



GTP for Limit Test for *Staphylococcus Aureus* and *Pseudomonas aeruginosa*

REAGENTS AND CULTURE MEDIUM:

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

1. Soyabean – Casein Digest Medium.
2. Vogel-Johnson Agar Medium.
3. Mannitol salt agar medium.
4. Baird Parker agar medium
5. Cetrimide Agar Medium.
6. Rabbit or Horse plasma.
7. Pseudomonas Agar Medium for detection of fluorescin.
8. Pseudomonas Agar Medium for detection of Pyocyanin.
9. Oxidase reagent

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.

2.0 Test For *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*:

2.1 TEST PREPARATION:

To 10 gm or 10 ml of sample add 90 ml of Fluid Soyabean Casein digest medium.



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2.2 POSITIVE CONTROL:

Take 0.1ml from suspension of *S. aureus* and *P. aeruginosa* and add into 90mL of Soyabean casein digest medium.

2.3 Incubate at 30 to 35°C for 24 to 48 hours. After incubation proceed as directed in individual test for *S. aureus* and *P. aeruginosa*.

3.0 Test For *Pseudomonas Aeruginosa*:

3.1 Streak a portion of soyabean casein digest medium on the surface of plates of Cetrimide Agar by taking a loop full of inoculum from sample and positive control Soyabean Casein Digest medium respectively.

3.2 Cover and invert the dishes and incubate at 30 to 35°C for 24 to 48 hours. After incubating, examine the plates.

3.3 INTERPRETATION:

Positive control should show the characteristics given in table-1.

If the colonies on the sample plate do not match as per characteristics given in table-1 that indicates that the sample meets the requirements for the absence of *Pseudomonas aeruginosa*. If the colonies on the sample plate match to the characteristics given table-1, then proceed for oxidase and Pigment Test.

Table 1. Morphologic Characteristic of *Pseudomonas aeruginosa* on selective and Diagnostic Agar Medium

Selective Medium	Characteristic Colonial Morphology	Fluorescence in Ultraviolet Light	Oxidase test	Gram Stain
Cetrimide Agar Medium	Generally Greenish	Greenish	Positive	Negative rod
<i>Pseudomonas</i> Agar Medium for detection of fluorescin.	Generally colourless to yellowish	Yellowish	Positive	Negative rod
<i>Pseudomonas</i> Agar Medium	Generally Greenish	Blue	Positive	Negative rod

4.0 PIGMENT TEST:

4.1 With the aid of an inoculating loop, streak suspect colonies from the surface of cetrimide agar medium *Pseudomonas* Agar (Pyocyanin) and *Pseudomonas* Agar (Fluorescin).

4.2 If numerous colonies are to be transferred, divide the surface of each plate into quadrants, each of which may be inoculated from a separate colony.

4.3 Also transfer colony from positive control plates of cetrimide agar on the surface of



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Pseudomonas Agar (Pyocyanin) and Pseudomonas Agar (Fluorescin).

- 4.4 Cover and invert the inoculated media, and incubate at $35\pm 2^{\circ}\text{C}$ for not less than 72 hours.
- 4.5 Examine the streaked surfaces under ultraviolet light.

4.6 Interpretation:

Positive control should show the characteristics given in table-1.

If colonies do not match as per characteristics given in table-1 that indicates the sample meets the requirements for absence of *Pseudomonas aeruginosa*. If colonies on the sample plate match to the characteristics given table-3 then proceed for confirmation test.

5.0 OXIDASE TEST:

- 5.1 Place or transfer colonies taken from test sample and positive control plates of Pseudomonas Agar (for Pyocyanin), Pseudomonas Agar (for fluorescin) to disks or strips of filter paper, impregnated with N1N-dimethyl-p- phenylenediamine dihydrochloride.

5.2 INTERPRETATION:

Colonies of positive control should show the development of pink color, changing to purple.

If there is no development of pink color, changing to purple shown by colonies of test sample plates, the specimen meets the requirements for the absence of *Pseudomonas aeruginosa*.

6.0 Test For *Staphylococcus Aureus*:

- 6.1 Streak a portion from soyabean casein digest medium on the surface of the plates of Vogel-Johnson Agar medium or Mannitol-salt Agar medium or baird-parker Agar medium by taking loop full of Inoculum from sample and Positive control vessel of Soyabean Casein digest medium flasks respectively.
- 6.2 Cover and invert the dishes and incubate at 30 to 35°C for 24 to 48 hours. After incubating, examine the plates.
- 6.3 **Interpretation:**

Positive control should show the colony characteristics as described in table- 2.

If the colonies on the sample plate does not match with the colony characteristic



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given table-2 that indicates that the sample meets the requirements for the absence of *Staphylococcus aureus*. If the colonies on the sample plate matches with the colony characters given table-2 then proceed for Coagulase Test for confirmation.

Table-2 Morphologic Characteristics of *Staphylococcus aureus* on Selective Agar Media

Selective Medium	Characteristic Colonial Morphology	Gram Stain
Vogel-Johnson Agar Medium	Black Surrounded by yellow zone	Positive Cocci (in clusters)
Mannitol Salt Agar Medium	Yellow colonies with yellow zones.	Positive Cocci (in clusters)
Baird-Parker Agar Medium	Black, shiny, surrounded by clear zones 2 to 5 mm.	Positive Cocci (in clusters)

7.0 Coagulase Test (For *Staphylococcus aureus*)

- 7.1 Take 0.5mL of mammalian, preferably rabbit or horse, plasma with or without suitable additives into each tube.
- 7.2 With the aid of an inoculating loop, transfer representative suspect colonies from the Agar surfaces of the Vogel-Johnson Agar Medium (or baird-Parker Agar Medium or Mannitol-Salt Agar Medium) to individual tubes containing mammalian plasma.
- 7.3 Also inoculate the plasma tube with colonies of positive control plates.
- 7.4 Incubate in a water bath at 37°C, examining the tubes at 3 hours and subsequently at suitable intervals upto 24 hours.
- 7.5 **INTERPRETATION:**

Positive control tubes should show coagulation

If no coagulation in any degree is observed, the specimen meets the requirements of the test for absence of *Staphylococcus aureus*. If coagulation is observed, the specimen does not meet the requirements for the absence of *Staphylococcus aureus*.

NOTE: PROCEDURE FOR RETESTING:

For the purpose of confirming a doubtful result by any of the procedures outlines 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.