## CHN PB-SAM 001: CHAIN PB-SAM preparation of freezing mixture SOP

**Purpose**

The purpose of this SOP is to describe the standard procedures involved in preparation and QC of freezing mixture used to store wet rectal swab (R2) samples

**Responsibility**

This SOP applies to any study laboratory staff. It is the responsibility of those users to follow the guidelines stipulated herein.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The study laboratory coordinator is responsible for answering questions you may have about the content of this SOP and any other relevant study documentation. Please contact the study laboratory coordinator through your site lab-coordinator.

Main CHAIN PB-SAM laboratory coordinator: Caroline Tigoi (email: ctigoi@kemri-wellcome.org or rmusyimi@kemri-wellcome.org).

**Abbreviations/Definitions**

**SOP** Standard Operating Procedure

**PI** Principal Investigator

**FM** Freezing mixture

**QC** Quality control

**R2** Rectal swab (wet)

**Required material**

1. -80oC freezer
2. Nunc vial
3. Tryptone Soy Broth, Oxoid CM0129
4. Glycerol, Sigma G5516

**Methods**

1. **General considerations**
	1. FM prepared using tryptone soya broth and 15% is a standard media for preservation of micro-organisms in rectal swabs and culture.
	2. This is a general purpose medium, containing two peptones, is suitable for the cultivation of both aerobes and anaerobes.
	3. The media must be sterilized at 1210 C for 15 minutes and stored at 2 – 80 C to maintain sterility.
2. **Methodology**
	1. In a conical flask, dissolve 3.0g of TSB powder in 85mls of distilled water.
	2. Add 15mls of glycerol and distribute 1ml volumes of the mixture in Nunc vials arranged in a rack.
	3. Recap the vials loosely and sterilize by autoclaving at 121oC for 15 minutes. Incorporate autoclave indictors to monitor sterility.
	4. Tighten the caps after sterilization and place the vials to cool on the bench in the media preparation room.
	5. Label the rack (freezing mixture), date of preparation and expiry and then place it in a fridge at 2 – 8o C.
	6. Shelf life of vials is 3 months from date of preparation.

**References**

N/A

**3.0 Document history**

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| **Version** | **Author** | **Approved by** | **Signature** | **Dated** |
| 1.01 CHAIN PB-SAM preparation of freezing mixture SOP | Robert Musyimi | Caroline Tigoi |  | June, 3rd 2021 |
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**6.0 Site training record**

All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

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| **Document History** |
| **Version No.** | **Trained staff initials** | **Signature of trained staff** | **Date** | **Trainer’s Initials** |
| **1.01** | **KDT** | **Example row** | **1st Jan 2016** | **DM** |
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