1.0 Objective

1.1 The objective of this SOP is to follow the Microbiological Best Laboratory Practices.

2.0 Scope

2.1 The scope of this document is applicable for Microbiology Laboratory at pharma micro hub Private Limited.

3.0 Responsibilities

3.1 All microbiologists are responsible to follow the SOP.

3.2 Microbiology Head / Designee is responsible to ensure compliance of SOP.

4.0 Accountability

4.1 Head / Designee – Microbiology

5.0 Procedure

5.1 Media preparation:

5.1.1 While preparation of media shall follow as per manufacturer's instructions by following the certificate of analysis describing expiry date and recommended storage conditions.

5.1.2 For media preparation water for injection/purified water shall be used.

5.1.3 Volume of the water and quantity of weighing of dehydrated media or media supplements shall be recorded.

5.1.4 Calibrated balance with the appropriate weight range for the ingredients shall be used.

5.1.5 For media preparation cleaned weighing containers and tools shall be used. For cleaning of glassware purified water shall be used for rinsing.

5.1.6 Sterilization of media shall be performed for the parameters provided by the manufacturer and as validated by the user.

5.1.7 Calibrated pH meter shall be used to verify the pH of the media prior to before sterilization and after sterilization. Flat pH probe for agar surfaces immersion probe for liquids shall be used.

5.1.8 Prepared media shall be checked for the following quality and integrity checks:

5.1.9 Cracked containers or lids

- Unequal filling of containers
- Dehydration resulting in cracks or dimpled surfaces on solid media
- Excessive darkening or colour change

- Excessive number of bubbles
- Lot number and expiration date
- Sterility
- Cleanliness of plates (lid should not stick to dish)

5.1.10 Ready to use media shall provide documentation of the sterilization method that was used.

5.2 Media Storage:

5.2.1 Media shall be stored at prescribed conditions as per manufacturer recommendations. Media prepared in-house should be stored under validated conditions.

5.2.2 Label the Media properly with batch or lot numbers, preparation and expiration dates, and media identification.

5.2.3 Do not store agar at or below 0°C as freezing could damage the gel structure.

5.2.4 Protect stored media from exposure to bright illuminate light, excessive temperature and to retard moisture loss.

5.2.5 Perform re-melting of an original container of solid media only once to avoid media overheating or potential contamination.

5.2.6 Hold the molten agar medium in a monitored water bath at a temperature of 45-50°C for not more than 6 hours.

5.2.7 Precautions shall be taken while pouring the media from the container immersed in water bath to prevent water mixing with sterile media by careful wiping of exterior of container.

5.2.8 Decontamination and Disposal of used cultured media and as well as for expired media shall be performed as per validated parameters.

5.3 Reference cultures:

5.3.1 Use only certified reference materials with international standards and pharmacopoeia reference.

5.3.2 Cultures shall not be used for more than four passages from the original reference strain.

5.4 Maintenance of equipment's:

5.4.1 All Equipment's / Instruments shall be calibrated and validated as per respective procedures and regular schedule.

5.4.2 All Equipment's / Instruments shall be monitored as per respective preventive maintenance procedures by following regular schedule.

5.4.3 Status of calibration, validation and preventive maintenance shall be clearly mentioned on status label of equipment or instrument as per respective format.

5.4.4 Regular cleaning of equipment's shall be performed to minimize the potential for contamination and frequency of cleaning shall be defined in respective SOPs.

5.4.5 All activities related to equipment's shall be documented in respective log books and records.

5.5 Personnel training:

5.5.1 Personnel engaged in all phases of testing shall have the education, training and experience to perform job responsibilities.

5.5.2 Job responsibilities should be defined with respect to their level of skill and experience.

5.5.3 Analysts shall perform analysis independently only after they are qualified to perform the test.

5.5.4 Periodic training and qualification of analysts including regular vision checks shall be performed to limit the variability of microbiological test results with respect to handling and reading of test results.

5.5.5 Current job descriptions for all personnel involved in Analysis, calibrations, validations and verifications shall be maintained.

5.5.6 personnel should follow aseptic behaviour in production areas

5.6 Microbial Testing Practices:

5.6.1 Wear hand gloves and sanitize hands with 70% IPA and shall start the testing's.

5.6.2 Growth promotion test shall be performed for all prepared medias and ready to use media.

5.6.3 After preparation of media plates, pre-incubate the plates in biological incubators with proper labelling. (E.g. Name of the media, Pre incubation date).

5.6.4 Initiate an investigation to identify the cause if a batch of media does not meet the requirements of growth-promotion testing. This investigation should include a corrective action plan to prevent the recurrence of the problem.

5.6.5 Subject the media for pre-incubation and 100% inspection prior to use.

5.6.6 Method suitability and validations shall be performed prior to regular testing of samples.

5.6.7 Negative control shall be performed during the analysis to demonstrate the aseptic techniques followed in microbiology laboratory.

5.6.8 Incubation times for microbiological tests of less than 3 days shall be expressed in hours and tests longer than 72 hours shall be expressed in days.

5.6.9 For incubation times expressed in hours incubate for minimum specified time and for incubation times expressed in days shall be concluded at the same time of day as incubated.

5.6.10 During incubation if any excursions occurs, impact assessment shall be performed to determine potential impact on test samples.

5.7 Maintenance of laboratory records:

5.7.1 Documentation in microbiology laboratory shall demonstrate that the testing was performed in laboratory and by methods under control includes but not limited to:

- Microbiologist training and qualification
- Equipment calibration, validation and maintenance
- Equipment monitoring
- Media preparation and Growth promotion tests
- Media inventory, quality and shelf-life testing
- Methods as per test procedure
- Data and calculation verification
- Review of reports
- Investigation of data deviations

5.7.2 At a minimum, the laboratory records should include the following: Date of activity, Material tested, Microbiologist's name, Procedure number, Document test results, Deviations (if any), Documented parameters (equipment used, microbial stock cultures used, media lots used), Second review signature.

5.7.3 Every activity which is being performed in laboratory shall be performed as per respective approved procedures and shall document in the respective records.

5.7.4 All laboratory records shall be documented and archived as per procedure.

5.7.5 Reports from contract laboratory shall include complete analytical worksheets from the analysis performed.

5.7.6 All the data used for calculations, including all positive (growth promotion results) and negative controls during analysis shall include in the final report provided. Relevant method suitability test results shall also be available to support the testing.

5.7.7 Whenever calibration certificates and COAs received from External agency or vendor, shall be stamped as reviewed and signed.

5.8 Data integrity:

5.8.1 Ensure all microbiologists are understood and following the Good documentation practices as SOP.

5.8.2 Equipment related if any Chart, Graph or Print outs shall be duly sign and date.

5.8.3 All documents shall be reviewed by a qualified analyst or supervisor.

5.8.4 Reviewer shall verify the testing activity is correctly performed, reading of the results is correctly executed and correctly transcribed to raw data sheet. If any of the discrepancies observed while verifying the plates, tubes and documents, shall inform to Microbiology Head or Designee.

5.8.5 While reporting the results for count of CFU shall not report in decimals, i.e. Rounding off shall be done while considering the counts of more number of plates.

5.8.6 Colony counts shall be considered strictly based on the incubation times and temperature mentioned in the approved test procedure.

5.8.7 Microbiological results shall be reviewed and trended as per prescribed schedules to assess the measures to control contamination and quality of products.

5.8.8 If results do not conform to the established acceptance criteria, shall raise deviation and investigation shall be performed to identify the root cause by selection of proper risk management tool.

6.0 Abbreviations

6.1 IPA - Iso Propyl Alcohol

6.2 SOP - Standard Operating Procedure

6.3 CFU - Colony Forming Units

6.4 MB-Microbiology

6.5 PMH-Pharma Micro Hub

7.0 References

7.1 USP <1117> - Microbiology best microbiology practices.

8.0 Annexures

8.1 NIL

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