PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Evaluation of disinfectant and cleaning solution	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1. **Purpose:** The purpose of this SOP to define the procedure for evaluation of disinfectant and cleaning solution against micro-organism.

2. **Scope:** This procedure is applicable to all disinfectant and cleaning solution which are used in production area and in Microbiology Laboratory at ……

3. **References, Attachments & Annexures:**
   - 3.1 **References:** In- House
   - 3.2 **Attachments:**
     - 3.2.1 Attachment-1:Protocol For Evaluation of Disinfectant/Cleaning solution
     - 3.2.2 Attachment-2:Hold time efficiency of Sanitization/Cleaning solution
   - 3.3 **Annexures:** None

4. **Responsibilities:**
   - 4.1 **Microbiologist:**
     - 4.1.1 To perform the activity as per SOP.
     - 4.1.2 To maintain the records as per SOP.
   - 4.2 **QC Head:**
     - 4.2.1 To check the SOP.
     - 4.2.2 To give the training to all concern persons..
   - 4.3 **Quality Assurance:**
     - 4.3.1 To check the SOP
     - 4.3.2 To ensure proper implementation of SOP.
   - 4.4 **Regulatory Affairs, Quality Head , Plant Head:**
     - 4.4.1 To review and approve the SOP.

5. **Distribution:**
   - 5.1 Quality control (microbiology)

   - 5.2 **Abbreviations & Definition of Terms:**
     - 5.2.1 TFC : Total fungal count
     - 5.2.2 CFU : Colony forming unit
     - 5.2.3 RODAC : Replicate organism detection and counting.
     - 5.2.4 TBC : Total bacterial count
     - 5.2.5 SOP : Standard Operating Procedure
     - 5.2.6 SCDA : Soya casein digest medium

   - 5.3 **Definition of Terms : None**

6. **Procedure:**
   - 6.1 Evaluation of disinfectant/cleaning solution is one time study, but if the disinfectant/cleaning solution change, evaluation should be done for that disinfectant/cleaning solution.
   - 6.2 **Evaluation of disinfectant/cleaning solution shall be done in three steps:**

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6.2.1 Initial count study
6.2.2 Recovery study of microorganisms using different type of plates like epoxy, or cota stone.
6.2.3 Study of reduction/inhibition in count after applying of disinfectant/cleaning solution.

6.3 **Isolation of house organism:**
6.3.1 Select isolated colonies from environmental monitoring plates.
6.3.2 Take a loopful of isolated colony and streak on the surface of sterile (per incubated) Soyabean casein digest agar plate and incubate at 30-35°C for 24 hours.

6.4 **Identification of house organism:**
6.4.1 Select any one colony and note down the colony character like size, shape, elevation, color, margin etc.
6.4.2 After characterization perform gram staining and note down the result.
6.4.3 Identify the isolated organism with the help of identification kit.

6.5 **Bacterial culture suspension preparation, dilution and cell enumeration:**
6.5.1 Take 10 ml of Soyabean casein digest medium and inoculate a loopful of the bacterial strains separately in 10 ml of Soyabean casein digest medium.
6.5.2 Incubate the tubes at 30-35^{0}C for 18-24hrs.
6.5.3 Observe the tubes for turbidity.

6.6 **Preparation of serial dilution of the bacterial cultures:**
6.6.1 Aseptically transfer 1ml of [NMT 24hrs] broth cultures in to 9ml of sterile normal saline.
6.6.2 Vortex the test tubes for about 30 seconds to homogenate the contents.

6.7 **Enumeration of culture suspension:**
6.7.1 Transfer aseptically 1ml of (10^{-4}) dilution of isolated organism in to empty sterile Petri plates in duplicate.
6.7.2 Add about 15 to 20 ml of previously melted and cooled to approximately 45^{0}C Soyabean casein digest agar , mix the culture suspension with agar and allow to solidify at room temperature
6.7.3 Repeat above step for remaining four dilution i.e. 10^{-5}, 10^{-6},10^{-7} 10^{-8}
6.7.4 Repeat above procedure for remaining other organism.
6.7.5 Immediately store the culture suspension in the freeze at 2-8^{0}C.
6.7.6 Invert the petri plates and incubate the plates at 30-35°C for 2-5 days (20-25°C for 5-7 days in case of fungi).
6.7.7 Observe the plates and report number of cfu/plate.
6.7.8 Select the dilution which is having the colonies in the range of 30 to 300 per ml (10 to100for fungi) . Following dilutions are used for Recovery study and inhibition study.

6.8 **Recovery study of micro organisms using different types of plates like epoxy, cota stone etc.**
6.8.1 Take 4 to 6 sterile Epoxy floor piece or cotastone having marking of a square dimensions of 15x15 mm.
6.8.2 Mark the square with number 1 to 5.

6.8.3 Identify each piece e.g. (1) Recovery study (2) Zero minute study (3) 5 minutes study (4)10minutes study and (5) 15 minutes study along with the name of organism to be used for study.

6.8.4 Take 1ml of the culture suspension having the dilution of 10^{-7} .

6.8.5 Spread the suspension uniformly on the epoxy piece /cota stone or floor.

6.8.6 Let it get air dried. Take RODAC plates from epoxy sheet no.(1) .

6.8.7 Incubate it at 30-35°c for 24-48 hrs.

6.8.8 Report the microbial counts observed on the RODAC, take the total cfu by summation of all RODAC plate counts.

6.8.9 Extrapolate the results with the area of epoxy sheet and get the total recovery of microorganisms per ml. against the initial count.

6.8.10 It should be 80-120% of the initial count study.

6.8.11 Calculate the results with the help of following formula;

Area of RODAC Plate = πr^2 =3.14x2.75x2.75cm =23.746 cm

Total cfu recovered =___ cfu in(23.746 x_____) = ____ cm area

So, in _____cm area _____cfu (_____x_____/ ____) per ml

6.9 **Study of reduction/inhibition in count after applying of sanitizing/cleaning solution:**

6.9.1 Prepare in use concentration of sanitizing/cleaning solution using purified water and mix well. Take unfiltered solution in a sterile container.

6.9.2 Take a sterile sponge sheet dip it in to the unfiltered sanitization/cleaning solution and mope the other four epoxy sheets and note down the time of moping started.

6.9.3 Immediately after sanitization take RODAC plate from epoxy sheet No.2 (zero minute) At the time interval of 5 minutes of contact period, take RODAC plates from epoxy sheet no. 3 and note down the time of sampling after 5 minutes.

6.9.4 At the time interval of 10 minutes of contact period, take RODAC plate from the epoxy sheet no.4 and note down the time of sampling (10minutes).

6.9.5 Same way take RODAC plate from the sheet no.5 after 15 minutes and note down the time of sampling (15 minutes).

6.9.6 Incubate the plates at 30-35°C (20-25°C for fungi) for 48-72 hours (5 days in case of fungi).

6.9.7 Calculate the results with the help of following formula;

Area of RODAC Plate = πr^2 =3.14x2.75x2.75cm =23.746 cm

Total cfu recovered =___ cfu in(23.746 x_____) = ____ cm area

So, in _____cm area _____cfu (_____x_____/ ____) per ml

6.10 Record the results in approved protocol.

6.11 **Acceptance criteria:** Three-Log reduction in cell density within 15 minutes.

6.12 **Determining the hold time efficiency of sanitization/cleaning solution**

6.12.1 With the help of an micropipette, pipette out 1.0 ml of solution and filter through a 0.45 µ membrane.

6.12.2 Give three washings of 100 ml each with 1 % sterile peptone water.

6.12.3 After filtration, with the help of a sterile forcep take the membrane filter and place the membrane filter on a SCDA agar plate and Incubate the plate for bacterial count and fungal count

6.12.4 After incubation count the number of colonies present on the membrane filter.

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Attachment – 1

Activity	Sign & Date
Study design : Part-1 Initial count study Part-2 Recovery study of microorganisms using different types of plates like epoxy, cota stone etc. Part -3 Study of reduction/inhibition in count after application of disinfectant/cleaning solution.	
Incubator ID No: (30-35°C)_____ Calibration valid up to: _____	
Incubator ID No: (20-25°C)_____ Calibration valid up to: _____	
Micro pipette No:_____ Calibration valid up to:_____	
LAF ID NO:_____ Calibration valid up to:_____	
Part – 1 Initial Count Study	
Bacterial culture suspension preparation, dilution and cell enumeration.	
Bacterial suspension preparation.	

Organism to be used	Working Culture ID	
Inoculate a loopful of the bacterial strains separately in 10 ml of Soyabean casein digest medium. Medium preparation Lot No:_____.		
Incubate the tubes at 30-35^{0}C for 18-24hrs.		
Incubation date _____ Time_____ Incubation out Date:_____Time:_____		
Observe the tubes for growth (Turbidity). Record the details in the following table.		
Organism Used	Growth observed / Not observed	
Preparation of serial dilution of the bacterial cultures.		
Aseptically transfer 1ml of (NMT 24hrs) broth cultures in to 9ml of sterile normal saline.		
Vortex the test tubes for about 30 seconds to homogenate the contents.		
Enumeration of culture suspension		

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Transfer aseptically 1ml of (10^{-4}) dilution of isolated organism in to empty sterile Petri plates in duplicate

Add about 15 to 20 ml of previously melted and cooled to approximately 45^0c Soyabean casein Digest agar , mix the culture suspension with agar and allow to solidify at room temperature

Repeat above step for remaining four dilution i.e. 10^{-5}, 10^{-6},10^{-7} 10^{-8}

Repeat above procedure for remaining other organisms.

Immediately store the culture suspension in the freeze at 2-8^0c.

Invert the petri plates and incubate the plates at 30-35^0C for 2-5 days.

Observe the plates and report number of cfu/plate and details in below table.

Name of Microorganism : _____

Dilution	**No of cfu /plate**		
	Plate1	Plate2	Cfu/plate Mean
10-4			
10-5			
10-6			
10-7			
10-8			

Name of Microorganism _____

Dilution	**No of cfu /plate**		
	Plate1	Plate2	Cfu/plate Mean
10-4			
10-5			
10-6			
10-7			
10-8			

Select the dilution which is having the colonies in the range of 30 to 300 per ml (10 to100for fungi) . Following dilutions are used for Recovery study and inhibition study.

Dilution used for recovery for study			Dilution used for inhibition study	
Name of organism	Dilution used	Total no. of cfu/ml	Dilution used	Total no. of cfu/ml

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		_____Cfu/ml		_____Cfu/ml	
		_____Cfu/ml		_____Cfu/ml	
		_____Cfu/ml		_____Cfu/ml	

Part – 2 Recovery study of micro organisms using different types of plates like epoxy ,cota stone etc.

Take 4 to 6 sterile Epoxy floor piece or cota stone having marking of a square dimensions of 15x15 mm. Mark the square with no1 to 5.Identify each piece e.g. (1) Recovery study (2) Zero minute study (3) 5 minutes study (4)10minutes study and (5) 15 minutes study along with the name of organism to be used for study. Take 1ml of the culture suspension of selected dilution and spread the suspension uniformly on the epoxy piece/cota stone. Let it to air dry. Take RODAC plates from epoxy piece or cota stone no.(1) .Incubate it at 30-35°c for 24-48 hrs. Report the microbial counts observed on the RODAC,take the total cfu by summation of all RODAC plate counts. Extrapolate the results with the area of epoxy sheet and get the total recovery of microorganisms per ml against the initial count .It should be 80-120% of the initial count study.

Name of organism	cfu per plate					Total cfu	Total recovery on epoxy/cota piece
	1	2	3	4	5		

Calculation: (1) Name of organism:_____

Area of RODAC Plate = πr^2 =3.14x2.75x2.75cm =23.746 cm

Total cfu recovered =_____ cfu in(23.746 x_____) = _____ cm area

So, in _____cm area _____cfu (_____x_____/ _____) per ml

(2)Name of organism:_____

Area of RODAC Plate = πr^2 =3.14x2.75x2.75cm =23.746 cm

Total cfu recovered =_____ cfu in(23.746 x_____) = _____ cm area

So, in _____cm area _____cfu (_____x_____/ _____) per ml

Part 3 : Study of reduction / inhibition in count after applying of sanitizing solution

Preparation of sanitation solutions : Prepare in use concentration of sanitizing solution using purified water and mix well. Take unfiltered solution in a sterile container.

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Name of Sanitation solution :_____ Lot No . _____

Procedure :Take a sterile sponge sheet deep it in to the unfiltered sanitization solution and mope the other four epoxy sheets and note down the time of moping started. Immediately after sanitization take RODAC plate from epoxy sheet No.2 (zero minute)

At the time interval of 5 minutes of contact period, take RODAC plates from epoxy sheet no. 3 and note down the time of sampling.(5 minutes)
At the time interval of 10 minutes of contact period, take RODAC plate from the epoxy sheet no.4 and note down the time of sampling(10minutes). Same way take RODAC plate from the sheet no.5 after 15 minutes and note down the time of sampling (15 minutes).

Incubate at 30-35°C (20 to 25 °C for fungi) for 48 to 72 hours (5 days in case of fungi),
Date In_____Time_____
Date Out _____ Time _____

Observe for CFU and record in the table given.

S.No.	Name of Organism	0 min	5 min	10 Min	15 min	Name of Organism	0 min	5 min	10 min	15 min
1										
2										
3										
4										
5										
	Total					Total				

Calculation: (1) Name of organism:_____
Area of RODAC Plate = πr^2 =3.14x 2.75 x2.75 cm =23.746cms
Total Count =_____ cfu in 23.746 x_____ = _____cms area
So, in _____cms area _____cfu (_____x_____/_____) = _____cfu
obtained after exposure to sanitization solution for 15 minutes.
This is _____times reduction in count compared to the initial count

(2)Name of organism:_____
Area of RODAC Plate = πr^2 =3.14x 2.75 x2.75 cm =23.75cms

Total Count =_____ cfu in 23.75 x_____ = _____cms area
So, in _____cms area _____cfu (_____x_____/_____) = _____cfu
obtained after exposure to sanitization solution for 15 minutes.
This is _____times reduction in count compared to the initial count

Acceptance Criteria: There should be at least three log reduction of initial count achieved within 15 minutes of contact time

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Conclusion: Reduction is achieved/not achieved, so the sanitation solution _____is effective against organisms _____ and _____ when _____ minutes contact time is allowed as three log reduction from initial count is achieved.

Sample destroyed after analysis. Done By/Date: _____

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

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Attachment-2

Sanitization/cleaning solution:
Testing Method: Membrane Filtration
Lot/batch No. of sample:
Sample received date:
Hold time period of solution:

Date of Testing:
Media Used:

Date of observation:
Lot No. of Media:

Temperature:35±2^{0}C 48 hrs & 25±2^{0}C for further 72 hrs

S.No.	Solution Name	Count after 72 hrs (TFC)	Count after 48 hrs (TBC)	Total counts

Analyzed by: Date:	Checked by: Date:	Approved by: Date:

7. **History:**

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