GTP for Limit Test for Total Combined Molds & Yeasts count

Reagents:

1. **Sodium hydroxide TS:** Dissolve 4.0 g of sodium hydroxide in water to make 100 mL.

2. **pH 7.2 Phosphate Buffer Stock Solution :** Dissolve 34 g of monobasic potassium phosphate in about 500mL of water contained in a 1000mL volumetric flask. Adjust to pH 7.2 ± 0.1 by addition of sodium hydroxide TS (about 175mL), add water to volume, and mix. Dispense and sterilize in an autoclave at 121°C for 30 minutes. Store under refrigeration.

3. **pH 7.2 Phosphate Buffer:** Dilute the pH 7.2 Phosphate Buffer Stock Solution with water in the ratio of 1 to 800, and sterilize in an autoclave at 121°C for 30 minutes.

   Reconstitute the following dehydrated media as directed by the manufacturer and sterilize in an autoclave at 121°C for 15 minutes.

4. Soyabean-Casein Digest Medium

5. Sabouraud Chloramphenicol Agar Medium

1.0 SAMPLE PREPARATION:

1.1 Prepare the specimen to be tested, by treatment that is appropriate to its physical characteristics and that does not alter the number and kind of microorganisms originally present, in order to obtain a solution or suspension of all or part of it in a form suitable for the test procedure(s) to be carried out.

1.2 For a solid that dissolves to an appreciable extent but not completely: Reduce the substance to a moderately fine powder, suspend it in the vehicle specified.

1.3 For a fluid specimen that consists of a true solution, or a suspension in water or a hydro alcoholic vehicle containing less than 30 percent of alcohol, and for a solid that dissolves readily and practically completely in 90 mL of pH 7.2 Phosphate Buffer or the media specified, for water-immiscible fluids, ointments, creams, and waxes: Prepare a suspension with the aid of a minimal quantity of a suitable, sterile emulsifying agent (such as one of the polysorbates), using a mechanical blender and warming to a temperature not exceeding 45°, if necessary.

1.4 Using above treatment dissolve or suspend 10.0 g of the specimen if it is a solid, or 10 mL, accurately measured, if the specimen is a liquid, in pH 7.2 Phosphate Buffer or Fluid Soybean-Casein Digest Medium, to make 100 mL. For viscous specimens that cannot be pipeted at this initial 1:10 dilution, dilute the specimen until a suspension is obtained, i.e., 1:50 or 1:100, etc., that can be pipetted.

2.0 PLATE METHOD:

2.1 Dilute further, if necessary, the fluid so that 1mL will be expected to yield between 30

and 300 colonies.

2.2 Pipet 1mL of the final diluted sample into each of two sterile petri dishes.

2.3 Pipette 1mL of sterile pH 7.2 phosphate buffer into sterile petridish as negative control.

2.4 Transfer to each dish 15 to 20mL of Sabouraud Chloramphenicol Agar Medium that previously has been melted and cooled to approximately 45°C.

2.5 Cover the petri dishes, mix the sample with the agar by tilting or rotating the dishes, and allow the contents to solidity at room temperature.

2.6 Invert the petri dishes, and incubate at 20° - 25°C for 5 days. Following incubation, examine the plates for growth.

3.0 INTERPRETATION:

3.1 Negative control should not show any colony.

3.2 Count the number of colonies and determine the average number of colonies in two plates. Multiply the average count with dilution factor and express the count in terms of the number of micro-organisms per g or per mL of specimen.

3.3 If no microbial colonies are found from the dishes representing the initial 1:10 dilution of the specimen, express the results as ‘less than 10 micro-organisms per g or per mL of specimen”.

NOTE: PROCEDURE FOR RETESTING:

1. For the purpose of confirming a doubtful result by any of the procedures outlined in the foregoing tests following their application to a 10.0 g specimen, a retest on a 25 g specimen of the product may be conducted.

   Proceed as directed under Procedure, but make allowance for the larger specimen size.