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Title: Blood Culture Processing

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1. Purpose and scope

This document sets out the steps for blood culture processing at the Fleming Fund supported clinical microbiology laboratories that use the BACTEC blood culture system.

2. Principle/Clinical application

Blood is a sterile fluid which is collected for culture to assist in the detection of bacteraemia and fungaemia ('BSI'=bloodstream infection). The BSI may be a primary disseminated infection without an obvious focus or entry point (e.g. sepsis from *Neisseria meningitidis*, 20% of *Staphylococcus aureus* BSI events or enteric fever (typhoid) caused by *Salmonella* Typhi) or be secondary to infection in a specific body location or in association with a medical device such as an intravenous line or indwelling urinary catheter.

The cultured pathogen species often provides a likely indication of the infection source – e.g. an *E. coli* BSI usually arises from a urinary, biliary tract or bowel source whereas a *Staphylococcus aureus* BSI frequently arises from skin, bone or deep soft tissue/muscle sources.

Table1: Bacteria and fungi isolated from blood cultures

Gram Positive	**Gram Negative**
Staphylococcus aureus	*Escherichia coli*
Coagulase negative *staphylococci (*CoNS*)**	*Klebsiella species*
Viridans group *streptococci **	*Proteus species*
Streptococcus pneumoniae	*Salmonella* Typhi and Paratyphi
Streptococcus pyogenes and other beta-haemolytic species (groups B, C and G)	Non-typhoidal *Salmonella* species
Enterococcus faecalis and *E. faecium*	*Pseudomonas aeruginosa*
*Anaerobic streptococci**	*Acinetobacter baumannii*
Clostridium perfringens and related species*	*Haemophilus influenzae*
Listeria monocytogenes	*Neisseria meningitidis*
Bacillus species*	Environmental oxidase positive GNB*
	Brucella species
Fungi	*Bacteroides* species (anaerobic GNB)
Cryptococcus neoformans	*Campylobacter* (microaerophilic)
Candida species	*Burkholderia pseudomalleii*

* Indicates a species that frequently represents contamination but may also cause infection – to confirm actual infection, isolation of the same species from more than one culture set is required.

Blood is inoculated aseptically into the culture bottles at the point of care. The inoculated culture bottles are held at room temperature and transported without delay to the laboratory.

The bottles are placed in the BD BACTEC instrument for incubation and regular periodic reading.

- Each bottle contains a sensor, which responds to the concentration of CO_2 produced by the metabolism of microorganisms or the consumption of oxygen needed for the growth of microorganisms.
- The instrument monitors the sensor every ten minutes for an increase in its fluorescence, which is proportional to the increasing amount of CO_2 or the decreasing amount of O_2 present in the bottle.
- A positive reading indicates the presumptive presence of viable microorganisms in the bottle. This usually occurs within 12 hours for Gram negatives, from 10-18 hrs for Gram positives and from 48-72 hrs for yeasts / fungi..
- Contaminated cultures with bacteria often take > 48 hrs to flag indicating a low initial inoculum.

The blood culture bottle is subcultured onto agar media for incubation and a Gram stain is performed to determine a presumptive initial identification.

See also
- Blood culture Gram stain work instruction.
- Information sheet: Common blood culture Gram stains and their clinical implications

3. Responsibilities

3.1 Staff performing blood culture require specific training and demonstrated competency.

3.2 Accession new cultures, process, test and record results without delay- blood cultures have high priority.

3.3 Notify all positive Gram stains, cultures and susceptibility results to the responsible Medical Officer without delay: these results enable correct treatment and better outcomes of patients (see below for process).

4. Specimen

4.1 Inoculated BACTEC blood culture bottles (aerobic, anaerobic, PEDS+) are the received specimen.

4.2 Expected volumes: Adults: 16-20mLs (divided between two bottles); Children: 4-10mLs (single Aerobic bottle) (volume=age in years) Infants- 1-3mLs (PEDS+ bottle) Neonate 0.5-

1mL (PEDS+ bottle). *See also Document G_90_Info_3_A, Blood culture collection work instruction.*

5. Reagents, Materials and Equipment

4.1 Equipment:

- BD BACTEC™ instrument
- BD BACTEC Bottles, paediatric, aerobic and anaerobic
- Class II Biosafety Cabinet
- Bacticinerator
- Heating block
- CO_2 incubator (35ºC) or candle jar and candle
- Room air Incubator (35ºC)
- Microscope

4.2 Reagents:

- Optochin discs
- Gram stain reagents, crystal violet, iodine, decoloriser, safranin
- Culture plates (Blood agar, MacConkey and Chocolate agar)
- Trypticase Soy Broth (TSB) with 20% glycerol for isolate storage
- Rabbit plasma – coagulase test

4.3 Materials:

- Alcohol swabs
- Venting needles
- Glass slides
- Nichrome wire loops (10 ul)
- Tweezers
- Immersion Oil

6. Procedure

6.1 **Accessioning of received inoculated BACTEC bottles**

<u>Pathology Reception desk</u>

6.1.1 Check labels vs request form
6.1.2 Enter specimen and patient details onto the LIS
6.1.3 Print specimen labels and worksheet
6.1.4 Affixed worksheet to request form and place labels on request form and bottles
6.1.5 Place bottles, labels and worksheet onto tray for transport to microbiology lab

Micro laboratory

6.1.6 Weigh each bottle in grams and enter weight onto worksheet. Place worksheet in the "Active blood culture folder" next to BACTEC-FX.

6.1.7 Entering Blood Culture Vials into the Bactec-FX

1. Before inserting vials into the stations, visually inspect all vials for evidence of microbial growth such as dark or black blood in non-lytic (aerobic/Paeds) bottles, haemolysis, turbidity and excess gas pressure (causing the vial septum to bulge outward) – these vials should be treated as positive and gram stained/subcultured
2. Fully open the drawer of the cabinet by pulling the drawer directly forward
3. Scan the patient accession lab number and the bottle bar code by placing the appropriate bar code in front of the laser beam of that cabinet (a vial entry page will appear on the screen for that cabinet).
4. Insert the bottle into any available station (indicated by a green light) ensuring it reaches the back of the station
5. If you accidentally place a vial into a blocked station, the vial entry tone does not sound and the barcode scanner remains off. Remove the vial and repeat steps 1-4 (entering the bottle in a different station.
6. After all the bottles have been scanned and placed in the machine close the drawer of the cabinet; a click will sound to confirm that the doors have been shut properly. If the drawer is not properly closed a beeping alarm will sound.

Note: The sequence of scanning of the lab number and bottle numbers is not important.

6.1.8 Entering Blood Culture Vials with Damaged Barcodes

If the bottle barcode has been damaged and cannot be scanned, a new generic barcode must be adhered over it; these labels are found on the accession bench.

1. Scan the lab number using the laser beam at the open cabinet.
2. Scan the new bottle barcode, and then identify the bottle type (i.e. aerobic, anaerobic or paediatric) by selecting the appropriate type displayed on the screen of the cabinet.
3. Enter the bottle into the station and close the drawer.

6.1.9 Delayed Blood Culture Vial Entry

Delayed blood culture vials are defined as:

- Preincubated bottles (at 35±1°C) >20 hours post collection
- non-pre-incubated bottles (i.e. held at room temperature) >48 hours post collection
- For delayed blood culture vials, perform a gram stain on receipt:
  - If organisms seen in Gram, follow procedure for setup below
  - If no organisms seen, follow procedure below in table 6.3

6.1.10 Removal of Negative Bottles (after 4 days incubation)

1. Remove bottle out of the BACTEC FX station indicated by green light flashing above it.
2. Check name and accession number labelling when removing the bottles.
3. Record negative results on the specimen worksheet.

6.1.11 BACTEC Errors:

If the instrument encounters any errors it will display a yellow light on the front panel of affected drawer, indicating a 'system alert'.

The System Alert display shows a list of system alerts that have occurred. (Workflow messages are not displayed.) The last 100 alerts are shown in the display, from the most recent (top) to oldest (bottom). The list is updated dynamically. Any currently active alerts are indicated by an exclamation mark at the left side of the display. Active alerts cannot be deleted from the listing until the alert condition is cleared.

To select a message to view a detail window or delete the message, tap the message in the scrollable alert window.

To deal with any 'system alerts' consult page 5.3.5 of the BD BACTEC™ FX Instrument User's Manual

6.1.12 Anonymous Vials

If the error is identified as an anonymous bottle, then the affected station will have a yellow flashing light above it. If the anonymous vial has been identified as positive then the station will flash alternately yellow and red.

1) Remove the vial from the flashing station
2) The ID Anonymous display will appear and the barcode scanner will be activated
3) Scan the vial sequence number and accession numbers
4) Modify the protocol length at this point if required
5) Return the vial to the instrument, placing it in the flashing green station (same station from which it was pulled)
6) If the vial is not being returned to the instrument tap the 'save' button

6.2 Removal of Positive Bottles, initial Gram stain and notification

Positive Blood cultures are a critical sample and must be processed ASAP (within 30 mins).

6.2.1 Remove positive bottles from the drawer of the BACTEC machine displayed by a red light on the front panel of that drawer. A beeping sound will also signal a positive bottle.
6.2.2 Remove the Positive vial from the BACTEC machine and place in the Class II Biosafety Cabinet for processing.
6.2.3 Check name and laboratory number when removing the bottles.
6.2.4 Turn on Biosafety Cabinet and wait 5 minutes for cabinet to purge air. Put on gloves and protective eye wear.
6.2.5 Gently mix the bottle, disinfect the rubber seal with 70% alcohol.
6.2.6 Label a glass slide for gram stain.
6.2.7 After 30 seconds, place a venting unit into the rubber seal.
6.2.8 Place a drop of blood culture broth onto the smear.
6.2.9 Allow the smear to air dry and place the smear on heating block to fix.
6.2.10 Place a daily gram stain control slide (mixed *Staph. aureus* and *E. coli* on slide) on heating block at same time.
6.2.11 Place a drop of blood culture broth onto appropriately labelled (use barcoded lab number label) Blood Agar, Chocolate Agar and MacConkey agar and streak plates.
6.2.12 Remove the venting needle and dispose into a sharps container.
6.2.13 Incubate the Blood and Chocolate in CO2 and the MacConkey in O2 for up to 96 hrs (daily reading).
6.2.14 Once the smear is dry, heat fix and perform a Gram stain. Also stain the control slide.
6.2.15 Examine the smear under 100x oil objective.

Note: Report as Gram positive cocci (GPC) in clusters, GPC in chains or pairs, Gram negative rods (GNR), Gram negative cocco-bacilli, Gram negative cocci (GNC), Gram positive bacilli (GPB) or no organisms seen.

6.2.16 Record the Gram stain results onto the specimen worksheet.
6.2.17 Record the control Gram stain results into the daily QC workbook.

Gram stain notification

6.2.18 **Lab scientist (all hours)** notifies patient name, labnumber and Gram stain finding onto the WhatsApp lab group (includes assigned micro path registrar, micro HOD, other micro scientists, infection control nurse and medical microbiologist) for action. Record date, time, name of person notified on the worksheet.

6.2.19 **Registrar or microbiologist (in hours 0800hr to 1600hr**) calls the treating clinician by and acknowledges with details back to WHATSAPP as a **reply** to the original notification.

6.2.20 **Lab scientist (after hours),** ring the phone number on the request form to notify the result (record details back to WhatsApp as a **reply** to the original notification).

6.3 Notes for pathology registrars (PMGH):

- Working days, in addition to keeping an eye on WHATSAPP, the registrar on for microbiology (backup ClinChem registrar) should check for new results relevant to blood cultures (new positives and follow-up results) at 9 am and 3pm.
- New Gram stain results can be notified to clinician at those times if not already done so.
- Record clinical details, including current antibiotic treatment on a WHATSAPP reply.
- Followup ID result and basic AST results added as well and notified as appropriate to clinician – signify whether isolate is MSSA or MRSA (for *S.aureus*), ESBL, CRE (for GNR) or VRE (*enterococci*). This will then serve as notification to infection control as well.
- After hours and on weekends, responsibility for notification to WHATSAPP and clinician falls to the SO on call.

6.4 Specific setup required as indicated by Gram stain (in BSCII cabinet):

Gram stain result (predominant)	**Media**	**Notes**
Gram positive cocci in clusters (Staph)	HBA – $35^0C/CO_2$ MAC – $35^0C/O_2$	WHATSApp Direct coagulase test from broth ***Positive coagulase – WHATSApp result against the original posting.*** Document result as presumptive *S. aureus* on the specimen worksheet
Gram positive cocci in chains or pairs (i.e (*Strep.* or *Enterococcus*)	HBA – $35^0C/CO_2$ Choc – $35^0C/CO_2$	WHATSApp Place an optochin disc in the 2nd quadrant after streaking
GPB (GPR)	HBA – $35^0C/CO_2$	WHATSAPP

GNR	HBA – $35^0C/CO_2$ MAC – $35^0C/O_2$	WHATSApp
"tiny" GNR's & GNCB, gram variable bacilli	HBA – $35^0C/CO_2$ MAC – $35^0C/O_2$ CHOC – $35^0C/CO_2$	WHATSApp Seal plates with masking tape before incubation
Campylobacter or spiral shaped GNR	Nil possible	WHATSApp
Yeast	SAB+A – $35^0C/O_2$	WHATSApp
No organisms seen (false positive blood culture)	CHOC – $35^0C/CO_2$ HBA – $35^0C/CO_2$	WHATSApp Perform wet film for presence of motile organisms. If organisms are present, repeat the Gram stain on a fresh slide and subculture. If Nil organisms again, return bottle to Bactec-FX within 1hr (otherwise it will reset). See also below

6.5 Removal of Negative BACTEC bottles (at 4 days)

When negative bottles are ready for removal a drawer of the BACTEC FX a green light will be displayed on the front panel of that drawer.

6.5.1 Each negative vial ready for removal will have a green light flashing above it.
6.5.2 Bottles are removed simply by pulling the bottle out of the station (negative bottles do not need to be scanned out). See Hunter Bacteriology Accessions Manual (TM-28945) for disposal of bottles.
6.5.3 Be careful to remove all negatives and avoid removing bottles still in protocol
6.5.4 Once the last bottle has been removed wait for the machine to "beep" to confirm the update is complete.
6.5.5 The instrument will automatically make each negative slot available for new bottles.
6.5.6 Once instruments have been unloaded retain all removed bottles for 30 minutes in case error messages occur. E.g. - "Anonymous vial" - removal of vial missed or "Vial Missing" - vial accidently removed before completion of protocol.

6.6 Culture reading, identification and AST

6.6.1 Lab notifies the registrar of preliminary ID AND final ID and AST results via WhatsApp as replies to the original posting.

6.6.2 For significant culture results, registrar notifies the responsible clinician using an ISBAR approach – suggested- https://idmic.net/2015/11/11/isbar-an-essential-process-for-improving-clinical-handover-and-liaison-by-pathology-services/

6.6.3 Day 2 (18-24hrs)

- Remove plates from incubators
- Record colony morphology on worksheet
- Perform routine tests to identify organism as per SOP.
- Set up AST as indicated as per SOP.
- Repeat coagulase if indicated (Staph with initial negative result).
- Document further items on worksheet as indicated
- If required, re-incubate all initial subculture plates to allow for optimal growth of any slow growing organisms and to allow colony morphology to form.
- Finalise worksheet for LIMS entry if indicated

6.6.4 Day 3

- Remove plates from incubators
- Record identification and susceptibilities on worksheet.
- Finalise worksheet for LIMS entry

6.6.5 Organism notes

1) Gram negative cocci
    a. Immediately notify WHATSAPP for registrar attention
    b. Consider *N. meningitidis* – must perform culture manipulation in the safety cabinet
    c. Day 2 – In the safety cabinet - record colony morphology and perform routine tests to identify organism. Notify registrar of preliminary results.
    d. Do not set up susceptibilities if suspect N. meningitidis or N. gonorrhoeae. Other organisms to consider are *Moraxella* spp. and *Acinetobacter* spp.

2) No organisms seen (false positive blood culture)
    a. If bottles flag positive for a second time repeat microscopy procedures, culture and incubate the bottle in the blood culture O^2 incubator for a terminal subculture after 3 days.
    b. Both BA/ Choc subculture plates are checked for growth every day for 5 days.

3) GPR
    a. Organisms such as *Corynebacterium* spp. and *Bacillus* spp. are always almost contaminants.

b. Perform tests to confirm/rule out *Listeria* spp. and *B. anthracis*. Notify WHATSAPP immediately if these organisms are suspected.
c. Some *Clostridium* spp. will flag positive in the anaerobic BACTEC bottle but will not grow on culture (strict anaerobes). Others will grow on culture.

6.6.6 Storage of organisms

All non-contaminant isolates from blood and sterile fluids must be stored frozen. If unsure discuss with the medical microbiologist. Procedure:

1) Inoculate the storage broth (20% glycerol in nutrient broth) heavily with the organism to be frozen in a 2 ml cryo tube.
2) Place the printed barcode label and on the cryo-tube then place tube in the next spot in the current storage box.
3) Record the box and position number on the LIMS and also on the manual register form e.g. 2010-60/88. (YYYY-BOX/POSITION)
4) Return box to freezer (-20DegC)

6.7 Entry of results onto LIMS and issuing of reports

6.7.1 Data entry to LIMS- these elements entered:

- Weights of BACTEC bottles
- Gram stain results
- Notification details
- Culture results
- AST results
- Predefined comments as directed by the culture and AST result

6.7.2 Reporting

- Inadequate sample comment: auto added in according with calculated inoculation for adult bottles (<5mL) only for negative cultures in adults:
  "There was an inadequate volume of patient blood added to the blood culture bottle. This reduces the sensitivity of detection for bacteria. Adult BACTEC blood culture require 8-10mLs of patient blood sample; avoid overfilling."

- Negative report:
  - "No growth at 48 hours. Culture will continue for 2 further days. If growth is detected the requesting clinician will be notified."
  - Lab use blood culture result flag remains at default setting of "Negative" unless the bottle flagged as positive and had no organisms on Gram stain or cultures – in that case switch flag to "false positive"

- Negative culture report (positive Gram stain result)
    - "No growth at 48 hours. Culture will continue for 2 further days. If growth is detected the requesting clinician will be notified."
    - Lab use blood culture result flag remains at default setting of "Negative"
    - Add comment:

    "The appearance of organisms on Gram stain may implicate prior antibiotic treatment and/or presence of anaerobic organisms that cannot be currently cultured by the laboratory. Please discuss with the medical microbiologist if necessary"

- Blood culture showing significant growth:
    - Report the number of positive bottle(s), bottle(s) collected and the length of incubation. e.g. "Growth in 1 of 2 bottle collected after 2 days"
    - Gram stain: e.g. Gram-negative rods
    - Culture: species identification, e.g. Escherichia coli
    - AST results released as per SOP
    - Lab use blood culture result flag set to indicate significant bacterial, fungal growth

- Blood culture showing growth of a probable contaminant (Coagulase negative *staph*, *Corynebacterium*, *Bacillus* species etc) :
    - Report the number of positive bottle(s), bottle(s) collected and the length of incubation. e.g. "Growth in 1 of 2 bottle collected after 2 days"
    - Gram stain: Organism, e.g. Gram positive cocci (staph)
    - Culture: Identification, e.g. Coagulase negative staph
    - Lab use blood culture result flag set to indicate contamination for that bottle set
    - Contamination comment reported:

    "This result usually indicates contamination and treatment is not indicated. Contamination can be avoided through care with asepsis during collection and inoculation of the blood culture bottle. If significant infection is suspected, collect another blood culture set before starting antibiotics and/or contact the medical microbiologist to discuss."

7 Interpretation (recorded on worksheet and LIS)

7.1 Blood culture result flag categories are a) negative, b) significant bacterial pathogen, c) significant fungal pathogen (takes priority), d) potential contaminant isolate(s), e) false positive.

7.2 Infection control to record event metadata for each significant event –(e.g. whether healthcare associated, principle clinical manifestation, IV device association, clinical service/ICU association)

8 Safety

Any culture plates from patients with suspected class 3 pathogens (e.g. Brucella or Burkholderia pseudomallei) should be processed in the BSC-II, taped with masking tape and labelled "infection risk"

8.1 Wash hands before and after processing positive blood cultures
8.2 Gloves, safety glasses and gown to be worn for processing blood culture bottles. Closed toe shoes.
8.3 Trained staff member to process all positive bottles in Class II Biosafety Cabinet
8.4 Do not remove venting units with fingers- use forceps/tweezers.
8.5 Clean any spills from surfaces in the cabinet with 70% alcohol
8.6 Do not sniff plates when reading cultures.
8.7 Dispose of all positive and negative blood culture bottles, culture plates and contaminated materials into contaminated waste bins.

9 Quality Control

9.1 Reagent QC required at least weekly; AST and Media QC as per those SOPs
9.2 Daily BACTEC-FX checks (see Form):

- Digital readout of temperature for each drawer of each cabinet daily.
- Temperature of each probe within each drawer of each cabinet daily.
- Check red and green LED display for each drawer of each cabinet.
- For each cabinet check front panel lights and audible alarm.
- Temperature probe positions are changed regularly.
- Filters should be removed and washed once a week and recorded.

10 Related documents - refer to https://path-png.org/microbiology-sops-fleming-fund/ for access

Info	ACORN Specimen Collection Poster- adults	G_90_Info_1
Info	ACORN Specimen Collection Poster- paediatrics	G_90_Info_2
Info	BACTEC Blood Culture Collection Procedure	G_90_Info_3
Info	Common blood culture Gram stain appearances and clinical implications	G_90_Info_4
SOP	Antibiotic disc susceptibility testing	G_90_SOP_6
SOP	Use and Maintenance of Class I and Class II Biological Safety Cabinets	G_90_SOP_8
WS	Daily BACTEC checks	G_90_WS_5
WS	Bench Reagent QC Worksheet	G_90_WS_1
WS	Blood culture statistics	G_90_WS_6

11 References

- Pathology North, NSW Blood culture SOP
- PMGH 2018 Blood culture SOP
- Diagnostic Microbiology Development Program documents