

Protocol for assessing the sensitivity of hygiene test systems for live microorganisms and food residues

WJ Simpson, JL Archibald & CJ Giles

Cara Technology Limited, Leatherhead Enterprise Centre, Randalls Road, Leatherhead, Surrey, KT22 7RY, UK

Report 120906, 13 October 2006

Summary

We have developed a protocol for comparing hygiene test systems and used it to assess the performance of two systems (Biotrace Uni-Lite® NG / Clean-Trace® and the Hygiena systemSURE II™ ATP Hygiene Monitoring system / Ultrasnap™ ATP Swab). The protocol evaluates the systems' ability to detect three types of microorganisms and two types of food residue. Using the criteria for 'fail', 'caution' and 'pass' recommended by each supplier as the basis for evaluation, the Biotrace system failed 64.5% of all samples tested ($n = 400$) and the Hygiena system failed about a quarter of the samples (26.5%). The Hygiena system passed a majority (62.3%) of the samples, while the Biotrace system passed a quarter of them (26.5%). The results indicate that the test protocol provides an objective basis for system comparison, the Biotrace system being found to have better sensitivity and repeatability for residues of food and live microorganisms.

Introduction

Assessment of surface hygiene by measurement of adenosine 5'-triphosphate (ATP) is widespread. Many test systems are available but objective studies of their performance are few. While tests with solutions of ATP are easy to carry out, they provide only part of the picture. We aimed to develop a robust method for comparing the sensitivity of hygiene test systems which makes use of commonly-encountered microorganisms and foodstuffs. Here we report on the results obtained using this method with two commercially-available hygiene test systems.

Assessment protocol

In designing the protocol we aimed to challenge the systems with a range of widely-available sample types, representing Gram-positive and Gram-negative bacteria, a food-spoilage yeast, a live dairy product, and a yeast-derived food product – differentiated in terms of cultivation and storage times. Such samples span the range of residues likely to be encountered in many testing situations. The protocol is simple to use, and requires only a basic knowledge of statistics. A detailed work instruction for the protocol is available from us on request.

Two types of luminometer (Biotrace Uni-Lite® NG [Biotrace International plc, UK] and Hygiena systemSURE II™ ATP Hygiene Monitoring Device [Hygiena Limited, UK]) and test devices (Clean-Trace® from Biotrace and Ultrasnap™ ATP Swabs from Hygiena) were obtained *via* different industrial 'Mystery Shoppers'. These systems represent two different types of light detection technologies, *viz* photomultiplier and photodiode. Test devices from the same manufacturer were from the same batch in each case. Devices were stored at 4 – 8°C and brought to room temperature (controlled at 20°C) for 1 hour before use. Systems were used strictly in accordance with the manufacturers' instructions. Samples consisted of serial dilutions in sterile water of *Staphylococcus aureus* ATCC 6538 (Oxoid Culti-Loop® culture, grown for 1 - 4 days at 37°C in Tryptone Soya broth), *Citrobacter freundii* ATCC 8090 (Oxoid Culti-Loop® culture grown for 1 - 4 days at 37°C in Tryptone Soya broth), *Zygosaccharomyces bailii* NCYC 730 (grown for 1 - 4 days at 25°C in YM broth), a commercially available yeast extract (Vegemite, Kraft Foods, UK – stored for 1 - 4 days at 20°C prior to dissolving 2 g in 10 ml hot water and cooling prior to diluting, referred to as 'neat' below), and a commercially available probiotic yoghurt drink (Actimel, Danone, UK – stored for 1 - 4 days at 20°C prior to dilution). Samples were prepared by centrifuging 5 ml of broth culture (4000 g, 5 min, 4°C) in a centrifuge tube. The supernatant was removed and the pellet washed and reconstituted with autoclaved de-ionized water (5 ml). A dilution series down to 10^{-3} (or 10^{-4} in the case of *Zygosaccharomyces bailii*) was prepared in autoclaved de-ionized water. Food samples were prepared by direct dilution into such water. Samples (10 µl) were added to the mid section of the swab of each device. Devices were not rotated during application of the sample. Five replicate measurements of each sample type by dilution and time point were made. Tests were alternated between test systems to eliminate time-dependent effects. Device performance was checked with pure ATP-free water and solutions of ATP. Repeatability was tested by making alternate replicate measurements on a dilution of *Citrobacter freundii* at one time point (2 days).

Results

The samples used represent those likely to be found in industry, except insofar that variability due to sample pick-up from surfaces has been eliminated by their direct application to the test devices. In many ways they represent a best case concerning how the systems might perform in practice. The responses differ from system to system since light is measured in 'Relative Light Units' (RLU). As the choice of scale is arbitrary, no advantage is conferred by systems having high or low response values. Figure 1 shows the distribution of 30 replicate measurements on cells (*ca* 6×10^5 per swab) of *Citrobacter freundii*. The results, presented in the form of frequency histograms, were standardized to provide a common basis for comparison. They revealed differences in system performance: the Biotrace system was more repeatable ($cv = 7.8\%$ at a mean value of 8679 RLU) than the Hygiena system ($cv = 59.8\%$ at a mean value of 69.5 RLU) for identical samples.

Figure 2 shows the results of 400 tests on each system. Using the criteria for 'fail', 'caution' and 'pass' recommended by each supplier as the basis for the evaluation, the Biotrace system failed 64.5% of all samples ($n = 400$) with the Hygiena system failing less than half that number (26.5%). Sixty-two *per cent* of samples were passed by the Hygiena system, while the Biotrace system passed just over a quarter (26.5%). The remaining samples were at 'caution' levels. Since all the samples contained levels of viable or non-viable contaminants at or near the level which a food or beverage producer might initiate re-cleaning, the results suggest that the Biotrace system has better sensitivity for the types of residues encountered in food and beverage processing operations. Compared to the Biotrace equipment, the Hygiena system produced almost 60% false negative results.

Figure 1. Comparison of the repeatability of the Biotrace and Hygiena test systems for detection of live cells of *Citrobacter freundii*.

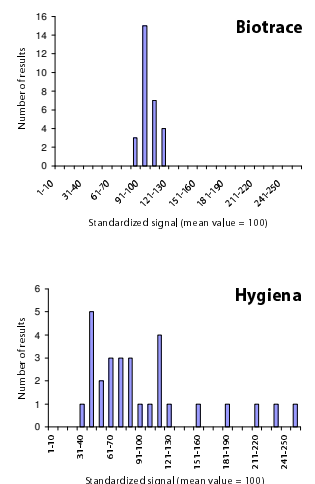


Figure 2. Comparison of the sensitivity of the Biotrace and Hygiene test systems. Table on left shows mean values for each sample (including all time points) by dilution for each test system. Tables on the right show the results for all 400 samples for each system (five replicates per sample). Pie charts summarize the ability of each system to detect contamination in the 400 samples presented.

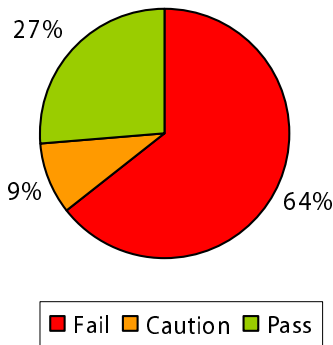
Sample	Staphylococcus aureus		Citrobacter freundii		Zygosaccharomyces bailii		Yeast extract		Yoghurt drink	
	Biotrace	Hygiene	Biotrace	Hygiene	Biotrace	Hygiene	Biotrace	Hygiene	Biotrace	Hygiene
Dilution 1	47282	197	51879	586	27556	467	1213	1	8162	17
Dilution 2	7969	22	8188	106	3401	41	272	2	3609	11
Dilution 3	880	4	999	21	448	8	69	2	596	19
Dilution 4	133	2	143	3	77	3	46	1	120	4

KEY	pass	caution	fail
Biotrace	≤150	151-299	≥300
Hygiene	≤10	11-29	≥30

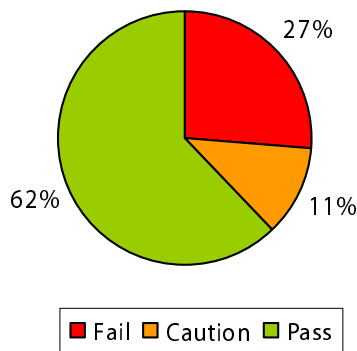
Note. Samples were diluted in 10-fold increments. Thus, differences in detection by dilution for each sample type are highly significant from a practical perspective.

Tests with ATP solutions showed that 'fail' levels for both systems relate to approximately 30 fmol ATP.

Biotrace - all samples



Hygiene - all samples



Number of samples found positive by Biotrace system	258
Minimum number of positive samples (assuming no false positives with Biotrace)	258
Number of samples found positive by Hygiene system	106
Number of samples passed by Hygiene system though failed by Biotrace system	152
Percentage of false negatives for Hygiene system compared to Biotrace system	58.9%

Sample type: Staphylococcus aureus		Incubation conditions: static culture @ 37°C									
Time (d)	Dilution	Biotrace Clean-Trace ⁺					Hygiene Ultrasnap TM				
		1	Neat	121273	74249	31833	47602	55904	103	100	53
	10-1	16290	17532	13659	19096	15405	13	15	9	9	13
	10-2	1586	1807	1939	1183	1853	2	2	3	2	2
	10-3	184	213	180	212	203	3	3	2	1	1
2	Neat	61038	53985	53037	47866	54912	442	608	650	503	296
	10-1	5382	5538	5859	5331	5715	34	34	47	15	38
	10-2	697	629	571	592	638	3	6	6	6	5
	10-3	104	102	110	111	95	3	3	2	3	2
3	Neat	43836	34529	33794	35365	33250	124	157	60	83	130
	10-1	3229	3887	3363	3117	3267	28	19	16	22	28
	10-2	491	480	474	409	477	5	4	5	4	4
	10-3	123	121	106	120	138	2	2	4	4	4
4	Neat	32631	36297	30317	34271	27645	72	82	113	50	124
	10-1	6534	7051	5944	6869	6317	26	18	16	25	12
	10-2	742	656	743	834	790	3	5	4	3	4
	10-3	109	115	106	113	93	1	0	1	0	2

Sample type: Citrobacter freundii		Incubation conditions: static culture @ 37°C									
Time (d)	Dilution	Biotrace Clean-Trace ⁺					Hygiene Ultrasnap TM				
		1	Neat	91135	79083	78110	62630	89257	1668	354	1064
	10-1	12526	14223	12336	12663	10880	79	285	65	160	110
	10-2	1834	1791	2152	2662	2367	14	77	36	25	22
	10-3	284	321	313	280	356	3	8	3	5	4
2	Neat	60781	75881	72543	83707	72525	409	155	714	640	386
	10-1	8450	9075	9230	9036	9347	22	57	70	15	94
	10-2	592	653	709	616	566	4	4	3	3	3
	10-3	42	50	53	48	52	2	2	2	2	2
3	Neat	35069	29865	31437	25054	31085	373	518	334	1376	598
	10-1	7975	8806	7946	8916	7880	206	103	107	183	141
	10-2	660	803	832	869	968	27	28	40	42	35
	10-3	158	167	154	149	112	9	5	2	3	2
4	Neat	18958	27331	28065	22306	22755	166	713	521	252	323
	10-1	2735	2744	2837	2967	3179	91	92	55	52	134
	10-2	373	368	394	393	374	4	9	8	28	11
	10-3	67	61	67	65	67	1	2	2	2	3

Sample type: Zygosaccharomyces bailii		Incubation conditions: static culture @ 25°C									
Time (d)	Dilution	Biotrace Clean-Trace ⁺					Hygiene Ultrasnap TM				
		1	10-1	15546	12211	9708	14383	11200	53	354	212
	10-2	955	1476	1155	1408	1451	62	30	46	7	82
	10-3	157	210	164	164	183	5	15	2	9	5
	10-4	67	48	70	53	40	2	4	2	3	2
2	10-1	24413	19129	9892	15515	12687	53	116	154	65	881
	10-2	3266	2512	2378	1158	4162	36	63	20	8	7
	10-3	311	362	207	409	472	3	6	3	3	3
	10-4	45	56	77	42	53	4	3	5	3	3
3	10-1	22736	49103	53534	33698	41648	481	244	181	309	99
	10-2	5786	2206	6219	5726	6148	11	11	2	3	13
	10-3	726	688	975	536	709	2	8	15	2	27
	10-4	87	87	222	123	107	2	1	5	1	1
4	10-1	49317	43151	44191	44643	24421	1020	2194	55	2057	156
	10-2	3112	4306	3752	4571	6273	139	14	232	31	9
	10-3	646	454	587	418	589	21	11	5	14	10
	10-4	44	65	96	79	70	2	2	1	2	4

Sample type: Yeast extract		Incubation conditions: stored @ 20°C									
Time (d)	Dilution	Biotrace Clean-Trace ⁺					Hygiene Ultrasnap TM				
		1	Neat	1207	1105	1328	1201	1241	0	0	0
	10-1	262	263	329	249	233	0	0	0	0	0
	10-2	58	56	61	66	79	0	0	0	0	0
	10-3	71	54	48	40	38	0	0	0	0	0
2	Neat	1080	1265	1296	1362	1268	1	2	2	2	2
	10-1	290	251	285	287	281	2	2	2	2	2
	10-2	67	74	77	58	63	2	2	2	2	3
	10-3	49	32	27	35	32	1	1	1	1	1
3	Neat	1119	1188	1243	1311	1260	2	2	2	2	2
	10-1	287	291	292	275	291	3	3	3	3	3
	10-2	63	67	76	75	67	3	3	3	4	4
	10-3	56	51	45	56	57	2	2	2	2	2
4	Neat	1236	1161	1135	1165	1084	1	2	2	2	2
	10-1	263	254	264	215	277	2	3	3	3	3
	10-2	78	81	71	58	79	3	3	3	3	3
	10-3	48	37	37	52	45	2	2	2	2	2

Sample type: Yoghurt drink		Incubation conditions: stored @ 20°C									
Time (d)	Dilution	Biotrace Clean-Trace ⁺					Hygiene Ultrasnap TM				
		1	Neat	11087	22011	25736	18236	18503	38	38	33
	10-1	6043	6769	5967	6295	6551	25	19	33	32	23
	10-2	899	730	881	861	710	49	54	58	28	42
	10-3	82	65	76	83	73	4	4	4	3	4
2	Neat	3619	3799	2605	4460	4103	11	9	8	10	7
	10-1	2284	2237	2396	1937	2479	5	4	5	5	5
	10-2	365	373	365	344	374	2	3	4	5	4
	10-3	79	69	70	76	87	3	2	3	3	3
3	Neat	3578	4976	4887	4750	4699	6	6	7	7	8
	10-1	2616	2996	3108	2956	2251	5	5	7	7	6
	10-2	435	450	508	428	525	7	7	5	7	4
	10-3	77	73	70	84	80	3	4	3	4	3
4	Neat	7189	4442	4338	5351	4850	7	7	8	9	9
	10-1	2826	2867	3145	2882	3579	7	7	8	9	9
	10-2	796	722	763	720	682	17	20	26	23	20
	10-3	84	100	99	107	85	5	5	5	6	5

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