Title

Blood Culture

Test Summary

Blood culture processing assists in the diagnosis of bacteraemia and sepsis. Pathogens include: *Staphylococcus aureus*, *E.coli*, *Klebsiella pneumoniae* and other *Enterobacteriaeae*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. *Salmonella typhi*, other *Salmonella* and *Burkholderia pseudomallei* are important blood culture isolates seen in Cambodia.

Principle

Blood is a sterile fluid. Inoculation of blood in prepared media bottles and incubation may detect the presence of organisms in the blood. Growth of an organism allows us to perform identification and susceptibility testing.

Specimen Handling and Preparation

Blood samples are inoculated into blood culture media bottles.

It is encouraged to collect two separate venipuncture collections per patient.

10ml blood for each adult collection (20ml total in 2 bottles) and 2 to 5ml blood for a pediatric collection in 1 bottle).

It is important to use aseptic technique when collecting blood for blood culture so that skin flora or environmental organisms are not introduced into the bottles.

Refer to blood culture collection procedures.

Quality Control (QC)

Appropriate QC testing should be performed for all media used, biochemical testing, Gram stain and AST performed according to the laboratory QC procedures.

Reagents, Materials and Equipment

Adult and Pediatric blood culture bottles

Venting needles

Media for subculture of blood from blood culture bottles:

Sheep Blood Agar (BAP)

Chocolate Agar (CHOC)
MacConkey Agar (MAC)

Glass slides for Gram stain preparation

Usual laboratory equipment and consumables

**Procedure**

Assign each blood culture bottle a separate Lab number. (If each blood culture bottle is from a separate venipuncture).

Record patient details on to the worksheet.

Clean the top of the blood culture bottle with 70% alcohol.

Aseptically insert a venting needle.

Incubate bottles for 7 days at 35-37°C.

Keep the venting needle in place for the full 7 days of incubation.

Do not disturb the bottle but check the bottles daily for signs of growth: turbidity, bubbles, hemolysis. If none seen, gently rotate the bottle on the bench to resuspend the blood and mix it. Reincubate until the next day.

Hold for 7 days before reporting final report as ‘negative’.

Gram stain and subculture all bottles that show signs of growth.

Subculture all bottles after 1 nights incubation. (If not already subcultured)

Subculture:

Place 2 drops of blood to BAP and prepare a Gram stain.

If Gram positive cocci are detected on the Gram stain add an optochin disc to the 2nd quadrant of the BAP plate.

If Gram negative bacilli are detected on Gram stain subculture blood to a MAC plate.

If Gram negative diplococci or Gram negative coccobacilli are detected ensure that a subculture to CHOC agar is performed.

Incubate BAP and CHOC plates in a candle jar. Incubate the MAC plate and the candle jar for 3 days at 35-37°C.

Read all agar plates daily for 3 days
After the visual checks of blood culture bottles, gently invert several times before reincubation.

A blind subculture should be performed on 5% of blood culture bottles (1 bottle in 20).

Record daily on the specimen worksheet all actions performed.

**Reading Gram stains**

Read the Gram stains the same day that they are prepared.

A positive Gram stain result is a critical result. The result may have direct impact on the treatment of the patient. Record all Gram stain results on the specimen worksheet.

Phone the result to the clinician or ward. Record notification on the worksheet.

Prepare a Primary Report in CAMLIS.

**Reading Cultures**

Read all plates daily and record results on the specimen worksheet.

If growth is detected perform Identification and susceptibility testing.

Refer to Dr E.J Baron’s Flow Charts for pathogen identification.

Refer to the current AST Guidelines to help choose appropriate antibiotic discs for testing.

Keep plates until a final report has been sent to the clinician.

Inoculate a broth for storage of all blood culture isolates at -20°C.

**Result Reporting**

**Blood culture showing ‘Growth’**

Primary Report: Gram stain result. Example: Gram negative bacilli


Final Report: add Antibiotic Susceptibility Test result

Refer to the current AST Guidelines to help choose appropriate antibiotic discs for reporting.

In some cases the significance of the isolate will not be clear.

Add the following comment: **Significance depends on clinical assessment**

**Blood culture showing ‘NO Growth’**
No Primary report is issued.

The clinicians should be informed that any results of a positive blood culture will be immediately communicated.

Final Report: If the blood culture bottle is clear on Day 7 of incubation:

‘No growth at 7 days’

Note: If there is any doubt about the blood culture being clear or turbid a final Gram stain must be performed

Expected Values

Blood is a sterile fluid.

Growth from a blood culture may indicate bactereamia or sepsis.

Limitations

It is important to inoculate the correct volume of blood into a blood culture bottle. Follow the collection procedure. Insufficient blood volume decreases the sensitivity of this test.

Sometimes contaminants from the environment or from skin flora may be introduced to the blood culture bottles. Always report positive growth. Add the comment: ‘Significance depends on clinical assessment’.

On visual inspection bottles may appear turbid but show no growth on subculture.

In these circumstances always carefully examine the Gram stain. If there is any doubt repeat the Gram stain and subculture.

Some fastidious bacteria may not grow under the usual culture conditions. Some bacteria require a microaerophilic atmosphere (Campylobacter, Helicobacter)

In Cambodia we do not use anaerobic blood culture bottles or culture for anaerobes

Sometimes a bottle may not appear turbid but the subculture will be positive.

References

Dr E.J.Baron’s Flow Charts

Manual of Clinical Microbiology 9\textsuperscript{th} Edition P.R.Murray, E.J.Baron, J.H.Jorgensen, M.L.Landry, M.A.Pfaller