Innovate™ AutoSampler III has a Low Sample Carryover Impact that Is Not Affected by Sample Viscosity

Automated, high-throughput sample preparation for ATP-bioluminescence-based microbial screening

Introduction

Adenosine triphosphate (ATP) bioluminescence technology has become the industry standard for rapid microbial screening of ultra-high temperature (UHT) and extended shelf-life (ESL) dairy and beverage products. Hygiena's Innovate™ Rapid Microbial Screening System (i.e., luminometer and RapiScreen Kits) provides an effective method of incorporating ATP bioluminescence testing into food safety programs. When combined with the Innovate Autosampler III automated sampling platform, high throughput bottling and aseptic packaging facilities can streamline product sample preparation, increasing the efficiency and consistency of setting up the testing plates for the RapiScreen Kits.

The Innovate System is increasingly used to test a wide variety of package types containing dairy, foods and beverage products, such as syrups, soups and broths, brewed tea, sports and nutritional drinks, pudding, infant formula, nut milks, fruit and vegetable juices, condiments and sauces. To ensure that the automation platform yields accurate and reproducible results, extensive testing was conducted to ensure that the washing protocol between sampling was sufficient to minimize carryover contamination between samples. Here, we focus on two trials using samples with varying viscosities to examine potential carryover effects and one larger study to examine potential carryover effects during routine testing.

Methods

Samples

For the trials, the following ultra-high temperature (UHT)-treated samples were chosen because of their varying viscosity. Test samples were either unspiked or spiked with ATP (200 nM).

Table 1. Study Samples

UHT Samples	Viscosity
Infant formula	Low
Strawberry fiber drink	Medium
Protein shake	High

For the larger study, a variety of nutritional drinks were tested for contamination during the manufacturing of consumer products.

Sample Preparation

The AutoSampler III (Product no. MCH4003) processed samples alternating between spiked and unspiked samples and using the optimized, preset workflows for serial sample collection and processing.

Sample Testing

The RapiScreen Dairy Kit (Product no. KIT4015) was used as instructed to screen the samples for the presence or absence of ATP. The Innovate luminometer (Product no. MCH4000) was used as instructed to measure ATP bioluminescence in relative light units (RLUs).

Results and Discussion

Product Viscosity is Not a Driver of Carryover Between Samples

The highest contamination levels were seen with samples in Experiment 2, where we observed carryover rates that demonstrated product viscosity is not a significant driver of carryover as previously hypothesized. The percent carryover was low: less than 1% for most samples and around 1% for high-viscosity samples (Table 2).

Table 2. Carryover Effects Measured in Experiment 2.

	High Viscosity	Medium Viscosity	Low Viscosity
Average Carryover (RLU)	298	145	43
% Carryover from Spiked Sample	1.077%	0.961%	0.181%

Data recorded from Experiment 1 demonstrated a similar but reduced carryover effect with medium-viscosity products (Table 3).

Table 3. Comparison of Carryover Effects from Experiments 1 and 2.

Average RLU Next Well Carryover (Carryover/Previous Contaminated Sample)	Experiment 1	Experiment 2
Low Viscosity	0.251%	0.471%
Medium Viscosity	1.501%	1.086%
High Viscosity	4.490*	0.871%

* Denotes lower RLUs with higher variability.

Use of Appropriate Cutoff Values Minimizes False Negatives

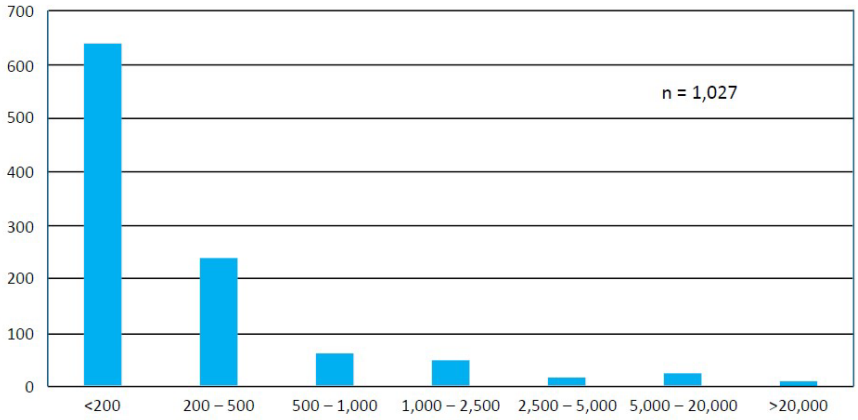
Based on the carryover levels observed, Hygiena scientists determined that with a cutoff value of 50 RLUs, only highly contaminated samples pose a risk of creating a false positive due to carryover. For example, for medium viscosity products, samples with RLUs of ≤4,605 (1.09% of 4,605 equals 50) will not pose a risk for carryover effects on the next sample (Table 4).

Table 4. Examples Showing Low Risks for False Positive Results Due to Carryover Contamination.

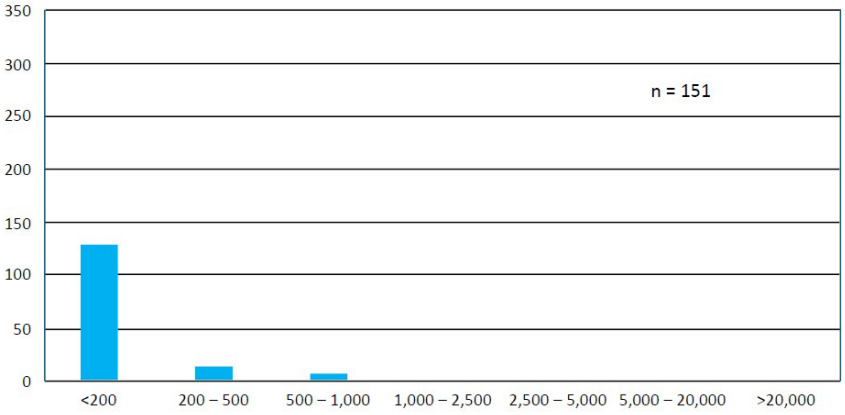
Carryover (RLU)	Pass/Fail: 50 RLU Cutoff	Viscosity	% Carryover	Maximum RLU from a Contaminated Sample
50	Pass	Medium	1.09%	4,605
		High	0.81%*	6,179
		Low	0.47%	10,623

* Average carryover percent.

A global food producer shared data for 1,027 failed samples from approximately 800,000 routine test samples. The average contamination value of the failed samples was 767 RLUs (Figure 1A). When only considering the 151 samples that were subsequently found to be "false positives," the average RLU value for the first test was 114, and the majority of the RLU results (147 of 151) were <500 (Figure 1B).



A. Distribution of RLUs of the Failed Samples from Approximately 800,000 Total Samples.



B. Distribution of RLUs of the False Positives from the 1,027 Failed Samples.

Figure 1. Comparison of the RLUs from All Failed Samples with the Subset of False-Positive Samples.

Further analysis of the data from the 151 false-positive samples revealed that the highest RLU value was 946. Applying the carryover percentages observed to the sample with the highest RLU value shows that the carryover was in line with both retests performed on that specific sample (Table 5). If this sample was actually positive, it likely would not trigger a positive result in subsequent samples (e.g., Retest 2) when using a cut-off value of 50 RLUs.

Table 5. Example Focusing on the False Positive Sample with the Highest RLU Value.

Test	Actual Results (RLU)	Implied Carryover %	Implied Results (RLU)
First Test	949	0.47%	4.4
		0.81%	7.7
		1.09%	10.3
Retest 1	5		
Retest 2	6		

Conclusions

The AutoSampler III system, together with the Innovate System, is designed to yield accurate, repeatable results that consistently differentiate between contaminated and non-contaminated samples. Overall, there was ≤1% carryover impact from highly positive samples, independent of product viscosity. Based on the analysis of carryover assessments and contamination data, the Autosampler III is a sufficient, automated sample-preparation system for high-throughput screening for microbial contamination when used with the Innovate System and RapiScreen Kits. However, if sample carryover is suspected in two or more consecutive samples with high RLU values, Hygiena recommends retesting the high-RLU samples to confirm the results.

Companies that implement the rapid microbial screening technology offered by the AutoSampler III and the Innovate System can release products days sooner than traditional methods. Even in response to a contamination event, the total time products must be held in micro-hold to identify contamination and then to clear the replacement product is significantly shortened when using the Innovate System, which means companies can keep their safety standards high and their cost of quality low.