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TECHBRIEF
Environmental Technology Verification Program
ETV

Rapid Fungi and Bacteria Detection Technologies

Air and water pollution from microbes such as fungi and bacteria can cause significant health concerns. Certain species of fungi can produce allergens, irritants, and potentially toxic substances resulting in asthma, respiratory infections, and a variety of allergic reactions in humans. Some bacterial species contaminate water supplies causing gastrointestinal illnesses, liver damage or other negative health effects. Rapid and cost-effective detection technologies are needed to identify these microorganisms to document, control and prevent microbial outbreaks. In 2011, the U.S. EPA Environmental Technology Verification (ETV) Program's Advanced Monitoring Systems (AMS) Center, operated by Battelle under a cooperative agreement with EPA, evaluated the performance of two Mycometer, Inc. detection technologies, one for fungi in air samples and one for bacteria in water samples. The technologies provide semi-quantitative results of bacterial or fungal biomass. Technology descriptions are provided in Table 1.

Technology Description and Verification Testing

Bacteria Testing:

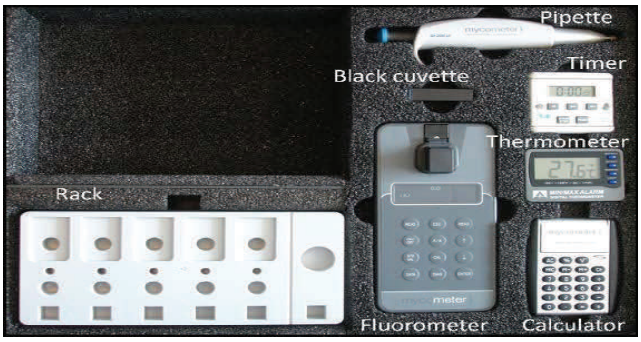
Battelle used indigenous lake water bacteria and a reference strain of *Pseudomonas aeruginosa* from the American Type Culture Collection (ATCC 27853) to compare the technology against the reference method of heterotrophic plate counts (HPC) in triplicate according to Standard Method (SM) 9215. For each type of bacteria, four different concentrations were sub-sampled five times. For each sub-sample, 250 mL of bacterial solution was filtered and processed using the technology kit. Linearity testing determined test kit accuracy by plotting the fluorescence readings for each sample against the HPC bacterial concentration. Repeatability and inter-assay reproducibility testing were also conducted using both indigenous lake water bacteria and the reference strain of *P. aeruginosa*. For both tests, two analysts each processed four water samples containing the bacteria using the technology kit and separate fluorometers.

Fungi Testing:

For linearity testing, Battelle used two different fungal cultures: *Cladosporium herbarum* ATCC 58927 and *Aspergillus flavus* ATCC 58870. Fungal stocks were diluted with dechlorinated tap water to form five replicates of four different concentrations of testing solution. Linearity was determined by plotting the fluorescence readings for each sample against the actual fungal concentration, measured by a hemocytometer following procedures in American Society for Testing and Materials (ASTM) D4300-01. Repeatability and inter-assay reproducibility were evaluated by producing controlled air samples containing one fungal stock, *A. flavus*, and sampling and analyzing the air with the technology kit. For repeatability, one analyst processed eight simultaneously-collected air samples using a fluorometer. For inter-assay reproducibility, two analysts processed eight simultaneously-collected samples (four each). Each analyst used a separate fluorometer.

Environmental and Regulatory Background of Bacteria and Fungi at a Glance

EPA has several current regulatory frameworks that aim to monitor and control bacterial and fungal outbreaks. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) is EPA's regulatory framework that allows EPA to monitor the sale and use of fungicides and pesticides. The Safe Drinking Water Act (SDWA) sets national health standards for drinking water to protect against naturally-occurring and man-made contaminants, Long Term 2 Enhanced Surface Water Treatment Rule (LT2) aims to reduce illnesses related to C*ryptosporidium* and other microbial contaminants and the Total Coliform Rule (TCR) sets maximum contaminant limits (MCLs) for total coliforms and *E. coli* in drinking water supplies.



Fluorometer kit components of a verified detection technology.

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[1]The ETV Program operates largely as a public-private partnership through competitive cooperative agreements with non-profit research institutes. The program provides objective quality-assured data on the performance of commercial-ready technologies. Verification does not imply product approval or effectiveness. EPA does not endorse the purchase or sale of any products and services mentioned in this document.

Table 1. Description of Bacteria and Fungi Detection Technologies		
Vendor and Technology	Type of Microorganism	Technology Descriptions (Provided by Vendors)
Mycometer, Inc. *Bactiquant®-test*	Bacteria	The Mycometer, Inc. *Bactiquant®-test* is a semi-quantitative test kit designed to provide a rapid method to estimate total bacterial biomass, based on the enzyme activity of viable and some non-viable bacteria, in water. Once samples are passed through a membrane filter to concentrate the bacteria, an enzyme substrate is then added to the filter. The bacterial enzyme hydrolyzes the enzyme substrate, cleaving the substrate into two molecules. One of these molecules absorbs UV light at 365 nm and re-emits visible light that can be measured by a fluorometer. The amount of fluorescence correlates to bacterial concentration. This technology can also be applied to surface and air samples.
Mycometer, Inc. *Mycometer®-test*	Fungi	The Mycometer, Inc. *Mycometer®-test* is designed to provide a rapid method to estimate total fungal biomass in air, on surfaces, or in bulk materials. The technology provides semi-quantitative results of viable and non-viable spores, hyphae, hyphael fragments, and microfragments. The *Mycometer®-test* operates under the same principles as the *Bactiquant®-test.* The technology measures the fluorescent product released from the enzyme substrate complex. The amount of fluorescence correlates to the amount of fungi present in the sample.

Selected performance results are provided in Table 2. Battelle also evaluated both fungi and bacterial detection technologies for data completeness and operational and sustainability factors. The analysis was completed at Battelle's headquarters in Columbus, Ohio. Additional information is available in the verification reports and statements on ETV's website at http://www.epa.gov/nrmrl/std/etv/vt-ams.html#refdtfb_air or http://www.epa.gov/nrmrl/std/etv/vt-ams.html#refdtfb_water.

Potential Outcomes of Verified Bacteria and Fungi Detection Technologies

Current bacterial and fungal detection techniques, such as HPC, direct microbial counts using epifluorescence, or real-time polymerase chain reaction, all detect bacteria or fungi but have long analysis times or are expensive. The verified detection technologies estimate fungal or bacterial biomass concentrations in a cost-effective manner, achieving on-site results in less than one hour. The ability of these technologies to quickly and affordably screen and monitor water and air quality can help to prevent and control microbial outbreaks, expedite remediation efforts, and protect public health.

Table 2. Selected Verification Results for Rapid Fungi and Bacteria Detection Technologies							
Microorganism	Test Organism	Linearity Testing			Interassay Reproducibility Testing		
		Concentration Range Tested (spores/mL or CFU/mL)	Range of Adjusted Fluorescence Units (fu)	Coefficient of Determination (R^2)	Concentration Tested	Relative Standard Deviation (RSD)[1,4]	Relative Percent Difference (RPD)[2]
Fungi	*A. flavus*	$3.1x10^5$ to $6.2x10^6$	$2.2x10^2$ to $4.3x10^3$	0.9979	$6.2x10^3$ (spore/L of air)	6.7%	5.3%
	C. herbarum[3]	$4.8x10^5$ to $9.6x10^6$	$1.3x10^2$ to $3.4x10^3$	0.9976	N/A	N/A	N/A
Bacteria	Lake water indigenous bacteria	$3.7x10^2$ to $6.0x10^3$	$2.4x10^3$ to $2.4x10^4$	0.9138	$3.7x10^2$ CFU/mL	4.5%	6.0%
	Lake water indigenous bacteria[3]	$3.7x10^2$ to $3.0x10^3$	$2.4x10^3$ to $1.7x10^4$	0.9689	N/A	N/A	N/A
	P. aeruginosa	$8.2x10^2$ to $8.0x10^3$	$1.3x10^3$ to $1.2x10^4$	0.9923	$4.7x10^3$ CFU/mL	3.1%	2.9%

1. RSD was not performed for *C. herbarum*
2. RPD was not performed for *C. herbarum*
3. Result from most concentrated test solution is not included
4. Result is the average RSD determined by two analysts using different fluorometers where n=4 for each analyst
5. Parameters are not available due to design of the experiment

References:

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