



One Health Diagnostics™

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INTRODUCTION:

MicroSnap® Total and Enhanced Nutrient Broth is a rapid bioluminogenic test for detection and enumeration of the total population of viable aerobic bacteria in a sample, providing results in 7 hours.

The two-step test procedure requires an enrichment step where the growth of bacteria occurs followed by a detection step. During the enrichment step, bacterial numbers increase, and sample interference is reduced. As bacteria grow, they use up available food resources in the media and generate biomarkers. The greater the number of bacteria in the sample, the higher the biomarker concentration, and the greater output of light. Once the enrichment step is done, an aliquot of enriched sample is transferred to the detection device, activated, mixed, and measured in a luminometer. Light output is directly proportional to initial starting concentration of bacterial contamination in pre-enriched samples.

Some of the product matrices may pose sample inhibition with the testing method.

PURPOSE:

To evaluate the matrix effects of flavored tea beverages and to detect aerobic spoilage bacteria using the MicroSnap Total Enrichment Device and Enhanced Nutrient Broth.

REGISTERED TRADEMARKS:

MicroSnap® is a registered trademark of Hygiena.

Detection of Spoilage Organisms in Tea Beverages Using Hygiena’s MicroSnap® Total Enrichment Device and Enhanced Nutrient Broth

MicroSnap®

METHOD:

20% dilutions of cranberry, grapefruit, sweet tea, blueberry, peach and strawberry tea were made in BPW. Serial dilutions of mixed cultures of bacteria were spiked into the diluted teas. 1 ml of each of these dilutions were pipetted into 1.2 mL enrichment devices, 9 ml enhanced nutrient broth vials and plated on TSA agar plates, respectively. Enriched samples were tested using the MicroSnap detection device at the end of 8 hours and colony counts were performed from the TSA plates after 24 hours.

RESULTS:

All the beverages had acidic pH ranges from 2.52- 4.23. Diluting the teas in BPW helped to neutralize the low pH and the background signal caused by nonmicrobial ATP present in the beverages. For blueberry, sweet tea, peach and cranberry flavored teas, correlation between RLUs and CFUs was ≥ 0.95 when tested with 1.2 ml MicroSnap Total enrichment device whereas correlation values for grapefruit and strawberry flavored beverages were 0.88 and 0.79, respectively. When testing 20% dilutions of flavored teas using 9 ml Nutrient broth, a correlation between RLU and CFU of ≥ 0.95 was seen for all the flavored beverages.

RESULTS:

Table 1: pH of Different Juices (Neat, Neat diluted in nutrient broth and 10% in BPW)

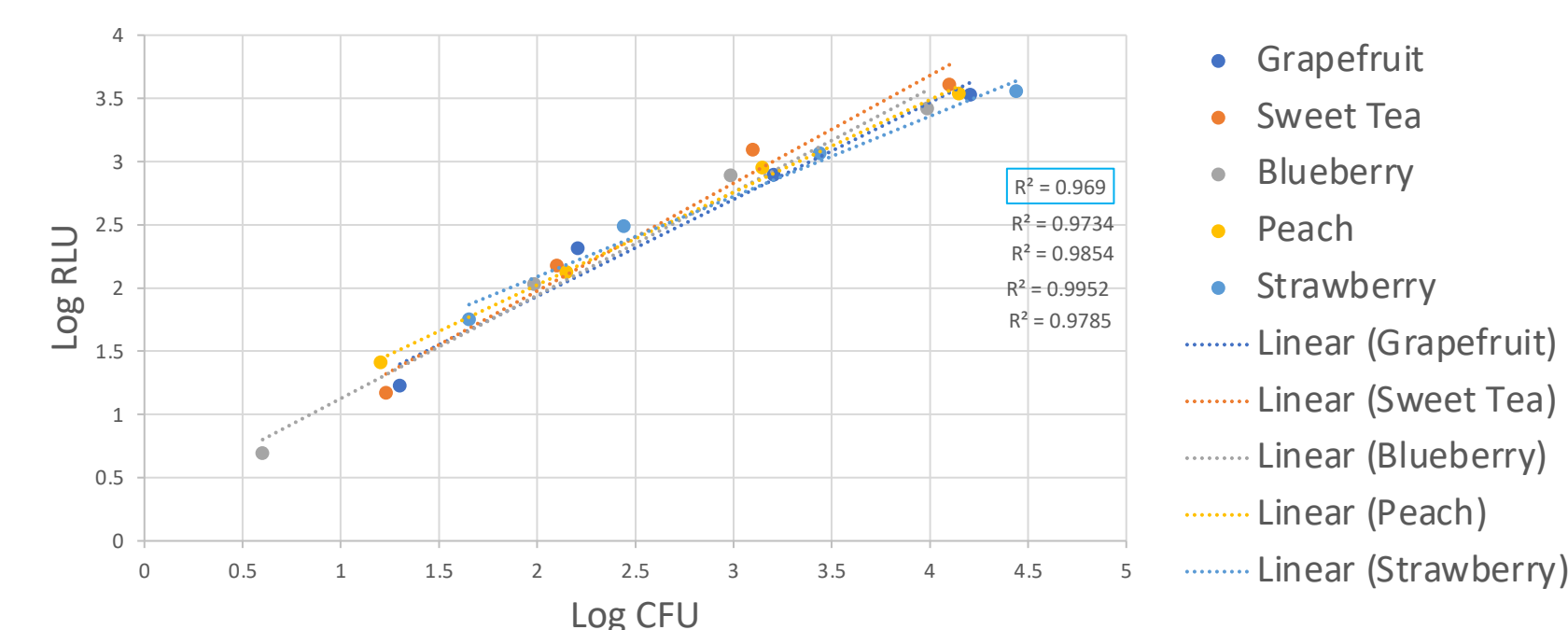
Beverage	Neat	Neat in Nutrient Broth	10% in BPW
Cranberry	2.52	5.02	6.81
Grapefruit	3.43	4.93	6.78
Sweet Tea	4.23	6.70	7.09
Blueberry	3.17	6.52	7.07
Peach	3.28	6.55	7.08
Strawberry	3.27	6.49	7.07

Table 2. Background Signal After 7 hours Incubation in MicroSnap Total Enrichment Swab Media

Beverage	Neat (CFU/mL estimate)	10% in BPW (CFU/mL estimate)
Cranberry	<10	965 ± 289*
Grapefruit	1126 ± 337	<10
Sweet Tea	1930 ± 579	<10
Blueberry	64 ± 19	<10
Peach	9037 ± 2711	<10
Strawberry	3248 ± 974	<10

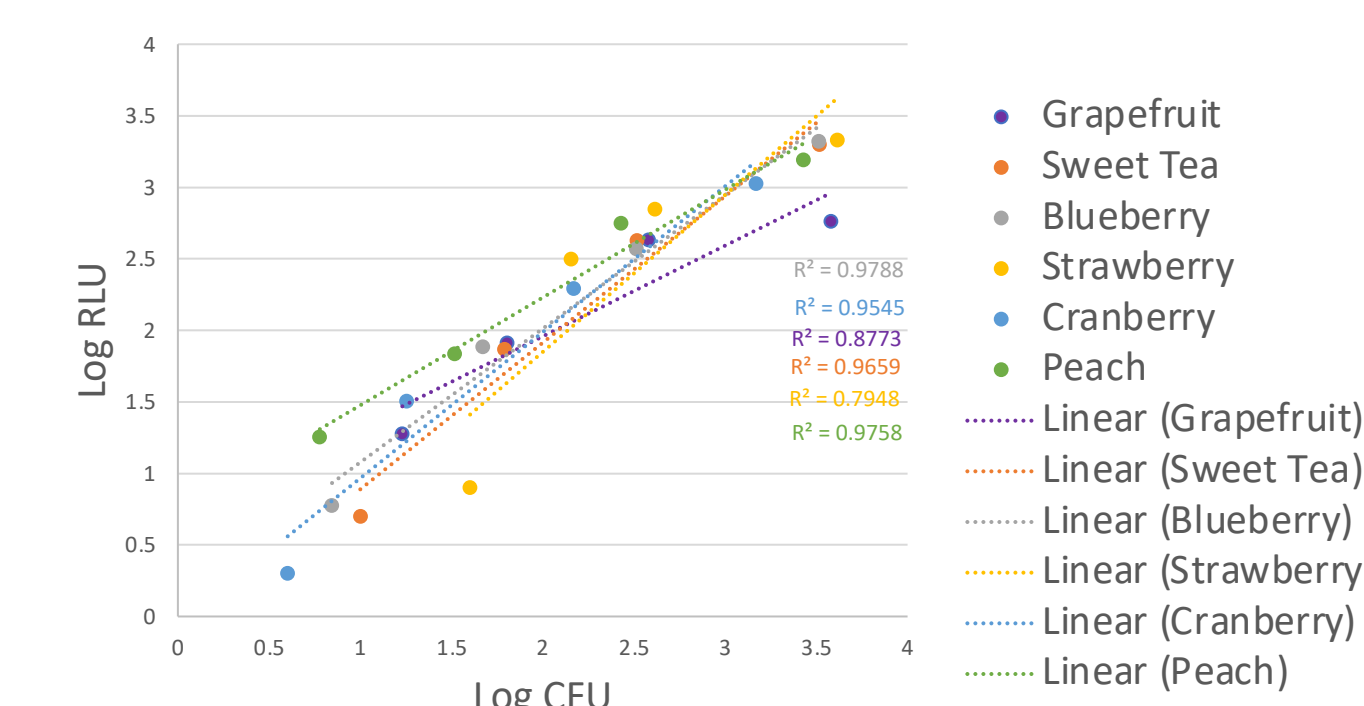
* Background signal for a 10% Cranberry beverage dilution in BPW decreased to <10 after 8-hour incubation

Graph 1: MicroSnap Total 8 hours Incubation – 10% dilutions

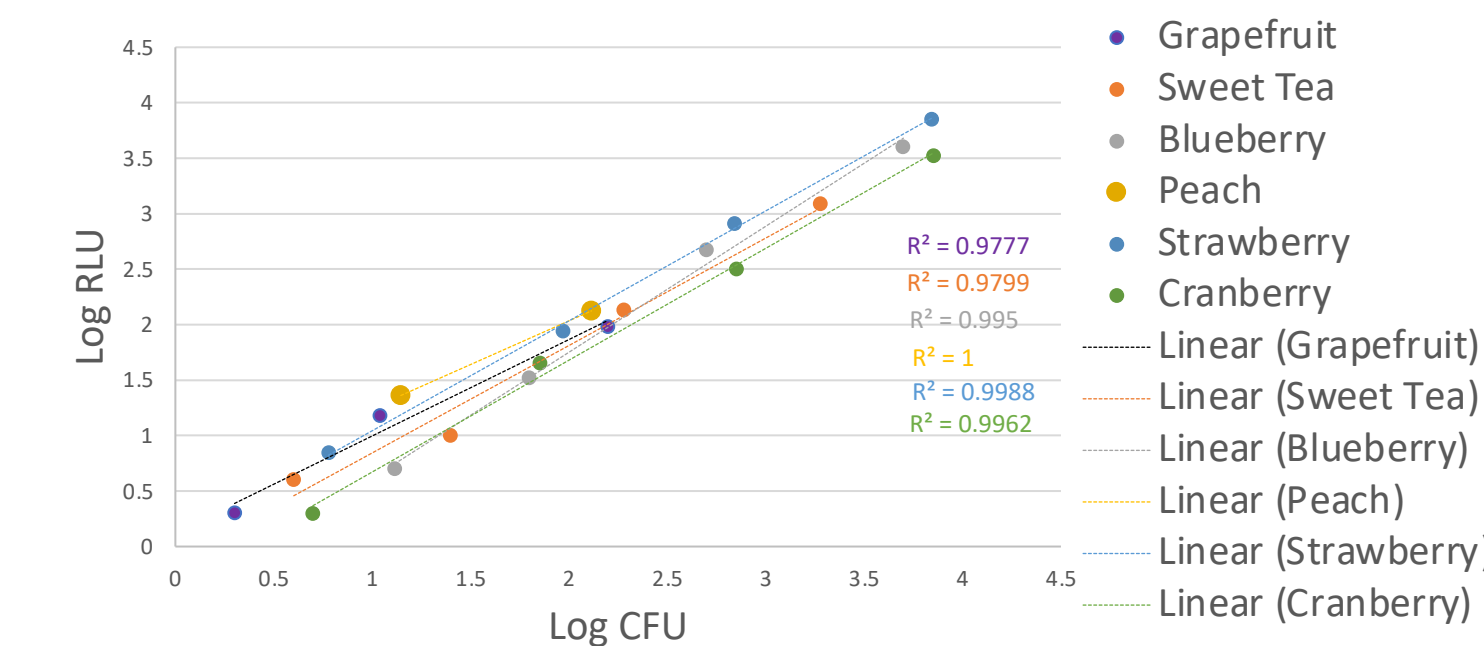


RESULTS:

Graph 2: MicroSnap Total 8 hours Incubation-20% dilutions



Graph 3: Nutrient Broth 8 hours Incubation- 20% dilutions



CONCLUSIONS:

Sample preparation is required for all beverages before analysis with MicroSnap Total or MicroSnap Enhance Nutrient Broth. The 1:5 dilution of each beverage in BPW allows for the neutralization of sample effects seen from the beverages. Incorporating the sample dilution step and incubating for 8 hours into the test protocol allows for the detection of ≥ 10 CFU/mL in the neat beverages. A product would be estimated to contain <10 CFU/mL if RLU results are below the threshold RLU found on Table 4. An RLU result equivalent to the Pass/Fail limit would merit a second reading with a new detection device to confirm first RLU result.

The MicroSnap System works well with the flavored tea samples, when the samples are first diluted in BPW. The validation has shown that there are subtle differences between the different flavored tea samples and hence for accuracy, each data set has been corrected using a multiplier. Overall, there is a good correlation between CFU and RLU when correction for unknown factor is accounted for using the multiplier.

The MicroSnap System works well with the flavored tea samples and can be used as a simple, rapid, accurate method to measure spoilage organisms from flavored tea beverages. Results can be obtained within 8 hours as compared to traditional methods which takes 24-48 hours.

