| STANDARD OPERATING PROCEDURE | |
|---|---|
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## 1.0 OBJECTIVE:

To lay down a procedure for Analyst Qualification in Microbiology Laboratory.

## 2.0 SCOPE:

This SOP is applicable to qualification of newly joined analyst, completing satisfactory training and to the qualified analyst annually, or earlier, if necessary in Quality Control Department.

## 3.0 RESPONSIBILITY:

Executive / Manager-QC

## 4.0 ACCOUNTABILITY:

Head-QC

## 5.0 ABBREVIATIONS:

| | |
|---|---|
| BET | Bacterial Endotoxin Test |
| E. Code | Employee Code |
| Ltd. | Limited |
| NMT | Not More Than |
| No. | Number |
| QA | Quality Assurance |
| QC | Quality Control |
| SOP | Standard Operating Procedure |

## 6.0 PROCEDURE:

Before start the analyst qualification training imparted to concern person of all required standard operating procedures and to be verify by the assigned supervisor.

Analyst Qualification shall be carried out in the following.

- Sterility Testing
- BET Analysis
- MLT
- GPT
- Microbial Assay

### 6.1 STERILITY TESTING:

**6.1.1** Following items shall be required before execution of Analyst Qualification for Sterility Testing:

**6.1.1.1** Sterile Water for Injection.

**6.1.1.2** Laminar Air Flow

**6.1.1.3** Sterile Forceps & Scissor

**6.1.1.4** Sterile Fluid Thioglycolate Medium.

**6.1.1.5** Sterile Soyabean casein Digest Medium.

**6.1.1.6** Membrane Filtration Assembly

**6.1.2** Verification of aseptic technique and practices (cleaning and sanitization, sterile material transfer and sample handling) is to be checked by the assigned supervisor who records the value /results checked during analysis.

**6.1.3** Take Prepared 3 x 100 ml Water for injection in tubes**.**

**6.1.4** Segregate the sterilized tubes of WFI**.**

**6.1.5** Perform the sterility test for all three trials of sterile water for injection using membrane filtration method as per SOP of Sterility Test.

**6.1.6** Perform the negative control with test.

**6.1.7** After completion of work transfer all inoculated media and other materials to dynamic pass box of incubation room and exit from sterility room as per SOP of Entry/ exit and Gowning Procedure for Sterility Room.

**6.1.8** Collect the FTM, SCM tubes from dynamic pass box of incubation Room.

**6.1.9** Incubate FTM tubes at 32.5°C ± 2.5°C and SCM tubes at 22.5°C ± 2.5°C for 14 days.

**6.1.10** Daily visual observation of all tested sample tubes including negative control of FTM & SCM for 14 days.

**6.1.11** Perform these tests with 3 trials on 3 consecutive days to confirm the reproducibility of the analyst performance.

**6.1.12** Record the observation of sterility test in format Titled **"Sterility Test Report by Membrane Filtration Method"**

**6.1.13** **Acceptance criteria**:

**6.1.13.1** The sterile water for injection samples should pass the sterility test in all three trials.

**6.1.14** After successful completion of the test, a certificate shall be prepared for the approval of analyst qualification as per **Format.**

**6.1.15** The Head Microbiology shall evaluate the analyst and the activity in which Qualification is required.

**6.1.16** The details of sample & Batch No. shall be recorded by Head Microbiology as per **Annexure-I**, Title **"Analyst Qualification Sample Log Book"**.

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**6.2 FOR BACTERIAL ENDOTOXIN TEST:**

**6.2.1** Following items shall be required before execution of Analyst Qualification for Bacterial Endotoxin Testing:

**6.2.1.1** LAL Reagent
**6.2.1.2** CSE
**6.2.1.3** LRW
**6.2.1.4** Cyclo Mixer
**6.2.1.5** Depyrogenated 10 x 75 mm glass test tubes
**6.2.1.6** 12 x 75 mm /16 x 75mm depyrogenated borosilicate glass tube
**6.2.1.7** Heating block
**6.2.1.8** Micropipette
**6.2.1.9** Endotoxin free tips
**6.2.1.10** Test tube stand etc.

**6.2.2** Perform the analyst qualification with labeled lysate sensitivity.

**6.2.3** Give the reagent for analyst qualification after confirmed Lysate Sensitivity.

**6.2.4** Prepare the Control Standard Endotoxin dilution up to 0.03 EU/ml (λ/4) as per SOP of Bacterial Endotoxin Test.

**6.2.5** Perform the labeled lysate sensitivity as per SOP of Bacterial Endotoxin Test.

**6.2.6** Analyst shall perform the lysate sensitivity test by using Gel-clot Method for analyst qualification for BET.

**6.2.7** Perform the lysate sensitivity test one time to confirm the analyst performance.

**6.2.8** Record the observation in format**,** Titled **"Lysate sensitivity record"**

**6.2.9** **Acceptance criteria**:

**6.2.9.1** The lysate sensitivity result should not less than λ/2 and not more than 2λ (2 fold variation).

**6.2.10** After successful completion of the test, a certificate shall be prepared for the approval of analyst qualification as per **Format.**

**6.2.11** The Head Microbiology shall evaluate the analyst and the activity in which Qualification is required.

**6.2.12** The details of sample & Batch No. shall be recorded by Head Microbiology as per **Annexure-I**, Title **"Analyst Qualification Sample Log Book"**.

**6.3 MLT Testing.**

**6.3.1** Following items shall be required before execution of Analyst Qualification for MLT Testing:

**6.3.1.1** Tested sample (product).

**6.3.1.2** Laminar Air Flow

**6.3.1.3** Culture media.

**6.3.1.4** Micropipette

**6.3.1.5** Tips

**6.3.1.6** Sterile petriplate

**6.3.2** Sample of known analytical results shall be identified by Quality control manager/designee for analysis.

**6.3.3** All the samples shall be issued to Microbiologist on the day of analysis along with the necessary information required for the analysis.

**6.3.4** The analysis skills, GLP adherence and SOP/STP compliance, verification of aseptic technique and practices (cleaning and sanitization, sterile material transfer and sample handling) is to be checked by the assigned supervisor who records the value /results checked during analysis.

**6.3.5** Performed the analysis as per SOP of Microbial Enumeration Test of Raw Materials, Finished Products and In Process Samples.

**6.3.6** Perform these tests on 3 consecutive days to confirm the reproducibility of the analyst performance.

**6.3.7** Record the observation in format Titled **"Microbial Enumeration Test Report".**

**6.3.8** **Acceptance criteria**:

**6.3.8.1** The results of Microbiologist shall be checked for cGLP compliance and compared with expected values.

**6.3.9** After successful completion of the test, a certificate shall be prepared for the approval of analyst qualification as per **Format.**

**6.3.10** The Head Microbiology shall evaluate the analyst and the activity in which Qualification is required.

**6.3.11** The details of sample & Batch No. shall be recorded by Head Microbiology as per **Annexure-I**, Title **"Analyst Qualification Sample Log Book".**

**6.4 GPT Testing.**

**6.4.1** Following items shall be required before execution of Analyst Qualification for GPT Testing:

**6.4.1.1** Micropipettes.

**6.4.1.2** Laminar Air Flow

**6.4.1.3** Sterile spreader

**6.4.1.4** Prepared plates of Soyabean casein Digest Agar and Selective agar media

**6.4.1.5** Broth Tubes.

**6.4.1.6** Microbial Culture Suspension

**6.4.2 Growth Promotion Test of Agar Medium:**

**6.4.2.1** Use the inoculums having microbial population of not more than 100 cfu/ml of appropriate microorganism. For recovery of microorganism either Spread Plate or Pour Plate Method used any one agar media for quantitative test.

**6.4.2.2 By Spread Plate Method: -** Take prepared agar media Petri plate in duplicate for selected microorganism and inoculate having not more than 100 cfu of appropriate microorganism after this spread the inoculum on agar surface by a sterile spreader aseptically.

**6.4.2.3 By Pour Plate Method: -** Take pre sterilized Petri plate in duplicate for selected microorganism and inoculate having not more than 100 cfu of appropriate microorganism after this, Pour 20-25 ml of agar media. Swirl the plate having media clock wise and anti-clock wise direction. Leave the petriplate in LAF until media gets solidify.

**6.4.2.4** Incubate all the inoculated petriplate at specified temperature for specified incubation period as per respective SOP of GPT.

**6.4.3 Growth Promotion Test of Selective Agar Medium:**

**6.4.3.1** Take any one selective agar media for characteristic growth test.

**6.4.3.2 By Spread Plate Method: -** Take prepared agar media Petri plate for any one selected microorganism and inoculate having not more than 100 cfu of appropriate microorganism after this spread the inoculums on agar surface by a sterile spreader aseptically.

**6.4.3.3 By Pour Plate Method: -** Take pre sterilized Petri plate for any one selected microorganism and inoculate having not more than 100 cfu of appropriate microorganism after this, Pour 20-25 ml of agar media. Swirl the plate having media clock wise and anti-clock wise direction. Leave the petriplate in LAF until media gets solidify.

**6.4.3.4** Incubate all the inoculated Petri plate at specified temperature for specified incubation period as per respective SOP of GPT.

**6.4.3.5** After completion of incubation period, observe characteristic Growth of microorganism on the selective agar Media.

**6.4.4 Growth Promotion Test of Broth Medium:**

**6.4.4.1** Take any one broth media for Growth promotion Test.

**6.4.4.2** Take prepared Broth Media tubes and inoculate the inoculums having not more than 100 cfu/ml of appropriate microorganism. Incubate all the inoculated Broth media at specified temperature for specified incubation period as per respective SOP of GPT.

**6.4.4.3** Also carry out the negative control for each of culture media testing:

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**Table-1**

| Specified Microorganism | Media Name | Positive Growth Characteristics |
|---|---|---|
| *E. coli* | MacConkey Agar | Pink/red coloured non-mucoid colonies. |
| | MacConkey Broth | Medium colour turns to yellow. |
| *Salmonella* | Rappaport Vasilliadis Salmonella Broth | Medium colour turns to light green. |
| | Xylose lysine Deoxycholate Agar | Red colonies with or without black centers. |
| *Pseudomonas aeruginosa* | Cetrimide Agar | Greenish yellow colonies. |
| *Staphylococcus aureus* | Mannitol Salt Agar | Yellow colonies surrounded by yellow zones. |
| *Bile Tolerant Gram Negative Enterobacteria* | Enterobacteria Enrichment Broth, Mossel | Medium colour turns to yellow. |
| | Violet Red Bile glucose Agar | Pink/red colonies |

**6.4.5 Test for Inhibitory Properties of Agar Medium:**

**6.4.5.1** This test is performed for any one agar medium, intended for the detection of specified microorganisms.

**6.4.5.2** **By Spread Plate Method: -** Take prepared agar media Petriplate for any one selected microorganism and inoculate having at least 100 cfu of appropriate microorganism after this spread the inoculums on agar surface by a sterile spreader aseptically.

**6.4.5.3** **By Pour Plate Method: -** Take pre sterilized Petri plate for any one microorganism and inoculate 1.0 ml having at least 100 cfu of appropriate microorganism after this, Pour 20-25 ml of agar media. Swirl the plate having media clock wise and anti clock wise direction. Leave the petriplate in LAF until media gets solidify.

**6.4.5.4** Incubate all the inoculated petriplate at specified temperature for specified Incubation period as per respective SOP of GPT.

**6.4.5.5** After completion of incubation period, observe characteristic Growth of microorganism on the agar Media.

**6.4.6 Test for Inhibitory Properties of Broth Medium:**

**6.4.6.1** This test is performed for any one broth medium, intended for the detection of specified microorganisms.

**6.4.6.2** Take prepared selective Broth Media and inoculate the inoculums at least 100 cfu of appropriate microorganism after this Incubate all the inoculated Broth media at specified temperature for the specified Incubation period as per respective SOP of GPT.

**6.4.6.3** Observe the GPT and inhibitory test results and record the observations in format Titled **"Growth Promotion Test report of Culture Media"**

**6.4.7** **Acceptance criteria**:

**6.4.7.1** In the test for Growth Promoting Properties of agar media microbial recovery for agar media growth obtained must not differ by a factor greater than 2 (divided by 2 and multiplied by 2) from the calculated value for a standardized Inoculum.

**6.4.7.2** In the test for Growth Promoting Properties for selective media colony morphology and indication reaction should be obtained characteristic growth as per table-1.

**6.4.7.3** For broth culture media luxurious growth of microorganism should be observed.

**6.4.7.4** In the test for Inhibitory Properties there should be no any growth of the microorganisms.

**6.4.8** Perform these tests on 3 consecutive days to confirm the reproducibility of the analyst performance.

**6.4.9** After successful completion of the test, a certificate shall be prepared for the approval of analyst qualification as per **Format**.

**6.4.10** The Head Microbiology shall evaluate the analyst and the activity in which Qualification is required.

**6.4.11** The details of sample & Batch No. shall be recorded by Head Micro as per **Annexure-I**, Title **"Analyst Qualification Sample Log Book"**.

## 6.5 MICROBIAL ASSAY TESTING:

**6.5.1** Following items shall be required before execution of Analyst Qualification for Microbial Assay Testing:

- Analytical balance
- Micropipette
- Glass pipette
- LAF, Incubator
- Sonicator
- Vortex
- Water bath
- Autoclave
- Borer
- Sterile 0.9% Sodium chloride
- Culture media
- Measuring cylinder
- Conical flask

- Pipette
- Volumetric flasks
- Sterile petri plates

**6.5.2** Sample of known analytical results shall be identified by Quality control manager/designee for analysis.

**6.5.3** All the samples shall be issued to Microbiologist on the day of analysis along with the necessary information required for the analysis.

**6.5.4** The analysis skills, GLP adherence and SOP/STP compliance is to be checked by the assigned supervisor who records the value /results checked during analysis.

**6.5.5** Perform the Microbial Assay of following method.

- Cup plate Method
- Serial dilution Method

**6.5.6** Perform the test for all trials using Microbial Assay Testing Method as per respective GTP/STP.

**6.5.7** Perform these tests on 3 consecutive days to confirm the reproducibility of the analyst performance.

**6.5.8** Record the activity as per Format of general work sheet and approved excel sheet.

**6.5.9** **Acceptance criteria:**

**6.5.9.1** All three trials should be complies with in limit.

**6.5.10** After successful completion of the test, a certificate shall be prepared for the approval of analyst qualification as per **Format.**

**6.5.11** The Head Microbiology shall evaluate the analyst and the activity in which Qualification is required.

**6.5.12** The details of sample & Batch No. shall be recorded by Head Microbiology as per **Annexure-I**, Title **"Analyst Qualification Sample Log Book".**

**6.5.13** **Re- Qualification procedure:**

**6.5.13.1** Analyst not involve in analysis activity more than one year.

**6.5.13.2** The Head Microbiology shall brief the analyst regarding the importance of qualification, acceptance / Re-Approval criteria.

**6.5.13.3** In case of failure with the Acceptance criteria for qualification the Analyst shall be Retrained / Revaluated.

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**6.5.13.4** In case the analyst is not performing the task / test for which he / she is qualified for a period of more than one year then he / she shall undergo re-qualification prior to performing the task / test.

**6.5.14** Microbial Limit Test Qualified Analyst also qualified for water analysis.

## 7.0 ANNEXURES:

| ANNEXURE No. | TITLE OF ANNEXURE | FORMAT No. |
|---|---|---|
| Annexure-I | Analyst Qualification Sample Log Book | |
| Annexure-II | Analyst Qualification Report | |
| Annexure-III | Analyst Qualification Acceptance Criteria Guidelines | |
| Annexure-IV | Analyst Qualification Certificate | |

**ENCLOSURES:** SOP Training Record

## 8.0 DISTRIBUTION:

- Controlled Copy No. 01 — Quality Assurance
- Controlled Copy No. 02 — Microbiology Laboratory
- Master Copy — Quality Assurance

## 9.0 REFERENCES:

Not Applicable

## 10.0 REVISION HISTORY:

### CHANGE HISTORY LOG

| Revision No. | Change Control No. | Details of Changes | Reason for Change | Effective Date | Updated By |
|---|---|---|---|---|---|
| | | | | | |

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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## ANNEXURE-I
## ANALYST QUALIFICATION SAMPLE LOG BOOK

| S.No. | Date | Name of Analyst | E. Code | Name of Sample | Batch No. | Reviewed by Sign & Date |
|---|---|---|---|---|---|---|
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PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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## ANNEXURE –II
## ANALYST QUALIFICATION REPORT

| | | | |
|---|---|---|---|
| **Name of Analyst** | | **Employee Code No.** | |
| **Name of Sample** | | **Batch No.** | |
| **Sample Issued By** | | **Reference GTP/STP No.** | |
| **Date of Sample Received** | | **Reference SOP No.** | |
| **Qualification Start Date** | | **Qualification End Date** | |

| S.No. | Parameter | Remarks |
|---|---|---|
| **1.** | Adherence to SOP | Yes / No / NA |
| **2.** | Adherence to Testing Method | Yes / No / NA |
| **3.** | Correctness of Culture Media used/Reagent | Yes / No / NA |
| **4.** | Usage of Calibrated Instruments | Yes / No / NA |
| **5.** | GLP in Handling of Samples | Yes / No / NA |
| **6.** | Correct usage of Membrane Filter, Instruments etc. | Yes / No / NA |
| **7.** | GLP in Performing Dilutions | Yes / No / NA |
| **8.** | Correctness of Calculations | Yes / No/NA |
| **9.** | Correctness in Reporting of Results | Yes / No / NA |
| **10.** | Good Documentation Practices | Yes / No / NA |
| **11.** | Verification of aseptic technique and practices | Yes / No / NA |

| **Results of Analyst under Certification Sample** | | | | **Complies / Does Not Comply** |
|---|---|---|---|---|
| **Sample** | **Results** | | | |
| | **Trial-1** | **Trial-2** | **Trial-3** | |
| **Sample-A** | | | | |
| **Sample-B** | | | | |
| **Sample-C** | | | | |

**Remarks (if any):**

_____

_____

_____

**Conclusion:** The analyst is Qualified / Not Qualified for carrying out the above Analysis independently.

**Evaluated By:** **Date of Qualification:**

**Sign & Date**

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## ANNEXURE-III
## ANALYST QUALIFICATION ACCEPTANCE CRITERIA GUIDELINES

| S.No. | TEST | ACCEPTANCE CRITERIA |
|---|---|---|
| | **MICROBIOLOGICAL TESTING** | |
| **1.** | BET | The lysate sensitivity result should not less than λ/2 and not more than 2λ (2 fold variation) |
| **2.** | MLT | The results shall be checked for cGLP compliance and compared with expected values. |
| **3.** | Sterility | The sterile water for injection samples should pass the sterility test in all three trials. |
| **4.** | GPT | In the test for Growth Promoting Properties of agar media microbial recovery for agar media growth obtained must not differ by a factor greater than 2 (divided by 2 and multiplied by 2) from the calculated value for a standardized Inoculum.<br>In the test for Growth Promoting Properties for selective media colony morphology and indication reaction should be obtained characteristic growth as per Table-1<br>For broth culture media luxurious growth of microorganism should be observed.<br>In the test for Inhibitory Properties there should be no any growth of the microorganisms. |
| **5.** | **Microbial Assay** | All three trials should be complies as per results tested by qualified analyst. |

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**ANNEXURE –IV**

***ANALYST QUALIFICATIONCERTIFICATE***

**Name of Analyst : _____**
**Department : _____**
**Designation : _____**
**E. Code : _____**

**This is to Certify that Mr./Ms._____dated_____has been qualified to**

**Carryout ----------Analysis.**

**Head of Department _____**

**(Date & Sign)**