Poster #019

Rapid and Sensitive Detection of *Bacillus anthracis* Toxin Lethal Factor Direct from Blood Sample

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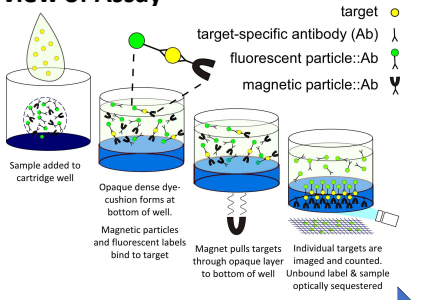
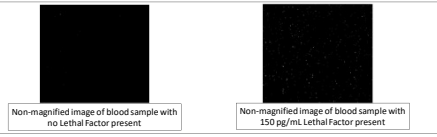
Abstract

Secreted Lethal Factor (LF), a subunit of Lethal Toxin, is the earliest known biomarker of *Bacillus anthracis* infection, making it a logical target for diagnostics to detect exposure to this potentially lethal pathogen[1] (Boyer 2009). There are currently no commercial methods for anthrax LF detection that are rapid (time to result of fewer than 30 minutes), simple enough to use in a physician's office laboratory, and sensitive enough to detect low concentrations of LF (<100 pg/mL) early in infection. In this report, we detail the analytical performance of the MultiPath™ Anthrax Test, a novel immunoassay that is being developed by First Light Biosciences on behalf of Biomedical Advanced Research and Development Authority (BARDA), on the MultiPath platform. The MultiPath Anthrax test can be performed on a small volume (<60 µL) of venous or finger stick whole blood added to the disposable cartridge with no sample preparation. Once loaded into the analyzer, the test proceeds automatically without further user input. The time from sample loading to diagnostic result is fewer than 20 minutes and as many as 20 samples can be processed simultaneously on the platform. The limit of detection (LoD) of the assay, determined using whole blood samples spiked with pure LF protein, is <60 pg/mL. The dynamic range of the assay covers 5 logs of LF concentration, an important performance metric given the broad range of LF concentrations observed over the course of anthrax infections. With its ease of use, rapid time to result and high sensitivity the MultiPath Anthrax Test potentially fills an important gap in the toolkit for anthrax diagnostics.

Overview of Assay



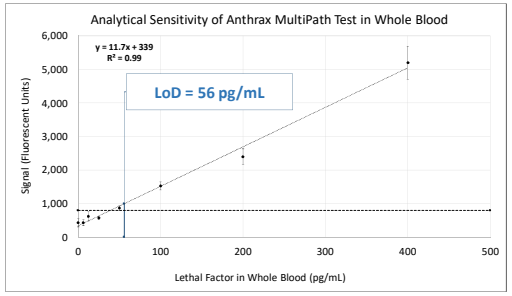


Workflow

The MultiPath platform currently being developed requires no sample preparation. Venous whole blood or finger-stick blood is added to a sample diluent stored in the cartridge. Cartridges are loaded onto the MultiPath Analyzer. The analyzer provides a diagnostic readout in <20 minutes.

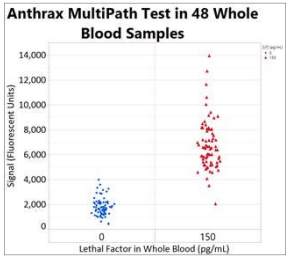
Analytical Sensitivity

Lethal Factor was serially diluted into venous whole blood samples, then run through the MultiPath platform. Limit of the Blank was determined as 3 standard deviations above the mean of 24 independently prepared blank samples. The analytical sensitivity was determined by determining the lowest interpolated concentration of Lethal Factor at which 95% of data points are expected to reside above the limit of the blank. Comparable performance is seen across a panel of blood samples.



Detection of Lethal Factor Spiked into Whole Blood Samples

48 negative individual patient samples of whole venous blood were run on the MultiPath platform in duplicate. Lethal Factor was then spiked into the same 48 samples at 150 pg/mL and run in duplicate on the MultiPath platform. The distribution of signal of the population of un-spiked and spiked samples is shown below, as is the presumptive diagnostic performance using a signal cutoff of 4000 fluorescent units.



	MultiPath Positive	MultiPath Negative
Spiked	94	2
Unspiked	1	95

Diagnostic Performance	
Positive % Agreement	98%
Negative % Agreement	99%
Accuracy	99%

Robustness to Microbial Interference

The ability of the MultiPath system to correctly identify the presence or absence of Lethal Factor was tested in the presence of various common microbial organisms, which were spiked into whole blood at 1E7 cfu/mL, as per CLSI guidelines. The MultiPath system was able to correctly detect the presence or absence of Lethal Factor in all cases.

Potential Microbial Interferent (at 1E7 cfu/mL)	0 pg/mL	150 pg/mL
Enterobacter cloacae	No LF Detected	LF Present
Staphylococcus epidermis	No LF Detected	LF Present
Bacillus licheniformis	No LF Detected	LF Present
Klebsiella oxytoca	No LF Detected	LF Present
Candida albicans	No LF Detected	LF Present
Staphylococcus aureus	No LF Detected	LF Present
Escherichia coli	No LF Detected	LF Present
Pseudomonas pneumonia	No LF Detected	LF Present
Klebsiella pneumonia	No LF Detected	LF Present
Enterococcus faecalis	No LF Detected	LF Present

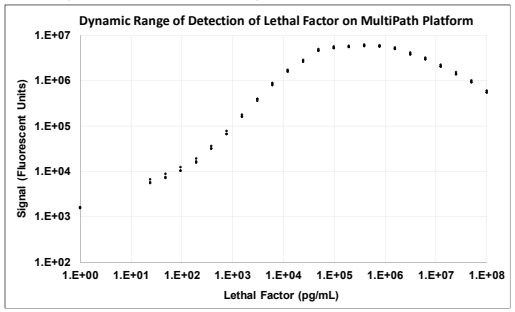
Robustness to Chemical Interference

The ability of the MultiPath platform to correctly identify the presence or absence of Lethal Factor was tested in the presence of various potential interfering substances. Common potentially interfering endogenous and exogenous substances were tested at recommended concentrations as per CLSI guidelines. The MultiPath platform was able to correctly detect the presence or absence of Lethal Factor in all cases.

Potential Interferent	0pg/mL	150pg/mL	300pg/mL
Acetaminophen (0.2 mg/mL)	No LF Present	LF Detected	LF Detected
Amoxicillin (0.08 mg/mL)	No LF Present	LF Detected	LF Detected
Ascorbic Acid (0.06 mg/mL)	No LF Present	LF Detected	LF Detected
Albuterol (0.4 ug/mL)	No LF Present	LF Detected	LF Detected
Cefotaxime (0.31 mg/mL)	No LF Present	LF Detected	LF Detected
Ciprofloxacin HCl (0.01 mg/mL)	No LF Present	LF Detected	LF Detected
Doxycycline (0.03 mg/mL)	No LF Present	LF Detected	LF Detected
EDTA (1 ug/mL)	No LF Present	LF Detected	LF Detected
Glucose (1.2 mg/mL)	No LF Present	LF Detected	LF Detected
Erythromycin (0.06 mg/mL)	No LF Present	LF Detected	LF Detected
Ethanol (3.5%)	No LF Present	LF Detected	LF Detected
N acetylcysteine (1.66 mg/mL)	No LF Present	LF Detected	LF Detected
Gentamicin (0.01 mg/mL)	No LF Present	LF Detected	LF Detected
Heparin (0.02 mg/mL)	No LF Present	LF Detected	LF Detected
Ibuprofen (0.5 mg/mL)	No LF Present	LF Detected	LF Detected
Naproxen (0.5 mg/mL)	No LF Present	LF Detected	LF Detected
Rifampicin (0.06 mg/mL)	No LF Present	LF Detected	LF Detected
Sulfamethoxazole (0.28 mg/mL)	No LF Present	LF Detected	LF Detected
Tetracycline (0.02 mg/mL)	No LF Present	LF Detected	LF Detected
Isopropanol (0.32%)	No LF Present	LF Detected	LF Detected
Trimethoprim (0.04 mg/mL)	No LF Present	LF Detected	LF Detected
Acetone (0.03%)	No LF Present	LF Detected	LF Detected
Chloroquine (0.05 mg/mL)	No LF Present	LF Detected	LF Detected
Albumin (50 mg/mL)	No LF Present	LF Detected	LF Detected
Streptomycin (0.35 mg/mL)	No LF Present	LF Detected	LF Detected
Sodium citrate (38 mg/mL)	No LF Present	LF Detected	LF Detected
SPS (0.5 mg/mL)	No LF Present	LF Detected	LF Detected
Cromolyn sodium (0.04 mg/mL)	No LF Present	LF Detected	LF Detected
Flunisolide (0.84 ug/mL)	No LF Present	LF Detected	LF Detected
Tobramycin (0.02 mg/mL)	No LF Present	LF Detected	LF Detected
Triglycerides (5mg/mL)	No LF Present	LF Detected	LF Detected
Human IgG (36 mg/mL)	No LF Present	LF Detected	LF Detected
DMSO (1.1%)	No LF Present	LF Detected	LF Detected
NH4OH (0.45%)	No LF Present	LF Detected	LF Detected
Bleach (1%)	No LF Present	LF Detected	LF Detected
Bilirubin (0.15 mg/mL)	No LF Present	LF Detected	LF Detected
Cholesterol (2.5 mg/mL)	No LF Present	LF Detected	LF Detected
Hemoglobin (2 mg/mL)	No LF Present	LF Detected	LF Detected
Acid-citrate-dextrose (10%)	No LF Present	LF Detected	LF Detected
Acid-citrate-dextrose (1%)	No LF Present	LF Detected	LF Detected

Dynamic Range

The MultiPath platform has a dynamic range of over 1E5 pg/mL of Lethal Factor. Lethal Factor was serially diluted from 1 µg/mL to 100 pg/mL and spiked into pooled human plasma. The samples were then run in triplicate for each spike level on the MultiPath platform.



Summary

The MultiPath Anthrax test run on the MultiPath platform requires minimal sample preparation, returns a result in <20 minutes, and can detect Lethal Factor over a large dynamic range down to <60 pg/mL in whole blood while demonstrating robustness to commonly interfering substances and organisms, offering health care providers a rapid testing solution at the point of medical need in the event of public exposure to *Bacillus anthracis*.

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References: 1. Boyer AE, Quinn CP, Hoffmaster AR, Kozel TR, Saile E, Marston CK, Percival A, Plikaytis BD, Woolfitt AR, Gallegos M, Sabourin P, McWilliams LG, Pirkle JL, Barr JR. 2009. Kinetics of lethal factor and poly-D-glutamic acid antigenemia during inhalation anthrax in rhesus macaques. Infect Immun. 77(8):3432–3441