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| MC 1.07 Anaerobic Culture |
| **Purpose** | This procedure provides instruction for the Anaerobic Culture for the Microbiology laboratory. |
| **Principal and Clinical Significance** | Anaerobic bacteria are a significant component of the normal microbiota of the human host. There are anaerobes present on most body surfaces and mucous membranes; they exist in large numbers throughout the entire gastrointestinal tract, from the mouth to the colon, with the exception of the stomach and esophagus; they are found in large numbers in the female genitourinary tract.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 an sinusitis, bacteremia, endocarditis, myonecrosis; gas gangrene; and dental and oral infections. Incidence is reported as 0.5-9%, depending on location, age and demographics of the population. Proper collection of specimens to avoid contamination with organisms of the normal microbiota and prompt transport to the laboratory for processing are essential.Interpretation of the culture results should be based on the aerobic culture gram stain and number and type of anaerobes isolated.

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| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | ANAC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram Stain reagents
* MALDI reagents
* Vitek
 | * Glass slide
* MALDI slides
* Palladox catalyst
* Anaerobic Gas Pack
 | * Ambient Air Incubator
* Anoxomat
* Anoxomat jars
* Anaerobic GasPak EZ Bag
* Anaerobic Jar/GasPak
* Incinerator
* Inoculating loop
* Microscope
* MALDI
* Vitek 2XL
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| Refer to the Sunquest specimen label for media information. The specimen site determines appropriate media. Media code in parentheses.  |

* CDC Anaerobe Blood agar (ASB2)
* Laked Kanamycin

Vancomycin agar (AKV) * Phenylethyl Alcohol Agar (PLAA)
* Bacteroides Bile Esculin Agar (BBE)
* Enriched Thioglycolate broth (THIO)

Aerotolerance testing media* Chocolate (CHOC)
* Anaerobic SB (ASB1)
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| **Specimen** | 1. Acceptable specimens:
2. Aspirates or biopsy from deep wound, abscess, lung, bone or joint.
3. Suprapubic aspirate.
4. Body fluids.
5. Specimens submitted on swabs in Port-a-cul anaerobic transport media.
6. Refer to [Lab Test Directory Anaerobic Culture](https://www.childrensmn.org/References/Lab/microbioviral/anaerobic-culture.pdf) for collection and transport instructions.
7. DO NOT refrigerate specimens.
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| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling.1. [*Biohazard Containment*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20201%20%20%20Biohazard%20Containment.doc)
2. [*Biohazardous Spills*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20204%20%20%20Biohazardous%20spills.doc)
3. [*Safety in the Microbiology Laboratory*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20202%20%20%20Safety%20in%20the%20Microbiology%20Lab%20Policy.doc)
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| **Procedure** | A. Inoculation

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1. Allow all media to come to room temperature before inoculation. Use media that has been pre-reduced for 24 hours in the nitrogen holding tank.
* Store 5 sets of anaerobic media in the nitrogen holding tank.
1. Label all plates, tubes and slides properly with the patients name, accession number and date. Write tech code on one of the labels.
2. Inoculate the media in the order of the least selective first to prevent carryover of inhibitory substances to another medium. Refer to the Sunquest specimen label for the order of inoculation.
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|  | B. Specimen processing1. Specimens received on swabs:
2. Remove swab from port-a-cul tube and roll directly onto plates, covering one third of each plate.
3. Place swab in Enriched Thioglycolate media.
4. Aspirates, tissue, bone and fluids:
5. If specimen is received in a syringe, transfer the entire amount into a sterile tube and mix thoroughly. Vortex on low speed to limit introduction of oxygen.
6. If the specimen is received in a syringe and the volume is small, rinse syringe with a small amount of THIO or sterile saline (SLNE) to remove the specimen from the syringe. Mix well.
7. Tissues should be stomached in a stomacher bag with 0.5 ml sterile saline.
8. Grind bone with grinder in 0.5 ml sterile saline.
9. If specimen is clotted, put clot in stomacher bag and add 0.5ml sterile saline and gently homogenize to disperse clot and release bacteria.
10. Place 1-2 drops directly on each plate and into a THIO.
11. Streak plates semi-quantitatively for primary isolation.
12. Sterilize the inoculating loop in the incinerator for 5 to 10 seconds. Allow loop to cool.
13. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately 1/4 of the plate.
14. Flame the loop, turn the plate a quarter turn and pass the loop through the edge of the first quadrant 4 times while streaking into the second quadrant. Continue streaking in the second quadrant without going back into the first quadrant 3-4 times.
15. Flame loop again, turn the plate another quarter of a turn, and pass the loop through the edge of the second quadrant approximately four times while streaking into the third quadrant. Continue streaking in the third quadrant without going back into the second quadrant 4-5 times. See Figure 1 for an illustrative example.

  Figure 1. Semi-quantitative plate streaking.C. Incubation1. Using the Anoxomat instrument, close plates in jar directly after processing, using the Anaerobic setting.
2. If Anoxomat jar is not available, place anaerobic plates into GasPak EZ bag or GasPak jar. Each bag can hold up to 4 plates. Open sachet and place in bag and immediately seal bag.
3. Incubate all plates in anaerobic jar or bag at 35o C for 48 hours before initial opening and reading. (Anaerobes are most sensitive to oxygen during the log phase of growth.)
4. Incubate Thioglycolate broth in ambient air at 35o C with cap tight.

 D. Culture examination1. Day 2 from set up.
2. Examine anaerobic plates after 48 hours incubation. Exposure of media plates to air should be kept to a minimum.
3. Plated media
4. Examine each plate carefully for different colony types.
* PEA-pick colonies that are different from the colonies isolated in the CDC anaerobe SB agar.
* BBE-pick all the different colonies growing that are >1 mm in diameter. Record the Esculin hydrolysis reaction (black=positive).
* LKV-pick all colony types.
1. Note characteristics such as pitting, swarming, hemolysis, pigment and greening of the agar.
2. **If predominant and well isolated**, set up definitive biochemical or identification procedures on significant organisms (i.e. VITEK MS, VITEK2) after performing a gram stain.
3. If not isolated, select a single colony of each morphology type to subculture for **aero tolerance (ATT)** and **Gram stain**.
* Subculture each morphology type to CHOC agar and anaerobic SB (ASB1) and streak for isolation.
* Incubate the CHOC plate at 35°C in a CO2 incubator for 24 hours.
* Incubate anaerobic SB (ASB1) in anaerobic conditions for 24 hours. Slow growing organisms should be incubated for 48 hours.
1. Correlate colony types with the direct Gram stain from the aerobic culture.
2. Use the initial aerobic Gram stain and culture source to help determine the extent of work-up required on the culture. The presence of many WBC’s indicates an infectious process.
3. Re-incubate remaining plates anaerobically.
4. Keep all original and subculture plates together, in the same anaerobic jar.
5. THIO broth
6. Visually inspect THIO daily until there is growth on plated media. The THIO can be discarded (code TOSS) when growth is observed on plates. Hold THIO if *Actinomyces* is suspected or other special requests have been made.
7. IF plates are negative, subculture and Gram stain at 7 days. Subculture aerobically and anaerobically to CHOC, ASB2, LKV, PEA, BBE.
8. Perform gram stain and subculture the THIO only if plated media reveals no growth or if the anaerobic jar or bag failed. Never rely on broth exclusively for isolation of anaerobes.
9. Day 3 from set up.
10. Examine CHOC aerotolerance plate. Growth on CHOC indicates the organism is not an anaerobe. Correlate with corresponding aerobic culture to confirm same growth.
11. Examine growth on ASB1 aerotolerance plate. If there is good growth, proceed with identification. Slow growing organisms should be incubated for 48 hours.
12. Continue to monitor growth on anaerobic plates on days 3, 5 and 7. Discard any selective agar, such as, LKV, PEA and BBE at 5 days, as these plates lose their selectivity. Continue to incubate and examine remaining plates once every 48 hours.
13. Examine culture and work up any additional isolates as needed.
14. Plates may be held for up to 14 days by special request if *Actinomyces* species or *Cutibacterium* species are suspected.

 1. Additional days
	1. Complete identification procedures until all significant isolates are finished.
	2. Send updated report and finalize.
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| **Method Performance Specifications** | 1. Perform definitive identifications using the following guidelines.
2. Single isolates from any source.
3. Predominate pathogen with mixed anaerobes and aerobes. Do identification on predominate anaerobic organism and report others as “MIXED FLORA” (MF) and/or “MIXED ANAEROBIC FLORA: (MIXA)
4. Rule out *Bacteroides fragilis* group and *Clostridium perfringens* in a mixed culture with no organisms predominant.
5. Cultures with 3 or more anaerobic organisms present can be reported as “MIXED ANAEROBIC FLORA, No further identification” (MIXA).
6. The clinical value of reporting the presence of anaerobic bacteria is directly related to the speed of reporting those results. Also, reporting mixed anaerobic flora from sites such as rectal fistula and intra-abdominal abscess, may be of clinical utility, and no further identification is necessary.
7. Consult with physician on identification and susceptibility testing. Susceptibilities will be done on physician request only. Organisms will be sent to U of M lab for susceptibility testing. Bill as appropriate according to referral testing.
8. CDC Anaerobe Blood agar with 5% sheep blood supplemented with vitamin K and hemin for the isolation of most organisms.
9. Phenylethyl alcohol (PEA)-sheep blood for the inhibition of enteric and other facultative anaerobic Gram-negative bacilli that may overgrow the anaerobes. PEA also reduces the spreading or swarming characteristic of some anaerobes.
10. Kanamycin-vancomycin-laked blood agar for the selection of pigmented *Prevotella* and other *Bacteroides* spp.
11. Bacteroides Bile Esculin agar for the selection of *Bacteroides fragilis* group organism and *Bilophila wadsworthia*. *Fusobacterium mortiferum/varium* group organisms may also grow on the media. *Bacteroides fragilis* group organisms form brown to black colonies. *Bilophila wadsworthia* produces small, transparent colonies with black dot in the center.
12. Use CHOC only for aerotolerance testing. *Haemophilus* spp will grow anaerobically on an ASB1 and therefore will be mistaken for anaerobic Gram-negative rods if CHOC is not used. Incubate on CO2 to detect slow-growing aerobic organisms such as *Capnocytophaga,* *Haemophilus*, *Actinobacillus and* *Eikenella* spp.
13. Anaerobic organism clues from primary culture plates

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| **Result Reporting** | 1. **Culture results**: Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box. Report results semi quantitatively, i.e., 1+, 2+, 3+ or 4+.

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| Quantity | 1st quadrant# colonies | 2nd quadrant# colonies | 3rd quadrant# colonies |
| 1+ | <10 |  |  |
| 2+ | >10 | <5 |  |
| 3+ | >10 | >5 | <5 |
| 4+ | >10 | >5 | >5 |

1. **No growth cultures**: Update culture status in the Observation result box (*Culture Entry* tab), by using the “No Growth” update key (‘). Report as “No growth “*x*” days". Final ( / )
2. **Positive cultures:**

Observations: 1. 4+ BACTEROIDES FRAGILIS Workups: Wkup #1 Workup Components Med :ASB2 GMS :GNR Desc :GR ATT :ANA Id :BAFR MSID :1  1. If growth is only in the THIO, report as:

Observations: 1. GRAM NEGATIVE RODS ISOLATED FROM BROTH ONLY  Further identification to follow (**GNR-BO-FID**)   Workups: Wkup # 10 Workup Components  Med : THIO SC : SB MAC  Desc : CLDY GMS : GMNR  ID : GNR 1. Add comment code **BRPA** (*Bacteroides* species are intrinsically resistant to penicillin and ampicillin) when *Bacteroides* species is isolated.
2. Add comment **CSPC** (*Cutibacterium* species is susceptible to penicillin and cefotaxime and most are susceptible to clindamycin) when *Cutibacterium* speciesis isolated.
3. Review Culture Summary for accuracy before filing report.
4. If a culture requires a correction, the code **CORR** (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to policy MCVI 5.1 LABELING ERRORS/SPECIMEN MIXUP for Sunquest report entry information.
5. If additional testing is requested after the culture is finalized, remove the final status and send out a supplementary report. Do this by adding the code SRPT to either the SREQ or Culture Observations along with an explanation, ie susceptibilities requested or further identification needed. Refinal the culture once testing is complete, leaving the SRPT code in place.
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| **References** | 1. Leber, Amy, Clinical Microbiology Procedures Handbook, 4th Ed. Vol 1, 2016. American Society for Microbiology, Washington D.C., Sections 4, Anaerobic Bacteriology.
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Susan DeMeyere  | 1/10/2023 | Initial Version |
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