**MICRO.PROC.2.0 INITIAL PROCESSING, INOCULATION, AND INCUBATION**

**PRINCIPLE**

When the specimen arrives in the laboratory it is judged for acceptability of specimen, labeling and collection and accessioned into the laboratory information system based on criteria set forth in procedure MICRO.PROC.1.0, *Specimen Receipt, Accessioning and Acceptability*. From there processing the specimen properly involves many important steps, including specimen preparation (tissue grinding, swab elution, cytospinning), inoculation of media and slides, correct incubation, and safe disposal. This procedure addresses these steps.

**OWNERS**

Manager, Regional Microbiology

Microbiology and Molecular Best Practice Team

**SPECIMEN**

Any specimen for microbiological analysis

**REAGENTS**

A. Albumin

B. Sterile saline

**EQUIPMENT**

A. Tissue grinding kit

B. Sterile swabs

C. Inoculating loops, disposable and wire

D. Biobags

E. AnaeroPack System, Anoxomat anaerobic system, Anaerobe Chamber

F. Incubators (CO2 and non-CO2 at various temperatures)

G. Centrifuge

**QUALITY CONTROL**

Refer to procedure MICRO.GEN.7.0, *Microbiology Quality Control and Quality Improvement Plan*, for quality control performance on plated and tubed media.

**PROCEDURE**

A. Timing—The most important specimens must be inoculated to media first. Look through the cultures and pull out all those that are invasively collected. The preferred order of setting up cultures is listed below.

1. STATS: specimens from surgery and normally sterile sites are processed before STATs from nonsterile body sites.

2. *N. gonorrhoeae* cultures submitted on plates or unpreserved swabs.

3. CSF

4. Tissues

5. Body fluids and unpreserved urine specimens

6. Abscesses

7. Unpreserved stools for culture

8. Sputum and other lower respiratory cultures

9. Blood

10. Swabs in transport tubes

11. Group A and B streptococcal cultures

12. Preserved urine

B. Specimen inoculation:

1. Catheter tips:

a. Using sterile forceps, remove the catheter tip from the transport tube.

b. Using sterile forceps roll tip back and forth 4 times across the surface of a blood agar plate.

c. Incubate plates at 35-37°C in CO2.

2. Bone, granules, and hardware:

NOTE: Always examine these specimens for the presence of any soft tissue. If you find any such material, remove it with a sterile surgical scalpel, and follow step 6 to process it by tissue grinder.

a. If specimen is small, place into Thioglycollate (THIO) broth medium directly, incubate immediately at 35-37°C in a CO2 incubator. Subculture broth after 48 hours.

b. If prosthesis specimens are large and are received in sterile urine or sputum containers, place 10 to 20 ml of Thioglycollate broth into the container and incubate at 35-37°C in a 5-7% CO2 incubator.

3. Intrauterine devices:

a. Place 10 ml. of Thioglycollate broth into the container. Seal container securely, and vortex for 30 seconds.

b. Remove broth to a sterile tube, and centrifuge for 15 minutes at 2500 to 3000 rpm.

c. Process sediment for Gram stain and plate onto solid media. Include a Thayer Martin plate. Incubate specimens in a 5-7% CO2 incubator at 35-37°.

4. Specimens received in syringes:

NOTE: The needle must have been removed from the syringe before being sent to the laboratory. If not, handle with extreme caution and follow up with client to prevent repeat occurrence.

a. If specimen is more than 1mL centrifuge the specimen and use the sediment to inoculate the plated media and make smears for appropriate stains. Streak plates for isolation.

5. Specimens received on swabs:

a. Direct inoculation (for swabs received in transport media):

i. Roll the swab directly onto the agar creating the primary quadrant.

ii Using a sterilized inoculating loop, streak the plate for isolation.

b. Swab elution:

i. Immerse the specimen swab in a tube of 0.5 ml sterile saline and vortex for 3-5 seconds.

ii. Using the initial swab, inoculate all plates required for the culture.

iii. Dispose of the swab and saline tube in a suitable biohazard container.

c. Notes:

i. Do not pre-treat anaerobic culture swabs.

ii. When a gram stain is required in addition to the culture from a single swab, sterilize a slide by holding the back of the slide to incinerator for 3-4 seconds, allow slide to cool and roll swab over the surface of the slide.

6. Tissues   
a. Place a portion of specimen into a tissue grinding tube.

b. Add a small amount of sterile saline, proportional to the size of the piece of tissue.

c. Using a circular motion, homogenize the specimen.

d. Remove the specimen by using a sterile pipette, and proceed with culturing.

NOTE: If tissue is being processed for fungal culture, do not use homogenized (ground) specimen. Using sterile scalpel mince tissue into 1mm cubes and place the fragments directing on the agar, submerging them slightly beneath the surface.

7. Fluids, except urine

a. Normally sterile body fluids

i. If the volume of the fluids is > 1 ml, centrifuge the specimen to sediment the bacteria. Using a sterile pipette remove the sediment and use to make smears, as well as to inoculate the appropriate media as outlined in Table 1 or use Cytospin to make smear.

ii. Specimens of < 1 ml should be plated directly. Use the cytocentrifuge to concentrate smears from clear fluids.

b. For other liquid specimens, inoculate plates and smears using a swab or pipette dipped into the liquid.

C. Gram stain preparation:

1. Perform Gram stains on lower respiratory and wound cultures, all cultures from normally sterile sites; urine and genital cultures are performed on request. Gram stains are not performed on throat, nasal, or catheter tip cultures. Specific requirements for each culture type can be found in Table 1.

2. Refer to procedure MICRO.STAIN. 1.0 for smear preparation.

3. Smears from normally sterile body fluids, especially CSF, should be prepared by centrifugation or cytocentrifugation.

D. Plate Streaking Techniques

1. Streaking for Isolation:

a. Roll the swab directly onto the agar or use the specimen to inoculate a quarter-sized area of the plate.

b. Sterilize the inoculating loop in the incinerator for 5-10 seconds. Allow to cool. Sterile disposable loops may be used as an alternative.

c. Using gentle pressure, streak the specimen into quadrants as shown below in Figure a. *Note: The streak lines should overlap only 2-3 times from one quadrant to another. It is not necessary to sterilize the loop between quadrants. For Throat cultures, stab the agar 2-3 times in the primary quadrant of the BAP.*

2. Streaking for Quantitation (Urines and Broncheoalveolar Lavages)

a. Mix specimen well (do not shake).

b. Immerse a 0.001 mL or 0.01mL calibrated loop vertically below the surface of the specimen, being careful to avoid bubbles. *Note: routine urine cultures require use of a 0.001 mL calibrated loop. Suprapubic and bladder urines require the use of a 0.01 mL and 0.001 mL calibrated loop. Broncheoalveolar lavages are setup using both calibrated loops. Duodenal aspirates (small bowel contents) are set up using 0.1 mL, 0.01 mL and 0.001 mL calibrated loops.*

c. Deliver a loopful of specimen onto the plate by making a straight line down the center of the plate.

d. Streak the specimen by making a series of passes at 90° angles through the primary inoculum as depicted in Figure b.

Fig. b Streaking for quantitation

Primary

inoculum

Primary

inoculum

Fig. a Streaking for isolation

E. Incubation of specimens (Refer to Tables 1 and 2 for detailed incubation requirements for each specimen type):

1. Temperature and humidity:

a. Incubate most routine cultures at 35-37°C. Other incubators are maintained for Campylobacter species (42°C), fungus cultures and some atypical mycobacteria (30°C). Temperatures are monitored daily.

b. Humidity is kept high by a humidifier (in the walk-in incubator), pans of water or water jacketing of the incubator itself.

2. Atmospheric gas environment:

a. CO2 incubators are maintained at an atmosphere of 5-10% CO2.

b. Anaerobe jars, boxes, or chamber are used for anaerobe plates. See procedure MICRO.PROC.9.0 Processing and Incubation for growth of anaerobes.

**PROCEDURE NOTES**

A. Patient name and specimen type must be verified prior to plating.

B. Specimens received from clients that have already been plated may be able to be evaluated for growth. If the specimen was inoculated on the day prior to receipt, give the inoculated plates to a technologist for evaluation. If the specimen was inoculated on the day of receipt, incubate the plates appropriately according to culture type.

**REFERENCE**

A. Isenberg, H.D. et al., Clinical Microbiology Procedures Handbook. ASM Press, 2007, Washington D.C., Chapter 3.3.1

B. Sautter, R.L. Yeakle, R., Bihl, J., Increased isolation of pathogenic microorganisms utilizing a saline rinse of routine culture swabs, Abstracts of the Annual Meeting of the American Society for Microbiology, 1985, Washington, D.C., Abstract C291, p. 348.

**SPECIMEN SETUP FOR BACTERIAL CULTURES (TABLE 1)**

**Routine Bench Tests (Aerobic cultures, Anaerobic cultures, Gram stains)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Specimen** | **Aerobic** | **Anaerobic?** | **Broth?** | **Gram Stain?** | **Incubator\*\*** | **Comments** |
| **Fluids** | CSF | CHOC, BAP  (spin if >1ml) | No | THIO if from shunt | Yes, sediment (>1ml) or cytospin (<1ml) | CO2, Routine | Before inoculation, wipe slide with alcohol swab.  If only India Ink is ordered, reflex fungus culture. |
| Bone marrow | CHOC | ABAP | No | No | CO2, Routine |  |
| Pleural, Pericardia,  Thoracentesis,  Synovial (joint) | CHOC, BAP  (spin if >1ml) | ABAP | No | Yes, sediment (>1ml) or cytospin (<1ml) | CO2, Routine | Any body fluid specimen in blood culture bottles is acceptable. Add comment in LIS to denote that bottles were received and loaded onto BACTEC blood culture instrument. |
| Peritoneal,  Abdominal,  Ascites,  Bile | CHOC, BAP,  MAC, CNA | ABAP, LKV  PEA | No | Yes | CO2, Routine |
| Peritoneal  dialysate | ----------------- | ----------------- | Blood culture bottles | Yes | ---------------- | Incubated in BACTEC blood culture instrument |
| Unkown Fluid  (treat any unknown fluid as non-sterile) | CHOC, BAP,  MAC, CNA | ABAP, LKV  PEA | No | Yes | CO2, Routine |  |
| Prostatic fluid | ---------------- | ---------------- | -------------- | ---------------- | ---------------- | Refer to Genital |
| **Swabs,**  **Wounds,**  **Tissues** | Wound  (in aerobic transporter) | CHOC, BAP,  MAC, CNA | No | No | If ordered | CO2, Routine |  |
| Wound  (in anaerobic transporter) | CHOC, BAP,  MAC, CNA | ABAP, LKV  PEA | No | Yes | CO2, Routine | Only set up anaerobic culture if ordered. |
| Sterile sites  (includes surgical sources) | CHOC, BAP | ABAP | No | Yes | CO2, Routine | Reflex anaerobic culture if not ordered. |
| Tissue, from organs or internal source above the waist (ie. Heart, lung, brain, etc.) | CHOC, BAP | ABAP | THIO | Yes | CO2, Routine | Reflex anaerobic culture if not ordered, |
| Tissue, from internal source at or below the waist or from skin or extremities (ie. Abdomen, hand, toe, foot) | CHOC, BAP,  MAC, CNA | ABAP, LKV  PEA | THIO | Yes | CO2, Routine | Reflex anaerobic culture if not ordered. |
| Boils, pustules, skins | CHOC, BAP,  MAC, CNA | No | No | If ordered | CO2, Routine |  |
| Abscess | CHOC, BAP,  MAC, CNA | ABAP, LKV  PEA | No | Yes | CO2, Routine |  |
| Decubitus (bedsore) | CHOC, BAP,  MAC, CNA | No | No | No | CO2, Routine |  |
| **Bones, granules, hardware** | No soft tissue attached |  |  | YES  THIO only | NO | CO2, Routine |  |
| Soft tissue attached | CHOC, BAP | ABAP | THIO | Yes | CO2, Routine | Grind up soft tissue |
| **Gastric** | Gastric aspirate | CHOC, BAP,  MAC, CNA | No | No | If ordered | CO2, Routine |  |
| **Duodenal** | Duodenal Aspirate (small bowel contents) | 3xBAP, 1xHEK | 3xABAP | No | No | Non-CO2 | Use 0.1 mL, 0.01 mL, and 0.001 mL loops |
| **Gallbladder** | Bile or gallbladder | CHOC, BAP,  MAC, CNA | ABAP, LKV  PEA | No | Yes | CO2, Routine |  |
| **Genital** | Vag, cervix | ----------------- | ----------------- | ------------- | --------------- | ----------------- | Change the order to Genital culture using clarification code. |
| Pelvic absc., uterus, placenta, Bartholin, perineum, scrotum, prostatic fluid, | TM/CHOC bi,  BAP, MAC, CNA | ABAP, LKV  PEA | No | Yes | CO2, Routine |  |
| vag cuff  Labia  Vulva  Penis  urethra | TM/CHOC bi,  BAP, MAC, CNA | Yes only if ordered | No | Yes only if ordered | CO2, Routine |  |
| **Ear** | From surgery | CHOC, BAP | ABAP | No | Yes | CO2, Routine |  |
| All others | CHOC, BAP,  MAC, CNA | No | No | If ordered | CO2, Routine |  |
| **Eye** | Cornea | ----------------- | ----------------- | THIO | No | CO2, Routine | If specimen is eye culturette swab, emulsify swab in a tube containing 0.5 ml sterile saline. Vortex well. |
| From surgery | CHOC, BAP | ABAP | No | Yes | CO2, Routine |
| Conjunctiva, | CHOC, BAP, | No | No | If ordered | CO2, Routine |
| **Mouth** | Call the doctor or floor and ask what organism(s) to rule out. | | | |  |  |  |
| **Lower Respiratory** | Lung aspirate or  Lung biopsy | CHOC, BAP | ABAP | THIO | Yes | CO2, Routine |  |
| Protected brush | CHOC, BAP | ABAP | No | Yes | CO2, Routine |  |
| Trans-tracheal aspirate | CHOC, BAP, MAC | ABAP, LKV, PEA | No | Yes | CO2, Routine |  |
| **Sinus** | All sinuses | CHOC, BAP, MAC & CNA | ABAP, LKV  PEA | No | Yes | CO2, Routine | In SQ, order as aerobic/anaerobic cultures. |
| **Anaerobic**  **Culture only** | Any source | Add BAP write on “Anaerobic only” | Setup according to source | No | No | CO2, Routine | Except Anaerobic culture only from **Putnam Co**. |

\* All specimens submitted from the anatomic site of “foot” should be considered non-sterile and both selective and non-selective media should be used.

**Stool Bench Tests (Stool related cultures, MRSAS, RES, GC cultures, and genital cultures)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Specimen** | **Aerobic** | **Anaerobic?** | **Broth?** | **Gram Stain?** | **Incubator** | **Comments** |
| **Stool** | Stool, rectal swab | BAP, MAC, HEK, CAMPY (CSC) | No | Selenite-F  (SEL)  GN broth | If ordered | Non-CO2 | Incubate Campy at 42oC using an Anaeropak microaerophilic gas generator.  Selenite broths are subcultured to HEK after 12-18 hours incubation.  GN broths are tested for Shiga toxin following 16-24 hours incubation. |
| Vibrio requested | Add TCBS to stool setup | No | No | No | Non-CO2 |  |
| E. coli O157 culture | SMAC (MAC with sorbitol) | No | No | No | Non-CO2 |  |
| Yersinia culture | CIN | No | No | No | Incubate at room temperature (on the bench) |  |
| GC swab | Jembec or TM or  TM/CHOC bi | No | No | No | CO2, Genital |  |
| Occult Blood or  Hemoccult card | ------------------- | ----------------- | ------------- | --------------- | ----------------- | Perform the test underneath the processing hood |
| **Gastric Fluid** | Gastricoccult | ------------------- | ----------------- | ------------- | --------------- | ----------------- | Perform the test underneath the processing hood |
| **Genital** | Vag, penis, cervix, urethra | TM/CHOC bi,  BAP, MAC, CNA | No | No | If ordered | CO2, Genital | When penis or urethra is ordered for aerobic culture, leave the order to be aerobic culture. |
| GC culture | Jembec or TM or  TM/CHOC bi | No | No | If ordered | CO2, Genital |  |
| **MRSA**  **Screen**  **(MRSAS)**  **(MRSIF)** | Swab | ChromID MRSA | No | No | No | Non-CO2 | MRSAP is not a microbiology test. Please give specimen to molecular. |
| **S. aureus screen (QLS only)** | Swab | BAP, CNA | No | No | No | Non-CO2 |  |
| **RES**  **(VRE screen)** | Preferred: Rectal Swab  Other source:  Stool | CNAV (CNA with vancomycin) or ChromID VRE (SVEV) | No | No | No | Non-CO2 | **Any source suspected of harboring VRE is acceptable.** |

**Urine Bench Tests**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Specimen** | **Aerobic** | **Anaerobic?** | **Broth?** | **Gram Stain?** | **Incubator** | **Comments** |
| **Urine** | CCMS, Cath | BAP, MAC with 0.001 mL loop only. | No | No | If ordered, mix the urine well and put a drop on slide. | Non-CO2 | Streak for quantitation, or use automated specimen processor |
| Bladder, cytoscopy | BAP, MAC with 0.01 mL & 0.001 mL loop. | No | No | Non-CO2 |
| Kidney, suprapubic | BAP, MAC with 0.01 mL and 0.001 mL loop | ABAP with 0.01 mL and 0.001 loop. | No | Non-CO2 |
| Foley cath tip | ------------------- | ----------------- | ------------- | --------------- | ----------------- | Not acceptable specimen. |

**Respiratory Bench Tests**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Specimen** | **Aerobic** | **Anaerobic?** | **Broth?** | **Gram Stain?** | **Incubator** | **Comments** |
| **Throat** | Throat culture (SQ)  Strep Grp. A (TL) | SSA | No | No | No | Non-CO2 | Put **SXT disk\*** on BAP plate. Stab agar 2-3 times in primary quadrant.  SVEV uses SXT disk only if SSA not available. |
| Comprehensive throat culture | CHOC, BAP | No | No | No | CO2, RESP |
| For GC | Jembec or TM or  TM/CHOC bi | No | No | If ordered | CO2, RESP |  |
| For thrush, yeast, or fungus | Order a fungus culture |  |  |  |  |  |
| **Nasal/NP** | Routine | CHOC, BAP | No | No | If ordered | CO2, RESP |  |
| Pertussis/  Bordetella | BAP,  Regan Lowe | No | No | No | Non-CO2 | A Pertussis smear (red slide) may or may not be ordered along with the culture. Check with the order. |
| **Lower Respiratory** | Sputum, trach,  Bronch washing | CHOC, BAP, MAC | No | No | Yes | CO2, RESP |  |
| Throat or sputum on cystic fibrosis patient | CHOC,  BAP, MAC, CNA,  PC agar | No | No | Yes | CO2, RESP |  |
| BAL, lavage | 2 sets of  CHOC, BAP,  MAC | No | No | Yes | CO2, RESP | Setup 2 sets of plates, one using 0.01 mL loop and one using 0.001 mL loop. Streak for quantitation. |
| **CTC** | IV Cath Tip (CTC) | BAP | No | No | No | CO2, RESP | Roll the tip on BAP |

**Miscellaneous Tests**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Specimen** | | **Aerobic** | **Anaerobic?** | **Broth?** | **Gram Stain?** | **Incubator** | **Comments** |
| **GBC**  **(Group B Strep. Screen)** | Vagino-Rectal | | No | No | Carrot broth | No | Non-CO2 | Subcultured to GBS agar if Carrot Broth negative after a minimum incubation of 6 hours. |
| **Blood and component products, transfusion reactions** | Sample pouch, units of blood or component | <=10 mL | 2 CHOC, BAP | ABAP | No | If ordered. | CO2, Routine | Incubate one CHOC plate at room temp. (on the R2 bench) |
| >10 mL | 2 Aerobic blood culture bottles (Blue) | 1 Anaerobic blood culture bottle (Purple) | No | If ordered | ----------------- | If bottles came positive, give the bottles to blood bench tech.  If they came negative, incubate 1 aero and 1 anaero in BACTEC. Incubate the other aero at room temp. (on the blood bench.) |

**\*\* SEL should always be incubated in non-CO2 incubator.**

Abbreviations:

CHOC = Chocolate agar, BAP = TSA with 5% sheep blood agar, MAC = MacConkey agar, CNA = Colistin-naladixic acid Columbia agar with 5% sheep blood, SMAC = MacConkey sorbitol agar, HEK = Hektoen agar, ABAP = Anaerobic blood agar (CDC), PEA = Phenylethyl alcohol with 5% sheep blood, LKV = Laked blood agar with KV, TM = Thayer Martin agar, HBT = Human blood-tween 80, SSA = Selective Strep Agar, THIO = Thioglycollate broth, GBS Agar = Group B Strep Detect agar.

**SPECIMEN SETUP FOR FUNGUS CULTURE BACKUP MEDIA**

**(Table 2) (Table 3)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Media** | **Incubator** |  | **Out of** | **Use** |
| CSF, sterile fluids, sterile tissues | BHIA, IMA | 30oC,  Mycology |  | BHIA | BAP |
| MYCO | SABHI |
| Exudates, drainage, pus, non-sterile tissues, sputum, stool, and specimens on swabs (other than skin or genital source) | BHIA, MYCO, IMA |
| IMA | SABHI |
| SABHI | BHIA only is acceptable |
| APEA or AKV | As long as there is an ABAP, it is acceptable. |
| Corneal scrapings or other eye sources | BHIA, IMA |
| ABAP | BAP |
| Skin, nails, hair, urine and genital sources (including swabs from these sites) | MYCO, IMA (embed fragments in media, if media not already received inoculated.) | CHOC/TM biplate | CHOC |
| HEK | MAC |
| SSA | BAP w/ SXT disk in primary quadrant |

Abbreviations: BHIA: Brain Hear Infusion Agar with 5% Sheep Blood, SABHI: Sabaroud dextrose Agar supplemented with BHI, MYCO: Mycosel/Mycobiotic Agar, IMA: Inhibitory Mold Agar