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INTRODUCTION:

MicroSnap® is based upon the biochemical detection of analytes produced by growing and actively respiring bacteria.

The Coliform and *E.coli* assay is based upon the detection of indicator enzymes beta-galactosidase and beta-glucuronidase respectively. These enzymes are produced and expressed by the bacteria intracellularly. Overexpression of these enzymes is prompted by the addition of inducers that have been added to the media. The enzyme activity is then measured using a pro-luciferin substrate that is cleaved, producing light as the bacteria grow.

The bacteria express beta-galactosidase and beta-glucuronidase, respectively, throughout their grow cycle, meaning the expression of the enzyme is proportional to bacteria CFU levels; this proportion has been calculated as being optimal at 6 hours at 37 °C. To continue the incubation to 8 hours starts to produce non-linear responses and presence-absence RLU levels.

MicroSnap could be utilized in the dairy industry to detect *E . coli* and Coliform within 8 hours compared to traditional methods which may take more than 24-48 hours.

PURPOSE:

To validate the following using MicroSnap *E . coli* and Coliform:

- 1) To estimate the enzymatic background of both the cheese products first using MicroSnap.
- 2) To establish a protocol for estimating Coliforms and *E . coli* in cream cheese and string cheese.

REGISTERED TRADEMARKS:

MicroSnap® is a registered trademark of Hygiena.
Petrifilm™ is a registered trademark of 3M.

Detection of Coliforms and *E. coli* in String Cheese and Cream Cheese Using Hygiena® MicroSnap®

MicroSnap®

METHOD:

The cheese products were first tested to determine their background enzymatic activities by testing the product at 0, 4, 6, 8 and 24 hours. They were then inoculated with a cocktail of Coliform species diluted in buffered peptone water (BPW). The cocktail included *Escherichia coli*, *Citrobacter freundii*, *Klebsiella oxytoca* and *Enterobacter*. Samples were analyzed via MicroSnap at 0, 6, 8, and 24 hours to determine if the matrix and starter culture would interfere with the assay and background signal, and to determine the level of detection at each timepoint. Samples were plated on selective agar to confirm growth of the target organisms.

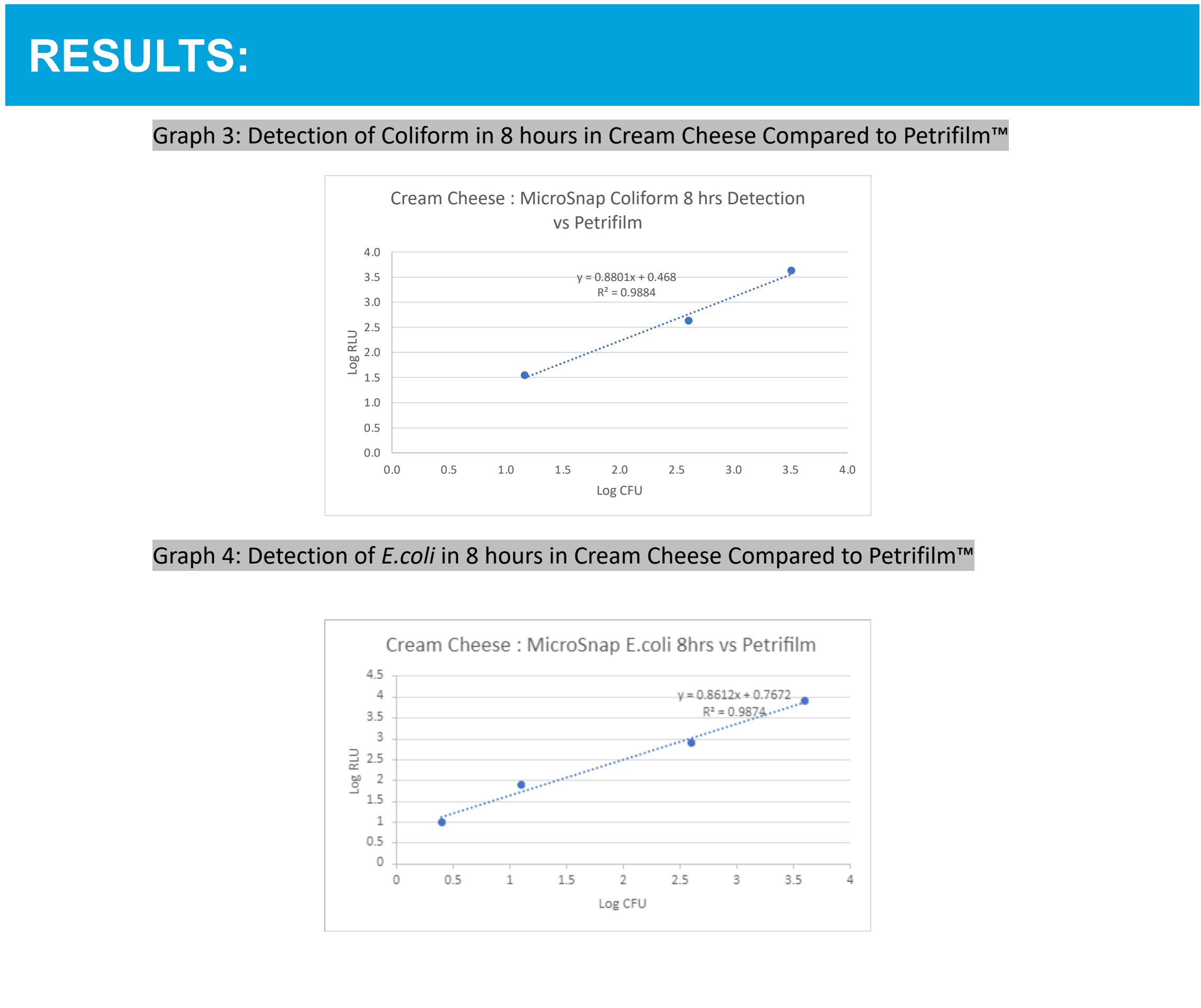
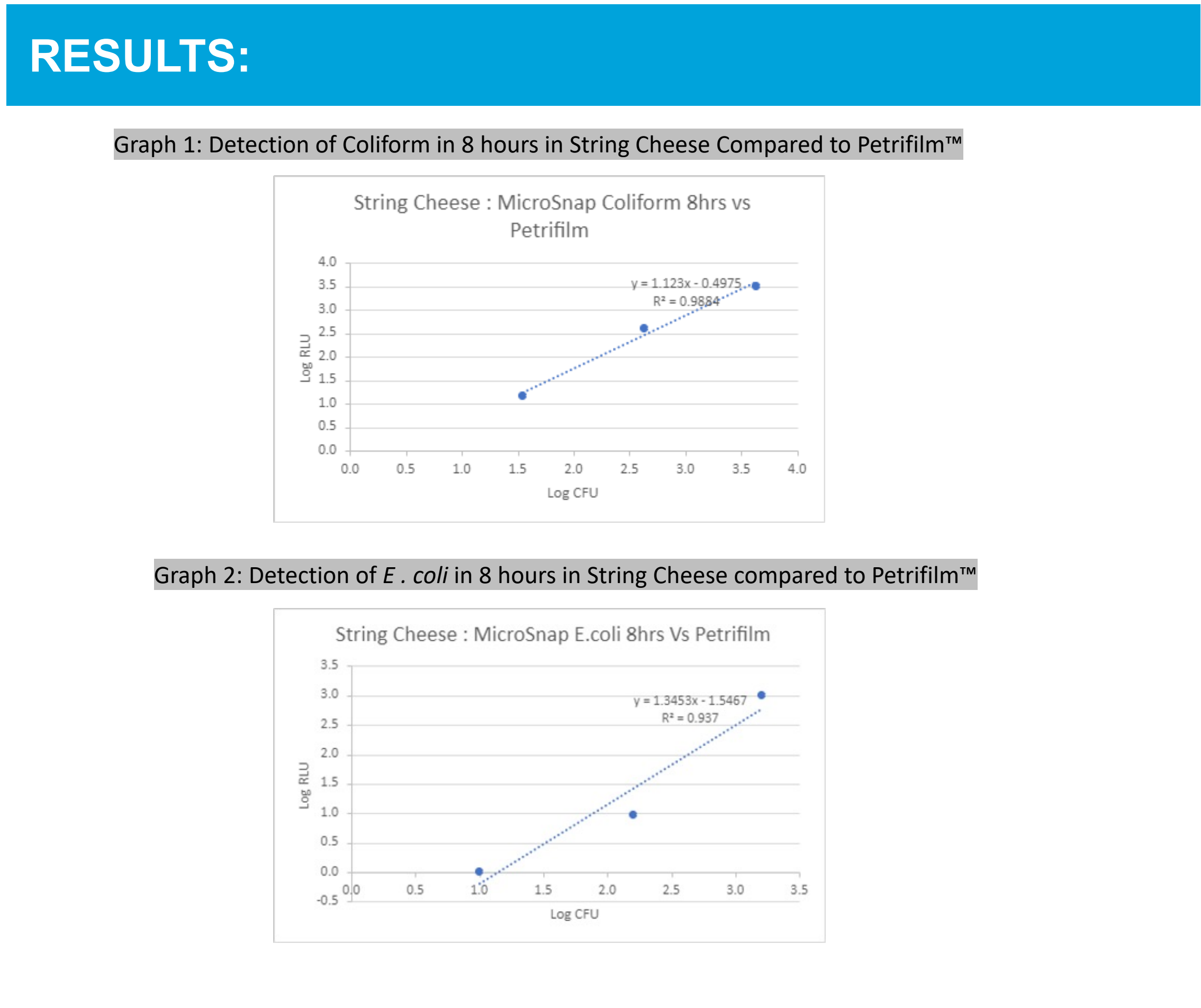
RESULTS:

There was no background enzymatic signal from any of the starter cultures. Growth was evident in the 1:10 product dilution and all spiked dilutions showed detectable growth as early as 6 hours of incubation. The LoD study showed that at 6 hours, the average LoD was 857 CFU (range of 50 to 1,480 CFU); at 8 hours, the LoD was 48 CFU (range of 10 to 90 CFU); and at 24 hours or less, LoD was 17 CFU (range of presence/absence to 50 CFU). The fractionality shows that the CFU added may go through attrition and be lower when growth and detection occurs; hence, the true LoD will be even lower.

CONCLUSIONS:

- ❑ **Background signal:** The results showed that there was no background signal from the cream cheese when tested with Coliform and *E . coli* detection devices but there was a background signal from the string cheese when tested with Coliform detection devices.
- ❑ **Detection in cream cheese:** Limit of detection was 50 CFU with detection at 8 hours for Coliform whereas the limit of detection was 75 CFU for *E . coli* at 8 hours. It was also concluded that the beta-glucuronidase signal was weak and hence, an extended time of 10-12 hours could help lower the limit of detection.
- ❑ **Detection in string cheese:** String cheese product had a background signal when tested with Coliform detection devices, but this signal remained stable over time indicating that signal was enzymatic. Limit of detection was calculated by subtracting the background signal from positive detection. Limit of detection for Coliform was at 8 hours. The beta-glucuronidase signal was weak, hence incubating the products further and reading the devices at 10-12 hours could help lower the limit of detection.

	Limit of Detection (CFU/ml)		
	6 h	8 h	24 h
Cream Cheese		Coliform Detection	Presence/Absence
	4,450	50	
		<i>E . coli</i> Detection	Presence/Absence
	7,500	75	
String Cheese		Coliform Detection	Presence/Absence
	4,300	35	
		<i>E . coli</i> Detection	Presence/Absence
	1500	155	



SIGNIFICANCE:

The MicroSnap technology demonstrates that Coliform and *E .coli* detection from cheeses is feasible within 8 hours. These results demonstrate that the technology can be used as a simple and accurate method to measure *E.coli* and Coliform within the 8-hour time frame when compared to traditional methods which take 24-48 hours .

MicroSnap technology can thereby be used as a simple, rapid, accurate method to measure coliforms and *E. coli* from cheese products within the same day shift.