

Protocol 905 _Culturing ANAEROBIC bacteria from frozen stocks

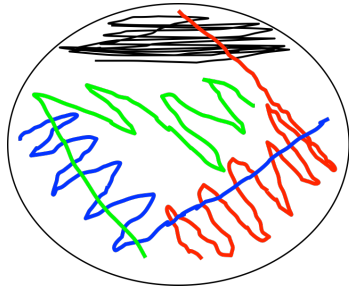
Reagents:

- Frozen bacterial stocks or caecal contents
- Styrofoam ice bucket
- 2mL plastic serological pipettes
- Appropriate Media:
 - ASF broth –reduce for at least 24hrs prior to use
 - MacConkey agar plates
 - LB agar plates
 - Anaerobic blood plates

Method:

1. Label all plates with strain, date and initials
2. Import labeled test tubes for broth into the anaerobic chamber
3. Fill with test tubes with 5mL of media and export
4. Sterilize hood and prepare all required reagents prior to removing stocks from freezer

5. Place frozen stocks on dry ice
6. Only open stocks in sterile hood (or anaerobic chamber). *If in anaerobic chamber ensure your ice bucket is small enough to fit in chamber ports.*
7. Lift sample off dry ice (briefly) careful not to place fingers on stock. *If you hold the tube with your fingers on the stock it will rapidly thaw from the heat released from your hands and the viability of the bacteria will dramatically decrease*
8. Use sterile 2ml plastic serological pipette to remove aliquot (enough so you can see it on the pipette) of frozen sample
9. Place aliquot on agar plate
10. Seal tube and place back in dry ice
11. If inoculating multiple plates use a sterile 2 ml plastic serological pipette every time you go into the stock
12. Once all plates are inoculated place samples back in -80°C
13. Streak out samples on plates by flame (see figure 1)
14. If any samples have been thawed during this process it should be noted!
15. Place MacConkey and LB plates at 37°C upside down in aerobic incubator
ASF media and blood plates are imported into anaerobic chamber
16. Check plates at 24 and 48 hours



- 1-throughly spread inoculum
 - 2-flame loop
 - 3-spread out sample diluting it across the plate
- Flame** loop each change in direction (colour change)

*if you cannot plate outside of the anaerobic chamber than switch 2 ml pipettes between each streak.