MicroSnap™ Coliform Method Validation for Dairy Products

Introduction

MicroSnap™ technology is based upon the biochemical detection of analytes produced by growing and actively respiring bacteria. Specifically, the MicroSnap™ Coliform assay is based upon the detection of the indicator enzyme, beta-galactosidase. This enzyme is produced and expressed by the bacteria intracellularly. The overexpression of this enzyme is prompted by the addition of inducers that have been added to the enrichment/incubation media. The enzyme is then measured using a pro-luciferin substrate that is cleaved by the producing light as the bacteria grow.

The bacteria express the beta-galactosidase throughout their growth cycle; therefore, enzyme expression is proportional to bacteria CFU levels. This value has been determined to be optimal following a 6 hour incubation at 37˚C. Incubating beyond this timepoint to an 8 hour timepoint is used to produce presence-absence RLU levels and is considered a non-linear response. This study evaluates results from dairy product testing.

Equipment, Supplies and Reagents

- MicroSnap™ Coliform Kit (containing Incubation and Detection Devices)
- Incubator – set at 37˚C ± 2˚C
- Buffered Peptone Water (BPW)
- Tryptone soy broth (TSB)
- Tryptone soy agar (TSA)
- Bacterial cultures
- Pipettes and tips
- Cell spreaders
- Vortex mixer

Procedure

Starter Cultures Testing

Part A: Protocol for Estimation of Backgrounds

Starter Cultures were prepared following the directions provided by an industry leading dairy manufacturer. The prepared Starter Cultures were then diluted as shown in the Table 1 (3 replicates (reps) per dilution and timepoint).

For each dilution, 1 mL of culture was added to a MicroSnap™ Coliform Incubation Device and incubated at 37˚C. Background detection of beta-galactosidase-like activity was performed at the start of incubation (T=0 hrs), and again after 6 hrs, 8 hrs and 24 hrs.

Table 1: Starter Culture Background Testing Plan

Starter Culture Dilution	0 hours	6 hours at 37˚C	8 hours at 37˚C	Overnight (18 - 24 hours) at 37˚C
Neat	3 reps	3 reps	3 reps	3 reps
1:10	3 reps	3 reps	3 reps	3 reps
1:100	3 reps	3 reps	3 reps	3 reps
1:1000	3 reps	3 reps	3 reps	3 reps

Part B: Protocol for Estimation of Detection of Coliforms in Starter Cultures

Starter Cultures were prepared following the directions provided by the customer. Specifically, four overnight cultures were produced of the following organisms:

a. *Escherichia coli*
b. *Citrobacter freundii*
c. *Klebsiella oxytoca*
d. *Enterobacter cloacae*

Following incubation (24 hours), the four overnight coliform cultures were mixed, and a dilution series was produced as shown below in Table 2.

Table 2. Coliform Inoculation Plan

Coliform Dilution Series	Estimated CFU/mL	Add 100 µL of each dilution to 1 mL Starter Culture, add to MicroSnap™ Incubation Devices	Estimated Coliforms per mL in Neat Starter Culture	Replicate Incubation devices to be incubated at 37°C
-1	1×10^{8}			
-2	1×10^{7}			
-3	1×10^{6}			
-4	1×10^{5}		10,000	x1
-5	1×10^{4}		1,000	x1
-6	1×10^{3}		100	x3
-7	1×10^{2}		10	x3
-8	1×10^{1}		1	x3
-9	1×10^{0}		0.1	x3
-10	1×10^{-1}		0.01	x3
Blank	0		0	x3
			Total	x20

Results

Starter Culture 1

Part A: Estimation of Background

Starter Culture Background Estimation - Starter Culture 1							
Dilution	**Device**	**Time (hours)**				**Brilliance™ Agar: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**0h**	**24h**
Neat	Blank	0	0	0	0	NG	NG
	+ve control	0	0	0	0	NA	+
1:10	Blank	0	3	0	0	NA	NG
	+ve control	0	136	10,610	11,951	NA	+
1:100	Blank	0	0	0	0	NA	NG
	+ve control	0	146	12,927	20,000	NA	+
Note:	+ve control (*E. coli* ATCC® 8739) inoculum of 64 CFU						

NG = no growth; + = confirmed growth. Brilliance™ is a trademark of Thermo Fisher Scientific

Observations and Summary

1. There was no background signal from Starter Culture 1.
2. Signal inhibition was noted when the product was spiked with 64 CFU at each of the dilution level. With Neat product, the signal inhibition was the most. The Neat Starter Culture may be very inhibitory to the growth of the added Coliform.
3. Diluting the product 1:10 and 1:100 helped to reduce the signal inhibition further.

Next Step

The 1:10 Dilution was chosen for the protocol in Part B - **Estimation of Detection of Coliforms in Starter Cultures.**

Starter Culture 1

Part B: Estimation of Detection of Coliforms in Starter Cultures

Coliform Detection - 1:10 Suspension of Starter Culture 1							
Dilution	**Device**	**Time (hours)**				**TSA: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**Inoculum**	**6h**
6	Rep 1	3	20	457	5,567	79	Confirmed growth
	Rep 2	0	27	726	5,613		
	Rep 3	0	16	620	11,225		
6.5	Rep 1	2	5	178	6,760	41	Confirmed growth
	Rep 2	0	11	436	4,876		
	Rep 3	3	4	252	6,940		
7	Rep 1	0	0	26	6,654	7	Confirmed growth
	Rep 2	2	2	112	7,197		
	Rep 3	0	0	8	7,085		
7.5	Rep 1	0	0	0	273	5	Confirmed growth
	Rep 2	0	0	0	34		
	Rep 3	0	3	113	6,425		
8	Rep 1	-	0	4	84	0	Confirmed growth
	Rep 2	2	0	0	236		
	Rep 3	0	0	0	64		
Blank PDT + BPW	Rep 1	4	0	0	0	0	-
	Rep 2	3	3	0	2		
	Rep 3	3	0	3	0		
Note:	Each inoculated replicate was streaked to confirm growth						

Observations and Summary

1. Growth is evident in the 1:10 product dilution of Starter Culture.
2. The spiked dilutions are growing and being detected at 6 hrs and 8 hrs.
3. Unspiked product sample (Blank) has no signal at any of the time points.
4. Limit of detection is 70 CFU with fractional detection at 8 hrs.

Starter Culture 2

Part A: Estimation of Backgrounds

Starter Culture Estimation of Backgrounds - Starter Culture 2							
Dilution	**Device**	**Time (hours)**				**Brilliance™ Agar: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**0h**	**24h**
Neat	Blank	4	0	0	0	NG	NG
	+ve control	3	0	0	0	NA	+
1:10	Blank	0	0	0	0	NA	NG
	+ve control	0	373	9,378	20,000	NA	+
1:100	Blank	0	0	0	0	NA	NG
	+ve control	0	144	11,203	20,000	NA	+
Note:	+ve control (*E. coli* ATCC® 8739) inoculum of 64 CFU						

Observations and Summary

1. There was no background signal from Starter Culture.
2. Signal inhibition was noted when the product was spiked with 64 CFU at each of the dilution levels. With Neat product the signal inhibition was the highest. The Neat Starter Culture may be very inhibitory to the growth of the added Coliform.
3. Diluting the product 1:10 and 1:100 helped to reduce the signal inhibition further.

Next Step

The 1:10 Dilution was chosen for the protocol in Part B - **Estimation of Detection of Coliforms in Starter Cultures**

Starter Culture 2

Part B: Estimation of Detection of Coliforms in Starter Cultures

Coliform Detection - 1:10 Suspension of Starter Culture 2							
Dilution	**Device**	**Time (hours)**				**TSA: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**Inoculum**	**6h**
6	Rep 1	2	110	1,106	13,485	79	Confirmed growth
	Rep 2	4	143	2,604	12,401		
	Rep 3	3	282	2,376	12,377		
6.5	Rep 1	3	70	1,347	20,000	41	Confirmed growth
	Rep 2	3	83	2,683	14,914		
	Rep 3	0	109	2,599	20,000		
7	Rep 1	3	17	282	13,763	7	Confirmed growth
	Rep 2	0	16	166	15,771		
	Rep 3	0	8	126	14,095		
7.5	Rep 1	2	15	165	14,255	5	Confirmed growth
	Rep 2	2	4	106	7,041		
	Rep 3	2	12	138	14,853		
8	Rep 1	0	0	0	1,013	0	Confirmed growth
	Rep 2	0	0	0	2,563		
	Rep 3	2	0	0	357		
Blank PDT + BPW	Rep 1	2	0	0	0	0	-
	Rep 2	2	0	0	0		
	Rep 3	0	0	0	0		
Note:	Each inoculated replicate was streaked to confirm growth						

Observations and Summary

1. Growth is evident in 1:10 product dilution of Starter Culture.
2. The spiked dilutions are growing and being detected at 6 hrs and 8 hrs.
3. Unspiked product sample (Blank) has no signal at any of the time points.
4. Limit of detection is 50 CFU with detection at 8 hrs and fractional detection starting at 6 hrs.

Starter Culture 3

Part A: Estimation of Backgrounds

Starter Culture Estimation of Backgrounds - Starter Culture 3						
Dilution	**Device**	**Time (hours)**				**MRS: CFU/mL**
		0	**6**	**8**	**24**	**0h**
Neat	Rep 1	2	0	0	0	NA
	Rep 2	2	0	0	0	NA
	Rep 3	0	0	0	0	NA
1:10	Rep 1	0	0	0	0	NA
	Rep 2	0	2	0	0	NA
	Rep 3	0	0	2	0	NA
1:100	Rep 1	0	0	0	0	NA
	Rep 2	0	0	0	0	NA
	Rep 3	0	0	0	0	NA
1:1000	Rep 1	0	0	0	0	NA
	Rep 2	3	0	0	0	NA
	Rep 3	0	0	0	2	NA
Blank	BPW only	0	0	0	0	NA

Observations and Summary

1. There was no background signal from Starter Culture.
2. The product was diluted 1:10 for the spiking study.

Starter Culture 3

Part B: Estimation of Detection of Coliforms in Starter Cultures

Coliform Detection - 1:10 Suspension of Starter Culture 3							
Dilution	**Device**	**Time (hours)**				**TSA: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**0h**	**6h**
4	Rep 1	0	12,678	20,000	NA	14,750	Confirmed growth
5	Rep 1	0	2,765	20,000	NA	1,475	Confirmed growth
	Rep 2	0	2,435	20,000	NA		
	Rep 3	0	2,762	20,000	NA		
6	Rep 1	0	76	12,833	20,000	148	Confirmed growth
	Rep 2	0	154	12,916	20,000		
	Rep 3	0	113	13,500	20,000		
7	Rep 1	0	8	2,780	20,000	15	Confirmed growth
	Rep 2	0	7	2,656	20,000		
	Rep 3	0	3	1,074	20,000		
8	Rep 1	0	0	33	9,886	2	Confirmed growth
	Rep 2	0	0	2	0		
	Rep 3	0	0	2	844		
9	Rep 1	0	0	2	0	<1	-
	Rep 2	0	0	0	0		
	Rep 3	0	0	0	0		
Blank PDT + BPW	Rep 1	0	0	3	3	0	-
	Rep 2	0	0	0	0		
	Rep 3	0	0	0	0		
Note:	Each inoculated replicate was streaked to confirm growth						

Observations and Summary

1. Growth is evident in 1:10 product dilution of the Starter Culture.
2. The spiked dilutions are growing and being detected at 6 hrs and 8 hrs.
3. Unspiked product sample (Blank) has no signal at any of the time points.
4. Limit of detection is 20 CFU with fractional detection at 8 hrs.

Starter Culture 4

Part A: Estimation of Backgrounds

Starter Culture Estimation of Backgrounds - Starter Culture 4							
Dilution	**Device**	**Time (hours)**				**Brilliance™ Agar: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**0h**	**24h**
Neat	Blank	0	0	0	0	NG	NG
	+ve control	0	0	0	0	NA	+
1:10	Blank	2	2	2	0	NA	NG
	+ve control	2	610	13,786	NA	NA	+
1:100	Blank	0	0	3	2	NA	NG
	+ve control	0	297	20,000	NA	NA	+
1:1000	Blank	0	3	4	0	NA	NG
	+ve control	4	376	20,000	NA	NA	+
Note:	+ve control (*E. coli* ATCC® 8739) inoculum of 84 CFU						

Observations and Summary

1. There was no background signal from Starter Culture.
2. Signal inhibition was noted when the Neat product was spiked with 84 CFU. The Neat Starter Culture may be very inhibitory to the growth of the added Coliform.
3. Diluting the product 1:10 and 1:100 helped to reduce the signal inhibition further.
4. 1:10 dilution was chosen as the next step for spiking study.

Starter Culture 4

Part B: Estimation of Detection of Coliforms in Starter Cultures

Coliform Detection - 1:10 Suspension of Starter Culture 4							
Dilution	**Device**	**Time (hours)**				**TSA: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**Inoculum**	**6h**
4	Rep 1	0	10,041	12,089	13,989	1,3550	Confirmed growth
5	Rep 1	3	1,280	13,512	13,489	1,355	Confirmed growth
	Rep 2	3	1,435	13,650	12,896		
	Rep 3	0	1,418	13,056	11,345		
6	Rep 1	2	33	7,327	13,056	121	Confirmed growth
	Rep 2	2	70	6,843	13,904		
	Rep 3	2	52	5,980	11,198		
7	Rep 1	0	7	185	20,000	15	Confirmed growth
	Rep 2	2	3	18	5,064		
	Rep 3	0	9	883	20,000		
8	Rep 1	2	0	3	0	5	-
	Rep 2	0	0	96	1,274		Confirmed growth
	Rep 3	0	0	6	5,758		Confirmed growth
9	Rep 1	0	5	3	0	1	-
	Rep 2	2	4	0	3,771		Confirmed growth
	Rep 3	2	0	4	0		-
Blank PDT + BPW	Rep 1	2	3	4	2	NG	-
	Rep 2	NA	0	0	2		
	Rep 3	NA	0	4	2		
Note:	Each inoculated replicate was streaked to confirm growth						

Observations and Summary

1. Growth is evident in 1:10 product dilution.
2. The spiked dilutions are growing and being detected at 6 hrs and 8 hrs.
3. Unspiked product sample (Blank) has no signal at any of the time points.
4. Limit of detection is 50 CFU with fractional detection at 8 hrs.

Starter Culture 5

Part A: Estimation of Backgrounds

Starter Culture Estimation of Backgrounds - Starter Culture 5							
Dilution	**Device**	**Time (hours)**				**Brilliance™ Agar: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**0h**	**24h**
Neat	Blank	0	0	0	0	NG	NG
	+ve control	3	0	0	0	NA	+
1:10	Blank	2	3	3	2	NA	NG
	+ve control	0	843	11,186	NA	NA	+
1:100	Blank	0	2	2	0	NA	NG
	+ve control	2	448	20,000	NA	NA	+
1:1000	Blank	2	0	0	2	NA	NG
	+ve control	0	384	20,000	NA	NA	+
Note:	+ve control (*E. coli* ATCC® 8739) inoculum of 84 CFU						

Observations and Summary

1. There was no background signal from Starter Culture.
2. Signal inhibition was noted when the Neat product was spiked with 84 CFU. The Neat Starter Culture may be very inhibitory to the growth of the added Coliform.
3. Diluting the product 1:10 and 1:100 helped to reduce the signal inhibition further.
4. The 1:10 dilution was chosen as the next step for spiking study.

Starter Culture 5

Part B: Estimation of Detection of Coliforms in Starter Cultures

Coliform Detection - 1:10 Suspension of Starter Culture 5							
Dilution	Device	Time (hours)				TSA: CFU/ 100 µL	Brilliance™ Agar Confirmation
		0	6	8	24	Inoculum	6h
4	Rep 1	3	4,897	13,870	12,287	13,550	Confirmed growth
5	Rep 1	2	1,017	12,413	20,000	1,355	Confirmed growth
	Rep 2	0	896	8,536	10,683		
	Rep 3	3	1,002	8,651	13,060		
6	Rep 1	0	45	2,214	13,707	121	Confirmed growth
	Rep 2	2	26	3,893	12,875		
	Rep 3	0	57	3,798	11,354		
7	Rep 1	2	5	672	12,025	15	Confirmed growth
	Rep 2	0	7	191	13,800		
	Rep 3	0	6	323	11,010		
8	Rep 1	0	6	139	13,235	5	Confirmed growth
	Rep 2	0	0	6	3		-
	Rep 3	0	5	2	0		-
9	Rep 1	2	0	5	0	1	-
	Rep 2	0	4	0	0		
	Rep 3	0	3	10	2		
Blank PDT + BPW	Rep 1	3	0	5	0	NG	-
	Rep 2	NA	0	0	3		
	Rep 3	NA	0	7	3		
Note:	Each inoculated replicate was streaked to confirm growth						

Observations and Summary

1. Growth is evident in 1:10 product dilution.
2. The spiked dilutions are growing and being detected at 6 hrs and 8 hrs.
3. Unspiked product sample (Blank) has no signal at any of the time points.
4. The limit of detection is 50 CFU with fractional detection at 8 hrs.

Starter Culture 6

Part A: Estimation of Backgrounds

Starter Culture Estimation of Backgrounds - Starter Culture 6							
Dilution	**Device**	**Time (hours)**				**Brilliance™ Agar: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**0h**	**24h**
Neat	Blank	0	0	2	0	NA	NG
	+ve control	0	0	0	0	NA	NG
1:10	Blank	2	0	3	0	NA	NG
	+ve control	3	65	5,323	10,507	NA	+
1:100	Blank	NA	NA	NA	NA	NA	NA
	+ve control	NA	NA	NA	NA	NA	NA
1:1000	Blank	NA	NA	NA	NA	NA	NA
	+ve control	NA	NA	NA	NA	NA	NA
Note:	+ve control (*E. coli* ATCC® 8739) inoculum of 78 CFU						

Observations and Summary

1. There was no background signal from Starter Culture.
2. Signal inhibition was noted when the Neat product was spiked with 78 CFU. The Neat Starter Culture may be very inhibitory to the growth of the added coliforms.
3. Diluting the product 1:10 helped to reduce the signal inhibition further.
4. The 1:10 dilution was chosen as the next step for spiking study.

Starter Culture 6

Part B: Estimation of Detection of Coliforms in Starter Cultures

Coliform Detection - 1:10 Suspension of Starter Culture 6							
Dilution	**Device**	**Time (hours)**				**TSA: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**Inoculum**	**6h**
6	Rep 1	0	11	1,815	13,379	87	Confirmed growth
	Rep 2	0	18	1,609	10,855		
	Rep 3	0	28	2,862	20,000		
6.5	Rep 1	0	9	1,201	11,408	57	Confirmed growth
	Rep 2	3	7	904	20,000		
	Rep 3	0	5	1,188	20,000		
7	Rep 1	0	2	119	11,101	9	Confirmed growth
	Rep 2	2	0	112	13,678		
	Rep 3	4	3	276	12,275		
7.5	Rep 1	2	0	21	7,733	1	Confirmed growth
	Rep 2	0	0	5	2,781		
	Rep 3	4	0	5	3,090		
8	Rep 1	0	0	5	1,831	0	Confirmed growth
	Rep 2	0	3	0	1,010		
	Rep 3	6	0	5	3,510		
Blank PDT + BPW	Rep 1	0	4	0	0	NG	NG
	Rep 2	NA	0	4	0		
	Rep 3	NA	4	2	7		
Note:	Each inoculated replicate was streaked to confirm growth						

Observations and Summary

1. Growth is evident in 1:10 product dilution.
2. The spiked dilutions are growing and being detected at 6 hrs and 8 hrs.
3. Unspiked product sample (Blank) has no signal at any of the time points.
4. The limit of detection is 10 CFU with fractional detection at 8 hrs.

Starter Culture 7

Part A: Estimation of Backgrounds

Starter Culture Estimation of Backgrounds - Starter Culture 7							
Dilution	**Device**	**Time (hours)**				**Brilliance™ Agar: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**0h**	**24h**
Neat	Blank	0	0	0	0	NA	NG
	+ve control	0	0	2	0	NA	NG
1:10	Blank	3	0	0	0	NA	NG
	+ve control	4	70	6,830	14,154	NA	+
1:100	Blank	NA	NA	NA	NA	NA	NA
	+ve control	NA	NA	NA	NA	NA	NA
1:1000	Blank	NA	NA	NA	NA	NA	NA
	+ve control	NA	NA	NA	NA	NA	NA
Note:	+ve control (*E. coli* ATCC® 8739) inoculum of 78 CFU						

Observations and Summary

1. There was no background signal from Starter Culture.
2. Signal inhibition was noted when the Neat product was spiked with 78 CFU. The Neat Starter Culture may be very inhibitory to the growth of the added coliforms.
3. Diluting the product 1:10 helped to reduce the signal inhibition further.
4. The 1:10 dilution was chosen as the next step for spiking study.

Starter Culture 7

Part B: Estimation of Detection of Coliforms in Starter Cultures

Coliform Detection - 1:10 Suspension of Starter Culture 7							
Dilution	**Device**	**Time (hours)**				**TSA: CFU/ 100 µL**	**Brilliance™ Agar Confirmation**
		0	**6**	**8**	**24**	**Inoculum**	**6h**
6	Rep 1	5	14	2,584	20,000	87	Confirmed growth
	Rep 2	2	33	3,585	20,000		
	Rep 3	0	11	1,239	20,000		
6.5	Rep 1	5	14	1,382	20,000	57	Confirmed growth
	Rep 2	2	16	1,069	20,000		
	Rep 3	0	11	908	20,000		
7	Rep 1	7	9	688	20,000	9	Confirmed growth
	Rep 2	2	4	43	12,320		
	Rep 3	0	6	39	13,690		
7.5	Rep 1	2	6	33	20,000	1	Confirmed growth
	Rep 2	4	5	3	6,109		
	Rep 3	0	8	34	12,156		
8	Rep 1	5	4	0	15	0	-
	Rep 2	0	0	0	2,071		Confirmed growth
	Rep 3	5	0	6	5,934		Confirmed growth
Blank PDT + BPW	Rep 1	3	0	0	5	NG	NG
	Rep 2	NA	0	0	0		
	Rep 3	NA	5	5	3		
Note:	Each inoculated replicate was streaked to confirm growth						

Observations and Summary

1. Growth is evident in 1:10 product dilution.
2. The spiked dilutions are growing and being detected at 6 hrs and 8 hrs.
3. Unspiked product sample (Blank) has no signal at any of the time points.
4. Limit of detection is 10 CFU with fractional detection at 8 hrs.

Condensed Whey Testing

In addtion to Starter Cultures, the dairy manufacturing customer asked us to help validate MicroSnap™ with other matrices, including whey and cream. What follows is information regarding those studies.

Procedure

1. Four overnight cultures were prepared of the following organisms:

   a. Escherichia coli *b. Citrobacter freundii* *c. Klebsiella oxytoca* *d. Enterobacter cloacae*

2. After 24 hours (the next day), the coliform cultures were mixed together and a dilution series was prepared in PBS. Accurate CFU/mL counts from dilutions were determined by plating the dilutions on TSA.
3. A 1:5 dilution of condensed whey was made in BPW and plated on Brilliance™ Agar and Violet Red Bile Glucose Agar (VRBGA).
4. 1 mL of diluted (1:5) condensed whey product was added to the MicroSnap™ Incubation Devices, followed by the addition of 100 µL of the coliform dilution series (as shown in the table below) and the devices were incubated at 37˚C.
5. The detection assay was run at 0 hrs, 6 hrs, 8 hrs and 24 hrs as shown below.
6. From each enrichment/incubation, after 6 hours, a streak confirmation was done on Brilliance™ agar/VRBGA.

Limit of Detection Results

1:5 Product Suspension of Condensed Whey LoD								
Dilution	**Device**	**Time (hours)**				**TSA: CFU/mL**	**Brilliance™ Agar Confirmation**	**VRBGA Confirmation**
		0	**6**	**8**	**24**	**0h**	**6h**	
	Rep 1	0	44	3,941	2,999		+	+
	Rep 2	0	42	3,334	3,862	556	+	+
	Rep 3	0	41	3,505	2,449		+	+
6	Rep 1	0	4	276	1,595		+	+
	Rep 2	0	0	360	2,102	55	+	+
	Rep 3	0	0	281	1,184		+	+
7	Rep 1	0	0	11	706		+	+
	Rep 2	0	0	25	527	10	+	+
	Rep 3	0	0	20	329		+	+
8	Rep 1	0	0	0	0		-	-
	Rep 2	0	0	0	2	1	-	-
	Rep 3	0	0	0	0		-	-
Blank	Rep 1	0	0	0	0	0	-	-

Observations and Summary

1. Condensed whey product was diluted to 1:5.
2. Growth is evident in the 1:5 product dilution.
3. The spiked dilutions are growing and being detected at 8 hrs at 50 CFU.
4. The unspiked sample (Blank) is not being detected after 24 hours. This shows that this product does not have indigenous beta-galactosidase producing bacteria.

Cream (Half & Half Cream) Testing

Procedure

1. Product was tested Neat or diluted 1:5 and 1:10 in BPW.
2. A mixture of coliform culture was produced.
3. The product suspensions were spiked with the diluted coliform mix to attain CFU/mL levels as shown in the table below.
4. The spiked suspensions were then plated onto Brilliance™ Agar.

Limit of Detection Results

Half & Half Cream LoD								
Dilution	**Device**	**Time (hours)**				**Post Dilution Inoculum (CFU/mL) Brillance™**	**TSA CFU/mL**	**Brilliance™ CFU/mL**
		0	**6**	**8**	**24**	**0h**	**0h**	**6h**
Neat	Rep 1	0	0	108	552	20	2,300	TNTC*
	Rep 2	0	0	425	685			TNTC
	Rep 3	0	0	180	422			TNTC
	Blank	0	0	0	0			TNTC
1:5	Rep 1	0	3	1,508	13,133	10		TNTC
	Rep 2	0	5	1,820	10,615			TNTC
	Rep 3	0	4	1,652	3,510			TNTC
	Blank	0	0	0	0			TNTC
1:10	Rep 1	15	13	3,139	20,000	50		TNTC
	Rep 2	0	34	6,987	20,000			TNTC
	Rep 3	2	15	3,575	20,000			TNTC
	Blank	0	0	0	0			TNTC

**TNTC = Too Numerous to Count*

Observations and Summary

1. For cream, growth is evident in Neat 1:5 and 1:10 product dilutions.
2. The spiked dilutions are growing and being detected at 6 hrs to 8 hrs at 20, 50 and 500 CFU.
3. The unspiked samples (Blank) are not being detected after 24 hours. This shows that this product does not have indigenous beta-galactosidase producing bacteria.

Summary

Starter Cultures

RLU Growth of Positive Control Spike					
	Background Enzyme	**Neat Product**	**1 in 10 Product (6 hours)**	**1 in 10 Product (8 hours)**	**Threshold RLU at Mean ± 6 std dev Blanks**
Starter Culture 1	NO	0 RLU	136 RLU @ 64 CFU	10,610 RLU @ 64 CFU	10.2
Starter Culture 2	NO	0 RLU	373 RLU @ 64 CFU	9,378 RLU @ 64 CFU	9.6
Starter Culture 3	NO	0 RLU	NA	NA	0
Starter Culture 4	NO	0 RLU	610 RLU @ 84 CFU	13,786 RLU @ 84 CFU	8.2
Starter Culture 5	NO	0 RLU	843 RLU @ 84 CFU	11,186 RLU @ 84 CFU	8.6
Starter Culture 6	NO	0 RLU	65 RLU @ 78 CFU	5,373 RLU @ 78 CFU	13.1
Starter Culture 7	NO	0 RLU	70 RLU @ 78 CFU	6,830 RLU @ 78 CFU	16.4

Limit of Detection (CFU/mL)				
	6 hours	**8 hours**	**24 hours**	**Threshold RLU at Mean ± 6 std dev Blanks**
Starter Culture 1	410 - 790 CFU	50 - 70 CFU (F)	<10 CFU	10.2
Starter Culture 2	50 - 70 CFU (F)	50 - 70 CFU	<10 CFU	9.6
Starter Culture 3	1,480 CFU	20 CFU (F)	20 CFU (F)	10*
Starter Culture 4	1,210 CFU (F)	50 CFU (F)	<10 CFU (F)	8.2
Starter Culture 5	1,210 CFU	50 CFU	50 CFU (F)	8.6
Starter Culture 6	870 CFU	10 - 90 CFU (F)	<10	13.1
Starter Culture 7	570 CFU	10 - 90 CFU (F)	<10 (F)	16.4
All Starter Cultures	Log 2.9 (857 CFU)	Log 1.68 (48 CFU)	Log 1.23 (17 CFU)	11.0 RLUs

Note: 10 is the assumed cutoff threshold RLU (Threshold RLU at mean ± 6 Std Dev for blanks) based on the background signal of the other Starter Cultures tested at time 0; (F) = fractional*

Results Demonstrate That:

- The MicroSnap™ system will detect Coliforms in the dairy Starter Cultures at 6, 8 and 24 hours.
- The limit of detection (LoD) average at 6 hours is 857 CFU (range of 50 to 1,480 CFU).
- The LoD average at 8 hours is 48 CFU (range of 10 to 90 CFU).
- The LoD average at 24 hours or less is 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 lower.
- The calculated thresholds across the Starter Cultures using mean blanks ± 6 std dev from Time 0. RLUs remains steady except Starter Culture 3 which had all 0 RLUs for blanks.

Whey and Cream Testing

Limit of Detection (CFU/mL)				
	6 hours	**8 hours**	**24 hours**	**Threshold RLU at Mean ± 6 std dev Blanks**
Condensed Whey	2,780 CFU	50 CFU	10 CFU	10*
Half & Half Cream	500 CFU	20 CFU	10 CFU	10*

Note: 10 is the assumed cutoff threshold RLU (Threshold RLU at mean ± 6 Std Dev for blanks) based on the background signal of the other Starter Cultures tested at time 0; (F) = fractional*

Results Demonstrate That:

- The MicroSnap™ system will detect Coliforms in the condensed whey cultures at 6, 8 and 24 hours.
- The MicroSnap™ system will detect Coliforms in the half & half cream cultures at 6, 8 and 24 hours when diluted at least 1:5 in BPW. In Neat samples, Coliforms were detected at 8 and 24 hours.
- For whey, the limit of detection (LoD) average at 6 hours is 2,780 CFU and at 8 hours is 50 CFU.
- For cream, the LoD average at 6 hours is 500 CFU and at 8 hours is 20 CFU.
- The LoD average at 24 hours (or less) is 10 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 lower.
- The calculated thresholds across the whey cultures and cream cultures all had 0 RLUs for blanks.

Conclusions

For all Starter Cultures, growth was evident in the 1:10 product dilution of Starter Culture. In addition, all spiked dilutions showed detectable growth at 6 hrs and 8 hrs of incubation, while unspiked product samples (Blanks) had no signal at any of the time points.

For condensed whey, growth was evident in the 1:5 product dilution in PBS. In addition, all serial dilutions to -7 showed detectable growth at 8 hours, while unspiked samples (Blanks) had no signal at any of the time points.

For half & half cream, growth was evident at 8 hours in Neat 1:5 and 1:10 dilutions. Furthermore, growth was detected at 6 hours in cultures diluted 1:10 in BPW.

The only variability observed for the seven Starter Cultures was in the limit of detection, but values were within 7-fold of each other (ranging from 10 to 70 CFU). In most cases, fractional detection of Starter Cultures was observed at 8 hours. For whey and cream, fraction detection was observed at 8 hours and in some cases, 6 hours.

These results demonstrate that MicroSnap™ Coliform can be used as a simple, rapid, accurate method to measure bacterial levels. Therefore, when paired with EnSURE™ Touch, MicroSnap™ Coliform can be used as an alternative method to plating, providing bacterial counts in less than 8 hours, rather than 24 - 48 hours.