

Suggested procedure for HANDLING OF REFERENCE STRAINS

Upon reception of a certified culture, record all relevant information (e.g. date of reception, strain number, strain ID (type), supplier and batch number).

Always handle reference strains in aseptic conditions (e.g. LAF-bench).

As indicated in the flow sheet (Figure 1), open lyophilised cultures from Czech Collection of Microorganisms (CCM) by following guidelines from CCM available at <http://www.eurl-ar.eu/208-eqas.htm>, add 1 mL broth (e.g. nutrient broth) to the lyophilised material, incubate 1 hour at relevant temperature, transfer 1-3 drops to four agar plates (e.g. blood agar) and streak out using a loop. Or, if handling a culture from an agar stick, streak the culture on four agar plates.

After overnight incubation at relevant temperature, add 4 mL LB+15% glycerol to each of the four agar plates (=> in all, 16 mL) and transfer 1 mL to 11 individual cryo tubes. Perform a purity control from each agar plate by collecting some remaining broth with a loop and streaking a new agar plate (e.g. blood agar). After overnight incubation at relevant temperature, check for purity. If the culture is not pure, discard all the cryo tubes made from that plate. Record the results for QA purposes.

Mark two of the tubes "REF". These are passage (subculture) number 1 and will be used for production of a new set of 'green' cryo tubes (reference strains for working cultures). Store cryo tubes marked "REF" at -80°C in red (or otherwise easily distinguishable) boxes labelled "Reference strains".

Store the remaining cryo tubes at -80°C in green (or otherwise easily distinguishable) boxes labelled "Reference strains for working cultures".

Working cultures are produced from tubes contained in the green box (specifics regarding handling of working cultures is described elsewhere, e.g. CLSI M07-09 Appendix E):

- Scrape material from the still-frozen culture with a loop, streak an agar plate (e.g. blood agar), put the still-frozen culture back into the freezer
- Incubate overnight at relevant temperature
- Label the plate with strain identity, subculture date and name of the persons performing the procedure
- Register that a working culture is produced (for the QA-system)

When starting to use the last cryo tube of a reference strain from the green box, subculture a 'REF' cryo tube for that strain to four agar plates (e.g. blood agar). Produce nine new cryo tubes by following the procedure described above (including purity control). Place the original "REF" tube in the red box again after use.

A frozen reference strain may be subcultured maximum four times (five passages from the ATCC reference culture) to keep the ATCC-number, therefore, keep track of the number of times the reference strain has been subcultured by keeping relevant records.

Figure 1: Flowsheet

