ISO 16140-2:2016 Validation of Hygiena's Innovate™ RapiScreen™ Dairy Kit as an Alternative Method for Commercial Sterility Testing in Nutraceutical Products

Mat Lovesmith and Bernard Linke
Hygiena® International Ltd., Guildford, United Kingdom

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INTRODUCTION:

The nutraceuticals market is rapidly growing within the healthcare sector and from consumption by an increasingly health-conscious general public. Stringent sterility requirements for nutraceutical products consumed by high-risk patients puts more importance on microbial release testing, requiring commercial sterility testing with long incubation times.

PURPOSE:

This study demonstrates that the Innovate™ Rapid Microbial Screen System and the RapiScreen™ Dairy Kit are suitable for commercial sterility testing in UHT nutraceutical products using a validation guided by ISO16140-2:2016. The 30-minute method enables reduced time to results compared to standard plate-based commercial sterility testing.

REGISTERED TRADEMARKS:

METHODS:

Microbial cultures were prepared in Tryptic Soy (TSB) or Sabouraud Dextrose (SDB) broth with incubation at 37 °C for 24 hours. Overnight cultures were heat-shocked at 55 °C for 10 minutes before dilution to the desired spike level in maximum recovery diluent. Organisms were spiked into product packs (200 - 500 mL) at low (1 - 1.5 CFU/pack) and high (5 - 11 CFU/pack) levels. Spiked and uninoculated product packs were incubated for 14 days at 30 or 55 °C, depending on the tested organism. After incubation, aliquots were tested using the Innovate System and standard plate media (Tryptic Soy agar, Potato Dextrose agar, Orange Serum agar). Data from both methods was used to calculate the relative limit of detection (RLOD).

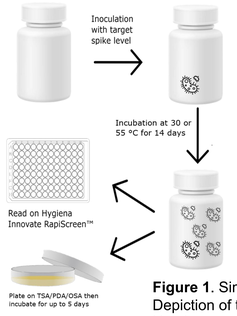

Figure 1. Simplified Depiction of the Protocol Used.

RESULTS:

High Protein & Energy Supplement

Organism	Spiked CFU per pack	Data type	Timepoints (days) 7	10	12	14
A. aceti	1.2	No. of Positives	0/30	1/30	9/30	10/30
		Average +ve RLU	N/A	25	400	1,534
	11	No. of Positives	0/5	5/5	5/5	5/5
		Average +ve RLU	N/A	63	2,037	1,101
L.brevis	1.5	No. of Positives	1/30	12/30	20/30	20/30
		Average +ve RLU	75	98	557	679
	7	No. of Positives	0/5	5/5	5/5	5/5
		Average +ve RLU	N/A	213	1,262	621
S. cerevisiae	1	No. of Positives	20/30	23/30	23/30	24/30
		Average +ve RLU	5,649	1,176	847	1,877
	5	No. of Positives	5/5	5/5	5/5	5/5
		Average +ve RLU	9,468	2,306	burst	burst
Negative control	0	No. of Positives	0/5	0/5	0/5	0/5
		Average RLU	3	3	4	5

Peptide-based Tube Feed

Organism	Spiked CFU per pack	Data type	Timepoints (days) 7	10	12	14
A. aceti	1	No. of Positives	0/30	0/30	17/30	18/30
		Average +ve RLU	N/A	N/A	61	348
	9.4	No. of Positives	0/5	0/5	5/5	5/5
		Average +ve RLU	N/A	N/A	286	1,674
L.brevis	1.5	No. of Positives	8/30	6/30	N/A	23/30
		Average +ve RLU	28	108	N/A	1,676
	7	No. of Positives	0/5	1/5	N/A	5/5
		Average +ve RLU	N/A	439	N/A	1,506
S. cerevisiae	1	No. of Positives	24/30	24/30	burst	burst
		Average +ve RLU	15,317	3,540	burst	burst
	5	No. of Positives	5/5	5/5	burst	burst
		Average +ve RLU	20,365	burst	burst	burst
Negative control	0	No. of Positives	0/5	0/5	0/5	1/5
		Average RLU	6	5	5	59

Iso-osmolar Carbohydrate Drink

Organism	Spiked CFU per pack	Data type	Timepoints (days) 7	10	12	14
B. cepacia	1.2	No. of Positives	8/30	8/30	8/30	8/30
		Average +ve RLU	511	510	236	542
	7	No. of Positives	5/5	5/5	5/5	5/5
		Average +ve RLU	618	586	285	781
Negative control	0	No. of Positives	0/5	0/5	0/5	0/5
		Average RLU	2	2	2	2

Amino Acid Ready-to-feed (RTE) Formula

Organism	Spiked CFU per pack	Data type	Timepoints (days) 7	10	12	14
A. aceti	1	No. of Positives	0/30	6/30	13/30	20/30
		Average +ve RLU	N/A	4,094	2,302	5,068
	9.4	No. of Positives	0	4/30	4/30	4/30
		Average +ve RLU	N/A	6,171	7,057	8,489
	0	No. of Positives	0/5	0/5	0/5	0/5
		Average RLU	21	10	10	11
L.brevis	1.5	No. of Positives	0/30	0/30	5/30	8/30
		Average +ve RLU	N/A	N/A	1,346	2,149
	7	No. of Positives	0/5	2/5	4/5	4/5
		Average +ve RLU	N/A	290	1,842	3,424
	0	No. of Positives	0/5	0/5	0/5	0/5
		Average RLU	7	17	11	11
			Timepoints (days) 2	5	7	14
S. cerevisiae	1	No. of Positives	0/30	1/30	7/30	14/30
		Average +ve RLU	N/A	714	6,961	157,478
	5	No. of Positives	0/5	2/5	2/5	4/5
		Average +ve RLU	N/A	392	49,345	102,826
	0	No. of Positives	0/5	0/5	0/5	0/5
		Average RLU	19	13	11	20

Tables 1 - 4. Summary of the Results of the ISO 16140-2:2016 Studies Performed Using Microbes Found to Grow in the Nutraceuticals by Showing Positives Obtained from the Innovate System and Their Average RLUs.

Product Type	Product Background at 168 h	Product Background at 168 h	Threshold RLU
Iso-osmolar carbohydrate drink	2	7	2
Amino acid ready-to-feed formula	986	13	39
Peptide-based tube feed	99	9	26
High protein & energy supplement	99	7	21

Table 5. RLU Values Used to Examine the External ATP Clearance and Calculate an Appropriate Contamination Threshold

Table 6. Statistical (RLOD) Analysis of the Day 14 Results Across the Examined Products.

Time	Product	Organism	RLOD	RLODL	RLODU	b=ln (RLOD)	sd(b)	z-Test statistic	p-value
Day 14	Iso-osmolar carbohydrate drink	*B. cepacia*	1.000	0.403	2.484	0.000	0.455	0.000	1.000
	Amino acid ready-to-feed formula	*A. aceti*	0.926	0.500	1.716	-0.077	0.308	0.249	1.197
		L. brevis	1.000	0.403	2.484	0.000	0.455	0.000	1.000
		S. cerevisiae	0.848	0.417	1.726	-0.165	0.355	0.465	1.358
	Peptide-based tube feed	*A. aceti*	1.000	0.518	1.930	0.000	0.329	0.000	1.000
		L. brevis	1.000	0.540	1.852	0.000	0.308	0.000	1.000
		S. cerevisiae	n/a						
	High protein & energy supplement	*A. aceti*	1.000	0.436	2.295	0.000	0.415	0.000	1.000
		L. brevis	1.000	0.530	1.887	0.000	0.318	0.000	1.000
		S. cerevisiae	1.000	0.541	1.850	0.000	0.308	0.000	1.000

DISCUSSION:

Four organisms grew in the nutraceutical products: *Acetobacter aceti*, *Burkholderia cepacia*, *Lactobacillus brevis* and *Saccharomyces cerevisiae*. All four organisms were detectable using the Innovate System after 14 days incubation with an RLOD of ≤1 CFU per pack. Four other organisms used in preliminary studies showed no growth in the nutraceutical products: *Bacillus coagulans*, *Bacillus licheniformis*, *Geobacillus stearothermophilus* and *Lactococcus lactis*.

SIGNIFICANCE:

Although nutraceutical products are inhospitable to many microorganisms, those capable of growth (risking spoilage or infection) are detected by the Innovate RapiScreen Dairy Kit, decreasing the time to results in commercial sterility testing.

REFERENCES:

1. International Organization for Standardization. 2016. ISO 16140-2:2016 - Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method. iTeh Standards.