



National Department of Health

Title: Bacterial Isolate Preparation for Storage and or Transport

ID: G_90_SOP_5

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Date	

NB. Printed copies of this document for local laboratory use require endorsement on the front page and recording on the Printed Controlled Document Log sheet, G_10_WS_5.

1. Purpose/Scope

This procedure outlines the process for the preparation, storage and maintenance of bacterial cultures in a way that maintains long-term viability, integrity and traceability in the clinical microbiology laboratory environment within and between CPHL, Human Health and Animal Health Fleming Fund AMR surveillance laboratories.

2. Principle/Clinical application

A biobank is an organized collection of biological material and associated information for one or more public health and/or research purposes. In the field of microbiology, biobanking refers to the process of storing biological cultures



Title: Bacterial Isolate Preparation for Storage and or Transport

ID: G_90_SOP_5_A

Revision: A

Issue date: 12/4/22

Page 2 of 8

and linked data (i.e., sample and patient information, laboratory results, etc.) in a manner that:

- Ensures long-term viability of the bacterial culture without changing key characteristics (e.g. antibiotic susceptibility) of the strain
- Traceable record to link cultures and data (i.e., to serve as a trusted database for antimicrobial resistance (AMR) reporting)

A microbial biobank can also serve as a place to master cultures of important organisms including:

- microorganisms that have defined characteristics and are required to verify and validate culture media and laboratory tests (generally ATCC strains)
- materials sourced from external quality assurance programs
- from routine testing that may also be utilized if they are well characterized and where no other reference material is available with the required characteristics.

3. Responsibilities

3.1. FF AMR Surveillance HH Labs in PNG (PMGH, Goroka, Mt Hagen, Angau and Nonga)

Role	Person Responsible
Culture, identification and performance of AST on isolates for key surveillance pathogens	Microbiology trained and competent scientist
Notify possible MDROs isolated each day to the Microbiology Quality Officer and Sectional Head daily	Microbiology trained and competent scientist on each bench
Prepare isolates for storage and or referral	Microbiology Quality officer and Sectional Head daily
Record and store at - 20°C (short term) and at -80°C (long term)	Microbiology Quality officer and Sectional Head daily

4. Specimen

Bacterial isolates (fresh culture 18-24 hours)



Title: Bacterial Isolate Preparation for Storage and or Transport

ID: G_90_SOP_5_A

Revision: A

Issue date: 12/4/22

Page 3 of 8

5. Equipment/Materials

Equipment	Materials	Reagents/media
- 20 °C freezer - 80 °C freezer Biosafety cabinet (BSCII)	Cryovial (tube) storage box Isolated colonies from 18–24-hour cultures from non-selective media; Blood agar plate (BAP) or Chocolate agar plate (CAP) Sterile loop Inventory system (paper-based log – G_90_WS_2A for locally stored isolates, G_90_9_A for referred isolates)	Trypticase Soy broth plus 20% glycerol broth (Freeze) NA slopes/Broth (Room Temp)



Title: Bacterial Isolate Preparation for Storage and or Transport

ID: G_90_SOP_5_A

Revision: A

Issue date: 12/4/22

Page 4 of 8

6. Section criteria for bacterial isolates for storage and referral

Fresh subcultures of isolates specified below are to be stored in glycerol broth at -20 deg C pending transport in the monthly shipment to the NRL at CPHL/PMGH. For each referred isolate, complete the Inventory of stored bacterial and fungal isolates form (G_90_WS_9) and record on LIMS.

1. Any species of the Enterobacterales family (*E. coli*, *Klebsiella*, *Enterobacter* etc) isolated from any specimen site that tests as resistant to meropenem. **Biobank: all presumptive Carbapenemase producing Enterobacterales (CPE) – flag is meropenem resistance**

2. Bloodstream isolates as specified:

Use 14 day cut-off – i.e. repeat isolates of same species from the same patient not sent if < 14 days.

- *Staphylococcus aureus* (VRSA, MRSA or MSSA)
- Other *Staphylococcus* species (coagulase negative) – maximum 5 isolates per month
- Enterococcal species (*E. faecium* or *E. faecalis*)
- *Streptococcus pneumoniae*
- *Streptococcus Viridans* (other alpha-haemolytic strep. species) isolated from 2 or more different blood culture collections from same patient within 2 days
- Penicillin resistant beta-haemolytic *Streptococci* groups A, B, C or G (PEN1 disc zone < 18mm)
- *E. coli*, *Klebsiella*, other Enterobacterales species, *Salmonella* (Biobank: ESBL isolates)
- *Acinetobacter* or *Pseudomonas* species (see below) **Biobank: *Acinetobacter baumannii* complex**
- *Neisseria meningitidis*
- Other Gram negative diplococci/coccobacilli (unidentified)

3. CSF isolates:

- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- Any other isolate obtained from a turbid CSF sample

4. Faeces

- *Vibrio cholerae*
- *Salmonella* or *Shigella* species

5. Isolates from other specimens

- ESBL *E. coli*, *Klebsiella* species from urine if isolate is resistant to either ceftriaxone OR ceftazidime on disc testing
- Up to 10 other Gram negative isolates of any species type from urine per month
- *Acinetobacter* species (presumptive) isolates from blood, sterile site (i.e. pleural, peritoneal, joint fluid etc) or ET aspirate (ventilated patient – PMGH only) **Biobank: *Acinetobacter baumannii* complex only**
- *Pseudomonas aeruginosa* - isolates from blood, sterile site or ET aspirate (ICU) **Biobank: meropenem-resistant isolates**
- *Neisseria gonorrhoeae*
- All suspected *Burkholderia pseudomallei* or *Bacillus anthracis*



6.3. GLASS Pathogens

GLASS priority specimens and selected pathogens (WHO, 2016 – 2017)

Specimen	Laboratory case definition	Surveillance type and sampling setting	Selected pathogens for surveillance
Blood	Isolation of pathogen from blood ^a	Selected sites or national coverage Continuous Patients in hospitals and the community	Acinetobacter spp. E. coli K. pneumoniae Salmonella spp. S. aureus S. pneumoniae
Urine	Significant growth in urine specimen ^b	Selected sites or national coverage Continuous Patients in hospitals and the community	E. coli K. pneumoniae
Stool	Isolation of Salmonella spp. ^c or Shigella spp. from stool	Selected sites or national coverage Continuous Patients in hospitals and the community	Salmonella spp. Shigella spp.
Urethral and cervical swabs	Isolation of <i>N. gonorrhoeae</i> from urethral and cervical swabs	Selected sites or national coverage Continuous Patients in hospitals and the community	<i>N. gonorrhoeae</i>

a. Any pathogen isolated from a blood culture may be significant for surveillance locally and nationally; only the prioritised pathogens for global surveillance are listed here.

b. Culture according to local laboratory practice. Catheter samples should be excluded if possible.

c. Diarrhoeal surveillance is for non-typhoid salmonella species; for local clinical purposes, typhoid and paratyphoid should be included.



Title: Bacterial Isolate Preparation for Storage and or Transport

ID: G_90_SOP_5_A

Revision: A

Issue date: 12/4/22

Page 6 of 8

7. Procedure

7.1. Isolate storage requirements for peripheral FF labs

7.1.1. Use fresh colonies from purity agar subculture

7.1.2. Inoculate the organism into the storage tube with a loop, with care to avoid contamination

7.1.3. Store a sweep of the isolate into glycerol broth at -20°C until referred to CPHL

7.1.4. Label the storage tube with the patient's name, date of specimen, lab number and the isolate species; it is important not to miss out any of these details!

7.1.5. Document in the isolate inventory worksheet (form). Refer to Document G_90_WS_2 below.

7. Safety

For safety instructions, please review this document G_10_Info_3 Laboratory Biosafety.

8. Quality Control

Not applicable

9. Related documents

For access, refer to <https://path-png.org/microbiology-sops-fleming-fund/>

Inventory of stored bacterial and fungal isolates	G_90_WS_2
Bacterial Isolates for referral (jobaid)	G_90_J_10

10. References

[https://www.who.int/docs/default-source/searo/amr/global-antimicrobial-resistance-surveillance-system-\(glass\)-report-early-implementation-2016-2017.pdf?sfvrsn=ea19cc4a_2](https://www.who.int/docs/default-source/searo/amr/global-antimicrobial-resistance-surveillance-system-(glass)-report-early-implementation-2016-2017.pdf?sfvrsn=ea19cc4a_2)



Title: Bacterial Isolate Preparation for Storage and or Transport

ID: G_90_SOP_5_A

Revision: A

Issue date: 12/4/22

Page 7 of 8

Acronyms

AH – Animal Health

AMR – Antimicrobial Resistant

AST – Antimicrobial Susceptibility Testing

ATCC – American Type Culture Collection

BA – Blood Agar

CA – Chocolate Agar

ET – Endotracheal aspirate

CPE- carbapenemase-producing Enterobacterales

CRE- carbapenem-resistant Enterobacterales

CPHL – Central Public Health Laboratory

CSF – Cerebral Spinal Fluid

FF – Fleming Fund

HH – Human Health

ICU – Intensive Care Unit

LIS – Laboratory Information System

MALDI-TOF- Matrix-Assisted Laser Desorption/Ionization-Time Of Flight

MRDOs – Multi Drug Resistant Organisms

MRSA – Methicillin Resistant *Staphylococcus aureus*

MSSA – Methicillin Sensitive *Staphylococcus aureus*

PMGH- Port Moresby General Hospital

PPE – Personal Protective Equipment

TSB – Tryptone Soy Broth

WGS – Whole Genome Sequencing

WHO – World Health Organization



Annex A.

Bacterial Isolates Referral Flowchart

Isolate storage requirements for peripheral FF HH labs

