LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

UCRL-TR-233904

LLNL Summer 2007 Internship Experience

Alika Ah New

August 22, 2007

This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

LLNL Summer 2007 Internship Experience

Alika Ah New

Project:

Since the 2001 anthrax attacks involving the US postal service, there have been increased efforts to study more advanced methods of decontamination and detection of viable *Bacillus anthracis* before and after decontamination efforts. Current methods for sample processing and viability analysis are low throughput (~30-40 per day) requiring several manual steps, with confirmed results obtained days later. The group I am working with has developed more rapid, high throughput methods using automation to process surface samples combined with a time-course real-time Polymerase Chain Reaction (PCR) approach to determine the presence of viable *B. anthracis* spores. This process is referred to as Rapid Viability (RV)-PCR. These methods based on an observable change in PCR response during culturing showed detection of low numbers of bacterial pathogens in hours compared to days required for conventional culture analysis.

In this project, we are studying detection limits, growth inhibition and PCR inhibition of a modified real-time PCR-based automated method of detecting *B. anthracis* Sterne (non-infectious variant) in various environmental samples containing levels of background debris expected during sampling. In order to decrease the detection limit, additional clean-up steps are employed. Since *B. anthracis* spores are very resilient to solvents, ethanol treatment can also be used to kill other bacteria (vegetative cells) in the sample. Finally, dilution of the sample may be useful to dilute out contaminants. Using commercially available robotics (Figure 1), each of these treatment steps can be automated, allowing processing of 100-200 swabs per day, with

quantitative results obtained within 24 hours. Automation also reduces the risk of pathogens since no manual liquid handling steps and no plating or centrifugation is required. Traditional viability analysis uses manual steps for sample processing including performing dilutions, plating onto solid media, counting colonies and confirming the presence of *B. anthracis* using biochemical tests. The RV-PCR approach uses specific detection via real-time PCR so that additional verification of the pathogen is unnecessary. The RV-PCR method is based on a significant shift in real-time PCR response curve over time (ΔCt), but also is dependent on Ct_0 and Ct_{final} (Figure 2). Criteria were developed to accurately distinguish live cells from dead spores by testing with thousands of samples containing low levels (1-10) of live spores in background of 106 dead spores and/or background debris and high populations of non-target bacteria. Finally, a Most Probable Number (MPN) method was combined with the RV-PCR approach to yield a quantitative method to estimate the number of spores in the sample. In this study, the automated MPN RV-PCR method has been optimized to accommodate high amounts of debris from real-world samples.

Figure 1: Multiprobe II on the left and V-Prep on right used for sample preparation

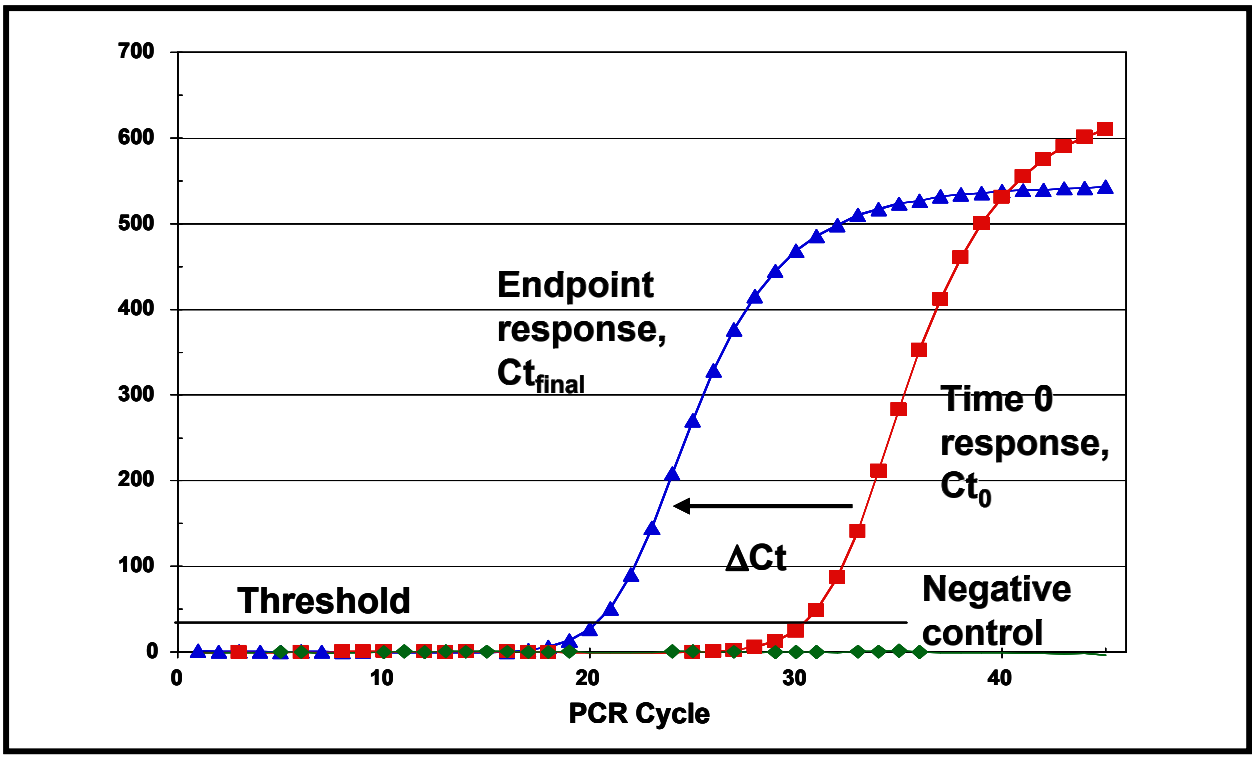

Figure 2: PCR response curves showing shift in response (ΔCt)

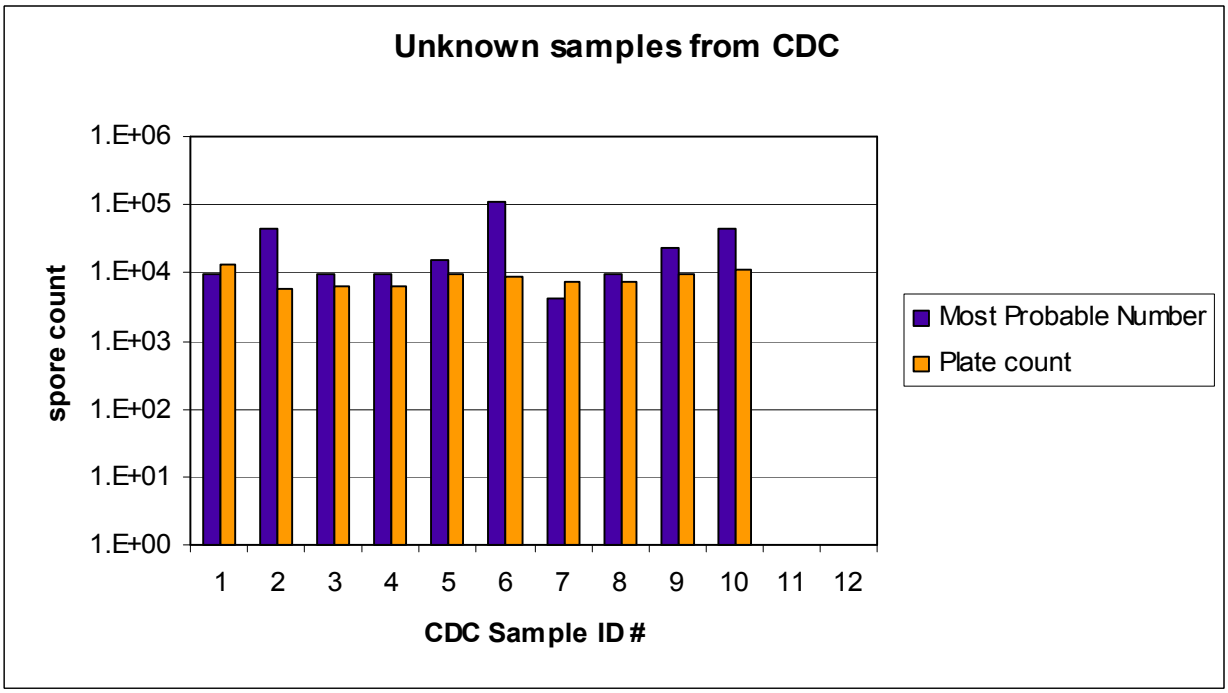

Figure 3: Comparison of methods in analysis of CDC blind samples

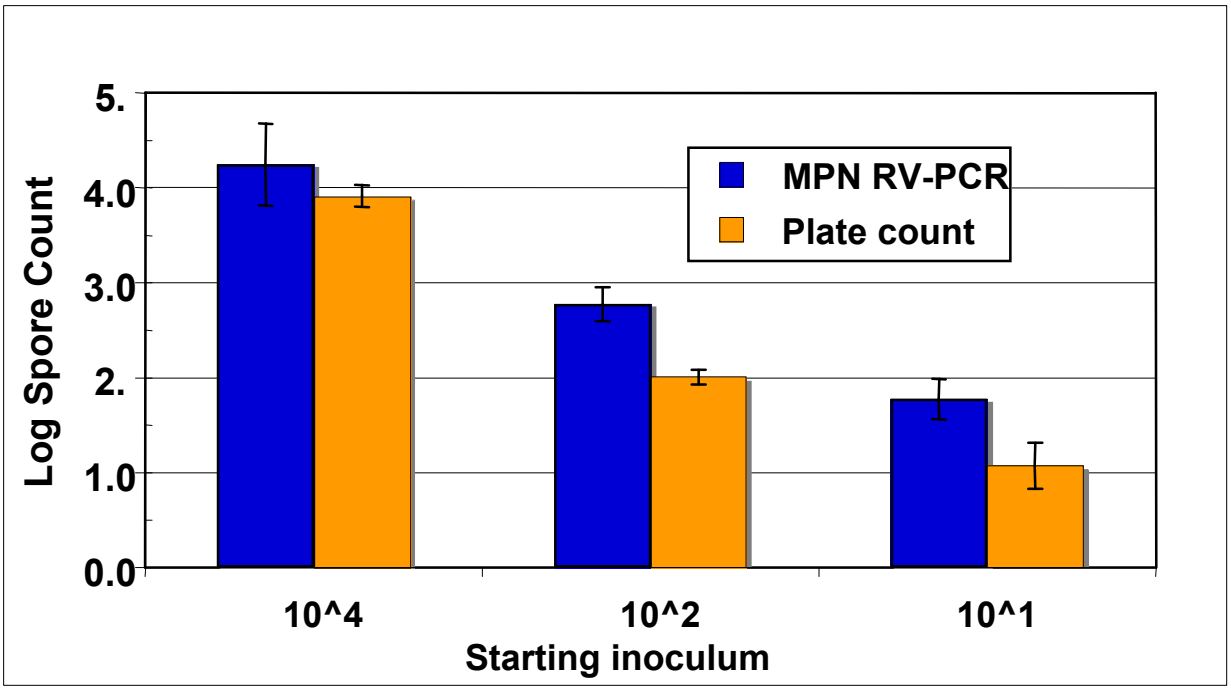

Figure 4: Comparison of methods in swabs with dirt

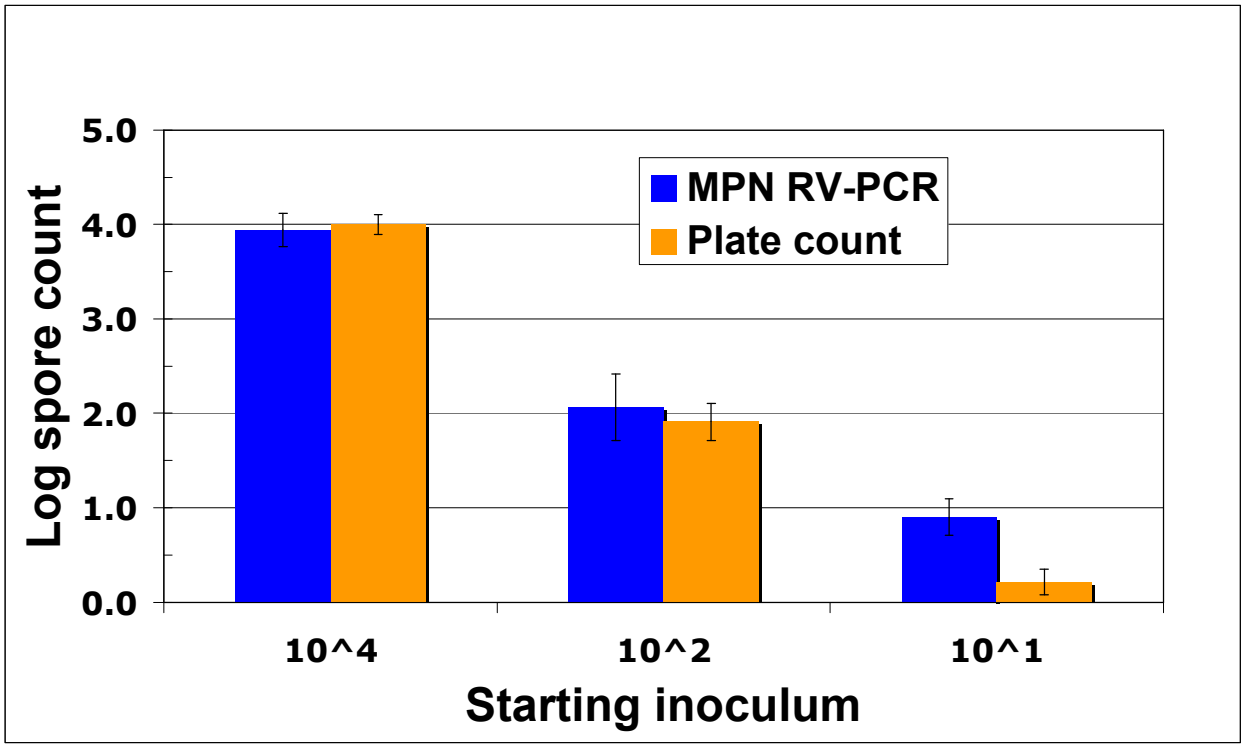

Figure 5: Comparison of methods in swabs without dirt

Unknown samples from the CDC (Figure 3) along with swabs with dirt (Figure 4) and swabs without dirt (Figure 5) were processed using both the MPN RV-PCR method and the traditional culturing analysis. The results were compared for recovery efficiency, detection limits, and potential growth and/or PCR inhibition. A comparison of spore counts from Rapid Viability PCR (calculated as Most Probable Number) and conventional plate counts shows statistically similar results (based on t-test analysis). We can also see that there were more differences between methods for swabs containing dirt and for low inoculum densities. Based on analysis of colony counts at the endpoint, there was little indication of growth inhibition for swabs with dirt. PCR inhibition was evident, but could be overcome with a 1:10 dilution. Also, it is important to note that the negative samples were confirmed in the unknown CDC samples study, which shows that there is no cross-contamination in the process.

In testing soil samples, we noticed that the slowest step in the automated process was the washing steps. In this step, the soil samples are arrayed into a 96-well filter block, and 4 mL of liquid wash is vacuum filtered through the samples. The high amounts of soil in the samples are deposited on the filter, slowing this process down. For samples containing 1 gram of soil, this step can take over two hours. In an effort to significantly decrease the time it takes to prepare these samples, we are in the process of testing a new method for handling soil samples. The idea is that we can suspend the sample in a high density (1.22 g/mL) sucrose solution and centrifuge it at a low speed. When this low-speed centrifugation is complete, the soil (which is higher in density than the sucrose solution) should be in a pellet at the bottom and the spores (which are less dense than the sucrose solution) should be suspended in the supernatant. This

supernatant is then collected and centrifuged at a high speed to pellet the spores. The supernatant is discarded, and ideally we should be left with only spores in the pellet, which can then either be put through the normal RV-PCR routine or go through a bead-beating process to isolate the spore DNA. This way, we should have a very low amount of soil in our sample and the processing time will be drastically shorter. Initial testing indicates that this process can be useful for samples containing up to 2 grams of soil. Further testing is being done in an effort to make this process fully automated and more high-throughput, as well as to determine the rate of recovery and detection limits.

Impact on my career:

Being a mechanical engineer, I was a little worried at first when I realized this project was so heavy on microbiology. Luckily, my advisor and coworkers were very patient with me and they were able to teach me everything I needed to know to get me running experiments in a short period of time. After a couple weeks I was competent enough in the lab to be able to actively participate in all aspects of the project. Once I reached that point where I was able to understand what was going on, it felt really good to be in the lab doing and learning something besides engineering.

The robotic liquid handling equipment in the lab was very impressive. Working on this project definitely increased my interest in automation and robotics. I am now very excited to continue doing research to improve upon and develop new robotic systems like these. Throughout my engineering education, I have always been the one trying to design robots, and it was nice to see the robots from the user's side too. Hopefully this

will help me in future design projects to give more consideration to the user's point of view.

The lectures and tours that I attended at LLNL were very interesting. The tours of the National Ignition Facility, Joint Genome Institute, and Site 300 were incredible and I had no idea that some of the things they are doing were even possible. I really enjoyed the "Destination Space Station" presentation by Jeff Wisoff. It brought back memories of when I was a kid and dreamed of being an astronaut. Of course the poster symposium (my first poster presentation) was a great experience. I learned an important lesson from the DHS weekly seminars. Many of the speakers mentioned that DHS is having a problem with scientists designing products that the end-users have trouble using. The engineers and scientists have brilliant, elaborate ideas for products, but the people in the field have trouble using them. Maybe the product is too complicated to use or it is just not practical. I had never thought of this before, but I realized how important it is. Being an engineer interested in mechanical design, I hope I can always remember this lesson.

This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48. This research was performed under an appointment to the Department of Homeland Security (DHS) Scholarship and Fellowship Program, administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and DHS. ORISE is managed by Oak Ridge Associated Universities (ORAU) under DOE contract number DE-AC05-06OR23100. All opinions expressed in this paper are the author's and do not necessarily reflect the policies and views of DHS, DOE, or ORAU/ORISE.