Ministry of Health

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Contents Table:

Acknowledgment

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Acronyms:

ABC	Aerobic blood culture
ANBC	Anaerobic blood culture
PEAD	Pediatric blood culture
BA	Blood agar
CA	Chocolate agar
MAC	MaConkey
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
LIS	Laboratory information system
IQC	Internal Quality Control
MDRO	Multidrug Resistant Organism
MRSA	Methicillin Resistant Staph. Aureus
SOP	Standard operating procedure
TAT	Turnaround time
WHO	World Health Organization

1. Purpose

This document describes the procedure describes the processing and microbiological investigation of blood cultures and aims to set standards for processing and reporting blood cultures. It addresses the general principles involved in the diagnosis of bacteraemia and fungaemia from blood cultures. It does not address the detection of parasites, viruses or *Mycobacterium* species.

2. Scope

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. Definitions

3.1 sepsis: potentially life-threatening condition that occurs when the body's response to an infection damages its own tissues. When the infection-fighting processes turn on the body, they cause organs to function poorly and abnormally

3.2 Bacteraemia: The presence of bacteria in blood stream that induces an inflammatory response that is responsible of producing the symptoms of a bloodstream infection (ie, fever and chills).

4. Procedure

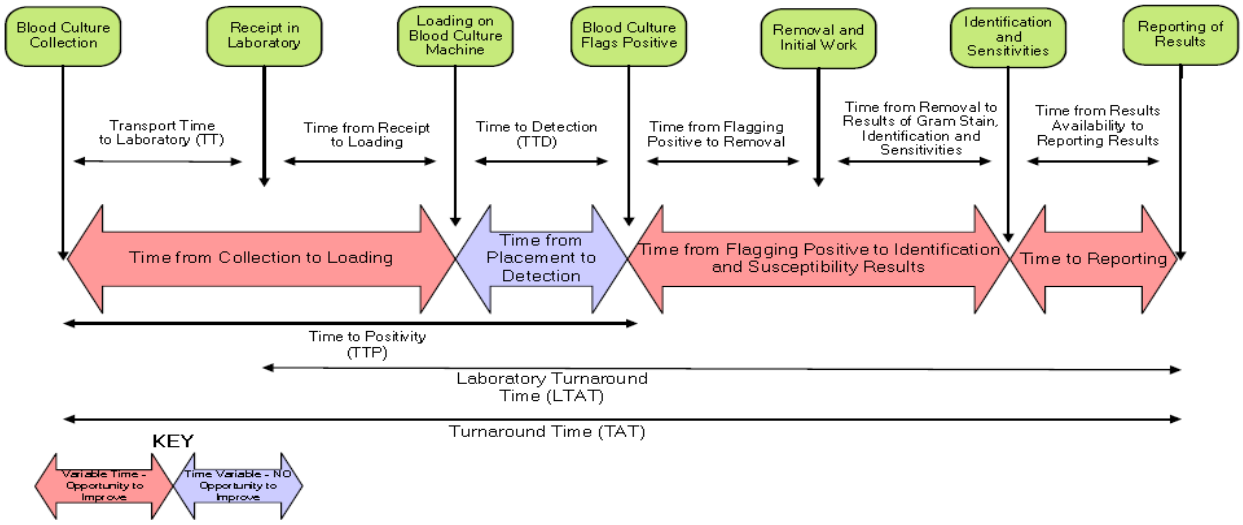
4.1. Clinical background:

- Blood stream infection is caused by bacteria (bacteremia) or fungi (fungaemia) in the blood and may be transient, intermittent or continuous. Blood culture is considered to be the “gold standard” investigation for the detection of micro-organisms in blood. Blood cultures should always be requested when a bloodstream infection or sepsis is suspected.
- Clinical symptoms in a patient which may lead to a suspicion of a bloodstream infection are: undetermined fever (≥38°C) or hypothermia (≤36°C) raised heart rate low or raised blood pressure raised respiratory rate, chills, rigors severe local infections (meningitis, endocarditis, pneumonia, pyelonephritis, intra-abdominal suppuration).
- To optimize the ability to detect bacteremia, blood cultures should be obtained before the initiation of antimicrobial therapy whenever possible. The yield of blood cultures would also depend on several factors including the number of cultures

taken and the volume of blood obtained. There are three stages that has to be taken into account for blood culture investigation (figure 1):

- **Pre-analytical:** The pre-analytical stage from collection to loading into incubator including: Transport time (TT) and time from receipt to loading.
- **Analytical:** From loading to identification and susceptibility results including: Time to Detection (TTD), time from flagging positive to removal and time from removal to results of gram stain, identification and susceptibility.
- **Post-analytical:** From identification and susceptibility to reporting.

Figure 1: Blood culture investigation stages



- Early identification and antibiotic susceptibility results for blood culture isolates provide valuable diagnostic information on which appropriate antimicrobial therapy can be based, so helping to reduce morbidity and mortality, improve patient care and reduce healthcare costs.

4.2. Principle:

If microorganisms are present in the test sample inoculated into a blood culture vial, CO_2 will be produced when the organisms metabolize the substrates present in the vial. Increases in the fluorescence of the vial sensor caused by the higher amount of CO2 are monitored by the blood culture incubator fluorescent series instrument. Analysis of the rate and amount of CO_2 increase enables the fluorescent series instrument to determine if the vial is positive which means that the test sample contains viable organisms.

4.3. Pre – analytical stage:

4.3.1. Sample:

- Samples should be taken as soon as possible after a spike of fever.
- Blood samples should be taken prior to antimicrobial treatment.
- For adults: collect 8 to 10 ml of peripheral venous blood.
- For pediatric: collecting depends on weight of the child as per the following table:

Table 1: Blood volumes suggested for cultures from infants and children

Adapted from Kellogg *et al*. Frequency of low-level bacteremia in children from birth to fifteen years of age. J Clin Microbiol. 2000; 38:2181-2185.

Weight of patient		Patient's total blood volume (ml)	Recommended volume of blood for culture (ml)		Total volume for culture (ml)	% of patient's total blood volume
kg	lb		Culture no.1	Culture no.2		
≤1	≤2.2	50-99	2		2	4
1.1-2	2.2-4.4	100-200	2	2	4	4
2.1-12.7	4.5-27	>200	4	2	6	3
12.8-36.3	28-80	>800	10	10	20	2.5
>36.3	>80	>2,200	20-30	20-30	40-60	1.8-2.7

- Changing needles between venipuncture and inoculation of the bottles is not recommended because this carries a risk of needle stick injury.
- Two blood culture sets are recommended

4.3.2. Material:

Consumables	**Equipment**	**Reagents**
disposable gloves, alcohol wipes needles and syringes, sterile saline, glass microscope slides,	Automated blood culture incubator instrument Microscope Incubators (Aerobic, CO2, Anaerobic, Anaerobic jars), Class II biological safety cabinet.	Aerobic bottles, Anaerobic bottles, Pediatric bottles. gram stain reagents culture media (Blood, Chocolate, Maconkey, Sabouraud, Muller Hinton, Muller Hinton with blood), Antimicrobial susceptibility testing methods.

4.3.3. Safety precaution:

- All specimens need to be treated as potentially infectious.
- Always wear gloves when handling blood culture bottles as blood cultures from patients may harbor blood borne pathogens
- Positive blood cultures should always be processed inside the biological safety cabinet class II.
- Never recap needles when you transfer blood
- Dispose needles/syringes in puncture proof containers
- Suspected *Neisseria* species, *Brucella* species, *Francisella* species etc. should be sealed by parafilm and processed inside the biological safety cabinet.

4.3.4. Quality control:

- Follow the quality control procedure for Blood culture incubator instrument as recommended by the manufacturer.
- Monitor and record the instrument temperature daily as a part of QC checkup.
- Check the alarms, lights.
- Calibration: if recommended by manufacture, calibrate the cells when required, if the cell calibration failed or not done, the cell will be disabled.
- Follow the quality control procedure for culture media, biochemical, identification & susceptibility systems
- Vial QC check:
    - Evaluate each shipment of blood culture vials before distribution to different wards.
    - Evaluate QC performance for each type of bottles (aerobic, anaerobic and pediatric) through the use of a positive and negative vial test.
    - The positive vial should be inoculated with 1.0 ml of a 0.5 McFarland Standard of Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 prepared in sterile distilled water from a fresh 18-24 h culture.
    - Culture the suspension on blood agar to verify the purity of the inoculated strain.
    - The negative vial or the un- inoculated vial should be logged into the instrument and tested for sterility.

- The inoculated vial should be detected as positive by the instrument within 72 hours.
- The negative control vials should remain negative throughout the entire testing protocol. This verifies that the media were not subject to adverse storage or shipping conditions prior to receipt in the laboratory.
- Record all the Blood Culture bottles QC evaluation results in Annexes: 8.1.
- If the QC checkup is accepted, call the medical store to distribute the shipment.
- If the QC checkup is unaccepted, verify that there are no laboratory errors by checking the overall process.
- Call the stores to inform the issue and fill the laboratory supply quality report form.

4.4. Analytical stage:

4.4.1. Sample receipt:

- Check proper labeling of blood culture bottles (Patient name, ID number, Patient date of birth (Dob), Barcode, etc.).
- Check expiry date of blood culture bottles.
- Check acceptance criteria of the blood culture bottles (e.g. collection time, leaking, cracks).
- Receive the blood culture into Al Shifa system in Laboratory Information System (LIS). If no request in the system calls the concern department and the laboratory shall add Non Entered test name/request (MOH code 72902) and release with comment.
- Label or mark the bottle as infectious (e.g. Brucellosis) if indicated on the patient clinical notes.
- Load all blood culture bottles automated system according to manufacturer's instructions (35 °C for 5 days total).
- Extended incubation (>5 days) maybe performed upon microbiologist request.
- Incubation times may be extended for 7 days or as per instructed by microbiologist, for specific clinical situations (when known): endocarditis, fungal infection, transfusion unit, bone marrow samples, brucellosis /

tularaemia and further processing of the samples is discussed with microbiologist.

4.4.2. Positive blood culture:

- After the alert, remove positive vials from the instrument to safety cabinet.
- Confirm that patient's details on the bottle match the ones in the request.
- Record the following blood culture details into the blood culture log book. Date, laboratory episode number and vial type.
- Print extra sticker and stick it to the positive blood culture worksheet.
- The worksheet sheet should be signed by laboratory technician and microbiologist.
- Ensure that the blood culture is adequately mixed.
- Rub an alcoholic wipe over the septum.
- Vent the vial’s cap with the available device.
- Dispense one drop from the bottle to culture each plate, and then microscopic slide to avoid the contamination.
- Withdraw a small volume of blood for gram stain and 1-2 drops into the following listed media with appropriate incubation conditions:

BLOOD CULTURE					
Clinical specimen	**Media**	**Incubation**			**Target Organism(S)**
All	Blood agar **BA**	35-37ºC	CO_2	2days* (read daily)	Any organism may be significant
	Chocolate blood agar **CHOC**	35-37ºC	CO_2	2days* (read daily)	
	Anaerobe agar +	35-37ºC	anaerobic	2days* (read daily)	
	MacConkey agar	35-37ºC	O_2	2days* (read daily)	
***Based on Gram stain findings , the bellow culture media can be added accordingly**					
Microscopy suggestive of mixed infection	*Neomycin fastidious anaerobe agar with metronidazole 5µg disc **NEO**	35-37	anaerobic	2days* (read daily)	
	*Staph/strep selective agar	35-37	air	2days* (read daily)	
Fungal infection suspected clinically Microscopy suggestive of fungal infection	Fungal selective agar	35-37	air	5days (read at 2d and 5d)	
Primary subculture negative but positive growth curve ** (1) Incubate all primary plates for total of 5 days (2)Subculture all bottles to additional media here	Campylobacter selective agar	35-37	micro-aerobic	5days (read at 2d and 5d)	*Campylobacter sp*
	Anaerobe agar	35-37	5-10% CO_2	5days (read at 2d and 5d)	Cysteine-dependent organisms

- Using a wood sticks, spread the blood in the slide and allow to heat dry in hot pot (temperature >50 C).
- Perform the Gram stain immediately and add the Gram stain as a reflex test (as Blood Culture Gram stain). Report it.

- Dispense blood culture directly to the media (4-5 drops for Gram positives and 2-3 drops for Gram Negative bacteria). Use this as the inoculum for any further cultures and susceptibility tests.
- For gram positive cocci in cluster add direct tube coagulase test (5 drops of blood into 1ml of rabbit plasma
- For gram positive cocci in chain, include the Optochin disc to direct susceptibility.
- If no organism seen, prolonged incubation is required following subculture into culture media. If organisms are not seen on microscopy, check the growth curve (automated systems).
- Examine culture media at 24 hour of incubation.
- If no growth, re-incubate the plates for 48 hrs and consult the microbiologists for further actions.
- Susceptibility testing of all isolates should be performed as advised by the microbiologists.

4.4.3. Negative blood culture:

- Remove Final negative vials at the end of their incubation when indicated in the instrument.

4.5. Post – analytical stage:

4.5.1. Interpretation / Results / Alerts:

- Assigned microbiologist would be responsible to verify the gram stain results and communicate findings with the concerned physicians
- If microbiologist is not around, laboratory technician would be responsible to verify the gram stain results and communicate with concern ward staff.
- All phone calls should be recorded in the laboratory remarks (Gram stain result, name of staff informed, ward and time/ date, read back).

4.5.2. Reporting:

- Blood culture gram stains should be entered in LIS.
- Identify isolates using the identification systems available.

- Final positive culture reports are entered in the Al Shifa LIS by the laboratory technician, and then verified and authorized by the medical microbiologist/ senior laboratory technologist.
- Negative cultures at 48 hrs will be reported as:

"No pathogen grown after 48 hours of incubation, this report will be amended only if found positive within 5 days".

  - All clinically significant isolates should be identified to species level.
  - Note: Any organism considered to be a contaminant may not require identification to species level.
- Positive blood culture reports:
  - Record organism seen in microscopy.
  - Record organism identification and any antimicrobial susceptibility results
  - Record the doubtful clinical significance of skin contamination.

4.5.3. Limitation:

- False negatives may occur if inadequate blood culture volumes are submitted.
- Skin contamination.

5. Responsibilities

5.1. Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels of communication once needed

5.2. Quality manager /officer

- To follow up the implementation of the procedure
- To ensure QC of each lot of blood culture bottles before distribution to the wards.
- monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended

- To report test failures or incident

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Public Health England. Investigation of blood cultures (for organisms other than *Mycobacterium* species).	UK Standards for Microbiology Investigation	(2019)	B 37
https://www.eucast.org/rapid_ast_in_blood_cultures/	eucast.org		

8. Annexes:

8..1. **Blood Culture bottles QC evaluation:** Each shipment of vials MUST BE evaluated for QC performance before use for patients.

Vials	Inoculation	Incubation time
Negative vial	Un-inoculated	Should stay negative for **5** days
Positive vial	inoculated with 1.0 ml of a 0.5 McFarland Standard of either Escherichia coli or Staphylococcus prepared from a fresh 18-24 h culture.	should be detected as positive by the instrument within **72** hours

Vials	Received by	Batch #	Time/date loaded	Time/date alarmed	Performance Pass / fail	Signature	Medical stores informed?
Negative vial	Time……. Date…… Sign……	…….	Time……... Date…… Sign……	Should not alarm before 5 days ……..	……	…….	……..
Positive vial	Sign……	……	Time…… Date……	Time……. Date……	……	Sign……	……..