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. These assays for detection of E. coli O157:H7, Salmonella, and Listeria in food and environmental samples previously received AOAC R.I. PTM approval. These kits were originally packaged in a Low Volume (LV) format. In an effort to reduce setup time and increase sample throughput, development of High Volume (HV) kits with the ability to test up to 8 samples from a single vial was initiated. These kits can test 160 samples thus reducing packaging substantially.

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 reaction vial from one to eight and decreasing the reaction volume. The reaction volume was scaled 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. This screening was performed 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 sensitivity. 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 creating crossing point and maximum fluorescence using both purified nucleic acid and live organism. Results demonstrated 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 and 1 kit can test 160 samples thus reducing packaging substantially. In addition, HV kits were developed to contain the same reagent chemistry. Higher throughput was achieved by increasing the number of reactions in a reaction vial from one to eight and decreasing the reaction volume. The reaction volume was scaled 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. This screening was performed 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 sensitivity. 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.

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 statistical screen was performed and evaluated for the sample with the lowest crossing point (Cp) and lowest maximum fluorescence (Fmax) values. This sample was then run on a split rotor in both 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.

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 specific amount of time in commercially available media, performing mechanical cell lysis to release the DNA, rehybridization 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.

ACCESSORIES

In an effort to accommodate the increased throughput that the 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 tube deck. Blast tube data are also conveniently numbered to eliminate the need for labeling. Following the mechanical lysis step, the bead tube 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.

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.

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 statistical screen was performed and evaluated for the sample with the lowest crossing point (Cp) and lowest maximum fluorescence (Fmax) values. This sample was then run on a split rotor in both 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.

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 listing methods and McFarland in city square (c) dilutional analysis was applied to each data set. Representative results for select assays are summarized in Table 1 and shown in Figure 4.