PROCEDURE: PLANTING PROCEDURE

I. PRINCIPLE

The primary processing of clinical specimens for pathogen identification is critical to the detection and identification of pathogenic organisms. It is a complex process which involves several decisions dependent on the specimen source, as well as the method of collection. The nature of the specimen will also determine any additional processing of the specimen for anaerobic bacteria, viral agents, yeast, and mold. Furthermore, the need for direct testing (i.e. Gram stain) must also be considered when making decisions in regard the processing of clinical specimens.

To effectively isolate and detect the pathogenic content of a clinical specimen, colony growth must be achieved. This is dependent on the utilization of strong microscopy skills, aseptic techniques, and the selection of appropriate plate and broth media. The following will provide a clear and concise guide for the primary processing of the array of clinical specimens encountered.

II. AVAILABILITY

Primary planting procedures are performed in the Microbiology Laboratory 24/7.

III. TEST CODE

CX??? – Refer to the Lifespan Laboratory Guide for specimen and pathogen specific test codes.

IV. SPECIMEN

For specimen specific guidelines, refer to the Order Entry chart, Department of Pathology Guide to Laboratory Services, Specimen Management Manual, and Hood Planting Notes.

V. MATERIALS AND EQUIPMENT

A. Materials
    1. Equipment
        a. Class II, Type A2 Biological Safety Cabinet
        b. Slide Warmer – Set to 40°C
        c. Cytospin 4 – Cyto-centrifuge
        d. Vortex
    2. Planting Supplies
        a. Calibrated Disposable Inoculating Loops
            i. Green, 1 µL
            ii. Blue, 10 µL
        b. Sterile Transfer Pipettes
        c. Tissue Grinders, 15ml and 50ml
        d. Sterile, Graduated Glass Pipettes (For Legionella Cultures)
        e. Sterile Polyester Tipped Applicator
        f. Shrink Seals (For Mycology Plates)
        g. Ethanol, 70% (140 Proof)
        h. Glass Coplan Jar
        i. Glass Slides
            i. Copan WASP Microscope Slides
            ii. Ultrafrost Microscope Slides (Frosted)
            iii. SUPERFROST PLUS Microscope Slides (Circle)
        j. Disposable Cytology Funnel
        k. Cytoclip
        l. Saline Tube (0.85%)
        m. Potassium Chloride Tube pH 2.2 (0.2M) (For Legionella Cultures)

n. Sterile Conical Tube – 15mL & 50mL
o. Sterile Suture Kit
p. Sterile Alcohol Pad 2x2
q. Parafilm

3. Media

	NAME	CODE	PURPOSE	INCUBATION TEMP	INCUBATION ENVIRONMENT
Aerobic Media	Trypticase™ Soy Agar with 5% Sheep Blood	BAP	General, Enriched, Differential	35±2˚C	5% CO^2 / Ambient for Urines
	MacConkey II Agar	MAC	Selective for Gram-negative organisms, Differential	35±2˚C	5% CO^2 / Ambient for Urines
	Chocolate II Agar	CHOC	Fastidious organisms, Enriched, Non-Selective	35±2˚C	5% CO^2
	Columbia CNA Agar with 5% Sheep Blood	CNA	Selective for Gram-positive organisms, Differential	35±2˚C	5% CO^2
	Modified Thayer-Martin Agar	MTM	Enriched, Selective for *Neisseria species*	35±2˚C	5% CO^2
Anaerobic Media	Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K1	BRUC	Enriched, Non-selective, Anaerobes	35±2˚C	Anaerobic Jar (0% O^2)
	Laked Sheep Blood Agar w/ Kanamycin and Vancomycin	KV	Enriched, Selective for Anaerobic Gram-negative Bacilli	35±2˚C	Anaerobic Jar (0% O^2)
	5% Sheep Blood Agar with Phenylethyl Alcohol	PEA	Enriched, Selective for Obligate Anaerobes, Fastidious and Slow growing	35±2˚C	Anaerobic Jar (0% O^2)
Fungal Media	Sabouraud Dextrose Agar, Emmons	SAB	Growth of pathogenic and nonpathogenic fungi	30˚C	Ambient
	Inhibitory Mold Agar	IMA	Selective	30˚C	Ambient
	Mycosel Agar	MYC	Selective	30˚C	Ambient
	Dermatophyte Test Medium	DTM	Selective for Dermatophytes from cutaneous sources	30˚C	Ambient
Screening Media	CHROMagar™ MRSA II	CHROM	Selective for MRSA, Differential	35±2˚C	Ambient, in Dark
Stool Media	BHIsulfate Citrate Bile Salts Sucrose Agar	TCBS	Selective for *Vibrio sp.*	35±2˚C	Ambient
	CIN (cefsulodin-Irgasan™-novobiocin) Agar	CIN	Selective for *Yersinia enterocolitica*	Room Temp	Ambient
	Campy CVA Agar	CAMPY	Selective for *Campylobacter jejuni* from stool specimens	42˚C	Microaerophilic Jar (5% O^2)
Mycobacterium Media	Middlebrook 7H11 Agar	7H11	Isolation of *Mycobacterium species*	35±2˚C	5% CO^2
	Mitchison 7H11 Selective Agar	MITCH	Isolation of *Mycobacterium species* from specimens containing mixed flora	35±2˚C	5% CO^2
Broth Media	Lim Broth	LIM	Enriched, Selective for group B streptococci	35±2˚C	Ambient
	BHI Medium, with Vitamin K1 and Hemin	BHI	Enriched, General	35±2˚C	Ambient
	GN (Gram Negative) Broth	GN	Enriched, Selective for Gram-negative Enteric Organisms	35±2˚C	Ambient
Respiratory Media	Buffered Charcoal Yeast Extract Agar	BCYE	Non-selective, Isolation of *Legionella species,* Nocardia	35±2˚C	5% CO^2
	BCYE Selective Agar w/ Polymyxin B, Anisomycin, Vancomycin (PAV)	BCYEP	Selective, Isolation of *Legionella species*	35±2˚C	5% CO^2
	Burkholderia cepacia Selective Agar	BCSA	Differential, Selective for *Burkholderia cepacia*	35±2˚C	Ambient

VI. STORAGE AND HANDLING

A. Culture media will be stored in the temperature and environment specified by the manufacturer[CEA1][CTL2]. A small supply of room temperature media will be kept by the planting hood.
B. Culture media will be incubated at the temperature and environmental conditions specified by the manufacturer.

VII. QUALITY CONTROL

A. The laboratory performs quality control on all media based on manufacturer's guidelines, CLSI guidelines, and the laboratories own IQCP. Specific instructions are provided in detail in the ***Procedure**: Quality Control for Media, Reagents, Kits, and Stains* procedure.

VIII. TEST PROCEDURE

A. Pre-planting
    1. Evaluate the specimen for:
        a. Proper labeling, collection, and transportation requirements
        b. Appropriate specimen type for test order
        c. Sufficient volume to perform all requested test orders
    2. Acquire media labels from the printer and match patient information of specimen.
    3. Label the appropriate agar and tube media for the requested test.

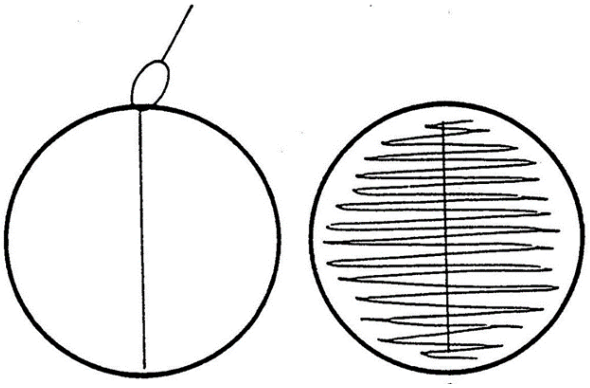
B. Inoculation
    1. Using a sterile swab (if the specimen is not collected on one), or a sterile pipette, inoculate all of the media requested.
        a. If using a swab, roll the swab back and forth over the agar.
        b. If using a pipette, inoculate with one free-falling drop of specimen.
    2. Aerobic specimens collected in an ESwab™ or urines collected in a grey or yellow vacutainer must be loaded onto the WASP for automated planting. Manual processing is only to be done when the WASP is not operating properly, the specimen collection device is not compatible, or when the quantity of specimen is insufficient. Refer to the *WASP Procedure* for further instruction.
        a. ESwab™ collections that are rejected by the WASP may be planted manually.
            i. Vortex tube for 5 seconds to release sample from the swab tip and evenly resuspend the patient specimen in the liquid transport medium.
            ii. Unscrew the cap to remove the swab applicator.
            iii. Roll the tip of the applicator onto the surface of the first quadrant of the media plate to provide the primary inoculum.
            iv. If it is necessary to inoculate additional media plates, return the applicator to the tube to absorb and recharge the applicator tip with transport medium/patient sample, then repeat Step 3.
            NOTE: A 0.01 (blue) disposable loop can be used in placed of the collection swab. Vortex the specimen and proceed to remove the swab. Dip the loop into the liquid media and inoculate the plates.

C. Slide Preparation
    1. All slides should be labeled with patient demographics. Apply the GS media label to slide. If a label is not available there should be a minimum of the patient's name, source of specimen & accession number on slide.
    2. All slides must be decontaminated before inoculated by using 70% ethanol. Slides should be placed in Coplan jar filled with ethanol. Jar should be empty and refilled each shift. Slides are then placed in an empty Coplan jar to dry.

3. All slides must be methanol fixed after slide is adequately dried on the slide warmer.

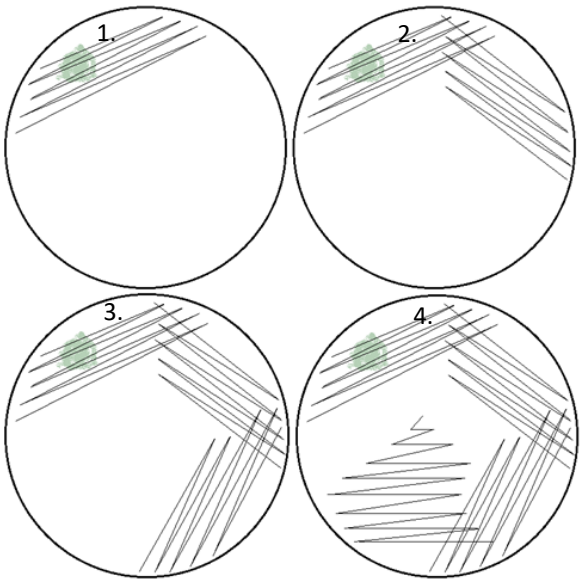
4. Cytospin slide:
    a. Place a clean, sterile SUPERFROST circle slide into a clean cytoclip.
    b. Place a disposable cytofunnel over the slide and close the cytoclip.
    c. Using a sterile pipette, place three drops of specimen into the cytofunnel and cap. Place the cytofunnel into the cytospin.
    d. When spin cycle is complete, remove the funnel and dispose in biohazard waste. The slide should be placed on the 40^0C slide warmer found within the planting hood.
    e. Cytoclips are to be decontaminated after every use by soaking in a container of 10% bleach solution and then rinsing by submerging in DI water. Both are found next to the Gram stain sink and replaced daily.
    f. If the quality of the specimen seems to be too viscous to provide a good quality gram stain smear, a 1:20 dilution of the specimen will be created using 85% sterile saline. Repeat the process (Steps a-d) with the diluted specimen.
5. Direct slide
    a. Using a sterile swab (if the specimen is not collected on one), or a sterile pipette, inoculate a WASP slide.
        i. If using a swab, roll the swab back and forth within the circle
        ii. If using a pipette, inoculate with one free-falling drop of specimen.
6. Calcoflour slide
    a. Using a sterile swab (if the specimen is not collected on one), or a sterile pipette, inoculate ULTRA FROST slide.
        i. If using a swab, roll the swab back and forth in the center
        ii. If using a pipette, inoculate with one free-falling drop of specimen.

D. Streaking Protocols
    1. Quantitation
        a. Using the appropriate sterile plastic loop (0.01 or 0.001), transfer one loopful of specimen to the agar plate (**A**)
        b. Cross streak through the primary streak line by moving the loop back and forth perpendicular to the initial streak (**B**).



A **B**

2. Isolation
    a. Using a sterile plastic loop, pass the loop back and forth through the inoculum of the first quadrant several times.
    b. Turn the plate and pass the loop through the edge of the first quadrant 2-3 times while streaking into the second quadrant, ensuring to not overlap any streaks. Continue streaking into the second quadrant without returning to the first.
    c. Turn the plate and repeat the previous step for quadrant three as well as quadrant four.
    d. Repeat this process for all media plates.



E. Media Inoculation Sequence
    1. Gram stain slide
    2. Broth media
    3. Plated media starting with the least inhibitory
        a. Plates should be streaked out in the same order.
            i. Bacterial media: BAP, CHOC, BRUC, CNA, MAC, KV, PEA
            ii. Fungal media: SAB, IMA, MYC
        b. After streaking, place all plate and broth media in the appropriate incubation environment (See Materials & Equipment section for specific incubation requirements)

F. Specimen aliquots
    a. Print additional patient labels for appropriate aliquot tubes
    b. Ensure primary and secondary labels match
    c. Label secondary containers
    d. To prevent cross-contamination, aliquot specimens into secondary containers one at a time and never return specimen back to primary container once removed
    e. Initial all secondary container labels for any necessary follow-ups

ACID FAST BACILLI CULTURES

Order: CXAFB
Media for AFB culture: N/A
Streaking Protocol: N/A
Special Instructions:

- All specimens must be parafilmed after processing. Media labels should be kept with specimen container and placed in refrigerator.

GASTRIC ASPIRATES

Order: CXAFB
Media for AFB culture: N/A
Special Instructions:

- Specimen must be neutralized upon receipt (and within 4 hours of collection) by adding pre-aliquoted sodium bicarbonate to the specimen container and vortexing.
- Label container as “Neutralized”. Parafilm specimen. Media labels should be kept with specimen container and placed in refrigerator.

BLOOD ISOLATOR SPECIMENS

Must be completely filled. Requires 1 tube per test. Requires ID approval. Initial all media.
Order: ISAFB
Media for AFB culture: 7H11, BD Myco Bottle
Streaking Protocol: N/A
Special Instructions:

- Plant within 16 hours of collection. Spin tube at 3500 rpm for 30 minutes. Re-spin if sitting more than 30 minutes.
- Iodine top of tube and place white cardboard sleeve on top. Place tube onto Isostat Press and pull down lever until fully punctured.
- Squeeze bulb of pipette with larger bulb, insert into the hole and release bulb to suck up supernatant. Discard.
- Vortex remaining concentrate. Using smaller pipette, transfer concentrate to conical tube.
- Using needle and syringe, put 2-3 drops (or until 1ml of specimen is left in syringe) into Middlebrook 7H11 slant. Alcohol top of myco bottle and add the remaining specimen (1ml or less).
- Alcohol myco bottle and put connector on. Put on AFB Versatrek. Place slant in incubator in AFB.
- If received in a Pedi isolator, disinfect top to tube with iodine. Using a needle and syringe, inoculate media. Place “PEDI” media label on one of the plates.

Order: ISFUN
Media for Fungal culture: IMA, IMA2, SAB, SAB2
Streaking Protocol: Quantitation
Special Instructions:

- Plant within 16 hours of collection. Spin tube at 3500 rpm for 30 minutes. Re-spin if sitting more than 30 minutes.
- Iodine top of tube and place white cardboard sleeve on top. Place tube onto Isostat Press and pull down lever until fully punctured.
- Squeeze bulb of pipette with larger bulb, insert into the hole and release bulb to suck up supernatant. Discard.
- Vortex remaining concentrate. Using smaller pipette, add 3-5 drops of specimen to the top of each plate. Tip plate upwards to create a line of specimen down the middle. Be careful to avoid getting any specimen on the edge of the plate.
- Using a loop, streak for quantitation. Let plates completely dry before parafilming.

- If received in a Pedi isolator, disinfect top to tube with iodine. Using a needle and syringe, inoculate media. Place “PEDI” media label on one of the plates.

ANAEROBES

ABSCESS
Order: CXANA or CXANO if from OR, CXFUS
Media for Aerobic/Anaerobic culture: BAP, CHOC, MAC, CNA, BRUC, KV, PEA, BHI, GRAM
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Upon request from Orthopedic doctor, plates can be held for 14 days.

BODY FLUID (STERILE)
Order: CXANA or CXANO if from OR, CXFUS
Media for Aerobic/Anaerobic culture: BAP, CHOC, MAC, CNA, BRUC, KV, PEA, BHI, GRAM (BAP, CHOC, BRU, BHI and GRAM if CXANO is ordered)
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Cytospin using 3 drops of specimen. If viscous, dilute 1:20 for cytospin smear.
- If received in blood culture bottles, mark with tape indicating BF, result and credit gram stain.
- Upon request from Orthopedic doctor, plates can be held for 14 days.

DIALYSATE FLUID
Order: CXDIA, CXFUS
Media for Aerobic/Anaerobic culture: BAP, CHOC, MAC, CNA, BRUC, KV, PEA, BHI, GRAM
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Cytospin using 3 drops of undiluted specimen.
- If not received in blood culture bottles, inoculate both Aerobic and Anaerobic bottles and load onto automated blood culture instrument using standard labels. [BMN3][CTL4]
- If sterile specimen not received, be sure to result and credit gram stain.

TISSUE
Bone, Solid Organs, Soft tissue
Order: CXANA or CXANO if from OR, CXFUS
Media for Aerobic/Anaerobic culture: BAP, CHOC, MAC, CNA, BRUC, KV, PEA, BHI, GRAM
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Choose the appropriate tissue grinder (15 ml or 50 ml) depending on the size of the tissue specimen being processed. Label the tube of the tissue grinder with an extra media label and initial.
- When a large piece of tissue is received, identify necrotic portions and use a sterile scalpel or sterile scissors to remove the area for processing. Place tissue in tube. A few drops of saline can also be added to the tube if needed. Do not overfill the tube. Hold the tube upright and insert the pestle. Screw on cap. While applying adequate pressure, turn and rotate the handle to grind the tissue specimen. Avoid plunging the pestle up and down. Remove the cap and dispose of pestle, ensuring that all tissue specimen is left in container.
- With a sterile pipette or swab inoculate proper plates for isolation.
- For solid bone specimens, refer to foreign body/hardware special instructions.
- Upon request from Orthopedic doctor, plates can be held for 14 days.
- Tissue specimens where a request to rule out Mucormycetes had been made should not be ground. Instead slice or mince tissue with a sterile scalpel and place on fungal media. An

additional sabouraud plate should be added and incubated at 37°.

CORNEA
Order: CXCOR, CXFUS
Media for Aerobic/Anaerobic culture: CHOC, BRUC, BHI
Media for Fungal culture: IMA, CALCO
Streaking Protocol: Isolation
Special Instructions:

- CHOC and IMA plates are received already inoculated. Two slides should also be inoculated at bedside for gram stain and Calcoflour.
- Use provided Eswab to inoculate BRUC and BHI.
- For donor Cornea, alcohol top of BHI and place ring in. Use transport fluid to inoculate plates.

VITREOUS FLUID/WASHINGS/AQUEOUS HUMOR
Order: CXVIT, CXFUS
Media for Aerobic/Anaerobic culture: CHOC, BRUC, BHI, GRAM
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Cytospin slide must be made with undiluted specimen.

FOREIGN BODY/HARDWARE
Order: CXANA or CXANO if from OR, CXFUS
Media for Aerobic/Anaerobic culture: BAP, CHOC, MAC, CNA, BRUC, KV, PEA, BHI
Media for Fungal culture: SAB**,** IMA, MYC
Streaking Protocol: Isolation
Special Instructions:

- Foreign body specimens are only processed if no other specimens are submitted.
- If the foreign body/bone specimen is small enough, using a sterile set of forceps, the specimen can be placed into a BHI liquid media tube.
- If the specimen is too large to place inside the BHI tube, pour enough BHI broth into the collection container to cover the foreign body/bone. This may require several BHI broths. The specimen cup will serve as the BHI portion of the culture.
- Thoroughly vortex the specimen container. With a sterile pipette, draw up a sufficient volume of the solution and inoculate the plate media.

BONE MARROW
Order: CXBM*,* CXFUS
Media for Aerobic culture: BAP, CHOC, MAC, BRU, BHI, GRAM
Media for Fungal culture: 2 IMA, 2 SAB, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Collect or transfer to pedi isolator tube before processing.
- Disinfect top of tube with iodine. Using a needle and syringe, inoculate media.

BLOOD

TRANSFUSION REACTION
Order: CXBLD
Media: 2 Aerobic BC Bottles
Special Instructions:
Refer to ***Transfusion Reaction Cultures*** procedure for further instruction.

CEREBRAL SPINAL FLUID

LUMBAR PUNCTURE
Order: CXCSF, CXFUS
Media for Aerobic culture: BAP, CHOC, BHI, GRAM
Media for Fungal culture: SAB, IMA, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Cytospin slide must be made with undiluted specimen.
- EV PCR must be aliquoted before planting.

SHUNT FLUID
Order: CXSHN, CXFUS
Media for Aerobic culture: BAP, CHOC, BRUC, BHI, GRAM
Media for Fungal culture: SAB, IMA, CALCO
Streaking Protocol: Isolation
Special Instructions:

- BRUC plate is placed directly into anaerobic glove box.
- Cytospin slide must be made with undiluted specimen.

HAIR, SKIN, NAIL FUNGAL SPECIMENS

Order: CXFNS
Media for fungal culture: IMA, DT, CALCO
Special Instructions:

- If specimen is not received on swab, place in Mycology bucket near fungal incubator with media labels attached. Mycology will process and grind specimen.

EAR SPECIMENS

Order: CXEAR, CXEAG
Media for aerobic culture: BAP, CHOC, MAC, CNA (IF CXEAG ordered, GS will be added)
Streaking Protocol: Isolation
Special Instructions: N/A

EYE SPECIMENS

Order: CXEYE, CXEYG
Media for aerobic culture: BAP/SS, CHOC, MAC, CNA (IF CXEYG ordered, GS will be added)
Streaking Protocol: Isolation
Special Instructions:

- Inoculate BAP with three Staph streaks, bisecting the first, second, and third quadrant.

RESPIRATORY SPECIMENS

BRONCHO ALVEOLAR LAVAGE (BAL) (Quantitative)
Order: CXBQT, CXFUS
Media for Aerobic culture: BAP, CHOC, MAC, CNA, GRAM
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Quantitation
Special Instructions:

- Cytospin 3 drops of specimen.

- Use .01 blue loop for quantitative streaking. Mark fungal media with “0.01”

BRONCH WASH
Order: CXBRO, CXFUS
Media for Aerobic culture: BAP/SS, CHOC, MAC, CNA, GRAM
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Inoculate BAP with three Staph streaks, bisecting the first, second, and third quadrant.

BRONCHIAL BRUSH
Order: CXBRS, CXFUS
Media for Aerobic culture: BAP/SS, CHOC, MAC, CNA, GRAM
Media for Fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Place brush into 1ml of sterile saline, vortex, then plate using saline.
- Inoculate BAP with three Staph streaks, bisecting the first, second, and third quadrant.

SPUTUM CYSTIC FIBROSIS
Order: CXCF, CXFUS
Media for aerobic culture: BAP/SS, CHOC, MAC, CNA, BCSA, GRAM
Media for fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Inoculate BAP with three Staph streaks, bisecting the first, second, and third quadrant.
- Put BCSA plate in ambient incubator

SPUTUM CYSTIC FIBROSIS ON SWAB
Order: CXCF, CXFUS
Media for aerobic culture: BAP/SS, CHOC, MAC, CNA, BCSA
Media for fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Source should be “SPSW”, this will ensure no gram stain is ordered.
- Inoculate BAP with three Staph streaks, bisecting the first, second, and third quadrant.
- Put BCSA plate in ambient incubator

SPUTUM (Expectorated/Induced/Tracheal aspirate)
Order: CXSPT, CXFUS
Media for aerobic culture: BAP/SS, MAC, CHOC, CNA, GRAM
Media fungal culture: SAB, IMA, MYC, CALCO
Streaking Protocol: Isolation
Special Instructions:

- Inoculate BAP with three Staph streaks, bisecting the first, second, and third quadrant.

RESPIRATORY CULTURE
Media for aerobic culture: BAP/SS, CHOC, MAC, CNA
Streaking Protocol: Isolation
Special Instructions:

- Edit source in OE. Use Micro OE comment for R/O Organisms (ie Nasals for r/o MRSA). Reorder all Sinus specimens as CXANA

SCREENS

MRSA SCREEN

Order: CXMRS
Media: CHROM or CNA
Streaking Protocol: Isolation
Special Instructions:

- Plant all Eswab specimens on the WASP.
  - Exceptions include all sources planted on CNA including urine, rectal, sputum
  - MRSA's received in Copan Double Swabs will be manually processed.
- CHROM plates are light sensitive and need to be incubated in dark incubator.

ACINETOBACTER SCREEN

Order: CXACA
Media: MAC
Streaking Protocol: Isolation
Special Instructions: N/A

GROUP A SCREEN

Order: CXGAL (Rectal) or CXGAG (Genital)
Media: CNA
Streaking Protocol: Isolation
Special Instructions: N/A

GROUP B SCREEN

Order: CXGRB
Media: BAP
Special Instruction:

- Only performed at the request of the doctor if the patient is allergic to penicillin.

THROAT CULTURE

Order: CXTHR
Media: BAP w/ ATAB
Special Instructions:

- Only performed when Group A Strep PCR results as invalid twice.
- Can be performed upon doctor request for susceptibilities.
- BAP plate is stored anaerobically.

URINE SPECIMENS

Order: CXURN
Media for aerobic culture: BAP, MAC
Media for fungal culture: SAB, IMA, MYC
Streaking Protocol: Quantitation
Special Instructions:

- Inoculate media with a 0.001µl (green) loop.
- Invasively collected specimens i.e., Straight Catheter, cystoscopy, PCN-Percutaneous Needle Aspirate and Suprapubic Needle Aspirate, [CTL5]will have an additional BAP inoculated using a 0.01 (blue) loop.
- Add GRAMO for pediatric patients <6 months in Hasbro ER.

WOUND SPECIMENS

Order: CXWND, CXFUS
Media for aerobic culture: BAP, CHOC, MAC, CNA, GRAM
Media for fungal culture: SAB, IMA, MYC, CALCO
Streaking protocol: Isolation
Special Instructions:

- Specimens received on Eswabs must be processed on WASP. Manual processing is only to be done when the WASP is not operating properly, the specimen collection device is not compatible, or when the quantity of specimen is insufficient.
- Source of wound is required.
- Add MTM to any genital sources.

CATHETER TIP

Media for Aerobic culture: BAP
Streaking Protocol: Semi-quantitative
Special Instructions:

- Using a pair of sterile disposable forceps, remove the tip from the collection container. Place the tip onto the agar media and roll it across as much of the agar surface as possible. Avoid touching the catheter to the side of the agar dish. Replace tip into collection container.
- Refer to: Catheter Tip Protocol procedure for list of acceptable Type of tips to be processed in this manner:
    - Note: Cordis Sheath (This tip is placed in BHI broth after plate inoculation)

LEGIONELLA CULTURES

Sputum, BAL, Bronch, Tissue, Body Fluid
Order: CXLEG
Media for aerobic culture: 2 BCYE, 2 BCYEP, BAP/SS
Streaking Protocol: Isolation
Special Instructions:

- Draw up 0.5 ml of specimen using a sterile, graduated glass pipette. Dispense specimen into a 4.5 ml tube of KCl. Vortex specimen and incubate at room temperature for 5 minutes.
- While waiting for the acid-treated specimen, inoculate BAP, BCYE and BCYEP plates with 0.1 ml of specimen. Inoculate BAP with three Staph streaks, bisecting the first, second, and third quadrant.
- After 5 minutes have passed, inoculate the two remaining Legionella media plates, BCYEA and BCYPA, with 0.1 ml of the acid-treated specimen using a sterile, graduated glass pipette.
- Label the plates with an out-date that is 7 days from the inoculation date.

CREUTZFELDT-JAKOB DISEASE

- For more details, refer to the **Microbiology Safety Guidelines** procedure.

II. NOTES

A. A small volume of plate media will be kept at room temperature. Fastidious organisms such as Haemophilus and Neisseria may be killed by inoculation onto cold media. Also, plate media containing antibiotics are labile when left at room temperature for extended periods of time.
B. Specimens should be planted as soon as they are received in the lab.
C. Visually inspect specimens for contamination and ensure they meet collection and transportation requirements. Refer to Criteria for Rejection in the Laboratory Administrative Manual for further guidance.
D. ALL specimens are planted inside a biosafety cabinet. The use of a face shield to plant specimens is not acceptable.
E. Do not inoculate the media for multiple cultures simultaneously. Only one specimen is to be in the hood at a time. Specimens are to be completely processed before moving on to the next culture.
F. The alcohol in the Coplan jars used for decontamination of slides is replaced at the end of every shift.
G. For manually planted cultures, gram stain slides are labeled with a printed media label. The label will need to be cut to fit onto the slide and should not fall below the frosted portion the slide.
H. Accession numbers are assigned automatically by the computer system when the order is placed in the LIS.
I. 1N NAOH is available for sterilization of specimens possibly contaminated with Creutzfeldt–Jakob virus.
J. Gram stains of clinical specimens will be saved for 2 weeks.
K. Primary collection containers are saved (even when empty) in the microbiology lab for 7 days. Urine cultures are saved in the chemistry lab for 24 hours. Specimens will be delivered to chemistry at the end of every shift.

III. REFERENCE

A. Garcia, L. S. (Ed.). (2010). *Clinical Microbiology Procedure Handbook* (3rd ed., Vol. 1). Washington, DC: ASM Press.
B. Becton, Dickinson and Company. (2016) *Liquid Amies Elution Swab (ESwab™) Collection and Transport System [package insert].* Sparks, MD.
C. **PACKAGE INSERTS**

IV. REVISIONS

A. 12/09/2022 Reformatted procedure to include special instructions for various specimen types.