National Department of Health

Title: Maintenance of Cultures Used for Quality Control Testing

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1. Purpose

To provide a stock culture maintenance protocol and relevant regulatory requirements for quality control organisms.

2. Scope

This document lays out the processes for manipulating and storing QC control organisms across laboratories that require these strains.

3. Principle/Clinical application

Quality control organisms are a fundamental part of any quality assessment program for microbiology laboratories. Predominantly they are used for verifying performance of culture media, reagents, kits and systems to identify specified microorganisms, including a key role ensuring acceptable performance of susceptibility testing.

Proper maintenance of these organisms is critical to achieving accurate control results for culture media and reagents. This document highlights industry guidelines and best practices to ensure compliance to various standards, at the same time as making optimum use of materials.

4. Responsibilities

a. Master culture management and preparation of monthly stock cultures for peripheral labs is performed by the reference lab (CPHL/PMGH).

b. The laboratory quality officer at each lab site is responsible for implementation of this SOP and management of stock cultures, replacing them on a monthly basis.

5. Equipment & Materials

- ATCC strain master cultures as per Appendix
- glycerol broth vials
- agar media as appropriate for subculture
- Freezer -20degC for working stock culture vials (peripheral sites)
- Freezer -80degC for master strain vials (reference lab site)
- IATA compliant packaging for stock culture vials sent annually to peripheral sites (on ice)

6. Definitions

Reference Culture: This is a freeze-dried isolate from a recognised culture collection or a supplier that can demonstrate traceability to a recognised culture collection (e.g., ATCC: American Type Culture Collection).

Master Culture: This is prepared from the freeze-dried isolate. The Master Culture is the primary source of the organism in the culture collection. Frozen in glycerol broths at -80^{0}C for up to 5 years.

Stock Culture: This is prepared from the Master Culture on an annual basis or as required. Frozen in glycerol broths in laboratory freezer for up to 1 year.

Working Culture: This is prepared from the Stock Culture for routine use, or as required, in test and media quality control. This working culture can be sub cultured from plate to plate for one month. At the end of the month, discard plate and remove a stock culture vial from the freezer to prepare a new working culture.

Wild strain: This is a culture from a clinical or other source. It may be recovered in one's own lab or referred by another lab.

Control Culture Data sheet: This is the record of the culture identity, its optimal storage and growth conditions, source, date of acquisition and expected results. It lists all worksheets used to record sub-culture details.

Overview of culture management

MASTER CULTURE
Stored in glycerol broths at -80^{0}C for up to 5 years

↓ Once per year

Subculture to agar plate and incubate for 24-48 hours
STOCK CULTURE
Glycerol broth aliquots x 12 prepared for each lab
Store in -20^{0}C laboratory freezer for use over the next year

↓ Once per month

Remove 1 vial of stock culture from freezer and subculture to agar plate
WORKING CULTURE
Agar plate used for QC. Store in fridge for 1 week. This working culture can be subcultured from plate to plate for one month. At the end of the month, discard plate and remove a stock culture vial from the freezer to prepare a new working culture.

7. Procedure

7.1. Preparedness prior to receiving reference cultures

7.1.1. The Laboratory Manager/Microbiology Sectional Head/Microbiology Quality Officer should notify the staff the expected date of arrival of control strains from the shipper.

7.1.2. The staff must be made aware of processes involved in receiving and storing of the ATCC strains in the absence of the Microbiology Sectional Head and Microbiology Quality Officer

7.1.3. The Microbiology Sectional Head and Microbiology Quality Officer must ensure that all necessary requirements for the maintenance of the ATCC strains are readily available

7.2. Reception of reference cultures (master and stock cultures)

7.2.1. Documentation – in QC strain log book for each lab – use this format:

Date received	Delivered by (Print name & sign)	Received by (Print name & sign)	Physical description and number of item(s) received	Condition of the item (Good or Bad)

7.2.2. Open the package and ensure that the information and reference culture organisms correspond.

7.2.3. Notify shipper that the item has been received (if necessary)

7.3. Preparation, Confirmation and Maintenance of Master strain isolate.

Isolates may be supplied in freeze-dried form or on an agar slope. The type of preparation for the culture strain ordered relates to the number of passages from the original source.

7.4. Rehydration of Freeze-Dried Cultures in Rubber Stopper Vials

Safety alert:

Aerosol risk: Work within a BSC

Sharps risk: Care must be taken when using a syringe. Never recap a syringe. Dispose of syringes directly in a sharps container.

7.4.1. Use forceps to remove metal or plastic wrapping around top of vial

7.4.2. Sterilize rubber stopper with alcohol

7.4.3. Rehydrate as per manufacturer's instructions

Note: If no specific instructions have been provided, proceed as follows:

7.4.4. Use a sterile syringe to collect 1ml of an appropriate broth (15% glycerol) broth is used as a default

7.4.5. Secure the vial within a rack

7.4.6. Puncture the rubber stopper with the needle and allow the broth medium to be sucked into the vial (the vial; is under vacuum this should occur without the need to use the plunger)

Note: If the broth is not sucked into the vial, this may indicate an ineffective seal and possible contamination.

7.4.7. Leaving the needle in the vial, gently swirl the contents and then allow 5-10 minutes for the material to rehydrate

7.4.8. Withdraw enough inoculum from the vial to inoculate the number of plates required

7.4.9. Inoculate the suspension, using the syringe, onto the surface of an appropriate non-selective medium such as blood or chocolate agar

7.4.10. Streak the plate

7.4.11. Incubate at 35^{o}C as per Appendix A

Note:

- Some cultures may require special growth media- refer to Appendix A.
- Ideally, a minimum of one whole plate per vial is prepared. (one plate per site plus one extra for master stock)
- **SAFETY: Dispose the needle and syringe with the into the sharps container with the needle attached**

8. References

Nil

9. **Related documents –** to access , refer to https://path-png.org/microbiology-sops-fleming-fund/

Inventory of stored bacterial and fungal isolates	G_90_WS_2

Appendix A: ATCC master strains

ATCC Master Strain	Growth Media	Incubation Condition	Character
Pseudomonas aeruginosa ATCC® 27853	Maconkey (MA)	35^{0}C (O_2)	Susceptible wild type
Salmonella sp ATCC® 29890	XLD/DCA	35^{0}C (O_2)	
Streptococcus epidermidis ATCC 12228	Blood agar (BA)	35^{0}C (O_2)	Negative control for coagulase
Enterococcus faecalis ATCC® 51299	BA	35^{0}C (O_2)	Vanc resistant *vanB* high level aminoglycoside resistant
Escherichia coli ATCC® 25922	MA	35^{0}C (O_2)	Susceptible wild type
Escherichia coli ATCC® 35218	MA	35^{0}C (O_2)	TEM-1 betalactamase (non-ESBL)
Klebsiella pneumoniae ATCC® 700603	MA	35^{0}C (O_2)	ESBL strain (SHV-18)
Staphylococcus aureus ATCC® 29213	BA	35^{0}C (O_2)	mecA negative, weak betalactamase positive
Enterococcus faecalis ATCC® 29212	BA	35^{0}C (O_2)	Susceptible wild type
Streptococcus pneumoniae ATCC® 49619	Chocolate agar (CA)	35^{0}C with 5% (CO_2)/ candle jar	Reduced susceptibility to penicillin
Staphylococcus aureus NTCC 12493	BA	35^{0}C (O_2)	MRSA mec A positive- from PPTC
Haemophilus influenzae ATCC 49766	CA	35^{0}C with 5% (CO_2)/ candle jar	Susceptible wild type