GTP FOR SALMONELLA SPECIES Ph. Eur.

Reagents:

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

(1) Tetrathionate bile brilliant green broth

(2) Xylose Lysine Desoxycholate Agar

(3) Deoxy cholate citrate Agar.

(4) Triple Sugar Iron Agar

(5) Casein Soyabean Digest Broth

Sample Preparation:

Prepare the sample preparation as directed in the individual Standard Test Procedure.

(1) Test preparation:

(a) Homogenize the sample preparation and incubate at 35°C to 37°C for 18 hours to 24 hours.

(b) Transfer 1 ml of the sample preparation to 10 ml of Tetrathionate bile brilliant green broth.

Preparatory testing:

Perform the test for absence of inhibitory (antimicrobial) properties as described below:

Note: Preparatory testing shall be performed for initial 3 batches and if the results are found satisfactory, subsequent batches need not be tested.

(1) **10^{-1}dilution of 24hr broth cultures**: Pipet 10ml of 24hour broth cultures of *Escherichia Coli*, *Salmonella* species, *Psuedomonas aeruginosa* and *Staphylococcus aureus* to separate 100ml volumetric flasks and make up to volume with sterile buffered sodium Chloride - Peptone Solution pH 7.0.

(2) **10^{-3} dilution of 24hr broth cultures:** Pipet 1ml of 10^{-1} dilution of 24h broth cultures of *Escherichia Coli*, *Salmonella* species, *Psuedomonas aeruginosa* and *Staphylococcus aureus* to separate 100ml volumetric flasks and make up to volume with sterile buffered Sodium Chloride - Peptone solution pH 7.0.

(3) Mix 10ml of 10^{-3} dilution of 24hour broth cultures of *Escherichia Coli*, *Salmonella* species, *Psuedomonas aeruginosa* and *Staphylococcus aureus*.

(4) Take 0.4ml of mixed suspension in a 100ml volumetric flask; add 10ml of the prepared sample

and make up to volume with Tetrathionate Broth.

(2) Negative control:

Inoculate 100 ml of Tetrathionate Broth with 0.4 ml of buffered Sodium Chloride pH 7.0 solutions.

(3) Incubate sample, negative and preparatory testing flasks at 41°C to 43°C for 18 to 24hours.

(4) After incubation examine the flasks.

(5) Interpretation:

(a) If growth is present in the preparatory testing flask, that indicates the absence of inhibitory substances in the sample. If the growth is absent, that indicates the presence of inhibitory substances in the sample.

(b) Negative control flask should not show any growth.

(c) Proceed for subculturing of the sample flask.

(6) Subculturing:

(a) Subculture on Sample, Negative on atleast two different agar media of Deoxycholate Citrate agar or Xylose Lysine Deoxycholate Agar or Brilliant green, phenol red, lactose monohydrate sucrose agar plates by taking inoculum from sample, negative tetrathionate bile brilliant green broth.

(b) Incubate the plates at 35°C to 37° C for 18 to 72 hours.

(c) Examine the plates after incubation

(d) Interpretation:

(i) If colonies on sample plates matches as below, then proceed for the confirmation test. If colonies on the sample plates do not match as below that means sample meets the requirements for the absence of *Salmonella* species.

(1) Deoxycholate Citrate Agar

(2) Xylose Lysine Desoxychlolate Agar

(3) Brilliant green, phenol red, lactose monohydrate, sucrose agar (ii) Negative control plates should not show any colonies.

(7) Confirmation test:

(a) Prepare Triple Sugar Iron Agar slants by taking 10ml into each tube.

(b) Transfer representative suspected colonies and colonies from sample plates individually by means of inoculating wire to the sample tubes of Triple Sugar Iron Agar slants by first streaking the surface of the slant and then stabbing the wire well beneath the surface.

(c) Maintain negative control without streaking and stabbing medium.

(d) Incubate 30-35°C for 24hrs.

(e)Examine the tubes for growth.

(f) **Interpretation:**

(a) Negative control should not show any growth.

If the sample tubes show a change of color from red to yellow and usually a formation of gas with or without production H2S in deep inoculation (but not on the surface of agar medium) that means, sample does not meet the requirements for the absence of *salmonella* species. If the sample tube does not show any change that means, sample meets the requirement for the absence of *Salmonella* species.

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.

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