkaline pH suppresses commensals. Subcultured to TCBS.

continued...

Special Bacteriologic Media *continued*

MEDIUM	USE	OTHER
Thiosulfate citrate bile salts sucrose (TCBS) agar	Selective for *Vibrio*	High pH inhibits most bacteria. *V. cholerae* ferments sucrose, produces yellow colonies. *V. parahaemolyticus* & *V. vulnificus* don't ferment sucrose; usually produce blue-green colonies.
Bordet-Gengou agar	Selective enrichment medium for isolation of *Bordetella pertussis*	Potato-glycerol-based medium enriched with blood. Contaminants inhibited by methicillin. "Cough plate." *Bordetella* colonies resemble mercury droplets.
Regan-Lowe agar	Selective for *B. pertussis*	Charcoal agar supplemented with horse blood, cephalexin, & amphotericin B.
Buffered charcoal-yeast extract (BCYE) agar	Enrichment medium for isolation of *Legionella*	Yeast extract & L-cysteine enhance growth of *Legionella*. Charcoal absorbs toxic compounds.
Human blood bilayer Tween (HBT) agar	Selective & differential for *G. vaginalis*	Incubate in ↑ CO_2 for 48 hr. Colonies are beta hemolytic.

Aerotolerance Test

CLASSIFICATION	BLOOD AGAR INCUBATED AEROBICALLY	BLOOD AGAR INCUBATED ANAEROBICALLY	CHOCOLATE AGAR INCUBATED IN CO_2 INCUBATOR
Aerobe	Growth	No growth	Growth
Capnophilic aerobe	No growth	No growth	Growth
Facultative anaerobe	Growth	Growth	Growth
Obligate anaerobe	No growth	Growth	No growth
Aerotolerant anaerobe	No growth	Growth	Growth

Organisms Requiring Incubation in Increased CO_2

- *Campylobacter*
- *Haemophilus*
- *Helicobacter*
- *Moraxella catarrhalis*
- *Mycobacterium*
- Pathogenic *Neisseria*

Hemolytic Reactions on Sheep Blood Agar

HEMOLYSIS	DESCRIPTION	EXPLANATION	EXAMPLES
Alpha	Green zone around colony. May be narrow or wide.	Partial lysis of RBCs	*Streptococcus pneumoniae*, viridans streptococci, some enterococci
Beta	Clear zone around colony. May be narrow or wide.	Complete lysis of RBCs	Group A strep, group B strep, *Listeria monocytogenes*
Gamma (nonhemolytic)	No zone of hemolysis.	No lysis of RBCs	Some enterococci

ORGANISM	PATHOGENICITY	GRAM STAIN	COLONIES ON SBA	KEY CHARACTERISTICS	OTHER
Staphylococcus spp	See specific organisms below	GPC, usually in clusters	1–3 mm, round, smooth, convex, glistening, opaque, entire edge, butyrous (butter-like).	Catalase pos. Fermentative. Microdase (modified oxidase) neg. Resistant to bacitracin. Susceptible to furazolidone & lysostaphin.	Normal flora of skin, mouth, pharynx, vagina, urethra, GI tract. Facultative anaerobe. Grows on most nonselective media. Salt tolerant.
S. aureus	Causes suppurative cutaneous infections, toxic shock syndrome, food poisoning	GPC, usually in clusters	Most are beta hemolytic (small zone). May be golden.	Coagulase pos. Ferments mannitol (yellow colonies on mannitol salt agar). Usually DNase & thermonuclease pos.	10%–60% are carriers. Spread by direct contact. 85%–90% resistant to penicillin.
Coagulase-negative staphylococci (CNS)	Opportunistic pathogen. Common cause of hospital-acquired UTI	GPC, usually in clusters	White, usually nonhemolytic.	Coagulase neg. Grow on mannitol salt agar but don't ferment mannitol. Sensitive to novobiocin.	Normal on skin & mucous membranes. Often contaminant. Usually only speciated if from normally sterile site. 50%–80% are *S. epidermidis*.

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Staphylococci *continued*

ORGANISM	PATHOGENICITY	GRAM STAIN	COLONIES ON SBA	KEY CHARACTERISTICS	OTHER
S. saprophyticus	UTI in young sexually active females, urethritis & prostatitis in males	GPC, usually in clusters	White to slightly yellow. Non-hemolytic.	Coagulase neg. Resistant to novobiocin. May ferment mannitol.	Novobiocin only performed when CNS isolated from urine of female.
Micrococcus	Usually nonpathogenic. Found in environment & on skin, mucous membranes, oropharynx	Large GPC in pairs, tetrads (predominant arrangement), clusters	Often pigmented (bright yellow, orange, pink, tan). High-domed colonies.	Catalase pos. Coagulase neg. Oxidative. Microdase (modified oxidase) pos. Susceptible to bacitracin. Resistant to furazolidone & lysostaphin.	Not commonly isolated. Must differentiate from staph. Usually only grow aerobically.

UTI = urinary tract infection.

Summary of Tests for Identification of Staphylococci

TEST	PRINCIPLE	KEY REACTIONS	OTHER
Catalase	Enzyme catalase converts 3% hydrogen peroxide (H_2O_2) to oxygen & water. Immediate bubbling.	Staphylococci pos. Streptococci & enterococci neg	Bubbles after 20–30 sec aren't considered pos rxn. Catalase from RBCs in blood agar may produce weak bubbles.
Coagulase	Enzyme coagulase causes coagulation (tube test) or agglutination (slide test) in plasma.	*S. aureus* pos	Slide test is screening test. Detects bound coagulase (clumping factor). If neg, tube test should be performed. Detects free coagulase. Largely replaced by latex agglutination tests.
Slide agglutination tests for *S. aureus*	Agglutination of latex beads coated with fibrinogen & abs to protein A (protein in cell wall of *S. aureus*).	*S. aureus* pos	Most labs report pos organisms as *S. aureus*.
Mannitol salt agar (MSA)	Fermentation of mannitol results in color change from pink to yellow.	*S. aureus* pos	7.5% salt inhibits most organisms other than staph. All staph can grow on MSA. Rarely used for ID of *S. aureus* today because other species ferment mannitol.
Novobiocin susceptibility	Organisms resistant to novobiocin grow to edge of disk.	*S. saprophyticus* is resistant. Other CNS susceptible	Performed on CNS isolated from urine.

ORGANISM	PATHOGENICITY	GRAM STAIN	COLONIES ON SBA	KEY CHARACTERISTICS	OTHER
Streptococcus spp	See specific organisms below	Oval GPC in chains & pairs	<1 mm, white to gray, translucent, or semiopaque. Variable hemolysis.	Catalase neg	Facultative anaerobes. Require enriched media. Chaining best in broth cultures.
Group A streptococci (GAS)	Causes 90% of strep infections. Strep sore throat, rheumatic fever, glomerulonephritis, scarlet fever (scarlatina), erysipelas, puerperal sepsis, impetigo	Oval GPC in chains	Pinpoint. Grayish white. Translucent. Usually beta hemolytic (wide zone).	Sensitive to bacitracin. Resistant to SXT. PYR pos	Most common is *S. pyogenes*. Hemolysis due to O_2-stable streptolysin S & O_2-labile streptolysin O. To detect species that produce streptolysin O only, stab into agar, place coverslip over inoculum, or incubate anaerobically.

continued...

Streptococci/Enterococci *continued*

ORGANISM	PATHOGENICITY	GRAM STAIN	COLONIES ON SBA	KEY CHARACTERISTICS	OTHER
Group B streptococci (GBS)	Normal flora of female genital tract. Most common cause of neonatal septicemia & meningitis	Oval GPC in chains	Slightly larger than GAS. Gray-white. Narrow zone of diffuse beta hemolysis. May be nonhemolytic.	Resistant to SXT & bacitracin. Sodium hippurate pos. CAMP pos	*S. agalactiae*. Vaginal & rectal swabs collected from pregnant women at 35–37 wk gestation. Inoculated in selective broth, e.g., LIM, GBS broth, StrepB Carrot Broth.
Group D streptococci, nonenterococci	Normal in GI tract. Causes nosocomial UTI, wound infections, bacteremia	Oval GPC in chains	Usually nonhemolytic. May be alpha hemolytic.	Hydrolyzes esculin	Most common is *S. gallolyticus* (formerly *S. bovis*).
Enterococcus	Normal in mouth, GI tract, female genital tract. Causes nosocomial UTI, wound infections, bacteremia	Oval GPC in pairs & chains	Usually alpha or nonhemolytic. Rarely beta.	Hydrolyzes esculin. Grows in 6.5% NaCl broth. PYR pos	Lancefield group D. 80% are *E. faecalis*, 15% *E. faecium.*

continued...

Streptococci/Enterococci *continued*

ORGANISM	PATHOGENICITY	GRAM STAIN	COLONIES ON SBA	KEY CHARACTERISTICS	OTHER
Streptococcus pneumoniae	Normal in upper respiratory tract of some. Most common cause of community-acquired pneumonia. Major cause of otitis media, meningitis in adults. Infects sinuses, eyes	Football-shaped (lancet-shaped) GPC. Usually in pairs. May be single or in short chains. Frequently encapsulated	Round, translucent, glistening, dome shaped when young. Central depression with age (umbilicate) due to autolysis. Alpha hemolytic. Encapsulated strains are mucoid	Bile solubility pos. Sensitive to optochin	No Lancefield group. >80 serotypes based on capsular antigens.
Viridans streptococci	Normal in oral, respiratory, GI mucosa. Opportunistic pathogens. Frequent cause of subacute bacterial endocarditis. Gingivitis & dental caries (cavities)	GPC in chains	Usually alpha hemolytic, but may be beta hemolytic or nonhemolytic.	Resistant to optochin. Bile solubility neg	

continued...

ORGANISM	PATHOGENICITY	GRAM STAIN	COLONIES ON SBA	KEY CHARACTERISTICS	OTHER
Microaerophilic *Streptococcus* spp (formerly *S. milleri* group)	Normal flora of mouth & GI, GU tracts. Infections associated with trauma or surgery. Most common sites: abdomen, head, neck	GPC in pairs, chains	Colonies are half the size of other strep. Usually beta hemolytic but can be alpha hemolytic or nonhemolytic. Butterscotch or caramel odor.	Usually Lancefield group F. PYR neg. VP pos	3 species: *S. anginosus, S. constellatus, S. intermedius*. In viridans group. Require 10% CO_2. Grow better anaerobically.

Tests for Identification of Beta-Hemolytic Streptococci

TEST	PRINCIPLE	KEY REACTIONS	OTHER
Bacitracin disk	Zone of inhibition after overnight incubation = susceptibility to bacitracin.	GAS susceptible. GBS resistant	Also known as A disk. Performed on SBA. No longer recommended because some groups C & G are susceptible. Replaced by PYR & serogrouping by latex agglutination.
Trimethoprim-sulfamethoxazole (SXT) disk	Organisms resistant to SXT grow up to disk.	GAS & GBS resistant. Group C & G strep susceptible	Used in conjunction with bacitracin disk to differentiate GAS from group C or G. Requires overnight incubation.
Pyrrolidonase (PYR) test	If PYR is hydrolyzed, red color after addition of color developer.	GAS pos (also enterococci)	More specific than bacitracin for GAS. GAS is only beta-hemolytic strep that's PYR pos. Disk test only takes minutes.
CAMP test	GBS produces extracellular protein that enhances hemolysis of beta-hemolytic *S. aureus* on SBA.	GBS pos	Classic method: unknown streaked perpendicular to streak of β-lysin producing *S. aureus*. Incubated in ambient air overnight. False pos in CO_2. Arrowhead hemolysis where inoculum lines meet. Disks containing β-lysin can be used instead of *S. aureus*. Rapid test: drop of β-lysin on colonies on SBA. Only requires 20-min incubation.

continued...

Tests for Identification of Beta-Hemolytic Streptococci *continued*

TEST	PRINCIPLE	KEY REACTIONS	OTHER
Hippurate hydrolysis	Organisms that produce hippuricase (hippurate hydrolase) hydrolyze sodium hippurate to benzoate & glycine.	GBS pos	Alternative to CAMP. 2-hr test available.
Slide agglutination tests	Latex particles coated with group-specific antibodies agglutinate in presence of bacterial antigens	Organisms agglutinate in corresponding antisera	Tests commercially available for rapid ID of groups A, B, C, D, F, G strep & *S. pneumoniae*. (Most labs only use for ID of beta hemolytic.)

Tests for Identification of Alpha-Hemolytic Streptococci

TEST	PRINCIPLE	KEY REACTIONS	OTHER
Optochin disk	Zone of inhibition ≥14 mm with 6-mm disk or ≥16 mm with 10-mm disk = susceptibility to optochin	*S. pneumoniae* susceptible. Viridans strep resistant	Also known as P disk. Contains ethylhydrocupreine hydrochloride. Placed on lawn of inoculum on SBA. Plates incubated overnight in ↑ CO_2. If zone of inhibition <14 mm, ID organism as *S. pneumoniae* only if bile soluble.
Bile solubility	Bile salts (e.g., sodium deoxycholate) cause lysis of some organisms	*S. pneumoniae* pos	Can be performed in broth or on colony. Pos = clearing of broth or disappearance of colony. Results in 30 min or less.

Tests for Identification of Nonhemolytic Streptococci/Enterococci

TEST	PRINCIPLE	KEY REACTIONS	OTHER
Pyrrolidonase (PYR) test	See "Tests for Identification of Beta-Hemolytic Streptococci"	Enterococci pos (also GAS)	
Bile-esculin agar	Organisms that can grow in 40% bile & produce esculinase hydrolyse esculin, producing black precipitate	Group D strep & enterococci pos	Bile inhibits GPs other than group D strep & enterococci.
Esculin test	Organisms that produce esculinase hydrolyze esculin on disk, producing dark spot	Group D strep & enterococci pos	Doesn't test for bile tolerance.
6.5% NaCl broth	Organisms that can grow in 6.5% NaCl produce turbidity	Enterococci pos (also GBS). Group D strep neg	Requires overnight incubation.

Antibiograms of Gram-Positive Cocci

ORGANISM	SUSCEPTIBILITY	ROUTINE SUSCEPTIBILITY TESTING?
Staphylococcus aureus	Significant resistance to multiple antibiotics, including vancomycin. Usually resistant to penicillin & ampicillin. Methicillin-resistant *S. aureus* (MRSA) resistant to all β-lactams.	Yes
Group A streptococci	Universally susceptible to penicillin, cephalosporins, vancomycin.	No
Group B streptococci	Susceptible to penicillin, cephalosporins, vancomycin.	No (may be done when isolated from baby)
Streptococcus pneumoniae	Increasingly resistant to penicillin & other antibiotics. Susceptible to vancomycin.	Yes
Group D streptococci	Susceptible to penicillin, vancomycin.	Yes
Enterococci	Usually resistant to penicillin & several other commonly used antibiotics. Most are susceptible to vancomycin, but vancomycin-resistant enterococci (VRE) are becoming more common in hospitals.	Yes

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS	OTHER
Bacillus spp	Usually contaminants.	See *B. anthracis* below.	Large spreading beta-hemolytic colonies with irregular edges (Medusa head). Whitish gray. May be pigmented.	Catalase pos. Most are motile.	Must rule out *B. anthracis.*
Bacillus anthracis	One of most highly pathogenic microorganisms. Causes anthrax. Contracted from contaminated hides, wool, meat. Rare in U.S.	Large with square ends. May be in chains. Oval, central to subterminal spores that aren't swollen. Looks like bamboo. Spores may not be seen in direct smear.	Large, adherent, nonhemolytic, flat to slightly convex, irregular border, ground-glass appearance, comma-shaped projections. Stands up like beaten egg white when touched with loop.	Nonhemolytic. (Beta hemolysis rules out.) Catalase pos. Nonmotile. Capsules seen in CSF & blood smears.	Potential bioterrorism organism. Handle in BSC. Submit to public health lab for confirmation.
Bacillus cereus	Often environmental contaminant. Can cause local & systemic infections, food poisoning.	Same as *B. anthracis.*	Same as *B. anthracis* except hemolytic.	Hemolytic. Motile.	2nd most important pathogen in genus.

Aerobic Non–Spore-Forming Gram-Positive Rods

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS	OTHER
Corynebacterium spp	Normal on skin & mucous membranes. Opportunistic pathogens.	Irregular, slightly curved, nonparallel sides, club-shaped ends. Chinese letter or picket fence formation (palisades).	Facultative anaerobes. Grow on most media.	Catalase pos. Nonmotile.	Called diphtheroids because resemble *C. diphtheriae*.
Corynebacterium diphtheriae	Diphtheria. Rare in U.S.	Same as above.	Gray-black colonies with brown halos on Tindale agar. Black colonies on cystine tellurite. Loeffler medium stimulates growth & production of metachromatic granules.	Catalase pos. Nonmotile. Toxin producing.	ID requires demonstration of toxin production. Modified Elek immunoprecipitation test. PCR for *TOX* gene.

continued...

Aerobic Non–Spore-Forming Gram-Positive Rods *continued*

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS	OTHER
Corynebacterium jeikeium	Hospital-acquired pathogen.	Same as above.	Same as other commensal corynebacteria.	Catalase pos. Rapid sucrose urea (RSU) neg.	Most commonly isolated diphtheroid. Highly resistant to antibiotics.
Listeria monocytogenes	Meningitis & septicemia in newborns & immunocompromised. Food poisoning.	Parallel sides, rounded ends, coccobacillary. Singles, chains, or diphtheroid arrangement.	Tiny colonies with narrow zone of indistinct beta hemolysis. Translucent, gray.	Catalase pos. Hippurate hydrolysis pos. Esculin pos. CAMP pos (hemolysis looks like shovel, not arrowhead). Tumbling motility on wet mount. Umbrella growth in motility agar at RT but not at 35°C.	Grows from 0.5°–45°C. Cold enrichment may be used. Catalase differentiates from GBS. Motility differentiates from diphtheroids.
Nocardia	Immunocompetent: skin infections. Immunocompromised: invasive pulmonary & disseminated infections.	Fine branching filaments with fragmentation. Often beaded.	Slow-growing. On SBA wrinkled, dry, crumbly, chalky white to orange-tan, beta hemolytic.	Catalase pos. Partially acid fast. Sulfur granules.	*N. brasiliensis* most common species to cause skin infections. *N. asteroides* most common species to cause lung infections.

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS	OTHER
Neisseria spp	See individual organisms below	GNDC, coffee-bean shaped	Aerobic or facultative. Pathogens are capnophilic.	Catalase pos. Oxidase pos.	
N. gonorrhoeae	Gonorrhea, salpingitis, ophthalmia of the newborn	Intracellular & extracellular GNDC. Diagnostic in urethral discharge from symptomatic males. Culture confirmation required for females	Requires ↑ CO_2. Usually doesn't grow on SBA. Grows on CHOC & *Neisseria*-selective media. Colonies are small, grayish white or tan. 5 different colony types. May look like mixed culture.	Carbohydrate utilization: glucose only. Superoxol pos. Can ID with monoclonal antibodies.	Susceptible to drying & cold. Don't refrigerate specimens. Molecular methods for genital specimens. Culture preferred for nongenital specimens & those from children. Should confirm by different method.

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ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS	OTHER
N. meningitidis	Can be normal flora in upper respiratory tract. Common cause of meningitis in young adults	Intracellular & extracellular GNDC	Grows on SBA, CHOC, & *Neisseria*-selective media. Colonies are bluish gray or tan. May be mucoid.	Carbohydrate utilization: glucose & maltose. ONPG neg.	Don't refrigerate. Handle in BSC. Serogrouping by slide agglutination. Bacterial antigen test available for detection but poor sensitivity. Shouldn't replace culture. Vaccine available.
Commensal *Neisseria* spp	Normal in upper respiratory tract. Rarely cause disease	GNDC	Grow on SBA & CHOC at RT, nutrient agar at 35°C. Some grow on *Neisseria*-selective media. Don't require ↑ CO_2. May be pigmented.	Varies with species.	Not speciated if from respiratory specimens. Must differentiate from pathogenic *Neisseria* when isolated on selective media or from normally sterile body site.

continued...

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS	OTHER
N. lactamica	Normal in upper respiratory tract of children. Rare in adults. Rarely causes disease	GNDC	Will grow on *Neisseria*-selective media. Resembles *N. meningitidis* but smaller.	Carbohydrate utilization: glucose, maltose, & lactose (slow). ONPG pos.	One of commensal *Neisseria*. Easily misidentified as *N. meningitidis*. May react with meningococcal typing sera. ONPG differentiates.
Moraxella catarrhalis	Normal in upper respiratory tract. Causes respiratory infections in young, old, & compromised; otitis media & sinusitis in children	GNDC	Grows on SBA & CHOC. Some may grow at RT &/or on *Neisseria*-selective media. "Hockey puck colonies" (colony can be pushed over agar surface with loop).	Catalase pos. Oxidase pos. Carbohydrate utilization: neg for all sugars & ONPG. DNase pos. Butyrate esterase pos.	DNase & butyrate esterase differentiate from *Neisseria* spp.

GNDC = gram-negative diplococci.
Note: Gram-negative rods *Kingella* & *Acinetobacter* may grow on *Neisseria*-selective media.

Characteristics of Enterobacteriaceae

Gram stain	Non–spore-forming gram-negative rods
Growth characteristics	Facultative anaerobes
Colonies on sheep blood agar	Most are large, dull, gray, nonhemolytic
Colonies on MacConkey	Lactose fermenters pink Non–lactose fermenters colorless
Biochemicals	Ferment glucose Oxidase neg Most reduce nitrates to nitrites Most are catalase pos

Biochemical Tests for Identification of Enterobacteriaceae

TEST	PRINCIPLE	INTERPRETATION	OTHER
Oxidase	Tetramethyl-para-phenylene-diamine dihydrochloride reacts with cytochrome C to produce blue or purple color.	Pos = blue or purple	Good test to differentiate Enterobacteriaceae from nonfermenters. False pos from iron-containing wire. Use platinum wire or wooden stick.
Nitrate reduction	If organism reduces nitrates to nitrites, red color develops when sulfanilic acid & *N,N*-dimethyl-L-naphthylamine added.	Pos = red, or no color after addition of zinc dust	If no color develops, zinc dust added. Reduces nitrates. Red color after zinc dust indicates presence of residual nitrates, i.e., neg rxn. No color after zinc dust means nitrates reduced to N_2 or NO_2, i.e., pos rxn.
Carbohydrate fermentation	When carbohydrate fermented, acidic end products cause color change in pH indicator.	With phenol red indicator, change from red to yellow	Frequently tested carbohydrates are glucose, lactose, sucrose, mannose, sorbitol, mannitol, xylose, adonitol, cellobiose, dulcitol, trehalose. All Enterobacteriaceae ferment glucose.
ONPG	ONPG is changed to orthonitrophenol by beta-galactosidase.	Pos = yellow	Test for slow lactose fermentation. Helpful in differentiating *Citrobacter* (pos) from most *Salmonella* (neg).

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Biochemical Tests for Identification of Enterobacteriaceae *continued*

TEST	PRINCIPLE	INTERPRETATION	OTHER
H_2S production	Organisms that possess H_2S-producing enzymes produce colorless H_2S gas from sulfur-containing compounds. H_2S reacts with iron salt in medium to form black ferrous sulfide.	Black precipitate	Sulfur-containing compounds = sodium thiosulfate, cystine, methionine. Good test to differentiate *Salmonella* (H_2S pos) from *Shigella* (H_2S neg).
Triple sugar iron (TSI) agar	Sugar fermentation produces acid, changes color of pH indicator.	Yellow = acid (A). Pink = alkaline (K). Pink butt = glucose not fermented. Yellow butt = glucose fermented. Pink slant = lactose/sucrose not fermented. Yellow slant = lactose and/or sucrose fermented. Black precipitate = H_2S produced. Bubbles = gas production	Contains 0.1% glucose, 1% lactose, 1% sucrose, phenol red, sodium thiosulfate, iron salt. Leave cap slightly loose. Record as slant/butt, e.g., K/A. (Some record NC [no change] for pink butt.) Kligler's iron agar (KIA) is same except no sucrose.
Indole	Tryptophanase deaminates tryptophan. Indole produced. Kovacs's reagent (paradimethylaminobenzaldehyde) forms pink-colored complex with indole.	Pos = pink	Spot indole test commercially available. Need source of tryptophan. Use colonies from SBA or CHOC agar, not MacConkey. Pos = blue. *E. coli* & *Proteus vulgaris* are pos.

continued...

Biochemical Tests for Identification of Enterobacteriaceae *continued*

TEST	PRINCIPLE	INTERPRETATION	OTHER
Methyl red (MR)	Acid products formed when glucose is metabolized by mixed acid fermentation pathway. Color change in pH indicator.	Pos = red color after addition of methyl red (pH indicator)	
Voges-Proskauer (VP)	Acetoin is produced from alternate pathway for glucose metabolism.	Pos = red color when alpha-naphthol & KOH added	Little acid produced by this pathway. Organisms that are VP pos are usually methyl red neg & vice versa.
Citrate	If organism can use citrate as sole source of carbon, pH ↑, pH indicator changes color.	Pos = green to blue, or growth	False neg if cap not loose. *Klebsiella* & *Enterobacter* are pos.
Urease	Urease breaks down urea. Ammonia released, pH ↑, pH indicator changes color.	Pos = yellow to pink	*Proteus* & *Morganella* are rapid urease producers.
Phenylalanine deaminase (PD)	Phenylalanine deaminase deaminates phenylalanine to phenylpyruvic acid, which reacts with ferric chloride to produce green color.	Pos = green color after addition of ferric chloride	*Proteus, Providencia, Morganella* are pos.

continued...

Biochemical Tests for Identification of Enterobacteriaceae *continued*

TEST	PRINCIPLE	INTERPRETATION	OTHER
Decarboxylase reactions	If organism has enzyme to decarboxylate amino acid (e.g., ornithine, lysine, arginine), pH ↑, pH indicator changes color.	Pos = yellow to purple	Ornithine decarboxylase (ODC) differentiates *Klebsiella* (neg) & *Enterobacter* (pos).
Motility	Motile organisms grow away from stab line in motility medium.	Pos = movement away from stab line or hazy appearance throughout medium after overnight incubation	Most Enterobacteriaceae are pos, except *Klebsiella* & *Shigella*. Differentiates *Klebsiella* (neg) & *Enterobacter* (pos).

Antigens of Enterobacteriaceae

ANTIGEN	ALTERNATE NAME	LOCATION	CHARACTERISTICS	OTHER
O antigen	Somatic antigen	Cell wall	Lipopolysaccharide, heat stable	Used for serological grouping of *Salmonella* & *Shigella*.
H antigen	Flagellar antigen	Flagella	Proteins, heat labile	Used to serotype *Salmonella*.
K antigen	Capsular antigen	Capsule	Polysaccharide, heat labile, may mask O antigen. Removed by heating	Role in preventing phagocytosis. ↑ virulence. Vi antigen is K antigen produced by *S. typhi*.

		KEY REACTIONS		
ORGANISM	**PATHOGENICITY**	**POSITIVE**	**NEGATIVE**	**OTHER**
Escherichia coli	UTI, septicemia, neonatal sepsis & meningitis, diarrhea (some)	Lactose, gas, indole, MR, motility	H_2S, VP, citrate, PD, urease	Predominant aerobe in GI tract. Most common cause of UTI. Green metallic sheen on EMB. Presumptive ID: lactose pos, dry colony on MacConkey, oxidase neg, indole pos. *E. coli 0157:H7* doesn't ferment sorbitol; colorless colonies on SMAC.
Shigella	Dysentery (shigellosis). Most communicable of bacterial diarrheas. Found primarily in crowded or substandard conditions, e.g., day-care centers, jails, prisons	MR	Lactose, gas, H_2S, VP, citrate, PD, urease, motility	Blood, mucus, polys in stool. Rarely disseminates. Fragile organism. *S. dysenteriae* most severe. *Shigella sonnei* most common in U.S. Serogrouped by O antigens. Serogroups A, B, C, D. Closely related to *Escherichia* on molecular basis.
Edwardsiella tarda	Opportunistic. Bacteremia, wound infections	Gas, H_2S, indole, MR, motility	Lactose, VP, citrate, PD, urease	Chief reservoirs are reptiles & freshwater fish. Infections often involve aquatic environments. Pos indole differentiates from *Salmonella*.

continued...

Commonly Isolated Enterobacteriaceae *continued*

KEY REACTIONS

ORGANISM	PATHOGENICITY	POSITIVE	NEGATIVE	OTHER
Salmonella	Typhoid (enteric) fever, bacteremia, enterocolitis	H_2S, MR, motility, lysine decarboxylase (LDC)	Lactose, indole, VP, PD, urease, ONPG	Found in poultry. May be transmitted by reptiles. *S. typhi* has Vi antigen, only trace H_2S, citrate neg. Grouped by O antigens (e.g., A, B, C), serotyped by H antigens (e.g., 1, 2).
Citrobacter freundii	Nosocomial infections	Gas, H_2S, MR, citrate, motility, ONPG	VP, PD, LDC	Lactose variable. ONPG & LDC differentiate from *Salmonella*.
Klebsiella pneumoniae	Pneumonia, UTI, septicemia	Lactose, gas, VP, citrate, urease (slow)	H_2S, indole, MR, PD, motility, ornithine decarboxylase (ODC)	Encapsulated. Colonies usually mucoid. Some strains hydrolyze urea slowly. *K. oxytoca* is similar to *K. pneumoniae* except indole pos. Motility & ODC differentiate from *Enterobacter*.
Enterobacter aerogenes & cloacae	Opportunistic & nosocomial infections. UTI, RTI, & wound infections	Lactose, gas, VP, citrate, motility, ODC	H_2S, indole, MR, PD	Colonies may be mucoid. Same IMViC reactions as *Klebsiella*.

continued...

Commonly Isolated Enterobacteriaceae *continued*

KEY REACTIONS

ORGANISM	PATHOGENICITY	POSITIVE	NEGATIVE	OTHER
Serratia marcescens	Opportunistic pathogen. Pneumonia & septicemia in immunosuppressed	VP, citrate, motility	Lactose, H_2S, indole, PD, urease	Some produce red pigment when incubated at RT.
Proteus vulgaris & *mirabilis*	UTI, wound infections, septicemia	H_2S, MR, PD, urease, motility	Lactose	Swarming. Burned chocolate odor. *P. mirabilis* is most common & indole neg. *P. vulgaris* indole pos, A/A on TSI because of sucrose fermentation.
Morganella morganii	Mainly nosocomial infections. UTI, wound infections.	Indole, MR, PD, urease (weak), motility, ODC	Lactose, H_2S, VP, citrate	
Providencia	UTI, diarrhea	Indole, MR, citrate, PD, motility	Lactose, H_2S, VP	*P. rettgeri* is urease pos.
Yersinia enterocolitica	Diarrhea	MR, urease	Lactose, H_2S, VP, citrate, PD	GN coccobacilli. Bipolar staining. Optimal temperature 25°–30°C. Motile at 25°C but not 35°C. CIN agar is selective. Incubate 48 hr. Red "bull's-eye" colonies surrounded by colorless halo. *Y. pestis* causes plague.

UTI = urinary tract infection; RTI = respiratory tract infection; IMViC = indole, methyl red, Voges-Proskauer, citrate.

Summary of Key Reactions for Enterobacteriaceae

LACTOSE NEGATIVE	H_2S POSITIVE	VP POSITIVE	PD POSITIVE	UREASE POSITIVE	NONMOTILE AT 35°C
Shigella *Edwardsiella* *Salmonella* *Citrobacter* (some) *Serratia* *Proteus* *Morganella* *Providencia* *Yersinia*	*Edwardsiella* *Salmonella* *Citrobacter* *Proteus*	*Klebsiella* *Enterobacter* *Serratia*	*Proteus* *Morganella* *Providencia* *Klebsiella* (slow)	*Proteus* *Morganella* *Providencia rettgeri*	*Shigella* *Klebsiella* *Yersinia* (motile at 22°C)

Appearance of Enterobacteriaceae on Selected Media

ORGANISM	TSI	MacCONKEY	HEKTOEN ENTERIC	XLD
Escherichia coli	A/A, gas	Flat, dry pink colony with darker pink halo	Yellow	Yellow
Shigella	K/A	Colorless	Green	Colorless
Edwardsiella	K/A, gas, H_2S	Colorless	Colorless	Red, yellow, or colorless with or without black centers
Citrobacter	A/A or K/A, gas, with or without H_2S	Colorless at 24 hr. May become pink at 48 hr	Colorless	Red, yellow, or colorless with or without black centers
Salmonella	K/A, gas, H_2S	Colorless	Green	Red with black center
Klebsiella	A/A, gas	Pink, mucoid	Yellow	Yellow
Enterobacter	A/A, gas	Pink. May be mucoid	Yellow	Yellow
Serratia	K/A	Colorless at first, turning pink. *S. marcescens* may have red pigment at RT	Colorless	Yellow or colorless
Proteus	K/A (*mirabilis*) A/A (*vulgaris*), gas, H_2S	Colorless. May swarm	Colorless	Yellow or colorless, with or without black centers
Morganella	K/A, gas	Colorless	Colorless	Red or colorless
Providencia	K/A	Colorless	Colorless	Yellow or colorless
Yersinia	Yellow/orange	Colorless to peach	Salmon	Yellow or colorless

Diarrheagenic *Escherichia coli*

GROUP	PATHOGENICITY	TRANSMISSION	DISEASE MECHANISM	GRAM STAIN OF STOOL	OTHER
Enterohemorrhagic (EHEC). Also known as Shiga toxin–producing (STEC) or verotoxin-producing (VTEC)	Diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS). Most common cause of renal failure in children in U.S. May be fatal, especially in young or elderly	Undercooked meat, raw milk, apple cider	Toxins (vertoxins or Shiga toxins)	RBCs but usually no polys	*E. coli 0157:H7* is most common isolate of group & pathogen most often isolated from bloody stools. Non-0157 STEC also causes disease. DNA probes can ID genes that code for toxins. Report to public health.
Enterotoxigenic (ETEC)	Traveler's diarrhea, diarrhea in infants	Contaminated food or water	Toxins	No polys or RBCs	Profuse, watery stool. DNA probes to detect toxins or toxin genes.
Enteroinvasive (EIEC)	Bloody diarrhea. Dysentery-like. Usually in young children in areas of poor sanitation.	Contaminated food or water	Invasiveness	Polys, RBCs, mucus	

continued...

Diarrheagenic *Escherichia coli* *continued*

GROUP	PATHOGENICITY	TRANSMISSION	DISEASE MECHANISM	GRAM STAIN OF STOOL	OTHER
Enteropathogenic (EPEC)	Diarrhea in infants. Major pathogen in infants in developing countries	Formula & food contaminated with fecal material	Adherence-attachment	No polys or RBCs	Watery diarrhea with mucus.
Enteroaggregative (EAEC)	Diarrhea in developing countries. Chronic diarrhea in HIV-infected patients	Nosocomial & community acquired	Adherence-attachment		Most labs can't detect.
Diffusely adherent (DAEC)	Diarrhea & UTI. Most common in children in developing countries	Little known about epidemiology	Adherence-attachment		Not well studied.

Characteristics of Nonfermenting Gram-Negative Rods

- Obligate aerobes.
- Don't ferment carbohydrates. K/K on TSI.
- May be oxidizers or nonoxidizers (asaccharolytic).
- Oxidation-fermentation (OF) medium: either open tube pos/closed tube neg (oxidizer) or open tube neg/closed tube neg (nonoxidizer).
- Grow on SBA & CHOC in 24–48 hr.
- Most grow on MAC. Appear as non–lactose fermenter.
- Most are oxidase pos. Differentiates from Enterobacteriaceae.
- Resistant to variety of antibiotics.

Commonly Isolated Nonfermenting Gram-Negative Rods

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS
Pseudomonas aeruginosa	Usually not normal flora. Important cause of nosocomial infections, e.g., burn, wound, RTI, UTI, bacteremia. Causes swimmer's ear & contact lens keratitis.	Long, thin, pale-staining GNR. Slightly pointed or rounded ends.	On SBA: flat spreading colonies. Usually beta hemolytic. Dull gray or blue-green. Metallic sheen. Grows on MAC & EMB (lactose neg).	Oxidase pos, catalase pos, motile, grows at 42°C. Grape-like odor. Only nonfermenter to produce pyocyanin. (4% don't.) Also produces pyoverdin (fluorescent pigment). Resistant to many antibiotics.
Acinetobacter spp	Part of normal flora of skin, pharynx in some. Opportunistic pathogen. Nosocomial infections, e.g., UTI, pneumonia, septicemia, meningitis. 2nd to *P. aeruginosa* in frequency. *A. baumannii* is most common.	Pleomorphic GNCB in singles, pairs, short chains. Can be confused with *N. gonorrhoeae*, *Moraxella*. May retain crystal violet in broths & direct smears & be confused with GPC.	Can grow on most media, including MAC. Some produce purplish colonies (might be mistaken for lactose fermenter).	Oxidase neg (differentiates from *N. gonorrhoeae*). Catalase pos. Nonmotile. Resistant to many antibiotics.
Stenotrophomonas maltophilia	Not part of normal flora. Colonizes immunocompromised & cystic fibrosis patients. Common in hospital. Nosocomial infections, e.g., pneumonia.	Straight or slightly curved slender GNR in singles or pairs.	On SBA: large, nonhemolytic. May be light yellow. Agar may have lavender-green discoloration in areas of heavy growth. Grows on MAC.	Oxidase neg. Catalase pos. Motile. Rapid oxidation of maltose, weaker oxidation of glucose. Ammonia odor. Resistant to many antibiotics. Disk diffusion can give false results. Broth dilution recommended.

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS
Campylobacter jejuni	Most common cause of bacterial diarrhea. Sources of infection: chickens, raw milk, pets	Curved, slender, GNR. "Seagulls," loose spirals, & S shaped. Stain faintly.	Microaerophilic & capnophilic. Grown on Campy-BAP at 42°C in ↑ CO_2. (Can grow at 37°C; normal enteric flora inhibited by 42°C incubation.) Slow growing. Hold plates 3 days.	Darting corkscrew motility. Oxidase, catalase, & hippurate hydrolysis pos.
Campylobacter coli	Similar to *C. jejuni*, but less severe. Usually foodborne	Same as above.	Same as above.	Rarely differentiated from *C. jejuni*. Hippurate hydrolysis neg.
Campylobacter fetus	Causes bacteremia in immunocompromised & elderly. Uncommon stool isolate	Same as above.	Most often isolated in blood cultures. Hold for 2 wk. Inhibited on Campy agar. Grows on routine media at 37°C, not at 42°C.	Oxidase & catalase pos. Hippurate hydrolysis neg.
Helicobacter pylori	Gastritis, duodenal & peptic ulcers. Possible risk factor for gastric carcinoma	Curved, slender, GNR.	Grows on nonselective media incubated at 37°C in same atmosphere as Campy. Doesn't grow at 42°C. Slow growing.	Rarely cultured. Rapid urease tests on gastric biopsy, urea breath test, histology, PCR, serology.

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS
Vibrio cholerae	Cholera (acute diarrhea, dehydration, electrolyte imbalance). Transmitted by contaminated water, seafood. Uncommon in U.S., but may be seen in coastal areas.	Small comma-shaped GNR in direct smears; straight pleomorphic GNR in culture.	Nonhalophilic (doesn't require NaCl for growth). Grows on SBA, CHOC, MAC (NLF). Large yellow colonies on TCBS (ferments sucrose). Alkaline peptone water (APW) can be used for enrichment.	"Rice water" stools. Oxidase pos. Motile. Serological ID with antisera to O ag. 01 & 0139 strains cause epidemics. El Tor biotype causes most cases worldwide. Notify public health department, send for confirmation.
Vibrio vulnificus	2nd most serious type of vibrio infection. Immunocompromised or individuals with liver disease: septicemia following consumption of raw oysters, water-associated wound infections. Healthy individuals: gastroenteritis. Seen in U.S.	Straight or curved GNR.	Halophilic (salt loving, requires addition of Na^{+}). Most are green on TCBS; some are yellow. May look like enteric on MAC because some are lactose pos.	Oxidase pos. Motile.

continued...

Vibrio and Related Organisms *continued*

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS
Vibrio parahaemolyticus	2nd most common *Vibrio* to cause gastroenteritis. Contaminated seafood. Water-associated wound infections. Seen in U.S.	Straight or curved GNR.	Requires 1% NaCl for growth. Grows on SBA, MAC (NLF). Blue-green colonies on TCBS (doesn't ferment sucrose).	Oxidase pos. Motile.
Aeromonas spp	Gastroenteritis & wound infections, often related to aquatic exposure. Septicemia, meningitis.	Straight or curved GNR.	Grows on routine media. Most are beta hemolytic on SBA, NLF on MAC. Doesn't grow on TCBS. CIN & APW can be used for selective isolation.	Oxidase pos (differentiates from Enterobacteriaceae). Motile.
Plesiomonas shigelloides (now included in Enterobacteriaceae)	Gastroenteritis from contaminated water or seafood. Bacteremia & meningitis in immunocompromised & neonates.	Pleomorphic GNR in singles, pairs, short chains, or long filaments.	Grows on SBA, CHOC. Most grow on MAC, appear as NLF. Doesn't grow on TCBS.	Biochemical & antigenic similarities to *Shigella*. Oxidase pos. Motile.

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	OTHER
H. influenzae	Normal flora of upper respiratory tract. Causes sinusitis, otitis media, pneumonia, bronchitis, often in elderly or compromised. Type b common cause of pneumonia & meningitis in children where Hib vaccine not available.	Small, pleomorphic GNCB to long filaments. Capsules may be seen.	Grows on CHOC in 5%–10% CO_2. Translucent, moist, tannish colonies. Encapsulated strains form larger & more mucoid colonies. Mousy or bleach-like odor. May demonstrate satellitism with staph on SBA.	Should be serotyped.
H. influenzae biotype *aegyptius* & *H. aegyptius*	Both cause conjunctivitis (pink eye). *H. influenzae* biotype *aegyptius* also causes Brazilian purpuric fever.	Same as above.	Same as above.	Characteristics are similar to *H. influenzae*. Difficult to differentiate.
H. parainfluenzae, H. haemolyticus, & *H. parahaemolyticus*	Normal flora of upper respiratory tract. Low incidence of pathogenicity.	Small, pleomorphic GNCB to long filaments.	Colonies are larger, dry, & tannish.	
H. ducreyi	Never normal flora. Causes chancroid (sexually transmitted disease).	Small GNCB, bipolar staining. May resemble schools of fish or railroad tracks.	Difficult to culture.	Can ID by PCR.

Speciation of *Haemophilus*

SPECIES	REQUIRES X FACTOR (HEMIN)	REQUIRES V FACTOR (NAD)	HEMOLYSIS ON RABBIT OR HORSE BLOOD AGAR	PORPHYRIN/ALA
H. influenzae & *H. aegyptius*	+	+	0	0
H. parainfluenzae	0	+	0	+
H. haemolyticus	+	+	+	0
H. parahaemolyticus	0	+	+	+
H. ducreyi	+	0	0	0

Note that porphyrin/ALA reactions & X factor requirement are opposite, i.e., species that are pos for porphyrin/ALA don't require X factor. Species with "para" in name only require V factor & are porphyrin/ALA pos. Species with "haemolyticus" in name are hemolytic on rabbit & horse blood agar. Multitest biochemical systems available for ID.

ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS
Bartonella	Trench fever, relapsing fever, bacteremia, endocarditis, cat-scratch disease	Intracellular GNCB	Not practical. Takes 9–40 days to grow.	Dx usually by serological or molecular methods.
Bordetella pertussis	Whooping cough in children & adults	Small GNCB	Grows on Bordet-Gengou & Regan-Lowe (charcoal, horse's blood) after 3–7 days.	Fluorescent antibody stain. DTaP vaccine (diphtheria, tetanus, pertussis).
Brucella	Brucellosis (undulant fever). From unpasteurized milk or contact with infected goats, cows, hogs, dogs	Tiny, faintly staining GNCB	Most often isolated from blood cultures or biopsies of reticuloendothelial (RE) tissue. Blood cultures incubated in ↑ CO_2 for 3 wk. Culture not sensitive.	Oxidase & urease pos. Level 3 pathogen. Potential bioterrorism agent. Reportable disease. Serological tests are primary means of Dx.
Francisella	Tularemia (rabbit fever). Man infected by tick or handling infected animal	Pale-staining, small, pleomorphic, intracellular GNCB with bipolar staining	Grows on special media enriched with glucose & cystine (e.g., blood cysteine glucose agar), TM, & BCYE. Small, transparent colonies after 3 days.	Level 3 pathogen. Direct fluorescent ab methods for ID. Send to public health lab for confirmation. Serological tests.

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ORGANISM	PATHOGENICITY	GRAM STAIN	CULTURE	KEY CHARACTERISTICS
Gardnerella	Normal flora of female genital tract. Associated with bacterial vaginosis (BV) when it & anaerobic GNR are predominant & *Lactobacillus* is absent. Linked to maternal & neonatal infections, bacteremia, rarely UTI	Small, pleomorphic gram-variable rods	Not recommended for Dx of BV. Grows on SBA, PEA, CNA, human blood Tween (HBT) agar, V agar. Produces diffuse beta hemolysis only on media containing human blood. Requires ↑ CO_2 & 48–72 hr incubation.	Catalase & oxidase neg. Tests for Dx of BV: clue cells (vaginal epithelial cells covered with gram-variable rods), whiff test (10% KOH added to vaginal secretion → fish-like odor).
Legionella	Legionnaire's disease, Pontiac fever. Found in water (e.g., air conditioners, showerheads, whirlpools). Contracted by inhalation of aerosol	Small, pleomorphic, weakly staining GNR	Grows on BCYE in 3–4 days. Pale yellow-green fluorescence with Wood's lamp.	Oxidase pos. ID by immunofluorescent stain. Serology.
Pasteurella multocida	Contracted by bite or scratch of cat or dog or contact with infected carcass. Wound & respiratory tract infection	Pleomorphic GNCB with bipolar staining	Grows on SBA & CHOC, but not MAC. Musty odor.	Oxidase, catalase, & indole pos.

TM = Thayer-Martin; BCYE = buffered charcoal yeast extract.

Specimens for Anaerobic Culture

ACCEPTABLE	UNACCEPTABLE
Bile	Expectorated sputum
Blood	Feces
Body fluids	Gastric juice
Bone marrow	Swabs
Percutaneous lung aspirate or biopsy	Voided or catheterized urine
Suprapubic bladder aspirates	Bronchial washings (unless obtained with a double-lumen plugged catheter)
Tissue	
Transtracheal aspirate	
Wound	

Media for Culture of Anaerobes

MEDIUM	USE
Anaerobic blood agar (CDC)	Nonselective enrichment medium that grows obligate & facultative anaerobes. Contains yeast extract, L-cysteine, hemin, & vitamin K.
Bacteroides bile–esculin (BBE) agar	Selective differential medium for *Bacteroides fragilis*. Bile salts & gentamicin act as inhibitors. *B. fragilis* colonies black with dark halos due to esculin hydrolysis.
Brucella blood agar	Enriched medium that grows obligate & facultative anaerobes.
Colistin–nalidixic acid (CNA) blood agar	Selective medium that grows obligate anaerobes & GP facultative anaerobes.
Cycloserine cefoxitin fructose egg yolk (CCFA) agar	Selective & differential for *Clostridium difficile.* Colonies are yellow due to fermentation of fructose. Chartreuse fluorescence.
Egg-yolk agar (EYA)	For determination of lecithinase & lipase production by clostridia & fusobacteria.
Kanamycin-vancomycin laked blood (KVLB) agar	Also known as laked blood kanamycin-vancomycin (LKV) agar. Most commonly used selective medium for isolation of anaerobic GNRs, especially *Bacteroides* & *Prevotella.*
Phenylethyl alcohol (PEA) agar	Selective medium that inhibits enteric GNRs & grows obligate anaerobes & GP facultative anaerobes.
Thioglycolate (THIO) broth	All-purpose medium that supports growth of most aerobes & anaerobes. Can be used as backup broth to detect organisms present in small numbers or anaerobes. Thioglycolate acts as reducing agent. Aerobes grow at top, strict anaerobes at bottom, facultative anaerobes throughout. Store at RT. Boil & cool before use.

Anaerobic Environment

Ideal atmosphere	80%–90% N_2, 5% H, 5%–10% CO_2
Methods	
Anaerobic transport media	Tubes of O_2-reduced agar, tubes with anaerobic environment inside, or gas-impermeable bags with gas-generating environment. Most have indicator that changes color in presence of O_2. Can maintain anaerobes for 24–72 hr.
Self-contained anaerobic agar	*Brucella*-based blood & LKV agars contain enzyme that reduces O_2. Lid creates airtight seal.
Anaerobic generating systems	Plates in bag or container. Packet or ampule added. Chemical reaction removes O_2.
Anaerobic chamber	"Glove box." Gold standard. Workbench in gas-tight cabinet. Anaerobic atmosphere created/maintained by gas tank, palladium catalysts, & desiccants. Work done through gloved or gloveless openings.
Quality control	
Methylene blue strip	Blue = O_2 present, white or colorless = no O_2.
Resazurin	Pink = O_2 present, colorless = no O_2.
Oxygen analyzers	More expensive. Real-time monitoring.

Methods to Identify Anaerobes

METHOD	EXPLANATION
Gram stain	Gram reaction; morphology; presence, location, & shape of spores provide clues to ID. Some GN anaerobes stain faintly with safranin. Recommended to extend time of counterstaining to 3–5 min or use 0.1% basic fuchsin. Some GP anaerobes, e.g., *Clostridium*, may stain pink.
Growth on media	Which media organism grows on, pigmentation, hemolysis, & colonial morphology provide clues to ID.
Special-potency antimicrobial disks	Kanamycin, vancomycin, & colistin disks can be used to differentiate anaerobes & ensure that over-decolorized *Clostridium* is not misidentified as GNR. Disks placed on 1st quadrant of plate. After incubation, observe if organism is susceptible or resistant.
Rapid tests	For presumptive ID, e.g., fluorescence; catalase; spot indole; urease; motility; SPS, nitrate, & bile disks; lecithinase, lipase, & proteolytic rxn on egg-yolk agar.
Conventional tubed biochemicals	Test tubes containing variety of media inoculated & incubated in anaerobic environment. Rxn leads to change in pH. Expensive & time consuming. Largely replaced by multitest systems.
Biochemical multitest systems	Trays or strips are inoculated & read after 24–48 hr incubation in anaerobic environment. Code number is obtained & ID determined from codebook. Only contains codes for most commonly isolated anaerobes.
Preformed enzyme-based systems	Detect preexisting enzymes. Panels or cards are inoculated & incubated in room air. Color changes are read in 4 hr. Code number obtained & ID determined from codebook. Only contains codes for most commonly isolated anaerobes.

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Methods to Identify Anaerobes *continued*

METHOD	EXPLANATION
Gas-liquid chromatography (GLC)	Analysis of metabolic end products or cellular fatty acids.
16S ribosomal RNA gene sequencing	DNA extracted from organism, amplified by PCR, sequenced on automated sequencer. Nucleotide sequence compared with known sequences in database.

Anaerobic Gram-Positive Cocci

ORGANISM	PATHOGENICITY	GRAM STAIN	OTHER
Finegoldia magna (formerly *Peptostreptococcus magnus*)	Normal flora on skin & in mouth, intestines, female genital tract. Most commonly isolated & most pathogenic anaerobic GPC. Associated with skin infections, decubitus ulcers, septic arthritis, bone infection following orthopedic surgery, oral & female genital tract infections, bacteremia.	GPC in singles, pairs, tetrads, clusters. Resembles staph.	Small colonies. May take 48 hr to grow. Catalase & indole neg. Resistant to SPS.
Peptostreptococcus anaerobius	Normal on skin & in mouth, GI, & GU tracts. Mixed infections of skin, soft tissues, GI tract, female genital tract, bones, joints, lungs, brain.	Tiny GPC in chains. Resembles strep.	Small gray-white colonies in 24-48 hr. Sweet odor. Sensitive to SPS.
Peptoniphilus asaccharolyticus (formerly *Peptostreptococcus asaccharolyticus)*	Normal on skin & in GI & GU tracts. Associated with obstetric & gynecological infections.	GPC in pairs, short chains, tetrads, clusters.	Yellow colonies. Musty odor. Resistant to SPS. Indole pos.

	PATHOGENICITY	GRAM STAIN	OTHER
Actinomyces	Infects brain, face, lungs, genitals	Short or long GPR. Branched or unbranched. Banded appearance. Can break into club-shaped rods resembling diphtheroids.	Sulfur granules may be seen in discharge. Crush & stain to reveal characteristic Gram-stain morphology. "Molar tooth" colonies.
Clostridium botulinum	Botulism due to ingestion of toxin in inadequately cooked or improperly canned foods. Infant botulism due to ingestion of spores in honey. Wound botulism from injection drug use	GPR with oval subterminal spores.	Reportable disease. Toxin testing at public health labs.
Clostridium difficile	Antibiotic-associated diarrhea, pseudomembranous colitis	Thin GPR. May form chains. Rare oval subterminal spores.	Yellow ground-glass colonies on cycloserine cefoxitin fructose agar (CCFA). Usually not cultured. Must demonstrate toxin production. Toxins A & B. Tissue culture or EIA.
Clostridium perfringens	Normal in GI tract. Causes gas gangrene, food poisoning	Large GPR with blunt ends in chains. "Box cars." Tendency to stain gram neg. Usually no spores seen.	Most commonly isolated *Clostridium*. Double zone of beta hemolysis on SBA.

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Anaerobic Gram-Positive Rods *continued*

	PATHOGENICITY	GRAM STAIN	OTHER
Clostridium tetani	Causes tetanus. Deep wounds infected with soil	GPR with swollen terminal spores. "Drumstick." "Tennis racquet." Becomes gram neg after 24 hr.	Rare in U.S. because of DTaP vaccine. Lab confirmation rarely required.
Propionibacterium	Normal skin flora. Common contaminant of blood cultures. Causes acne; infections associated with artificial joints, catheters, shunts, artificial heart valves; keratitis; bacteremia; endocarditis	Club-shaped, pleomorphic GPR. Diphtheroid-like.	Most common anaerobic GPR. Catalase & indole pos.
Lactobacillus	Normal in mouth, GI tract. Predominant flora of vagina during reproductive years. Produces lactic acid, maintains vaginal pH. Absence in vagina predisposes to bacterial vaginosis & yeast infections. Rare cause of endocarditis, septicemia in immunocompromised	Pleomorphic, long, thin, non–spore-forming GPR, often in chains. Chains of rods in THIO.	Aerotolerant anaerobes. Grow better under anaerobic conditions. Colonies vary greatly. May resemble *S. viridans*. Catalase neg.

Gram-Negative Anaerobes

ORGANISM	PATHOGENICITY	GRAM STAIN	OTHER
Gram-negative cocci *Veillonella*	Normal flora of upper respiratory tract, GI & GU tracts. Usually in mixed culture. Can cause infections in immunocompromised	Tiny GNDC in pairs, clusters, short chains. Can resemble *Neisseria*	May show weak red fluorescence under UV light. Doesn't grow on KVLB. Usually doesn't reduce nitrates.
Gram-negative rods *Bacteroides fragilis*	Mixed infections below diaphragm	Pleomorphic, pale, irregularly staining GNR	Most common anaerobe isolated. Resistant to bile. Hydrolyses esculin. Turns BBE brown.
Fusobacterium	Pulmonary infections, brain abscesses, oral lesions	GNR. *F. nucleatum* is long & thin with tapered ends (spindle shaped)	Indole pos. Most fluoresce chartreuse (green-yellow) under UV light.
Porphyromonas	Head, neck, & pleuropulmonary infections	GNCB	Slow growing. Brown to black on SBA. Some fluoresce brick-red to orange under UV light. Doesn't grow on KVLB.
Prevotella	Head, neck, & pleuropulmonary infections	GNCB	Slow growing. Some are pigmented. Brown to black on SBA & KVLB. Some fluoresce brick-red to orange under UV light. Doesn't grow on BBE.

Lab safety	*Mycobacterium tuberculosis* transmitted by inhalation of aerosols. Low infective dose. Separate room, if possible, with non-recirculating ventilation system & negative air pressure. BS-2 practices. Use of BSC for all procedures that might generate aerosols. Electric incinerators instead of flames to sterilize wire loops. Slide-warming trays instead of flames to fix slides. Tuberculocidal disinfectants to clean equipment & BSC, e.g., 1:10 dilution of household bleach made fresh daily. UV light in BSC (only when not in use). Annual testing of employees for exposure, e.g., Mantoux skin test with PPD.
Specimens requiring digestion/decontamination	Sputum & other specimens with normal flora such as gastric lavage, urine, feces.
Specimens not requiring digestion/decontamination	Tissue or body fluids collected aseptically.
Digestion/decontamination methods	Sodium hydroxide (NaOH), *N*-acetyl-L-cysteine (NALC)/NaOH, benzalkonium chloride, oxalic acid. NaOH is digestant & decontaminating agent. NALC is liquefying agent.
Staining	Cell walls have high lipid content (mycolic acids). Difficult to stain. Resist decolorization by acid alcohol (acid-fast). Gram stain poorly. Use carbolfuchsin or fluorochrome acid-fast stains.
Media	Agar-based (Middlebrook 7H10 & 7H11), egg-based (Löwenstein-Jensen, Petragani, American Thoracic Society), liquid (Middlebrook 7H9). Combination of a solid-based medium & a liquid-based medium recommended for primary isolation.

PPD = purified protein derivative

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Laboratory Identification of Mycobacteria *continued*

Incubation	35°C in 5%–10% CO_2. (Exception: If *M. marinum, M. ulcerans,* or *M. haemophilum* suspected, incubate at 25°–30°C.)
Rate of growth	Slow. On solid media, most require 2–6 wk incubation; rapid growers 2–3 days. More rapid growth in liquid media.
Automated systems for recovery	Liquid broth inoculated, placed in blood culture instrument for automatic or continuous monitoring. Growth indicated by consumption of O_2 or production of CO_2. Earlier detection than manual methods.
Methods for identification	Colony morphology, growth rate, optimum temp for growth, photoreactivity, biochemical tests, chromatography, nucleic acid hybridization, PCR, automated DNA sequencing.

Acid-Fast Stains

STAIN	PRIMARY STAIN	DECOLORIZER	COUNTERSTAIN	APPEARANCE OF AFB	OTHER
Ziehl-Neelsen	Carbolfuchsin	Acid alcohol	Methylene blue	Red, slightly curved, beaded rods (2–8 μm). Blue background.	Requires heat. Examine at least 300 OI fields. Wipe lens after pos smears to avoid cross-contamination & false pos.
Kinyoun	Carbolfuchsin	Acid alcohol	Methylene blue	Red, slightly curved, beaded rods (2–8 μm). Blue background.	Cold stain. Examine at least 300 OI fields. Wipe lens after pos smears to avoid cross-contamination & false pos.
Fluorochrome	Auramine- rhodamine	Acid alcohol	Potassium permanganate or acridine orange	Yellow-orange rods against dark background.	More sensitive than carbolfuchsin. Faster to read. Can examine at 250×. Read immediately or store at 2°C–8°C in dark to avoid fading. Examine at least 300 fields. Pos should be confirmed with carbolfuchsin stain. Rapid growers might not stain.

AFB = acid fast bacilli. OI = oil immersion.

Classification of Mycobacteria Based on Pathogenicity

GROUP	PATHOGENICITY	SPECIES
Mycobacterium tuberculosis complex ("tubercle bacilli")	Cause human tuberculosis (TB).	*M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti*
Atypical mycobacteria, nontuberculous mycobacteria (NTM), or mycobacteria other than tubercle (MOTT) bacilli	Some cause pulmonary infection similar to TB but not transmitted person to person. Usually in immunocompromised. Contracted from environment (soil, water).	*M. avium, M. intracellulare, M. kansasii, M. malmoense, M. chelonae, M. xenopi, M. gordonae*

Classification of Nontuberculous Mycobacteria Based on Physiology

RUNYOUN GROUP	CHARACTERISTICS	EXAMPLES
Photochromogens	Yellow pigment when exposed to light after being grown in dark. >7 days to appear on solid media.	*M. marinum, M. kansasii*
Scotochromogens	Yellow pigment when grown in light or dark. >7 days to appear on solid media.	*M. gordonae*
Nonphotochromogens	No pigment produced in light or dark. >7 days to appear on solid media.	*M. avium complex, M. ulcerans*
Rapid growers	<7 days to appear on solid media.	*M. smegmatis*

ORGANISM	PATHOGENICITY	KEY POINTS
M. tuberculosis	Tuberculosis	Slow growth. Average recovery time by conventional methods 21 days. Rough, dry, buff-colored (no pigmentation) colonies. Serpentine cording on smear from culture. Niacin & nitrate pos. 68°C catalase neg.
M. bovis	GI infection following consumption of unpasteurized milk	Rare in U.S. Bacille Calmette-Guérin (BCG) is attenuated strain used in vaccine in some parts of world. Causes false-pos PPD test.
M. africanum	Causes lung infection. Disseminated disease in immunocompromised	Spread by respiratory droplets. Endemic in equatorial Africa. Rare in U.S.
M. avium complex (MAC)	Most common NTM to cause lung disease in U.S. Most common systemic bacterial infection in AIDS patients. Cervical lymphadenitis in children	*M. avium* & *M. intracellulare.* Usually not differentiated. Contracted from environment. Nonphotochromogens.
M. hemophilum	Skin, joint, bone, lung infections in immunocompromised. Lymphadenitis in children	Requires hemin for growth. Grows best at 30°C. Nonphotochromogen.
M. ulcerans	Chronic infection of skin & subcutaneous tissue. Buruli ulcers	Endemic in tropics. 3rd most common mycobacterial disease after TB & leprosy. Grows best at 30°C. Nonphotochromogen.

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Medically Important Mycobacteria *continued*

ORGANISM	PATHOGENICITY	KEY POINTS
M. kansasii	2nd most common NTM to cause lung disease. Causes skin & soft tissue infections, lymphadenitis. Can disseminate in immunocompromised	Photochromogen. Can ID by 16sRNA probe.
M. marinum	Skin infections	Contracted from swimming pools, aquariums. Grows best at 30°C. Photochromogen.
M. scrofulaceum	Common cause of cervical lymphadenitis in children in Africa	Scotochromogen.
M. gordonae	Rarely causes infection	"Tap-water bacillus." Laboratory contaminant. Scotochromogen. Can ID by PCR & nucleic acid probes.
M. abscessus	In water, soil, dust. Contaminant of medical devices. Skin & soft tissue infections. Lung infections in those with chronic lung disease, e.g., cystic fibrosis. Disseminated infection in immunocompromised	Tap water is reservoir. Rapid grower.
M. chelonae	Disseminated cutaneous infections in immunocompromised, infections of lungs, bone, central nervous system, prosthetic heart valves	Rapid grower.

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ORGANISM	PATHOGENICITY	KEY POINTS
M. fortuitum	Infections of skin, soft tissues, IV & injection sites, surgical wounds	Common in environment. Rapid grower. Weakly gram pos. Weakly acid fast. Stains with carbolfuchsin, but may not with fluorescent stain.
M. leprae	Leprosy (Hansen's disease)	Endemic in Southern hemisphere. <100 cases per year in U.S. Most in TX, CA, LA, HI, PR. Armadillos may be reservoir. Doesn't grow on artificial media. Can be grown in footpads of mice & armadillos. Dx by acid-fast stain of tissue. Less acid fast than *M. tuberculosis*.

Chlamydia and *Chlamydophila*

ORGANISM	PATHOGENICITY	LABORATORY DIAGNOSIS	OTHER
Chlamydia trachomatis	Most common sexually transmitted bacterial infection in U.S. Trachoma, lymphogranuloma venereum, nongonococcal urethritis, pelvic inflammatory disease. Pneumonia & conjunctivitis in newborns.	Giemsa stain, direct fluorescent antibody stain, cell culture, rapid antigen assay (EIA), nucleic acid amplification tests (NAATs), DNA probes, serological tests for antibodies	Obligate intracellular parasite. Need epithelial cells for culture. Wooden swabs are toxic. Cell culture was gold standard; NAAT now preferred for genital specimens. Commercial systems available for simultaneous detection of *N. gonorrhoeae*.
Chlamydophila (Chlamydia) pneumoniae	3rd most common cause of acute RTI.	Serological tests are method of choice.	Obligate intracellular parasite. Risk factor for Guillain-Barré syndrome. May be risk factor for cardiovascular disease.
Chlamydophila (Chlamydia) psittaci	Psittacosis ("parrot fever"). Rare in U.S.	Serological tests.	Obligate intracellular parasite. Spread by birds.

ORGANISM	PATHOGENICITY	LABORATORY DIAGNOSIS	OTHER
Borrelia recurrentis	Relapsing fever	Giemsa- or Wright-stained blood smears.	Transmitted by ticks & lice.
Borrelia burgdorferi	Lyme disease (erythema chronicum migrans, neurological & cardiac abnormalities, arthritis)	Grows on modified Kelly medium. Serology is most common method.	Most commonly reported tick-borne infection in U.S. Vector = *Ixodes* tick.
Treponema pallidum subspecies *pallidum*	Syphilis	Doesn't grow on artificial media. Darkfield microscopy, fluorescent stain. Serology is most common method.	Worldwide.
Leptospira interrogans	Weil's disease (infectious jaundice, leptospirosis)	Culture. Growth = turbidity below surface of semisolid Fletcher's medium. Hold cultures for 6 wk. Serological tests, PCR.	Zoonotic disease. Transmitted by urine of infected animal. Organism in blood & CSF during first week, then urine.

Mycoplasma/Ureaplasma

ORGANISM	PATHOGENICITY	OTHER
Mycoplasma pneumoniae	Primary atypical pneumonia (walking pneumonia)	Smallest free-living cells. Lack cell wall. Pleomorphic. Not visible by Gram staining. Difficult to grow. Culture rarely performed. May take 21 days or more. Usually Dx by serology.
Mycoplasma hominis	Urogenital tract disease	Only species that will grow on SBA & CHOC but may require 4 days. Pinpoint translucent colonies easily overlooked. "Fried-egg" colonies seen with stereomicroscope after staining with methylene blue. Serological methods available.
Ureaplasma	Urogenital tract disease	Granular brown appearance on A8 agar because of urease production. Serological methods available.

GENUS	PATHOGENICITY	LABORATORY DIAGNOSIS	OTHER
Rickettsia	Rocky Mountain spotted fever, rickettsial pox, epidemic typhus, murine typhus, scrub typhus	Don't grow on artificial media. Grow in lice, ticks, tissue culture, eggs. Usually Dx by serology. Immunohistologic & molecular methods available.	Obligate intracellular parasite. Transmitted by ticks, mites, lice, fleas. Rocky Mountain spotted fever is most common rickettsial infection in U.S. Caused by *R. rickettsii*. BSL-3 biohazard.
Coxiella	Q fever	Grows in cell culture. Usual Dx by serology. Immunohistologic & molecular methods available.	Obligate intracellular parasite. Zoonotic disease. Found in cattle, sheep, goats. Highly contagious. BSL-3 biohazard. Reportable disease. Potential bioterrorism agent.
Ehrlichia	Ehrlichiosis	Morulae (clusters of organisms that resemble blackberry) in WBCs. Usual Dx by serology.	Obligate intracellular parasite. Infects WBCs. Transmitted by ticks. Disease similar to Rocky Mountain spotted fever.

SPECIMEN	MEDIA	NORMAL FLORA	COMMON PATHOGENS	OTHER
Throat/nasopharynx	SBA, CHOC. *Streptococcus*-selective agar may be used	Alpha & gamma strep, commensal *Neisseria*, CNS, diphtheroids, *S. pneumoniae*, *Candida*	GAS (*S. pyogenes*)	Stab streaking to detect beta strep that only produce streptolysin O. Direct antigen tests for GAS. Negs should be cultured.
Sputum, bronchial washings/aspirates, transtracheal aspirates	SBA, CHOC, MAC	CNS, non–beta-hemolytic strep, diphtheroids, commensal *Neisseria*, *Haemophilus*, yeast. Aspirates: none	*S. pneumoniae*, *H. influenzae*, *S. aureus*, Enterobacteriaceae, *Pseudomonas*, *M. catarrhalis*, *C. albicans*	Gram stain. Acceptable sputum: <10 epithelial cells/LPF, >25 polys/LPF. Note: Neutropenic patients may not produce polys.
Urine	SBA, MAC. Use 0.01-mL or 0.001-mL calibrated loop	Clean catch: few skin flora. Catheterized or aspirate: none	*E. coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Enterococcus*, *Pseudomonas*, *S. aureus*, *S. saprophyticus*, CNS, GBS	Rapid screen for UTI: 1 or more bacteria/OI field on Gram stain of uncentrifuged urine. Interpretive guidelines vary. Low counts are significant in catheterized urines & aspirates. 3 or more organisms with none predominant = probable contamination.

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Routine Culture Setup and Interpretation *continued*

SPECIMEN	MEDIA	NORMAL FLORA	COMMON PATHOGENS	OTHER
Stool	SBA, MAC or EMB, XLD or HE, Campy blood. Some labs also use SMAC, CIN, and/or TCBS	Anaerobes, Enterobacteriaceae, enterococci, strep, yeast	*Salmonella, Shigella, Campylobacter,* diarrheagenic *E. coli, Aeromonas, Plesiomonas, Y. enterocolitica, Vibrio*	Polys = invasive diarrhea; e.g., *Salmonella, Shigella, Campylobacter*, EIEC, *C. difficile*. No polys = enterotoxin-mediated diarrhea; e.g., ETEC, *Vibrio*. RBCs with EHEC.
Genital	SBA, CHOC, MAC, Thayer-Martin. Add THIO & anaerobic media for tissue & aspirates	Urethra: CNS, diphtheroids. Vagina, prepubescent/postmenopausal: yeast, GNR, GPC. Vagina, reproductive years: *Lactobacillus*, GPC. Surgical/aspirates: none	*N. gonorrhoeae, C. albicans,* GBS, *S. aureus.* Surgical/aspirates: also anaerobes	Gram stain
CSF	SBA, CHOC. MAC. Some add THIO	None	GBS (infants), *Listeria* (infants, elderly), *H. influenzae* (unimmunized children), *N. meningitidis, S. pneumoniae,* GNR. Any isolate significant	Use tube 2. Handle stat. Don't refrigerate. Use of cytocentrifuge to concentrate specimen increases sensitivity of Gram stain. Use slides that

continued...

Routine Culture Setup and Interpretation *continued*

SPECIMEN	MEDIA	NORMAL FLORA	COMMON PATHOGENS	OTHER
				have been alcohol dipped & flamed or autoclaved. If pos, call. ID all isolates. Direct antigen tests of limited value.
Blood	Most labs inoculate an aerobic & anaerobic bottle. Some use 2 aerobic bottles, no anaerobic	None Common contaminants: CNS, *Micrococcus,* alpha strep, diphtheroids, *P. acnes*	Any isolate potentially significant. GPC are most common	Skin prep: 80%–95% ethanol or isopropyl alcohol followed by povidone-iodine or tincture of iodine. Optimum volume: adult, 20 mL; child, 1 mL per yr of life. blood-to-broth ratio 1:5 to 1:10. Usually draw 2 sets in 24 hr. Automated systems widely used for monitoring. Growth indicated by consumption of O_2 or production of CO_2. Gram stain & subculture still required for ID.

continued...

Routine Culture Setup and Interpretation *continued*

SPECIMEN	MEDIA	NORMAL FLORA	COMMON PATHOGENS	OTHER
Wound	SBA, CHOC, MAC. Surgical, aspirate, or tissue: add anaerobic blood, KVLB, THIO	CNS, diphtheroids, *P. acnes*. Surgical, aspirate, or tissue: none	*S. aureus*, beta-hemolytic strep, Enterobacteriaceae, *P. aeruginosa*. Surgical, aspirate, or tissue: also anaerobes. Human bite: alpha-hemolytic strep, *S. aureus, S. pyogenes, Eikenella corrodens*, anaerobes	Gram stain.
Eye	SBA, CHOC	CNS, diphtheroids, *P. acnes*	*H. influenzae, S. pneumoniae, S. aureus,* Enterobacteriaceae, *Pseudomonas*	Gram stain. If pos, call. Keratitis is emergency. Loss of eye can occur.
Ear	SBA, CHOC, MAC	Normal skin flora	*S. pneumoniae, H. influenzae,* GAS, *M. catarrhalis, S. aureus, Pseudomonas*	Gram stain.

Practices vary. Follow laboratory's SOP.

Fecal Pathogens

OXIDASE POSITIVE	OXIDASE NEGATIVE
Campylobacter	*Escherichia coli*
Vibrio	*Salmonella*
Aeromonas	*Shigella*
Plesiomonas	*Yersinia*

Major Classes of Antibiotics

CLASS	REPRESENTATIVE ANTIBIOTICS	MODE OF ACTION	SPECTRUM OF ACTIVITY	OTHER
Natural penicillins	Penicillin V, penicillin G	Inhibit cell wall synthesis	Narrow. GPs other than staph, some GN.	β-Lactam. Bactericidal. Allergic rxn common side effect.
Synthetic penicillins	Methicillin, oxacillin, ampicillin, carbeni-cillin, piperacillin	Inhibit cell wall synthesis	Broader spectrum of activity than natural penicillins. GPs (not MRSA) & GNs.	β-Lactam. Bactericidal. Penicillinase-resistant penicillins = oxacillin, methi-cillin, nafcillin, cloxacillin, dicloxacillin. Drug class of choice for staph infections.
Cephalosporins	Cephalexin, cefo-toxin, ceftriaxone, cefepime	Inhibit cell wall synthesis	GPs & GNs. Each genera-tion has broader spectrum of activity than previous.	β-Lactam. Bactericidal.
Carbapenems	Imipenem	Inhibit cell wall synthesis	GPs & GNs, including *P. aeruginosa* & anaerobes, but not MRSA or VRE.	β-Lactam. Bactericidal. Reserved for IV use in serious infections.
Monobactams	Aztreonam	Inhibit cell wall synthesis	*P. aeruginosa* & other GNR. Low activity against GPs & anaerobes.	β-Lactam. Bactericidal.

continued...

Major Classes of Antibiotics *continued*

CLASS	REPRESENTATIVE ANTIBIOTICS	MODE OF ACTION	SPECTRUM OF ACTIVITY	OTHER
Glycopeptides	Vancomycin	Inhibit cell wall synthesis	GPs. Drug of choice for MRSA. Some strains of *Enterococcus* are now resistant (VRE).	Bactericidal. Used for serious infections.
Macrolides	Erythromycin, clarithromycin, azithromycin	Inhibit protein synthesis	GPs & some GNs, intracellular organisms, atypical mycobacteria.	Bacteriostatic.
Tetracyclines	Tetracycline, doxycycline	Inhibit protein synthesis	GPs & GNs, intracellular organisms, spirochetes, MRSA, VRE.	Bacteriostatic. Used less often than β-lactams. Not given to children or pregnant women due to staining of teeth, abnormal bone growth.
Aminoglycosides	Gentamycin, tobramycin, amikacin	Inhibit protein synthesis	Used primarily for GNs. Ineffective against anaerobes.	Bactericidal. Toxic to kidneys & ears. Only used to treat serious GN infections.

continued...

CLASS	REPRESENTATIVE ANTIBIOTICS	MODE OF ACTION	SPECTRUM OF ACTIVITY	OTHER
Sulfonamides	Sulfamethoxazole (SMZ), trimethoprim (TMP)	Inhibit folic acid synthesis	Broad spectrum of activity against Enterobacteriaceae.	Bacteriostatic. Used primarily for UTI.
Quinolones	Ciprofloxacin, levofloxacin, ofloxacin	Inhibit DNA synthesis	Broad spectrum.	Used to treat serious infections. Bactericidal. Ciprofloxacin used for anthrax.

MRSA = methicillin-resistant *S. aureus*; VRE = vancomycin-resistant *Enterococcus.*

Disk Diffusion Susceptibility Method (Kirby Bauer)

Organisms	Rapidly growing aerobes & facultative anaerobes. Not for slow growers, anaerobes, or fastidious organisms (except with modifications).
Inoculum	18–24 hr pure culture. 1.5×10^8 CFU/mL. Compare to 0.5 McFarland standard. Too heavy = smaller zones/false resistant. Too light = larger zones/false susceptible.
Medium	Mueller-Hinton agar. Swab entire surface evenly to achieve solid lawn of growth.
Disks	Store in refrig or freezer in sealed, desiccated container. Warm to RT before use. Check expiration dates. Place within 15 min of inoculation. No more than 12 per 150-mm plate.
Incubation	Within 15 min of disk placement. Inverted. Ambient air. 35°C for 16–18 hr. $<$16 hr = ↑ zone, false susceptible. $>$18 hr = ↓ zone, false resistant. (Exception: MRSA may require 24 hr.)
Modifications for fastidious bacteria	*Streptococcus:* Use MH supplemented with 5% sheep blood. Incubate in 5%–7% CO_2. *Haemophilus:* Use *Haemophilus* test medium (HTM). Incubate in 5%–7% CO_2.
Reading	Routine: Measure zones from back side of plate using ruler or calipers. Media containing blood: Read from top with lid removed.
Situations/actions	Sparse growth: Repeat with standardized inoculum. Mixed culture: Repeat with pure inoculum. Colonies within zone: Subculture & retest. Slight growth in zone for trimethoprim & sulfonamides: Disregard. Swarming of *Proteus* into zone: Ignore.

continued...

Disk Diffusion Susceptibility Method (Kirby Bauer) *continued*

Reporting	Resistant, intermediate, or susceptible based on zone of inhibition in mm. Refer to CLSI interpretive tables.
Quality control	Test QC strains of *E. coli, S. aureus, P. aeruginosa, Enterococcus faecalis* for 20–30 consecutive days. If results are acceptable, frequency can be reduced to weekly.

CFU = colony-forming units; CLSI = Clinical and Laboratory Standards Institute.

Other Susceptibility Tests

TEST	EXPLANATION
Minimal inhibitory concentration (MIC)	Standard inoculum added to serial dilutions of antibiotics & incubated. MIC = least amount of antibiotic (highest dilution) that prevents visible growth. Can be done in tubes, microtiter trays, or automated analyzers.
Minimal bactericidal concentration (MBC)	Clear tubes from MIC are subcultured to agar. MBC = concentration of antibiotic that results in 99.9% ↓ in CFU per mL.
Serum bactericidal test (Schlicter test)	Serial dilutions of patient's peak & trough specimens inoculated with standardized amount of patient's pathogen & incubated overnight. Serumstatic titer = highest dilution that inhibits growth. Clear tubes subcultured. Serumcidal titer = highest dilution with 99.9% ↓ in CFU/mL. Rarely performed.
β-Lactamase	Detects enzyme that cleaves β-lactam ring, rendering penicillin & cephalosporins ineffective. Test *Haemophilus, N. gonorrhoeae, Moraxella*. Bacteria applied to moistened disk impregnated with cephalosporin nitrocefin (cefinase disk). Red color if β-lactam ring is broken, i.e., organism produces β-lactamase.
Screen for penicillin susceptibility in *S. pneumoniae*	Test with oxacillin disk instead of penicillin disk. More sensitive in detecting resistance. Mueller-Hinton with 5% sheep blood inoculated with organism. Oxacillin disk (1 µg) placed. Incubated overnight in 5%–7% CO_2. Zone of inhibition ≥20 mm = susceptible to penicillin. <20 mm, perform MIC.

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TEST	EXPLANATION
Tests for oxacillin- (methicillin-) resistant *S. aureus* (MRSA)	Oxacillin used as class representative for penicillinase-resistant penicillins. Organism resistant to it is resistant to all. (Oxacillin-resistant *S. aureus* referred to as methicillin-resistant for historical reasons.) Oxacillin screen plate: Mueller-Hinton with 4% NaCl & oxacillin (6 μg /mL) inoculated & incubated overnight. Any growth = resistant. Not suitable for testing coag-neg staph (CNS). CLSI recommends cefoxitin disk diffusion method for both *S. aureus* & CNS. Many labs use PCR. Can be done directly on specimen. Faster results.
Vancomycin screen	For detection of vancomycin-resistant enterococci (VRE). Brain-heart infusion agar plus 6 μg vancomycin/mL inoculated & incubated overnight. Growth = resistance. Can also be used for *S. aureus.*
E test	Plastic strip containing antibiotic concentration gradient placed on inoculum lawn on Mueller-Hinton plate & incubated overnight. MIC = point where border of growth inhibition intersects strip. Combines convenience of disk diffusion with ability to generate MIC data. Useful for testing fastidious organisms such as *S. pneumoniae*, other strep, *H. influenzae*, & anaerobes.
D test	To detect inducible clindamycin resistance in MRSA isolates that are resistant to erythromycin & susceptible to clindamycin on initial testing. Erythromycin & clindamycin disks placed 15–26 mm apart on Mueller-Hinton agar inoculated with organism. After overnight incubation, flattened zone between disks (D-shaped zone of inhibition around clindamycin disk) means erythromycin induces clindamycin resistance. Clindamycin reported as resistant. Similar procedure for beta-hemolytic strep except Mueller-Hinton with sheep blood used & disks placed 12 mm apart.

Automated Identification and Susceptibility Testing

Identification	Disposable cards or microtiter plates with freeze-dried conventional or fluorogenic substrates are inoculated & incubated. Detection based on turbidity, colorimetry, or fluorescence. Panels available for ID of Enterobacteriaceae & other GNR, *Neisseria*, *Haemophilus*, streptococci, enterococci, staphylococci, yeast, anaerobes.
Susceptibility	Broth with various dilutions of antibiotics are inoculated & incubated. Growth is determined by photometry, turbidity, or fluorescence, depending on system.
Features	Walk-away capability, LIS interface, generation of institutional antibiograms (susceptibility data for most commonly isolated organisms), epidemiology reports.
Advantages	Accuracy, shorter turnaround time, frees techs for other duties.
Limitations	Only for rapidly growing organisms. Not all microorganisms are in databases.

Examples of Quality Control in Microbiology Labs

PARAMETER	REQUIREMENT(S)
Autoclave	Spore test weekly *(B. stearothermophilus)*. Must reach 121°C/15 psi.
Balances	Check for accuracy annually.
Biosafety cabinet	Check airflow annually.
Centrifuges	Check rpm every 6 months.
CO_2 incubator	Check CO_2 & temp daily.
Gas pack	Check for anaerobiosis with methylene blue strip each use (white = no O_2, blue = O_2).
Microscopes	Clean & adjust 4 times/yr.
Temperatures	Daily checks of incubators, heating blocks, water baths, refrigerators, freezers.
Thermometers	Must be checked against reference thermometer from National Bureau of Standards.
Instrument logs	Document routine function checks, preventive maintenance service. Maintain for life of instrument.
Gram stain	Control slide weekly (*E. coli* & *S. aureus*).
Reagents	Most are tested each day of use with pos & neg controls.

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Examples of Quality Control in Microbiology Labs *continued*

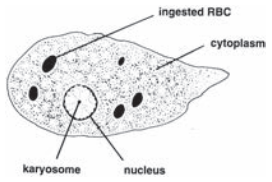
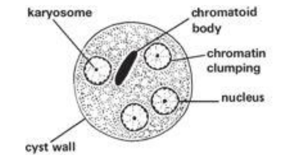
PARAMETER	REQUIREMENT(S)
Media	Most exempt from retesting if purchased from manufacturer who follows CLSI guidelines. Obtain & retain statement of QC. Retesting required for CHOC, selective media for *Neisseria* & *Campylobacter*.
Procedure manual	Must be reviewed & signed annually by lab director. Changes must be approved & signed by lab director. Obsolete procedures must be removed & retained for 2 years.
QC manual	Record all QC results & document any corrective action. Forms should be reviewed & initialed by supervisor monthly. Maintain for at least 2 years.
Employee competency	Verification on hiring & annually.
Proficiency testing	Test unknowns exactly as patient specimens. Must maintain an average score of 80%. Document corrective action.

Stool Specimens for Ova and Parasites

CONSISTENCY	MAXIMUM TIME BETWEEN COLLECTION AND EXAMINATION	PARASITES RECOVERED
Liquid	30 minutes	Trophozoites, helminth eggs, larvae
Semisolid	1 hour	Trophozoites, cysts, helminth eggs, larvae
Formed	24 hours	Cysts, helminth eggs, larvae

Ova and Parasite Examination

Number of specimens	1–3
Interfering substances	Antibiotics, antiseptics, barium, laxatives, mineral oil, soap, water, urine
Preservation	Polyvinyl alcohol (PVA), 5%–10% formalin, MIF, sodium acetate–acetic acid–formalin (SAF). Formed stools may be refrigerated for 1–2 days
Direct smear, saline	On fresh liquid stools. To observe motility of trophs
Direct smear, iodine	1% D'Antoni or Lugol's iodine. To observe nuclei of cysts. Trophs will be killed
Concentration	To concentrate parasites & separate from fecal debris
Sedimentation method	Formalin-ethyl acetate. Recovers eggs, larvae, cysts
Flotation method	33% zinc sulfate. Specific gravity 1.18. Parasites float to top. May miss operculated eggs, unfertilized *Ascaris*
Permanent stained slides	For Dx of protozoa. PVA fixed or fresh specimen. Iron hematoxylin or trichrome stain

AMEBA	NUCLEUS	TROPH	CYST	PATHOGENICITY	OTHER
Entamoeba histolytica	Round. Fine, even peripheral chromatin. Small, delicate, central karyosome.	10–60 μm, average 20 μm. 1 nucleus. Delicate, finely granular cytoplasm. Ingested RBCs. Seldom contains bacteria. Single, long, finger-like pseudopod. Directional motility.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:91.)*	Round. 10–20 μm. 2–4 nuclei. 10% have cigar-shaped chromatoid bodies. May have glycogen vacuoles.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:91.)*	Causes acute amebic dysentery. Can cause extraintestinal amebiasis.	Only ameba that ingests RBCs. EIA for antigen detection. Molecular testing available.

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AMEBA	NUCLEUS	TROPH	CYST	PATHOGENICITY	OTHER
Entamoeba hartmanni	Like *E. histolytica*.	Like *E. histolytica*, but 5–12 µm & ingests bacteria.	Like *E. histolytica,* but smaller. 5–9 µm.	Nonpathogenic.	
Entamoeba coli	Coarse, irregular peripheral chromatin. Eccentric karyosome.	15–50 µm. Coarse cytoplasm with many vacuoles & ingested bacteria. Short, blunt, multiple pseudopods. Sluggish nondirectional motility.	Round. 10–35 µm. 2–8 nuclei. Occasional chromatoid bodies with splintered ends. May have glycogen vacuoles.	Nonpathogenic.	
Endolimax nana	No peripheral chromatin. Large, irregular karyosome.	2–12 µm. Single nucleus. Finely granular vacuolated cytoplasm. Blunt pseudopods. Sluggish, nonprogressive motility.	5–10 µm. Round to oval. Usually 4 nuclei. No chromatoid bodies. May have poorly defined glycogen mass.	Nonpathogenic.	One of smallest amebae.
Iodamoeba bütschlii	No peripheral chromatin. Large karyosome surrounded by layer of small granules.	8–20 µm. Coarsely granular cytoplasm with vacuoles & bacteria. Blunt pseudopod. Sluggishly progressive motility.	5–20 µm. Ovoid. 1 nucleus. Prominent glycogen vacuole. "Iodine cyst."	Nonpathogenic.	Only ameba with just one nucleus in cyst.

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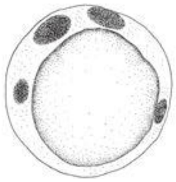
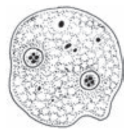
AMEBA	NUCLEUS	TROPH	CYST	PATHOGENICITY	OTHER
Blastocystis hominis	Up to 4, pushed to the side.	Round. 6–40 µm. Large central body.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:95.)*	None	Uncertain.	Should be quantitated. Formerly classified as yeast; now considered an ameba.

All amebae are transmitted by ingestion of fecally contaminated food or water.

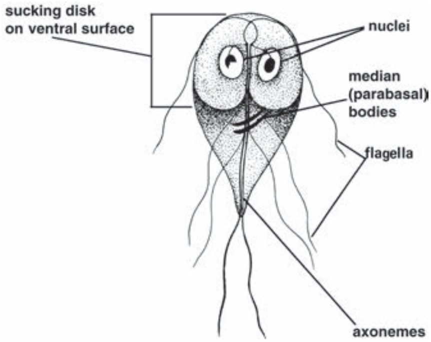
Flagellates of the Intestinal and Urogenital Tracts

FLAGELLATE	TROPH	CYST	TRANSMISSION	PATHOGENIC?	OTHER
GI tract					
Dientamoeba fragilis	5–15 µm. 80% have 2 nuclei. No peripheral chromatin. Karyosome is mass of 4–8 granules.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:101.)*	None	Fecal-oral, oral-anal	Yes	Flagella only visible with electron microscope. Common in U.S.

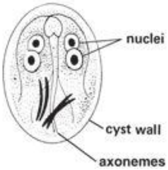
continued...

Flagellates of the Intestinal and Urogenital Tracts *continued*

FLAGELLATE	TROPH	CYST	TRANSMISSION	PATHOGENIC?	OTHER
Giardia lamblia (also known as *G. intestinalis* or *G. duodenalis)*	Pear-shaped. 10–20 µm. 2 nuclei with large central karyosomes. 4 pairs of flagella. Anterior & ventral sucking disks. 2 axostyles. 2 median bodies. "Old man in glasses." Falling leaf motility.	Ovoid. 8–19 µm. Thick double wall. Cytoplasm shrinks away from cell wall. 2–4 nuclei at anterior end. Median bodies & axostyles.	Ingestion of cyst in contaminated water, oral-anal, pets	Yes	Common in U.S. Enterotest: Patient swallows capsule attached to string. String withdrawn after 4 hr. Mucus examined. Antigen detection.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:100.)



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:100.)

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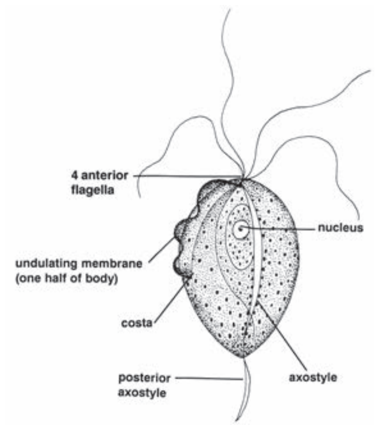
Flagellates of the Intestinal and Urogenital Tracts *continued*

FLAGELLATE	TROPH	CYST	TRANSMISSION	PATHOGENIC?	OTHER
Chilomastix mesnili	6–24 µm. Broad anterior, tapering posterior. 3 flagella at anterior, one at posterior. Spiral groove running length of body. Prominent cytostome bordered by fibrils. Single nucleus at anterior. Stiff rotary motility.	6–10 µm. Round with bulge at anterior. Single large nucleus. Hourglass-shaped cytostome & prominent fibrils. "Lemon cyst."	Ingestion of cyst	No	
Trichomonas hominis	6–14 µm. Prominent axostyle extending through posterior. 4 anterior flagella. Undulating membrane entire length of body along costa. 1 oval nucleus with small karyosome.	None	Ingestion of fecally contaminated material	No	Easier to ID living organism. Rotary motion.

continued...

Flagellates of the Intestinal and Urogenital Tracts *continued*

FLAGELLATE	TROPH	CYST	TRANSMISSION	PATHOGENIC?	OTHER
Urogenital tract *Trichomonas vaginalis*	5–18 µm. 4 anterior flagella, undulating membrane that extends half the body length.	None	Sexual	Yes	Only flagellate in urogenital tract. Dx by wet mount (jerky motility), culture, rapid antigen tests, PCR.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:103.)

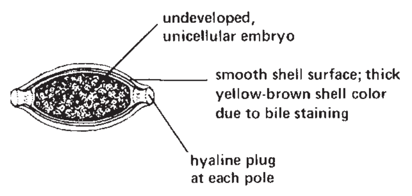
Intestinal Ciliates

CILIATE	TROPH	CYST	TRANSMISSION	PATHOGENIC?	OTHER
Balantidium coli	Ovoid. Usually 40–50 µm, but can reach 200 µm. Covered with cilia. Funnel-shaped cytostome. Macronucleus & micronucleus. Vacuoles. Rapid rotary motion.	Round. 45–65 µm. Thick refractile cell wall. No cilia.	Ingestion of cyst	Yes	Only ciliate pathogenic to humans. Largest protozoan of humans. Common in pigs.

Intestinal Sporozoans

ORGANISM	TRANSMISSION	DIAGNOSIS	OTHER
Isospora belli	Ingestion of oocysts in fecally contaminated food or water	Oocysts in feces. 30 × 12 µm. Transparent. 1–2 sporoblasts.	Human is definitive host. Causes anorexia, nausea, abdominal pain, diarrhea, possible malabsorption.
Cryptosporidium parvum	Ingestion of oocysts from food or water contaminated with animal feces, oral/anal route, direct contact with infected individual or animal	Modified acid-fast stain of feces. Red spherical bodies 3–6 µm. 4 sporozoites. Immunoassay kits.	Important opportunistic infection in AIDS patients. Causes chronic diarrhea in immunosuppressed, acute self-limited diarrhea in immunocompetent.
Cyclospora cayentanensis	Contaminated food & water	Oocysts in direct wet mount. 8–10 µm. Variable staining with modified acid-fast stain. Autofluorescence under UV light. Bright blue at 365 nm, mint green at 450–490 nm.	Prolonged diarrhea.
Microsporidia	Ingestion of spores	Chromotrope or calcofluor white stain of formalin-preserved stool. Spores are 1.5–4.0 µm.	Obligate intracellular parasites. Opportunistic pathogens, mainly in AIDS patients. Cause prolonged diarrhea. Can disseminate. Molecular studies indicate related to fungi.

NEMATODE	ROUTE OF INFECTION	DIAGNOSTIC STAGE	OTHER
Trichuris trichiura	Ingestion of infective egg.	Barrel-shaped ovum. 50 × 23 µm. Brown or yellow. Clear plug at either end.	Whip worm. Eosinophilia. Eggs require developmental period in warm soil before infective.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:17.)

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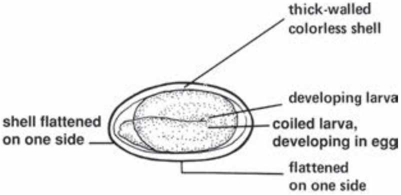
Intestinal Nematodes *continued*

NEMATODE	ROUTE OF INFECTION	DIAGNOSTIC STAGE	OTHER
Enterobius vermicularis	Ingestion of infective egg.	Ovoid ovum. 50 × 20 µm. 1 side flattened. Colorless. Larva inside.	Pinworm. Common in U.S. Common in children. Resistant eggs in home can infect other family members. Itching when adult female migrates out of anus at night to deposit eggs. Diagnose by pinworm paddle or cellophane tape prep.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:15.)

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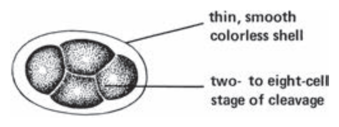
Intestinal Nematodes *continued*

NEMATODE	ROUTE OF INFECTION	DIAGNOSTIC STAGE	OTHER
Necator americanus	Skin penetration or ingestion of filariform larva.	Ovoid ovum. 40 × 60 µm. Colorless. Contains segmented embryo 2- to 8-cell stage surrounded by clear outer zone.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:22.)*	American hookworm. "Ground itch." Larval migration through lungs. Eosinophilia. Can lead to anemia.
Ancylostoma duodenale	Same as *Necator.*	Same as *Necator.*	Old World hookworm.

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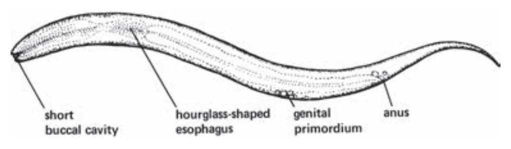
NEMATODE	ROUTE OF INFECTION	DIAGNOSTIC STAGE	OTHER
Strongyloides stercoralis	Skin penetration by filariform larva. Sexual transmission. Autoreinfection.	Rhabditiform larvae. 200–250 µm long × 16 µm in diameter. Short buccal cavity. Large bulb in esophagus. Prominent genital primordium.	Threadworm. Larval migration through lungs. Duodenal aspirates and/or Entero-test may help Dx. Eosinophilia. Can disseminate & be fatal in immunocompromised. Infectious to laboratorians. Must differentiate from hookworm larvae.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:24.)

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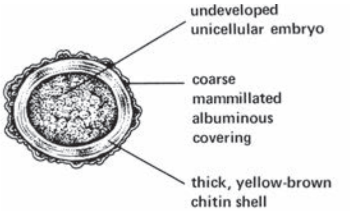
NEMATODE	ROUTE OF INFECTION	DIAGNOSTIC STAGE	OTHER
Ascaris lumbricoides	Ingestion of infective ovum.	Fertilized ovum: ovoid. 50–75 μm × 40–60 μm. Yellow to brown. Granular yolk. Clear zones at either end. May have mammillated covering or be decorticated.	Most common helminth worldwide. Often seen with *Trichuris*. Eggs require several weeks in soil to become infective. Larval migration through lungs. Adult resembles earthworm. May pass from anus, mouth, or nose. Eosinophilia.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:20.)

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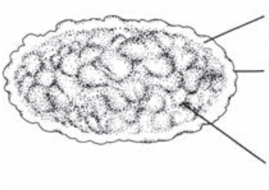
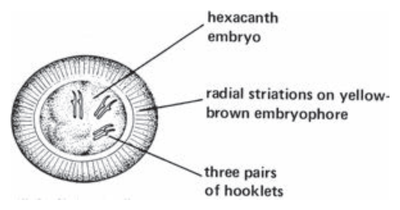
NEMATODE	ROUTE OF INFECTION	DIAGNOSTIC STAGE	OTHER
		Nonfertilized ovum: larger & more elongated. Thinner shell. No concentric clear zones. Can be mammillated or decorticated.	



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5e. Philadelphia: FA Davis; 2002:20.

CESTODE	SCOLEX	GRAVID PROGLOTTID	OVUM	INTERMEDIATE HOST	TRANSMISSION	DIAGNOSTIC STAGE	OTHER
Taenia saginata	Tetragonal. 4 cup-shaped suckers. No hooklets.	Longer than wide. 15–30 uterine branches per side. Irregularly alternating genital pores.	Spherical. 35–45 µm. Yellow or brown. Thick radially striated shell.	Cow	Ingestion of larva in under-cooked beef	Ova in feces	Beef tape-worm. Ova are infective for cattle, but not humans.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:49.)

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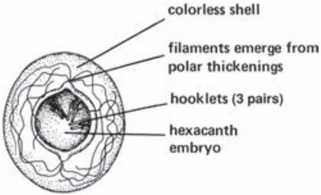
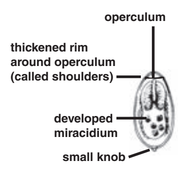
CESTODE	SCOLEX	GRAVID PROGLOTTID	OVUM	INTERMEDIATE HOST	TRANSMISSION	DIAGNOSTIC STAGE	OTHER
Taenia solium	4 cup-shaped suckers. 2 rows of hooklets around rounded rostellum.	Longer than wide. 7–13 uterine branches per side. Regularly alternating genital pores.	Same as *T. saginata*.	Pig	Ingestion of larva in under-cooked pork	Ova in feces	Pork tape-worm. Ova infective to humans. Causes cysticercosis.
Diphyllobothrium latum	Almond-shaped. Lat-eral groove on each side. No hooklets.	Wider than long. Central rosette-shaped uterus.	Ovoid. 45 × 70 µm. Yellow-brown. Oper-culum. Small knob at posterior.	1st: crustacean. 2nd: fish	Ingestion of larva in under-cooked freshwa-ter fish	Ova in feces	Fish tape-worm. Can cause vitamin B_{12} deficiency.

continued...

CESTODE	SCOLEX	GRAVID PROGLOTTID	OVUM	INTERMEDIATE HOST	TRANSMISSION	DIAGNOSTIC STAGE	OTHER
Hymenolepis nana	Diamond-shaped. 4 suckers. Single row of hooklets around short rostellum.	Wider than long. Uterine branches obliterated by eggs. 3 ovoid irregularly spaced testes.	Round. 40 µm. Colorless. 2 distinct walls. Slight bulge at each pole of inner wall with 4–8 hair-like polar filaments.	None	Ingestion of eggs in feces of infected mice/rats	Ova in feces	Dwarf tapeworm. Adult 2.5–4 cm long. Most common tapeworm. Only tapeworm without intermediate host. Mainly in children. Growth retardation.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:47.)

TREMATODE	ROUTE OF INFECTION	LOCATION OF ADULT	DIAGNOSTIC STAGE	OTHER
Fasciolopsis buski	Ingestion of metacercariae on aquatic plants	Duodenum	Ova in feces. 135 × 80 µm. Yellow-brown. Thin shell. Small operculum. Granular contents evenly distributed. Clear zone between shell & yolk.	Largest trematode.
Fasciola hepatica	Ingestion of metacercariae on aquatic plants	Bile duct	Similar to *F. buski*.	Sheep liver fluke.
Clonorchis sinensis	Ingestion of metacercariae in raw, undercooked, dried, salted, or pickled fish	Bile ducts, gallbladder, pancreatic ducts	Ova in feces. 29 × 16 µm. Bulbous. Opercular shoulders. Small comma-shaped protuberance at posterior. Thick shell with tiny spines. Ciliated miracidium inside.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:67.)*	Chinese liver fluke. One of the most frequent infections seen in Asian refugees. Egg is one of smallest passed by humans.

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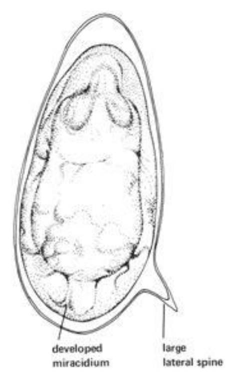
TREMATODE	ROUTE OF INFECTION	LOCATION OF ADULT	DIAGNOSTIC STAGE	OTHER
Paragonimus westermani	Ingestion of under-cooked crabs or crayfish	Lungs	Ova in feces or sputum. 80–120 μm × 48–60 μm. Yellow-brown. Thick shelled. Flattened operculum. "Chinese vase."	
Schistosoma mansoni	Skin penetration	Intestinal venules *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:74.)*	Ova in feces. Elongated. 155 × 65 μm. Pronounced lateral spine.  	Blood fluke

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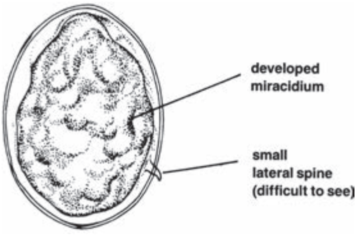
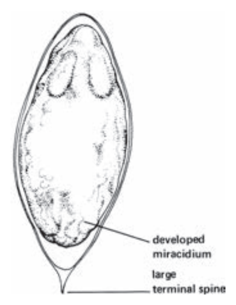
TREMATODE	ROUTE OF INFECTION	LOCATION OF ADULT	DIAGNOSTIC STAGE	OTHER
Schistosoma japonicum	Skin penetration	Intestinal venules	Ova in feces. Ovoid. 90 × 70 µm. Minute lateral spine.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:74.)*	Blood fluke.

continued...

TREMATODE	ROUTE OF INFECTION	LOCATION OF ADULT	DIAGNOSTIC STAGE	OTHER
Schistosoma haematobium	Skin penetration	Bladder venules	Ova in urine. Elongated. 140 × 60 µm. Terminal spine.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:75.)*	Blood fluke. Only parasite of urinary system.

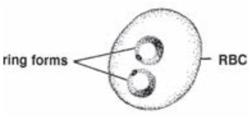
Note: All trematodes have a snail as an intermediate host.

ORGANISM	CLASSIFICATION	TRANSMISSION	DIAGNOSIS	OTHER
Acanthamoeba	Ameba	Swimming in contaminated water, inadequately disinfected contact lenses	Stained smears of culture material. Histological examination of brain. Trophs & cysts in corneal scrapings.	Causes granulomatous amebic encephalitis (GAE) & ulcerative acanthamoeba keratitis in contact lens wearers. Usually in immunocompromised or debilitated.
Naegleria fowleri	Ameba	Swimming in contaminated ponds or streams	Stained smears of culture material. Trophs in CSF.	Causes primary amebic meningoencephalitis (PAM).
Plasmodium	Sporozoan	Anopheles mosquito	Wright's-stained thick & thin blood smears. Draw blood just before paroxysm.	Causes malaria. Parasites in RBCs.

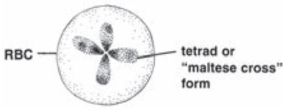
continued...

Blood and Tissue Protozoa *continued*

ORGANISM	CLASSIFICATION	TRANSMISSION	DIAGNOSIS	OTHER
Babesia	Sporozoan	Tick bite, blood transfusion	Wright's-stained thick & thin blood smears. Parasites in RBCs. 2–4 µm. Pear-shaped. Usually in pairs or tetrads (Maltese cross).	Symptoms resemble malaria.



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:121.)



(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:121.)

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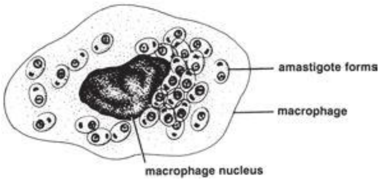
ORGANISM	CLASSIFICATION	TRANSMISSION	DIAGNOSIS	OTHER
Toxoplasma gondii	Coccidian	Ingestion of undercooked meat or oocysts from cat feces, transplacental, organ transplants	Serological tests. PCR.	Cat is definitive host. Congenital infection causes birth defects & mental retardation. Major cause of encephalitis in AIDS patients.
Leishmania	Flagellate	Sand flies	Amastigote forms in WBCs & cells of RE system.  (From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:111.)	Obligate intracellular parasite. Causes kala azar, cutaneous & mucocutaneous leishmaniasis.

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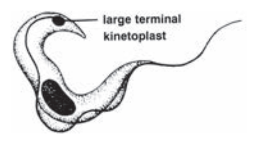
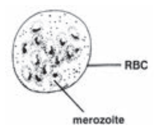
ORGANISM	CLASSIFICATION	TRANSMISSION	DIAGNOSIS	OTHER
Trypanosoma	Flagellate	*T. gambiense* & *rhodesiense*: tsetse fly. *T. cruzi*: reduviid or kissing bug.	*T. gambiense* & *rhodesiense*: trypomastigote in blood, lymph nodes, CSF. *T. cruzi*: trypomastigote in blood; amastigote in RE cells, myocardium, CNS.  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:109.)*	*T. gambiense* & *rhodesiense*: Sleeping sickness. *T. cruzi*: Chagas disease; found in southern U.S.

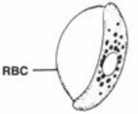
Differentiation of *Plasmodium*

	P. FALCIPARUM	*P. MALARIAE*	*P. OVALE*	*P. VIVAX*
Name of disease	Malignant malaria	Quartan malaria	Ovale malaria	Tertian malaria
Paroxysm cycle	36–48 hr	72 hr	48 hr	44–48 hr
Infected RBCs	Not enlarged	Not enlarged	Sometimes enlarged. Frequently oval with ragged margins. Schüffner's dots	Enlarged. Schüffner's dots
Stages seen	Ring forms (trophs) & gametocytes	All	All	All
Merozoites per mature schizont	Not seen in peripheral blood	6–12	8–12	12–24 

(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:119.)

continued...

Differentiation of *Plasmodium* *continued*

	P. FALCIPARUM	*P. MALARIAE*	*P. OVALE*	*P. VIVAX*
Shape of gametocytes	Sausage- or crescent-shaped  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:118.)*	Ovoid  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:118.)*	Round	Round

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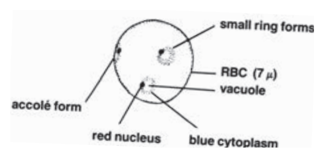
Differentiation of *Plasmodium* *continued*

	P. FALCIPARUM	P. MALARIAE	P. OVALE	P. VIVAX
Multiple parasites per cell?	Yes  *(From Leventhal R, Cheadle RF. Medical Parasitology: A Self-Instructional Text, 5th ed. Philadelphia: FA Davis; 2002:118.)*	No	No	Rare
Double chromatin dots?	Yes	No	No	Rare
Other characteristics	High mortality. Medical emergency	Rarely fatal. Band-shaped trophs	Least common. Rarely fatal. May cause relapses	Most common. Rarely fatal. May cause relapses

Note: A 5th species, *P. knowlesi*, now recognized. Can resemble *P. malariae* or *P. falciparum*. Differentiate by PCR.

Blood and Tissue Helminths

ORGANISM	CLASSIFICATION	TRANSMISSION	DIAGNOSIS	OTHER
Toxocara	Nematode (in dogs/cats)	Ingestion of eggs from dog/cat feces	Serological tests.	Humans are accidental hosts. Larvae in tissues. Visceral & ocular larva migrans (toxocariasis).
Trichinella spiralis	Nematode	Undercooked pork, walrus, bear	Larvae encysted in muscle. Serological tests.	Eosinophilia, edema of upper eyelids, fever, myalgia.
Wuchereria bancrofti	Nematode	Mosquitoes	Microfilariae in blood. Giemsa-stained thick & thin smears.	Elephantiasis (lymphatic filariasis).
Brugia malayi	Nematode	Mosquitoes	Microfilariae in blood. Giemsa-stained thick & thin smears.	Elephantiasis. Nocturnal periodicity.
Loa loa	Nematode	Mango fly (*Chrysops*)	Microfilariae in blood. Giemsa-stained thick & thin smears.	Loiasis. Nocturnal periodicity.
Onchocerca volvulus	Nematode	Black fly	Microfilariae from skin snips or aspirates of nodules	River blindness.
Echinococcus granulosus	Cestode	Ingestion of fecal material from infected dog	Radiologic ID of cysts or ID of scoleces in needle biopsy material from cyst (hydatid sand). Serological methods available.	Humans are accidental intermediate host. Cysts develop in liver or lungs.

Stains Used in Mycology

STAIN	USED FOR	OTHER
KOH	Skin, hair, nails	Dissolves keratin. Makes fungal elements more visible.
Calcofluor white	Tissues, sputum, body fluids, skin & corneal scrapings	Fluorescent stain.
Lactophenol cotton blue	Microscopic exam of fungal culture	Lactic acid preserves. Phenol kills. Cotton blue stains.
Gram stain	Yeast	Fungi stain gram pos.
Acid-fast	*Nocardia*	*Nocardia* is partially acid-fast.
India ink	CSF sediment if *Cryptococcus* is suspected	Halos around yeast due to wide capsule. Insensitive. Direct antigen test preferred.
Wright/Giemsa	Blood & bone marrow	Useful for *Histoplasma.*

Fungal Culture Media

MEDIUM	USE
For isolation	
Sabouraud dextrose agar (SDA)	General medium. pH 5.6 inhibits bacteria. Grows most molds & yeast.
Sabouraud dextrose agar with antibiotics	Antibiotics inhibit fungal contaminants & bacteria. Dermatophytes & most fungal pathogens grow.
Brain-heart infusion agar	For isolation & conversion of dimorphic fungi from mold to yeast phase. Used for normally sterile specimens.
Brain-heart infusion agar with antibiotics	Selective medium used for isolation of pathogenic fungi from specimens contaminated with bacteria.
Inhibitory mold agar	For recovery of fungi from specimens contaminated with bacteria. Contains chloramphenicol & gentamicin to inhibit bacteria.
Dermatophyte test medium	For recovery of dermatophytes from skin, hair, nails. Turn agar from yellow to red. Antibiotics inhibit bacteria.
For identification	
Potato dextrose agar	Stimulates sporulation of molds. Good for slide cultures.
Cornmeal agar with Tween 80	To differentiate *Candida* species. *C. albicans* produces chlamydospores.
Urea agar	Detection of urease production by *C. neoformans.*
Birdseed agar (niger seed or caffeic agar)	Isolation of *C. neoformans*. Black-brown colonies in 4–7 days.

DERMATOPHYTE	INFECTS	MACROCONIDIA	MICROCONIDIA	OTHER CHARACTERISTICS	MOST COMMON SPECIES
Microsporum	Skin, hair. Rarely nails.	Large, spindle or cylinder shaped, thick walled, multiseptate, rough, spiny. Borne singly on short conidiophores.	Few or absent. Small, club shaped.	Ectothrix hair invasion. Some species cause hair to fluoresce.	*M. caris, M. gypseum, M. audouinii*
Trichophyton	Skin, hair, nails.	Rare. Pencil shaped, multiseptate, thin walled, smooth, borne singly on conidiophore.	Predominant. Spherical, tear shaped, or clavate.	Hair infections endothrix or ectothrix. Hair usually doesn't fluoresce. May have spirals, nodular bodies, chlamydospores, faviform mycelia. *T. rubrum* colonies are red on reverse side.	*T. mentagrophytes, T. rubrum, T. tonsurans* (primary cause of tinea capitis in U.S.)
Epidermophyton	Skin, sometimes nails. Rarely hair.	Club shaped, septate, thin walled, smooth. Borne in singles or clusters of 2–3 on conidiophore.	Absent.	Numerous chlamydospores.	*E. floccosum* is only species

Dimorphic Fungi

FUNGUS	INFECTION	MOLD PHASE	YEAST PHASE	OTHER
Blastomyces dermatitidis	North American blastomycosis (Gilchrist's disease)	White to gray-brown colony. Hyaline, septate hyphae with small oval conidia borne singly at tips of conidiophores. "Lollipops."	8–15 µm. Round, thick walled. Single bud connected by wide neck.	Endemic to Ohio & Mississippi River valleys. Usually in farmers who contract from soil. Begins in lungs. May become systemic.
Paracoccidioides brasiliensis	South American blastomycosis	Resembles *Blastomyces*	10–30 µm. Round, double walled. Multiple buds pinched at attachment. "Mariner's wheel."	Mainly in Brazil, Argentina, S. Mexico. Begins in lungs. Can become systemic.
Coccidioides immitis	Valley fever	Fluffy or powdery white to gray-tan colonies. Barrel-shaped arthrospores.	Spherule 20–80 µm containing numerous endospores.	Found in desert of Southwest U.S. More common in dark-skinned men. Spores are inhaled or contaminate injured skin. Affects respiratory tract 1st. Can spread to other organs. Caution: Arthrospores highly infectious. Serological tests available.

continued...

Dimorphic Fungi *continued*

FUNGUS	INFECTION	MOLD PHASE	YEAST PHASE	OTHER
Histoplasma capsulatum	Histoplasmosis (spelunker's disease, Darling's disease)	Silky, white to gray-tan colonies. Thick-walled spherical macroconidia, 7–16 µm, surrounded by finger-like projections. "Tuberculate chlamydospores"	1–4 µm round to oval. Intracellular in phagocytes of blood or bone marrow.	Endemic in Ohio & Mississippi River valleys. Soil saprophyte. Found in manure of chickens, pigeons, bats. Acute pulmonary disease that can be self-limited or spread. Predilection for RE system. Tissue phase may be confused with *Leishmania*. Serological tests & nucleic acid probes available.
Sporothrix schenckii	Sporotrichosis (rose gardener's disease)	White to yellow colonies. Clusters of pear-shaped conidia at tips of conidiophores. "Flowers"	Gram pos cigar-shaped cells. Usually not seen in direct smears unless by immunofluorescence.	Found in Mississippi & Missouri River valleys. Found on rose bushes, barberry bushes, sphagnum moss, mulch. Usually infects farmers or florists. Introduced by trauma, usually to hand. Cutaneous lesions spread along lymphatics.

Yeast

YEAST	INFECTIONS	IMPORTANT CHARACTERISTICS	OTHER
Candida albicans	Thrush (mouth), vulvovaginitis, diaper rash, onychomycosis (nails), paronychomycosis (cuticles), endocarditis, meningitis, UTI, pulmonary infections, fungemia	Grows on SBA, EMB (spider-like projections), SDA. Gram-pos cells, 2–4 µm. Blastoconidia or pseudohyphae (no constrictions). Produces germ tubes in serum, round terminal chlamydospores on cornmeal agar.	Most frequently isolated yeast. Normal flora in GI tract, mucocutaneous areas. Serious infections most often in immunocompromised. Onychomycosis, esophagitis common in AIDS.
Candida tropicalis	Vaginitis, UTI, GI, pulmonary, & systemic infections	Blastoconidia produced randomly along pseudohyphae on cornmeal agar.	May produce constricted germ tubes. (True germ tubes lack constrictions.) Usually infects immunosuppressed.
Geotrichum candidum	Uncommon cause of wound infections & oral thrush	Forms hockey stick–shaped arthroconidia on cornmeal agar.	No blastoconidia.
Cryptococcus neoformans	Lung infection that can disseminate to brain	Irregularly sized, spherical cells surrounded by capsule. Maroon to brown-black colonies on niger seed agar. Urease pos, phenol oxidase pos.	In bird & bat droppings, decaying vegetation, fruit, milk. Capsule seen with India ink prep in about 50% of cases. Cryptococcal antigen test preferred. Disseminated cryptococcosis common in AIDS.

continued...

Yeast *continued*

YEAST	INFECTIONS	IMPORTANT CHARACTERISTICS	OTHER
Malassezia furfur	Tinea versicolor. Systemic infection in children given lipid replacement therapy	Oval or bottle-shaped budding yeast, 3–8 µm in diameter. Characteristic collar between mother & daughter cells. Chains of short, slightly curved septate hyphae may be seen in skin scrapings.	In skin infections, usually not cultured. Identified by KOH prep of skin scrapings. “Spaghetti & meatball” appearance. In systemic infections, recovered from blood cultures. Will only grow on media overlaid with fatty acid, e.g., sterile mineral or olive oil.
Pneumocystis jiroveci	Atypical interstitial plasma cell pneumonia (PCP)	Cysts are 4–12 µm spheres with intracystic bodies.	Can’t be cultured. Dx by histopathological staining. Opportunistic infection in AIDS & other immunocompromised patients. Formerly *P. carinii* & classified as a protozoan.

Yeasts can be identified by carbohydrate assimilation.

FUNGUS	CLASSIFICATION	SIGNIFICANCE	COLONIAL MORPHOLOGY	MICROSCOPIC MORPHOLOGY
Rhizopus	Zygomycetes	Common laboratory contaminant. Propensity to attack vascular system of immunocompromised. Rapidly spreading. Often fatal.	Mature within 4 days. "Lid lifter." Front is dense cotton candy–like growth, white at first, turning gray or yellowish brown. Reverse is white.	Hyphae: large, broad, nonseptate. Produces horizontal stolons that attach by rhizoids. Sporangiophores in clusters opposite rhizoids. Terminate in dark, round sporangia containing sporangiospores.
Mucor	Zygomycetes	Common laboratory contaminant. Propensity to attack vascular system of immunocompromised. Rapidly spreading. Often fatal.	Mature within 4 days. "Lid lifter." Front is dense cotton candy–like growth, white at first, turning gray. Reverse is white.	Like *Rhizopus,* but sporangiophores may be branched. No rhizoids.
Aspergillus	Hyaline	Common contaminant. Can cause invasive infection, colonization, toxicosis, allergy. *A. fumigatus* is most common.	Mature within 3 days. Front is fluffy, granular, or powdery texture. White at first, then color depends on species: *A. fumigatus* white to blue-green. *A. niger* black. *A. flavus* yellow to green. *A. terreus* tan to cinnamon. Reverse is white, goldish, or brown.	Septate hyphae, branching at 45° angle. Unbranched conidiophore arises from foot cell. Expands into large, spherical vesicle covered with phialides that produce chains of round conidia.

continued...

FUNGUS	CLASSIFICATION	SIGNIFICANCE	COLONIAL MORPHOLOGY	MICROSCOPIC MORPHOLOGY
Acremonium	Hyaline mold	Can be a contaminant. Can cause mycetoma, corneal & nail infections.	Mature within 5 days. Front is white, spreading, moist, colorless. Becomes cottony with gray top. Reverse is yellow or rose.	Small, hyaline, septate hyphae. Unbranched phialides. Oblong, 1- to 2-celled conidia in clusters at tips of phialides.
Fusarium	Hyaline mold	Can be a contaminant. Can cause eye, skin, nail, systemic infections.	Mature within 4 days. Front is white & cottony, developing pink or violet center. Reverse is light.	Septate hyphae. Unbranched conidiophores. Large, canoe-shaped, multiseptate macroconidia. Small 1- to 2-celled oval or cylindrical conidia in singles or clusters on simple conidiophores.
Penicillium	Hyaline mold	Can be contaminant. Can cause keratitis, external ear infections, endocarditis with artificial heart valves.	Mature within 4 days. Front is white at first. Becomes powdery, blue-green with white border. Reverse is usually white.	Septate hyphae. Branched or unbranched conidiophores. "Brush-like." Flask-shaped phialides bearing unbranched chains of round conidia.

continued...

Contaminants/Opportunistic Fungi *continued*

FUNGUS	CLASSIFICATION	SIGNIFICANCE	COLONIAL MORPHOLOGY	MICROSCOPIC MORPHOLOGY
Alternaria	Dematiaceous	Can be contaminant. Can cause subcutaneous infection.	Mature within 5 days. Front is gray-white & wooly at first. Becomes green-black or brown with light border. Reverse is black.	Dark septate hyphae. Conidiophores of variable length, sometimes branched. Large brown, drumstick-shaped conidia with transverse & longitudinal septations, in singles or chains.
Cladosporium	Dematiaceous	Nonpathogenic.	Mature within 7 days. Front is green-brown or black with velvety nap. Becomes heaped & slightly folded. Reverse is black.	Dark septate hyphae. Dark branching conidiophores producing 2 or more chains of oval brown conidia.
Curvularia	Dematiaceous	Can be a contaminant. Can cause sinusitis, keratitis.	Mature within 5 days. Front is dark olive green to brown or black with pink-gray wooly surface. Reverse is black.	Dark septate hyphae. Simple or branched conidiophores, bent where conidia attach. Large, 4-celled, curved conidia. Central cell is larger & darker.

Fungal Pathogens by Site

SITE	POSSIBLE PATHOGENS
Blood/bone marrow	*Histoplasma capsulatum, Cryptococcus neoformans, Candida albicans, Blastomyces dermatitidis, Malassezia furfur*
Cerebrospinal fluid	*Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Candida* spp
Hair	*Trichophyton, Microsporum*
Nail	*Trichophyton, Epidermophyton, Candida, Aspergillus*
Sputum, bronchial washings, transtracheal aspirates	*Candida, Aspergillus, Rhizopus, Mucor, Penicillium, Blastomyces dermatitidis, Coccidioides immitis, Paracoccidioides brasiliensis, Histoplasma capsulatum, Sporothrix schenckii*
Skin	*Candida, Trichophyton, Microsporum, Epidermophyton*
Throat	*Candida albicans, Geotrichum candidum*
Urine	*Candida, Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Cryptococcus neoformans*
Vaginal/cervical	*Candida*

Viral Structure

TERM	EXPLANATION
Virion	Complete virus particle.
Nucleocapsid	Nucleic acid & capsid.
Nucleic acid	DNA or RNA. Single- or double-stranded. Linear or circular.
Capsid	Protein coat that encloses genetic material. May be helical (rod-like) or icosahedral (cuboid). Composed of protein subunits called capsomers. Protects nucleic acid, enables virus to attach to & enter host cell.
Envelope	Outer membrane surrounding capsid in some viruses. Aids in attachment to host cell. Viruses without called naked nucleocapsids.

Viral Replication

TERM	EXPLANATION
Adsorption	Attachment of virus to host cell receptor.
Penetration	Virus enters host cell by direct penetration, endocytosis (entering in a vacuole), or fusion with cell membrane.
Uncoating	Loss of capsid. Genome enters cytoplasm (most RNA viruses) or nucleus (most DNA viruses).
Eclipse/synthesis	Eclipse: several hr during which virions can't be detected. Synthesis: mRNA is produced. Directs synthesis of viral particles.
Maturation/release	Genetic material assembled into protein coat. Virions migrate to cytoplasmic membrane. Released by budding off, leaking out, or lysing host cell with enzymes.

Human DNA Viruses

COMMON FAMILY NAME	REPRESENTATIVE VIRUSES	INFECTION(S)
Adenoviruses	Adenoviruses serotypes 1–489	Respiratory, urinary tract, GI, & eye infections
Hepadnaviruses	Hepatitis B virus (HBV)	Hepatitis B
Herpes viruses	Herpes simplex viruses (HSV-1, HSV-2) Varicella-zoster virus (VZV) Epstein-Barr virus (EBV) Cytomegalovirus (CMV) Human herpesviruses 6–8	Oral, genital, neonatal, & ocular herpes, HSV encephalitis Chicken pox (varicella), shingles (zoster) Infectious mononucleosis Infections in newborns & immunocompromised Roseola, Kaposi sarcoma
Papillomaviruses	Human papilloma virus (HPV)	Warts, including genital warts that are linked to cervical cancer
Parvoviruses	Parvovirus B–19	Fifth disease (erythema infectiosum)
Poxviruses	Variola	Smallpox

Human RNA Viruses

COMMON FAMILY NAME	REPRESENTATIVE VIRUSES	INFECTION(S)
Arenaviruses	Lymphocyte choriomeningitis virus (LCM) Lassa fever virus	Aseptic meningitis or meningoencephalitis Lassa fever
Astroviruses	Astrovirus	Gastroenteritis in children
Bunyaviruses	Arboviruses (California encephalitis, LaCrosse virus) Hantaviruses (Sin Nombre virus)	Encephalitis, hepatitis Hantavirus pulmonary syndrome (HPS)
Caliciviruses	Noroviruses	Most common cause of infectious gastroenteritis in U.S.
Coronaviruses	Coronavirus	Severe acute respiratory syndrome (SARS), cold-like infections, pediatric diarrhea
Filoviruses	Marburg & Ebola viruses	Hemorrhagic fever
Flaviviruses	Arboviruses (yellow fever virus, St. Louis encephalitis virus, dengue virus, West Nile virus) Hepatitis C virus (HCV)	Yellow fever, St. Louis encephalitis, dengue fever, West Nile virus infection Hepatitis C
Orthomyxoviruses	Influenza A, B, & C	Influenza

continued...

Human RNA Viruses *continued*

COMMON FAMILY NAME	REPRESENTATIVE VIRUSES	INFECTION(S)
Paramyxoviruses	Measles virus Mumps virus Parainfluenza virus Respiratory syncytial virus (RSV) Human metapneumovirus (HMPV)	Measles (rubeola) Mumps RTI in children RTI in infants, elderly, immunocompromised RTI
Picornaviruses	Enteroviruses (polioviruses, coxsackieviruses A & B, echoviruses, enteroviruses) Hepatitis A virus (HAV) Rhinovirus	Polio, hand-foot-mouth disease, aseptic meningitis, others Hepatitis A Common cold
Reoviruses	Rotavirus	Most common cause of gastroenteritis in infants & children
Retroviruses	Human immunodeficiency viruses (HIV-1, HIV-2) Human T-lymphotropic viruses (HTLV-1, HTLV-2)	AIDS T-cell leukemia & lymphoma, tropical spastic paraparesis
Rhabdoviruses	Rabies virus	Rabies
Togaviruses	Rubella virus Eastern, Western, & Venezuelan equine encephalitis viruses	Rubella (German measles) Eastern, Western, & Venezuelan encephalitis

RTI = respiratory tract infection.

Common Viruses by Site

SITE OF INFECTION	SPECIMENS	COMMON VIRUSES
Central nervous system	CSF, throat swab, stool, brain tissue, blood	Enteroviruses, HSV, arboviruses
Eye	Conjunctival swab, corneal scraping	HSV, adenoviruses
Genital tract	Genital swab, vesicle swab or fluid, lesion biopsy	HSV, HPV
GI tract	Stool, rectal swab	Adults: noroviruses, adenoviruses, enteroviruses. Infants/children: rotavirus, adenoviruses
Respiratory tract	Nasal aspirate, throat swab, nasopharyngeal swab, bronchoalveolar lavage, lung biopsy	Influenza A & B, parainfluenza virus, adenoviruses, RSV, HMPV, rhinovirus, enteroviruses
Skin	Vesicle fluid or scrapings	HSV, VZV, measles, rubella, enterovirus, parvovirus B19
Urinary tract	Urine	Adenovirus, HSV, CMV

Viral Specimen Collection and Transport

Time of Collection	During acute phase (1st 3–5 days).
Site of collection	Site of infection, entry & exit sites.
Collection containers	Sterile, leak-proof, non-breakable.
Swabs	Dacron, rayon, or other polyester tips. Plastic or aluminum shafts. Calcium alginate, cotton, wood are inhibitory for some viruses.
Transport media	Viral transport medium (VTM), 2-sucrose phosphate (2-SP), Amies or Stuart's transport media. Not required for blood, CSF, urine.
Transport	Deliver immediately. If not possible, keep at 2°–8°C & deliver within 2 hr. Exception: Keep whole blood at room temp.
Storage	Best to process upon arrival. If not possible, hold at 2°–8°C for up to 48 hr. >48 hr, freeze at –70°C. (Not recommended.)

Cytology/histology	Microscopic examination of specimen for viral cytopathic effect (CPE).
Electron microscopy	Rarely used. Labor intensive, expensive.
Direct fluorescent antibody stain	Fluorescent-labeled antibody added to patient cells fixed to slide. If viral antigen present, antibody binds. Fluorescence seen with fluorescent microscope.
Antigen detection	Solid-phase & membrane ELISAs.
Cell culture	Different viruses grow in different cell lines. Growth may take 1–28 days. Examine microscopically for cytopathic effects (CPE): cell rounding, clumping, vacuolation, granulation, giant multinucleate cells, cell fusion, syncytial formation, cell lysis, plaques (groups of killed cells), inclusion bodies. Not all viruses produce CPE. Immunofluorescent stains may be used for confirmation.
Shell vial culture	Rapid modification of conventional cell culture. Detection in 1–2 days. Specimen centrifuged onto monolayer of cells growing on coverslip. Coverslips stained with viral-specific immunofluorescent conjugate. Used primarily for viruses that are slow to produce CPE.
Molecular methods	PCR, real-time PCR, branched DNA, nucleic acid hybridization. Faster & more sensitive than cell culture. Can detect viruses that can't be cultured, multiple viruses simultaneously.
Serology	Detects antibodies in serum. Useful in evaluating immune status or diagnosing viral infections where culture is difficult or impossible. Presence of antibodies isn't always indicative of current infection.

Cell Cultures

CELL LINE	DERIVATION	EXAMPLES	FOR ISOLATION OF
Primary	Cells from mammalian tissue	Primary monkey kidney (PMK) cells	Most viruses
Finite (diploid)	Cultures of connective tissue cells	Human neonatal lung (HNL)	CMV (only type of cell culture that can be used)
Continuous (immortal, heteroploid)	Malignant or transformed cells	Hep2, A549, Vero	HSV, RSV, enteroviruses, adenoviruses

No single cell type grows all viruses. Several types should be inoculated.

	BACTERIA	CHLAMYDIA	MYCOPLASMA	RICKETTSIAE	VIRUSES
Size	0.4–2 µm	0.3–0.5 µm	300 nm	0.3–0.5 µm	25–300 nm
DNA & RNA?	Both	Both	Both	Both	Either
Ribosomes?	Yes	Yes	Yes	Yes	No
Reproduce by binary fission?	Yes	Yes	Yes	Yes	No
Cell wall?	Yes	Yes	No	Yes	No
Growth on nonliving medium?	Yes	No	Yes	No	No
Obligate intracellular parasite?	Most are not	Yes	No	Yes	Yes
Gram stain?	Yes	No	No	No	No
Susceptible to antibiotics?	Yes	Yes	Yes	Yes	No

SECTION

4

Hematology Review

Blood Cells

CELL	FUNCTION	SITE OF PRODUCTION
Erythrocytes	O_2 transport	Bone marrow
Granulocytes	Defense against bacterial infection	Bone marrow
Lymphocytes	Cellular & humoral immunity	Lymphoid tissue
Platelets	Coagulation	Bone marrow

Comparison of Conventional and SI Units for Adult Reference Ranges

	CONVENTIONAL UNITS	SI UNITS
WBC	4.5–11.5 × 10^3/⊠L	4.5–11.5 × 10^9/L
RBC	Male: 4.6–6 × 10^6/⊠L Female: 4–5.4 × 10^6/⊠L	Male: 4.6–6 × 10^{12}/L Female: 4–5.4 × 10^{12}/L
HGB	Male: 14–18 g/dL Female: 12–15 g/dL	Male: 140–180 g/L Female: 120–150 g/L
HCT	Male: 40%–54% Female: 35%–49%	Male: 0.40–0.54 L/L Female: 0.35–0.49 L/L
MCV	80–100 fL	80–100 fL
MCH	27–31 pg	27–31 pg
MCHC	32%–36%	32–36 g/dL
PLT	150–450 × 10^3/⊠L	150–450 × 10^9/L

SI = Syst' me International d'Unit"s (international system of units).

	BIRTH	1–2 MO	1–3 YR	8–13 YR	ADULT	COMMENTS
RBCs ($\times 10^{12}$/L)	4.10–6.10	3.4–5	3.4–5.2	4–5.4	M: 4.6–6 F: 4–5.4	
HGB (g/dL)	16.5–21.5	10.6–16.4	9.6–15.6	12–15	M: 14–18 F: 12–15	Preterm infants: About 1 g lower than full-term
HCT (%)	48–68	32–50	38–48	35–49	M: 40–54 F: 35–49	
MCV (fL)	95–125	83–107	78–94	80–94	80–100	Macrocytes 1st 5 days. MCV higher in preterm infants
RDW (%)	14.2–19.9		11.4–14.5	11.5–14.5	11.5–14.5	
Retic (%)	1.5–5.8	0.8–2.8	0.5–1.5	0.5–1.5	0.5–1.5	Newborns: ↑ polychromasia
NRBCs (/100 WBCs)	2–24	0	0	0	0	Preterm infants: Up to 25 for >1 wk

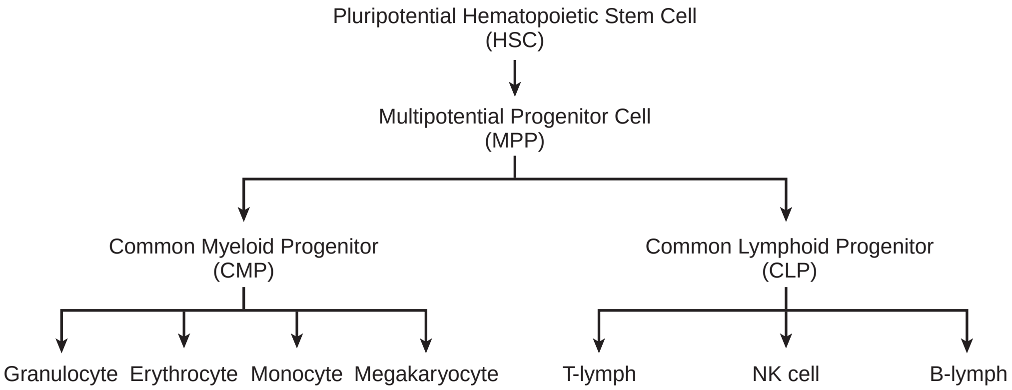
Note trends and comparisons to adult values.

Reference Ranges for Leukocytes and Platelets

	BIRTH	1–2 MO	1–3 YR	8–13 YR	ADULT	COMMENTS
WBCs (× 10^9/L)	9–37	6–18	5.5–17.5	4.5–13.5	4.5–11.5	
Segs (%)	37–67	20–40	22–46	23–53	50–70	
Bands (%)	3–11	0–5	0–5	0–5	0–5	Newborns: Occasional metas & myelos. More immature grans in preterm infants.
Lymphs (%)	18–38	42–72	37–73	23–53	18–42	Newborns: A few benign immature B cells may be seen ("baby" or "kiddie" lymphs).
PLT (× 10^9/L)	150–450	150–450	150–450	150–450	150–450	Newborns: Variation in size & shape.

Note trends and comparisons to adult values.

Hematopoietic Cell Differentiation



Note that this algorithm does not show all differentiation steps.

Erythropoiesis

PERIOD	SITE(S)	COMMENTS
1–2 mo of gestation	Yolk sac & aorta-gonads-mesonephros (AGM) region	Primitive erythroblasts. Embryonic hemoglobin (Gower I, Gower II, Portland).
3–6 mo of gestation	Liver, spleen	Liver is primary site.
7 mo of gestation—age 4 yr	Bone marrow	All marrow is active.
Adult	Bone marrow	Only active sites are pelvis, vertebrae, ribs, sternum, skull. Shafts of long bones filled with fat. Fatty marrow may be reactivated to compensate for anemia. Liver & spleen may be reactivated (extramedullary hematopoiesis) if bone marrow fails to keep up with demand.

Changes During Cell Maturation

CHARACTERISTIC	CHANGE(S) WITH MATURATION
Size	Becomes smaller.
N:C ratio	Becomes smaller.
Cytoplasm	Less basophilic due to loss of RNA. Granulocytes produce granules. Erythrocytes become pink due to Hgb production.
Nucleus	Becomes smaller. Nuclear chromatin condenses. Nucleoli disappear. In granulocytic series, nucleus indents, then segments. In erythrocytic series, nucleus is extruded.

N:C ratio = nucleus to cytoplasm ratio

Erythrocytic Developmental Series

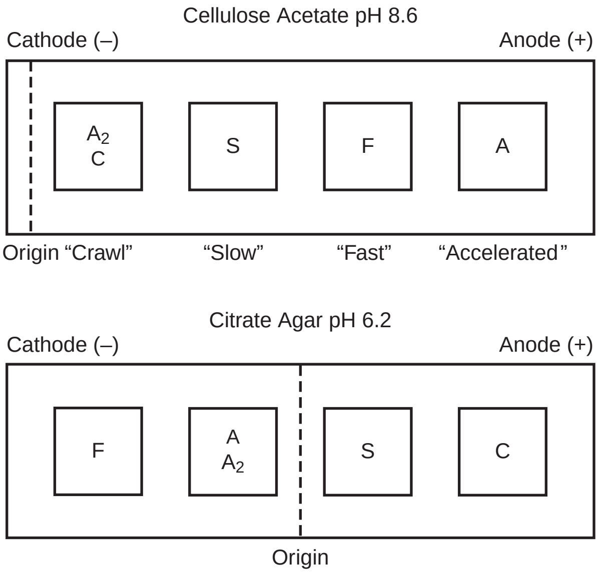
RUBRIBLAST TERMINOLOGY	NORMOBLAST TERMINOLOGY	KEY CHARACTERISTICS
Rubriblast	Pronormoblast	14–24 µm. N:C ratio 8:1. Royal blue cytoplasm. Fine chromatin. 1–2 nucleoli. Normally confined to bone marrow.
Prorubricyte	Basophilic normoblast	12–17 µm. N:C ratio 6:1. Chromatin is coarser with slightly visible parachromatin. Nucleoli usually not visible. Normally confined to bone marrow.
Rubricyte	Polychromatophilic normoblast	10–15 µm. N:C ratio 4:1. Cytoplasm is polychromatophilic due to hemoglobin production. Chromatin is clumped with distinct areas of parachromatin. Last stage to divide. Normally confined to bone marrow.
Metarubricyte	Orthochromic normoblast	8–12 µm. N:C ratio 1:2. Nucleus is pyknotic. Last nucleated stage. Normally confined to bone marrow.
Reticulocyte	Polychromatophilic erythrocyte	7–10 µm. No nucleus. Cytoplasm is diffusely basophilic (bluish tinge). Reticulum seen with supravital stain. 0.5%–1.5% of RBCs in adult peripheral blood.
Mature erythrocyte	Mature erythrocyte	7–8 µm. Biconcave disk. Reddish-pink cytoplasm with area of central pallor 1/3 diameter of cell.

Asynchronous Erythropoiesis

TYPE	CAUSE	EXPLANATION	CHARACTERISTICS	EXAMPLE
Megaloblastic	Vitamin B_{12} or folic acid deficiency	Nucleus lags behind cytoplasm in maturation. Cells grow larger without dividing.	Oval macrocytes	Pernicious anemia
Iron deficiency	Iron deficiency	Cytoplasm lags behind nucleus in maturation due to inadequate iron for hgb synthesis.	Microcytic, hypochromic RBCs	Iron deficiency anemia

Hemoglobin

HEMOGLOBIN	MOLECULAR STRUCTURE	ADULT REFERENCE VALUE	NEWBORN REFERENCE VALUE
A	2 α, 2 β chains	>95%	20%
A_2	2 α, 2 δ chains	1.5%–3.7%	<1%
F	2 α, 2 γ chains	<2%	50%–85%
S	Valine substituted for glutamic acid in 6th position of β chain	0	0
C	Lysine substituted for glutamic acid in 6th position of β chain	0	0


Cellulose Acetate pH 8.6
Cathode (–)
Anode (+)
A2
C
S
F
A
Origin "Crawl"
"Slow"
"Fast"
"Accelerated"
Citrate Agar pH 6.2
Cathode (–)
Anode (+)
F
A
A2
S
C
Origin

Hemoglobin Derivatives

HEMOGLOBIN	CAUSE	EFFECT	NORMAL % OF TOTAL HGB	OTHER
Methemoglobin	Iron oxidized to ferric (Fe^{3+}) state. Usually acquired from exposure to oxidants. Rarely inherited.	Can't bind O_2. Cyanosis, possibly death.	≤1%	Heinz bodies. Treat with methylene blue.
Sulfhemoglobin	Sulfur bound to heme. Acquired from exposure to drugs & chemicals.	O_2 affinity 1/100th normal. Cyanosis.	0	Can't be converted back to normal hemoglobin. Not detected in cyanmethemoglobin method.
Carboxyhemoglobin	Carbon monoxide bound to heme.	↓ O_2 to tissues. Can be fatal.	<1 %	Affinity of hgb for CO is 200× greater than for O_2. Skin turns cherry red.

Hemoglobin derivatives are quantitated by differential spectrophotometry.

ABNORMALITY	DESCRIPTION	SIGNIFICANCE
Size		
Anisocytosis	Variation in size.	Seen in many anemias.
Macrocytes	RBCs >9 µm.	Megaloblastic anemias, liver disease, reticulocytosis. Normal in newborns.
Microcytes	RBCs <6 µm.	Iron deficiency anemia, thalassemia, anemia of chronic infections.
Shape		
Poikilocytosis	Variation in shape.	Seen in many anemias.
Elliptocytes/ovalocytes	Oval or pencil/cigar shaped.	Membrane defect. Hereditary ovalocytosis, various anemias.
Crenated RBCs	Round cell with knobby, uniform projections.	Osmotic imbalance. If seen in most cells in thin part of smear, don't report. Probably artifact due to excess anticoagulant or slow drying.
Burr cells (echinocytes)	Round cell with evenly spaced blunt or pointed projections.	Membrane defect. Uremia, pyruvate kinase deficiency. May be drying artifact. A few can be present in healthy individuals.
Acanthocytes (spur cells)	Small, dense cells with irregularly spaced projections of varying length.	Membrane defect. Severe liver disease, abetalipoproteinemia.

continued...

RBC Morphology *continued*

ABNORMALITY	DESCRIPTION	SIGNIFICANCE
Schistocytes	RBC fragments	RBCs split by fibrin strands. Microangeopathic hemolytic anemias (DIC, TTP, HUS), prosthetic heart valves.
Sickle cells (drepanocytes)	Crescent, S or C shaped, boat shaped, oat shaped	Sickle cell anemia.
Hemoglobin C crystals	Blunt, 6-sided, dark-staining projection. "Bar of gold." "Washington monument"	Hemoglobin C disease.
Hemoglobin SC crystals	Glove-like intracellular crystals	Hemoglobin SC disease.
Teardrops (dacryocytes)	Teardrop shaped	Myelofibrosis, thalassemia & other anemias.
Staining Hypochromia	Central pallor >1/3 cell diameter	Iron deficiency anemia, thalassemia.
Anisochromia	Mixture of normochromic & hypochromic RBCs	Dimorphic anemia, post-transfusion.
Polychromasia	Bluish-gray color	Young RBCs. Retics with supravital stain. Sign of active erythropoiesis. 1%–2% in normal adult. ↑ with acute blood loss, hemolytic anemia, following treatment for iron deficiency or pernicious anemia.

continued...

RBC Morphology *continued*

ABNORMALITY	DESCRIPTION	SIGNIFICANCE
Target cells (codocytes)	Bull's-eye, "Mexican hat cell"	Hemoglobinopathies, thalassemia, liver disease. May be artifact if observed in only 1 part of smear.
Stomatocytes	RBC with slit-like central pallor	Hereditary stomatocytosis, hereditary spherocytosis, thalassemia, alcoholic cirrhosis, Rh null disease. May be artifact in parts of smear that are too thin or too thick.
Spherocytes	Small, dark-staining RBCs without central pallor	Membrane defect. Hereditary spherocytosis, autoantibodies, burns, hemoglobinopathies, hemolysis, ABO HDN, incompatible blood tf, tf of stored blood. A few are normal due to aging of RBCs.
Arrangement		
Rouleaux	RBCs resemble stack of coins	Serum protein abnormality; e.g., ↑ globulins or fibrinogen. Seen in multiple myeloma & macroglobulinemia. May be artifact due to delay in spreading drop of blood or smear that's too thick.
Agglutination	RBCs in irregular clumps	Autoantibodies, cold autoagglutinin

DIC = disseminated intravascular coagulation
TTP = thrombotic thrombocytopenic purpura
HUS = hemolytic uremic syndrome

RBC Inclusions

INCLUSION	STAIN	DESCRIPTION	EXPLANATION	SIGNIFICANCE	CONDITIONS
Basophilic stippling	Wright's & new methylene blue	Multiple, irregular purple inclusions evenly distributed in cell	Aggregation of RNA (ribosomes)	Coarse: exposure to lead. Fine: young RBC	Exposure to lead, accelerated or abnormal hemoglobin synthesis, thalassemia
Howell-Jolly bodies	Wright's & new methylene blue	Round, purple, 1–2 µm in diameter. Usually only 1 per cell	Nuclear remnants (DNA)	Usually pitted by spleen. Seen with accelerated or abnormal erythropoiesis	Postsplenectomy, thalassemia, hemolytic & megaloblastic anemias, sickle cell anemia
Cabot rings	Wright's	Reddish purple rings or figure-8s	May be part of mitotic spindle, remnant of microtubules, or fragment of nuclear membrane	Rapid blood regeneration, abnormal erythropoiesis	Megaloblastic anemia, thalassemia, postsplenectomy
Pappenheimer bodies	Wright's (siderotic granules with Prussian blue stain)	Small purplish blue granules. Vary in size, shape, #. Usually in clusters at periphery	Iron particles	Faulty iron utilization	Sideroblastic anemias, postsplenectomy, thalassemia, sickle cell anemia, hemochromatosis

continued...

RBC Inclusions *continued*

INCLUSION	STAIN	DESCRIPTION	EXPLANATION	SIGNIFICANCE	CONDITIONS
Siderotic granules	Prussian blue	Blue granules of varying size & shape	Aggregates of iron particles	Faulty iron utilization in hgb synthesis	Sideroblastic anemias, postsplenectomy, thalassemia, sickle cell anemia, hemochromatosis
Reticulocytes	New methylene blue (polychromasia on Wright's stain)	Blue-staining network	Residual RNA (ribosomes)	$>2\% = \uparrow$ erythropoiesis $<0.1\% = \downarrow$ erythropoiesis	Hemolytic anemia, blood loss, following treatment for iron deficiency or megaloblastic anemia
Heinz bodies	Supravital stain, e.g., crystal violet, brilliant cresyl blue, methylene blue	Round blue inclusions, varying sizes, close to cell membrane. May be >1	Precipitated, oxidized, denatured hemoglobin	Normal during aging but pitted by spleen	G6PD deficiencies, unstable hemoglobins, chemical injury to RBCs, drug-induced hemolytic anemia

Staining of RBC Inclusions

INCLUSION	WRIGHT'S STAIN	NEW METHYLENE BLUE STAIN	PRUSSIAN BLUE STAIN
Reticulum	Cell appears polychromatophilic	Yes	No
Howell-Jolly bodies	Yes	Yes	No
Pappenheimer bodies	Yes	Yes	Yes
Siderotic granules	Yes, but called Pappenheimer bodies	Yes	Yes
Heinz bodies	No	Yes	No

INDEX	DEFINITION	MANUAL CALCULATION	REFERENCE RANGES	COMMENTS
Mean corpuscular volume (MCV)	Average volume of RBC	$MCV = \frac{HCT\ (\%) \times 10}{RBCs\ (\times 10^{12}/L)}$	80–100 fL	Used to classify anemias. Normal MCV = normocytic. MCV >100 = macrocytic. MCV <80 = microcytic. MCV is an average. Combination of microcytes & macrocytes may result in normal MCV.
Mean corpuscular hemoglobin (MCH)	Average weight of hgb in individual RBCs	$MCH = \frac{HGB\ (g/dL) \times 10}{RBC\ (\times 10^{12}/L)}$	27–31 pg	Varies in proportion to MCV.
Mean corpuscular hemoglobin concentration (MCHC)	Average concentration of hgb per dL of RBCs	$MCHC = \frac{HGB\ (g/dL) \times 100}{HCT\ (\%)}$	32–36 g/dL	RBCs with normal MCHC = normochromic (area of central pallor 1/3 diameter of cell). MCHC ↓ in hypochromic cells (↑central pallor). 50% of hereditary spherocytosis patients have MCHC ≥36. MCHC >37 may indicate problem with specimen (hyperlipidemia, cold agglutinins) or instrument.

Hemoglobinopathy Versus Thalassemia

	HEMOGLOBINOPATHY	THALASSEMIA
Abnormality	Qualitative abnormality. Abnormality in amino acid sequence of globin chain, not in amount of globin produced.	Quantitative abnormality. Amino acid sequence of globin chains is normal, but underproduction of 1 or more globin chains.
Examples	Sickle cell anemia & trait, hemoglobin C disease & trait.	β-thalassemia major & minor.

Note: Some hematologists refer to all qualitative & quantitative hemoglobin abnormalities as hemoglobinopathies.

ANEMIA	ETIOLOGY	BLOOD SMEAR	HEMOGLOBIN ELECTROPHORESIS	OTHER
Sickle cell anemia (SS)	Inheritance of sickle cell gene from both parents. Valine substituted for glutamic acid in 6th position of β chain.	Aniso, poik, sickle cells, target cells, nRBCs, HJ bodies, basophilic stippling, siderotic granules, polychromasia.	≥80% S, 1%–20% F, normal A_2, no A	Hgb S polymerizes under ↓ O_2 & ↓ blood pH. Disease not evident in newborn because of ↑Hgb F. Pos solubility test. Retics 10%–20%. May have ↑ WBC with shift to left & ↑ PLT. Moderate to severe anemia.
Sickle cell trait (AS)	Inheritance of sickle cell gene from 1 parent.	Occasional target cells. No sickle cells unless hypoxic.	50%–65% A, 35%–45% S, normal F, N to slightly ↑ A_2	No anemia. Pos solubility test. Important to Dx for genetic counseling.
Hemoglobin C disease (CC)	Inheritance of gene for Hgb C from both parents. Lysine substituted for glutamic acid in 6th position of β chain.	Many target cells, folded cells, occasional Hgb C crystals.	>90 C, <7% F, no A	Mild to moderate anemia.

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Normocytic Anemias *continued*

ANEMIA	ETIOLOGY	BLOOD SMEAR	HEMOGLOBIN ELECTROPHORESIS	OTHER
Hemoglobin C trait (AC)	Inheritance of gene for Hgb C from 1 parent.	Many target cells.	60%–70% A, 30%–40% C	
SC disease (SC)	Inheritance of 1 sickle cell gene & 1 Hgb C gene.	Many target cells. Folded & boat-shaped cells, occasional SC crystals (finger-like projections, "Washington Monument" crystals).	>S than C, normal to 7% F, no A	Pos solubility test. Mild to moderate anemia.
Hereditary spherocytosis	Defect of cell membrane.	Spherocytes, polychromasia.	Normal	MCHC usually >36 g/dL. ↑ retics, ↑ osmotic fragility.
Autoimmune hemolytic anemia	Autoantibodies.	Polychromasia, spherocytes, nRBCs.	Normal	↑ retics, ↑ indirect bili, ↓ haptoglobin, pos DAT.

N = normal, nRBCs = nucleated red blood cells, DAT = direct antiglobulin test.

Macrocytic Anemias

ANEMIA	ETIOLOGY	BLOOD SMEAR	HEMOGLOBIN ELECTROPHORESIS	OTHER
Megaloblastic Folate deficiency	Nutritional deficiency, ↑ cell replication (e.g., hemolytic anemias, myeloproliferative diseases, pregnancy), malabsorption, drug inhibition. Deficiency impairs DNA synthesis.	Oval macrocytes, Howell-Jolly bodies, hypersegmentation, aniso, poik	Normal	Pancytopenia, ↑ LD
Vitamin B_{12} deficiency	Nutritional deficiency, malabsorption, impaired utilization, parasites. Deficiency impairs DNA synthesis.	Same	Normal	Same. Pernicious anemia is most common type. Autoimmune disease. Gastric atrophy leads to ↓ intrinsic factor needed for B_{12} absorption
Nonmegaloblastic	Alcoholism, liver disease, ↑ erythropoiesis.	Round macrocytes, no hypersegmentation	Normal	WBC & PLT normal

Microcytic, Hypochromic Anemias

ANEMIA	ETIOLOGY	BLOOD SMEAR	OTHER
Iron deficiency anemia (IDA)	Insufficient iron for hgb synthesis.	Aniso, poik, hypochromic microcytes	Most common anemia.
Sideroblastic anemia	Enzymatic defect in heme synthesis.	Dual population of RBCs (normocytic & microcytic), Pappenheimer bodies, basophilic stippling	RBC indices usually normal. Ringed sideroblasts in marrow.
β–thalassemia major	↓ β-chain production.	Marked aniso & poik, hypochromic microcytes, target cells, ovalocytes, nRBCs, basophilic stippling	Homozygous. Little or no Hgb A, 95%–98% F, 2%–5% A_2. Severe anemia. MCV <67 fL.
β–thalassemia minor	↓ β-chain production.	Aniso, poik, hypochromic microcytes, target cells, basophilic stippling	Heterozygous. >90%–95% Hgb A, 3.5%–7% A_2, 2%–5% F. Mild anemia.
Anemia of chronic inflammation*	Acute phase reactants (e.g., hepcidin) affect iron absorption & release. Iron in bone marrow macrophages is not released to developing RBCs.	60%–70% of cases have normocytic normochromic RBCs; 30%–40% microcytic hypochromic	Associated with chronic infections & inflammation, malignancies, autoimmune diseases. 2nd most common anemia after IDA. Most common anemia in hospitalized pts.

*Formerly known as anemia of chronic disease. More often normocytic normochromic but included here because must be considered in differential Dx of microcytic anemia.

Differentiation of Microcytic Hypochromic Anemias

ANEMIA	RBCS	RDW	SERUM IRON	TIBC	SERUM FERRITIN	HGB A_2
Iron deficiency anemia	↓	↑	↓	↑	↓	N
Sideroblastic anemia	↓	↑	↑	N	↑	N
β-thalassemia minor	↑	N	N	N	N	↑
Anemia of chronic inflammation	↓	N	↓	↓	↑	N

TIBC = total iron binding capacity.

Acute Versus Chronic Blood Loss

	ACUTE BLOOD LOSS	CHRONIC BLOOD LOSS
Definition	Rapid loss of >20% blood volume.	Loss of small amounts of blood over extended period of time
RBCs	Normocytic, normochromic. May be transient macrocytosis when ↑ retics reach circulation.	Microcytic, hypochromic (due to iron deficiency)
WBCs	↑ (up to 35×10^9/L) with shift to left for about 2–4 days.	Normal
Retics	↑ in 3–5 days. Peak around 10 days.	Normal or slightly ↑
HGB/HCT	Steady during 1st few hr due to vasoconstriction & other compensatory mechanisms. Can be 48–72 hr before full extent of hemorrhage is evident (after fluid from extravascular spaces moves into circulation to expand volume).	↓
Other	Immediate fall in PLT, followed by ↑ within 1 hr.	↓ serum iron & ferritin

Granulocytic Maturation

STAGE	KEY CHARACTERISTICS
Myeloblast	15–20 μm. Small amount of dark blue cytoplasm. Usually no granules. Nucleus has delicate chromatin with nucleoli.
Promyelocyte	12–24 μm. Similar to myeloblast but has primary (nonspecific) granules.
Myelocyte	10–18 μm. Secondary (specific) granules (eosinophilic, basophilic, or neutrophilic). Last stage to divide.
Metamyelocyte	10–18 μm. Nucleus begins to indent.
Band	10–16 μm. Nuclear indentation is more than half.
Segmented neutrophil	10–16 μm. 2–5 nuclear lobes connected by thin strands of chromatin.

Normal Leukocytes of the Peripheral Blood

CELL	SIZE	NUCLEUS	CYTOPLASM	ADULT REFERENCE RANGE: RELATIVE (%)	ADULT REFERENCE RANGE: ABSOLUTE ($\times 10^9$/L)
Segmented neutrophil	10–16 μm	Segmented. 2–5 lobes connected by thread-like filament of chromatin.	Pinkish tan with neutrophilic granules	50–70	2.4–7.5
Band	10–16 μm	Horseshoe shaped. Parallel sides with visible chromatin in between. No filament.	Pinkish tan with neutrophilic granules	2–6	0.1–0.6
Eosinophil	10–16 μm	Band shaped or segmented into 2 lobes.	Large red granules	0–4	0–0.4
Basophil	10–16 μm	Usually difficult to see because of overlying granules.	Dark purple granules	0–2	0–0.2
Monocyte	12–18 μm	Round, horseshoe shaped, or lobulated. Convoluted. Loose strands of chromatin.	Gray-blue with indistinct pink granules. Vacuoles. Occasional pseudopods	2–9	0.1–0.9
Lymphocyte	7–15 μm	Round or oval. Dense blocks of chromatin. Indistinct chromatin/parachromatin separation.	Sparse to abundant. Sky blue. May contain a few azurophilic granules	20–44	1.2–3.4

Note: Automated analyzers do not differentiate between bands and segs.

ABNORMALITY	DESCRIPTION	SIGNIFICANCE
Shift to the left	Presence of immature granulocytes in peripheral blood	Bacterial infection, inflammation.
Toxic granulation	Dark-staining granules in cytoplasm of neutrophils	Infection, inflammation.
Dö hlebodies	Light blue patches in cytoplasm of neutrophils	Infection, burns.
Vacuolization	Phagocytic vacuoles in cytoplasm of neutrophils	Septicemia, drugs, toxins, radiation.
Hypersegmentation	>5 % of segs with 5-lobed nuclei or any with >5 lobes	One of 1st signs of pernicious anemia.
Pelger-Huët anomaly	Most neutrophils have round or bilobed nuclei	Inherited disorder. No clinical effect. May be misinterpreted as shift to left.
Auer rods	Red needles in cytoplasm of leukemic myeloblasts & occasionally promyelocytes & monoblasts	Rules out lymphocytic leukemia. Seen in up to 60% of patients with AML. From abnormal fusion of primary granules.
Variant lymphocytes (atypical or reactive)	1 or more of following: large size, elongated or indented nucleus, immature chromatin, ↑ parachromatin, nucleoli, ↑ cytoplasm, dark blue or very pale cytoplasm, peripheral basophilia, scalloped edges due to indentation by adjacent RBCs, frothy appearance, many azurophilic granules	Viral infections (e.g., IM, CMV).

IM = infectious mononucleosis, CMV = cytomegalovirus.

Quantitative Abnormalities of Leukocytes

ABNORMALITY	ASSOCIATIONS
Neutrophilia	Bacterial infection, inflammation, hemorrhage, hemolysis, stress
Neutropenia	Acute infection, antibodies, drugs, chemicals, radiation
Lymphocytosis	IM, CMV, whooping cough, acute infectious lymphocytosis
Monocytosis	Convalescence from viral infections, chronic infections, TB, subacute bacterial endocarditis, parasitic infections, rickettsial infections
Eosinophilia	Allergies, skin diseases, parasitic infections, CML
Basophilia	Chronic myelogenous leukemia, polycythemia vera

Hematopoietic Neoplasms*

DISORDER	EXPLANATION	EXAMPLE(S)	OTHER
Myeloproliferative neoplasms (MPN) or myeloproliferative disorders (MPD)	Premalignant hematopoietic stem cell disorders involving overproduction of 1 or more myeloid (nonlymphocytic) cell lines. Bone marrow & peripheral blood show ↑ RBCs, granulocytes, &/or platelets, with 1 cell line usually predominate. Normal maturation & morphology.	Polycythemia vera, chronic myelogenous leukemia, essential thrombocythemia, primary myelofibrosis	Usually in older adults. Caused by mutations in hematopoietic stem cells. Primarily chronic but can transform into acute leukemia. Splenomegaly, extramedullary hematopoiesis common.
Myelodysplastic syndromes (MDS)	Premalignant hematopoietic stem cell disorders involving ineffective hematopoiesis in 1 or more myeloid cell lines. Hypercellular bone marrow with maturation abnormalities (dysplasias). Peripheral blood cytopenias (↓ counts) & morphologic abnormalities.	Refractory anemia, refractory neutropenia, refractory thrombocytopenia	More common in elderly. May be due to exposure to chemicals, radiation, chemotherapy, viral infections. Can transform into acute leukemia.
Myelodysplastic/ myeloproliferative disorders (MDS/MPN)	Premalignant neoplasms with both myeloproliferative & myelodysplastic features.	Chronic myelomonocytic leukemia (CMML)	

continued...

Hematopoietic Neoplasms* *continued*

DISORDER	EXPLANATION	EXAMPLE(S)	OTHER
Leukemia	Malignant neoplasms involving unregulated proliferation of hematopoietic stem cells. Abnormal cells in bone marrow & peripheral blood.	Acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL)	Classified as acute or chronic, & lymphoid or myelogenous. See "Common Leukemias" card for more detail.
Lymphoma	Malignant neoplasm of lymphoid cells in lymphatic tissues or lymph nodes.	Hodgkin lymphoma, non-Hodgkin lymphoma	Solid tumors. Classified as B or T cell. Can spread to bone marrow, then malignant cells can be present in peripheral blood (leukemic phase).

*Neoplasm = new growth; unregulated growth of a single transformed cell; may be benign or malignant. A benign neoplasm can progress to a malignant neoplasm.

	FRENCH-AMERICAN-BRITISH (FAB)	WORLD HEALTH ORGANIZATION (WHO), 2008
Criteria	Morphology, cytochemistry, immunophenotyping	Morphology, cytochemistry, immunophenotyping, cytogenetics, clinical features
Major groups	Myeloproliferative disorders (MPD) Myeloplastic syndromes (MDS) Acute leukemias (AL)	Myeloproliferative neoplasms (MPN) Myeloid & lymphoid neoplasms associated with eosinophilia & abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1** Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) Myelodysplastic syndromes (MDS) Acute myeloid leukemia (AML) & related neoplasms Acute leukemias of ambiguous lineage B-lymphoblastic leukemia/lymphoma T-lymphoblastic leukemia/lymphoma
Criteria for diagnosis of AML	≥30% blasts	≥20% blasts
Use	1st system. Still used by some but being replaced by WHO.	Widely used

**PDGFRA, PDGFRB,* & *FGFR1* are genes that code for production of platelet-derived growth factor receptor (alpha & beta types) & fibroblast growth factor receptor 1. Abnormalities in these genes are a factor in selection of drug therapy.

Acute Versus Chronic Leukemia

	ACUTE	CHRONIC
Age	All ages, with peaks in 1st decade & after 50 yr	Adults
Onset	Sudden	Insidious
Median survival time, untreated	Weeks to months	Months to years
WBC	$\uparrow$, N, or $\downarrow$	$\uparrow$ (may be >50,000)
Differential	Blasts usually present	More mature cells
Anemia	Mild to severe	Mild
Platelets	Mild to severe $\downarrow$	Usually N
Other	Usually lymphoid in children, myeloid in adults	Myeloid mostly in young to middle-aged, lymphoid in older adults. Most go into blast crisis
Methods used to diagnose	Peripheral blood smear, bone marrow examination, cytochemical stains, immunophenotyping, cytogenetics, molecular genetics	Same but less use of cytochemical stains

Common Leukemias

TYPE	OTHER NAMES	WBC ($\times 10^9$/L)	BLOOD SMEAR	OTHER
Acute myeloid leukemia (AML)	Acute myelocytic, acute myelogenous, acute nonlymphocytic leukemia (ANLL)	Usually 5–30 but can range from 1–200	≥20% blasts. May have Auer rods, pseudo-Pelger-Huët cells, Howell-Jolly bodies, Pappenheimer bodies, basophilic stippling, nRBCs, hypogranular or giant PLT.	Most common type in children <1 yr & adults. Rare in older children & teens. ↑ uric acid & LD from ↑ cell turnover.
Acute lymphoblastic leukemia (ALL)	Acute lymphocytic	↑ in 50% of patients. Can be N or ↓	Small, homogeneous blasts in children; larger, heterogeneous blasts in adults. Many do not have circulating blasts.	Peak incidence 2–5 yr. Smaller peak in elderly. ↑ uric acid & LD. Spreads to central nervous system. Immunophenotyping to determine lineage (T or B). Cytogenetics & molecular analysis for prognosis.
Chronic myelogenous leukemia (CML)	Chronic granulocytic, chronic myeloid	Usually >100	All stages of granulocytic maturation. Segs & myelocytes predominant. ↑ eos & basos. Pseudo-Pelger-Huët cells (hyposegmentation of neutrophil nuclei), NRBCs, abnormal PLT may be seen.	Most common MPD. Most common after age 55 yr. Philadelphia (Ph) chromosome. ↓ LAP. Eventually becomes AML or ALL.

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Common Leukemias *continued*

TYPE	OTHER NAMES	WBC ($\times 10^9$/L)	BLOOD SMEAR	OTHER
Chronic lymphocytic leukemia (CLL)		30–200	80%–90% small, mature-looking lymphs. May have hypercondensed chromatin & light-staining parachromatin ("soccer ball appearance"), few prolymphocytes. Smudge cells.	Most common type of leukemia in older adults. Proliferation of B lymphs.

Cytochemical Stains for Differentiation of Acute Leukemia

STAIN	AML	ALL
Myeloperoxidase	Pos	Neg
Sudan black	Pos	Neg
Napthol AS-D chloroacetate esterase (specific esterase)	Pos	Neg
Periodic acid–Schiff (PAS)	Neg or diffusely pos	Pos (coarse granular or block-like)

Leukemoid Reaction Versus Chronic Myelogenous Leukemia

	LEUKEMOID REACTION	CML
WBC count	High	High
Peripheral blood smear	Shift to left (blasts rare), toxic granulation, Döhle bodies	Shift to left with blasts, eosinophilia, basophilia
Leukocyte alkaline phosphatase (LAP)	High	Low
Ph chromosome	Neg	Pos

DISORDER	KEY CHARACTERISTICS
Multiple myeloma	Malignant plasma cells in marrow. Normocytic, normochromic anemia. Rouleaux on blood smear. ↑ ESR due to ↑ globulins. M spike on serum protein electrophoresis (monoclonal gammopathy). May have Bence Jones proteinuria. Lytic bone disease.
Plasma cell leukemia	Form of multiple myeloma. Plasma cells in peripheral blood. Pancytopenia. Rouleaux. Monoclonal gammopathy.
Waldenströ m's macroglobulinemia	Malignant lymphocyte–plasma cell proliferative disorder. Monoclonal gammopathy due to ↑ IgM. Rare plasmacytoid lymphocytes or plasma cells on peripheral smear. Rouleaux. May have Bence Jones proteinuria & cryoglobulins.

ESR = erythrocyte sedimentation rate.

Manual Hematology Procedures

TEST	PURPOSE	METHOD	COMMENTS
Manual WBC count, CSF	Differential Dx of meningitis	CSF loaded into Neubauer hemacytometer. WBCs counted in all 9 squares of each side under 10×.	Acetic acid can be used to lyse RBCs, if necessary. Disposable 1-piece hemacytometers available. Most labs perform counts on hematology analyzers today. Manual counts are no longer performed on blood.
Microhematocrit (packed cell volume, PCV)	Screening for anemia	Microhematocrit tubes centrifuged at 10,000-15,000 rpm for 5 min. % of total volume occupied by RBCs determined.	Values may be slightly higher than calculated values from automated analyzers.
Reticulocyte count	Assess rate of erythropoiesis	Blood smear stained with new methylene blue. 1,000 RBCs counted. % containing reticulum determined.	Miller ocular can be used to facilitate counting. Adult reference range = 0.5%–1.5%. ↑ with ↑ erythropoiesis, e.g., blood loss, hemolytic anemia, following treatment of pernicious or iron deficiency anemia. Most retic counts are performed on automated analyzers today.
Erythrocyte sedimentation rate (ESR)	Screen for inflammation	Whole blood added to Westergren tube & placed in vertical rack. Height of RBC column read after 1 hr.	Nonspecific. CRP preferred. Reference ranges: males 0–15 mm/hr; females 0–20. ↑ with inflammation. Automated methods available with results in <60 min.

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TEST	PURPOSE	METHOD	COMMENTS
Tube solubility screening test for Hemoglobin S	Screening for Hgb S	Blood mixed with reducing agent, e.g., sodium dithionite. Hgb S is insoluble, produces turbid solution that obscures black lines behind tube.	Not specific for Hgb S. Doesn't differentiate SS from AS. Follow up with hgb electrophoresis.
Osmotic fragility	Dx of hereditary spherocytosis	Blood added to serial dilutions of NaCl & incubated. Amount of hemolysis determined by reading absorbance of supernatant from each tube.	↑ in hereditary spherocytosis. ↓ with target cells, sickle cell anemia, iron deficiency anemia, thalassemia.
Donath-Landsteiner (DL) test	Dx of paroxysmal cold hemoglobinuria	Blood collected in 2 clot tubes. Tube 1 incubated at 4°C, then 37°C. Tube 2 incubated at 37°C only. Pos = hemolysis in Tube 1, none in Tube 2.	Rare autoimmune hemolytic anemia due to biphasic antibody (autoanti-P) that binds complement to RBCs in capillaries at <20°C & elutes off at 37°C. Complement remains attached & lyses cells.

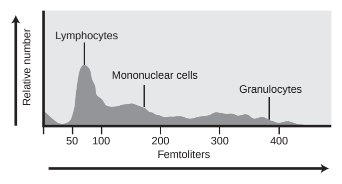
CRP = C-reactive protein.

Changes in Blood at Room Temperature

PARAMETER	CHANGE
MCV	↑ due to RBC swelling
HCT	↑ due to ↑ MCV
MCHC	↓ due to ↑ HCT
ESR	↓ (swollen RBCs don't rouleaux)
Osmotic fragility	↑
WBC	↓
WBC morphology	Necrobiotic cells, karyorrhexis (nuclear disintegration), degranulation, vacuolization

Methods of Automated Cell Counting & Differentiation

METHOD	PRINCIPLE	APPLICATION
Electrical impedance (Coulter principle)	Low-voltage direct current (DC) resistance. ↑ resistance (impedance) when nonconductive particles suspended in electrically conductive diluent pass through aperture. Height of pulses indicates cell volume, # pulses indicates count.	Cell counting & sizing
Radio frequency (RF)	High-frequency electromagnetic probe measures conductivity. Change in RF signal provides information about nucleus-to-cytoplasm ratio, nuclear density, granularity.	WBC differential
Optical light scattering (flow cytometry)	Hydrodynamically focused stream of cells passes through quartz flow cell past light source (tungsten halogen lamp or laser light). Scattered light is measured at different angles. Provides information about cell volume & complexity, e.g., granularity.	Cell counting & sizing, WBC differential

GRAPH	INFORMATION	USE
Histogram  *(From Ciesla B. Hematology in Practice, 2nd ed. Philadelphia: FA Davis; 2012:323.)*	Size distribution graph that plots cell size (*x* axis) vs. relative number (*y* axis). Size thresholds separate cell populations.	RBC, WBC, & PLT

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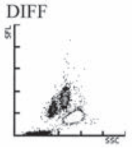
Graphic Representations of Cell Populations *continued*

GRAPH	INFORMATION	USE
Scatterplot or cytogram  *(From Ciesla B. Hematology in Practice, 2nd ed. Philadelphia: FA Davis; 2012:329.)*	Cells are plotted based on 2 characteristics, e.g., volume vs. light scatter. Separates cells into distinct populations.	WBC differential

Technologies Used in Automated Hematology Analyzers

MANUFACTURER	TECHNOLOGIES
Impedance instruments Beckman Coulter (LH Series)	Cell counting & sizing: electrical impedance WBC differential: VCS technology
Sysmex (X-Series)	Impedance, radiofrequency, absorption spectrophotometry, & flow cytometry with fluorescent dyes
Abbott (CELL-DYN)	Impedance, fluorescence staining, flow cytometry, multiangle polarized scatter separation (MAPSS)
Light-scattering instruments Siemens (Advia)	Light scattering & cytochemical analysis

Beckman Coulter (LH Series), VCS technology:

PARAMETER	MEASUREMENT	INFORMATION
Volume	Direct current (DC) impedance	Cell volume
Conductivity (opacity)	Radiofrequency (RF)	Cell size & internal structure
Scatter	Light scatter as cells pass through laser beam	Cell surface structure & cellular granularity

PARAMETER	VARIOUS METHODS USED
Cell counts	Impedance Light scatter
WBC differential	VCS technology (volume, conductivity, scatter) Fluorescent flow cytometry & light scatter MAPSS technology (multiangle polarized scatter separation) Cytochemistry (peroxidase) & optical flow cytometry
HGB	Cyanmethemoglobin method Modified cyanide-free cyanmethemoglobin method Sodium lauryl sulphate (SLS-hgb) method
HCT	Calculated from RBC & MCV Cumulative pulse heights detection
MCV	Mean of RBC volume histogram Calculated from HCT & RBC
MCH	Calculated from HGB & RBC
MCHC	Calculated from HGB & HCT

continued...

Automated CBC *continued*

PARAMETER	VARIOUS METHODS USED
RDW	CV of RBC histogram
Retics	Staining with new methylene blue; VCS technology Staining with auramine O; fluorescence detection Staining with fluorescent dye; light scatter & fluorescence detection Staining with oxazine-750; optical scatter & absorbance

QA/QC for Automated Hematology Analyzers

BEFORE IMPLEMENTATION	AFTER IMPLEMENTATION
Verification of accuracy & precision	Periodic calibration with stabilized whole blood calibrators
Linearity studies to verify analytic measurement range	Periodic calibration verification
Correlation studies to compare new method to current method	Analysis of at least 2 levels of control material each day of testing
	Instrument maintenance
	Participation in proficiency testing program

Flow Cytometry

Principle	Measurement of physical, antigenic, functional properties of cells suspended in fluid
Measurements	**Fluorescence**: Cells stained with antibodies conjugated to specific fluorochrome pass 1 by 1 in front of laser light source. Electrons of fluorochrome raised to higher energy state; emit light of specific wavelength as they return to ground state. Emitted light detected by photodetectors for specific wavelengths. **Forward scatter (FS)**: Photodetector in line with laser beam measures forward scatter (FS). Proportional to volume or size. **Side scatter (SS)**: Photodetector to side measures side scatter (SS). Reflects surface complexity & internal structures. FS, SS, fluorescence displayed simultaneously on screen. Cell populations with similar characteristics form clusters on dot plot. Specific populations can be selected with cursor (gating).
Applications	Immunophenotyping: Differentiating cells on basis of surface & cytoplasmic markers. Can determine lineage & maturity of cells in hematologic malignancies. Useful for Dx, follow-up, & prognosis. Certain immunophenotypes associated with specific cytogenetic abnormalities. Dx & monitoring of immunodeficiencies Dx of paroxysmal nocturnal hemoglobinuria Enumeration of stem cells Quantitation of fetal hemoglobin

FORMULA	EXAMPLE CALCULATION
$\text{Retic \%} = \dfrac{\text{retics per 1,000 RBCs}}{10}$	What is the retic count if reticulum is observed in 15 of 1,000 RBCs? $\text{Retic \%} = \dfrac{15}{10} = 1.5$
Reticulocyte % using Miller Disc = $\dfrac{\text{retics in square A} \times 100}{\text{RBCs in square B} \times 9}$	What is the retic count if 60 retics are counted in square A & 300 RBCs are counted in square B? $\text{Retic \%} = \dfrac{60 \times 100}{300 \times 9} = 2.2$
Absolute retic count (ARC) ($\times 10^9$/L) = $\dfrac{\text{retic \%} \times \text{RBC } (10^{12}/\text{L}) \times 1{,}000}{100}$	What is the absolute retic count if the retic count is 2% & the RBC is 5.2×10^{12}/L? $\text{ARC} = \dfrac{2 \times 5.2 \times 1{,}000}{100} = 104 \times 10^9/\text{L}$
Corrected retic count (CRC) = $\text{retic \%} \times \dfrac{\text{HCT(\%)}}{45}$	What is the corrected retic count if the uncorrected retic count is 5% & the HCT is 36%? $\text{CRC} = 5 \times \dfrac{36}{45} = 4\%$

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Hematology Calculations *continued*

FORMULA	EXAMPLE CALCULATION
Retic production index (RPI) = $\frac{\text{corrected retic count}}{\text{maturation time correction factor*}}$	What is the RPI if the corrected retic is 5% & the HCT is 35%? (maturation time correction factor for HCT of 35% is 1.5)? $$\text{RPI} = \frac{5}{1.5} = 3.3\%$$
$\text{MCV} = \frac{\text{HCT (\%)} \times 10}{\text{RBC } (10^{12}/\text{L})}$	Calculate the MCV if the RBC is 3×10^{12}/L, the HGB is 6 g/dL, & the HCT is 20%. $$\text{MCV} = \frac{20 \times 10}{3} = 66.7 \text{ fL}$$
$\text{MCH} = \frac{\text{HGB (g/dL)} \times 10}{\text{RBC } (10^{12}/\text{L})}$	Calculate the MCH if the RBC is 3×10^{12}/L, the HGB is 6 gm/dL, & the HCT is 20%. $$\text{MCH} = \frac{6 \times 10}{3} = 20 \text{ pg.}$$
$\text{MCHC} = \frac{\text{HGB (g/dL)} \times 100}{\text{HCT (\%)}}$	Calculate the MCHC if the RBC is 3×10^{12}/L, the HGB is 6 g/dL, & the HCT is 20%. $$\text{MCHC} = \frac{6 \times 100}{20} = 30\%$$

*The maturation time correction factor is based on the patient's HCT & is obtained from a maturation timetable.

continued...

FORMULA	EXAMPLE CALCULATION
Rules of Three: $$RBC \times 3 = HGB \pm 0.5$$ $$HGB \times 3 = HCT \pm 3\%$$	What should the HGB be if the RBC is 4.1×10^{12}/L? $$HGB = 4.1 \times 3 = 12.3 \pm 0.5 = 11.8\text{–}12.8 \text{ g/dL}$$ What should the HCT be if HGB is 12.3 g/dL? $$HCT = 12.3 \times 3 = 36.9 \pm 3 = 33.9\%\text{–}39.9\%$$
Manual Cell Count: $$\text{Cells/mm}^3\ (\mu L) = \#\text{ cells counted} \times \text{depth factor (always 10)} \times \text{reciprocal of dilution} \times \text{reciprocal of area counted (mm}^2)$$	Calculate the CSF WBC count if 18 WBCs are counted in 9 mm^2 on 1 side of a Neubauer hemacytometer, using undiluted CSF. $$\text{WBCs/}\mu L = 18 \times 10 \times 1 \times 1/9 = 20$$
Absolute WBC = total WBC × relative count (% from differential)	Calculate the absolute lymphocyte count if the total WBC is 10×10^9/L & there are 70% lymphocytes. $$\text{Absolute count} = 10 \times 0.70 = 7 \times 10^9/L$$
Corrected WBC = $$\frac{\text{uncorrected WBC} \times 100}{100 + \text{NRBCs per 100 WBCs}}$$	The automated hematology analyzer reports a WBC of 30×10^9/L. The technologist counts 115 NRBCs per 100 WBCs while performing the differential. What is the corrected WBC? $$\text{Corrected WBC} = \frac{30 \times 100}{100 + 115} = 14 \times 10^9/L$$

Overview of Hemostasis

Primary hemostasis	• Vasoconstriction • Platelet adhesion • Platelet aggregation to form primary hemostatic plug at injury site
Secondary hemostasis	• Interaction of coag factors to produce fibrin (secondary hemostatic plug) • Fibrin stabilization by factor XIII
Fibrinolysis	• Release of tissue plasminogen activator • Conversion of plasminogen to plasmin • Conversion of fibrin to fibrin degradation products

DISORDER	EXPLANATION	CLINICAL MANIFESTATIONS	LAB TESTS
Thrombocytopenia	↓ production (e.g., aplastic anemia, myelodysplastic syndromes), ↑ destruction (e.g., immune thrombocytopenic purpura, drugs, DIC, mechanical destruction by artificial heart valves), splenic sequestration, massive transfusion (dilution effect)	$<30 \times 10^9$/L: petechiae, menorrhagia, spontaneous bruising. $<10 \times 10^9$/L: severe spontaneous bleeding	PLT $<150 \times 10^9$/L
Primary thrombocytosis	Unregulated production of megakaryocytes in bone marrow, e.g., essential thrombocythemia, other myeloproliferative disorders	Thrombosis or hemorrhage	PLT usually $>1{,}000 \times 10^9$/L. Platelet aggregation may be abnormal
Secondary or reactive thrombocytosis	↑ PLT due to another condition, e.g., hemorrhage, surgery, splenectomy	Thrombosis or hemorrhage infrequent	PLT $>450 \times 10^9$/L but usually $<1{,}000 \times 10^9$/L

Qualitative Platelet Disorders

DISORDER	EXPLANATION	LABORATORY TESTS
Inherited Bernard-Soulier syndrome	Lack of functional glycoprotein (GP) Ib/IX/V on plt surface prevents interaction with VWF. Abnormal plt adhesion to collagen.	Giant plts with dense granulation. ↑ closure time (PFA). Abnormal aggregation with ristocetin.
Glanzmann's thrombasthenia	Deficiency or abnormality of plt membrane GP IIb/IIIa. Fibrinogen can't attach to plt surface & initiate plt aggregation.	↑ closure time (PFA). Abnormal aggregation with ADP, collagen, epinephrine.
δ-storage pool disorder	Dense granule deficiency. Lack of ADP release.	Abnormal secondary aggregation with ADP & epinephrine.
Acquired	Functional plt disorders occur with chronic renal failure, myeloproliferative disorders, cardiopulmonary bypass, use of aspirin & other drugs. Mechanisms vary.	Abnormal plt aggregation.

All can result in serious bleeding.
VWF = Von Willebrand factor.

Tests of Platelet Function

TEST	METHOD	CLINICAL SIGNIFICANCE
Platelet aggregation	Aggregating agent (e.g., ADP, collagen, ristocetin, epinephrine) added to plt suspension. As plts aggregate, ↑ in light transmittance. Plt aggregation curves generated (time vs. % transmittance).	Abnormal curves with plt dysfunctions such as von Willebrand disease, Bernard-Soulier syndrome, plt storage pool defects, idiopathic thrombocytopenia purpura, drugs.
Platelet function assay (PFA)	Citrated whole blood drawn through capillary tubes coated with ADP/collagen or epinephrine/collagen. Plts adhere & aggregate when exposed to collagen. Closure time = length of time for plts to form platelet plug & close aperture of capillary tube.	Screening test for qualitative plt defects. Replaces bleeding time. Von Willebrand disease: prolonged with collagen/ADP & collagen/epinephrine. Defects related to drugs (e.g., aspirin): normal with collagen/ADP, prolonged with collagen/epinephrine.
VWF:Ag	Immunologic tests (e.g., EIA) using monoclonal antibodies to VWF.	VWF connects plts to collagen. ↓ in von Willebrand disease, so plts don't function normally.

Coagulation Factors

	NAME	PATHWAY	INHERITED DEFICIENCY	OTHER
I	Fibrinogen	I, E, C	Rare	Converted to fibrin by thrombin.
II	Prothrombin	I, E, C	Rare	Precursor of thrombin.
III	Tissue factor (TF)	E		Phospholipid released from injured vessel wall. Not normally in blood.
IV	Ca^{2+}	I, E, C		Bound by anticoagulant sodium citrate. In assays using citrated plasma, must be supplied by reagents.
V	Labile factor (proaccelerin)	I, E, C	Rare	Deteriorates rapidly.
VII	Stable factor (proconvertin)	E	Rare	
VIII	Antihemophilic factor	I	Common (hemophilia A)	Circulates in association with von Willebrand factor (VWF). VIII:C = coagulant portion. Extremely labile.

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Coagulation Factors *continued*

	NAME	PATHWAY*	INHERITED DEFICIENCY	OTHER
IX	Christmas factor (plasma thromboplastin component)	I	Common (hemophilia B).	
X	Stuart factor	I, E,C	Rare.	
XI	Plasma thromboplastin antecedent	I	Rare (hemophilia C). May or may not cause bleeding.	
XII	Hageman factor (contact factor)	I	No bleeding.	Glass activation factor. Not part of in vivo coagulation.
XIII	Fibrin stabilizing factor	I, E, C	Rare. Poor wound healing.	Stabilizes fibrin clot.
HMWK	High molecular weight kininogen (Fitzgerald factor)	I	Rare. No bleeding.	Not part of in vivo coagulation.
PK	Prekallikrein (Fletcher factor)	I	No bleeding.	Not part of in vivo coagulation.

*I = intrinsic, E = extrinsic, C = common

Functional Classification of Coagulation Factors

	EXPLANATION	FACTORS
Substrate	Substance changed by an enzyme.	Fibrinogen
Cofactor	Protein that accelerates enzymatic reactions. No enzymatic activity of its own.	V, VIII (V is cofactor for Xa; VIII is cofactor for IXa)
Enzyme	Protein that catalyzes a change in specific substrate. Secreted in inactive form (proenzyme, zymogen). Must be activated to function.	Serine proteases: thrombin (IIa), VIIa, IXa, Xa, XIa, XIIa, prekallikrein Transglutaminase: XIIIa

Letter "a" following Roman numeral indicates activated form of enzyme.

	FACTORS	EXPLANATION
Contact group	PK, HMWK, XII, XI	Factors involved in initiation of intrinsic pathway.
Prothrombin group	II, VII, IX, X	Vitamin K–dependent factors.
Fibrinogen group	I, V, VIII, XIII	Factors acted on by thrombin (V, VIII, & XIII are activated; I is converted to fibrin). All are high molecular weight proteins.
Factors in extrinsic pathway	TF, VII	
Factors in intrinsic pathway	PK, HMWK, XII, XI, IX, VIII	
Factors in common pathway	X, V, II, I	
Extrinsic tenase complex	VIIa/TF	Acts on X.
Intrinsic tenase complex	IXa/VIIIa	Acts on X.
Prothrombinase complex	Xa/Va	Acts on prothrombin.
Factor VIII complex	VIII:C & von Willebrand factor (VWF)	VIII:C is the procoagulant; VWF is the carrier protein.
Produced in liver	All	

continued...

Summary of Coagulation Factors *continued*

	FACTORS	EXPLANATION
Require vitamin K for synthesis	II, VII, IX, X	
Affected by Coumadin (warfarin)	II, VII, IX, X	All that require vitamin K. Warfarin is a vitamin K antagonist.
Consumed during clotting	I, II, V, VIII, XIII	Not present in serum.
Labile factors	V, VIII	

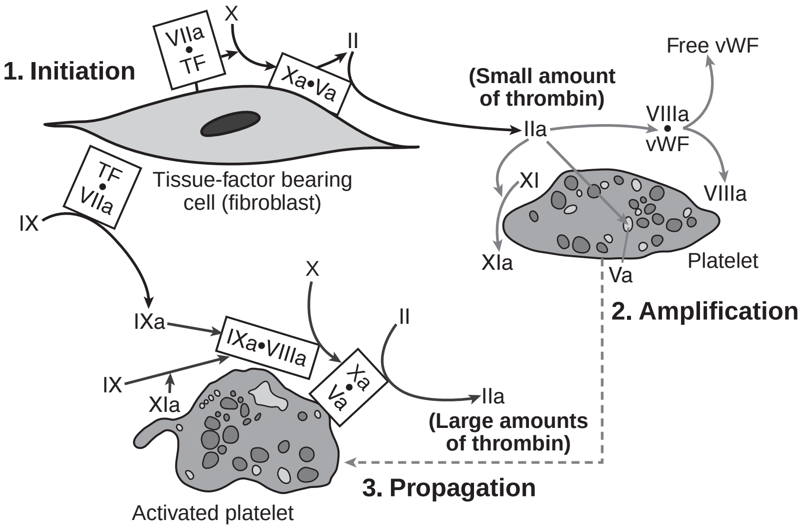
	CASCADE MODEL*	CELL-BASED OR PHYSIOLOGICAL MODEL
Overview	Focuses on role of coagulation factors. Sees coagulation as chain rxn in which each coag factor is converted to active form by preceding factor. Intrinsic & extrinsic pathways converge on common pathway.	Focuses on role of receptors for coagulation factors on surface of tissue factor (TF)–bearing cells (e.g., fibroblast or monocyte) & platelets. Sees coagulation as 3 overlapping phases that begin with small amount of thrombin formation on surface of TF-bearing cells, followed by large-scale thrombin production on platelet surface.
Steps	Extrinsic pathway (TF, factor VII): • TF from injured blood vessel wall activates factor VII. • TF:VIIa activate factor X.	Initiation (on surface of TF-bearing cell): • Break in vessel wall exposes extravascular TF-bearing cell to plasma. • Factor VII binds to TF on cell membrane. • TF:VIIa activates factors IX & X. • Factor Xa combines with factor Va. • Xa:Va generates small amount of thrombin, but no fibrin formed at this point.
	Intrinsic pathway (factors XII, XI, IX, VIII): • Factor XII activated by exposure to collagen. • Factor XIIa, HMWK, & PK activate factor XI. • Factor XIa activates factor IX. • IXa:VIIIa activates factor X.	Amplification: • Thrombin & collagen activate platelets. • Platelets release factor V from granules. • Thrombin activates factors V, VIII, & XI. • Factor XIa supplements activation of factor IX.

continued...

Coagulation Theories *continued*

	CASCADE MODEL*	CELL-BASED OR PHYSIOLOGICAL MODEL
	Common pathway (factors X, V, II, I): • Xa:Va converts prothrombin (II) to thrombin (IIa). • Thrombin cleaves fibrinogen (I) into fibrin & activates factor XIII to stabilize clot.	Propagation (on surface of activated platelet): • Factor Xa binds to factor VIIIa on platelet. • IXa:VIIIa activates factor X. • Xa:Va converts prothrombin (II) to thrombin (IIa). • Thrombin cleaves fibrinogen (I) into fibrin & activates factor XIII to stabilize clot.
Comments	"Classic" theory. Explains in vitro coag (PT & APTT tests) & helps ID factor deficiencies, but doesn't fit current understanding of coag in vivo. Pathways don't operate independently.	To compare models, think of extrinsic pathway as occurring on TF-bearing cell & intrinsic pathway (without factor XII, HMPK, PK) on platelet surface. (Factor XII, HMPK, PK aren't needed in vivo because factor XI is activated by thrombin produced in initiation phase.)

*For simplicity, Ca^{2+} & platelet factor 3 not shown.



(From Harmening DM. Clinical Hematology and Fundamentals of Hemostasis, 5th ed. Philadelphia: FA Davis; 2009:569.)

Prothombin Time (PT) and Activated Partial Thromboplastin Time (APTT)

	PT	APTT
Purpose	To detect deficiencies in extrinsic & common pathways & to monitor coumadin (warfarin) therapy.	To detect deficiencies in intrinsic & common pathways & to monitor unfractionated heparin therapy.
Reagent(s)	Thromboplastin reagent (thromboplastin, phospholipid, Ca^{2+}).	Activated partial thromboplastin reagent (phospholipid, activator), $CaCl_2$.
Prolonged results	Coumadin therapy; deficiency of VII, X, V, II, or I; circulating inhibitors.	Heparin therapy; deficiency of HMWK, PK, XII, XI, IX, VIII, X, V, II, or I; circulating inhibitors.
Other	Report INR (international normalized ratio). INR = [patient PT/mean normal PT]^{ISI}. ISI = international sensitivity index. Supplied by manufacturer. Be sure to use ISI for current lot of thromboplastin & analyzer being used. Therapeutic range for most situations is 2–3. For patients with artificial heart valves, 2.5–3.5.	

Interpretation of PT/APTT

PT	APTT	POSSIBLE DEFICIENCY
Prolonged	Normal	VII
Normal	Prolonged	HMWK, PK, XII, XI, IX, VIII
Prolonged	Prolonged	X, V, II, I

Other Coagulation Tests

TEST	DISCUSSION
Mixing studies	Follow up to abnormal PT or APTT. Test is repeated on 1:1 mixture of pt plasma & normal plasma. If pt has factor deficiency, time will be corrected because normal plasma supplies missing factor. If time is not corrected, an inhibitor is present, e.g., antibody or anticoagulant.
Activated clotting time (ACT)	Whole blood clotting method using point-of-care analyzer. Used in cardiac care units & during cardiac surgery to monitor heparin.
Thrombin time (TT)	Measures time required for thrombin to convert fibrinogen to fibrin. Prolonged with hypo- or dysfibrinogenemia, heparin, FDPs.
Reptilase time	Similar to TT except uses reptilase (snake venom enzyme) instead of thrombin. Prolonged results with afibrinogenemia & most congenital dysfibrinogenemias. Variable results with hypofibrinogenemia. Not affected by presence of heparin.
Fibrinogen	Estimation of fibrinogen level by modified TT. Thrombin added to dilutions of pt plasma. Results obtained from calibration curve prepared from testing dilutions of a fibrinogen standard. Normal: 200–400 mg/dL.
Factor assays	% of factor activity determined by amount of correction of PT or APTT when dilutions of pt plasma are added to factor-deficient plasma.
Factor XIII screening test	Pt's platelet-rich plasma mixed with $CaCl_2$. Clot placed in urea or monochloroacetic acid & incubated at 37°C. With XIII deficiency, clot dissolves within 24 hr.
Anti-Factor Xa assay	Test to monitor therapy with low molecular weight heparin. Can also be used instead of APTT to monitor therapy with unfractionated heparin. Pt plasma added to excess factor Xa & substrate specific for factor Xa. Heparin in sample forms complex with AT & inhibits factor Xa. Residual factor Xa cleaves substrate to produce colored product whose intensity is inversely proportional to concentration of heparin.

Most Common Inherited Coagulation Disorders

DISEASE	DEFICIENCY	CLINICAL FINDINGS	LABORATORY FINDINGS	OTHER
Von Willebrand disease	VWF	Mucocutaneous bleeding ranging from mild to severe	PLT: N Closure time (PFA): N or ↑ Platelet aggregation: abnormal with ristocetin PT: N APTT: N or ↑ Factor VIII: N or ↓ VWF:Ag: ↓	Most common inherited bleeding disorder. Autosomal dominant. Both sexes affected. Plts can't adhere to collagen to form plt plug. Lab results vary.
Hemophilia A	Factor VIII	Varies from asymptomatic to crippling bleeding into joints, muscles, & fatal intracranial hemorrhage	PLT: N PT: N APTT: ↑ Factor VIII: ↓	2nd most common inherited bleeding disorder. Sex-linked recessive. Occurs primarily in males. Mothers are carriers.
Hemophilia B (Christmas disease)	Factor IX	Same as hemophilia A	PLT: N PT: N APTT: ↑ Factor IX: ↓	Sex-linked recessive.

Acquired Factor Deficiencies

CONDITION	EXPLANATION
Liver disease	Coagulation proteins are synthesized in liver.
Vitamin K deficiency	Vitamin K is needed for synthesis of II, VII, IX, X.
Disseminated intravascular coagulation (DIC)	Uncontrolled formation & lysis of fibrin in blood vessels. Fibrinogen, II, V, VIII, XIII, & plts are consumed.
Primary fibrinolysis (fibrinogenolysis)	Plasminogen activated to plasmin, degrades fibrinogen, V, VIII, XIII. No fibrin formation.
Acquired inhibitors (circulating anticoagulants)	Antibodies against coagulation factors. Inhibitors to VIII & IX are most common & usually in pts who have received replacement therapy for hemophilia A or B. Occasionally associated with other diseases or in normal individuals.

Tests of Fibrinolytic System

TEST	EXPLANATION	METHOD(S)	CLINICAL SIGNIFICANCE
D-dimer	Fragment that results from lysis of fibrin by plasmin	Latex agglutination using monoclonal antibodies against D-dimer, ELISA	Marker for DIC. Also pos with deep vein thrombosis, pulmonary embolism, & after lytic therapy. Neg in primary fibrinolysis.
Fibrin(ogen) degradation products (FDP)	Product of action of plasmin on fibrin or fibrinogen	Latex agglutination using antibodies against FDP	Sign of ↑ fibrinolytic activity. Doesn't differentiate between fibrin degradation products & fibrinogen degradation products. Present in DIC, primary fibrinolysis, deep vein thrombosis, pulmonary embolism, & after lytic therapy.

Disseminated Intravascular Coagulation vs. Primary Fibrinolysis

	DIC	PRIMARY FIBRINOLYSIS
PT	Prolonged	Prolonged
APTT	Prolonged	Prolonged
Fibrinogen	↓	↓
Platelets	↓	Normal
FSP	Present	Present
D-Dimer	Pos	Neg
RBC morphology	Schistocytes	Normal

TEST	SIGNIFICANCE	ASSAYS
Antithrombin (AT, formerly antithrombin III)	Plasma inhibitor that neutralizes all serine proteases, including thrombin. Deficiencies associated with ↑ risk of thrombosis.	Chromogenic substrate assay, immunologic assay, nephelometry.
Protein C	Coagulation inhibitor. Inactivates Va & VIIIa. Deficiencies associated with ↑ risk of thrombosis.	Immunologic assay, chromogenic substrate assay, clot based assay.
Protein S	Cofactor for protein C.	Clotting assay, immunologic assay.
Factor V Leiden	Most common cause of hereditary activated protein C resistance (APC). Mutation that makes V resistant to activity of activated protein C. ↑ risk of thrombosis.	APC resistance assay is most frequent screening test. Patient plasma diluted in V-deficient plasma. Activated protein C added. APTT or dilute Russell viper venom time (dRVVT) performed. Abnormals must be confirmed by molecular testing (e.g., PCR, restriction fragment length polymorphism).
Lupus anticoagulants	Risk factor for thrombosis & recurrent spontaneous abortion. Acquired antiphospholipid antibodies that interact with phospholipid in APTT reagent & prolong time. In vitro phenomenon. Patient doesn't have factor deficiency or bleeding. Present in patients with lupus, other autoimmune diseases, neoplasms, infections, drugs. Also present in some normal individuals.	Detected by unexplained prolongation of APTT that isn't corrected by addition of equal volume of normal plasma. No definitive assay.

Anticoagulant (Antithrombotic) Therapy

	COUMADIN (WARFARIN)	UNFRACTIONATED HEPARIN (UFH)	LOW MOLECULAR WEIGHT HEPARIN (LMWH)
Administration	Oral	IV	Subcutaneous
Action	Vitamin K antagonist	Catalyzes inhibition of thrombin, Xa, & IXa by AT	Catalyzes inhibition of Xa by AT
Effect	Slow acting	Immediate	Immediate
Duration	Long	Short	Longer than UFH; shorter than warfarin
Test(s) for monitoring	PT	APTT, ACT (point of care), anti–factor Xa	Monitoring usually not required. If needed, anti–factor Xa should be used.
Other	Decreases production of II, VII, IX, X	Requires AT to be effective	APTT is insensitive to LMWH

ENDPOINT DETECTION	PRINCIPLE
Mechanical	Change in electrical conductivity between 2 probes or change in movement of steel ball when clot forms
Photo-optical	↓ in light transmittance as fibrin forms
Chromogenic	↑ in light absorbance at 405 nm as para-nitroaniline (pNA) is cleaved from synthetic substrate by coag enzyme
Immunologic	↑ in light absorbance as latex particles coated with specific antibody are agglutinated by antigen

Some analyzers have multiple detection methods.

Examples of Criteria for Specimen Rejection in Coagulation Testing

- Improper labeling
- Delay in delivery to lab
- Exposure to extremes of temperature
- Tube <90% full
- Specimen clotted
- Specimen hemolyzed

Sources of Error in Coagulation Testing

ERROR	COMMENT
Incorrect anticoagulant	3.2% sodium citrate should be used. Labile factors are preserved better.
Drawing coagulation tube after other anticoagulant tubes	Contamination with other anticoagulants can interfere.
Probing to find vein	Tissue thromboplastin activates coagulation & ↓ times.
Incorrect ratio of blood to anticoagulant	Need 9:1 blood to anticoagulant ratio. Tubes <90% full will have longer times.
Failure to mix anticoagulant with blood	Blood will clot.
Polycythemia	HCT >55% leads to longer times. Anticoagulant must be reduced.
Heparin contamination from catheter or heparin lock	Will prolong times. Lines must be flushed with saline, first 5 mL drawn discarded.
Hemolysis	Hemolyzed RBCs may activate clotting factors. Hemolysis may interfere with photometric reading.
Lipemia	May interfere with optical methods. Test by mechanical method.

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Sources of Error in Coagulation Testing *continued*

ERROR	COMMENT
Improper storage of specimen	Should be stored in vertical position at RT with stopper on to prevent change in pH. Specimens for PT must be tested within 24 hr of collection, APTT within 4 hr. (If APTT is for monitoring heparin, must be centrifuged within 1 hr of collection.)
Improper storage or reconstitution of reagents	Run normal & abnormal controls every 8 hr & with each change of reagents to verify system performance.
Equipment malfunction, e.g., temperature, timer, detector, volumes dispensed	Run normal & abnormal controls every 8 hr & with each change of reagents to verify system performance.

SECTION

5

Immunology Review

Immunology Terminology

TERM	EXPLANATION
Acute phase reactants	Proteins that ↑ due to infection, injury, trauma (e.g., C-reactive protein, alpha-1 antitrypsin, haptoglobin, fibrinogen, ceruloplasmin, alpha-1 acid glycoprotein, complement).
Alloantibody	Antibody formed in response to antigens from individuals of same species.
Antigen (ag)	Foreign substance that stimulates antibody production. Large, complex molecules (MW >10,000), usually protein or polysaccharide.
Antibody (ab)	Immunoglobulin produced by plasma cells in response to ag.
Autoantibody	Ab against self.
Avidity	Strength of bond between ag & ab.
Chemokines	Cytokines that attract cells to a particular site. Important in inflammatory response.
Chemotaxis	Migration of cells toward chemokine.
Clusters of differentiation (CD)	Antigenic features of leukocytes.
Cytokines	Chemicals produced by activated immune cells that affect function of other cells. Includes interferons, chemokines, tumor necrosis factors, transforming growth factors, colony stimulating factors, interleukins.
Epitope	Determinant site on ag.

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Immunology Terminology *continued*

TERM	EXPLANATION
Hapten	Low molecular weight substance that can bind to ab once it's formed, but is incapable of stimulating ab production unless bound to larger carrier molecule.
Histamine	Vasoactive amine released from mast cells & basophils during allergic rxn.
Hypersensitivity	Heightened state of immune responsiveness that causes tissue damage in host.
Immunity	Resistance to infection.
Immunogen	Any substance capable of inducing immune response.
Immunoglobulin (Ig)	Antibody.
Immunology	Study of reactions of host when exposed to foreign substances.
Inflammation	Cellular & humoral mechanisms involved in reaction to injury or infection.
Interferons	Cytokines with antiviral properties. Also active against certain tumors & inflammatory processes.
Interleukins	Cytokines produced by leukocytes that affect inflammatory response through ↑ in soluble factors or cells.
Ligand	Molecule that binds to another molecule of complementary configuration; the substance being measured in an immunoassay.

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Immunology Terminology *continued*

TERM	EXPLANATION
Lysozyme	Enzyme found in tears & saliva that attacks cell walls of microorganisms.
Major histocompatibility complex (MHC)	System of genes that control expression of MHC molecules found on all nucleated cells; originally referred to as human leukocyte antigens (HLA).
Monoclonal antibody	Ab derived from a single B-cell clone.
Opsonin	Serum proteins that attach to foreign substance & enhance phagocytosis.
Phagocytosis	Engulfment of cells or particulate matter by neutrophils & macrophages.
Plasma cells	Transformed B cells that secrete ab.
Polyclonal antibody	Ab produced by many B-cell clones.
Postzone	Reduced ag/ab complexes due to ag excess. Can cause false neg in serological test for ab. Repeat test in 1–2 wk.
Prozone	Reduced ag/ab complexes due to ab excess. Can cause false neg in serological test for ab. Dilute serum & retest.
Seroconversion	Change of serological test from neg to pos due to development of detectable ab.

continued...

Immunology Terminology *continued*

TERM	EXPLANATION
Serum sickness	Type III hypersensitivity reaction that results from buildup of abs to animal serum used in some passive immunizations.
Thymus	Small, flat bilobed organ found in thorax; site of T-lymph development. One of the primary lymphoid organs.
Titer	Means of expressing ab concentration; reciprocal of highest dilution with pos rxn.
Vaccination	Injection of immunogenic material to induce immunity.
Zone of equivalence	When # of multivalent sites of ag & ab are approximately equal. Results in optimal precipitation.

Branches of the Immune System

BRANCH	DEFINITION	DEFENSE AGAINST	CELLS INVOLVED	EXAMPLES
Cellular	Cell mediated	Viruses, fungi, mycobacteria, other intracellular pathogens, tumor cells	T lymphs, macrophages	Graft rejection, hypersensitivity rxn, elimination of tumor cells
Humoral	Antibody mediated	Bacteria (extracellular)	B lymphs, plasma cells	Ab production

Types of Immunity

TYPE	EXPLANATION	COMPONENTS	MEMORY?
Natural or innate	Defense mechanisms present at birth. Not ag specific.	External defense system: intact skin, mucous membranes, cilia & mucus in respiratory tract, stomach acid, flushing of urine, lactic acid in vagina, lysozyme in tears & saliva, normal flora Internal defense system: neutrophils, macrophages, acute phase reactants, complement	No
Acquired or adaptive	Defense mechanisms that are antigen specific.	T cells, B cells, plasma cells, antibodies, cytokines	Yes

TYPE	EXPLANATION	EXAMPLE	SPECIFIC?	IMMEDIATE?	LONG-TERM?
Naturally acquired active immunity	Individual infected with microorganism produces ab	Clinical or subclinical infection	Yes	No	Yes
Artificially acquired active immunity	Individual exposed to ag through vaccine develops immunity without having infection	DTaP, MMR, polio, tetanus, Hib vaccine	Yes	No	Yes
Naturally acquired passive immunity	Individual protected by abs produced by another person	Maternal abs that cross placenta & are present in breast milk	Yes	Yes	No
Artificially acquired passive immunity	Individual receives immune globulin containing abs produced by another person	Rh immune globulin, HBIG, antitoxins	Yes	Yes	No

Cells of the Natural Immune System

CELL	FUNCTION	COMMENTS
Granulocytes		
Neutrophils	Phagocytosis, inflammatory response	Respond to chemotaxins. Granules contain bactericidal enzymes.
Eosinophils	Neutralization of basophil & mast cell products Destruction of some helminths	Some phagocytic ability.
Basophils	Hypersensitivity rxn	Granules contain histamine, heparin, eosinophil chemotactic factor A. In allergic rxn, bind IgE. Granules release contents in presence of ag.
Mononuclears		
Monocytes	Phagocytosis	Migrate to tissues, become macrophages. Respond to chemotaxins.
Tissue cells		
Mast cells	Hypersensitivity rxn	Connective tissue cells. Resemble basophils but larger & more granules. Bind IgE.
Macrophages	Phagocytosis; elimination of bacteria, intracellular parasites, tumor cells; secretion of cell mediators; ag presentation	Activated by contact with microorganisms or cytokines from T lymphs.
Dendritic cells	Phagocytosis, presentation of ag to helper T lymphs in blood & lymphoid organs	Initiate acquired immune response.

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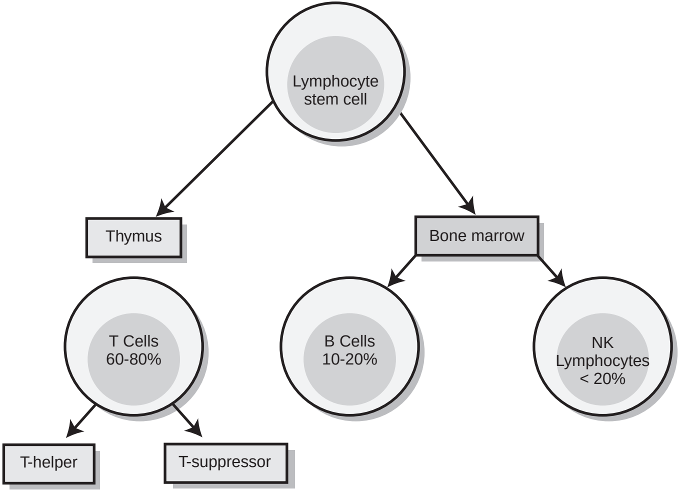
CELL	FUNCTION	COMMENTS
Lymphocytes Natural killer (NK) cells	1st line of defense against tumor cells & cells infected with viruses	Lymphs without T or B markers. No unique surface antigens, but $CD16^+$ & $CD56^+$. Bridge between innate & acquired immunity. Lack specificity. Stimulated by cytokines. Respond early in infection. Provide time for T & B cells to be activated. <20% of lymphs.

Cells of the Acquired Immune System

CELL	FUNCTION	COMMENTS
T lymphs	Cell-mediated immunity.	Derived from cells in bone marrow. Develop T-cell–specific surface ags in thymus. 60%–80% of lymphs.
Helper/inducer T cells	Orchestrate cell-mediated immunity. Activate B cells, cytotoxic cells, & NK cells.	$CD4^{+}$. 2/3 of peripheral T cells. Normal CD4 = 1,000/μL. In AIDS, <200/μL.
Cytotoxic/suppressor T cells	Suppressor cells inhibit helper T cells. Cytotoxic cells kill other cells.	$CD8^{+}$. 1/3 of peripheral T cells. (Normal CD4/CD8 ratio 2:1. In AIDS, <0.5:1).
T regulatory cells	Suppress immune response to self.	$CD4^{+}$ & $CD25^{+}$.
B lymphs	After antigenic challenge, transform into blasts that give rise to plasma cells & memory cells.	Develop in bone marrow. When mature, have surface Igs (IgM, IgD) that act as receptors for ags. 10%–20% of lymphs.
Plasma cells	Ab production.	In peripheral lymphoid organs. Nondividing. Die in a few days.
Memory cells	Respond to ag when encountered again with ↑ speed & intensity.	In peripheral lymphoid organs. Live months to years.

Subpopulations of Lymphocytes



(From Ciesla B. Hematology in Practice, 2nd ed. Philadelphia: FA Davis; 2012:136.)

Lymphoid Organs

PRIMARY	SECONDARY
Bone marrow Thymus	Spleen Lymph nodes Tonsils Appendix Cutaneous-associated lymphoid tissue Mucosal-associated lymphoid tissue (MALT), including Peyer's patches in lower ileum

Isolation	Density gradient centrifugation with Ficoll-Hypaque. Layers from top to bottom: plasma, mononuclear cells, Ficoll-Hypaque, RBCs, & granulocytes.
Identification	Flow cytometry. Fluorescent-labeled monoclonal abs against specific surface ags. Each ab has different fluorescent tag. Light scattering measured as cells flow through laser beam. Common T-cell ags tested: CD2, CD3, CD4, CD7, CD8; B-cell ags: CD19, CD20, CD22, surface Ig.

Immunoglobulin (Ig) Structure

Basic structure	2 heavy (H) chains & 2 light (L) chains held together by disulfide (S-S) bonds.
Heavy (H) chains	γ, α, μ, δ, ε. Determine Ig class (IgG, IgA, IgM, IgD, IgE). 2 H chains in Ig always same.
Light (L) chains	κ or λ. Both found in all classes of Igs, but only 1 type per molecule. Free L chains are known as Bence Jones proteins.
Fab fragment	Fragment antigen binding. Consists of 1 L chain & 1/2 H chain held together by S-S bonds. 2 per Ig. Each can bind ag.
Fc fragment	Fragment crystallizable. Carboxy-terminal halves of 2 H chains held together by S-S bonds. Role in opsonization & complement fixation.
Constant region	Carboxy-terminal ends of H & L chains where amino acid sequence is same for all chains of that type.
Variable region	Amino-terminal ends of H & L chains where amino acid sequence varies. Also known as antigen-recognition unit. Responsible for Ig specificity.
Hinge region	Flexible portion of H chain between 1st & 2nd constant regions. Allows molecule to bend so that 2 ag-binding sites can operate independently.
Joining chain	Glycoprotein that links Ig monomers in IgM & secretory IgA.

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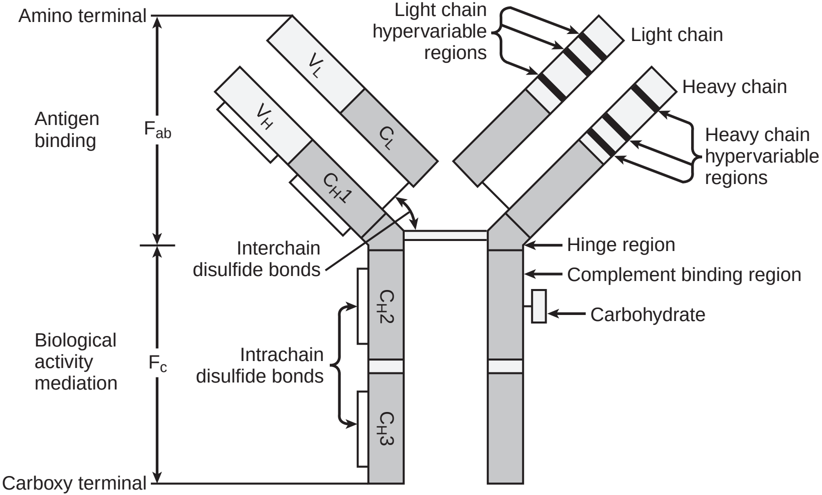

Structure of the basic immunoglobulin unit. VH = variable region heavy chain; VL = variable region light chain; CH = constant region heavy chain; CL = constant region light chain. *(From Harmening DM. Clinical Hematology and Fundamentals of Hemostasis, 5th ed. Philadelphia: FA Davis; 2009:502.)*

Immunoglobulins

	IgG	IgM	IgA	IgD	IgE
Form	Monomer	Pentamer	Monomer & dimer	Monomer	Monomer
Molecular weight (daltons)	150,000	900,000	160,000 or 400,000	180,000	190,000
Heavy chain	Gamma (γ)	Mu (μ)	Alpha (α)	Delta (δ)	Epsilon (ε)
Light chain	Kappa (κ) or lambda (λ)	κ or λ	κ or λ	κ or λ	κ or λ
% of total Ig	70–75	10	10–15	<1	0.002
Serum concentration (mg/dL)	800–1,600	120–150	70–350	1–3	0.005
Antigen-binding sites	2	10	2 or 4	2	2
Complement fixation	Yes	Yes	No	No	No

continued...

	IgG	IgM	IgA	IgD	IgE
Crosses placenta?	Yes	No	No	No	No
Role(s)	Defense against bacteria & viruses. Neutralizes toxins. Opsonin. Passive immunity in newborns.	Neutralizes toxins. Opsonin.	1st line of defense. Patrols mucosal surfaces. Prevents adherence of bacteria and neutralizes toxins.	May play role in B-cell maturation.	Role in allergic rxn. Binds to basophils & mast cells. When 2 adjacent molecules on mast cell bind ag, degranulation of cell with release of histamine & heparin.
Other	More efficient at precipitation than agglutination	First Ig produced in immune response. Only Ig produced by newborn. Most efficient Ig at initiating complement cascade. More efficient at agglutination than IgG. Destroyed by sulfhydryl compounds.	In tears, sweat, saliva, respiratory & GI mucosa, breast milk.	On surface of B lymphs.	Type I immediate hypersensitivity rxn.

Complement

Definition	Group of >30 proteins involved in phagocytosis & clearance of foreign antigens. Most are inactive enzyme precursors that are converted to active enzymes in precise order (cascade).
Functions	Inflammation, opsonization, chemotaxis, cell lysis.
Classical pathway*	Triggered by ag/ab rxn. IgM is most efficient activator. Single molecule attached to 2 adjacent ags can initiate cascade. IgG1, 2, & 3 can activate complement but at least 2 molecules required. Recognition unit: C1 (first to bind). Activation unit: C4, C2, C3. Membrane attack complex: C5, C6, C7, C8, C9 (cell lysis).
Alternative pathway*	Ab independent. Activated by bacteria, fungi, viruses, tumor cells, some parasites.
Lectin pathway*	Ab independent. Initiated by mannose-binding lectin (MBL). Nonspecific recognition of sugars on microorganisms. Important defense mechanism in infancy.
Present in highest concentration in plasma	C3
Key component of all pathways	C3
Ions required	Ca^{2+}, Mg^{2+}

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Complement *continued*

Deficiencies	↑ susceptibility to infection. Accumulation of immune complexes, which can lead to glomerulonephritis.
Inactivation	56°C for 30 min.

*All 3 pathways converge at point of cleavage of C3.

Hypersensitivity Reactions

	TYPE I: ANAPHYLACTIC	TYPE II: CYTOTOXIC	TYPE III: IMMUNE COMPLEX	TYPE IV: T-CELL DEPENDENT
Key reactant(s)	IgE	IgG, IgM, complement, cellular antigens	IgG, IgM, complement, soluble antigens	T cells
Mechanism	Release of mediators from mast cells & basophils	Cytolysis due to ab & complement	Deposits of ag-ab complexes in tissues	Release of cytokines
Onset of symptoms	Immediate	Immediate	Immediate	Delayed (sensitization after 1st contact with ag, symptoms upon reexposure).
Examples	Anaphylaxis, hay fever, asthma, food allergies	Transfusion reactions, hemolytic disease of newborn, autoimmune hemolytic anemia	Arthus rxn, serum sickness, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA)	Contact dermatitis, hypersensitivity pneumonitis, tuberculin skin test

Agglutination Methods

METHOD	PRINCIPLE	APPLICATION
Direct agglutination	Naturally occurring ags on particles (e.g., bacterial ags). Particles agglutinate in presence of corresponding ab.	Widal test for typhoid fever. Salmonella O & H ags used to detect abs in patient serum. Test no longer common in U.S.
Hemagglutination	Ag-ab rxn that results in clumping of RBCs.	ABO slide typing.
Passive (indirect) agglutination	Soluble ags bound to particles, e.g., latex. Particles agglutinate in presence of corresponding ab.	Rheumatoid factor, antinuclear antibody.
Reverse passive agglutination	Ab attached to carrier particles. Particles agglutinate in presence of corresponding ag.	Rapid ID of bacteria.
Agglutination inhibition	Competition between particulate ag (reagent) & soluble ag (in specimen) for sites on reagent ab. Lack of agglutination is pos result.	Detection of illicit drugs.
Hemagglutination inhibition	Detects abs to certain viruses that agglutinate RBCs. In presence of ab, virus is neutralized & hemagglutination doesn't occur.	Rubella & other viruses.
Coagglutination	Reagent ab attached to carrier bacteria. (*Staphylococcus aureus* most frequently used as carrier. Protein A binds Fc portion of reagent ab.) Visible agglutination in presence of corresponding ag.	Rapid ID of bacteria.
Antiglobulin-mediated agglutination	Detection of nonagglutinating ab by coupling with 2nd ab (antihuman globulin).	Direct & indirect antiglobulin tests.

Notes: Rheumatoid factor can cause false-pos rxn in agglutination tests because it reacts with any IgG. Heterophile antibodies can cause false-pos rxn in hemagglutination tests.

Precipitation Methods

METHOD	PRINCIPLE	APPLICATION
Precipitation	Soluble ag combines with soluble ab to produce visible complexes. Less sensitive than agglutination.	See examples below.
Ouchterlony double diffusion	Ags & abs diffuse from wells in gel & form precipitin lines where they meet.	Fungal antigens, extractable nuclear antigens.
Radial immunodiffusion (RID)	Ag diffuses out of well in gel containing ab. Precipitin ring forms. Diameter proportional to concentration of ag.	No longer commonly performed except for low-volume testing of IgD & IgG.
Rocket immunoelectrophoresis	Electrical charge applied to RID to facilitate migration of ag into agar. Height of rocket-shaped precipitin band proportional to concentration of ag.	Igs, complement, alpha-fetoprotein.
Immunoelectrophoresis (IEP)	Proteins separated by electrophoresis then double diffusion with reagent abs in trough in agar. Shape, intensity, & location of precipitin arcs compared with normal control.	Serum proteins, including Igs. Largely replaced by immunofixation electrophoresis.
Immunofixation electrophoresis (IFE)	Proteins separated by electrophoresis. Antiserum placed directly on gel. Ag-ab complexes precipitate.	ID of Igs in monoclonal gammopathies, Bence Jones proteins. Western blot is an adaptation.
Nephelometry	Light scattering by ag-ab complexes.	Igs, complement, C-reactive protein.

Immunoassay Terminology

TERM	EXPLANATION
Ligand	Substance being measured in immunoassay. Can be ag or ab.
Isotopic	Immunoassay that uses radioisotope as label. Rarely used today.
Nonisotopic	Immunoassay that uses something other than radioisotope as label, e.g., enzyme, fluorochrome, chemiluminescent molecule.
Competitive	Immunoassay in which patient ag & labeled reagent ag compete for binding sites on reagent ab.
Noncompetitive	Immunoassay that doesn't involve competition for binding sites. More sensitive than competitive assays.
Heterogeneous	Immunoassay with separation step to remove free from bound analyte. More sensitive than homogeneous assays.
Homogeneous	Immunoassay that doesn't require separation step. Easier to automate.

Enzyme Immunoassay (EIA) Terminology

TERM	EXPLANATION
EIA	Any immunoassay that uses an enzyme as label. A substrate is added to measure enzyme activity.
Direct EIA	1st type of EIA developed. Competitive. Enzyme-labeled reagent is part of initial ag-ab rxn. All reactants added at same time. 1 incubation & 1 wash.
Indirect EIA	Noncompetitive EIA. Enzyme-labeled reagent isn't involved in initial ag-ab rxn. 2 incubations & 2 washes. More sensitive than direct assays. Also known as enzyme-linked immunosorbent assay (ELISA). See next card for more details.
Solid phase	Reagent ag or ab bound to support medium, e.g., polystyrene test tubes, microtiter plates, cellulose membranes, glass beads.

METHOD	DESCRIPTION	PRINCIPLE	OTHER
EIA	Heterogeneous, competitive, direct	Enzyme-labeled ligand & unlabeled patient ligand compete for binding sites on ab attached to solid phase. Free labeled ligand removed by washing. Substrate added. Color inversely proportional to concentration of ligand in specimen.	Original EIA. Used to measure small relatively pure ags, e.g., insulin, estrogen.
Enzyme-linked immunosorbent assay (ELISA)	Heterogeneous, noncompetitive, indirect	Ag attached to solid phase. Ab in specimen attaches. Unbound ab removed by washing. Enzyme-labeled antiglobulin added. Attaches to ab on solid phase. Substrate added. Color directly proportional to ab concentration. More sensitive than competitive EIA. One of most common immunoassays.	Used to detect abs to viruses, e.g., HIV, HAV, HCV, EBV.
Sandwich ELISA or capture assay	Heterogeneous, noncompetitive, indirect	Ab attached to solid phase. Ag in specimen attaches. Enzyme-labeled ab added, attaches to different determinant. Enzymatic activity directly proportional to amount of ag in sample.	Ags must have multiple determinants. Used to measure Igs, hormones, proteins & detect tumor markers, viruses, parasites, fungi. High concentration of ag can cause hook effect. Too much ag for binding sites so undiluted sample has lower absorbance than dilutions.

continued...

Enzyme Immunoassays (EIA) Formats *continued*

METHOD	DESCRIPTION	PRINCIPLE	OTHER
Rapid ELISA	Membrane based	Reagent ag or ab bound to membrane in single use cassette. Sample added. Presence of ag-ab complex indicated by colored rxn.	May have built in control. Usually qualitative.
Enzyme-multiplied immunoassay technique (EMIT)	Homogeneous	Ag in specimen & enzyme-labeled ag compete for binding sites on reagent ab. When enzyme-labeled ag binds, enzyme activity inhibited. Enzyme activity is directly proportional to concentration of ag in specimen.	Used for determination of low molecular weight analytes not readily measured by other methods, e.g., hormones, therapeutic drugs, drugs of abuse. Automated.

Fluorescent Immunoassays (FIA)

METHOD	PRINCIPLE	OTHER	EXAMPLES OF ANALYTES
Direct fluorescent antibody (DFA) staining	Specimen on glass slide overlaid with fluorescein-labeled ab. If corresponding ag present, labeled ab binds. Fluorescence observed with fluorescent microscope.	Detects ags. Fluorescent labels: fluorescein isothiocyanate or rhodamine B isothiocyanate.	Bacterial, viral antigens
Indirect fluorescent antibody (IFA) staining	Reagent ag on glass slide overlaid with patient serum. If corresponding ab present in serum, attaches to ag. When fluorescein-labeled antihuman globulin added, attaches to ab. Fluorescence observed with fluorescent microscope.	"Sandwich technique." Detects abs in serum.	Fluorescent antinuclear antibody (FANA), fluorescent treponemal antibody (FTA)
Fluorescence polarization immunoassay (FPIA)	Labeled ag competes with ag in specimen for sites on reagent ab. Free labeled ag rotates rapidly, emits little polarized light. Bound labeled ag rotates more slowly, emits more polarized light. Amount of polarized light is inversely proportional to concentration of ag in specimen.	Competitive. Homogeneous. Automated.	Therapeutic drugs, hormones

Comparison of Labeled Immunoassays

	RIA	EIA	FIA	CHEMILUMINESCENT IMMUNOASSAY (CIA)
Labels	^{125}I, ^{131}I, ^{3}H.	Alkaline phosphatase, horseradish peroxidise, -D-galactosidase, glucose-6-phosphate dehydrogenase.	Fluorescein, rhodamine.	Luminol, acridium esters, ruthenium derivatives, nitrophenyl oxalates.
Detection	Radioisotopes emit radioactivity.	Enzymes react with substrate to produce color change.	Fluorochromes absorb energy from light source, convert to longer wave-length (lower energy).	Chemiluminescent mole-cules produce light from chemical rxn.
Type(s) of assays available	Competitive/ heterogeneous.	Mostly noncompetitive now. Heterogeneous & homogeneous.	Usually competitive. Heterogeneous & homogeneous.	Competitive & noncompet-itive. Heterogeneous & homogeneous.
Advantages	Sensitivity. Specificity.	Sensitivity. Specificity. No health hazard or disposal problems. Reagents with long shelf life. Can be automated.	Same as EIA. Automated.	Same as EIA. Automated.

continued...

Comparison of Labeled Immunoassays *continued*

	RIA	EIA	FIA	CHEMILUMINESCENT IMMUNOASSAY (CIA)
Disadvantages	Radiation hazard. Short shelf life of reagents. Disposal problem. Licensing & federal regulations.	Natural inhibitors in some specimens. Nonspecific protein binding.	Autofluorescence from organic substances in serum. Nonspecific binding to substances in serum. Expensive, dedicated instrumentation.	Quenching of light emission by some biological materials.
Use	Not common.	Common.	Common.	Common.

Nontreponemal Tests for Syphilis

	VDRL	RPR
Method	Flocculation	Flocculation.
Detects	Reagin (ab against cardiolipin that is in serum of pts with syphilis)	Reagin.
Antigen	Cardiolipin	Cardiolipin with charcoal.
Positive reaction	Microscopic clumps	Macroscopic agglutination.
Specimen(s)	Inactivated serum, CSF	Serum (inactivation not required), plasma.
Reactivity during disease	May be neg in primary stage. Titers usually peak during secondary or early late stages. Titers in late stage, even when untreated. More rapid decline with treatment. Becomes nonreactive in 1–2 yr following successful treatment.	Same as VDRL.
False positives	Biologic false pos with infectious mononucleosis (IM), infectious hepatitis, malaria, leprosy, lupus erythematosus, rheumatoid arthritis, advanced age, pregnancy. Reactive in other treponemal infections such as yaws & pinta.	Same as VDRL.
Other	Screening test. Reactives should be confirmed by treponemal test. Replaced by RPR for serum. Still performed on CSF for Dx of neurosyphilis.	Used for screening & treatment monitoring. Screening test. Reactives should be confirmed by treponemal test.

	FLUORESCENT TREPONEMAL ANTIBODY ABSORPTION (FTA-ABS)	*TREPONEMA PALLIDUM* PARTICLE AGGLUTINATION (TP-PA)	ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)
Detects	Antibody to *T. pallidum*	Antibody to *T. pallidum*	Antibody to *T. pallidum.*
Reagent(s)	Sorbent (nonpathogenic treponemes—Reiter strain), slides with Nichols strain of *T. pallidum*, fluorescein-labeled antihuman globulin	Colored gelatin particles coated with treponemal ag	Enzyme-labeled treponemal ag.
Positive reaction	Fluorescence	Agglutination of sensitized gel particles. Smooth mat over surface of well	Color development following addition of substrate.
Specimen(s)	Serum, CSF	Serum	Serum
Reactivity during disease	Usually pos before reagin tests. Some false negs in primary syphilis. Usually pos for life	Not as sensitive in primary syphilis as FTA. Sensitivity close to 100% in secondary syphilis. Usually pos in late stages	High sensitivity in primary syphilis. Decreases in later stages.

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Treponemal Tests for Syphilis *continued*

	FLUORESCENT TREPONEMAL ANTIBODY ABSORPTION (FTA-ABS)	*TREPONEMA PALLIDUM* PARTICLE AGGLUTINATION (TP-PA)	ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)
False positives	Fewer than nontreponemal tests. Reactive with other treponemal diseases, e.g., yaws, pinta	Fewer than nontreponemal tests	Similar to other treponemal tests.
Other	Sorbent removes nonspecific antibodies. Used to confirm reactive nontreponemal test. Not good for treatment monitoring	Sorbent removes nonspecific antibodies. Used to confirm reactive nontreponemal test. Not good for treatment monitoring	Can be automated. Used for screening & for confirmation of reactive nontreponemal test. When used for screening, reactives should be confirmed with nontreponemal test (reverse sequence screening), followed by TP-PA or FTA-ABS if nontreponemal test is nonreactive. IgM capture assay for Dx of congenital syphilis.

Interpretation of Syphilis Test Results

RESULTS	INTERPRETATION
RPR reactive FTA reactive	Pos for syphilis
RPR reactive FTA nonreactive	Neg for syphilis
ELISA reactive RPR reactive	Pos for syphilis
ELISA reactive RPR nonreactive FTA-ABS reactive	Late, latent, or previous syphilis

Serological Tests for Other Bacterial Infections

TEST	DIAGNOSIS	COMMON METHOD(S)	COMMENTS
Anti-streptolysin O (ASO)	Sequelae of group A strep (GAS) infection: rheumatic fever, post-streptococcal glomerulonephritis	Nephelometry.	Uses recombinant streptolysin ag. If ab present, ag-ab complexes form & ↑ light scatter. Replaces classic neutralization method.
Anti-DNase B	Sequelae of GAS infection: rheumatic fever, glomerulonephritis following skin infection, i.e., impetigo	EIA, nephelometry.	Highly specific. May be pos when ASO is neg.
Streptozyme	Sequelae of GAS infection	Slide agglutination.	Uses sheep RBCs coated with several streptococcal ags. More false pos & false negs. Should be used in conjunction with ASO & anti-DNase. Serial titers should be performed.
Helicobacter pylori antibodies	Gastric & duodenal ulcers caused by *H. pylori*	Method of choice: ELISA. Rapid tests, PCR available.	Most tests detect IgG. 25% ↓ in titer = successful treatment. Abs remain for years. Pos rapid tests should be confirmed by ELISA.
Mycoplasma pneumoniae antibodies	Primary atypical pneumonia (PAP)	Most common: EIA. Also agglutination, IFA. Molecular methods available.	Can test for IgM & IgG abs. Replaces cold agglutinin, which was nonspecific.
Rickettsial antibodies	Typhus, Rocky Mountain spotted fever, other rickettsial infections	Gold standard: IFA, micro-IF. PCR available.	Organism specific assays replace Weil-Felix rxn, which was nonspecific.

Serological Tests for Infectious Mononucleosis (IM)

	HETEROPHILE ANTIBODIES*	EBV-SPECIFIC ANTIBODIES
Specificity	Nonspecific abs that agglutinate horse, sheep, & bovine RBCs. Heterophile abs are abs that react with similar ags from different species.	Specific abs against Epstein-Barr virus (EBV) ags present in different phases of infection—early: early ag (EA), late: viral capsid ag (VCA), latent: EBV nuclear ag (EBNA).
Occurrence	90% of patients develop in 1st month of illness. Can persist for 1 yr. Neg in 10% of adults & up to 50% of children with IM. If symptomatic & heterophile neg, test for EBV-specific abs.	Anti-VCA IgM appears at onset of symptoms, disappears in 3 mo. Anti-VCA IgG appears at onset of symptoms, persists for life. Anti-EBNA present during convalescence. Acute infection: Anti-VCA IgM, anti-VCA IgG, anti-EA. Past infection: Anti-EBNA, anti-VCA IgG, neg anti-VCA IgM.
Tests	Rapid latex agglutination, solid-phase immunoassays. Ag is purified bovine RBC extract. Screening tests.	Indirect immunofluorescent assays (IFA), ELISA, chemiluminescent assays. IFA is gold standard but time consuming & harder to interpret. Molecular tests can be used for immunocompromised patients who don't produce abs.

Hepatitis Tests

TEST	SIGNIFICANCE	COMMENTS
Hepatitis A		
Total anti-HAV	Past infection & immunity	
IgM anti-HAV	Acute infection	
HAV RNA	Current infection	Used to detect HAV in food & water.
Hepatitis B		
HBsAg	Acute or chronic infection, infectivity	1st serological marker to appear. Used to screen donor blood. Pos should be confirmed by repeat testing & another assay such as HBsAg neutralization or HBV DNA.
HBeAg	Acute or chronic infection	Indicates high degree of infectivity.
Total anti-HBc	Current or past infection or carrier	Predominantly IgG, which persists for life.
IgM anti-HBc	Current or recent infection	1st ab to appear. Useful for detecting HBV infection when HBsAg is no longer detectable ("window period"). Used to screen blood donors.
Anti-HBe	Recovery, reduced infectivity	
Anti-HBs	Recovery & immunity	Ab that develops following immunization.

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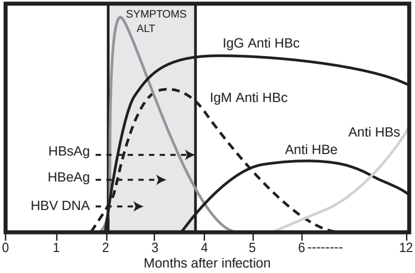
Hepatitis Tests *continued*

TEST	SIGNIFICANCE	COMMENTS
HBV DNA	Current infection	Detectable 21 days before HBsAg. Used to monitor viral load & therapy.
Hepatitis C Anti-HCV	Acute, chronic, or previous infection	Pos should be confirmed by recombinant immunoblot assay (RIBA) or molecular method.
HCV RNA	Current infection	Used for viral load testing, blood/organ donor screening. HCV genotyping to determine optimal treatment.
Hepatitis D (delta hepatitis) IgM anti-HDV	Acute or chronic infection	HDV is a defective virus that can only occur in presence of HBV.
IgG anti-HDV	Recovery or chronic infection	
HDV RNA	Current infection	Marker of active viral replication. Used to monitor therapy.
Hepatitis E		Tests are currently not approved by FDA for use in U.S.

Most serological tests for hepatitis are by EIA or CIA. Molecular methods detect infections earlier.

Hepatitis Serological Profiles

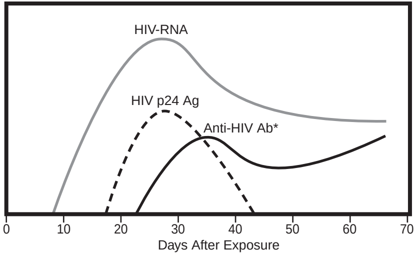
ACUTE HEPATITIS A	RECOVERY FROM HEPATITIS A	ACUTE HEPATITIS B	RECOVERY FROM HEPATITIS B	CHRONIC HEPATITIS B/ CARRIER	HEPATITIS B IMMUNIZATION
IgM anti-HAV +	Total anti-HAV +	HBsAg + Total anti-HBc + IgM anti-HBc + Anti-HBs –	HBs Ag – Total anti-HBc + Anti-HBs +	HBsAg + Total anti-HBc + IgM anti-HBc – Anti-HBs –	HBsAg – Anti-HBc – Anti-HBs +



Clinical indications of hepatitis B virus over time. *(From Arneson W, Brickell J. Clinical Chemistry: A Laboratory Perspective. Philadelphia: FA Davis; 2007:258.)*

Appearance of HIV Markers

MARKER	COMMENTS
Viral RNA	Detectable within days of infection.
p24 ag	Core coat for nucleic acids. Detectable in 2–3 wk. Becomes undetectable as abs develop, then detectable again in late stages as immune system fails & virus replicates.
IgM ab	Usually detectable in 2–8 wk. Transient. Peaks in about 1–2 wk, undetectable about 1–2 wk later.
IgG ab	Detectable shortly after IgM. Gradual ↑ in titer over several months. Long lasting.



*By 3rd generation EIA. IgM first, then IgG.

Appearance of HIV markers after exposure (exact times vary with virus, patient immune response, and tests used).

HIV Screening Tests

TEST	DETECTS	WINDOW PERIOD*	COMMENTS
EIA/ELISA			
1st generation	IgG ab to HIV-1	6–12 wk	
2nd generation	IgG ab to HIV-1/2	6–12 wk	
3rd generation	IgG & IgM ab to HIV-1/2	3–4 wk	
4th generation	IgG & IgM ab to HIV-1/2 & p24 ag	2 wk	EIA or CIA. P24 ag without HIV ab = acute infection. P24 ag & HIV ab = established infection. Currently not approved for screening blood donors.
Rapid tests	IgG & IgM ab to HIV	4–12 wk	Immunochromatographic assays. Can be performed on whole blood, serum, oral fluid.
Nucleic acid amplification testing (NAAT)	HIV RNA	5 days	Not cost effective for screening in most settings. Used for screening blood donors & infants. (Ab tests aren't reliable in children <18 months of age. Abs from infected mother can be present even if child isn't infected.)

All specimens with pos screening tests must be tested again by same or different screening test. If repeatedly pos, a confirmatory/supplemental test must be done.
*Time between exposure & pos test result.

False Positives and Negatives with HIV-Antibody ELISA Testing

CAUSES OF FALSE POSITIVES	CAUSES OF FALSE NEGATIVES
Heat inactivation of serum	Blood drawn before seroconversion (window period)
Repeated freezing/thawing of serum	Hypogammaglobulinemia
Autoantibodies	Immunosuppressive therapy
Multiple pregnancies	Strain of HIV not detected by assay
Liver disease	Technical errors
Administration of immunoglobulins	
Administration of certain vaccines	
Some malignancies	

False neg infrequent. False pos more common in low-risk populations.

HIV Confirmatory/Supplemental Tests

TEST	DETECTS	COMMENTS
Western blot (WB)	Ab to HIV	Traditional confirmatory test but not as sensitive as 4th-gen EIA or NAAT. Interpretation is controversial but most labs report pos if at least 2 of the following 3 bands are present: p24, gp41, gp120/160. NAAT required following neg or indeterminate results. Time-consuming, difficult to interpret.
Indirect immunofluorescent assays (IFA)	Ab to HIV	Sensitivity & specificity comparable to Western blot. Not frequently used. Expensive, subjective.
NAAT	HIV RNA	Qualitative test used for confirmation.

More specific than screening tests. Fewer false pos.

Tests to Stage and Monitor HIV

TEST	COMMENTS
CD4 T-cell count	HIV infects CD4 cells. # declines as disease progresses. <200/μL defines AIDS according to CDC. Also used to monitor therapy. Perform every 3–6 mo. Flow cytometry is gold standard.
HIV-1 viral load assays	Quantitative NAAT to determine plasma HIV RNA. Used to predict disease progression, determine when to start antiretroviral therapy, & monitor response to therapy. Test 2–8 wk after start of therapy & then every 3–4 mo. Same assay should be used in order to assess changes.

Screening Tests for Systemic Lupus Erythematosus (SLE)

TESTS	SENSITIVITY	SPECIFICITY	COMMENTS
Fluorescent antinuclear antibody (FANA)	High. 95%–100% of SLE patients test pos.	Low. Abs present in other autoimmune diseases, 2% of healthy individuals, 75% of elderly. Dilutions tested to eliminate low titer rxn in normal population. Cutoff dilution to report pos usually ≥1:80. End-point titer may be reported. Generally higher in SLE.	Indirect immunofluorescence (IIF). Substrate is human epithelial tumor cell line (HEp-2). Detects abs to >100 autoantigens. Staining patterns reported but not considered as significant as in past. Labor intensive. Subjective. Still considered gold standard by rheumatologists.
EIA antinuclear antibody (EIA-ANA)	May not be as sensitive as IIF.	Low.	Easier, less expensive. Can be automated. Interpretation not subjective. Not as many antigens as IIF.

Tests for Specific Antinuclear Antibodies (ANA)

TESTS	SENSITIVITY FOR SLE	SPECIFICITY FOR SLE	COMMENTS
Anti-dsDNA	Low	High. Uncommon in other diseases or normal individuals.	Most specific ab for SLE. Titers correlate with disease activity. Peripheral or homogeneous fluorescent pattern with indirect immunofluorescence (IIF). Other methods: EIA, immunoblotting, immunodiffusion.
Anti-Sm	Low	High. Uncommon in other diseases or normal individuals.	Coarsely speckled pattern with IIF. Other methods: EIA, immunoblotting, immunodiffusion.
Antihistone, anti-DNP, anti-SS-A/Ro, anti–SS-B/La, anti-nRNP	Low	Low.	Generally not useful for Dx of SLE. Used to Dx other connective tissue diseases. Methods: IIF, EIA, immunoblotting, immunodiffusion.
Extractable nuclear antigen (ENA) antibiotics	Low	Anti-Sm is specific for SLE.	Immunodiffusion (Ouchterlony double diffusion) test panel that typically tests for abs to Sm, SS-A/Ro, SS-B/La, RNP. Precipitin lines of identity/nonidentity. New method: multiplex bead assay. Immunoassay using specific ag-coated beads & flow cytometry to detect multiple (currently 6–13) ANAs simultaneously.

Serological Tests for Rheumatoid Arthritis (RA)

TEST	COMMON METHOD(S)	COMMENTS
Rheumatoid factor (RF)	Agglutination, ELISA, nephelometry	Autoantibody (usually IgM) against IgG. Pos in 70%–80% of patients with RA. Not specific for RA. Present with other autoimmune diseases, infections, & in some normal individuals. Agglutination tests only detect IgM RF. ELISA & nephelometry can also detect IgA & IgG classes of RF. Automated methods more common.
Anti–cyclic citrullinated peptide antibody (Anti-CCP)	ELISA	More specific for RA than RF. Associated with more severe form of RA.

Interpretation of Serological Tests

- ≥4-fold increase in titer from acute to convalescent specimen drawn 10–14 days later is diagnostic.
- IgM ab is sign of recent infection.
- IgG ab is sign of immunity.
- IgG ab in newborn is maternal ab.

Serology Calculations

1. How would you prepare a 5% suspension of human group O RBCs?
   5% = 5 mL per 100 mL
   Mix 5 mL of packed RBCs + 95 mL of buffer, or for a smaller amount, 0.5 mL of packed RBCs + 9.5 mL buffer. Any 1:20 dilution could be used. (5:100 = 1:20)

2. How would you prepare 5 mL of a 1:10 dilution of serum?
   $$\frac{1}{10} = \frac{x}{5}$$
   $10x = 5$
   $x = 0.5$ mL
   0.5 mL diluted to 5 mL is a 1:10 dilution, so mix 0.5 mL of serum + 4.5 mL of buffer.

3. How would you prepare 10 mL of a 1:100 dilution from a 1:10 dilution?
   A. Determine the dilution factor to make a 1:100 dilution from a 1:10 dilution:
   $1/10 \times 1/x = 1/100$
   $1/10x = 1/100$
   $10x = 100$
   $x = 10$
   A 1:10 dilution of a 1:10 dilution yields a 1:100 dilution. ($1/10 \times 1/10 = 1/100$).
   B. Determine how to make 10 mL of a 1:10 dilution:
   1:10 dilution is 1 part + 9 parts. To make 10 mL, mix 1 mL of solution + 9 mL of buffer.
   To make 10 mL of a 1:100 dilution from a 1:10 dilution, mix 1 mL of the 1:10 dilution + 9 mL of buffer.

4. What is the dilution in tube 4 of a twofold serial dilution, if tube 1 is undiluted?
   $1 \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8}$

SECTION 6

Immunohematology Review

Criteria for Whole Blood Donors (AABB)

	ALLOGENEIC	AUTOLOGOUS
Age	≥16 or as allowed by state law	As determined by medical director.
HGB/HCT	HGB ≥12.5g/dL or HCT ≥38%	HGB ≥11.0 g/dL or HCT ≥33%.
Temperature	≤37.5°(99.5°F)	As determined by medical director. Bacteremia is cause for deferral.
Venipuncture site	No infectious skin disease or scars indicative of drug use	As determined by medical director.

Current as of date of publication. Check current AABB Technical Manual for updates.
Autologous–No donations within 72 hr of surgery.

Donor Deferrals (AABB)

DEFERRAL	CONDITION
2 days	Aspirin, if donor is sole source of platelets
2 weeks	Measles (rubeola), mumps, polio, typhoid, yellow fever vaccines
4 weeks	Rubella, chicken pox (varicella-zoster) vaccine
6 weeks	Pregnancy
8 weeks	Whole blood donation
12 months	Syphilis Gonorrhea Mucous membrane exposure to blood Skin penetration with sharp contaminated with blood or body fluids Household or sexual contact with individual with hepatitis Sexual contact with individual with HIV or at high risk Incarceration in correctional facility for >72 consecutive hr Travel to Iraq or area endemic for malaria Recipient of blood, blood components, plasma-derived clotting factor concentrates, or transplant
3 years	Malaria, or from an area endemic for malaria

continued...

Donor Deferrals (AABB) *continued*

DEFERRAL	CONDITION
Permanent	Parenteral drug use Family history of Creutzfeldt-Jakob disease Treatment with pituitary growth hormone of human origin Viral hepatitis after 11th birthday Confirmed positive HBsAg Repeatedly reactive anti-HBc on >1 occasion Repeatedly reactive HTLV on >1 occasion Present or past clinical or laboratory evidence of infection with HIV, HCV, HTLV History of babesiosis or Chagas' disease

Not all inclusive. Current as of date of publication. Check current AABB Technical Manual for updates.

Collection of Whole Blood

Skin preparation	Aseptic method, e.g., povidone-iodine scrub & prep solution.
Volume of blood routinely collected	450 mL ± 10% or 500 mL ± 10%, depending on collection bag.
Maximum volume	10.5 mL of blood per kg of donor's weight, including samples for testing (e.g., 473 mL from 100 lb [45 kg] donor).
Low-volume collections	300–404 mL in 450-mL bag or 333–449 mL in 500-mL bag. Labeled "low-volume." RBCs may be transfused, but other components shouldn't be prepared because of abnormal anticoagulant-to-plasma ratio.
Volumes of anticoagulant	63 mL anticoagulant for 450-mL collection, 70 mL for 500-mL collection.
Time of collection	Usually <10 min. If >15–20 min, unit may not be suitable for preparation of platelets or plasma.
Samples for testing	From diversion pouch or by 2nd phlebotomy.
Storage temperature of unit between collection & processing	20°–24°C if platelets are to be prepared; otherwise 1°–6°C.

Apheresis

Explanation	Automated blood collection system that allows removal of 1 or more components from blood & return of remainder to donor.
Advantages	Allows collection of larger volume of specific components. Can reduce # of donors to which pt is exposed.
Donor requirements	Vary with procedure.
Components collected	RBCs (2 units can be collected at same time from donors who are larger & have higher HCT. 16 wk between donations). Platelets. (Plateletpheresis. Can collect HLA matched for pts who are refractory to random plts. Can be leukoreduced during collection. Contain $\geq 3 \times 10^{11}$ plt.) Plasma (Plasmapheresis). Granulocytes. (Leukapheresis. Not widely used to date.) Stem cells. (For bone marrow reconstitution in pt with cancer, leukemia, lymphoma. Autologous or HLA matched.)
Therapeutic uses	Therapeutic plasmapheresis (plasma exchange) used to remove abnormal plasma proteins & replace with crystalloid, albumin, or FFP. Therapeutic cytapheresis used to remove cellular elements, e.g., abnormal # platetets (to ↓ risk of hemorrhage or thrombosis), leukemic WBCs, lymphocytes (to induce immunosuppression).

Donor Testing Required by AABB and/or FDA

Typing ABO Rh (including weak D)	
Antibody screen	
Syphilis testing Antibodies to *Treponema pallidum* or nontreponemal serological test for syphilis, e.g., RPR	
Hepatitis testing HBsAg Anti-HBc Anti-HCV HCV RNA (NAT)	
HIV testing Anti-HIV-1/2 HIV-1 RNA (NAT)	
Other infectious disease testing Anti-HTLV-I/II West Nile virus RNA (NAT) Anti–*Tryanosoma cruzi* (FDA recommends 1-time donor screening)	
Test to detect bacterial contamination of platelets Culture of platelets or FDA-approved rapid test (e.g., Pan Genera Detection [PGD] test)	

For autologous donations, complete donor testing required if tf will occur outside collection facility; otherwise only ABO & Rh required.

Anticoagulant/Preservative Solutions

NAME	ABBREVIATION	RBC SHELF LIFE	COMMENTS
Acid citrate-dextrose (Formula A)	ACD-A	21 days	Citrate prevents coagulation by chelating Ca^{2+}. Dextrose (glucose) supports ATP generation. Used for apheresis.
Citrate-phosphate-dextrose	CPD	21 days	Higher pH preserves 2,3-DPG better. Better O_2 delivery.
Citrate-phosphate-double-dextrose	CP2D	21 days	Contains 100% more glucose than CPD.
Citrate-phosphate-dextrose with adenine	CPDA-1	35 days	Adenine increases ADP, which increases synthesis of ATP. Contains more glucose to sustain cells during longer storage.

Additive Solutions

Purpose	Extend shelf life of RBCs to 42 days.
Constituents	Glucose for energy, adenine to support ATP levels.
Procedure	Plasma expressed from WB. 100–110 mL additive transferred from attached satellite bag to RBCs within 72 hr of collection or per manufacturer's instructions.
Final hematocrit	55%–65% (HCT of RBCs without additive 65%–80%). Lower viscosity facilitates tf.
Examples	Adsol (AS-1), Nutricel (AS-3), Optisol (AS-5)

Open and Closed Systems

	EXPLANATION	EFFECT ON EXPIRATION DATE OF COMPONENT
Open system	Seal on unit is broken to attach external transfer bag. Exposure to air poses threat of bacterial contamination.	Components stored at 1°–6°C must be used within 24 hr after system opened; components stored at 20°–24°C within 4 hr.
Closed system	Sterility maintained through use of attached satellite bags or sterile connecting device that welds tubing from 1 bag to another. No exposure to air.	No change.

Blood Components—RBCs

COMPONENT	PREPARATION	STORAGE TEMPERATURE	SHELF LIFE	INDICATIONS	OTHER
RBCs	Separated from WB by centrifugation or sedimentation any time before expiration date of WB, or collected by apheresis.	1°–6°C	35 days in CPDA-1	Inadequate tissue oxygenation	Should have HCT ≤80%; otherwise not enough preservative to support RBCs. 1 unit should ↑ HGB 1 g/dL or HCT 3%.
RBCs adenine, saline added	Additive solution added to RBCs following removal of most plasma.	1°–6°C	42 days	Same	Most commonly used RBC product.
RBCs frozen	Frozen in glycerol within 6 days of collection. High glycerol (40%) method most commonly used.	Frozen in high glycerol: ≤65°C. After deglyc (washing in ↓ concentration of saline): 1°–6°C	Frozen: 10 yr. After degylc: 24 hr (unless closed system used)	Same	Osmolality to monitor glycerol removal. Virtually all plasma, anticoagulant, WBCs, & platelets removed. Safe for IgA-deficient pt. Used to store rare cells.

continued...

Blood Components—RBCs *continued*

COMPONENT	PREPARATION	STORAGE TEMPERATURE	SHELF LIFE	INDICATIONS	OTHER
Washed RBCs	RBCs washed with saline.	1°–6°C	24 hr after washing	History of severe allergic rxn (e.g., IgA, other plasma proteins)	Not a substitute for leuko-reduced RBCs. About 20% of RBCs lost in process.
RBCs leukocytes reduced	Filtration or apheresis processing.	1°–6°C	Closed system: same as RBCs. Open system: 24 hr	History of febrile rxn	Must retain 85% of original RBCs. $<5 \times 10^6$ WBCs.
RBCs irradiated	Irradiation at 2,500 cGy.	1°–6°C	Original outdate or 28 days from irradiation, whichever comes 1st	Immunodeficiency, malignancy, bone marrow transplant, tf with blood from blood relative, intrauterine & neonatal tf	For prevention of graft-vs.-host disease. Kills donor T cells.

COMPONENT	PREPARATION	STORAGE TEMPERATURE	SHELF LIFE	INDICATIONS	OTHER
Fresh frozen plasma (FFP)	Plasma separated from WB & frozen within 8 hr of collection	Frozen: ≤–18°C. After thawing: 1°–6°C	Frozen: 12 mo. After thawing: 24 hr	Deficiency of coag factors	Contains all coag factors. Check for evidence of thawing & refreezing. Thawed at 30°–37°C or by FDA-approved microwave.
Cryoprecipitate	Prepared by thawing FFP at 1°–6°C, removing plasma, & refreezing within 1 hr	Frozen: ≤–18°C. After thawing: RT	Frozen: 12 mo. After thawing: single units 6 hr, pools 6 hr if sterile connecting device used, otherwise 4 hr	Fibrinogen & factor XIII deficiencies	Used for hemophilia A & von Willebrand disease only if factor VIII concentrate or recombinant factor preparations not available. Should contain ≥80 IU of factor VIII & ≥150 mg of fibrinogen.

COMPONENT	PREPARATION	STORAGE TEMPERATURE	SHELF LIFE	INDICATIONS	OTHER
Platelets	Centrifugation of WB at RT within 8 hr of collection. 1st soft spin yields platelet-rich plasma. 2nd hard spin separates plt from plasma.	20°–24°C	5 days from collection, with agitation. After pooling, 4 hr	Severe thrombocytopenia or abnormal platelet function	40–70 mL plasma. $\geq 5.5 \times 10^{10}$ plt. pH ≥ 6.2. 1 unit should ↑ plt by 5,000–10,000/µL in 75-kg recipient. Shouldn't be used if visible aggregates present. May contain residual RBCs. Usually pooled (4–6 units).
Apheresis platelets	Apheresis.	20°–24°C	5 days with agitation	Same	$\geq 3.0 \times 10^{11}$ plt. Equivalent to 4–6 units. Exposes recipient to fewer donors.
Leukocyte-reduced platelets	WBCs removed by filtration or during apheresis processing.	20°–24°C	Open system—4 hr. Apheresis—5 days	Recurrent febrile rxn & to ↓ risk of CMV transmission or HLA alloimmunization	
Prestorage pooled platelets	4–6 ABO-identical plt pooled using closed system.	20°–24°C	5 days from collection	Same	Extended outdate overcomes need for tf service to pool just before administration.

Labeling Requirements for Blood and Components

- ISBT 128 bar-code symbology
- Name of product
- Method of preparation (whole blood, apheresis)
- Storage temperature
- Preservatives/anticoagulant
- Number of units in pooled components
- Name, address, registration number, & license number of collection & processing facility
- Expiration date (& time if applicable). When expiration time not indicated, expiration is at midnight.
- Identification number for unit or pool
- Donor category (volunteer, autologous)
- ABO group & Rh type, if applicable.
- Special handling information
- Statements regarding recipient identification, *Circular of Information*, infectious disease risk, & prescription requirement
- Autologous units: “For Autologous Use Only.” Biohazard label if any infectious disease markers are pos or “Donor Untested” if testing not performed.

Leukocyte Reduction (Leukoredution)

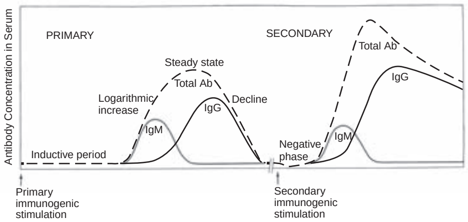
Purpose	To ↓ WBCs to ↓ febrile nonhemolytic transfusion reactions, transmission of CMV, & HLA alloimmunization
WBCs	$<5 \times 10^6$
Methods	• By apheresis processing • By filtration during manufacture of components or after storage. Prestorage leukocyte reduction is most effective. WBCs removed before they release cytokines • Use of filter during infusion

RBC Storage Lesion

INCREASED	DECREASED	OTHER CHANGES
Lactic acid Plasma K^+ Plasma hemoglobin Microaggregates	ATP 2,3-DPG pH Glucose Viable cells Labile coagulation factors	Shift to left of O_2 dissociation curve (↑ HGB/O_2 affinity, ↓ O_2 delivery to tissues)

Primary Versus Secondary Response

	PRIMARY	SECONDARY (ANAMNESTIC)
Stimulus	1st exposure to ag	Subsequent exposure to ag
Lag phase	Days to months	Hours
Type of antibody	IgM at first. May switch to IgG after 2–3 weeks (isotype switching)	IgG
Titer	Rises slowly, peaks, then declines	Rises faster & higher, stays elevated longer



Schematic representation of primary and secondary antibody responses. Note the enhanced antibody production and expanded antibody-producing cell population during the secondary antibody response.
(From Herscowitz HB. Immunophysiology. In Bellanti JA [ed]: Immunology III. Philadelphia: WB Saunders; 1985:117, with permission.)

IgG Versus IgM

	IgG	IgM
Structure	Monomer	Pentamer
Number of ag-binding sites	2	10
Type of ab	Immune	Naturally occurring
Optimum temperature of reactivity	37°C	25°C or lower
Reacts in saline?	No	Yes
Reacts best by IAT?	Yes	No
Complement fixation	Moderate	Strong
Causes transfusion reactions?	Yes	Not usually, except ABO
Crosses placenta?	Yes	No
Causes hemolytic disease of the newborn/fetus?	Yes	No
Destroyed by sulfhydryl compounds (dithiothreitol [DTT], 2-mercaptoethanol [2-ME])?	No	Yes

Factors That Affect Agglutination in Tube Testing

FACTOR	COMMENTS
Sensitization stage	**Attachment of ab to ag**
Temperature	Clinically significant abs react best at 37°C.
pH	Most abs react at pH 5.5–8.5.
Ionic strength	Reducing ionic strength of medium facilitates interaction of ab with ag (e.g., low ionic strength solution [LISS]).
Ag/ab ratio	Too much ab can cause prozone (false neg). Optimum serum-to-cell ratio is 80:1. Usually 2 drops serum to 1 drop of 2%–5% RBCs. Follow manufacturer's directions.
Incubation time	Depends on medium. Usually 10–30 min. Follow manufacturer's directions.
Agglutination stage	**Formation of ag-ab bridges between RBCs**
Type of ab molecule	IgM is larger, can span distance between RBCs more easily.
Density of ags & location on RBC surface	Affects ease of attachment of abs.
Zeta potential	Difference in charge between neg-charged RBC surface & cloud of pos ions that surround RBCs. Reducing zeta potential allows RBCs to move closer together (e.g., enzyme treatment of test cells).

Comparison of Tube, Gel, and Solid-Phase Testing

	TUBE TESTING	GEL TESTING	SOLID-PHASE TESTING
Reaction container	Glass test tubes	Plastic microtube containing dextran-acrylamide gel.	Microplate with RBC membranes bound to surface of wells.
Principle	Abs attach to corresponding ags on RBCs, forming bridges between cells. RBCs agglutinate.	Ag-ab rxn results in agglutinated RBCs. Gel acts as sieve. Large agglutinates can't pass through, remain at top. Small agglutinates pass into gel. Unagglutinated cells go to bottom.	Abs in sample attach to RBC ags on surface of wells. After incubation, unbound ab removed by washing. Anti-IgG-labeled indicator RBCs added. Attach to abs bound to reagent RBC ags during centrifugation.
Pos rxn	Agglutinated RBCs or hemolysis	Agglutinated RBCs suspended in gel. Position indicates strength of rxn. Larger agglutinates at top.	Indicator RBCs adhere diffusely to surface of well.
Neg rxn	No agglutinated RBCs or hemolysis	Button of unagglutinated RBCs in bottom of microtube.	No adherence of RBCs. Button of RBCs in bottom of well.
Adaptable to automation?	No	Yes	Yes
Advantages	Low cost	Standardized. More sensitive than tube testing. Rxn stable 2–3 days; can be captured electronically. AHG tests don't require washing or control cells.	Standardized. More sensitive than tube testing. Rxn stable 2 days.

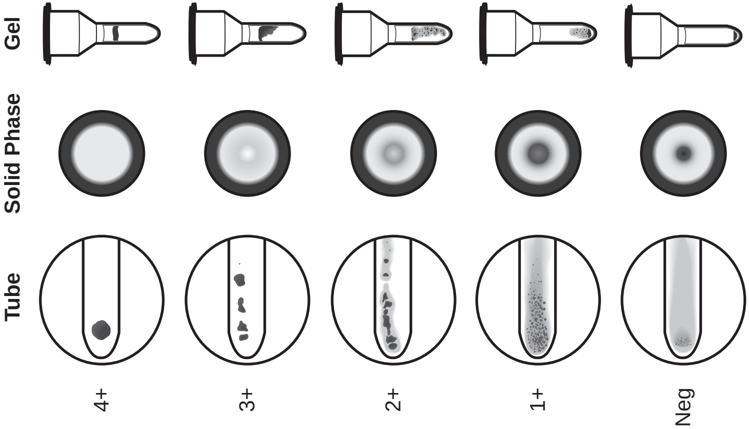
Note: A cell button in tube testing is a solid agglutinate—4+ rxn. A cell button in solid phase is unagglutinated cells—neg rxn.

Grading Reactions—Tube Versus Gel

	TUBE*	GEL
4+	One solid agglutinate	Solid band of agglutinated RBCs at top
3+	Several large agglutinates	Band of agglutinated RBCs near top with a few staggered below
2+	Medium-sized agglutinates, clear background	Agglutinates throughout
1+	Small agglutinates, turbid background	Agglutinates predominantly in lower half of column with some RBCs at bottom
Mixed field	Some agglutinated RBCs in sea of free RBCs	Layer of agglutinated RBCs at top & pellet of unagglutinated RBCs at bottom
Neg	No agglutinates	Well-defined pellet of unagglutinated RBCs at bottom

*In tube testing, hemolysis is also a pos rxn.

Comparison of Tube, Gel, and Solid-Phase Reactions



ABO Genotypes and Phenotypes

PHENOTYPE	GENOTYPE(S)
A	*AA, AO*
B	*BB, BO*
AB	*AB*
O	*OO*

Using Punnett Square to Predict ABO Type

		Mother's genotype	
		A	**O**
Father's genotype	**B**	AB	BO
	O	AO	OO

Frequency of ABO Types

TYPE	WHITES (%)	BLACKS (%)	HISPANICS (%)	ASIANS (%)
O	45	49	57	40
A	40	27	31	27
B	11	19	10	25
AB	4	4	2	7

ABO System

GROUP	ANTIGEN(S) ON RBC	ANTIBODY(S) IN SERUM
O	Neither	Anti-A, Anti-B
A	A	Anti-B
B	B	Anti-A
AB	A & B	Neither anti-A nor anti-B

ABO Typing

FORWARD GROUPING		REVERSE GROUPING		TYPE
ANTI-A	ANTI-B	A CELLS	B CELLS	
0	0	+	+	0
+	0	0	+	A
0	+	+	0	B
+	+	0	0	AB

Use of anti-A,B & A_2 cells optional.

ANTI-A	ANTI-B	A_1 CELLS	B CELLS	POSSIBLE CAUSE*	RESOLUTION
0	0	0	0	Missing isoagglu-tinins in group O	Incubate reverse grouping at RT for 30 min. If still neg, incubate at 4°C for 15–30 min. Include controls: pt RBCs in 4% albumin, pt serum with O cells.
4+	0	1+	4+	A_2 with anti-A_1	Type RBCs with anti-A_1 (*Dolichos biflorus* lectin). Test serum with several additional A_1, A_2, & O cells.
4+	4+	2+	2+	Rouleaux	Use washed RBCs suspended in saline for forward grouping. Perform saline replacement technique in reverse grouping.
4+	4+	2+	2+	AB with cold alloantibody	Perform ab panel at RT. If cold alloantibody identified, repeat reverse grouping with A_1 & B cells that lack corresponding ag.
3+	4+	1+	0	A_2B with anti-A_1	Type cells with anti-A_1 (*Dolichos biflorus* lectin). Test serum with several additional A_1, A_2, & O cells.
4+	2+	0	4+	Acquired B antigen	Check medical history for infection by GI bacteria (some have enzymes that convert A ag to B-like ag.) Retype RBCs with different monoclonal anti-B or acidified human anti-B (pH 6.0; doesn't react with acquired B ag).

*Other explanations may be possible.

Rh Genotypes and Phenotypes

PHENOTYPE	GENOTYPE(S)
Rh positive	*DD, Dd*
Rh negative	*dd*

Using Punnett Square to Predict Rh Type

		Mother's genotype	
		D	**d**
Father's genotype	**d**	Dd	dd
	d	Dd	dd

Rh Antigens

FISHER-RACE	WEINER	ROSENFIELD
D	Rh_0	Rh1
C	rh′	Rh2
E	rh″	Rh3
c	hr′	Rh4
e	hr″	Rh5

Frequency of Rh Antigens

ANTIGEN	WHITES (%)	BLACKS (%)
D	85	92
C	68	27
E	29	22
c	80	96
e	98	98

Note which antigens are the most and least common.

Frequency of Rh Genes

GENE	ANTIGENS	WHITES (%)	BLACKS (%)	ASIANS (%)
Rh⁰ → Rh^0	Dce	4	44	3
Rh^1	DCe	42	17	70
Rh^2	DcE	14	11	21
Rh^z	DCE	≤0.01	≤0.01	1
rh	dce	37	26	3
rh′	dCe	2	2	2
rh″	dcE	1	≤0.01	≤0.01
rh^y	dCE	≤0.01	≤0.01	≤0.01

Note which genes are the most and least common and which show marked racial differences.

Breaking the Rh Code

SEE	THINK	EXAMPLE(S)
r before h	"big"	rh′ = C
h before r	"little"	hr′ = c
R	presence of D	Rh_1 = DCe
r	absence of D	rh′ = dCe
1 or ′	C (if no 1 or ′, then c)	Rh_1 = DCe rh′ = dCe Rh_0 = Dce
2 or ″	E (if no 2 or ″, then e)	Rh_2 = DcE rh″ = dcE Rh_0 = Dce
0	c + e	Rh_0 = Dce
Z or y	C + E	rh^y = dCE

Notes: Italics & superscripts denote genes, e.g., *Rh′*.
Standard type is used for agglutinogens (antigens).
Subscripts are used with R, e.g., Rh_0.
Superscripts are used with r, e.g., rh′.
The h's are left out in shorthand notation, e.g., $Rh_0 = R_0$.

Rh Typing Sera

	HIGH-PROTEIN ANTI-D	LOW-PROTEIN ANTI-D
Source	Prepared from pools of human sera (immunized Rh-neg individuals).	Mixture of monoclonal IgM & monoclonal or polyclonal IgG.
Control	Same ingredients as reagent, except no anti-D. Should be purchased from same manufacturer as anti-D.	Any neg typing rxn serves as control, e.g., neg rxn with anti-A or anti-B. When RBCs react with all antisera (i.e., AB pos), run control recommended by manufacturer. (Usually pt RBCs with autologous serum or 6% or 8% albumin.)
Comments	More false-pos than low-protein reagents, e.g., RBCs with pos DAT.	Most widely used. Lower rate of false pos with Ig-coated RBCs.

Interpretation of Rh Typing

ANTI-D	CONTROL	Rh TYPE
+	0	Pos
0	0	Neg*
+	+	Invalid

*Test for weak D if donor or infant of mother being evaluated for RhIG.

Causes of False Rh Typing Results

FALSE POSITIVE	FALSE NEGATIVE
Warm or cold autoagglutinins	Failure to add reagent
Rouleaux	RBC suspension too heavy
Polyagglutinable RBCs	Resuspending cell button too vigorously
Nonspecific agglutination due to ingredient in reagent, e.g., dye, preservative	Contaminated or incorrect reagent
Contaminated or incorrect reagent	Blocking of ag sites by abs, e.g., severe HDFN due to anti-D

Weak D Testing and Interpretation

When performed	When anti-D & Rh control are neg in Rh typing of donor or infant of mother being evaluated for RhIG.
Reagent	Not all anti-D reagents are appropriate for use. Refer to manufacturer's package insert.
Method	Incubate Rh typing tubes at 37°C for 15–60 min & carry through AHG.*
Interpretation	

ANTI-D	Rh CONTROL	Rh TYPE
Pos	Neg	Rh pos
Pos	Pos	Invalid
Neg	Neg	Rh neg

*RBCs with pos DAT will react in any IAT test.

Selection of Rh Type for Transfusion

RECIPIENT TYPE	Rh TYPE PATIENT CAN RECEIVE
Rh pos	Rh pos or Rh neg
Weak D	Rh pos or Rh neg
Rh neg	Rh neg only, especially women of childbearing age. (If Rh pos must be given in emergency, RhIG can be given to prevent immunization.)

Frequency of Other Selected Blood Group Antigens

ANTIGEN	WHITES (%)	BLACKS (%)
K	9	2
k	99.8	100
Fy^a	68	13
Fy^b	80	23
Jk^a	76	92
Jk^b	74	48
M	79	74
N	70	75
S	55	30
s	90	92
Le^a	22	23
Le^b	72	55
P_1	79	94

Note which antigens are the most and least common and which show marked racial differences.

I System

	I ANTIGEN	i ANTIGEN
Adult cells	Much	Trace
Cord cells	Trace	Much
i adult cells	Trace	Much

Antibody Characteristics

Naturally occurring	ABO, Lewis, P_1, MN, Lu^a
Clinically significant	ABO, Rh, Kell, Duffy, Kidd, SsU
Warm antibodies	Rh, Kell, Duffy, Kidd
Cold antibodies	M, N, P_1
Usually only react in AHG	Kell, Duffy, Kidd
Can react in any phase of testing	Lewis
Detection enhanced by enzyme treatment of test cells	Rh, Lewis, Kidd, P_1
Not detected with enzyme treatment of test cells	M, N, Duffy
Enhanced by acidification	M
Show dosage	Rh other than D, MNS, Duffy, Kidd
Bind complement	I, Kidd, Lewis
Cause in vitro hemolysis	ABO, Lewis, Kidd, Vell, some P_1
Labile in vivo & in vitro	Kidd
Common cause of anamnestic response (delayed tf rxn)	Kidd

continued...

Antibody Characteristics *continued*

Associated with paroxysmal nocturnal hemoglobinuria	Anti-P
Associated with cold agglutinin disease & *Mycoplasma pneumoniae* infections	Anti-I
Associated with infectious mononucleosis	Anti-i

Antigen-Antibody Enhancement

REAGENT	EXPLANATION
Albumin	22% bovine serum albumin. Reduces net neg charge of RBCs, allowing them to come closer together.
Low ionic strength solution (LISS)	Lowers ionic strength of suspending medium, allowing ags & abs to move closer together more rapidly. Reduces incubation time for IAT.
Polythyleneglycol (PEG)	Increases ab uptake. Used for detection & ID of weak IgG abs.
Enzymes	Ficin & papain most commonly used. Reduce RBC surface charge by cleaving sialic acid molecules. M, N, S, Fy^a, Fy^b antigens destroyed.

Antihuman Globulin Serum

TYPE	DETECTS	COMMENTS
Polyspecific (broad spectrum)	IgG & C3d	Used for direct antiglobulin test (DAT). Some labs use for xmatch & ab detection. If pos, monospecific sera used for follow-up testing. Advantage: May detect complement-binding ab more readily. Disadvantage: Rxn due to complement binding by clinically insignificant cold ab.
Monospecific Anti-IgG	IgG	Can be used for routine compatibility tests & ab ID. Detects clinically significant abs.
Anti-C3d or anti-C3b-C3d	Complement	Helpful in investigation of immune hemolytic anemia.

Antiglobulin Testing

	DIRECT (DAT)	INDIRECT (IAT)
Detects	In vivo sensitization of RBCs by IgG ab	In vitro sensitization of RBCs by IgG ab
Specimen	EDTA red cells preferred	Serum, plasma, RBCs
Incubation	None required	Serum or plasma with reagent RBCs or RBCs with reagent antiserum
Application	HDFN, tf rxn, autoimmune hemolytic anemia, drug-induced hemolytic anemia	Ab screen, xmatch, RBC phenotyping, weak D testing
False positives	Complement binding in vitro if RBCs are taken from red-top tube & broad-spectrum AHG used Septicemia Contamination of specimen Wharton's jelly in cord blood Over-reading Overcentrifugation	Cells with pos DAT Overcentrifugation
False negatives	Interruption in testing Contamination, improper storage, or outdating of AHG Failure to add AHG Neutralization of AHG from inadequate washing Dilution of AHG by residual saline Over- or undercentrifugation	Same as DAT, plus over- or underincubation

Antibody Identification

REACTIONS	POSSIBLE EXPLANATION
Same strength & in 1 phase only	Suggestive of single ab
Varying strength	Multiple abs, abs exhibiting dosage, ags of differing strength
In different phases	Combination of warm & cold abs, ab with wide thermal range
All cells in AHG, autocontrol negative	Multiple abs, ab to high frequency ag
All cells in AHG, autocontrol positive	Warm autoantibody
All cells at 37°C, neg in AHG, autocontrol positive	Rouleaux

Rule out (cross out)	1. On row of ags at top of antigram, cross out those that are present on cells that didn't react in any phase. (Some blood bankers only cross out homozygous ags to avoid missing a weak ab that displays dosage.) 2. Circle ags that aren't crossed out. An ab to 1 or more of these is present. 3. Look for matching patterns, e.g., if serum only reacted with cells # 3 & # 5, & E ag is only on cells # 3 & # 5, ab is most likely anti-E. An ab will react with all cells that possess the corresponding ag (except for abs that demonstrate dosage & only react with homozygous cells). An ab won't react with any cells that lack the correspon-ding ag.
Testing with selected cells	If other abs can't be ruled out, further testing with selected cells might be required. Cells selected for testing should be pos for only 1 ag corresponding to abs in question, e.g., if pattern of reactivity matches anti-Jk^a, but anti-K & anti-S can't be ruled out, serum should be tested with cells of the following type: • Jk(a–), K+, S– • Jk(a–), K–, S+ • Jk(a+), K–, S– Homozygous cells are preferred to avoid missing weak abs that demonstrate dosage.
Confirmation	Conclusive ID requires testing sufficient # of cells that are pos & neg for corresponding ag, e.g., 3 pos & 3 neg. Some labs use other combinations that provide same probability (*p*) value of $\leq$0.05 (i.e., 95% confidence level that ab ID is correct). Once ab is identified, type pt RBCs for corresponding ag. Should be neg.

Cold Antibodies

Anti-A_1	Only found in subgroups of A. Agglutinates A_1 & A_1B cells, but not A_2 or O.
Anti-I	Agglutinates all adult cells, except i adult. Doesn't agglutinate cord cells.
Anti-i	Agglutinates cord cells more strongly than adult cells.
Anti-H	Most common in A_1 & A_1B. Agglutinates O cells most strongly, followed by A_2 & B; then A_1 & A_1B.
Anti-IH	Most common in A_1 & A_1B. Agglutinates cells that possess both I & H. Agglutinates adult O cells most strongly. Weaker rxn with A_1 cells. Doesn't agglutinate cord cells.

Compatibility Testing

- Specimen collected within 3 days of tf if pt has been pregnant or transfused in preceding 3 months
- Confirmation of identifying information on request form & specimen
- Check of blood bank records
- Repeat ABO type on donor
- Repeat Rh type on donor if unit is labeled Rh neg (weak D not required)
- ABO type on recipient
- Rh type on recipient (weak D not required)
- Antibody screen on recipient
- Xmatch recipient serum & donor RBCs
- Retain pt specimen & unit segment at 1°–6°C for 7 days after tf

Crossmatches

	EXPLANATION	WHEN PERFORMED	COMMENTS
Antiglobulin xmatch	Recipient serum & donor RBCs tested through AHG	If recipient has or had clinically significant ab	
Immediate spin (IS) xmatch	Recipient serum & donor RBCs tested in IS only	If recipient doesn't have & never had clinically significant ab	Detects ABO incompatibility. Ab screen carried through AHG must be performed & must be neg.
Computer (electronic) xmatch	Computer check of donor ABO & Rh type & recipient ABO & Rh type	If recipient doesn't have & never had clinically significant ab	Detects ABO incompatibility. ABO typing of recipient must be done × 2. Ab screen carried through AHG must be performed & must be neg. Computer system must be validated to prevent release of ABO-incompatible blood & must alert user to discrepancies & incompatibilities.

The Major Crossmatch*

WILL	WILL NOT
Detect ABO incompatibility Detect most abs against donor cells	Detect all ABO typing errors Detect most Rh typing errors Detect all abs Detect platelet or leukocyte antibodies Prevent immunization Ensure normal survival of RBCs

*Recipient serum & donor RBCs.

Examples of Incompatible Crossmatches

REACTIONS	POSSIBLE CAUSE*	RESOLUTION
Neg ab screen, incompatible IS xmatch	ABO incompatibility	Retype donor & recipient. Xmatch with ABO-compatible donor.
1 ab screening cell & 1 donor pos in AHG	Alloantibody	Identify ab. Xmatch units neg for corresponding ag.
Ab screening cells & all donors except 1 neg at 37°C & in AHG. 1 donor pos in AHG only	Positive DAT on donor	Perform DAT on unit. If pos, return to collecting facility.
Ab screening cells, donors, & autocontrol pos in AHG	Warm autoantibody	Best not to tf. If unavoidable, find "least incompatible" unit.
Ab screening cells, donors, & autocontrol pos at 37°C, neg in AHG	Rouleaux	Saline replacement technique.

*Other causes are possible.

Transfusion of Non-Group-Specific RBCs

PATIENT TYPE	RBC TYPE PATIENT CAN RECEIVE	PLASMA TYPE PATIENT CAN RECEIVE
O	O only	O, A, B, AB
A	A, O	A, AB
B	B, O	B, AB
AB	AB, A, B, O	AB

Pretransfusion Testing

COMPONENT	CROSSMATCH?	OTHER
RBCs	Yes	Can be computer xmatch if neg ab screen & no record of previously detected abs.
Plasma	No	Must be ABO compatible with recipient RBCs.
Cryoprecipitate	No	All ABO groups acceptable. Rh type not considered.
Platelets	No*	Any ABO group acceptable. Compatible with recipient RBCs recommended. ABO-identical preferred.

*For apheresis platelets containing >2 mL RBCs, ABO compatibility & xmatch required.

Conditions for Reissue of RBCs

- Maintained at 1°–10°C. (RBCs at RT reach 10°C in 30 min.)
- Closure wasn't broken.
- At least 1 sealed segment remains attached to unit.
- Unit is inspected before release.
- Records indicate that blood has been inspected & is acceptable for reissue.

Emergency Transfusions

- If time allows for typing, give ABO & Rh compatible; otherwise give O-neg RBCs.
- Label must indicate that xmatch wasn't completed.
- Physician must sign emergency release.
- Routine testing must be completed & physician notified immediately of any incompatibility.

Transfusion-Associated Infections

INFECTION	PREVENTION	COMMENTS
HIV	Donor testing	Estimated risk: 1 in 1,467,000 transfusions. With NAT, window period 9 days.
Hepatitis B	Donor testing	Estimated risk: 1 in 280,000 transfusions.
Hepatitis C	Donor testing	Estimated risk: 1 in 1,149,000 transfusions. With NAT, window period 7.4 days.
HTLV-I and -II	Donor testing	Rare in U.S.
Syphilis	Donor testing	Limited risk in refrigerated or frozen components. Spirochetes can't survive in cold. Highest risk from platelets.
Malaria	Donor history	*Plasmodium* is transmitted in RBCs.
Babesiosis	Donor history	*Babesia* is transmitted in RBCs.
Chagas' disease	Donor history	*Trypanosoma cruzi* is transmitted in blood. Potential risk in donors from Central & S. America.
Cytomegalovirus	Selected donor testing (not routine). Use of CMV-neg or leukoreduced components for pts at risk.	CMV transmitted in WBCs. Risk to CMV-neg immunocompromised pts & premature infants of CMV-neg mothers.

continued...

Transfusion-Associated Infections *continued*

INFECTION	PREVENTION	COMMENTS
Sepsis	Donor screening (history, temp), aseptic technique, use of diversion pouches (1st 30–45 mL of blood goes into pouch so that skin plug doesn't enter unit), tests to detect bacterial contamination of platelets (e.g., culturing 24 hr after collection)	Highest risk from platelets because of RT storage.

Acute Immunologic Transfusion Reactions*

TYPE	CLINICAL SIGNS	CAUSE	LABORATORY FINDINGS	OTHER
Hemolytic, intravascular	Fever; chills; shock; renal failure; DIC; pain in chest, back, or flank	Immediate destruction of donor RBCs by recipient ab	In post-tf specimens: HGB in urine & serum; mixed-field DAT (unless donor cells are all destroyed); ↓ haptoglobin, HGB, & HCT	Most serious rxn. May be fatal. Usually due to tf of ABO-incompatible blood.
Febrile	Temp ↑ ≥1°C or 2°F during or shortly after tf, with no other explanation	Anti-leukocyte abs or cytokines	None	Common. Most often in multiply transfused pts or women with multiple pregnancies. Future tfs should be with leukoreduced components. Antipyretics (aspirin, acetaminophen) can be used to premedicate.
Allergic	Hives (urticaria), wheezing	Foreign plasma proteins	None	Common. Treat with antihistamines. Tf rxn investigation not required.
Anaphylactic	Pulmonary edema, bronchospasms	Anti-IgA in IgA-deficient recipient	None	Rare, but dangerous. Treated with epinephrine. Transfuse with washed products.

continued...

Acute Immunologic Transfusion Reactions* *continued*

TYPE	CLINICAL SIGNS	CAUSE	LABORATORY FINDINGS	OTHER
Transfusion-related acute lung injury (TRALI)	Fever, chills, coughing, respiratory distress, fluid in lungs, ↓ BP within 6 hr of tf. Life-threatening.	Unknown. Possibly donor abs to WBC ags	None	Most common cause of tf-related deaths in U.S. Related to infusion of plasma. All components have been implicated. Reduced use of plasma from female donors appears to be reducing TRALI fatalities.

*Occurring <24 hr after tf.

Acute Nonimmunologic Transfusion Reactions

TYPE	CLINICAL SIGNS	CAUSE	LABORATORY FINDINGS	OTHER
Sepsis	Fever, chills, ↓ BP, cramps, diarrhea, vomiting, muscle pain, DIC, shock, renal failure	Bacterial contamination	Pos Gram stain & culture on unit	
Transfusion-associated circulatory overload (TACO)	Coughing, cyanosis, difficulty breathing, pulmonary edema	Too large a volume or too rapid rate of infusion	None	Problem in children, cardiac & pulmonary patients, elderly, & those with chronic anemia
Nonimmune hemolysis	Variable	Destruction of RBCs due to extremes of temp, addition of meds to unit	Hemoglobinuria, hemoglobinemia	
Hypothermia	Cardiac arrhythmia	Rapid infusion of large amounts of cold blood	None	Use blood warmer for rapid infusions

Delayed Transfusion Reactions*

TYPE	CLINICAL SIGNS	CAUSE	LABORATORY FINDINGS	OTHER
Immunologic Hemolytic, extravascular	Fever, anemia, mild jaundice 2–14 days after tf.	Donor RBCs sensitized by recipient IgG ab & removed from circulation.	↑ bili, mixed-field DAT, ↓ haptoglobin, ↓ HGB & HCT, pos ab screen	May be due to anamnestic response. Kidd antibodies most common cause. Usually not life-threatening.
Alloimmunization	None, unless subsequently exposed to same foreign ags.	Development of abs to foreign RBCs, WBCs, platelets, plasma proteins following tf.	Ab to RBCs detected in ab screen. Others require specialized testing.	Use leukoreduced components for pts with WBC abs.
Transfusion-associated graft-vs.-host disease (TA-GVHD)	Rash, nausea, vomiting, diarrhea, fever, pancytopenia. Usually fatal.	Viable T lymphs in donor blood attack recipient.	None	Irradiate components for premature infants, intrauterine or exchange transfusion, stem cell or bone marrow transplants, recipients of blood from a 1st-degree relative, immunocompromised, pts with leukemia or lymphoma.

continued...

Delayed Transfusion Reactions* *continued*

TYPE	CLINICAL SIGNS	CAUSE	LABORATORY FINDINGS	OTHER
Nonimmunologic				
Iron overload	Diabetes, cirrhosis, cardiomyopathy	Build up of iron in body.	↑ serum ferritin	Problem for pts receiving repeated tf over long period of time, e.g., pts with thalassemia, sickle cell anemia, other chronic anemias.

*Occurring >24 hr after tf.

Signs & symptoms of possible transfusion reaction	Fever; chills; respiratory distress; hyper- or hypotension; back, flank, chest, or abdominal pain; pain at site of infusion; hives (urticaria)*; jaundice; hemoglobinuria; nausea/vomiting; abnormal bleeding; oliguria/anuria.
Specimens needed	Pre-tf blood. Post-tf blood. Post-tf urine. Segment from unit. Blood bag with administration set & attached IV solutions.
Immediate steps	Stop tf. Check all IDs & labels. Repeat ABO on post-tf sample. Visual check of pre- & post-tf samples for hemolysis. DAT on post-tf sample. If pos, perform on pre-tf sample.
Signs of hemolytic reaction	Hemolysis in post-tf sample, but not in pre-tf sample. Mixed field agglutination in DAT on post-tf sample, but not on pre-tf sample.
Further steps if signs of possible hemolytic reaction	Check HGB in first voided urine after tf. Repeat ABO & Rh on pre- & post-tf samples & unit. Repeat antibody screen on pre- & post-tf samples. AHG xmatch with pre- & post-tf samples.

continued...

Additional tests that may be performed	Haptoglobin (↓ with hemolysis). Gram stain & culture of unit. Bilirubin 5–7 hr after tf (sign of extravascular hemolysis). BUN & creatinine (sign of renal involvement).
Reporting of transfusion-related fatalities	Must be reported to FDA Center for Biologics Evaluation & Research (CBER) by phone or e-mail ASAP.

*If symptoms dissipate after treatment with antihistamines, tf may be resumed, & investigation isn't required.

Testing of Neonates*

	SPECIMEN	COMMENTS
ABO & Rh	Cord blood, capillary, or venous blood	ABO forward grouping only. Only required once per admission.
Antibody screen	Serum or plasma of mother or baby	Only required once per admission
Crossmatch	Serum or plasma of mother or baby	If ab screen pos, perform AHG xmatch on units neg for corresponding ag. If ab screen neg, xmatch not required.

*Younger than 4 months.

Hemolytic Disease of the Fetus and Newborn (HDFN)

	ABO	Rh
Mothers at risk	Usually group O	Rh neg
First child affected?	Yes	Not usually
Frequency	Common	Uncommon
Severity	Mild	Can be severe
DAT*	Weak pos or neg	Strong pos
Spherocytes?	Yes	Rare
Predictable?	No	Yes (maternal ab screen)
Preventable?	No	Yes (RhIG)

*Strength of rxn doesn't correlate with severity of disease.

Rh Immune Globulin (RhIG) Workup

Prenatal evaluation	ABO & Rh (weak D not required). If Rh pos, woman isn't candidate for RhIG. Ab screen. (Don't use IS or RT incubation or AHG with anticomplement activity.) If pos, ID antibody. If anti-D present, woman isn't candidate.
Postpartum evaluation	ABO & Rh, including weak D, on baby. If baby is Rh neg, mother isn't candidate. If baby is Rh pos, draw mother's blood after delivery & perform rosette test to screen for large fetal bleed. Mother's RBCs incubated with anti-D. Anti-D coats fetal D-pos RBCs. Indicator D-pos RBCs added. Attach to anti-D on fetal D-pos RBCs, forming rosettes. If rosette test pos, quantitate fetal bleed by flow cytometry or Kleihauer-Betke acid-elution test. Fetal cells resist acid elution; stain pink. Adult cells lose hgb; appear as "ghosts."

Rh Immune Globulin (RhIG)

Composition	Anti-D derived from pools of human plasma
Purpose	Prevent immunization to D
Administration	Antepartum: To Rh neg woman at 28 wk of gestation Postpartum: Within 72 hr of delivery when Rh-neg woman delivers Rh-pos baby Other obstetric events: To Rh-neg woman after spontaneous or therapeutic abortion, ectopic pregnancy, amniocentesis, chorionic villus sampling, antepartum hemorrhage, or fetal death Note: May also be administered to Rh-neg recipients of Rh-pos blood or components
Dose	1 dose per 15 mL of D-pos fetal RBCs (30 mL of fetal WB). In calculating dose, if # to right of decimal point is ≥0.5, round up to next whole # & add 1 vial, e.g., 1.6 vials calculated = 2 + 1 = 3. If # to right of decimal point is <0.5, don't round up; just use whole # & add 1 vial, e.g., 1.4 vials calculated = 1 + 1 = 2.

Examples of Equipment/Reagent Quality Control

Blood storage refrigerators & freezers, platelet incubators	System for continuous temp monitoring & audible alarm.
Temperature recorder	Compare against thermometer daily. Calibrate as necessary.
Alarms	Check high & low temp of activation quarterly.
Waterbaths	Check temp daily.
Heat blocks	Check temp daily. Periodically check each well.
Centrifuges	Determine optimum speed & time for different procedures upon receipt, after repairs, & periodically. Check timer every 3 mo, RPM every 6 mo (with tachometer).
Cell washers	Check tube fill level daily, AHG volume monthly. Verify time & speed quarterly.
Pipettes	Calibrate quarterly.
Antisera	Test with pos & neg controls each day of use. Use heterozygous cells for pos controls.
Reagent cells	Check for hemolysis. Test each day of use with pos & neg controls.
Antihuman globulin	Check anti-IgG activity each day of use by testing Rh-pos cells sensitized with anti-D.

Not all inclusive. Follow manufacturer's guidelines & lab's Standard Operating Procedures Manual.

SECTION

7

Urinalysis and Body Fluids Review

Urine Specimens

TYPE	USE	COLLECTION	COMMENTS
Random	Routine urinalysis (UA)	Anytime	Not ideal since urine may be dilute & contaminated.
First am	Routine UA	Upon awakening	Best for screening. Most concentrated.
2-hr postprandial	Diabetes mellitus monitoring	2 hr after eating	Best for detecting glycosuria.
24-hr	Quantitative chemical tests	Discard 1st void on day 1 & note time. Collect all urine for next 24 hr, including first void at same time on day 2	Improper collection is common source of error. Refrigerate or keep on ice. Preservatives required for some tests.
Clean catch	Routine, culture	Cleanse external genitalia & collect midstream in sterile container	Less contamination. Do culture before UA.
Catheterized	Culture	Catheter inserted into urethra	Avoids contamination.
Suprapubic aspiration	Culture	Needle inserted through abdomen into bladder	Avoids contamination.

Urine Volume

Normal daily volume	600–2,000 mL (average 1,200–1,500mL)
Normal day-to-night ratio	2:1–3:1
Diuresis	↑ urine production
Polyuria	Marked ↑ in urine flow Adult: >2,500 mL/day Children: 2.5–3 mL/kg/day
Oliguria	Marked ↓ in urine flow Adult: <400 mL/day Children: <0.5 mL/kg/hr Infants: <1 mL/kg/hr
Anuria	No urine production

Note: Urine volume ↑ with diabetes mellitus (solute diuresis) & diabetes insipidus (lack of ADH).

Urine Color

Normal	Yellow due to urochrome
Dilute urine	Colorless, pale yellow
Concentrated urine	Dark yellow, amber
Bilirubin	Amber, orange, yellow-green; yellow foam on shaking
Urobilin	Amber, orange; no yellow foam on shaking
Homogentisic acid	Normal on voiding; brown or black on standing
Melanin	Brown or black on standing
Methemoglobin	Brown or black
Myoglobin	Red; brown on standing
Blood/hemoglobin	Pink or red when fresh; brown on standing
Porphyrin	Port-wine
Drugs, medications, food	Green, blue, red, orange
***Pseudomonas* infection**	Green, blue-green

Changes in Unpreserved Urine at Room Temperature >2 hr

	CHANGE	CAUSE
Turbidity	↑	Multiplication of bacteria, precipitation of amorphous crystals
pH	↑	Conversion of urea to ammonia by bacteria
Glucose	↓	Metabolism by bacteria
Ketones	↓	Volatilization of acetone, breakdown of acetoacetate by bacteria
Bilirubin	↓	Oxidation to biliverdin
Urobilinogen	↓	Oxidation to urobilin
WBCs, RBCs, casts	↓	Lysis in dilute or alkaline urine

Chemical Urinalysis by Reagent Strip

TEST	NORMAL	PRINCIPLE	SIGNIFICANCE	COMMENTS
pH	First am: 5–6 Random: 4.5–8	Double indicator system	Acid-base balance, management of urinary tract infection (UTI)/renal calculi	Acid with protein/meat diet; alkaline with vegetarian diet. pH 9 = improperly preserved specimen.
Protein	Neg–trace	Protein error of indicator	Possible renal disease	Buffered to pH 3. Most sensitive to albumin. Orthostatic proteinuria—benign condition, protein is neg in 1st am specimen, pos after standing.
Glucose	Neg	Glucose oxidase/peroxidase	Possible diabetes mellitus	Specific for glucose. More sensitive & specific than copper reduction. Normal renal threshold = 160–180 mg/dL.
Ketones	Neg	Sodium nitroprusside rxn	↑ fat metabolism (uncontrolled diabetes mellitus, vomiting, starvation, low carb diet, strenuous exercise)	Most sensitive to acetoacetic acid. Less sensitive to acetone. Doesn't react with beta-hydroxybutyric acid.
Blood	Neg	Pseudoperoxidase activity of hgb	Hematuria, hemoglobinuria, myoglobinuria	Uniform color = hgb or myoglobin. Speckled = RBCs.

continued...

Chemical Urinalysis by Reagent Strip *continued*

TEST	NORMAL	PRINCIPLE	SIGNIFICANCE	COMMENTS
Bilirubin	Neg	Diazo reaction	Liver disease, biliary obstruction	Only conjugated bilirubin is excreted in urine.
Urobilinogen	1 Ehrlich unit or 1 mg/dL	Ehrlich's aldehyde rxn or diazo rxn	Liver disease, hemolytic disorders	Reagent strips don't detect absence of urobilinogen, only ↑.
Nitrite	Neg	Greiss reaction	UTI	Some bacteria reduce nitrates to nitrites. 1st am specimen best. ↑ sensitivity when urine in bladder at least 4 hr.
Leukocyte esterase	Neg	Leukocyte esterase rxn	UTI	Longest rxn time. Detects intact & lysed grans & monos, not lymphs. Can be used with nitrite to screen urines for culture.
Specific gravity (SG)	Random specimen: 1.003–1.030	pKa change of polyelectrolyte	Indication of kidney's concentrating ability & state of hydration. ↑ in diabetes mellitus due to glucose. ↓ in diabetes insipidus due to ↓ ADH	Only measures ionic solute. Not affected by urea, glucose, radiographic contrast media, plasma expanders. Not always same as SG by refractometer.

General Sources of Error with Reagent Strip Testing

	POSSIBLE EFFECT	COMMENTS
Failure to test within 2 hr of collection or to preserve correctly	Changes in chemical composition	
Failure to bring refrigerated specimens to RT before testing	False-neg enzymatic rxns	
Failure to mix specimen well	False-neg leukocyte, blood	WBCs, RBCs settle out.
Failure to follow manufacturer's instructions	Erroneous results	
Failure to dip all test pads in urine	False-neg rxns	
Prolonged dipping	False-neg rxns	Reagents may leach from pads.
Failure to remove excess urine from strip	Runover of chemicals to adjacent pads, distortion of colors	
Failure to read at recommended time	Erroneous results	
Failure to compare to color chart or read in good light	Erroneous results	
Failure to store strips properly	Erroneous results	Store in capped original container at RT.

continued...

General Sources of Error with Reagent Strip Testing *continued*

	POSSIBLE EFFECT	COMMENTS
Failure to perform quality control	Erroneous results	Run pos & neg control every 24 hr & when opening new container.
Expired strips	Erroneous results	
Highly pigmented urine	Atypical colors, false-pos rxns	Pigment masks true rxns. Test by alternate method.

Specific Sources of Error with Reagent Strip Testing

	INCREASED OR FALSE POSITIVE	DECREASED OR FALSE NEGATIVE
pH	Improperly preserved specimen	Acid runover from protein square.
Protein	Highly buffered alkaline urine, prolonged dipping, contaminated container, ↑SG	Proteins other than albumin.
Glucose	Contamination with peroxide or bleach	Unpreserved specimen, ↑ascorbic acid, ↑SG, ↓temp.
Ketones	Red pigments, dyes, some meds	Improper storage. Acetone is volatile. Bacteria break down acetoacetic acid.
Blood	Menstruation, oxidizing agents, bacterial peroxidase	↑ascorbic acid, ↑nitrite, ↑SG (crenated RBCs), unmixed specimen.
Bilirubin	Highly pigmented urine	Exposure to light, ↑ascorbic acid, ↑nitrite.
Urobilinogen	Highly pigmented urine	Improperly preserved specimen (oxidation to urobilin), formalin.
Nitrite	Highly pigmented urine, improperly preserved specimen (contaminating bacteria produce nitrites)	Non-nitrate-reducing bacteria, inadequate time in bladder, reduction of nitrites to N_2, ↓dietary nitrate, antibiotics, ↑ascorbic acid, ↑SG.

continued...

Specific Sources of Error with Reagent Strip Testing *continued*

	INCREASED OR FALSE POSITIVE	DECREASED OR FALSE NEGATIVE
Leukocyte esterase	Highly pigmented urine, oxidizing agents, formalin, nitrofurantoin, vaginal discharge	↑glucose, ↑protein, ↑ascorbic acid, ↑SG; antibiotics; reading too soon.
Specific gravity	↑ protein	Alkaline urine. (Add 0.005 if pH is 6.5 or higher. Correction is made by automated readers.)

Sources of error vary with brand of reagent strip. Refer to manufacturer's package insert.

Other Urine Chemistry Tests

TEST	DETECTS	METHOD(S)	SOURCES OF ERROR	COMMENTS
Microalbumin	Albumin in low concentration	Immunoassay on 24-hr urine or albumin-to-creatinine ratio (ACR) on random sample. Dipsticks available for ACR.		Not detected by most urine dipsticks. 50-200 mg/24 hr or ACR ≥2.8 for males, ≥2.0 for females predictive of diabetic nephropathy. Strict control of glucose & blood pressure can prevent progression to end-stage renal disease.
Sulfosalicylic acid (SSA)	All proteins, including Bence Jones proteins	Acid precipitation	False pos: radiographic dyes, tolbutamide, some antibiotics, turbid urine False neg: highly buffered alkaline urine	Centrifuge & test supernatant. No longer used to confirm pos protein by dipstick.
Clinitest	Reducing substances	Copper reduction	False pos: ↑ascorbic acid False neg: "pass through" (Due to ↑ concentration. Goes through orange, returns to green. Repeat using 2-drop method)	Watch rxn to avoid missing pass through. Not as sensitive or specific for glucose as reagent strip. Reacts with other reducing sugars. Used to detect galactosemia in children <2 yr.

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Other Urine Chemistry Tests *continued*

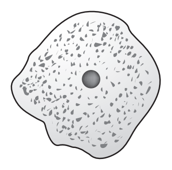
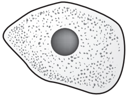
TEST	DETECTS	METHOD(S)	SOURCES OF ERROR	COMMENTS
Acetest	Ketones	Sodium nitroprusside reaction	False neg: Improperly stored specimen (Acetone is lost into air if sample is left at RT. Bacteria break down acetoacetic acid.)	Most sensitive to acetoacetic acid. Can be used on urine, serum, plasma.
Ictotest	Bilirubin	Diazo reaction	False pos: Urine pigments False neg: Exposure to light, improperly stored specimen, ↑ascorbic acid, ↑nitrite	More sensitive than reagent strip. Less affected by interfering substances.

Glucose Oxidase Versus Copper Reduction

GLUCOSE OXIDASE (REAGENT STRIP)	COPPER REDUCTION (CLINITEST)	MOST LIKELY INTERPRETATION*
+	+	Glucose
+	0	Glucose below sensitivity of Clinitest
0	+	Non-glucose-reducing substance
0	0	No glucose or other reducing substances

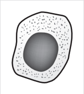
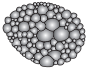
*Defective reagents and presence of interfering substances should be ruled out.

Epithelial Cells in the Urine Sediment

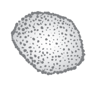
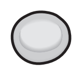
CELL	DESCRIPTION	ORIGIN	CLINICAL SIGNIFICANCE	COMMENTS
Squamous epithelial cell 	40–50 µm. Flat. Prominent round nucleus.	Lower urethra, vagina	Usually none	↑ numbers usually seen in urine from females. May obscure RBCs & WBCs. Reduced by collecting midstream clean-catch specimen.
Transitional epithelial cell 	20–30µm. Spherical, pear-shaped, or polyhedral. Round central nucleus.	Renal pelvis, ureters, bladder, upper urethra	Seldom significant	May form syncytia (clumps)

continued...

Epithelial Cells in the Urine Sediment *continued*

CELL	DESCRIPTION	ORIGIN	CLINICAL SIGNIFICANCE	COMMENTS
Renal tubular epithelial cell 	Slightly larger than a WBC (12 μm). Round. Eccentric round nucleus.	Renal tubules	Tubular necrosis, toxins, viral infections, renal rejection	Add 2% acetic acid to visualize nucleus & differentiate from WBC
Oval fat body 	Renal tubular epithelial cell containing fat droplets.	Renal tubules	Same as renal tubular epithelial cells	Maltese crosses with polarized light

Blood Cells in the Urine Sediment

CELL	DESCRIPTION	ORIGIN	CLINICAL SIGNIFICANCE	COMMENTS
White blood cell (WBC) 	Usually polys. About 12 µm. Granular appearance.	Kidney, bladder, or urethra	Cystitis, pyelonephritis, tumors, renal calculi.	Normal: 0–8/HPF. Clumps of WBCs associated with acute infection.
Glitter cell	WBC with Brownian movement of granules. Stain faintly or not at all.	Same as WBC	Same as WBC.	Seen in hypotonic urine.
Red blood cell (RBC) 	Biconcave disk, about 7 µm. Smooth. Non-nucleated.	Kidney, bladder, or urethra	Infection, trauma, tumors, renal calculi. Dysmorphic RBCs indicate glomerular bleeding.	Normal: 0–3/HPF. Crenate in hypertonic urine. Lyse in hypotonic urine & with 2% acetic acid.

Normal Crystals Found in Acid or Neutral Urine

CRYSTAL	DESCRIPTION	COMMENTS
Amorphous urates 	Irregular granules.	Form pink precipitate in bottom of tube. May obscure significant sediment. Dissolve by warming to 60°C.
Uric acid 	Pleomorphic. 4-sided, 6-sided, star-shaped, rosettes, spears, plates. Colorless, red-brown, or yellow.	Birefringent. Polarizes light.
Calcium oxalate 	Octahedral (8-sided) envelope form is most common. Also dumbbell & ovoid forms.	Occasionally found in slightly alk urine. Monohydrate form may be mistaken for RBCs. Most common constituent of renal calculi. From oxalate-rich foods.

From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:113.

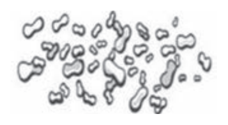
Normal Crystals Found in Alkaline Urine

CRYSTAL	DESCRIPTION	COMMENTS
Amorphous phosphates 	Irregular granules	Form white precipitate in bottom of tube. Dissolve with 2% acetic acid.
Triple phosphate 	"Coffin-lid" crystal	
Ammonium biurate 	Yellow-brown "thorn apples" & spheres	Seen in old specimens.

From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:113.

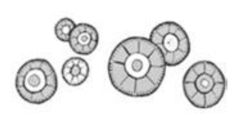
continued...

Normal Crystals Found in Alkaline Urine *continued*

CRYSTAL	DESCRIPTION	COMMENTS
Calcium phosphate 	Needles, rosettes, "pointing finger"	Only needle form seen in alkaline urine.
Calcium carbonate 	Colorless dumbbells or aggregates	

From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:113.

Abnormal Crystals*

CRYSTAL	DESCRIPTION	SIGNIFICANCE	COMMENTS
Leucine 	Yellow, oily-looking spheres. Radial & concentric striations.	Severe liver disease	Often seen with tyrosine.
Tyrosine 	Fine yellow needles in sheaves or rosettes.	Severe liver disease	Often seen with leucine.
Cystine 	Hexagonal (6-sided).	Cystinuria	Must differentiate from uric acid. Doesn't polarize light. Confirm by cyanide-nitroprusside test.

From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:117.

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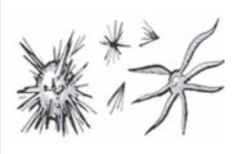
Abnormal Crystals* *continued*

CRYSTAL	DESCRIPTION	SIGNIFICANCE	COMMENTS
Cholesterol 	Flat plates. Notched out corners. "Stair-steps."	Nephrotic syndrome	Birefringent.
Bilirubin 	Yellowish brown needles, plates, granules.	Liver disease	Chemical tests for bilirubin should be pos.

*Abnormal crystals are found in acid or neutral urine.

From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:117.

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Casts

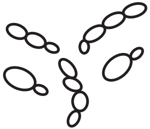
TYPE	DESCRIPTION	SIGNIFICANCE	COMMENTS
Hyaline	Homogeneous with parallel sides, rounded ends.	Normal: 0–2/LPF. ↑ with stress, fever, trauma, exercise, renal disease.	Most common type. Least significant. Tamm-Horsfall protein only. Dissolve in alk urine. Same refractive index as urine; may be overlooked with bright light.
Granular	Same as hyaline, but with granules.	Normal: 0–1/LPF. ↑ with stress, exercise, glomerulonephritis, pyelonephritis.	From disintegration of cellular casts.
RBC	RBCs in cast matrix. Yellowish to orange color.	Acute glomerulonephritis, strenuous exercise.	IDs kidneys as source of bleeding. Most fragile cast. Often in fragments.
Blood	Contain hemoglobin. Yellowish to orange color.	Same as RBC cast.	From disintegration of RBC casts.
WBC	WBCs in cast matrix. Irregular in shape.	Pyelonephritis.	IDs kidneys as site of infection.
Epithelial cell	Renal tubular epithelial cells in cast matrix.	Renal tubular damage.	Transitional & squamous epithelial cell aren't seen in casts. Distal to renal tubules & collecting ducts where casts are formed.

continued...

Casts *continued*

TYPE	DESCRIPTION	SIGNIFICANCE	COMMENTS
Waxy	Homogeneous, opaque, notched edges, broken ends.	Urinary stasis.	From degeneration of cellular & granular casts. Unfavorable sign.
Fatty	Casts containing lipid droplets.	Nephrotic syndrome.	Maltese crosses with polarized light if lipid is cholesterol. Sudan III & Oil Red O stain triglycerides & neutral fats.
Broad	Wide. May be cellular, granular, or waxy.	Advanced renal disease.	Formed in dilated distal tubules & collecting ducts. "Renal failure casts."

Miscellaneous Urine Sediment

STRUCTURE	DESCRIPTION	SIGNIFICANCE	COMMENTS
Bacteria	Rods, cocci.	UTI or contaminants.	If clinically significant, WBCs present (unless patient is neutropenic).
Yeast 	5–7 µm. Ovoid, colorless, smooth, refractile. May bud & form pseudohyphae.	Usually due to vaginal or fecal contamination. May be due to kidney infection. Often in urine of diabetics.	Add 2% acetic acid to differentiate from RBCs. RBCs lyse; yeast don't. Pseudohyphae indicate severe infection. WBCs are present in true yeast infections.
Sperm	4–6 µm head, 40–60 µm tail.	Usually not significant in adult. May be sign of sexual abuse in child.	

continued...

Miscellaneous Urine Sediment *continued*

STRUCTURE	DESCRIPTION	SIGNIFICANCE	COMMENTS
Trichomonas	Resembles WBC. Flagella & undulating membrane. Rapid, jerky, nondirectional motility.	Parasitic infection of genital tract.	More common in females. Don't report unless motile.
Mucus	Transparent, long, thin, ribbon-like structure with tapering ends.	None.	May be mistaken for hyaline casts.

Renal Disorders

DISORDER	CAUSE	REAGENT STRIP	SEDIMENT	OTHER
Acute glomerulonephritis	Inflammation & damage to glomeruli	Protein, blood	RBCs (some dysmorphic), WBCs, hgb casts.	Frequently follows untreated group A strep infection
Nephrotic syndrome	Increased glomerular permeability	Protein (large amount)	Casts (all kinds), free fat & oval fat bodies.	Hypoproteinemia, hyperlipidemia
Pyelonephritis	Kidney infection	Protein, leukocyte esterase, nitrite	WBCs, WBC casts, bacteria.	
Cystitis	Bladder infection	Leukocyte esterase, nitrite	WBCs, bacteria, possibly RBCs. No casts.	

Urinalysis Correlations

PARAMETERS	RELATIONSHIPS/CONSIDERATIONS
pH/microscopic	Ck pH when identifying crystals. RBCs, WBCs, & casts lyse at alk pH.
pH/nitrite/leukocyte esterase (LE)/microscopic	With bacterial UTI, usually have pos nitrite, pos LE. Ck for bacteria & WBCs. Bacteria convert urea to ammonia, ↑ pH.
Protein/microscopic	Protein may indicate renal disease. Ck for casts.
Protein/blood/microscopic	Large amounts of blood or myoglobin can cause pos protein. Ck for RBCs. Present with hematuria, not with hemoglobinuria or myoglobinuria.
Protein/specific gravity (SG)	↑SG can cause false-pos trace protein. Trace protein more significant in dilute urine.
Glucose/ketones	Ketones present with uncontrolled diabetes mellitus.
Glucose/microscopic	Yeast thrives in ↑glucose. WBCs should be present if true yeast infection.
Glucose/protein/microscopic	Renal disease is common complication of diabetes mellitus. Ck for casts.
Blood/microscopic	Pos blood, no RBCs: Blood rxn could be due to hemoglobin or myoglobin, or false pos due to bacterial peroxidase. Ck for bacteria. Neg blood, RBCs seen: Could be false-neg blood due to ↑ascorbic acid. Yeast, or monohydrate calcium oxalate crystals could be misidentified as RBCs.

continued...

Urinalysis Correlations *continued*

PARAMETERS	RELATIONSHIPS/CONSIDERATIONS
Bilirubin/urobilinogen	Liver disease: bili pos or neg, urobili ↑. Biliary obstruction: bili pos, urobili N. Hemolytic disorder: bili neg, urobili ↑.
LE/microscopic	Can have pos LE without WBCs (WBCs lysed). Can have pos LE & WBCs without bacteria (*Trichomonas*).
Nitrite/LE/microscopic	With pos nitrite, usually have pos LE, WBCs, & bacteria. Can have pos LE, WBCs, bacteria, & neg nitrite (non-nitrate-reducing bacteria).
SG/microscopic	RBCs & WBCs lyse in dilute urine. With ↑SG, RBCs & WBCs may crenate, cause false-neg blood & LE.

Cerebrospinal Fluid

	NORMAL	ABNORMALITIES	COMMENTS
Color	Colorless	Xanthochromia = slight pink, orange, or yellow due to oxyhemoglobin or bilirubin. Seen with subarachnoid hemorrhage. Traumatic tap = red or pink, decreasing from tube 1–3.	Examine within 1 hr of collection to avoid false pos from lysis of RBCs. Centrifuge CSF & examine against white background.
Clarity	Clear	Cloudy with infection or bleeding.	
WBC	Adult: 0–5/µL Newborn: 0–30/µL (mononuclear)	↑ in meningitis.	Cells begin to lyse within 1 hr. Perform cell count within 30 min of collection. Traditionally performed in hemacytometer, following lysis of RBCs with 3% glacial acetic acid. Can be performed on automated analyzers of sufficient sensitivity. Differential on stained smear following concentration (cytocentrifugation).
RBC	0	↑ with subarachnoid hemorrhage, traumatic tap.	Limited diagnostic value.
Glucose	60%–70% of blood glucose	↓ in bacterial meningitis.	Blood glucose method.

continued...

Cerebrospinal Fluid *continued*

	NORMAL	ABNORMALITIES	COMMENTS
Protein	15–45 mg/dL	↑ in meningitis & with traumatic tap.	Biuret method not sensitive enough. Use trichloracetic acid.
Cells	Lymphs, monos, ependymal cells, choroid plexus cells	Siderophages with subarachnoid hemorrhage. Blasts with leukemia.	Nucleated RBCs may be seen due to bone marrow contamination. Cartilage cells may be seen.

Differential Diagnosis of Meningitis

	BACTERIAL	VIRAL	MYCOBACTERIAL	FUNGAL
WBC	↑	↑	↑	↑
Differential	Polys	Lymphs	Lymphs, monos	Lymphs, monos
Protein	↑	↑	↑	↑
Glucose	↑	N	↑	N or ↑
Lactate	↑	N	↑	↑
Other	Pos Gram stain, bacterial antigen		Weblike clot or pellicle	Pos India Ink and/or latex agglutination test with *Cryptococcus neoformans*

Body Fluids

TERM	DEFINITION
Effusion	Abnormal accumulation of fluid in body cavity. Classified as transudate or exudate.
Serous fluid	Fluid contained in pericardial, peritoneal, & pleural cavities.
Pericardial fluid (pericardiocentesis fluid)	Fluid surrounding heart.
Peritoneal fluid (abdominal fluid, ascitic fluid)	Fluid in abdominal cavity.
Pleural fluid (chest fluid, thoracentesis fluid, empyema fluid)	Fluid surrounding lungs.
Synovial fluid (joint fluid)	Fluid in joints.

Differentiation of Transudates and Exudates

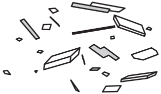
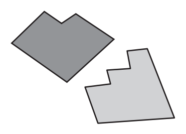
	TRANSUDATE	EXUDATE
Etiology	Systemic disorder affecting fluid filtration & reabsorption (congestive heart failure, hypoalbuminemia, cirrhosis). Problem originating outside body cavity.	Condition involving membranes within body cavity (infection, malignancy, inflammation, hemorrhage)
Type of process	Noninflammatory	Inflammatory
Color	Colorless	Yellow, brown, red, green
Clarity	Clear	Cloudy
Specific gravity	<1.015	>1.015
Protein	<3 g/dL	>3 g/dL
Fluid-to-serum protein ratio	<0.5	>0.5
Glucose	Equal to serum level	30 mg or more < than serum level
Spontaneous clotting	No	Yes
LD	<60% of serum	>60% of serum
WBC	<1,000/µL	>1,000/µL
Differential	Predominantly mononuclears	Predominantly polys

Synovial Fluid

	NORMAL	NONINFLAMMATORY	INFLAMMATORY	INFECTIOUS	CRYSTAL INDUCED	HEMORRHAGIC
Etiology		Degenerative joint disease	Rheumatoid arthritis, lupus erythematosus, gout, pseudogout	Bacterial infection	Gout, pseudogout	Trauma, coagulation abnormality
Color	Pale yellow to colorless	Yellow	Yellow	Yellow-green	Yellow, white	Pink, red, red-brown
Clarity	Clear	Clear	Cloudy, turbid	Cloudy, turbid	Cloudy, milky	Cloudy
Viscosity	Good	Good	Poor	Poor	Poor	Poor
WBCs/µL*	<200	<2,000	2,000–100,000	50,000–200,000	500–200,000	50–10,000
Polys	<25%	<30%	>50%	>90%	<90%	<50%
Other				Pos culture (*S. aureus*, *N. gonorrhoeae* most common)	Crystals	

*Considerable overlap among disorders. Differential is more helpful for diagnosis.

Synovial Fluid Crystals

CRYSTAL	DESCRIPTION	SIGNIFICANCE
Monosodium urate 	1–30 µm long needles. Intra- or extracellular. Strongly birefringent. Yellow when long axis of crystal is parallel to slow wave of red compensator; blue when perpendicular.	Gout
Calcium pyrophosphate 	1–20 µm long, 4 µm wide. Rod-shaped, rectangular, or rhomboid. Intracellular. Weakly birefringent. Blue when long axis of crystal is parallel to slow wave of red compensator; yellow when perpendicular.	Pseudogout
Cholesterol 	Large rectangle with notched-out corner. Extracellular.	Chronic effusions (rheumatoid arthritis)

From Strasinger SK, Di Lorenzo MS. Urinalysis and Body Fluids, 5th ed. Philadelphia: FA Davis; 2008:215.

Semen Analysis

	FERTILITY TESTING	POST-VASECTOMY
Specimen collection	Collect in sterile container, without condom, after 3-day abstinence. Keep at RT. Deliver to lab within 1 hr of collection.	Condom can be used. Time & temp not critical. Test monthly beginning 2 months after vasectomy. Continue until 2 consecutive specimens are without sperm.
Liquefaction	Don't analyze until specimen is liquified. (Normally within 30 min of collection.)	Don't analyze until specimen is liquified.
Volume	Normal: 2–5 mL	NA.
Motility	Observe within 3 hr of collection. 50%–60% of sperm should show at least fair motility.	NA.
Cell count	Dilute & count in Neubauer hemacytometer. Normal: >20 million per mL.	Examine undiluted & following centrifugation. Even 1 sperm is significant.
Morphology	Stain & examine at least 200 cells. Normal: oval head (3×5 µm) with long tapering tail. Abnormalities: double heads, giant heads, amorphous heads, pinheads, tapering heads, constricted heads, double tails, coiled tails, large numbers of spermatids (immature forms). Normal = <30% abnormal forms.	
pH	7.3–8.3	NA.

Amniotic Fluid Tests

TEST	ASSESSMENT/DIAGNOSIS OF	METHOD	COMMENTS
Lecithin-to-sphingomyelin (L/S) ratio	Fetal lung maturity	Thin-layer chromatography	Reference method. L/S ratio ≥ 2.0 signifies maturity. False ↑ with blood or meconium contamination.
Phosphatidylglycerol (PG)	Fetal lung maturity	Immunologic agglutination	Last surfactant to rise. Not affected by presence of blood or meconium.
Foam stability index (shake test)	Fetal lung maturity	Shake with increasing amounts of 95% ethanol	Index is highest concentration of ethanol that supports ring of foam after shaking. Index of 0.48 is comparable to L/S ratio of 2.0. Blood and meconium interfere.
Lamellar body count	Fetal lung maturity	Count in platelet channel of hematology analyzers	Number correlates with amount of phospholipid present in fetal lungs. Sample must be free of blood or meconium.
Amniotic fluid bilirubin	Hemolytic disease of the newborn/fetus	Direct spectrophotometric scan from 365–550 nm	Bilirubin has peak absorbance at 450 nm. Baseline is drawn connecting 365- & 550-nm points. ΔA_{450} compared to gestational age to determine severity of disease. Specimen must be protected from light. Blood & meconium interfere.
Alpha-fetoprotein (AFP)	Neural tube defects	Immunologic method	High levels with anencephaly, spina bifida.

SECTION 8

Molecular Diagnostics (MDx) Review

Nucleic Acid Terminology

TERM	EXPLANATION
Nucleic acid	DNA & RNA
Deoxyribonucleic acid (DNA)	Nucleic acid that carries genetic information. In eukaryotes, located in chromosomes in nucleus & small amount in mitochondria. Also present in bacteria & DNA viruses.
dsDNA	Double-stranded DNA.
ssDNA	Single-stranded DNA.
Genomic DNA	DNA in chromosomes.
Mitochondrial DNA	Circular piece of DNA found in mitochondria of eukaryotes. Codes for polypeptides involved in oxidative phosphorylation. Transmitted by maternal inheritance (mitochondria in ova, not sperm). Used in forensics, lineage & population studies.
Chromosome	Piece of coiled DNA containing many genes. Humans have 23 pairs of chromosomes. 1 set from mother, 1 from father. 22 pairs of autosomes, 1 pair sex chromosomes (X,Y).
Gene	Specific sequence of nucleotides (1,000–4,000) at particular location on chromosome. Starts at 5′ end with promoter region that initiates transcription & ends at 3′ end with terminator sequence that ends transcription. Humans have about 30,000 genes. DNA sequence in genes is 99.9% the same in all people.
Exons	DNA sequences on chromosomes that code for amino acids/proteins.

continued...

Nucleic Acid Terminology *continued*

TERM	EXPLANATION
Introns	Noncoding regions between exons.
Genome	All hereditary information for an individual.
Genetic code	Relationship between sequence of bases in DNA or its RNA transcript & sequence of amino acids in proteins.
Ribonucleic acid (RNA)	Nucleic acid that converts genetic information from DNA into specific proteins. Some has regulatory or structural function. Present in nucleus & in cytoplasm where it's associated with ribosomes (free or attached to endoplasmic reticulum). Source of genetic information in RNA viruses (retroviruses).
Nucleoside	Pentose sugar with nitrogen base attached. Dephosphorylated nucleotide. Adenosine, guanosine, cytidine, thymidine.
Nucleotide	Building blocks of DNA & RNA. A pentose sugar with nitrogen base attached to 1′C & 1–3 phosphate groups attached to 5′C. Nucleotide sequences always written in 5′ to 3′ direction, e.g., 5′ATCGAACAGTAC 3′
Pentose	Sugar with 5 carbons (C). Cs are numbered 1′–5′ starting with C to right of oxygen & going clockwise. Superscript (prime) differentiates Cs in sugar from Cs in bases, which are numbered 1–9.
Ribose	5-C sugar found in RNA.
Deoxyribose	5-C sugar found in DNA. Similar to ribose, but with 1 less oxygen.

continued...

Nucleic Acid Terminology *continued*

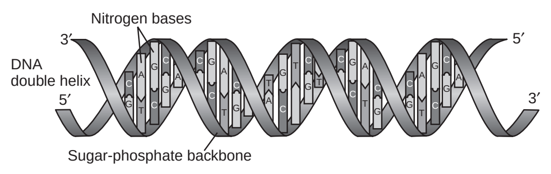
TERM	EXPLANATION
Nitrogen bases	Carbon-nitrogen ring structures attached to 1′C of sugar in DNA & RNA: adenine (A), guanine (G), cytosine (C), thymine (T), & uracil (U). A, G, C, T are in DNA. U replaces T in RNA. T & U are similar except that U lacks methyl group.
Base pairs	Purine from 1 strand of nucleic acid & pyrimidine from another strand joined by hydrogen (H) bonds. A forms 2 H bonds with T or U. G forms 3 H bonds with C.
Complementary	Opposite or partner base in base pair, e.g., A is complementary to T or U, G is complementary to C.
Pyrimidine	Nitrogen bases with single C-N ring (C, T, U).
Purine	Nitrogen bases with 2 C-N rings (A, G).
Phosphodiester bonds	Bonds that join nucleotides in nucleic acid. 5′ phosphate group of 1 sugar attaches to 3′ hydroxyl group of adjacent sugar. A molecule of H_2O splits off.

Comparison of DNA and RNA

	DNA	RNA
Function	Carries genetic information. Serves as template for synthesis of RNA.	Converts genetic information from DNA into proteins. Some has regulatory or structural function. Source of genetic information in RNA viruses.
Location	Nucleus (except mitochondrial DNA).	Nucleus & cytoplasm.
Composition	Repeating nucleotides linked by phosphodiester bonds between 5′ phosphate group of 1 sugar & 3′ hydroxyl group of next.	Repeating nucleotides linked by phosphodiester bonds between 5′ phosphate group of 1 sugar & 3′ hydroxyl group of next.
Sugar	Deoxyribose.	Ribose.
Pyrimidines	C, T.	C, U.
Purines	A, G.	A, G.

continued...

Comparison of DNA and RNA *continued*

	DNA	RNA
Usual structure	Double stranded. Each strand has 5′ end (phosphate group attached to 5′C) & 3′ end (OH group attached to 3′C). Strands are antiparallel, i.e., one in 3′ to 5′ direction, other 5′–3′. 2 strands coil around each other to form double helix with alternating sugar & P groups on exterior & bases on interior. Strands held together by hydrogen (H) bonds between complementary bases.  *(From Buckingham L. Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications, 2nd ed. Philadelphia: FA Davis; 2012:3.)*	Single stranded. Shorter than DNA. Irregular 3-D structure.

Nitrogen Bases

	PURINES	PYRIMIDINES	BASE PAIRING	# H BONDS IN BASE PAIR
DNA & RNA	A, G	C	G-C	3
DNA only		T	A-T	2
RNA only		U	A-U	2

A = adenine, G = guanine, C = cytosine, T = thymine, U = uracil.

Nucleotides

SUGAR	BASE	PHOSPHATES	NUCLEOTIDE
Ribose	Adenine	1 2 3	Adenosine monophosphate (AMP) Adenosine diphosphate (ADP) Adenosine triphosphate (ATP)
Ribose	Guanine	1 2 3	Guanosine monophosphate (GMP) Guanosine diphosphate (GDP) Guanosine triphosphate (GTP)
Ribose	Cytosine	1 2 3	Cytidine monophosphate (CMP) Cytidine diphosphate (CDP) Cytidine triphosphate (CTP)
Ribose	Uracil	1 2 3	Uridine monophosphate (UMP) Uridine diphosphate (UDP) Uridine triphosphate (UTP)
Deoxyribose	Adenine	1 2 3	Deoxyadenosine monophosphate (dAMP) Deoxyadenosine diphosphate (dADP) Deoxyadenosine triphosphate (dATP)*
Deoxyribose	Guanine	1 2 3	Deoxyguanosine monophosphate (dGMP) Deoxyguanosine diphosphate (dGDP) Deoxyguanosine triphosphate (dGTP)*

continued...

Nucleotides *continued*

SUGAR	BASE	PHOSPHATES	NUCLEOTIDE
Deoxyribose	Cytosine	1 2 3	Deoxycytidine monophosphate (dCMP) Deoxycytidine diphosphate (dCDP) Deoxycytidine triphosphate (dCTP)*
Deoxyribose	Thymine	1 2 3	Deoxythymidine monophosphate (dTMP) Deoxythymidine diphosphate (dTDP) Deoxythymidine triphosphate (dTTP)*

*One of the 4 dNTPs used in PCR.

DNA Replication Terminology

TERM	EXPLANATION
Replication	Reproduction of DNA during cell division.
Semiconservative replication	Newly synthesized strand of DNA consists of 1 strand of original DNA & new daughter strand. Original DNA is conserved.
Helicase	Enzyme that unwinds section of DNA by breaking bases' hydrogen bonds to form 2 single-stranded templates for replication.
Replication fork	Location where dsDNA separates into ssDNA & synthesis of DNA begins.
Template	Strand of DNA that serves as pattern for new strand of DNA or RNA.
Primase	RNA polymerase that synthesizes RNA primers needed to initiate replication.
RNA primer	Segment of RNA formed on DNA template to start replication. Provides 3′OH needed for phosphodiester bond formation with incoming nucleotide.
DNA polymerase	Enzyme that synthesizes daughter DNA by reading code on DNA template. Can only synthesize DNA from 5′ end to 3′ end because 3′OH needed to receive incoming nucleotides & form phosphodiester bonds. Can only extend a nucleotide chain; can't start one. Needs primer to start.
Continuous synthesis	Synthesis of daughter strand of DNA from 3′–5′ target strand. DNA polymerase proceeds in linear fashion, assembling series of joined nucleotides in leading strand.

continued...

DNA Replication Terminology *continued*

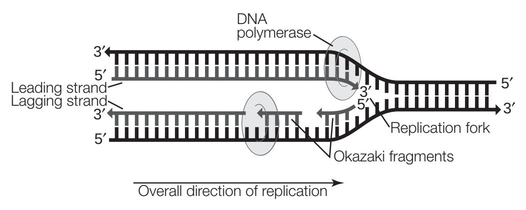
TERM	EXPLANATION
Discontinuous synthesis	Synthesis of daughter strand of DNA from 5′–3′ target strand. DNA polymerase must read target in 3′ to 5′ direction, so must proceed in nonlinear fashion, reading a section, assembling appropriate nucleotides, then moving back upstream to read another section in 3′ to 5′ direction. New strand called lagging strand. Consists of unconnected DNA fragments called Okazaki fragments separated by RNA primers.
Leading strand	Daughter strand synthesized continuously from 3′–5′ template.
Lagging strand	Daughter strand synthesized discontinuously from 5′–3′ template.
Okazaki fragments	Short fragments of unconnected DNA formed during replication of 5′–3′ template.
RNase H	Enzyme that removes RNA primers from daughter strands of DNA.
Ligase	Enzyme that joins DNA fragments together.

DNA Replication

STEP	EXPLANATION
Strand separation	Helicase unwinds section of dsDNA to form replication forks. Both original strands of DNA will serve as templates. Replication will proceed simultaneously on both strands, in opposite directions.
Synthesis of primers	Primase reads section of code on DNA, attaches complementary nucleotides to form RNA primer that provides 3′OH end needed to grow nucleotide chain.
Extension of primers	DNA polymerase reads template, attaches complementary nucleotides starting at 3′ OH of primer. Code is read in 3′ to 5′ direction. Synthesis on 3′–5′ template is continuous, forming leading strand. Synthesis on 5′–3′ template is discontinuous, forming lagging strand of disconnected Okazaki fragments.
Removal of primers	RNase H removes RNA primers.
Replacement of primers	DNA polymerase places nucleotides where primers used to be.
Joining of Okazaki fragments	DNA ligase joins DNA fragments together.

continued...

DNA Replication *continued*



(From Buckingham L. Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications, 2nd ed. Philadelphia: FA Davis; 2012:9.)

Primer Extension

Template DNA: 3′ AGCA 5′
→(DNA polymerase reads template, attaches nucleotides in this direction
Daughter strand: 5′ TCGT 3′

Polymerase reads template in 3′ to 5′ direction. New strand grows in 5′ to 3′ direction

In example above:
1. Polymerase reads A on template, adds T to daughter strand
2. Polymerase reads G on template, adds C to daughter strand
3. Polymerase reads C on template, adds G to daughter strand
4. Polymerase reads A on template, adds T to daughter strand

5′ P of incoming nucleotide attaches to 3′ OH of last nucleotide on strand, forming phosphodiester bond

Discontinuous Synthesis of DNA on Lagging Strand

Nucleotide position		1	2	3	4	5	6	7	8	
Template DNA	5′	A	G	C	T	T	G	A	C	3′
					¬ polymerase first pass				¬ polymerase second pass	
Daughter DNA	3′	T	C	G	A	A	C	T	G	5′
5′–3′ DNA template is read in 3′ to 5′ direction. DNA daughter strand is synthesized in 5′ to 3′ direction.										
First pass: polymerase reads positions 4 to 1 on template—TCGA, assembles AGCT on daughter strand.										
Second pass: polymerase reads positions 8 to 5 on template—CAGT, assembles GTCA on daughter strand.										

Gene Expression Terminology

TERM	EXPLANATION
Gene expression	Synthesis of specific proteins based on DNA code.
Messenger RNA (mRNA)	RNA that transcribes DNA code in nucleus & carries it to cytoplasm where it's translated into protein. About 1%–2% of total cellular RNA.
Ribosomal RNA (rRNA)	RNA associated with ribosomes, site of protein synthesis.
Transfer RNA (tRNA)	RNA that reads code in mRNA & transports amino acids to growing polypeptide chain.
Micro RNA (miRNA)	Tiny regulatory RNAs involved in control of gene expression.
Transcription	Conversion of genetic information from DNA into complementary strand of mRNA. If DNA sequence is AGCT, mRNA sequence will be UCGA.
Translation	Conversion of genetic information from mRNA into specific sequence of amino acids to form specific protein. Takes place on ribosomes in cytoplasm. tRNA reads mRNA & assembles amino acids.
Sense strand	Strand of DNA that doesn't serve as template for synthesis of mRNA. Its sequence is same as mRNA that is translated into protein (except it has T where RNA has U).
Antisense strand	Strand of DNA that serves as template for synthesis of mRNA.
Promoter	Region of DNA that binds RNA polymerase, initiating transcription.

continued...

Gene Expression Terminology *continued*

TERM	EXPLANATION
Codon	3 nucleotides that code for an amino acid, e.g., GCC codes for alanine. 64 codons code for 21 amino acids. 2–6 codons for each, except methionine & tryptophan which only have 1.
Start codon	AUG, codes for methionine.
Termination codons	UAA, UAG, UGA. Not associated with any amino acid. Signal to stop protein synthesis.
Anticodon	3-nucleotide sequence on tRNA that attaches to codon on mRNA, bringing with it specified amino acids.
Ribosomes	Organelles in cytoplasm & on surface of rough endoplasmic reticulum. Made of rRNA & protein. Site of protein synthesis.

Protein Synthesis

STEP	EXPLANATION
Transcription	1. RNA polymerase binds to promoter region of gene. 2. DNA separates into 2 single strands. 3. RNA polymerase adds complementary nucleotides to produce strand of mRNA. If DNA sequence is 3′ ATTCGA 5′, mRNA will be 5′ UAAGCU 3′. 4. mRNA is released from DNA. 5. DNA strands reanneal.
Translation	1. mRNA associates with ribosomes in cytoplasm. 2. Ribosome moves along mRNA, reading genetic code. Always begins with codon AUG. 3. tRNA with complementary 3-nucleotide sequence (anticodon) attaches to codon on mRNA, bringing with it amino acid specified by codon, e.g., anticodon UAC on tRNA attaches to codon AUG on mRNA, bringing with it amino acid methionine. 4. As ribosome moves along mRNA, other tRNAs bring in other amino acids. 5. Peptide bonds form between amino acids. 6. Process continues until ribosome reaches stop codon. 7. Protein is released.

Basic MDx Terminology

TERM	EXPLANATION
Molecular diagnostics	Analysis of DNA & RNA; nucleic acid testing (NAT).
Extraction	Isolation of DNA/RNA from other cellular components.
RNase	Enzyme that degrades RNA. Ubiquitous in environment.
DNase	Enzyme that degrades DNA.
Target	Specific section of DNA under investigation.
Hybridization	Pairing of complementary strands of nucleic acid, 1 from sample & 1 a reagent. Resulting hybrids can be DNA:DNA, DNA:RNA, or RNA:RNA.
Hybrid	Product of hybridization.
Duplex	Hybrid.
Stringency	Stability of bonding during hybridization. Based on degree of match & base composition. Influenced by temp, pH, & salt concentration. ↑ temp, ↓ salt ensure only most perfectly matched strands will remain paired.

continued...

Basic MDx Terminology *continued*

TERM	EXPLANATION
Nucleic acid probe	Short strand of DNA or RNA with known base sequence. Isolated from organisms, cloned in bacteria, or synthesized. Labeled with fluorescent or chemiluminescent dyes, enzymes, or radioisotopes to produce visible sign of hybridization. Used in many molecular methods, e.g., Southern blot, Northern blot, in situ hybridization, restriction fragment length polymorphism, conventional & real time PCR. If complementary base sequence is present, probe attaches to target.
Homologous	Matching, e.g., a homologous nucleic acid segment is 1 whose nucleotides are in correct order to hybridize with another nucleic acid segment, such as a primer or probe. The base pairs of 1 strand are complementary to the base pairs of the other. Term also used in reference to chromosome pairs, e.g., maternal copy of chromosome 17 and homologous paternal copy.

Overview of MDx

Specimen collection
Specimen processing
Nucleic acid extraction
Nucleic acid amplification (optional)
Nucleic acid detection/analysis by: • Gel electrophoresis • Hybridization with labeled probes • Southern or Northern blotting • Restriction enzyme mapping • DNA sequencing • Real-time PCR

Blood Collection Tubes for MDx

STOPPER COLOR	CONTENT	USE
Lavender	EDTA	Isolation of DNA & detection of viruses. 1 of preferred anticoagulants for blood & bone marrow.
White	K_2EDTA & gel barrier	Isolation of plasma. Gel forms barrier between plasma & cells.
Blue/black	Sodium citrate, gel, density gradient fluid	Isolation of mononuclear cells. Gel forms barrier between mononuclears in plasma & RBCs/granulocytes.
Yellow	Acid citrate dextrose (ACD)	Enhanced recovery of WBCs for several days after collection. 1 of preferred anticoagulants for blood & bone marrow.
Green	Heparin	Generally not recommended. Heparin inhibits polymerase; unacceptable for testing that involves PCR.

Specimens for MDx

SPECIMEN	USE/COMMENTS
Whole blood/plasma	Nucleated cells are source of DNA. Some recent techniques can detect non-nuclear DNA in plasma. Nucleic acid of infectious agents often detected in plasma, sometimes in host DNA. Hemolysis can interfere with amplification.
Bone marrow	DNA obtained from nucleated cells.
Urine	Detection of *Chlamydia*, CMV, & BK virus (associated with rejection of transplanted kidney).
Feces	Detection of intestinal pathogens. Analysis of stool DNA proposed for detection of colorectal cancer.
Cerebrospinal, synovial, pleural, pericardial, ascetic fluids	Detection of infectious agents & cancer cells.
Amniotic fluid, choronic villus sampling (CVS)	Dx of sickle cell anemia, Tay-Sachs disease, thalassemia, other chromosomal abnormalities & inherited disorders. Usually grown in tissue culture to ↑ # of cells for DNA extraction.
Buccal cells	Good source of DNA, especially for genotyping bone marrow recipients & patients who have been recently transfused. Avoid use of mouthwash with phenol or ethanol. Use sterile Dacon or rayon swabs with plastic shafts. Calcium alginate swabs & wooden shafts may contain substances that inhibit PCR.

continued...

Specimens for MDx *continued*

SPECIMEN	USE/COMMENTS
Tissue	Formalin-fixed, paraffin embedded tissue: DNA degraded to low molecular weight (MW) fragments. Can usually be amplified but Southern blot can't be used (requires high MW DNA). Fresh tissue, snap-frozen tissue, or tissue frozen at –80°C in optimal cutting temperature compound (OCT): yields higher MW DNA. OCT must be completely removed before DNA extraction.
Hair/nails	Forensic analysis.

Specimen Processing

SAMPLE	PREPARATION
Blood or bone marrow	Whole blood can be stored at 2°–25°C for 24–48 hr after collection. Separate WBCs by: 1. Differential lysis in hypotonic buffer or water (RBCs lysed, WBCs precipitated by centrifugation) or 2. Density gradient centrifugation in Ficoll to isolate mononuclears WBCs can be stored at –70°C for up to a year*
Plasma	Remove from RBCs promptly & hold at –20°C or below. *
Tissue	Fresh or frozen: grind, homogenize, or mince Embedded: deparaffinize in xylene, rehydrate in ↓ concentrations of ethanol
Bacteria/fungi	Break cell walls (enzymes, detergents, NaOH, boiling, or glass beads). Enzyme treatment—less damage to chromosomal DNA. NaOH & boiling yield ssDNA. Not suitable for restriction enzyme analysis. OK for amplification.

*Avoid frost-free freezers because of temp variation. Thawed samples should be thoroughly mixed before testing.

Isolation of DNA

STEP	EXPLANATION
Isolation of nucleated cells (if necessary)	Centrifugation of whole blood to obtain buffy coat, differential lysis with water or hypotonic solution to separate WBCs from RBCs, density gradient centrifugation in Ficoll to obtain mononuclear cells (settle below plasma, above polys/RBCs).
Release of DNA from cells	Cells ruptured by mechanical or chemical methods.
Separation of DNA from cellular debris, protein, lipids	Liquid-phase extraction: use of solvents. Multiple manual steps. Limited sample throughput. Phenol & chloroform are hazardous. Solid-phase extraction: DNA adsorption onto silica on membrane filters or magnetic particles. More commonly used. Easier, can be automated.
Concentration	Precipitation in ethanol or isopropanol in high salt concentration. Resuspension in H_2O or TE buffer. Handle with care to avoid breaking DNA.
Storage	Extracted DNA can be stored at RT for several months, refrigerated for 1 yr, or frozen at –20°C to –70°C for up to 10 yr.

Comparison of RNA & DNA Isolation

Method	Similar. Lysis of cells, isolation by phenol-chloroform extraction or binding to silica, precipitation in alcohol.
Stability	RNA less stable. Process specimens promptly. Can be stored suspended in ethanol for several months at –20°C or long term at –70°C.
Contamination	RNA more susceptible to degradation. Use RNase decontamination solution to clean gloves, benchtops, equipment; RNase-free reagents & plasticware; nuclease-free water; DNase I to digest DNA.

Assessment of Nucleic Acid Yield/Quality

	METHOD	EXPLANATION	ASSESSMENT
Yield	Spectrophotometry: Absorbance @ 260 nm	Nucleic acids absorb light at 260 nm.	DNA µg/mL: A260 × 50 RNA µg/mL: A260 × 40
	Gel electrophoresis/densitometry	Intensity of bands is proportional to nucleic acid concentration. Compare to standard.	Brighter bands = higher yield
Purity	Spectrophotometry: Absorbance @ 260 nm/absorbance @ 280 nm	Nucleic acids absorb light at 260 nm. Absorption peak for protein is 280 nm.	DNA A260/A280 ratio: 1.6–2.0 = good quality <1.6 = protein contamination, specimen must be reprocessed >2 = possible contamination with RNA RNA A260/A280 ratio: ≥2 = good quality
	Gel electrophoresis/densitometry	Position of bands is related to MW. Lower MW fragments migrate furthest from point of application.	DNA: high MW fragments = good quality RNA 28S/18S ratio: 2 = good quality <2 = smaller fragments due to RNase degradation

Amplification Terminology

TERM	EXPLANATION
Amplification	Technique to ↑ (amplify) amount of nucleic acid in sample, probe, or signal so that very small amounts of nucleic acid can be detected.
Target amplification	Technique to ↑ amount of target nucleic acid in sample through in vitro replication, e.g., polymerase chain reaction (PCR), transcription mediated amplification (TMA).
Probe amplification	Technique to ↑ amount of probe bound to target so very small amounts of nucleic acid can be detected, e.g., ligase chain reaction.
Signal amplification	Technique to ↑ signal generated so that very small amounts of nucleic acid can be detected, e.g., branched chain signal amplification (bDNA), hybrid capture assay (HCA).
Nucleic acid amplification testing (NAAT)	Term used in blood banks to describe tests that detect bloodborne pathogens by presence of nucleic acids rather than antibodies. Provides earlier detection.
Polymerase chain reaction (PCR)	1st & most widely used amplification method. Specific primers & thermostable DNA polymerase make copies of target DNA by repeated cycles of denaturation, annealing, & extension. Can produce a million copies in less than an hour.
Denaturation	Separation of ds DNA into 2 strands using heat or alkali.
Melting	Use of heat to separate strands of DNA.

continued...

Amplification Terminology *continued*

TERM	EXPLANATION
Melting temperature (Tm)	Temperature required to denature 50% of dsDNA. Depends in part on GC content. G-C pair harder to break than A-T pair because 3 H-bonds instead of 2.
Anneal	Binding to complementary strand of nucleic acid.
Polymerase	Enzyme that assembles nucleotides to produce new strand of nucleic acid.
DNA polymerase	Enzyme that synthesizes DNA using existing strand of DNA as template. Can only add to existing piece of DNA so needs primer to start.
Taq polymerase	Heat-stable DNA polymerase used to catalyze synthesis of DNA in PCR. Originally isolated from bacterium *Thermus aquaticus* in hot springs of Yellowstone National Park. Can with-stand high temp required for denaturation.
Oliogonucleotides	Short fragments of ssDNA that are synthesized for specific purpose, e.g., oligonucleotide primers.
Primers	Short segments of DNA designed to anneal to DNA target in PCR. Provide free 3′-OH group to which DNA polymerase can add dNTP.
Deoxynucleotide triphosphates (dNTPs)	Building blocks from which DNA polymerase synthesizes new strands of DNA. Deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP), deoxycytidine triphosphate (dCTP).

continued...

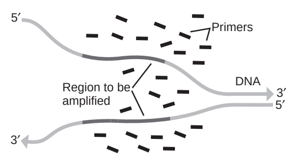
Amplification Terminology *continued*

TERM	DEFINITION
Thermocycler	Instrument that rapidly changes temperatures during amplification cycle.
Amplicon	Product of amplification.
Melting curve analysis	PCR amplicons are slowly heated in presence of dyes specific for dsDNA. At low temp, signal is high. As temp ↑, DNA strands separate & signal ↓. Fluorescent signal patterns vary depending on nucleotide sequence.
Reverse transcriptase	Enzyme used in vitro to make DNA from RNA. Isolated from retroviruses.
Copy or complementary DNA (cDNA)	DNA copied from RNA by reverse transcriptase.
Ligate	To join together by chemical process.
DNA ligase	Enzyme that links DNA strands by joining 5′ end of one to 3′ end of another.

Components of Polymerase Chain Reaction (PCR)

COMPONENT	ROLE
Template	DNA region to be amplified. From patient's genomic or mitochondrial DNA or from microorganisms.
2 oligonucleotide primers	Short segments of ssDNA designed to hybridize to template strands & provide starting point for synthesis of daughter strands. 1 designed to hybridize adjacent to region of interest on 3′ end of 1 strand, other designed to hybridize adjacent to region of interest on 3′ end of other strand. Segment to be amplified falls between 2 primers.
Thermostable DNA polymerase	E.g., *Taq* polymerase. Extends primers on each template strand by adding dNTPs & catalyzing formation of phosphodiester bonds.
Deoxynucleotide triphosphates (dNTPs)	Building blocks from which DNA polymerase synthesizes a new strand of DNA: dATP, dGTP, dTTP, dCTP.

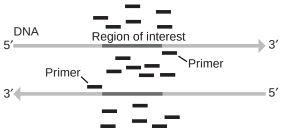
Steps in PCR

STEP	TEMP/TIME*	EXPLANATION
1. Denaturation (melting)	90°–96°C/ 20–60 sec.	dsDNA separated into 2 strands by heat. Each strand serves as template for synthesis of new strand.  *(From Buckingham L. Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications, 2nd ed. Philadelphia: FA Davis; 2012:133.)*

continued...

Steps in PCR *continued*

STEP	TEMP/TIME*	EXPLANATION
2. Annealing (hybridization)	50°–70°C/ 20–90 sec.	Primers attach to both template strands by binding with complementary bases on 3′ side of region to be amplified.



(From Buckingham L. Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications, 2nd ed. Philadelphia: FA Davis; 2012:134.)

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Steps in PCR *continued*

STEP	TEMP/TIME*	EXPLANATION
3. Extension (polymerization)	68°–75°C/ 10–60 sec.	DNA polymerase attaches to ends of primers, adds dNTPs that are complementary to template, synthesizing new strands of DNA.  *(From Buckingham L. Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications, 2nd ed. Philadelphia: FA Davis; 2012:134.)*
Cycle repeated 15–40 times		Each cycle results in doubling of DNA, yielding millions of copies of original DNA segment. Amplicon can be analyzed by variety of methods, e.g., gel electrophoresis, hybridization.

*Exact temperatures & times vary.

PCR Controls

CONTROL	DESCRIPTION	PURPOSE	EXPECTED REACTION
Positive control	Contains nucleic acid sequence of interest	Ensures that polymerase is active, primers & thermocycler working properly.	Target sequence detected
Negative control (contamination control, reagent blank, or no target control)	Contains all PCR components except DNA	Checks for contamination with target DNA or amplicon from previous amplification.	Target sequence not detected
Negative template control	Contains DNA but not target DNA	Ensures that primers aren't binding to wrong DNA sequences.	Target sequence not detected
Internal control (amplification control)	Contains 2nd set of primers & unrelated target (housekeeping gene or other nucleic acid target introduced in sample before nucleic acid extraction or during PCR)	Demonstrates that PCR is working. Differentiates true neg from false neg due to amplification failure. Detects PCR inhibitors.	Housekeeping gene detected

Other Target Amplification Methods

METHOD	EXPLANATION
PCR modifications	
Nested PCR	Uses 2 pairs of primers & 2nd round of amplification to ↑ sensitivity & specificity.
Real-time PCR	Simultaneous amplification & detection (fluorescence) in sealed tube. Less subject to contamination. Eliminates need for analysis of product by gel electrophoresis. Quantitative. Commonly used for detection of microorganisms.
Multiplex PCR	Uses more than 1 set of primers so multiple targets can be amplified in same tube.
Reverse transcriptase PCR (RT-PCR)	Method to amplify RNA (mRNA or microbial RNA). Same procedure as PCR except RNA is 1st converted to cDNA by reverse transcriptase. Used to detect HIV & HCV, measure viral loads in HIV & HCV infections, measure gene expression.
Transcription-based amplification systems (TAS)	
Self-sustaining sequence replication (3SR) Transcription-mediated amplification (TMA) Nucleic acid sequence-based amplification (NASBA)	Non-PCR methods to amplify target RNA. Similar methods, different manufacturers. cDNA is formed from target RNA. Millions of copies of RNA produced by transcription of cDNA. Assays to detect RNA viruses, *Mycobacterium tuberculosis, Chlamydia trachomatis.* TMA & NASBA are isothermal rxn. Don't require thermocycler.

Other Amplification Methods

METHOD	EXPLANATION	APPLICATIONS
Probe amplification		
Ligase chain reaction (LCR)	Pair of probes designed to hybridize to opposite ends of target DNA. DNA ligase will join pair together if there is an exact match to target bases, then probe acts as template for additional replication. Ligation doesn't occur if there are any base mismatches.	Was used to detect *C. trachomatis* & *Neisseria gonorrhoeae* in clinical specimens but taken off market.
Strand-displacement amplification (SDA)	Isothermal rxn involving amplification of probes/primers.	ID of *Legionella pneumophila*, *C. trachomatis*, *N. gonorrhoeae*.
Signal amplification		
Branched chain DNA (*b*DNA)	Probes capture target sequence to solid support. Extender, preamplifier, & amplifier probes hybridize sequentially. Final probe is branched & carries signal-generating enzymes that act on chemiluminescent substrate.	Detection of HBV, HCV, CMV; HIV viral loads.
Hybrid capture assay	Complexes containing target DNA bind to antibodies on solid support. Detected by enzyme-labeled antibodies.	Detection of HPV.
Cleavage-based amplification (Invader technology)	Isothermal method that uses primary probe, invader probe, reporter probe. Enzyme cleavage amplifies reporter probe bound to DNA target.	Detection of cystic fibrosis, factor V Leiden, HPV.

Hybridization Assays

ASSAY	PRINCIPLE	APPLICATION
Solid-phase hybridization	Dot/slot blot. Sample applied to membrane. Membrane heated to denature DNA. Labeled probe added. Unhybridized probe washed away. Hybridized probe visualized by autoradiography or enzyme assay. Pos rxn indicates presence of target sequence.	Detection of mutations associated with genetic diseases, e.g., sickle cell anemia
	Sandwich hybridization. Unlabeled probe bound to membrane or well surface (capture probe). Sample applied. If target sequence is present, anneals to capture probe. Labeled probe (signal-generating probe) that anneals to different site on target added. More specific than dot blot. Can be carried out in microtiter plates, so adaptable to automation.	
	Line probe assays (LiPA). Probes attached to strips, amplified target added. Unbound target washed away. Hybridized complexes visualized with Biotin-Streptavidin method.	Infectious diseases, e.g., HCV genotyping, *Mycobacteria* speciation
Liquid-phase hybridization	Target nucleic acid & probe interact in aqueous solution. Rapid & more sensitive than solid phase. Uses smaller sample size. Adaptable to automation.	Detection of bacterial rRNA in clinical specimens or cultures

continued...

Hybridization Assays *continued*

ASSAY	PRINCIPLE	APPLICATION
In situ hybridization	Target nucleic acid detected in intact cells. Tissue (fresh, frozen, or paraffin-embedded) or cells fixed on glass slide. Probe added. Attaches to complementary DNA sequences. If fluorescent-labeled probe used, known as fluorescent in situ hybridization (FISH). Viewed with fluorescent microscope.	Cytogenetic studies, e.g., *HER2/neu* oncogenes in breast cancer, chromosome translocations in leukemia
DNA chip technology (microarrays)	Multiple probes (up to 10^5–10^6) immobilized at specific locations on solid support (glass slide, silicone chip). After amplification, sample & control nucleic acids labeled with 2 different fluorescent dyes & loaded onto chip. Sample & control compete to hybridize to chip based on relative amounts. Unbound strands of target washed away. Hybridized nucleic acid detected with fluorescent detector. Relative colors measured. Thousands of reactions can be performed at same time.	Gene profiles associated with certain cancers/diseases, classification of leukemias, tumor staging, determination of drug resistance in HIV

Gel Electrophoresis

Principle	Nucleic acids migrate to pos pole (anode) in electrical field due to neg charge on phosphate group. Molecules with lowest MW travel furthest from point of application. Traditional method to detect/analyze nucleic acids following isolation.
Matrices	Agarose, polyacrylamide.
Buffers	Tris-acetate-EDTA (TAE), tris-borate-EDTA (TBE).
Tracking dyes	Bromphenol blue, xylene cyanol.
Stains	Ethidium bromide (fluorescent DNA-binding dye, mutagen, handle with care), silver, Coomassie blue.
Variations	Polyacrylamide gel electrophoresis (PAGE): Used when DNA fragments are small. High degree of resolution. Can differentiate fragments that differ by just 1 base pair. Capillary electrophoresis: Performed in thin capillary tubes using high voltage. Used in DNA sequencing & fragment analysis. Pulsed-field gel electrophoresis: Alternates direction of current. Used in epidemiology of infectious diseases. Denaturing gel electrophoresis: Uses chemicals to denature secondary structures. Used most often in electrophoresis of RNA.
Applications	PCR/RFLP, RT-PCR fragment analysis, Southern & Northern blotting, DNA sequencing.

Blotting

Principle	Nucleic acids that have been separated by electrophoresis are transferred to solid support membrane.
Southern blot	Used to analyze DNA. Useful for identification of DNA fragments that don't amplify well, for polymorphisms & structural alterations. 1. DNA extracted from sample. 2. DNA cut into fragments with restriction endonucleases. 3. Fragments separated by gel electrophoresis. 4. dsDNA fragments denatured into single strands by treatment with base. 5. Single-stranded fragments transferred (blotted) to solid support medium by capillary action. (Newer methods use vacuum & pressure to ↑ speed of transfer.) 6. DNA immobilized by baking or UV light. 7. Labeled DNA probe applied. 8. Excess probe washed away. 9. Visible bands seen where probe hybridized to complementary DNA fragments. Labor intensive. Rarely used.
Northern blot	Similar method used to analyze RNA. Digestion step isn't required because RNA is short. Used to determine if a gene is being expressed by measuring amount of a specific mRNA. Primarily a research tool.

DNA Sequencing/Variation Terminology

TERM	EXPLANATION
Sequencing	Determination of order of nucleotides in DNA molecule. Used to detect mutations, type microorganisms, establish treatment for HIV & HCV.
Dideoxy chain termination sequencing	Sanger method. Most widely used method to determine DNA sequence.
Dideoxynucleotides (ddNTPs)	ddATP, ddGTP, ddCTP, or ddTTP. Chain-terminating nucleotides used in DNA sequencing. Lack 3′-OH group required for formation of phosphodiester bonds between nucleotides. When incorporated into growing DNA strand, extension stops.
Sequencing ladder	4-lane gel electrophoresis pattern obtained from dideoxy chain termination sequencing.
Cycle sequencing	Most commonly used method for DNA sequencing in clinical lab. Based on Sanger method. Amplification by PCR, denaturation, annealing of primer, extension of new strand by DNA polymerase. Extension stops when ddNTP is incorporated. ddNTPs are labeled with different fluorescent tags, detected by capillary electrophoresis & fluorescent detector.
Pyrosequencing	Method to determine DNA sequence in short segments based on generation of light. Doesn't require electrophoresis or sequencing ladder.
Mutation	Permanent change in nucleotide sequence. May or may not cause disease.
Point mutation	Mutation involving single nucleotide pair.

continued...

DNA Sequencing/Variation Terminology *continued*

TERM	EXPLANATION
Polymorphism	Variation in DNA sequence that occurs in at least 1% of population. Some cause susceptibility to disease, affect response to drugs.
Single nucleotide polymorphism (SNP)	"Snip." Variation in single base. Most common type of variation in human genome. May or may not cause disease.
Restriction endonucleases	Bacterial enzymes used to cut DNA at specific sites. Used to produce short fragments for electrophoresis.
Restriction site (recognition site)	Nucleotide sequence recognized by restriction endonuclease. Most are palindromes.
Palindrome	Nucleotide sequence that reads same in both directions, e.g., the complementary strand to 5′-CATG-3′ is 3′-GTAC-5′. Since reading is from 5′ to 3′ direction, both are read as CATG.
Restriction digest	DNA fragments formed by action of restriction endonucleases on DNA.
Restriction pattern (RFLP profile)	Pattern of DNA fragments produced following digestion with restriction endonucleases. Fragments separated by gel electrophoresis. Stained with ethidium bromide & viewed under UV light or blotted onto nitrocellulose membrane & detected with labeled probe (Southern blot).
Restriction fragment length polymorphisms (RFLPs)	Differences in restriction patterns due to variations in nucleotide sequences that change where restriction enzymes cleave DNA. Useful in forensic & paternity testing, detection of genes associated with specific diseases, ID of microorganisms.

Sanger Dideoxy Chain Termination Method

Principle	Modification of DNA replication that incorporates labeled dideoxynucleotides (ddNTPs, chain terminating nucleotides) in reaction mixture. Similar to dNTPs except lack 3′ OH needed for phosphodiester bond formation. When incorporated into growing DNA chain, elongation is terminated.
Components	Template, primer, DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), ddNTPs (ddATP, ddCTP, ddGTP, ddTTP)
Procedure	1. DNA template amplified. 2. 4 reaction tubes containing: a. template b. primer c. DNA polymerase d. all 4 dNTPs e. only 1 ddNTP (ddATP, ddCTP, ddGTP, or ddTTP) 3. Amplicon denatured. 4. Primer hybridizes to target. 5. DNA polymerase extends primer, occasionally incorporating ddNTP that stops further extension. Resulting fragments are of various lengths. All fragments in tube end with same labeled ddNTP. 6. Products electrophoresed in 4 separate lanes labeled A, C, G, T, corresponding with the ddNTP in tube. Fragments separate according to size. 7. Gel dried & exposed to x-ray film, producing sequencing ladder. Band furthest from origin is smallest, fastest migrating fragment & ends in the 1st nucleotide in the sequence, e.g., if band furthest from origin is in lane A (lane from tube that contained ddATP), then 1st nucleotide in sequence is A. Ladder is read from bottom to top to determine entire nucleotide sequence.

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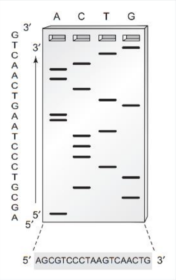
Sanger Dideoxy Chain Termination Method *continued*



(From Buckingham L. Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications, 2nd ed. Philadelphia: FA Davis; 2012:229.)

Automated sequencing	Uses fluorescent labels & capillary gel electrophoresis. Fragments migrate through gel according to size, pass by laser beam & detector. Bases identified by color of fluorescence. Results in electropherogram rather than gel pattern.
Application	Detection of mutations, genotyping HIV to determine drug resistance & HCV to determine therapy & prognosis.

Setup of Sanger Dideoxy Chain Termination Method

	TUBE A	TUBE C	TUBE G	TUBE T
Template	√	√	√	√
Primer	√	√	√	√
Polymerase	√	√	√	√
4 dNTPs	√	√	√	√
ddATP	√			
ddCTP		√		
ddGTP			√	
ddTTP				√
Terminal ddNTP of all resulting fragments	ddATP	ddCTP	ddGTP	ddTTP

Strengths and Limitations of Molecular Testing

Strengths	High sensitivity High specificity Ability to predict some diseases before they are manifested in patient Ability to detect mutations that predict response to some therapies Ability to detect nonviable, fastidious, slow-growing, & nonculturable microorganisms Adaptability to automation (high throughput, rapid turnaround time)
Limitations	Potential for nucleic acid contamination Data not always clinically significant, e.g., presence of microbial nucleic acid doesn't always indicate disease, unable to differentiate viable & dead microorganisms Cost

Causes and Prevention of False Results in MDx

CAUSES	PREVENTION
False positives	
Contamination from other specimens	Use of plugged pipet tips & screw cap tubes to minimize sample aerosols
Contamination with amplicons from previous target amplification	Use of closed tube (real-time) PCR or signal or probe amplification methods; separate areas for sample prep & amplification with no movement of equipment or reagents from amplification area to sample prep area; use of dUTP-UNG system to destroy amplicons from previous amplifications
Environmental contamination	Use of 10% bleach or alcohol to clean benches, hoods; UV light to decontaminate sample prep areas (interferes with DNA replication by breaking sugar-phosphate backbone)
Contamination by testing personnel	Use of lab coats, gloves
False negatives	
Inhibitors in sample, e.g., heparin, hemoglobin, lactoferrin	Proper specimen collection (correct anticoagulant, no hemolysis), use of internal control or split sample testing (1 aliquot with target added)
Degradation of nucleic acids during transport/handling	Use of transport media, ice, dry ice, or freezing (depending on specimen), collection tubes designed to stabilize RNA (PAXgene Blood RNA System, BD Diagnostics), prompt processing or preservation, inhibition of DNases & RNases

continued...

Causes and Prevention of False Results in MDx *continued*

CAUSES	PREVENTION
Degradation of nucleic acids during processing	Use of gloves when handling samples, standards, controls to protect against nuclease degradation. RNase-free conditions for isolation of RNA
Degradation of nucleic acids during storage	Storage at proper temp: • Specimens: snap freeze & store at −70°C • DNA: RT a few months or 1 yr in refrig, −20°C to −70°C for 10 yr • RNA: −20°C few months, −70°C longer. Avoid repeated freeze/thaw cycles & frost-free refrigerators. Can damage DNA.

Clinical Applications of Molecular Diagnosis

DISCIPLINE	APPLICATIONS
Microbiology	Detection of microorganisms, e.g., *Mycobacterium tuberculosis*, *Legionella*, *Chlamydia trachomatis*, *N. gonorrhoea*, *Gardnerella*, *Trichomonas vaginalis*, *Candida*, groups A and B streptococci, HIV, HBV, HCV, CMV, HPV, HSV Viral load testing, e.g., HIV, HCV, HBV, CMV, EBV Genotyping to determine antimicrobial resistance, e.g., methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *M. tuberculosis*, HIV, HCV Epidemiology Classification
Blood banking	Screening donors for bloodborne pathogens: HIV, HBV, HCV, West Nile virus
Coagulation	Detection of factor V Leiden, von Willebrand's disease
Hematology	Dx & classification of B- & T-cell malignancies, non-Hodgkin's lymphomas; monitoring of therapy & remission
Immunology	Dx of HIV, HBV, HCV, CMV infections, measurement of cytokine activity
Cytogenetics	Dx of genetic diseases, e.g., sickle cell anemia, cystic fibrosis, Huntington's chorea, Duchenne-type muscular dystrophy, hemochromatosis. Dx of carrier state, e.g., Duchenne-type muscular dystrophy, von Willebrand's disease.
Histocompatibility	HLA typing, detection of polymorphisms in major histocompatibility complex (MHC) genes

Clinical Applications of Molecular Diagnosis *continued*

DISCIPLINE	APPLICATIONS
Oncology	Dx of cancer, e.g., breast, bladder, lung, colon. *KRAS* mutation in colon cancer predicts lack of response to certain therapies.
Other	Paternity testing, forensics (DNA fingerprinting), pharmacogenomics ("personalized medicine," selection of optimal drug therapy based on patient's genotype), fetomaternal medicine (circulating DNA & RNA)

SECTION 9

Management & Education Review

Management Skills and Styles

SKILLS

Organizational	Ability to apply management process, systematize workflow, make decisions, communicate with coworkers.
People	Understanding theories of human needs & work motivation.
Financial	Effective use of & accounting for company's monetary assets.
Technical	Skills to transform resources into products/services.

STYLES

Authoritarian	Manager makes all decisions without input from others. Quick decision-making. Least acceptance & commitment from staff. Poorest quality decisions.
Democratic	Manager makes decisions after polling staff. Better quality & acceptance. Decisions take longer. Those in minority might feel ignored.
Consensus	Manager tries to get at least partial agreement from all staff. Everyone has input. Highest quality decisions. Good acceptance & commitment. Time-consuming.
Laissez-faire	Manager leaves decision to staff. Least effective approach. Manager abdicates responsibility.

Management Principles

FOUNDATIONS

Mission	Organization's purpose
Goals	Organization's broad, long-term ambitions
Objectives	Directives that describe how a goal will be achieved. Should be SMAART: Specific Measurable Achievable Agreed-upon Realistic Time-bound

PROCESSES

Planning	Establishing goals & objectives, formulating policies to carry out objectives.
Organizing	Coordinating resources to achieve plans. Defining working relationships, including line of authority & workflow.
Directing	Communicating, motivating, delegating, & coaching. Creating a climate that meets the needs of individuals & the organization.
Controlling	Defining standards of performance, developing a reporting system, & taking corrective action when necessary.

TITLE	RESPONSIBILITY	FOCUS
Director	Establishes goals & priorities. Broad policy making.	Organizational goals
Administrator	Runs organization within framework of policies given to him/her.	Organizational goals
Manager	Oversees activity to achieve goal or purpose.	Work environment
Supervisor	Oversees activities of others to help them accomplish specific tasks.	People, operations

Maslow's Hierarchy of Needs

NEED	DEFINITION	WORKPLACE COUNTERPART(S)
Physiological	Survival needs. Food, water, air, rest	Income
Safety	Physical & psychological security	Insurance, safe work environment, job security
Social	Sense of belonging, acceptance, affection	Social relationships with coworkers
Esteem	Respect, independence, appreciation, recognition	Job title, privileges, respect of colleagues
Self-actualization	Realization of full potential	Challenging work, autonomy, professional growth

Personnel Required in High-Complexity Laboratories Under CLIA '88

TITLE	RESPONSIBILITIES
Laboratory director	Overall operation & administration of lab.
Technical supervisor	Technical & scientific oversight of lab. Must be available on as-needed basis to provide on-site, telephone, or electronic consultation.
Clinical consultant	Consultation regarding appropriateness & interpretation of tests.
General supervisor	Day-to-day supervision of lab.
Testing personnel	Specimen processing, test performance, & reporting of test results.

Employee Performance Appraisal

COMPONENTS

Job description	Basis for evaluation.
Standards/criteria	What is expected. Should be objective & measurable.
Measurement instrument	Instrument to compare actual performance with desired performance.
Evaluator	Person trained in use of instrument, familiar with intricacies of job, time to dedicate to process.
Feedback mechanism	Plan for sharing results of review, taking corrective action, planning for future.

EVALUATION ERRORS

Error of central tendency	Everyone is rated toward middle of scale.
Contrast error	An individual is rated lower than justified because of comparison with another exceptional individual. (The opposite may also occur.)
Error of leniency	Everyone is rated high. (The opposite may also occur.)
Halo effect	Good performance in one area influences evaluation in other areas.
Reverse halo effect	Poor performance in one area influences evaluation in other areas.
Recency phenomenon	Judgments are made based on recent events or unusual incidents.

Testing Personnel Competency Assessment

Frequency	Semiannually during 1st yr, annually thereafter, & whenever there's a change in test methodology or instrumentation
Methods	Direct observation of routine patient test performance Monitoring of recording/reporting of test results Review of intermediate test results or worksheets, QC records, proficiency testing results, preventive maintenance records Direct observation of performance of instrument maintenance/function checks Assessment of test performance through testing of previously analyzed specimens, internal blind testing samples, or external proficiency testing samples Assessment of problem-solving skills

Required by CLIA '88 for moderate & high complexity labs.

Laboratory Operating Costs

	DEFINITION	EXAMPLES
Fixed costs	Expenses that don't fluctuate when volume of work changes over short term	Instrument leases, maintenance contracts, computer services, equipment costs, facilities upkeep, management salaries, custodial salaries, employee benefits, depreciation, lease payments, rent, taxes
Variable costs	Expenses that fluctuate directly with change in work load	Labor costs, supplies, reagents, disposables
Direct costs	Costs associated with performance of a test	Supplies, reagents, controls, standards, disposables, equipment costs, equipment maintenance contracts, equipment depreciation, technical & supervisory labor
Indirect costs	Overhead	Administration, plant maintenance, security, utilities, building depreciation, rent, taxes, insurance, housekeeping, purchasing, billing, regulatory expenses, laboratory information system (LIS) expenses
Unit cost/cost per test	Total of direct & indirect expenses of producing a test result	

Break-Even Points

ANALYSIS	FORMULA
Break-even price per test	$\dfrac{\text{Annual fixed costs} + \text{variable costs}}{\text{Test volume}}$
Break-even test volume	$\dfrac{\text{Total fixed costs}}{\text{Average revenue per test} - \text{variable cost per test}}$
Break-even revenue	$\dfrac{\text{Total fixed costs}}{(\text{Average revenue per test} - \text{variable cost per test})/\text{average revenue per test}}$

Quality Management

SYSTEM	HIGHLIGHTS
GP26-A4: *Quality Management System: A Model for Laboratory Services*	Quality management system developed by Clinical & Laboratory Standards Institute (CLSI) to organize all policies, processes, & procedures for preanalytic, analytic, & postanalytic activities. Based on 12 quality systems essentials (QSEs): organization, customer focus, facilities & safety, personnel, purchasing & inventory, equipment, process management, documents & records, information management, nonconforming event management, assessments, continual improvement. Similar to ISO 15189.
ISO 15189: *Medical laboratories-Particular requirements for quality & competence*	Quality management system developed specifically for clinical labs by the International Organization for Standardization (ISO). Based on ISO 17025 (*General requirements for the competence of testing and calibration laboratories*) and ISO 9001 (*Quality management systems-Requirements*). Accreditation to ISO 15189 is mandatory in some countries, but currently voluntary in U.S. The College of American Pathologists (CAP) offers an ISO 15189 accreditation program. It doesn't replace the CAP Laboratory Accreditation Program.
Lean	System developed by Toyota to improve quality by improving workflow & eliminating waste. Focuses on equipment layout, standardization of processes, cross-training, inventory management. Turnaround times are improved by grouping automated analyzers in core lab & replacing batch processing with single-piece flow.

continued...

Quality Management *continued*

SYSTEM	HIGHLIGHTS
Six Sigma	System developed by Motorola to improve quality by determining & eliminating causes of defects/errors & reducing variability in processes. Uses DMAIC methodology (define, measure, analyze, improve, control) to improve processes & statistical methods to measure quality improvements. Six sigma = only 3 errors per million tests. Select employees are trained to become experts & lead efforts, e.g., "Green Belts, Black Belts."
Lean Six Sigma	Quality improvement system that combines principles of Lean & Six Sigma.
Tracer Methodology	Internal audit tool to evaluate quality of patient care by following a specimen through preanalytic, analytic, & postanalytic phases of testing. Used by The Joint Commission & CAP as part of accreditation.

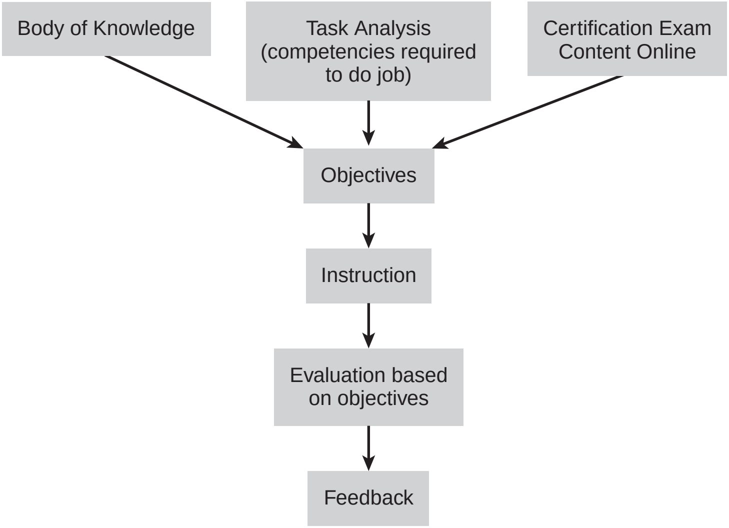
Sentinel Events: The Joint Commission (TJC)

Definition	Unexpected event involving death or serious physical or psychological injury, or risk thereof
Example	Administration of ABO-incompatible blood
Goals	Improved patient care
Requirements	1. Root cause analysis: Analysis of why event happened. Examines proximate causes, e.g., personnel, equipment, environment, leadership, corporate culture, communication, external factors. Focuses on systems/processes, not individuals. 2. Action plan: Establishes risk reduction strategies & measures of effectiveness. Should delineate responsibilities for implementation/oversight & establish time lines. 3. Implementation. 4. Monitoring.
Reporting	Reporting to TJC is optional but encouraged so that event can be added to database & used as educational tool to help others avoid similar events. Confidentiality is maintained.

Point-of-Care Testing (POCT)

Definition	Testing performed at site of patient care. Also known as decentralized, bedside, or near-patient testing.
Goal	Provide rapid test results where immediate medical action is required, e.g., emergency department, intensive care units.
Common POCT tests	Urine reagent strips, glucose, electrolytes, blood gases, activated clotting time (ACT), PT, APTT, hemoglobin.
Regulations	Determined by test complexity. May operate under clinical laboratory's CLIA certificate or separate CLIA certificate.
Optimal staffing	Director: MLS or pathologist responsible for administrative, financial, & technical decisions. Point-of-Care Coordinator (POCC): Oversees testing & responsible for compliance, training, QC, proficiency testing (if required). Designated contact/trainer at each testing site: Liaison between POCC & testing personnel, assists with training/competency assessment. Testing personnel: Qualifications determined by test complexity of testing. Usually phlebotomists, lab assistants, nurses, respiratory therapists.
Considerations	Cost, performance specifications, ease of use, turnaround time, impact on quality & cost of patient care, data management/connectivity/interface capabilities.

Competency-Based Instruction



ABCs of Writing Behavioral Objectives

	COMPONENT	QUESTION(S) ANSWERED	EXAMPLES
A	Audience	Who?	The MLT student; the phlebotomist; the laboratory assistant
B	Behavior	What?	Perform; demonstrate; explain; list; outline; label
C	Criteria	Under what conditions? When? How?	Following the directions in the Blood Bank Procedure Manual; after a lecture, demonstration, & practice session; without use of notes; within 30 min; without coaching
D	Degree	Expected standard of performance. How many? How much? How well?	10 normal & 3 abnormal differentials; in complete agreement with the instructor; with 90% accuracy; within ±15% of reported value; within ±2 SD of instructor

VAK Learning Style Model

STYLE	PREFERENCE	EFFECTIVE TECHNIQUES
Visual	Seeing	Reading assignments, pictures, slides, diagrams, drawings, graphs, videos, demonstrations.
Auditory	Hearing	Lectures, tapes, discussions. Stimulated by changes in vocal tone, pitch, pacing.
Kinesthetic	Doing	Laboratories, role-play, group work.

Domains of Learning

DOMAIN	ELEMENTS	KNOWLEDGE LEVEL	APPLICATION LEVEL	PROBLEM-SOLVING LEVEL
Cognitive	Facts, knowledge	Recall & comprehend facts/information	Apply information in concrete situations	Manipulate information in new situation or context. Analyze, synthesize, evaluate.
Psychomotor	Physical skills	Observe & imitate a skill	Practice a skill	Adapt existing skills to meet new demands or originate new procedures.
Affective	Attitudes, feelings, values	Receive & respond to information about attitudes/feelings	Assess attitudes/feelings	Organize & internalize values into system that guides behavior.

Bloom's Cognitive Taxonomy

LEVEL	ABILITY TO	EXAMPLES OF VERBS FOR OBJECTIVES AT DIFFERENT LEVELS
Knowledge	Recall specific facts	Cite, define, identify, label, list, match, name, pronounce, quote, recite, reproduce, state
Comprehension	Grasp meaning of material	Change, describe, explain, give examples, give main ideas, illustrate, interpret, paraphrase, rephrase, summarize
Application	Use material in new & concrete situations	Apply, classify, compute, demonstrate, predict, prepare, present, show, solve, utilize
Analysis	Break down material into component parts	Analyze, associate, conclude, determine, diagnose, diagram, differentiate, discriminate, distinguish, examine, infer, outline
Synthesis	Put elements together to form new whole	Combine, compile, compose, create, design, develop, devise, generalize, invent, modify, originate, plan, propose, project, revise, rewrite, synthesize, theorize
Evaluation	Judge value of material for given purpose	Appraise, assess, compare, conclude, contrast, critique, deduce, evaluate, judge, weigh

Instructional Methods

INSTRUCTOR CENTERED	STUDENT CENTERED
Lectures	Labs
Demonstrations	Discussions
	Role playing
	Simulations
	Case studies
	Cooperative learning groups
	Writing assignments
	Presentations

Testing at Different Cognitive Levels

LEVEL	DEFINITION	EXAMPLE
Recall	Recognizing or remembering isolated information	Which enzymes are elevated with liver disease?
Application	Interpreting or applying limited data	15 nRBCs per 100 WBCs were observed on a differential. The automated analyzer reported the total WBC as 15×10^6/L. What is the corrected WBC count?
Analysis	Evaluating data, solving problems, or fitting together a variety of elements into a meaningful whole	A patient's RBCs agglutinated in anti-A, but not in anti-B. His serum agglutinated A_1 cells & B cells. What might account for these results & how should you proceed?

Test-Taking Tips

- Set a study schedule & stick to it. Frequent, short study sessions are more productive than long, irregular ones.
- Spend the most time on your weak areas.
- Look at the exam content outline. Don't spend an inordinate amount of time on topics that represent a low percentage of test items.
- If possible, drive to the examination site ahead of the exam so that you know how to get there & how long it takes.
- Don't cram the night before the exam.
- Get a good night's sleep the night before the exam.
- Eat a good breakfast the morning of the exam.
- Review the examination instructions & make sure you have the documentation & materials required.
- Allow plenty of extra time to get to the examination site so that you will be relaxed when you arrive.
- Read the instructions carefully before beginning the test.
- Wear a watch & budget your time. Don't spend an inordinate amount of time on any one question. If you aren't sure of an answer, mark that question & come back to it. Sometimes a later question will jog your memory or provide a clue.
- Read the question & try to think of the answer without looking at the choices, then look for that answer among the choices.
- Read all choices before answering.
- Answer all questions. You have a 25% chance of getting the question right by guessing. You can increase your odds by taking an "educated guess." Eliminate choices that are obviously incorrect.
- Read questions carefully looking for key words such as "best," "most likely," "least likely," and "not."
- Choices with absolute words like "never," "none," "always," "every," or "all" are usually not the correct answer.
- Options "all of the above" or "none of the above" are often the correct answer.
- When all of the choices are numbers, the probability of guessing the right answer is increased by eliminating the highest and lowest numbers.
- Correct options are often longer or more detailed than distracters.
- It is usually best not to change answers.