

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION  
PROGRAM



ETV Verification Statement

<b>TECHNOLOGY TYPE:</b>	<b>RAPID FUNGI DETECTION</b>	
<b>APPLICATION:</b>	<b>ANALYSIS OF FUNGI IN AIR</b>	
<b>TECHNOLOGY NAME:</b>	<b>Mycometer<sup>®</sup>-test</b>	
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The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at [www.epa.gov/etv](http://www.epa.gov/etv).

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permittees), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field and laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six verification centers under ETV, is operated by Battelle in cooperation with EPA's National Risk Management Research Laboratory. The AMS Center evaluated the performance of a rapid fungi detection technology. This verification statement provides a summary of the test results for Mycometer<sup>®</sup>-test developed by Mycometer A/S and distributed in the United States by Mycometer, Inc.

## **VERIFICATION TEST DESCRIPTION**

Rapid technologies (results available same day of testing) to detect fungi from matrices such as surfaces, bulk material, air, or water are of interest to improve the efficiency of delineating and documenting fungal contamination in buildings and water systems, and for monitoring progress during cleanup and remediation processes. Traditional methods of analysis can take up to seven days for results. Technologies providing same day or near “real-time” results indicating changes in air quality would help to control diseases associated with fungal outbreaks, expedite remediation efforts, and protect public health.

The verification test of the Mycometer<sup>®</sup>-test technology was conducted from May 19 through June 9, 2011 at Battelle in Columbus, Ohio. Technology operation, sample handling, and analyses were performed according to the vendor’s instructions.

For this verification, the Mycometer<sup>®</sup>-test technology was verified for repeatability and inter-assay reproducibility by detecting fungi in air samples. Linearity was assessed using dilutions of stock cultures in dechlorinated tap water. The linearity test for fungi was a modification of test procedures in place for air and surface samples. In addition, sustainable operational factors such as ease of use, required reagents, analysis time, laboratory space, and utilities required were reported.

QA oversight of verification testing was provided by Battelle and EPA. Battelle and EPA QA staff conducted technical systems audits of the testing and Battelle QA staff conducted a data quality audit of at least 10% of the test data. This verification statement, the full report on which it is based, and the quality assurance project plan for this verification test are available at [www.epa.gov/etv/centers/center1.html](http://www.epa.gov/etv/centers/center1.html).

## **TECHNOLOGY DESCRIPTION**

The Mycometer<sup>®</sup>-test rapid fungi detection technology is based on fluorogenic detection of enzyme activities found predominantly in a taxonomic group of organisms. A sample (e.g., filter or swab) is contacted with a test solution containing a synthetic enzyme substrate. The enzyme present in the fungal cells hydrolyzes the synthetic enzyme substrate. When the synthetic substrate molecule is cleaved into two molecules by the enzyme, one of the molecules can be made to fluoresce upon excitation with ultraviolet (UV) light (365 nanometers). The amount of fluorescence is measured using a handheld fluorometer after processing for a reaction time based on the ambient temperature. This fluorescence semi-quantitatively correlates to a measure of the fungal biomass. Fluorescence measurements can be captured electronically and may be downloaded to a computer or can be transcribed by hand. The sample preparation and analyses can be performed on site in less than one hour.

According to the vendor, the Mycometer<sup>®</sup>-test for fungi is designed to measure both viable and non-viable spores, hyphae and fungal particles such as hyphal fragments in air, on surfaces, or in bulk materials to give a representation of the contamination in the environment. Although the Mycometer<sup>®</sup>-test cannot distinguish between fungal genera or viable/non-viable fungi, it provides a semi-quantitative measure of the total fungal biomass present. Air samples can be collected with traditional air sampling pumps onto filter media. Typically 300 liters of air are collected by sampling 20 liters per minute (LPM) for 15 minutes, or 15 LPM for 20 minutes. Surface samples are collected by swabbing a nine square centimeter area and bulk material samples are weighed. Enzyme substrate is added to the filter, swab, or bulk material and the fungal enzyme reacts with the substrate to release a fluorescent product. The amount of fungi in the sample is estimated by measuring the fluorescence produced. For the Mycometer<sup>®</sup>-test, the type and material of the air sampling filter cartridge are critical for both sampling and the enzyme reaction (which take place directly on the filter). It is important, therefore, to use the filters provided by the vendor. The vendor provides a proficiency certification training program that is included with the fluorometer kit (on a flash drive) and is mandatory for use of their technology to document understanding and proper training.

## VERIFICATION RESULTS

Table 1 summarizes the linearity results for Mycometer<sup>®</sup>-test using two fungal cultures in water, *Aspergillus flavus* ATCC 58870 and *Cladosporium herbarum* ATCC 58927.

**Table 1. Linearity Results for Mycometer<sup>®</sup>-test Adjusted Fluorescence vs. Total Spores Tested**

Test Organism	Total Spores Tested	Range of Average Adjusted Fluorescence Units (fu)	Slope	Y-intercept	Coefficient of Determination (R <sup>2</sup> )
<i>A. flavus</i> ATCC 58870	3.1 x 10 <sup>5</sup> to 6.2 x 10 <sup>6</sup>	218 to 4322	0.0007	20.637	0.9979
<i>C. herbarum</i> ATCC 58927	4.8 x 10 <sup>5</sup> to 9.6 x 10 <sup>6</sup>	125 to 3389	0.0004	-135.25	0.9976

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading

Table 2 summarizes the repeatability results for Mycometer<sup>®</sup>-test using eight replicates of one fungal culture in air, all analyzed by one person.

**Table 2. Mycometer<sup>®</sup>-test Repeatability: Air Containing *A. Flavus***

Test Iteration	Adjusted Fluorescence Units (fu)
	<i>A. flavus</i> (6.2 x 10 <sup>3</sup> spores/L) n=8
Average	334
Standard Deviation	27
RSD (%)	8.0

Table 3 summarizes the inter-assay reproducibility results for Mycometer<sup>®</sup>-test using eight replicates of one fungal culture in air split into four samples each for analysis by two people with two different fluorometers.

**Table 3. Mycometer<sup>®</sup>-test Inter-Assay Reproducibility: Air Containing *A. Flavus***

Test Iteration	Adjusted Fluorescence Units (fu)	
	<i>A. flavus</i> (6.2 x 10 <sup>3</sup> spores/L) n=4	
	Analyst 1	Analyst 2
Average	291	307
Standard Deviation	25	15
RSD (%)	8.7	4.7
RPD (%)	5.3	

**Operational Factors.** The verification staff found that the Mycometer<sup>®</sup>-test was easy to use. A Mycometer A/S representative came to Battelle to train the verification staff in the use of the Mycometer<sup>®</sup>-test reagents and operation of the fluorometer. This training lasted one day and staff felt it was more than sufficient to be comfortable using the reagent kits and fluorometer without assistance. This on-site training focused on the technology operating protocols for air and water matrices. While the operational aspects of this training were similar to the proficiency certification program, the proficiency certification program also focuses on understanding the principles behind the technology as well as additional applications.

The fluorometer is provided in a hard-cover carrying case. The carrying case has dimensions of 45 cm wide × 15 cm deep × 32 cm high (17.5 in wide × 6 in deep × 12.5 in high) and weighs approximately 7.2 kilograms (16 pounds). Included with the fluorometer is a black calibration cuvette, a 100 µL automatic pipette, a timer, two test racks, a calculator, a thermometer, and training materials. The fluorometer operates on four AAA batteries and has push-button operation. Testing staff found that the display was easy to read and surfaces were easy to wipe clean. The fluorometer required a calibration check once daily with the black cuvette provided with the fluorometer and a calibration standard provided in the reagent kit. Both an instruction manual and a quick reference card were provided for the Mycometer<sup>®</sup>-test. Verification staff found that the instructions provided were not always consistent between the manual and the quick reference. For example, the manual indicated that the blank sample for air testing was to be a blank filter processed alongside the test filters, while the quick reference guide indicated that the blank was to be an aliquot of the substrate combined with the developer.

The Mycometer<sup>®</sup>-test reagents are sold in lots of 10 for air assays and lots of 20 for surface assays. Each reagent kit included the sampling media (filter for air samples), enzyme substrate, developer, and calibration standard, all of which were clearly labeled for identification and storage conditions. Syringes and cuvettes used for processing were also included. All containers and packaging were easy to open; however, verification staff found there was packaging waste involved with the different components, particularly if multiple kits were needed to analyze the required number of samples. All reagents were ready for use with the exception of the enzyme substrate which required re-hydration. Each sample resulted in approximately 5 mL of liquid waste from the substrate and developer used to process the sample. Based on the expiration date stamped on the kits, the shelf life of the kits received for testing was over one year from receipt date. Several kit components required refrigeration. Once rehydrated, the enzyme substrate could be stored in a refrigerator for up to one week or at -18 °C for up to 6 months. All components needed to prepare and analyze a sample were included either in the reagent kit or the fluorometer kit. No other laboratory equipment was needed for processing air samples. For air sample collection, however, a sampling pump must be obtained. The recommended air sampling pumps (Gast 3-30 LPM IAQ Pump w/Tubing & Rotameter) are commercially available. Pricing for the fluorometer and reagent kits can be obtained from the provided vendor contacts. Verification testing staff found they were able to collect and analyze eight air samples in one hour, given the availability of enough air sampling pumps to generate eight air samples simultaneously.

For data reduction, a laptop or personal computer is needed. Mycometer provides an Excel spreadsheet for quantification of mold/fungi in air that converts fluorescence unit values into a 'Mycometer-Air' value and provides suggested interpretation guidelines based on the resulting value obtained. The Mycometer-Air value calculation converts the fluorescence reading to fluorescence units (fu) per volume of air measured in cubic meters. This can be used to standardize the results for consistent comparison and interpretation if there are slight variations in the air volume sampled. Because all sample volumes used in verification testing were the same, conversion of results to a Mycometer-Air value were not needed for verification testing. In addition, the interpretation guidelines associated with the Mycometer-Air values were not verified as part of this test.

Signed by Tracy Stenner

01/06/2012

Signed by Cynthia Sonich-Mullin 02/01/2012

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Date

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