



GTP FOR LIMIT TESTS FOR SALMONELLA SPECIES AND ESCHERICHIA COIL

REAGENTS:

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

1. Fluid Lactose Medium
2. Fluid Selenite-Cystine Medium
3. Fluid Tetrathionate Medium
4. Brilliant Green Agar Medium
5. Xylose-Lysine-Desoxycholate Agar Medium
6. Bismuth Sulfite Agar Medium
7. Triple Sugar Iron-Agar Medium
8. MacConkey Agar Medium
9. Levine Eosin-Methylene Blue 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.



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2.0 Test For *Salmonella Species and Escherichia Coli*:

2.1 Test Preparation:

To 10gm or 10ml of specimen add 90 mL of fluid lactose medium.

2.2 Positive Control :

Take 0.1 ml of E. coli and Salmonella culture suspension in to 90 mL of fluid lactose medium.

2.3 Incubate at 30 to 35°C for 24 to 48 hours. After incubation proceed as directed in individual test for E. coli and Salmonella.

3.0 Test For *Salmonella Species*:

3.1 Pipette 1-mL portions from fluid lactose medium, in to tubes containing 10 mL of Fluid Selenite-Cystine Medium and Fluid Tetrathionate Medium from test and positive control vessel respectively, mix, and incubate at 30-35°C for 12 to 24 hours. (Retain the remainder of the Fluid Lactose Medium).

3.2 By means of an inoculating loop streak portions from both the selenite cystein medium and tetrathionate medium on the surface of Brilliant Green Agar , Xylose-Lysine – Desoxycholate Agar and Bismuth sulfate Agar.

3.3 Cover and invert the dishes and incubate at 30 to 35°C for 24 to 48 hours.

3.4 Interpretation:

Positive control plates of Brilliant green Agar, Xylose-Lysine-Desoxycholate Agar, Bismuth sulfate Agar should show colonial morphology as described in table - 1.

If colonies on the sample plates of Brilliant green Agar, Xylose-Lysine-Desoxycholate Agar, Bismuth sulfate Agar does not match to the description given in the table-1, then the sample meets the requirements for the absence of Salmonella species. If colonies of gram negative rods matches to the description given in the table -1 then proceed for the confirmation test.

Table-1. Morphologic Characteristics of *Salmonella Species* on Selective Agar Medium

Selective Medium	Characteristic Colonial Morphology
Brilliant Green Agar Medium	Small, transparent, colorless or pink to white opaque (frequently surrounded by pink to red zone)
Xylene-Lysine-Desoxycholate Agar medium	Red, with or without black centers
Bismuth Sulfite Agar Medium	Black or green



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4.0 Confirmation Test:

- 4.1 Transfer representative suspected colonies, individually by means of an inoculating wire, to a butt - slant tube of Triple Sugar Iron Agar medium by first streaking the surface of the slant and then stabbing the wire well beneath the surface.
- 4.2 Maintain positive control by inoculating colony from positive plate by streaking and stabbing medium.
- 4.3 Incubate at 30 to 35°C for 24 to 48 hours. Examine the tubes after incubation.

4.4 Interpretation:

Positive control should the characteristics as described below.

If the sample tube show alkaline (red) slants and acid (yellow) butts (with or without concomitant blackening of the butt from hydrogen sulfide production), that indicates the sample does not meet the requirements for the absence of *Salmonella species*. If the sample tube does not show above characteristics, that indicates sample meets the requirements for the absence of *Salmonella species*.

5.0 Test For *Escherichia Coli*:

- 5.1 By means of inoculating loop, streak a portion from the remaining fluid Lactose medium of test and positive control on the surface of Macconkey Agar medium.
- 5.2 Cover and invert the dishes, and incubate at 30 to 35°C for 24 to 48 hours. After incubating, examine the plates.
- 5.3 **Interpretation:**

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

Colonies in the sample plate should not match as given in table 2 for the absence of *Escherichia Coli*. If it matches with the colony characteristics described in table - 2, proceed to confirmation test.

6.0 Confirmation test:

- 6.1 Transfer the suspected colonies from the plate of MacConkey to Eosin-methylene blue agar plates. Cover and invert the plates and incubate at 30 to 35°C for 24 to 48 hours.
- 6.2 Also inoculate EMB plates with colony picked from positive pate of MacConkey agar as positive control. After incubating, examine the plates.



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6.3 Interpretation :

Colonies of Positive control plates should show the characteristics as described below.

If the colonies on sample plate exhibit both the characteristic metallic sheen under reflected light and blue-black appearance under transmitted light, that indicates, the sample does not meet the requirements for the absence of *Escherichia Coli*. If the colonies sample plate do not match to above said characteristics that indicate, sample meets the requirements for the absence of *Escherichia Coli*.

Table 2. Morphologic Characteristics of *Escherichia Coli* on MacConkey Agar Medium

Gram Strain	Characteristic Colonial Morphology
Negative rods (cocco-bacilli)	Brick –red; may have surrounding zone of precipitated bile.

NOTE: PROCEDURE FOR RETESTING:

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.