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APPLICATION NOTE

Sample Pooling Increases Throughput and Decreases Per-Sample Cost

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You no longer have to compromise between speed, accuracy and cost for food safety testing. InstantLabs' pooled sample methodology increases throughput by 400% and decreases costs by 75% - while maintaining the Hunter® platform's renowned speed, accuracy and ease-of-use.

Introduction

Food safety contamination continues to create headlines worldwide and headaches for the companies involved. To address these issues and to protect their brand, food processors seek fast and easy ways to accurately test for dangerous food pathogens at reasonable cost. Traditional plate-based microbiology is accurate and inexpensive, but time is wasted waiting for results. This delays the time-to-market and increases spoilage and storage costs. Real-time polymerase chain reaction (RT-PCR) technology is the undisputed gold-standard for accurate and fast results (hours versus days for traditional plating), but at increased cost.

InstantLabs has developed and tested a simple and efficient sample pooling protocol for use with the Hunter® Real-Time PCR system that increases throughput and reduces testing costs dramatically. Pooling is particularly beneficial when the percentage of contaminated samples is low; as is typical with most food samples. This protocol was tested using InstantLabs' *Salmonella* Species and *E. coli* 0157:H7 Food Safety kits and confirms that even at low levels of inoculation we can accurately detect harmful food pathogens in pooled samples accurately and cost-effectively.

Materials and Methods

Validation of the pooling method was conducted using rigorous microbiology laboratory techniques. Ground beef and ground chicken food samples were tested against two of the most common and prevalent food pathogens; *E. coli* O157:H7 and *Salmonella* species. For each food type and pathogen combination, 16 individual food samples were prepared resulting in a total of 64 samples for enrichment. One out of each set of 16 samples was inoculated with fewer than 10 cells of the target organism. Samples were blind coded and then enriched according to the corresponding InstantLabs Food Safety Pooling protocol. The pooling protocol increases standard enrichment times by one hour to offset any potential reduction in sensitivity caused by dilution of a contaminated sample. Aliquots of each individual enrichment culture were labeled and stored for future analysis.

The 16 enrichment samples resulting from each of the four food type and pathogen combinations tested were randomly combined into 4 sets of 4 samples each. A pooled sample was created by taking 25 µl from each of the four samples in each set. These aliquots were mixed together in a new micro-centrifuge tube. These pooled sets

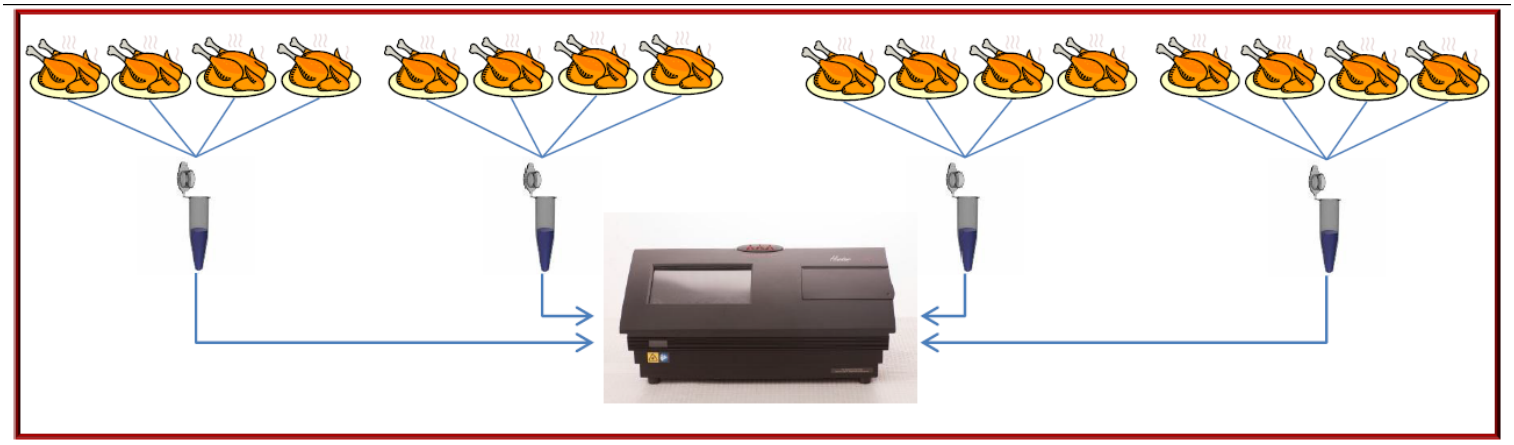

Figure 1. Representation of the pooling approach using four samples per pool.

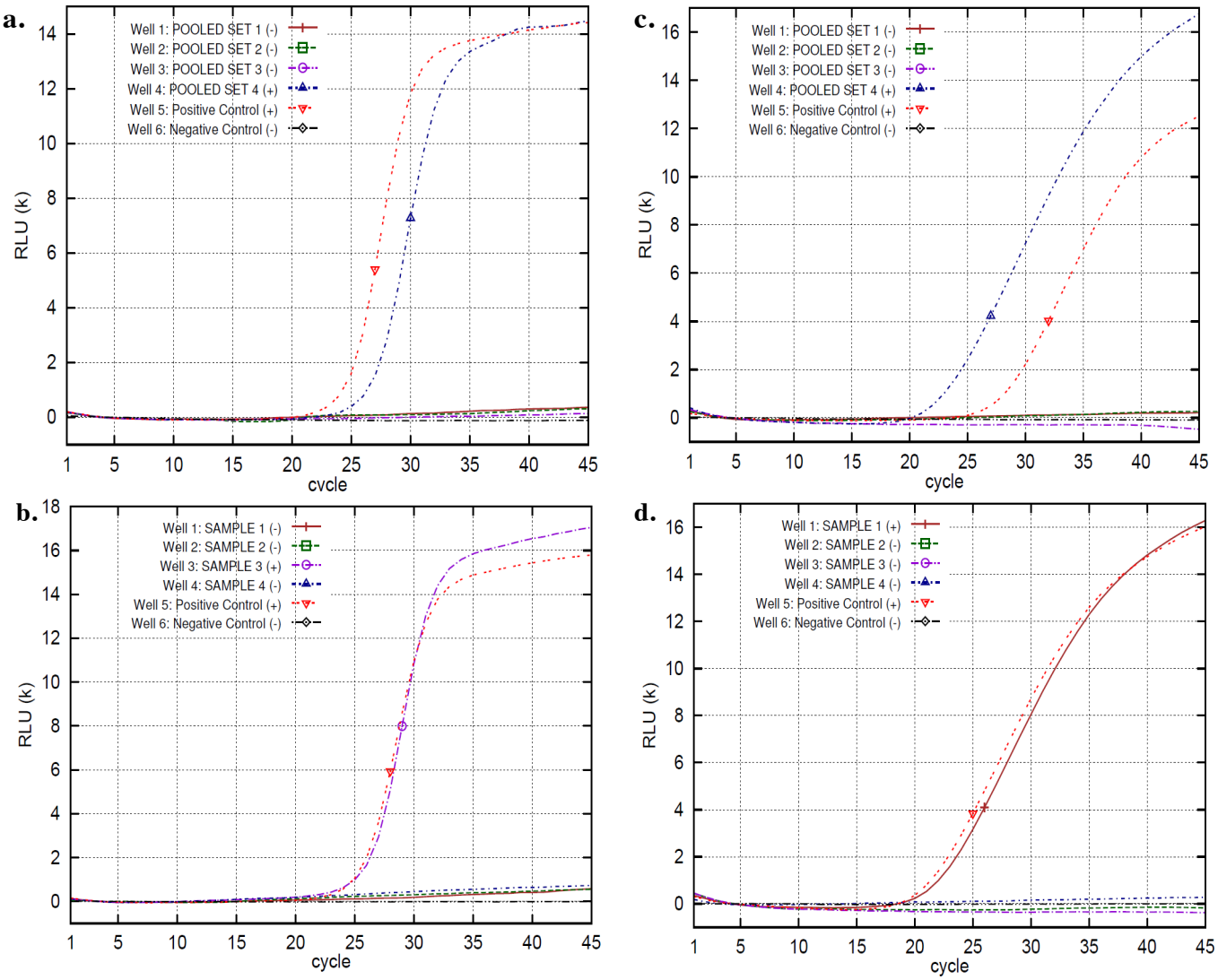

Figure 2. Hunter data for *E. coli* O157:H7 (left) and *Salmonella* species (right) ground beef samples. Data not shown for ground chicken. A) Pooled sets of ground beef enrichments, one of which was inoculated with *E. coli* O157:H7. It was determined that pooled set 4 contained the blind coded positive sample. B) Deconvolution of ground beef pooled set 4 (positive *E. coli* O157:H7), as seen in A. Individual sample 3 of pooled set 4 was positive for *E. coli* O157:H7. C) Pooled sets of ground beef enrichments, one of which was inoculated with *Salmonella* species. It was determined that pooled set 4 contained the artificially inoculated and blind coded positive sample. D) Deconvolution of samples making up ground beef pooled set 4 (*Salmonella* species), as seen in C. After further testing, it was determined individual sample 1 of pooled set 4 was positive for *Salmonella* species.

were then tested using the corresponding InstantLabs Food Safety Kit protocol.

After sample preparation and DNA extraction, the 16 pooled samples were run on the Hunter® RT-PCR device.

Results

One pooled set of samples from each food and pathogen combination tested positive on the Hunter® instrument for the target pathogen. For the ground chicken inoculated with *E. coli* O157:H7, this was pooled set 2, while for the ground chicken inoculated with *Salmonella* species, this was pooled set 3. It was determined that for both the *E. coli* O157:H7 and *Salmonella* species inoculated ground beef, the positive pooled set was pooled set 4.

The samples composing the pooled sets that tested positive were then tested individually on the Hunter instrument to determine the offending "lot". For the ground beef samples, it was determined that samples 3 and 1 were positive for *E. coli* O157:H7 and *Salmonella* species, respectively. For ground chicken, sample 1 of pooled set 2 contained *E. coli* O157:H7 and sample 2 in pooled set 3 was contaminated with *Salmonella* species. See Figure 1 and Tables 1 and 2. The test results correlated perfectly with the expected results following decoding of the blind-coded results. It does not appear that diluting equal parts of 3 negative samples and 1 positive sample in the pooled sets had any effect on the ability of the Hunter system to detect a contaminated sample.

Food-Pathogen	Hunter® Pooled Well Result					
	1	2	3	4	Negative Control	Positive Control
Ground Chicken - *E. coli* O157:H7		+			-	+
Ground Chicken - *Salmonella* species			+		-	+
Ground Beef - *E. coli* O157:H7				+	-	+
Ground Beef - *Salmonella* species				+	-	+

Table 1. Pooled sample runs for each of the tested food type and pathogen combination resulted in one and only one positive well.

Food-Pathogen-Pooled Sample	Hunter® Individual Well Result					
	1	2	3	4	Negative Control	Positive Control
Ground Chicken - *E. coli* O157:H7 – Well 2	+				-	+
Ground Chicken - *Salmonella* species – Well 3		+			-	+
Ground Beef - *E. coli* O157:H7 – Well 4			+		-	+
Ground Beef - *Salmonella* species – Well 4	+				-	+

Table 2. Each pooled set that tested positive was deconstructed to determine which individual sample within the set was contaminated.

Discussion

The experimental outcomes coincide with the expected results. Pooling increased throughput and had no negative effect on sensitivity.

Sample pooling is a simple, effective, time- and cost-saving technique that food quality professionals can employ to increase testing throughput. This protocol is especially useful when few samples will be contaminated. This allows for more efficient and effective clearance of food production lots. When combined with the InstantLabs Food Safety Kit rapid PCR test protocol, results are obtained in a total of 10 - 24 hours. This fast and easy testing technique can decrease the amount of time that food products spend in the warehouse, reducing cost and bringing fresher foods to the supermarket for the consumer without sacrificing safety or quality.

ORDERING INFORMATION

Catalog #	Item
9034-0090-0000	Hunter® Accelerated PCR Instrument
9034-0600-0003-10	InstantLabs® *Salmonella* Species Food Safety Kit
9034-0600-0017-10	InstantLabs® *E. coli* STEC Screening Food Safety Kit
9034-0600-0019-10	InstantLabs® *E. coli* STEC Verification Food Safety Kit
9034-0600-0001-10	InstantLabs® *E coli* 0157:H7 Food Safety Kit
9034-0600-0010-10	InstantLabs® *Listeria monocytogenes* Food Safety Kit
9034-0600-0015-10	InstantLabs® *Listeria* Species Food Safety Kit
9034-0600-0018-10	InstantLabs® *Vibrio* Species Food Safety Kit
9034-0600-0016	InstantLabs® Hunter® Instrument Validation Kit
9034-0700-5192	Buffered Listeria Enrichment Broth (BLEB)
9034-0700-4332	InstantLabs® FASTGRO SE™ Enrichment Broth