STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Operation, Cleaning and Calibration of Bacterial Identification System	**Effective Date:**
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1.0 OBJECTIVE

1.1 To lay down the Procedure for Operation, Cleaning and Calibration of Bacterial Identification System.

2.0 SCOPE

2.1 This procedure is applicable for Operation, Cleaning and Calibration of Bacterial Identification System for Microbiology department.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible for performing Operation of Bacterial Identification System.

4.0 ACCOUNTABILITY

4.1 Head Microbiology

5.0 EHS CONSIDERATIONS

5.1 NA.

6.0 PROCEDURE

6.1 **Equipment Details:**

Equipment Name	Equipment ID	Make
Bacterial Identification System		

6.2 **Introduction of Equipment:**

6.2.1 The BBL Crystal MIND System for Microbial Identification is designed to identify wide variety of significant bacterial strains. The purpose of this software is to provide the identification of the unknown organism run in a panel by mathematically interpreting the BBL Crystal profile number and off-line test results and evaluating the result against the organisms contained in the appropriate BBL Crystal database. By providing the interpretative component of the BBL Crystal ID System in this manner, the user has access to the proper interpretation of all BBL Crystal profile numbers. The user has full access to the calculated parameters used in the ID process.

6.3 **Inoculation of Panels:** The requirement for panel inoculation are as follows

6.3.1 BBL Crystal E/NF ID Panels

6.3.2 BBL Crystal GP ID Panels

6.3.3 BBL Crystal ANR ID Panels

6.3.4 E/NF ID Broth

6.3.5 GP ID Broth

6.3.6 ANR ID Broth.

6.3.7 Sterile Cotton swab or loop
6.3.8 24 hrs. old bacterial culture on Non-Selective Culture Media.
6.3.9 Oxidase Test Reagents
6.3.10 Indole Test Reagents
6.3.11 Catalase Test Reagents.
6.3.12 Gram Staining Reagents.
6.3.13 Discard Container with proper disinfectant.

6.4 **Reagent Storage:** BBL Crystal E/NF kit shall be stored at 2-25^{0}C and GP and ANR kit stored at 2-8^{0}C.

6.5 **Procedure for Inoculation of Panels:**

6.5.1 Remove lids from pouch. Discard desiccant. Once removed from the pouch, covered lids should be used within 1 hour.

6.5.2 Do not use the panel if there is no desiccant in the pouch.

6.5.3 Take an inoculum tube/ plate and label with isolate number. Using aseptic technique, with the tip of a sterile cotton swab (do not use a polyester swab) or a disposable plastic loop, pick one well isolated large (2 − 3 mm or larger in diameter) colony (or 4 − 5 smaller colonies of the same morphology) from appropriate culture media.

6.5.4 Suspend colonies in a tube of **BBL CRYSTAL** Inoculum Fluid.
(Note: - Please use designated inoculum fluid for different panel)

6.5.5 Recap tube and vortex for approximately 10 − 15 seconds.

6.5.6 Then adjust the turbidity with McFarland standard as mentioned below.

Panel Type	McFarland Standard
E/NF & GP	McFarland 0.5
Anaerobic	McFarland 4

6.5.7 If the inoculum concentration is in excess of the recommended McFarland standard, one of the following steps are recommended:

1. With a fresh tube of inoculum fluid, prepare a new inoculum equivalent to required McFarland standard.

2. If additional colonies are unavailable for preparation of a new inoculum, using aseptic techniques, dilute the inoculum by adding the minimum required volume (not to exceed 1.0 ml) of 0.85% sterile saline to bring down the turbidity equivalent to required McFarland standard. Remove the excess amount added to the tube with a sterile pipette, so that the final volume of inoculum is approximately equivalent to that of the original volume in tube.

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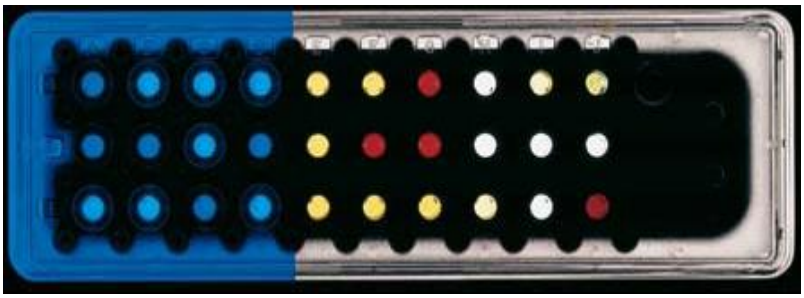
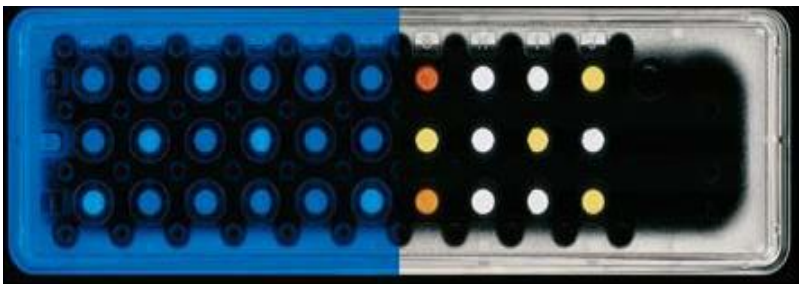
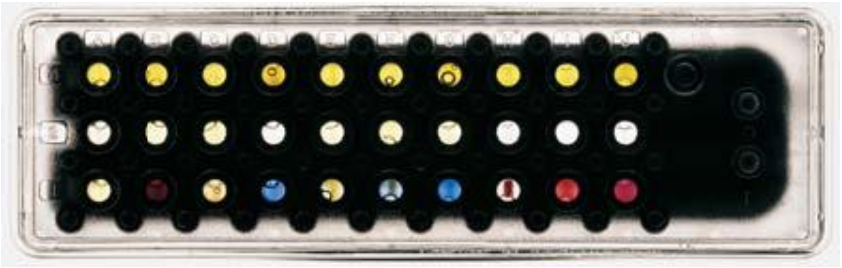


(Figure-1 Gram Positive Panel)



(Figure-2 Anaerobic Panel)



(Figure-3 Gram Negative Panel)

6.5.8 Take a base, and mark the specimen number on the side wall.

6.5.9 Pour entire contents of inoculum fluid into target area of base.

6.5.10 Hold base in both hands and roll inoculum gently along the tracks until all of the wells are filled. Roll back any excess fluid to the target area and place the base on a bench top.

6.5.11 Align the lid so that the labeled end of the lid is on top of the target area of the base.

6.5.12 Push down until a slight resistance is felt. Place thumb on edge of lid towards middle of panel on each side and push downwards simultaneously until the lid snaps into place (listen for two “clicks”).

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(Figure-4 Panel Fitting in the Base)

6.6 **Purity Plate:**

6.6.1 Using a sterile loop, recover a small drop from the inoculum fluid tube either before or after inoculating the base and inoculate an agar slant or plate (any appropriate media) for purity check.

6.6.2 Discard inoculum fluid tube and cap in a biohazard disposal container. Incubate the slant or plate for 18 − 24 h at 30 − 35°C in incubator. The purity plate or slant may also be used for any supplementary tests, if required.

6.7 **Incubation:**

6.7.1 Place inoculated panels in incubation trays. Ten panels can fit in one tray (5 rows of 2 panels).

6.7.2 All panels should be incubated face down (larger windows facing up; label facing down) in a non-CO_2 incubator with 40 - 60% humidity.

6.7.3 Trays should not be stacked more than two high during incubation. The incubation time for E/NF 18-20 hours, GP panel's 18 - 24 hrs and ANR panels 4hrs at 30 - 35°C.

(Figure-5 Panel incubation Tray)

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6.8 Machine Operation:

6.8.1 Reference Test:

6.8.1.1 This test has to be performed before routine identification work. Select the Reference Test menu item from the Main Menu.

6.8.1.2 The Auto Reader drawer automatically opens and a screen is displayed instructing you to place the Reference Panel in the Auto Reader.

6.8.1.3 Once you have placed the panel in the Auto Reader, click the "Scan" button to initiate the scanning process.

6.8.1.4 When the scan of the panel is complete, a message appears that indicates the success or failure of the Reference Panel Test.

6.8.1.5 Click On "Close" button.

(Figure-6 Auto reader)

6.8.1.6 Data Entry And Panel Reading :

6.8.1.7 Select the Data Entry menu from Main Menu bar.

6.8.1.8 Enter the Accession Number.

6.8.1.9 Enter Patient ID and Patient Name. (Mention Sample ID and Sample name)

6.8.1.10 Select Type of panel.

6.8.1.11 Enter required off-line tests.

6.8.1.12 Click on "Scan" button.

6.8.1.13 Click on "Add" button.

6.8.1.14 Click the "ID" button and then click on review menu.

6.8.2 **Review Result:**

6.8.2.1 Click on "Review" menu from "Main Menu bar".

6.8.2.2 At the top of the screen, all the panels that have not been printed and have been Batch Id or ID processed are displayed in a scrollable work-list.

6.8.2.3 If want to see all result click on "Display the Profile already printed" radio button.

6.8.3 **Reports Printing:**

6.8.3.1 Go to the "Review" screen.

6.8.3.2 Select any panel from Work-list.

6.8.3.3 Click On "Print" button.

6.8.4 **Recapitulative Reports:-**

6.8.4.1 Select "Report" menu from "Main menu".

6.8.4.2 From them select "Recapitulative Report".

6.8.4.3 Once you select the report it will open a window asking for start and end date.

6.8.4.4 Put desired start and end date and click on OK.

6.8.5 **Cleaning of the Reader**

6.8.5.1 **Cleaning of the Diffuser Filter:**

The Diffuser Filter is the white glass plate on the bottom of the Auto Reader drawer. This filter will need to be cleaned periodically to insure accurate panel readings. Prior to cleaning the filter, you must power off the Auto Reader and unplug it from the wall. You can clean it with either a lint-free cloth or lens paper moistened with water or commercial lens cleaning solution.

6.8.5.2 **Cleaning the Exterior Surfaces of the Reader:-**

The external surface of the reader shall be cleaned with lint free cloth soaked with disinfectant solution.

6.8.5.3 **Removing a Jammed Panel:-**

If a panel should get jammed in the Auto Reader, turn off the power to the reader and remove the panel. Turn the power back on and re-scan the panel.

6.8.6 **Preparation of Standard McFarland Turbidity solution:** The steps in creating the McFarland Turbidity Standards are given below:

6.8.6.1 Make a 1% solution (w/v) of anhydrous barium chloride ($BaCl_2$).

6.8.6.2 Make a 1% solution (v/v) of sulfuric acid (H_2SO4).

6.8.6.3 Mix these two solutions using this ratio to obtain desired McFarland scale.

6.8.6.4 McFarland Scale No._____ Amount of 1% BaCl (ml)_____Amt. of 1% H_2SO4 (ml)

McFarland Scale No.	Amt. of 1% BaCl (ml)	Amt. of 1% H_2SO_4 (ml)
0.5	0.05	9.95
1	0.1	9.9
2	0.2	9.8
3	0.3	9.7
4	0.4	9.6
5	0.5	9.5
6	0.6	9.4
7	0.7	9.3
8	0.8	9.2
9	0.9	9.1
10	1.0	9.0

6.8.6.5 Tightly seal these tubes and store them at room temperature in the dark. These would remain stable for at least 6 months.

6.9 **Performance verification (Calibration):**

6.9.1 Check the performance of microbial identification system by evaluating / Analyzing with reference & known ATCC strains of the below mentioned organisms.

- *Staphylococcus aureus* (ATCC No: 6538)
- *Bacillus subtilis (ATCC No: 6633)*
- *Escherichia coli (ATCC No: 8739)*
- *Clostridium sporogenes (ATCC No: 11437)*

6.10 **Acceptance criteria:** When challenged with above known & reference culture strains, organisms shall be identified up to species levels.

6.11 **Frequency:** Once in Six months or in case of any major maintenance.

7.0 DEFINITIONS AND ABBREVIATIONS

7.1 QC : Quality Control
7.2 SOP : Standard Operating Procedure
7.3 No. : Number
7.4 Temp. : Temperature
7.5 ATCC : American Type culture collection
7.6 ml : Milliliter
7.7 w/v : Weight/ Volume
7.8 °C : Degree Celsius

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8.0 REFERENCE

8.1 Operational Manual of BBL Crystal.

9.0 ANNEXURES

9.1 Annexure I- : Bacterial Identification System Usage Record

9.2 Annexure II- : Bacterial Identification System Performance verification Record

10.0 DISTRIBUTION DETAILS

10.1 Controlled copy of this SOP shall be distributed to Quality Assurance and Microbiology.

11.0 REVISION HISTORY

Supersedes SOP No.	Change Control No.	Reason for revision	Effective date

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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Annexure I
BACTERIAL IDENTIFICATION SYSTEM USAGE RECORD

S.No.	Date	Purpose	Usage on	Completed on	Done By	Reviewed By	Remarks

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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Annexure II
BACTERIAL IDENTIFICATION SYSTEM PERFORMANCE VERIFICATION RECORD

S.No.	Date	Name of Organism	Culture ref. no.	Result (%)	Done by	Reviewed by	Remarks