An Integrated PCR-Microarray Lab-on-Chip Assay for Detection and Identification of 4 Category A Biological Agents (Anthrax, Plague, Small Pox, Tularemia)

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Introduction

Current fears over the threat of bioterrorism attacks using Category A biological agents has necessitated the need for the rapid detection and identification of 4 common biological agents: *Bacillus anthracis*, *Yersinia pestis*, *Variola* and *Franciscella tularensis*. Here we describe the utility of an integrated PCR-Microarray platform, the VereID™ Biosystem with the VereThreat™ Chip, which can enhance biological agent surveillance efforts. The platform is designed around a miniaturized silicon chip that integrates a PCR reactor with a customizable low-density DNA Microarray. As a result, the VereID™ Biosystem platform is able to perform multiplexing PCR and detection of the PCR products on a low-density DNA microarray, on the same chip. The probes of the microarray were designed to identify any or all 4 biological agents in one single test.

Materials and Methods

Positive controls preparation. The target genes of the biological agents are: a) *rpoB*, *pag* and *capB* genes for *Bacillus anthracis*; b) *pla* and *caf1* genes for *Yersinia pestis*; c) HA gene for *Variola* virus and; d) *tul4* and *fopA* genes for *Franciscella tularensis*. The target region of the genes (~200bps) were synthesized, cloned into plasmids and used as surrogates for the analytical specificity and sensitivity tests.

PCR. For each biological agent, a PCR reaction of 25µl was setup with 10^2 copies of its corresponding genes and pipetted into the VereThreat™ Chip. The chip is placed into the VereID™ Biosystem for PCR amplification.

Chip Hybridization. Hybridization buffer was added to the amplicons and hybridization occurred for 30min at 62ºC. After hybridization, chips were washed in SSC buffers and dried before imaging.

Microarray Imaging and Analysis. Hybridized samples were scanned using the Optical Reader. Signals were considered to be positive when its fluorescence intensity was more than three times that of the background.

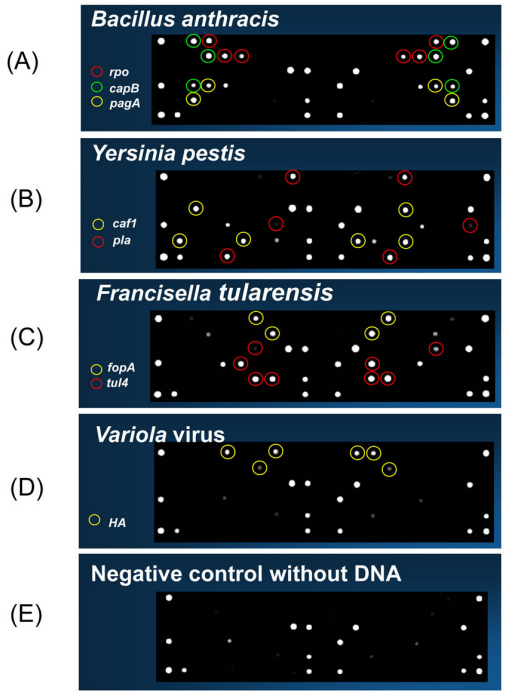
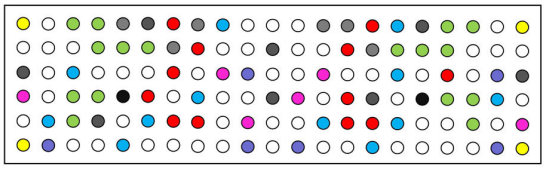


FIG. 1 Typical microarray images demonstrating correct detection of (A) *B.anthracis*, (B) *Y.pestis*, (C) *F.tularensis* and (D) *Variola* virus. (E) Negative control without DNA.

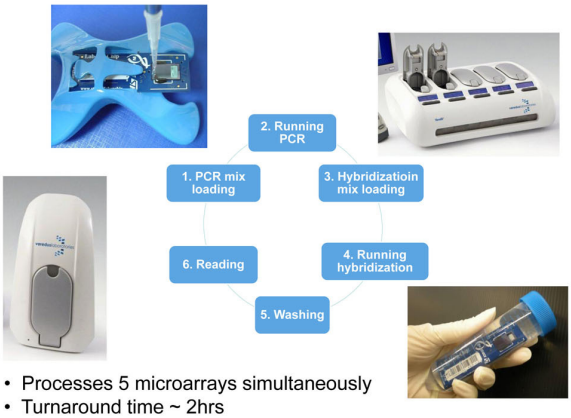
VereThreat™ Chip Probe Layout



Capture probes for:

- ***B. anthracis***
- ***Y. pestis***
- ***F. tularensis***
- ***Variola* virus**
- Orientation Probes
- Positive control
- Negative Control
- Hybridization control
- Binding Probes
- Probe location left empty

VereID™ VereThreat™ Chip operational procedure



- Processes 5 microarrays simultaneously
- Turnaround time ~ 2hrs

Result and conclusion

Detection of biological agents. Analysis of the fluorescent images of the microarray showed that the VereThreat™ Chip was able to identity and discriminate the 4 targeted biological agents.

No cross-reactivity of the hybridization probes between the targets was observed.

With this Lab-on-Chip approach, results can be obtained rapidly, within 2 hours and is an invaluable tool for the rapid identification of *Bacillus anthracis*, *Yersinia pestis*, *Variola* and *Franciscella tularensis.*

References

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Keiko Tomioka, Michael Peredelchuk, Xiangyang Zhu, Roberto Arena, Dmitri Volokhov, Angamuthu Selvapandiyan, Katie Stabler, Jenny Mellquist-Riemenschneider, Vladimir Chizhikov, Gerardo Kaplan, Hira Nakhasi and Robert Duncan . 2005. A Multiplex Polymerase Chain Reaction Microarray Assay to Detect Bioterror Pathogens in Blood. J. of Molecular Diagnostics, Vol 7, No. 4: 486-94