Detection of Beverage Spoilage Organisms in Low pH Juice Products Using Hygiena's Innovate™ System and RapiScreen™ Beverage Kit

One Health Diagnostics™

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INTRODUCTION:

Low pH juice products have a unique set of issues when testing for contaminants. The initial low contamination levels coupled with a difficult growth environment can mean that traditional detection methods may miss spoilage events. Growth of contaminating organisms often leads to severe spoilage and can burst containers.
Hygiena's Innovate™ System and RapiScreen™ Beverage Kit reagent technology is a high-throughput microbial screening system designed to meet the needs of today's fast paced beverage industry. The robust ATP bioluminescence detection system overcomes issues with inhibition in difficult beverage matrices, often producing a faster time-to-result than traditional plating methods. This study looked at a wide variety of organisms (including bacteria, yeast, and mold) that were spiked and recovered from 6 beverage products: Sports Drink, Mixed Berry, Apple, Orange 10%, Orange 85% and Red Berry.

PURPOSE:

This study set out to demonstrate the applicability of the Innovate System and RapiScreen Beverage Kit reagents for use in commercial sterility testing in the beverage industry. The panel of beverage products tested included a wide range of types and challenges, including low pH (3.09-4.08), varying sugar levels, and varying amounts of pulpy fruit.

REGISTERED TRADEMARKS:

METHODS:

Dekkera bruxellensis, Talaromyces pinophilus, Lactobacillus fructivorans, Saccharomyces cerevisiae, Alicyclobacillus acidoteresstris, Candida orthopsilosis and *Byssochlamys fulva* were acid adapted by dilution in a TSB product mixture to strengthen the organism and better recreate natural contamination events. The organisms were then individually grown in media and spiked into each product pack individually at <50 CFU per pack. The packs were run in triplicate and incubated at 25 °C; after 48 to 168 hours, samples were drawn, ATP measured on the Innovate System and confirmation plates produced.

RESULTS:

Saccharomyces and *Candida* were detected in all beverages by 48 hours with RLUs of 9,120, 49, 21, 629, 38, 46, 291, 2,026, 50, 160, 1,361 and 33 respectively. Sports Drink did not support the growth of *Saccharomyces* or *Candida*. The negative RLUs ranged from 1 to 5. *Talaromyces*, *Byssochlamys* and *Dekkera* were detected at 96 to 120 hours in all products with RLUs of 75, 54, 5,198 and 116, 77, 16,838 and 136, 572, 216 and 154, 56, 3,255 and 643, 174, 64. Sports Drink only supported the growth of *Byssochlamys* at 168 hours with an RLU value of 59,099. *Lactobacillus* and *Alicyclobacillus* did not grow in any of the products except *Lactobacillus* in Orange 85%, detected by ATP and plate methods at 168 hours incubation. Confirmation plates used typically showed growth 48 hours behind the detectable ATP levels.

RapiScreen Beverage Kit	Background RLU		Baseline RLU	
	Average RLU	Standard Deviation	Average RLU	Standard Deviation
Sports Drink	3	2	3	1
Apple Juice	6,015	122	4	3
Mixed Berry	1,533	179	2	1
Orange Juice	878,304	13,106	32	89
Red Berry Mix	4,618	295	2	1
Tropical Juice	249,740	43,135	49	222

Table 1. The average RLU and standard deviation results from background and baseline testing of all study products. Product packs were incubated for 72 hours at 30 ± 2 °C prior to testing on the Innovate Sytem with the RapiScreen Beverage Kit

RapiScreen Beverage Kit	Baseline RLU	Caution RLU	Fail RLU
Sports Drink, Apple Juice, and Berry Mixes	5	11	16
Orange Juice	32	64	95
Tropical Juice	49	97	146

Table 2. Average baseline, caution, and fail RLU values used for analysis of the spike study data. Sports Drink and Top Fruit products (excluding Orange) were grouped together with common cutoff values. Orange Juice and Tropical Juice gave higher and more variable RLU baselines and each have specific cutoff levels. Caution RLU = 2 x Baseline RLU, Fail RLU = 3x Baseline RLU.

Product	Organism	Spike Level (CFU/Pack)	48 h	72 h	96 h	120 h	168 h	TTD on confirmation plates
Sports Drink (pH 3.25)	*Alicyclobacillus acidoteresstris*	16	1	1	3	1	1	No Growth
	Talaromyces pinophilus	40	1	14	24	261	758	96 h
	Byssochlamys fulva	10	1	4	2	3	59,099	168 h
Mixed Berry (pH 3.75)	*Lactobacillus fructivorans*	12	2	3	2	2	2	No Growth
	Saccharomyces cerevisiae	8	9,120	Burst Pack				72 h
	Talaromyces pinophilus	37	5	3	75	469	967	96 h
	Dekkera bruxellensis	13	2	3	54	1,134	21,542	168 h
	Byssochlamys fulva	10	1	3	3	5,198	22,770	120 h
	Candida orthopsilosis	2	49	18,937	30,207	15,484	42,747	72 h
Apple Juice (pH 3.69)	*Lactobacillus fructivorans*	12	3	7	5	3	3	No Growth
	Saccharomyces cerevisiae	8	21,629	Burst Pack				72 h
	Talaromyces pinophilus	37	27	25	116	2,701	2,697	96 h
	Dekkera bruxellensis	13	3	6	77	1,873	59,368	168 h
	Byssochlamys fulva	10	3	3	3	16,838	77,836	120 h
	Candida orthopsilosis	2	38	15,192	36,365	61,742	60,048	72 h
Orange Juice (pH4.08)	*Lactobacillus fructivorans*	12	23	41	28	23	342	168 h
	Saccharomyces cerevisiae	<300	46,291	477,536	329,617	175,953	Burst Pack	72 h
	Talaromyces pinophilus	37	25	29	15	47	28	168 h
	Dekkera bruxellensis	13	28	15	62	572	31,038	168 h
	Byssochlamys fulva	10	35	44	37	44	2,478	268 h
	Candida orthopsilosis	2	2,026	1,493	10,074	15,390	56,338	72 h
Red Berry Mix (pH3.09)	*Lactobacillus fructivorans*	49	4	2	2	2	2	No Growth
	Saccharomyces cerevisiae	8	50	9,135	62,171	106,795	Burst Pack	72 h
	Talaromyces pinophilus	37	6	63	154	1,343	5,102	96 h
	Dekkera bruxellensis	13	14	4	56	1,093	31,994	168 h
	Byssochlamys fulva	10	3	4	8	3,255	2,828	120 h
	Candida orthopsilosis	2	160	45,831	31,895	26,007	10,568	72 h
Tropical Juice (3.38)	*Lactobacillus fructivorans*	12	8	13	15	13	18	No Growth
	Saccharomyces cerevisiae	8	1,361	79,661	48,141	233,740	Burst Pack	72 h
	Talaromyces pinophilus	37	5	130	643	560	785	96 h
	Dekkera bruxellensis	13	4	21	177	3,452	56,938	168 h
	Byssochlamys fulva	10	12	5	2	12	150	120 h
	Candida orthopsilosis	4	33	1,680	27,244	72,456	91,809	72 h

Table 3. Spike study average RLU results for each product, organism, and incubation time tested. Values highlighted in green indicate a result below the caution levels as shown in table 3. Values highlighted in yellow indicate a value between the caution RLU and the fail RLU. Finally, values highlighted in red indicate values above the fail RLU value.

CONCLUSION:

Alicyclobacillus acidoterrestris was the only organism tested in Sports Drink that was not detected within 168 hours incubation at 25 °C. There was also no growth on any of the confirmation plates. This indicates that the microorganism either died or was not able to grow to a detectable level in either culture methods or the Innovate system.
Lactobacillus fructivorans was only detected in Orange Juice after 168 hours incubation at 25 °C. No colonies were seen on any confirmation plates for any other product spiked with *Lactobacillus fructivorans*. This again indicates that the microorganism either died or was not able to grow to a detectable level in the product for either detection by culture methods or using the Innovate System.
Candida orthopsilosis and *Saccharomyces cerevisiae* were detected in every product after 48 hours incubation at 25 °C. Confirmation plates all showed growth when checked after 24 hours incubation, for a total detection time of 72 hours.
Talaromyces pinophilus spores and *Dekkera bruxellensis* cells were detected in each product tested within 120 hours of incubation at 25 °C except for Orange Juice, where *T. pinophilus* did not seem to be detected. In this case, a single colony was seen on PDA from one of two replicate spiked products after 120 hours of product incubation. Plates from the 168-hour incubated products showed no growth. As such, it can be assumed that although the microorganism didn't die in the product, the growth was stunted and slow.
Byssochlamys fulva was detected in every product after 168 hours incubation at 25 °C. Orange Juice was tested again after 240 hours incubation (data not shown). After 168 hours, only one replicate from the two spiked products gave an RLU result above the fail threshold. Additionally, no growth was seen on plates from the 168-hour incubated product samples. After 240 hours, there was detection on the Innovate System from one of the sample replicates. There was growth on confirmation plates from both replicates. As *Byssochlamys* forms fungal masses within the product itself, it provides a challenge during sampling. Additionally, it's likely there was a low concentration of *Byssochlamys* in the product. Due to these two factors, detection on the Innovate System was inconsistent and growth on plates resulted in low CFU counts.

In conclusion, in virtually every instance of testing a spiked sample, if there was growth in the product, as indicated by growth of the organism on the confirmation plates, the Innovate System detected this within the 96-168 hours incubation. Additionally, the Innovate System had a quicker or matching time to detection when compared to the confirmation plates.

SIGNIFICANCE:

Early and significant detection of acid-adapted organisms in low pH beverage products is rapid using ATP methods. This allows quicker release of products and shorter hold times. This demonstrates that the Innovate System and RapiScreen Beverage Kit reagents can be effective in commercial sterility testing in the beverage industry, reducing both product hold times and operator hands on time.

REFERENCES:

1. *Microbiology of the food chain - Method validation - Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory.*