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

APPLICATION ::: NOTE

Purpose

The purpose of this study was to look at the field usability and the efficacy of Idaho Technology Inc.'s (ITI) RAZOR system to detect inactivated organisms for select Bio-terror agents. The organisms tested were *Bacillus anthracis* (Anthrax), *Fransicella tularensis* (Tularemia), *Yersinia pestis* (Plague), and *Clostridium botulinum* Type A and *Brucella spp*.

The testing took place on two consequtive days at the Health Canada National Microbiology Laboratory in conjunction with the Royal Canadian Mounted Police in Winnipeg, Canada: Monday July 18 and 19, 2005. Eight different runs were completed in the two days examining a variety of parameters. Four runs were completed on the 18th and another four on the 19th.

The testing used standard Biothreat Pathfinder freeze dried pouches from Idaho Technology (Biothreat Screen 1 and Biothreat Screen 2). Each Pathfinder™ 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 one tests for the presence of Anthrax, Brucella, and F. tularensis DNA. Biothreat Screen 2 tests for Anthrax (a second gene target), Y. pestis, and C. botulinum Type A DNA.

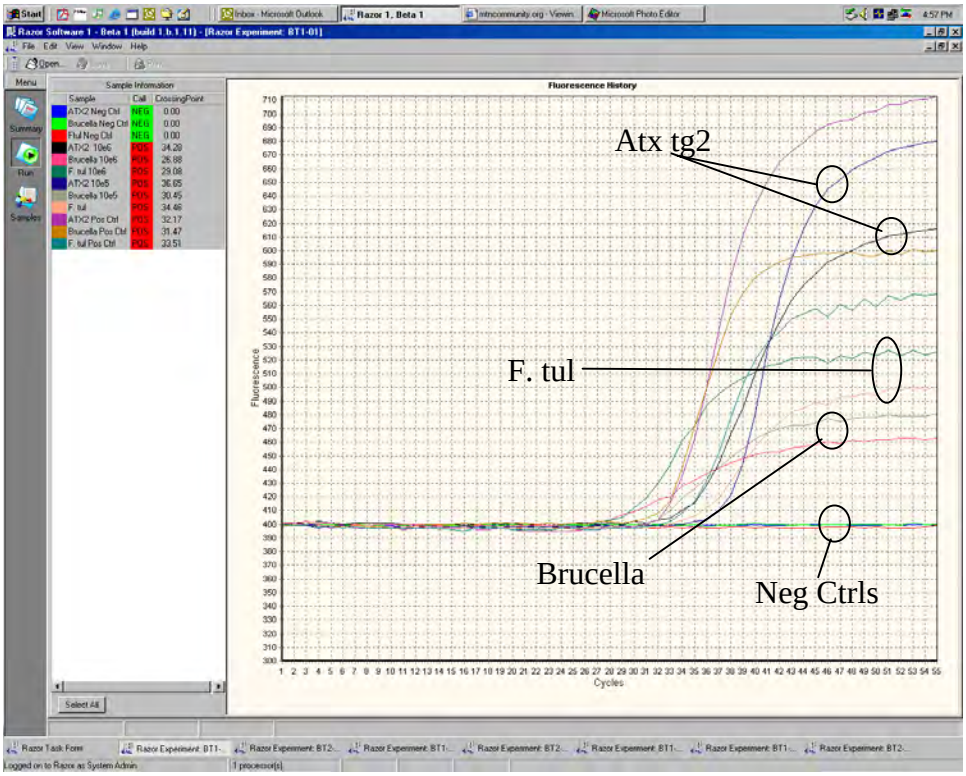
Results

Run 1

Biothreat Screen 1 was used to test for *B. anthracis* (PXO1), *Brucella spp*, and *F. tularensis*. A mixture of *F. tularensis*, *Y. pestis*, Wild type Anthrax, *Brucella*, and Botulinum toxin A was prepared. All bacteria were at a concentration of 10e7 cfu/ml. The Botulinum toxin was not quantified and had previously not shown reactivity to antibody based testing. The mixture was diluted 1:10 and 1:100 using the RAZOR sample preparation kit. Final sample concentration was 10e6 – sample numbers 4, 5, and 6 - and 10e5 – sample numbers 7, 8, and 9). Samples 1-3 are negative controls while samples 10-12 are positive controls.

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 then 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 ealier).

The data was downloaded from the RAZOR and is presented in the screen shot below. All three targets (Anthrax tg2, Brucella, and F. tul) for the two dilutions were reported positive.



Crossing points for the reactions were as follows –

Target	CFU/ml	Crossing point
Anthrax tg2	10e6	34.28
	10e5	36.65
Brucella	10e6	26.88
	10e5	30.45
F. tularensis	10e6	29.08
	10e5	34.46

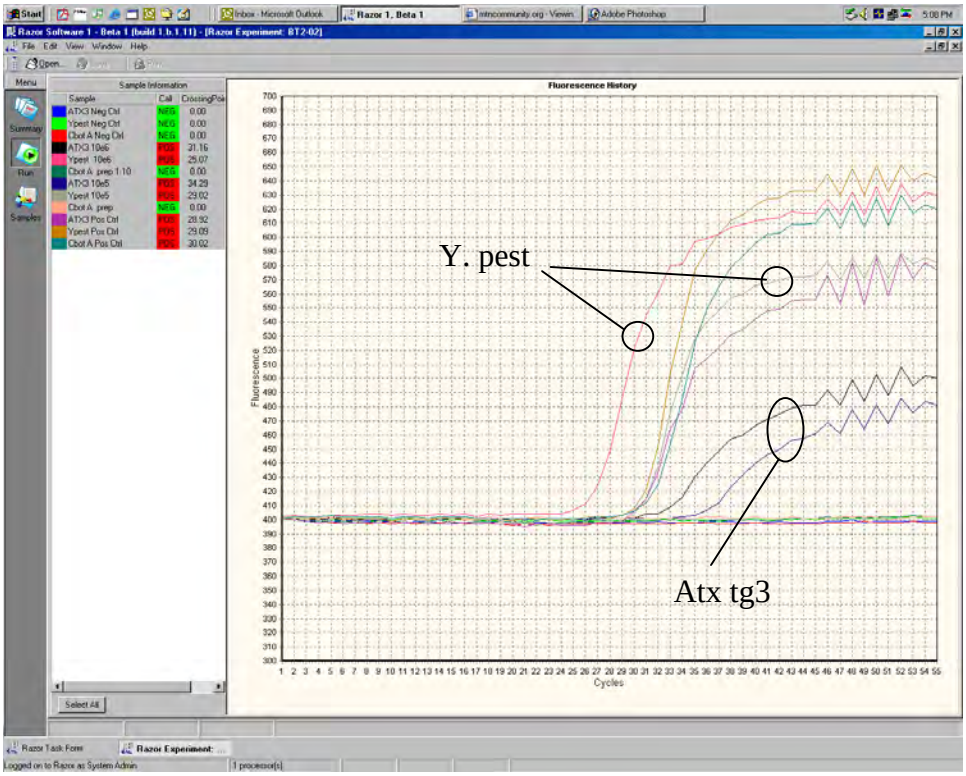
Run 2

Biothreat Screen 2 was used to test for *B. anthracis* (PXO2), *Y. pestis*, and C.botulinum type A. The mixture of *F. tularensis*, *Y. pestis*, Wild type Anthrax, *Brucella*, and Botulinum toxin A from run 1 was used. All bacteria were at a concentration of 10e7 CFU/ml. The Botulinum toxin was not quantified and had previously not shown reactivity to antibody based testing. The mixture was diluted 1:10 and 1:100 using the RAZOR sample preparation kit. Final sample concentration was 10e6 – sample numbers 4, 5, and 6 - and 10e5 – sample numbers 7, 8, and 9). Samples 1-3 are negative controls while samples 10-12 are positive controls.

The pouch was loaded and the sprayed with 10% bleach solution (0.5% hypochlorate). This was then put in a plastic bag and stored for 20 minutes to examine the feasibility of decontaminating the Pathfinder pouch in order to keep the instrument away from the hotzone while maintaining an acceptable bio-safety level with materials removed from the hotzone.

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. Two of the three targets (Anthrax tg3, and Y. pestis) for the two dilutions were reported positive. The *Clostridium botulinum* type A toxin that had previously presented negative for antibody testing did not show a positive result. The decontamination of the pouch appeared to have no negligible effect on the reaction or results.



Crossing points for the reactions were as follows –

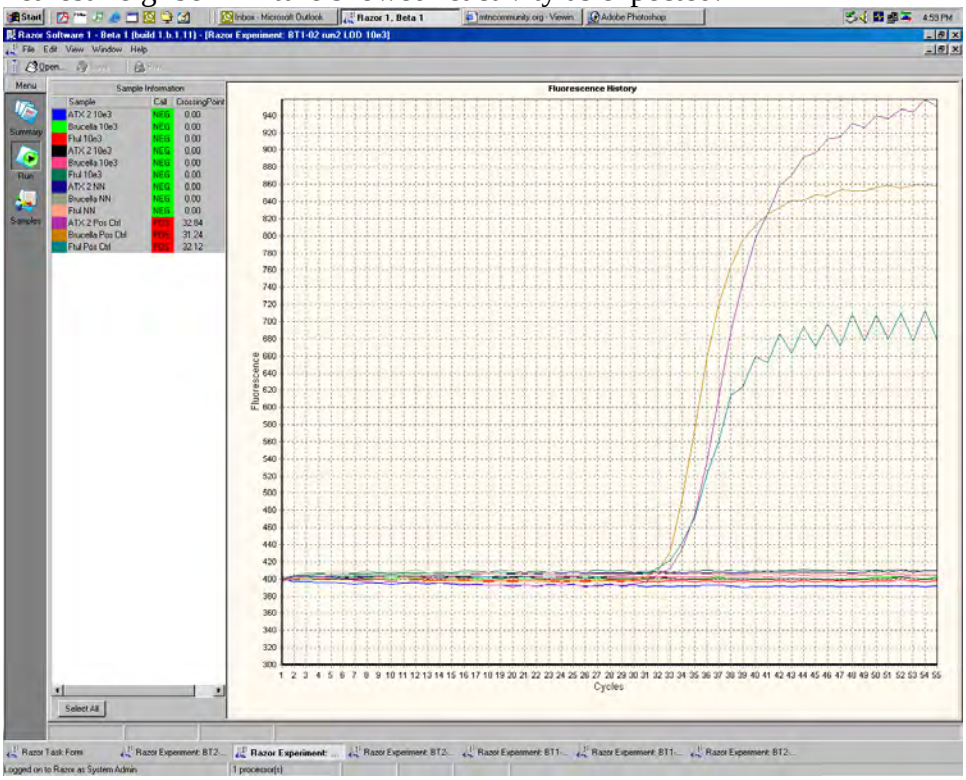
Target	CFU/ml	Crossing point
Anthrax tg3	10e6	31.16
	10e5	34.29
Y. pestis	10e6	25.07
	10e5	29.02

Run 3
Biothreat Screen 1 was used.

The test was done in real-time mode (connected to a PC).

The negative control was used as an unknown. A mixture of wild type Anthrax, *Y. pestis*, and *F. tularensis* at 10e3 cfu/ml was used for this experiment in lanes 1-6. Lanes 7-9 were used with a mixture of nearest neighbors – *Bacillus megaterium*, *B. cereus*, *Francisella philomiragia*, and *Yersinia pseudotuberculosis*.

The data from the RAZOR and is presented in the screen shot below. None of the samples from the 10e3 dilution were positive. This is near the LOD for the reagents and did not yield the desired results. None of the nearest neighbor mixture showed reactivity as expected.

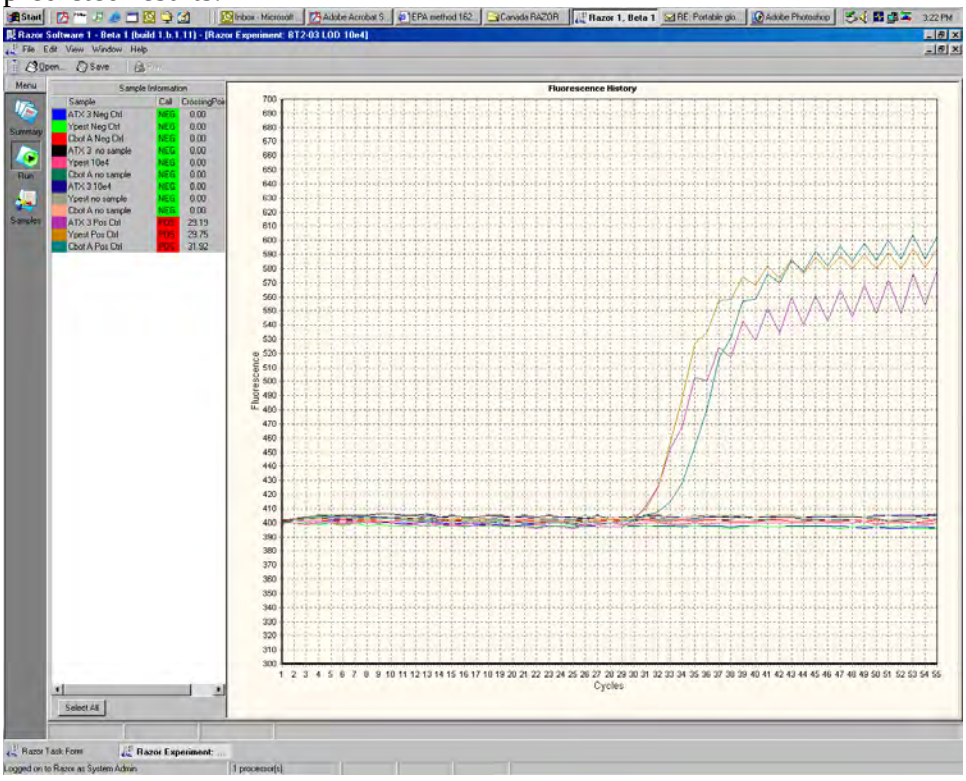


Run 4
Biothreat Screen 2 was used.

The test was done in real-time mode (connected to a PC).

A mixture of wild type Anthrax, *Y. pestis*, and at 10e4 cfu/ml was used for this experiment.

The data from the RAZOR is presented in the screen shot below. None of the samples from the 10e4 dilution were positive. This is above Idaho Tech's predicted LOD for the reagents and did not yield the desired results. Fresh dilutions will be tried in subsequent runs that yielded predicted results.



Note: Runs 5-8 were completed on the second day of testing. Dilutions of the agents was confirmed by conventional real time PCR (see table).

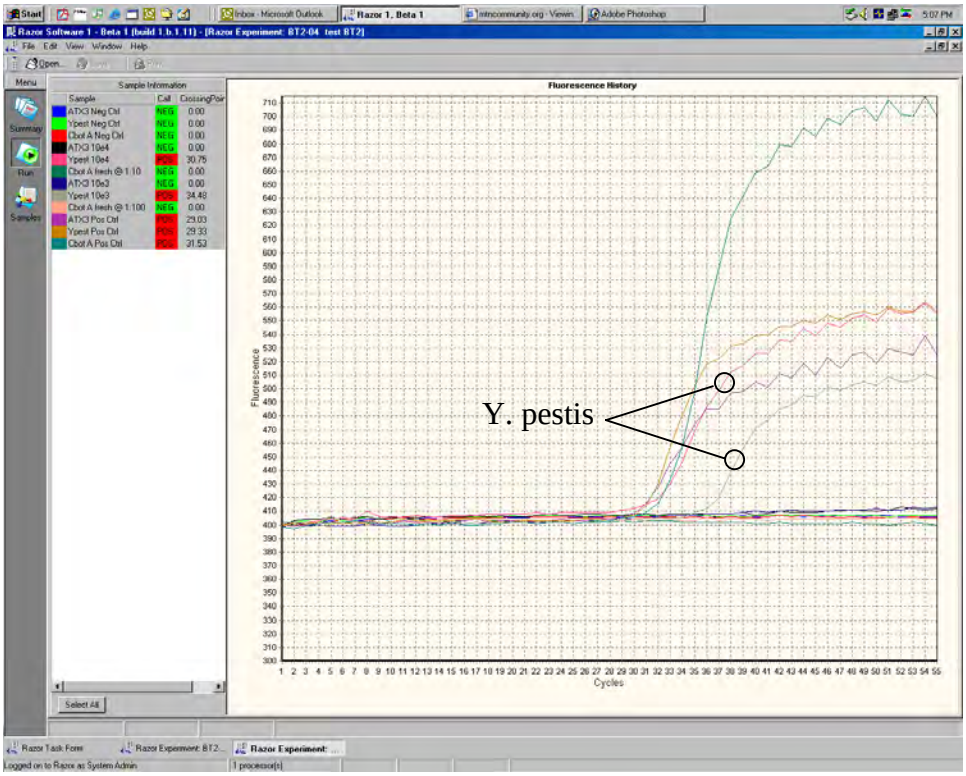
Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	Roche DNA FastStart 10/30	BA 103	UNKN		OK	POS	38.31
A2	Roche DNA FastStart 10/30	BA 104	UNKN		OK	POS	33.68
A3	Roche DNA FastStart 10/30	BA 105	UNKN		OK	POS	31.39
A4	Roche DNA FastStart 10/30	YP 103	UNKN		OK	POS	36.78
A5	Roche DNA FastStart 10/30	YP 104	UNKN		OK	POS	32.82
A6	Roche DNA FastStart 10/30	YP 105	UNKN		OK	POS	30.12
A7	Roche DNA FastStart 10/30	FT 103	UNKN		OK	POS	31.61
A8	Roche DNA FastStart 10/30	FT 104	UNKN		OK	POS	28.65
A9	Roche DNA FastStart 10/30	FT 105	UNKN		OK	POS	24.74
A10	Roche DNA FastStart 10/30	16s BA103	UNKN		OK	POS	29.34
A11	Roche DNA FastStart 10/30	16S BA104	UNKN		OK	POS	29.11
A12	Roche DNA FastStart 10/30	16S BA 105	UNKN		OK	POS	28.75
A13	Roche DNA FastStart 10/30	16S YP103	UNKN		OK	POS	29.12
A14	Roche DNA FastStart 10/30	16S FT 103	UNKN		OK	POS	28.8
A15	Roche DNA FastStart 10/30	16S YP 104	UNKN		OK	POS	27.88
A16	Roche DNA FastStart 10/30	16S YP 105	UNKN		OK	POS	25.28
B1	Roche DNA FastStart 10/30	16S FT 104	UNKN		OK	POS	26.92
B2	Roche DNA FastStart 10/30	16S FT 105	UNKN		OK	POS	23.63

Run 5
Biothreat Screen 2 was used.

The test was done in real-time mode (connected to a PC).

Three samples were tested – A true unknown *Bacillus* from a real world sample, a fresh preparation of *C. botulinum* organism from the same source as the *C. botulinum* toxin from the previous day, and a fresh dilution of *Y. pestis* with a starting concentration of 10e5 CFU/ml. The sample preparation kit was used and the samples were run at 1:10 and 1:100 dilutions.

The data from the RAZOR is presented in the screen shot below. Both of the samples from the 10e4 and 10e3 CFU/ml dilutions were positive for *Y.pestis*. This coincides with Idaho Tech's predicted LOD for the reagents. The unknown Bacillus was negative for all three tests and the fresh preparation of the previously noted *C. botulinum* Type A also did not react giving a negative result.



Crossing points for the reactions were as follows –

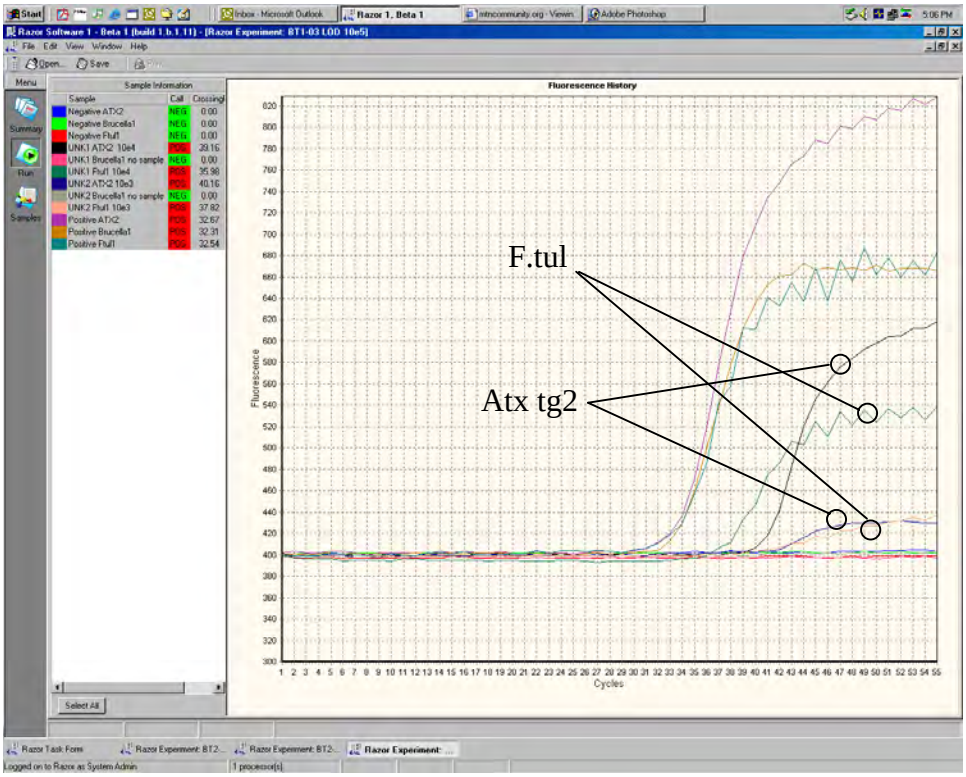
Target	CFU/ml	Crossing point
Y. pestis	10e4	30.75
	10e3	34.48

Run 6
Biothreat Screen 1 was used.

The test was done in real-time mode (connected to a PC).

A mixture of wild type Anthrax and *F. tularensis* at 10e5 cfu/ml each was used for this experiment. The samples were diluted 1:10 and 1:100.

The data from the RAZOR is presented in the screen shot below. Both Anthrax and *F. tularensis* were detected at the 10e4 and the 10e3 CFU/ml concentrations.



Crossing points for the reactions were as follows –

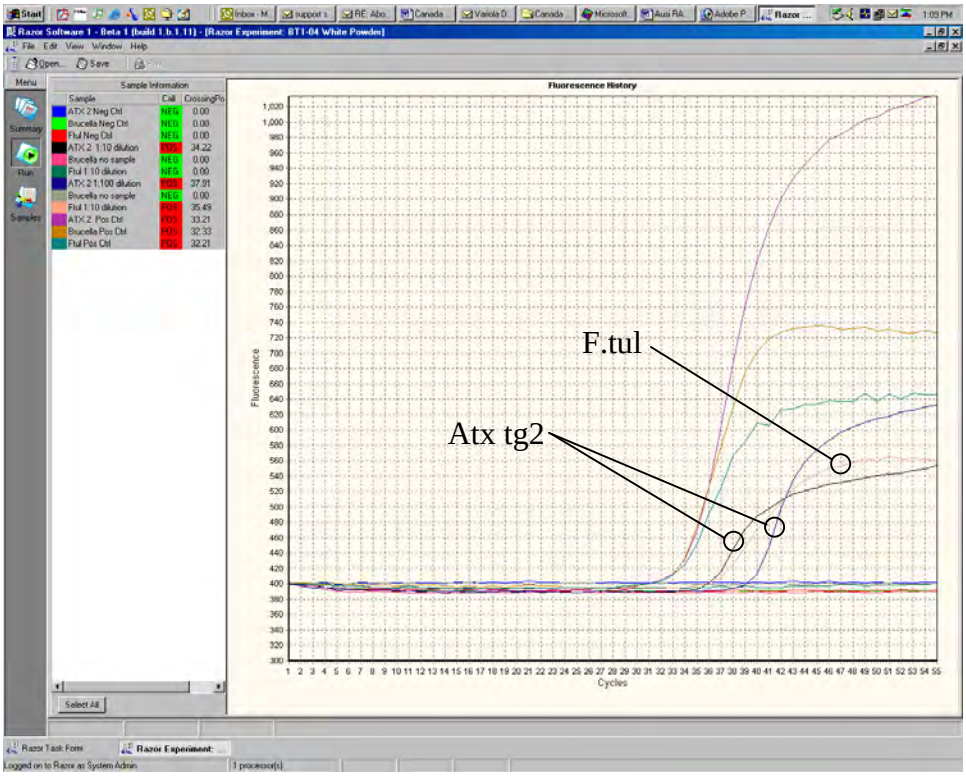
Target	CFU/ml	Crossing point
Anthrax tg2	10e4	39.16
	10e3	40.16
F. tularensis	10e4	35.98
	10e3	37.82

Run 7
Biothreat Screen 1 was used.

The test was done in real-time mode (connected to a PC).

A mixture of wild type Anthrax and *F. tularensis* at 10e7 cfu/ml each was used for this experiment. This mixture was spiked into an uncharacterized white powder with no final concentration per gram of white powder determined. A cloudy mixture was prepared and diluted using the sampling kit. The samples were diluted 1:10 and 1:100.

The data from the RAZOR is presented in the screen shot below. Both Anthrax and *F. tularensis* were detected at the 1:100 dilution. Only Anthrax was detected at the 1:10 dilution.



Crossing points for the reactions were as follows –

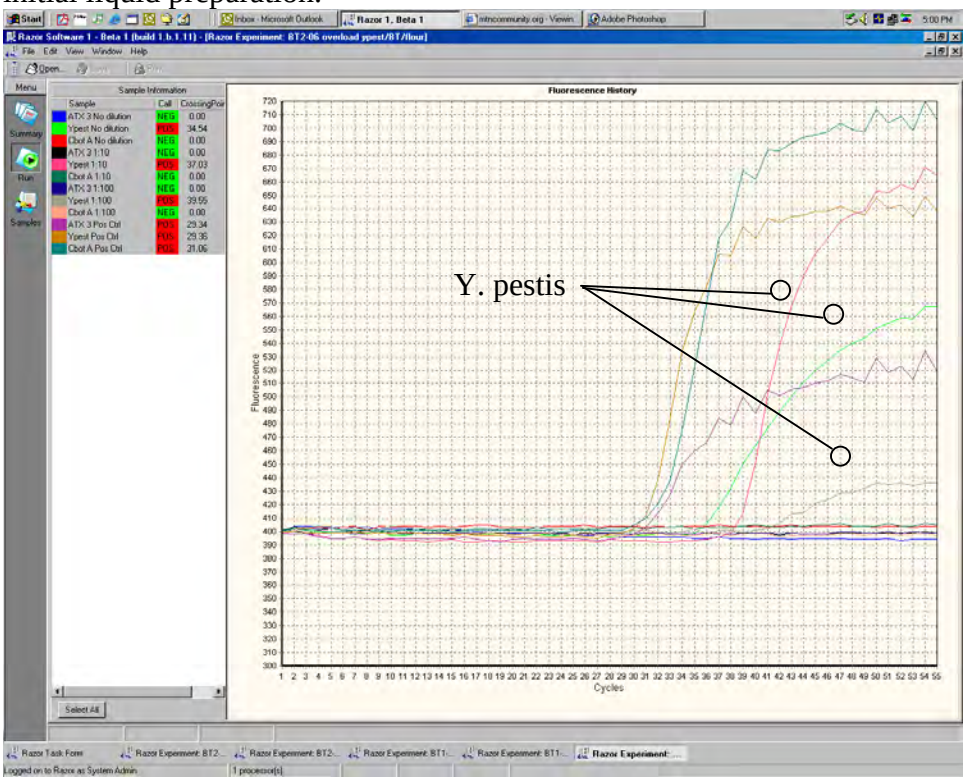
Target	CFU/ml	Crossing point
Anthrax tg2	1:10	34.22
	1:100	37.91
F. tularensis	1:100	35.49

Run 8
Biothreat Screen 2 was used.

The test was done in real-time mode (connected to a PC).

A mixture of powdered *Bacillus thuringensis*, baking flour, and *Yersinia pestis* spiked into the mixture at 10e7 cfu/ml with no final concentration per gram of white powder determined. A cloudy mixture was prepared by taking an excessive amount of the mixture with a sampling swab and placing it into a sample vial from the sampling kit. This liquefied sample was then diluted using the sampling kit. The samples were diluted 1:10 and 1:100. In this experiment the negative control was used as an unknown for the first liquid preparation. The dilutions were used as unknowns one and two.

The data from the RAZOR is presented in the screen shot below. *Y. pestis* was detected at all three concentrations including the extremely "raw" initial liquid preparation.



Crossing points for the reactions were as follows –

Target	CFU/ml	Crossing point
Y. pestis	"X"	34.54
	1:10	37.03
	1:100	39.55

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

Additional Information:

Address: 390 Wakara Way, Salt Lake City, Utah 84108 USA
Phone: 1-800-735-6544 or + 801-736-6354
Email: it@idahotech.com
Website: www.idahotech.com