



Preservation of Bacterial Isolates/ Control Strains

Standard Operating Procedure

**National Programme on Antimicrobial Resistance Containment
National Centre for Disease Control, India
September 2020**

National Centre for Disease Control (NCDC) - National Programme on Antimicrobial Resistance (AMR) containment		
Document Type : SOP- Bacteriology- Preservation of bacterial Isolates/Control Strains	Version No.: 1	
Approved date: 31 August 2020	Effective Date: 4 September August 2020	Page: 2 of 6
Prepared by: Programme Officer	Approved by: Incharge, National Programme on AMR Containment	

National Centre for Disease Control (NCDC) - National Programme on Antimicrobial Resistance (AMR) containment		
Document Type : SOP- Bacteriology- Preservation of bacterial Isolates/Control Strains	Version No.: 1	
Approved date: 31 August 2020	Effective Date: 4 September August 2020	Page: 3 of 6
Prepared by: Programme Officer	Approved by: Incharge, National Programme on AMR Containment	

Contents

I.	Abbreviation	Error! Bookmark not defined.
II.	Objectives and Scope.....	4
III.	Preparation of Glycerol Stocks (long term storage)	4
A.	Preparation of 30 % Glycerol (100ml)	4
B.	Preparation of glycerol stocks of control strains/bacterial isolates for EQAS and AMR alert strains.....	4
C.	Retrieval of Stocked strains	4
IV.	Preparation of Agar Media Stabs (short term storage (< 1 year).....	5
A.	Media Preparation.....	5
B.	Preparation of Agar Media Stabs.....	5
C.	Retrieval of Stock Strains	5

National Centre for Disease Control (NCDC) - National Programme on Antimicrobial Resistance (AMR) containment		
Document Type : SOP- Bacteriology- Preservation of bacterial Isolates/Control Strains		Version No.: 1
Approved date: 31 August 2020	Effective Date: 4 September August 2020	Page: 4 of 6
Prepared by: Programme Officer	Approved by: Incharge, National Programme on AMR Containment	

I. Objectives and Scope

The aim of this SOP is to preserve and maintain the bacterial pathogens of significance for public health. In addition, the emerging resistant strains and Alert strains must be preserved for further gene characterization.

II. Preparation of Glycerol Stocks (long term storage)

For preparation of 15% glycerol stocks of isolates to be preserved, the following procedure may be used:

A. Preparation of 30 % Glycerol (100ml)

1. Mix 30 ml of sterilized glycerol and 70 ml of sterilized saline to make 30% glycerol
2. Aliquot 0.5ml of 30% glycerol in autoclavable 2 ml cryotubes and slightly loosened the screw caps.
3. Sterilize the aliquoted cryotubes by autoclaving.
4. Once cool, tighten the screw caps and store the cryotubes in refrigerator.

B. Preparation of glycerol stocks of control strains/bacterial isolates for EQAS and AMR alert strains

1. Ideally for each isolate prepare minimum 2 stocks, one to be stored at -20°C and other at -80°C (if available).
2. From an overnight pure growth of the isolate on an appropriate non-selective medium, inoculate 4-5 colonies in 5ml of suitable broth medium like Tryptic Soya Broth (TSB) and incubate at 37°C . (Fig. 1)
3. After 4-5hrs of incubation when the growth is in mid-log phase, take 0.5ml of broth and inoculate cryotubes containing 0.5ml of 30% glycerol (prepared at A above)
4. Vortex the tubes to make the suspension homogenous
5. Label properly with isolate inventory Id, name of pathogen, date of stock preparation etc.
6. Store the cryotubes in deep freezer as stated at B.1.

C. Retrieval of Stocked strains

1. Remove a portion of the frozen bacterial suspension with a loop and inoculate appropriate liquid broth or solid media. Return the cryotube immediately to the freezer.
2. Alternatively, remove cryotubes and thaw contents rapidly in warm water. Once thawed do not refreeze the tube. Use contents to subculture to solid medium for isolation of pure bacterial colonies.

National Centre for Disease Control (NCDC) - National Programme on Antimicrobial Resistance (AMR) containment		
Document Type : SOP- Bacteriology- Preservation of bacterial Isolates/Control Strains		Version No.: 1
Approved date: 31 August 2020	Effective Date: 4 September August 2020	Page: 5 of 6
Prepared by: Programme Officer	Approved by: Incharge, National Programme on AMR Containment	

III. Preparation of Agar Media Stabs (short term storage (< 1 year))

Stab cultures at room temperature (use for non-fastidious organisms like *Staphylococcus* and Enterobacteriaceae)

A. Media Preparation

1. Prepare carbohydrate free agar media like nutrient agar or tryptic soy agar as per manufacturer's instructions and autoclave.
2. After autoclaving, pour 1ml of media in autoclaved/sterile screw capped 2 ml tubes and let them set as agar deeps (higher volumes may be poured depending on capacity of storage tubes, tubes may be filled till 2/3rd of capacity).
3. Date the batch of tubes and store in cool place

B. Preparation of Agar Media Stabs

1. Label each tube with isolate inventory ID, lab or Sample ID/Lab code/month/Year and date of inoculation. For e.g. sample ID is 18004325; Lab code is GMC; the organism was submitted in the month of October 2019 (October/2019); then the label on the strain should be 18004325/GMC/OCT/2019.
2. Stab inoculate the media at several places with overnight growth of the organism on plate cultures using a sterile straight wire. (Fig. 1)
3. Incubate overnight at 37°C with slightly loose screw caps.
4. After incubation, check the surface of the agar stab for growth, tighten screw caps and seal with Para film or molten paraffin wax.
5. Store at room temperature or 4°C for up to 1-2 years.

C. Retrieval of Stock Strains

1. Remove a portion of the visible growth using a sterile inoculating loop or straight wire and inoculate into liquid culture medium. Incubate at the optimum growth temperature and streak onto fresh culture plates.

For transport of bacterial isolates for EQAS or AMR alerts to National Reference Laboratory at NCDC, the agar stabs must be prepared as above and for transport refer to the document on Guidance for submission of EQAS and Alert Strains¹ which is available on NCDC website.

An inventory register may be maintained with details of isolates stored, site where stored etc. Format for inventory register may be seen at Annexure 1.

¹ Guidance for Submission of AMR Surveillance Isolate for External Quality Assessment and Reporting Emerging AMR Alerts

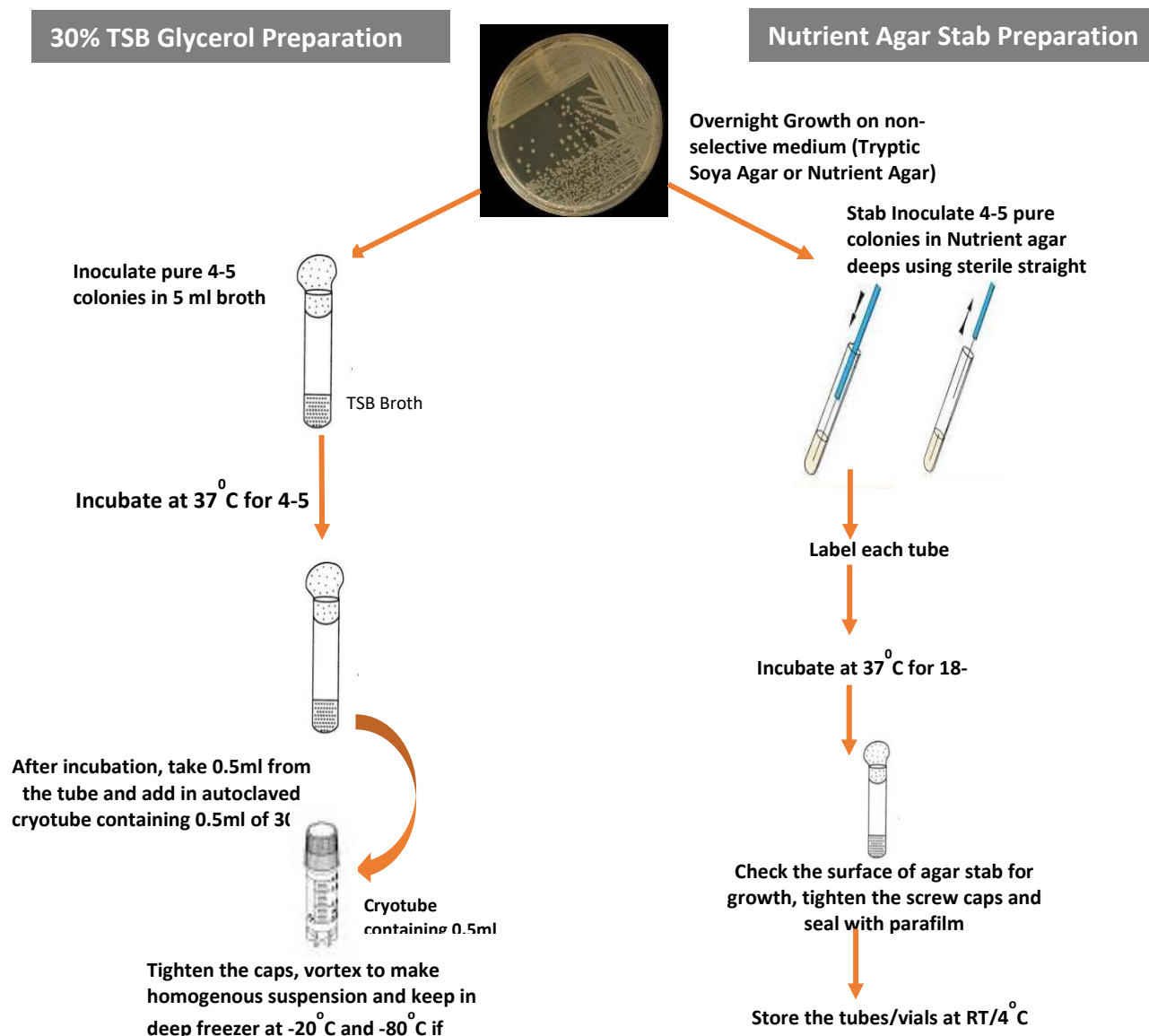


Fig 1. Diagrammatic representation of preparation of 30% TSB Glycerol and Nutrient Agar Stabs for preservation of isolates.

National Centre for Disease Control (NCDC) - National Programme on Antimicrobial Resistance (AMR) containment		
Document Type : SOP- Bacteriology- Preservation of bacterial Isolates/Control Strains		Version No.: 1
Approved date: 31 August 2020	Effective Date: 4 September August 2020	Page: 7 of 6
Prepared by: Programme Officer	Approved by: Incharge, National Programme on AMR Containment	

Annexure 1

Format for inventory of preserved isolates

Strain inventory Id	Date Stock Created	Name of the strain	Isolate Details (like serotype etc)	Specimen Type	Location			Date Discarded
					Facility (Room and/or Building)	Storage Device (Freezer etc)	Storage Details (box, shelf)	