Microbiology Overview

Interpretation of Preliminary Microbiology Data

Gram-positive cocci	**Gram-negative cocci**
Aerobic In clusters ● Coagulase (+): *Staphylococcus. aureus* ● Coagulase (-):*Staphylococcus lugdunensis* or other coagulase-negative Staphylococcus In pairs/chains ● Optochin sensitive: *Streptococcus pneumoniae* ● Alpha-hemolytic: Viridans group *Streptococcus*, *Enterococcus species* ● Beta-hemolytic: ○ Group A Strep (*Streptococcus pyogenes)* ○ Group B Strep (*Streptococcus agalactiae)* ○ Group C, D, G Strep **Anaerobic**: *Peptostreptococcus spp.* and many others	**Aerobic** ● Diplococcus: *Neisseria meningitidis, N. gonorrhoeae, Moraxella catarrhalis* ● Cocco-bacillus: *Haemophilus influenzae, Acinetobacter,* HACEK organisms **Anaerobic:** *Veillonella* spp.
Gram-positive rods	**Gram-negative rods**
Aerobic ● Large: *Bacillus spp* ● Cocco-bacillus: *Listeria monocytogenes, Lactobacillus spp* ● Small, pleomorphic: *Corynebacterium spp* ● Branching filaments: *Nocardia spp, Streptomyces spp* **Anaerobic** ● Large: *Clostridium spp* ● Small, pleomorphic: *P. acnes, Actinomyces spp*	**Aerobic** Lactose fermenting (Lactose positive): ● *Enterobacter spp, Escherichia coli, Klebsiella spp* ● *Citrobacter spp*, Serratia spp** Non lactose-fermenting (Lactose negative): ● Oxidase (-): *Acinetobacter* spp*, Burkholderia spp, E. coli*, *Proteus* spp, *Salmonella* spp, *Shigella spp, Serratia spp*, Stenotrophomonas maltophilia* ● Oxidase (+): *P. aeruginosa, Aeromonas spp.* **Anaerobic**: *Bacteroides spp, Fusobacterium spp, Prevotella spp.*

**Serratia* and *Citrobacter* spp can appear initially as non-lactose fermenting due to slow fermentation.

Interpretation of Key Phrases

- “Gram positive cocci in clusters” may suggest *Staphyloccocus* species.
- "Gram positive cocci in pairs and chains" may suggest *Streptococcus* species or *Enterococcus* species.
- “Gram negative coccobacilli” may suggest *Haemophilus* species.
- “Lactose-positive gram negative rods” may suggest *E. coli Klebsiella* or *Enterobacter spp.*
- “Lactose-negative gram negative rods” may suggest *Pseudomonas*
- “Branching Gram positive rods, modified acid fast stain positive” may suggest *Nocardia* or *Streptomyces* species.
- “Acid fast bacilli” indicates *Mycobacterium* species.
- “Yeast” suggests *Candida* spp. “Round Yeast” suggests *Cryptococcus* spp
- “Fungal elements or hyphal elements” suggest filamentous fungi (moulds).

Quantitation values (rare/few/moderate/many) are reported on some cultures, and indicate the number of a specific bacterium present in the culture. The interpretation of these values depend on a number of factors including: source of the culture, Gram stain results, organism, likelihood that the culture was contaminated based on the organisms that are isolated, number of organisms that grow, and patient gender. When a report says “rare gram-negative rod,” it does not mean an unusual bacterium, it means it was present in low numbers.

Susceptibility Testing

The UCLA microbiology laboratory utilizes standard reference methods for determining susceptibility. Urine isolates are tested by an automated system.

The minimal inhibitory concentration (MIC) represents the concentration of the antimicrobial agent that inhibits the growth of the organism *in vitro*.

The MIC of each antibiotic tested against the organism is reported with one of five interpretations: S (susceptible), I (intermediate), S-DD (susceptible dose-dependent), R (resistant) or NS (nonsusceptible). These interpretations are based on the serum achievable concentration of antibiotic.

The “susceptible” category implies the isolate is inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used.

The “resistant” category implies the isolate is not inhibited by these usually achievable concentrations, OR that the organisms might express a resistance mechanism.

The “intermediate” category indicates that the MIC is approaching the usually attainable concentration, but that response rates may be lower than for a susceptible isolate. Clinical efficacy can potentially be expected in body sites where the drug is concentrated (e.g. aminoglycosides and beta-lactams in the urine) or when a higher dose of the drug can be used (e.g. beta-lactams).

The “susceptible dose dependent” category indicates that the MIC is within the usually attainable concentration, but only if using a dosing regimen that results in higher drug exposures (i.e. by higher doses, more frequent doses, or both, within FDA-cleared prescribing information)

Finally, the “non-susceptible” category is reserved for isolates that only have had “S” criteria assigned, but that have an MIC isolate about this “S” value. A “NS” value does not necessarily mean that the isolate has a resistance mechanism, but rather that it has an unusually high MIC.

MICs which are ½ to ⅛ the breakpoint MIC are more frequently utilized to treat infections where antibiotic penetration is variable or poor (endocarditis, meningitis, osteomyelitis, pneumonia). Similarly, some organisms yielding antibiotic MICs at the breakpoint frequently possess or have acquired a low-level resistance determinant with the potential for selection of high-level expression and resistance. This is the most notable with cephalosporins and *Enterobacter*, *Serratia*, *Morganella, Providencia, Citrobacter,* and *Pseudomonas* spp. These organisms all possess a chromosomal beta-lactamase which frequently will be overexpressed during therapy despite initial *in vitro* susceptibility.

MIC values are interpreted using the Clinical Laboratory Standards Institute (CLSI) breakpoints, which are published yearly. These interpretive standards are based on many factors, including clinical, pharmacokinetic, pharmacodynamic, and microbiological studies. It is important to be aware that although there are many examples of bacteria and antibiotics for which we have CLSI breakpoints (particularly for the most common pathogens), there are some bacteria and antibiotics for which there are no breakpoints. Consultation with the Microbiology Laboratory or Infectious Diseases is strongly encouraged when seeking and interpreting MIC data in these circumstances.

On the microbiology report, these results are interpreted with the % sign, indicating that no breakpoint exists for that drug/bug combination

NOTE: MIC values vary from one drug to another and from one bacterium to another, and thus the MIC values are NOT comparable between antibiotics or between organisms. Do not be tempted to select an antibiotic solely because the MIC is lower than other options.

Selection of Antimicrobial Agents for Testing/Reporting

The laboratory chooses agents to routinely test and report based on:

- Clinical appropriateness for treating infections caused by the species
- Known inherent resistance of some bacteria to some agents
- Body site from which the organism was isolated
- Overall antimicrobial susceptibility profile
- Agents available on the UCLA formulary
- Selective reporting, where results of broad spectrum agents are withheld if narrow spectrum agents within a given class are active
- Cost and toxicity issues

The laboratory only reports results on antimicrobial agents that are documented to be clinically appropriate for the species tested.

Bacteriology

Contaminant vs Pathogen

Source	Pathogens	Likely Contaminants / Normal Flora
Blood - normally sterile Note: The number of cultures drawn versus the number of positive bottles, and the patient's clinical syndrome must be considered when evaluating blood culture results. Multiple positive bottles drawn from a single venipuncture or sequentially through one line are not considered separately when evaluating the potential significance of a likely contaminant.	Any organism isolated	● Coagulase-negative staphylococci ● Alpha-hemolytic (viridans) streptococci ● *Bacillus* spp. ● *Corynebacterium* spp. (**Except *C. jeikeium)*** ● *Propionibacteirum acnes* ● *Micrococcus*
Tissue and body fluids - normally sterile	Any organism isolated; use judgment to evaluate the possibility of normal flora being present in relation to the source of the specimen.	Eye/Ear ● Coagulase-negative staphylococci ● Non-hemolytic streptococci ● Alpha-hemolytic streptococci ● *Corynebacterium* Skin ● Coagulase-negative staphylococci ● *P. acnes* ● *Corynebacterium* ● Alpha-hemolytic streptococci ● *Bacillus* spp.
Urine - normally sterile. Significance of organism is determined by colony count. Urine from stomas/conduits is not sterile	*Enterobacteriaceae* *Enterococcus* spp *Pseudomonas* spp Group B streptococci (in pregnancy) *S. aureus* *S. saprophyticus* Yeast	**Significance determined by colony count** *Corynebacterium* Coagulase-negative staphylcocci Alpha-hemolytic streptococci *Lactobacillus* spp Gram-negative rods *Bacillus* spp

Gastrointestinal Tract	*Salmonella* spp *Shigella* spp *Campylobacter jejuni* *Aeromonas/Plesiomonas* *Yersinia enterocolitica* *Vibrio* spp	*Enterobacteriaceae* *Staphylococcus* spp *Streptococcus* spp *Enterococcus* spp *Pseudomonas* spp Anaerobes Yeast
Respiratory Tract Amount of organism present, source of culture, presence of endotracheal tube or tracheostomy, immune status, and patient age may determine significance as a pathogen.	Group A streptococci *Streptococcus pneumoniae** *S. aureus* (many) *H. influenzae** *Neisseria meningitidis* *Enterobacteriaceae* (many) *Pseudomonas (*many) *Nocardia spp* *Moraxella catarrhalis** (many)	*Staphylcoccus* spp Alpha-hemolytic streptococci Gram-negative rods Beta-hemolytic streptococci other than Group A Saprophytic *Neisseria* spp *Enterococcus* spp *Corynebacterium* spp *Bacillus* spp Yeast Anaerobes *Haemophilus* spp *Micrococcus* spp *Stomatococcus* spp (*Rothia)*

**S. pneumoniae, H. influenze,* and *M. catarrhalis* are all members of the normal respiratory flora and the presence of these organisms in a respiratory culture alone does not necessarily indicate infection.

Specific Cultures

Stool cultures

Stool culture for bacterial pathogens:

- If a stool culture is ordered, the laboratory will screen for *Salmonella*, *Shigella* spp, *Campylobacter* spp.
- The most commonly isolated bacterial pathogen causing bacterial gastroenteritis at UCLA is *Campylobacter jejuni.* If *Yersinia enterocolitica* or *Vibrio* sp are suspected, alert the laboratory as specialized media are required to optimize recovery of these organisms.
- A total of three specimens received on separate days will increase the probability of isolating the etiologic agent in greater than 95% of the cases.
- It is inappropriate to order a stool culture on patients who develop diarrhea after >3 days in the hospital; in these situations, studies have shown that the most common pathogen is *C. difficile,* and the *C. difficile* nucleic acid amplification test (NAAT) should be ordered.

***Clostridium difficile* DNA assay**

- The *C. difficile* testing is performed by nucleic acid amplification test (NAAT) at UCLA.
- This test is >99% sensitive and specific, therefore empiric therapy for patients with negative *C. difficile* NAAT should be avoided.
- Due to the high sensitivity and specificity, repeat testing is unnecessary.

- This test is not indicated for test of cure, as patients may remain positive for 30 days following clinical cure. Many patients will continue to carry the organism without any clinical manifestations of colitis and need no further treatment.
- Only one liquid stool specimen per 7 day period will be accepted for testing.
- Following a positive test result, replicate specimens will only be tested after 10 days.

Infants <1 year of age may be asymptomatically colonized with C. difficile and testing is not performed on this patient population

Blood Cultures

- A minimum of two sets (one set = one anaerobic and one aerobic bottle) should always be obtained. The minimum volume of blood needed per bottle for adults is 10 ml. Thus, the minimum volume of blood per set is 20 ml.
- Ordering one set may lead to confusion if the culture is positive for an organism that is commonly a contaminant. For example, if one set is ordered and is positive for coagulase-negative staphylococci, a common contaminant, it is impossible to determine if this represents contamination or infection. However, if two sets are ordered, and only one is positive for coagulase-negative staphylococci, this most likely represents contamination.
- Multiple sets drawn from a single venipuncture or from a single line draw should not be considered separately.
- Cultures are monitored continuously by the laboratory using automated instrumentation.
- A preliminary report is available as soon as the specimen becomes positive; a final negative report in 5 days.
- Ideally, blood cultures should be drawn before the first dose of antibiotics, but antibiotics should not be withheld because of a delay in getting blood drawn.
- Although it is common practice to wait 30-60 minutes between blood culture draws, there are few data to support this practice. Rather, multiple draws from different venipuncture sites may be indicated.
- If a patient is persistently febrile without a defined source of infection, obtain two sets of cultures per day for 48-72 hours. Do not continue drawing daily blood cultures beyond 72 hours.
- If a vascular catheter is thought to be a potential site of infection, blood should be drawn from the catheter and the periphery. Site and time of phlebotomy should always be noted.

Respiratory Cultures

Lower respiratory tract: Appropriate specimens to identify pathogens causing disease of the lower respiratory tract (tracheitis, bronchitis, pneumonia, lung abscess, and empyema) include expectorated and induced sputum, endotracheal tube aspirations, bronchial brushings, washes, or alveolar lavages collected during bronchoscopy and pleural fluid.

Upper respiratory tract: Appropriate specimens to identify pathogens causing respiratory tract infections include samples from the nasopharynx, throat, oral ulcerations. Nares swabs and nasopharyngeal swabs and washes are not acceptable for routine bacterial culture. If *Bordatella pertussis* or *B. parapertussis* is suspected, a nasopharyngeal specimen should be submitted for *B. pertussis/parapertussis* PCR.

Lower respiratory tract specimens (particularly sputum) are assessed for quality (lack of contaminating oral respiratory tract flora and epithelial cells) through a Gram stain. If the specimen shows a lack of PMNs but many epithelial cells and oropharyngeal flora, the specimen will be rejected by the laboratory and another specimen must be collected for culture.

Mycobacteriology

General: *Mycobacterium* spp. are typically placed into one of three groups based upon their growth characteristics and, in some cases, their phylogenetic relatedness.

1. *M. tuberculosis* complex: This group includes *M. tuberculosis*, *M. bovis, M. africanum, M. microti, M. pinnipedii,* and *M. canettii*. These organisms, which grow very slowly (14-21 days), are of extreme public health importance due to person-to-person spread.
2. Slowly growing nontuberculous mycobacteria: This group includes, among others, the *M. avium* complex (MAC), *M. kansasii,* and *M. ulcerans.*
3. Rapidly growing mycobacteria: This group includes *M. fortiuitum, M. abscessus,* and *M. chelonae.*

Staining: Due to the high mycolic acid content in their cell wall, *Mycobacterium* spp. stain poorly by the Gram stain method. Therefore, AFB smears for mycobacteria are screened by fluorescent Auramine O stain and confirmed positive by the Ziehl-Neelsen stain. Bacteria that stain positive with the Ziehl-Neelsen stain are called acid-fast bacteria (AFB) because they resist decolorization with acidified organic solvents and retain the carbol fuschisin dye, appearing red. The positive acid-fast stain by Ziehl-Neelsen should not be confused with a positive “modified” acid-fast stain, which is used to detect partial or weakly acid-fast aerobic actinomycetes including *Nocardia, Gordonia, Rhodococcus*, and *Tsukamurella*.

Specimen requirements: Typical specimens submitted for *Mycobacteria* spp isolation include sputum, tissue, or sterile body fluid (including blood). It should be noted that specimens submitted on swabs or contaminated with tap water are not acceptable for isolation of *Mycobacterium* spp. Ordering physicians must report positive results to the LA County Public Health Dept.

Timing: Specimens collected for AFB culture are processed at 10 am Mon-Sat by the Microbiology laboratory. This process takes 3-4 hours to perform. Following decontamination, a direct smear is performed on each specimen, and cultures are set up. The smear refers to the acid fast stain, which is also known as a Ziehl-Neelsen stain. Positive smears are called to the ordering physician by the laboratory. The smear is only ~30% sensitive, and many results must await the growth of the organism, which may take up to 8 weeks. Positive cultures are first tested using a DNA probe test (not a PCR-based approach) to detect *Mycobacterium tuberculosis* complex, *M. avium*-complex, and *M. gordonae*. All other *Mycobacterium* species are identified using DNA sequencing.

Susceptibility testing: Susceptibility testing for slow-growing mycobacterial isolates is a sendout test. Susceptibility testing is routinely performed on *M. tuberculosis*. Susceptibilities are <u>not</u> routinely performed on non-*M. tuberculosis* isolates unless requested by the clinician. Turn around time is typically 2-4 weeks. A molecular beacon test is also available for the screening of isonizid (INH) and rifampin resistance in *M. tuberculosis*, and associated with a 3-5 day turn around time; this test is also a send-out. Susceptibility testing is routinely performed in-house for the rapidly growing *Mycobacteria*.

Tuberculosis Detection by PCR: A PCR test is for detection of *M. tuberculosis* complex directly from patient specimens. Approved specimens include sputum and BAL. It is recommended that all patients with a high clinical suspicion of TB be tested by this method because of the high sensitivity/specificity.

Virology

Virus detection: At UCLA there are three general ways in which viral infections can be detected: direct viral antigen detection, serology, or nucleic acid detection.

Nucleic Acid Amplification Test: Viruses that cause respiratory infections can be detected using an FDA-approved Respiratory Viral Panel test. This test is capable of simultaneous detection and identification of eighteen respiratory viruses using a qualitative nucleic acid multiplex PCR assay. Viruses detected by this methodology include Influenza A/B, Respiratory Syncytial Virus (RSV), Human Metapneumovirus, Rhinovirus, Adenovirus, and Parainfluenza virus. The acceptable specimen is a nasopharyngeal swab placed in viral transport media, or 2-3 ml of nasopharyngeal wash, or bronchoalveolar lavage (BAL). The test is performed Tuesdays and Fridays, with additional days added during respiratory virus season. The test should be reserved for patients in whom detection of these viruses will impact clinical management, such as critically ill patients or transplant patients. This test should not be used as a test of cure.

Direct viral antigen detection: HSV and VZV can be detected directly from skin and mucous membrane lesions using a direct fluorescent antibody (DFA) test. Specimens should be collected during the acute phase of the disease. For best results, samples should be collected from early stage vesicular lesions rather than ulcerative or crusted lesions. Information on how to collect an appropriate specimen can be found at http://www.crlonline.com/crlsql/servlet/crlonline, or by phoning the laboratory at extension 42767.

Serology: Both IgM (acute) and IgG antibody titers can be assessed for a number of viral infections including: Epstein Barr Virus (EBV), CMV, measles, mumps, and West Nile Virus (sent out). Detection of antibody to HIV is by chemiluminescent assay and confirmed by Western blot. Contact the laboratory at 42767 for specific questions.

Specimen collection: With a few exceptions (listed below) specimens sent to the laboratory for viral culture should be collected either using a swab transported in viral transport medium or within viral transport media. For instance, cultures for HSV and VZV from lesions should be collected using a swab and send to the laboratory in viral transport medium. It is important to note that these swabs are distinct from the typical swab for bacterial culture. All tissue specimens should be placed in viral transport medium--it is best not to use a swab when collecting tissue. The two exceptions when viral cultures do NOT need to be in viral transport medium are: 2) blood for CMV, EBV; and 3) sterile fluid (such as CSF) where the specimen should not be diluted. Sterile fluids not placed in viral transport media should be kept at 4°C until transported to the microbiology laboratory. However, if both bacterial and viral identification is requested on a sterile body fluid (eg, CSF), the specimen must NOT be refrigerated as the loss of bacterial viability is likely.

Mycology

Specimen collection: The ideal specimens for fungal isolation are tissue, sterile body fluid, or blood. If a tissue specimen is to be tested for the presence of fungi, it is critical that part of the specimen is sent to the microbiology laboratory **before** the specimen is fixed in formalin for histological examination. Sending tissue specimens to both pathology and the microbiology greatly improve the diagnostic value of both findings.

Blood to be tested for fungi should be added to a separate blood culture bottle (Bactec Myco/F Lytic bottle) that is specially formulated for fungal and mycobacterial growth. Note that *Candida* species will grow well in standard conventional bacterial blood culture media and special fungal blood cultures are not necessary. It is important to note that the growth of moulds from specimens that originate from non-sterile sites should be interpreted with caution. In many cases, saprophytic moulds are contaminants.

Timing of reports: Moulds may take 3-4 weeks to grow, whereas yeasts grow rapidly and can usually be identified within 3-5 days. Tissue, biopsy, bone marrow, and autopsy specimens will be finalized at 6 weeks; all other specimens will be finalized at 4 weeks.

Susceptibility testing: Yeast susceptibility testing is performed using a microtiter broth dilution method. It is used to determine antifungal susceptibility (MIC) of rapidly growing yeasts including *Candida* species from sterile sites including blood, CSF, pleural fluid, etc. Mould isolates may be tested for antimicrobial susceptibility in extreme conditions only, and are sent to the San Antonio Fungal Testing reference laboratory for evaluation when requested. Please call the Mycology laboratory if susceptibility testing is desired on a particular isolate (310-794-2770)

Parasitology

Ova and Parasites: An O&P test should be ordered on patients presenting with a history of chronic diarrhea (>10 days), especially if there is appropriate epidemiologic history. It is rarely appropriate to order an O&P test if the patient develops diarrhea while in the hospital. Physicians must report the following organisms to the Public Health Department: *Entamoeba histolytica*, *Entamoeba histolytica/E.dispar*, *Giardia lamblia*, and *Cryptosporidium*. One negative result does not rule out the possibility of parasitic infestation. Up to three specimens collected on separate days should be submitted. Routine ova and parasites is not optimal for *Giardia, Cryptosporidium*, *Isospora*, *Cyclospora*, or microsporidia. Separate tests must be requested for these organisms.

Malaria: Thick and thin blood smears are prepared and evaluated for blood parasites such as *Plasmodium* and *Babesia*. Please call the Parasitology laboratory at 310-794-2770 with any questions. Consultation with the microbiologist on call is recommended in order to ensure the optimal recovery and interpretation of blood smears. STAT smears are available with a 4-hour turn-around time. Note that one negative blood exam does not rule out an infection due to blood parasites. *Babesia* sp and *Plasmodium* sp must be reported to the LA County Public Health Department.

Ectoparasites: The microbiology laboratory identifies insect vectors associated with human disease (e.g. lice, ticks, scabies). Physicians must report positive results for *Sarcoptes scabiei* to the LA County Public Health Department.