



**REPUBLIC OF THE PHILIPPINES
DEPARTMENT OF HEALTH
NATIONAL REFERENCE LABORATORY
EAST AVENUE MEDICAL CENTER**



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**Instructions for Preparation of PT Sample
Water Microbiology PT**

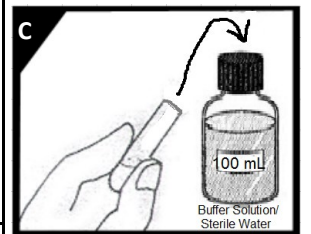
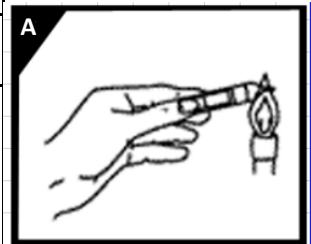
MATERIALS NEEDED:

- 1 PT culture in screw cap vials (included in PT package)
- 2 Bottle containing volume of sterile water/Buffer Solution* suitable to method of choice *
- 3 Sterile Pasteur pipette*

BEFORE YOU BEGIN:

- The bacteria are in freeze-dried form and each sample must be rehydrated per the following instructions prior to analysis.
- NRL recommends that the quality control guidelines in Standard Methods of Examination of Water and Wastewater (SMEWW), be followed to determine media acceptability prior to analysis.
- The glass vials containing the bacteria should be stored at 2-8 degrees Celcius (Ref Temp)
- The hydrating fluid can be stored at Room Temperature.
- This PT sample contains viable microorganisms and should be analyzed immediately after being hydrated.

| | | Sample Code | Sample Code | Sample Code |
|---|--|-------------|-------------|-------------|
| INSTRUCTIONS (mark adjacent box with ✓ after finishing each step.) | | | | |
| 1 | Prepare the work area as PT cultures packaged in glass vials need to remain in a sterile environment. | | | |
| 2 | Prepare three (3) sterile water with volume suitable to the method of choice (for MTFT/Ezyme Substrate Method prepare 100 ml; for MFT use 200 ml). Label the bottle with corresponding sample ID codes. (Note: Do not use more than 400 ml sterile water to suspend cultures.) | | | |
| 3 | Remove the vials from refrigeration and allow them to warm to stand at room temperature. | | | |
| 4 | Open the bacteria sample vial and aseptically rehydrate culture by adding 5-10 drops of the sterile water prepared in step 2 using a sterile Pasteur pipette. Rehydrate drop per drop until the vial become clear. (Figure A and B) | | | |
| 5 | Transfer rehydrated culture into the corresponding sterile water/ Buffer Solution. (Figure C) | | | |
| 6 | With the bottle tightly closed (prepared in step 2), shake the sample 25 times in preparation for the analysis. | | | |
| 7 | Process the samples according to Standards Methods of Examination of Water and Wastewater (SMEWW). | | | |
| 8 | Properly disinfect and sterilize used vials by autoclaving. Submit the vials to NRL with the hardcopy of results. | | | |



* Supplies not included in the PT package and which are to be provided by participating laboratories.

Analyst(s): _____ Date Ended: _____
 Comment(s): _____ Time Ended: _____