

College of Physicians & Surgeons of Saskatchewan
Laboratory Quality Assurance Program

Procedures/Guidelines for the Microbiology Laboratory



January 2010

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QUALITY CONTROL IN MICROBIOLOGY LABORATORIES

Microbiology laboratories shall use quality control procedures to ensure the accuracy, reliability and reproducibility of the various tests used in the isolation, identification and antimicrobial susceptibility testing of microorganisms, and in the performance of serological testing. The extent of quality control testing done will be determined by the scope of clinical testing performed in each laboratory.

All laboratories performing microbiology shall have appropriate internal quality control procedures using CLSI guidelines for antibiotic susceptibility testing and quality control of media.

- a) The most recent update of the following CLSI documents should be followed:
 - (i) **M22** Quality Assurance for Commercially Prepared Microbiological Culture Media
 - (ii) **M100** Performance Standards for Antimicrobial Susceptibility Testing
 - (iii) **M2** Performance Standards for Antimicrobial Disk Susceptibility Tests
 - (iv) **M7** Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically
 - (v) **M11** Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria
- b) Identification panels should be quality controlled according to the manufacturer's recommendations.
- c) ATCC organisms shall be used for proper quality control of media and of antimicrobial susceptibility testing.
CLSI: Clinical and Laboratory Standards Institute
ATCC: American Type Culture Collection

All laboratories performing microbiology shall demonstrate and document their efforts to meet the standards established by CLSI guidelines and quality control using appropriate organisms.

GUIDELINE FOR QUANTITATIVE INTERPRETATION OF GRAM STAINS

Background:

Microscopic examination remains the initial diagnostic test in the processing of specimens in the clinical microbiology laboratory. The timely report of a Gram stain result gives the physician important information about the presence and cause of infection. The Gram stain has a broad staining spectrum and classifies bacteria as either Gram-positive or Gram-negative. Despite the clinical importance of the Gram stain, there are few available standards for the reading and interpretation of this test. The assignment of semi-quantitative and quantitative values to the number of cells and bacteria seen is clearly arbitrary, since published criteria for general use vary dramatically⁽¹⁻⁵⁾. Although laboratories may report only semi-quantitative Gram stain criteria, it is clinically important to have a standardized schema for assigning quantities of cells and bacteria to individual semi-quantitative scores. Since several clinical studies have shown that the presence of moderate to heavy amounts of pus and/or the presence of bacteria on Gram stains correlates with the presence of infection⁽⁶⁻⁸⁾, information reported from Gram stains of specimens needs to be accurate.

Principle:

To review current practice in Canada and evidence from the literature to establish a current best practice approach to the quantitative interpretation of Gram stains for all types of clinical specimens.

Recommendation:

Although there are several published Gram stain reporting criteria, the Canadian Coalition for Quality Laboratory Medicine recommends that:

- Laboratories use:
 - One set of interpretive criteria for all types of clinical specimens except for sputa and vaginal smears for bacterial vaginosis.
 - Separate published specimen rejection criteria for sputa.
 - Standard criteria for diagnosis of bacterial vaginosis^(9, 10).
- Laboratories specify the criteria used when reporting Gram stain results to facilitate interpretation by physicians.

Gram Stain Quantification Criteria:

1) Grading System for Assessing Sputum Quality*:

i) Q score₁:

Type of cell and #/low power field (x100)	Grade
Neutrophils: <10 10 - 25 >25	0 +1 +2
Mucous present	+1
Squamous Epithelial cells: 10-25 >25	1 2

Rejection criteria: Reject specimens with an average score of 0 or less.

To determine average score, count the number of neutrophils and epithelial cells in up to 20 fields and assign the appropriate positive or negative grade for each cell type in each field. Total the individual grades and divide by the number of fields examined to arrive at the average score.

*Grading system is only applicable to sputa from adults (>14 years)^{12, 13}. Patients who are neutropenic may not demonstrate purulence and a clinical history shall be provided to the laboratory so that sputum specimens are not rejected.

ii) Number of Squamous epithelial cells (SEC)₁₁.

Examine 20-40 fields under low power. Average the number of cells in representative fields that contain cells.

Rejection criteria: 10 SEC / LPF

Acceptable: <10 SEC / LPF

NOTE: if number of PMN is 10X the number of SECs and there is 3-4+ of a single morphotype accept the specimen for culture.

2) All Other Specimen Types:

Epithelial or Polymorphonuclear (PMN) Cells		Microorganisms (bacteria, yeast/fungi)	
Description	Number/LPF	Description	Number/OIF
1+, rare	<1	1+, rare	<1
2+, few	1-10	2+, few	1-10
3+, moderate	11-25	3+, moderate	11-25
4+, many	>25	4+, many	>25

Low-power field (LPF) ($\times 100$); high-power oil immersion field (OIF) ($\times 1000$).
Numbers are based on an average of 10 fields.

A thorough examination of all areas of the Gram-stained smear should be made microscopically first under LPF ($\times 100$) to observe any inflammatory cells and then under OIF ($\times 1000$) to observe bacteria.

NOTE: For fluid specimens where cytopspin or similar preparations are used, the quantification guidelines do not apply.

3) QA Criteria for Gram Stain Interpretation:

For scoring Gram stains a standard error classification schema is recommended as follows:

MINOR ERROR:

- Report the presence of polymorphs when not present

MAJOR ERROR:

- Report the presence of an organism not present or;
- Failure to report the presence of 1+ to 2+ polymorphs

VERY MAJOR ERROR:

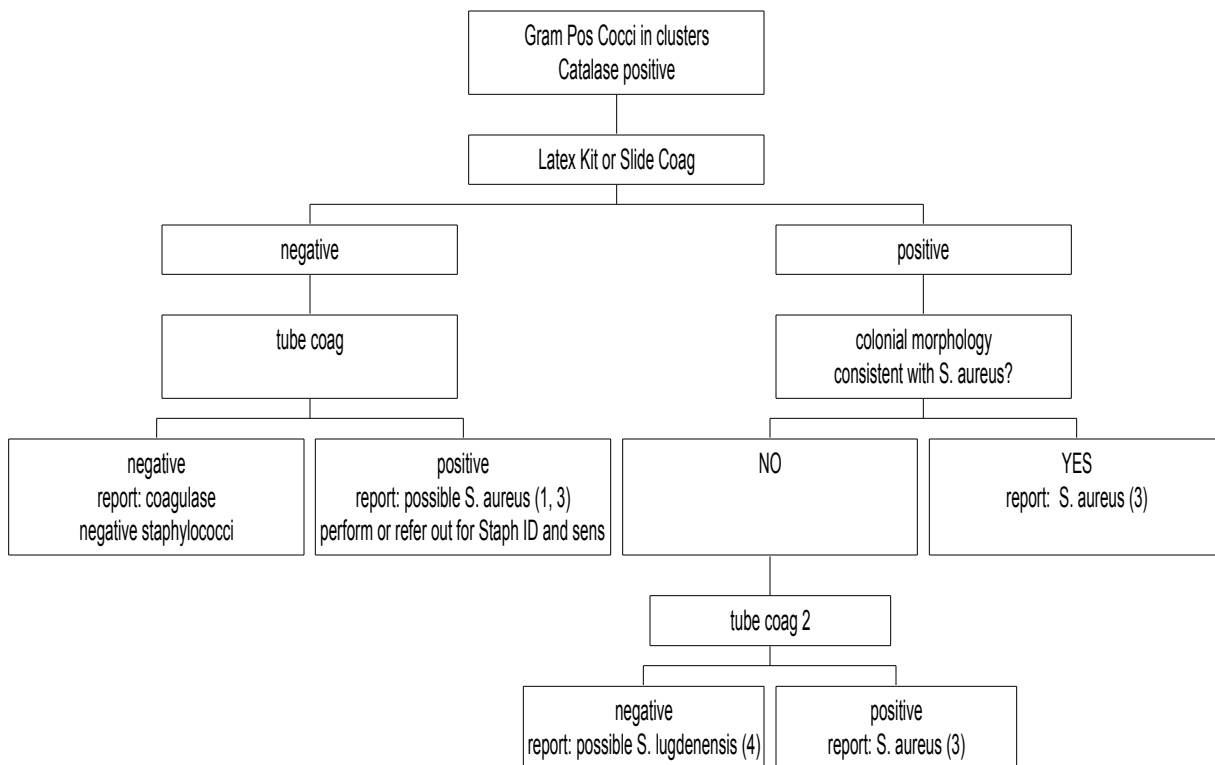
- Failure to report the presence of a microorganism
- Failure to report the presence of 3+ to 4+ polymorphs

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Staphylococcus Identification Scheme



1. Some colonies of MRSA and *S. intermedius* are clumping factor negative.
2. “False” positive Staph aureus results can be obtained with *S. saprophyticus*.
3. Remember to do an Oxacillin plate to rule out MRSA.
4. Refer for full staph identification.

COAGULASE NEGATIVE STAPHYLOCOCCUS SPECIES (CNS)

Background:

Traditionally, medical technology students were taught that there were two types of staphylococci. The first group was coagulase positive (ie. *S. aureus*) and caused infection. The second group was coagulase negative staphylococci (CNS), and with the exception of *S. saprophyticus* and *S. epidermidis*, did not cause infection.

As with all traditions, time brings change. Advances in medical technology have led to more invasive techniques. Patients may have one or more of a variety of medical devices, such as catheters and prosthetic device implants. Chemotherapy and immunodeficient diseases have led to an increase in the number of immunocompromised patients. IV drug users are at increased risk of infection due to CNS. These and other factors have allowed CNS to take on a new role as a major nosocomial pathogen.

The majority of CNS isolates are found in hospitalized patients and are often resistant to antibiotics. Methicillin resistant CNS are more frequently isolated than MRSA in a facility. CNS is a predominant part of the normal skin flora, and may be present in a specimen as a contaminant or a pathogen. Some species of CNS produce a slime layer, allowing them to adhere to medical devices such as catheters, shunts, heart valves, graft material, implants and artificial joints. These factors also make the organism more difficult to treat, and difficult to determine clinical significance when isolated in a specimen.

S. epidermidis is the most common nosocomial CNS. Infections include bacteremia, post-operative cardiac infections, and infections of shunts, orthopedic devices and prosthetic implants. It has been isolated in patients undergoing Continuous Ambulatory Peritoneal Dialysis. There seems to be no clear standard on the reporting of *S. epidermidis* in urines. It is a recognized pathogen in patients with prostatic infection, prosthetic devices or post transplantation. It rarely causes urinary tract infections in females.

S. saprophyticus is an important urinary pathogen, especially in young, sexually active females. Males may also develop nonspecific urethritis. It can usually be quickly differentiated from other CNS by using a novobiocin disk. *S. saprophyticus* is resistant, while other CNS are sensitive.

S. lugdenensis was first described in 1988 in France. It is a CNS that can be misidentified as *S. aureus* if the slide coagulase test is the only test used. The organism is slide coagulase positive, tube coagulase negative, PYR positive and ornithine decarboxylase positive. It is also very susceptible to penicillins. It closely resembles *S. aureus*, in that it has similar virulence factors and produces similar types of infections. Most reported infections are skin and soft tissue infections, and blood and vascular catheter infections. The organism is part of the normal skin flora.

S. schleiferi can also be misidentified as *S. aureus* if only the slide coagulase test is used. It is slide coagulase positive, tube coagulase negative, PYR positive, and ornithine decarboxylase negative. It too appears to be a significant opportunistic pathogen associated with empyema, wound and IV catheter infections and bacteremia. As with *S. lugdenensis*, many affected patients have defective immune systems due to underlying disease.

S. haemolyticus has been reported to be the second most commonly isolated CNS and has been isolated in endocarditis, septicemia, UTIs, bone, wound and joint infections.

Other CNS less commonly isolated include *S. hominis*, *S. warneri*, *S. capitis*, *S. simulans*, *S. cohnii*, *S. xylosum*, and *S. saccharolyticus*.

Procedure:

A review of the literature suggests that CNS be identified to species:

- when isolated from normally sterile sites-blood, CSF and joint and other fluids.

Regarding blood cultures, a CNS isolated from one bottle only is considered to be a contaminant. It is not necessary to identify or report susceptibility results. Laboratories may store the isolate in case further testing is requested. Isolates from several bottles are identified to species level. If the same species is isolated, it is a significant finding and susceptibility testing should be performed. If different species are identified, it is probably a contaminated set of cultures and susceptibility testing should not be performed. CNS can be contaminants in CSF and other sterile fluids, therefore always correlate with clinical information and direct gram smear results to determine the significance of the isolate.

- when repeatedly isolated from wound specimens with a good gram smear (showing the presence of GPC and WBC).
- when isolated from shunts, catheters, or prosthetic devices.
- to differentiate CNS from *S. saprophyticus* in midstream urines.
- when isolated from catheter or cystoscopy urine specimens

It is not appropriate or cost effective to do identification and susceptibility testing on every CNS isolated. Each laboratory should look at their particular situation and establish policies specific to their needs. If the CNS is isolated as part of the normal flora, then it should be reported as such-normal flora. Although a review of the literature and laboratory practice shows that there is consensus that full ID & susceptibility testing is not always required, there is no absolute consensus as to when ID & susceptibility testing should be performed. It remains the responsibility of the laboratory to look at patient demographics, type of service, etc and decide on their policies. It is advisable to consult with a Medical Microbiologist or Infectious Disease specialist when developing these policies.

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BACTERIOLOGY CULTURE PLANTING PROCEDURES

Media Use:

Most media used in clinical bacteriology laboratories are commercially available, either completely prepared or as dehydrated powder. The number and types used vary from laboratory to laboratory depending on budgets, personnel and personal bias. Media selection is governed by the specimen source. Media that allow growth of the majority of potential pathogens from a particular source are used to recover the pathogen(s).

Inoculation and Isolation Techniques:

Traditional identification methods depend on obtaining isolated colonies. Many clinical specimens contain a mixture of bacteria; therefore specimens are streaked to produce single colonies of each organism type present. Isolation techniques include streaking the inoculum with a wire loop or spreading with a glass rod, or, inoculating pour plates. The choice depends on the material to be cultured, the result desired, and the laboratory's preference.

Streak Plate:

The streak plate method is practical and fast. All streak plate methods are designed to provide a continuous dilution of the specimen in order to give isolated colonies. Either a stainless steel or nickel and chromium (Nichrome) wire or a plastic bacteriologic loop is acceptable, although Nichrome should not be used for anaerobic bacteria. In the most common method a portion of the specimen is deposited in one quadrant of an agar plate free of moisture.

The method of transferring the inoculum to the plate depends on the specimen type. Liquid specimens are usually inoculated by transfer with a sterile pipette or syringe and needle. One or 2 drops are placed in the first quadrant of the plate. Urine is an exception and must be plated quantitatively. (see below)

Specimens received on swabs can be planted directly by rolling the swab over an area about 2 cm in diameter in the first quadrant of the plate.

Faeces and sputum are most easily inoculated by dipping a swab into the specimen rather than attempting to use a loop to inoculate plates.

After implantation of the inoculum, a wire loop is flamed and cooled, or a sterile plastic disposable loop is selected. The loop is held between the thumb and index finger and passed at a 90 degree angle several times through the initial inoculum into the second quadrant of the plate as illustrated in Figure 1 (streak area 1). The plate is turned 90 degrees, and the process is repeated, streaking into the third quadrant (streak area 2), and finally – after another 90 degree turn – into the fourth quadrant (streak area 3). The loop is flamed between quadrants unless the inoculum is light or the medium is selective or inhibitory. When streak plates are used, the laboratory can report relative numbers of

bacteria present, which may be helpful to the clinician, particularly if different organism types are present.

Several methods of semi-quantitation are used (Figure 2a & b). Some laboratories use very few, few, moderate or many; others use 1+ to 4+, based on the following criteria:

Score	Number of Colonies in Streak Area		
	1	2	3
1+	<10		
2+	<10	<5	
3+	>10	<5	>5
4+	>10	<5	>5

Quantitative Methods for Urine:

To determine urinary tract infection, the actual colony count of a urine specimen is necessary (one colony equals one colony-forming unit, or CFU). For a colony count a calibrated loop is used.

A calibrated loop of 1 or 10 µl is dipped vertically into a well-mixed specimen. One loopful is streaked down the center of a plate. Without flaming, cross-streaks at a 90 degree angle are made perpendicular to the original streak. Cross-streaks should be close together and extend toward the edge of the plate.

1 µl loop is commonly used in Clinical Microbiology laboratory for urine culture. In some situations, as in urines obtained during cystoscopy, or by suprapubic aspirate (SPA) culture using a 10µl loop will be needed in order to identify a lower count of bacteria. The number of colonies growing on the agar plate multiplied by the dilution factor (10^3 if a 1 µl loop is used and 10^2 for 10µl loop) equals the number of CFU per millimetre in the original specimen.

Culture Incubation:

Inoculated plates are incubated inverted to prevent the water of condensation from dropping onto the agar surface, which can result in the colonies coalescing and is a safety hazard.

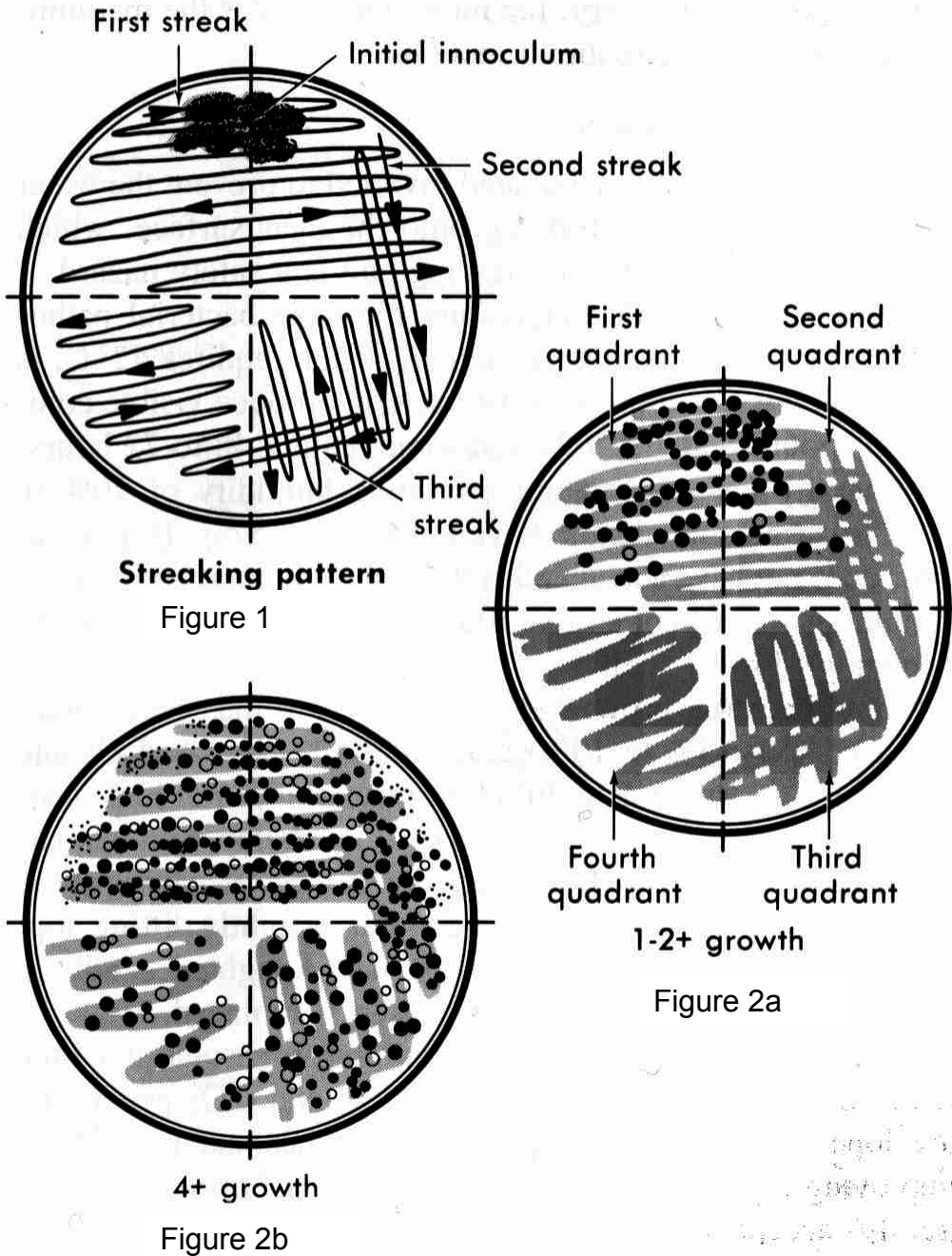
The optimal growth temperature for most bacterial pathogens is 35°C. Most bacteria produce visible colonies in about 18 hours, although some require 48 to 72 hours.

Generally bacteria require a relative humidity of 70 to 80%. Most new incubators have a water reservoir. If an incubator does not have a mechanism for humidity regulation, a pan containing water kept at a constant level can be placed on the incubator's lowest shelf.

Many bacteria grow well in air but some need either increased CO₂ (5 to 10%) (capnophilic), decreased O₂ and increased CO₂ (microaerophilic), or anaerobic

conditions. Carbon dioxide may be provided by a CO₂ incubator, a candle jar, or a container with a CO₂ –generating device. A candle jar is simply a wide-mouthed container with a tightly fitting top. Inoculated media are placed in the jar. A lighted candle is placed on top of the media, and the top is replaced.

Reference:
Clinical and Pathogenic Microbiology



URINE SPECIMEN PROCESSING

Background:

Urinary tract infections (UTI) are caused by a range of common bacteria, often derived from the enteric or skin flora. Diagnosis of UTI is accomplished by semi-quantitative culture of urine and antimicrobial susceptibility testing of potential pathogens present in significant numbers. This procedure describes the collection of appropriate specimens and their transport to the laboratory.

Specimen Collection and Transport:

Patient preparation: Instruct the patient to collect a mid-stream specimen of urine into the specimen container provided. Mid-stream urine is used because the first few ml of urine passed will contain skin flora from the urethral orifice and will yield potentially misleading results. Pre-cleaning of the periurethral area is not recommended (Manual of Clinical Microbiology 9th Ed.). The optimal timing for specimen collection is at the first voiding of urine in the morning.

Collection, Handling, Storage: Urine is collected into sterile containers. These may be sterile plain bottles or sterile bottles containing boric acid, which is bacterostatic and limits overgrowth during transport. Specimens should be transported immediately to the laboratory. If the delay between collection and culturing is more than 2 hr, specimens not in preservative must be refrigerated.

Acceptable specimens: Midstream urines, catheterized urines, suprapubic bladder aspirates and cystoscopy specimens, properly labeled and received within 24 hr of collection.

Reject the following specimens, which are unsuitable for culture:

1. Bedpan urines
2. Condom/Texas catheter urines
3. Specimen received in non-sterile container
4. Bag urine
5. Unlabelled and improperly labelled specimens
6. Specimens that have leaked out of the container

Inform the ward or clinic when rejecting specimens.

Procedure/Media:

Media Plating Procedure:

1. Mix urine well.
2. Hold calibrated loop vertically and immerse the loop just below the surface of the urine.

3. Spread the urine over the surface of the agar plate as described in Bacteriology Culture Planting Procedure. *Media generally used are Blood Agar and MacConkey agar or a single media that will support the growth of uropathogens.*
4. Incubate plates aerobically overnight at 35°C.

Specimen Type	Calibrated Loop	Media	Incubation
MSU	1_1 loop	Blood Agar (BA)	O ₂ , 35°C x 24 hrs
Catheterized urine	1_1 loop	MacConkey Agar (MAC)	O ₂ , 35°C x 24 hrs
Bladder, Suprapubic or Cystoscopy Urine	10_1 loop		

Dip 'n' Count Procedure:

1. Mix urine well.
2. Remove lid with the agar paddle from the dipslide container. Do not touch the agar surfaces.
3. Immediately (within 1 hour) dip paddle of dipslide into the urine so both sides of agar paddle are immersed (fully covered). Alternately pour urine over both sides of the agar paddle to cover both agar surfaces.
4. Drain excess urine from the agar paddle by touching edge of paddle against the edge of the collection container or drain by touching a filter/blotting paper.
NOTE: The agar on the paddle should not touch any other surface. DO NOT put urine into the dipslide container. Excess urine in the container can re- inoculate the dipslide, causing falsely increased bacterial counts.
5. Replace lid and paddle into dipslide container. Label container.
6. Send dipslide to the laboratory as soon as possible.

Interpretation of Cultures:

1. Inspect the plates and dipslides for growth and determine the colony count (refer to Standardization of the Colony Count for Urine Cultures).
2. Identify isolates of potential pathogens present in significant numbers according to laboratory identification protocol.
3. Perform antimicrobial susceptibility tests according to the Antibiotic Reporting Guideline and laboratory protocol.

Potential Pathogens in Urine Specimens:

Enterobacteriaceae such as *E. coli*, *P. mirabilis*, *Klebsiella sp.*
Pseudomonas aeruginosa
Staphylococcus aureus
Staphylococcus saprophyticus
 Coagulase Negative Staphylococcus
 -haemolytic Streptococci
Streptococcus pneumoniae

<i>Enterococcus</i> spp. <i>Listeria monocytogenes</i> Yeast <i>Candida</i> species

These organisms are normally considered contaminants, but may be significant upon repeat isolation:
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<i>Lactobacillus</i> spp. -haemolytic Streptococci Most Gram positive rods such as Diphtheroids

NOTE: ANY FEMALE WITH GBS BACTERIURIA IN ANY CONCENTRATION DURING HER PREGNANCY SHOULD RECEIVE INTRAPARTUM ANTIBIOTICS. (SOGC Clinical Practice Guideline No. 149, J. Obstet. Gynaecol. Can. 2004; 26:826-832)

References:

Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller, Manual of Clinical Microbiology, 9th ed.: American Society for Microbiology, 2007.

STANDARDIZATION OF THE COLONY COUNT FOR URINE CULTURES

In order to standardize the interpretation of the colony count, the Microbiology Quality Assurance committee recommends the adoption of the following criteria, as minimum guidelines. These are based on the information gathered from the survey process, and from the references provided.

Recommended Standardization of the Colony Count:

Detected Growth	In-dwelling Catheter Midstream Urine 1µl loop	In & Out Catheter 1µl loop	Bladder Urine Suprapubic or Cystoscopy 10 µl loop
1 organism isolated	<10 ⁷ cfu/L No significant <u>growth</u> ** 10 ⁷ cfu/L ID & Sens	ID & Sens any amount of growth	ID & Sens any amount of growth
2 organisms isolated	10 ⁷ cfu/L 2 probable pathogens in equal amounts ID & Sens both	≥10 ⁷ cfu/L ID & Sens both probable pathogens	ID & Sens any amount of growth
2 organisms with one predominate organism	ID & Sens predominant org only 1 org >10 ⁷ cfu/L 2 nd org <10 ⁷ cfu/L	1 org >10 ⁷ cfu/L 2 nd org <10 ⁷ cfu/L	ID & Sens any amount of growth
>2 organisms	Mixed growth - probable contamination	Mixed growth - probable contamination	ID & Sens any amount of growth

**Exceptions include symptomatic males, adult females with urethritis, patients on antibiotic therapy, patients with chronic or reoccurring infection, & children under 12 years of age. Isolates from specimens with positive leukocytes and/or nitrites will be worked up when urine strip values are provided.

Calculation for the Interpretation of the Colony Count:

The colony count is multiplied by 1000 (when 1µl calibrated loop is used) or 100 (when 10 µl calibrated loop is used) to provide an estimate of the number of colonies per ml. This number is multiplied by 1000 to equal the number of Colony Forming Units per litre.

References:

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THROAT SWABS

Background:

Throat swab specimens are most commonly collected to diagnose infections caused by *S. pyogenes* (Group A haemolytic streptococcus) and occasionally sequelae and should be reported.

Throat swabs can also yield large (not pinpoint) colony-forming beta-hemolytic streptococci harboring the Lancefield Group C or Group G antigen belonging to *Streptococcus dysgalactiae* subsp. *equisimilis*. They are associated with upper respiratory tract infections similar to *S. pyogenes*, infections. Cases of glomerulonephritis and acute rheumatic fever have been reported. In work up of throat swabs submitted for acute pharyngitis, Group C and Group G need to be reported.

Culture of any other suspected agents of disease such as: *Arcanobacterium hemolyticum*, *N. gonorrhoeae*, *C. diphtheriae* shall be performed only at the request of the ordering physician.

Specimen Collection:

A sterile swab is used to collect the throat swab from the posterior pharynx, tonsils and/or inflamed areas. The swab is placed into the appropriate transport media, for C & S use Modified Amies.

Storage Requirements: Place specimens at room temperature if to be processed within 2 hours, if processing to be delayed place into 4° C fridge.

Procedure\Media:

A. Processing of Specimens:

Culture: On the following media, and incubate plate as indicated:

<u>Media</u>	<u>Incubation</u>
Blood Agar(BA) or selective media such as Beta Strep Agar	CO ₂ 35°C x 48 hours
	AnO ₂ 35°C x 48 hours or /
	AnO ₂ 35°C x 24 hours and then CO ₂ 35°C x 24 hours

* Some institutions have better recovery incubating anaerobically.

Commercial kits for Group A streptococcus (rapid direct tests) are also available. If a laboratory implements the rapid direct test, two throat swabs should be collected. If the rapid test is positive the second swab may be discarded; but if the rapid test is negative the second swab should be cultured, because the sensitivity of these direct tests are generally <100%.

B. Interpretation of Cultures:

The medium used to isolate *S.pyogenes* and other beta-hemolytic streptococci is sheep blood agar with 5% blood, or selective agar containing agents that will suppress the normal flora of the throat. Anaerobic incubation of the plates may enhance the beta-hemolysis, thereby making the colonies more easily detectable. If there are no colonies demonstrating beta-hemolysis after 24 hours incubation, reincubate plates for another 24 hours. If beta-hemolysis is present, perform catalase and if negative, perform PYR & latex agglutination testing.

C. Susceptibility Testing:

Antimicrobial susceptibility testing of these isolates is not required unless the patient is known to be allergic to penicillin. Perform antimicrobial susceptibility tests according to the Antibiotic Reporting Guideline and laboratory protocol.

Reporting Results:

Culture: Quantitate all significant isolates.

- i. If culture is negative, report out as 'No Group A, C or G Streptococcus Isolated'.
- ii. If culture is positive for Group A Streptococcus, report 'Group A Streptococcus Isolated'.
- iii. If either Group C or Group G Streptococcus is positive report out as 'Group C Streptococcus' or 'Group G Streptococcus Isolated'.

References

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CONJUNCTIVAL SWABS

Background:

Conjunctival swabs are collected for the diagnosis of conjunctivitis. Swabs should be sent from both the infected and non-infected eye. This allows comparison of flora and helps determine pathogenicity of any organisms isolated.

Specimen Collection and Transport:

Swabs should be collected prior to the administration of drugs such as topical anesthetics or antibiotics. The swab should be sent in Amies transport medium.

Procedure\Media:

A. Processing of Specimens:

- a) Direct Examination of Gram stained smear – Note the presence of polymorphonuclear cells and organisms. Quantitate as per Guideline for Quantitative Interpretation of Gram Stains.
- b) Culture on the following media, and incubate the plates as indicated.

Media	Incubation
Blood Agar (BA)	CO ₂ , 35°C x 48 hours
Chocolate Agar (CHOC)	CO ₂ , 35°C x 48 hours
G. C. Selective Agar (neonates)	CO ₂ , 35°C x 72 hours

Interpretation of Cultures:

Examine the BA and CHOC plates after 24 hours and 48 hours incubation and the G.C. plate after 48 and 72 hours incubation. Compare growth from infected and non-infected eye cultures. This comparison will often provide hints of which organisms are pathogens. Potential pathogens: *S aureus*, *H.influenzae*, *M. catarrhalis*, *N. gonorrhoeae*, *S. pyogenes*, *S. pneumoniae*, *Moraxella* species, and *P. aeruginosa* . For other organisms, a significant result is determined by the isolation of a moderate or heavy growth of an organism that is seen as the predominant organism on the Gram stain. There should be $\geq 1+$ polymorphonuclear cells on the Gram smear. Full identification is required to the species level for all significant organisms.

B. Susceptibility Testing:

Perform antimicrobial susceptibility tests according to the Antibiotic Reporting

Guideline and laboratory protocol.

NOTE: There is no method for testing of topical agents.

Reporting Results:

- a) Gram stained smear: Report with quantitation the presence of polymorphonuclear cells and organisms.
- b) Culture: Quantitate all significant isolates and report with antimicrobial susceptibilities. If normal flora is also present, report with quantitation.

References:

1. Cumitech BA. Sep 1994. ASM Press, Laboratory Diagnosis of Ocular Infections
2. Bailey and Scott's Diagnostic Microbiology, 11th Edition 2002. Mosby Inc.

SPUTUM /LOWER RESPIRATORY CULTURE

Background:

Lower respiratory tract specimens are primarily submitted for the detection of the causative agent of pneumonia. The most common specimen type is expectorated or induced sputum. Bronchial lavage, washes, brushings and transtracheal aspirates may also be received but require additional handling procedures. The most common causative agents of bacterial pneumonia are: *S. pneumoniae*, *H. influenzae*, *S. aureus*, *M. catarrhalis*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*.

Specimen Collection and Transport:

Collect expectorated or induced sputum into a sterile sputum container (early morning specimens generally more suitable).

Storage Requirements: Specimens should be delivered promptly (within 2 hours) to the lab. If a delay in processing is unavoidable the specimen must be refrigerated at 4 °C.

Procedure/Media:

A. Processing of Specimens:

- a. Assess the quality of the specimen as per Guideline for Quantitative Interpretation of Gram Stains to determine acceptability for culture.
- b. Culture on the following media, and incubate plate as indicated.

Media	Incubation
Blood Agar(BA)	CO ₂ , 35°C x 48 hours
MacConkey Agar (MAC)	O ₂ /CO ₂ , 35°C x 48 hours
Chocolate Agar (CHOC)	CO ₂ , 35°C x 48 hours

Using a sterile swab or loop enter the specimen container and obtain a purulent portion of the specimen. Plant each plate with the specimen following Bacteriology Culture Planting Procedures.

B. Interpretation of Cultures:

After 24 hours incubation the plates are examined and if needed, tests are performed to determine the organism(s) present. Any plates with significant growth of a potential pathogen must be quantified, identified, and have susceptibility testing done. Plates showing only normal flora are then reincubated for another 24 hours and re-read the

following day. If any changes are noted do the appropriate testing required to identify the organism(s) and perform sensitivity testing.

C. Susceptibility Testing:

Perform antimicrobial susceptibility tests according to the Antibiotic Reporting Guideline and laboratory protocol.

Reporting Results

- a) Gram stained smear: If the specimen is suitable for culture, report the gram stain smear result. Report with quantification the presence of polymorphonuclear cells, squamous epithelial cells and organisms.

If the specimen is unsuitable for culture according to the Q score, report the gram smear result and also comment: "Gram smear indicates the sputum specimen is not suitable for culture. Please resubmit". (*refer to: Guideline for Quantitative Interpretation of Gram Stains*)

- b) Culture: Quantify all significant pathogens and report with appropriate antimicrobial susceptibilities. If only normal oral flora present, report with quantification: "Growth of Oropharyngeal Flora".

Limitations Of The Procedure:

- 1) Poor quality specimen will not be cultured.
- 2) Procedure does not allow for isolation of other agents such as *Legionella* sp. Submit urine specimen for antigen detection to the Saskatchewan Disease Control Laboratory (SDCL). SDCL will culture *Legionella* sp. and/or bronchoalveolar lavage (BAL).

References:

Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller, Manual of Clinical Microbiology, 9th ed., 2007: American Society of Microbiology

EAR SWABS

Background:

Ear swabs are collected for the diagnosis of otitis externa. Otitis externa is a bacterial infection of the external auditory canal usually caused by *P. aeruginosa*, *S. aureus*, Group A streptococcus, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* or fungus/yeast. Tympanocentesis fluid is required for the diagnosis of otitis media, which is cultured as a deep wound.

Specimen Collection and Transport:

The ear swab should be collected using sterile swab and sent in Amies transport medium.

Procedure\Media:

A. Processing of Specimens:

- a. Direct examination: Gram stained smear – note presence of polymorphonuclear cells organisms. Quantitate as per Guideline for Quantitative Interpretation of Gram Stains.
- b. Culture on the following media, and incubate plate as indicated.

<u>Media</u>	<u>Incubation</u>
Blood Agar(BA)	CO ₂ , 35°C x 48 hours
MacConkey Agar (MAC)	O ₂ /CO ₂ , 35°C x 48 hours
Fungal Plate (ie: Sabouraud Dextrose Agar, Fungal Selective Agar, Phytone)	O ₂ , 30°C x 48 hours optional up to 4 weeks
Chocolate Agar (CHOC)	CO ₂ , 35°C x 48 hours

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 hours incubation. Potential pathogens are *S. aureus*, *P. aeruginosa*, Group A streptococcus, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* or yeast. For specimens from neonates only, identify and report Gp. B. Strep. For other organisms, a significant result is determined by the presence of a moderate to heavy growth of an organism which is seen as the predominant organism on the Gram smear. The Gram smear should also show 1+ polymorphonuclear cells. Fungal plates may be incubated at 30° for 4 weeks.

C. Susceptibility Testing:

Perform antimicrobial susceptibility tests according to the Antibiotic Reporting Guideline and laboratory protocol.

Reporting Results:

- a) Gram Stained smear: Report with quantitation the presence of polymorphonuclear cells and organisms.
- b) Culture: Quantitate all significant isolates and report with appropriate antimicrobial susceptibilities. If normal skin flora is also present, report as normal.

References:

1. Cumitech 10. Dec 1979. ASM. Press, Laboratory Diagnosis of Upper Respiratory Tract Infection
2. Bailey and Scott's Diagnostic Microbiology, 11th Edition 2002. Mosby Inc.
3. see Antibiotic Reporting Guideline - www.quadrant.net/cps

GENITAL TRACT SPECIMENS

Background:

Genital specimens are usually taken to diagnose infection or to rule out specific sexually transmitted diseases. Certain pathogens are known to be associated with specific types of genital infections. The physician will base the selection of culture requests on the type and location of infection.

Specimen Collection and Transport:

Cervical Swabs:

Specimens for *N. gonorrhoeae* are collected from the endocervical canal using a clean, sterile swab, and are transported in Amies Charcoal transport medium.

Urethral Swabs:

Exudate from the urethra should be collected at least one hour after urination, using a clean, sterile swab and transported in Amies Charcoal transport medium.

Vaginal Swabs:

Swabs from the posterior vaginal vault or vaginal orifice are collected and transported in Amies transport medium. Vaginal swabs are not recommended for isolation of *N. gonorrhoeae* from adults, but are indicated for the detection of Bacterial Vaginosis and *Candidal* infections.

Group B Streptococcus (*Streptococcus agalactiae*) Screening for Pregnant Women:

A swab obtained from the combined vaginal and anorectal areas should be collected in Amies transport medium. Cervical swabs are not recommended for isolation of this organism.

Procedure/Media:

A. Processing of Specimens:

Direct Smear Examination (Gram Stain):

Gram stain of a vaginal swab is the most reliable method for the detection of Bacterial Vaginosis, and is also sensitive for the detection of yeast. Follow the Guideline for Laboratory Processing and Interpretation of Vaginal Specimens in the Diagnosis of Bacterial Vaginosis and note presence or absence of yeasts.

Other vaginal swab specimens (post op, post partum, recurrent – no yeast): note presence of polymorphonuclear cells and organisms. Quantitate as per Guideline for Quantitative Interpretation of Gram Stains. Note the presence or absence of yeast.

Urethral: reliable for detection of *Neisseria gonorrhoeae*. Note the presence of

polymorphonuclear cells and intracellular gram negative diplococci. Quantitate as per Guideline for Quantitative Interpretation of Gram Stains.

Cervix: gram stain not indicated for *Neisseria gonorrhoeae* due to the presence of normal flora.

Specimen Type	Media	Incubation
Vaginal - BV and yeast	Not cultured: direct stain	
Vaginal	Blood Agar (BA) Sabouraud's Agar with Chloramphenicol (Phytone) Other media (Choc, MML) added per clinical information – post partum, post op, recurrent vaginitis	CO ₂ , 35°C x 48 hours O ₂ , 30°C x 48 hours
Genital child	Blood agar Sabouraud agar Chocolate Agar (CHOC)	CO ₂ , 35°C x 48 hours O ₂ , 30°C x 48 hours CO ₂ , 35°C x 72 hours
	Modified Martin Lewis (MML)	CO ₂ , 35°C x 72 hours
Cervix	Chocolate Agar (CHOC)	CO ₂ , 35°C x 72 hours
Urethral Rectal Throat	Modified Martin Lewis (MML)	CO ₂ , 35°C x 72 hours
Combined Vaginal/Anorectal for Group B Streptococcus	Group B Streptococcus Broth Blood Agar (BA) subculture from broth	CO ₂ , 35°C x 24 hours CO ₂ , 35°C x 48 hours

B. Interpretation of Cultures:

Vaginal Cultures:

BA plates are examined at 24 and 48 hours: Growth of *N. gonorrhoeae* in any number is significant. The significance of other organisms, is determined by pure or heavy growth correlated with their presence as predominant organisms in the Gram smear. The Gram smear should also show the presence of polymorphonuclear cells.

Cervical and Urethral Cultures:

CHOC and MML plates are examined at 48 hours and 72 hours for *N. gonorrhoeae* colonies. Growth of *N. gonorrhoeae* in any number is significant.

Group B Streptococcus Screening:

The swab is inoculated into the GBS broth and incubated for 24 hours. Subcultures are made from the broth to the BA plate and the plate incubated for 24 hours. The BA plates are examined for strep-like colonies at 24 and 48 hours and the organism identified using the laboratory protocol.

C. Susceptibility Testing:

Susceptibility testing is not performed for *N. gonorrhoeae*, *Group B Streptococcus* and *Yeast*. For other organisms, follow the antimicrobial susceptibility tests according to the Antibiotic Reporting Guideline and laboratory protocol, where appropriate.

All isolates of *N. gonorrhoeae* are referred to Saskatchewan Disease Control Laboratory for susceptibility testing.

Isolates of Group B. Streptococcus from patients with a history of penicillin allergy will need to be tested for susceptibility to erythromycin.

Reporting Results:

- a. Gram stained smear – report with quantitation of polymorphonuclear cells and organisms.
- b. Culture – Report pathogens and normal flora with quantitation. Report susceptibilities where appropriate.
- c. All isolates of *N. gonorrhoeae* must be confirmed by two methods.

References:

1. Bailey and Scott Diagnostic Microbiology, 11th Ed., Mosby Inc.
2. Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller, Manual of Clinical Microbiology, 9th ed., 2007: American Society of Microbiology

GUIDELINE FOR LABORATORY PROCESSING AND INTERPRETATION OF VAGINAL SPECIMENS
IN THE DIAGNOSIS OF BACTERIAL VAGINOSIS

Background:

Bacterial vaginosis (BV) as a specific clinical entity has been recognized since the 1950s and is characterized by increased, malodorous vaginal discharge. It is associated with marked changes in microbial flora often described as a change in local vaginal ecology, and a notable absence of inflammation. A reduction in *Lactobacillus spp.* and an increase in *Gardnerella vaginalis*, several anaerobic species including *Mobiluncus spp.*, *Prevotella bivia*, *Peptostreptococcus spp.* and others such as *Mycoplasma hominis* have been described. The stimuli for these changes are very poorly understood. While BV is often of only minor clinical consequence to the women affected, it is common and recent work has heightened the importance of its recognition. It is clearly associated with several obstetric and gynecologic conditions, most notably premature labour, and often responds to antimicrobial therapy.

Clinical diagnosis of BV remains difficult despite the use of formalized clinical criteria first proposed by Gardner and Dukes¹ and formalized by Amsell.² Criteria include visual change in discharge, increase (>4.5) in pH, presence of “clue cells” (epithelial cells coated with bacteria) in microscopic wet mount, and a fishy odor when discharge is mixed with 10% KOH. The difference between “Homogenous, grey-white adherent” discharge and “normal” discharge is often subtle and microscopy to detect clue cells is rarely performed in Canadian primary care settings.

Laboratory diagnosis is essential and in recent years has focused on the morphologic characteristics distinguishable on simple Gram’s stain of vaginal secretions.^{3,4} Culture for *Gardnerella vaginalis* was advocated for many years but has been found to be poorly predictive of clinical BV and culture for anaerobic organisms and *Mycoplasma spp.* is impractical in the routine diagnostic laboratory.

Purpose:

Specimen Acceptability:

- That the presence or absence of epithelial (clue) cells be reported to evaluate specimen acceptability. If epithelial cells are absent, the specimen should be considered inadequate or inappropriate for use.

Scoring System:

- That the Nugent numerical scoring system on Gram-stained smears of vaginal secretions be utilized.
- That there be an age cut-off of >12 years and < 55 years for application of the interpretive criteria of Bacterial Vaginosis by Q-score.
- That although the Q-score in the “pre-pubescent” and “post-menopausal” age

categories is of less value, if the microscopic picture is consistent with BV, but the specimen specified as having been collected from a prepubescent individual, further investigation is warranted and the results should not be ignored and be discussed with the physician.

NOTE: *The Nugent system was validated using smears made with a modified Gram Stain^{4,5,6}. This modified staining method recommended by Spiegel, utilizes a basic fuchsin counterstain to minimize over-decolorization of gram-positive bacteria and to enhance detection of gram-negative bacteria. It is acknowledged however, that the use of this modified Gram Stain is not common practice in Canada.*

NOTE: *The wording of the score interpretations was suggested by Nugent and subsequently endorsed.*

Nugent Scoring System:

Gram-stained slides are examined under oil immersion (×1000). Smears are observed and quantitated for the presence of the following morphotypes:

- Large gram-positive bacilli (*Lactobacillus* morphotypes)
- Small gram-variable bacilli (*Gardnerella* morphotypes)
- Curved gram-negative or gram-variable bacilli (*Mobiluncus* morphotypes)

The number of organisms seen is quantitated according to the following scale:

- 1 + = <1 organism per field
- 2 + = 1–4 organisms per field
- 3 + = 5–30 organisms per field
- 4 + = >30 organisms per field

A total numerical score is calculated by summing the scores for the three components as indicated in the following table:

Lactobacillus	Score	Gardnerella	Score	Mobiluncus	Score	
4+	0	4+	4	4+	2	
3+	1	3+	3	3+	2	
2+	2	2+	2	2+	1	
1+	3	1+	1	1+	1	
0	4	0	0	0	0	
		+		+		<u>= TOTAL SCORE</u>

The total score is interpreted and reported as follows:

0 - 3 “Gram stain indicates normal bacterial vaginal flora”

- 4 – 6 “Gram stain reveals altered vaginal flora that is not consistent with bacterial vaginosis. This frequently represents a transitional stage. If signs and/or symptoms persist, repeat testing is warranted”
- 7 – 10 “Gram stain consistent with bacterial vaginosis”

References:

1. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "nonspecific" vaginitis. *Am J Obstet Gynecol* 1955;69:962-976.
2. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;74(1):14-22.
3. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991;29(2):297-301.
4. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. *J Clin Microbiol* 1983;18(1):170-7.
5. Spiegel CA. Bacterial vaginosis. *Clin Microbiol Rev* 1991;4(4):485-502.
6. Spiegel CA. Bacterial vaginosis: changes in laboratory practice. *Clin Microbiol Newsletter* 1999;21:33-37.

STOOL CULTURES

Background:

Enteric pathogens include *Salmonella* and *Shigella* sp., *Campylobacter*, *E. coli* 0157:H7, *Yersinia enterocolitica*, *Clostridium difficile*, *Aeromonas* and *Plesiomonas*. *Vibrio* (*cholerae* and *parahemolyticus*) are an important cause of diarrhea in other parts of the world. *S. aureus* and *Candida albicans* may be found in pure cultures from patients on broad-spectrum antibiotic therapy.

In adult patients who develop diarrhea 3 days after hospitalization, *C. difficile* is the most common pathogen. Stools from these patients are tested for the presence of *C. difficile* toxin only.

Specimens from outbreaks, as defined by Public Health must be sent to Saskatchewan Disease Control Laboratory with the outbreak number.

When investigating patients suspected of being in a carrier state refer to the Saskatchewan Disease Control Laboratory.

Specimen Collection and Transport:

Freshly passed stool specimens should be transported to the laboratory within 30 minutes of collection, and processed in 2 hours. If there is a delay in transport and/or processing, the specimen should be transferred into a transport container with Cary-Blair medium. Specimens should be up to the “fill line” in the transport container. Rectal swabs may be adequate for the detection of pathogens in acute infections, but are NOT the preferred specimens. Ensure that rectal swabs are for C & S, and not for isolation of *N. gonorrhoeae*. Submit one specimen per stool sample, to a maximum of 2 stool samples, ideally on separate days.

Stools for *C. difficile* toxin testing should be sent in a sterile container without transport medium.

Unacceptable Specimens:

1. Specimens in containers with external contamination.
2. Specimens with contaminants such as urine or tissue paper.
3. Specimens not correctly identified will be rejected if the discrepancy cannot be rectified.
4. Stool in SAF.

In all cases the ward or physician will be notified and a repeat specimen requested.

Procedure/Media:

A. Processing of Specimens:

Culture on the following media, and incubate the plates as indicated.

Media	Incubation
MacConkey Agar (MAC)	O ₂ , 35°C x 24 hours
Xylose Lysine Deoxycholate (XLD), Hektoen, Salmonella-Shigella (SS), or Deoxycholate Agar (DCA) - selective for Salmonella	O ₂ , 35°C x 24 hours
Sorbitol MacConkey (SMA)	O ₂ , 35°C x 24 hours
Campylobacter Agar	Microaerophilic, 42°C x 48-72 hours

When requested or clinically indicated culture to:

Media	Incubation
Blood Agar (BA) or Aeromonas selective	O ₂ , 35°C x 24 hours
Thiosulfate Citrate – Bile Salts – Sucrose (TCBS) - selective for Vibrio	O ₂ , 35°C x 48 hours
Yersinia Agar (CIN)	O ₂ , 30°C x 72 hours

Suspicious colonies of enteric pathogens should be subcultured and identified using the laboratory identification system. i.e. conventional or automated methods as applicable.

Reporting Results:

Isolation of *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* 0157:H7, *Yersinia enterocolitica*, *Plesiomonas*, *Aeromonas*, and *Vibrio* are considered significant. A pathogen specific negative report should be provided for each pathogen ruled out.

Antibiotic susceptibility testing is not done on *E. coli* 0157:H7 since its treatment is associated with an increased risk of HUS and it is a self-limited disease. It is also not done on *Campylobacter* and *Yersinia sp*, *Aeromonas*, *Plesiomonas* and *Vibrio sp*. For *Salmonella sp*, the illness is also self-limited and the antibiogram is done and released only in specific circumstances (elderly, immunosuppressed, infant (<2 years), severe disease, presence of *S. typhi*).

If there is a concomitant isolate from a blood culture, antimicrobial susceptibilities should be reported on the blood isolate.

* see Antibiotic Guidelines for more information

References:

1. Bailey and Scott's Diagnostic Microbiology, 11th Edition., Mosby Inc.
2. Cumitech 12A Diagnosis of Bacterial Diarrhoea. 1992. ASM Press.

INVESTIGATION OF INFECTIOUS DIARRHEA

Diarrheal illness is a significant health problem. Guidelines for testing of stool specimens in the investigation of infectious diarrhea are based on current literature and on guidelines from other Provinces (Ontario, Alberta and British Columbia, most recently).

The most common guideline requires:

- the clinical history indicating symptoms;
- their duration;
- recent travel;
- antimicrobial use and;
- any underlying medical conditions.

Bacterial Infections

a) Stool for C&S

Initially a single stool should be ordered in patients with symptoms of < 5 days duration and where antibiotic associated diarrhea is not suspected. Stool for C&S will detect *Shigella sp*, *Salmonella sp*, *Campylobacter sp*, *Aeromonas* and *E. coli 0157*. If other pathogens e.g. *Vibrio sp* or *Yersinia enterocolitica* are suspected, the laboratory must be informed. If the first stool culture is negative, and symptoms persist, a second sample may be submitted. Up to two specimens may be collected on consecutive days. Stool for C & S should be submitted in enteric transport media or Carey Blair transport media.

- b) *C. difficile* toxin test should be ordered on patients who have diarrhea secondary to antimicrobial use. Antibiotic associated diarrhea may occur up to 8 weeks after antibiotics have been stopped. Testing should be performed on outpatients who have recently been on antibiotics. A single sample should be submitted. If results are negative but symptoms persist, a second sample may be submitted 5-7 days after the first.

C. difficile is the most common cause of infectious diarrhea in hospitalized patients who develop diarrhea >3 days after admission. *C. difficile* should be considered in other cases of persistent diarrhea, in which no other causative agent has been identified. Stool for *C. difficile* toxin testing should be transported in a sterile container without preservative.

C. difficile is not a cause of disease in children under the age of one-year; therefore testing should not be performed.

C. difficile toxin test should not be done as a test of cure.

- c) Routine culture of stool, and O&P examination are not indicated in patients who develop diarrhea > 3 days after admission to the hospital.

Ova and Parasites Investigation

- a) One stool sample is recommended for initial ova and parasite examination. Specimens should be transported in SAF preservative

- b) Additional samples may be examined, if clinically required, after the results of the first examination are known. In special circumstances, e.g. patients with history of travel to the tropics, immunocompromised patients, etc., please consult the laboratory.

Two stool samples may only be requested in the following high risk cases:

- patients with chronic diarrhea
- returning travellers with diarrhea
- other high risk categories of patients (e.g. immunosuppressed patients, patients on steroids, male homosexuals)

Exclusions

These guidelines may not apply to the following:

- To work up food borne outbreaks of diarrhea, need to refer to the provincial laboratory to cover such agents as *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens*. The initial specimen should be stool sample, taken as soon as possible. This result is used to determine the work up for food samples.
- Where an infectious etiology is not a consideration.

If a laboratory is not able to perform these tests as part of the work-up, then the stool should be referred to a reference laboratory to perform this level of testing.

References:

J. CLIN MICRO: Aug '93
Application of Rejection Criteria for Stool Cultures for Bacterial Enteric Pathogens. Fan et al. Pages 2233-2235.

JAMA Feb 16, 1990 Vol: 263 #7
Inappropriate Testing for Diarrhoeal Diseases in the Hospital.
Siegel D.K., Edelstein P.M.

Am. J. of INFECTION CONTROL: Dec 88 Vol 16 #16
Yield of Stool Culture, Ova and Parasite tests and *C. difficile* determinations in nosocomial diarrhoeas. Yanelli et al. Pages 246-249.

DEEP WOUND, ABSCESS AND PUS SPECIMENS

Background:

Infections in deep wounds and abscesses are often caused by a mixture of aerobic and anaerobic organisms.

Specimen collection and Transport:

Pus from an abscess or deep wound should be sent in a clean sterile container and/or an anaerobic transport container.

Procedure/Media:

A. Processing of Specimens:

Direct examination of Gram stained smear-Note the presence of polymorphonuclear cells, squamous epithelial cells and organisms. Quantitate as per Guideline for Quantitative Interpretation of Gram Stains.

If *Actinomyces sp.* or *Nocardia sp.* is requested or suggested on gram stain (branching or beaded gram positive organisms), the Modified Acid Fast stain should be performed. If the Modified Acid Fast stain is not performed in your laboratory, refer to a reference laboratory capable of isolating these organisms.

Culture on the following media, and incubate the plates as indicated.

Media	Incubation
Blood Agar (BA)	CO ₂ 35°C x 48 hrs
MacConkey Agar (MAC)	O ₂ /CO ₂ 35°C x 48 hrs
Chocolate Agar (CHOC)	CO ₂ 35°C x 48 hrs
Anaerobic Agar (BRUC)	AnO ₂ 35°C x 72-96 hrs

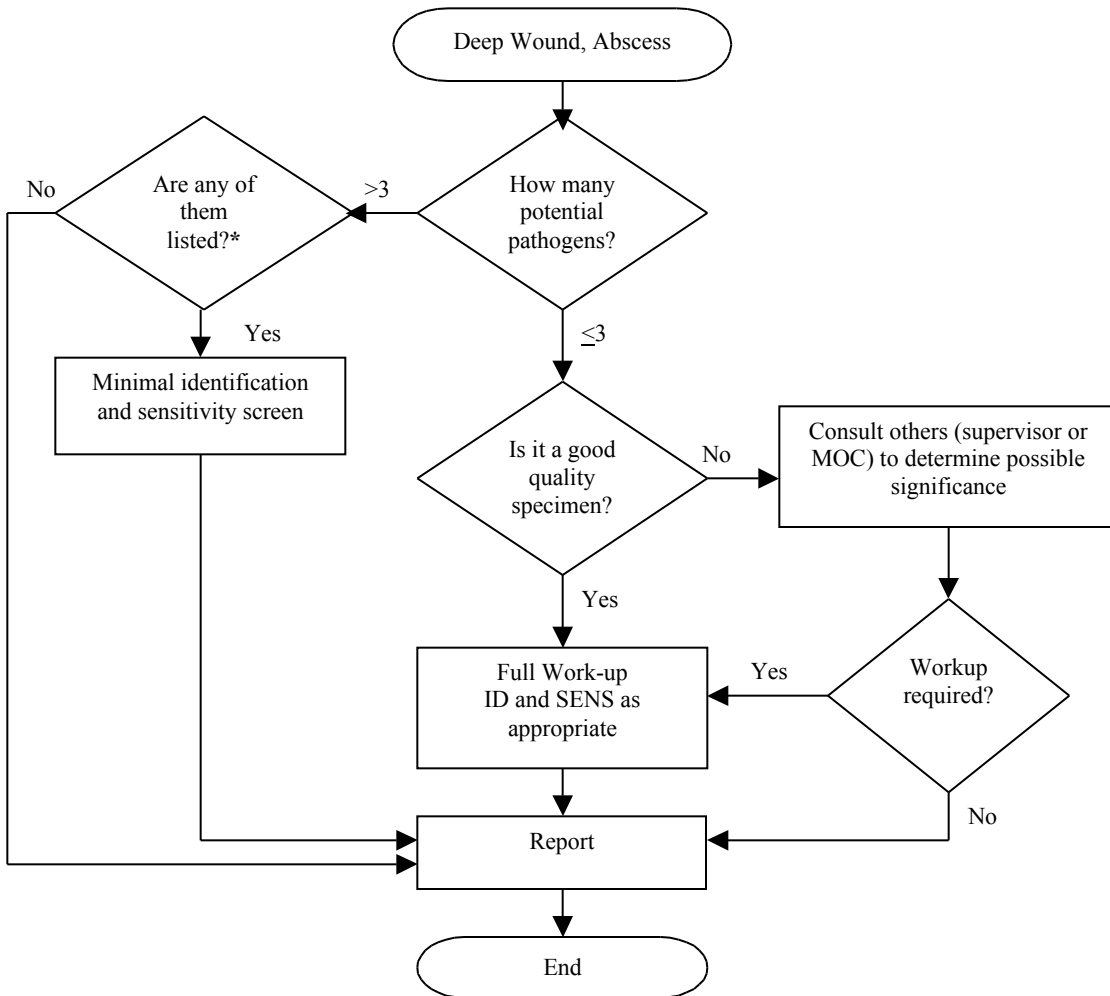
Other anaerobic agars as per laboratory protocol

If *Actinomyces sp.* requested or questioned on direct smear, incubate anaerobic media for 7 days.

B. Interpretation of Cultures:

Examine the aerobic plates daily for the total incubation time; and the anaerobic plates after 48 hours and 4 days. **Potential pathogens** include such organisms as *S. aureus*, *Beta haemolytic streptococci*, *Pasteurella sp.*, *Capnocytophaga sp.* (animal bites)

Eikenella sp. (human bites) , *Enterobacteriaceae*, *P. aeruginosa*, *anaerobes*. Correlate with the results of the direct gram smear.



<p><u>Potential Pathogens:</u> <i>S. aureus</i> <i>Beta haemolytic streptococci</i> <i>Pasteurella</i> sp. <i>Capnocytophaga</i> sp. (animal bites) <i>Eikenella</i> sp. (human bites) <i>Enterobacteriaceae</i>, <i>P. aeruginosa</i> <i>anaerobes</i></p>	<p><u>*Listed Organisms:</u> <i>S. aureus</i> - rule out MRSA beta-hemolytic streptococci (groups A, B, C, F, and G) Yeast only when isolated in moderate to abundant amounts (consult with MOC or supervisor before reporting) <i>Pseudomonas species</i> <i>Enterococcus species</i> – rule out VRE <i>Bacteroides fragilis</i> group <i>Actinomyces israelii</i> <i>Clostridium perfringens</i> - if predominant</p>
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C. Susceptibility Testing:

Perform antimicrobial susceptibility tests according to the Antibiotic Reporting Guideline and laboratory protocol.

Reporting Results:

Gram stained smear: Report with quantitation the presence of polymorphonuclear cells, squamous epithelial cells and organisms.

Culture:

- a. Negative report : No growth , Mixed aerobic flora, Mixed anaerobic flora, Mixed aerobic and anaerobic flora Including “Listed Organisms” or Type of flora, depending on site
- b. Positive report: Report potential pathogens and sensitivities (as determined by organism). Quantitate. Single isolates of anaerobes may require susceptibility testing.
- c. If organisms seen in the direct smear do not appear in culture, and anaerobic specimen was not submitted, it might suggest that the organisms seen failed to grow indicating the presence of fastidious or anaerobic organisms. It could also indicate that the gram stain was interpreted incorrectly. It is helpful to re-check the direct smear. If the gram stain is correct, the comment “Organism(s) seen in direct smear did not grow on aerobic culture, suggesting the presence of anaerobic or other fastidious organisms or previous antibiotic therapy.”

References:

1. Isenberg, H. 1992. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington DC.
2. Miller, J.M. 1996. A Guide to Specimen Management in Clinical Microbiology. American Society for Microbiology. Washington DC.
3. Murray, P.R. 1996. ASM Pocket Guide to Clinical Microbiology. American Society for Microbiology. Washington DC.
4. Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller, Manual of Clinical Microbiology, 9th ed., 2007: American Society of Microbiology Washington, DC.

SUPERFICIAL WOUND CULTURES

Background:

Superficial skin swabs are collected from lesions, open wounds, open abscesses, rashes. Usual skin flora can contaminate the specimen if care is not taken to properly collect the swab. Superficial swabs are not suitable for the recovery of anaerobes.

Specimen Collection and Transport:

Specimens should be collected following appropriate skin decontamination to reduce the presence of contaminating organisms. Specimens should be collected using a clean, sterile swab and sent in Amies transport medium.

Procedure/Media:

A. Processing of Specimen:

Direct examination: Gram stained smear - note the presence of polymorphonuclear cells, squamous epithelial cells and organisms. Quantitate as per Guideline for Quantitative Interpretation of Gram Stains.

		Quantity of cells per 10x (objective lens) microscopic field			
No. of neutrophils	Q-value for neutrophils	Q-value for squamous epithelial cells present in following no.			
		0	1-9	10-24	25
		0	-1	-2	-3
0	0	(1)	0	0	0
1-9	1+	1	0	-1	-2
10-24	2+	+2	+1	0	-1
25	3+	+3	+2	+1	0

Attach Q-values to squamous cell and neutrophil quantities. Add the two Q-values together. Specimens with positive Q-values are more likely to contain increased numbers of potential pathogens and decreased numbers of potential contaminants. Specimens with negative Q-values (or a Q-value of zero) are likely to be contaminated with the local flora. Specimens with no squamous cells or neutrophils are scored as 1, allowing neutropenic patients or those with necrotic or serous secretions to be processed as acceptable.

NOTE: The first row of numbers in the table is the Q-value for squamous epithelial cells only. The following four rows are the Q-values for the sum of the neutrophils and squamous epithelial cells.

Culture: On the following media, and incubate plate as indicated:

Media	Incubation
Blood Agar (BA)	CO ₂ 35°C x 48 hrs
MacConkey Agar (MAC)	O ₂ /CO ₂ 35°C x 48 hrs
Colistin Nalidixic Acid Agar (CNA)/PEA - optional	CO ₂ 35°C x 48 hrs
If tracheal swab or from chest tube site, add Chocolate Agar(CHO) - optional	CO ₂ 35°C x 48 hrs

B. Interpretation of Cultures:

Examine the plates after 24 and 48 hrs incubation. Potential pathogens include *S. aureus*, *Beta hemolytic Streptococcus species*, *P. aeruginosa*. A heavy, pure growth of other organisms that is consistent with the predominant organism seen in the direct smear is significant if there are 1+ polymorphonuclear cells seen.

C. Susceptibility Testing:

Perform antimicrobial susceptibility tests according to the Antibiotic Reporting Guideline and laboratory protocol.

Reporting Results:

A. Gram stained smear: Report with quantitation the presence of polymorphonuclear cells, squamous epithelial cells and organisms.

If Q-score has a negative value, add a report comment such as: “Microscopy of Gram-stained smear indicates potential contamination with skin flora.”

B. Culture:

a. Negative report: “No growth” or other normal type of flora dependent on site – as per laboratory protocol.

b. Positive report: Quantitate all significant isolates and report with appropriate antimicrobial susceptibilities. If normal skin flora also present, report with quantitation. Or, if other normal flora, report with quantitation.

References:

1. Isenberg, H. 1992. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington DC.
2. Miller, J.M. 1996. A Guide to Specimen Management in Clinical Microbiology, American Society for Microbiology. Washington DC.

3. Murray, P.R. 1996. ASM Pocket Guide to Clinical Microbiology, American Society for Microbiology. Washington DC.
4. Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller, Manual of Clinical Microbiology, 9th ed., 2007: American Society of Microbiology; Washington, DC.

BLOOD CULTURE PROCEDURE

Background:

The detection of microorganisms in a patient's blood has diagnostic and prognostic importance. When bacteria multiply at a rate that exceeds the capacity of the reticuloendothelial system to remove them, bacteremia results. Bacteria enter the blood from extravascular sites via lymphatic vessels. Blood cultures are essential in the diagnosis and treatment of the etiologic agent of sepsis.

Specimen Collection:

1. Specimens must be collected aseptically following the laboratory's venipuncture protocol.
2. A "set" is the total number of blood culture bottles drawn from two sites, with two bottles taken from site 1 and one bottle taken from site 2. All three bottles are taken during the same visit to the patient.
3. The table illustrates the optimum schedule.

	Bottles/Set	Volume/Bottle	# of Sets	Timing
Adult Routine or ANY adult on antibiotics	Site 1: 1 aerobic 1 anaerobic Site 2: 1 aerobic	10 ml 7 ml 10 ml	1	No time required between draws from site 1 and site 2
Adult Endocarditis FUO* on antibiotics	Site 1: 1 aerobic 1 anaerobic Site 2: 1 aerobic	10 ml 7 ml 10 ml	2	1 hour** The first set of three bottles is drawn as above, and an hour later, the second set of three bottles is drawn
Neonates Children < 4 yrs.	Site 1: 1 pediatric Site 2: 1 pediatric	0.5-3 ml 0.5-3 ml	1	
Request for yeast or candidemia	1 aerobic			

NOTE: * FUO – Fever of Unknown Origin (ie. 3 weeks of fever above 38°C)

** In severe cases antibiotics may need to be started immediately after collection of the first set.

It is recommended to collect the blood just prior to administration of antibiotics.

Blood drawn through an intravenous or intra-arterial catheter is acceptable only if the blood cannot be drawn from a peripheral venipuncture.

Volume:

1. The volume of blood drawn is dependent upon the blood culture system used. There is much published evidence that a total volume of 30 ml per febrile gives optimum recovery from adult patients.
2. The volume of blood is critical because the concentration of organisms in the majority of bacteremias is low. In infants and children, the concentration of microorganisms during bacteremia is higher than in adults; therefore, less blood is required for culture.
3. The recommended blood to broth ratio is 1:5 to 1:10. Dilutions beyond 1:10 may occur in blood cultures of infants and small children.
4. Collect a maximum of 30 mls for adults, maximum of 5 mls for children, and any obtainable amount for infants.

Unacceptable Specimens:

1. Bottles with external contamination.
2. Specimens incorrectly identified. In these cases the ward or physician must be notified immediately and a repeat specimen requested.

Incubation:

For patients presenting with an unknown fever and if endocarditis is suspected, up to 3 weeks incubation may be required.

Materials, Equipment and Reagents: As per your laboratory procedure.

Procedure:

A. Collection of Specimen

1. Remove flip caps from the blood culture vials and clean vial tops with an alcohol swab.
2. Select the vein and prepare the arm using a double application of 70% alcohol starting at the centre of the site and swabbing concentrically for 1 minute.
3. Allow the venipuncture site to dry.

4. Do not touch the venipuncture site after preparation or prior to phlebotomy.
5. Perform venipuncture.
6. Transport directly to laboratory.

In the Laboratory

Proceed with incubation and subculture of the bottles as per the manufacturer's instructions for the system involved.

B. Detection and Work-Up of Positive Cultures

Gram Smear:

All bottles flagged as "positive" by the automated system, or detected as positive by visual means such as turbidity, must be examined by preparation of a gram smear.

NOTE: Results of gram smears must be called to the ward/physician stat.

Subculture:

Media	Incubation
Blood Agar (BA)	CO ₂ , 35°C x 48 hours
Chocolate Agar (CHOC)	CO ₂ , 35°C x 48 hours
MacConkey Agar (MAC)	CO ₂ , 35°C x 48 hours
Anaerobic media eg. Brucella	AnO ₂ , 35°C x 72 hours

Identify isolates and perform susceptibility testing as per your laboratory protocol.

Reporting:

Negative – No growth after 5 days incubation, or as per manufacturers instructions.

Positive – Report organism with corresponding antimicrobial susceptibility.

NOTE: Organisms that are normal skin commensals such as coagulase negative staphylococci, may be contaminants. The isolation of this type of organism in one bottle/culture only may indicate contamination. This will need to be resolved by discussion with the clinician. Reporting of contaminants may result in the inappropriate use of antibiotics.

References:

1. CUMITECH 1C – Blood Cultures 111 (2005)
2. Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller, Manual of Clinical Microbiology, 9th ed., 2007: American Society of Microbiology
CEREBROSPINAL FLUID AND STERILE BODY FLUIDS

CEREBROSPINAL FLUID AND STERILE BODY FLUIDS

Background:

Bacterial meningitis is the result of infection of the meninges caused by aerobic or anaerobic organisms. The specimens cultured include cerebrospinal and ventricular shunt fluid.

Sterile body fluids include specimens such as joint, pleural, peritoneal and amniotic fluid. CSF and other sterile body fluids may contain very few organisms per milliliter of fluid and the concentration of the specimen is recommended. All isolates of sterile body fluids must be reported.

Specimen Collection and Transportation:

CSF and other sterile body fluids should be collected in a sterile tube. For sterile body fluids, if there is enough specimen, inoculate either an aerobic blood culture bottle or a pediatric blood culture bottle. Process blood culture according to laboratory procedure. Do not refrigerate the specimens collected.

PCR for *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* on CSF specimens may be submitted upon request and review of the MHO.

Procedure/Media:

A. Processing of Specimens:

a) Preparation of CSF specimens

1. Record volume and gross appearance of CSF, i.e., clear, bloody, cloudy, xanthochromic. If the specimen is cloudy, a gram stain should be done before the centrifugation.
2. Centrifuge the CSF specimen for Gram stain.
3. Centrifuge the specimen:
 - a. If the volume of fluid is >1 ml, centrifuge for 15 min. at 2500-3000 rpm (1500 x g). The higher speed is preferred to sediment the bacteria.
 - b. Remove supernatant and vortex the sediment vigorously for at least 30 secs. **This step is critical.** Do not use a pipette to mix the sediment, because the bacteria and cells may adhere to the sides of the tube and cause false-negative findings.
 - c. If 1 ml is received, do not centrifuge but vortex the specimen.
4. Using a sterile pipette, inoculate the media and streak for isolated colonies. In addition, add a single drop of fluid to the 4th quadrant of the plate and allow to air dry. Do not streak out this drop of fluid.

b) Preparation of Sterile Body Fluids

1. Briefly describe the appearance of the specimen, i.e., color, viscosity, light-transmitting properties, and presence of a clot.
2. Centrifuge the specimen if >5 ml is received.
 - a. If the volume of fluid is >5 ml, centrifuge for 15 min. at 2500-3000 rpm (1500 x g).
 - b. Remove supernatant and vortex the sediment.
 - c. If <5 ml is received, vortex.

- c) Process specimens (both CSF and Sterile body fluids) with large clots as follows:
- a. Pour sediment containing clotted material into a sterile tissue grinder. Add a small volume (<0.5 ml) of sterile nutrient broth to the grinder, and gently homogenize this mixture to disperse the clots and release any trapped bacteria.
 - b. **OR**, alternatively use a sterile stick, as clot adheres to stick, turn and wring out the clot.

NOTE: Do not grind the clots if a fungal culture is also requested on the sample. Tease the clots apart. Vigorous grinding can destroy fungal cells.

- d) Direct Examination of Gram stained smear - Note the presence of polymorphonuclear cells, epithelial cells and organisms. Quantitate as per Guideline for Quantitative Interpretation of Gram Stains.

- e) Culture on the following media, and incubate the plates as indicated.

Media	Incubation
Blood Agar (BA)	CO ₂ 35°C x 72 hrs
Chocolate Agar (CHOC)	CO ₂ 35° C x 72 hrs
MacConkey Agar (MAC)	O ₂ /CO ₂ 35°C x 72 hrs
Anerobic plate (BRUC)	CO ₂ 35°C x 72 hrs
Blood Culture Bottle	
* Thioglycolate broth (THIO)	CO ₂ 35°C x 7 days

* If CSF: add the broth only when working up shunt infections.

B. Interpretation of Cultures:

- a. CSF specimens: Examine the BA, MAC, CHOC and BRUC plates daily for 72 hours. In shunt infections, examine the THIO broth media daily for 7 days.
- b. Sterile Body Fluids: Examine the BA, MAC, CHOC and BRUC plates daily for 48 hours. Anaerobic jars - examine at 48 hours and 72-96 hours before discarding. Broth can be examined daily.

- c. Potential Pathogens include the organisms listed in the table below. Identify and provide antimicrobials susceptibility on all microorganisms, regardless of the amount isolated. Correlate with the results of the direct Gram smear.

CSF - Common organisms causing acute bacterial meningitis:

Age or condition	Organism(s)
Neonate < 30 d	<i>Escherichia coli</i> , Group B Streptococcus, <i>Listeria monocytogenes</i> , Gram-negative bacilli, <i>S. aureus</i>
1-3 months	Gr. B Streptococcus, <i>E.coli</i> , <i>Streptococcus pneumoniae</i> , <i>Neisseria meningitidis</i> , <i>Haemophilus influenzae</i> , <i>L. monocytogenes</i>
3 mo-18 yrs	<i>Streptococcus pneumoniae</i> , <i>Neisseria meningitides</i> , <i>Haemophilus influenzae</i>
Young adult	<i>Neisseria meningitidis</i>
Adult	<i>S. pneumoniae</i> , <i>N. meningitidis</i>
Elderly	<i>S. pneumoniae</i> , <i>N. meningitidis</i> , Gram-negative bacilli, <i>L. monocytogenes</i>
AIDS patient	<i>Cryptococcus neoformans</i> (fungi)
CNS Shunts	Coagulase-negative Staphylococci, <i>Corynebacterium</i> , <i>Propionibacterium</i> , Gram-negative bacilli

NOTE: For CSF and Sterile Body Fluids – Potential Pathogens include all isolates, especially in immunocompromised patients.

C. Susceptibility Testing

As per laboratory protocol. For CSF follow the Antibiotic Reporting Guideline from Microbiology Quality Assurance Committee.

Reporting Results:

- A. Gram stained smear: Report with quantitation the presence of polymorphonuclear cells, epithelial cells and organisms. Phone the results to the ward/doctor.
- B. Culture: Report all isolates with quantitation and with the appropriate antimicrobial susceptibilities. Note: subcultures from broths cannot be quantitated.

NOTE: A positive culture is a critical value, and must be phoned to the doctor STAT.

Procedure notes for sterile body fluids:

Although in the past the use of blood bottles for fluid collection has not been recommended, recent studies suggest that the large sample volume that can be cultured may improve the recovery of low numbers of organisms in fluids such as ascites. As with any broth system, however, the fastest growing organism is often the only one

isolated, jeopardizing the recovery of slow growers. When a broth is used, no direct smear information is available, and, therefore, no assessment of the initial distribution of organisms or inflammatory cells can be made. A smear can be prepared, however, at the time of specimen procurement and submitted with the broth medium. Also, unless a biphasic system is available, a broth culture delays by at least 24 h the preliminary workup of organisms possible only from growth on solid media.

Limitations of the Procedure:

This procedure is not suitable for optimal recovery of all pathogenic microorganisms found in sterile body fluids, including fungi, mycobacteria, parasites, or viruses.

Culture Negative CSF specimens from patients who have received antibiotics may be sent to Saskatchewan Disease Control Laboratory for PCR, upon request of the MHO.

References:

1. Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller, Manual of Clinical Microbiology, 9th ed., 2007: American Society of Microbiology
2. Meredith F.T., Phillips H.K, and L. B. Reller, Clinical Utility of Broth Cultures of Cerebral Spinal Fluid from Patients at Risk for Shunt Infections, 1997, JCM, 35:3109-3111
3. Dunbar, S.A., Eason R.A., Musher D.M., & J.E. Clarridge, Microscopic Examination and Broth Culture of cerebral Spinal Fluid in Diagnosis of Meningitis, 1998 JCM, 36:1617 – 1620.
4. Sturgis C.D., Petersen L.R. & J.R. Warren, Cerebral Fluid Broth Culture Isolates: Their Significance to Antibiotic Treatment, Am J Clin Pathol. 1997, 108(2): 217-221.
5. Performance Standards for Antimicrobial Susceptibility Testing; M100-S11 Vol. 21. No.1. CLSI.

ANTIBIOTIC REPORTING GUIDELINE

Organism	Site	Routine Report	Alternate
Staphylococcus species	Wound/Sputum	Penicillin, Oxacillin, Cefazolin, Erythromycin	SXT ¹ , Clindamycin ⁹ , Vancomycin
	Blood	Penicillin, Oxacillin	Vancomycin, Clindamycin ⁹
	CSF	Penicillin, Oxacillin	Vancomycin
	Urine	Penicillin, Oxacillin, Cefazolin, Nitrofurantoin	SXT, Fluoroquinolone ²
Streptococcus pneumoniae	Eye/ear ³	Penicillin, Erythromycin, SXT	Cefuroxime, Clindamycin
	Blood	Penicillin	Cefotaxime, Vancomycin
	CSF		Cefotaxime ⁸ , Vancomycin ⁸
	Sputum	Penicillin, Erythromycin, SXT, Fluoroquinolone ²	Cefuroxime, Clindamycin
Alpha hemolytic Streptococcus Non-Strep pneumoniae	Sterile sites ⁴	Penicillin, 3 rd Gen Cephalosporin	Vancomycin
Enterococcus species	Wound	Ampicillin	Vancomycin
	Blood	Ampicillin, Gentamicin synergy ¹² , Streptomycin synergy ¹²	
	Urine	Ampicillin, Fluoroquinolone ² , Nitrofurantoin	
Beta hemolytic Streptococcus (Groups A, B, C, F, G)	Wound	Penicillin, Clindamycin ⁹ , Erythromycin	Vancomycin
	Sputum		
	Blood	Penicillin, Clindamycin ⁹	
	CSF ⁵	Penicillin, Cefotaxime	
	Sterile sites	Penicillin, 3 rd Gen Cephalosporin, Clindamycin ⁹ , Erythromycin	
	Throat	Erythromycin ⁷	
	Vaginal/Rectal	Cefazolin ⁷	
Urine	Ciprofloxacin ⁷ , Norfloxacin ⁷ , Tetracycline ⁷ , Nitrofurantoin ⁷ , Cefazolin ⁷		
Enterobacteriaceae	Wound/Sputum	Ampicillin, Cefazolin and/or Cephalothin ⁶ , Gentamicin, SXT, Ciprofloxacin, Amoxicillin-clavulanic acid	Cefotaxime ⁵ , Meropenem, Piperacillin, Tobramycin, Pip-Tazobactam
	Blood	Ampicillin, Cefazolin and/or Cephalothin ⁶ , Gentamicin, SXT	
	Urine	Ampicillin, Cefazolin and/or Cephalothin ⁶ , Gentamicin, SXT, Ciprofloxacin, Nitrofurantoin	
	CSF	Ampicillin, Gentamicin, Cefotaxime	
Pseudomonas aeruginosa	All sites	Gentamicin, Fluoroquinolone ² , Piperacillin	Ceftazidime, Tobramycin, Meropenem, Pip-Tazobactam
Shigella species	Stool	Ampicillin, SXT, Fluoroquinolone ²	Cefixime if resistant to SXT
Salmonella and Shigella species	Systemic	Ampicillin, SXT, Fluoroquinolone ^{2, 10}	Cefotaxime
Haemophilus species	Sputum	Ampicillin, SXT, Cefuroxime, Amoxicillin-clavulanic acid	Fluoroquinolone ² , Clarithromycin
	Eye/Ear ³		
	Blood	Ampicillin, Cefotaxime	Meropenem
	CSF		
Stenotrophomonas maltophilia	CSF/Sterile sites	SXT ¹¹ , Ceftazidime, Ciprofloxacin	

NOTES:

- Alternate antibiotics should be reported, only if the organism is resistant to two or more first choice antibiotics, if the patient cannot tolerate those antibiotics or if the patient is already on the antibiotic of choice.
- For Enterococcus species, cephalosporins, aminoglycosides, clindamycin and trimethoprim/sulfamethoxazole may appear susceptible in vitro but are not effective clinically and should not be reported.
- Oxacillin agar screen plate should be used for detection of Methicillin Resistant Staphylococcus aureus (MRSA)
- Vancomycin agar screen plate should be used for detection of Vancomycin Resistant Enterococcus (VRE)
- All enteric pathogens other than Shigella and Salmonella typhi: do not report susceptibility, unless the patient is less than two years old, or is immunosuppressed.
- For Salmonella and Shigella species, first and second generation cephalosporins may appear susceptible in vitro but are not effective clinically and should not be reported.
- β -lactamase testing should be done on all isolates of: (1) Penicillin susceptible *Staph. aureus*; (2) *Haemophilus sp.*; (3) *N. gonorrhoeae*; (4) *M. catarrhalis*; (5) *Enterococcus sp.* isolated from sterile body sites; (6) anaerobic gram negative bacilli (*B. fragilis* group).

¹ SXT refers to trimethoprim/sulfamethoxazole

² Fluoroquinolones should not be reported for patients <17 years of age or pregnant women. Fluoroquinolones that may be used are Levofloxacin, Gatifloxacin, Ciprofloxacin, or Moxifloxacin. Ciprofloxacin is not the drug of choice for Strep. pneumoniae.

³ Guidelines for the interpretation of susceptibility testing of topical antibiotics are not available. However, testing of eye or ear isolates using serum breakpoints may be indicated as these infections may become systemic.

⁴ For Strep Viridans susceptibility testing must be done by an MIC method.

⁵ Potential inducible extended spectrum beta-lactamase producing species (ESBL), Klebsiella pneumoniae, K. oxytoca, Escherichia coli, and Proteus mirabilis (bacteremic isolates). These organisms produce beta lactamase enzymes that promote resistance to broad spectrum beta-lactam antimicrobials, such as cefotaxime (≤ 27 mm), ceftriaxone (≤ 25 mm), ceftazidime (≤ 22 mm), cefpodoxime (≤ 17 mm) and aztreonam (≤ 27 mm). Such potential ESBL strains should be screened using the recommended disk diffusion or MIC breakpoints. (MIC >1ug/ml for all antibiotics). The phenotypic confirmatory test using ceftazidime and cefotaxime alone and in combination with clavulanic acid will confirm ESBL producing strains. In all ESBL strains, the zone diameters should increase in the presence of clavulanic acid. For all confirmed ESBL producing strains, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam. CLSI M100 Table 2A

⁶ Enterobacteriaceae -parenteral (IV/IM) Cefazolin is generally used to treat in-patients, and oral Cephalothin is generally used to treat out-patients.

⁷ Consider these susceptibility tests for patients with known Penicillin allergies. Consider Cephalozin if patient is pregnant.

⁸ CLSI recommends that all CSF isolates of *S. pneumoniae* should immediately be tested by an MIC method for susceptibility for penicillin, 3rd gen Ceph and Vancomycin. Only report 3rd gen Ceph and Vancomycin when penicillin is resistant.

⁹ Strains of Staphylococci and Streptococcus that are susceptible to Clindamycin, but resistant to Erythromycin should be tested for inducible Clindamycin resistance using the D-zone test. Erythromycin should be included in testing for this purpose, even if not reported.

¹⁰ Resistance of Salmonella species to Nalidixic Acid may be used to predict the potential for resistance to Fluoroquinolones.

¹¹ Highly inherently resistant organism. SXT in high doses plus other antibiotics (if susceptible) used.

¹² Use of high-level aminoglycoside screening to predict synergy.

There are several reasons for prescribing two antimicrobial drugs together and one is to achieve a synergistic effect. Bacterial synergy is seen between two drugs, which individually are incompletely bactericidal, although acting together they completely kill the inoculum. Enterococci are inherently resistant to aminoglycosides. However, strains of enterococci with medium-level resistance ($MIC \leq 2,000 \mu\text{g/ml}$) show synergistic killing by a combination of penicillin/ampicillin and gentamicin. For enterococcal infections of sterile sites such as blood the recommended therapy is a combination of beta-lactam (e.g., ampicillin) or vancomycin combined with an aminoglycoside

(gentamicin or streptomycin). In the JCM 25:2443 - 2444 (1987) Zervos et al. demonstrated that growth in a single broth microdilution well containing gentamicin at a concentration of $500 \mu\text{g/ml}$ predicted high level resistance to gentamicin ($MIC \geq 2,000 \mu\text{g/ml}$). Antimicrobial testing panels use a single concentration high-level aminoglycoside as a synergy screen.

The ATB ENTEROC5 susceptibility test strip tests for high-level gentamicin and streptomycin sensitivity. The MicroScan choice is also based on the observation that in the presence of high-level gentamicin resistance many isolates can be sensitive to streptomycin. High-level gentamicin resistance indicates that there is resistance to synergy to gentamicin, tobramycin, sisomicin and netilmicin, due to the presence of the common bifunctional 6'-N-acetyltransferase and 2"-O-phosphotransferase enzyme. This enzyme does not affect streptomycin. Thus streptomycin has its own screen.

From the January 2005, Performance Standards for Antimicrobial Susceptibility Testing from CLSI (formerly CLSI) document M100 – S15; Table 2D recommends the following parameters for screening for high-level resistance

(HLR) to aminoglycosides among enterococci: (i) agar dilution concentrations gentamicin $500 \mu\text{g/ml}$ and streptomycin $2000 \mu\text{g/ml}$; endpoint any growth greater than 1 colony, (ii) broth microdilution, gentamicin $500 \mu\text{g/ml}$ and streptomycin $1000 \mu\text{g/ml}$; endpoint - any growth; (iii) disk diffusion concentrations gentamicin $120 \mu\text{g}$ disk and streptomycin $300 \mu\text{g/disk}$; endpoint $5\text{mm} = \text{HLR}$, $7\text{-}9 \text{ mm}$ inconclusive (use agar dilution method), $\geq 10 \text{ mm}$ sensitive. In summary, enterococci isolated from sterile sites should have antimicrobial susceptibility testing for ampicillin MIC, high-level screen for gentamicin and streptomycin, and vancomycin screen.

EXCEPTIONS

Any organism that does not present the typical antibiotic pattern for that organism should have repeat testing (identification and susceptibility) performed +/- or sent to reference laboratory (ie. SDCL).

New resistance in a species never reported before (e.g. *Streptococcus A* resistant to penicillin G).

Streptococci and enterococci:

Resistant to vancomycin

Do not need to re-test if isolate has intrinsic resistance (eg. *E. gallinarum*)

Staphylococcus aureus:

Susceptible or intermediate to penicillin G without checking β -lactamase

Resistant to oxacillin and sensitive to penicillin

Resistant to clindamycin and susceptible to erythromycin

Resistant to vancomycin

No need to re-test if resistant to oxacillin and susceptible to any or beta lactams. This is expected and is handled by reflexing the interpretation of those organisms based on the Oxacillin result.

Enterobacteriaceae:

Resistant to a third-generation cephalosporin when the strain is sensitive to cephalothin

Resistant to ampicillin and susceptible to ticarcillin and cephalothin (check ampicillin disk)

Salmonella:

Susceptible to ampicillin and resistant to cephalothin

Enterobacter and *Serratia*:

Susceptible to cephalothin, report as resistant

Klebsiella pneumoniae:

Susceptible to ampicillin, report as resistant

Pseudomonas aeruginosa:

Susceptible to cephalothin, nalidixic acid, or trimethoprim

Susceptible to ticarcillin and resistant to ceftazidime, or piperacillin

Haemophilus influenzae:

Susceptible or intermediate to ampicillin without checking β -lactamase

Proteus mirabilis and *Serratia marcescens*:

Susceptible to nitrofurantoin

LIMITATIONS

The following organisms do not have CLSI standards for susceptibility and interpretation. If testing is performed, an MIC method should be used, and all interpretations must be reported as "non-standardized".

These guidelines are for the use of various antibiotics in treatment of infections caused by these organisms.

Pasteurella multocida

Penicillin remains the drug of choice. Ampicillin, SXT, Cefuroxime, Amoxicillin-clavulanic Acid, Tetracycline and Fluoroquinolones also demonstrate good in vitro activity.

For *Pasteurella* species Oxacillin, Macrolides, Aminoglycosides and 1st gen Cephalosporins are not effective and should not be reported.

Listeria monocytogenes

Ampicillin, or Amoxicillin combined with an Aminoglycoside is the primary choice for treatment. Co-trimoxazole is the alternative drug. Resistant to Cephalosporins

Moraxella catarrhalis

Most *M. catarrhalis* strains are beta-lactamase positive. Ampicillin, amoxicillin, penicillin, and 1st generation cephalosporin therapy is not suggested. Recommended therapy: generally susceptible to macrolides, SXT, cefuroxime, ceftriaxone, quinolones, and amoxicillin/clavulanic acid. Fluoroquinolones are not recommended in pregnancy or individuals <17 years.

Bacillus sp.

Corynebacterium sp.

Eikenella sp.

Micrococcus sp.

INTRINSIC ANTIMICROBIAL RESISTANCE IN SOME COMMON *ENTEROBACTERIACEAE*

<u>Genus or species</u>	<u>Most strains are resistant to:</u>
<i>Buttiauxella</i> species.	Cephalothin
<i>Cedecea</i> species.	Polymyxins, ampicillin, cephalothin
<i>Citrobacter amalonaticus</i>	Ampicillin
<i>Citrobacter freundii</i>	Cephalothin
<i>Citrobacter diversus</i> (<i>C. koseri</i>)	Cephalothin, carbenicillin
<i>Edwardsiella tarda</i>	Colistin
<i>Enterobacter cloacae</i>	Cephalothin
<i>Enterobacter aerogenes</i> .	Cephalothin
Many other <i>Enterobacter</i> species.	Cephalothin
<i>Escherichia hermannii</i>	Ampicillin, carbenicillin
<i>Ewingella americana</i>	Cephalothin
<i>Hafnia alvei</i>	Cephalothin
<i>Klebsiella pneumoniae</i>	Ampicillin, carbenicillin
<i>Kluyvera ascorbata</i>	Ampicillin
<i>Kluyvera cryocrescens</i>	Ampicillin
<i>Proteus mirabilis</i>	Polymyxins, tetracycline, nitrofurantoin
<i>Proteus vulgaris</i>	Polymyxins, ampicillin, nitrofurantoin, tetracycline
<i>Morganella morganii</i>	Polymyxins, ampicillin, cephalothin
<i>Providencia rettgeri</i>	Polymyxins, cephalothin, nitrofurantoin, tetracycline
Other <i>Providencia</i> ^a species.	Polymyxins, nitrofurantoin
<i>Serratia marcescens</i> ^b	Polymyxins, cephalothin, nitrofurantoin
<i>Serratia fonticola</i>	Ampicillin, carbenicillin, cephalothin
Other <i>Serratia</i> species.	Polymyxins, ^c cephalothin

^a Most strains of *Providencia stuartii* are also resistant to cephalothin and tetracycline.

^b *Serratia marcescens* can also be resistant to ampicillin, carbenicillin, streptomycin, and tetracycline.

^c Most *Serratia* species are resistance to polymyxins, but some strains have unusual zones of inhibition, 10 to 12 mm or larger , even though they are resistant when tested by other methods.

Reference: Antibiotics in Laboratory Medicine, Lorian 4th Edition

PROCEDURE/METHOD STATISTICAL WORK-UP/VALIDATION STUDY GUIDELINES: MICROBIOLOGY

Work-up guidelines and definitions (change in method/instrument):

Work-up Element:	Definition: CLSI or International Federation of Clinical Chemistry (IFCC)	Minimum Data Requirements (where appropriate):	Applicability:	
			Qualitative	Quantitative
1. Imprecision <ul style="list-style-type: none"> ▪ Within run ▪ Between run 	The variation in analytical results demonstrated when a particular specimen or aliquot is analyzed multiple times or on multiple days. Imprecision is expressed quantitatively by a statistic such as standard deviation or coefficient of variation.	Within run – use preferably a patient sample or pool close to the decision levels with a minimum of 10 data points. Between run – 20 results from 20 separate runs on 2 levels over a 10-day minimum time period using appropriate QC material		–
2. Patient correlation	The correlation coefficient is a means to look for a relationship, not agreement, between pairs. Two methods may have a perfect correlation throughout the measuring range but may not agree in value i.e.: one may be double the value of the other.	40 data points are recommended with a minimum of 20 having 50% of the data points outside the reference intervals, if possible. Correlations should involve comparison with an acceptable reference method or laboratory.	n=20 –	n=40 –
3. Linearity	(IFCC) The range of concentration or other quantity in the specimen over which the method is applicable without modification (CLSI) when analytical results are plotted against expected concentrations; the degree to which the plotted curve conforms to a straight line is a measure of the system linearity.	4 data points each in duplicate as a minimum requirement, but 5 data points are preferred (over reportable range). Linearity studies are expected on an initial method work-up and further studies as defined by the College guidelines		–
4. Reference range validation	It is common convention to define the reference range or interval of a laboratory test as the central 95% interval bounded by the 2.5 and 97.5 percentiles of the selected patient population. Validation of an established reference range requires a minimum of 40 samples	The minimum requirement is 40 data points for confirmation of an established reference range and 120 for the establishment of a new reference range.		–
5. Accuracy	Closeness of the agreement between the result of	3 data points using acceptable	–	–

	a measurement and the accepted reference value (true value of the analyte).	reference material ie.: CEQAL or CAP.		
6. Sensitivity	Measure of the ability of an analytical method to detect small quantities of the measured component. When concern is performance at very low concentration it is useful to determine the detection limit as influenced by imprecision.	Sensitivity studies are only required for those method which have clinical relevance at values close to "0" eg. PCR	– when clinically relevant	– when clinically relevant
7. Specimen Stability	The conditions of handling and storage which permits the measurement and reporting of a clinically relevant result.	Data required for specimens known to be time sensitive. Usually associated with rejection criteria, eg. specimens >24 hrs in transit or arrived in inappropriate media.	– storage and transport dependent	– storage and transport dependent
8. Interference	The effect of any component of the sample on the accuracy of the measurement of the desired analyte	Document the manufacturer's interference information. The method should include a disclaimer or a process for dealing with a lipemic, icteric or hemolyzed sample.	– methods with known interferences	– methods with known interferences
9. Organism Identification		20 patient isolates worked up by the new identification test in parallel with an acceptable reference method or laboratory. In addition, for specimens likely to possess complex flora, up to 20 common contaminants should be included as negative controls. See patient correlation above.	–	– semi-quantitative only
10. Antibiotic sensitivity		Appropriate CLSI standard organisms should be tested in principle, eg. see accuracy above.	–	– semi-quantitative only

Quantitative:

Work-up requirements when an instrument is moved from site “A” to site “B”: (it is assumed that the instrument has been in recent use with acceptable performance).

Work-up Element:	Minimum data requirements:
1. Imprecision studies, QC only	As above
2. Patient correlation	10 data points, where feasible

Qualitative or semi-qualitative:
If site B has no previous experience

Work-up Element:	Minimum data requirements:
1. Organism Identification	10 patient isolates, up to 10 contaminants
2. Antibiotic susceptibility	As above