MEDLAB BASICS · STUDY SHEET

Specimens, Cultures and Susceptibility Testing

Collection, transport and rejection by site, blood, urine, CSF, respiratory, stool and genital cultures, AST methods and breakpoints, MRSA, D-test, ESBL, AmpC, carbapenemases, VRE, expected resistance and reporting

27 sources + more detail

medlabbasics.com/sheets/lab-micro-specimens-ast.html

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1 Collection and transport by specimen

SPECIMEN	COLLECT	TRANSPORT	REJECT OR REDIRECT
Blood culture	2-3 sets (up to 4) per episode, separate draws; adult 20-30 mL per set, 8-10 mL per bottle, aerobic + anaerobic	Room temperature, at once; never refrigerate	Under-filled bottles: culture, but tell the team; avoid draws through catheters (more contamination)
CSF	Tube 2 or 3 (not tube 1) for culture and molecular tests; 0.5-1 mL for bacteria, 5-10 mL for AFB or fungi	Room temperature, immediately; never refrigerate	Bacterial antigen testing is no longer recommended
Sterile fluids (pleural, peritoneal, synovial)	Aspirate in a sterile container; peritoneal fluid at least 10 mL before antibiotics; may also inoculate blood culture bottles	Room temperature, promptly	Swabs dipped in fluid; if only bottles are sent, ask for a tube for the Gram stain
Expectorated sputum	Deep cough, before antibiotics	Room temperature up to 2 h; 2-24 h at 4 °C	Fails the Gram stain screen (mostly saliva); bronchial washes are not for routine bacterial culture
Urine	Midstream clean catch, straight catheter, suprapubic aspirate, or a new catheter port (not the bag)	Culture within 30 min to 1 h, or refrigerate (4 °C), or use a boric acid preservative tube	Unrefrigerated, unpreserved urine past the limit; Foley catheter tips (no clinical value)
Stool, bacterial	Diarrheal stool; rectal swab only when stool cannot be had in time	Enteric transport medium (such as Cary-Blair) when delivery is delayed	Routine enteric culture on patients in hospital more than 3 days (C. difficile testing instead)
Stool, C. difficile	Unformed stool from a patient with at least 3 unformed stools in 24 h	Per assay	Formed stool (unless the physician notes ileus), patients on laxatives, test of cure, repeat within 6 days of a negative NAAT, children under 1 year
Anaerobic culture	Tissue or aspirate from a normally sterile site	Oxygen-free container, room temperature, at once	Sputum, voided urine, stool, superficial swabs; plain swabs when tissue or pus was available
N. gonorrhoeae culture	Urethral (men) or endocervical (women) swab; plate directly when possible	Room temperature, within 1 h; do not refrigerate; CO2 on arrival	Refrigerated or delayed swabs (NAAT remains the screening test)
IV catheter tip	Not recommended at routine removal	-	Do not culture a tip removed without signs of infection

EXAM TIP The two classic "never refrigerate" specimens are blood cultures and CSF for bacterial culture (Neisseria meningitidis and Haemophilus influenzae die in the cold). Gonococcal swabs are cold-sensitive too. Urine and sputum are the opposite: refrigerate them if they cannot be plated promptly.

2 Specimen quality and rejection

- Sputum screen (routine bacterial culture): Gram stain at low power (10x objective, 100x total). The adult ideal is fewer than 10 squamous epithelial cells and more than 25 neutrophils per LPF. Many squamous cells mean saliva: reject and ask for a new deep-cough specimen. Neutrophils cannot rescue a specimen that fails the squamous cell limit.
- The screen applies to routine bacterial culture; laboratories do not reject mycobacterial or Legionella requests on squamous cells.
- Swabs are poor specimens for tissue, fluids, abscesses and deep infections: few organisms, drying, and no anaerobic protection. Ask for tissue, fluid or an aspirate.
- Mislabeled or unlabeled specimens, leaking containers, wrong transport, and delays past the stability limit are rejected under written criteria.
- Before rejecting, call the care team: some specimens (CSF, tissue, operative samples) cannot be recollected. If an irreplaceable specimen is tested anyway, add a comment such as "Specimen cultured; volume inadequate for optimal recovery".

SAFETY Processing priority: CSF for bacterial culture and other irreplaceable specimens (tissue, sterile fluids) go to the front of the line. A positive CSF Gram stain is called to the provider at once.

3 Blood cultures

- Volume, not timing, matters most: adult bacteremia is often low grade. 20-30 mL per set split between an aerobic and an anaerobic bottle (8-10 mL each). If 10 mL or less is drawn, put it all in the aerobic bottle.
- Two or more sets from separate venipunctures let the reader tell infection from contamination. Single sets are discouraged.
- Skin antisepsis with an alcohol-containing agent (alcoholic chlorhexidine or iodine tincture), allowed to dry; wipe bottle tops with 70% isopropyl alcohol.
- Continuous-monitoring systems flag a positive bottle, which gets a Gram stain, a call, subculture and rapid ID or resistance tests.
- Contamination rate: 3% or less is the accepted benchmark; 1% or less has been proposed as the new standard. Initial specimen diversion devices lower it.
- Catheter-related infection: paired sets drawn at the same time through the catheter and by venipuncture. If both grow the same organism and the catheter set turns positive more than 2 h before the peripheral set, catheter-related bloodstream infection is likely (differential time to positivity).

ORGANISM	USUAL MEANING
Coagulase-negative staphylococci, Micrococcus, Corynebacterium, Bacillus (not B. anthracis), Cutibacterium acnes	Contaminant when one bottle or one set of several grows; true infection when several sets drawn in sequence grow the same organism (or with lines and prosthetic material)
Viridans streptococci, enterococci, Clostridium	Variable significance: judge by number of positive sets and the patient
S. aureus, beta-hemolytic streptococci, S. pneumoniae, Enterobacterales, P. aeruginosa, Candida	Not on the contaminant list: full workup and AST, even from one bottle

EXAM TIP For a probable contaminant the laboratory does an abbreviated workup with no susceptibility testing and a report comment (for example "Coagulase-negative Staphylococcus, probable contaminant; call within 2 days if further workup is needed").

4 Urine culture: what a colony count means

SPECIMEN OR SETTING	COUNT THAT MATTERS
Asymptomatic bacteriuria (voided), women	10^5 CFU/mL or more (10^8 CFU/L) of the same organism in 2 consecutive specimens
Asymptomatic bacteriuria (voided), men	10^5 CFU/mL or more in a single specimen
Indwelling catheter, asymptomatic	10^5 CFU/mL or more
Suprapubic aspirate or cystoscopic specimen (arranged with the laboratory)	Counts below 1,000 CFU/mL can matter; a single potential pathogen as low as 200 CFU/mL may be significant
Febrile infant (2-24 months), catheter or suprapubic specimen	More than 50,000 CFU/mL of a single organism (10^4 with reliable pyuria finds more cases)
Symptomatic patient, midstream specimen	Lower counts of one uropathogen can be significant; reports below 10,000 CFU/mL are arranged with the laboratory

- Three or more species usually means contamination at collection: report as mixed flora and ask for a new specimen rather than identifying each.
- Correlate with the urinalysis: pyuria and nitrite support the culture; many squamous cells and no pyuria point to collection carryover.
- A delay at room temperature lets a few contaminants multiply into a "significant" count. That is why the 30-minute to 1-hour limit exists.
- Main uropathogens in the ASCP outline: Enterobacterales, Enterococcus, S. saprophyticus, S. agalactiae, Candida.

5 Stool and genital specimens

TARGET	LABORATORY APPROACH
Salmonella, Shigella, Campylobacter, STEC	Routine on every stool culture; Vibrio and Yersinia usually only on request
STEC	Culture for O157 (sorbitol-MacConkey or chromogenic agar) and test for Shiga toxin or its genes, which also finds non-O157 strains; O157-only tests miss them
Multiplex GI PCR (culture-independent)	Results in hours, but detects DNA from dead organisms too: never a test of cure. Bacterial positives are cultured (here or at the public health laboratory) when an isolate is requested or required
C. difficile	NAAT or toxin-based algorithm on unformed stool only; a positive NAAT in a colonized patient misleads, so the acceptance rules protect its predictive value

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5 Stool and genital specimens CONTINUED

TARGET	LABORATORY APPROACH
N. gonorrhoeae and C. trachomatis	NAAT is the test: vaginal swab (women) and first-void urine (men) are optimal; rectal and pharyngeal swabs too. Culture is needed for susceptibility testing and treatment failure
Urethral Gram stain, symptomatic man	Neutrophils with intracellular gram-negative diplococci are diagnostic (sensitivity above 95%, specificity above 99%); not used for endocervical, pharyngeal or rectal specimens
Trichomonas vaginalis	NAAT is most sensitive; a saline wet mount (sensitivity roughly 40-80%) must be read within 30 min to 2 h
Bacterial vaginosis	Scored Gram stain (or Amsel criteria); culture is not recommended
Group B streptococcus, prenatal screen	Vaginal-rectal swab late in pregnancy (about 36-37 weeks) into selective enrichment broth, then subculture or NAAT from the broth; direct plating misses carriers

6 Susceptibility testing methods

METHOD	RESULT	KEY POINTS
Broth microdilution (reference)	MIC: the lowest concentration that inhibits visible growth	Twofold dilutions in microtiter wells; read after 16-24 h; automated systems (VITEK 2, Phoenix, MicroScan) are miniaturized broth methods
Agar dilution	MIC	Drug in the agar; many isolates per plate; reference method for some organisms
Disk diffusion (Kirby-Bauer)	Zone diameter (mm), read against breakpoints	Mueller-Hinton agar 4.0 ± 0.5 mm deep, 0.5 McFarland lawn, 35 °C; larger zone means a lower MIC
Gradient strip	MIC read where the ellipse meets the strip	Between two marks, read the higher value; beta-hemolysis can be misread as growth and give a falsely high MIC

CATEGORY	MEANING
S (susceptible)	Likely to respond at the standard dose
SDD (susceptible dose-dependent)	Susceptible if exposure is raised by higher or more frequent dosing
I (intermediate)	May work where the drug concentrates (such as urine) or at higher doses; also a buffer for technical variation
R (resistant)	Not inhibited at achievable concentrations
Errors	Very major: a resistant isolate called susceptible (the dangerous one). Major: susceptible called resistant. Minor: involves I

EXAM TIP Breakpoints change (CLSI M100 is revised every year) and the FDA does not always recognize them, so a commercial panel may run older breakpoints. Example from M100: piperacillin-tazobactam for Enterobacterales is S ≤ 8/4, SDD 16/4, R ≥ 32/4 µg/mL. EUCAST calls I "susceptible, increased exposure".

7 Detecting resistance

RESISTANCE	MECHANISM	LABORATORY TEST	REPORT
MRSA (and methicillin-resistant CoNS)	mecA encodes PBP2a, a penicillin-binding protein with low beta-lactam affinity	Cefoxitin 30 µg disk (S. aureus and S. lugdunensis: R ≤ 21, S ≥ 22 mm at 16-18 h; most other staphylococci: R ≤ 24, S ≥ 25 mm at 24 h) or oxacillin MIC (S. aureus and S. lugdunensis R ≥ 4, S ≤ 2; most other staphylococci R ≥ 1, S ≤ 0.5 µg/mL); PBP2a latex; mecA PCR	Oxacillin resistant: resistant to all beta-lactams except the anti-MRSA cephalosporins (ceftaroline, ceftobiprole)
Inducible clindamycin resistance	erm methylase alters the ribosome, switched on by a macrolide (inducible MLSB)	D-test: erythromycin 15 µg next to clindamycin 2 µg; flattening of the clindamycin zone toward erythromycin is a D	D positive: report clindamycin resistant. Round zone (efflux, msrA): clindamycin susceptible
ESBL	Plasmid enzymes (descended from TEM and SHV, among others) that hydrolyze penicillins, extended-spectrum cephalosporins and aztreonam, not cephamycins; inhibited by clavulanate	Screen: reduced susceptibility to cefotaxime, ceftriaxone, ceftazidime, cefpodoxime or aztreonam. Confirm: ceftazidime and cefotaxime with and without clavulanate; a zone increase of 5 mm or more	With current breakpoints report results as tested; M100 asks the laboratory to agree with stewardship whether ESBL results guide therapy
AmpC	Class C cephalosporinase: chromosomal and inducible (Enterobacter, C. freundii, K. aerogenes, Serratia, Morganella, Providencia, Hafnia) or plasmid-borne	Cefoxitin resistance suggests it (not specific); cloxacillin or boronic acid tests; not inhibited by clavulanate	Can mask an ESBL test. Derepressed mutants emerge on third-generation cephalosporins; cefepime is more stable
Carbapenemase (CP-CRE)	Serine: KPC (class A), OXA-48-like (class D). Metallo: NDM, VIM, IMP (class B, need zinc)	mCIM (meropenem disk after incubation with the isolate, on an E. coli ATCC 25922 lawn: 6-15 mm positive, ≥ 19 mm negative, 16-18 mm borderline and read by the M100 colony rule); eCIM adds EDTA (≥ 5 mm larger than mCIM = metallo; read only when mCIM is positive); Carba NP; PCR for blaKPC, blaNDM, blaVIM, blaIMP, blaOXA-48	Infection prevention at once; public health reporting and isolate submission per state rules
VRE	vanA: high-level vancomycin and teicoplanin R, transferable. vanB: vancomycin R, teicoplanin S. vanC: low-level, intrinsic in E. gallinarum and E. casseliflavus	MIC or screening agar; chromogenic agar; vanA/vanB PCR (other genera can carry van genes, lowering PPV)	Report the species: vanC is intrinsic to E. gallinarum and E. casseliflavus; follow the infection prevention policy on whether they count as VRE

8 Expected (intrinsic) resistance

ORGANISM	EXPECTED RESISTANT TO
Klebsiella pneumoniae, K. oxytoca, Citrobacter koseri	Ampicillin (and ticarcillin)
Enterobacter cloacae complex, K. aerogenes, Citrobacter freundii	Ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefoxitin (chromosomal AmpC)
Serratia marcescens	Ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefoxitin, cefuroxime, colistin, nitrofurantoin
Proteus mirabilis	Colistin, nitrofurantoin, tetracyclines
Proteus vulgaris, Morganella morganii, Providencia	Ampicillin, cefazolin, colistin, nitrofurantoin, tetracyclines; also cefuroxime (P. vulgaris) and amoxicillin-clavulanate (Morganella, Providencia)
All Enterobacterales	Benzylpenicillin, glycopeptides (vancomycin), macrolides (azithromycin aside, used for typhoid), clindamycin, linezolid, rifampin
Pseudomonas aeruginosa	Ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefotaxime and ceftriaxone, ertapenem, chloramphenicol, kanamycin, tetracyclines and tigecycline, trimethoprim
Acinetobacter baumannii	Ampicillin, amoxicillin-clavulanate, cefotaxime and ceftriaxone, aztreonam, ertapenem, trimethoprim, fosfomycin, tetracycline and doxycycline
Stenotrophomonas maltophilia	Carbapenems (imipenem, meropenem), aminoglycosides, trimethoprim alone (usually susceptible to trimethoprim-sulfamethoxazole)
Burkholderia cepacia complex	Aminoglycosides, polymyxin B and colistin, many beta-lactams
All gram-positive bacteria	Aztreonam, polymyxin B and colistin, nalidixic acid
Staphylococcus saprophyticus	Novobiocin, fosfomycin, fusidic acid
Enterococcus faecalis	Cephalosporins, clindamycin, quinupristin-dalfopristin, low-level aminoglycosides (synergy still possible)
Enterococcus faecium	Cephalosporins, low-level aminoglycosides
E. gallinarum, E. casseliflavus	Vancomycin (low level, vanC), cephalosporins, clindamycin
Leuconostoc, Pediococcus	Vancomycin and teicoplanin
Listeria monocytogenes	Cephalosporins

EXAM TIP An "unexpected" result (for example vancomycin-susceptible Leuconostoc, or a carbapenem-susceptible S. maltophilia) means check the identification and repeat the test before reporting. Drugs on the expected-resistant list are not tested; they are reported resistant or suppressed.

MORE CSF, sterile fluids and respiratory counts

- CSF: the Gram stain finds organisms in 60-80% of bacterial meningitis before antibiotics and 40-60% after. Draw blood cultures too. Correlate with cell count, glucose and protein (see the Body Fluids page).
- Shunt infections: coagulase-negative staphylococci, Corynebacterium and Cutibacterium are real pathogens here. Shunt or drain parts are cultured only when the patient has signs of CNS infection.
- Pleural fluid in blood culture bottles raises recovery by about 20%, but send a separate tube for the Gram stain and for non-bacterial pathogens.
- Quantitative lower respiratory cultures (ventilated patients): endotracheal aspirate 10^6 CFU/mL, bronchoalveolar lavage 10^4 CFU/mL, protected specimen brush 10^3 CFU/mL. Above the threshold favors pneumonia; below favors colonization. Report the count, not a yes or no.

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