Development of High Volume Reagent Kits for Idaho Technology's R.A.P.I.D.® LT Food Security System to Increase Sample Throughput

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ABSTRACT

Introduction and Rationale:
The Idaho Technology (IT) Food Security System (FSS) is a rapid detection method for food pathogens that combines sensitive, rapid, real-time polymerase chain reaction (PCR) and minimal sample preparation. Three assays for detection of *E. coli* O157:H7, *Salmonella*, and *Listeria* in food and environmental samples previously received AOAC RI PTM approval. These kits were originally packaged in a Low Volume (LV) format. LV reagent vials are designed to test a single sample, which has many benefits in low throughput settings, but becomes difficult to use when testing up to 32 samples at a time.

To address throughput concerns, IT developed High Volume (HV) reagent kits for use in high throughput food testing laboratories. HV kits were developed by minor volume modifications to the existing LV kits. These changes were subjected to validation to ensure that HV and LV kits achieve the same sensitivity and provide the same results. Results of this validation will be presented. Along with development of the HV reagents, a Workflow Center was developed to organize the preparation and setup process.

Materials and Methods:
HV kits were developed to contain the same reagent chemistry. Higher throughput was achieved by increasing the number of reactions in a reagent vial (from one to eight) and decreasing the reaction volume. The reaction volume decrease was tested extensively to ensure that the lower volume did not lead to reduced sensitivity. Much of the initial feasibility work for the High Volume kits focused on evaluating the performance of freeze-dried reactions run at the lower reaction volume with all three assays (*E. coli* O157:H7 LT, *Salmonella* LT, and *Listeria* LT). Performance was measured by comparing crossing points and maximum fluorescence values, as well as detection. Assay sensitivity, using purified nucleic acid, was evaluated for each assay target in the HV format. Finally, studies comparing HV and LV reactions were performed using live organisms inoculated at a fractional level in food matrices to evaluate the overall system performance.

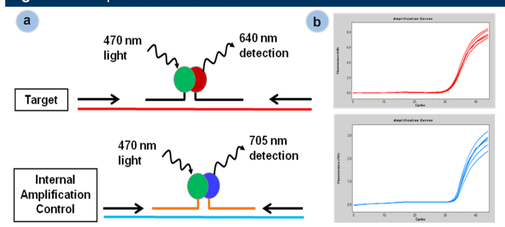
Results and Discussion:
HV kits detected samples spiked at fractional levels of organism as well as LV kits. Also, HV kits performed within the acceptable ranges defined for crossing point and maximum fluorescence using both purified nucleic acid and live organism. Results demonstrate that the HV kit format is as sensitive as the LV kit format.

Conclusion:
The new HV kits have the ability to test up to 8 samples from a single vial. In a HV kit, 160 samples can be tested. The High Volume kits reduce packaging and when used in conjunction with the Workflow Center can significantly reduce sample setup time and increase sample throughput. Results were reviewed by AOAC and HV kits have been included in the AOAC RI PTM approval. The High Volume kits are ideal for use in laboratories with a high sample throughput.

BACKGROUND

The R.A.P.I.D. LT Food Security System (FSS) is a PCR-based pathogen detection method used to detect pathogens from enriched food samples. In general, the method involves enriching a sample for a specified amount of time in commercially available media, performing mechanical cell lysis to release the DNA, rehydration of freeze-dried PCR reagents, DNA amplification and melting peak analysis in the R.A.P.I.D. LT instrument using glass capillaries, and automated data and results interpretation by the R.A.P.I.D. LT software.

Figure 1: Multiplex PCR detection Schematic



Sequence specific primers are used to amplify the target and internal amplification control DNA. Hybridization probes, utilizing fluorescence resonance energy transfer (FRET) chemistry, add specificity to the real-time PCR assay and are used for fluorescent detection of the amplified target and internal amplification control DNA at 640 nm and 705 nm respectively. a) Multiplex PCR b) Fluorescence at 640 nm vs. cycles and Fluorescence at 705 nm vs. cycles

ACCESSORIES

In an effort to accommodate the increased throughput that HV kits will allow, a Workflow Center and carousel centrifuge have been developed. The Workflow Center was designed to organize the reagents needed for 8 individual samples by holding 8 bead tubes, used for mechanical lysis, and 8 capillaries, used for PCR in the R.A.P.I.D. LT. The Workflow Center makes loading and unloading the Disruptor Genie™ simple, by transferring all 8 bead tubes at the same time using a single bead tube disk. Bead tube disks are also conveniently numbered to eliminate the need for labeling. Following the mechanical lysis step, the bead tubes can be transferred and spun in the carousel centrifuge. The carousel centrifuge enables the user to spin up to 16 bead tubes at one time.

Additional spaces have been provided in the Workflow Center to track each sample as it is added to the capillary. Full rotors of capillaries can then be spun in the carousel centrifuge prior to loading the R.A.P.I.D. LT instrument. Used together, these accessories help to organize samples, minimize sample set-up time and reduce capillary handling.

FEASIBILITY

Development of HV kits required evaluating smaller reaction volumes. Feasibility work comparing freeze-dried reagents run in HV (blue) and LV (green) formats were performed using fractionally inoculated food samples for the *Salmonella* LT, *Listeria* LT and *E. coli* O157:H7 LT kits. To do this, a fractional screen was performed and evaluated for the sample with the latest crossing point (Cp) and lowest maximum fluorescence (Fmax) values. This sample was then run on a split rotor in either HV (blue) and LV (green) format. These results are shown in Figure 2. These results show that samples spiked at fractional levels of organism can be detected equally well in either format.

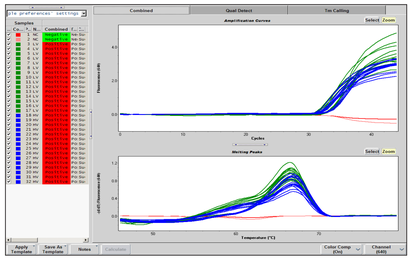


Figure 2A: ***Salmonella* LT**: Lettuce inoculated with *Salmonella* Sar A4 (150µL 10^{-8} =>~0.33 CFU/25 g), incubated in Lactose Broth at 37°C for 16 hours.

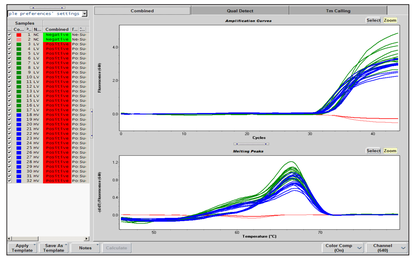


Figure 2B: ***Listeria* LT:** Turkey Deli Meat inoculated with *Listeria monocytogenes* ATCC 13932 (80µL 10^{-8} =>~3.33 CFU/25g), equilibrated at 4°C for ~ 48 hours, incubated in Oxoid ONE Broth at 30°C for ~26 hours.

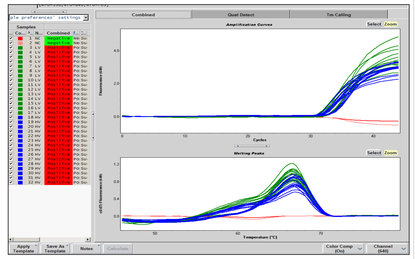


Figure 2C: ***E. coli* O157:H7 LT:** Spinach inoculated with *E. coli* O157:H7 ATCC 43895 (75µL 10^{-8} =>~0.33 CFU/25g), incubated in BPW at 42°C for 8 hours.

DEVELOPMENT

Assay sensitivity for each assay was evaluated in the HV format (eight 10 µL reactions per freeze-dried tube) using purified nucleic acid. The data for the limit of detection (LOD) evaluation is shown below for the *Salmonella* LT, *Listeria* LT and *E. coli* O157:H7 LT assays. The LOD is defined at 85% success and 90% confidence. The sensitivity of the assays in HV format was equivalent to the sensitivity in the LV format. (Low volume data not shown.)

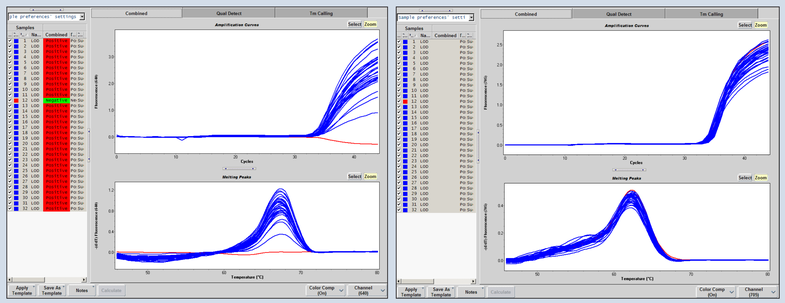


Figure 3A: *Salmonella* LT Lot # 242509 1x LOD

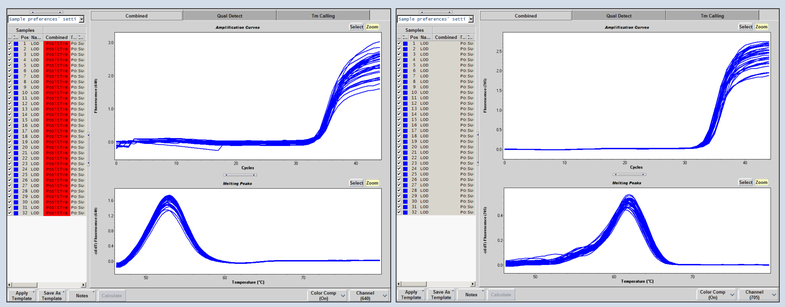


Figure 3B: *Listeria* LT Lot # 214309 1x LOD

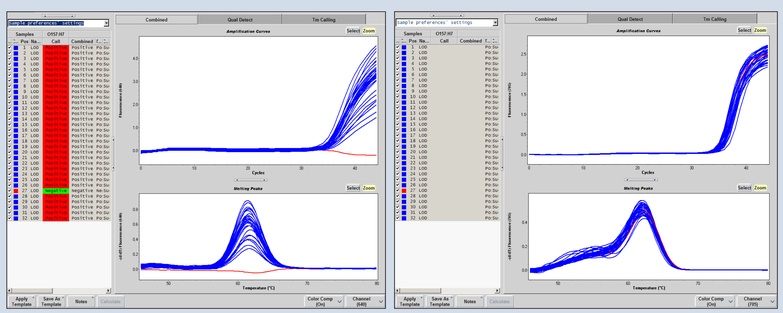


Figure 3C: *E. coli* O157:H7 LT Lot # 271609 1x LOD

FULL SYSTEM VERIFICATION

Fractional testing of food samples, using HV kits, was performed to evaluate the sensitivity of each assay at low inoculum levels. Raw chicken, Mexican-style soft cheese, and raw ground beef samples were inoculated at a fractional level to give 3–7 positive results out of 10 samples. Samples were enriched according to the AOAC PTM approved protocols appropriate for each assay. Samples were then tested using the appropriate HV kit. Samples were confirmed by standard plating methods and McNemar's chi square (χ^2) statistical analysis was applied to each data set. Representative results for select assays are summarized in Table 1 and shown in Figure 4.

Table 1

			Test Kit			Test Kit Performance			
Matrix	**Inoculating organism**	**No. test portions for each method**	**Presumptive Positive**	**Confirmed Positive**	**χ^2**	**Sensitivity**	**Specificity**	**False negative**	**False positive**
Fresh raw chicken	*Salmonella* Typhimurium Sar A4	10	5	5	0	100	100	0	0
Raw ground beef	*E. coli* O157:H7 EDL-933	10	3	3	0	100	100	0	0

Figure 4A: ***Salmonella* LT HV:** Fresh raw chicken inoculated with *Salmonella* Sar A4 (200µL 10^{-8} =>~0 CFU/25g), incubated in BPW at 37°C for ~16 hours. Samples were tested using *Salmonella* LT HV lot #242509. Samples transferred to Tetrathionate broth after 24 hours incubation then streaked to XLD after 24 hours of incubation.

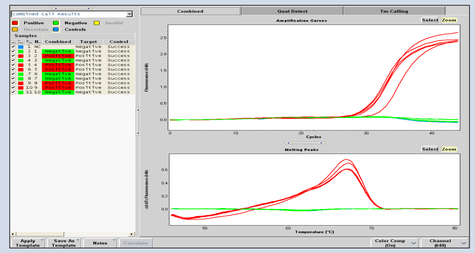
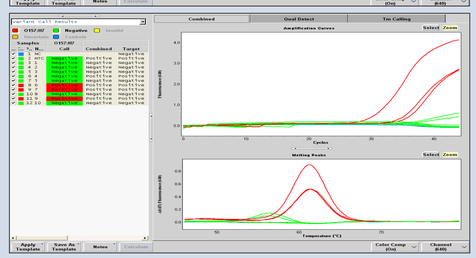


Figure 4B: ***E. coli* O157:H7 LT HV:** Raw ground beef inoculated with *E. coli* O157:H7 EDL-933 (~0.3 CFU/25g), incubated in BPW at 42°C for ~8 hours. Samples were tested using *E. coli* O157:H7 LT HV lot #271609. Samples streaked to CT-SMAC agar plates after 24 hours of incubation. The positive amplification curves and shifted melting peaks for samples in rotor positions 2 and 6 suggest that the ground beef was naturally contaminated with *E. coli* O157:non H7.



CONCLUSION

- The HV kits are ideal for use in laboratories with a high sample throughput.
- The new HV kits have the ability to test up to 8 samples from a single vial and 1 kit can test 160 samples thus reducing packaging substantially.
- The Workflow Center and carousel centrifuge can significantly reduce sample setup time, increase sample throughput and minimize capillary handling.
- HV kits are approved by the AOAC Research Institute Performance Tested Methods℠ Program.