

Test/QA Plan for Mold-Resistant Building Material Testing

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This document serves as the Test and Quality Assurance (QA) plan for mold-resistant building material testing. The Quality Management Plan (QMP) under which this work is conducted is the *Verification Testing of Air Pollution Control Technology Quality Management Plan*, Revision 2.2, February 17, 2005¹. This QMP was approved by EPA for the ETV program and fulfills the requirements of the ETV program-level QMP.

Acknowledgement

RTI would like to thank the stakeholders who input to this program.

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A3: Distribution List

EPA

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Mr. Michael Herman
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List of Acronyms/Abbreviations/Definitions

ACH	air changes per hour
ADQ	audit of data quality
ASTM	American Society for Testing and Materials
a _w	water activity
CFU	colony forming unit
DNPH	2,4-dinitrophenylhydrazine
DQO	data quality objective
EPA	U.S. Environmental Protection Agency
ESTE	environmental and sustainable technology evaluations
ERH	equilibrium relative humidity
ETV	environmental technology verification
g	gram(s)
GC/MS	gas chromatography/mass spectrometry
ISO	International Organization for Standardization
MC	moisture content
ML	microbiology laboratories
ML SOP	microbiology laboratory standard operating procedure
QA	quality assurance
QAM	quality assurance manager
QAPP	quality assurance project plan
QC	quality control
QMP	quality management plan
RH	relative humidity
RTI	Research Triangle Institute (RTI International)
sec	second(s)
SOP	standard operating procedure
spp	species
t	temperature
TOP	technical operating procedure
T/QAP	test/quality assurance plan
TSA	technical system audit
TVOC	total volatile organic compounds
VOC	volatile organic compounds
μg	microgram(s)
μm	micrometer(s)

SECTION A: PROJECT MANAGEMENT

A4: Project/Task Organization

The Environmental Technology Verification (ETV) / Environmental and Sustainable Technology Evaluations (ESTE) Program was established by the U.S. Environmental Protection Agency's Office of Research and Development to accelerate the development and commercialization of improved environmental technologies through third party verification and reporting of performance. RTI will perform the testing, evaluate the data, and prepare the verification reports. The various quality assurance (QA) and management responsibilities are divided between EPA and RTI key project personnel as defined below. The lines of authority between key personnel for this project are shown on the project organization chart in Figure 1.

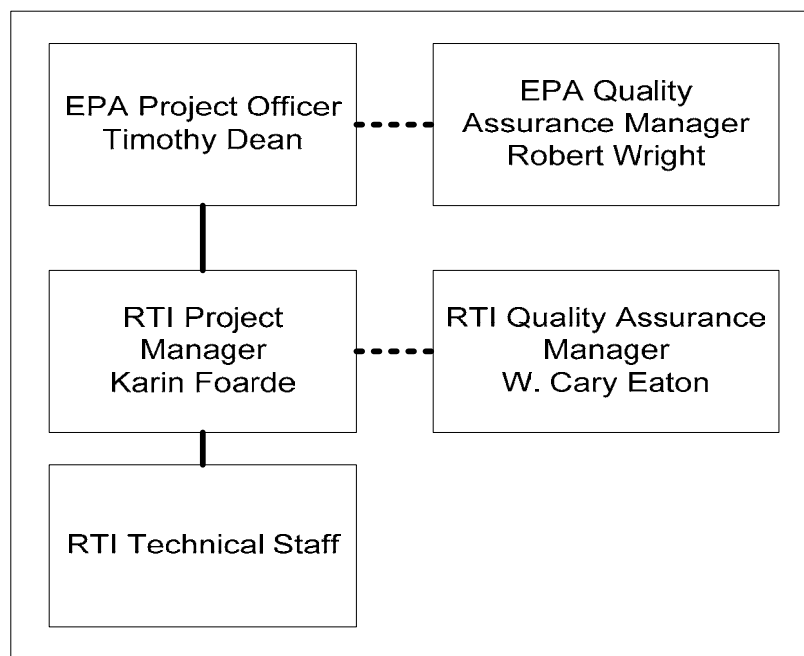


Figure 1. Organization Chart. Dotted lines indicate organizational independence.

A4.1: Management Responsibilities

Project management responsibilities are divided between EPA and RTI personnel as listed below.

A4.1.1: EPA Project Officer

Dr. Timothy Dean is the EPA Project Officer for the contract. He is responsible for oversight of this program.

A4.1.2: RTI Project Manager

The RTI Project Manager, Ms. Karin Foarde is responsible for task implementation and technical quality control. The RTI Project Manager is also responsible for the following:

- Update and deliver revisions of the Test/Quality Assurance Project Plan (T/QAP),
- Define task objectives in coordination with EPA,
- Develop a detailed work plan schedule,

- Halt testing if there is a quality or safety problem,
- Work with vendors and stakeholders,
- Review work progress to ensure that task budgets and schedules are met, and
- Prepare verification reports.

Ms. Foarde will also be the technical leader and will review and compare the results for consistency based on her experience with the respective components of the tests.

A4.2: Quality Assurance Responsibilities

QA responsibilities are divided between the EPA and RTI personnel as listed below.

A4.2.1: EPA Quality Assurance Manager

The EPA Quality Assurance Manager (EPA QAM), Mr. Robert Wright, will conduct audits of RTI's QA System¹ and of specific technical activities on the project as specified by EPA. He will be available to resolve any QA issues relating to performance and EPA's QA requirements. Specific functions and duties of the EPA QAM include approving the contents of this T/QAP and subsequent revisions, performing a TSA (technical system audit) on RTI, and reviewing QA reports prepared by RTI, including RTI's internal QA evaluations and audits.

A4.2.3: RTI Quality Assurance Manager

The RTI Quality Assurance Manager (RTI QAM), Dr. W. Cary Eaton, is organizationally independent of the RTI Project Manager and is responsible for ensuring that QA/quality control (QC) procedures described in this T/QAP are followed. In addition, the RTI QAM will:

- Maintain regular communication with the EPA QAM regarding QA issues,
- Report on the adequacy, status, and effectiveness of the QA program to the Project Manager,
- Conduct an internal TSA after samples have been received and cut for distribution. This will constitute a "readiness review." An audit of data quality (ADQ) will be conducted after results from day zero are in hand. Any TSA items not completed during "readiness review" will be completed at the time of the ADQ.
- Halt testing if a quality or safety problem is found in the TSA, after consultation with RTI program manager,
- Ensure that corrective action, if necessary, is properly implemented and documented,
- Review and approve test (including QC) reports, and
- Prepare the QA section of each verification report.

A5: Problem Definition/Background Information

Fungal growth and the resulting contamination of building materials is a well-documented problem, especially after the reports from New Orleans and the US Gulf Coast post Hurricane Katrina. However, contaminated materials have been recognized as important indoor fungal reservoirs for years. For example, contamination with fungi has been associated with a variety of materials including carpet, ceiling tile, gypsum board, wallpaper, flooring, insulation, and heating, ventilation and air conditioning components^{2,3,4,5}.

Exposure to fungi may result in respiratory symptoms of both the upper and lower respiratory tract such as allergy and asthma⁶. Everyone is potentially susceptible. However, of particular

concern are children with their immature immune systems and individuals of all ages that are immunocompromised^{7,8}.

One approach to limiting exposure is to reduce the levels of fungi in the indoor space. For some sensitive individuals, limiting exposure through avoidance is an effective control method; however, avoidance is not always possible or practical. The investigation, development, and application of effective source controls and strategies are essential to prevent fungal growth in the indoor environment. Mold resistant building material is a potentially effective method of source control.

A building is not a sterile environment, nor should it be. However, a building may serve as a reservoir for microorganisms. While many different types of microorganisms occupy indoor spaces, it is well-recognized that fungi can colonize and amplify on a variety of building materials if sufficient nutrients and moisture are present. These contaminated materials are known to be important indoor reservoirs. Fungal growth on natural and fabricated building materials can be a major source of respiratory disease in humans. Some common environmental fungi that have been isolated from contaminated materials include *Acremonium spp.*, *Alternaria spp.*, *Aspergillus spp.*, *Chaetomium spp.*, *Cladosporium spp.*, *Epicoccum spp.*, *Fusarium spp.*, *Penicillium spp.*, *Stachybotrys spp.*, and *Trichoderma spp.*

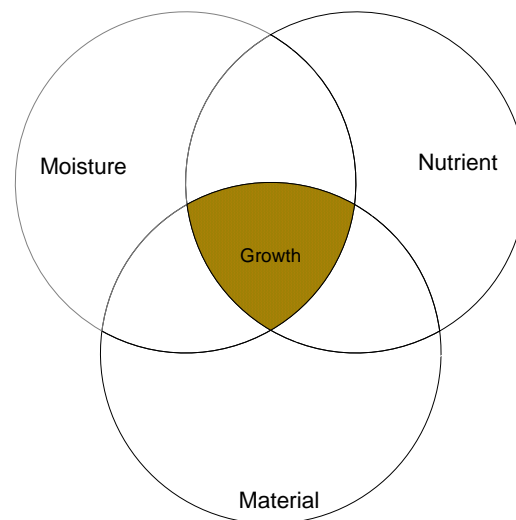


Figure 2. Diagram illustrating the conditions required for fungal growth on a material.

Figure 2 illustrates the combination of moisture and nutrients required for microbial growth on a material. Sufficient nutrients for growth may be provided by the material itself or through the accumulation of dust on or in the material. When sufficient nutrients are available, the ultimate determinate for microbial growth is availability of water. The more hygroscopic a material is, the more impact on the overall hygroscopicity the surface treatments may have.

This test plan addresses three specific characteristics of mold resistant building material: 1) mold resistance, 2) emissions of VOCs and aldehydes, and 3) moisture content. Mold resistance is the critical measurement, so the T/QAP is focusing on mold resistance. Moisture content and emissions of VOCs and aldehydes are ancillary tests and may or may not be performed depending upon the relevance to the test material. Information can be found in Appendix A and B, respectively. Other characteristics, such as fire resistance, are important and should be considered by users of the products, but are beyond the scope of this test plan.

A building is not a sterile environment, nor should it be. In fact, a building is frequently a reservoir for microorganisms. While many different types of microorganisms occupy indoor spaces, it is well-recognized that fungi can colonize and amplify on a variety of building materials if sufficient nutrients and moisture are present. These contaminated materials are known to be important indoor reservoirs. Fungal growth on natural and fabricated building

materials can be a major source of respiratory disease in humans. Some common environmental fungi that have been isolated from contaminated materials include *Acremonium spp.*, *Alternaria spp.*, *Aspergillus spp.*, *Chaetomium spp.*, *Cladosporium spp.*, *Epicoccum spp.*, *Fusarium spp.*, *Penicillium spp.*, *Stachybotrys spp.*, and *Trichoderma spp.*

Mold resistance testing will be performed following the guidelines outlined in ASTM 6329-98 (2008)⁹. This method was developed as part of a more comprehensive project to apply indoor air quality engineering to biocontamination in buildings. One of the primary goals was to provide a scientific basis for studying indoor air biocontaminants. Available methods, including those from ASTM, AATCC, and UL, for evaluating the resistance of a variety of materials to fungal growth were surveyed at the initial stages of that project. Although the basic principals were similar, a major concern was the way growth on the different materials was evaluated. Although quantitative methods for inoculation were employed, none assessed growth as the endpoint quantitatively. The strategy was to develop a method that would provide a quantitative endpoint for growth in a well-controlled environment to and to improve repeatability and comparability. The method has been successfully used to evaluate fungal resistance on a variety of materials including ceiling tiles and HVAC duct materials^{10,11,12,13}.

A6: ESTE Testing - Description and Schedule

The ESTE testing for mold-resistant building material will be performed under the US EPA's quality system whose goal is to ensure that environmental programs and decisions are supported by data of the type and quality needed and expected for their intended use, and that decisions involving environmental technology are supported by appropriate quality-assured engineering standards and practices. The implementation of the EPA Quality System is based on a graded approach, meaning that quality systems for different organizations and programs will vary according to the specific objectives and needs of the organization. EPA requires that contractors performing research or testing for EPA have both a quality management plan¹ and a quality assurance project plan (QAPP) (or test plan) – this document.

The ETV management plan states:

The quality system for the overall ETV program seeks to be consistent with industry consensus standards. Each verification organization shall implement a valid and approved quality system. The Agency's required quality system for cooperative agreements and contracts is ANSI/ASQC E4. Each verification test will be performed according to planned and documented, pre-approved test/QA plans. All technical statements in ETV verification reports shall be supported by the appropriate data¹⁴.

ETV requires that all test plans, data and results be reviewed by both the company (in this case, RTI) and the EPA project quality managers. In addition, both RTI and EPA perform audits of the RTI ESTE quality system and how the testing is being performed and how ETV quality management performs.

EPA uses the data quality objective (DQO) process as defined in Guidance on Systematic Planning using the Data Quality Objectives Process (QA/G-4)¹⁵. The DQO process is used to establish performance or acceptance criteria, which serve as the basis for designing a plan for collecting data of sufficient quality and quantity to support the goal of a study¹⁵. DQOs may

include specific values for accuracy and precision, but they will often provide one value that incorporates both entities.

EPA and ETV strongly support the use of international consensus standards, however, recognizes that a laboratory will need to supplement standards with their own SOPs. The following sections indicate which standards are used in developing this QAPP/test plan. This document is written in a format defined by EPA that includes sections not found in a standard test method. This document will indicate sections and document information when procedures are being used from those documents.

A6.1: Description of Testing

The ESTE verification test includes separate tests to be performed on each building material product submitted for testing:

- Mold resistance testing.
- VOC and aldehyde emissions testing, and
- Moisture content

Testing for VOC and aldehyde emissions and moisture content are ancillary tests which may or may not be performed depending upon the relevancy to the test material. Some materials have undergone GreenGuard testing for VOC emissions. For those products, the test report downloaded from the website will serve as the ancillary report. The use of this report falls under the use of existing data clause in the ETV management plan

The testing consists of the three steps summarized below:

- Acquiring the mold-resistant building material products for testing,
- Performing the testing of the products, and
- Preparing verification reports and summary statements.

Each of the three tests is discussed separately. The moisture test can be found in appendix A. The VOC and aldehyde emissions test can be found in appendix B. Both tests are considered optional and will be performed as appropriate for a test material.

A6.1.1: Identification and Acquisition of Mold-Resistant Building Materials

The companies will provide their building material product to RTI for testing. Tricia Schwartz will be the custodian at RTI and will be responsible for storage, labeling, etc. of the products. She is responsible for documentation showing chain of custody. Each piece of building material product will be labeled as will the samples cut from it. The products will be stored in the Building 11, Bay 1 storage room at RTI until used and will be retained until the verification report for the product is approved. All products will be logged in upon receipt and will be checked out for testing, then the unused portion will be returned to the storage room when testing is complete.

A6.1.2: Performance of Mold Resistance Testing

Mold resistance testing will be performed following the guidelines outlined in ASTM D6329-98 (2008)⁹. The specifics of the test and the related SOPs are discussed in further detail in Section B1.2. In overview, the test organisms are inoculated by pipette directly onto the surface of each building material piece in sufficiently high numbers to provide an adequate challenge, but at a

level that is realistic to quantitate. The tests will run for 12 weeks. Within the 12 weeks of the test, four test days, Day 0, Week 1, Week 6, and Week 12 will be evaluated. Day 0 will provide the baseline inoculum level. A sufficient number of test pieces will be inoculated simultaneously for all four test days. All of the pieces for one material and one test organism will be put in the same static chamber. Two test organisms, *Stachybotrys chartarum* and *Aspergillus versicolor* will be used. The chambers will be set to 100% ERH for the tests with *S. chartarum* and at 85% for *A. versicolor*. On each test day (including day 0), five replicates of the test material pieces are removed from the chamber, placed in sterile buffer, and extracted by shaking. The resulting suspension of eluted organisms is plated and microbial growth on materials quantitated by manually enumerating colony-forming units (CFU),

The numbers of CFU eluted on test days week 1, 6, and 12 and compared to the baseline at Day 0. The numbers of CFU are expressed as log₁₀. The results will be reported as the log change in CFUs between Day 0 and Week 1, Day 0 and Week 6, and Day 0 and Week 12.

A6.1.3: Preparation of Report

The final step is to complete the verification report and verification statement for each product tested and submit them to EPA for review.

A6.2: Schedule

The verification will begin when the T/QAP is approved by EPA and will continue at least until all three initial products are tested. Testing may continue beyond that if there are additional products and funding to test.

A7: Data Quality Objective and Criteria for Measurement Data

Data quality objectives (DQOs) are qualitative and quantitative statements designed to ensure that the type, quality, and quantity of data used are appropriate for the intended application. The DQO for the critical measurement, quantitation of fungal growth on an individual test date, is found in Table 2.

Table 2. Data Quality Objectives

Test	Parameter	DQO		
		Precision	Accuracy	Completeness
Mold Resistance	Quantitation of fungal growth on an individual test date	± 5-fold difference	10% of the plates will be counted by a second operator. ± 20% agreement between the operators	100%

All RTI data will be reviewed for accuracy (correctness) and reasonableness. If the results are deemed unreasonable (e.g., internally inconsistent), they will be discarded, the procedures reviewed, and the test repeated if necessary. Data points that are analyzed and determined to be obvious outliers will be discarded without requiring the entire test to be repeated.

A8: Special Training Requirements/Certification

There are no specialized certification requirements specified for these tests. The RTI Project Manager will be responsible for overseeing all work and ensuring that RTI personnel are fully trained in each operation for the microbial, VOC, and moisture testing. RTI personnel working with the mold resistance testing are trained to perform all of the required procedures, including determining microbial growth.

A9: Documentation and Records

This section identifies the documents and reports to be generated as part of the verification program and the information to be included in the verification reports. A description of the data management system established for this task is presented in Section B.10.

Requirements for record keeping and data management for the overall program are found in the Quality Management Plan (QMP) for the Verification Testing of Air Pollution Control Technology, Revision 2.2¹, including Table 5-1, Records Management Responsibilities for ETV. All ML SOPs and TOPs (Technical Operating Procedures) are maintained on file at RTI. Access to these files is permitted on-site at RTI and will be available for review during internal and external TSAs.

A9.1: Laboratory Documentation

The culturable test organism counts or CFUs from each test piece on each test day will be entered in the project notebook or recorded by a computer. If recorded to a computer, the file will be saved to the hard drive and later copied to a floppy disk or shared directory for backup.

A9.2: QA Reports

The RTI QAM will perform an internal TSA based on the approved T/QAP during the first month of verification; this is considered suitable because this testing program is using well-known measurement systems components. A report will be prepared for the Project Manager within 15 days of completion of the audit.

The RTI QAM will perform an ADQ of all RTI data. RTI will provide a report of the ADQ to EPA with the verification report for review.

RTI will cooperate with audits performed by the EPA Project Officer, EPA QAM, or their designee insofar as resources permit. EPA will perform a TSA for this project.

A9.3: Reporting

After the completion of tests, the control test data, sample inventory logs, calibration records, and certificates of calibration will be stored in the laboratories. Copies of these will be made for RTI and EPA QA. Calibration records will include such information as the instrument being calibrated, raw calibration data, calibration equations, analyzer identifications, calibration dates, calibration standards used and their traceabilities, identification of calibration equipment used, and the staff conducting the calibration. Final reports of self-assessments and independent assessments (i.e., technical systems audits, performance evaluations, and audits of data quality [TSAs and ADQs]) will be retained as required by ETV and RTI. Each verification report will contain a QA section, which will describe QA activities and the extent to which test data comply with DQOs.

A9.4: Verification Reports

Verification reports will be prepared by RTI personnel and reviewed by RTI's Project Manager and QAM prior to submittal to the EPA Project Manager for review.

There shall be a general discussion of the test process giving dates and other information as appropriate.

The microbial resistance will be reported as the log change in CFUs between the start date and each of the four test dates. Each test organism will be reported separately.

The DQO will be reported. The results of the ancillary moisture and emissions tests will also be included when performed.

A9.4.1 Environmental Sustainability Criteria

A section of the verification report and the verification statement will focus on environmental sustainability, with information provided by the vendor.

The verification organization will work with the vendor to estimate impacts on solid waste disposal from replacing building materials less frequently, using the test results for microbial resistance. If possible this should be done quantitatively with uncertainty estimates.

The vendor will supply the following:

- Information regarding the chemicals or other product characteristics that engender the product microbial resistant.
- For chemical additives that are claimed to confer microbial resistance, the vendor shall provide the identity of the chemical and a summary of toxicity information relative to the chemical. The quantity of the chemical used in the building material product shall also be provided. It is expected that this information will come from the MSDS sheet, which can be included as an appendix to the report.
- Additional information relative to the environmental sustainability of the product such as recyclability/reusability of the product and disposability of the product and use of renewable resources or other criteria the vendor deems relevant to the environmental sustainability of the product.

SECTION B: MEASUREMENT/DATA ACQUISITION

B1: Test Design

Mold resistance testing will be based on ASTM D6329-98 (2008)⁹, “Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chambers”.

B1.1 Static Chambers

Acrylic desiccators will serve as the static environmental chambers. The desiccators are sealed so there is no air exchange and serve as good static chambers. The chamber humidity will be maintained through the use of saturated salt solutions (ASTM E104-02¹⁶) or sterile water. Temperature control is externally controlled and maintained at room temperature. Prior to use, the chambers will be decontaminated following ML (microbiology laboratories) SOP #017 (Standard Operating Procedure for the Decontamination of Humidity Chambers). The chambers will be characterized following ML SOP #005 (Standard Operating Procedure for the Characterization of Relative Humidity Chamber) once prior to initiation of the testing.

The chambers will be set to 100% ERH for the tests with *Stachybotrys chartarum* and at 85% for *Aspergillus versicolor*. The ERH in each chamber will be monitored with a hygrometer.

B1.2 Test Organisms

Selecting the “correct” test organism is critical to any test; therefore selection criteria were developed. The selection criteria used to choose the appropriate test organisms for this study were:

- 1) the reasonableness or likelihood of the test material being challenged by that particular organism when in actual use, and
- 2) that they cover the range of ERHs to the needed to bracketing the ERHs where fungal growth can occur.

While exposure to many of the fungi can be considered problematic, none are as controversial as exposure to *Stachybotrys chartarum*^{17,18}. There are numerous reports demonstrating an association between exposure to *S. chartarum* and adverse respiratory health effects; but none is as compelling as the paper simply entitled *Stachybotrys* by Etzel¹⁹, which reviews what has been learned about *Stachybotrys* and its association with pediatric pulmonary disease. Pulmonary hemosiderosis among infants was reported in Cleveland, OH, in the early 1990s (Etzel²⁰ et al.(a)) although the findings were not sufficient to support an association between *Stachybotrys chartarum* on building materials and the disease (Montana²¹ et al., Etzel²² et al. (b); CDC MMWR²³). In another study, mycotoxin produced from *S. chartarum* on wetted wallboard has been found to be water-soluble and was toxigenic in vitro (Black²⁴ et al.).

Two fungi will be used as test organism, *Aspergillus versicolor* and *Stachybotrys chartarum*. Both are from the RTI culture collection (CC). The CC number for *S. chartarum* is 3075 and was received from EPA NERL. *A. versicolor* is CC #3348, and it is a field isolate. Prior to initiation of the testing, their identification will be confirmed by standard techniques. *Aspergillus versicolor* is recommended because it is a xerophilic fungus and capable of growing at lower relative humidities. *Stachybotrys chartarum* is recommended because it requires high levels of available water to grow and has been associated with a number of toxigenic symptoms. A.

versicolor is frequently reported as a causative agent of hypersensitivity pneumonitis and has been isolated from a number of problem buildings. Both have been reported as growing on building materials.

B1.3 Sample Preparation and Inoculation

Small (at least 4 cm x 4 cm), replicate pieces of test mold resistant building material will be prepared and inoculated. To minimize error and demonstrate reproducibility, five pieces of each sample type will be processed on each respective sampling day. Because there are four test dates, a minimum of 20 pieces will be prepared simultaneously. Each piece is placed on a separate labeled sterile petri dish.

Fungi media, cultures, spore isolation, suspension, quantitation, and inoculation on the test wallboard will follow: ML SOP #001 (Standard Operating Procedure for Media Preparation – Dehydrated); ML SOP #002 (Standard Operating Procedure for the Preparation of Sterile Water); ML SOP #003 (Standard Operating Procedure for the Preparation of Sterile Buffer); ML SOP #009 (Standard Operating Procedure for the Quantitative Evaluation of Microorganisms); ML SOP #012 (Standard Operating Procedure for the Quantitation of Viable Spores in Suspension Preparation); and ML SOP #058 (Standard Operating Procedure for Direct Inoculation of Materials with a Spore Suspension). A review of those steps follow.

The fungi challenge suspensions are prepared by inoculating the test organism onto solid agar media, incubating the culture at room temperature until mature, wiping organisms from the surface of the pure culture, and eluting them into sterile 18-Mohm distilled water to a known concentration to serve as a stock solution. The organism preparation is viewed microscopically to verify purity of spores (absence of hyphae). The suspension is diluted in sterile 18-Mohm distilled water if needed to a concentration of approximately $10^5 - 10^6$ CFU/mL. The test pieces are inoculated (usually with 5 $10\mu\text{L}$ spots in an X configuration) by pipet onto the surface of the building material test piece and allowed to dry in the biosafety cabinet. The goal is to load each of the individual test pieces with approximately 10^3 to 10^5 CFU/piece. The fungi spore suspension is quantified on appropriate media to enumerate the inoculum.

On each test day (including day 0), the test pieces will be removed from the static chamber, placed in approximately 30 mL sterile buffer, and extracted by shaking using a vortex or wrist action shaker. The extract is diluted if needed and plated on agar media to determine CFU.

B2: Sampling Methods Requirements

The mold resistance sampling method requirements and critical dimensions and configurations of the test chamber are specified in Foarde²⁵ et al. All sampling methodology will comply where appropriate. Static chambers are maintained in accordance with ML SOP #005 (Standard Operating Procedure for the Characterization of Relative Humidity Chamber), ML SOP #009 (Standard Operating Procedure for the Quantitative Evaluation of Microorganisms), ML SOP #017 (Standard Operating Procedure for the Decontamination of Humidity Chambers), and ML SOP #058 (Standard Operating Procedure for Direct Inoculation of Materials with a Spore Suspension). Where used, all equipment will be calibrated and operated according to the manufacturer's specifications.

Each SOP has its own QUALITY ASSURANCE/QUALITY CONTROL section, which includes the QC Checks, which are available at RTI. As one example, ML SOP #001 Standard Operating Procedure for Media Preparation – Dehydrated, the QC Checks are as follows:

- If balance has an internal calibration program, that may be used, or a check weight can be used as first and last weighing. Refer to the procedures outlined in the appropriate user's manual for more specific calibration instructions if needed.
- Balances are professionally calibrated annually.
- If the Manostat is used, the desired volume of media dispensed is checked with a graduated cylinder or tube before filling plates.
- Incubate plates at room temperature (on counter) for at least 3 days (no more than 5) prior to use so that contaminated plates may be identified (appearance of any colonies) and disposed of in an appropriate manner. Media for bacteria may be used if necessary after 1 day.
- Inoculate several plates (~2 out of 100) with a loop of appropriate organism to prove the media's ability to sustain growth (see supervisor to ascertain incubation time and temperature).
- Record the reaction in the media preparation notebook.
- After quality control tests have been completed, store bagged and labeled plates upside down in a refrigerator (2-5°C).

B3: Sample Handling and Custody Requirements

Sampling methods and laboratory procedures are described in specific laboratory SOPs. These SOPs address any anticipated failures and the methods that will be employed to overcome these failures. Most of the methods are well-known sampling methods; therefore, sampling failures are not anticipated. Any additional project-specific considerations will be addressed and included in an updated SOP. Supporting measurements, such as temperature, relative humidity or atmospheric pressure, will be recorded in laboratory data logs, run sheets or notebooks.

B4: Analytical Methods Requirements

The analytical method requirement for the mold resistance in static chamber test is described in ASTM D6329-98 (2008)⁹. The requirements for biological testing are described in the appropriate ML SOPs.

B5: Quality Control Requirements

The static chambers are decontaminated and cleaned in accordance with ML SOP #017 (Standard Operating Procedure for the Decontamination of Humidity Chambers). All media and reagents are QC'd as outlined in ML SOP #001 (Standard Operating Procedure for Media Preparation – Dehydrated) and ML SOP #003 (Standard Operating Procedure for the Preparation of Sterile Buffer). All laboratory surfaces are disinfected using ML SOP #023 (Standard Operating Procedure for Regular Disinfection of Laboratory Surfaces). All laboratory equipment including the autoclave sterilizer is verified using ML SOP #019 (Standard Operating Procedure for Maintenance and Record Keeping of Laboratory Equipment).

B6: Instrument/Equipment Testing, Inspection, and Maintenance Requirements

RTI ML instrument maintenance is done in accord with the ML's SOPs.

B7: Instrument Calibration and Frequency

Calibration will be performed in accordance with the manufacturer's recommendations or annually. Recommended instrument calibration frequencies are provided in the respective SOPs and/or manufacturer's manuals. Pipettes will be calibrated gravimetrically following ML SOP #013 (SOP for Pipet Calibration).

B8: Inspection/Acceptance Requirements for Supplies and Consumables

Chemicals, supplies, and other consumables will be purchased from sources that have provided high-quality products to the laboratory in the past. Materials such as growth media will be purchased from a single source to help ensure uniformity throughout the duration of the project. All supplies will be inspected by the lab personnel. RTI's purchasing department will assist with the return of any equipment or materials that do not meet project requirements. Items will be NIST traceable when possible.

B9: Data Acquisition Requirements (Non-direct measurements)

No types of data are needed for project implementation or decision making that would be obtained from non-measurement sources such as computer databases, programs, literature files, or historical databases.

Manual methods of primary data acquisition (e.g., visual CFU counting) are described in ML's SOPs, while automated data acquisition equipment (e.g., balances and environmental controls) is checked using procedures recommended by the manufacturer. Procedures for screening and verifying manually entered data are used to reduce input errors to a minimum through double checking each other. Non-experimental data, such as an MSDS, will be included in the project notebook and a copy maintained in the RTI Project Manager's project file.

B10: Data Management

Guidelines for data management in the ML include the description, location, format, and organization of all types of records. The RTI Project Manager will oversee all data management activities. This section identifies the activities and processes planned for documenting the traceability of the data, calibrations, and information in the verification report. Corrections to manual entries are made on the same line (where possible), are initialed and dated.

B10.1: Data Recording

Data for this task will be collected either by computer or by manual (handwritten) entries. Observations and records (e.g., sample description and collection information) will be recorded manually in lab notebooks kept exclusively for this task.

B10.2: Data Analysis

Analysis will be performed as defined in ASTM D6329-98 (2008), Section 12.3.3.

B10.3: Data Storage and Retrieval

Laboratory notebooks containing manually recorded information and data output generated from instrumentation will be stored in the custody of the Project Leader for the duration of the project. Access to the notebooks is controlled; any changes made are initialed and dated, thus maintaining a good audit trail.

Spreadsheet files including raw and calculated data will be stored on computers. The files will be downloaded to a network server backed up nightly on magnetic tape. Access to the files are controlled, with file names indicating date and initials of person creating file, thus maintaining a good audit trail.

Following ETV policy, data files will be archived for 10 years following the end of the project and reports will be kept in perpetuity. The records will not be destroyed without written approval from EPA.

SECTION C: ASSESSMENT/OVERSIGHT

C1: Assessments and Response Actions

C1.1: Audits

RTI will be subject to both external and internal audits as specified in the ETV QMP¹, especially Table 9-1, ETV Assessments. A subset of that table is shown below, Table 3. Audits based on this test/QA plan include Technical System Audits (TSAs). RTI raw and summary data are subject to Audits of Data Quality (ADQs). An external TSA will be conducted by EPA or a designated representative. Other external audits may be performed. The auditor(s) will document their findings and note where corrective actions are necessary. The auditor(s) will distribute audit reports to those listed in Section A3 as well as to the supervisor whose laboratory was audited. RTI will provide the QA reports, including the ADQ report, to EPA with the verification report for review.

Table 3. RTI's ETV Assessments

Assessment Tool	Assessors	Subject of Assessment	Minimum Frequency	Reason for Assessment	Report Reviewed by
Technical Systems Audits	<u>Self</u> RTI QAM <u>Independent</u> EPA QAM	Test/QA plan	<u>Self</u> Once per technology evaluation <u>Independent</u> Once per year, as applicable	Assess technical quality of evaluations	EPA Project Officer EPA QAM RTI Project Manager
Audits of Data Quality	<u>Self</u> RTI QAM <u>Independent</u> EPA QAM	Raw data and summary data	<u>Self</u> At least 10% of the data in each technology evaluation <u>Independent</u> Each technology evaluation, as applicable	Assess data calculations and reporting	EPA Project Officer EPA QAM RTI Project Manager

C1.2: Corrective Actions

Technical personnel will have the direct responsibility for ensuring that whenever accuracy or bias is outside the limits of the DQOs for the critical measurements that corrective actions are taken. Corrective action will be taken If procedures are found to be faulty, corrective action will also be taken.

Corrective actions include:

- Problem identification;
- Attempting to find the cause;
- Attempting immediate steps to remedy the situation (if possible);
- Reporting or documenting the problem;
- Planning for corrective action (if major repairs are needed);

- Checking that the problem was corrected;
- Documenting the corrective actions taken; and
- Recommending changes to instruments, SOPs, etc. to avoid similar future occurrences.

The RTI QAM and Project Manager will be jointly responsible for proper documentation of corrective actions. Minor corrective actions are to be recorded in the laboratory notebooks. Major problems will be addressed as outlined above. All corrective actions will be noted in the verification report. Depending on the time and expense involved with necessary corrective actions, it will be necessary to consult the Program Manager or the sponsor before implementing any changes in the planned activities.

C2: Reports to Management

The RTI Project Manager will notify the EPA Project Officer when testing under this project is being conducted. The RTI Project Manager will submit verification reports, as well as data, to the RTI QAM. The RTI QAM will submit reports of all technical assessments to the RTI Project Manager. The RTI Project Manager will submit verification reports to the EPA Project Officer.

TSA and ADQ reports will be sent to the EPA Project Officer and QAM.

SECTION D: DATA VALIDATION AND USABILITY

D1: Data Review, Validation, and Verification Requirements

The verification is acceptable if all the measured parameters fall within the DQO limits described in Table 2. The test operator and analyst are responsible for checking that all measured parameters fall within prescribed limits before continuing testing.

D2: Validation and Verification Methods

Both the test operator and the test analyst will verify that the test data have been correctly entered and processed and that all manual calculations are correct. They will verify all newly developed or modified software, including spreadsheets for correctness before using the software to process project data.

Each verification report will be reviewed by the RTI QAM for compliance with the applicable method and for the quality of the data reported.

The RTI QAM will check for the following:

- Data completeness,
- Initial and continuing calibrations, and
- QC reference and internal standards.

D3: Reconciliation with Data Quality Objectives

Each ETV verification report will present the critical and relevant ancillary measurements.

The program manager will be responsible for reconciling data sets with the DQOs. She will work with the QAM for the mold resistance testing and with RTI technical staff for the moisture and emissions testing. This will be done during the ADQ and will be reported in the ADQ report.

Actual data quality will be compared with the DQO specified in Section A7; if the data quality meets or exceeds the objectives and verification specifications have been met, the test data will be considered acceptable. If exceptions are identified, the issues will be investigated for impact on the credibility of the data, the EPA QAM will be consulted, and the verification results disposed of on the basis of this careful consideration. If the impact is not significant, the data will be reported with indication of the exceptions.

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RTI Operating Procedures Referenced in the Test/QA Plan

- ML SOP #001 Standard Operating Procedure for Media Preparation – Dehydrated
- ML SOP #002 Standard Operating Procedure for the Preparation of Sterile Water
- ML SOP #003 Standard Operating Procedure for the Preparation of Sterile Buffer
- ML SOP #005 Standard Operating Procedure for the Characterization of Relative Humidity Chamber
- ML SOP #007 Standard Operating Procedure for the Gravimetric Determination of Moisture Content
- ML SOP #009 Standard Operating Procedure for the Quantitative Evaluation of Microorganisms
- ML SOP #012 Standard Operating Procedure for the Quantitation of Viable Spores in Suspension Preparation
- ML SOP #013 Standard Operating Procedure for Pipet Calibration
- ML SOP #017 Standard Operating Procedure for the Decontamination of Humidity Chambers
- ML SOP #019 Standard Operating Procedure for Maintenance and Record Keeping of Laboratory Equipment
- ML SOP #023 Standard Operating Procedure for Regular Disinfection of Laboratory Surfaces
- ML SOP #058 Standard Operating Procedure for Direct Inoculation of Materials with a Spore Suspension

Appendix A Moisture Testing

Impact of Moisture on Building Materials

It is well established that molds can colonize and amplify on a variety of building materials if sufficient nutrients and moisture are present. Commonly, sufficient nutrients are available and water is usually the growth factor most limiting the establishment and growth of microbial populations. Sufficient moisture for growth may become available through water incursion from leaks and spills, condensation on cold surfaces, or ab- or adsorption directly from the indoor air. The amount of water required is not large, and materials that appear dry to cursory inspection may be capable of supporting microorganism growth. Because moisture is so important, knowing the amount of available moisture in a material is critical to preventing and controlling microbial growth and amplification on materials.

Performance of Moisture Testing

Moisture testing will also be performed following the guidelines outlined in ASTM 6329. Two types of measurements are commonly used to evaluate building material moisture: most engineers think in terms of moisture content (MC) and many microbiologists utilize water activity (a_w). MC, defined as mass of water per unit mass of dry material, is measured gravimetrically (West and Hansen²⁶; Foarde²⁷ et al.). It is a bulk measurement of the water in a sample of the material. The a_w , primarily used to relate the water content of foods to the ability of microorganisms to grow on them, is defined as the equilibrium RH (ERH) above a sample of a material, divided by 100 (Pitt²⁸).

In the test described in this document, the ERH is the RH in a closed chamber containing a material sample after the material and the air in the chamber have reached water equilibrium. Therefore, the a_w of the material that has been equilibrated in a closed chamber having an RH of 94% is 0.94. Corry²⁹ stated that a_w is the proportion of "available water for biological reactions." It is a useful laboratory measurement when RH conditions are known to be at equilibrium.

For porous materials, MC and ERH (or a_w) are related through the material's water adsorption isotherm, and different relationships are obtained for different materials. In this test, the MC for each test material will be determined at four ERHs, 100%, 94%, 85% and 62%. There is no need to test lower ERHs as microbial growth cannot occur below ~ 60% ERH.

For microbial growth, the a_w or ERH is the important number as it indicates how much water is available for growth. The MC will be determined for each test material, but will be reported only as a reference value. This reference value will provide two pieces of information. First, the actual MC at the time of the test. This may be useful if other sample are tested at a later date for comparison purposes. Additionally, while the microbial resistance testing will be performed with the test material at ERH, in buildings, the RH of a space and the materials in the space are not at equilibrium. Therefore, another measure such as MC would be important.

Test Design for Moisture Testing

In addition to ASTM D6329, ML SOP #007 (Standard Operating Procedure for the Gravimetric Determination of Moisture Content) will be followed. The details are described below.

Replicate small (at least 4 cm x 4 cm) pieces of the test material will be used. Pieces will be

placed on sterile dishes on the shelves in the static chamber. Equilibration time will depend upon both the material to be tested and the chamber relative humidity selected for the test and will be determined for each material prior to testing. Equilibration will be defined as when the bulk moisture content of the material reaches a constant value. A calibrated analytical balance will be used.

At least 5 chambers will be used. One each will be set at 100%, 94%, 85% and 62% ERH. The fifth chamber will contain a desiccant for a near 0% RH baseline comparison.

Data Analysis for Moisture Testing

Analysis will be performed as defined in ASTM D6329, Section 8.3.2.

Appendix B. Emissions Testing

Impact of Emissions of VOCs and Aldehydes from Building Materials

Volatile organic compounds (VOCs) and aldehydes are of a concern for indoor air quality because of adverse health effects. Many items in buildings may emit VOCs and aldehydes, including paints and lacquers, paint strippers, cleaning supplies, pesticides, building materials and furnishings, office equipment such as copiers and printers, correction fluids and carbonless copy paper, graphics and craft materials including glues and adhesives, permanent markers, and photographic solutions. Manufacturers of these items have been working since the early 1990s (or earlier) to reduce emissions from their products. EPA has sponsored research and testing on low emission products. Under an earlier project, RTI developed a test method for measuring VOC and aldehyde emissions from commercial furniture³⁰.

This test plan includes measurement of emissions of aldehydes and VOCs from building materials under conditions designed to simulate product use. Formaldehyde and total volatile organic compounds (TVOC) can be measured in addition to a range of other individual aldehydes and individual VOCs. Emissions levels are determined by placing a piece of the building material into a small environmental test chamber under specified test conditions, then measuring chamber air concentrations of aldehydes and VOCs at selected time intervals. Product-specific emission factors are calculated from the chamber air measurements. The method provides a standard test that reproducibly and accurately measures emissions from building material under controlled laboratory conditions.

Chemical testing of VOCs and aldehydes will be performed by RTI.

One of the provisions of the ETV program is the use of existing data. VOC and aldehyde testing of building materials is fairly common and there are a number of programs which perform VOC testing. If a test material has been previously tested by an organization using the ASTM method and the test results are publically available on the internet, RTI will evaluate the appropriateness of using the existing data rather than retesting. If after discussion with the EPA project officer, he is in agreement, the existing data will be used.

Performance of Emissions Testing

The emissions testing and analysis will be performed following the guidance of ASTM D5116-06³¹, "Standard Guide for Small Scale Environmental Chamber Determinations of Organic Emissions from Indoor Materials/Products"; ASTM, D6670-01³². A sample is placed into an environmental chamber. Measurements are taken over time for VOCs and aldehydes. Gas chromatography/mass spectrometry (GC/MS) is used to determine the quantity of VOCs and reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is used for aldehydes.

Test Design for Emissions Testing

The testing will be performed following the guidance of ASTM D5116-06³¹, Emissions levels are determined by placing the test objects into an environmental test chamber under specified test conditions then measuring chamber air concentrations of aldehydes and VOCs at selected time intervals. Product-specific emission factors are calculated from the chamber air measurements.

Aldehydes in chamber air samples are collected on silica gel cartridges coated with 2,4-dinitrophenylhydrazine (DNPH). The DNPH-aldehyde derivatives on the cartridges are eluted with acetonitrile, and the eluate is analyzed using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

VOCs in chamber air samples are collected on sorbent cartridges (tubes). VOCs trapped on the cartridges are thermally desorbed then analyzed by gas chromatography/mass spectrometry (GC/MS). Results of these analyses will be reported as mg/m^3 (or $\mu\text{g}/\text{m}^3$) and may be used to estimate concentrations in chamber air samples.

Sampling will be performed following the guidance of ASTM D5116-06³¹. Each sample will be accompanied by a chain of custody form that documents all sample handling, transfer, and storage prior to emissions testing.

Analysis for Emissions Testing

Chemical analysis for VOCs will be performed using GC/MS. The collected sorbent tubes will be subjected to thermal desorption using a Perkin Elmer ATD Turbomatrix system interfaced to an Agilent 6890 Plus GC and an Agilent 5973N MS.

Total VOCs (TVOC) will be calculated as the sum of those VOCs that elute between the retention times of n-hexane and n-hexadecane on a non-polar or equivalent capillary GC column and quantified based on the response factor for toluene. For individual VOCs, the analytical methods are based on ASTM D6196-03³³, "Standard Practice for Selection of Sorbents, Sampling, and Thermal Desorption Analysis Procedures for Volatile Organic Compounds in Air", EPA methods T017³⁴, "Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling onto Sorbent Tubes" and TO-1³⁴, "Determination of Volatile Organic Compounds in Ambient Air Using Tenax Adsorption and Gas Chromatography/Mass Spectrometry".

The analytical methods for formaldehyde, and other low molecular weight aldehydes are based on ASTM D5197-03³⁵, Standard Test Method for Determination of Formaldehyde and Other Carbonyl Compounds in Air (Active Sampler Methodology)".

Data analysis will be performed as defined in ASTM D5116-06³¹.

Quality Assurance and Control for Emissions Testing

QC requirements for emissions testing will be consistent with ASTM D5116-06³², and include the following:

1. Air change: 1.0ACH +/- 5.0%
2. Mixing: +/- 5.0%
3. Air Tightness: <0.03 ACH
4. Recovery*: 100% +/- 20%

*recovery is based on average recoveries from Toluene, Decane, and Formaldehyde.

All RTI staff are trained and knowledgeable for the work they perform; training files are maintained for each staff member. Calibrations for chemical analysis instruments are obtained using multi-point calibration curves prepared using pure compounds; a minimum of four points

shall be used. For those VOCs determined by GC/MS, target analytes are introduced onto sorbent tubes as gas or liquid standards and then analyzed using methods identical to those used for analysis of chamber samples. A calibration check standard is prepared and analyzed daily, at a minimum, to verify that calibration for each analyte is in control. Corrective action will be taken as needed to ensure quantitation accuracies.

Carbonyls analyzed by HPLC will be quantified based on a multipoint calibration curve prepared using dinotrophenyl hydrazone derivatives of the pure target analytes. Standards and unknown are analyzed using the same analytical conditions. A QC check standard will be analyzed every 10 injections to ensure accuracy. Re-calibration will be performed as needed.

All VOC data will be reviewed for accuracy (correctness) and reasonableness.

The emissions test results will be reported as mg/m^3 (or $\mu\text{g}/\text{m}^3$) TVOC and VOCs by species, unless all parties (EPA, RTI and vendor) have agreed that only TVOC shall be reported.