



STANDARD OPERATING PROCEDURE

Department: Microbiology

SOP No.:

Title: Microbial Limit Test of Raw Materials, Finished Products and In Process Sample

Effective Date:

Supersedes: Nil

Review Date:

Issue Date:

Page No.:

1.0 OBJECTIVE:

To lay down a procedure for Microbial Limit Test of Raw Materials, Finished Products and In-Process Samples.

2.0 SCOPE:

This SOP is applicable for Microbial Limit Test of Raw Materials, Finished Products and In-Process Samples of Quality Control.

3.0 RESPONSIBILITY:

Operating Person: Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

| | |
|------|--------------------------------|
| SOP | Standard Operating Procedure |
| QC | Quality Control |
| SCA | Soyabean Casein Digest Agar |
| SDA | Sabouraud Chloramphenicol Agar |
| SDB | Sabouraud Dextrose Broth |
| hrs | Hours |
| ml | Milliliter |
| UV | Ultra Violet |
| LAF | Laminar Air Flow |
| SCM | Soyabean Casein Digest Medium |
| TAMC | Total Aerobic Microbial count |
| TYMC | Total Yeast and Mold count |
| ML | Microbiology Laboratory |
| NG | No Growth Observed |
| G | Growth Observed |



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6.0 PROCEDURE:

Prerequisite for Microbiological analysis of Raw Materials, Finish Products and in process Sample:

| S.No. | Requirements |
|-------|--|
| 1. | Sample of Raw Material, Finish Product and In process. |
| 2. | Sample container/tray |
| 3. | Sporocidal disinfectant |
| 4. | Sterile Tips (20-200µl) (100 – 1000µl) (1000µl - 10000 µl) |
| 5. | Sterile Empty Beaker/ Bottle |
| 6. | Sterile Empty Petriplates |
| 7. | Sterile Scissor, Forcep |
| 8. | Preincubated Media Tubes and Media Plates |
| 9. | Weight Box |
| 10. | Filtered 70%IPA Solution |
| 11. | Sterile lint Free Cloth/Sterile Mopper |
| 12. | Calibrated Micropipette |
| 13. | Infrared Gun |
| 14. | Balance |

6.1 Pre-Treatment of Sample:

After receiving the samples for microbial limit test, record the details of samples in sample receiving record e.g. S.No., Date of receipt, Product Name, Batch No., Sample quantity, AR. No. etc. in Annexure-X, Titled “Sample Receipt/Analysis Record For Microbial Limit Test” Format.

Collect all samples to be tested for the microbial limit test, sanitize the external surface of all samples subjected for microbial limit test by using sporocidal disinfectant and put in previously sanitized sample container/tray. Transfer the sample through dynamic pass box to microbial limit test area.

Use specified quantity of sample as required and prepare the sample as follows:

6.1.1 Water-Soluble Products: Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in Soyabean Casein Digest Broth added with 0.1% polysorbate 80. Further dilution, where necessary, are prepared with the same diluent (**Solution A**).

6.1.2 Non-fatty Products Insoluble in water: Dissolve the product to be examined (usually a 1 in 10 dilution is prepared) in Soyabean Casein Digest Broth added with 0.1% polysorbate 80. Further dilution, where necessary, are prepared with the same diluent (**Solution A**).

6.1.3 Fatty Products: Dissolve in isopropyl myristate sterilized by filtration, or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface active reagent heated, if necessary, to not more than 40°C or in exceptional cases to not more than 45°C, Mix carefully and if necessary maintain the



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temperature in water bath. Add a sufficient quantity of pre-warmed chose diluents to make a 1 in10 dilution of the original product. Mix carefully while maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial 10 fold dilutions may be prepared using the chosen diluent containing a suitable concentration sterile polysorbate 80 or another non-inhibitory sterile surface active reagent. **(Solution A).**

6.1.4 Products which are insoluble/immiscible in water; should be appropriately treated (Crush with pestle mortar/heating at 37°C) to obtain a suspension.

6.2 Pour Plate Method for TAMC and TYMC:

6.2.1 Use two pre-sterilized petri plates (diameter 90mm) each for TAMC and TYMC.

6.2.2 For TAMC: Add 1 ml from solution A into two pre-sterilized petri plates and pour 20-25 ml sterilized soyabean casein digest agar (SCA) (cool up to 45°C, checks with IR gun) and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of sample.

6.2.3 Negative Control: Add 1 ml from the chosen diluent to sterile petriplate and pour 20-25 ml of sterile soyabean casein digest agar (SCA) (cool up to 45°C, checks with IR gun).

6.2.4 Allow the medium to solidify and incubate at 30-35°C for 3-5 days in inverted position.

6.2.5 For TYMC: Add 1 ml from solution A into two pre-sterilized petri plates and pour 20-25 ml sterilized sabouraud chloramphenicol agar (SDA) (cool up to 45°C,checks with IR gun) and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of sample.

6.2.6 Negative Control: Add 1 ml from the chosen diluents in to sterile petriplates and pour about 20-25 ml of sterile sabouraud chloramphenicol agar (SDA) (cool up to 45°C, checks with IR gun) in the petri dishes.

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

6.2.8 After completion of incubation calculate the CFU per gm or per ml of sample being examined and record the observation in Annexure-I, Titled “Microbial Limit Test Report”.

6.2.9 Negative control should not show any growth.

6.3 Interpretation of TAMC and TYMC Results:

6.3.1 After completion of incubation period; observe the plates and express the result as colony forming unit (CFU) per g/ml, by multiplying an average number of cfu/plate with dilution factor.

6.3.2 If no colonies are observed in both petri plates express the result as less than one and final results shall be express the number of colonies less than dilution factor.
i.e.



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Plate 1: No colony
Plate 2: No colony
Average Count: $(< 1 + < 1) / 2 = < 1$
Dilution factor: 10
Final Result: $< 1 \times 10 = < 10$ CFU/ml or gm

6.3.3 If no colony is observed in one petri plate and one colony is observed in other petri plate; average shall be calculate and express the result as one and final results shall be express the number of colonies multiply with dilution factor.

i.e.
Plate 1: No colony
Plate 2: 01 colony
Average Count: $(< 1 + 01) / 2 = 0.5 = 1$
Dilution factor: 10
Final Result: $01 \times 10 = 10$ CFU/ml or gm.

6.3.4 If one colony is observed in one petri plate and two colony is observed in other petri plate; average shall be calculate and express the result as two and final results shall be express the number of colonies multiply with dilution factor.

i.e.
Plate 1: 01 colony
Plate 2: 02 colonies
Average Count: $(01 + 02) / 2 = 1.5 = 02$
Dilution factor: 10
Final Result: $02 \times 10 = 20$ CFU/ml or gm

6.3.5 If colonies of fungi are detected on soyabean casein digest agar (SCA), they are counted as part of TAMC and if colonies of bacteria are detected on sabouraud chloramphenicol agar (SDA) they are counted as part of TYMC.

6.4 Tests for Specified Micro Organisms (Pathogens):

6.4.1 Bile-Tolerant Gram-Negative Bacteria (Enterobacteria):

6.4.1.1 Qualitative Test:

6.4.1.1.1 Incubate the solution A at 20-25°C for 2-5 hours.

6.4.1.1.2 After Incubation; transfer 10 ml from solution A to 90 ml pre-incubated Enterobacteria Enrichment Broth-Mossel medium (EEM) and Incubate the medium at 30-35°C for 24 to 48 hrs.

6.4.1.1.3 After Incubation of Enterobacteria Enrichment Broth-Mossel medium, Subculture a loop full on pre-incubated plates of Violet Red Bile Glucose Agar (VBA) and incubate the plate at 30 to 35°C for 18 to 24 hours in inverted position.



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6.4.1.1.4 After incubation completion; observe the plates for growth. If the above media shows violet colored colonies indicates the possible presence of Bile-Tolerant Gram-Negative Bacteria (Enterobacteria) which shall be confirmed by gram staining and Vitek-2 compact identification system.

6.4.1.1.5 If there are no growth observed; it indicates absence of Enterobacteria.

6.4.1.1.6 Process Negative Control: Take 10ml of preincubated soyabean casein digest broth containing 0.1% Polysorbate 80 and inoculate into 90 ml SCM and incubate at 20-25°C for 2-5 hrs. After incubation; shake the container, transfer 10 ml portion in to 90 ml of Pre-incubated Enterobacteria Enrichment Broth-Mossel and incubate at 30-35°C for 24-48 hours. After incubation completion; subculture a loop full on pre-incubated plates of Violet Red Bile Glucose Agar and incubate at 30-35°C for 18 to 24 hours in inverted position.

6.4.1.1.7 Negative control should not show any growth.

6.4.1.2 Quantitative Test:

6.4.1.2.1 Incubate solution A at 20 -25°C for 2-5 hours.

6.4.1.2.2 Inoculate suitable quantities of Enterobacteria Enrichment Broth Mossel with the Preparation as directed under sample preparation and Pre-incubation and/or dilutions of it containing respectively 0.1g, 0.01 g and 0.001 g (or 0.1ml, 0.01 ml and 0.001 ml) of the product to be examined. Incubate at 30-35°C for 24 to 48 hours. After incubation completion; subculture a loop full each of dilutions on pre-incubated plates of Violet Red Bile Glucose Agar and Incubate at 30-35°C for 18 to 24 hours in inverted position.

6.4.1.2.3 Interpretation-Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine the following table for the probable number of bacteria.

| Results for Each Quantity of the Product | | | |
|---|-------------------|---------------------|---|
| 0.1g or 0.1 ml | 0.01 g or 0.01 ml | 0.001 g or 0.001 ml | Probable Number of Bacteria per g or ml of product |
| + | + | + | More than 10 ³ |
| + | + | - | Less than 10 ³ and more than 10 ² |
| + | - | - | Less than 10 ² and more than 10 |
| - | - | - | Less than 10 |

6.4.1.2.4 Process Negative Control: Use 01 ml chosen diluent and inoculate in to 09 ml of Pre-incubated Enterobacteria Enrichment Broth-Mossel and serially dilute upto three tubes containing 09 ml Enterobacteria Enrichment Broth-Mossel and incubate at 30-35°C for 24-48 hours. After incubation completion; shake the test tubes & subculture a loop full on pre-



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incubated plates of Violet Red Bile Glucose Agar and incubate at 30-35°C for 18 to 24 hours in inverted position.

6.4.2 Test for Escherichia coli:

6.4.2.1 Sample Preparation: Transfer 10 ml from Solution A to 90 ml preincubated Soyabean Casein Digest Broth Medium (SCM) containing 0.1% polysorbate 80 and incubate at 30- 35°C for 18 to 24 hrs. (Solution B).

6.4.2.2 After incubation completion of Solution B, Shake the broth and transfer 1ml from Solution B to 100 ml of pre-incubated MacConkey Broth. Incubate at 42- 44°C for 24 to 48 hrs.

6.4.2.3 After Incubation completion of MacConkey Broth; shake the Broth and subculture a loop full on pre-incubated plates of MacConkey Agar and incubate at 30- 35°C for 18 to 72 hours in inverted position.

6.4.2.4 If the above media shows pink, non-mucoid colonies indicates the presence of *E. coli* which shall be confirmed by Vitek-2compact identification system.

6.4.2.5 If there are no growth observed it indicates absences of *E. coli*.

6.4.2.6 Process Negative Control: Take 10ml of preincubated soyabean casein digest broth containing 0.1% Polysorbate 80 in 90 ml SCM and incubate at 30-35°C for 18-24 hrs. After incubation completion; transfer 1 ml sample to sterile MacConkey broth and incubate at 42-44°C for 24-48 hrs. After incubation completion; subculture a loop full on pre-incubated plates of Mac Conkey agar and incubate at 30-35°C for 18-72 hours in inverted position.

6.4.2.7 Negative control should not show any growth.

6.4.3 Test for Staphylococcus aureus:

6.4.3.1 After incubation of Solution B, shake the broth and subculture a loop full on pre-incubated plates of Mannitol Salt Agar medium and incubate at 30-35°C for 18 to 72 hours in inverted position.

6.4.3.2 If the above media shows Yellow or white colonies surrounded by a yellow zone indicate the presence of *Staphylococcus aureus* which shall be confirmed by Vitek-2compact identification system.

6.4.3.3 If there are no growth observed it indicates absences of *Staphylococcus aureus*.

6.4.3.4 Process Negative Control: Take 10ml of preincubated soyabean casein digest broth containing 0.1% Polysorbate 80 in 90 ml SCM containing 0.1% polysorbate 80 and incubate at 30-35°C for 18-24 hrs. After incubation completion; subculture a loop full on pre-incubated plates of Mannitol Salt Agar medium and incubate at 30 to 35°C for 18 to 72 hours in inverted position.

6.4.3.5 Negative control should not show any growth.



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6.4.4 Test for *Pseudomonas aeruginosa*:

6.4.4.1 After incubation completion of Solution B, shake the broth and subculture a loop full on plates of pre-incubated Cetrimide Agar medium plate and incubate at 30 to 35°C for 18 to 72 hours in inverted position.

6.4.4.2 If the above media shows Green colonies indicates the presence of *Pseudomonas aeruginosa* which shall be confirmed by Vitek-2 compact identification system.

6.4.4.3 If there are no growth observed it indicates absences of *Pseudomonas aeruginosa*.

6.4.4.4 Process Negative Control: Take 10ml of preincubated soyabean casein digest broth containing 0.1% Polysorbate 80 in 90 ml SCM containing 0.1% polysorbate 80 and incubate at 30-35°C for 18-24 hrs. After incubation; subculture a loop full on pre-incubated plates of Cetrimide Agar medium (CTA) and incubate at 30-35°C for 18 to 72 hours in inverted position.

6.4.4.5 Negative control should not show any growth.

6.4.5 Test for *Clostridia*:

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

6.4.5.2 Transfer each of the homogenized portions in two tubes containing 90 ml pre-incubated Reinforced medium for Clostridia. Incubate the tubes under anaerobic condition at 30-35°C for 48 hrs.

6.4.5.3 After incubation completion; subculture a loop full from each container on pre-incubated plates of Columbia agar and incubate under anaerobic conditions at 30-35°C for 48 to 72 hrs.

6.4.5.4 The Presence of anaerobic growth of Gram positive bacilli with or without endospores, giving a negative catalase test indicates the possible presence of *Clostridia* which shall be confirmed by Vitek-2 compact identification system.

6.4.5.5 If there are no growth observed; it indicates absences of *Clostridia*.

6.4.5.6 Process Negative Control: Add 10 ml of chosen diluent in 90 ml of pre-incubated Reinforced medium for Clostridia. Incubate the tubes under anaerobic condition at 30-35°C for 48 hrs. After incubation completion; subculture a loop full on pre-incubated plates of Columbia agar. Incubate under anaerobic conditions at 30-35°C for 48-72 hrs.

6.4.5.7 Negative control should not show any growth.

6.4.6 Test for *Salmonella*:



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Preparation of sample: Dissolve 10g or 10ml of test sample in 90 ml of preincubated Soybean Casein Digest Medium (SCM) containing 0.1% polysorbate 80 and incubate at 30-35°C for 18-24 hours (**Solution C**).

6.4.6.1 Transfer 0.1 ml of the enrichment culture from solution C to 10 ml of pre-incubated Rappaport Vassiliadis Salmonella Enrichment Broth (RVS) and incubate at 30-35°C for 18-24 hours.

6.4.6.2 After incubation completion; shake the test tube and subculture a loop full on pre-incubated plates of Xylose lysine Deoxycholate Agar (XLD) and incubate at 30-35°C for 18-48 hours in inverted position.

6.4.6.3 If the above media shows well-developed red colonies with or without black center indicates the possible presence of *Salmonella* which shall be confirmed by Vitek-2compact identification system.

6.4.6.4 If there are no growth observed it indicates absence of *Salmonella*.

6.4.6.5 Process Negative Control: Take 10ml of preincubated soyabean casein digest broth containing 0.1% Polysorbate 80 in 90 ml SCM added with 0.1% polysorbate 80 and incubate at 30-35°C for 18-24 hrs. After incubation transfer 0.1 ml to 10 ml of sterile RVS Broth and incubate at 30-35°C for 18-24 hrs. After incubation completion; subculture a loop full on pre-incubated plates of Xylose lysine Deoxycholate Agar (XLD) and incubate at 30-35°C for 18-48 hours in inverted position.

6.4.6.6 Negative control should not show any growth.

6.4.7 Test for *Shigella*:

6.4.7.1 Transfer 1 ml of the enrichment culture from **Solution C** to 100 ml of pre-incubated GNB broth and incubate at 30-35°C for 24-48 hours. After incubation completion; shake the test tube & subculture a loop full on pre-incubated plates of Xylose Lysine Deoxycholate Agar (XLD) and Incubate at 30-35°C for 24-48 hours in inverted Position.

6.4.7.2 If the above media shows red color translucent colony without black center indicates the possible presence of *Shigella* which shall be confirmed by Vitek-2compact identification system.

6.4.7.3 If there are no growth observed it indicates absence of *Shigella*.

6.4.7.4 Process Negative Control: Take 10ml of preincubated soyabean casein digest broth containing 0.1% Polysorbate 80 in to 90 ml SCM added with 0.1% polysorbate 80 and incubate at 30-35°C for 18-24 hrs. After incubation transfer 1 ml to 100 ml of sterile GNB Broth and incubate at 30-35°C for 24-48 hrs. After incubation completion; subculture a loop full on pre-incubated plates of Xylose lysine Deoxycholate Agar (XLD) and incubate at 30-35°C for 24-48 hours in inverted position.

6.4.7.5 Negative control should not show any growth.



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6.4.8 Test for *Candida albicans*:

6.4.8.1 Take the 10 ml of sample from solution-A in 90 ml of Sabouraud Dextrose Broth (SDB) and incubate at 30 to 35°C for 3 to 5 days. **(Solution D)**

6.4.8.2 After Incubation completion; subculture a loop full from Solution D on pre-incubated plates of Sabouraud Chloramphenicol agar medium (SDA) and incubate the plate at 30- 35°C for 24 to 48 hours in inverted position.

6.4.8.3 If the above media shows cream colored colonies may indicate the possible presence of *Candida albicans* which shall be confirmed by Vitek-2 compact identification system.

6.4.8.4 If there are no growth observed it indicates absences of *Candida albicans*.

6.4.8.5 Process Negative Control: Take 10ml of preincubated soyabean casein digest broth containing 0.1% Polysorbate 80 in to 90 ml Sabouraud Dextrose Broth (SDB) and incubate at 30 to 35°C for 3 to 5 days. After incubation subculture a loop full on pre-incubated plates of Sabouraud Chloramphenicol Agar (SDA) and incubate at 30- 35°C for 24 to 48 hrs.

NOTE: *If there is a holiday on the day of transfer/release of media tubes and plates, observation of media plates and tubes shall be taken on next working day.*

6.5 Interpretation of Test Results of Specified Microorganism:

6.5.1 If no growth is observed express the results as no growth observed (NG).

6.5.2 If growth is observed express the results as growth observed (G) and identification tests are negative, than the product complies with the test for absence of specified organism.

6.6 Microbiological Limit Test shall be performed as following:

6.6.1 Raw materials or Bulk Sample: As per specification.

6.6.2 Finished Product:

- Process Validation Batch
- Every 10th Batch
- As per customer requirement

7.0 ANNEXURES:

| ANNEXURE No. | TITLE OF ANNEXURE | FORMAT No. |
|--------------|---|------------|
| Annexure-I | Microbial Limit Test Report | |
| 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
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- Master Copy Quality Assurance

9.0 REFERENCES:

- Indian Pharmacopoeia Chapter <2.2.9>
- British Pharmacopeia Chapter <Appendix XVI B>
- United State Pharmacopeia Chapter <61,62>
- WHO Technical Report Series, No. 961

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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



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ANNEXURE – I
MICROBIAL LIMIT TEST REPORT

| | | |
|---------------------|--------------------------|--|
| Product Name | A.R. No. | |
| Batch No. | Date of Release | |
| Balance ID.: | Micropipette ID.: | |

Preparation of Sample: Solution A - Dissolve _____ gm/mL of sample in ____ ml preincubated Soyabean Casein Digest Broth added with 0.1 % polysorbate 80.

Media Reference No. : SCM/

TOTAL AEROBIC MICROBIAL COUNT:

Name of Media : Soyabean Casein Digest Agar (SCA)
Volume of Sample : _____ ml from Solution _____
Media Reference No. : SCA/
Incubation Condition : 30-35°C for 3-5 days,
Incubator ID. :
Analyzed By :
Date :

| Observation Date | Observation | | Average Count | Average count X dilution factor | Total cfu/gm or ml of Sample | Observed By |
|------------------|-------------|---------|----------------|---------------------------------|------------------------------|-------------|
| | Plate 1 | Plate 2 | | | | |
| | | | + _____ = 2 | | | |

Negative Control:

TOTAL YEAST AND MOULD COUNT

Name of Media : Sabouraud Chloramphenicol Agar (SDA)
Volume of Sample : _____ ml from Solution _____
Media Reference No. : SDA/
Incubation Temp. : 20-25°C for 5-7 days,
Incubator ID. :
Analyzed By :
Date :

| Observation Date | Observation | | Average Count | Average count X dilution factor | Total cfu/gm or ml of Sample | Observed By |
|------------------|-------------|---------|----------------|---------------------------------|------------------------------|-------------|
| | Plate 1 | Plate 2 | | | | |
| | | | + _____ = 2 | | | |

Negative Control:

TEST OF SPECIFIED MICROORGANISMS:



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Enrichment of test for specified Microorganisms: Inoculate _____ ml sample from solution A into _____ ml pre-incubated SCM media added with 0.1% polysorbate 80 (**Solution B**) for *E. coli*, *S.aureus*, *P.aeruginosa*,
Incubator ID. No.:

| SCM/ | 30-35° for 18-24 Hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|------|-----------------------|----------------|-------------|------------------|-------------------|
| | | | | | |

Primary Test For Specified Micro organisms

Incubator ID. No.:

Incubator ID. No.:

| E.coli | MCB/ | 42-44°C for 24-48 hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|---------------------|------|------------------------|----------------|--------------------|-------------------------|-------------------|
| | | | | | | |
| S.aureus | MSA/ | 30-35°C for 18-72 hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| | | | | | | |
| P.aeruginosa | CTA/ | 30-35°C for 18-72 hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| | | | | | | |

Dissolve _____ gm/ml sample in _____ ml pre-incubated SCM Media added with 0.1 % polysorbate 80 (**Solution C**) For *Salmonella*, *Shigella*.

Incubator ID. No.:

| SCM/ | 30-35°C for 18-24 hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date | |
|-------------------|------------------------|-----------------------|----------------|--------------------|-------------------------|-------------------|
| | | | | | | |
| Salmonella | RVS/ | 30-35°C for 18-24hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| | | | | | | |
| Shigella | GNB/ | 30-35°C for 24-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| | | | | | | |

Bile Tolerant gram negative Bacteria: Incubate Solution A at 20-25° for 2 to 5 hours.

Incubator ID. No.:

| SCM/ | 20-25°C for 02-05hrs | Tested By/Date | | | |
|------|----------------------|----------------|--|--|--|
| | | | | | |

Qualitative Test: After incubation at 20-25° for 2 to 5 hours inoculate _____ ml sample in _____ ml pre-incubated Enteriobacteriaceae Enrichment broth mossel media for **Bile Tolerant Gram Negative Bacteria**.

Incubator ID. No.:

| Bile Tolerant Gram Negative Bacteria | EEM/ | 30-35°C for 24-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|--------------------------------------|------|-----------------------|----------------|-------------|------------------|-------------------|
| | | | | | | |

Quantitative Test: After incubation at 20-25° for 2 to 5 hours inoculate it containing respectively 0.1, 0.01, 0.001ml/gm of the product in _____ ml pre-incubated Enteriobacteriaceae Enrichment broth mossel Media for **Bile Tolerant Gram Negative Bacteria**.

Incubator ID. No.:

| 0.1g or 0.1 ml | EEM/ | 30-35°C for 24-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|----------------|------|-----------------------|----------------|-------------|------------------|-------------------|
| | | | | | | |



STANDARD OPERATING PROCEDURE

| | |
|--|------------------------|
| Department: Microbiology | SOP No.: |
| Title: Microbial Limit Test of Raw Materials, Finished Products and In Process Sample | Effective Date: |
| Supersedes: Nil | Review Date: |
| Issue Date: | Page No.: |

| | | | | | | |
|---------------------------|------|-----------------------|----------------|-------------|------------------|-------------------|
| 0.01g or 0.01 ml | EEM/ | 30-35°C for 24-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| 0.001g or 0.001 ml | EEM/ | 30-35°C for 24-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |

Take two equal portions of 10 ml from solution A in Sterile Test Tubes and heat one portion at 80°C for 10 minute and cool rapidly. Do not heat the other portion. Transfer each of the homogenized portions in two tubes containing 90 ml Pre-incubated Reinforced medium for Clostridia. Incubate the tubes under anaerobic condition at 30 to 35°C for 48 hours **for Clostridia.**

Incubator ID. No.:

| | | | | | | |
|-----------------------|------|--------------------|----------------|-------------|------------------|-------------------|
| Clostridia(I) | RMC/ | 30-35°C for 48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| Clostridia(II) | RMC/ | 30-35°C for 48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |

Inoculate _____ ml sample from solution A into _____ ml pre-incubated SDB Media (**Solution D**) for **Candida.**

Incubator ID. No.:

| | | | | | | |
|----------------|------|----------------------|----------------|-------------|------------------|-------------------|
| Candida | SDB/ | 30-35°C for 3-5 Days | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|----------------|------|----------------------|----------------|-------------|------------------|-------------------|

Secondary Test For Specified Micro organisms

Incubator ID. No.:

| | | | | | | |
|-------------------|------|------------------------|----------------|-------------|------------------|-------------------|
| E.coli | MCA/ | 30-35°C for 18-72 hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| Salmonella | XLD/ | 30-35°C for 18-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| Shigella | XLD/ | 30-35°C for 24-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |

Qualitative Test (Bile Tolerant Gram Negative Bacteria)

Incubator ID. No.:

| | | | | | | |
|---|------|-----------------------|----------------|-------------|------------------|-------------------|
| Bile Tolerant gram negative Bacteria | VBA/ | 30-35°C for 18-24hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|---|------|-----------------------|----------------|-------------|------------------|-------------------|

Quantitative Test (Bile Tolerant Gram Negative Bacteria)

Incubator ID. No.:

| Observation for each quantity of product | | | | | | | | |
|--|---------------|----------------|----------------------|-----------------------|-------------------|---------------------|--|-------------------|
| Bile Tolerant gram negative Bacteria | Media lot No. | Tested By/Date | Incubation condition | 0.1g or 0.1 ml | 0.01 g or 0.01 ml | 0.001 g or 0.001 ml | Probable Number of Bacteria per g or ml of product | Observed By/ Date |
| | | VBA/ | | 30-35°C for 18-24hrs. | | | | |



PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

| | |
|--|------------------------|
| Department: Microbiology | SOP No.: |
| Title: Microbial Limit Test of Raw Materials, Finished Products and In Process Sample | Effective Date: |
| Supersedes: Nil | Review Date: |
| Issue Date: | Page No.: |

Incubator ID. No.:

| Clostridia(I) | CLA/ | 30-35°C for 48-72hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|----------------|------|-----------------------|----------------|-------------|------------------|-------------------|
| | | | | | | |
| Clostridia(II) | CLA/ | 30-35°C for 48-72hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
| | | | | | | |

Incubator ID. No.:

| Candida | SDA/ | 30-35°C for 24-48hrs. | Tested By/Date | Observation | Negative Control | Observed By/ Date |
|---------|------|-----------------------|----------------|-------------|------------------|-------------------|
| | | | | | | |

NG : No Growth Observed

G : Growth Observed

Weight Detail Print:

Acceptance Criteria:

| Test | Test Performed | Limit | |
|-------------------------|--|--------------|----------|
| TAMC | Yes / No | Cfu/gm/ml | |
| TYMC | Yes / No | Cfu/gm/ml | |
| Pathogens | <i>Escherichia coli</i> | Yes / No | |
| | <i>Salmonella sps</i> | Yes / No | |
| | <i>Staphylococcus aureus</i> | Yes / No | |
| | <i>Pseudomonas aeruginosa</i> | Yes / No | |
| | Bile – Tolerant Gram Negative Bacteria | Qualitative | Yes / No |
| | | Quantitative | Yes / No |
| | <i>Shigella</i> | Yes / No | |
| | <i>Clostridia</i> | Yes / No | |
| <i>Candida albicans</i> | Yes / No | | |

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

Reviewed by
Date:

