PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE				
Title: Operation, Cleaning and Calibration of Autoclave (Automatic)				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 11	

1. **Purpose:** The purpose of this SOP is to describe the procedure for operation, cleaning and calibration of Autoclave (Automatic).

2. **Scope:** This SOP is applicable to autoclave.

Make	Model	Sr. No.	ID No.
Medica			

3. **References, Attachments & Annexures:**
   - 3.1. **References:**
     - 3.1.1. Instruction manual supplied by manufacturer.
     - 3.1.2. Receipt, storage, preparation, growth promotion and disposal of microbiological media.
     - 3.1.3. Maintenance of laboratory instrument.
     - 3.1.4. Preparation of internal and external (Third Party) calibration schedule and calibration practices.
     - 3.1.5. Handling of Out of Calibration.
   - 3.2. **Attachments:**
     - 3.2.1. Attachment-1: Sterilization record
     - 3.2.2. Attachment-2: Autoclave calibration template
   - 3.3. **Annexures:**
     - 3.3.1. Annexure-1 : Autoclave cleaning record

4. **Responsibilities:**
   - 4.1. **Microbiologist:**
     - 4.1.1. To follow and perform the activity as per SOP
     - 4.1.2. To calibrate the equipment as per SOP
     - 4.1.3. To maintain the calibration records
   - 4.2. **QC Head or designee:**
     - 4.2.1. To check the SOP.

4.2.2. To give training to all concerned persons before implementation of SOP
4.2.3. To ensure the operation and calibration of the equipment is carried out as per SOP
4.2.4. To initiate repairs or breakdown and to make alternative arrangements during the status of under maintenance
4.2.5. To execute the OOC in case of calibration failure and in case of breakdown intimate to the Quality Head

4.3. **Quality Assurance:**
4.3.1. To check the SOP
4.3.2. To ensure the implementation of system as per SOP

4.4. **Regulatory Affairs, Quality Head , Plant Head:**
4.4.1. To review and approve the SOP

5. **Distributions:**
5.1. Quality Assurance
5.2. Microbiology

6. **Abbreviations & Definitions of terms :**
6.1. **Abbreviations:**
6.1.1. Strt.: Start
6.1.2. lbs. : The traditional symbol stands for *libra*, the latin word for the unit-pound.
6.1.3. SCDM: Soybean casein digest medium
6.1.4. OOC : Out of calibration
6.2. **Definitions of terms :**None

7. **Procedure:**

7.1. **General:**

7.1.1. Maintain the internal and third party calibration schedule for the instrument as per SOP, “Preparation of internal and external (Third party) calibration schedule and calibration practices”.

7.1.2. In case of any maintenance of instrument, follow SOP on “Maintenance of laboratory instrument”.

7.1.3. In case of noticing any calibration results not within the specified limit, follow the SOP of "Handling Out of Calibration”.

7.2. **Precautions:**

7.2.1. Do not open the autoclave top lid during it's operation.

7.2.2. Use safety gloves during unloading of autoclave.

7.2.3. Before starting the autoclave check the water level upto mark.

7.3. **Operation:**

7.3.1. **Start up:**

7.3.1.1. Switch “ON” the power and after it switch “ON”power controller and the upper display will show actual temperature and lower display will show “strt” temperature. Switch ON/OFF rocker of the printer to get print (if printer is available). Printer will give print for each minutes of all three channel. If print is not required then switch off the printer.

7.3.1.2. To alter temperature or SOAK time set point, press SET key once, upper display will show “User” and lower display will show “list”. Press enter key then upper display will show 121.0 and lower display will show SP. To change the temperature set point press enter key to enable it, upper display start blinking,change value by pressing “UP and DOWN” arrow key. Press enter key to register. Display will change to Upper and show 20.0 i.e previous set time and lower display show SOAK. To change the SOAK time set point, press enter key to enable it; upper display will start blinking and change value by pressing UP and DOWN arrow key, press enter key to register.

7.3.1.3. Press SET key to return to main display.

7.3.1.4. Set the sterilization cycle for media, diluent, saline, empty glasswares at 121ºC/15 lbs. for 15 minutes or as per requirement.

7.3.1.5. At the end of cycle, display will show END. To start new cycle keep pressing ENTER for 3 seconds and lower display will show Strt. Now press ENTER.

7.3.2. **Sterilization run numbering:**

7.3.2.1. Allot sterilization run number using prefix ST (stand for sterilization), followed by last two digit of year (12 for 2013), and four digits serial number (0001).

For example: First sterilization run number in 2013 shall be numbered as ST/13/0001 and record the data in attachment-1.

7.3.3. **Operation:**

7.3.3.1. Open the autoclave by unscrewing the fly to access the sterilizing chamber.

7.3.3.2. Remove the baskets but leave the heater cover, 'X' shape stand inside.

7.3.3.3. Ensure that the drain valve at the bottom of the autoclave is closed.

7.3.3.4. Pour purified water direct into the autoclave, up to the heater cover 'X' shape stand.

7.3.3.5. Load the glassware/media/dresses/purified water/filtration assembly or other miscellaneous items to be sterilized in autoclave chamber as per calibration cycle. The maximum load volume of the autoclave is 70% of its drum / carrier size. Close the screw valves tightly. Turn “ON” the autoclave, using the 'MCB'.

7.3.3.6. Follow the load quantity and loading pattern as followed while external calibration of autoclave.

7.3.3.7. The set temperature and set time will be displayed on the microprocessor controller (µPC) in brackets. If acceptable, press START.

7.3.3.8. The solenoid valve will remain open up till the temperature reaches approximately 100 °C in order to purge air enclosed in the autoclave, after which it will automatically be closed.

7.3.3.9. After this the temperature and pressure will gradually rise, which will be indicated by the microprocessor controller and pressure gauge.

7.3.3.10. Just before the set temperature is attained the heater will be cut “OFF”and then gradually will attain the set temperature. The timer will start its countdown after the set temperature is attained. During the count down the temperature and pressure will be maintained by the microprocessor controller by switch “ON/OFF” the heater as required.

7.3.3.11. During this period of countdown the safety valve may leak slightly, which is normal. The start time of sterilization cycle will be checked manually and shall be recorded in attachment – 1

7.3.3.12. After the elapse of the set time a buzzer will sound for about 20 seconds, the heater will be cut “OFF” and the solenoid valve open to exhaust the pressure.

7.3.3.13. For faster evacuation of the pressure the manual steam release valve (exhaust valve) may be opened.

7.3.3.14. Unscrew the fly nuts only after the pressure gauge shows "0". Do not attempt to open the fly nuts at even 1 or 2 psi.

7.3.3.15. To maintain sterility, remove the sterilized material at the earliest after total exhaust of pressure. The purge valve cum vacuum breaker will allow air to enter inside the chamber to prevent a vacuum within. This air may re-contaminate the load.

7.3.3.16. To restart the cycle press the "START"switch. Be sure to top up the water level with purified water before starting a new cycle.

7.3.3.17. Keep one self-indicator strips (Steam-clox) or propper sterilization indicator (OK) or stick autoclave indicator tape in any material to be sterilized with every run and record as per attachment-1.

7.4. **Cleaning:**

7.4.1. To clean the chamber, a drain valve is provided at the bottom. Drain valve is attached with drainage line by rubber tubing. For cleaning open the drain valve. Place a vessel of adequate size bellow the drain valve.

7.4.2. After the chamber is empty, Pour adequate amount of cleaning solution (Hemac-4 10%) for cleaning and finally rinse the autoclave with purified water (all around) so that the residual matters/particles are flushed out and close drain valve.Pour purified water for next cycle. Change the water on daily basis and cleaning should be done on weekly basis.

7.4.3. Record the autoclave cleaning record as per annexure-1

7.5. **Calibration:**

7.5.1. **Internal:**

7.5.1.1. **Frequency :** Yearly ± 30 days or after any breakdown.

7.5.1.2. Ensure that temperature and pressure gauge are calibrated.

7.5.1.3. Keep three self-indicator strips (steam-clox) in any material to be sterilized in upper basket,one in lower basket and one in medium basket of the autoclave. Check steam-clox self-indicator strip for the color change from purple to green on the upper side of arrow.

7.5.1.4. Use biological indicator of *Geobacillus stearothermophilus.*

7.5.1.5. Keep 10 biological indicators in the autoclave at 10 different locations in upper and lower basket outside the load.

7.5.1.6. After completion of sterilization cycle indicators are removed and incubated in 100 ml sterile SCDM for 5-7 days at 55-60°C.

7.5.1.7. **For positive control:** A non-sterilized indicator should be incubated. In case of strip/ampoule inoculated in SCDM, for 5-7 days at 55-60°C, growth must be observed in positive control.

7.5.1.8. **Acceptance criteria:** If the strip/ampoule changes its color from purple to green (chemical indicator) and if strip/ampoule don't show any turbidity in

medium (biological indicator) after 5-7 days of incubation, it means the sterilization cycle is running properly.

7.5.1.9. But if the color of the strip/ampoule remains as initial that is purple (chemical indicator) and if strip/ampoule (biological indicator) show turbidity in medium, after 5-7 days of incubation, it means the sterilization cycle is not running properly.

7.5.1.10. Record the results as per attachment-2.

7.5.2. **External:**

7.5.2.1. **Frequency:** Yearly ± 30 days

7.5.2.2. Heat distribution and Heat penetration study is carried out by external agency as per approved protocol.

Attachment – 1
Sterilization Record

Date	Sterilization run no.	Materials sterilized	Cycle start Time	Cycle end Time	Done by	Checked By	Autoclave Indicator

Attachment – 2
Autoclave Calibration Template

Instrument ID No.	
Make and Model	
Calibration frequency	
Date of Calibration	
Next Calibration due on	
Temp. Gauge calibration due date	
Pressure gauge calibration due date	
Calibration aids	Chemical indicator and Biological indicator

Chemical Indicator (Steam-Clox™):

S.No.	Location of indicator	Observations	Acceptance criteria		
			Indicator part	From	To
1	Top		Three upper squares		
2	Middle		Circle		
3	Bottom				

Biological Indicator

Sterilizer:		Biological Indicator:		Lot No:	
Sterilization Temperature:		Spore density:	(Acceptance criteria: NLT 1.0 X 10^6 spores/ indicator)		
Date and Time of Incubation started:		Date and Time of Incubation completed:			

S.No.	Biological Indicator Position	Observation	Remarks

	Positive Control		
	Negative Control		

Acceptance criteria: If the strip/ampoule changes its color from purple to green (chemical indicator) and if strip/ampoule don't show any turbidity in medium (biological indicator) after 5-7 days of incubation, it means the sterilization cycle is running properly

Positive control: A non-sterilized indicators, should be incubated. In case of strip/ampoule inoculated in broth, for 5-7 days at 55-60°C , growth must be observed in positive control.

Negative control: Growth should not be observed in negative control.

Calibrated By: **Date:**	**Checked By:** **Date:**	**Approved By:** **Date:**

Annexure–1
Autoclave Cleaning Record

Autoclave ID No. : …..... **Frequency:** Weekly

Sr.No.	Date	Solution used	Cleaned by	Checked by	Remarks

8. **History:**

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