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Title:	Microbiological Testing of Non-Sterile Products	**Version:**	1.0
Company:	NovaThera Pharmaceuticals Pvt. Ltd.	**Effective Date:**	2025-01-01
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Microbiological Testing of Non-Sterile Products

Category: Quality Control Laboratory

Standard Operating Procedure (SOP)

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Department: Quality Control Laboratory

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1.0 PURPOSE

This procedure outlines the standardized method for performing microbiological testing of non-sterile pharmaceutical products manufactured at NovaThera Pharmaceuticals Pvt. Ltd. This ensures compliance with Good Manufacturing Practices (GMP) and relevant pharmacopeial standards, allowing for the determination of microbial content and the identification of objectionable microorganisms in pharmaceutical manufacturing.

2.0 SCOPE

This SOP applies to all non-sterile raw materials, in-process materials, and finished products manufactured at NovaThera Pharmaceuticals Pvt. Ltd. It covers the procedures for sample collection, preparation, and testing to determine total aerobic microbial count (TAMC), total yeast and mold count (TYMC), and the absence or presence of specific objectionable microorganisms, as defined in the relevant pharmacopeia (e.g., USP, EP, BP, IP). The SOP excludes the testing of sterile products.

3.0 RESPONSIBILITY

QC Inspector: Responsible for performing microbiological testing according to this SOP, accurately recording results, and reporting any deviations or out-of-specification (OOS) results to the QA Manager. The QC Inspector is also responsible for maintaining the cleanliness and proper functioning of the microbiology laboratory and associated equipment.

Production Supervisor: Responsible for ensuring that samples are collected from the production line according to the sampling plan and delivered to the Quality Control Laboratory in a timely manner, along with the necessary documentation. They are also responsible for informing the QC Inspector of any potential contamination issues in the production area.

QA Manager: Responsible for reviewing and approving test results, investigating OOS results, and ensuring that corrective and preventive actions (CAPA) are implemented as necessary. The QA Manager is also responsible for maintaining the validity and integrity of the microbiological testing data.

Head of QA: Responsible for the final approval of this SOP and any subsequent revisions. The Head of QA is also responsible for ensuring that the Quality Control Laboratory is adequately staffed and equipped to perform microbiological testing in compliance with GMP.

4.0 MATERIALS & EQUIPMENT

PPE:

- Laboratory coat
- Safety glasses
- Nitrile gloves
- Surgical mask

Equipment:

- Laminar Air Flow (LAF) hood (LAF-01)
- Incubators (INC-01, INC-02)
- Autoclave (AUT-01)
- pH meter (PHM-01)
- Water bath (WTB-01)
- Colony counter (CLC-01)
- Microscope (MIC-01)
- Vortex mixer (VXM-01)
- Stomacher (STM-01)
- Pipettes (various volumes)
- Petri dishes (sterile)
- Test tubes (sterile)
- Glassware (beakers, flasks, cylinders)
- Weighing balance (BAL-01)

Documentation:

- Microbiological Testing Request Form (F-QC-013-01)
- Microbiological Testing Record (F-QC-013-02)
- Equipment Logbook
- Media Preparation Logbook
- Incubator Temperature Monitoring Log
- Autoclave Cycle Record
- Deviation Report Form
- Out-of-Specification (OOS) Investigation Report

5.0 PROCEDURE

5.1 Sample Collection and Handling

5.1.1 The Production Supervisor shall collect samples according to the approved sampling plan, ensuring representative samples are obtained from the batch.

5.1.2 The Production Supervisor shall complete the Microbiological Testing Request Form (F-QC-013-01) with all relevant information, including the product name, batch number, date of sampling, and requested tests.

5.1.3 The Production Supervisor shall transport the samples to the Quality Control Laboratory in a timely manner, maintaining appropriate storage conditions to prevent microbial growth. Samples requiring refrigeration shall be stored at 2-8°C.

5.1.4 Upon receipt of the samples, the QC Inspector shall verify the sample integrity, ensure that the accompanying documentation is complete and accurate, and record the sample details in the Microbiological Testing Record (F-QC-013-02).

5.1.5 The QC Inspector shall assign a unique laboratory identification number to each sample.

5.2 Media Preparation

5.2.1 The QC Inspector shall prepare all media required for microbiological testing according to the manufacturer's instructions and the relevant pharmacopeial standards. Examples of media include:

- Soybean-Casein Digest Agar (SCDA) for TAMC
- Sabouraud Dextrose Agar (SDA) for TYMC
- Selective media for specific objectionable microorganisms (e.g., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species)

5.2.2 The QC Inspector shall document the preparation of each batch of media in the Media Preparation Logbook, including the date, media name, batch number, manufacturer, lot number, and the QC Inspector's initials.

5.2.3 The QC Inspector shall sterilize all media by autoclaving at 121°C for 15 minutes.

5.2.4 The QC Inspector shall perform a sterility test on each batch of prepared media by incubating a representative sample at 30-35°C for at least 48 hours. The media shall be considered sterile if no growth is observed.

5.2.5 The QC Inspector shall store prepared media at the recommended storage temperature, typically 2-8°C.

5.3 Sample Preparation

5.3.1 The QC Inspector shall prepare the samples for testing in the Laminar Air Flow (LAF) hood (LAF-01) to minimize the risk of contamination.

5.3.2 For solid samples (e.g., tablets, capsules, powders), the QC Inspector shall weigh a representative amount of the sample (typically 1-10 grams) and dissolve or suspend it in a sterile diluent (e.g., sterile saline, phosphate buffer). Use a sterile BLN-04 (Blender) or STM-01 (Stomacher) if required for proper homogenization.

5.3.3 For liquid samples (e.g., solutions, suspensions), the QC Inspector shall directly use an appropriate volume of the sample.

5.3.4 The QC Inspector shall perform serial dilutions of the sample preparation, as necessary, to obtain countable colonies on the agar plates.

5.4 Total Aerobic Microbial Count (TAMC)

5.4.1 The QC Inspector shall use the pour plate or spread plate method for determining TAMC.

5.4.2 For the pour plate method, the QC Inspector shall aseptically transfer 1 mL of the sample preparation or its dilutions into sterile Petri dishes.

5.4.3 The QC Inspector shall pour approximately 15-20 mL of molten Soybean-Casein Digest Agar (SCDA) (cooled to approximately 45°C) into each Petri dish.

5.4.4 The QC Inspector shall gently swirl the Petri dishes to mix the sample with the agar and allow the agar to solidify.

5.4.5 For the spread plate method, the QC Inspector shall aseptically transfer 0.1 mL of the sample preparation or its dilutions onto the surface of pre-poured SCDA plates.

5.4.6 The QC Inspector shall spread the inoculum evenly over the agar surface using a sterile spreader.

5.4.7 The QC Inspector shall incubate the Petri dishes at 30-35°C in INC-01 for 5 days.

5.4.8 After incubation, the QC Inspector shall count the number of colonies on each plate using the colony counter (CLC-01).

5.4.9 The QC Inspector shall calculate the TAMC per gram or milliliter of the sample, taking into account the dilution factor.

5.5 Total Yeast and Mold Count (TYMC)

5.5.1 The QC Inspector shall use the pour plate or spread plate method for determining TYMC, following the procedures described in section 5.4.

5.5.2 The QC Inspector shall use Sabouraud Dextrose Agar (SDA) as the culture medium.

5.5.3 The QC Inspector shall incubate the Petri dishes at 20-25°C in INC-02 for 5-7 days.

5.5.4 After incubation, the QC Inspector shall count the number of yeast and mold colonies on each plate using the colony counter (CLC-01).

5.5.5 The QC Inspector shall calculate the TYMC per gram or milliliter of the sample, taking into account the dilution factor.

5.6 Testing for Objectionable Microorganisms

5.6.1 The QC Inspector shall test for the presence of specific objectionable microorganisms as defined in the relevant pharmacopeia and product specifications.

5.6.2 The specific tests to be performed will depend on the product and its intended use. Examples of common objectionable microorganisms include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* species.

5.6.3 The QC Inspector shall follow the procedures described in the relevant pharmacopeia for each specific test. These procedures typically involve:

- Pre-enrichment of the sample in a non-selective broth medium
- Selective enrichment in a selective broth medium
- Plating onto a selective agar medium
- Incubation at the appropriate temperature and time
- Examination of the plates for the presence of characteristic colonies
- Confirmation of the identity of the microorganisms using biochemical tests or other identification methods.

5.6.4 The QC Inspector shall use appropriate positive and negative controls for each test to ensure the validity of the results.

5.6.5 Any suspect colonies should be further identified using standard microbiological techniques, including Gram staining, microscopic examination, and biochemical testing.

5.7 Data Analysis and Interpretation

5.7.1 The QC Inspector shall record all test results in the Microbiological Testing Record (F-QC-013-02).

5.7.2 The QC Inspector shall calculate the TAMC and TYMC per gram or milliliter of the sample.

5.7.3 The QC Inspector shall report the presence or absence of objectionable microorganisms.

5.7.4 The QC Inspector shall compare the test results to the acceptance criteria specified in the relevant pharmacopeia and product specifications.

5.7.5 If the test results meet the acceptance criteria, the QC Inspector shall report the results as "Pass."

5.7.6 If the test results do not meet the acceptance criteria, the QC Inspector shall report the results as "Fail" and immediately notify the QA Manager.

6.0 POST-TESTING ACTIVITIES

6.1 The QC Inspector shall properly dispose of all used culture media, samples, and other materials in accordance with the laboratory's waste disposal procedures. All potentially infectious materials must be autoclaved prior to disposal.

6.2 The QC Inspector shall clean and disinfect the Laminar Air Flow (LAF) hood (LAF-01) and all other equipment used during the testing process.

6.3 The QC Inspector shall review the Microbiological Testing Record (F-QC-013-02) to ensure that all data is complete and accurate.

6.4 The QC Inspector shall submit the completed Microbiological Testing Record (F-QC-013-02) to the QA Manager for review and approval.

6.5 The QA Manager shall review the test results and documentation to ensure that the testing was performed correctly and that the results are valid.

6.6 If the QA Manager approves the test results, they shall sign and date the Microbiological Testing Record (F-QC-013-02).

6.7 If the test results are OOS, the QA Manager shall initiate an OOS investigation according to the OOS Investigation SOP.

7.0 SAFETY PRECAUTIONS

7.1 Always wear appropriate PPE, including a laboratory coat, safety glasses, nitrile gloves, and a surgical mask, when performing microbiological testing.

7.2 Work in a Laminar Air Flow (LAF) hood (LAF-01) to minimize the risk of contamination.

7.3 Use aseptic techniques to prevent contamination of samples and media.

7.4 Handle all microorganisms as potentially infectious.

7.5 Autoclave all used culture media and other materials before disposal.

7.6 Clean and disinfect the work area and equipment after each use.

7.7 Wash hands thoroughly with soap and water after handling microorganisms and before leaving the laboratory.

7.8 In case of a spill of infectious material, immediately clean the spill with a disinfectant solution and notify the QA Manager.

7.9 Refer to the Material Safety Data Sheets (MSDS) for all chemicals used in the laboratory.

7.10 Exercise caution when working with hot agar and autoclaves to avoid burns.

8.0 APPROVALS

Prepared By: QC Inspector

Reviewed By: QA Manager

Approved By: Head of QA

Date: [Leave blank for manual completion]

Document Approval

Role	Name	Signature	Date
Prepared by:	_____	_____	_____
Reviewed by (QA):	_____	_____	_____
Approved by (Head QA):	_____	_____	_____

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