



Title:

**Technical Procedure for Freezing and
Reconstituting *Trichomonas vaginalis* and *Tritrichomonas
foetus* Using the InPouch™**

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EQUIPMENT/TOOLS/MATERIALS

1. Lab attire: lab coat, protective gloves, safety glasses
2. Biohazard container
3. BioSafety Level 2 hood
4. -70°C freezer
5. Pure Glycerol
6. Microscope with 10x objective (100x magnification)
7. Glass pipettes - sterile
8. Live InPouch™ culture
9. Hemacytometer
10. Mechanical tally counter
11. Cup, beaker, or rack to hold pouches upright
12. 37°C incubator
13. Microscope clip (optional)

METHOD FOR FREEZING

1. Count the live culture with hemacytometer to determine cell concentration
2. The live InPouch™ culture should be somewhere between $2.0 - 2.5 \times 10^5$ /mL at a minimum of 24 hours after initial inoculation.
3. Express the contents of the upper chamber into the lower chamber.
4. Open the live InPouch™ culture and insert 50-80 μ l (equivalent of two drops) of pure glycerol using a sterile glass pipette.
5. Gently pull the InPouch™ up and down across the edge of a table approximately 3-4 times to dispense the glycerol. **Avoid producing bubbles in the media.**
6. Roll the pouch down to the top of the label and fold the tabs over.
7. Label the InPouch™ culture.
8. Let the culture equilibrate for 10 minutes.
9. Place the culture into a -70°C freezer.

METHOD FOR RECONSTITUTION

1. Remove the InPouch™ culture from the -70°C freezer and immediately incubate it (vertically) at 37°C for 24 hours.
2. Before reading the InPouch™ culture, mix it by gently pulling the InPouch™ up and down across the edge of a table approximately 3-4 times.
3. (Optional) Place the bottom of the lower chamber on the raised platform of the open microscope clip, and then close the clip over the pouch.
4. Observe microscopically under low power (10x). The best location in the pouch to find trichomonads is slightly above the bottom edge of the pouch. (NOTE: you might only observe 1-2 viable organisms at this time).
5. Further incubate the pouch vertically at 37°C for another 24 hours.
6. Repeat step #2
7. Repeat step #3
8. Repeat Step #4 - Now you should see more live trichomonads.
9. Subculture your live culture.

Reference:

Dr. Kenneth A. Borchardt, PhD

CAMT, San Francisco State University, San Francisco, CA.

Dr. Borchardt developed, successfully tested and documented the procedure for freezing and reconstituting live TV and TF organisms using BioMed Diagnostics' InPouch™. Other species of trichomonads can also be frozen and reconstituted using this procedure. Using this procedure, Dr. Borchardt observed no morphological or antigenic changes in frozen trichomonads.