PROTOCOL

FOR

PROCESS SIMULATION STUDY

(MEDIA FILL)

FOR DRY POWDER INJECTION

SUPERSEDES PROTOCOL No.	
DATE OF VALIDATION	
VALIDATION BATCH NUMBER	
VALIDATION BATCH SIZE	

PROTOCOL CONTENTS

1.0 PROTOCOL APPROVAL:

PREPARED BY:

DESIGNATION	NAME	SIGNATURE	DATE
OFFICER/EXECUTIVE (QUALITY ASSURANCE)			

REVIEWED BY:

DESIGNATION	NAME	SIGNATURE	DATE
EXECUTIVE/MANAGER (QUALITY ASSURANCE)			
HEAD (PRODUCTION)			
HEAD (ENGINEERING)			
HEAD (QUALITY CONTROL)			

APPROVED BY:

DESIGNATION	NAME	SIGNATURE	DATE
HEAD (QUALITY ASSURANCE)			

2.0 OBJECTIVE:

- Process Simulation Study (Media Fill) is carried out to simulate the whole Aseptic Process in order to evaluate the Sterility Confidence of the Process.
- Prospective as well as Re-Validation of Aseptic Process provides the necessary level of assurance for aseptically produced products.
- Simulations are made to ensure that the regular process for commercial batches repeatedly and reliably produces the finished product of required quality.
- To establish documented evidence that the whole process is capable of performing as per specified acceptance criteria and is adequate to provide the aseptic assurance for which the process is intended.

3.0 SCOPE:

- The Scope of this protocol is to lay down the process which includes exposing the Microbiological Growth Support Medium (MGSM) to Product Contact Surfaces of Equipment, Container Closure System, Critical Environments, and Process Manipulations to closely simulate the same exposure that the product itself will undergo.
- This Protocol is applicable for performing Process Simulation Study (Media Fill) at Dry Powder Injection facility.

4.0 RESPONSIBILITY:

DEPARTMENTS	RESPONSIBILITIES
Quality Assurance	• Preparation, Review and Approval of Process Simulation Study (Media Fill) Protocol. • To Evaluate Protocol Completeness and Technical Accuracy. • Protocol Training • To Co – Ordinate and schedule with other departments for carrying out Media fill as per protocol. • To monitor all Process Simulation Study Activities and ensure Media fill as per Protocol. • To review and compile the Media Fill data.
Production	• To Review the Protocol. • To schedule the Process Simulation Study Activity. • To assist in the preparation and execution of the process.
Quality Control	• To Review the Protocol. • To provide all applicable Analytical Procedures and Documentation. • To carry out Microbiological Test / Sampling as per Sampling Plan mentioned in Media Fill Protocol. • To incubate and monitor the Media Filled Vials. • To analyze the sample collected and provide all analysis data during Media Fill.
Engineering	• To Review the Protocol. • To Co-Ordinate and support the Process Simulation Study Activity. • To provide engineering support during Process Simulation Study (Media Fill).

5.0 TRAINING

- Training shall be conducted on Aseptic process simulation activity. All persons shall attend training session on aseptic process simulation protocol from the respective departments production, warehouse, QA, QC and engineering personnel's who is not authorized to enter in aseptic area shall enter in aseptic area during aseptic process simulation escorted by authorized person for microbiological sampling of aseptic area entry authorization, after the successful training on the aseptic process simulation activity for the protocol as well as BMR training evaluation of the each involved persons shall be done through **training attendance record** and same shall be attached with the aseptic process simulation report.

6.0 RE-QUALIFICATION CRITERIA:

- Any major modification to any of the existing equipment, system or area after the Process Simulation Study (Media Fill), that may affect the quality of the product as intended, shall be documented through a Change Control Procedure and shall be subjected for Re-qualification.

The Re qualification shall be performed due to any of the following reasons:

- As per Validation Frequency.
- Any major modification to any of the existing Equipment, System or Area.
- Change in Environment, Disinfection Procedures, Equipment Cleaning and Sterilization (Including Containers and Closures).
- Major Maintenance and Qualification of Equipment, e.g. Autoclave, Depyrogenating Tunnel, Vial Washing Machine, HVAC (Heating, Ventilation and Air Conditioning) System, water system Pure Steam Generation System, etc.

7.0 FREQUENCY OF VALIDATION:

- The frequency of Periodic Validation for Process Simulation Study (Media Fill) shall be Twice in a year ± 30Days for existing system as per Validation Master Plan or as and when required as per point No.6.0

8.0 NUMBER OF RUNS:

- The Production Line is initially qualified by conducting Individual Media Fills for at least Three Consecutive Separate Successful Runs to ensure that the results are consistent and meaningful.
- For routine Semi-Annual Qualification / Re-Qualification conducted for DPI Line of through validation matrix pack size for only single (one) run.

9.0 DURATION OF RUNS:

- The duration of Media Fill run shall be the time it takes to incorporate Aseptic Manipulations and Interventions, as well as appropriate consideration of duration of the Actual Aseptic Processing Activity / Operation. **Media Filling Duration Time NLT 24 hrs.**

10.0 SIZE OF RUN :

- The batch size of single run shall be not less than 10,000 vials for minimum & maximum pack size.

11.0 EQUIPMENT QUALIFICATION/INSTRUMENT CALIBRATION VERIFICATION:

All Major Equipments used for Process, Facility and Utility as listed below shall be verified for their Performance Qualification and Calibration. Record the details in Media Fill Report.

Calibration details of all the Critical Instruments used in the Manufacturing Process shall also be verified for calibration. Record the details in Exhibits.

- Vial Washing Machine
- Depyrogenating Tunnel
- Autoclave cum Bung processor
- Vial Filling and Bunging Machine
- Vial Sealing Machine
- Weighing Balance (s)
- LAF Filling Room
- LAF Sealing Room
- LAF Sterile Sampling Room
- LAF Cooling Zone
- Dynamic Garment Storage Cabinet
- Dynamic Pass Box

- HVAC System
- Water System (Purified Water & WFI)
- Compressed Air System
- Pure Steam Generation System

12.0 DESCRIPTION OF PROCESS SIMULATION STUDY METHODOLOGY:

- Only after the Pre-Approval, the Protocol shall be executed.
- Process Simulation Study includes Formulation and Filling with Suitable Media.
- During the course of Process Simulation Study (Media Fill) the Documentation System, Manufacturing Procedure, Laboratory Controls, In Process Checks and Media Filled Vials shall be evaluated.
- The Process Simulation Study shall be carried out for Three Consecutive run of selected pack size to Assess the process consistency.
- The Process Simulation Study (Media Fill) methodology consists of following basic parts –
  - Process Parameters Monitoring.
  - Incubation of Filled Vials at Specific Temperatures for 14 days.
  - Visual Inspection of the Vials after **1st 7 days of Incubation at 20^0C to 25^0C** for observing **Fungal Growth or Turbidity** (If any).
  - Visual Inspection of the Vials after **Next 7 days of Incubation at 30^0C to 35^0C** for observing **Bacterial Growth or Turbidity** (If any).
- QC shall analyze all samples and the data will be recorded / attached with the report. Where applicable The graph and data print outs of critical process parameters shall be obtained and attached.
- All Parameters and Process Details shall be recorded in relevant records (e.g. Formats, and Media Fill Record etc.)
- All the personnel qualified for aseptic area in Media fill activity shall be documented in Media fill Report.
- A Summary Report shall be finally prepared summarizing the data obtained from the Process Simulation Study for only single (one) run of selected pack size, Conclusions Drawn and Recommendations, if any.

13.0 LINE SPEED:

- Three runs of variable line speed for each pack size:
- Initial Stage of Filling during Process Simulation Study (Media Fill) with Slow Line Speed,
- Middle Stage of Filling during Process Simulation Study (Media Fill) with Optimum Line Speed
- End Stage of Filling during Process Simulation Study (Media Fill) with Fast Line Speed.

14.0 SELECTION OF MEDIA:

- The Criteria for selection of Microbiological Growth Support Medium (MGSM) include: low selectivity, clarity, medium concentration and filterability.
- **Ability to support growth of a wide range of microorganisms:** The medium should have a low selectivity i.e. be capable of supporting growth of a wide range of microorganisms such as Bacillus subtilis, Staphylococcus aureus, Candida albicans, Aspergillus brasilliensis, Pseudomonas aeruginosa (e.q. Soya Bean Casein Digest Medium) & in house flora (i.e. Micrococcus luteus, Lactococcus lactis, Streptococcus salvarius, Streptococcus agalactiae, Streptococcus sanguinis).
- Selection of medium should also consider in-house flora (e.g. Isolates from Environmental Monitoring etc).
- Growth Promotion Test (GPT) to demonstrate that the medium clearly growth of microorganisms should be observed.
- Growth Promotion Testing of the media used in simulation studies to be carried out on completion of the incubation period to demonstrate the ability of the media to sustain growth if contamination is present. Growth should be demonstrated within 5 days at the same incubation temperature as used during the simulation test performance.
- **Clarity:** The medium should be clear to allow for ease in observing turbidity.
- **Medium Concentration:** Recommendations of the supplier shall be followed unless alternative concentrations are validated to deliver equal results.
- **Filterability:** If a filter is used in the Aseptic Manufacturing Process, the medium should be capable of being filtered through the same grade as used in production.
- Soya Bean Casein Digest Medium is used as Microbiological Growth Support Medium (MGSM) for Process Simulation Study.
- The concentration of Soya Bean Casein Digest Medium (SCDM) is selected 3% w/v in Water for Injection on the basis of above selection parameters and Pre GPT Studies performed.

15.0 SELECTION OF PROCESS SIMULATION POWDER / DILUENT:

- A Process Simulation Powder / Diluent is the material which enhances the Growth Promotion Properties of a Microbiological Growth Support Medium.
- Various Process Simulation Powders / Diluents are available which can be used for Process Simulation Study (Media Fills) of Dry Powder Injection e.g. Polyethylene Glycol, Lactose, Mannitol etc.
- Lactose has been selected as diluents for Microbiological Growth Support Medium (MGSM) i.e. Soya Bean Casein Digest Medium.

16.0 STERILITY TEST OF STERILIZED LACTOSE:

- The Sterilized Lactose shall be subjected for Sterility Test Simultaneously while performing Media Fill runs by performing sterility test on a sample collected from each container just before loading the sterilized lactose.(Gama Irradiated)
- The detail of Sterility Test shall be recorded in Media Fill Record.

17.0 STERILITY TEST AND GPT OF COMBINATION OF STERILIZED LACTOSE AND MEDIA (SCDM) SOLUTION:

- The Sterilized Lactose powder shall be mixed with Predefined Concentration of Media Solution under Aseptic Conditions in Microbiology Lab. To result a clear solution.
- The above solution shall be subjected for Sterility and GPT. Only after successful Sterility Test and GPT results of combination solution the dispensing for Media Shall be performed.
- The Sterility and GPT results of combination solution shall be recorded in Media Fill Report.

18.0 INTERVENTIONS (WORSE CASE STUDY):

Interventions shall be recorded in Media Fill Record and Media Fill Report specifying the Types of Interventions, Duration of Intervention providing for consistent production practices and assessment of these practices during Media Fills.

- **The Filled units after different Interventions shall be kept separately for Incubation with Proper Status Label.**
- **NO N_2 flushing shall be done in place of N_2 filtered Compressed air shall be used for pre & post N_2 flushing also Compressed air shall be used to push the material from the doser to vial.**

18.1 TYPES OF INTERVENTION:

18.1.1 Routine Interventions:

Routine Interventions are activities that are inherent parts of the Aseptic Process and integral parts of every batch. Typical Routine Interventions include:

- Aseptic assembling of the equipment and initial product connection or introduction.
- Aseptic Transfer of sterile material from powder container to hopper of filling machine.
- Charging of rubber stopper in hopper.
- Initial Fill weight adjustment.
- Environmental Monitoring with active air sampling
- Environmental Monitoring with Passive Air Sampling (Settle Plate)
- During Filling Fill weight adjustment
- Purging of filtered compressed air at pre filling & post filling stage.
- Machine run at Optimum speed
- Machine run at maximum speed
- Machine run at Minimum speed
- Product sampling
- Operator Break for Lunch/Dinner
- Operator staff changes during Shift Change over
- Tea break for 15 min.
- Removal of fallen rubber stoppers from the line with the help of forceps
- Glove Replacement
- Pushing of rubber stopper from the chute with the help of forceps
- Compressed air sampling by microbiologist
- Fallen or broken vials removal from turn table
- Stoppering star wheel adjustment
- Out Feed Track Adjustment
- Stopper Lock Adjustment
- Stopper Bowl Adjustment
- No Vial No Fill sensor adjustment
- Turn Table Vial absence Sensor adjustment
- Fallen Vials remove from conveyor
- Swing Conveyor movement to enter either side of the machine

18.1.2 Non-Routine Interventions:

Non routine interventions are activities that are predominantly corrective and may not be a part of every batch. Non routine interventions may not be necessary during the aseptic process, in practice such interventions are almost always required to correct some anomaly. Some common Non routine interventions involve:

- Filling room door open for 2 min.
- Machine Break down activities for 15 Minutes (MINOR)
- Machine Break down activities for 60 Minutes (MAJOR)
- Increase in No. of Persons for 15 Minutes (Not more than 7 persons)
- Power Failure for 10 Minutes
- AHU OFF for 5min
- Operator Fatigue
- Replace 1 piston

19.0 INCUBATION AND EXAMINATION OF MEDIA-FILLED VIALS:

- Before Incubation, the Containers with the Microbiological Growth Support Medium shall be inverted or otherwise manipulated to ensure that all surfaces including the Internal Surface of Container and Closure are thoroughly wet by the Medium.
- The Containers shall not be completely filled with medium in order to provide Sufficient oxygen for growth of obligate aerobes.
- Media units shall be incubated under conditions adequate to detect microorganisms that might otherwise Be difficult to culture.
- Damaged Media Filled units shall not be kept for incubation and shall be destroyed as per SOP for Destruction of Media.

19.1 INCUBATION TEMPERATURE:

- Incubation Temperature for **Ist 7 days** suitable for **Fungal Growth: 20^{0}C to 25^{0}C.**
- Incubation Temperature for **Next 7 days** suitable for **Bacterial Growth: 30^{0}C to 35^{0}C.**

20.0 INTERPRETATION OF DATA AND ACCEPTANCE CRITERIA:

20.1 INTERPRETATION OF DATA:

- After the Incubation Period of Media-Filled Containers, they shall be visually examined for Microbial Growth. Contaminated containers shall be examined for evidence of container / closure **damage which Might compromise the integrity of the packaging system. Damaged container shall** not be included

as failures (positives) when evaluating results.

- Each Media-Filled unit shall be examined for contamination by Microbiologist / Trained QA Person with appropriate Education, Training, and Experience in inspecting Media Filled Units for Microbiological Contamination. All suspected units identified during the examination shall be brought to the immediate attention of the Head – QA.

20.2 ACCEPTANCE CRITERIA:**

- The number of containers used for Media Fills should be sufficient to enable a valid evaluation.

When filling fewer than 5000 units, no contaminated units should be detected.

❖ One or more contaminated unit should results repeat Media fill following investigation.

When Filling 5,000 to 10,000 units:

❖ One (1) contaminated unit should result in an investigation, including consideration of a repeat Media fill following investigation.

❖ Two (2) contaminated units are considered cause for revalidation, following investigation.

When filling more than 10,000 units:

❖ One (1) contaminated unit should result in an investigation;

❖ Two (2) contaminated units are considered cause for revalidation, following investigation.

**** Reference:** PIC/S Guide PI007-6

- Any contaminated unit shall be considered Objectionable and Investigated. The Microorganisms shall be identified up to Species Level. The investigation shall survey the possible causes of contamination. In addition, any failure investigation shall assess the impact on commercial drugs produced on the Production Line since last Media Fill.

21.0 ANALYTICAL SUPPORT:

- Growth Promotion Test (GPT) of Microbiological Growth Support Medium (MGSM).
- Solubility Study of Lactose in Predefined Concentration and Volume of Microbiological Growth Support Medium (MGSM).
- Sterility Test of Sterile Lactose after Sterilization with Gamma Radiation.
- Sterility and GPT of Combination of Sterilized Lactose and Solution of Predefined Media Concentration
- Bulk Solution Sampling after sterilization of media for GPT & Sterility Test.

- MLT and Bacterial Endotoxin Test of WFI used for Media Solution Preparation.
- Sterility Test and BET of Rubber Plug & Empty vials after depyrogenation.
- Growth Promotion Test (GPT) of incubated media filled Vials.
- Growth Promotion Test (GPT) of Deactivated media solution.

22.0 ENVIRONMENTAL MONITORING:

Media Fill shall be adequately representative of the conditions under which actual manufacturing operations are conducted. Following Environmental Conditions shall be monitored during Process Simulation Study (Media Fill):

- Temperature, RH & Differential Pressure.
- Passive Air Sampling (Settle Plate) Before, During and After Filling and Sealing of Media Filled Vials.
- Active Air Sampling Before, During and After Filling and Sealing of Media Filled Vials.
- Microbiological Swab of Walls, Floor and Machine Surface Before, During and After Filling and Sealing of Media Filled Vials.
- Sterility Test of Compressed Air.
- Microbiological Swab of Aseptic Area Garments Before, During and After Filling and Sealing of Media Filled Vials.
- Personal Monitoring by RODAC Plate & Finger Dab of all persons involved in Media Fill (After completion of Media Fill).

23.0 TRAINING DETAILS:

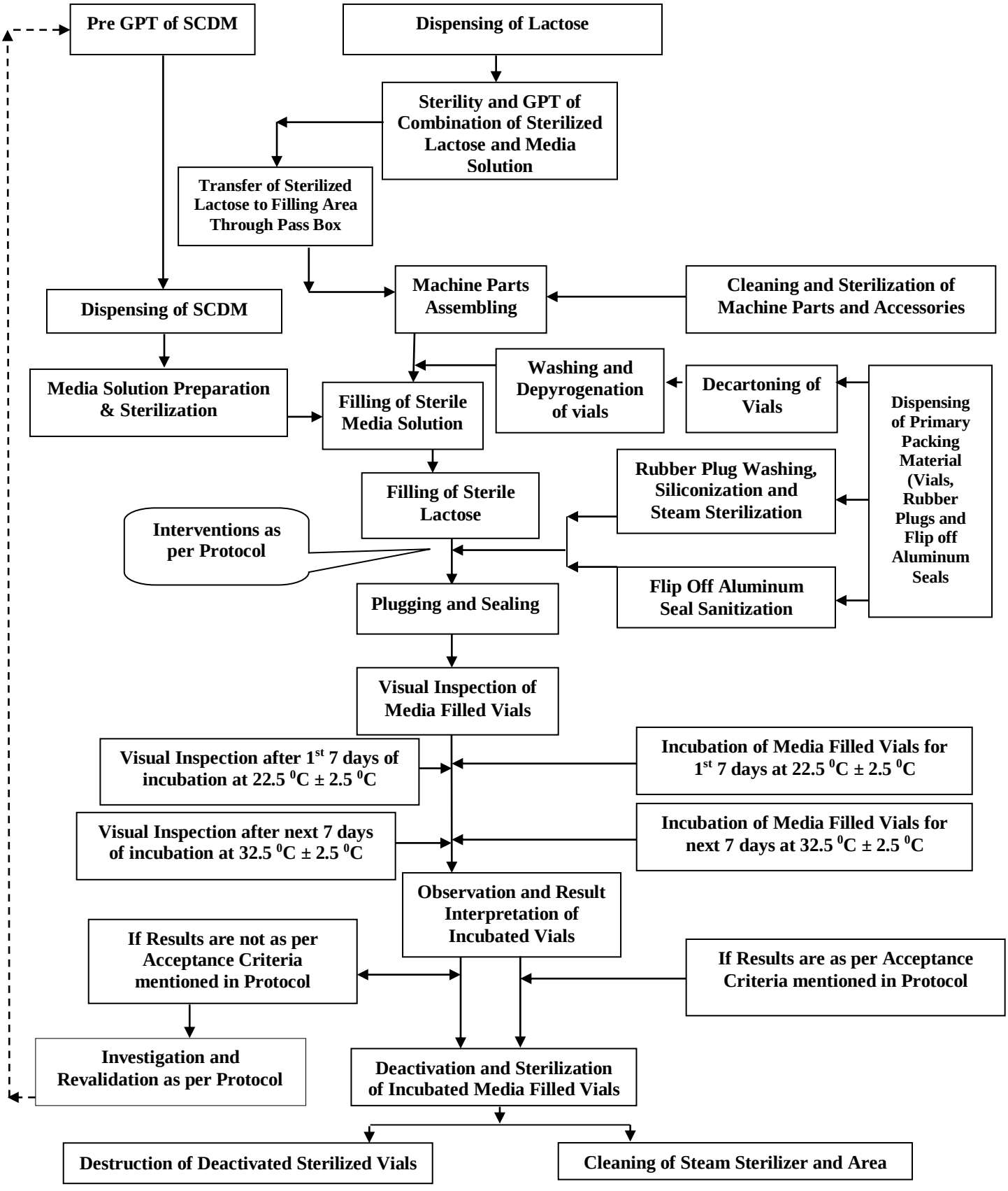
- All the personnel involved in the Process Simulation Study (Media Fill) shall be appropriately trained both in their job related activities and on the Process Simulation Study (Media Fill) Protocol.
- Photocopy of Training record of the persons involved in the Process Simulation Study (Media Fill) shall be attached and recorded in Process Simulation Study (Media Fill) Report.
- All details of Master Document Verification shall be recorded in Media Fill Report.
- All the details of Media, Lactose and Packaging Material shall be recorded in Media Fill Report.

24.0 EQUIPMENT QUALIFICATION / INSTRUMENT CALIBRATION & VERIFICATION:

- All Major Equipment used for Process, Facility and Utility as listed below shall be verified for their Performance Qualification and Calibration. Record the details in Media Fill Report.

- Calibration details of all the Critical Instruments used in the Manufacturing Process shall also be verified for calibration. Record the details in Exhibits.
- Vial Washing Machine
- Depyrogenating Tunnel
- Autoclave cum Bung processor
- Vial Filling and Bunging Machine
- Vial Sealing Machine
- Weighing Balance (s)
- LAF Filling Room
- LAF Sealing Room
- LAF Sterile Sampling Room
- LAF Cooling Zone
- Dynamic Garment Storage Cabinet
- Dynamic Pass Box
- HVAC System
- Water System (Purified Water & WFI)
- Compressed Air System
- Pure Steam Generation System

25.0 PROCESS FLOW:



26.0 PROCEDURE:

26.1 DISPENSING / ISSUANCE OF MEDIA (SCDM):

- Only after successful Growth Promotion Test results of Media (SCDM), the Media shall be dispensed / issued for Media Fill Activity.
- The required quantity of Media shall be transferred from Microbiology Lab. To the Production Floor and the media issuance details shall be recorded in Media Fill Record.

26.1.1 STERILITY TEST OF STERILISED LACTOSE:

- The Sterilized Lactose shall be subjected for Sterility Test. Simultaneously when gamma radiated containers opened for use.
- The detail of Sterility Test and the report shall be recorded in Media Fill Record.

26.1.2 DISPENSING OF LACTOSE:

- The Lactose shall be dispensed as per Media Fill Record.

26.1.3 STERILITY AND GPT OF COMBINATION OF STERILIZED LACTOSE AND MEDIA (SCDM) SOLUTION:

- Mix the Sterilized Lactose Powder with Predefined Concentration 3 % of Media Solution under aseptic condition to result a clear solution. The above solution shall be subjected for Sterility and GPT.
- The Sterility and GPT results shall be recorded in Media Fill Record and the reports shall be attached with the Media Fill Report.

26.1.4 VERIFICATION OF DISPENSED QUANTITY OF VIALS, RUBBER BUNGS AND FLIP – OFF ALUMINUM SEALS:

- Check the quantity of Vials, Rubber Bungs and Flip off Seals and verify respective Analytical Reference No. (A.R. No.) From the Material Requisition Slip, record the details in Media Fill Record.

26.1.5 WASHING, SILICONIZATION, STERILIZATION AND DRYING OF RUBBER BUNGS:

- Open the Poly Bags and transfer the Rubber Bungs to Bung Processor cum Autoclave for Washing and Siliconization.
- Run the cycle for Bung Processing as per SOP.
- Unload the Rubber Bungs from the cool zone after completion of Sterilization Cycle.
- Steam Clox Indicator and Thermograph shall be attached with the Media Fill Record.
- Record the details of Rubber Bung Autoclave Cycle in Media Fill Record.

26.1.6 CLEANING AND SANITIZATION OF FLIP OFF ALUMINIUM SEALS:

- Sanitize the Flip off Aluminium Seal using 70% IPA Solution.

26.1.7 CLEANING AND STERILIZATION OF MACHINE PARTS AND ACCESSORIES:

- Clean the Machine Parts and Accessories.
- Sterilize the Machine Parts in Autoclave as per the Loading Pattern.
- Thermograph and Steam Clox indicator should be attached with the Media Fill Record.
- Record the details of Machine Parts Autoclave Cycle in Media Fill Record.

26.1.8 MEDIA SOLUTION PREPARATION AND STERILIZATION:

- The Media Solution shall be prepared and sterilized as per SOP.
- Ensure that the temperature of WFI is above 80 °C.
- Collect approximately 60.0 Liters of WFI in clean Manufacturing Vessel.
- Add dispensed quantity of Media in Pressure Vessel under continuous stirring.
- Check and ensure that the solution is clear+recorded in Media Fill Record.
- For worst case condition Filling Operation shall be performed with different Interventions as per Protocol.

Note: Compressed Air shall be used for Dosing in place of N_2 gas to prevent the formation of inert Atmosphere inside the vials during Filling.

26.2 VISUAL INSPECTION OF FILLED AND SEALED VIALS:

- Perform the Visual Inspection of all vials for Clarity, Breakage, Quality of Sealing and any other rejects.
- Record the observations during Visual Inspection in Media Fill Record.

26.3 TRANSFER OF VISUALLY INSPECTED FILLED AND SEALED VIALS FOR INCUBATION:

- Send the Visually Inspected Good Vials for Incubation.
- Label the individual tray.
- Collect the vials of Worse Case Study, Stage Wise, label them and arrange accordingly in the Incubation Room / Chamber.

26.4 VISUAL INSPECTION OF FILLED VIALS AFTER INCUBATION:

26.4.1 VISUAL INSPECTION AFTER 7 DAYS OF INCUBATION AT 20^0C to 25^0C.

- Visually inspect the vials after 7 days of incubation to observe any Fungal Growth or Turbidity.

- If any Fungal Growth or Turbidity observed, inform Head – QA for further action.
- Record the Visual Inspection Observations in Observation sheet and attach to the Media Fill Record.
- After visual inspection of vials transfer the vials into Incubator at 30°C to 35°C.
- Post cleaning swab & rinse sampling to assure cleanliness of Autoclave & aseptic area to prevent carryover for next batch.

26.4.2 VISUAL INSPECTION AFTER NEXT 7 DAYS OF INCUBATION AT 30^0C to 35^0C.

- Visually inspect the vials after next 7 days of incubation to observe any Bacterial Growth or Turbidity.
- If any Bacterial Growth or Turbidity observed, inform Head – QA for further action.
- Record the Visual Inspection Observations in Observation sheet and attach to the Media Fill Record.

26.4.3 DEACTIVATION OF MEDIA FILLED VIALS AFTER INCUBATION:

- Perform the Post GPT after completion of incubation period of media filled vials.
- Collect all the vials from Incubation Chamber for deactivation (By Moist Heat Sterilization Method) and Destruction.
- Ensure that the Autoclave Chamber is clean.
- Load the Media solution in Autoclave and run the Cycle for Sterilization.
- Unload the Deactivated Media solution & perform the GPT of the same.
- Attach the Post Sterilization / Deactivation GPT report to the Media Fill Report and record the details in Media Fill Record.
- Send the Vials for Destruction as per SOP.
- Record the Destruction details in Media Fill Record.

26.4.4 POST MEDIA FILL CLEANING:

26.4.5 CLEANING OF AUTOCLAVE:

- Clean the Chamber and Drain Line of Autoclave with WFI.
- Run the Standard Cycle to ensure complete cleanliness and Sterilization of the Chamber and the Drain Line.
- Take the Rinse Water Sample from the Drain Point and send to QC for Microbiological Analysis.
- Take the Swab of the Autoclave Chamber and send to QC for Microbiological Analysis.
- In case any Microbial Growth is observed, again perform the cleaning of Autoclave and send the sample to QC for Microbiological Analysis.

- Proceed for Commercial Production Activity only, if no Microbiological Growth is observed.

26.4.6 CLEANING OF ASEPTIC AREA:

- Clean the Aseptic Area with WFI immediately after Media fill.
- Wipe the Walls, Floor, and Machine Surfaces with WFI, followed by cleaning with Schedule Disinfectant.
- Take the Microbiological Swab of Walls, Floor, and Machine Surfaces.
- Perform the Environmental Monitoring (Active Air Sampling, Passive Air Sampling) for consecutive 7 days and observe the results.
- Proceed for Commercial Production Activity only, if no Microbiological Growth is observed.

26.4.7 MONITORING OF PROCESS PARAMETERS:

S.No.	PROCESS STEPS	MONITORING PARAMETERS
1.	**Rubber Bung Washing**	Washing start time
		Washing end time
		Rinsing with Purified water Start Time Stop Time
		Rinsing with WFI Start Time Stop Time
		Siliconization Start Time Stop Time
2.	**Sterilization of Rubber Bungs and Accessories**	Sterilization Time
		Sterilization Temperature (Min. & Max.)
		Vacuum Drying Time
3.	**Vial Washing**	Differential Pressure of Area
		Speed of Vial Washing Machine
		Recycled WFI Pressure (Min. & Max.)
		Water For Injection Pressure (Min. & Max.)
		Compressed Air Pressure (Min. & Max.)
4.	**Vial Depyrogenation**	Tunnel Conveyor Belt Speed
		Temperature of Sterile Zone Entry
		Temperature of Sterile Zone exit
		Drying Zone Manometer reading
		Sterilization Zone Manometer reading
		Cool Zone Manometer reading
5.	**Vial Filling & Sealing**	Differential Pressure in Sterile Area
		Temperature
		Area Humidity
		Filling machine speed
		Sealing Inspection
		Clarity
		Leak test

27.0 CRITICAL PARAMETERS AND ACCEPTANCE CRITERIA:

27.1 RUBBER BUNG WASHING & STERILIZATION:

- Rubber Bungs should comply Sterility Test.
- Endotoxin should be NMT 0.25 EU/ml.
- Moisture content should be not more than 0.1%.

27.2 MACHINE PARTS:

- Final washed and rinsed (With WFI) Machine Parts and Accessories should be visually clean.

27.3 VIAL WASHING & DEPYROGENATION:

- The Washed Vials after washing should comply Clarity test.
- The Depyrogenated Vials should comply Test for Sterility and BET.
- Endotoxin should be NMT 0.25 EU / vial.

27.4 VIAL FILLING:

- Average Fill Weight shall not vary by more than 2% of Theoretical Fill Weight and the Media Solution and lactose Filled Vial should cover NMT 70% of the Vial Size.

27.5 SAMPLING PLAN:

Collect the samples as per the Sampling Plan at different Operations as mentioned. Label each Sampled Container indicating Product Name, Batch No., Sample No., Date of Sampling and Name of Person who sampled.

S. No.	Sample	Stage & Frequency of sampling	Location	Quantity	Test to be Performed	Responsibility
PRE MEDIA FILL PLAN						
01	Pre GPT of media	Before media fill execution (Once)	Micro Lab	---------	GPT	Micro
02	Combination of Sterilized Lactose And Media (SCDM)	Before media fill execution (Once)	Micro Lab	-------	Sterility Test And GPT	Micro
MEDIA FILL SAMPLING PLAN						
03	Filtered IPA 70%	After Disinfectant Filtration (Once)	From disinfectant	100 ml	Sterility	Micro

			filtration area			
04	Rinse water of machine Parts	After cleaning and before sterilization	Unit Preparation area	100ml + 100 ml	Description, Ph, Conductivity	Micro
05	WFI for media preparation	Before media preparation (once)	Manufacturing Area	500ml + 500ml	Complete analysis (Chemical + Micro)	Chemical & Micro
06	Compressed Air	Before ,During and End Stage of Filling	Filling Room	1000 L at Each stage of filling from user point	Sterility	Micro.
07	Compressed Air	Before Filling	From Unit Preparation Room	1000 L	Sterility	Micro.
08	Sterilized Lactose from Hopper during Transfer	Start of filling	Filling room	6.0 gm.	Sterility	Micro
09	Media Bulk Solution	Before Sterilization	Unit Preparation area	200 ml	Description, Ph, Bioburden	Micro
10	Media solution	Before Filling (Once)	From Filling room	800 ml	Ph, GPT + Sterility	Micro
11	Left Over media Solution	After Filling (Once)	From Filling Room	800 ml	Ph, GPT + Sterility	Micro
12	Rubber Stopper	After sterilization Initial, Middle & End Stage of filling	Cooling Zone	50 Rubber Stopper	Sterility BET & Moisture Content	Micro + Chemical
13	Vial After Washing	Initial, Middle & End Stage of Washing	From Vial Washing AREA	90 VIALS (30X3)	Bioburden, Clearity & LBPC	Micro
14	Vial after Depyrogenation	Initial, Middle & End Stage of Depyrogenation	From Filling Room	75 vials (25x3)	Sterility & BET	Micro
15	Filled & Sealed Vials	Initial, Middle & End Stage of Filling	From Packing Area	90 Vials (30X3)	Description, Fill Volume, Ph, Sterility	Micro
16	Clarity Test & leak test	Initial, Middle, & End Stage of Filling	From Packing Area	08 Nos. (from each head)	Leak Test	IPQA
POST MEDIA FILL PLAN						
17	Swab sample of Machine	After media fill (once)	Filling area	------	Bioburden by swab	Micro

	Parts				method	
18	Swab sample of wall, floor & machine surface	After media fill (once)	Filling area	------	Bioburden by swab method	Micro
19	Environmental monitoring (Active & Passive air sampling)	After media fill (once)	Filling area	------	By Settle plate & air sampling Method	Micro
20	Post GPT of Incubated Vials (Once)	After Completion of incubation of media fill vials of each batch of media fill (Once)	Micro lab	------	GPT	Micro
ENVIROMENTAL MONITORING PLAN						
21	Temperature , % RH & Differential Pressure	Before Media Fill	During Media fill		After media fill	IPQA
22	Online Non-Viable Particle count of filling area	During media operation	Grade-A		----------	IPQA
23	Offline Non-Viable Particle count of filling area	Initial & end stage of media fill	Grade-B		----------	IPQA
24	Passive air sampling (Settle Plate)	Before Media fill	During Media fill		After media fill	Covering each shift by Micro
25	Active air sampling	Before Media fill	During Media fill		After media fill	Covering each shift by micro
26	Microbiological swab of walls, floor and machine surface	----------	----------		After media fill	Micro
27	Personnel Monitoring by RODAC plate & finger Dab of all persons involved	After media fill				Micro

28.0 DEVIATIONS:

- All protocol deviation, non-conformances and out of specification results obtained shall be investigated in accordance with corresponding SOP's and documented in Process Simulation Study (Media Fill) report.

29.0 DEFINITIONS:

- **Action Level:** Established criteria, e.g. microbial or particulate levels, requiring immediate follow-up and corrective action if exceeded.
- **Alert Limits (Environmental Monitoring):** Established microbial or particulate levels giving early warning of potential drift from normal operating conditions which are not necessarily grounds for definitive corrective action but which require follow-up investigation.
- **Alert Limits (Media Fill):** Established levels or numbers of positive media filled units, the cause of which should be investigated, but which are not necessarily grounds for definitive corrective action.
- **Aseptic Processing:** Operation whereby the product is sterilized separately then filled and packaged using sterilized containers and closures in critical processing zones.
- **Bioburden**: Total number of viable microorganisms on or in pharmaceutical product prior to sterilization.
- **Environmental Monitoring Programme**: Defined documented programme which describes the routine particulate and microbiological monitoring of processing and manufacturing areas, and includes a corrective action plan when action levels are exceeded.
- **Growth Promotion Test (GPT):** Test performed to demonstrate that media will support microbial growth.
- **High Efficiency Particulate Air (HEPA) Filter**: Retentive matrix designed to remove a defined percentage of particulate matter of a defined size.
- **HVAC**: Heating, ventilation and air conditioning
- **Integrity Test:** Test to determine the functional performance of a filter system.
- **Media Fills:** Method of evaluating an aseptic process using a microbial growth medium. (Media fills are understood to be synonymous to simulated product fills, broth trials, broth fills etc.).
- **Sampling Frequency:** Established period for collecting samples.
- **Shift:** Scheduled periods of work or production, usually less than 12 hours in length, staffed by alternating groups of workers.

- Sterile: Free from any viable organisms. (In practice, no such absolute statement regarding the absence of microorganisms can be proven).
- **Sterilization:** Validated process used to render a product free of viable organisms.
- **Sterility Assurance Level (SAL):** Probability of product is sterile. (SAL is expressed as 10^{-n}).
- **Sterility Test:** Test performed to determine if viable microorganisms are present.

30.0 REFERENCES:

- Pharmaceutical Inspection Convention (Pharmaceutical Inspection Co-Operation Schemes) (PIC/S) PI 007-6, “Recommendation on the Validation of Aseptic Processes”.
- WHO Technical Report Series – 961
- United States Pharmacopoeia – 37
- Validation Master Plan.
- SOP Entitled “Process Simulation Study (Media Fill)” Sop.

31.0 ABBREVIATIONS:

SOP	:	Standard Operating Procedure
Temp.	:	Temperature
NLT	:	Not Less than
NMT	:	Not More Than
LAF	:	Laminar Air Flow
No.	:	Number
min.	:	Minimum
max.	:	Maximum
QA	:	Quality Assurance
QC	:	Quality Control
EU	:	Endotoxin Unit
WFI	:	Water for Injection
SS	:	Stainless Steel
A.R. No.	:	Analytical Report Number
kg	:	Kilogram
mg	:	Milligram
Qty.	:	Quantity
VMP	:	Validation Master Plan
GPT	:	Growth Promotion Test
PIC/S	:	Pharmaceutical Inspection Convention OR Pharmaceutical Inspection Co-Operation Scheme
GMP	:	Good Manufacturing Practice
SCDM	:	Soya Bean Casein Digest Medium
MLT	:	Microbial Limit Test
USP	:	United States Pharmacopoeia
HVAC	:	Heating, Ventilation and Air Conditioning

32.0 REVISION HISTORY:

Revision No.	Change Control No.	Details of Changes	Reason of Changes	Effective Date	Done By