

PROCEDURE: ANAEROBIC CULTURE

I. PRINCIPLE

Anaerobic bacteria are a significant component of the normal microbiota of humans. There are anaerobes present on most body surfaces and mucous membranes. Some examples include: large numbers throughout the entire gastrointestinal tract, from the mouth to the colon- apart from the stomach and esophagus; or even found in large numbers in the female genitourinary tract. In most areas, a true symbiotic relationship exists. Humans supply the environment for the anaerobes to live and multiply in the presence of food, water, and a “friendly” atmosphere. This allows the bacteria to digest food for metabolism, thus preventing attachment of more virulent microbes by their presence in very large numbers. In addition, these organisms benefit the human body because they are a major component of the innate immunity of the host. Anaerobes can cause infections when they increase in number, when normally they play a harmonious role as part of the microbiota. This can happen when introduced into a new site in the body or when a non-normal microbiota anaerobic bacterium gains entrance into the host via penetrating wounds because of trauma or surgery. Some instances of entry occur with abscesses of the liver, brain, lung, and other local sites; appendicitis; peritonitis; chronic otitis media and sinusitis; endophthalmitis; bacteremia; endocarditis; myonecrosis; gas gangrene; and dental and oral infections.

To optimally recover anaerobic bacteria, appropriate collection, transport, and processing procedures must be followed. The proper transport for fluids, aspirates, and tissues is in a sterile container and brought immediately to the lab for processing. Collection of swabs for anaerobic culture is not optimal and not routinely permitted. Swabs have very small surface area and are prone to be used in areas where anaerobic normal microbiota organisms predominate and therefore can provide a skewed culture result of what is the true pathogen, and do not provide an appropriate anaerobic atmosphere for adequate transport.

II. AVAILABILITY

7 days a week; all shifts

III. TEST CODE

CXANA – AEROBE, ANAEROBE CULTURE
CXANO – OR-SURGICAL AEROBE, ANAEROBE CULTURE
CXBM – BONE MARROW CULTURE/GS
CXCOR – CORNEA CULTURE
CXDIA – DIALYSATE CULTURE
CXVIT – VITREOUS FLUID CULTURE

IV. SPECIMEN

- A. Refer to the [Specimen Managment](#) procedure for complete acceptance/rejection criteria.
- B. The best specimen for the recovery of clinically significant anaerobes is a needle aspiration of an abscess/body fluid or collection of a tissue sample.
- C. Specimens that have been improperly collected or transported are unacceptable for anaerobic culture.
- D. Specimens with the presence of epithelial cells seen in the gram stain (apart from OR/IR specimens) are unacceptable for anaerobic culture and must be reordered as CXWND. Refer to [Gram Stains Procedure](#) for protocol regarding presence of epithelial cells in anaerobic cultures.
- E. Specimen collection and processing instructions
 1. Specimen should be transported to the Microbiology laboratory and processed immediately upon receipt.
 2. Specimens submitted with an “OR EXTEND” sticker should be held for 14 days.

3. Specimens submitted from orthopedic surgeons may be extended to 14 days upon physician's request.
4. Extended cultures should receive colored dots on solid media/thioglycolate broth and media \$EXAN should be added.
5. Cultures to rule out *Actinomyces* should be incubated for 10 days.
6. Cultures to rule out *Nocardia* should have a CXFUN ordered at the time of processing and BCYE media added to the culture plates.
7. Fluid specimens received in VersaTREK REDOX bottles should be ordered as a CXANA. The ABOT and NBOT must be ordered in Result Entry worksheet or in Order Entry as media. The media labels must be used to scan the specimen on to the VersaTREK instrument. If only bottles are received on an anaerobic culture, a Gram-stain is not performed. The Gram-stain should be resulted using the statement "NOT PERFORMED" and credited in Order Entry.
8. Specimens received in VersaTREK REDOX bottles for CXDIA can be loaded on the VersaTREK instrument using the specimen labels.
9. Fluid bottles should be marked as such with tape before being loaded onto the VersaTREK instrument.
10. Specimens should be collected in sterile container without any additive.

V. MEDIA AND EQUIPMENT

A. Media

1. Aerobic
 - a. Blood Agar with 5% sheep blood (BAP) – General purpose medium. Hemolysis can be observed.
 - b. Colistin Nalidixic Acid with 5% sheep blood (CNA) – Selective agar that inhibits most Gram-negative organisms.
 - c. Chocolate Agar (CHC) - Enriched agar suitable for supporting the growth of *Haemophilus*, *Neisseria gonorrhoeae*. CHC should be used as the aerobic plate for aerotolerance testing of potential anaerobic bacteria.
 - d. MacConkey Agar (MAC) – Selective agar that inhibits most Gram-positive organisms.
2. Anaerobic
 - a. Brucella (BRU) agar with 5% sheep blood, Vitamin K and hemin – enriched media for the growth of all clinically significant anaerobes
 - b. Phenylethyl alcohol (PEA)-sheep blood agar – Promotes the growth of anaerobes. Used for inhibition of enteric and certain facultative anaerobic Gram-negative rods. PEA can reduce spreading and swarming of some *Proteus* and *Clostridium* species. PEA should not be incubated for longer than 7 days.
 - c. Laked Kanamycin-vancomycin (LKV) agar – for selection of *Prevotella*, *B. fragilis* group and occasional *Fusobacterium*. Inhibits many Gram-positive and most Gram-negative anaerobes. LKV should not be incubated for longer than 7 days.
 - d. Brain Heart Infusion Broth (BHI)– enrichment broth that supports the growth of various fastidious and nonfastidious bacteria; enriches the growth of small number of organisms or inhibited organisms; provides a back-up to solid media culture plates; antibiotic diffuser.

B. Equipment

1. Glove Box
 - a. (Gas tanks: Nitrogen and Mixed anaerobic: 5% Hydrogen; 10%CO₂; 85% Nitrogen)
 - b. Culture plates and supplies may be transported in and out of the glove box through the Entry Port located at the front right side of the box. Make sure that both the inner and outer doors are securely locked.

- c. Press the “Starts Cycle” button to purge and replace the gas in the interchange chamber. When cycle is complete, the anaerobic indicator light turns on.
 - d. Concept 400 (older model at ANA 2 bench) – Enter the glove box by inserting hands and arms into sleeves and using the vacuum followed by gas exchange foot pedal. Repeat this exchange three times. Unlock and enter the chamber.
 - e. Concept 400 (newer model at ANA 1 bench) – Enter the glove box by inserting hands and arms into sleeves and evacuate the air on both the right and left side completely. Unlock and enter the chamber.
 - f. Transfer of plates and supplies can occur at this point by using the inner access door.
 - g. Culture plates should be kept organized by hospital and sequence order.
 - h. Anaerobic indicators need to be checked and replaced daily.
 - i. Water levels need to be checked daily and the drip tray emptied and wiped. Water reserve can be checked on the left side of the box. The level should always be maintained between the low and high markings. Any deviation can cause the instrument to alarm.
 - j. The glove boxes need to be cleaned with 70% ethanol, rinsed with distilled or de-ionized water, and wiped dry daily.
 - k. After a tank is changed, the “Com. Cycle Active” indicator light will turn on and possibly blink. The glove box only needs to go through a commission cycle if the anaerobic environment has been lost. If the anaerobic environment has been maintained the “commission override” button can be pressed and held until this cycle stops. The button is located on the right top of the indicator under a white sliding door.
 - l. One extra emergency tank is kept in the back room of the Main Microbiology Laboratory.
2. Anoxomat Jars
- a. (Gas tank: 10% Hydrogen; 10% CO₂; 80% Nitrogen)
 - b. Indicator strips must be monitored daily for each jar.
 - c. Jars require cleaning after each use with of Halamid, rinsed with water, and wiped dry.
 - d. Refer to [Anoxomat Procedure](#) for full instructions.

VI. STORAGE AND HANDLING OF CULTURES

- A. The aerobic plates must incubate at 35°C in 5% CO₂ for at least 18-24 hours before the first examination. They must incubate for at least 48 hours to determine no aerobic growth.
- B. Anaerobic plates are incubated in the Anoxomat jars for the first 48 hours before examination/comparison to aerobic plates. These plates are then transferred to the anaerobic glove box where they are incubated for an additional three days (for 5-day cultures) or 12 days (14-day cultures).
- C. **Anaerobic plates are NOT to be exposed to ambient air for more than 15 minutes. Exposure for anything longer risks compromising the growth of obligate anaerobic organisms. Set a timer if necessary.**
- D. CNA and MAC plates can be discarded if there is no growth after 48 hours.
- E. For extended anaerobe cultures, the BAP, CHC, PEA, and LKV can be discarded if no growth after 5 days. Continue to incubate the BRU and THIO for the duration of the culture. If *C. acnes* is isolated before the full 14-day incubation is complete, then the culture can be finalized.

VII. QUALITY CONTROL

- A. Quality Control organisms (*Cutibacterium (Propionibacterium) acnes*, *Clostridium perfringens* and *Bacteroides fragilis*, *Fusobacterium mortiferum*, *Fusobacterium nucleatum*,

Porphyromonas levii, *Peptostreptococcus anaerobius* & *Prevotella melaninogenica*) are to be re-streaked on Tuesdays and Fridays.

- B. Anaerobic indicator strips in Anoxomat jars for the anaerobe and blood benches are to be checked daily. An anaerobic environment is indicated by a white colored tip. The presence of oxygen is indicated by a blue colored tip.
- C. Anaerobic indicator pads in the anaerobic chambers are to be checked, recorded and changed daily. An anaerobic environment is indicated by a white color. The presence of oxygen is indicated by a pink color.
- D. The maintenance checklist should be reviewed and recorded daily.
- E. Media and reagents are quality controlled according to IQCP and CLIA guidelines. Refer to the IQCP and Quality Control binders for complete procedures.

VIII. ANAEROBIC CULTURE PROCEDURE (Work-Flow)

- A. Print Worklists (anaerobes, shunts and autopsies).
- B. Beginning with the oldest cultures, examine the aerobic plates and record new growth on worklist. Growth on this media needs to be compared to the organisms growing on anaerobic media. Compare growth/no growth on the solid media to Gram-stain result and appearance of THIO. Refer to the interpretation section for site/source-based work-up guidelines in section X.
- C. For cultures that are 48 hours old, retrieve the Anoxomat jars by opening only one jar at a time. ***The anaerobic media should not be left in ambient air for longer than 15 minutes.*** Only work with what is required and deliver to the glove box within this time. Set a timer if needed to keep track of time. Examine the indicator strip to ensure an anaerobic environment was maintained in the jar. Compare the growth on the anaerobic plates to the aerobic plates. Compare growth to Gram-stain and appearance of THIO. Work-up the THIO and perform a Gram-stain review if needed. Record the testing in the computer. Refer to the interpretation section for site/source-based work-up guidelines in section X.
- D. Examine all new 24-hour old cultures for growth. Record testing in the computer. Refer to the interpretation section for site/source-based work-up guidelines in section X.
- E. Bring your worklist and any anaerobic plates into the glove box. Examine all the oldest anaerobic media. Work-up of anaerobic organisms can take 2 main routes. Primary identification methodology is always MALDI.
- F. If MALDI fails, proceed to VITEK ANC card. See Chart below:

Identification of anaerobes – Tests required are located under specific method	
MALDI IDENTIFICATION	VITEK ANC IDENTIFICATION
Gram Stain	Gram Stain
Colony Morphology/ Plate growth	Aero-tolerance
Spot Tests / Fluorescence	Spot Tests / Fluorescence
Specimen source	Special Potency Disks
	Specimen Source

- G. Record the work-ups on your worklist to be entered into the computer later. Re-streak any QC organisms at this time, if necessary. Check the anaerobic indicator to ensure that an anaerobic environment was maintained. Remove all of the oldest culture plates from the glove box that have reached the end of their incubation. Refer to the interpretation section for site/source-based work-up guidelines in section X.
- H. If the source/site-based work-up cannot be interpreted, using this procedure as a guide, bring up on Rounds.
- I. Completely enter work-ups written on your worklist into computer.
- J. Reincubate re-streak and primary aerobic plates as necessary. Reincubate the BAP and CHC for the entire duration of the culture to consider the possibility of fastidious organisms.

- K. Save BAP and CHC on benchtop when aerobic growth work-up is complete and until the culture is finalized. Most clinically important aerobic organisms will grow within 48 hours.
- L. Set-up susceptibilities and stocks. Refer to [Isolate Stocking Procedure](#) for more detailed instructions.
- M. THIO of finalized cultures are stored in racks located beside the ANA 2 glove box. These racks should be rotated.
- N. Save a representative plate for any culture that did not receive full identification and susceptibility performed. Save for 7 days in the appropriate environment.
- O. Record your QC and maintenance in binder.
- P. Clean Anoxomat jars.
- Q. Clean bench and record.
- R. Prepare printed worklist on Fridays and record work-ups needing attention over the weekend.
- S. Cultures held for extended periods should have their BRU agar observed for growth over the weekend.

IX. ANAEROBE IDENTIFICATION GUIDE – [Refer to Appendix AP13](#)

- A. Gram stain – Any organism suspected to be an anaerobe needs a Gram-stain.
- B. Vitek MS (MALDI) – Primary identification method for anaerobic organisms. Always consider Gram reaction, colony morphology, spot tests/florescence and specimen source for final identification. Refer to [Vitek MS Procedure](#) for complete instructions.
- C. Vitek 2 ANC card – Second (back-up) system for the identification of anaerobic organisms. Always consider Gram reaction, colony morphology, spot tests/fluorescence, special potency disks and specimen source for final identification. Refer to [Vitek 2 Procedure](#) for complete instructions.
- D. Aerotolerance test – Any suspected anaerobe identified by ANC card needs to be re-streaked to a CHC plate (incubated 35°C in 5% CO₂) and BRU plate (incubated in glove box). Growth on the two plates is compared. No growth or less growth on the CHC plate indicates an anaerobic organism or facultative organism.

X. INTERPRETATION

- A. Before work-up begins, analysis of culture type and quality of anaerobic culture should be made by the following below to determine the extent of your workup on days 1 & 2:
 - 1. Identify where the specimen was taken (site).
 - 2. Consider the source (tissue, abscess, body fluid).
 - 3. Review the original Gram-stain (were there PMNs, Epithelial cells, multiple different organisms seen?)
- B. Culture Considerations
 - 1. Cultures that were planted after 15:00 should remain in the incubator until needed.
 - 2. Growth on plate must be compared to direct Gram-stain and THIO appearance. Refer to [Quality Assessment and Accurate Result Reporting Procedure](#) for complete instructions for Gram-stain reviews.
 - 3. THIO should be subbed when appropriate. This should only be done after the first 48 hours of the culture. This should include a CHC in 5% CO₂ and BRU in glove box at minimum. Selective media should be added if there are different morphologies observed in the Gram-stain. The specimen type can also be considered when supplementing with selective media. Subbing of THIO can be necessary in the following circumstances:
 - a. Appears cloudy/turbid but there is no growth on the primary plates.
 - b. If bacteria were seen in the direct Gram-stain, but there is no growth on the culture plates. This is done regardless of its physical appearance.
 - c. If there is a question of potential contamination of the primary media.
 - d. Subbing of thio, when considering obvious contaminants (growth on side edge of plate or growth not on a streak line) should be ignored. Questionable contaminants should be brought up on Rounds.

4. The number of different colony types growing in a culture and the source/site of the specimen are needed to direct a consistent and meaningful work-up of the culture.
- C. Organism Work-Up
1. Beta-hemolytic *Streptococci* group A or B, *S. aureus*, and *Pseudomonas aeruginosa* are **ALWAYS** reported as pathogens (Table 1) and should be worked-up regardless of multiple organisms present in the culture.
 2. Predominant Potential Pathogens (PPP) - any organism is a potential pathogen in an anaerobic culture if pure or predominant compared to other growth. Table 2 (not an exhaustive list) illustrates organisms that have significance when isolated from specific sources.
 3. Other organisms will be worked up (full ID/SUS) only if there are ≤ 3 different bacterial types or if there is a predominant organism in a mixed culture.
 - a. In general, all organisms may be pathogens in culture. When a collection is from a site that is adjacent to mucosal surface or enteric area, the specimen may be contaminated with commensal flora (Table 3). In these cultures, the predominance of one or two organisms is to be considered. Organisms not fully reported may be grouped together as “mixed aerobic, mixed anaerobic, or mixed aerobic/anaerobic” as appropriate, and include an appropriate canned message (Table 4).
 - i. In these specimen sites, there are potentially reasonable amounts of anaerobes to be present. A generic report (example: 3+ mixed aerobic flora; Organisms suggest mixed enteric flora) can be expanded to list the organisms once the presence/absence of anaerobic organisms is assessed.
 - b. A culture with a positive Gram-stain where multiple organisms seen should begin with its work-up (isolation, ID/SUS) **but do not report until the anaerobic plates are reviewed**. Report: “(Quant) mixed aerobic flora – Further report to follow” until anaerobic plates are examined, and the correct workup is established for the culture.
 - c. With any questions consult with ROUNDS.

Table 1.

PATHOGENS* – Always work-up
<i>Staphylococcus aureus</i>
<i>Streptococcus pyogenes</i>
<i>Streptococcus agalactiae</i>
<i>Pseudomonas aeruginosa</i>

Table 2. This is not a complete list. Questionable organisms should be brought on Rounds.

Source/Site	Potential pathogens (PPP) **
Bites	<i>Eikenella</i> and <i>Pasteurella</i>
Throat/Pharyngeal	<i>Fusobacterium</i>
Abscess	<i>Streptococcus anginosus</i> group
Jaw/Mouth	<i>Actinomyces</i> (10 day culture when requested)
Orthopedic	<i>Cutibacterium (Propionibacterium) acnes</i> (14 day culture when requested)
ANY	<i>Staphylococcus lugdenensis</i>
Myonecrosis	<i>Clostridium</i> (often <i>perfringens</i>)
Pelvic/GU	<i>B. fragilis</i> group

Table 3.

Location	Indigenous Flora
Skin	Cutibacterium (Propionibacterium), Finegoldia, Anaerococcus, Peptostreptococcus, <i>Peptiniphilus</i> , <i>Peptococcus</i> , Coagulase negative <i>Staphylococci</i> , <i>Corynebacterium</i> , <i>Micrococcus</i> , <i>Bacillus</i> (not <i>B. anthracis</i>), alpha hemolytic streptococci, Nonpathogenic <i>Neisseria</i>
Oral, Nasopharynx, Respiratory	Coagulase negative staphylococci, <i>Corynebacterium</i> species, alpha hemolytic streptococci (not <i>S. pneumoniae</i>), <i>Enterococcus</i> spp., yeast, nonpathogenic <i>Neisseria</i> species, <i>Haemophilus</i> species (not influenza) and mixed anaerobes including <i>Lactobacillus</i> species, <i>Cutibacterium (Propionibacterium)</i> , and <i>Actinomyces</i> .
Stomach, Upper GI, Abdominal	Coagulase negative staphylococci, <i>Corynebacterium</i> , alpha hemolytic streptococci, <i>Enterococcus</i> , yeast, nonpathogenic <i>Neisseria</i> , <i>Enterobacteriaceae</i> , <i>Proteus</i> and mixed anaerobes including <i>Lactobacillus</i> , <i>Clostridium</i> (not <i>C. perfringens</i>). <i>Bacteroides</i> is often a part of the intestinal and perineal microbiota, but rarely found in the stomach and upper GI.
Lower GI	Coagulase negative staphylococci, <i>Corynebacterium</i> , alpha hemolytic streptococci, <i>Enterococcus</i> , yeast, nonpathogenic <i>Neisseria</i> , <i>Enterobacteriaceae</i> , <i>Proteus</i> and mixed anaerobes including <i>Lactobacillus</i> , <i>Clostridium</i> , <i>Bacteroides</i> , <i>Parabacteroides</i> , <i>Prevotella</i> , <i>Actinomyces</i> , <i>Bifidobacterium</i> and <i>Eubacterium</i> .
Vaginal/Perineum	Coagulase negative staphylococci, <i>Corynebacterium</i> , alpha hemolytic streptococci, non-hemolytic streptococci, nonpathogenic <i>Neisseria</i> , <i>Enterobacteriaceae</i> , <i>Proteus</i> and Mixed anaerobes including <i>Lactobacillus</i> .

Table 4.

Code	Canned Message
&OISC	Organisms isolated suggest mixed cutaneous flora
&OISG	Organisms isolated suggest mixed GI flora
&OISR	Organisms isolated suggest mixed respiratory flora
&OISO	Organisms isolated suggest mixed oral flora
&OISV	Organisms isolated suggest mixed vaginal flora
&OISU	Organisms isolated suggest mixed genital/urinary flora

4. If there are ≤ 3 Types of Organisms:

- a. Work-up any organism that grows on the primary media. An identification and susceptibility test should be performed (when appropriate for the organism- Refer to [Organisms & AST Procedure](#)).
- b. If growth is pure and on original media always consider the organism significant.
- c. Coagulase-negative *Staphylococcus*; Alpha-hemolytic *Streptococcus*, or *Corynebacterium* species should always be listed generically by genus, unless growing in multiple sets:
 - i. Identifications and/or susceptibilities must be compared when growing in multiple cultures.
 - ii. If identifications do not correlate amongst sets, apply the comment: **&DIDC**
 - a) "Identifications differ between corresponding cultures"
 - iii. If identifications match, perform susceptibility testing to further compare.

- a) If susceptibilities match, release susceptibility results.
 - b)** If susceptibilities differ, do not report/suppress results and add the following isolate comment: **&DSUC**
"Susceptibilities differ between corresponding cultures"
- d. Growth from Broth Only
- i. Coagulase-negative *Staphylococcus*; Alpha-hemolytic *Streptococcus*, or *Corynebacterium* species
 - a) If growing from a single specimen, report generically with comments **&BROT** "Organism growing "From Broth Only" usually indicates a low bacterial burden.", **&PROC** "Probable contamination" & **&NFW** "No Further Workup".
 - b) If growing from multiple specimens, perform identifications/susceptibilities for comparison
 - ii. Potential Pathogen
 - a) If growing only from a single specimen add comments **&BROT** "Organism growing "From Broth Only" usually indicates a low bacterial burden." & **&CONS** "Consult Required for Further Workup".
 - b) If growing from multiple specimens perform full ID/AST, if appropriate for organism
 - c) If questioning potential pathogen, bring up on Rounds.
5. If there are ≥ 4 Types of Organisms:
- a. Work up Pathogens* and report Predominant Potential Pathogens** if present and/or one of the following:
 - i. Mixed Aerobic Flora (mixaer) – Mixture of aerobic organisms, excluding predominant pathogens.
 - ii. Mixed Anaerobic Flora (mixana) - Mixture of anaerobic organisms, excluding predominant pathogens.
 - iii. Mixed Aerobic/Anaerobic Flora (mixaeae) – Mixture of aerobic and anaerobic organisms, excluding predominant pathogens.
 - iv. Add a mixed code to describe the mixed flora that is growing (Table 4)

XI. RESULTING (the number of days can be changed when needed for extended cultures)

NOTE: Cultures that are positive for an organism from a sterile body site (ie: CSF, Tissue, and Body Fluids) that was not seen in the initial Gram-stain are to be called. Refer to [Critical Results Notification](#) Procedure.

A. Preliminary report for a sterile culture at 24 hours:

No aerobic organisms isolated to date Culture in Progress Culture held for 5 days

B. Interim report for a sterile culture at 48 hours:

No aerobic organisms isolated to date No anaerobic organisms isolated to date Culture in Progress Culture held for 5 days
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C. Final report for a sterile culture at 5 days

No Aerobic Organisms isolated
 No Anaerobic Organisms Isolated
 No growth at 5 days

D. If blood culture bottles are drawn for an anaerobic culture and flag as positive, report the Gram-stain to that bottle in either of the following situations:

1. Culture is currently "No growth to date"
2. Gram stain from the bottle differs from what currently grows in the culture
3. In the Test Comment section use the following comment **JGSBR** to report Gram-stain of bottle:

Gram stain of broth culture shows: (insert Gram stain morphology)
 Restreak of broth culture in progress
 Called to and Read back by: (insert person called, date and time)

4. Follow the Critical

Reporting Policy in the [Critical Results Notification Procedure](#)

5. Document work in worksheet
 6. When organism grows, the "Restreak of broth culture in progress" should be hidden and replaced with "From Broth Only" as the quantitation, if that is the case for each organism isolated.
- E. Corneal scrapings are not quantitated.
- F. If a Potential Pathogen was already worked up, but then determined to be part of the mixed flora because of additional growth during incubation; report according to the example below:

*** Aerobe Anaerobe Cult** FINAL 12/01/17 16:27

1+ Staphylococcus aureus 2+ Pseudomonas aeruginosa 3+ Mixed Aerobic/Anaerobic Flora	
<i>Organisms isolated suggest mixed enteric flora.</i>	
<i>No further workup</i>	
Klebsiella pneumoniae	
<i>Component of the mixed flora. No further workup.</i>	
Escherichia coli	
<i>Component of the mixed flora. No further workup.</i>	

- G. *B. fragilis* group should be ruled out in pelvic specimens belonging to a female. Apply the comments **&BLPS** and **&NFW**, and report according to the example below:

*** Aerobe Anaerobe Cult** FINAL 12/01/17 16:16

1+ Staphylococcus aureus 2+ Pseudomonas aeruginosa 3+ Mixed Aerobic/Anaerobic Flora	
<i>Including B. fragilis group - All isolates should be considered Beta-Lactamase positive.</i>	
<i>Organisms isolated suggest mixed GI flora.</i>	
<i>No further workup</i>	

H. If commensal flora is reported, a designated isolate comment must be included.

1. *S. aureus*, *P. aeruginosa*, or Beta-hemolytic *Streptococcus* Group A or B **ISOLATED:**
 - a. Add isolate comment: **&NFW – No further workup**

* OR-Surgical Aerobe Anaerobe Culture FINAL 02/19/19 15:14

4+ Staphylococcus aureus

Oxacillin-susceptible staphylococci are susceptible to nafcillin, oxacillin, cephalosporins, carbapenems (meropenem) and B-lactam/B-lactamase inhibitor combinations.

3+ Mixed Aerobic/Anaerobic Flora

No further workup

2. *S.aureus*, *P. aeruginosa*, or Beta-hemolytic *Streptococcus* Group A or B **NOT ISOLATED:**

- a. Add isolate comment: **&NSPS – No Staphylococcus aureus, Pseudomonas aeruginosa or Beta-hemolytic Streptococcus Group A and B isolated.**

* OR-Surgical Aerobe Anaerobe Culture FINAL 02/19/19 15:10

2+ Mixed Aerobic/Anaerobic Flora

*Organisms isolated suggest mixed GI flora.
No Staphylococcus aureus, Pseudomonas aeruginosa or Beta-hemolytic Streptococcus Group A and B isolated.*

* OR-Surgical Aerobe Anaerobe Culture FINAL 02/19/19 15:12

4+ Streptococcus constellatus

1+ Mixed Aerobic/Anaerobic Flora

No Staphylococcus aureus, Pseudomonas aeruginosa or Beta-hemolytic Streptococcus Group A and B isolated.

I. Extended Incubation Requests

1. Add \$EXAN media upon an extended request.
2. All culture plates are observed daily for 5 days.
3. After the culture has been observed and worked up for the initial 5-day period, check off the R box next to the \$EXAN media. The culture will transfer to the Extended anaerobic queue.
4. After day 5, discard all aerobic plates if work up is complete, and any anaerobic selective plates with no growth. Continue incubating the brucella for the entire 14-day hold, observing daily or until *C. acnes* is isolated.

XII. LIMITATIONS

- A. Improper collection and/or transport may inhibit the growth of fastidious organisms.
- B. Loss of anaerobic environment will not allow strict anaerobes to grow.
- C. Failure to perform aerotolerance on colonies may result in falsely identifying an aerobic organism as an anaerobe (when Vitek ANC is used).

XIII. NOTES

- A. Routine anaerobic specimens not finalized by Day-7 or extended anaerobe cultures not finalized by Day-14 should be brought up on rounds.
- B. Any questionable cultures should be brought up on Rounds.
- C. If isolated from a sterile site, *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pyogenes* must be submitted to the RI Department of Health.
- D. All isolates of: *Legionella*, *Listeria monocytogenes*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, *Campylobacter*, *Mycobacterium*, Vancomycin resistant / Vancomycin intermediate *Staphylococcus aureus*, *Candida auris*, and suspected agents of bioterrorism must be sent to the RI Department of Health.

XIV. TECHNICAL SUPPORT

- A. Glove Box
 - 1. The Baker Corporation 1-800-992-2537
 - 2. Technical Service Rep (Chris) 1-207-608-8307
- B. Anoxomat
 - 1. Technical Service 1-800-225-4034 x2123
- C. Gases (Refer to [Appendix AP16](#): Ordering Gas Tanks)
 - 1. Airgas Account #329-0233 1-401-732-2920

XV. REFERENCES

- A. Clinical Microbiology Procedures Handbook Fourth Edition 2016, Editor in Chief: Amy L. Leber; Section Editor: Gerri S. Hall.
- B. Mount Sinai Hospital, Department of Microbiology Wounds/Tissues/Aspirates Culture Manual, Version 17. www.mountsinai.on.ca/education/staff-professionals/...manual/WOU-TIS-AS.doc
- C. Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline. M56-A; Clinical and Laboratory Standards Institute; July 2014. <http://www.health.ri.gov/diseases/infectious/resultsreportable.php>

XVI. REVISIONS

- A. May 23, 2019
 - 1. Updated testing and reporting guidelines for CoNS, AHS, *Corynebacterium* sp, and *B. fragilis*
- B. 02/24/2023: Updated call requirements to align with Critical Call Procedure.