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CERTIFICATION

AOAC Research Institute
Performance Tested Methods^{SM}

Certificate No.
091601

The AOAC Research Institute hereby certifies the method known as:

AccuPoint® Advanced ATP Hygiene Monitoring System

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*^{SM} Program and found to perform as stated in the applicability of the method. 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.

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

Issue Date	January 15, 2024
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METHOD NAME
AccuPoint® Advanced ATP Hygiene Monitoring System

CATALOG NUMBERS
9903, 9903RFID, 9904A, 9904E, 9904P

INDEPENDENT LABORATORY
NSF International
789 N Dixboro Rd
Ann Arbor, MI 48105 USA

APPLICABILITY OF METHOD
Target organism – Adenosine triphosphate (ATP).

Matrixes – stainless steel (4 x 4 in)

Performance claims – As determined by linear regression and other statistical approaches, the AccuPoint® Advanced ATP Hygiene Monitoring System method is effective at determining the presence of ATP on stainless steel surfaces in food processing and food service facilities at an LOD of 1.85 femtomoles (fmol) ATP/assay.

ORIGINAL CERTIFICATION DATE
September 09, 2016

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **January 2018 Level 1**
2. **December 2018 Level 1**
3. **October 2020 Level 2**

SUMMARY OF MODIFICATION
1. **Editorial changes.**
2. **Editorial changes to add PTM certification mark to labels.**
3. **Validation of AccuPoint Advanced Next Gen luminometer.**

Under this AOAC *Performance Tested Methods*[SM] License Number, 091601 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)

The AccuPoint Advanced test system utilizes an ATP-induced bioluminescence reaction to determine the cleanliness of test samples. ATP is a chemical compound found in all living cells, including bacteria, yeast and mold, and food debris. Bioluminescence is a chemical reaction that produces light. ATP bioluminescence occurs when ATP from a sample comes into contact with luciferase, an enzyme found in fireflies, and luciferin, a substrate. The amount of light emitted in this reaction is proportional to the amount of ATP detected in a sample. After a sample is taken, the sampler is pressed into its cartridge, breaking its seal and initiating the mixing of reagents. The reaction takes place within the cartridge, and a detector measures the amount of light produced. The reading is displayed on the liquid crystal display (LCD) screen in RLU. According to preset limits, an icon is displayed indicating a pass, marginal or fail result. These limits are defined by the operator based on internal validation data or by using the system presets. Customers should validate the AccuPoint Advanced test system to determine safety thresholds and applicability for their facilities. Also, the system is designed to record the time, date, specific test-site location and site group information in addition to the recorded RLU and result. This information can be uploaded to the Data Manager software for additional review and reporting. Up to 999 unique testing sites can be tracked with each AccuPoint instrument using radio-frequency identification tags. Results from up to 4,000 tests can be retained at any time and are not lost if the instrument loses power. Four rechargeable, nickel metal hydride (NiMH) batteries provide power. Training in the use of the AccuPoint Advanced ATP Hygiene Monitoring System is available through Neogen.

DISCUSSION OF THE VALIDATION STUDY (1)

The performance validation of the AccuPoint Advanced ATP Hygiene Monitoring System produced results that support kit claims in providing a useful way to monitor the effectiveness of sanitation programs. Pure analyte was used to determine the LOD of the assay. A RLU dose-response to ATP concentration was observed, and the LOD was determined to be 10.1 fmol ATP in the internal evaluation and 6.2 fmol ATP in the independent laboratory study. At these low ATP levels, the minor difference in results between labs provides evidence of good agreement. To determine the feasibility of detecting food matrix residues on stainless steel surfaces, the surface was treated with dilutions of matrix. Measurable results were observed in all food matrixes tested. Flour showed the least reactivity, which may be attributed to the nature of flour processing. Highly refined foods may contain little residual ATP, which could have contributed to the low response to flour (7). As expected, ground beef, deli turkey, orange juice, and yogurt showed a response to varying degrees, but greater than that observed with flour. To determine the feasibility of detecting microorganisms from stainless steel surfaces, the surface was treated with organisms representing those found in food processing facilities. Like the matrix studies, RLU responses to organism concentration were observed and was dependent on the organism. *Pseudomonas aeruginosa* was the least reactive and required the highest cell density to achieve measurable results. A possible explanation for this observation is that peak ATP levels in *Pseudomonas* occur in log phase and decrease in stationary phase to enhance stationary survival (7). It has been shown that peak ATP levels in *P. aeruginosa* were within 6 hours after incubation, and the cells used in this study protocol were harvested after approximately 24 h (8). Specificity results show that the test can discriminate between ATP and dATP, when tested at the same concentration, and does not react with similar compounds. Additionally, these compounds do not interfere with the response to ATP with the exception of dATP which was tested at 100X the ATP concentration. Regardless, these data provide evidence that the method can detect ATP from a variety of sources.

Collectively, the results in this validation report provide evidence that the AccuPoint Advanced ATP Hygiene Monitoring System produce consistent and reliable data for evaluating sanitation program effectiveness on stainless steel surfaces in food processing and food services facilities.

Table 2. Replicate RLU, mean RLU, standard deviation of repeatability (s$_r$), and calculated probability (p) of the AccuPoint Advanced method determined with various matrixes (1)

Matrix	Dilution	Replicate RLU					Mean RLU[a]	s$_r$[b]	p[c]
		1	2	3	4	5			
Ground Beef	Background	29	6	72	1	53	32	30	NA[d]
	10^{-1}	3598	4201	3498	1806	2812	3183	914	0.0000
	10^{-2}	184	163	215	161	13686[e]	181	25	0.0001
	10^{-3}	132	304[e]	145	160	103	135	24	0.0005
	10^{-4}	19	0	0	4	35	12	15	0.1061
	10^{-5}	5	18	17	265[e]	29	17	10	0.1904
Deli Turkey	Background	20	10	0	0	0	6	9	NA
	10^{-1}	996	1246	925	729	908	961	187	0.0000
	10^{-2}	1528	1152	967	883	1023	1111	253	0.0000
	10^{-3}	62	149	37	5	141	79	64	0.0177
	10^{-4}	7	27	0	13	32	16	11	0.1059
	10^{-5}	12	0	289[e]	0	0	3	6	0.2926
Orange Juice	Background	29	6	72	1	53	32	30	NA
	10^{-1}	89199	95923	53350[e]	97764	100466	95838	4803	0.0000
	10^{-2}	27141	19708	18042	10932	25397	20244	6442	0.0001
	10^{-3}	2684	2066	1910	1511	5720[e]	2042	487	0.0000
	10^{-4}	176	227	15	283	3708[e]	175	115	0.0153
	10^{-5}	129	37	43	93	100	80	39	0.0309
Yogurt	Background	0	10	0	23	23	11	12	NA
	10^{-1}	11672	11711	14238	9308	12918	11969	1822	0.0000
	10^{-2}	4732	4280	2130	4820	6564	4505	1588	0.0001
	10^{-3}	646	446	557	603	781	607	123	0.0000
	10^{-4}	103	91	92	76	67	86	14	0.0000
	10^{-5}	65	60	100	36	118	76	33	0.0016
Flour	Background	20	10	0	0	0	6	9	NA
	10^{-1}	81	96	169	168	265	156	73	0.0009
	10^{-2}	23	129	82	138	146	104	51	0.0015
	10^{-3}	0	0	0	34	31	13	18	0.2276
	10^{-4}	0	0	15	114	112	48	59	0.0776
	10^{-5}	31	31	0	31	125[e]	23	15	0.0365

[a]Mean RLU calculated from 5 replicate coupons per dilution.
[b]s$_r$ calculated from 5 replicate coupons per dilution.
[c]Calculated probability using a one-tailed, t-test.
[d]Not applicable.
[e]Excluded from data analysis based on Grubbs' test.

Table 3. Replicate RLU, mean RLU, standard deviation of repeatability (s$_r$), and calculated probability (p) of the AccuPoint Advanced method determined with various microorganisms (1)

Organism	CFU/mL[a]	Replicate					Mean RLU[b]	s$_r$[c]	p[d]
		1	2	3	4	5			
S. cerevisiae	Background	31	13	17	23	4	18	10	NA[e]
	10^0	32	0	18	21	31	20	13	0.3568
	10^1	47	0	50	71	34	40	26	0.0536
	10^2	24	97[f]	35	16	18	23	8	0.2028
	10^3	96	93	81	90	85	89	6	0.0000
	10^4	670	661	772	780	625	702	70	0.0000
	10^5	8947	7161	8562	8186	6167	7804	1131	0.0000
P. aeruginosa	Background	21	29	31	20	17	24	6	NA
	10^5	28	69	55	19	60	46	22	0.0270
	10^6	100	181	193	237	265	195	63	0.0002
	10^7	1563	1786	1841	1044	3230	1893	811	0.0004
	10^8	14089	12846	8334	14550	17960	13556	3479	0.0000
S. aureus	Background	48	66	55	60	60	58	7	NA
	10^0	49	59	65	76	68	63	10	0.1663
	10^1	77	0	11	37	38	33	30	0.0511
	10^2	22	40	30	52	78	44	22	0.1134
	10^3	0	34	5	48	1433[f]	22	23	0.2044
	10^4	110	76	121	129	163	120	31	0.0013
	10^5	270	396	517	442	484	422	96	0.0000
	10^6	5537[f]	7458	7790	7550	8139	7734	304	0.0000

[a]The actual amount of organism added to the coupon was 250 µL of the CFU/mL.
[b]Mean RLU calculated from 5 replicate coupons per dilution.
[c]s$_r$ calculated from 5 replicate coupons per dilution.
[d]Calculated probability using a one-tailed, t-test.
[e]Not applicable
[f]Excluded from data analysis based on Grubbs' test.

Table 4. Results of specificity testing for the AccuPoint Advanced method. (1)

Abbreviation[a]	Name	RLU at 0 fmol ATP and 6000 fmol compound	RLU at 60 fmol ATP and 6000 fmol compound
NA[b]	analyte-free water	0	NA
ATP	Adenosine 5'-triphosphate sodium salt hydrate	NA	300
dATP	2'-deoxyadenosine 5'-triphosphate sodium salt	2915	3433
UTP	Uridine 5'-triphosphate trisodium salt	0	242
GTP	Guanosine 5'-triphosphate sodium salt	0	292
TTP	Thymidine 5'-triphosphate sodium salt	0	313
dUTP	2'-Deoxyuridine 5'-triphosphate sodium salt	0	300
CTP	Cytidine 5'-triphosphate	0	325
dGTP	2'-deoxyguanosine 5'-triphosphate trisodium salt	0	299
ITP	Inosine 5'-triphosphate trisodium salt	0	241
dIMP	2'-deoxyinosine 5'-monophosphate sodium salt	0	255
dCTP	2'-deoxycytidine 5'-triphosphate disodium salt	0	269

[a]Abbreviation for each compound in the Name column.
[b]Not applicable.

Table 12. Independent laboratory mean RLU and standard deviation of repeatability (s_r) of the AccuPoint Advanced method determined with various matrixes. (1)

Matrix	Dilution	Replicate 1	2	3	4	5	6	Mean RLU[a]	s_r[b]	p[c]
Deli Turkey	Background	19	0	0	0	0	NA	4	17	NA[d]
	10^{-1}	2203	2182	2378	1655	2540	2590	2258	340	0.0000
	10^{-2}	864	253	356	502	736	451	527	232	0.0004
	10^{-3}	0	0	43[e]	5	8	0	3	4	0.3898
	10^{-4}	0	0	0	0	0	0	0	0	0.1483
	10^{-5}	0	0	0	0	0	0	0	0	0.1483
Orange Juice	Background	0	0	0	0	0	NA	0	0	NA
	10^{-1}	172090	139043	147392	191360	195420	131258	162761	27433	0.0000
	10^{-2}	20564	23843	29695	18756	19357	25841	23009	4262	0.0000
	10^{-3}	2784	3072	1823	3539	2262	2086	2594	651	0.0000
	10^{-4}	478	407	349	197	168	346	324	120	0.0001
	10^{-5}	0	2	13	15	27	21	13	11	0.0115

[a]Mean RLU calculated from 5 replicate coupons per dilution.
[b]s_r calculated from 5 replicate coupons per dilution.
[c]Calculated probability using a one-tailed, t-test.
[d]Not applicable.
[e]Excluded from data analysis based on Grubbs' test.

DISCUSSION OF THE MODIFICATION APPROVED OCTOBER 2020 (8)

An LOD of 1.85 fmol ATP/assay was calculated with the AccuPoint Advanced Next Gen, compared to 6–10 fmol/assay with the current AccuPoiont Advanced reader. No significant differences in results were observed between the instruments for the low standard. Statistical differences were seen at the medium and high standards but did not exceed a 7% difference in RLU.

Conclusion and Highlights: Based on the data collected in this study, the performance of the AccuPoint Advanced Next Gen system was comparable to the original instrument and is an acceptable alternative for use with the AccuPoint Advanced Hygiene Monitoring System.

Table 1: RLU measurement of pure analyte ATP added to AccuPoint samplers and read in an AccuPoint Advanced Next Gen luminometer (8)

| Replicate	RLU at Applied ATP Concentrations/assay 0[a]	1.6[a]	3.1[a]	6.3[a]	12.5[a]	25[a]	100[a]	1000[a]
1	0	0	13	22	61	168	572	5353
2	0	0	16	20	54	148	481	4569
3	0	8	6	23	82	154	713	7060
4	0	0	13	29	66	180	617	5602
5	0	0	7	40	68	158	622	6343
6	0	0	9	23	66	181	693	5227
7	0	9	16	33	66	168	756	5606
8	0	0	3	11	78	185	662	6808
9	0	0	9	18	79	154	801	6428
10	0	0	14	21	78	150	671	6103
Mean RLU[b]	**0**	**2**	**11**	**24**	**70**	**165**	**659**	**5910**
s_r[c]	0	4	4	8	9	14	92	773
LOD (RLU)[d]	6.51							
LOD (fmol)[e]	1.85							

[a]ATP (femtomole/assay) quantity applied to sampler pad.
[b]Average RLU from 10 replicates per ATP level.
[c]s_r calculated from 10 predicted replicates per RLU level.
[d]LOD in RLU calculated using regression analysis of s_r against mean interpolated ATP.
[e]LOD in fmol calculated using regression analysis of s_r against mean interpolated ATP.

AccuPoint Advanced Next Gen RLU vs. Known ATP Concentrations

$y = 5.908x + 7.7736$

$R^2 = 0.9812$
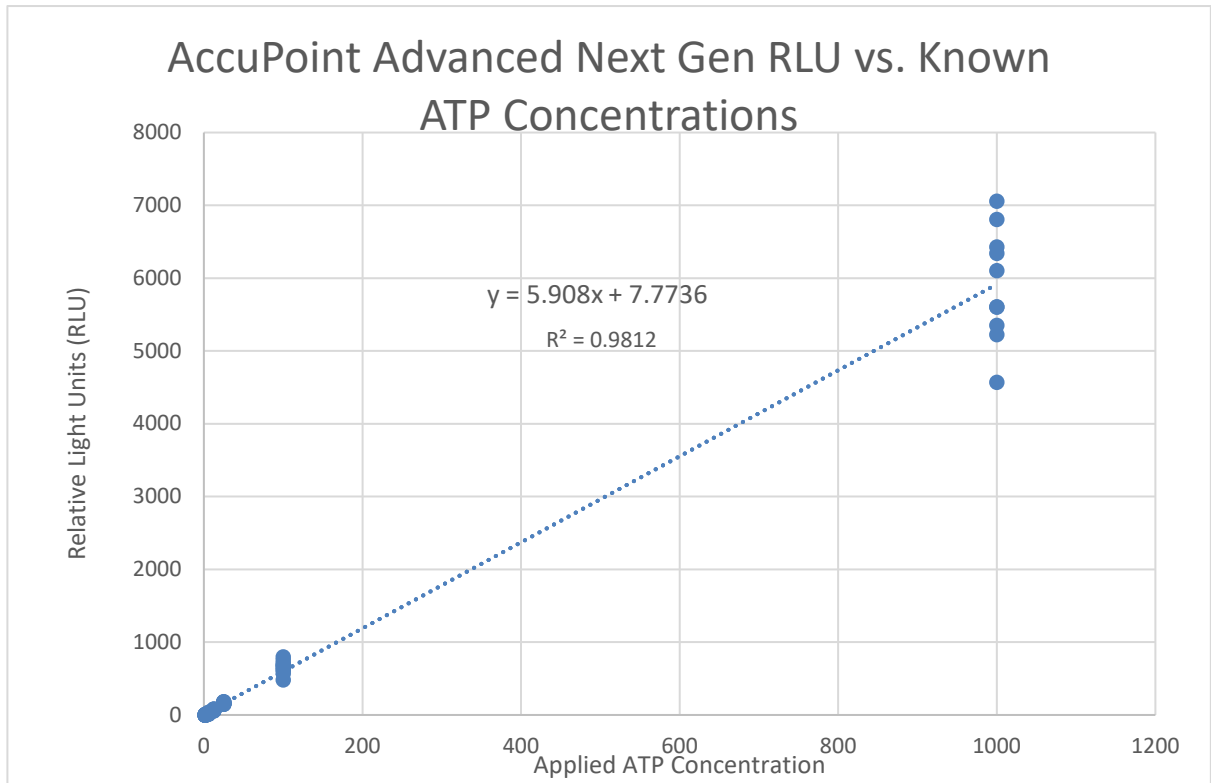
Figure 1: Relationship of AccuPoint Advanced Next Gen (10 replicates) to pure analyte ATP concentration measured in an AccuPoint Advanced Next Gen luminometer (8)

Table 2: Interpolated pure analyte ATP concentrations measured using AccuPoint Advanced Next Gen luminometer (8)

Replicate	Interpolated ATP at Applied ATP Concentrations (femtomoles/assay)[a]						
	1.6	3.1	6.3	12.5	25	100	1000
1	-1.32	0.89	2.41	9.01	27.12	95.50	904.74
2	-1.32	1.39	2.07	7.82	23.74	80.10	772.04
3	0.04	-0.30	2.58	12.56	24.75	119.37	1193.67
4	-1.32	0.89	3.59	9.86	29.15	103.12	946.89
5	-1.32	-0.13	5.46	10.19	25.43	103.97	1072.31
6	-1.32	0.21	2.58	9.86	29.32	115.98	883.42
7	0.21	1.39	4.27	9.86	27.12	126.65	947.57
8	-1.32	-0.81	0.55	11.89	30.00	110.74	1151.02
9	-1.32	0.21	1.73	12.06	24.75	134.26	1086.70
10	-1.32	1.05	2.24	11.89	24.07	112.26	1031.69
Mean fmol[b]	**-1.32**	**0.48**	**2.75**	**10.5**	**26.55**	**110.19**	**999.01**
s_r[c]	0.61	0.75	1.38	1.54	2.33	15.57	130.92

[a]Corresponding RLU values taken from Table 1 were converted into femtomoles/assay of ATP using the line equation $y = 5.908x + 7.7736$ generated from Figure 1.

[b]Average ATP (femtomoles/assay) from 10 theoretical replicates per ATP level.

[c]s_r calculated from 10 predicted replicates per RLU level.
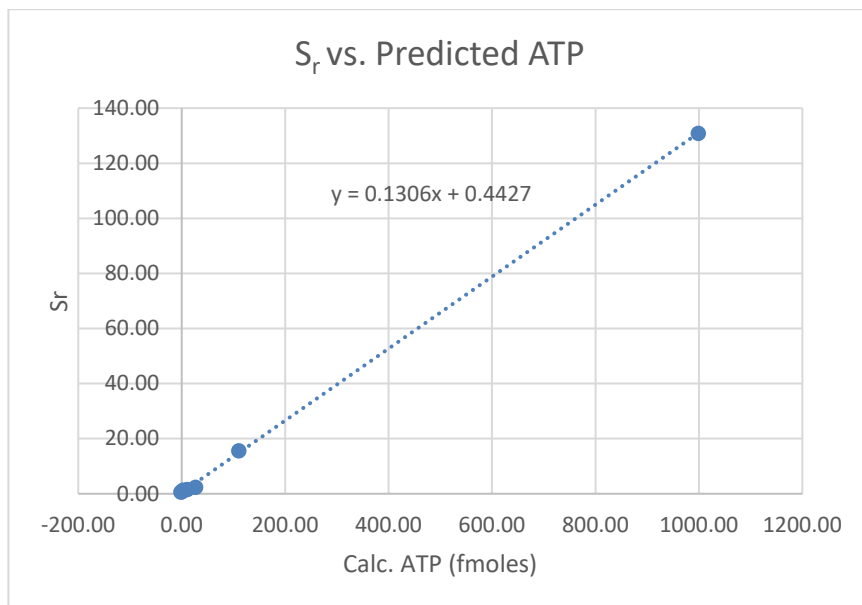
Figure 2: Relationship between Sr and mean of interpolated ATP concentrations. (8)

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