SOP-M023

<u>Glycerol Stocks (Bacteria) for -80° Freezer (Amended)</u>

Objective: To store bacteria using 15% glycerol + Broth in the -80° freezer

Products:

Cryule Vial Polypropylene Sterile: Wheaton Cat#982745 (50pcs)
Disposable inoculating Loop. (VWR cat#90001-100 BD Difco Blue 10ul)
Sterile cotton wool swab
Glycerol broth: 15% Solution w/v in TSA broth (autoclaved)

Procedure:

1. Make a new streak plate of the bacteria you want to check for viability from recent or older plates or slants. If you get growth from the streak, take a few colonies and do a serial dilution to grow single colonies. Subculture as required to obtain sufficient growth for -80ºC storage. This will vary depending on the bacterium you wish to store. Prolific growers (eg. *E. coli*) can be stored using a few colonies; fastidious organisms (eg. *Campylobacter, Arcobacter)* may require from one or even several whole plates!

2. Spray a tray with 70% ethanol and place in the sterile hood. In that tray, put as many Cryule vials as you need for each bacteria. Label each vial with the bacteria PU number, type and date.

3. In each Cryule vial, put 1.5mls of the Broth under aseptic conditions.

4. From your original streak of the bacteria, use the sterile cotton swab and remove sufficient growth so as to make the glycerol broth in the tube cloudy. The more organisms present, the better the chance of recovery after freezing. You want the concentration to be high.

5. Using the swab, press it firmly against the side of the Cryule vial and gently rotate using your thumb and forefinger to remove most of the colonies. Be sure it is well blended in the glycerol mixture.

6. Repeat steps 2 through 5 for each of the different bacteria samples you want to store in the -80° freezer.

7. Place the Cryule vials in a freezer box and label the outside with the necessary information. Plate this box in the -80° freezer for long term storage.

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Verified by:_____Date:____11/1/19_____

Print Name   Sign Name