1.0 PROTOCOL APPROVAL PAGE

Name	Designation	Department	Signature/Date
Prepared By:			
Reviewed By:			
Approved By:			

2.0 OBJECTIVE

The objective of this validation study is to define the shelf-life period for sterilized glassware and other accessories used in Microbiology laboratory.

3.0 SCOPE

This Protocol is applicable for sterilized glassware and other accessories in Microbiology laboratory at PMH Pvt Limited.

4.0 RESPONSIBILITY

- **Microbiology:** Preparation & Review of the Protocol; Training of the protocol to all the executers and reviewers; Execution of the study, compilation of the data related to testing; Prepare, review of the summary report.
- **Head Microbiology:** Review of the protocol; Review of the study outcome of the summary.
- **Quality Assurance:** Review and pre-approval of the protocol; Review of the study outcome and final approval of the summary report.

5.0 SAFETY PRECAUTIONS: Not applicable.

6.0 TRAINING RECORD: All the concern personnel shall be trained and record the training details in training attendance sheet.

7.0 PROCESS METHOD DESCRIPTION

Before usage of sterilized glassware and other accessories in routine usage, the shelf life period shall be evaluated for sterilized glassware and other accessories used in Microbiology laboratory.

- **7.1 Equipment/ Materials**
- 7.1.1 Biosafety cabinet/Laminar air flow unit
- 7.1.2 BOD Incubator (20-25°C)
- 7.1.3 BOD Incubator (30-35°C)
- 7.1.4 Calibrated Colony Counter
- 7.1.5 Horizontal Autoclave
- **7.2 Elements of validation**
- 7.2.1 Direct inoculation method.
- 7.2.2 Surface swab method.
- **7.3 Materials**
- 7.3.1 Soybean Casein Digest Medium (SCD)
- 7.3.2 Tip bottle (0.1mL and 1.0mL tips)
- 7.3.3 Glass test tubes and Glass Bottles
- 7.3.4 Filter caps
- 7.3.5 Manifold
- 7.3.6 Sampling bottles
- 7.3.7 Aspirator bottle
- 7.3.8 Autoclavable petri plates

8.0 VALIDATION TESTS & RESULTS:

8.1 METHODOLOGY

8.1.1 MONITORING PLAN AND RATIONALE

Name of Articles	Method Type	Rationale			
		Initial	3rd day	7th day	
Pipette tips (1.0 and 0.1mL)	D	√	√	√	Critical contact with samples / test activity
Pipette (10.0mL)	D	√	√	√	
Sampling bottles	D	√	√	√	
Aspirator bottle	D	√	√	√	
Rubber tube	S	-	-	√	
Manifold	S	-	-	√	
Filtration funnels	S	-	-	√	

Name of Articles	Method Type	Rationale			
		Initial	3rd day	7th day	
Autoclavable petri plates	S	-	-	√	
Pipette tips (1.0 and 0.1mL)	D	√	√	√	
Pipette (10.0mL)	D	√	√	√	
Sampling bottles	D	√	√	√	
Aspirator bottle	D	√	√	√	

- **Note:** As per Garment accessories load pattern Aspirator bottle-01 No, Manifold-01 No and Rubber tube-02 No's, Aspirator bottle and Manifold shall be performed on 7th day, Rubber tube shall be performed on initial and 7th day.
- **Method Type D:** Direct inoculation of the article into the sterile media or direct addition of the sterile media in to the article.
- **Method Type S:** Swab the articles and transfer to the media for qualitative testing.

8.1.2 Procedure for Preparation of Glassware and Other Accessories:

- Glassware and other accessories shall be prepared and sterilized as per the SOP as per validation load pattern.
- Unload all the sterile articles as per the routine procedure and hold them under the Buffer zone area.
- Use Prepared Soya bean casein digest medium or use ready to use Soya bean casein digest medium for the study.
- Sample the test articles as per the intervals mentioned in Table-1.
- Perform the entire testing under the aseptic conditions.
- **Method Type D:** Unwrap the barrier paper under the LAF / BSC. Transfer the sterile articles aseptically in to the tube containing 10 / 15 mL of the pre-sterilized Soya bean casein digest medium.
- In case of sample bottles, transfer about 100 / 150 mL of the pre-sterilized Soya bean casein digest medium in to the bottle and close the lid.
- Take 100 / 150 mL Soya bean casein digest medium kept for negative control.
- **Method Type S:** Unwrap the barrier paper under the LAF conditions. Remove the swab from the tube, squeeze the bud in the inner side of tube so as to remove the excess liquid aseptically. Swab the maximum area from the article to be tested. Hold the sampled swab bud in to a 100 / 15 mL tube containing the Soya bean casein digest medium.

8.1.3 Acceptance Criteria:

- There shall not be any turbidity in the sample tubes.
- The negative control shall not show any turbidity.
- The media used in the study shall pass the Growth promotion test.
- There shall be no growth of microorganisms. A failed negative control requires an investigation.
    - **Note:** During intervals if any holidays fallen down activity can be performed next working day.

9.0 RE-VALIDATION CRITERIA:

- Re-validation shall be performed whenever change in load pattern.

10.0 DETAILS OF DISCREPANCIES

- If any discrepancy observed during qualification period initiate the deviation as per SOP.

11.0 SUMMARY / CONCLUSION:

- Summary report shall be prepared upon completion of validation study.

12.0 REFERENCES:

- SOP on “Operation of Horizontal autoclave”
- SOP on “Decontamination and disposal of used media.
- SOP on Operation of B.O.D incubators.
- SOP on Cleaning, Drying and Sterilization of Microbiology Laboratory Glassware and Other Accessories.

13.0 ANNEXURES:

- Report for sterilized glassware and other accessories hold time study.

Report for sterilized glassware and other accessories hold time study.

Start Date		A.R No:			
Activity Status		Department			
Media Used		Activity start time			
Colony counter ID		Activity end time			
Sampling details	**Intervals: 0, 3, 7, 11 Days**				

Incubation conditions	Incubator ID	Incubation Start Date & time	Sign& Date	Incubation end Date & time	Sign& Date

S.No	Name of Article	Method	Day 0	Day 3	Day 7	Day 11	Total Result
1	Pipette tips (0.1mL & 1.0mL)						
2	Glass test tubes & Bottles						
3	Forceps						
4	Sampling / Aspirator Bottles						
5	Pipette (10mL)						
6	Lint free cloth / Coupons						
7	Silicon tubes						
8	Air Sampler Lids / Scissors						
9	Spray Bottles / L-Spreaders						
10	Rubber tube / Manifold						
11	Filtration Funnels / Plates						

Negative control		Limit	No growth / no turbidity observed		
Performed By:		Checked By:		Remarks :	

14.0 ABBREVIATIONS:

Abbreviation	Description
SOP	Standard Operating Procedure
MB	Microbiology
SCD	Soybean Casein digest Broth
BOD	Bacterial Oxygen Demand

Summary Report for Sterilized Glassware and Other Accessories Hold Time Study

1.0 OBJECTIVE:

The objective of this summary report is to present shelf life of prepared sterilized glassware and other accessories.

2.0 SCOPE:

This summary report is applicable for prepared sterilized glassware and other accessories at Pharma.

3.0 VALIDATION PROCEDURE AND OBSERVATIONS:

- **3.1 Preparation and Analysis of Glassware and other accessories:**
- 3.1.1 Glassware and other accessories prepared and sterilized as per the SOP as per validated load pattern.
- 3.1.2 After completion of sterilization unloaded all the sterilized articles as per the routine procedure and held them in the Cool zone area.
- 3.1.3 Performed the analysis for sterilized glassware and other accessories at different time intervals at 0th day, 3rd day, 7th day and 11th day.
- 3.1.4 Performed the hold time activity for below mentioned articles:

Table-1

Name of Articles	Method Type
Pipette tips (1.0 and 0.1mL)	D
Pipette (10.0mL)	D
Sampling bottles	D
Aspirator bottle	D
Rubber tube	S
Manifold	S
Filtration funnels	S
Autoclavable petri plates	S
Pipette tips (1.0 and 0.1mL)	D

Name of Articles	Method Type
Pipette (10.0mL)	D
Sampling bottles	D
Aspirator bottle	D

3.1.5 Method Type D: Direct inoculation of the article into the sterile media (or direct addition of the sterile media in to the article).

3.1.6 After completion of each time interval pipette tips (0.1 and 1.0mL) and Forceps articles were taken and directly transferred to 10mL of soybean casein digestive medium. Taken 10mL of soybean casein digestive medium added to glass tube and 100 mL soybean casein digestive medium added to glass bottle.

3.1.7 The Negative control was kept along with above samples.

3.1.8 Method Type S: Swab the articles and transfer to the media for qualitative testing.

3.1.9 After completion each time interval taken sterile swab and performed swabbing on pipette tip (10mL), lint free cloth, coupons, silicon tubes, air sampler lids, scissors, spray bottles and L-spreaders, after swabbing each swab transferred to 10mL of soybean casein digestive medium.

3.1.10 One unused swab kept for negative control.

3.1.11 After completion of activity incubated all the media tubes, bottles and negative controls at 20-25°C for 3 days and then followed by 30-35°C for next 2 days.

3.1.12 Observed the results for all the media tubes and bottles after completion of incubation conditions.

3.1.13 Refer below mentioned table for each article observations at different time intervals.

Name of the articles	0th Day	3rd Day	7th Day	11th Day
Pipette tips (0.1mL)	No Growth	No Growth	No Growth	No Growth
Pipette tips (1.0mL)	No Growth	No Growth	No Growth	No Growth
Pipette tips (10mL)	No Growth	No Growth	No Growth	No Growth
Glass test tubes	No Growth	No Growth	No Growth	No Growth
Glass bottle	No Growth	No Growth	No Growth	No Growth
Lint free cloth	No Growth	No Growth	No Growth	No Growth
Coupons	No Growth	No Growth	No Growth	No Growth
Silicon tubes	No Growth	No Growth	No Growth	No Growth
Forceps	No Growth	No Growth	No Growth	No Growth
Air Sampler Lids	No Growth	No Growth	No Growth	No Growth
Scissors	No Growth	No Growth	No Growth	No Growth

Name of the articles	0th Day	3rd Day	7th Day	11th Day
Spray Bottles	No Growth	No Growth	No Growth	No Growth
L-Spreaders	No Growth	No Growth	No Growth	No Growth
Negative Control	No Growth	No Growth	No Growth	No Growth
Swab Negative Control	No Growth	No Growth	No Growth	No Growth

4.0 CONCLUSION:

- 4.1 Based on the review of the results, No growth was observed in all sterilized glassware and other accessories at all time intervals.
- 4.2 All the negative controls did not shows any growth during study period.
- 4.3 **Recommendation:** It is concluded that sterilized glassware and other accessories were stable for period of 11 days stored at NMT 25°C (Cool Zone area). However routine usage purpose Sterilized articles and other accessories can be used for period of 7 days from the date of sterilization.

*****END OF DOCUMENT*****