

Evaluation of Multiple PCR Assays for the Detection of Bacteria from Liquid Infant Formula

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Introduction

Rapid and reliable detection of potentially pathogenic bacteria from liquid infant formula is vital to ensure safe consumption and rapid product release.

This study evaluated the performance of five PCR assays for the detection of diverse bacteria from two liquid infant formula products, demonstrating that a harmonized sample preparation protocol can be used for *Salmonella*, *Cronobacter* and *Escherichia coli*, saving cost and time to the end user (Figures 2 and 4).

Materials and Methods

The following Thermo Scientific™ PCR kits and bacterial isolates were used in the study:

PCR Assay	To detect...
Thermo Scientific™ SureTect™ Salmonella species PCR Assay	Salmonella Typhimurium
Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit	
Thermo Scientific™ SureTect™ Cronobacter species PCR Assay	Cronobacter sakazakii
Thermo Scientific™ SureTect™ Staphylococcus aureus PCR Assay	Staphylococcus aureus
Applied Biosystems™ TaqMan™ Escherichia spp Assay Beads	Escherichia coli

Performance was assessed by artificially contaminating eighteen 25 mL sample of two liquid infant formula products with 3-7 colony forming units (CFU) of target organisms and conducting their recommended detection workflows.

To assess if a harmonized workflow could be used for four of the PCR assays, the nucleic acid extraction method for the TaqMan E. coli PCR Assay (Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit) (Figure 1) was compared to the SureTect lysis protocol. The SureTect S. aureus PCR Assay was not included in this comparison.

Figure 1: PrepSEQ Rapid Spin Kit (left) and SureTect PCR Assay (right)



Figure 2: Harmonized enrichment and lysis protocol for the SureTect Salmonella spp., Cronobacter spp., and E. coli PCR Assays

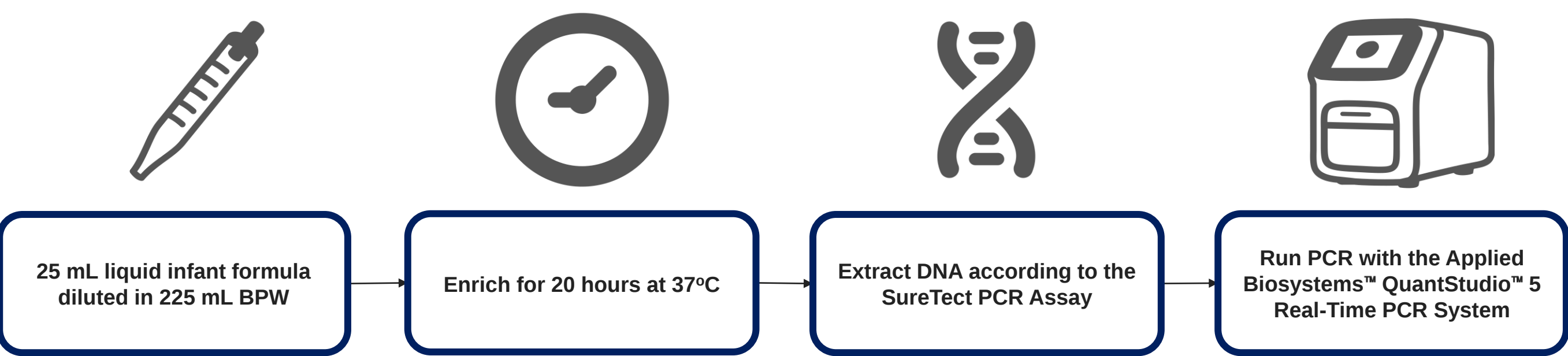
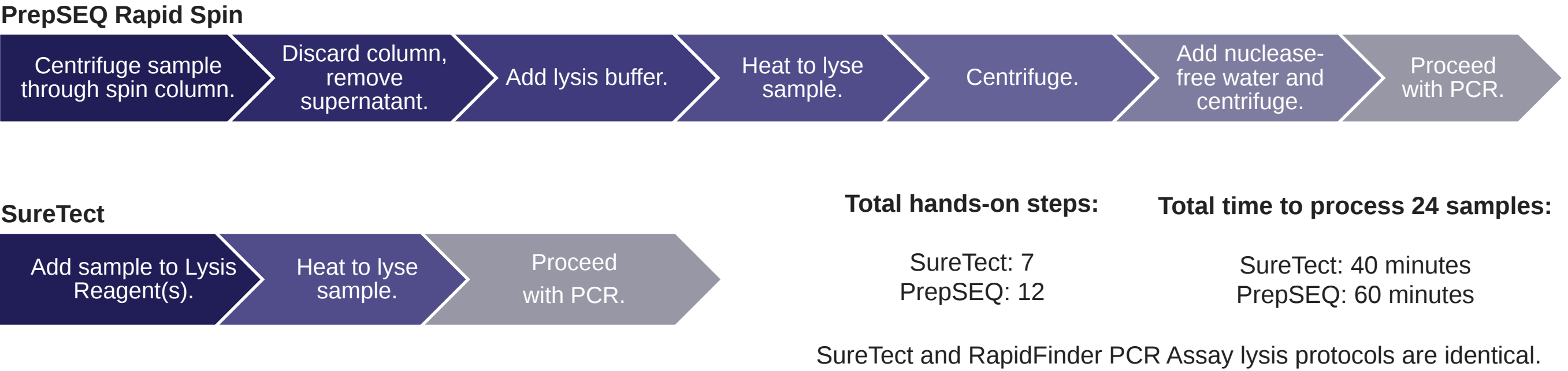


Figure 3: Workflow comparison of PrepSEQ Rapid Spin DNA extraction and SureTect PCR Assay lysis methods



Results

Figure 4: PCR and culture confirmation results

PCR Assay	Enrichment	Lysis Protocol	PCR positive	Culture confirmation
SureTect Salmonella spp.	BPW, 20 hours at 37°C	SureTect	36/36	36/36
RapidFinder Salmonella Multiplex			36/36	36/36
SureTect Cronobacter spp.			36/36	36/36
TaqMan E. coli		PrepSEQ Rapid Spin	36/36	36/36
SureTect Staphylococcus aureus	Giolitti-Cantoni Broth, 22 hours at 37°C	SureTect	36/36	36/36

Results showed that all PCR assays were able to detect the target isolates with 100% of the PCR assay results matching the culture confirmation results (Figure 4).

A harmonized enrichment protocol with BPW demonstrated suitability for four of the PCR assays, saving time and cost (Figure 2). The harmonized lysis workflow for *Escherichia coli* allows for further harmonization opportunity to run this PCR assay on Thermo Scientific™ RapidFinder™ Analysis Software with the other PCR assays.

The SureTect lysis protocol was compatible with the TaqMan E. coli PCR assay, allowing for harmonized enrichment and lysis with the *Salmonella* and *Cronobacter* workflows (Figure 4).

A workflow comparison of the DNA extraction and lysis protocols demonstrated that the SureTect workflow has fewer handling steps and faster time to result (Figure 3).

Both the SureTect and RapidFinder Salmonella PCR Assays had 100% agreement, demonstrating that both PCR Assays can be used for the detection of *Salmonella* from liquid infant formula (Figure 4).

Conclusions

Reliable

- All tested PCR assays can reliably detect low levels of target bacteria from liquid infant formula.

Harmonized

- Harmonized enrichment and lysis protocol for *Salmonella*, *Cronobacter* and *E. coli*, saving time and cost for the end user.

Flexible

- The SureTect Salmonella or the RapidFinder Salmonella multiplex PCR Assay can both be used for the detection of *Salmonella* from liquid infant formula.

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