STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbial Enumeration Test of Raw Materials and Finished Products	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE:

To lay down a procedure for Microbial Enumeration Test of Raw Materials and Finished Products.

2.0 SCOPE:

This SOP is applicable for Microbial Enumeration Test of Raw Materials and Finished Products of Quality Control area.

3.0 RESPONSIBILITY:

Officer / Executive - Microbiologist

4.0 ACCOUNTABILITY:

Head – QC

5.0 PROCEDURE:

5.1 PRETREATMENT OF SAMPLE:

Use specified quantity of sample for each test specified in the monograph and pretreat the sample as follows:

5.1.1 Water Soluble Products: Dissolve 10gm or dilute 10 ml of sample in buffered sodium chloride-peptone solution pH 7.0 and adjust the volume to 100 ml. **(Solution A).**

5.1.2 Water Insoluble Products: Suspend 10 gm or 10 ml sample in buffered sodium chloride peptone solution pH 7.0 and 0.1 % w/v polysorbate 80 and adjust the volume to 100 ml with same medium **(Solution A).**

5.1.3 Fatty Products: Homogenize 10 gm or 10 ml of the sample with 5 gm of polysorbate 20 or polysorbate 80, if necessary to heat not more than 40°C .Mix carefully add 85 ml of buffered sodium chloride peptone solution pH 7.0. **(Solution A).**

5.2 FOR TAMC AND TYMC:

5.2.1 MEMBRANE FILTRATION METHOD:

5.2.2 For TAMC, from above pretreated sample transfer 10ml to Membrane filtration assembly and Filter it. Repeat the process for TYMC. Wash both time Membrane Filters with 3 X 100 ml of suitable solution such as Sterilized Sodium Chloride-Peptone Solution pH 7.0.

5.2.3 Transfer one Membrane Filter to Pre incubated Soyabean Casein Digest Agar (SCA) medium and other Membrane Filter to Sabouraud Dextrose Agar (SBD) Plate.

5.2.4 **Negative Control:** Add 10 ml of the chosen diluent to Membrane filtration assembly and Filter it. Repeat the process for TYMC. Wash both time Membrane Filters with 3 X 100 ml of suitable solution such as Sterilized Sodium Chloride-Peptone Solution pH 7.0.Transfer one Membrane Filter to Pre incubated Soyabean Casein Digest Agar (SCA) medium and other Membrane Filter to Sabouraud Dextrose Agar (SBD) Plate.

5.2.5 **Positive Control:** Performed positive control as per SOP.

5.2.6 Incubate Soyabean Casein Digest Agar (SCA) Plates at 30°C to 35ºC for 5 days and Sabouraud Dextrose Agar (SBD) plates at 20 °C to 25 °C for 7 days.

5.2.7 After Incubation calculate the CFU per gm or per ml of the Sample being examined.

5.3 POUR PLATE METHOD:

5.3.1 Stir the pretreated sample on a vortex mixer.

5.3.2 Use two presterilized Petri plates (diameter 90-100 mm) each for TAMC and TYMC.

5.3.3 For TAMC, pour 1 ml of each pretreated sample into two presterilized Petri plates and than pour 20-25 ml sterilized Soyabean casein Digest Agar (cool up to 45°C) and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of sample.

5.3.4 **Negative Control:** Add 1 ml of the chosen diluent into sterile petriplates and add about 15 ml of liquefied sterile Soyabean casein Digest Agar (cool up to 45°C) the Petri dishes and allow the medium to solidify.

5.3.5 **Positive Control:** Performed positive control as per SOP

5.3.6 Allow to solidify and than incubate at 30-35^{0}C for 5 days in inverted position.

5.3.7 For TYMC, pour 1 ml each of pretreated sample into two presterilized Petri plates and than pour 20-25 ml sterilized Sabouraud Dextrose Agar or Sabouraud Chloramphenicol Agar (cool up to 45°C) and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of sample.

5.3.8 Allow to solidify and Incubate all the plates at 20°C to 25°C for 5-7 days in inverted position.

5.3.9 After incubation calculate the CFU per gm or per ml of the Sample being examined.

5.3.10 **Negative Control:** Add 1 ml of the chosen diluent to sterile petriplates and add about 15 ml of liquefied sterile Sabourand Dextrose agar (cool up to 45°C) in the Petri dishes and allow the medium to solidify.

5.3.11 **Positive Control:** Performed positive control as per SOP.

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5.4 TESTS FOR SPECIFIED MICRO ORGANISMS (PATHOGENS):

Sample Preparation: Transfer 10 mL of **Solution A** to 90 mL Soyabean Casein Digest Broth Medium and incubate at 30° to 35ºC for 18 to 24 hrs. **Solution B.**

5.4.1 Test for *Escherichia coli*:

5.4.1.1 After incubation of Solution B, Shake the broth and transfer 1ml to 100 ml of Preincuabted MacConkey Broth. Incubate at 42 °C to 44 °C for 24 to 48 hrs.

5.4.1.2 Subculture on a plate of Preincuabted MacConkey Agar plate and incubate at 30 °C to 35 °C for 18 to 72 hrs. The Growth of pink, non-mucoid colonies indicates the possible presence of *E. coli.* This should confirmed by identification test.

5.4.1.3 If the above media shows pink non-mucoid colonies indicates the presence of *E. coli* which is confirmed by Indole production test.

5.4.1.4 Confirmation Test: Add 0.1 ml of contents of Mac Conkey broth to 5 ml of sterile 1% peptone water. Add 0.5 ml of Kovac's reagent to the test tube, shake well and allow to stand for one minute. If a cherry red color ring is observed at upper layer of the reagent, it indicates presence of *E. coli.*

5.4.1.5 Negative Control: Use 1 ml chosen diluent to inoculate in 100 ml of sterile MacConkey broth and incubate at 42- 44°C for 24-48 hrs. After incubation subculture on plates of Mac Conkey agar and incubate at 30-35°C for 18-72 hrs.

5.4.1.6 Positive Control: Performed positive control as per SOP.

5.4.2 Test for *Salmonella:*

(If Limit is Absent/10 g)

5.4.2.1 Preparation of sample: Dissolve 10g of test sample 100ml of sterile Soybean Casein Digest Medium. Homogenize and incubate at 30-35°C for 18 - 24hours (**Solution C**).

5.4.2.2 Transfer 0.1 ml of the enrichment culture to 10 ml of Preincuabted Rappaport Vassiliadis Salmonella Enrichment Broth and Incubate at 30-35°C for 18-24 hours. After incubation shake the test tube & subculture on a sterile petriplate of Preincuabted Xylose lysine Deoxycholate Agar and incubate at 30-35°C for 18-48 hours. Growth of well developed red colonies with or without black centers indicates the possibility of *Salmonella.*

5.4.2.3 Conformation Test: Prepare the slant of Triple Sugar Iron Agar and after sterilization allow to solidify. Stab the suspected colony by means of inoculating needle in to the butt and streak the surface of slant. Possible presence of *Salmonella* is indicated by black butt and yellow slant.

5.4.2.4 Add 0.1ml of culture detected on Triple Sugar Iron Agar in 5ml sterile urea broth and incubate at 35-37°C for 18-24 hours. Acid & gas formation (color of the broth change from yellow to red) confirmed the presence of *Salmonella*. Product passes the test for Absence of *Salmonella* if no acid & gas formation occur in urea broth.

5.4.2.5 **(If Limit is Absent /g)** Transfer 0.1 ml of the enrichment culture from **Solution B** to 10 ml of Preincuabted Rappaport Vassiliadis Salmonella Enrichment Broth and Incubate at 30-35°C for 18-24 hours. After incubation shake the test tube & subculture on a sterile petriplate of Preincuabted Xylose lysine Deoxycholate Agar and incubate at 30-35°C for 18-48 hours. Growth of well developed red colonies with or without black centers indicates the possibility of *Salmonella.*

5.4.2.6 **Conformation Test:** Prepare the slant of Triple Sugar Iron Agar and after sterilization allow to solidify. Stab the suspected colony by means of inoculating needle in to the butt and streak the surface of slant. Possible presence of *Salmonella* is indicated by black butt and yellow slant.

5.4.2.7 Add 0.1ml of culture detected on Triple Sugar Iron Agar in 5ml sterile urea broth and incubate at 35-37°C for 18-24 hours. Acid & gas formation (color of the broth change from yellow to red) confirmed the presence of *Salmonella*. Product passes the test for Absence of *Salmonella* if no acid & gas formation occur in urea broth.

5.4.2.8 **Negative Control:** Use 1 ml chosen diluents to inoculate in 10 ml of sterile RVS Broth and incubate at 30- 35°C for 24-48 hrs. After incubation subculture on plates of Preincuabted XLD (Xylose lyseine Deoxychocolate Agar) and incubate at 30-35°C for 18-48 hrs.

5.4.2.9 **Positive Control:** Performed positive control as per SOP.

5.4.3 ***Staphylococcus Aureus:***

5.4.3.1 After incubation of Solution B,, shake the broth and subculture a loop full growth on the surface of Preincuabted Mannitol Salt Agar medium and Incubate at 30 °C to 35°C for 18 to 72 hrs.

5.4.3.2 If the above media shows Yellow or white colonies surrounded by a yellow zone indicates the presence of *Staphylococcus aureus* which is confirmed by coagulase test.

5.4.3.3 **Confirmation Test:** Add 2 to 3 drops of *Staphylococcus aureus* culture + 0.5ml of mammalian rabbit plasma. Incubate in water bath at 35-37°C, examine the tube at 3 hrs & subsequently at suitable interval upto 24 hrs. If white precipitate formation started after 3 hrs shows the presence of *Staphylococcus aureus*.

5.4.3.4 **Negative Control:** Use blank Preincuabted Mannitol Salt Agar medium plate and incubate at 30 to 35 °C for 18 to 72 hrs.

5.4.3.5 **Positive Control:** Performed positive control as per SOP.

5.4.4 *Pseudomonas aeruginosa*:

5.4.4.1 After incubation of Solution B, shake the broth and Subculture on Preincuabted Cetrimide Agar medium plate and incubate at 30 to 35 °C for 18 to 72 hrs.

5.4.4.2 Presence of growth on agar media indicates the possibility of presence of *Pseudomonas aeruginosa*. If there are no such types of growth, or identification test are negative, it indicates absences of *Pseudomonas aeruginosa*.

5.4.4.3 If the above media shows Green colonies indicates the presence of *P.aeruginosa* which is confirmed by confirmation test.

5.4.4.4 Confirmation Test: Place a suspected colony by means of needle on to **Oxidase Disc**. If the disc turns to blue in colour, it confirms the presence of *Pseudomonas aeruginosa.*

5.4.4.5 Negative Control: Use blank Preincuabted Cetrimide Agar medium plate and incubate at 30 to 35 °C for 18 to 72 hrs.

5.4.4.6 Positive Control: Performed positive control as per SOP.

5.4.5 Bile-Tolerant Gram-Negative Bacteria (*Enterobacteria*):

5.4.5.1 Prepare the sample Take Solution B, mix well and keep at 20 to 25 °C for 2 to 5 hrs.

5.4.5.2 Transfer 10 ml of solution A to 90 ml Preincuabted *Enterobacteria* Enrichment Broth-Mossel medium and Incubate the medium at 30 to 35 °C for 24 to 48 hrs.

5.4.5.3 After Incubation, Subculture on the Plate of Preincuabted Violet Red Bile Glucose Agar and incubate the plate at 30 to 35 °C for 18 to 24 hrs.

5.4.5.4 After incubation observe the plates and carryout the gram staining. If there are no such types of growth, or does not show gram negative bacteria, it indicates absences of Enterobacteria.

5.4.5.5 Negative Control: Use 1 ml chosen diluents to inoculate in to 100 ml of Preincuabted *Enterobacteria* Enrichment Broth-Mossel broth and Incubate at 30-35°C for 24-48 hours. After incubation shake the test tube & subculture on a plate of Preincuabted Violet Red Bile Glucose Agar and Incubate at 30-35°C for 18 to 24 hrs.

5.4.5.6 Positive Control: Performed positive control as per SOP.

5.4.6 Test For *Shigella:*

5.4.6.1 Transfer 1 ml of the enrichment culture from **Solution C** to 100 ml of Preincuabted GN broth and Incubate at 30-35°C for 24-48 hours. After incubation shake the test tube & subculture on a

plate of Preincuabted Xylose Lysine Deoxycholate Agar and Incubate at 30-35°C for 24-48 hours.

5.4.6.2 The probable presence of *Shigella* is indicated by the growth of a red color translucent colony without black centre. Product passes the test for Absence of *Shigella* if no growth of a red color translucent colony without black centre observed.

5.4.6.3 **Negative Control:** Use 1 ml chosen diluents to inoculate in to 100 ml of Preincuabted GN broth and Incubate at 30-35°C for 24-48 hours. After incubation shake the test tube & subculture on a plate of Preincuabted Xylose Lysine Deoxycholate Agar and Incubate at 30-35°C for 24-48 hours.

5.4.6.4 **Positive Control:** Performed positive control as per SOP.

5.4.7 ***Clostridia*:**

5.4.7.1 Take two equal portions of 10 ml from solution A and heat one portion at 80°Cfor 10 minute and cool rapidly. Do not heat the other portion.

5.4.7.2 Transfer each of the homogenised portion in two tubes containing 100 ml Preincuabted Reinforced medium for Clostridia. Incubate the tubes under anaerobic condition at 30 to 35°C for 48 hrs.

5.4.7.3 After incubation, make sub-subculture from each container on Preincuabted Columbia agar plates. Incubate under anaerobic conditions at 30 to 35°C for 48 hrs.

5.4.7.4 The Presence of anaerobic growth of Gram positive bacilli with or without endospores, giving a negative catalase test indicates the possibilities of presence of *Clostridia*. If there are no such types of anaerobic growth on Columbia agar or identification test are negative, it indicates absences of *Clostridia*.

5.4.7.5 **Negative Control:** Use 1 ml chosen diluents to inoculate in to 100 ml of Preincuabted Reinforced medium for Clostridia Incubate the tubes under anaerobic condition at 30 to 35°C for 48 hrs. After incubation, make sub-subculture from each container on Preincuabted Columbia agar plates. Incubate under anaerobic conditions at 30 to 35°C for 48 hrs.

5.4.7.6 **Positive Control:** Performed positive control as per SOP.

5.4.8 ***Candida albicans*:**

5.4.8.1 Dissolve 10 g of sample in 100 ml of Sabouraud Dextrose Broth and incubate at 30 to 35 ^{0}C for 3 to 5 days. **(Solution D)**

5.4.8.2 After Incubation, Subculture from solution D on the Plate of Sabouraud Dextrose agar medium and incubate the plate at 30 ^{0}C to 35 ^{0}C for 24 to 48 hrs.

5.4.8.3 Growth of cream coloured colonies may indicate the possibility of presence of *C.albicans*, this is confirmed by identification test. If there are no such types of growth, or identification test are negative, it indicates absences of *C.albicans.*

5.4.8.4 Negative Control: Use blank Preincuabted Sabouraud Dextrose agar medium plate and incubate at 30 ^{0}C to 35 ^{0}C for 24 to 48 hrs.

5.4.8.5 Positive Control: Performed positive control as per SOP.

5.4.9 Microbiological Limit Test shall be performed:

5.4.9.1 Raw materials or Bulk Sample: As per specification.

5.4.9.2 Finished Product:

- First three batch of new product
- Every 10th Batch
- As per customer requirement

6.0 REFERENCES:

Indian Pharmacopoeia
United state Pharmacopeia

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure-I	Microbial Enumeration Test Record	
Annexure-II	Microbial Enumeration Test Report For *Escherichia Coli*	
Annexure-III	Microbial Enumeration Test Report For *Salmonella*	
Annexure-IV	Microbial Enumeration Test Report For *S.aureus*	
Annexure-V	Microbial Enumeration Test Report For *P.aeruginosa*	
Annexure-VI	Microbial Enumeration Test Report For *Bile-Tolerant Gram Negative Bacteria (Enterobacteria)*	
Annexure-VII	Microbial Enumeration Test Report For *Shigella*	
Annexure-VIII	Microbial Enumeration Test Report For *Clostridia*	
Annexure-IX	Microbial Enumeration Test Report For *Candida albicans*	
Annexure-X	Sample Receipt/Analysis Record For Microbial Limit Test	

ENCLOSURES: SOP Training Record

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8.0 DISTRIBUTION:

- Controlled Copy No. 01 — Quality Assurance Department
- Controlled Copy No. 02 — Quality Control Department
- Master Copy — Quality Assurance Department

9.0 ABBREVIATIONS:

hrs	Hours
LAF	Laminar Air Flow
ml	Milliliter
QC	Quality Control
SOP	Standard Operating Procedure
SCA	Soyabean Casein Digest Agar
SBD	Sabouraud Dextrose Agar
SCM	Soyabean Casein Digest Medium
UV	Ultra Violet
TAMC	Total Aerobic Microbial count
TYMC	Total Yeast and Mold count.

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Details of Changes	Reason for Change	Effective Date	Updated By

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ANNEXURE – I
MICROBIAL ENUMERATION TEST REPORT

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Balance I.D.			
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample: Dissolve _____gm/ mL of sample in sodium chloride-peptone solution pH 7.0 +0.1 % w/v polysorbate 80 and adjust the volume to 100 ml. (**Solution A**)

OBSERVATIONS TABLE FOR TOTAL BACTERIAL COUNT

Date	Daily Observation Plate Count		Average count	Average count X dil. factor	Total cfu/gm or ml of Sample
	Plate 1	Plate 2			
+ ve Control:	**Passage No.**	**Count CFU/ml**			
- ve Control:					

OBSERVATIONS TABLE FOR TOTAL YEAST /MOULD COUNT

Date	Daily Observation Plate Count		Average count	Average count X dil. factor	Total cfu/gm or ml of Sample
	Plate 1	Plate 2			
+ ve Control:	**Passage No.**	**Count CFU/ml**			
- ve Control:					

Remarks: The above sample is complies/does not complies as per IP/BP/USP/IH specification.

Analyzed By:
Date:

Checked By:
Date:

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ANNEXURE – II
MICROBIAL ENUMERATION TEST REPORT FOR *ESCHERICHIA COLI*

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample:

Solution B: Transfer _____ml of Solution A into _____ ml Soyabean Casein Digest Medium.

Autoclave Media Reference No. :
Incubation Temp. : 30-35^{0}C for 18-24 hours
Date of Incubation :

1.0 Test for *Escherichia coli* :
(Limit: Should be absent)

Name of Media : **Soyabean Casein Digest Medium**
Volume of Sample : ____ ml from solution ___
Autoclave Media Reference No. :
Incubation Temp. : 30-35^{0}C for 18-24 hours
Date of Incubation :

(I)

Name of Media : **MacConkey Broth**
Autoclave Media Reference No. :
Incubation Temp. : 42-44^{0}C for 24 to 48hours
Date of Incubation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Turbidity Observed	

(II) Secondary Test:

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Name of Media : **MacConkey Agar**
Autoclave Media Reference No. :
Incubation Temp. : 30^0C - 35^0C 18 to 72 hours.
Date of Incubation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Cfu/ml	

Confirmatory test:
Observation:
Remarks: *E. coli* is present / absent in above sample.

Analyzed By:
Date:

Checked By:
Date:

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ANNEXURE – III
MICROBIAL ENUMERATION TEST REPORT FOR *SALMONELLA*

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample: Dissolve _____gm/ mL of sample in ____mL Soyabean Casein Digest Broth Medium and adjust the volume to 100 ml. Incubate at 30-35°C for 18 to 24 hours. **(Solution C)**

Test for *Salmonella*:
(Limit: Should be absent)

(I)
Name of Media : **Rappaport Vassiliadis *Salmonella* Enrichment Broth**
Autoclave Media Reference No. :
Volume of Sample : ____ ml from solution ___
Incubation Temp. : 30^0C - 35^0C 18 to 24 hours.
Date of Incubation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Turbidity Observed	

(II)
Name of Media : **Xylose Lysine Deoxycholate Agar**
Autoclave Media Reference No. :
Incubation Temp. : 30^0C - 35^0C 18 to 48 hours
Date of Incubation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Cfu /ml	

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Confirmatory test:
Observation:
Remarks: *Salmonella* is present / absent in above sample.

Analyzed By:
Date:

Checked By:
Date:

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ANNEXURE – IV
MICROBIAL ENUMERATION TEST REPORT FOR *STAPHYLOCOCCUS AUREUS*

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample:
Solution B: Transfer _____ml of Solution A into _____ ml Soyabean Casein Digest Medium.
Name of Media : Soyabean Casein Digest Medium
Autoclave Media Reference No. :
Incubation Temp. : 30-35^{0}C for 18-24 hours
Date of Incubation :
Test for *Staphylococcus aureus*:
(Limit: Should be absent)
(I)
Name of Media : Mannitol Salt Agar
Autoclave Media Reference No.:
Incubation Temp. : 30^{0}C - 35^{0}C 18 to 72 hours.
Date of Incubation :
Date of Observation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Cfu /ml	

Confirmatory test:
Observation:
Remarks: *Staphylococcus aureus* is present / absent in above sample.

Analyzed By: **Checked By:**
Date: **Date:**

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ANNEXURE – V
MICROBIAL ENUMERATION TEST REPORT FOR *PSEUDOMONAS AERUGINOSA*

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample:

Solution B: Transfer _____ml of Solution A into _____ ml Soyabean Casein Digest Medium.

Name of Media : Soyabean Casein Digest Medium

Autoclave Media Reference No. :

Incubation Temp. : 30-35^{0}C for 18-24 hours

Date of Incubation :

Test for *Pseudomonas aeruginosa*:

(Limit: Should be absent)

(I)

Name of Media : Cetrimide Agar

Autoclave Media Reference No. :

Incubation Temp. : 30^{0}C - 35^{0}C 18 to 72 hours.

Date of Incubation :

Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Cfu /ml	

Confirmatory test:

Observation:

Remarks: *Pseudomonas aeruginosa* is present / absent in above sample.

Analyzed By: **Checked By:**

Date: **Date:**

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ANNEXURE – VI
MICROBIAL ENUMERATION TEST REPORT FOR BILE – TOLERANT GRAM NEGATIVE BACTERIA (ENTEROBACTERIA)

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample:

Solution B: Transfer _____ml of Solution A into _____ ml Soyabean Casein Digest Medium.

Name of Media : Soyabean Casein Digest Medium
Autoclave Media Reference No. :
Incubation Temp. : 30-35^{0}C for 18-24 hours
Date of Incubation :
Test for Bile – Tolerant Gram Negative Bacteria (Enterobacteria):
(Limit: Should be absent)

Name of Media : Enterobacteria Enrichment Broth
Volume of Sample : _____ ml from solution _____
Autoclave Media Reference No. :
Incubation Temp. : 30-35^{0}C for 24-48 hours
Date of Incubation :

Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Turbidity Observed	

Name of Media : Violet Red Bile Glucose Agar
Autoclave Media Reference No. :
Incubation Temp. : 30^{0}C - 35^{0}C 18 to 24 hours.
Date of Incubation :
Date of Observation :
Observation Table:

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Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Cfu/ ml	

Confirmatory Test:
Observation:
Remark: The Bile – Tolerant Gram Negative Bacteria (Enterobacteria) is present / absent in above sample.

Analyzed By:
Date:

Checked By:
Date:

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ANNEXURE – VII
MICROBIAL ENUMERATION TEST REPORT FOR *SHIGELLA*

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample: Dissolve _____gm/ mL of sample in ____mL Soyabean Casein Digest Broth Medium and adjust the volume to 100 ml. Incubate at 30-35°C for 18 to 24 hours. **(Solution C)**

Test for *Shigella*:
(Limit: Should be absent)

Name of Media : **GN Broth**
Volume of Sample : _____ ml from solution _____
Autoclave Media Reference No. :
Incubation Temp. : 30-35^{0}C for 24-48 hours
Date of Incubation :
Date of Observation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Turbidity Observed	

Name of Media : **Xylose Lysine Deoxycholate Agar**
Autoclave Media Reference No. :
Incubation Temp. : 30^{0}C - 35^{0}C 24 to 48 hours
Date of Incubation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Turbidity Observed	

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Confirmatory test:
Observation:
Remarks: *Shigella* is present / absent in above sample.

Analyzed By: **Checked By:**
Date: **Date:**

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ANNEXURE – VIII
MICROBIAL ENUMERATION TEST REPORT FOR *CLOSTRIDIA*

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample:

Solution B: Transfer _____ml of Solution A into _____ ml Soyabean Casein Digest Medium.

Name of Media : **Soyabean Casein Digest Medium**
Autoclave Media Reference No. :
Incubation Temp. : 30-35^{0}C for 18-24 hours
Date of Incubation :

Test for *Clostridia*:
(Limit: Should be absent)

Water Bath ID No. :
Heat Shock Temp. : 80^{0}C for 10 minutes

Name of Media : **Reinforced Medium for Clostridia**
Autoclave Media Reference No. :
Incubation Temp. : 30 – 35^{0}C for 18 to 48 hours in Anaerobic Condition
Date of Incubation/Inoculation :
Observation Table:

Date of Observation	Observation		Positive Control		Negative Control
	Tube 1	Tube 2	Passage No.	Turbidity Observed	

Name of Media : **Columbia Agar with Gentamicin (20 mg /ltr)**
Autoclave Media Reference No. :
Incubation Temp. : 30^{0}C - 35^{0}C for 18 to 48 hours in Anaerobic Condition
Date of Incubation :
Date of Observation :

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Observation Table:

Date of Observation	Observation		Positive Control		Negative Control
	Plate 1	Plate 2	Passage No.	Cfu / ml	

Catalase Test :
Confirmatory test:
Observation:
Remarks: *Closridia* is present / absent in above sample.

Analyzed By:
Date:

Checked By:
Date:

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ANNEXURE – IX
MICROBIAL ENUMERATION TEST REPORT FOR *CANDIDA ALBICANS*

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt.**	
Sampled Qty.		**Sampled On**	
Sampled By		**Date of testing**	
Analysed By		**Date of release**	
Media Reference No.		**Incubator ID No.**	
Method use			

Preparation of Sample: Dissolve _____gm/ mL of sample in ____mL Sabouraud Dextrose Broth Medium and adjust the volume to 100 ml. Incubate at 20^0C - 25^0C 72 to 120 hours. **(Solution D)**

Test for *Candida albicans*:
(Limit: Should be absent)
Name of Media : Sabouraud Dextrose Broth
Autoclave Media Reference No. :
Incubation Temp. : 20^0C - 25^0C 72 to 120 hours.
Sample Qty :
Date of Incubation :
Date of Observation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Turbidity Observed	

Name of Media : Sabouraud Dextrose Agar/Sabouraud Chloramphenicol Agar
Autoclave Media Reference No. :
Incubation Temp. : 30^0C - 35^0C 24 to 48 hours.
Date of Incubation :
Date of Observation :
Observation Table:

Date of Observation	Observation	Positive Control		Negative Control
		Passage No.	Cfu/ ml	

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Confirmatory test:
Observation:

Remarks: *Candida albicans* is present / absent in above sample.

Remarks: The above sample is complies/does not complies as per IP/BP/USP/IH specification.

Analyzed By:
Date:

Checked By:
Date:

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ANNEXURE – X
SAMPLE RECEIPT/ANALYSIS RECORD FOR MICROBIAL LIMIT TEST

S. No.	Receipt Date	Product /Material Name	Batch No.	Mfg. Date	Exp. Date	Sample Qty. Received	A. R. No.	Analyzed By Sign/date	Status Released/ not Released	Release Date	Released By (Sign/Date)	Checked By (Sign/ Date)	Remarks