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technical BRIEF

Rapid Viability PCR Method for Detection of Live *Bacillus anthracis* Spores

Introduction

To protect human health and ensure that the environment and facilities are remediated following a biothreat agent release, the U.S. Environmental Protection Agency (EPA) needs rapid viability testing methods to evaluate contaminants. In the event of a biothreat agent release, hundreds to thousands of environmental samples of diverse types (aerosol, surface, water, etc.) could need to be processed. Decision-makers need rapid results to determine whether facilities and areas can be re-opened, or if additional decontamination is needed. Current viability test methods are too labor-, space-, and time-intensive to be able to meet the need for rapid analysis. With current methods, only 30-40 samples may be processed each day per laboratory with confirmation not available until days later.

EPA's Homeland Security Research Program (HSRP) develops products based on scientific research and technology evaluations. Our products and expertise are widely used in preventing, preparing for, and recovering from public health and environmental emergencies that arise from terrorist attacks. Our research and products address biological, radiological, or chemical contaminants that could affect indoor areas, outdoor areas, or water infrastructure. HSRP provides these products, technical assistance, and expertise to support EPA's roles and responsibilities under the National Response Framework, statutory requirements, and Homeland Security Presidential Directives.

The EPA's Office of Emergency Management within the Office of Solid, Waste, and Emergency Response has established the Environmental Response Laboratory Network (ERLN), a network of laboratories to analyze environmental samples following biological, chemical, or radiological attacks. Validated, rapid viability test protocols are needed as part of the ERLN capabilities. This critical need was highlighted during the response to the 2001 anthrax attacks in which clearance sampling and analysis required excessive time prior to facilities re-opening.

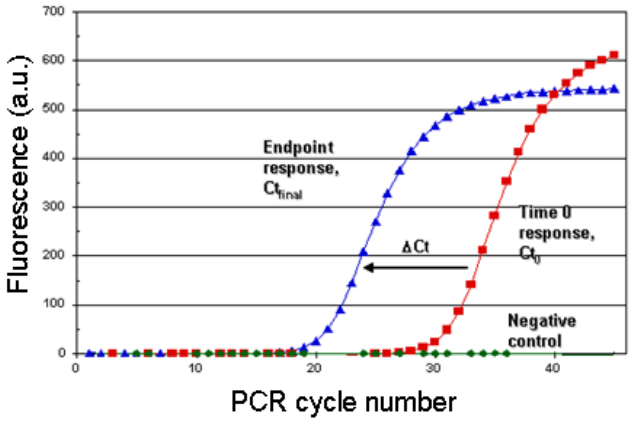
Rapid Viability PCR (RV-PCR)

The EPA-NHSRC developed Rapid Viability PCR (RV-PCR) method determines the presence or absence of live *B. anthracis* spores, which is a key analytical requirement during the cleanup phase of a response. This method can be more sensitive than the traditional culture-based method because RV-PCR uses the whole sample for analysis. RV-PCR is relatively rapid and cost-effective. It is also less labor-intensive, less prone to inhibition by environmental matrices, and less prone to interference from other biological material in the sample than the culture method. It also provides higher-throughput and generates significantly less bio-hazard and other laboratory wastes than the culture-based method.

The RV-PCR method combines broth culture and real-time PCR, a highly sensitive and specific analytical method to identify bioagents. The broth culture part of the method allows the germination of *B. anthracis* spores recovered from a sample. The real-time PCR part of the method offers a rapid determination of the identity of *B. anthracis* bacteria.

The first step of the protocol involves multiple extraction and wash steps through which spores are recovered in filter cups. Next, a growth media is added to the spores in filter cup for optimum growth of *B. anthracis*. An aliquot is withdrawn before incubating the broth culture in the filter cup. This is the Time Zero (T0) aliquot. After 9 hours of incubation another aliquot is withdrawn. This is the T9 aliquot. The DNA extracts of the T0 and T9 DNA aliquots, are then analyzed via real-time PCR to detect the presence of *B. anthracis*. The PCR cycle-threshold (Ct) number for both the T0 and T9 aliquots are recorded and compared. A change (decrease) in the Ct number for the T9 aliquot relative to the Ct number for the T0

aliquot is calculated. A delta Ct ≥ 9 with an endpoint PCR Ct of ≤36 for the T9 aliquot, represents an increase in *B. anthracis* concentration at T9 relative to T0 as a result of viable spores in the sample that germinated and grew during the 9 hr of incubation for growth. Depending upon the end user requirement and the phase of response during an event, a lower delta Ct(≥ 6, to represent at least a two log difference in DNA concentration), and a corresponding higher end point (PCR Ct of ≤ 39) could be set. It should be noted that the current version of the method provides qualitative (presence/absence) detection. However, introduction of the Most Probable Number (MPN) steps in the method could provide some quantitative analysis.

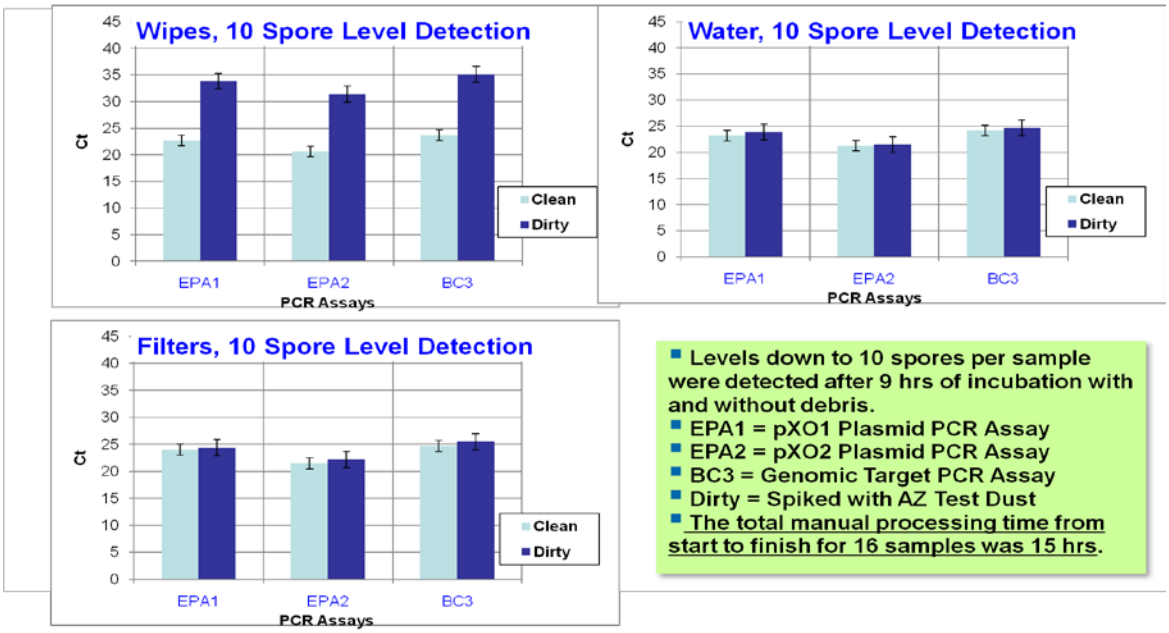


- The RV-PCR method is based on a shift in PCR Ct value indicating an increase in DNA due to growth of viable organisms
- The method accurately distinguishes live cells from dead spores/cells based on Ct_0, Ct_{final} and ΔCt
- **ΔCt = 9 for a positive result**

The method has significant advantages over the traditional plate method:

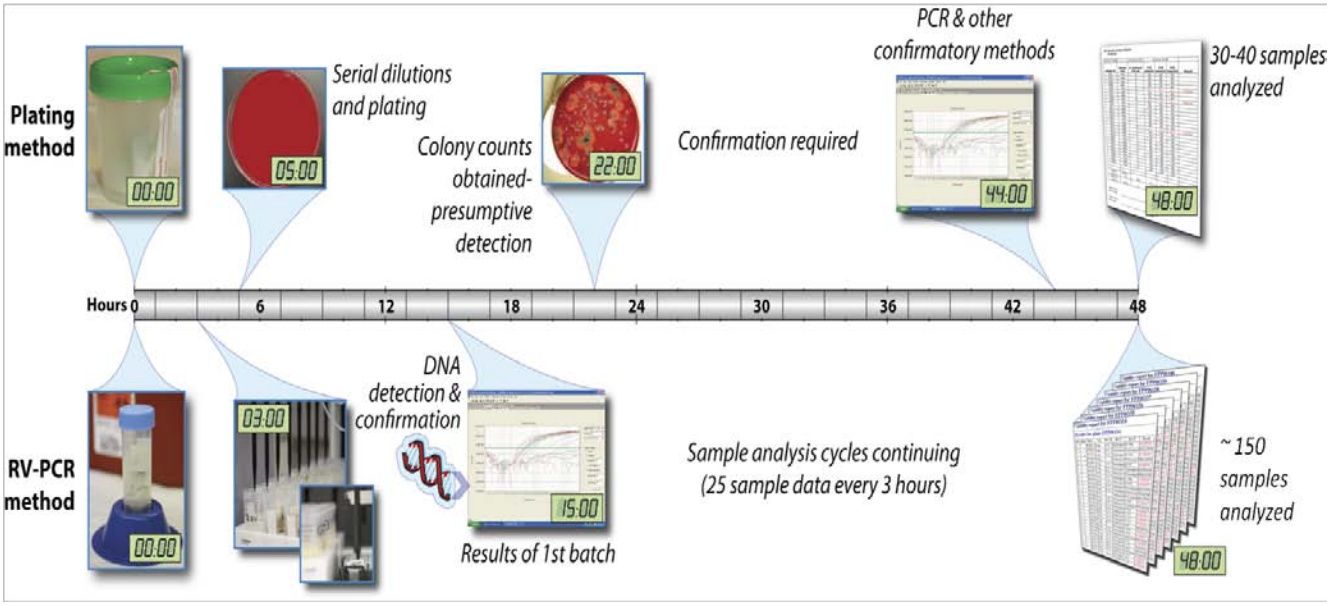
- Higher sensitivity (> 10x improvement over plate method)
- No interference with live environmental background (selective method)
- May be fully automated (high-throughput)
- Built-in PCR confirmation
- Faster turn-around time for results

Limit of Detection with RV-PCR



- Levels down to 10 spores per sample were detected after 9 hrs of incubation with and without debris.
- EPA1 = pXO1 Plasmid PCR Assay
- EPA2 = pXO2 Plasmid PCR Assay
- BC3 = Genomic Target PCR Assay
- Dirty = Spiked with AZ Test Dust
- The total manual processing time from start to finish for 16 samples was 15 hrs.

Comparison of RV-PCR with Traditional Microbiological Culture (Plating) Method



The RV-PCR method is being transitioned to the OEM (ERLN) and the capability has been established at the OPP Microbiology Lab at Ft. Meade, MD. Also, the Lawrence Livermore National Laboratory at Livermore, CA, who developed the RV-PCR method under the IA with NHSRC, is also an ERLN member lab to perform RV-PCR based sample analyses. Additionally, the RV-PCR capability will be established at a laboratory of University of Cincinnati, Cincinnati, OH, and the NEIC at Denver, CO,

The development of RV-PCR methods for Yersinia pestis and Francisella tularensis is also in progress.

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