PROTOCOL

FOR

PROCESS SIMULATION STUDY

(MEDIA FILL)

FOR LIQUID VIAL LINE

SUPERSEDE PROTOCOL No.	
DATE OF VALIDATION	
VALIDATION BATCH NUMBERS	
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. Process Simulation studies include Formulation (Compounding), Filtration and Filling with suitable media.
- 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) for Vial Line.

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 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.
- After introduction of any new pack size with subjected to three consecutive media fill run.
- Any Major Modification to any of the existing Equipment, System or Area.
- Change in Environment, Disinfection Procedures, Equipment Cleaning and Sterilization.
- Major Maintenance and Re-Qualification of Equipments, e.g. Autoclave, Vial Filling & sealing Machine, HVAC (Heating, Ventilation and Air Conditioning) System, Water System, etc.

6.0 FREQUENCY OF VALIDATION:

- The frequency of Periodic Validation for Process Simulation Study (Media Fill) shall be Twice in a year for existing system as per Validation Master Plan or as and when required as per point No.5.0

7.0 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 Re-Qualification, each media fill shall be conducted with single size of selected Vial size in rotation and all selected Vial size shall be covered.

8.0 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 Actual Aseptic Processing Activity / Operation.

9.0 SIZE OF RUN:

- The batch size of single run shall be 10,000 vials for minimum & maximum pack size.

10.0 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 selected should be capable of supporting a wide range of microorganisms, which might reasonably be encountered and be based also on the in house flora (e.g. isolates from monitoring etc.).
- 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.

11.0 INTERVENTIONS (WORSE CASE STUDY):

Interventions shall be recorded in 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.

11.1 TYPE OF INTERVENTIONS:

11.1.1 Aseptic Manipulation / Interventions:

11.1.1.1 Routine Interventions:

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

1. Aseptic Assembly of the Equipment and Initial Product Connection or Introduction
2. Initial Fill Volume Adjustment
3. Periodic Fill Volume Checking & Verification
4. Maximum Filling Speed
5. Optimum Filling Speed
6. Minimum Filling Speed
7. Rubber bung charging in hopper
8. Rubber stoppering machine chute adjustment for 2 min.
9. Handling of Vials by using forceps
10. Operator Breaks & Meals
11. Defective seals on container
12. Operator Shift Changes
13. Environmental Monitoring with active air sampling
14. Environmental Monitoring with Passive Air Sampling (Settle Plate)

11.1.1.2 Non-Routine Interventions:

15. Sensor Adjustment or Replacement
16. AHU of Filling Area OFF for 5 min.
17. Machine break down activity for 15 min (MINOR).
18. Machine break down activity for 60 min (MAJOR).
19. Power Failure for 10 minutes.

20. No. of Persons increased (Not more than 7 persons) in Filling and Sealing area for 15 minutes during filling.
21. Filling room door Open
22. Operator Fatigue

Note: - If any additional interventions shall be observed during commercial batches manufacturing, then those interventions shall be covered during next media fill execution.

12.0 INCUBATION AND EXAMINATION OF MEDIA-FILLED VIALS:

- The vials shall not be completely filled with medium (NMT 70% of overflow capacity of vial) in order to provide sufficient oxygen for growth of obligate aerobes.
- Each Media-Filled unit shall be examined for microbiological contamination by trained Microbiologist / trained visual inspectors and Training shall be provided before starting of visual inspection of incubated vials.
- Integral media filled units shall be incubated under conditions adequate to detect microorganisms that might otherwise be difficult to culture.
- Non integral media filled units shall not be kept for incubation and shall be destroyed as per SOP for Destruction of Media.

12.1 INCUBATION TEMPERATURE:

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

13.0 INTERPRETATION OF DATA AND ACCEPTANCE CRITERIA:

- 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.

13.1 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.

14.0 ANALYTICAL SUPPORT:

- Pre Growth Promotion Test (Pre GPT) of Microbiological Growth Support Medium (MGSM).
- MLT Test & BET of WFI used for Media Manufacturing.
- Bulk Solution Sampling for Bio burden before Filtration.
- Bulk Solution Sampling after Aseptic Filtration for GPT & Sterility Test.
- Bulk Solution Sampling after Aseptic Filtration for GPT & Sterility test at different time interval.
- BET & Sterility Test for Depyrogenated Vials.
- Swab Sampling from each Machine Part and accessories Before Sterilization for Bioburden.
- Sterility Test of 70% Filtered IPA.

- Sterility Test of Compressed Air.
- Growth Promotion Test (Post GPT) of Media Filled vials.
- Growth Promotion Test (GPT) of Deactivated Media solution.

15.0 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.
- Active Air Sampling Before, during and After Filling and Sealing of Media Filled vials.
- Passive Air Sampling (Settle Plate) Before, During and After Filling and Sealing of Media Filled Vials.
- Non Viable Particle Count of Filling and Sealing Area in Static Condition and Dynamic Condition (During Operation) once in a shift.
- Sterility Test of Compressed Air.
- Microbiological Swab of Walls, Floor and Machine Surface After Filling and Sealing of Media Filled Units.
- Microbiological Swab of Aseptic Area Garments after Filling and Sealing of Media Filled units.
- Personal Monitoring by RODAC Plate & Finger Dab of all persons involved in Media Fill (After completion of Media Fill).

16.0 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 Packaging Material shall be recorded in Media Fill Report.

17.0 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
- LAF Filling Room
- LAF Sealing Room
- LAF Sterile Sampling Room
- LAF Cooling Zone
- Manufacturing & Holding Tanks
- Dynamic Garment Storage Cabinet
- Dynamic Pass Box
- HVAC System
- Water System (Purified Water & WFI)
- Compressed Air System
- Pure Steam Generation System

18.0 DESCRIPTION OF PROCESS SIMULATION STUDY METHODOLOGY:

- Only after the Pre Approval, the Protocol shall be executed.
- Process Simulation Study includes Formulation, Filtration 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.

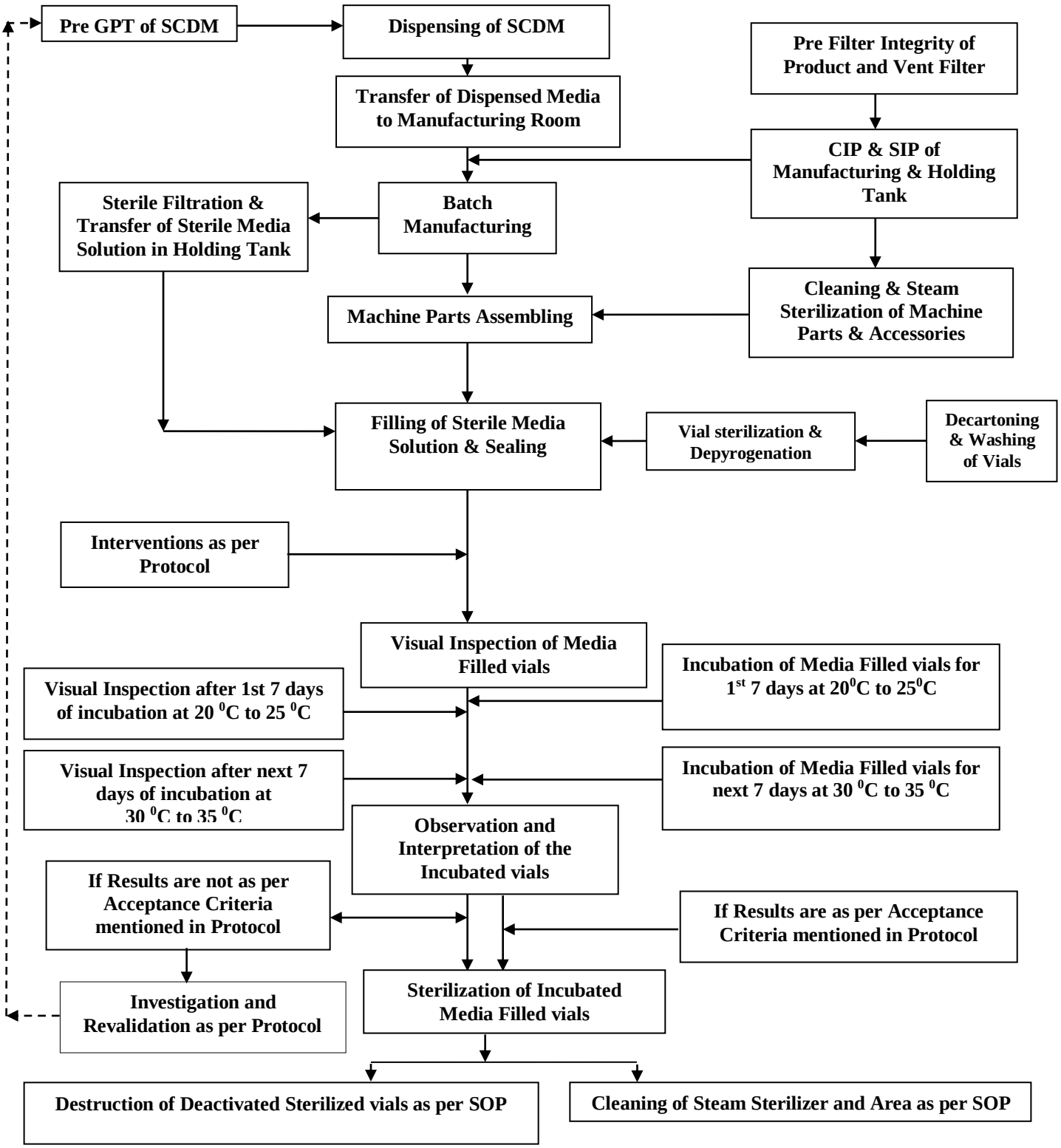
➢ Incubation of Filled Vials at Specific Temperatures for 14 days.

➢ 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. Exhibits, 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 Three consecutive runs of selected pack size, Conclusions Drawn and Recommendations, if any.

19.0 MEDIA FILL PROCESS FLOW FOR LIQUID VIAL LINE:-



20.0 PROCEDURE:

20.1 DISPENSING 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.

20.2 CIP & SIP OF MANUFACTURING & HOLDING TANK:

- Ensure that the Pre – filter Integrity of Product and Vent Filter has been performed as per SOP and is Pass before performing the CIP & SIP of the Manufacturing and Holding Tanks.
- Perform the CIP & SIP of the Manufacturing and Holding Tank as per SOP.

20.3 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.

20.4 CLEANING AND SANITIZATION OF FLIP OFF ALUMINIUM SEALS:

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

20.5 CLEANING AND STERILIZATION OF MACHINE PARTS AND ACCESSORIES:

- Clean the Machine Parts and Accessories as per SOP.
- 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.

20.6 MEDIA SOLUTION PREPARATION AND FILTRATION:

- Dispensed quantity of Soya Bean Casein Digest Medium (SCDM) is dissolved in Water for Injection at 50°C – 60°C and mixed for 30 minutes maintaining the temperature to obtain a 3% w/v Clear Solution.
- The prepared media solution shall be purged with compressed air for 10 minutes.

  Note: Compressed Air shall be used for Purging in place of N_2 gas.
- Before transferring the media, adequate quantity should be sampled for Pre-filtration bio-burden (For information only).
- The liquid growth medium should be sterilized by passing through sterilizing grade filters in a manner similar to the production process being simulated.
- After sterilization, the growth medium should be passed through the equipment line in same manner as followed during actual commercial production.
- After filtration of the media, adequate quantity should be sampled for Growth Promotion Test (GPT) & Sterility of the filtered media.
- Perform the Post - Filter Integrity of the Product filter and Air Vent Filter with WFI (Bubble Point Test).

20.7 HOLD TIME STUDY OF MEDIA SOLUTIONJ AFTER FILTRATION IN HOLDING TANK:

- After sterilization, hold the filtered media solution in holding tank up to 24 hrs. Aseptically.
- Take the adequate quantity of samples for GPT, pH, Sterility & BET from holding tank.
- The growth medium should be passed through the equipment line in same manner as followed during actual commercial production.
- Perform the post – filter integrity of the product filter and air vent filter.

20.8 DECARTONING OF VIALS:

- Transfer the dispensed quantity of vials in to Decartoning room As per SOP.
- De-carton the vials & transfer to the Washing area As per SOP.

20.9 VIAL WASHING & DEPYROGENATION:

- Feed the good vials on the conveyor of Vial Washing Machine.
- Vials are washed from inside and outside with compressed air in First Cycle followed by Recycled water-I (internal & external) in second cycle.
- Check and ensure all the Parameters for operating Vial Washing Machine are as per Acceptance Limit.
- Vials are further washed from inside with compressed air in third Cycle followed by fourth wash with Recycled water-II.
- Finally vials are washed from inside with fresh WFI followed by compressed Air (internal & external).
- Washed vials are passed to the conveyor of Depyrogenation Tunnel.
- Vials are depyrogenated at 330°C ± 10°C for at least 3 minutes in the Tunnel.
- Depyrogenated vials are Cooled, Stabilized inside the tunnel only and then are passed directly to the Aseptic Filling Area.
- A copy of Temperature Print Out should be attached with Media Fill Record.
- Record the details of Vial Washing & Depyrogenation in Media Fill Record

20.10VIAL FILLING & SEALING

- Assemble the "Manifolds" to the needle with silicon tubes & another end of Manifolds to Filling Vessel.
- Adjust the rack of machine as per the requirement and adjust the center of needle rack for smooth run of needle without touching neck of Vial during filling.
- Adjust the flame and sealing height of the ampoule.
- Adjust the volume of filled solution from each needle respectively at the start of filling and ensure that it is within permissible volume limit.
- Clarity & Volume Variation Test shall be performed at initial middle and end stage of filling and observations shall be recorded.
- 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 inert atmosphere inside the Vials during Filling.

20.11 VISUAL INSPECTION OF FILLED AND SEALED MEDIA FILL VIALS:

After visual inspection of media filled vials all integral vials shall be collected in tray and transferred for incubation and non-integral vials shall be destroyed. Integral and non-integral vials categorized as follows:

- **Integral Vial –** All good vials, Sealed ampoule, black particle vials, fibers particle vials, glass particle vials, rough surface, volume variation, moulding defect, etc.

  (All above specified integral vials shall be incubated after completion of visual inspection)
- **Non-Integral Vial –** Damaged vials / Cracked vials, major sealing defects Vial Record the visual inspection observation of media filled units in respective Media Fill BMR.

Note: Step wise reconciliation of Media Fill vials shall be recorded in respective Media Fill BMR.

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

- Send all the integral vials for Incubation at inverted condition where after 07 days of incubation vials will be incubated vertically.
- Label the Individual Tray.
- Collect the Vials of Worse Case Study, Stage Wise, Label them and arrange acc. in the Incubation Room.

20.13 VISUAL INSPECTION OF FILLED VIALS:

20.14 VISUAL INSPECTION OF FILLED VIALS AFTER INCUBATION:

During visual inspection of incubated Vials, each Media-filled unit shall be examined for microbiological contamination by trained Microbiologist / trained visual inspectors and Training shall be provided before starting of visual inspection activity of incubated vials.

20.14.1 (VISUAL INSPECTION AFTER 1st & 7 DAYS OF INCUBATION AT 20^0C to 25^0C)

- Visual Inspection of inverted incubated Media Fill vials after 1st & 7th days of incubation to be perform.

20.14.2 (VISUAL INSPECTION AFTER 14th DAYS OF INCUBATION AT 30^0C to 35^0C)

- Visual Inspection of vertically incubated Media Fill vials after 14th days of incubation to be perform.

20.15 DEACTIVATION OF MEDIA FILLED VIALS AFTER INCUBATION:

- After Incubation and Inspection of the filled Vials, Media Filled Vials shall be destroyed as per SOP.
- Collect all the Vials from Incubation Room / Chamber for deactivation and Destruction.
- Each Vial shall be carefully opened and the Media Solution shall be collected in SS container.

- Load the SS container for Deactivation of Media Solution. Load in Vertical Autoclave and run the Cycle for Sterilization.
- Unload the Sterilized Media Solution and send the Sample of Sterilized Media Solution for GPT.
- The Post Sterilization / Deactivation GPT report shall be attached in the Media Fill Record.
- Empty Vials shall be deactivated by using 10% NaOH Solution and destroyed as per SOP.
- Record the Destruction details in Media Fill Record.

21.0 POST MEDIA FILL CLEANING:

21.1 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) and observe the results.
- Proceed for Commercial Production Activity only, if no Microbiological Growth is observed.

22.0 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**	Sterilization Time

S. No.	PROCESS STEPS	MONITORING PARAMETERS
	and Accessories	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

23.0 CRITICAL PARAMETERS AND ACCEPTANCE CRITERIA:

23.1 STERILIZED VIALS:

- Should comply Sterility Test & BET Test.

23.2 VIAL FILLING & SEALING:

- Average fill volume shall not vary by more than 2% of theoretical fill volume and the Media Filled Vial should cover over 70% of the Vial size.

23.3 MACHINE PARTS:

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

24.0 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						
1.	Pre GPT of media	Before media fill execution (Once)	Micro Lab	---------	GPT	Micro
MEDIA FILL SAMPLING PLAN						
2.	Filtered IPA 70%	After Disinfectant Filtration (Once)	From disinfectant filtration area	100 ml	Sterility	Micro
3.	Rinse water of machine Parts	After cleaning and before sterilization	Unit Preparation area	100ml + 100 ml	Description, pH, Conductivity	QC
4.	Wash Water of CIP	After CIP (once)	From Manufacturing tank, holding tank, buffer tank	100 ml+ 100 ml + 100 ml	Description, pH, Conductivity, Bioburden	Chemical & Micro
5.	WFI for media preparation	Before media preparation (once)	Manufacturing Area	500ml + 500ml	Complete analysis (Chemical + Micro)	Chemical & Micro
6.	Compressed air	Before ,During and End Stage of Filling	Filling Room	1000 L at Each stage of filling from user point	Sterility	Micro.
7.	Compressed Air	Before Filling	From Manufacturing room	1000 L	Sterility	Micro.
8.	Media Bulk Solution	Before Sterilization	From Manufacturing room	200 ml	Description, pH, Bioburden	Micro

9.	Media bulk solution	After sterilization	From Filtration room	800 ml	pH, GPT + Sterility	Micro
10.	Washed vials	Initial, Middle & End Stage of washing	From Filling Room	20 Nos. at each Stage	Clarity Test	Chemical
11.	Sterilized vials	Initial, Middle & End Stage of sterilization & Depyrozenation	From Filling Room	20 Nos. +05 Nos. at each Stage	Sterility + BET	Micro
12.	Rubber Bung	After sterilization Initial, Middle & End Stage of filling	Cooling Zone	30 Bungs at each Stage	Sterility BET & Moisture Content	Micro + Chemical
13.	Filled & Sealed vials	Initial, Middle & End Stage of Filling	From Packing Area	40 vials at each Stage	Description, Fill Volume, pH, Sterility, clearity	Micro
14.	Clarity Test & leak test	Initial, Middle, & End Stage of Filling	From Packing Area	08 Nos. at each Stage	Leak Test	IPQA
15.	Left Over media Solution	After Filling (Once)	From Filling Room	800 ml	pH, GPT + Sterility	Micro
POST MEDIA FILL PLAN						
16.	Swab sample of Machine Parts	After media fill (once)	Filling area	------	Bioburden by swab method	Micro
17.	Swab sample of wall, floor & machine surface	After media fill (once)	Filling area	------	Bioburden by swab method	Micro
18.	Environmental monitoring (Active & Passive air sampling)	After media fill (once)	Filling area	------	By Settle plate & air sampling Method	Micro
19.	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					
20.	Temperature , % RH & Differential Pressure	Before Media Fill	During Media fill	After media fill	IPQA
21.	Non-Viable Particle count of filling area	Before Media fill (Static Condition)	During Media fill	----------	IPQA
22.	Passive air sampling (Settle Plate)	Before Media fill	During Media fill	After media fill	Covering each shift by Micro
23.	Active air sampling	Before Media fill	During Media fill	After media fill	Covering each shift by micro
24.	Microbiological swab of walls, floor and machine surface	----------	----------	After media fill	Micro
25.	Personnel Monitoring by RODAC plate & finger Dab of all persons involved	After media fill			Micro

25.0 DEVIATIONS:

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

26.0 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 Filling:** 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.
- **Compounding:** A process wherein bulk drug substance is combined with another bulk drug substance and/or one or more excipients to produce a drug product.
- **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.
- **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 Test:** Test performed to determine if viable microorganisms are present.
- **Vent Filter:** Hydrophobic Non-shedding porous material capable of removing viable and non-viable particles from gases passing in and out of a closed vessel.

27.0 REFERENCES:

- Pharmaceutical Inspection Convention (Pharmaceutical Inspection Co-Operation Schemes) (PIC/S) PI 007-6, "Recommendation on the Validation of Aseptic Processes".
- USFDA Guidelines for Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practices.
- WHO Technical Report Series - 961
- United States Pharmacopoeia – 37
- Validation Master Plan.
- SOP Entitled "Process Simulation Study (Media Fill)" Sop.

28.0 ABBREVIATIONS:

SOP	:	Standard Operating Procedure
Ster.	:	Sterilization
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
MLT	:	Microbial Limit Test
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
PDA	:	Parentral Drug Association, INC.
USP	:	United States Pharmacopoeia
HVAC	:	Heating, Ventilation and Air Conditioning
QAO	:	Quality Assurance Officer
QAE	:	Quality Assurance Executive

29.0 REVISION HISTORY:

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