SANITATION PROTOCOLS AND METHODS OF EVALUATION

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ABSTRACT

The use of ATP bioluminescence systems for sanitation checks has become very popular and gives a quick assessment of gross contamination, if any. However, one drawback in this method of assessment is that it does not give information on contamination of specific microbial types; for instance coliforms, yeast and mold. Therefore, other test methods need to be carried out to obtain this information. Some of the methods used in these studies were the ATP bioluminescence systems "Biotrace" and "AccuPoint" and different types of swab methods "RediSwab™," Hygiene SwabCheck, Coliform SwabCheck, and CultureSwabs which were used in conjunction with microbial plate checks for validation. These assessments were carried out at the East Grand Forks (EGF) and Moorhead (MHD) factories after extract tank and syrup truck sanitation protocols had been carried out.

At the Moorhead factory some discrepancies between luminometer readings and microbial plate checks were observed. This was mainly due to spore formers of the Bacillus spp. This problem was overcome not only by mandating more rigid cleaning protocols at truck wash stations but by lowering the pass/fail limits on the luminometer to <100 RLU (relative light units). The coliform SwabCheck technique was found to be another useful quick method that could be employed if rapid plating techniques were not readily available for coliform assessment.

Preliminary results with the use of the Hygiene Swab system were promising. The advantage in use of this method in extract tank sanitation over regular plate checks is the quick turn-around time, 8-24 hrs for a color change (red to yellow) versus 2-3 days for counts from microbial plate checks.

In extract tank sanitation checks we had previously found the use of ATP bioluminescence systems not to be reliable. This was probably due to the pitted nature of the steel tanks in use (2). Therefore, the Hygiene SwabCheck once its reliability is further validated will be a boon in hygiene monitoring of these tanks or may be used in conjunction with microbial plate checks for quick assessment. This is of particular importance at American Crystal Sugar Company (ACS) where about 5-10 million dollars worth of extract is stored in each tank over a period of 7-9 months during the beet sugar campaign.

INTRODUCTION

American Crystal Sugar Company began the use of ATP (Adenosine Tri Phosphate) bioluminescence systems about 8-10 years ago. This was begun after several different ATP systems available at the time were evaluated in-house for reliability, ease of use, ruggedness, etc. This instrument (Biotrace Uni-*Lite*® Xcel) was frequently used at the company's Moorhead factory for hygiene and sanitation checks on syrup trucks unloading into the factory and brown sugar production lines. At the beginning of every month a double set of swabs (ATP swabs and CultureSwabs) were taken from each location to make sure the ATP system was working appropriately. The CultureSwabs taken were plated on appropriate microbiological media and compared to RLU values obtained. Last year the vendor at the time switched the ATP system on hand to a new ATP system. About this time we began to see unacceptable growth on swabbed plates. This was after sanitizing protocols had been completed and the ATP system on hand

gave zero RLU values. Therefore, further in-depth scrutiny into sanitizing protocols and different methods of evaluation were begun.

At EGF the ATP bioluminescence systems were found not to be reliable in extract tank sanitation checks. Therefore a RediSwab™ technique followed by microbial plate checks was carried out. This method though reliable gave results in 2-3 days. Therefore other potential techniques such as the Hygiene SwabCheck system were evaluated to obtain quicker turn around time if possible.

MATERIALS AND METHODS

1. **ATP Bioluminescence Instruments**
   a. Biotrace Uni-*Lite*® Xcel – with Clean-Trace® ATP Swabs from Biotrace International BioProducts, Bothwell, WA 98041-0746 was used.
   b. AccuPoint – with AccuPoint ATP Surface Samplers from Neogen Corporation, 620 Lesher Place, Lansing, MI 48912 was used.
   c. Biotrace Uni-*Lite*® NG – with Clean-Trace® ATP Swabs from Biotrace International BioProducts, Bothwell, WA 98041-0746 was used.

2. **Swab Types**
   a. BBL™ CultureSwab™ – with Liquid Stuart's (220099) from Becton Dickinson & Co., Sparks, MD 21152 was used.
   b. RediSwab™ – with Letheen Broth (RS-960-10 LET) from International BioProducts Inc., Bothwell, WA 98041-0746 was used.
   c. Hygiene SwabCheck – from Schleicher & Schuell MicroScience, 950 Congress Ave., Riviera Beach, Florida 33404 was used.
   d. Coliform SwabCheck – from Schleicher & Schuell MicroScience, 950 Congress Ave., Riviera Beach, Florida 33404 was used.

3. **Sanitizers**
   a. Ster-Bac (150-200 ppm) – a Quaternary Ammonium Sanitizer, with an active ingredient of n-alkyl dimethyl benzyl ammonium chloride (10%) from Ecolab Inc., 370 N. Wabasha, St. Paul, MN 55102-1390 was used.
   b. Vortexx (2600 ppm) – an Acid Sanitizer, with active ingredients of hydrogen peroxide (6.9%), peroxy acetic acid (4.4%), octanoic acid (3.3%), from Ecolab Inc., 370 N. Wabasha, St. Paul, MN 55102-1390 was used.

4. **Microbiology**
   a. Sample collection from syrup tanks and brown sugar production lines:
   The truck outlet lines and hoses were sanitized with Ster-Bac or Vortexx. This was followed by taking a BBL™ CultureSwab™, Clean-Trace® ATP Swab, and/or monitored with an AccuPoint ATP Surface Sampler. The CultureSwabs were replaced in the transport medium and transported on ice, in a cooler, to the ACS Technical Services Center Microbiology Lab for further assessment. The Clean-Trace® ATP Swab was placed in the Biotrace Uni-*Lite*® Xcel or Biotrace Uni-*Lite*® NG luminometer and a relative light unit (RLU) value was measured. Likewise the AccuPoint ATP Surface Sampler was placed in the AccuPoint luminometer and an RLU value was obtained. This process was repeated until acceptable RLU values were obtained on both luminometers.

b. Sample collection from extract tanks:
A tank sanitation survey was carried out after cleaning and sanitizing protocols were completed and the tank was dry. The samples were obtained using a 50 cm^2 template and swabbing the surface within the template with a RediSwab™ in three different directions. The RediSwab™ was replaced in the Letheen broth and transported on ice in a cooler to the ACS Technical Services Center Microbiology Lab for further assessment. A second assessment with a Hygiene SwabCheck system was also made by swabbing the same area within the 50 cm^2 template at each region in the tank. The Hygiene Swab was then placed in the culture tube and transported as before to the Microbiology Lab for further assessment.

c. Mesophiles, yeast, mold, and coliform counts on BBL™ CultureSwab™:
Prepoured plates of plate count agar (PCA) for mesophiles, potato dextrose agar (PDA) for yeast and mold, and violet red bile agar (VRBA) for coliforms were used. The three types of plates were dried to remove moisture from the inner surface of the lid. Each plate was divided in half, numbered, and then swabbed in a close Z pattern with the BBL™ CultureSwabs™ brought to the lab. The plates were incubated at 35°C for mesophiles per 48 hours, 30°C for yeast and mold per 96 hours, and at 35°C for coliforms per 24 hours. The acceptability of swabs were dependent on the score given relative to growth on the plate (this ranged from: zero = no growth on plate, Acceptable to ++++ = solid growth on plate, Unacceptable). See tables for a key with detail.

d. Coliform SwabCheck:
These swabs were only assessed at the ACS Technical Services Microbiology Lab due to swabs being on back order and subsequent subzero frigid weather conditions.

e. Mesophilic counts on RediSwab™:
Appropriate serial dilutions were made, and decimal dilutions of samples were pipetted into labeled sterile Petri plates. A pour plate technique with tempered plate count agar (PCA) was used. The plates were incubated at 35°C for mesophiles per 48 hours, and counts were made.

f. Regular and osmophilic yeast and mold counts on RediSwab™:
Appropriate serial dilutions were made using Butterfield's phosphate buffer for regular yeasts and molds and the same buffer with 40% sucrose for osmophilic yeast and molds. The microbial counts were obtained using the Hydrophobic Grid Membrane Filter (HGMF) method or ISO-GRID Method, with use of 0.45 μm membrane filters and YM-11 agar with chlortetracycline-HCl supplement for regular yeasts and molds. The medium used for osmophilic yeasts and molds had 40% sucrose added to the YM-11 agar with chlortetracycline-HCl. The YM-11 plates were incubated at 28°C per 48 hours and the YM-11 sucrose plates at 30°C per 72 hours and counts were made.

g. Hygiene SwabCheck and Coliform SwabCheck Assessment:
Samples taken using the Hygiene SwabCheck or the Coliform SwabCheck system were taken to the ACS Technical Services Center Microbiology Lab and incubated at 37°C and observed for a color change as given below:

Swab Type	Detection	Incubation Time	Color Change
Hygiene SwabCheck	Total colony count	8-24 hrs	red → yellow
Coliform SwabCheck	E. coli/coliforms	18-24 hrs	purple → yellow

resulting in unnecessary rework. It is also puzzling how ATP can be lost within seconds of recheck on the AccuPoint system. Assessments carried out by factory personnel also showed that readings on the AccuPoint swabs were inconsistent on repeated checks. This could be due to the difference in swab types (cotton on Clean-Trace® swabs used in the Biotrace luminometer and sponge-like surface of the AccuPoint Surface Sampler used in the AccuPoint luminometer).

About this time we encountered another problem. This was due to the ATP values obtained on the luminometer used at the time (AccuPoint) not correlating with microbial assessment (Table 6 and Table 7, location #7 and #3d respectively). In this case we had solid growth on microbial plate checks with zero or acceptable RLU reading on the luminometer. Further studies were carried out on the isolates obtained on the plates from 8/9/04 (Table 7), and two species of Bacilli namely Bacillus licheniformis (99%) and Bacillus laevolacticus (72%) were identified using the Biolog MicroLog™ 3 Identification System.

Bacilli are gram-positive, endospore forming facultatively anaerobic bacteria. The resistance of its spore to a number of adverse conditions has resulted in widespread distribution of the organism. These spores are more resistant than vegetative cells to heat, drying, food preservatives, and other environmental challenges. Their hydrophobic nature coupled with presence of appendages on their surface, enables spores to adhere to several types of surfaces, including epithelial cells. This adhesiveness makes them difficult to remove during cleaning and sanitation of food contact surfaces. When spores are located in the space between seals and seal surfaces where water may be excluded, their heat resistance increases significantly (1). In addition, the isolate from the truck pump outlet on 9/28/04 was identified as Bacillus amyloliquefaciens B (99%), and the isolate from the truck tank outlet on 9/17/04 was identified as Serretia plymuthica (90.1%). These identifications were again carried out due to high microbial scores (+++) obtained on mesophilic plates with correspondingly low RLU values on the luminometer. See Table 8 for details on bacterial isolates from trucks.

The above situation was of concern. Therefore, a number of other possibilities leading to this problem – such as types of sanitizer used, employee sanitation protocols, trucking company, and truck wash station sanitation protocols – were investigated. We were using a quaternary ammonium sanitizer Ster-Bac from Ecolab at the time, and we looked at the value in switching to an acid sanitizer such as Vortexx and some comparative studies were run. However, no distinct differences were observed in the two sanitizers and therefore we decided to continue with the use of the quat sanitizer Ster-Bac. Investigation of employee sanitation protocols on syrup trucks showed that sometimes resanitizing of truck equipment had to be carried out 4-5 times before luminometer readings were at sufficiently low acceptable levels (Tables 7 & 9). The truck equipment also varied with the carrier. In addition truck wash station sanitation protocols were investigated by Jenny Kjos (Moorhead Packaging/Warehouse Supervisor) and stricter protocols were mandated and documented. The Moorhead factory also went back and forth with two different trucking companies at the time to see what served our needs best. Another problem we encountered with the AccuPoint Surface Sampler was that it was fairly short as compared to the Clean-Trace® ATP Swab used in the Biotrace Uni-*Lite*® Xcel and NG Systems, and therefore needed the use of an extender. This was an additional step in the sanitation process and more time consuming and not acceptable.

Additional work using the Accupoint, Biotrace Uni-*Lite*® Xcel and Biotrace Uni-*Lite*® NG were carried out and gave similar results. However we were still being tormented by bacillary spore formers which are very difficult to remove by any type of sanitizer. Therefore, about the same time that more rigid cleaning practices were enforced at the truck wash stations (180°F for 15 min.), we also decreased the pass/fail limits on the luminometer from 250 RLU to 100 RLU. Several weeks and months of evaluations followed with the use of the Biotrace NG system, and we have had no problems with discrepancies between microbial scores on plates and acceptable luminometer values since.

On a routine basis coliform testing is also carried out alongside mesophilic and yeast and mold assessments on syrup truck and brown sugar line sanitation checks. BBL CultureSwabs taken are swabbed on violet red bile agar plates which are checked the following day (24 hrs) after incubation. However, another method, the Coliform SwabCheck system, was also assessed. These assessments were only carried out at the ACS Technical Services Center Microbiological Lab due to swabs being on back order and subsequent subzero frigid weather conditions. The color change on these types of swabs (purple → yellow) occurred in 18-24 hrs and therefore is another rapid method that can be employed if quick microbial plating techniques are not available.

b) Molasses desugarization (MDS) extract tank sanitation survey and comparion of different evaluation methods

This sanitation check was carried out after cleaning and sanitation protocols had been completed in one of the steel tanks used for storage of extract at the East Grand Forks (EGF) molasses desugarization (MDS) plant. The usual protocol we had established was the use of a RediSwab™ technique with the use of a 50 sq. cm. template followed by microbial plate examination (2). This was because we had previously found that ATP bioluminescence systems were not reliable in the steel extract tank sanitation evaluations versus stainless steel surfaces. This was probably due to the pitted nature of these tank surfaces. However, though this method was reliable, results were obtained in 2-3 days. Therefore a second type of swab, the Hygiene SwabCheck, was also taken from the same region as the RediSwab™ sample was taken for comparison purposes. The samples were brought back on ice to the Technical Services Microbiology Lab, and the RediSwab™ samples were plated for mesophiles, osmophilic, and non-osmophilic yeast and mold. The Hygiene SwabCheck samples were incubated overnight at 37°C and observed for a color change (red to yellow) over a 8-24 hr. period.

Results obtained showed all negative samples (remain red at 24 hrs) and partially red at 24 hrs (+) gave zero or acceptable low microbial counts. However, positive yellow swabs (++ to +++++ = <19 hrs to 24 hrs) did not compare to microbial numbers in all cases (except for sample numbers 15 and 19). See Table 10 for further detail. Therefore this test method needs to be evaluated further but preliminary results are promising as no false negatives were obtained and some positives compared. Therefore due to quick turn around time for results the Hygiene SwabCheck method might be a boon to American Crystal Sugar Company which stores 5-10 million dollars worth of extract in each tank over a period of 7-9 months during the beet sugar campaign. In addition, this method could be used in conjunction with microbial plate checks for quick preliminary results followed by plate assessments for further reliability.

CONCLUSION

These studies have shown that:

a) In truck cleaning and sanitation protocols the key facet for having a clean product is to start clean at the truck wash station. Therefore, very rigid cleaning and sanitizing protocols have to be established initially at this stage as later on in the process once cane syrup is brought to the factory there are very few options for getting rid of spore formers such as Bacilli. Also quick detection methods with luminometers may not be reliable. Therefore at American Crystal we were able to overcome this problem not only with mandating more rigid cleaning protocols at truck wash stations but also by lowering the pass/fail limits on the luminometer. The Coliform SwabCheck technique was found to be another useful quick method that could be employed if rapid plating techniques were not readily available for coliform assessment.

b) Preliminary studies with the Hygiene SwabCheck technique has shown promise in extract tank sanitation checks. This is advantageous due to quick turn around time of 8-24 hours for a color change on swab samples versus 2-3 days for counts from microbial plate checks.

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Table 1

SANITATION SURVEY
ACCUPOINT, BIOTRACE, AND CULTURESWABS (5-19-04)

		LUMINOMETERS		BBL CULTURESWAB SCORES			
		Biotrace	AccuPoint	Mesophiles	Yeasts	Molds	Coliforms
#	LOCATION	RLU	RLU	PCA	PDA	PDA	VRBA
1	John Challey's desk	1130 (F)	46 (P)	++	0	0	0
2	Clean up west wall sink	14,217 (F)	23,443 / 687 (F)	++++	0	0	0
3	Drip pan below Ind. Brown S. end	94 (P)	129 / 145 (P)	++	+	0	0
4	Clean up W. wall sink (after Ster-Bac)	45 / 50 (P)	236 (P)	0	0	0	0
5	Micro bench – by balance	2432 / 2431 (F)	15,955 / 6009 (F)	+	0	0	0
6	Micro bench – by laser scanner	2012 / 2224 (F)	32, 005 / 48,995 (F)	++	0	0	0
7	Micro bench – by balance – sanitized 1	893 /914 (F)	10,213 / 1825 (F)	0	0	0	0
8	Unused swab	7	2	0	0	0	0
9	Micro bench – by balance – sanitized 2	328 / 440 (F)	3291 / 637 (F)	0	0	0	0
10	Same as 9 with 100% ETOH (1)	169 / 158 / 149 (P)	3504 / 49 / 0 (F/P/P)	0	0	0	0
11	Same as above – swab 2 (Baxter)	"	"	0	0	0	0
12	Micro bench – by scanner (10% bleach)	34 / 40 (P)	398 / 111 (F/P)	0	0	0	0
13	Same as 12 – swab 2 (Baxter)	"	"	+	0	0	0

NOTE: Luminometer value, <250 RLU=Pass (P), > 250 RLU = Fail(F)

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	Solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

BROWN SUGAR – HAYSSEN & INDUSTRIAL LINES (5-19-04) ACCUPOINT, BIOTRACE, AND CULTURESWAB COMPARISON

		Luminometers		BBL CultureSwab Scores			
		Biotrace	AccuPoint	Mesophiles	Yeasts	Molds	Coliforms
#	LOCATION	RLU	RLU	PCA	PDA	PDA	VRBA
1	Big Holder	14	0	0	0	0	0
2	Industrial Scale	48	0	0	0	0	0
3	Main Feed Auger	13	0	+	0	0	0

NOTE: Luminometer value, <250 RLU=Pass (P), > 250 RLU = Fail(F)

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	Solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

BROWN SUGAR PRODUCTION (5-24-04)
ACCUPOINT, BIOTRACE, AND CULTURESWAB COMPARISON

BOSCH EAST

		Luminometers		BBL CultureSwab Score			
#	LOCATION	Biotrace RLU	AccuPoint RLU	Mesophiles PCA	Yeasts PDA	Molds PDA	Coliforms VRBA
1	Large tubing pan	9	0	0	0	0	0
2	Vibrator trays	9	0	0	0	0	0
3	Vibrator trays	14	0	0	0	0	0
4	Vibrator trays	9	0	0	0	0	0
5	Scale buckets	10	0	0	0	0	0
6	Scale buckets	10	0	0	0	0	0
7	Scale buckets	12	0	0	0	0	0
8	Dumper	14	0	0	0	0	0
9	Distributor cone	15	31	0	0	0	0

BOSCH WEST

		Luminometers		BBL CultureSwab Score			
#	LOCATION	Biotrace RLU	AccuPoint RLU	Mesophiles PCA	Yeasts PDA	Molds PDA	Coliforms VRBA
1	Large tubing pan	10	0	0	0	0	0
2	Vibrator trays	12	0	0	0	0	0
3	Vibrator trays	15	0	0	0	0	0
4	Vibrator trays	14	0	0	0	0	0
5	Scale buckets	12	0	0	0	0	0
6	Scale buckets	11	0	0	0	0	0
7	Scale buckets	10	0	0	0	0	0
8	Dumper	10	0	0	0	0	0
9	Distributor cone	12	0	0	0	0	0

NOTE: Luminometer value, <250 RLU=Pass (P), > 250 RLU = Fail(F)

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	Solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

Table 4

BROWN SUGAR PRODUCTION (5-24-04)
ACCUPOINT, BIOTRACE, AND CULTURESWAB COMPARISON

BROWN SUGAR – HAYSSEN & INDUSTRIAL

		Luminometers		BBL CultureSwab Score			
		Biotrace	AccuPoint	Mesophiles	Yeasts	Molds	Coliforms
#	LOCATION	RLU	RLU	PCA	PDA	PDA	VRBA
1	Big Holder	20	0	0	0	0	0
2	Industrial Scale	45	0	0	0	0	0
3	Main Feed Auger	35	0	0	0	0	0

BROWN SUGAR BLENDING

		Luminometers		BBL CultureSwab Score			
		Biotrace	AccuPoint	Mesophiles	Yeasts	Molds	Coliforms
#	LOCATION	RLU	RLU	PCA	PDA	PDA	VRBA
1	Shell of blender – West Bosch line	86	79	0	0	0	0
2	Ribbon of blender – West Bosch line	49	117	0	0	0	0
3	Shell of blender – East Bosch line	76	68	0	0	0	0
4	Ribbon of blender – East Bosch line	64	113	0	0	0	0
5	Shell of blender – Industrial line	9	71	0	0	0	0
6	Ribbon of blender – Industrial line	86	0	0	0	0	0
7	Belt from blender to Industrial line	137	42	0	0	0	0

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	Solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

Table 5

BROWN SUGAR PRODUCTION (5-25-04) ACCUPOINT, BIOTRACE, AND CULTURESWAB COMPARISON

BROWN SUGAR BLENDING

		Luminometers		BBL CultureSwab Score			
		Biotrace	AccuPoint	Mesophiles	Yeasts	Molds	Coliforms
#	LOCATION	RLU	RLU	PCA	PDA	PDA	VRBA
1	Shell of blender – Hayssen line	12	0	0	0	0	0
2	Ribbon of blender – Hayssen line	21	45	0	0	0	0
3	Belt from blender to Hayssen line	46	419 / 440 / 34 / 140	0	0	0	0

BROWN SUGAR BLENDING
From different area of the blender #3

		Luminometers		BBL CultureSwab Score			
		Biotrace	AccuPoint	Mesophiles	Yeasts	Molds	Coliforms
#	LOCATION	RLU	RLU	PCA	PDA	PDA	VRBA
1	Shell of blender – Hayssen line	10	0	0	0	0	0
2	Ribbon of blender – Hayssen line	17	0	0	0	0	0
3	Bottom of blender to Hayssen line	17	0	0	0	++	0

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	Solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

Table 6

BROWN SUGAR SYRUP TRUCK SANITATION SWABS (7-6-04)
Luminometer and Microbial Score Comparison

#	LOCATION	AccuPoint (RLU)	Mesophiles	Yeasts	Molds	Coliforms
1	Truck tank outlet	8	0	0	0	0
2	Truck pump inlet	157	0	0	0	0
3	Truck pump outlet	7	+	0	0	0
4	Connector from truck to pump	4	0	0	0	0
5	Connector from hose to syrup line	0	0	0	0	0
6	Truck hose from pump	0	0	0	0	0
7	Truck hose to connector	0 (P)	++++	+++	0	0
8	In-house syrup line	0	0	0	0	0

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

Table 7

BROWN SUGAR SYRUP TRUCK SANITATION SWABS (8-9-04)
AccuPoint (ATP Swabs) and CultureSwab (agar plate) comparison

#	LOCATION	AccuPoint (RLU)	Mesophiles	Yeasts	Molds	Coliforms
1	Truck tank outlet	48 (P)	0	0	0	0
2a	Truck pump inlet	4486 (F)	0	0	0	0
2b	Truck pump inlet	269 (F)	0	0	0	0
2c	Truck pump inlet	13 (P)	+	0	0	0
3a	Truck pump outlet	750 (F)	++++	0	0	0
3b	Truck pump outlet	3025 (F)	++++	0	0	0
3c	Truck pump outlet	457 (F)	++++	0	0	0
3d	Truck pump outlet	**100 (P)**	**++++**	0	0	0
4	Connector from truck to pump	00 (P)	0	0	0	0
5	Connector from hose to syrup line	00 (P)	0	0	0	0
6	Truck hose from pump	00 (P)	0	0	0	0
7	Truck hose to connector	00 (P)	0	0	0	0
8	Positive control (lab counter surface)	-	++	0	++	0

(F) = Fail = > 250 RLU, (P) = Pass = Zero to <250 RLU

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

Table 8

GRAM POSITIVE & GRAM NEGATIVE BACTERIAL IDENTIFICATIONS (Biolog and API RapiD 20E Systems)

MHD Syrup Truck Gram positive isolates	Truck Unload Date
Bacillus licheniformis (99%)	8-9-04
Bacillus laevolacticus (72%)	8-9-04
Bacillus amyloliquefaciens B (99%)	9-28-04

MHD Syrup Truck Gram negative isolate	Truck Unload Date
Serratia plymuthica (90.1%)	9-17-04

Table 9

BROWN SUGAR SYRUP TRUCK SANITATION SWABS (8-16-04) Luminometer and Microbial score comparison

#	LOCATION	AccuPoint (RLU)	Mesophiles	Yeasts	Molds	Coliforms
1	Truck tank outlet	13	0	0	0	0
2a	Truck pump outlet	**3349 (F)**	0	0	0	0
2b	Truck pump outlet	**1508 (F)**	0	0	0	0
2c	Truck pump outlet	**0 (P)**	0	0	0	0
3	Connector from truck to pump	00	0	0	0	0
4	Truck hose from pump	00	0	0	0	0
5	Truck hose to connector	100	0	0	0	0
6	In-house syrup line	00	0	0	0	0

Key for CultureSwabs

Score	Amount of Growth	Colony Count
++++	solid growth on plate	very high (unacceptable)
+++	moderate growth	high (unacceptable)
++	sparse growth	low (acceptable)
+	1-3 colonies	very low (acceptable)
0	no presence	acceptable

Table 10

EAST GRAND FORKS EXTRACT TANK #2
REDISWAB SAMPLES (11-29-04)

#	Sample I.D.	Hygiene swab color change	LOG COUNT (cfu/50 sq. cm) Mesophiles	Yeast	Mold	Osmophilic Yeast	Osmophilic Mold
1	Floor by pillar #1	-	0.00	0.00	0.00	0.00	0.00
2	Pillar #1 base	-	0.00	0.00	0.00	0.00	0.00
3	Pillar #1 base	-	0.00	0.00	0.00	0.00	0.00
4	Center pillar base	-	0.00	0.00	0.00	0.00	0.00
5	Center pillar	-	0.00	0.00	0.00	0.00	0.00
6	Center inlet pipe (inside)	-	1.48	0.00	0.00	0.00	0.00
7	Inlet pipe surface (3' to center)	+	1.00	0.00	1.00	0.00	0.00
8	#10 pillar base (sticky)	-	1.00	0.00	0.00	0.00	0.00
9	Rust puddle on floor	-	1.60	0.00	0.00	0.00	0.00
10	East sample flange (wet)	-	0.00	0.00	0.00	0.00	0.00
11	E. sample point (sticky)	+++++	1.60	0.00	0.00	0.00	0.00
12	Wall – East sample point	-	2.75	0.00	0.00	0.00	0.00
13	Pillar #9 floor juice	++	1.30	0.00	0.00	0.00	0.00
14	Rust below NE Manway	-	0.00	0.00	0.00	0.00	0.00
15	NE Manway – wet spot	+++++	**5.11**	**2.49**	1.30	**2.18**	1.30
16	Wall to Right of NE manway	-	2.49	0.00	0.00	0.00	0.00
17	North flange	-	1.48	0.00	0.00	0.00	0.00
18	N. spigot – sticky	++++	0.00	0.00	0.00	0.00	0.00
19	NW manway	+++++	3.05	**1.48**	1.00	0.00	**1.60**
20	NW manway gasket	+++++	2.64	**1.78**	1.00	**1.00**	**1.70**
21	Pillar #4 base	+	1.00	0.00	0.00	0.00	1.00
22	Pillar #4 base	-	1.30	0.00	0.00	0.00	0.00
23	West flange (sticky)	++	1.00	0.00	0.00	0.00	0.00
24	W. spigot	+++	0.00	0.00	0.00	0.00	0.00
25	Wall (left of west flange)	++	**4.10**	0.00	0.00	0.00	0.00
26	Pillar #7 base (sticky hard)	+++	0.00	0.00	0.00	0.00	0.00
27	Sticky floor near sump	-	1.48	0.00	0.00	0.00	0.00
28	Heat exchanger floor drip	++	**4.06**	0.00	0.00	0.00	0.00
29	Heat exchanger surface	-	1.70	0.00	0.00	0.00	0.00
30	South manway floor	++++	2.04	0.00	0.00	0.00	0.00
31	South manway	no swab	0.00	0.00	0.00	0.00	0.00

Key for Hygiene SwabCheck color change: = Red to Yellow (within 2-24 hrs)
+++++ = < 19 hrs, ++++ = 19 hrs, +++ = 21 hrs, ++ = 24 hrs, + = 24 hrs (partial change)