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| Related Documents | | | |  |  |  | |  |
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**PRINCIPLE:** Anaerobic bacteria can cause a wide variety of infections, including wound infections as a result of trauma or surgery; 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.

Anaerobic cultures will be performed upon proper collection and request from the physician. The exact source of the specimen must be stated to allow for optimal selection of culture media. Specimens should also be cultured simultaneously for aerobic organisms for complete culture study and evaluation.

The goal of processing primary culture plates is to isolate significant anaerobic organisms present in the original specimen for identification and when susceptibility testing is indicated. The original Gram stain of the specimen is critical. At that time all morphological types should be carefully described and recorded. When evaluating culture plates, all morphotypes observed in the original Gram stain should match. It is important to remember that anaerobic culture media permit facultatively anaerobic bacteria to grow. All bacterial isolates must be subjected to aerotolerance testing before being designated anaerobes.

Special precautions must be taken to avoid exposure of culture plates to oxygen during examination. Even a 10-min exposure will kill some oxygen-sensitive anaerobes (*Fusobacterium* spp., *Porphyromonas* spp., and anaerobic cocci). In addition, reduced culture media will become oxidized when exposed to air, making the media unsuitable for isolation of fastidious anaerobes.

**SPECIMEN:** The minimum standards for the evaluation of deep wound cultures require adequate procedures for the collection, recovery and identification of clinically relevant pathogens. This includes aerobic, facultatively anaerobic, and strictly anaerobic organisms, when indicated and when the submitted material is of sufficient quality to provide an interpretable result.

* Specimens for anaerobic culture must be collected properly and transported to the laboratory immediately in the appropriate transport system.
* The nursing unit should be provided transport media and instructions when collecting anaerobe cultures.
* Fluids, aspirates and tissues are preferred. Aspirated fluids or exudates may be transported in syringes, capped using an acceptable mechanical recapping device. **Needles must be removed from syringes before transporting.**
* SWABS are sub-optimum for collection of anaerobe specimens.

**UNACCEPTABLE SPECIMENS:** The following specimens are **not acceptable** for anaerobic culture:

* + expectorated sputum or tracheal aspiration
  + vaginal/cervical secretions
  + throat and nasopharyngeal swabs
  + placenta, unless from C-section
  + large bowel contents; feces; ileostomy, colostomy effluents; rectal swabs (except for C. difficile)
  + bronchial washing (protected brush specimens are acceptable)
  + urine, other than supra-pubic aspiration
  + gingival swabs or any other oral cavity swabs
  + superficial swabs from decubitus ulcers, perirectal abscesses, foot ulcers, exposed wounds, pilonidal sinuses and other sinus tracts
  + any material adjacent to a mucous membrane that has not been adequately decontaminated

**Specimen preparation**

1. Vortex grossly purulent specimens in the anaerobic transport vial to ensure even distribution of microorganisms.
2. Grind bone or tissue with approximately 1 ml of liquid medium (THIO) to make a thick paste.
3. Wring out swabs in 0.5 ml of liquid medium (THIO or chopped meat), and then treat them as a liquid specimen. Alternatively, plant swabs directly onto appropriate media, but this option is less desirable because the loss of organisms on each medium will result in a poorer specimen for Gram stain.
4. Centrifuge large volumes of nonpurulent material. Use the sediment to inoculate the media and to prepare the Gram stain.

**SETUP:**

* BBE/PEA bi-plate (pre-reduced)
* Brucella Blood Agar (pre-reduced)
* Enriched Thiogylocolate Broth with Vitamin K and Hemin
* Chocolate (CO2) – Label as “Aero Control”
* Gram Stain\*\*

\*\*Gram Stains are typically performed and reported via the corresponding aerobic culture. If an anaerobic culture is ordered without a corresponding aerobic culture, a gram stain must be performed and reported.

**MICROSCOPIC EXAMINATION**

A direct smear can be gently heat fixed or fixed in absolute methanol for 1 min and then stained by standard Gram stain procedure and reagents. Gram stain reveals the types and relative numbers of microorganisms and host cells present and serves as a QC measure for the adequacy of anaerobic techniques. Correlation of specimen type with bacterial morphology on the Gram stain can provide the clinician with rapid presumptive information about the identity of the bacteria present.

1. Large gram-positive rods with boxcar-shaped cells and no spores usually indicate *Clostridium perfringens.* Some *C. perfringens* cells may appear gram negative but may have the same morphology as the gram-positive cells within the same microscopic field
2. Gram-negative coccobacillary forms suggest pigmenting *Prevotella* group or *Porphyromonas* group.
3. Thin gram-negative bacilli with tapered ends suggest *Fusobacterium nucleatum.*
4. Pleomorphic pale-staining gram-negative bacilli suggest *Bacteroides* spp.
5. Very small gram-negative cocci suggest *Veillonella* spp.
6. Results of the Gram stain may indicate the need for additional media or special stains. Aspirated material from a lung nodule, for example, may reveal long, thin, branching gram-positive bacilli. These bacilli suggest the possibility of *Actinomyces* or *Nocardia* spp. The addition of a modified Kinyoun acid-fast stain may quickly provide useful information for the clinician.

**INCUBATION:**

1. Incubate anaerobic plates and broth in 37°C incubator under anaerobic conditions. Depending on the facility, anaerobe bags, jars and/or an anaerobe chamber may be used. Anaerobic bags are typically used for newly setup specimens where jars or the chamber are used for ongoing cultures.
2. Incubate chocolate plate in the anaerobic designated section of the CO2 incubator

**QUALITY CONTROL:**

* Anaerobic media used is Exempt from User Performed QC
* Anaerobic conditions are monitored with each patient culture via appropriate anaerobic indicator for each anaerobic system being used. Document QC for each anaerobic system each day of use.

**PROCEDURE**:

1. Perform the initial plate examination 24 h after plate inoculation if an anaerobic chamber is used. Delay this first examination until 48 h after inoculation if anaerobic jars or bags are used.
2. Carefully examine the anaerobic blood agar plate (anaBAP). Record a detailed description of each colony type, noting such characteristics as pitting, swarming, hemolysis, pigment, “greening” of the medium, etc. These colony characteristics can provide valuable clues to the identity of the isolates when used in conjunction with rapid identification tests and Gram stain.
3. Select a single, well-isolated colony of each morphological type observed that cannot be definitively associated with aerobic growth from the aerobically incubated chocolate plate. Touch each colony with a loop or sterile stick, subculture it onto anaBAP and Chocolate agar and Gram Stain as appropriate.
4. Anaerobic BAP
5. Streak the subcultured organism in four quadrants to obtain isolated colonies.
6. Chocolate Agar
7. Divide this plate into quadrants, and subculture up to four organisms onto each plate.
8. Incubate at 35 to 37°C in a 5%CO2 environment for 24 to 48 h to detect slow-growing aerobic organisms such as *Capnocytophaga, Actinobacillus,* and *Eikenella* spp.
9. Use only CHOC for aerotolerance testing. *Haemophilus* spp. will grow anaerobically on BAP and therefore will be mistaken for anaerobic gram negative rods if CHOC is not used.
10. Gram stain
11. Air dry the smear. It is preferred not to heat fix the slide as heat can distort the morphology of many anaerobes.
12. Perform the normal Gram stain procedure which includes methanol fixation of the smear. Examine selective media such as PEA & BBE.
13. PEA media inhibits facultative anaerobic organisms and prevents swarming of Clostridium spp. and Proteus spp.
14. BBE media demonstrates an organism’s ability to grow in the presence of 20% bile. Most aerobes and anaerobes are inhibited on BBE except for *Bacteroides fragilis* group, *Bilophila wadsworthia,* and some *Fusobacterium* spp.
    1. Pick any colonies on PEA that are different from the colonies isolated on the anaBAP. The PEA plate may be used in place of the anaBAP if the culture is overgrown with swarming *Clostridium* spp., *Proteus* spp., or other organisms. PEA may also provide earlier detection of pigmented anaerobic organisms (*see Table*).
    2. Pick all the different colonies growing on BBE that are >1 mm in diameter. Record the esculin hydrolysis reaction (black = positive), and perform a spot catalase test on each colony type.
15. Broth culture
    * + 1. If No growth on original plates
    1. If anaerobes are suspected based on initial specimen gram stain and the anaerobic broth is turbid, prepare a Gram stain and subculture onto anaBAP and Chocolate agar. Add a comment of Broth only to the culture report if anaerobes grow from the broth sub.
    2. Incubate negative broth cultures for 5 days, examine visually, and discard appropriately.

**Note:** Subcultures of broth backups is only when anaerobic systems fail or when primary plates are negative but the broth is turbid.

* + - 1. If Growth on original plates

If there is growth on the primary plates, subculture of the backup broth is generally not helpful and can lead to needless duplication. If, on the other hand, *Actinomyces* is suspected, the backup broth can be occasionally helpful to recover this slow-growing organism, which may not grow readily on solid media.

1. Subsequent plate examination
2. Incubate primary anaBAP for 5 days. Examine the primary plates daily and isolate and perform aerotolerance tests on any new colony types that appear.
3. Pigmented *Prevotella* spp., *Porphyromonas* spp., and *Actinomyces* spp. commonly appear after 2 to 3 days of incubation. Examine the primary anaBAP for pigmented organisms.
4. *Bilophila wadsworthia* commonly appears after 3 to 4 days of incubation, generally first observed from the BBE medium. This organism appears as small, translucent colonies with a black center and can resemble “fish eyes.”

**Anaerobic organism clues from Primary Culture Plates and use of Supplemental Media**

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| **Colony morphology** | **Possible identification** | **Supplemental medium** |
| Agar pitting | *Bacteroides ureolyticus group* | n/a |
| Black or tan pigmentation | *Porphyromonas* spp. or pigmented *Prevotella* spp. | n/a |
| Brick red fluorescence | *Porphyromonas* spp. or pigmented *Prevotella* spp. (*Porphyrmonas gingivalis* does not fluoresce) | n/a |
| Chartreuse fluorescence (gram-negative rod) | *Fusobacterium* spp | n/a |
| Chartreuse fluorescence (gram-positive rod) | *Clostridium difficile* or *Clostridium innocuum* | n/a |
| Double zone of beta hemolysis | *Clostridium perfringens* | n/a |
| “Fried egg”*,* | *Fusobacterium necrophorum Fusobacterium varium* | BBE for bile growth |
| “Greening” of medium | *Fusobacterium* spp. | n/a |
| Large with irregular margin | *Clostridium* spp | n/a |
| “Medusa-head” | *Clostridium septicum* | PEA |
| “Molar tooth” | *Actinomyces* spp. | n/a |
| Pink to red colony (gram positive rod) | *Actinomyces odontolyticus* | n/a |
| Speckled or “breadcrumb” | *Fusobacterium nucleatum* | n/a |
| Swarming growth | *Clostridium septicum Clostridium sordellii*  *Clostridium tetani* | PEA to prevent swarming |

**EXTENT OF WORKUP**

1. For blood cultures and sterile sites that contain an anaerobe, perform a complete identification. An exception, however, could be when one finds that only one of many blood draws is positive for the anaerobe. This could represent contamination or transient bacteremia, in particular, if the anaerobe is a usual component of the skin microbiota, such as a gram positive coccus or *Propionibacterium* sp. (including *Propionibacterium acnes*). This does not mean that these organisms are never related to bacteremia, but in general one would expect growth from many or all of the patients’ blood cultures, not just an isolated draw, unless the patient has already been placed on broad-spectrum antimicrobial agents.
2. Wound samples, if collected on swabs, are less than optimal specimens, easily contaminated with anaerobic skin microbiota organisms and not appropriate for demonstration of the true pathogen in most situations. Workup of the specimen should be directed in light of the direct Gram stain results, and if the specimen has mixed aerobes and anaerobes, consider ruling out the *B. fragilis* group and *C. perfringens*; if these are present, consider reporting their presences and signing out that they were found in conjunction with mixed aerobic and anaerobic organisms. A comment on the result that swabs are inappropriate for optimal anaerobic recovery would be helpful as an educational tool for your clinical colleagues.
3. If an aspirate or a large fluid of body volume is sent, treat like an abscess. If there are many aerobes and anaerobes, there is rarely a clinical need to work everything up—look for more of the typical pathogens (*B. fragilis* group, *Bilophila wadsworthia,* and *C. perfringens*) and then morphologically describe others. Again, use the Gram stain to determine a correlation to the specimen contents that were sent to the lab initially.
4. Requests for *Actinomyces* spp. should result in a mechanism in the laboratory for holding the culture longer, at least 10 to 14 days, and an indication to the technologist to work up branching gram-positive bacilli resembling *Actinomyces* spp. with this specific request in mind. In addition, the thioglycolate broth that is set up with this specimen should be subbed for *Actinomyces* spp. Specifically before the culture is finalized. Sites where one might want to consider looking for *Actinomyces* spp. would include mandibular (jaw) abscess, neck abscess, sinus (collected on other than a swab), lacrimal gland specimens, tonsillar area, intrauterine device-related specimens, and sites where the Gram stain demonstrates “sulfur granules” and the presence of gram-positive branching bacilli.

**CULTURE REPORTING**

1. Report Anaerobic Cultures at Day 3 and Day 5.
2. Report as *No Growth* if there is no aerobic or anaerobic growth.
3. Report as *No Anaerobes Isolated* if there is aerobic growth but no anaerobic growth.
4. Positive anaerobe cultures will be reported as soon as detected.
5. Cultures that grow in broth only will be noted with a BROTH only comment. No quantitation is entered if recovered in broth only.

**SUSCEPTIBILITY**

1. Gram-negative and gram-positive anaerobes should be screened with a nitrocefin / cefinase disk test to rule out the presence of a β-lactamase enzyme. Note: B. fragilis group are usually β-lactamase positive and routine beta-lactamase testing is not necessary.
2. Non-sterile site cultures will have drug of choice information attached to the report with the comment to Notify Microbiology if susceptibility testing is desired.

*\* Susceptibility testing is not routinely performed on anaerobes. The following antimicrobic agents are generally active against common anaerobes:*

*Metronidazole Cefoxitin*

*Imipenem Ertapenem*

*Ampicillin/Sulbactam Piperacillin/Tazobactam*

*Most Anaerobic Infections are polymicrobic and may require combination drug therapy. Please notify the Microbiology Laboratory within 7 days if treatment failure occurs and infection persists. The isolate will be referred for susceptibility testing.*

1. Sterile Site cultures, including clinically relevant blood culture anaerobes, require anaerobe sensitivity testing.
2. Susceptibility testing of anaerobes, besides for β-lactamase production, is not performed on site. Send isolate in pure form to appropriate Reference Laboratory for susceptibility.

**REFERENCES**

1. Examination of Primary Culture Plates for Anaerobic Bacteria. Clinical Microbiology Procedures Handbook. March 2007. Section 4.4.
2. Clinical Laboratory Standards Institute M100S23. January 2013.