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

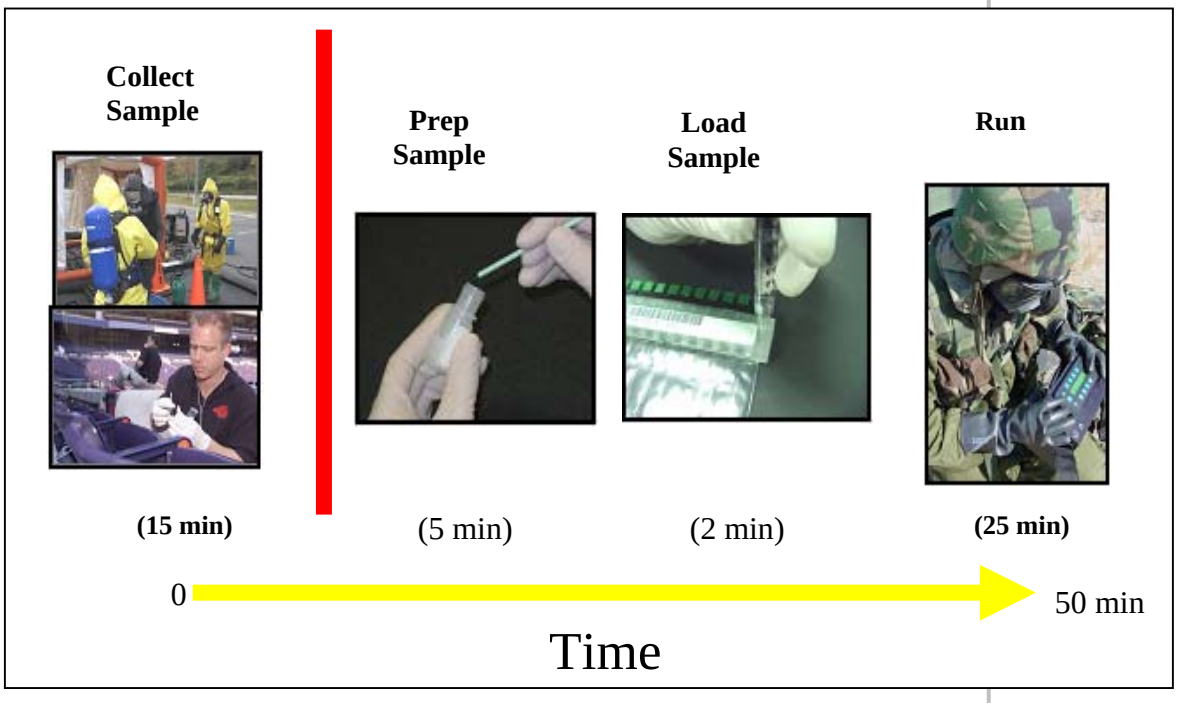
APPLICATION ::: NOTE

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* (source of Botulism toxin).

The demonstrations and testing took place on two separate days in two separate locations: Monday March 21st, 2005 at the Defense Science and Technology Organization (DSTO) in Melbourne, AU and on Wednesday March 23rd, 2005 at the Queensland Health – Pathology and Scientific Services laboratory in Brisbane, AU.

General workflow for the RAZOR is highlighted in the following diagram. All tests for this study were conducted in a laboratory setting.



Results

DSTO testing March 21st – Melbourne

The first agent test was done at the DSTO on DNA originally obtained from the Queensland Health lab. The two DNA samples were for *Bacillus anthracis* (Anthrax) at two different concentrations. A 45 cycle default protocol was used to analyze the two samples on the RAZOR™ instrument which was run independently of a PC.

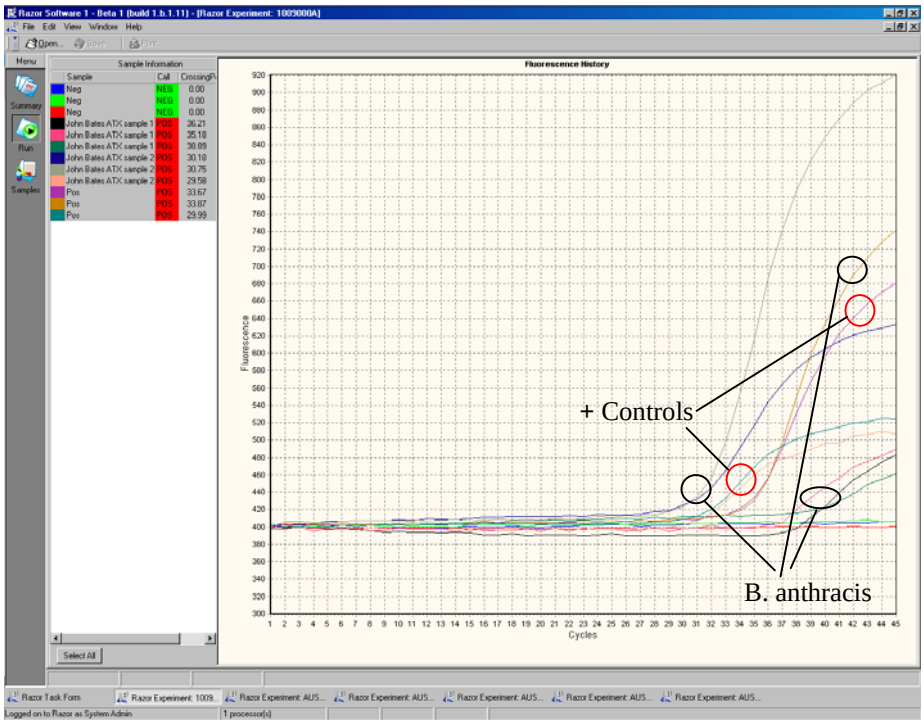
There was no sample preparation for this experiment. Samples were taken straight from the provided vials with a syringe and loaded into the reagent pouch.

A three Target *B. anthracis* Pathfinder™ Pouch Reagent kit (lot# P053033) 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 for Anthrax (two unknown inlet ports, one negative control port, and one positive control port). Results are shown below. Both samples were identified as positive by the RAZOR instrument.

RAZOR run name

RAZOR calls for 1009000A

Samples 1-3 are negative controls, samples 4-6 is the first *B. anthracis* DNA solution tested for three different gene targets, samples 7-9 is the second *B. anthracis* DNA solution tested for three different gene targets, and samples 10-12 are the positive controls.



Screen shot of downloaded data from the RAZOR™ software.

Queensland Health – Testing March 23rd - Brisbane

Testing in Brisbane was done on heat-inactivated agent (outside the PC3/BSL3) with the intent to identify samples from culture for *Bacillus anthracis*, *Yersinia pestis*, *Clostridium botulinum*, and *Fransicella tularensis*. Other strains of closely related bacteria were also tested for cross-reactivity.

For all subsequent tests, three straight culture samples previously taken from a plate and suspended in water were run in each test (test with the available test slots were run at multiple dilutions). No other sample preparation was performed. A 55 cycle protocol was used to analyze the three samples. All tests were run with the RAZOR™ in real-time configuration attached to the laptop and RAZOR software while running. All sample information was blinded until after the test was run.

Austest1

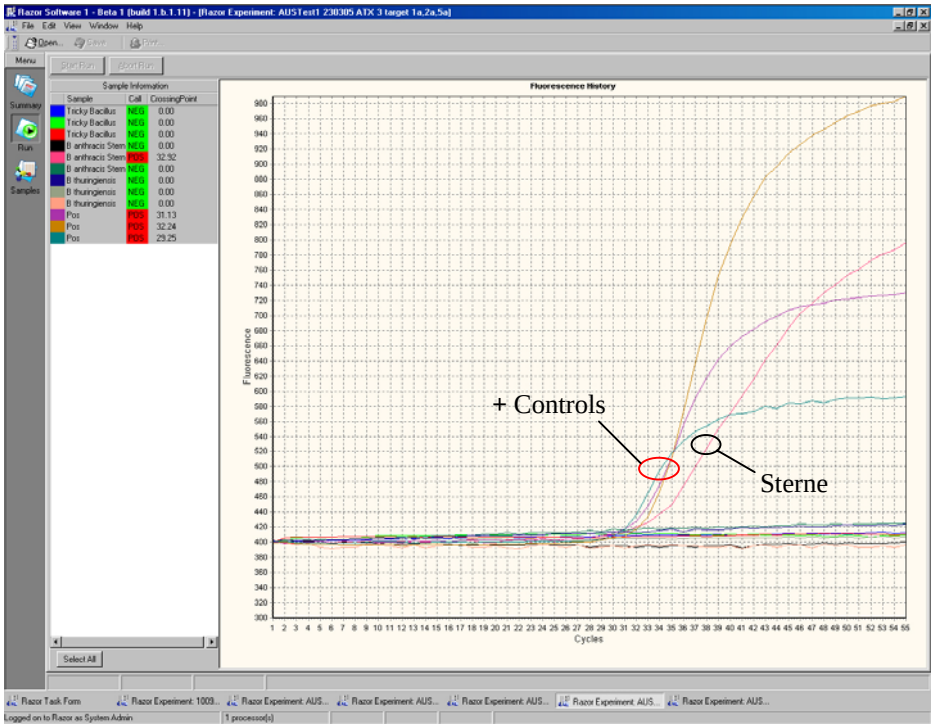
Test1 was for the presence of *Bacillus anthracis* using a three Target *B. anthracis* Pathfinder™ Pouch Reagent kit 4 x 3 (lot# P053033). The three samples were 5A "Tricky" bacillus (an aberrant bacillus strain grown by the lab), 1A *B.anthracis* Sterne strain, and 2A *B.thuringiensis* on the RAZOR instrument. Pouches were loaded using 1 ml syringes loading approximately 400ul of the samples into each inlet port and run. Each sample is channeled to three freeze-dried reagent channels in the Pathfinder pouch – each one a different gene target for Anthrax (two unknown inlet ports, one negative control port, and one positive control port). Results are shown below.

RAZOR run name

RAZOR calls for AUSTEST1

No negative control was run in this set. Samples 1-3 are 5A Tricky Bacillus, samples 4-6 is 1A *B.anthracis Sterne* strain tested for three different gene targets, samples 7-9 is the second 2A *B.thuringiensis* tested for three different gene targets, and samples 10-12 are the positive controls.

It was expected that two of the three gene targets for the Sterne strain would be positive. Only one target was positive. It is suspected that the samples were of very high concentration of raw bacteria (they were a cloudy bacterial suspension) and inhibited one of the reactions. This also explains the late cycle threshold for the second gene target in the presence of such a high concentration of agent. This would be mitigated by a dilution of the sample, but was not done at this time due to time constraints. This was the only sample to see such inhibition and give an unexpected result.



Screen shot of downloaded data from the RAZOR™ software.

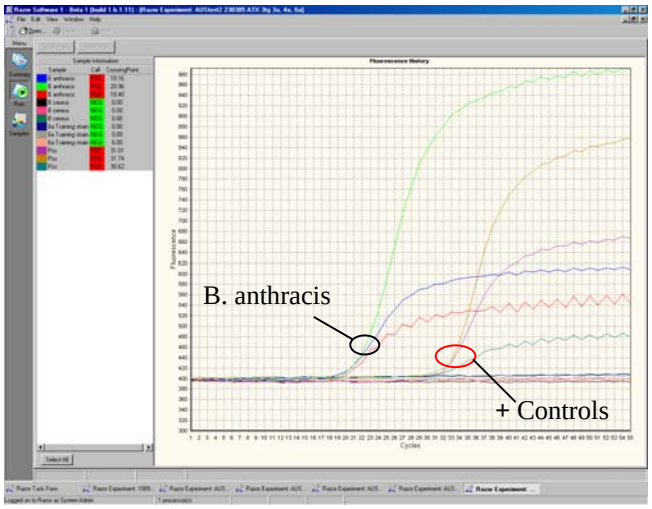
Austest2

Test2 was for the presence of *Bacillus anthracis* using a 3 Target *B. anthracis* Pathfinder™ Pouch Reagent kit (lot# P053033). The three samples were 3A *Bacillus anthracis* (Anthrax), 4A *Bacillus cereus*, and 6A an unidentified training strain reported to contain only the PXO2 plasmid on the RAZOR™ instrument. Pouches were loaded using 1 ml syringes loading approximately 400ul of the samples into each inlet port and run. Each sample is channeled to 3 freeze-dried reagent channels in the Pathfinder pouch – each one a different gene target for Anthrax (2 unknown inlet ports, 1 negative control port, and 1 positive control port). Results are shown below.

RAZOR run name

RAZOR calls for AUSTEST2

No negative control was run in this set. Samples 1-3 are 3A *Bacillus anthracis* (Anthrax), samples 4-6 is 4A *Bacillus cereus* tested for 3 different gene targets, samples 7-9 is 6A the unidentified training strain tested for 3 different gene targets (this was reported to be of very low concentration from a previous test done by the lab on a gel), and samples 10-12 are the positive controls. 3A *Bacillus anthracis* (sample #s 1-3) was the only sample to test positive with these reagents.



Screen shot of downloaded data from the RAZOR software.

Austest3

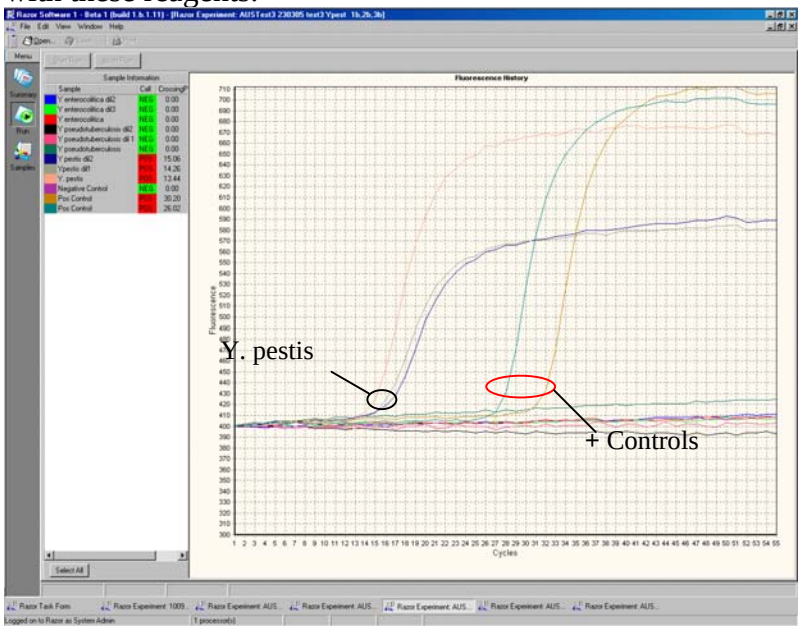
Test3 was for the presence of *Yersinia pestis* (Plague) using a 12 x 1 custom configuration pouch (Lot# P053036) against a single gene target for *Yersinia pestis*. Samples 1B *Yersinia pestis*, 2B *Y. pseudotuberculosis*, and 3B *Y. enterocolitica* were tested for cross reactivity.

Each sample was run three times at three concentrations from the culture suspension in water (No dilution, approx 1:1 and approximately 1:2 diluted with water).

RAZOR run name

RAZOR calls for AUSTEST3

Samples 1-3 are the 3 different dilutions of 3B – *Y. enterocolitica*, samples 4-6 are the 3 different dilutions of 2B – *Y. pseudotuberculosis*, samples 7-9 are the 3 different dilutions of 1B – *Y. pestis*, sample 10 is a negative control 11 and 12 are the positive controls at two different concentrations. 1B – *Yersinia pestis* (sample #s 7-9) was the only sample to test positive with these reagents.



Screen shot of downloaded data from the RAZOR™ software.

Austest4

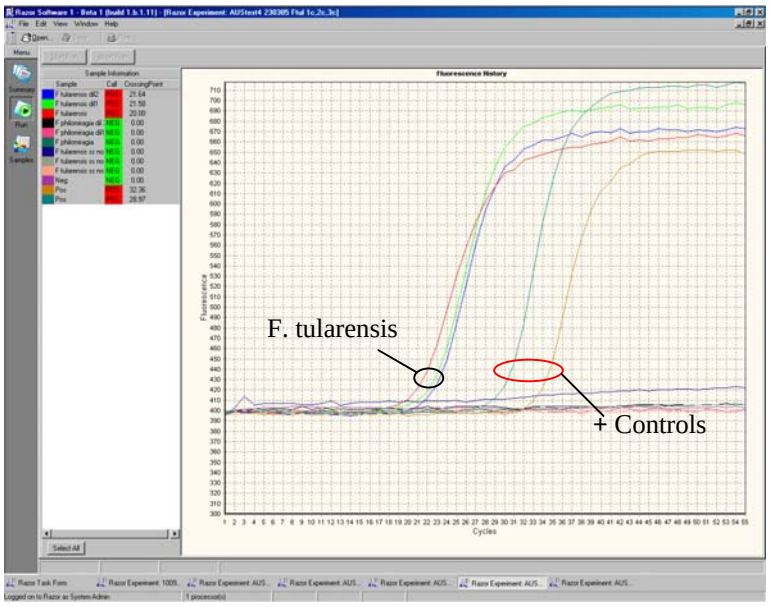
Test4 was for the presence of *Fransicella tularensis* (Tularemia) using a 12 x 1 custom configuration pouch (Lot# P053035) against a single gene target for *Fransicella tularensis*. Samples 1C - *F. tularesnsis* ss novicida (a non-virulent lab strain), 2C - *F. philomiragia*, and 3C - *F. tularensis* were tested for cross-reactivity.

Each sample was run three times at three concentrations from the culture suspension in water (No dilution, approx 1:1 and approximately 1:2 diluted with water).

RAZOR™ run name

RAZOR calls for AUSTEST4

Samples 1-3 are three different dilutions of 3C *F. tularensis*, samples 4-6 are three different dilutions of 2C *F. philomiragia*, samples 7-9 are three different dilutions of 1C *F. tularesnsis* ss novicida, sample 10 is a negative control 11 and 12 are the positive controls at two different concentrations. 3C – *Fransicella tularensis* (sample #s 1-3) was the only sample to test positive with these reagents.



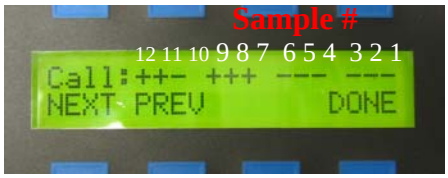
Screen shot of downloaded data from the RAZOR software.

Austest5

Test5 was for the presence of *Clostridium botulinum* typeA using a 12 x 1 custom configuration pouch (Lot# P053034) against a single gene target for *Clostridium botulinum*. Samples 1D – *C. botulinum*, 2D – *C. perfringens*, and 3D – *C. sporogenes* were tested for cross reactivity.

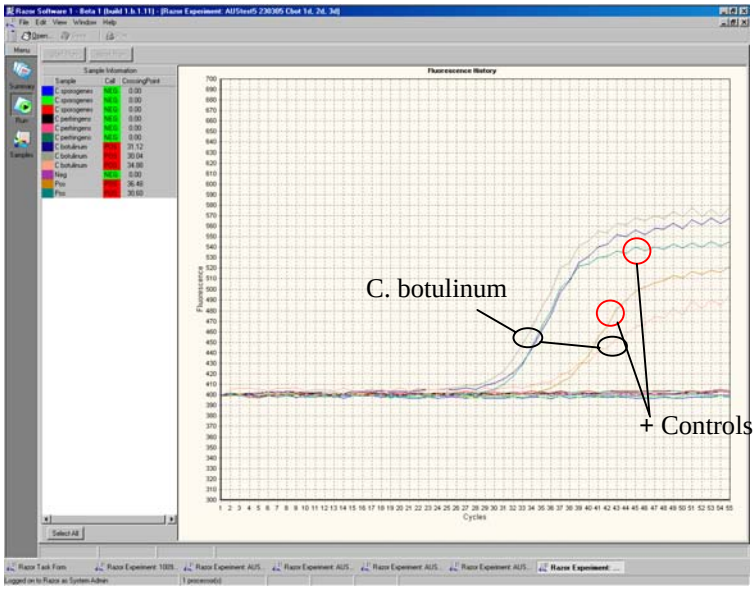
Each sample was run three times at three concentrations from the culture suspension in water (No dilution, approx 1:1 and approximately 1:2 diluted with water).

RAZOR run name



RAZOR calls for AUSTEST5

Samples 1-3 are three different dilutions of 3D – *C. sporogene*s, samples 4-6 are three different dilutions of 2D – *C. perfringens*, samples 7-9 are three different dilutions of 1D – *C. botulinum*, sample 10 is a negative control 11 and 12 are the positive controls at two different concentrations. 1D – *C. botulinum* (sample #s 7-9) was the only sample to test positive with these reagents.



Screen shot of downloaded data from the RAZOR™ software.

Conclusion

Most every aspect of the trials went as expected. There was good correlation between raw bacterial cultures and results by PCR with little or no sample preparation other than dilution. The reagents designed to detect *Bacillus anthracis* (Anthrax), *Fransicella tularensis* (Tularemia), *Yersinia pestis* (Plague), and *Clostridium botulinum* (source of Botulism toxin) reacted with the expected agents and showed no cross reactivity with the closely related organisms used in this study.

ITI would like to thank all those who participated in this study (without naming them specifically). 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