# Aim

To describe how to store bacterial / fungal isolates at -80°C for archiving purposes.

# Principle

Isolates are stored in cryovials containing a croypreservative (STGG; skim milk-tryptone-glucose-glycerol broth). A suspension of organism is made in the STGG, which is subsequently frozen at -80°C. If sub-culture is required, a small amount of the frozen bacterial suspension may be scraped from the surface of the STGG using a plastic loop and inoculated on to an agar plate. Using STGG, fastidious organisms such as *Streptococcus pneumoniae* have been maintained for more than ten years without interim subculture and refreezing.

# Method

## Method for the long term storage of isolates

Culture the organism on non-selective agar (e.g. Blood, Chocolate, or Columbia agar).

Ensure that the culture is pure: if mixed, pick off a well isolated colony and re-culture.

Perform the following steps in the class II biosafety cabinet:

* Using a sterile cotton swab, harvest the entire growth from the young culture plate and dispense into a labeled cryotube containing 1ml STGG medium.

Store in the -80°C freezer.

Record the details in the isolate logbook and laboratory database.

## Method for the recovery of frozen isolates

Remove the appropriate cryotube from the freezer and place on wet ice.

In the class II biosafety cabinet, remove the screw cap and, using a sterile plastic loop, inoculate a small amount of frozen material into broth or onto an appropriate solid medium.

Return the tube to the -80°C freezer immediately.

# Quality assurance

Only use STGG that has passed QC following production: the shelf-life of STGG is 6 months.

Do not use STGG tubes that are macroscopically turbid at time of intended use.

# Limitations

STGG is well validated for storage of bacterial and yeast isolates. Data on storage of other fungal isolates is not available, although there is published evidence that storage of dermatophytes and moulds at -70°C in commercially-produced cryovials is acceptable.

# References

Gibson LF, Khoury JT. Storage and survival of bacteria by ultra-freeze. Lett Appl Microbiol. 1986;3:127-9.

O'Brien KL, Nohynek H. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. Pediatr Infect Dis J. 2003;22(2):e1-11.

# Risk assessment

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| **COSHH risk assessment - University of Oxford COSHH Assessment Form** | |
| **Description of procedure**  Storage of bacteria and fungi at -80°C | **Substances used**  1. Bacterial / fungal cultures  2. STGG transport medium  3. Ultra-low temperature freezer |
| **Quantities of chemicals used**  Small | **Frequency of SOP use**  Daily |
| **Hazards identified**  1. Potentially pathogenic bacteria  2. Ultra-low temperatures (-80°C) | **Could a less hazardous substance be used instead?**  No |
| **What measures have you taken to control risk?**  1. Training in good laboratory practices (GLP)  2. Appropriate PPE (lab coat, gloves (incl. padded / thermal protection gloves), eye protection)  3. Use of biosafety cabinet for reading of plates / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) | |
| **Checks on control measures**  Observation and supervision by senior staff | |
| **Is health surveillance required?**  No | **Training requirements:**  GLP |
| **Emergency procedures**:  1. Report all incidents to Safety Adviser  2. Use eyewash for splashes  3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:  1. Sharps discarded into appropriate rigid containers for incineration  2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration |