Estimation of Viable Spores in Powder , Air and Water Samples in 5 Minutes

Rolf A. Deininger
JiYoung Lee
Department of Environmental Health Sciences
The University of Michigan
Ann Arbor, MI 48109

Introduction

Shortly after the anthrax scare in 2001, government laboratories received tens of thousands of powder samples. Until shown to be harmless, these samples need processing and are treated as if they are biologically active. It is estimated that 99.99+ % of the samples are harmless. If a rapid detection method could rule out the vast majority of the samples onsite, the workload would be significantly reduced and decontamination procedures would be lessened.

The purpose of the method described below is to allow a first responder to test the biological activity of a powder onsite in less than 5 minutes.

Materials and Methods

A group of whitish powders which have been used in hoaxes is shown in Figure 1. They include flour, baking soda, baby powder, foot powder, dry milk powder, and powdered sugar. Dipel 150 Dust and Milky Spore, however, contain spores and thus are biologically active. Dipel contains *Bacillus thuringiensis* (0.064% B/W) and Milky Spore contains *Bacillus popillae(0.02% B/W)*. These spore-containing powders were used in the experiments because they are not pathogenic to humans and can be used as surrogates for *B. anthracis*.

Figure 1. Various powders tested in this study

The test procedure is to induce the spores in the powder to germinate, lyse the vegetative cells and then determine their ATP using New Horizons Diagnostics PROFILE luminometer (Figure 2). The necessary equipment for the analysis fits onto a clipboard and is very portable.

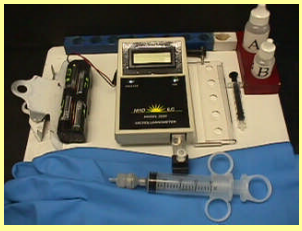

Figure 2. New Horizons PROFILE luminometer and necessary equipment.

Powder Samples

Insert a filtravette into the drawer of the luminometer. Add 100 µl of warm solution A to the filtravette. Solution A is a mixture of amino acids that have been heated to a specific temperature for optimal germination. Add a visible amount of powder (~1mg) to the solution in the filtravette. Close the drawer and wait for 4 minutes. After 4 minutes open the drawer. Add 100 µl of solution B to the filtravette. Solution B is a mixture of a bacterial cell releasing agent and luciferin/luciferase. Record the light development after the 15 seconds integration time. Redo the reading three more times. Sum up the four readings. The magnitude of each reading should decrease (R1>R2>R3>R4). Estimate the number of viable spores from Table 1.

Table 1. RLUs vs. spore count

RLU	spore count
500	380
1000	790
5000	4010
10000	8030
100000	80500
1000000	805000

Water Samples

Draw up 10 to 20 ml of water into a syringe. Attach the filter holder with a filtravette in it to the syringe. Press the sample through the filter. Remove the filtravette and press out the remaining water. Add solution A and B as described before. If there are 10,000 spores in a glass of water (200ml), a sample of 20 ml will deposit 1000 spores on the filter. They can be detected by the method above

Air Samples

In Figure 3 a novel air sample is shown. It has a rotating cup with 1 ml of PBS in it. Air particles are directly deposited into the liquid. Assuming an air concentration of 20 spores per liter, sampling at 5 liters/min will capture in 10 minutes about 1000 spores that are detectable by the method above.

Figure 3. Novel Personal air sampler CIP-10M

Results

The germination of the spores depends on many factors. The major ones include temperature, the mixture of amino acids and time. At higher temperatures and higher nutrient concentrations, the germination happens faster. In Figure 4, the germination development is shown. Generally speaking, under optimal conditions most of the germination happens in the first 5 minutes. Longer incubation times will lead to more vegetative bacilli, but it is not necessary to wait for a complete germination.

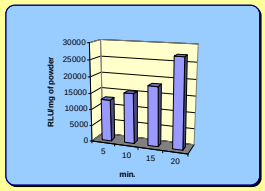

Figure 4. Germination of spores vs. time

Similarly, the measurement of the light development happens very rapidly. The luminometer in its present form has a fixed light integration time of 15 seconds. By opening and closing the drawer 3 more times, an additional light development can be measured. If one plots the total light development over a period of time it is clear that most of the light development happens in the first 15 seconds and that later time intervals contribute less. This is shown in Figure 5 where the cumulative light units are plotted over total time.

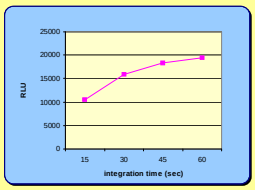

Figure 5. Cumulative light development in the luminometer (each point is the average of 12 tests)

The sensitivity of the method can be increased by both lengthening both the incubation time and the integration time for light measurement. However, speed in the analysis is critical, and the chosen times are a good compromise between analysis time and sensitivity. Based on a comparison of the light development and the count of colonies on an agar plate, it appears that the method has a detection limit of about 300 spores.

Conclusions

This method has been tested in the laboratory and in the field wearing protective gloves. The number of tests has been small and needs to be extended. The procedure needs to be optimized with regard to sample size, nutrient concentrations, germination times and light integration times.

rad@umich.edu, jylee@umich.edu