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-02-06

Date Revised: 05-11-17

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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:	_____ Print Name: _____
SOP Reviewer	_____ Print Name: _____
Quality Assurance Unit	_____ Print Name: _____
Branch Chief	_____ Print Name: _____

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

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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 QC-22: VITEK 2 Compact.
5. Sample Handling and Storage	Not Applicable
6. Quality Control	For quality control purposes, the required information is documented 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. Media must pass sterility and performance assessment prior to use.
8. Non-conforming Data	1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports.
9. Data Management	1. Data will be archived consistent with SOP ADM-03, Records and Archives.
10. Cautions	1. Ensure plates are opened for the specified timeframe to avoid flawed results.
11. Special Apparatus and Materials	1. Trypticase Soy Agar (TSA) plates or TSA with 5% sheep's blood. 2. Sabouraud Dextrose Agar (SDA).
12. Procedure and Analysis	1. The assay is conducted to investigate environmental sources of contamination. 2. In this method, general growth media are exposed to the environment to monitor the occurrence of airborne microorganisms (e.g., bacteria, mold and yeast). This is a passive air sampling method. The test is performed on an as needed basis, if contamination is detected in a test system or whenever construction, airflow or other environmental conditions change in the laboratory. 3. Petri plates containing TSA and/or SDA are exposed for a specific period of time at various sites in a laboratory (sample locations include

	bench tops, incubators and biosafety cabinets etc).
12.1 Conducting the Assay	a. Locations to be assayed are identified 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. c. Record the sites within the laboratory that will be evaluated on the corresponding form (see section 14). d. Determine the exposure period for plates. The time frame of exposure should be between 15, 30, 45 or 60 minutes. All exposed plates should be left 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 the plates at the desired locations and remove the covers. A TSA and SDA plate should be placed at each location. 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. Incubate the plates at 36±1°C (for TSA) and at 30±1°C (for SDA) for 2 to 7 days. Wrap plates in parafilm after 48 hours of incubation to prevent dehydration Plates may be observed daily for growth. j. 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. Refer to section 12.2 for interpretation of results.
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 sq. foot. b. Calculate the number of CFU per plate. If results indicate that the final number of contaminants per plate exceeds 15 CFU, then 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, then the BSC is not used 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 affected BSC. iii. Do not use the BSC until the air monitoring indicates an acceptable level of microbial counts. iv. Inform the ESC Facility Manager in the event the repeat air monitoring test continues to provide negative results (i.e. presence of unacceptable level of contamination).
12.3 Identification and Confirmation of Contaminants	a. Conduct a Gram stain on representative colonies from the TSA and SDA plates. b. Further presumptive identification may be conducted by plating onto general and/or selective media. c. VITEK identification may be conducted, if necessary. *Note: It may be necessary to use a specific growth medium and incubation conditions if one is attempting to identify the presence of a more fastidious microbe.*
13. Data Analysis/ Calculations	1. Determine the number of CFUs per 15 × 100 mm plate per 15-minute period (or multiply with the factor if the exposure time is more than 15 minutes, e. g., the number of CFUs be multiplied with 2 if the exposure time is 30 minutes (see section 15). Final number of contaminant/plate: $(x\ CFU) \times (y) = z$ Where x = number of CFU/plate, y = exposure time multiplying factor and z = final 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. In this case y = 2 since the exposure time was 30 minutes; y is always 1 if the exposure time is 15 minutes, as described in 13.1. 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-02-06_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. Rice E. W., Baird, R.B, Eaton, A.D., Clesceri, L.S., eds. 2012. Standard Methods for the Examination of Water and Wastewater, (Page 9-4), 22nd Edition. American Public Health Association, American Water Works Association, Water Environment Federation