

Test Report - Idaho Technology Inc.'s RAZOR™ System

APPLICATION ::: NOTE



Purpose

The purpose of this testing was to look at the efficacy of Idaho Technology Inc.'s (ITI) RAZOR system to detect DNA for organisms of select Bio-terror agents. The organisms tested were *Bacillus anthracis* (Anthrax), *Fransicella tularensis* (Tularemia), and *Yersinia pestis* (Plague).

The testing took place one day at the La Marinosa Laboratory outside of Madrid, Spain: Tuesday February 13, 2007. Three different runs were completed – 2 runs using Idaho Technology Inc.'s (ITI) freeze dried reagent pouches and one run using La Marinosa's home brewed detection reagents.

The testing used ITI's standard Biothreat Pathfinder freeze dried pouches from Idaho Technology (Biothreat Screen 1 and Biothreat Screen 2). Each RAZOR®Pouch Reagent kit was loaded using 1 ml syringes with approximately 400ul of the samples and run. Each sample is channeled to three freeze-dried reagent channels in the Pathfinder pouch – each one a different gene target specific to the organism of interest (two unknown inlet ports, one negative control port, and one positive control port). Biothreat Screen 1 tests for the presence of Anthrax, Ricin, and *F. tularensis* DNA. Biothreat Screen 2 tests for Small pox, *Y. pestis*, and *C. botulinum* Type A DNA. La Marinosa's home brewed detection reagents consisted of assays for *Bacillus anthracis*, Vaccinia, and *Yersinia pestis*.

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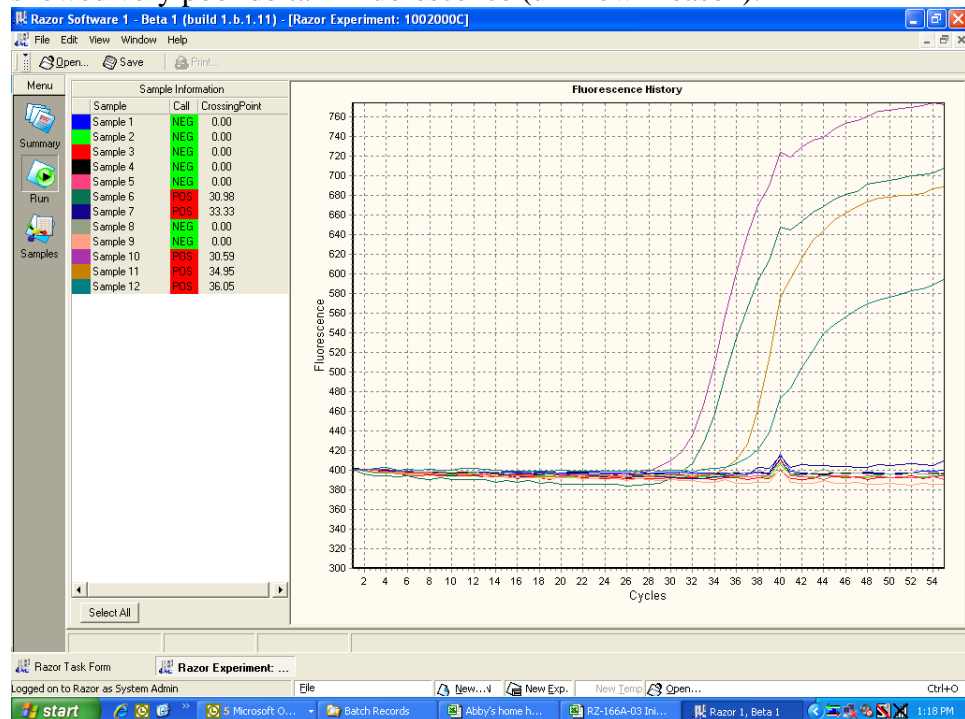
Results

Run 1

Biothreat Screen 1 was used to test for *B. anthracis* (PXO1), Ricin, and *F. tularensis*. Purified DNA for *F. tularensis* (Sample 1) and *B. anthracis* (Sample 2) were prepared at a concentration of 20 pg/ul that was then diluted 1:5.

The test was done in stand-alone mode (RAZOR unit only, not connected to a PC). An example of the results from the RAZOR is shown below. Analysis is done “on-the-fly” at the end of each cycle (this begins after cycle 10). Runs take approximately 25 minutes to complete, but results may be seen earlier depending on sample concentration (a concentrated sample will present positive at an earlier cycle).

The data was downloaded from the RAZOR and is presented in the screen shot below. The two individual targets (Anthrax and *F. tul*) for the two samples were reported positive. For an unknown reason the anthrax amplification curve, though positive at an early crossing threshold, showed very poor delta in fluorescence (unknown reason).



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Crossing points for the reactions were as follows –

Biothreat Screen 1

Slot 6 for RAZOR pouch (F. tul) – **30.98**

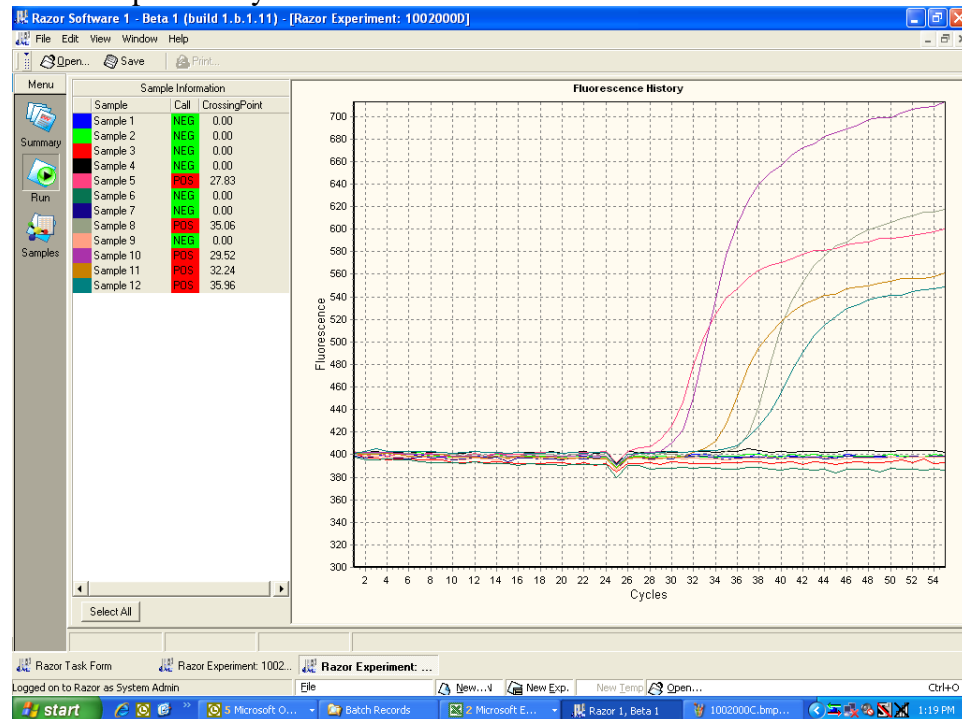
Slot 7 for RAZOR pouch (B. ant) – **33.33**

Run 2

Biothreat Screen 2 was used to test for Small pox, *Y. pestis*, and *C. botulinum* Type A DNA. Two samples of purified DNA from *Y.pestis* were prepared – Sample 1 approximately 20pg/ul diluted 1:5 and Sample two approximately 0.012pg/ul.

The test was done in stand-alone mode (RAZOR unit only, not connected to a PC).

The data was downloaded from the RAZOR and is presented in the screen shot below. The two samples were positive for *Y.pestis* at the two dilutions previously noted.



Crossing points for the reactions were as follows –

Biothreat Screen 2:

Slot 5 for RAZOR pouch (*Y. pestis*) – **27.83**

Slot 8 for RAZOR pouch (*Y. pestis*) – **35.06**

Run 3

La Marinosa’s home brewed detection reagents consisting of assays for *Bacillus anthracis*, *Vaccinia*, and *Yersinia pestis*. used.

The test was done in stand-alone mode (RAZOR unit only, not connected to a PC).

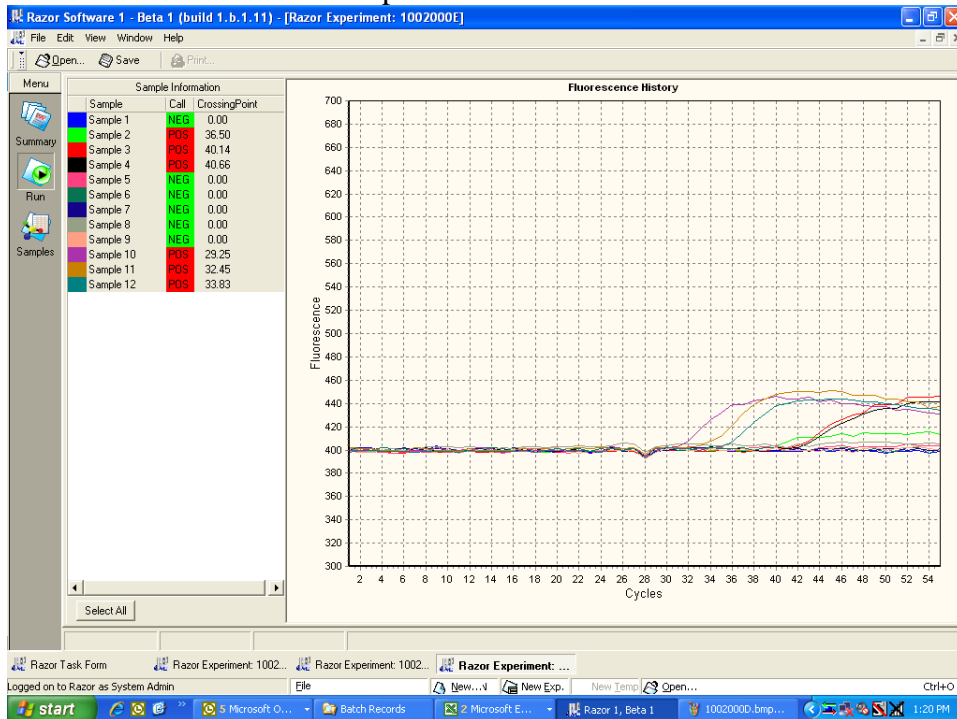
A 12x1 empty pouch was prepared with the home brewed reagents. 4 slots for each target were prepared. 1 negative, and 3 positives of varying concentrations (10 e-4, 10 e-5, 10 e-6/ml organism genomic equivalent of DNA) of purified DNA for each organism.

Sample 1 – BA Negative		
Sample 2 – BA 10 e-4	Cp Value -	36.50
Sample 3 – BA 10 e-5	Cp Value -	40.14
Sample 4 – BA 10 e-6	Cp Value -	40.66
Sample 5 – Vaccinia Negative		
Sample 6 – Vaccinia 10 e-4		
Sample 7 – Vaccinia 10 e-5		
Sample 8 – Vaccinia 10 e-6		
Sample 9 – <i>Y. pestis</i> Negative		
Sample 10 – <i>Y. pestis</i> 10 e-4	Cp Value -	29.25
Sample 11 – <i>Y. pestis</i> 10 e-5	Cp Value -	32.45
Sample 12 – <i>Y. pestis</i> 10 e-6	Cp Value -	33.83

This was a rough experiment. Due to missing hardware with the instrument (this was a demo instrument that had traded hands many times over the course of the last couple of years), the default protocol on the RAZOR for cycling was used (2 minute initial denature @ 93 deg C then cycling @ 91 deg C 3, 61 deg C for 15 seconds for 55 cycles was used. Roche Faststart Taq was used and required a 10minute hotstart (this was accomplished by starting, stopping and restarting the instrument 5x before allowing it to cycle).

The BA and *Y. pestis* samples with DNA all were positive on the RAZOR. The *Vaccinia* samples remained negative as expected (annealing temperature of primers is 50 deg C – below the cycling parameters of the

run). Though the amplification curves showed low overall fluorescence change, they were still favorable results considering no optimization was done and the hot start was improvised.



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ITI would like to thank all those who participated in this trial. All the personnel participating La Marinosa were very accommodating, cooperative and professional. The facilities were more than adequate and the testing went smoothly and quickly.

Additional Information:

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