PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Limit Test of Primary Packing Material	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down procedure for Microbiological Limit Test of Primary Packaging Material.

2.0 SCOPE:

This SOP is applicable to for all primary packaging material. (PVC, Plain Aluminium Foil & Blister Aluminium Foil, Printed Foil & Glass Bottle).

3.0 RESPONSIBILITY:

Microbiologist - Quality Control

4.0 ACCOUNTABILITY:

Head – QC

5.0 PROCEDURE:

5.1 SELECTION OF CONSIGNMENT OF PACKAGING MATERIALS

5.1.1 Bottles: As per party requirement

5.1.2 Aluminum Foil/PVC**:** As per party requirement.

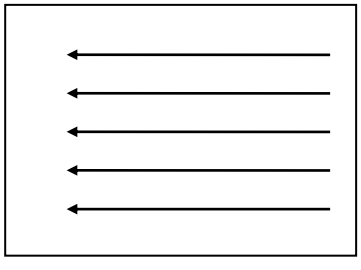
5.2 SAMPLING AND SWAB TESTING FOR TAMC AND TYMC:

5.2.1 Aluminum Foil/PVC:

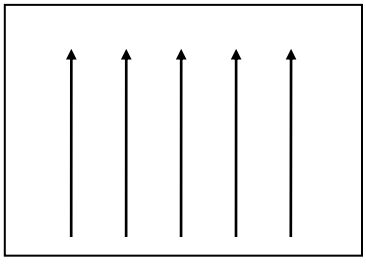
5.2.1.1 The area that swabbed shall define with a sterile template of 25 cm^2.

5.2.1.2 Microbiologist shall take swab sample as per defined location using sterile swab stick soaked in 10 ml of sterile buffered sodium chloride peptone solution.

5.2.1.3 Microbiologist shall take the swab sample in square of 5 cm x 5 cm with the help of Teflon template as per following pattern.



or



5.2.1.4 After taking the swab, label the swab properly. Swab tubes shall be transferred to Microbiology Laboratory in S.S. container disinfected by filtered 70% IPA.

5.2.1.5 Connect Filtration assembly with vacuum pump, place the 0.45 µm membrane aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder.

5.2.1.6 Wet the membrane filter with sterile 10 ml of 0.1% peptone water, vortex the swab for 1 minute and filter the 10 ml of sample fixed sterilized funnel.

5.2.1.7 Rinse the membrane with 100 ml of sterile 0.1% peptone water.

5.2.1.8 After filtrations remove the membrane aseptically with the help of sterilized forceps and transfer on Pre-incubated Soyabean Casein Digest Agar Plates.

5.2.1.9 Incubate the plates in upright position at 22.5°C±2.5°C for 72 hours followed by 32.5°C±2.5°C for 48 hours.

5.2.1.10 Count the number of colonies in the plates and expressed the result in CFU/ 25 cm^2.

5.2.1.11 **Negative Control:** Add 10 ml of sterile buffered sodium chloride peptone solution to Membrane filtration assembly and Filter it. Repeat the process for TYMC. Wash twicely Membrane Filters with 3 X 100 ml of suitable solution such as sterile 0.1% peptone water. Transfer one Membrane Filter to Pre incubated Soyabean Casein Digest Agar (SCA) medium.

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

5.2.2 **Bottles/Caps:**

5.2.2.1 Take random 10 bottles and add 10 ml of sterile buffered sodium chloride peptone solution in each bottle & after rinsing collect the 100 ml sterile buffered sodium chloride peptone in a sterile flask.

5.2.2.2 Connect filtrations assembly with vacuum pump, place the 0.45 µm membrane filter aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder.

5.2.2.3 Wet the membrane filter with approx sterile 10 ml of 0.1% peptone water, vortex the swab for 1 minute and filter the 1 ml of sample fixed sterilized funnel.

5.2.2.4 Rinse the membrane with100 ml of the sterile 0.1 % peptone water.

5.2.2.5 After filtration remove the membrane aseptically with the help of sterilized forceps and transfer on pre-incubated Soyabean Casein Digest Agar Plates.

5.2.2.6 Incubate the plates in upright position at 22.5°C ±2.5°C for 72 hours followed by 32.5°C ± 2.5°C for 48 hours days.

5.2.2.7 Count the number of colonies in the plate and express the result as CFU/ml.

5.2.2.8 **Negative Control**: Add 10 ml of sterile buffered sodium chloride peptone solution 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 sterile 0.1% peptone water. Transfer one Membrane Filter to Pre incubated Soyabean Casein Digest Agar (SCA) medium.

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

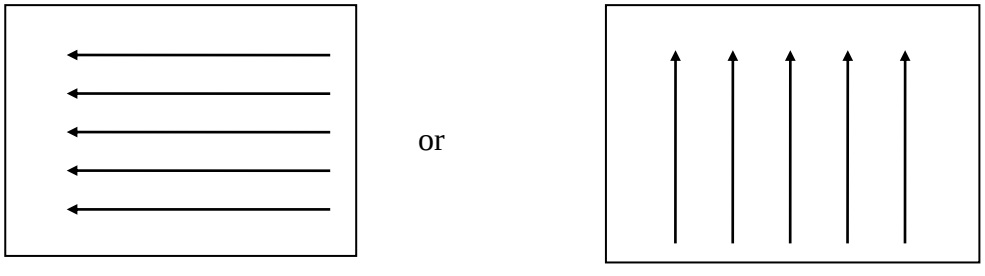
5.3 SAMPLING AND TESTING FOR PATHOGENS:

5.3.1 Aluminum Foil/PVC:

5.3.1.1 The area that swabbed shall define with a disinfectant Teflon template of 25 cm^2.

5.3.1.2 Microbiologist shall take swab sample as per defined location using sterile swab stick soaked in 10 ml of sterile buffered sodium chloride peptone solution.

5.3.1.3 Microbiologist shall take the swab sample in square of 5 cm x 5 cm with the help of disinfectant Teflon as per following pattern and take to Microbiology Laboratory for analysis.



5.3.1.4 After taking the swab, labeled the swab properly. Swabs tubes shall be transferred to Microbiology Laboratory in S.S. container disinfected by filtered 70% IPA.

5.3.1.5 Connect Filtration assembly with vacuum pump, place the 0.45 µm membrane aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder.

5.3.1.6 Wet the membrane filter with sterile 10 ml of 0.1% peptone water, and filter the 10 ml of sample fixed sterilized funnel.

5.3.1.7 Rinse the membrane with 100 ml of sterile 0.1% peptone water.

5.3.1.8 After filtrations remove the membrane aseptically with the help of sterilized forceps and transfer on Pre-incubated Soyabean Casein Digest Broth Medium and incubate at 30 to 35 ˚C for 18 to 24 hrs. (Solution A)

5.3.1.9 **Negative Control:** Add 10 ml of sterile buffered sodium chloride peptone solution 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 sterile 0.1% peptone water. Transfer one Membrane Filter to Pre incubated Soyabean Casein Digest Broth medium.

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

5.3.2 **Bottles/Caps:**

5.3.2.1 Take random 10 bottles extraction with sterile buffered sodium chloride peptone solution collect extract in volumetric flask make up volume 100 ml.

5.3.2.2 Connect filtrations assembly with vacuum pump, place the 0.45 µm membrane filter aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder.

5.3.2.3 Wet the membrane filter with approx sterile 10 ml of 0.1% peptone water, vortex the swab for 1 minute and filter the 10 ml of sample by fixed sterilized funnel.

5.3.2.4 Rinse the membrane with100 ml of the sterile 0.1 % peptone water.

5.3.2.5 After filtration remove the membrane aseptically with the help of sterilized forceps and transfer on pre-incubated Soyabean Casein Digest Broth Medium and incubate at 30 to 35 ˚C for 18 to 24 hrs. (Solution B)

5.3.2.6 **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 sterile 0.1% peptone water. Transfer one Membrane Filter to Pre incubated Soyabean Casein Digest Agar (SCA) medium.

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

5.3.3 **Test for *Escherichia coli*:**

5.3.3.1 After incubation of Solution A/Solution B, Shake the broth and transfer 1ml to 100 ml of Preincuabted MacConkey Broth. Incubate at 42 ºC to 44 ºC for 24 to 48 hrs.

5.3.3.2 Subculture on a plate of Preincuabted MacConkey Agar plate and incubate at 30 ºC to 35 ºC 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.

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

5.3.3.4 **Confirmation Test**: Add 0.1 ml of contents of MacConkey 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.

5.3.3.5 **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.

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

5.3.4 **Test for *Salmonella*:**

5.3.4.1 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.*

5.3.4.2 **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.

5.3.4.3 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.

5.3.4.4 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.

5.3.4.5 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.

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

5.3.5 **Staphylococcus aureus:**

5.3.5.1 After incubation of Solution A/Solution A1, 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.

5.3.5.2 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.

5.3.5.3 **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.

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

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

5.3.6 **Pseudomonas aeruginosa:**

5.3.6.1 After incubation of Solution A/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.

5.3.6.2 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.

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

5.3.6.4 **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.

5.3.6.5 **Negative Control:** Use blank Preincuabted Cetrimide Agar medium plate and incubate at 30 to 35 ºC for 18 to 72 hrs.

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

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Issue Date:	**Page No.:**

LIMITS FOR PACKING MATERIAL

Microbial Limit Test **Limit**

(A) Total viable aerobic count

1. **(For PVC/Aluminum Foil)**

i) Bacteria **:** 1000 cfu/ 25cm^2

ii) Fungi **:** 100 cfu/ 25cm^2

2. **(For Bottles/Caps)**

i) Bacteria **:** 1000 cfu/ 10 units

ii) Fungi **:** 100 cfu/ 10 units

(B) Pathogens

i) *E.coli* Should be absent
ii) *Salmonella* spp. Should be absent
iii) *Staphylococcus aureus* Should be absent
iv) *Pseudomonas aeruginosa* Should be absent

6.0 REFERENCES:

Not applicable

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure-I	Microbial Analysis Record	
Annexure -II	Total Aerobic Microbial Count Report	

ENCLOSURES: SOP training record

8.0 DISTRIBUTION:

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

9.0 ABBREVIATION:

No. Number

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Limit Test of Primary Packing Material	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Limit Test of Primary Packing Material	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE – I
MICROBIAL ANALYSIS RECORD

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

Test for Specified Microorganisms:

Filter the of sample and rinse the membrane with 100 ml of sterile 0.1% peptone water than filter paper transfer on Pre-incubated Soyabean Casein Digest Broth Medium and incubate at 30 to 35 ˚C for 18 to 24 hrs. **(Solution A /Solution B)**

Test For E.coli:
I)

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

Date of Observation	Observation	Positive Control	Negative Control

(II) Secondary Test:

Name of Media : **MacConkey 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

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

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Limit Test of Primary Packing Material	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Test for *Salmonella*:

(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 24 to 48 hours.
Date of Incubation :
Observation Table :

Date of Observation	Observation	Positive Control	Negative Control

(II)

Name of Media : **Xylose Lysine Deoxycholate Agar/Wilson Blair's BBS 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

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

Test for *Staphylococcus aureus*:

(I)

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

Date of Observation	Observation	Positive Control	Negative Control

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

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Limit Test of Primary Packing Material	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Test for *Pseudomonas aeruginosa*:
(I)
Name of Media : Cetrimide 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

Confirmatory test:
Observation:
Remarks: *Pseudomonas aeruginosa* 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:

STANDARD OPERATING PROCEDURE	
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Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE – II
TOTAL AEROBIC MICROBIAL COUNT REPORT

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

Preparation of Sample: Filter the of sample and rinse the membrane with 100 ml of sterile 0.1% peptone water than filter paper transfer on Pre-incubated Soyabean Casein Digest Agar and Incubate the plates in upright position at 22.5°C ±2.5°C for 72 hours followed by 32.5°C ± 2.5°C for 48 hours..

OBSERVATIONS TABLE FOR TOTAL BACTERIAL AND YEAST /MOULD COUNT

S.No.	Name of Rinse sample / Location of Swab sample	No. of CFU observed per plate		Total cfu / 25cm^2 or 10 units
		Incubation at 22.5°C ±2.5°C for 72 hours	Incubation at 32.5°C ±2.5°C for 48	
Negative Control				
Positive Control				

+ ve Control: **- ve Control:**

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

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