PURPOSE: A guide to the handling of specimens submitted for bacterial culture including:

1. Description of media used
2. Gram stain smear labeling
3. Gram stain smear preparation
4. Media inoculation technique for swab and fluid specimens
5. Media inoculation technique for urine specimens
6. Setting up anaerobic jars for fluid and deep wound specimens

REAGENTS and/or MEDIA:

- Blood agar (BA)
- MacConkey agar (MAC)
- Chocolate agar (CHO)
- Colistin-nalidixic acid agar (CNA)
- Brucella agar (BRU)
- Laked blood and KV agar (KV)
- Thioglycollate broth (THIO)
- Thayer Martin agar (TM)
- Sabouraud agar (SAB)
- StrepB*Select* agar (GBS)
- LIM broth (LIM)
- Uri*Select* 4 agar (URI)
- Denim Blue agar (DEN)
- Colorex VRE (VRE)

SUPPLIES:

- Disposable 1 µL and 10 µL loops
- Disposable needles
- Glass microscope slides
- Ringed cytology slides
- Alcohol swabs
- Sterile pipettes
- Sterile swabs
- Anaerobic trays and jars
- Anaerobic indicators
- AnaeroGen packs
- Blood culture subculture vents

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

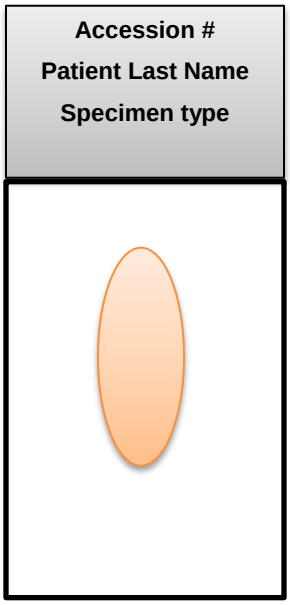
- Refer to MIC60010 – Microbiology Quality Control procedure.
- Refer to MIC60040 – Culture Media Quality Control procedure.

1. DESCRIPTION OF MEDIA USED:

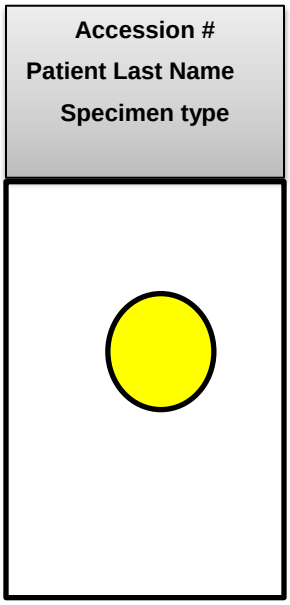
AGAR	TYPE	ATMOSPHERE	PUROSE
Blood (BA)	• Enrichment	CO_2 - wounds and fluids AnO_2 - throats	To grow most bacteria and determine the type of hemolysis. Will not support *N.gonorrhoeae* or *Haemophilus* spp.
Chocolate (CHO)	• Enrichment	CO_2 - from all sources	To grow most bacteria, including *N.gonorrhoeae* and *Haemophilus* spp.
MacConkey (MAC)	• Selective • Differential	O_2 - from all sources	Gram-negative enteric agar that inhibits the growth of Gram-positive organisms and yeasts. Differentiates lactose-positive organisms and lactose-negative organisms.
Colistin-nalidixic acid (CNA)	• Selective	CO_2 - from all sources	Inhibits most Gram-negative bacteria.
Brucella (BRU)	• Enrichment	AnO_2- from all sources	To allow growth of all clinically significant anaerobes.
Brucella with laked blood and KV (KV)	• Selective • Differential	AnO_2- from all sources	Selection of Gram-negative anaerobic organisms.
Thioglycollate broth (THIO)	• Enrichment	O_2 - from all sources	To enrich the growth of anaerobic organisms.
Thayer Martin (TM)	• Selective	CO_2 - from all sources	To select for *Neisseria gonorrhoeae* in mixed cultures.
Sabouraud agar (SAB)	• Selective	Room temperature	For the selective isolation of Yeast
LIM broth (LIM)	• Selective • Enrichment	CO_2	For the selective enrichment of group B *Streptococci*.
MRSA/VRE/GBS (DEN/VRE/GBS)	• Selective • Differential	O_2	For the selective and differential isolation of MRSA, VRE and GBS.
Uri*Select* 4 agar (URI)	• Differential	O_2	For the differential isolation of urinary tract pathogens

2. GRAM STAIN SMEAR LABELING:

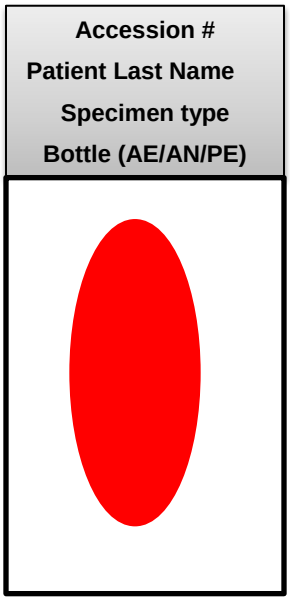
Swab specimen slide



Fluid specimen slide



Blood culture slide



3. GRAM STAIN SMEAR PREPARATION:

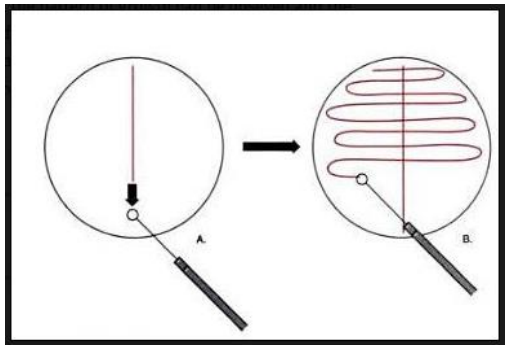
1. For direct smears, prepare a monolayer of organism sufficiently dense for easy visualization but sparse enough to reveal characteristic arrangements. As a guideline, newspaper print should be visible through the smear.
2. Specimens received on swabs:
   - Roll the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements.
3. Body fluids including CSF:
   - Use a sterile pipette to transfer 1 or 2 drops of the specimen directly to a sterile, ringed cytology slide. Allow the drop(s) to form one large drop. Do not spread the fluid.
4. Blood Cultures:
   - Using the sub-culturing vent, apply 1 to 2 drops of the specimen directly to a sterile slide. Spread the blood with a disposable loop to obtain a thin smear.
5. Sputum specimens:
   - Select purulent or blood-tinged portions of sputum with a sterile swab.
   - Spread the sample over a large area of the slide to form a thin film.
6. If there is insufficient specimen, omit the smear rather than the culture.

4. MEDIA INOCULATION TECHNIQUE FOR SWAB AND FLUID SPEICMENS:

1. Label media plates with LIS media labels.
2. Inoculate onto plate by touching specimen to one quadrant with swab or adding one to two drops of fluid with a sterile pipette.
3. Streak with gentle pressure onto one-fourth to one-third of the culture plate using a sterile loop or needle, with a back and forth motion several times and without entering the area that was previously streaked. Avoid touching the sides of the media.
4. Turn the plate a quarter turn. Pass the loop or needle through the edge of the first quadrant approximately three times, while streaking into the second quadrant. Continue streaking in the second quadrant without going back to the first quadrant.
5. Rotate the plate another quarter turn and repeat the above procedure until one or two additional quadrants are streaked.

5. MEDIA INOCULATION TECHNIQUE FOR URINE SPECIMENS:

1. Label media plate with LIS media label.
2. Using a disposable 1 µL calibrated loop, hold the loop vertically and immerse just below the surface of a well-mixed, un-centrifuged urine specimen. Avoid bubbles.
3. Deliver a loopfull of well-mixed urine onto the Uri*Select* 4 plate and using the loop, make a straight line down the center of the plate and streak the urine by making a series of passes at 90° angles through the inoculum.



6. SETTING UP ANAEROBIC JARS FOR FLUID AND DEEP WOUND SPECIMENS:

1. ASAP after planting, set up anaerobic tray or jar.
2. Place anaerobic plates into anaerobic tray or jar.
3. Tear open anaerobic indicator (kept in bucket with anaerobic packs) and withdraw wick 1 cm.
4. Place in small rectangle in anaerobic tray or in indicator holder in anaerobic jar.
5. Add AneroPouch pack to anaerobic tray or AnaeroGen pack to anaerobic jar.
6. Close the jar or tray. Label with 48 hour date (2 days from day jar or tray set up).
7. Place tray or jar on bottom shelf of O_2 incubator labelled AnO_2 Jars/Trays.

LIMITATIONS:

- False-positive cultures result from specimen mix-up and from contamination of media used for culture.
- False-negative results are due to improper collection, delays in culture inoculation, inappropriate medium usage and inappropriate incubation conditions.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven