GTP FOR TOTAL AEROBIC MICROBIAL COUNT

REAGENTS AND CULTURE MEDIA:

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 500 mL of water contained in 1000-mL volumetric flask. Adjust to pH 7.2 ± 0.1 by the addition of sodium hydroxide TS (about 175 mL), 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.

4. **Soyabean-Casein Digest Agar Medium:** Reconstitute dehydrated media as directed by the manufacturer and sterilize in autoclave at 121°C for 15 minutes.

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SAMPLE PREPARATION:

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. 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.

2. 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.

3. 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 pipette.

   For specimens that are sufficiently soluble or translucent to permit use of the Plate Method, use that method; otherwise use the Multiple-tube Method.

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A. PLATE METHOD:

Dilute further, if necessary, the fluid so that 1 mL will be expected to yield between 30 and 300 colonies.

1.0 Test Preparation:

1.1 Pipet 1 mL of the final diluted sample into each of two sterile petri dishes. Transfer to each dish 15 to 20 mL of Soyabean -Casein Digest Agar Medium that previously has been melted and cooled to approximately 45°C.

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

1.3 Invert the petri dishes, and incubate at 30 to 35°C for 48 to 72 hours. Following incubation, examine the plates for growth.

2.0 Negative Control :

2.1 Pipette 1 mL of sterile pH 7.2 phosphate buffer into a sterile petridish.

2.2 Transfer 20 mL of Soyabean -Casein Digest Agar Medium that previously has been melted and cooled to approximately 45°C.

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

2.4 Invert the petri dishes, and incubate at 30 to 35°C for 48 to 72 hours. Following incubation, examine the plates for growth.

3.0 Interpretation:

3.1 Negative control should not show any colony

3.2 Following incubation, count the number 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 number of microorganisms per gram or per mL of specimen.

1. If no microbial colonies are recovered from the dishes representing the initial 1:10 dilution of the specimen, express the results as "less than10 micro-organisms per g or per mL of specimen".

B. MULTIPLE - TUBE METHOD:

1. Into each of 15 test tubes of similar size place 9.0 mL of sterile Fluid Soyabean- Casein Digest Medium

2. Arrange twelve of the tubes in four sets of three tubes each.

1. Test Preparation:

1. Into each of the three tubes of one set ("100") and into a fourth tube (A) pipet 1 mL of the final diluted sample and mix.

2. From tube A, pipet 1 mL of its contents into the one remaining tube (B) not included in a set, and mix. These two tubes contain 100 mg (or 100 µl) and 10 mg (or 10 µL) of the specimen, respectively.

3. Into each of the second set ("10") of three tubes pipet 1 mL from tube A, and into each tube of the third set ("1") pipet 1 mL from tube B.

4. Discard the unused contents of tubes A and B.

2. Negative control:

1. Add 1 mL of sterile pH 7.2-phosphate buffer into negative control tubes.

2. Close well, and incubate all the tubes. Following the incubation, period, examine the tubes for growth.

3. Interpretation:

Negative control should not show any growth.

Observations in the tubes containing the specimen, when interpreted by reference to Table 1 indicate the most probable number of microorganisms per g or per mL of specimen.

Table 1. Most Probable Total Count by Multiple-tube Method

Observed Combinations of numbers of Tubes showing Growth in No. of mg (or mL) of Specimen per Tube			Most probable Number of Microgranism per gm or per mL
100 (100µL)	10 (10µL)	1 (1µL)	
3	3	3	> 1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

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.