STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 Objective

To lay down a procedure for the Preparation of Standardized Cell Suspension.

2.0 Scope

This Standard Operating Procedure is applicable for formulation plant.

3.0 Responsibility

Executive/Officer-Microbiology : Shall be responsible for following the SOP for Preparation of Standardized cell suspension.

Head-QC/Designee : Shall be responsible for compliance of this SOP.

4.0 Abbreviations and Definitions

SOP	: Standard Operating Procedure
QC	: Quality Control
SCDM	: Soyabean Casein Digest Medium
SCDA	: Soyabean Casein Digest Agar
SDA	: Sabouraud Dextrose Agar
CFU	: Colon Forming Unit
ml	: Milliliter
L	: Liter

5.0 Procedure

5.1 Prepare the required quantity of Soyabean Casein Digest media (SCDA) and Sabouraud Dextrose Agar (SDA) media in a conical flask.

5.2 Reconstitute the media with the required volume of purified water.

5.3 Boil the media in the water bath to uniformly dissolve the media.

5.4 Dispense 15 ml of the media in a clean 18 mm rimless test tube.

5.5 Plug the tubes with cotton plug and wrap the cotton plug of the tube with crepe paper and label the tubes for type of media, autoclave lot no and date of sterilization.

5.6 Similarly prepare normal saline solution (0.9% w/v sodium chloride solution) for harvesting of bacterial cultures, and buffered sodium chloride peptone solution containing 0.5% Tween 80 solution for harvesting of fungal and yeast cultures.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

5.7 Transfer 9 ml of the normal saline solution and buffered sodium chloride peptone solution in required number of test tubes and label the tubes for type of solution, autoclave lot no and date of sterilization.

5.8 Plug the test tube with cotton plug and wrap the plug with crepe paper.

5.9 Steam sterilize the media slants, normal saline solution tubes and peptone solution tubes as per the validated autoclave cycle.

5.10 After steam sterilization remove the media slants, normal saline solution tubes and peptone solution tubes from the autoclave.

5.11 Place the media tubes under laminar airflow (LAF) at approximately 30º from the surface.

5.12 Allow the media to solidify.

5.13 After the solidification of the media slants, transfer the SCDA slants, normal saline solution tubes and peptone solution tubes to the incubator for incubation at 30 to 35ºC for 48 hours and SDA slants at 20 to 25ºC for 48 hours for checking of any contamination.

5.14 Streak the surface of the SCDA slant with the bacterial culture and SDA slant with fungal culture.

5.15 Incubate the SCDA slants at 30 to 35ºC for 48 hours and SDA slants at 20 to 25ºC for 48 hours for *Candida albicans* and *Aspergillus niger* culture for 3- 5 days.

5.16 Prepare culture suspension by washing and scraping the surface of the slant by means of sterile inoculating loop in 10 ml of 0.9% saline for Bacterial, and buffered sodium chloride peptone solution containing 0.5% Tween 80 solution for yeast and fungal culture.

5.17 Transfer the culture suspension in a sterile test tube.

5.18 Vortex the culture suspension to obtain a uniform suspension.

5.19 Carry out serial dilution so as to obtain a culture suspension of 10-100 cfu/ml by following the steps given below.

5.19.1 Transfer 1 ml of the suspension to 9 ml sterile normal saline solution – 10^{-1} Dilution.

5.19.2 1 ml of 10^{-1} Dilution to 9 ml sterile normal saline solution – 10^{-2} Dilution.

5.19.3 1 ml of 10^{-2} Dilution to 9 ml sterile normal saline solution – 10^{-3} Dilution.

5.19.4 1 ml of 10^{-3} Dilution to 9 ml sterile normal saline solution – 10^{-4} Dilution.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

5.19.5 1 ml of 10^{-4} Dilution to 9 ml sterile normal saline solution – 10^{-5} Dilution.

5.19.6 1 ml of 10^{-5} Dilution to 9 ml sterile normal saline solution – 10^{-6} Dilution.

5.19.7 1 ml of 10^{-6} Dilution to 9 ml sterile normal saline solution – 10^{-7} Dilution.

5.19.8 1 ml of 10^{-7} Dilution to 9 ml sterile normal saline solution – 10^{-8} Dilution.

5.20 Pipette out 1 ml of the each microbial inoculum from last three dilution tubes into sterile petriplate in duplicate.

5.21 Incubate the SCDA plates at 30 to 35ºC for 48 hours for bacterial cultures and SDA plates at 20 to 25ºC for 48 hours for *Candida albicans* and for 3-5 days for *Aspergillus niger* culture.

5.22 Till the observation of the microbial counts preserve all the dilution tubes at 2 to 8ºC.

5.23 After incubation count the colonies and note the microbial count in the format attached as Annexure I.

5.24 Note the dilution, which is giving a microbial count in between 10 to 100 CFU/ml.

5.25 Preserve the previous dilution which is giving 10 to 100 CFU/ml. This dilution shall be preserved for microbial inoculum and from this dilution 100µl of the suspension shall be used for testing. For example if the 10^{-7} dilution is giving microbial count in between 10 to 100 CFU/ml the 10^{-6} dilution tubes shall be preserved and 100µl of the suspension shall be used to give microbial count in between 10 to 100 CFU/ml.

5.26 Label the suspension tubes as per the label given below

CULTURE SUSPENSION	
Name of Microorganism	:
Strain N o	:
Counts	:
Dilution	:
Prepared on	:
Prepared By	:
Use before	:

5.27 Preserve the diluted suspension in refrigerator at temperature 2- 8ºC for not more than15 days.

5.28 The frequency for preparation of the microbial suspension shall be weekly.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

6.0 Forms and Records

6.1	Serial Dilution Chart	:	Annexure-1
6.2	Observation and Results	:	Annexure-2

7.0 Distribution

7.1	Master Copy	:	Documentation Cell (Quality Assurance)
7.2	Controlled Copies	:	Quality Control, Quality Assurance,

8.0 History

Date	Revision Number	Reason for Revision
	00	New SOP

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE I

SERIAL DILUTION CHART

HARVESTED CULTURE SUSPENSION

↓

10^{-1}

↓

10^{-2}

↓

10^{-3}

↓

10^{-4}

↓

10^{-5}

↓

10^{-6}

↓

10^{-7}

↓

10^{-8}

Serial dilution shall be done by adding 1 ml of previous dilution to the 9 ml of sterile normal saline solution starting from harvested culture suspension.

Last 3 dilutions i.e. 10^{-6}, 10^{-7} and 10^{-8} shall be plated for enumeration of standardized cell suspension.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE II
OBSERVATION & RESULTS

1.0 Preparation of Slant:

Name of Media Used _____ **Autoclave Lot No of media** _____

Incubator ID (20-25 °C)_____ **Incubator ID (30-35 °C)**_____

S.No.	Name of culture	Streaked On	Incubation		Done By & Date
			At Temperature (°C)	Duration (Hrs / Days)	

Reviewed By:

Date :

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

2.0 Harvesting Record:

Name of Harvesting Media Used _____ Autoclave Lot No of Harvesting Media _____

S.No.	Name of Culture	Harvesting Date	Harvesting Media	Volume (ml)	Done By & Date

Reviewed By:

Date :

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Standardized Cell Suspension	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

3.0 Enumeration Record:

Name of Media Used ______________________ **Autoclave Lot no of media** __________

Incubator ID (20-25 °C) ____________________ **Incubator ID (30-35 °C)** _____________

S.No.	Name of Culture	Inoculated On	Done By & Date	Dilution Tubes	Observations			Observed By & Date
					Plates (CFU/ml)			
					I	II	Avg. Count	

Reviewed By:

Date :