Detection of Coliforms in Dairy Starter Cultures Using Hygiena's MicroSnap® Coliform Assay

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MicroSnap®

INTRODUCTION:

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

The Coliform assay is based upon the detection of indicator enzyme beta-galactosidase, this enzyme is produced and expressed by the bacteria intracellularly. The over-expression of this enzyme is initiated by the addition of inducers to the media. The enzyme is then measured using a pro-luciferin substrate, cleaved by beta-galactosidase producing light.

The bacteria produce beta-galactosidase throughout their grow cycle, this means the expression of 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.

PURPOSE:

To validate the following using Hygiena's MicroSnap Coliform Assay:

- To estimate the background signal from the seven starter cultures.
- To establish a protocol for estimating the detection of Coliforms for all seven starter cultures.

REGISTERED TRADEMARKS:

METHOD:

The detection assay for background beta-galactosidase was performed by diluting the starter cultures and taking readings from the incubated MicroSnap devices set at 37 °C for 0 , 6 , 8 , and 24 hours. The limit of detection (LoD) of coliforms in the starter cultures were performed by spiking the starter cultures with four overnight cultures of *Escherichia coli*, *Citrobacter freundii*, *Klebsiella oxytoca* and *Enterobacter cloacae.* For each culture, the detection assay was run on selected dilutions and compared to standard plate counts with readings taken at 24 hours.

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.

RESULTS:

Table 1: Background Enzymatic Activity and Detection of Spiked Coliform in Seven starter Culture and RLUs Taken at 6 and 8 Hours.

	Background Enzyme	RLU Growth of Positive Control Spike			
		Neat Product	1 in 10 Product (6 hours)	1 in 10 Product (8 hours)	Threshold RLU at Mean + 6 sd 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

*Starter Culture 3: Positive control wasn't spiked into the product

- There was no background signal from any of the seven starter cultures.
- 1:10 dilution was chosen for spiking study.
- Growth was evident in the diluted product when spiked with mixed coliform cultures as shown in Table 1 (readings taken at 6 hours and 8 hours).
- Threshold RLU was calculated from the blanks by averaging the mean ± 6 standard deviations.

RESULTS:

Table 2: Limit of Detection (CFU/ml) in Seven Starter Cultures Taken at 6, 8 and 24 Hours.

	Limit of Detection (CFU/mL)			
	6 hours	8 hours	24 hours	Threshold RLU at Mean + 6 sd 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)	0 (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 SCs	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 at time 0; (F) = fractional

- The MicroSnap system will detect Coliforms in the starter cultures at 6, 8 and 24 hours
- The LoD at 6 hours is 857 CFU (range 50 to 1,480 CFU)
- The LoD at 8 hours is 48 CFU (range 10 to 90 CFU)
- The LoD at 24 hours is 17 CFU (range presence/absence)
- 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
- Longer incubation of devices (8 hrs is the shortest time to result) will give better resolution

SIGNIFICANCE:

The study demonstrated that the MicroSnap system will detect Coliforms from starter cultures as early as 6 hours and a lower limit of detection can be achieved by incubating the devices for longer times (8-24 hours).

The MicroSnap technology demonstrates that Coliform detection from dairy starter cultures is feasible from the product within 8 hours. These results shows that the technology can be used as a simple and accurate method to measure Coliforms from starter cultures within the 8 hours time frame when compared to traditional methods which take 24-48 hours.