**[lab name]**

**STANDARD OPERATING PROCEDURE**

 **Microbiological Work Area Air Density Measurements**

**[SOP ID]**

**Based on V1M2 5.3.1**

**VERSION #1.0 Effective date: January 1, 2024**

**APPROVED BY**

**Signature**

 **[name] Technical Manager**

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**New version**

**Revision History**

|  |  |
| --- | --- |
| Version number and effective date | Revisions made |
| V 1.0 January 1, 2024 | Conforms to 2016 TNI quality system requirements. |
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# INTRODUCTION AND SCOPE

TNI V1M2 5.3.1 requires that laboratory spaces and facilities monitor for any condition that may adversely affect analytical performance or quality. In the list of suggested items, sterility is mentioned as one of the conditions to be checked.

This method is performed only if there is no testing for heterotrophic organisms via the pour plate method (Std. Methods 9215B or equivalent) at all, or during months when that method is not conducted.

# SUMMARY OF METHOD

A plate containing a non-selective medium has the cover removed for 15 minutes. At the end of the time, the cover is replaced, and the plate incubated for 48 hours. The number of colonies on the plate is recorded.

# Analytes to be Measured

Number of generic bacteria that settle on the plate while it is uncovered.

# Applicable Matrices

Air.

# SAFETY AND ENVIRONMENTAL

Always refer to the laboratory Health and Safety manual, SDS, Waste Handling and Disposal Plan, and the Chemical Hygiene Plan for general and specific requirements. In general, normal attire associated with working in the microbiology area is to be worn.

## Safety

There are no use of reagents, chemicals, or standards. Tempering the agar involves hot water. Use gloves and tongs when handling melted and tempered medium.

## Waste Minimization, Disposal and Pollution Prevention

Dispose of used plates as contaminated medium by autoclaving before disposal.

# EQUIPMENT, APPARATUS, INSTRUMENTATION, GLASSWARE/PLASTICWARE AND OTHER MATERIALS

All equipment and materials are selected to meet the specifications found in the method. All operating and maintenance manuals are stored nearby for quick reference. Important instructions, maintenance, and assessment criteria are contained in this SOP.

All volumetric syringes, pipettors, pipettes, mechanical volumetric devices, and other volumetric dispensing devices are calibrated per the procedures found in the Quality System manual.

## Equipment/Apparatus/Instrumentation

*Incubator*: capable of providing temperatures at 35oC+0.5oC. The standard incubator used for coliform analyses is sufficient.

*Quebec colony counter* or stereoscopic microscope capable of 20x magnification.

*Hand tally counter*

## Glassware/Plasticware/Other Materials

*Vendor provided sterile glass or plastic plates with non-selective medium* (see below).

# REAGENTS AND STANDARDS

Commercially prepared solutions of any of those cited in this SOP are satisfactory if the formulation matches that cited in this SOP. Follow all proper storage, usage, and disposal practices stated in this SOP or by the manufacturer of the solution. All solutions are discarded after the expiration date stated here in this SOP or as provided by the manufacturer. Concentrated acids and bases used are stored in proper and separate locations. Reagents requiring refrigeration are stored in a designated refrigerator.

Commercially prepared media will be checked for sterility on receipt or before first use. There are no Positive or Negative cultures for non-selective media.

Use reagent grade water for making all solutions.

*TSA or RHIA non-selective medium*

# PROCEDURE

**NOTE**: This procedure is required only if the laboratory does not perform heterotrophic plate counts by the pour plate method at least once a month. If this test is conducted at least once a month, then proceed to Section 11 below.

Disinfect the bench and surrounding area with disinfectant. Remove one specified plate stored in the coliform refrigerator, allow to come to room temperature, and make sure the media has not expired. More than one plate may be used. See below for data handling.

Wipe any condensation off the outside of the bottom of the plate and label the plate(s) “Air Density Settle Plate” with initials and the date on the outer edge. On the Air Density Settle Plate Form in the Microbiology Quality Control Log, record the analyst, date of analysis, vendor-prepared plate lot number and expiration date.

Take the lid off the plate and set the lid face down on the disinfected surface with the exposed plate next to it. Record the start time. Expose the media to the air for 15 minutes, then replace the lid. Record the end time. If the exposure exceeds 15 minutes, then correct the results as stated in Section 10.1.

Invert the plate and incubate at 35.0°C ± 0.5°C for 48 hours ± 2 hours. After 48 hours of incubation, remove the plate from the incubator.

Using a Quebec counter or stereoscopic microscope, slowly scan the plate for any bacterial or fungal colonies. Count all colonies observed and record the count. If additional plates are included, count them as well and record their individual counts.

## Calculations

If more than one plate is examined, then average the colony counts.

# DATA ASSESSMENT AND Reporting

## Calculations

The standard is 15 colonies/160cm2 per fifteen minutes. If the plate area or the exposure time are different, then correct this value as follows.

Equation computation of maximum allowed colonies based on plate areas and exposure times

$$Corrected colonies=15×\frac{actual plate area}{160 cm^{2}}×\frac{15 minutes}{actual time exposed}$$

Example. The plate is 78cm2. The exposure time is 20 minutes.

$$15×\frac{78}{160}×\frac{15}{20}=5.5 or 6 colonies$$

# Reporting

Report all actual counts and exposure times. Average the colony counts first before applying any correction factors. Round all values to the nearest even number.

Final counts over the allowed level are not themselves catastrophic, but the procedure needs to be repeated within a week. If the count still exceeds the limit, then repeat the next day, and examine the laboratory space for possible sources. If the counts remain over the limit for the third successive test, then stop all bacterial analyses and thoroughly examine and clean the laboratory.

# REFERENCES

* *Management and Technical Requirements for Laboratories Performing Environmental Analyses*, The NELAC Institute (TNI), Rev 2.1, September 1, 2016
* Laboratory QSM

# DEFINITIONS AND ACRONYMS

All definitions, unless stated below, are as found in the reference method or in TNI 2016, V1M2 section 3.

Acronyms not explicitly stated in the method or of general understanding are listed below.

## Definition

## Acronym