SPECIMEN OPTIMAL SAMPLING

MANUAL BLOOD CULTURE SPECIMEN PROCESSING (NON-AUTOMATED)

Purpose: To provide steps on how to process blood culture specimens using the manual method (non-automated).

Principle: Blood is a sterile fluid. The presence of microorganisms in the blood may give rise to bacteremia (transitory phase) or septicemia (infective phase). In suspected cases of septicemia, blood is inoculated into an enrichment broth to maximize quick recovery of microorganisms.

PROCEDURE: STEP BY STEP

Day 1

Within 4 hours of sample being collected, blood culture bottles should be placed in an O_2 incubator at 35-37°C overnight.

Day 2

Remove all bottles from the incubator and examine macroscopically for any evidence of turbidity, haemolysis, gas production or bacterial colonies on agar slope.

Subculture all samples, even those with no evidence of bacterial growth.

Use Class 1 cabinet, in case of potential Advisory Committee on Dangerous Pathogens (ACDP) category 3 or 4 organisms

Procedure

- Disinfect the cap with 70% alcohol then use a sterile needle and syringe to draw 1ml of the broth culture.
- Inoculate onto Blood, Chocolate and MacConkey agar plates.
- Prepare a smear on the glass slide. Perform a Gram strain and report the preliminary results immediately.
- Incubate blood and chocolate plates at 35-37°C in the CO_2 incubator and the MacConkey agar plates in the O_2 incubator for 18-24 hours.
- **Re-incubate all culture bottles in an O_2 incubator at 35-37°C overnight**

If anaerobic incubation conditions are available, inoculate another blood agar plate or an anaerobic agar plate (e.g. Fastidious Anaerobic Agar (FAA)) and incubate at 35-37°C in ANO_2 conditions

Day 3

- Examine all culture plates from the previous day. Pick colonies from pure cultures to set up biochemical tests and perform antibiotic susceptibility testing (AST), incubate accordingly.
- Where available, use automated methods (i.e. Vitek® 2 for ID and AST)
- Ensure appropriate quality controls are in place for ID and AST
- Set up purity plates on appropriate media and incubate in appropriate conditions
- **Remove culture bottles of all samples that have positive growth from the incubator, keep bottles at room temperature for a duration documented in local standard operating procedure (SOP) then discard in accordance with local procedures**

Day 4

Identify the organism by reading and interpreting biochemical results. Interpret antibiotic susceptibility results using the standard guidelines such as CLSI or EUCAST.

Report and send out the results immediately.

Day 5-7

- On each day, perform macroscopic examination and repeat procedures outlined on day 2, 3 and 4.
- **Perform blind sub-culturing every 48 hours in bottles showing no evidence of bacterial growth**
- During repeated sub-culturing, ensure aseptic techniques are maintained to reduce chances of contamination
- **Perform a final sub-culture of all incubated blood culture bottles, even those with no evidence of bacterial growth on day 7.**
- Read plates on day 8 and report accordingly.
- Report all negative cultures as “No growth” detected after 7 days incubation.

On day 7, remove all blood culture bottles from the incubator, keep bottles at room temperature for a duration documented in local standard operating procedure (SOP) then discard in accordance with local procedures

References: Best Practices of Blood Culture in Low- and Middle-Income Countries: https://doi.org/10.3389/fmed.2019.00131
District Laboratory Practice in Tropical Countries Part 2: Monica Cheesebrough

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