

Standard Operating Procedure

Title: Maintenance of Culture Collection



5.2.3. Break the ampoule, applying gentle pressure away from the body and keeping fingers away from file marks.

5.2.4. Using a sterile 1 ml pipette remove a few drops of TSB from a MacCartney bottle/test tube and add to the ampoule. Use SDA for bacterial and fungal cultures.

5.2.5. Dissolve the pellet by gently washing with TSB/SDA.

5.2.6. Aseptically transfer all the TSB/SDA from the ampoule into the MacCartney bottle/test tube from step

5.2.4. Label the bottle with culture name, number and date.

5.2.7. Incubate broths for 24-48 hours at a temperature specified for each organism.

5.3. Subculturing

Culture collection is subcultured on a bi-monthly basis onto a fresh set of agar slopes. Subcultures can be streaked from master cultures up to five times after which a new freeze-dried ampoule must be opened.

5.3.1. Establish viability and purity of all master cultures by streaking onto Tryptone Soya Agar (TSA) plates for bacteria and Sabouraud Dextrose Agar (SDA) plates for fungi. Incubate bacterial plates for 24-48 hours and fungal plates for 5-7 days at a temperature specified for each organism.

5.3.2. If culture is impure proceed as in 5.4.

5.3.3. If culture is pure, streak onto 2 slopes of TSA or SDA (as applicable) and inoculate 1 MacCartney bottle of Tryptone Soya Broth (TSB) if required. Ensure the new white Master slope is inoculated first, followed by the new routine slope and broth.

5.3.4. Label all slopes and broths as in section 5.5.

5.3.5. Incubate slopes and broth as in 5.3.1.

5.3.6. When cultures have grown, check purity of white Master slope by streaking onto TSA or SDA plates. Incubate plates at a temperature recommended for each organism. Check culture performance by streaking (inoculating) diagnostic media.

5.3.7. If culture is impure, proceed as in 5.4.

5.3.8. If culture is pure, document and store as described in the standard operating procedure.

5.3.9. For the first and fifth subculture obtain a biochemical profile for bacterial cultures via an API identification system (if possible).

5.4. Isolation of Pure Culture

5.4.1. Pick a single isolated colony and streak onto TSA/SDA plate.

5.4.2. Incubate for 24-48 hours at a required temperature.