



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

041901

The AOAC Research Institute hereby certifies the method known as:

Clean-Trace[®] Hygiene Monitoring System

manufactured by

Neogen Corporation

620 Leshar Place

Lansing, Michigan 48912

USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read 'Bradley A. Stawick'.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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December 05, 2024

Expiration Date

December 31, 2026

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METHOD NAME Neogen® Clean-Trace® Hygiene Monitoring System Formerly 3M™ Clean-Trace™ Hygiene Monitoring System	CATALOG NUMBER LM 1 (Clean-Trace Luminometer), UXL100 (ready-to-use devices)	
INDEPENDENT LABORATORY NSF International – Applied Research Center 789 N. Dixboro Rd. Ann Arbor, MI 48015 USA	APPLICABILITY OF METHOD Analyte: Adenosine triphosphate (ATP) Matrixes – stainless steel, ceramic, and plastic Performance claims – Clean-Trace Hygiene Monitoring and Management System (Clean-Trace) was shown to be effective for detecting ATP from food and microbial sources on stainless steel, plastic, and ceramic environmental surfaces. The LOD of pure analyte ATP was determined to be 3.03 femtomoles ATP/swab based on linear regression.	
ORIGINAL CERTIFICATION DATE April 28, 2019	CERTIFICATION RENEWAL RECORD Renewed through December 2026.	
METHOD MODIFICATION RECORD 1. January 2020 Level 1 2. November 2021 Level 1 3. January 2024 Level 1	SUMMARY OF MODIFICATION 1. Editorial changes. 2. Non-method performance software changes. 3. Editorial changes to rebrand method from 3M to Neogen Corporation.	
Under this AOAC Performance Tested MethodsSM License Number, 041901 this method is distributed by: NONE	Under this AOAC Performance Tested MethodsSM License Number, 041901 this method is distributed as: NONE	

PRINCIPLE OF THE METHOD (1)

The Neogen® Clean-Trace® Hygiene Monitoring and Management System consists of the following: Clean-Trace LM1 Luminometer (LM1 luminometer), Clean-Trace ATP Tests and Clean-Trace Hygiene Monitoring and Management Software (Version 1.0.7.0 or higher). This system detects Adenosine triphosphate (ATP) on surface or water samples and is used to support hygiene monitoring and management for food and beverage industries. The Clean-Trace ATP Tests use Luciferin/Luciferase bioluminescence assay to produce light that is directly proportional to the amount of ATP present. The LM1 luminometer measures the intensity of the light emitted from a sample, and it displays the test result in Relative Light Units (RLU) within 10 seconds. As with any method, users should conduct a validation study on their surface or water samples to determine the RLU test result for pass, caution and fail thresholds. The rapid test results provided by the LM1 luminometer provide data to verify cleaning effectiveness and support a food and beverage manufacturers decision to begin or delay production. The LM1 luminometer is a battery operated luminometer utilized for objective and sensitive determination of the result of ATP tests. The LM1 luminometer power supply is provided to charge the battery. The Clean-Trace Hygiene Monitoring and Management Software is designed for the transfer, organization, analysis and storage of data generated from the Clean-Trace ATP Tests.

Although the Clean-Trace Hygiene Monitoring and Management System is very sensitive, surfaces or solutions should not be considered sterile based on results. The Clean-Trace Hygiene Monitoring and Management System is not intended to be used in the diagnosis of conditions in humans or animals. The user is responsible to know and follow applicable workplace regulations. The system does not differentiate the source or type of contamination.

DISCUSSION OF THE VALIDATION STUDY (1)

The experiments conducted in this validation study demonstrate the ability of the Clean-Trace Hygiene Monitoring and Management System to detect low levels of ATP as pure analyte and from food and microbial sources on environmental surfaces (stainless steel, ceramic and plastic) relevant to food processing facilities. The LOD of the method was determined to be 3.03 fmole of ATP applied directly to the test swab in a volume of 20 µL using the formula described in Definitions section (d). However, a statistically significant difference is seen between 0 fmole and 1 fmole data sets using ANOVA (p=0.000). Related compounds did not interfere with ATP detection, even when tested at 100 times the ATP concentration. Two compounds, dATP and CTP, applied at 2500 fmole, gave RLU readings similar to ATP applied at 25 fmole, but did not interfere with ATP detection.

For the food matrix and microbial evaluations, the Clean-Trace method was able to detect ATP from a variety of food and microbial sources. The s_r and RSD_r values were relatively higher in the matrix study than in the microbial and pure analyte studies, which could indicate that complete homogenization of the foods and subsequent dilution and application onto the surface may have been more difficult because of the food particles. Variability in surface swabbing technique may have also played a role. For the matrix evaluation, there was a higher prevalence of outliers from the ceramic surface samples (approximately 7.5%), as opposed to the other two surfaces (approximately 5% for each surface). It is unknown whether this was due to the surface type or an artifact of the study design and testing. Outliers were less prevalent in the microbial study than from the food matrix study (less than 5% for each surfaces), and no increase in prevalence was observed from the ceramic surface. In all cases, the Clean-Trace RLU readings from the surface testing were in proportion to the dilution of food or organism type. Although inhibition (15.34–18.32%) was observed in the three classes of sanitizers, ATP was successfully detected on the Clean-Trace Hygiene Monitoring and Management System. Most decisions on ATP data are made in the range of 0–50 fmole ATP where the impact of sanitizers was 6.11% or less for this system. For this study, the sanitizers were not rinsed off the surfaces prior to obtaining the test sample. Common industry practice is to rinse after sanitizing, then proceed to collecting the surface test sample.

Table 2. ATP predicted result converted from RLU, standard deviation of repeatability (s_r), and LOD of the Clean-Trace method as determined with pure analyte

Replicates	ATP predicted result (fmole) ^a						
	0	1	2.5	5	10	25	100
1	-0.69	0.30	1.04	4.49	5.48	20.8	61.5
2	-1.19	0.79	0.79	2.02	7.21	20.8	136
3	-0.44	1.53	1.04	4.00	6.72	21.8	38.6
4	0.05	0.30	1.78	4.99	13.9	29.9	59.6
5	-0.94	0.54	2.52	4.49	15.4	27.9	104
6	-0.69	1.04	3.26	5.73	13.4	28.7	91.2
7	0.05	1.28	1.53	6.72	10.7	22.3	123
8	0.05	1.04	1.53	5.98	12.9	29.9	124
9	0.05	0.79	2.77	5.48	12.6	29.4	130
10	0.05	0.54	3.26	4.99	8.20	26.9	130
Mean fmole ATP ^b	-0.37	0.81	1.95	4.89	10.6	25.9	99.8
s _r ^c	0.48	0.41	0.93	1.29	3.48	3.95	35.2
LOD ^e	3.03 fmole ATP						

^aATP predicted results converted from RLU using equation from Figure 1.

^bMean ATP was calculated from 10 replicate test swabs per level.

^cStandard deviation of repeatability (s_r) was calculated from 10 replicate test swabs per level.

^eCalculated using a linear curve of the s_r versus RLU data, and RLU versus ATP concentration.

Figure 1. Clean-Trace mean RLU dose response to pure analyte ATP concentration (fmole)

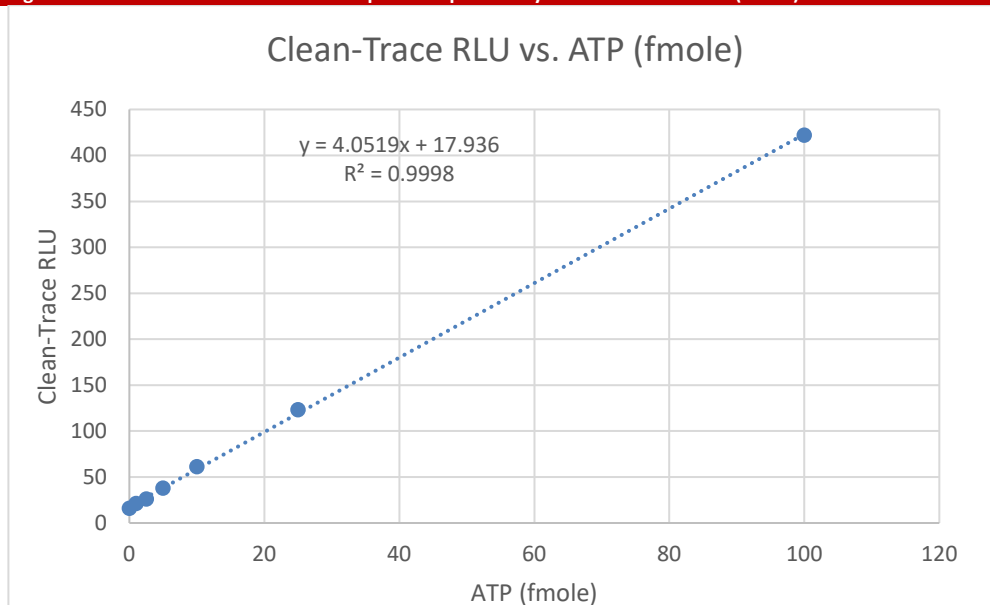


Table 3. Matrix study on stainless steel for the Clean-Trace Hygiene Monitoring and Management System (1)

Matrix	Dilution ^a	RLU ^b													Mean RLU ^c	s _r ^d	RSD _r ^e	
		1	2	3	4	5	6	7	8	9	10	11	12	13				14
Orange juice	1:12500	1252	563	1628	2051	669	574	694	287	1172	587	ND ^f	ND	ND	ND	948	531	56.0
	1:25000	221	285	205	188	363	304	231	38	367	521	ND	ND	ND	ND	272	130	47.6
	1:50000	67	86	38	167	295	138	257	218	202	171	ND	ND	ND	ND	164	83.2	50.7
	1:125000	50	37	37	69	44	41	40	50	134	81	ND	ND	ND	ND	58	28.7	49.5
	1:1250000	34	17	20	81 ^g	46	16	18	21	18	20	ND	ND	ND	ND	23	10.0	43.6
Apple turnover	1:100	486	649	1165	437	594	446	660	1643	991	582	1277	1295	ND	ND	852	405	47.6
	1:200	247	487	341	328	428	1002	488	952	757	642	2073 ^g	607	253	ND	544	255	46.9
	1:500	179	148	257	68	148	240	372	203	203	377	336	322	ND	ND	238	97.9	41.1
	1:2500	43	47	34	55	50	62	64	40	41	41	59	46	ND	ND	49	9.59	19.6
	1:5000	36	38	41	50	34	56	38	41	31	34	45	50	49	ND	42	7.64	18.2
Yogurt	1:500	422	307	618	1114 ^g	437	534	591	983 ^g	274	199	408	419	718	415	445	150	33.6
	1:1000	331	206	203	64	258	154	149	192	301	388	137	ND	ND	ND	217	95.0	43.8
	1:1500	120	97	169	195	298	568	116	114	316	314	236	ND	ND	ND	231	140	60.4
	1:2500	83	58	73	32	45	36	59	53	62	47	ND	ND	ND	ND	55	15.8	28.7
	1:5000	28	26	11 ^g	44 ^g	25	24	20	23	26	30	26	ND	ND	ND	25	2.87	11.5
	1:12500	17	24	16	34 ^g	21	15	16	18	18	21	ND	ND	ND	ND	18	2.96	16.5
Raw ground beef	1:5000	313	597	474	632	700	255	736	415	213	280	540	525	ND	ND	473	178	37.7
	1:10000	51	137	172	37	86	114	245	279	192	112	97	ND	ND	ND	138	76.7	55.6
	1:12500	61	58	28	49	69	83	59	42	92	47	43	79	ND	ND	59	18.9	32.0
	1:25000	31	23	41	24	58	20	42	26	25	31	38	27	ND	ND	32	10.8	33.9
	1:50000	26	21	21	25	25	24	25	28	22	32	19	26	ND	ND	25	3.50	14.0
Sliced deli turkey	1:300	407	417	324 ^g	411	380	318 ^g	412	405	633 ^g	413	ND	ND	ND	ND	406	12.3	3.0
	1:500	307	312	302	363	247	191	425 ^g	230	272	249	ND	ND	ND	ND	275	51.7	18.8
	1:1500	225	198	138	165	52	66	81	65	109	99	190	ND	ND	ND	126	60.5	48.0
	1:2500	65	54	44	71	59	107 ^g	48	33	46	52	ND	ND	ND	ND	52	11.5	22.1
	1:25000	15	19	54 ^g	19	15	18	35	16	25	22	ND	ND	ND	ND	20	6.37	31.8

^aDilution = Final dilution of matrix applied to 10 cm x 10 cm surface area.

^bRLU = Data are expressed in relative light units (RLU).

^cMean RLU = Average of the number of replicate surface areas tested per dilution.

^ds_r = Standard deviation of repeatability based on the number of replicate surface areas tested per dilution.

^eRSD_r = Relative standard deviation of repeatability, (s_r/mean x 100), expressed as a percentage.

^fND = Not done. Ten replicates were required for the study. In some cases, more replicates were tested.

^gOutlier = Data point determined to be an outlier based on Grubb's test and removed from the statistical analysis.

Table 4. Matrix study on ceramic for the Clean-Trace Hygiene Monitoring and Management System (1)

Matrix	Dilution ^a	RLU ^b													Mean RLU ^c	s _r ^d	RSD _r ^e
		1	2	3	4	5	6	7	8	9	10	11	12	13			
Orange juice	1:12500	1265	564	406	1797	673	614	1395	775	883	2930 ^f	ND ^g	ND	ND	930	458	49.3
	1:25000	1625	130	2584	67	1718	816	481	679	484	673	ND	ND	ND	926	800	86.5
	1:50000	60	259	162	216	230	164	205	188	251	131	ND	ND	ND	187	60.3	32.3
	1:125000	108	124	140	427 ^f	73	86	66	242 ^f	109	99	ND	ND	ND	101	25.1	24.9
	1:250000	246	142	78	95	257	3124 ^f	839	106	87	98	492	ND	ND	244	245	101
1:1250000	28	87	633 ^f	25	25	200 ^f	22	47	35	19	ND	ND	ND	36	22.4	62.2	
Apple turnover	1:200	2209	1044	2888	628	822	1436	289	2109	829	881	704	661	ND	1208	789	65.3
	1:500	139	630	515	930	622	563	541	848	153	519	589	1100	371	578	274	47.3
	1:2500	262	114	83	109	148	135	64	63	65	1697 ^f	131	546 ^f	ND	117	59.8	50.9
	1:5000	68	83	68	48	84	47	166 ^f	61	80	75	48	40	ND	64	16.0	25.0
	1:10000	45	39	38	54	53	41	38	36	35	57	58	ND	ND	45	8.88	19.8
Yogurt	1:250	798	2545 ^f	1365 ^f	504	596	688	898	600	728	429	873	ND	ND	679	161	23.8
	1:500	381	321	393	319	327	936 ^f	350	397	246	301	445	ND	ND	34	57.3	16.5
	1:1000	201	143	230	156	127	54	164	121	374	224	303	ND	ND	191	89.9	47.1
	1:1500	326	183	161	97	382	474	1338 ^f	133	58	71	33	ND	ND	192	151	78.7
	1:5000	23	28	44	40	97 ^f	30	35	39	25	30	26	28	ND	32	6.86	21.7
	1:12500	91 ^f	26	54 ^f	29	21	24	27	21	24	23	ND	ND	ND	24	2.83	11.6
Raw ground beef	1:5000	137	151	183	212	334	396	322	361	603	537	261	267	ND	314	146	46.4
	1:10000	180	108	95	98	82	173	188	351 ^f	120	121	123	296	ND	144	62.0	43.0
	1:12500	62	67	52	81	99	34	34	64	64	35	50	72	ND	60	19.8	33.3
	1:25000	30	38	29	37	72 ^f	32	28	52 ^f	38	26	36	ND	ND	33	4.66	14.3
	1:50000	23	30	20	18	19	27	30	25	27	31	31	27	ND	26	4.70	18.3
Sliced deli turkey	1:300	909	424	275	494	563	575	970	549	297	951	ND	ND	ND	601	258	43.0
	1:500	325	452	396	482	187	509	200	480	322	148	ND	ND	ND	350	135	38.5
	1:1500	145	85	180	131	77	72	141	102	62	ND	ND	ND	ND	111	40.4	36.5
	1:2500	37 ^f	97	76	59	252 ^f	85	135 ^f	96	84	80	ND	ND	ND	8	12.9	15.7
	1:25000	32	36	39	32	40	39	34	29	35	ND	ND	ND	ND	35	3.76	10.7

^aDilution = Final dilution of matrix applied to 10 cm x 10 cm surface area.

^bRLU = Data are expressed in relative light units (RLU).

^cMean RLU = Average of the number of replicate surface areas tested per dilution.

^ds_r = Standard deviation of repeatability based on the number of replicate surface areas tested per dilution.

^eRSD_r = Relative standard deviation of repeatability, (s_r/mean x 100), expressed as a percentage.

^fOutlier = Data point determined to be an outlier based on Grubb's test and removed from the statistical analysis.

^gND = Not done. Ten replicates were required for the study. In some cases, more replicates were tested.

Table 5. Matrix study on plastic for the Clean-Trace Hygiene Monitoring and Management System (1)

Matrix	Dilution ^a	RLU ^b														Mean RLU ^c	s ^d	RSD ^e
		1	2	3	4	5	6	7	8	9	10	11	12	13	14			
Orange juice	1:12500	1834	2081	903	225	1341	1740	415	1613	1247	1009	ND ^f	ND	ND	ND	1240	609	49.1
	1:25000	1136	653	592	579	395	660	526	1535	1838	2214	ND	ND	ND	ND	1013	637	62.9
	1:50000	315	77	204	211	486	358	258	407	319	497	ND	ND	ND	ND	313	132	42.0
	1:125000	341	105	130	145	151	72	343	143	58	459	ND	ND	ND	ND	193	136	69.8
	1:250000	33	90	41	119	100	112	104	43	100	151	ND	ND	ND	ND	89	38.4	43.0
	1:1250000	39	27	47 ^g	27	28	29	26	26	33	33	ND	ND	ND	ND	30	4.38	14.7
Apple turnover	1:200	797	1714	487	381	1030	587	1522	310	943	597	1303	69	ND	ND	ND	506	62.3
	1:500	592	609	267	317	633	802	448	182	541	439	633	527	ND	ND	ND	176	34.3
	1:2500	88	76	111	70	114	101	76	51	38	57	59	110	ND	ND	ND	24.5	31.0
	1:5000	410 ^g	61	74	56	59	93	40	66	44	48	107	52	ND	ND	64	20.7	32.5
	1:10000	46	38	39	54	31	48	41	32	39	45	45	ND	ND	ND	42	6.85	16.4
Yogurt	1:500	486	246	455	388	490	385	789 ^g	542	563	283	ND	ND	ND	ND	426	110	25.8
	1:1000	344	111	331	263	469	184	499	246	255	186	ND	ND	ND	ND	289	124	42.9
	1:1500	196	155	117	181	124	141	182	215	162	41	209	237	64	198	159	56.8	35.8
	1:5000	24	34	47 ^g	34	30	27	30	29	28	50 ^g	34	ND	ND	ND	30	3.50	11.7
	1:12500	22	23	20	30 ^g	22	18	16	25	22	23	ND	ND	ND	ND	21	2.77	13.1
Raw ground beef	1:5000	631	1111 ^g	196	540	544	434	153	302	299	145	243	361	239	ND	341	163	47.8
	1:10000	119	254	895 ^g	592	403	349	121	144	105	111	80	94	158	ND	211	159	75.5
	1:12500	98	250	169	80	544 ^g	74	76	190	153	91	96	ND	ND	ND	128	59.9	46.9
	1:25000	84	40	48	73	143 ^g	39	78	39	27	38	26	ND	ND	ND	49	21.2	43.2
	1:50000	29	30	103 ^g	59 ^g	38	17	27	32	22	35	28	ND	ND	ND	29	6.36	22.2
Sliced deli turkey	1:300	1348 ^g	1395 ^g	802	610	551	550	473	137	559	523	ND	ND	ND	ND	526	185	35.2
	1:500	558	766	159	227	380	330	244	985	667	511	ND	ND	ND	ND	483	265	55.0
	1:1500	225	329	258	257	236	147	114	104	102	120	ND	ND	ND	ND	189	81.3	43.0
	1:2500	144	96	272	363	273	82	73	58	77	127	ND	ND	ND	ND	157	107	68.3
	1:25000	37	72 ^g	75 ^g	26	31	25	20	30	28	35	ND	ND	ND	ND	29	5.50	19.0

^aDilution = Final dilution of matrix applied to 10 cm x 10 cm surface area.

^bRLU = Data are expressed in relative light units (RLU).

^cMean RLU = Average of the number of replicate surface areas tested per dilution.

^ds_r = Standard deviation of repeatability based on the number of replicate surface areas tested per dilution.

^eRSD_r = Relative standard deviation of repeatability, (s_r/mean x 100), expressed as a percentage.

^fND = Not done. Ten replicates were required for the study. In some cases, more replicates were tested.

^gOulier = Data point determined to be an outlier based on Grubb's test and removed from the statistical analysis

Table 6. Microbial study on stainless steel for the Clean-Trace Hygiene Monitoring and Management System (1)

Matrix	Dilution ^a	CFU/mL ^b	RLU ^c											Mean RLU ^g	s _r ^e	RSD _r ^f	
			1	2	3	4	5	6	7	8	9	10	11				
<i>Pseudomonas aeruginosa</i>	1:100	1x10 ⁶	1379	1217	ND ^g	ND	ND	ND	ND	ND	ND	ND	ND	ND	1298	81.0	6.2
	1:250	4x10 ⁵	970	647	1359	1356	633	1253	417	392	973	605	601	837	362	43.3	
	1:500	2x10 ⁵	497	425	465	283	379	609	262	428	366	336	455	410	99.6	24.3	
	1:1000	1x10 ⁵	111	72	488 ^h	99	126	192	202	261	96	148	47	135	65.8	48.7	
	1:2500	400	44	75	99	60	36	27	89	22	157	61	ND	67	40.7	60.7	
	1:5000	200	27	29	32	26	26	50	24	69	46	39	27	36	14.0	38.9	
	1:10000	100	23	22	35	35	40	19	39	36	45	25	29	32	8.50	26.6	
	1:50000	20	20	34	25	28	29	27	24	25	27	21	19	25	4.37	17.5	
<i>Staphylococcus aureus</i>	1:1250	800	31	63	108	136	41	131	156	135	112	162	ND	108	46.8	43.3	
	1:2500	400	38	55	97	66	37	42	69	86	69	29	810 ^h	59	22.5	38.1	
	1:11000	91	32	27	37	45	42	22	38	37	23	24	22	30	8.51	28.4	
	1:20000	50	32	37	15	38	26	13	28	24	22	32	18	26	8.46	32.5	
	1:101000	10	15	22	28	20	23	20	17	18	19	16	20	20	3.63	18.1	
<i>Saccharomyces cerevisiae</i>	1:16550	60	66	120	99	80	36	33	26	78	91	78	ND	71	30.7	43.2	
	1:49550	20	39	22	24	46	52	33	28	98 ^h	51	68	36	40	14.4	36.1	
	1:247750	4	76 ^h	26	29	53	25	22	32	26	45	23	25	29	10.3	35.4	
	1:495500	2	22	20	25	31	27	21	23	20	24	24	26	24	3.30	13.8	
	1:39283240	0	18	19	18	24	19	24	17	19	23	18	15	19	2.94	15.5	

^aDilution = Final dilution, made from 10⁶ CFU/mL cell suspensions of each organism, applied to 10 cm x 10 cm surface area.

^bCFU/mL = Estimated cell concentration applied to the surface, based on 10⁶ CFU/mL cell suspension starting material.

^cRLU = Data are expressed in relative light units (RLU).

^dMean RLU = Average of the number of replicate surface areas tested per dilution.

^es_r = Standard deviation of repeatability based on the number of replicate surface areas tested per dilution.

^fRSD_r = Relative standard deviation of repeatability, (s_r/mean x 100), expressed as a percentage.

^gND = Not done. Ten replicates were required for the study. In some cases, more replicates were tested.

^hOutlier = Data point determined to be an outlier based on Grubb's test and removed from the statistical analysis

Table 7. Microbial study on ceramic for the Clean-Trace Hygiene Monitoring and Management System (1)

Matrix	Dilution ^a	CFU/mL ^b	RLU ^c												Mean RLU ^d	s _r ^e	RSD _r ^f
			1	2	3	4	5	6	7	8	9	10	11	12			
<i>Pseudomonas aeruginosa</i>	1:500	2x10 ³	301	499	604	1041	710	798	398	840	824	1011	ND ^g	ND	703	236	33.6
	1:1000	1x10 ³	425	629	120	115	196	512	330	549	252	564	ND	ND	369	192	52.1
	1:2500	400	317	200	189	33	153	135	120	175	288	72	ND	ND	168	87.7	52.2
	1:10000	100	69	76	81	86	51	53	41	76	48	46	ND	ND	63	16.6	26.3
	1:250000	4	56	58	37	40	33	97 ^h	36	37	34	84	ND	ND	46	17.0	36.0
<i>Staphylococcus aureus</i>	1:2050	488	265	309	178	183	313	81	127	120	175	244	251	ND	204	77.8	38.1
	1:6050	165	85	87	86	48	130	92	35	117	78	13	71	51	74	33.3	45.0
	1:25250	40	50	30	30	73	29	30	27	64	44	38	24	29	39	15.8	40.5
	1:50500	20	17	30	104 ^h	45	20	42	32	25	22	33	24	ND	29	9.23	31.8
	1:510050	2	20	20	17	18	32	19	31	27	13	33	21	ND	23	6.78	29.5
<i>Saccharomyces cerevisiae</i>	1:25250	40	83	46	82	37	42	85	75	65	63	66	ND	ND	64	17.6	27.5
	1:50500	20	35	44	46	44	37	62	31	36	60	36	97 ^h	ND	43	10.6	24.5
	1:134667	7	27	24	16	27	25	21	54 ^h	20	25	23	22	19	23	3.44	15.1
	1:510050	2	42	15	14	28	25	17	28	37	26	20	17	17	24	8.91	37.1
	1:56615550	0	18	35 ^h	62 ^h	17	16	22	17	17	14	16	21	15	17	2.50	14.7

^aDilution = Final dilution, made from 10⁶ CFU/mL cell suspensions of each organism, applied to 10 cm x 10 cm surface area.

^bCFU/mL = Estimated cell concentration applied to the surface, based on 10⁶ CFU/mL cell suspension starting material.

^cRLU = Data are expressed in relative light units (RLU).

^dMean RLU = Average of the number of replicate surface areas tested per dilution.

^es_r = Standard deviation of repeatability based on the number of replicate surface areas tested per dilution.

^fRSD_r = Relative standard deviation of repeatability, (s_r/mean x 100), expressed as a percentage.

^gND = Not done. Ten replicates were required for the study. In some cases, more replicates were tested.

^hOulier = Data point determined to be an outlier based on Grubb's test and removed from the statistical analysis

Table 8. Microbial study on plastic for the Clean-Trace Hygiene Monitoring and Management System (1)

Matrix	Dilution ^a	CFU/mL ^b	RLU ^c													Mean		
			1	2	3	4	5	6	7	8	9	10	11	12	13	RLU ^d	s _r ^e	RSD _r ^f
<i>Pseudomonas aeruginosa</i>	1:250	4x10 ³	842	1781	569	903	1237	1147	1358	1861	1887	314	ND ^g	ND	ND	1189	517	43.5
	1:500	2x10 ³	190	1232 ^h	392	403	784 ^h	352	433	468	292	466	ND	ND	ND	375	94.9	25.3
	1:1000	1x10 ³	159	225	308	183	158	114	102	257	253	267	ND	ND	ND	203	69.5	34.2
	1:2500	400	77	91	101	68	67	88	68	60	109	66	ND	ND	ND	80	16.8	20.9
	1:250000	4	49	33	53	43	42	39	37	43	50	39	ND	ND	ND	43	6.27	14.6
<i>Staphylococcus aureus</i>	1:1375	727	385	111	89	75	199	232	136	324	78	875	487	488	408	299	231	77.4
	1:2750	364	225	166	294	75	177	149	266	388	47	109	111	97	ND	175	101	57.7
	1:5500	182	55	71	60	59	94	68	22	18	106	174 ^h	76	ND	ND	63	27.6	43.8
	1:20350	50	40	30	34	33	40	32	24	42	22	26	22	55	66 ^h	33	9.75	29.5
	1:616050	2	24	21	35 ^h	23	42 ^h	17	15	13	18	20	21	17	ND	ND	3.51	18.5
<i>Saccharomyces cerevisiae</i>	1:15150	66	112	128	78	70	97	102	156	167	79	157	79	ND	ND	111	35.6	32.1
	1:50500	20	46	51	56	63	45	53	31	72	41	50	57	ND	ND	51	11.0	21.6
	1:101000	10	38	25	33	38	24	33	30	29	38	32	27	43	35	33	5.66	17.2
	1:510050	2	19	39 ^h	26	19	21	34	26	24	20	19	ND	ND	ND	19	5.01	26.4
	1:51515050	0	21	28	17	20	17	19	24	30	23	18	ND	ND	ND	22	4.52	20.6

^aDilution = Final dilution, made from 10⁶ CFU/mL cell suspensions of each organism, applied to 10 cm x 10 cm surface area.

^bCFU/mL = Estimated cell concentration applied to the surface, based on 10⁶ CFU/mL cell suspension starting material.^cRLU = Data are expressed in relative light units (RLU).

^dMean RLU = Average of the number of replicate surface areas tested per dilution.^es_r = Standard deviation of repeatability based on the number of replicate surface areas tested per dilution.

^fRSD_r = Relative standard deviation of repeatability, (s_r/mean x 100), expressed as a percentage.

^gND = Not done. Ten replicates were required for the study. In some cases, more replicates were tested.

^hOulier = Data point determined to be an outlier based on Grubb's test and removed from the statistical analysis

Table 10. Effect of common sanitizers on the Clean-Trace Hygiene Monitoring and Management System

Sanitizer	Mean RLU ^a								Percent Inhibition ^f		
	C ^d	S ^e	50 fmole ATP ^b		125 fmole ATP		500 fmole ATP		50 fmole ATP	125 fmole ATP	500 fmole
			CA ^g	SA ^g	CA	SA	CA	SA			
Quaternary ammonium	14	22	299	289	813	698	2863	3202	6.11	15.3	-11.6
Peracetic acid	15	19	287	306	808	676	2850	3140	-5.59	17.2	-10.1
Sodium hypochlorite	33	41	302	319	725	606	2498	2166	-3.12	18.3	13.8

^aMean relative light units (RLU) calculated from replicate swabs per ATP (fmole) application.

^bATP = Adenosine triphosphate; 50, 125, or 500 fmole/20 µL, 20 µL applied directly to swab.

^cA negative percent inhibition correlates to an increase in signal. It was calculated using mean RLU and the following formula: % inhibition = 100 [1-((SA-S)/(CA-C))].

^dC = Signal from the control surface (analyte-free water dried onto the stainless-steel surface for 2 h, then swabbed).

^eS = Signal from the disinfectant sample control (disinfectant dried onto the stainless-steel surface for 2 h, then swabbed).

^fCA = Signal from the control surface plus ATP (analyte-free water dried onto the stainless-steel surface for 2 h, then swabbed, then ATP applied to the swab).

^gSA = Signal from the disinfectant sample plus ATP (disinfectant dried onto the stainless-steel surface then swabbed, then ATP applied to swab)



Table 15. Robustness study summary for the Clean-Trace Hygiene Monitoring and Management System

ATP ^a (fmole)	Test condition ^b	Mean RLU ^c	s _r ^d	RSD ^e
0	22°C; 5 s	11.5	1.50	13.1
	35°C; 15 s	9.48	1.76	18.6
	35°C; 2 s	8.40	1.32	15.7
	10°C; 15 s	7.88	0.93	11.8
	10°C; 2 s	5.76	1.45	25.2
10	22°C; 5 s	77.6	12.1	15.5
	35°C; 15 s	39.0	3.14	8.05
	35°C; 2 s	37.2	3.35	9.00
	10°C; 15 s	58.5 ^f	30.0	51.2
	10°C; 2 s	51.5	5.29	10.3
100	22°C; 5 s	699	38.9	5.56
	35°C; 15 s	282	39.9	14.2
	35°C; 2 s	273	39.8	14.6
	10°C; 15 s	495	72.5	14.7
	10°C; 2 s	431	81.9	19.0

^aATP = Adenosine triphosphate; 0, 10, or 100 fmole/20 µL, 20 µL applied directly to swab.

^bTest conditions are temperature of environment and test materials during testing and mix time once the swab is reinserted into the tube following sampling.

^cMean RLU = Data expressed as relative light units (RLU), based on the number of replicate swabs averaged across read times (0, 30, 60, 90 and 120 s).

^ds_r = Standard deviation of repeatability based on the number of replicate swabs averaged across read times (0, 30, 60, 90 and 120 s).

^eRSD_r = Relative standard deviation of repeatability, (s_r/mean x 100), expressed as a percentage.

REFERENCES CITED

1. Bakken, H., Yordem, B., and Monteroso, L, Evaluation of the 3M™ Clean-Trace™ Hygiene Monitoring and Management System, AOAC Performance Tested MethodsSM certification number 041901.