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-02-05

Date Revised: 04-15-14

SOP Number	QC-02-05
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Date SOP withdrawn:	

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. The Study Director and/or lead analyst should consult the Material Safety Data Sheet for specific hazards associated with products.
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 in advance of 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. If any unusual air handling events take place (e.g., significant laboratory air flow changes, construction within the facility), air monitoring may be conducted at any time to verify the quality of the lab's air according to procedures described in section 12.
11. Special Apparatus and Materials	1. Trypticase Soy Agar (TSA) plates or TSA with 5% sheep's blood.
12. Procedure and Analysis	1. The assay is conducted to investigate possible contamination sources. 2. Conduct the test only on the affected laboratory if possible. 3. 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 can be performed on an as needed basis or at least once a year for all

	laboratories. 4. Petri plates containing TSA and/or Sabouraud Dextrose Agar (SDA) are exposed to the environment for a specific period of time at various sites in a laboratory (sample locations include bench tops, incubators and biosafety cabinets etc). 5. The presence of contamination may be due to air flow changes, facility construction amongst others.
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 unusual air handling takes place. 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 contact time for plates to be exposed to the environment. The time frame of exposure should be between 15-60 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 desired locations and remove the covers sequentially at 15-30 second intervals. g. Expose the plates for the pre-determined amount of time (15-60 minutes). h. Replace the covers after exposure time is completed, in sequential order. Record exposure time on the appropriate form (see section 14). i. Incubate the plates at 36±1°C (for TSA) or at 24±1°C (for SDA) for 2 to 7 days. Wrap plates in parafilm to prevent dehydration during extended period of incubation (≥ 48 hours). Plates may be observed daily for growth. j. Count colonies and record the number of colonies per square foot (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. If results indicate contamination that exceeds 15 colonies/plate/15 minute (see section 15) or if the laboratory base line established on past

	historical data appears higher than normal, then the source of contamination is investigated. c. Perform general laboratory cleaning using an antimicrobial product, if necessary. d. Following the cleaning process, repeat the air monitoring procedure. Operation in a laboratory may be suspended until the problem is resolved. e. If one of the exposed plates, corresponds to a location inside a BSC and exhibits an unacceptable level of contamination (15 colonies/plate/15 minute or if laboratory base line established on past historical data appears higher than normal), then the BSC is not used. i. Inform the ESC Facility Manager. ii. Decontaminate the BSC if necessary, using an EPA registered disinfectant (the disinfectant is used following the label instructions for the determined amount of contact time). iii. Repeat the air monitoring test for the affected BSC. iv. Do not use the BSC until the air monitoring indicates acceptable level of microbial counts.
12.3 **Identification and Confirmation of Contaminants**	a. Conduct a Gram stain on contaminants. b. Further presumptive identification may be conducted by plating onto general and/or selective media, if necessary. c. Conduct VITEK identification if absolutely 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-02-05_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. Eaton, A.D., Clesceri, L.S., Rice E. W. eds. 2005. Standard Methods for the Examination of Water and Wastewater, (Page 9-4), 21st Edition. American Public Health Association, American Water Works Association, Water Environment Federation