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**GENERAL INSTRUCTIONS:**

1. Make sure you have the correct requisition label for the test ordered and that the information on the requisition label includes patient name, hospital number, room number, doctor’s name, and source.
2. Use isolation streaking technique on all plates unless otherwise specified.
3. Cultures are placed in the incubator in the order in which they were set up.
4. All slides for gram stain will be dipped in 95% ethyl alcohol and flamed before use. Using a pencil, label frosted end of slide with culture number, patient last name, and source of specimen.

**CULTURE PROCESSING**

1. **Evaluation of the specimen for adequacy**
2. The specimen must be properly labeled.
3. The transmittal must be submitted with the specimen, and the information on the transmittal must match the information on the specimen label.
4. The specimen must be submitted in the proper transport container.
5. The specimen volume must be adequate to perform all tests requested.
6. The specimen sent must be appropriate for the test ordered.
7. **Medium selection and labeling**
8. Select appropriate media for the tests ordered.
9. Examine all media for expiration date and contamination before inoculation.
10. Individually label all media with patient Meditech labels. Do not obscure the names and expiration dates of the media.
11. **Order of Media to Inoculate**
12. Inoculate the least selective medium first. This prevents any carryover of an inhibitory substance to another medium.
13. Arrange labeled plates in order from least to most selective.
14. Refer to media setup chart for the media used for the various culture types.

**IV. Procedure for Streaking Routine Culture plates for Primary Isolation**

1. Label plates with at least the identifying number, date of culture and at least one plate with the anatomic site and patient name. For samples with Meditech labels, use the large label on the TSA blood plate and smaller labels on other plates.
2. Generally inoculate onto plate by touching specimen to one quadrant with a swab, pipette, or sterile forceps containing the specimen.
3. Using a sterile plastic loop or stick streak with gentle pressure onto one-fourth to one-third of the culture plate using a sterile inoculating stick with a back-and-forth motion several times and without entering the area that was previously streaked. Avoid touching the sides of the petri dish.
4. Turn the plate a quarter turn. Pass the loop through the edge of the first quadrant approximately four times, while streaking into the second quadrant. Continue streaking in the second quadrant without going back to the first quadrant.
5. Turn the plate another quarter 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, approximately four times.
6. Continue streaking the rest of the culture media in the same manner.
7. If required, Inoculate nutrient broth with 1 to 2 loopfuls of specimen or swab.



**Isolation Streak – Whole Plate**

When using a biplate, as for inoculation of anaerobic media, follow the diagram below for isolation streaking.

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**Isolation Streak - Biplate**

**ANAEROBIC CULTURE**

**SPECIMEN:**

1. It is of great importance that specimens for anaerobic culture be collected properly and transported to the laboratory immediately.
2. The nursing unit should be given transport media and instructions when collecting anaerobes.
3. Swab collections are considered inferior to fluid or tissue specimens but when swabs are utilized for anaerobic culture collection the Eswab device can be used.

**SETUP:**

1. BBE/PEA Biplate
2. Brucella Blood Agar
3. Enriched Thio with Vitamin K and Hemin
4. Chocolate (CO2 incubator or CO2 pouch)
5. Gram Stain, if not already performed with aerobic specimen submission

**NOTES:**

1. If an eSwab is used vortex eSwab to release specimen from the flocked swab into the 1ml of liquid media in tube.
2. **Use 1-2 drops of liquid eluate** to make gram stain and setup media (1-2 drops per plate/broth used) using a sterile pipette.
3. **Alternately**, setup plates and gram stain slide **using the swab itself** *if sufficient material is present on the swab (visually apparent)* but continue to rewet the swab and inoculate plates in order of least inhibitory (Chocolate, TSA) to most inhibitory/selective (Hektoen, Mac, etc.).
4. Label chocolate as "aero control" and place in CO2 incubator on shelf labeled for “anaerobes”
5. Place the anaerobic plates into anaerobic atmosphere (bag/jar/chamber).

**BRONCH WASH, TRACH ASPIRATE, PLEURAL FLUID, GASTRIC, SINUS**

**OTHER RESPIRATORY SPECIMENS**

**SPECIMEN:** Use sterile swab or transfer pipette to obtain specimen from container. Try to use a good representative sample of entire specimen by going into different areas. **Refer to Respiratory Specimen Acceptability SOP** to determine specimen acceptability prior to culture setup (sputum and tracheal aspirate collections only).

**SETUP:**

1. TSA Blood
2. CNA (add optochin disk to quadrant 2)
3. MAC
4. Choc (CO2 incubator or CO2 pouch)
5. Gram stain

**NOTES:** Pleural fluid will have cytocentrifuged gram stain. Centrifuge the remaining fluid and use the sediment to plate the culture. Exception: extremely bloody fluid.

**BONE MARROW**

**SPECIMEN:** Collect 8-10 ml and place in blood culture bottle(s).

**PROTECTED SPECIMEN BRUSH - QUANTITATIVE RESPIRATORY CULTURE**

**SPECIMEN:** Protected specimen brush received in 1.0ml of TSB (BHI) with 5%Fildes. Vortex specimen thoroughly (at least 30 seconds) to release specimen material from brush.

**SETUP:**  Chocolate and TSA BAP 0.100 ml (100ul pipette)

 Chocolate and TSA BAP 0.010 ml (10 ul pipette)

 Chocolate and TSA BAP 0.001 ml (1ul loop)

Gram Stain - Remove brush from broth and make smear from brush.

**NOTES:**

1. Spread the inoculum evenly across each plate in the manner used for colony counts.
2. Incubate Chocolate plates in CO2 and Blood plates in ambient air incubator.
3. If quantitative anaerobic cultures are requested - use the same pipette sizes to streak inoculum on CDC Blood Agar and incubate anaerobically.

**CSF CULTURE**

**SPECIMEN:**

1. Tube #2 of CSF is preferred for culture.

2. Prepare a smear for Gram stain by cytocentrifuging 0.2ml of specimen.

3. Remaining CSF is centrifuged at 1500 G for 15 minutes, using the sediment to plate the culture.

**SETUP:**

1. TSA Blood
2. Choc (CO2 incubator or CO2 pouch)
3. Enriched Thio - only if from Shunt
4. BHI with X and V (or equivalent)- only if from Shunt
5. Gram Stain (CYTOSPIN SMEAR) – STAT

**NOTES:**

1. Processing of CSF is begun immediately upon receipt.
2. CALL “Positive” CYTOSPIN Gram stain report to physician STAT.
3. After centrifugation use a sterile pipette to transfer supernatant to a second sterile tube. Use button to set up culture.
4. Save any remaining specimen in appropriate rack for possible add-on testing.

**EAR CULTURE**

**SPECIMEN:** Aspirate or swab of ear fluid/discharge.

**SETUP:**

1. TSA Blood
2. CNA
3. MAC
4. Choc (CO2 incubator or CO2 pouch)
5. Gram Stain

**EYE CULTURE**

**SPECIMEN:** Aspirate or swab of eye fluid/discharge.

**SETUP:**

1. TSA Blood
2. CNA
3. MAC
4. Choc CO2 incubator or CO2 pouch)
5. Gram Stain

**NOTES:** Some ophthalmologists streak plates directly at patient bedside. The following

streak scheme is used:

R = Right eyelid

 L = Left eyelid

 C = Conjunctiva

 Squiggly = Corneal ulcer or parallel lines

**FUNGAL CULTURE**

**SPECIMEN:** All specimens ordered for mycology (fungus) cultures must be handled in the biological safety cabinet to prevent possible exposure of workers to aerosols. While fungal organisms in tissue pose very little threat, these specimens are often infected with AFB which is highly susceptible to being aerosolized.

**SETUP:** Sterile site specimens should be inoculated within 2 hours of receipt in lab or maintained at room temperature. Non-sterile site specimens can be refrigerated overnight until specimen processing can occur.

1. Mycosel – 30o C.
2. Sabhi -- 30o C.
3. KOH smear

**NOTES:**

1. All specimens ordered for fungus culture will also have a KOH smear done and reported immediately.
2. Culture tubes are placed in the 30o C incubator. Keep caps loose.
3. Tubes are labeled with the aliquot labels from the Meditech specimen label containing culture number and patient name.

**ASCITES, PERITONEAL OR OTHER STERILE SITE FLUID**

**SPECIMEN:**

1. Prepare a smear for Gram stain by cytocentrifuging 0.2ml of specimen.
2. Exception: extremely blood fluid.
3. Remaining fluid is centrifuged at 1500 G for 15 minutes, using the sediment to plate the culture.

**SETUP:**

1. TSA Blood
2. CNA
3. MAC
4. CHOC (CO2 incubator or CO2 pouch)
5. Gram Stain (CYTOSPIN smear)

 **NOTES:** 1. CALL “Positive” CYTOSPIN Gram stain report to physician/nurse STAT.

1. After centrifugation use a sterile pipette to transfer supernatant to a second sterile tube. Use button to set up culture.
2. Save any leftover specimen in refrigerator for 1 week.

**GC CULTURE**

**SPECIMEN:** Collected by physician or nurse using culturette or eSwab collection device. Process within 1 hour or leave at room temperature until setup can occur. DO NOT REFRIGERATE

**SETUP:**

1. Martin-Lewis (CO2 incubator or CO2 pouch)
2. Chocolate Agar (CO2 incubator or CO2 pouch)
3. Gram Stain\*\* (May be ordered with or without gram stain)

**NOTES:**

1. Roll eSwab over the surface of the media.

**UROGENITAL CULTURE**

**SPECIMEN:** Collected by physician or nurse using culturette or eSwab collection device

**SETUP:**

1. TSA Blood
2. CNA
3. Choc (CO2 incubator or CO2 pouch)
4. Martin-Lewis (CO2 incubator or CO2 pouch)
5. Gram Stain\*\* (May be ordered with or without gram stain)

**NOTES:**

1. Vortex the Eswab to release specimen from the flocked swab into the 1ml of liquid media in tube.
2. Use 1-2 drops of liquid eluate to make gram stain and setup media (1-2 drops per plate/broth used) using a sterile pipette.
3. Alternately, setup plates and gram stain slide using the swab itself *if sufficient material is present on the swab (visually apparent)* but continue to rewet the swab and inoculate plates in order of least inhibitory (Chocolate, TSA) to most inhibitory/selective.
4. Any specimen for Gonococcus should be set up immediately on media that is at room temperature. Roll the swab over the surface of the media.
5. If Group B Screen is desired – order Group B Strep Culture and setup LIM broth only.

**GENITAL GROUP B STREPTOCOCCUS CULTURE**

**SPECIMEN:** Vaginal and Anorectal Swabbing using culturette or eSwab collection device

**SETUP:** LIM Broth

**Procedure:**

1. After vortexing, inoculate eSwab directly into LIM broth, breaking off shaft and allowing swab to remain in the broth.
2. Incubate broth at 35oC until broth is sent to Core-Lab no later than 18-24 hours.

**INTRAVASCULAR CATHETER TIPS**

**SPECIMEN:** The actual tip of the catheter should be received in a sterile container. Do not accept a swab of the catheter tip. Two blood cultures collected from peripheral sites should accompany the catheter tip within 24 hours.

**SETUP:** TSA Blood Plate

**NOTES:**

1. Using sterile forceps, remove catheter tip from transport container. (If tubing is sent with catheter tip attached, use scissors sterilized by flaming with alcohol to cut tubing 2" above tip.)
2. Lay the tip on a TSA blood plate. Using sterile forceps, roll the tip over the entire plate surface.
3. Incubate plate at 35oC. (CO2 incubator or CO2 pouch)
4. DO NOT CULTURE URINARY CATHETER TIPS

**WOUND / ABSCESS / ASPIRATE**

**SPECIMEN:** These include but are not limited to decubitus ulcer, subcutaneous abscess, sinus tract, burn, surgical incision, cellulitis, and cutaneous fistula

**SETUP:**

1. TSA Blood
2. CNA
3. MAC
4. Gram Stain
5. Choc (CO2 incubator or CO2 pouch)

**NOTES:**

1. Please be sure site of wound or drainage is given.
2. Save any leftover abscess fluid or drainage in the refrigerator for 1 week. Swab collections do not require retention.
3. Deep wound cultures may also harbor anaerobic organisms. Refer to ANAEROBIC CULTURE section for media needed.

**STOOL**

**SPECIMEN:**

1. Sample representative portions of specimens, i.e. mucus flecks, blood and pus.
2. Inoculate media as soon as possible after receipt in the laboratory.
3. Only one specimen per day per patient should be accepted.
4. Check patient admission date - if >72 hours post- admission, reject stool culture.

**SETUP:**

1. TSA
2. MAC
3. CNA
4. HEK
5. GN Broth
6. MAC/Sorbitol
7. Campy (place in 42o C incubator in microaerophilic bag/jar)
8. MAC at room temp (*If Yersinia requested*)
9. Martin Lewis (*if Neisseria gonorrhea requested*, in CO2 incubator or CO2 pouch)

**NOTES:**

1. When Yersinia cultures are requested – Order CULYERSINIA.
2. Perform Shiga Toxin test on all stool cultures with growth in GN broth.

**SYNOVIAL, JOINT FLUID**

**SPECIMEN:** Fluid collection

1. Prepare a smear for Gram stain
2. Exception: extremely blood fluid.
3. Remaining fluid is centrifuged at 1500 G for 15 minutes, using the sediment to plate the culture.

**SETUP:**

1. TSA Blood
2. MAC
3. CNA
4. Choc (CO2 incubator or CO2 pouch)
5. Gram stain

**NOTES:**

1. Save any leftover specimen in the refrigerator for one week.
2. If *N. gonorrhoeae* culture is requested, set up Martin-Lewis and incubate in CO2 incubator.

**THROAT**

**SPECIMEN:**

1. Throat Swab collected via culturette or eSwab

**SETUP:**

1. After rapid A performance (if ordered and result is Negative) setup backup throat culture using 1-2 drops of liquid eluate from the Eswab device onto TSA Blood.
2. Place Bacitracin disc between 1st and 2nd quadrants.
3. Setup Choc (only if physician specifically requests screening for Haemophilus)
4. Setup Martin-Lewis (only if physician specifically requests screening for N. gonorrhoeae)

**NOTES:**

1. Place blood plate in ambient air incubator.
2. Place Chocolate, and Martin-Lewis, in CO2 incubator.
3. If the physician requests culture for other organisms, order a respiratory culture and setup appropriate media.
4. If physician specifically requests culture for *C. diphtheriae* – send specimen to Quest Diagnostics for culture.
5. Throat culture is required on all “Negative” Strep A antigen tests.

**TISSUE**

**SPECIMEN:**

1. Tissue specimens from surgery must be crushed using a sterile grinder (disposable) OR minced with scalpel.
2. Add a small amount of sterile non-inhibitory broth such as THIO to the tissue grinder to make a homogeneous suspension.

**SETUP:**

1. TSA Blood
2. CNA
3. MAC
4. CHOC (CO2 incubator or CO2 pouch)
5. Enriched Thio
6. Gram Stain (make smear using "touch prep" technique before grinding tissue.)

**NOTE:** Save any leftover specimen in the refrigerator for one week.

**URINE**

**SPECIMEN:**

1. Must be in sterile container
2. Mix urine thoroughly before sampling.

**SETUP:**

1. TSA Blood (colony count using .001 loop)
2. MAC
3. CNA

**V. Procedure for Streaking Urine Culture plates for Quantitation**

1. Use 0.001-ml calibrated loop for inoculating media for routine urine.
2. Transfer 1 loopful of urine to BAP and pull the loop down the surface of the agar to form a single streak down the plate.
3. Cross streak through the initial inoculum streak by moving the loop back and forth at perpendicular angles to initial streak.

**Urine Colony Count Streak**

**(TSA blood plate only)**



**NOTES:**

1. Refrigerate specimen if there is any delay in setup.
2. If the specimen is more than 2 hours old when received or has not been refrigerated during this time, call the unit and ask for a new specimen