# Aim

To describe the processing of blood to identify invasive bacterial infection.

# Principle

Culture of blood is the “gold standard” investigation for detection of micro-organisms in blood, although often sensitivity is poor. Blood stream infections may be transient, intermittent, or continuous and may be either community or healthcare acquired. Infecting organisms vary by patient age, immune status, and presence of comorbidities as well as site of primary infection. The bacterial load in paediatric infection is often greater than that in adult infections (>4 x103 cfu/L vs. <1 x103 cfu/L).

Common bacterial pathogens detected in blood cultures include:

|  |  |
| --- | --- |
| **Gram positive organisms** | **Gram negative organisms** |
| *Staphylococcus aureus* | *Escherichia coli* |
| *Streptococcus pneumoniae* | *Klebsiella* spp. |
| *S. pyogenes (group A)* | *Enterobacter* spp. |
| *S. agalactiae (group B)* | *Salmonella* spp. *(incl. S.* Typhi*)* |
| *Enterococcus* spp. | *Pseudomonas aeruginosa* |
| *Listeria monocytogenes* | *Neisseria meningitidis* |
|  | *Haemophilus influenzae*  *Burkholderia pseudomallei* |

In addition to bacteraemia, certain fungi may also cause blood stream infection (fungaemia), most notably *Candida* spp.

Contamination of the blood sample during sample collection may occur. Organisms which are often regarded as skin commensals and of unlikely significance include coagulase negative staphylococci (CoNS), diphtheroids (Gram positive rods with morphology similar to *Corynebacterium* spp.) and *Bacillus* spp. (excluding *B. anthracis*). Isolation of the same “contaminant” from multiple sets of blood cultures from the same patient should prompt re-evaluation of significance and further investigation.

# Method

## Specimen collection

Ideally specimens for blood culture should be collected prior to initiation of antimicrobial therapy and as soon as possible after a spike of fever.

The volume of blood cultured is the most significant factor affecting the detection of bacteraemia:

* For children, a minimum of 1 ml is recommended (and no more than 1% of total blood volume should be cultured)
* For adults, 20-30 ml of blood is recommended

Culture bottles may be weighed before and after inoculation to estimate volume of blood cultured (1g ≅ 1ml blood), taking into account the weight of the bottle cap.

For bottles containing 0.05% sodium polyanethol sulphonate (SPS), a blood to broth ration of 1:5 to 1:10 is optimal (e.g. 2–4ml of blood added to a 20 ml paediatric culture bottle).

Skin should be thoroughly cleaned with a 70% ethanol-2% chlorhexidine solution, and allowed to dry, prior to venepuncture. Following venepuncture using a non-touch technique, blood should be inoculated into the blood culture bottle prior to inoculation of other blood tubes (e.g. EDTA CBC tube). The rubber septum of the blood culture bottle must be disinfected with 70% ethanol prior to inoculation.

## Specimen transport and storage

After inoculation, bottles should be transported to the microbiology laboratory without delay in a sealed plastic bag or rigid specimen container. If a delay in processing is unavoidable, then inoculated bottles should be maintained at ambient temperature (and must not be refrigerated).

## Specimen processing

### Reception

Log the specimen in the appropriate specimen book and assign a specimen number.

Record the weight of the bottle and record the out-going weight and in-coming weight on the specimen request form / lab logbook.

### Culture

All blood culture bottle manipulations must be carried out in the Class II biosafety cabinet.

Venting of bottles:

* Disinfect the rubber septum with 70% ethanol and allow to dry
* Vent the bottle by inserting a sterile BD sub-vent needle through the septum, leaving the cap in place

The vented bottle is incubated at 35-37°C in air and inspected daily for seven days for signs of bacterial growth (turbidity or bowed septum).

Each day the bottle is moved to the appropriate tracking container (labelled Day 1 – 7) in the incubator.

Blind sub-culture at 24 hours and seven days are performed to detect early and late growth, respectively.

### Blind sub-culture

Gently mix the broth using a swirling motion.

Remove the cap of the sub-vent, being careful not to contaminate the vent.

Invert the bottle and allow a single drop of broth to inoculate the sub-culture media (do not allow the vent to touch the media or vigorously shake the bottle):

* At 24 hours, inoculate a blood (BA), chocolate (CA), and MacConkey (MAC) plate.
* At seven days, inoculate a CA plate only.
* Twenty four hour sub-culture plate may be divided into quarters and the seven day sub-culture plates may be divided into eighths.

Streak the inocula out using a sterile microbiological loop to obtain single colonies.

Incubate the plates for up to 48 hours at 35-37°C in air (BA / MAC) or 5-10% CO2 (CA), inspecting for growth daily.

### Processing of positive bottles

Perform a Gram stain.

Inoculate and incubate media as outlined in Table 1.

**Table 1.** Sub-culture media for positive blood culture broths

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gram stain result** | **Media** | **Incubation** | | | **Cultures read** | **Target organism(s)** |
| **Temp (°C)** | **Atmos** | **Time** |
| All | BA1 | 35-37 | 5-10% CO2 | 40-48h | Daily | Any |
| **Additional plates depending on the Gram stain** | | | | | | |
| GNC/GNCB | CA | 35-37 | 5-10% CO2 | 40-48h | Daily | *Haemophilus* spp.  *Neisseria* spp. |
| GNB | MAC | 35-37 | Air | 16-24h | ≥16h | Enterobacteriaceae  Non-fermenters  *Pseudomonas* spp. |
| Yeasts | SAB2 | 28-30 | Air | 5d | 2d & 5d | Fungi |
| **Other considerations** | | | | | | |
| No organisms seen | BA  CA | 35-37 | 5-10% CO2 | 40-48h | Daily | Any |
| Unclear whether GN or GP organism | Blood-CNA  MAC | 35-37 | Air | 16-24h | ≥16h | To help differentiate orgs with equivocal Gram results |
| ?Anaerobic infection | BA +  MTZ3 disc | 35-37 | Anaerobic | 40-48h | ≥40h | Anaerobes |
| Primary culture negative but positive growth signal / Gram stain | BA +  *S. aureus* streak | 35-37 | 5-10% CO2 | 40-48h | ≥40h | *Abiotrophia* spp. |
| MAC | 35-37 | Air | 16-24h | ≥16h | Cysteine-dependent organisms |

1 An optochin disc may be added if streptococci are seen on Gram stain

2 Sabouraud agar

3 5µg metronidazole disc

# Interpretation

## Minimum level of identification in the laboratory

All blood cultures are discussed on the daily board/bench round:

* Clinically significant isolates should be identified to species level (see SOPs MID-003/4)
* Any organism considered to be a contaminant may not require full species level identification

Store significant isolates at -80°C in a cryovial containing 1ml STGG and record the isolate details in the freezer logbook/database.

## Antimicrobial susceptibility testing

All significant isolates should have antimicrobial susceptibilities performed according to SOP MIC-001.

Direct susceptibility testing may be performed in certain circumstances (i.e. to improve turnaround time), although these results will need to be repeated if confluent growth is not obtained or the culture is mixed. For GNB, add 1-2 drops of blood culture broth to 2ml of sterile saline and use this as the inoculum for preparation of antimicrobial susceptibility testing media. A greater inoculum (or a drop of blood culture broth applied directly to the agar plate) may be required for Gram positive organisms.

## Reporting

Gram stain: report organism detected (by telephone / urgent ward review + hard copy).

Culture:

* All organisms isolated (with comment if isolate(s) are of doubtful significance)
* Absence of growth
  + Interim reports
    - “No growth at 24 hours” (based on no growth on 24 hour blind sub-culture)
    - “No growth at 48 hours” (based on negative 24 hour blind sub-culture AND clear broth at 48 hour inspection)
  + Final report: “No growth at 7 days”
* Do not use the terms “no significant growth” or “mixed growth of doubtful significance”

Antimicrobial susceptibility testing: report as clinically indicated.

# Quality assurance

Media and identification tests should be quality controlled according to the relevant MOPSOP.

# Limitations

Several factors may result in a false negative blood culture, including:

* Exposure to antimicrobial agents prior to blood sampling
* Inoculation of an inadequate volume of blood
* Intermittent bacteraemia sampled at the incorrect time point:

# References

1. Health Protection Agency, UK SOP B37: Investigation of Blood for organisms other than *Mycobacterium* species (Issue 7; March 2013).
2. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edition Update (2006). Cambridge University Press.
3. Standard Operating Procedures from LOMWRU, SMRU and AHC.

# Synopsis / Bench aid



# Risk assessment

|  |  |
| --- | --- |
| **COSHH risk assessment - University of Oxford COSHH Assessment Form** | |
| **Description of procedure**  Culture of blood to identify bacterial and fungal pathogens | **Substances used**  Variable, depending on organism cultured (may include Gram stain reagents; 3% hydrogen peroxide (catalase test); N,N,N',N'-tetramethyl-1,4-phenylenediamine (oxidase test); sodium deoxycholate (bile solubility test); bioMerieux API reagents) |
| **Quantities of chemicals used**  Small | **Frequency of SOP use**  Daily |
| **Hazards identified**  1. Autoclaved liquid  2. Potentially infectious material in sample  3. Potentially pathogenic bacteria  4. Chemical exposure form bacterial identification tests | **Could a less hazardous substance be used instead?**  No |
| **What measures have you taken to control risk?**  1. Training in good laboratory practices (GLP)  2. Appropriate PPE (lab coat, gloves, eye protection)  3. Use of biosafety cabinet for reading of plates until known not to be growing an HG3 organism / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) | |
| **Checks on control measures**  Observation and supervision by senior staff | |
| **Is health surveillance required?**  No | **Training requirements:**  GLP |
| **Emergency procedures**:  1. Report all incidents to Safety Adviser  2. Use eyewash for splashes  3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:  1. Sharps discarded into appropriate rigid containers for incineration  2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration  3. Chemical waste disposed of according to manufacturer’s instructions  4. Blood culture bottles are disposed of by autoclaving and subsequent incineration |