GENERAL STUDY PROTOCOL

FOR

DEVELOPMENT OF ENVIRONMENTAL AND WATER ISOLATES

PROTOCOL No.	
SUPERSEDES No.	NIL
EFFECTIVE DATE	

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1.0 Pre - Approval

The Protocol has been prepared, Reviewed and Approved for implementation by the under signed.

Microbiologist

PREPARED BY	SIGNATURE	DATE

Head Quality Control or Designee

REVIEWED BY	SIGNATURE	DATE

Head Quality Assurance or Designee

APPROVED BY	SIGNATURE	DATE

2.0 OBJECTIVE:

Objective of this protocol is to establish the database of normal microbial flora of Environment and water system.

3.0 SCOPE:

This document details the test procedure to establish the database of normal microbial flora of Environment and water system of …….. The same established data should be referred in future, whether isolates observed are part of the normal microbial flora or represent something different.

4.0 RESPONSIBILITY:

Department	Responsibility
User	• Preparation of validation protocol and of report. • Execution and recording of results. • Review of qualification protocol and report.
Quality Assurance	• Review of validation protocol and report in compliance to the requirements. • Archival of approved protocol and reports.

5.0 ABBREVIATIONS

5.1 Q.C : Quality Control

5.2 Q.A : Quality Assurance

5.3 ^{0}C : Degree Celsius

5.4 SOP: Standard Operating Procedure

5.5 SCDA : Soybean Casein Digest Agar

5.6 LAF : Laminar air flow

5.7 Ml : Milliliter

5.8 Hrs. : Hours

6.0 TRAINING TO PROTOCOL EXECUTOR(S)

Following persons have been trained on this approved protocol and will execute/ help in execution of this protocol.

Duration and time of training: _____

Venue of training: _____ Date of training: _____

S.No.	Name of Trainee	Designation of Trainee	Signature of Trainee	Evaluation OK/To be retrained	Signature of evaluator

Trainer details

Name	Designation	Signature

Verified by: _____ **Date:** _____

7.0 PRE-REQUISITES

7.1 Prior to start conducting/executing the analytical method validation protocol following things must be available

- Isolated Colony
- Inoculation Loops
- Petriplates with Media
- Staining Kit
- Microscope
- Phoenix Panels
- Phoenix ID Broth or Phoenix inoculum broth
- Phoenix panel closures
- Phoenix inoculation station
- Phoenix Transport Caddy
- BD PhoenixSpec Nephelometer
- 25 µl Pipettor and tips
- Incubators
- Biohazard Disposable Container.
- Marker
- Vortex Mixture

8.0 STUDY METHODOLOGY:

8.1 PRELIMINARY TESTING AND OBSERVATION:

8.1.1 Record the isolation details like date of isolation/isolation source/sampling method/sampling location/ and other details in Annexure –I and allot the isolate code number as per SOP.

8.1.2 Observe the colony morphology like size,colour,shape,elevation, edge,opacity,fluorescence and any other details and record the morphological characteristic in Annexure-I and take a photograph of colony. For colony shape ,elevation and Edge refer Figure-I (but not limited to)

Colony morphology

MARGIN	COLOUR	ELEVATION	TEXTURE	SHAPE
 Curled	 Orange	 Raised	Slimy, moist	 Round
 Entire (smooth)	 Red or pink	 Umbonate	Matte, brittle	 Punctiform
 Filamentous	 Black	 Flat	Shiny, viscous	 Rhizoid (root-like)
 Undulate (wavy)	 Brown	 Convex	Dry, mucoid	 Filamentous
 Lobate	 Opaque or white	 Pulvinate (Cushion-shaped)	Translucent	 Irregular
 **Erose (serrated)**	**Milky**	**Growth into culture medium**	**Iridescent (changes colour in reflected light)**	 **Spindle**

Source: MicroDok

Figure-I

8.2 Preparation of Pure Culture:

8.2.1 Pick the colony from environment monitoring sample or water sample which has to be identified.

8.2.2 Prepare the suspension of colony in a 10ml of sterile peptone water.

8.2.3 Take a loopful of prepared suspension and streak on the pre incubated agar Plate.

8.2.4 Streak the suspension in such a way to get a isolated colony

8.2.5 Plating of peptone water used for suspension should be done to confirm the sterility of the medium.

8.2.6 Incubate the streaked media plates at 30-35° for 18-24 hrs.

8.2.7 Incubate the peptone water plate at 30-35° for 5 days.

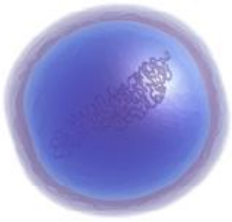
8.2.8 Record the subculturing details in Annexure-I.

8.3 STAINING AND MORPHOLOGY

8.3.1 Pick the pure colony and perform the staining as per SOP.

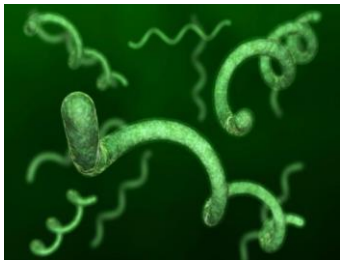
8.3.2 Observe the cell morphology like gram stain, spore former, cell shape, arrangement, motility and other details and record the cell morphology in Annexure-I and take the photograph of cell morphology.

8.3.3 For cell shape and cell arrangement refer the figure-II & figure-III (but not limited to)



Coccus

Bacillus

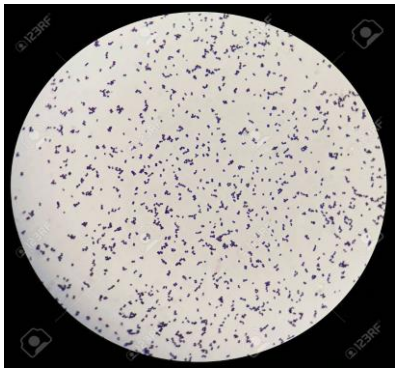


Spirillum

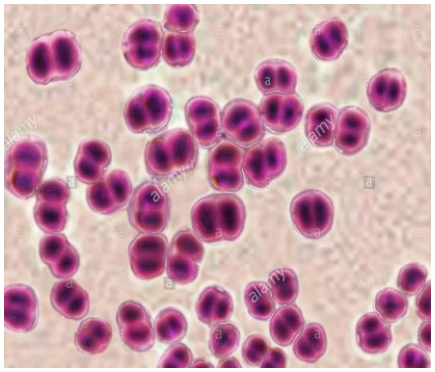
Vibrio

Figure-II: Shapes

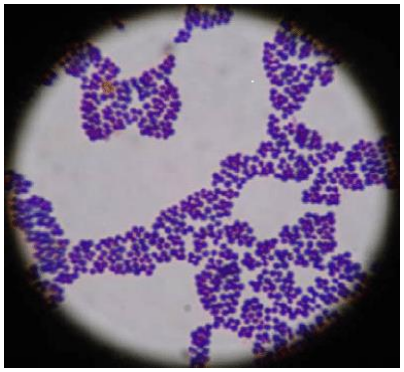
Arrangement of Cocci under Compound Microscope



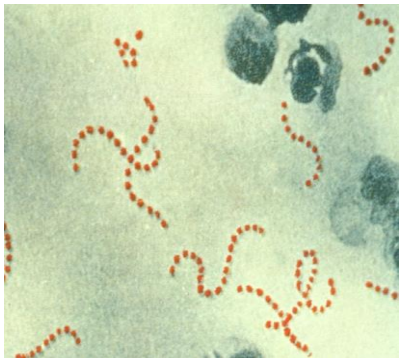
Coccus



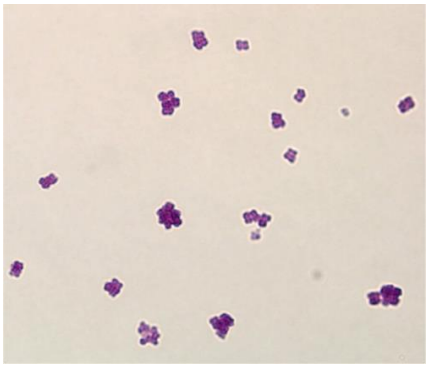
Diplococci



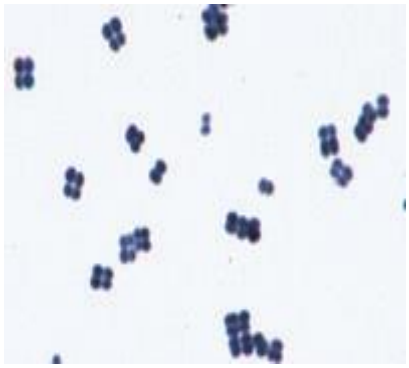
Staphylococci



Streptococcus

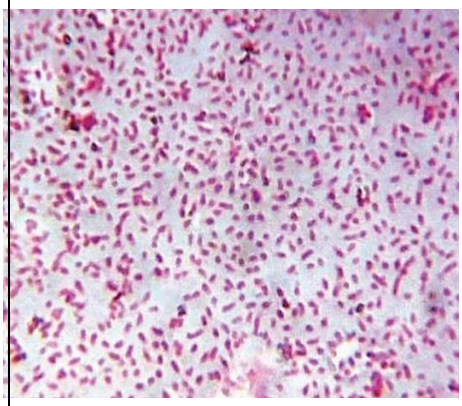


Sarcina

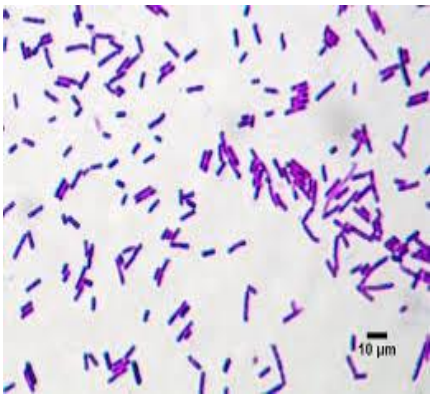


Tetrad

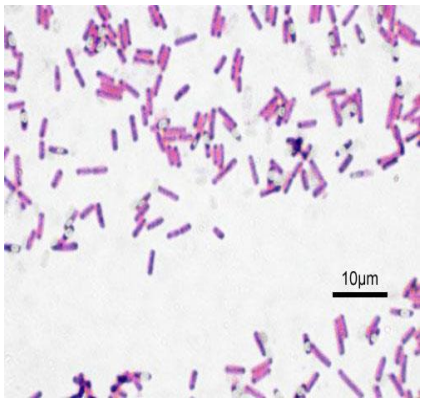
Arrangement of Bacilli under Compound Microscope



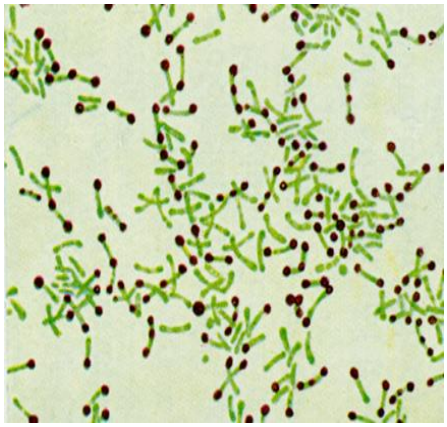
Coccobacillus



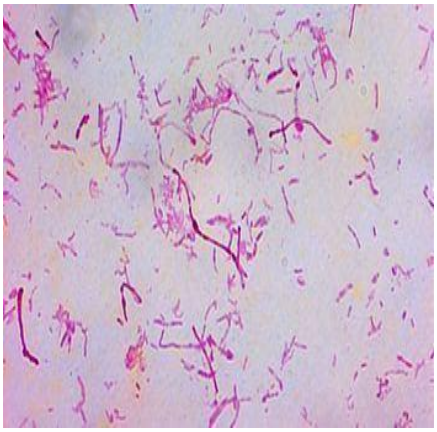
Bacilli



Diplobacilli



Palisades



Streptobacilli

Figure-III: Arrangements

8.4 Identifications

8.4.1 To identify the microbial strain follow the SOP " Operation & Maintenance of BD Phoenix™ 100 System".

8.5 Study Matrix

Following matrix shall be carried out as follows:

8.5.1 For Environment Isolates

Test Description	Facility Name	Frequency
Identification of Isolates	Manufacturing area	All isolates should be identifed once and this will be followed by identification of all morphologically distinct groups and further to acess the respective SOP.
Identification of Isolates	Controlled Area	
Identification of Isolates	Microbiology Lab	

8.5.2 For Water Isolates

Test Description	Water Type	Frequency
Identification of Isolates	Purified Water	All isolates should be identifed once and this will be Followed by identification of all morpho;ogically distinct groups and further to access the respective SOP.
Identification of Isolates	Potable Water	
Identification of Isolates	Bore Well Water	

9.0 OBSERVATION, RESULT, RECORDING & EVALUATION:

As per Annexure-1

10.0 ANNEXURE

10.1 Annexure – I: Identification of Microbial Isolates.

11.0 POST APPROVAL

It has been verified that all tests required by this protocol are completed complying as per the acceptance criteria.

Reviewed By:				
Department	**Name**	**Designation**	**Signature**	**Date**
Head Microbiology				
Head Quality Control				

Approved By:				
Department	**Name**	**Designation**	**Signature**	**Date**
Head – Quality Assurance				

Annexure – I
IDENTIFICATION OF MICROBIAL ISOLATES

A. Organism details:

Source	
Location	
Date of Isolation	
Date of Testing	
Laboratory code No.	

B. Preliminary Observation:

Test	Detail of observation
Name of Media	
Growth Temperature	
Colony color	
Colony shape	
Colony size	
Fluorescence	
Surrounding zone	
Other	
Done By	

C. Photograph

Plate:-

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Colony:-

D. Identification Details of Microorganism.

1.0 MORPHOLOGICAL CHARACTERISTICS

Test	Detail of observation
Gram staining	
Spore staining	
Cell shape	
Cell size	
Arrangement	
Motility	
Mycelium	

Cell Photograph

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Done By:

2.0 IDENTIFICATION WITH BD PHOENIX™ 100

Location	
Test Start on	
Test End on	
Identification Panel selected	
Lot No. of the Panel	
Expiry Date of the card	
LAF ID	
Isolated Organism Name	
Confidence Value	

Done By: **Checked By:**