Food testing

Performance Equivalency and Stability Analysis of Handling Improvements of the Thermo Scientific SureTect PCR Workflow

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Introduction

Real-time PCR detection of foodborne pathogens from food and environmental surfaces is a widely used principle in the food safety industry. The Thermo Scientific™ SureTect™ PCR Assay workflow is a real-time PCR detection method for a number of foodborne pathogens with over 13 assays currently available in the portfolio.

The importance of efficiency in process is a critical factor in food pathogen testing, therefore several handling improvements were identified and analysed for equivalency and stability (detailed below).

Blue dye relocation

- Blue dye currently located in Lysis reagent 1 (pre-filled into SureTect Lysis Tubes, was moved to the Proteinase K reagent to visually aid pipetting. New format shown in figure 2.

Pierceable Lysis Tube seal

- Used to seal the Lysis Tubes to enable transfer of Lysate to the SureTect PCR tubes without the need to uncap to streamline handling.

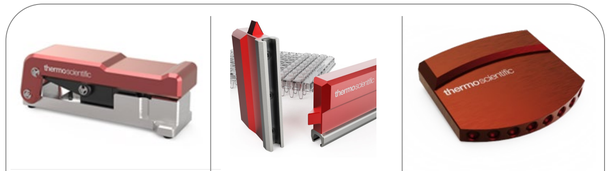
Improved plastics

- Rigid snappable PCR Assay tube frame to improve handling.
- Added color-coding of PCR Assay rube frame and orientation markers as visual aids.

New handling tools

- Cutting Tool compatible with new rigid PCR Assay tube plastic frame, allowing flexibility for number of tubes used.
- New and improved Lysis and PCR tube opening and closing tools.
- Handling tools were not analysed due to no impact to performance. (Shown below in figure 1).

Figure 1. New PCR handling tools



Strip Cutting Tool | **Uncapping Tool** | **Capping Tool**

Method and Results

Figure 2. SureTect PCR Assay workflow Improvement Equivalence and Stability Methods and Results

	Blue dye relocation	Pierceable lysis seal	Improved plastics

Method	Reagent stability testing
	• 37° C and 5° C, 0 weeks to 24 months
	• Two new batches analyzed with 3 assay-matrix combinations
	• Lysis enzymatic activity analysis
		• Equivalency testing with 1 assay-matrix combination
		• Contamination risk testing all 96 wells with
		  • 100 CFU/lysis
		  • 1,000 CFU/lysis
		  • Alternating columns of 0 CFU and 10,000 CFU/lysis
			• Lysis equivalency-11 assay-matrix combinations
			• PCR equivalency-11 assays using 100, 1,000 and 10,000 gDNA.
			• PCR stability testing 37° C and 5° C, 0 weeks to 24 months

Results	Equivalent performance in PCR and enzyme activity across all time points	Equivalent performance. No additional contamination risk.	Results: Equivalent performance for lysis and PCR, across all time points

Methods continued

Studies were designed in accordance with the manufacturing site quality system (ISO 13485:2016 certified) with added enhancements where required (detailed in figure 2). All studies included the following

- Applied Biosystems™ 7500 FAST Real-Time Food Safety PCR System
- Applied Biosystems™ SimpliAmp™ Thermal Cycler
- Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System

Acceptance criteria was based on the current variation between SureTect Assays (Ct value ±1.5 and dRn at ±50%).

Conclusion

The data show the SureTect PCR Assay workflow improvements offer increased efficiency and a reduced handling steps with no impact to performance or stability. SureTect PCR Assay workflow improvements have been certified by the following

- AOAC *Performance Tested Methods*℠
- AOAC *Official Method of Analysis*℠
- ISO 16140-2:2016 by AFNOR Certification
- ISO 16140-2:2016 by MicroVal certification

References

1. ISO 13485:2016 Medical devices — Quality management systems — Requirements for regulatory purposes

Trademarks/licensing

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