US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for
Monitoring of Laboratories for Airborne Contaminants

SOP Number: QC-02-08

Date Revised: 12-19-23

SOP Number	QC-02-08
Title	Monitoring of Laboratories for Airborne Contaminants
Revisions Made	• Minor editorial changes for clarification purposes. • Updated reference 15.1

SOP Number	QC-02-08
Title	Monitoring of Laboratories for Airborne Contaminants
Scope	This SOP describes a method for determining the occurrence (number and type) of airborne microorganisms in the laboratory.
Application	This procedure was designed based on references mentioned in section 15. Additional attributes have been added to detect airborne contamination in specific environments.

	Approval	Date
SOP Developer	REBECCA PINES Digitally signed by REBECCA PINES Date: 2023.12.20 16:23:01 -05'00'	12/20/23
	Print Name: Rebecca Pines	
SOP Reviewer	MICHELE COTTRILL Digitally signed by MICHELE COTTRILL Date: 2023.12.21 09:23:02 -05'00'	12/21/23
	Print Name: Michele Cottrill	
Quality Assurance Unit	Kiran Verma Digitally signed by Kiran Verma Date: 2023.12.21 10:23:35 -05'00'	12/21/23
	Print Name: Kiran Verma	
Branch Chief	REBECCA PINES Digitally signed by REBECCA PINES Date: 2023.12.21 10:28:04 -05'00'	12/21/23
	Print Name: Rebecca Pines	

Date SOP issued:	12/21/23
Controlled copy number:	0
Date SOP withdrawn:	

TABLE OF CONTENTS

1. Definitions	Abbreviations/definitions are provided in the text.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Refer to SOP QC-22, VITEK 2 Compact automated identification system.
5. Sample Handling and Storage	Not Applicable
6. Quality Control	For quality control purposes, document the required information on the appropriate form(s) (see section 14).
7. Interferences	1. Building construction, power outages, and equipment maintenance may cause transient aberrant counts. These events should be considered while interpreting results of the air testing and efficacy tests conducted during that time. Note these events in the comments section of the Air Monitoring Record Form (see section 14). 2. Do not conduct laboratory work while the air monitoring assay is being conducted.
8. Non-conforming Data	1. Manage non-conforming data consistent with SOP ADM-07, Non-Conformance Reports.
9. Data Management	1. Archive data consistent with SOP ADM-03, Records and Archives.
10. Cautions	1. Ensure plates are opened for the specified timeframe to avoid erroneous results.
11. Special Apparatus and Materials	Recovery media: 1. *Tryptic soy agar (TSA).* Used as general growth medium for bacteria. Purchase plates from a reputable source or prepare according to manufacturer's instructions. 2. *Sabouraud Dextrose Agar (SDA)*. Used as general growth medium for fungi. Purchase plates from a reputable source or prepare according to manufacturer's instructions.
12. Procedure and Analysis	1. Conduct the assay to investigate and determine possible environmental sources of contamination. The assay is conducted on an as needed basis, if recurring contamination is detected in a test system or whenever construction, airflow, or other environmental

	conditions change in the laboratory. 2. General growth media plates are exposed to the environment to monitor the occurrence of airborne microorganisms (e.g., bacteria, mold, and yeast). 3. This is a passive air sampling method. TSA and SDA Petri plates are exposed for a specific period of time at various sites in a laboratory (sample locations include bench tops, incubators, biosafety cabinets, etc).
12.1 Conducting the Assay	a. Identify locations to be assayed based on where the contaminant(s) were observed within a test system, assay, or routine laboratory work, or if disturbances to the laboratory environment occur. b. Determine the laboratory sites to be evaluated prior to commencing the assay. Record the sites within the laboratory that are being evaluated on the corresponding form (see section 14). c. Determine the exposure period for all plates. i. The exposure period may be determined based on various factors, such as: a) level of contamination observed, b) type of contamination (e.g., same contaminant, various contaminants, etc.), c) location of contamination within test systems (e.g., laboratory bench, incubator, BSC, etc.), and d) laboratory disturbances such as movement of equipment, laboratory construction, and laboratory upgrades. ii. The minimum exposure period is 15 minutes and should not exceed 60 minutes. d. Expose media plates for the pre-determined time period (i.e., 15 to 60 minutes). Leave all exposed plates uncovered for the same amount of time. e. Label plates in accordance with their locations where they are placed in a laboratory (room #, specific sites in a lab, etc.). f. Place a TSA and SDA plate at each location and remove the plate covers. g. Expose the plates for the pre-determined amount of time (15-60 minutes).

	h. Replace the covers after the exposure time is complete. Record the exposure time on the appropriate form (see section 14). i. Wrap plates in parafilm or bag plates to prevent dehydration. j. Incubate the plates at 36±1°C (for TSA) and at 30±1°C (for SDA). k. Monitor plates daily up to 7 days. Record final results on day 7 of incubation. Count colonies and record the number of colonies (up to 300 CFU per plate); counts > 300 CFU are recorded as too numerous to count or TNTC.
12.2 Interpretation of Results and Decontamination	a. The number of organisms which settle in 15 minutes of exposure on a Petri dish is equivalent to that for 1 square foot. b. Calculate the number of CFU per plate. If the final number of contaminants per plate exceeds 15 CFU/ft^2, perform general laboratory cleaning using an antimicrobial product. c. Following the cleaning process, repeat the air monitoring procedure. Work in a laboratory may be suspended until the problem is resolved. d. If one of the exposed plates corresponds to a location inside a BSC and exhibits an unacceptable level of contamination, do not use the BSC until the following corrective actions are conducted. i. Decontaminate the BSC using an EPA registered hospital disinfectant for the contact time specified on the label. ii. Repeat the air monitoring test for the impacted BSC. iii. Do not use the BSC until the air monitoring indicates an acceptable level of microbial counts of less than 15 CFU/ft^2 per exposed plate. iv. Inform the ESC Facility Manager if the repeat air monitoring test continues to indicate an unacceptable level of contamination.
12.3 Identification and Confirmation of Contaminants	a. Conduct a Gram stain on representative colonies from the TSA and lacto-phenol cotton blue (LPCB) stain from SDA plates. b. Conduct presumptive identification by performing streak isolations onto general and/or selective media. Use specific growth medium and incubation conditions if attempting to identify the presence of a more fastidious microbe.

	c. Use VITEK 2 automated identification system, if necessary.
13. Data Analysis/ Calculations	1. Determine the number of CFUs per 15 × 100 mm plate per 15-minute period. Multiply by the appropriate factor if the exposure time is greater than 15 minutes; e.g., multiply the number of CFUs by a factor of 2 if the exposure time is 30 minutes (see section 15.1). Total number of contaminants/plate: $(x\ CFU) \times (y) = z$ Where x = number of CFU/plate, y = exposure time multiplying factor (y is always 1 if the exposure time is 15 minutes), and z = total number of contaminants per plate. 2. For example: if a plate has 10 CFU and was exposed for 30 minutes then, 10 CFU × 2 = 20 CFU; y = 2 since the exposure time was 30 minutes. In conclusion, this plate indicates a higher than normal presence of contamination.
14. Forms and Data Sheets	1. Test Sheets. Test sheets are stored separately from the SOP under the following file names: Air Monitoring Record Form QC-02-08_F1.docx
15. References	1. Bordner, R.H., Winter, J.A., & Scarpino, P.V., eds. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. EPA 600/8-78-017, Part IV Quality Assurance, U.S. Environmental Protection Agency, Cincinnati, Ohio. 2. Lipps, W.C., Braun-Howland, E.B., & Baxter, T.E., eds. 2022. Standard Methods for the Examination of Water and Wastewater, 24th Edition. American Public Health Association, American Water Works Association, Water Environment Federation. Section 9020.