CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

101803

The AOAC Research Institute hereby certifies the method known as:

Hygiena UltraSnap ATP Kit

manufactured by

Hygiena LLC
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Camarillo, California 93012
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.

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

Issue Date December 04, 2024
Expiration Date December 31, 2025

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METHOD NAME
UltraSnap Surface ATP

CATALOG NUMBER
US2020

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

Matrixes – (10 x 10 cm) – stainless steel surfaces contaminated by raw lamb, ready-to-eat duck wrap, orange juice, yogurt, and doughnut residues

Performance claims – The UltraSnap Surface ATP test was proven to be effective at detecting the presence of ATP on stainless steel surfaces being representative of food processing and manufacturing facilities. Linear regression determined that the limit of detection (LOD) of UltraSnap swabs in the Ensure luminometer was 0.82 femtomoles of ATP.

ORIGINAL CERTIFICATION DATE
October 18, 2018

CERTIFICATION RENEWAL RECORD
Renewed through December 2025.

METHOD MODIFICATION RECORD
1. **December 2019 Level 2**
2. **December 2020 Level 1**
3. **December 2023 Level 1**
4. **December 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Incorporation of new reader, EnSURE Touch™ Luminometer.**
2. **Editorial changes.**
3. **Editorial changes.**
4. **Editorial changes.**

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PRINCIPLE OF THE METHOD (1)
UltraSnap Surface ATP Test is a self-contained device used with Hygiena luminometers. The test device and luminometer is a system used for monitoring the hygienic status of surfaces on processing equipment and other environments in a wide range of industries. The system measures adenosine triphosphate (ATP), the universal energy molecule found in all animal, plant, bacterial, yeast, and fungal cells. Product residues from organic matter left on surfaces contain ATP. After proper cleaning, all sources of ATP should be significantly reduced. When a sample is collected, any ATP is mixed with the unique liquid stable Luciferase/Luciferin reagent in the UltraSnap test device, light is emitted in direct proportion to the concentration of ATP present in the sample. The EnSURE luminometer measures generated light and reports results in Relative Light Units (RLU). RLU results provide information on the level of contamination within seconds. The higher the RLU number, the more ATP present, and the dirtier the surface. It is important to note that UltraSnap is designed to detect invisible/trace amounts of residue. Overloading the swab with physical matter by swabbing a visibly dirty surface will inhibit the bioluminescent reaction and produce non-proportional results.

To use the test, remove the UltraSnap swab device from the tube and swab a 10x10 cm surface. For optimal swabbing:

1. Do not touch swab or inside of sample device with fingers
2. Rotate swab while collecting sample to maximize sample collection on swab tip
3. Apply sufficient pressure to create flex in swab shaft
4. Swab in a crisscross pattern vertically, horizontally, and in both diagonal directions.

Replace the swab device into the tube, snap the bulb back and forth, then squeeze the bulb to expel the liquid reagents to the bottom of the tube. The activated device is then inserted into an EnSURE luminometer to measure the RLU output. Any ATP present on the swabbed surface will contribute to the RLU result.

DISCUSSION OF THE VALIDATION STUDY (1)

For an ATP test to function well as a rapid hygiene monitoring tool it must be sensitive to pure analyte, be capable of detecting ATP from and in the presence of food and microbial spoilage and must give proportional reproducible results. The pure analyte testing of UltraSnap demonstrated excellent sensitivity with LODs of 2.27RLU (Table 1) and 0.77RLU (Table 13), from the method developer and independent lab studies respectively. These RLU LODs correspond to 0.82 and 0.23 femtomoles of ATP converted using linear regression analysis. The RLU signal produced an accurate dose-response to the ATP concentration (R^2 value = 0.9992), at levels that are key to actionable pass/caution/fail results in hygiene monitoring applications.

The results from food matrix testing reveal that UltraSnap is capable of detecting ATP from, and in the presence of, surface soil by food and beverages. Detection of ATP was possible at dilutions of 1:1000 or lower for all matrixes tested (Tables 3 & 4). This sensitivity to low levels of food/beverage residue shows that ATP hygiene monitoring is a far more accurate check of cleaning performance than simply looking for a dirty surface. The five matrixes tested demonstrate the ability of UltraSnap to perform well across a broad range of industrial environments.

The results from microbial matrix testing validate the food testing data by showing that UltraSnap can successfully recover and measure microbial ATP from gram positive bacteria (*B. subtilis* – dry LOD = 30,694 CFU), gram negative bacteria (*P. aeruginosa* – dry LOD = 74,615 CFU), and yeast (*S. cerevisiae* – dry LOD = 1012 CFU). In each case there is a linear dose-response with correlations of >85% for all organisms tested in both wet and dry conditions.

An important point to draw from both the food and microbial matrix data is the successful detection of all matrixes after drying. Although the signal recovery is marginally lower from dried surfaces compared to wet surfaces, there is still a large amount of ATP signal from food and microbial ATP recovered in all cases. This demonstrates that there is not a dramatic decrease in ATP availability due to ATP instability on surfaces or when exposed to drying. ATP remains stable when dried on surfaces (3) and will not simply become negative over time when left without cleaning. To get a negative result by surface ATP hygiene monitoring the ATP must be physically removed by diligent cleaning, absence of regular well performed cleaning will lead to positive RLU results from surfaces monitored by ATP testing regardless of the industrial location. In both the food and microbial matrix data sets from surface testing, some results were identified as outliers by the Grubbs test and removed from the analysis. The experimental setup introduces various sources of variation that are likely to cause the observed outliers. There can be variation from sample homogenization, sample spreading, sample drying, sample swabbing, non-sample ATP from the environment, and differing environmental conditions during drying that all factor into the RLU results.

Three different classes of commonly used industrial sanitizers were tested for inhibition of the ATP signal from UltraSnap devices. The quaternary ammonium sanitizer increased the RLU value by 70-200%. The other two sanitizers both caused a reduction in RLU value; acid-anionic surfactant by -7-48% and peracetic acid by 15-48%. In the experimental design followed for this testing the sanitizers were not rinsed from the surfaces before sampling. In real world situations sanitizers would be rinsed from surfaces before testing, as a result we would expect to see reduced effect from all sanitizers tested here in real world situations.

Selectivity data shows that UltraSnap is highly selective for ATP over other similar nucleotides and not susceptible to competitive inhibition. Out of 12 different non-ATP analogues, only dATP and ADP give any signal at all at 2500fmoles (44 & 42 RLUs respectively - <2% of the ATP signal). In the presence of a 100x concentration of all 12 non-ATP analytes UltraSnap accurately measures 25 fmoles of ATP (Table 9).

Product consistency and stability was demonstrated through testing three batches of ULTRASNAP swabs manufactured over a period of 6 months. There was no statistical difference in the recorded RLU from 0, 10, and 100 fmoles of ATP across the three different ULTRASNAP batches.

Instrument Variation was tested by using three different EnSURE luminometers to measure ULTRASNAP RLU with three different levels of ATP. There was a statistically significant variation in the background readings from the three instruments, although this is largely due to the number of zero values obtained. This causes the variation (ranging from 0–3 RLU) to appear very large in comparison to the very low averages that are close to 0 (0.6, 1, and 2.8 RLU respectively). There was no statistical difference observed between the three instruments when reading UltraSnap devices containing 10 and 100 fmoles of ATP.

Robustness testing was carried out by altering the temperature of UltraSnap devices and the time between activating the UltraSnap device and reading it in the EnSure luminometer. Factorial ANOVA was performed on the data from the three ATP concentration levels tested (0, 10, 100 fmoles ATP). There was no statistically significant difference in the background readings when UltraSnap devices were time and temperature abused. However, there was a statistically significant difference between RLU readings in both the 10 fmole and 100 fmole ATP data sets. In both cases it was seen that RLU signal increases with time and temperature, with the largest effect coming from the temperature abuse of UltraSnap devices. These results show the importance of using the UltraSnap test according to the kit insert procedure.

Table 1: RLU measurement of pure analyte ATP added to UltraSnap devices and read in an Ensure luminometer. Results are given as raw, mean, s_r, RSDr, and LOD values. (1)

Replicates	RLU, at Applied ATP (femtomoles) [a]						
	0	**1**	**5**	**10**	**25**	**100**	**200**
1	1	3	7	14	37	156	301
2	1	1	7	19	55	228	463
3	0	2	7	21	38	257	551
4	1	2	7	22	45	265	542
5	1	2	7	20	57	278	568
6	0	1	7	20	53	260	526
7	1	1	7	25	58	196	425
8	1	2	8	20	33	190	435
9	0	1	7	16	46	213	406
10	0	2	6	32	39	235	532
Mean RLU [b]	**1**	**2**	**7**	**21**	**46**	**228**	**475**
s_r [c]	0.52	0.67	0.47	4.93	9.18	39.0	84.4
RSDr [d]	86.1%	39.7%	6.73%	23.6%	19.9%	17.1%	17.8%
LOD [e]	2.27RLU						

[a] ATP quantity added to swab tip.
[b] Average RLU from 10 replicates per ATP level.
[c] s_r calculated from 10 replicates per ATP level.
[d] RSDr calculated from 10 replicates per ATP level.
[e] LOD. calculated using regression analysis of RLU against ATP.

Table 2: Interpolated pure analyte ATP concentrations measured using UltraSnap in an EnSure luminometer. Corresponding RLU values taken from Table 1, were converted into femtomoles of ATP using the line equation y = 0.1758x + 0.3076, generated from Figure 2 above. (1)

	ATP (femtomoles) [a]						
Replicates	**0.0**	**1**	**5**	**10**	**25**	**100**	**200**
1	2	3	5	8	17	68	128
2	2	2	5	10	25	98	197
3	2	3	5	11	18	110	234
4	2	3	5	11	21	113	230
5	2	3	5	10	26	119	241
6	2	2	5	10	24	111	223
7	2	2	5	12	26	84	181
8	2	3	5	10	16	82	185
9	2	2	5	9	21	91	173
10	2	3	4	15	18	101	226
Mean RLU [b]	**2**	**3**	**5**	**11**	**21**	**98**	**202**
s_r [c]	0.22	0.28	0.20	2.07	3.86	16.4	35.5
RSDr [d]	9.94%	10.7%	4.07%	19.3%	18.1%	16.8%	17.6%

[a] Interpolated ATP concentration.
[b] Average ATP (femtomoles) from 10 theoretical replicates per ATP level.
[c] s_r calculated from 10 predicted replicates per RLU level.
[d] RSDr calculated from 10 predicted replicates per RLU level.

Table 3: Replicate RLU, mean RLU, Sr, and RSDr values of UltraSnap Surface ATP method used on wet food matrixes. All RLU values measured in an EnSURE luminometer. A negative control consisting of sterile analyte free water was used. (1)

	Dilution [a]	Replicate										Mean [b]	Stan. Dev. [c]	RSDr [d]
		1	2	3	4	5	6	7	8	9	10			
Lamb Leg Steak	**Sterile water**	0	0	0	0	0	0	0	0	0	0	**0**	**0.00**	**NA[e]**
	-5	2[e]	1	1	1	1	1	1	6[e]	1	1	**1**	**0.00**	**NA[f]**
	-3	44	32	34	28	22	18	18	21	25	32	**27**	**8.26**	**30.2**
	-2	298	508	731	569	638	441	533	584	507	473	**528**	**117**	**22.1**
	-1.75	1445	1433	1171	1648	1189	1835	1506	1280	2097	1004	**1461**	**330**	**22.6**
	-1.5	1568	1817	2396	1231	2161	3078	2784	1821	2158	2351	**2137**	**554**	**25.9**
Pink Icing Doughnut	**Sterile water**	1	0	0	0	0	5	3	1	2	4	**2**	**1.84**	**115**
	-6	2	3	6	3	8	4	1	3	1	0	**3**	**2.42**	**78.2**
	-4	5	6	7	9	6	8	12	16	12	7	**9**	**3.49**	**39.7**
	-3.5	64	39	47	52	49	46	70	73	40	41	**52**	**12.5**	**24.0**
	-3	74	80	60	70	127	63	68	39	102	129	**81**	**29.3**	**36.1**
	-2.5	342	336	271	337	226	563	441	515	312	260	**360**	**111.3**	**30.9**
Strawberry Yogurt	**Sterile water**	3	5	7	9	4	6	3	4	6	4	**5**	**1.91**	**37.5**
	-7	3	3	7	15[e]	6	3	3	3	2	2	**4**	**1.74**	**48.9**
	-3	6	5	8	16	12	5	15	8	15	14	**10**	**4.45**	**42.8**
	-2.75	40	22	37	39	47	28	39	38	75[e]	49	**38**	**8.40**	**22.3**
	-2.5	215	124	104	178	149	139	181	197	194	127	**161**	**37.1**	**23.1**
	-1	180	260	533[e]	306	226	203	286	233	207	368	**252**	**59.4**	**23.6**
Orange Juice (smooth)	**Sterile water**	2	2	1	2	1	1	2	3	9[e]	3	**2**	**0.78**	**41.4**
	-5	8	7	9	10	15	16	11	12	13	12	**11**	**2.91**	**25.7**
	-4.5	28	22	38	42	41	22	27	9	86[e]	14	**27**	**11.7**	**43.2**
	-4	23	37	14	51	22	37	16	16	15	18	**25**	**12.5**	**50.2**
	-3.5	67	58	140	172	133	119	155	62	74	73	**105**	**43.1**	**40.9**
	-3	209	168	246	183	124	233	153	148	231	204	**190**	**41.2**	**21.7**
Duck Wrap	**Sterile water**	5	4	4	6	3	3	5	7	7	6	**5**	**1.49**	**29.8**
	-7	4	7	5	8	11[e]	5	5	4	5	3	**5**	**1.54**	**30.1**
	-4.5	17	24	23	27	11	31[e]	26	26	16	14	**20**	**5.98**	**29.3**
	-4	38	65	48	50	58	59	70	50	68	61	**57**	**10.1**	**17.8**
	-3.25	222	159	135	129	209	110	52	97	168	94	**138**	**53.0**	**38.6**
	-3	659	505	444	467	377	272	425	337	283	375	**414**	**114.6**	**27.7**

[a] Dilution of the food matrix tested.

[b] The mean result of 10 wet replicate coupons per dilution.

[c] s_r calculated from 10 wet replicate coupons per dilution.

[f] Co-efficient of variance percentage calculated from 10 wet replicate coupons per dilution.

[e] Excluded due to Grubbs test.

[f] NA = Not applicable.

Table 4: Replicate RLU, mean RLU, s_r, and RSDr values of UltraSnap Surface ATP method used on dry food matrixes. All RLU values measured in an EnSure luminometer. A negative control consisting of sterile analyte free water was used. (1)

	Dilution [a]	Replicate										Mean [b]	Stan. Dev. [c]	RSDr [d]
		1	2	3	4	5	6	7	8	9	10			
Lamb Leg Steak	**Sterile water**	4	1	2	2	1	1	2	4	2	1	**2**	**1.15**	**57.7**
	-5	2	2	2	4	3	3	2	2	3	2	**3**	**0.71**	**28.3**
	-3	7	11	12	22	15	22	24	10	11	16	**15**	**5.87**	**39.1**
	-2	67	109	81	6	106	190	137	99	167	167	**113**	**55.0**	**48.7**
	-1.75	248	316[e]	195	231	154	217	213	170	220	217	**207**	**29.5**	**14.3**
	-1.5	331	673[e]	174	234	260	420	146	253	179	194	**243**	**86.8**	**35.6**
Pink Icing Doughnut	**Sterile water**	0	0	0	0	15[e]	3	0	0	2	0	**1**	**1.13**	**203**
	-6	0	0	0	0	0	3	1	2	1	1	**1**	**1.03**	**129.1**
	-4	3	2	3	3	4	2	6	3	4	5	**4**	**1.27**	**36.3**
	-3.5	9	9	3	6	12	10	7	15	2	14	**9**	**4.32**	**49.7**
	-3	66	50	8	23	21	22	39	67	110	47	**45**	**30.1**	**66.5**
	-2.5	713	308	177	492	415	430	165	545	274	249	**377**	**175**	**46.4**
Strawberry Yogurt	**Sterile water**	7	1	2	14[e]	1	2	27[e]	4	4	5	**3**	**2.12**	**65.3**
	-7	1	2	1	11[e]	1	0	4	2	3	3	**2**	**1.27**	**67.2**
	-3	4	22[e]	14	4	6	5	7	5	8	12	**7**	**3.56**	**49.3**
	-2.75	15	47	13	52	31	14	16	26	32	24	**27**	**13.8**	**51.0**
	-2.5	88	176	62	225	88	94	83	181	239	114	**135**	**64.4**	**47.7**
	-1	865	1070	1417	799	1919	1310	1503	1285	1052	1339	**1256**	**329**	**26.2**
Orange Juice (smooth)	**Sterile water**	1	9[e]	2	2	1	3	4	4	4	4	**3**	**1.30**	**46.9**
	-5	6	8	7	9	8	4	5	62[e]	4	5	**6**	**1.86**	**29.8**
	-4.5	16	7	14	6	9	16	6	12	6	13	**11**	**4.17**	**39.7**
	-4	8	8	6	5	18	20	13	8	15	12	**11**	**5.14**	**45.5**
	-3.5	46	28	99[e]	58	34	469[e]	50	19	22	15	**34**	**15.8**	**46.4**
	-3	134	55	64	65	40	172	381[e]	46	196	69	**93**	**58.3**	**62.4**
Duck Wrap	**- Sterile water**	2	4	3	2	11[e]	1	3	3	1	1	**2**	**1.09**	**49.2**
	-7	0	2	1	2	4	2	4	2	3	4	**2**	**1.35**	**56.2**
	-4.5	10	8	9	27[e]	4	1	4	3	6	5	**6**	**2.96**	**53.3**
	-4	14	14	9	16	21	6	16	26	15	23	**16**	**6.07**	**38.0**
	-3.25	33	32	106	37	42	14	16	21	47	129	**48**	**38.7**	**81.1**
	-3	107	166	69	99	148	31	351[e]	72	182	104	**109**	**49.1**	**45.2**

[a] The dilution of the food matrix tested.

[b] The mean result of 10 dry replicate coupons per dilution.

[c] Sr calculated from 10 dry replicate coupons per dilution.

[d] Coefficient of variance percentage calculated from 10 dry replicate coupons per dilution.

[e] Excluded due to Grubbs test.

[f] NA = Not applicable (the reading was incorrectly taken).

Table 9: A table showing the average RLU of thirteen pure analytes, plus a negative control, analyte free water at high concentrations (2500 fmol) with and without ATP present (25 fmol). Five replicates were tested for each condition, for each analyte. (1)

Abbreviation	Name	RLU at 2500 fmol compound, 0 fmol ATP	RLU at 2500 fmol compound, 25 fmol ATP
NA[a]	analyte-free water	**2**	**30**
ATP	Adenosine 5'-triphosphate sodium salt hydrate	**2537**	**2844**
dATP	2'-deoxyadenosine 5'-triphosphate sodium salt	**44**	**79**
UTP	Uridine 5'-triphosphate trisodium salt	**1**	**37**
GTP	Guanosine 5'-triphosphate sodium salt	**3**	**39**
TTP	Thymidine 5'-triphosphate sodium salt	**2**	**36**
dUTP	2'-Deoxyuridine 5'-triphosphate sodium salt	**1**	**33**
CTP	Cytidine 5'-triphosphate	**1**	**35**
dGTP	2'-deoxyguanosine 5'-triphosphate trisodium salt	**1**	**31**
ITP	Inosine 5'-triphosphate trisodium salt	**1**	**34**
dIMP	2'-deoxyinosine 5'-monophosphate sodium salt	**1**	**34**
dCTP	2'-deoxycytidine 5'-triphosphate disodium salt	**1**	**35**
ADP	adenosine diphosphate (bacterial origin)	**42**	**67**
AMP	adenosine monophosphate	**1**	**31**

[a]Not applicable.

Table 13: RLU measurement of pure analyte ATP added to UltraSnap devices and read in an Ensure luminometer. Results are given as raw, mean, s_r, RSDr, and LOD values. (1)

Replicates	**RLU, at Applied ATP (femtomoles) [a]**						
	0	**1**	**5**	**10**	**25**	**100**	**200**
1	0	1	4	7	19	88	140
2	0	0	5	6	12	98	178
3	0	0	4	5	22	73	163
4	0	1	4	6	17	93	179
5	0	1	3	5	15	94	148
6	0	1	3	8	20	87	226
7	0	1	4	6	18	109	181
8	0	1	3	7	19	115	158
9	0	1	5	6	22	95	198
10	0	1	4	8	20	100	151
Mean RLU [b]	**0.0**	**0.8**	**3.9**	**6.4**	**18.4**	**95.2**	**172.2**
s_r [c]	0	0.42	0.74	1.07	3.10	11.70	25.98
RSDr [d]	0	53	19	17	17	12	15
LOD [e]	0.77RLU						

[a] ATP quantity added to swab tip.
[b] Average RLU from 10 replicates per ATP level.
[c] Sr calculated from 10 replicates per ATP level.
[d] RSDr calculated from 10 replicates per ATP level.
[e] LOD. calculated using regression analysis of RLU against ATP.

Table 14: Interpolated pure analyte ATP concentrations measured using UltraSnap in an EnSure luminometer. Corresponding RLU values taken from Table 13, were converted into femtomoles of ATP using the line equation y = 0.88x - 0.4519, generated from Figure 7 above. (1)

	ATP (femtomoles) [a]						
Replicates	**0**	**1**	**5**	**10**	**25**	**100**	**200**
1	0.51	1.65	5.06	8.47	22.10	100.51	159.60
2	0.51	0.51	6.20	7.33	14.15	111.88	202.79
3	0.51	0.51	5.06	6.20	25.51	83.47	185.74
4	0.51	1.65	5.06	7.33	19.83	106.20	203.92
5	0.51	1.65	3.92	6.20	17.56	107.33	168.70
6	0.51	1.65	3.92	9.60	23.24	99.38	257.33
7	0.51	1.65	5.06	7.33	20.97	124.38	206.20
8	0.51	1.65	3.92	8.47	22.10	131.20	180.06
9	0.51	1.65	6.20	7.33	25.51	108.47	225.51
10	0.51	1.65	5.06	9.60	23.24	114.15	172.10
Mean RLU [b]	**0.5**	**1.4**	**4.9**	**7.8**	**21.4**	**108.7**	**196.2**
S_r [c]	0	0.48	0.84	1.22	3.52	13.29	29.53
RSDr [d]	0	34	17	16	16	12	15

[a] ATP quantity added to swab tip.
[b] Average RLU from 10 replicates per ATP level.
[c] Sr calculated from 10 replicates per ATP level.
[d] RSDr calculated from 10 replicates per ATP level.

Table 15: Replicate RLU, mean RLU, Sr, and RSDr values of UltraSnap Surface ATP method used on wet food matrixes. All RLU values measured in an EnSure luminometer. A negative control consisting of sterile analyte free water was used. (1)

Wet Food Matrix	**RLU Target Range**	**Replicate**										**Mean [a]**	**Stan. Dev. [b]**	**RSDr [c]**
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**			
Orange juice (smooth)	**0**	0	1	4	1	3	0	0	1	1	2	**1.3**	**1.34**	**103**
	0-10	25	12	6	14	12	21	5	18	27	11	**15.1**	**7.49**	**50**
	10-30	29	15	25	22	13	19	13	17	20	34	**20.7**	**6.95**	**34**
	30-50	21	67	30	67	68	49	105	30	46	61	**54.4**	**24.75**	**45**
	100-200	178	125	122	181	167	265	121	147	189	171	**166.6**	**43.11**	**26**
Duck wrap	**0**	0	0	1	1	0	0	0	0	0	1	0.3	0.5	161.0
	0-10	0	0	0	0	1	0	3	1	0	5[e]	0.6	1.0	182.5
	10-30	0	4	0	10	1	12	1	1	1	0	3.0	4.4	146.6
	30-50	1	1	2	7	4	12	1	3	18[e]	2	3.7	3.7	100.2
	100-200	9	91	27	7	27	80	43	7	6	10	30.7	31.4	102.3

[a] The mean result of 10 wet replicate coupons per dilution.
[b] Sr calculated from 10 wet replicate coupons per dilution.
[c] Co-efficient of variance percentage calculated from 10 wet replicate coupons per dilution.
[d] Excluded due to Grubbs test.

Table 16: Replicate RLU, mean RLU, s_r, and RSDr values of UltraSnap Surface ATP method used on dry food matrixes. All RLU values measured in an EnSure luminometer. A negative control consisting of sterile analyte free water was used. (1)

Dry Food Matrix	RLU Target Range	Replicate										Mean [a]	Stan. Dev. [b]	RSDr[c]
		1	2	3	4	5	6	7	8	9	10			
Orange juice (smooth)	**0**	1	1	1	1	1	2	1	1	1	1	**1.1**	**0.32**	**29**
	0-10	5	1	13	5	8	6	1	2	3	11	**5.5**	**4.12**	**75**
	10-30	9	6	10	1	9	8	10	12	6	8	**7.9**	**3.03**	**38**
	30-50	12	0	24	15	21	12	5	9	3	52	**15.3**	**14.94**	**98**
	100-200	46	13	56	57	49	51	7	27	132	50	**48.8**	**34.25**	**70**
Duck wrap	**0**	2	7	2	0	8	2	0	1	1	0	2.3	2.87	125
	0-10	3	2	1	3	5	0	3	29[d]	1	7	2.8	2.17	78
	10-30	3	2	1	7	6	2	2	3	50[d]	3	3.2	1.99	62
	30-50	1	1	3	562[d]	5	39	3	6	3	7	7.6	11.97	158
	100-200	285	43	76	12	78	179	21	51	163	192	110.0	89.81	82

[a] The mean result of 10 wet replicate coupons per dilution.

[b] Sr calculated from 10 wet replicate coupons per dilution.

[c] Co-efficient of variance percentage calculated from 10 wet replicate coupons per dilution.

[d] Excluded due to Grubbs test.

[a] The mean result of 10 wet replicate coupons per dilution.

DISCUSSION OF THE MODIFICATION DECEMBER 2019 (4)

For an ATP test to function well as a rapid hygiene monitoring tool it must be sensitive to pure analyte and must give proportional reproducible results. The pure analyte testing of UltraSnap demonstrated excellent sensitivity with an LOD of 2.05 RLU (Table 1). This LOD corresponds to 1.28 femtomoles of ATP converted using linear regression analysis. The RLU signal produced an accurate dose-response to the ATP concentration (R^2 value = 0.9935, Figure 2), at levels that are key to actionable pass/caution/fail results in hygiene monitoring applications.

Instrument Variation was tested by using three different EnSURE Touch luminometers to measure UltraSnap RLU with three different levels of ATP. There was no statistical difference observed between the three instruments when reading UltraSnap devices containing 0, 100 and 1000 fmoles of ATP (Table 3).

Table 1: RLU measurement of pure analyte ATP added to UltraSnap devices and read in an EnSURE Touch luminometer. Results are given as raw, mean, s_r, RSDr, and LOD values. (4)

Replicates	RLU at applied ATP concentrations (femtomoles) [a]						
	0	**0.5**	**1**	**5**	**10**	**50**	**100**
1	0	0	3	8	15	97	185
2	0	0	2	11	19	123	230
3	0	0	2	9	22	118	190
4	0	0	2	10	16	105	213
5	0	0	3	11	16	124	225
6	0	0	4	10	17	105	195
7	0	0	2	9	20	97	230
8	0	0	1	6	16	113	194
9	0	0	2	8	20	120	221
10	0	0	2	10	17	102	226
Mean RLU [b]	**0.00**	**0.00**	**2.30**	**9.20**	**17.80**	**110.40**	**210.90**
s_r [c]	0.00	0.00	0.78	1.47	2.18	9.94	17.06
RSDr [d]	N/A	N/A	33.96%	15.97%	12.26%	9.01%	8.09%
LOD	2.05RLU						

[a] ATP quantity added to swab tip.

[b] Average RLU from 10 replicates per ATP level.

[c] s_r calculated from 10 replicates per ATP level.

[d] RSDr calculated from 10 replicates per ATP level.

[e] LOD. calculated using regression analysis of RLU against ATP.

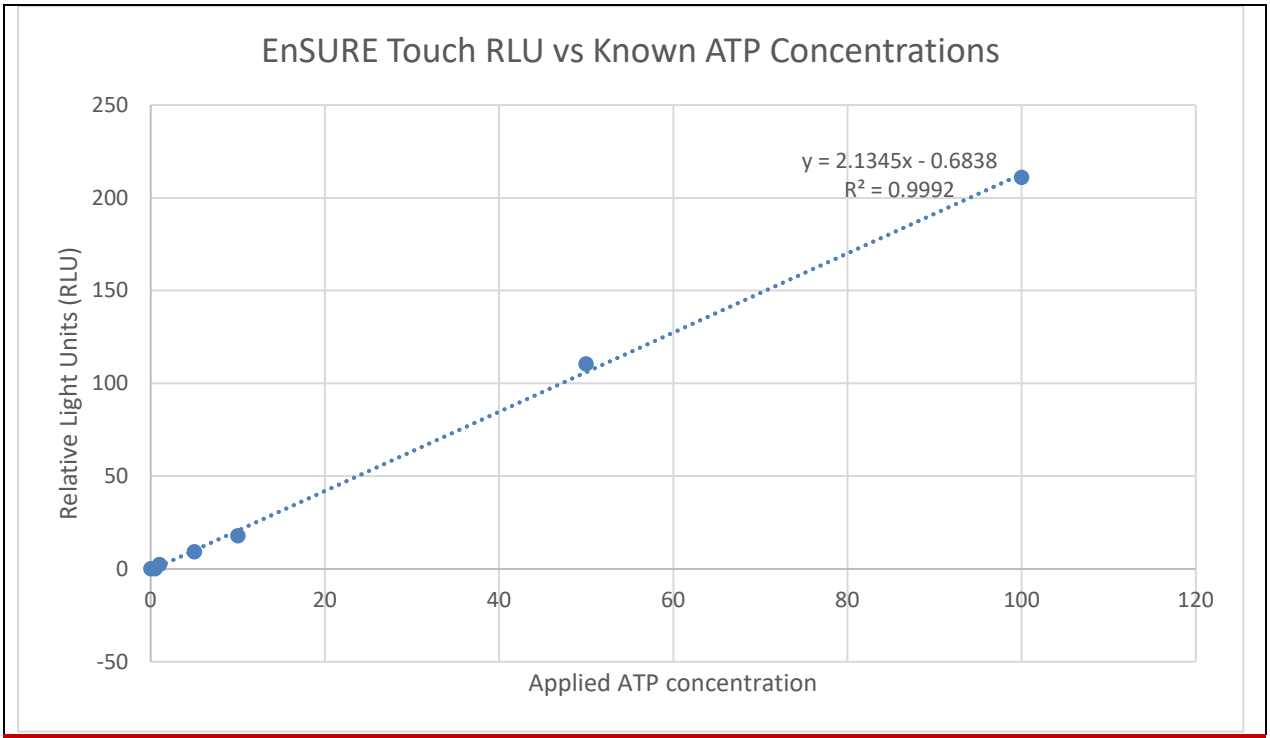

Figure 1: Relationship of mean UltraSnap RLU (10 replicates) to pure analyte ATP concentration measured in an EnSURE Touch luminometer. The correlation and line equation are shown on the graph.(4)

Table 2: Interpolated pure analyte ATP concentrations measured using UltraSnap in an EnSURE Touch luminometer. Corresponding RLU values taken from Table 1, were converted into femtomoles of ATP using the line equation y = 2.1345 – 0.6838, generated from Figure 1 above. (4)

Replicates	**Predicted RLU Vs Applied ATP (femtomoles) [a]**						
	0	**0.5**	**1**	**5**	**10**	**50**	**100**
1	0.32	0.32	1.73	4.07	7.35	45.76	86.99
2	0.32	0.32	1.26	5.47	9.22	57.95	108.07
3	0.32	0.32	1.26	4.54	10.63	55.60	89.33
4	0.32	0.32	1.26	5.01	7.82	49.51	100.11
5	0.32	0.32	1.73	5.47	7.82	58.41	105.73
6	0.32	0.32	2.19	5.01	8.28	49.51	91.68
7	0.32	0.32	1.26	4.54	9.69	45.76	108.07
8	0.32	0.32	0.79	3.13	7.82	53.26	91.21
9	0.32	0.32	1.26	4.07	9.69	56.54	103.86
10	0.32	0.32	1.26	5.01	8.28	48.11	106.20
Mean RLU [b]	**0.32**	**0.32**	**1.40**	**4.63**	**8.66**	**52.04**	**99.13**
s_r [c]	0.00	0.00	0.37	0.69	1.02	4.66	7.99
RSDr [d]	0.00%	0.00%	26.18%	14.87%	11.80%	8.95%	8.06%

[a] Interpolated ATP concentration.
[b] Average ATP (femtomoles) from 10 theoretical replicates per ATP level.
[c] s_r calculated from 10 predicted replicates per RLU level.
[d] RSDr calculated from 10 predicted replicates per RLU level.

Table 3: Consistency of three EnSURE Touch Luminometers (31018, 30913, 31022) when measuring RLU from UltraSnap Surface ATP devices at 0, 100 and 1000 femtomoles of ATP. Mean RLU and s_r are shown for the five replicates run, as well as the p-values calculated (using single factor ANOVA). (4)

ATP (fmol)	Etouch Luminometer	Replicates (RLU)										Average (RLU)	Standard Deviation	P-value (α = 0.05)
0	**31018**	0	0	0	0	0	0	0	3	0	0	0.30	0.90	0.59
	30913	0	0	0	0	0	0	0	0	2	0	0.20	0.60	
	31022	0	0	0	0	0	0	0	0	0	0	0.00	0.00	
100	**31018**	130	120	126	124	140	116	101	108	109	100	117.40	12.35	0.08
	30913	128	136	120	168	120	119	126	127	104	111	125.90	16.43	
	31022	117	111	118	106	119	119	99	123	125	145	118.20	11.66	
1000	**31018**	2450	2839	2561	2167	1995	2465	2741	2422	1846	2092	2357.80	307.31	0.36
	30913	2699	2602	2771	2276	2210	2445	2467	2629	2464	2417	2498.00	169.30	
	31022	2253	2176	2069	2221	2245	2496	2469	2068	2390	2145	2253.20	145.34	

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