FOOD BIOLOGICAL CONTAMINANTS

Validation of the MicroSnap Coliform and *E. coli* Test System for Enumeration and Detection of Coliforms and *E. coli* in a Variety of Foods

***Performance Tested Method*[SM] 071302**

Abstract

The MicroSnap Coliform and *E. coli* system was devised to give rapid enumeration and detection of coliforms and/or *Escherichia coli* strains in a sample of food within an 8 h working shift. The method measures β-galactosidase and β-glucuronidase enzymes using novel bioluminogenic substrates which develop an output light signal proportional to the concentration of enzyme discovered. The assay uses two different phases to determine the enzyme concentration. The first phase is an enrichment of the sample in a nutrient-rich broth device at 37 ± 0.5°C. After 6 or 8 h, an aliquot is taken from the enrichment device and injected into the Coliform Detection Device, which is assayed in a luminometer after 10 min of incubation at 37 ± 0.5°C. Samples testing positive in the Coliform Detection Device can be subsequently assayed specifically for *E. coli* using the *E. coli* Detection Device. The relative light unit output from the detection device is proportional to the bacterial concentration when the incubation was initiated, which is proportional to the contamination level in the matrix being assessed. The MicroSnap Coliform and *E. coli* system was evaluated for both quantitative and qualitative analysis of coliforms and *E. coli* in a variety of foods. Three different luminometers were used in the analysis, each of which has different functionalities and different sensitivities. The MicroSnap method showed good correlation with the appropriate corresponding reference method for enumeration of coliforms and *E. coli*. A statistically significant difference was seen in detection of *E. coli* in milk, as reported by the independent laboratory. The reference method reported higher mean Log_{10} CFU counts than the MicroSnap method; however, no significant differences were seen between the MicroSnap system and reference methods for any of the other matrixes. Inclusivity testing was conducted on 25 different non-*E. coli* coliforms and 25 different *E. coli* strains, and exclusivity testing was conducted on 30 different species of nontarget organisms. Two *E. coli* strains were not detected in the Coliform Detection Device after 8 h on one of the instruments. All other inclusivity strains tested were detected after 8 h of incubation. None of the exclusivity strains were detected. The lot-to-lot and kit stability studies showed no statistical differences between lots or over the term of the shelf-life. Robustness studies indicate that the timing of incubation for the detection phase is critical for correct system functioning.

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The method was independently tested, evaluated, and certified by the AOAC Research Institute as a *Performance Tested Method*[SM]. *See* http://www.aoac.org/testkits/steps.html for information on certification.

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Scope of Method

The MicroSnap Coliform and *E. coli* system is intended for the enumeration of coliforms and *E. coli* in a variety of foods. For coliform detection in fresh ground beef, fresh raw chicken, cooked chicken, liquid milk, fresh raw cod, fresh raw prawns, and bottled water (mineral, still). For *E. coli* detection in fresh ground beef, fresh raw cod, ready-to-eat (RTE) sandwiches, cooked chicken, liquid milk, fresh raw chicken and fresh raw prawns.

The method was shown to have a good correlation with AOAC *Official Method* **966.24** (1), and The U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) Chapter 4 (2) reference methods for enumeration and detection of coliforms and *E. coli* in the claimed matrixes. The method was shown to have an acceptable correlation with *Standard Methods for the Examination of Dairy Products* (SMEDP) Chapter 7 (3).

Table 1. Inclusivity for non-*E. coli* coliforms at 6 and 8 h incubation in the luminometers SS Plus and Pi102[a]

			6 h enrichment		8 h enrichment	
Organism	Source	Origin (if known)	SS Plus	Pi-102	SS Plus	Pi-102
Escherichia hermanii	Wild type	Minced beef	Positive	Positive	Positive	Positive
Enterobacter cloacae	Surrey University E002	Water	Positive	Positive	Positive	Positive
Enterobacter aerogenes[b]	Oxoid	ATCC 10006	Negative	Negative	Positive	Positive
Enterobacter cloacae	Surrey University E003	Rice	Positive	Positive	Positive	Positive
Enterobacter cloacae	Surrey University E004	Milk	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	Hospital KP13	ESBL urine	Positive	Positive	Positive	Positive
Klebsiella oxytoca[c]	Surrey University KO004	Food (unknown)	Positive	Negative	Positive	Positive
Citrobacter diversus	Surrey University C0011	Food (unknown)	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	HPA	ATCC 700603	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	Hospital KP9	ESBL feces	Positive	Positive	Positive	Positive
Citrobacter freundii	Surrey University C0012	Salad	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	HPA	NCTC 13438	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	HPA	NCTC 13465	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	HPA	NCTC 13443	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	HPA	NCTC 13439	Positive	Positive	Positive	Positive
Escherichia fergusonii	HPA	NCTC 12128	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	Surrey University K0015	Food (surface)	Positive	Positive	Positive	Positive
Escherichia hermanii	HPA	I73	Positive	Positive	Positive	Positive
Enterococcus sakazakii	Surrey University E0023	Baby milk	Positive	Positive	Positive	Positive
Enterococcus aerogenes	Oxoid	ATCC 13048	Positive	Positive	Positive	Positive
Citrobacter freundii[d]	Oxoid	ATCC 8090	Negative	Negative	Positive	Positive
Enterococcus sakazakii	HPA	NCTC 8155	Positive	Positive	Positive	Positive
E. coli O157 (NT)	Oxoid	ATCC 12900	Positive	Positive	Positive	Positive
Klebsiella oxytoca	Surrey University KO031	Salad	Positive	Positive	Positive	Positive
Enterococcus cloacae	Surrey University E0017	Salad	Positive	Positive	Positive	Positive

[a] Detection of approximately 10–100 CFU/mL using Coliform Detection Devices.

[b] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 1000 CFU/mL.

[c] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 410 CFU/mL.

[d] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 720 CFU/mL.

Definitions

Quantitative

Repeatability (s_r).—SD of replicates for each analyte at each concentration of each matrix for the MicroSnap.

Mean difference between candidate and reference methods.—Mean difference between MicroSnap and reference method-transformed results and its 95% confidence interval for each analyte at each concentration of each matrix.

T-test (two-tail unmatched).—Comparison of MicroSnap and reference method-transformed results and its 95% confidence interval for each analyte at each concentration of each matrix.

Qualitative

Probability of detection (POD).—Proportion of positive outcomes for MicroSnap for a given matrix at a given analyte concentration calculated by dividing the positive outcomes by the total number of trials and reported with a 95% confidence interval (4).

Difference of POD (dPOD).—Difference between any two POD values. For this study, dPOD was determined between the MicroSnap presumptive and confirmed results, and the MicroSnap confirmed results and the appropriate reference method. If the confidence interval of a dPOD does not contain a zero, the difference is statistically significant at the 5% level (5).

Principle

The MicroSnap method consists of two separate devices used in sequence to achieve a result from the assay. The first device (Enrichment Device) grows the bacteria from the sample under investigation. The sample is added to this device as a 1 mL aliquot of either a liquid or a 10% food suspension. This device contains a proprietary nutrient growth media with inducers of β-galactosidase and β-glucuronidase. During the incubation

Table 2. Inclusivity for *E. coli* coliforms at 6 and 8 h incubation in the luminometers SS Plus and Pi102[a]

			6 h enrichment		8 h enrichment	
Organism	Source	Origin (if known)	SS Plus	Pi102	SS Plus	Pi102
E. coli 63	Aberdeen University	Clinical isolate	Positive	Negative	Positive	Positive
E. coli 64[b]	Aberdeen University	Clinical isolate	Negative	Positive	Positive	Positive
E. coli 67[c]	Aberdeen University	Clinical isolate	Positive	Negative	Positive	Positive
E. coli 68	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli (EC 10)	Hospital	ESBL urine	Positive	Positive	Positive	Positive
E. coli 50	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 52[d]	Aberdeen University	Clinical isolate	Positive	Negative	Positive	Positive
E. coli 54	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 53	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 48	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 32	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 21	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli	Oxoid	ATCC 8739	Positive	Positive	Positive	Positive
E. coli	Oxoid	ATCC 25922	Positive	Positive	Positive	Positive
E. coli E0018	University Surrey	Unknown	Positive	Positive	Positive	Positive
E. coli E0019[e]	University Surrey	Food (unknown)	Negative	Negative	Positive	Positive
E. coli E0023[f]	University Surrey	Food (unknown)	Negative	Negative	Positive	Positive
E. coli E0026	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli E0025[g]	University Surrey	Food (unknown)	Negative	Negative	Negative	Positive
E. coli[h]	Oxoid	ATCC 13216	Negative	Negative	Negative	Positive
E. coli E0033	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli[i]	Oxoid	ATCC 35218	Negative	Negative	Positive	Positive
E. coli E0039	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli E0034	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli	Oxoid	ATCC 11775	Positive	Positive	Positive	Positive

[a] Detection of approximately 10–100 CFU/mL using Coliform Detection Devices.

[b] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 800 CFU/mL.

[c] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 210 CFU/mL.

[d] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 125 CFU/mL.

[e] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 1000 CFU/mL.

[f] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 110 CFU/mL.

[g] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 770 CFU/mL.

[h] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 100 CFU/mL.

[i] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 100 CFU/mL.

at 37±0.5°C the β-galactosidase and/or β-glucuronidase enzymes accumulate within the bacteria. Both enzymes become proportional to the number of bacteria in the starting inoculum at specific incubation times after the lag phase of the bacteria. These incubation times have been found to be 6 and 8 h at 37 ± 0.5°C. Because the bacteria do not readily export these enzymes into the media, each bacterium expresses a similar concentration of enzyme per bacterial cell; this is true for both enzymes. The concentration of enzyme expressed using the inducers is fairly consistent across the coliform group and *E. coli*, although exceptions do exist.

At 6 h of incubation, the dynamic range over which the test begins to function quantitatively begins at 100 CFU and upwards; this is expressed as a proportional rise in the relative light unit (RLU) measured in each sample in each luminometer. Longer incubation times push the lower level of detection downwards.

The second device is the Detection Device. Two separate specific detection devices are used, one for β-galactosidase detection and one for β-glucuronidase detection. These two devices can be used to verify the same enrichment device for coliforms and/or *E. coli*. Each detection device contain a lysis reagent in the tube portion with adenosine triphosphate, β-galactosidase substrate, or β-glucuronidase substrate and

Table 3. Inclusivity for *E. coli* at 6 and 8 h incubation in SS Plus and Pi102[a]

			6 h enrichment		8 h enrichment	
Organism	Source	Origin	SS Plus	Pi102	SS Plus	Pi102
E. coli 63	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 64[b]	Aberdeen University	Clinical isolate	Negative	Positive	Positive	Positive
E. coli 67	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 68	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli (EC 10)[b]	Hospital	ESBL urine	Negative	Negative	Positive	Positive
E. coli 50	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 52	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 54	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 53	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 48	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 32	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli 21	Aberdeen University	Clinical isolate	Positive	Positive	Positive	Positive
E. coli	Oxoid	ATCC 8739	Positive	Positive	Positive	Positive
E. coli[d]	Oxoid	ATCC 25922	Negative	Negative	Positive	Positive
E. coli E0018	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli E0019[e]	University Surrey	Food (unknown)	Negative	Negative	Positive	Positive
E. coli E0023[f]	University Surrey	Food (unknown)	Negative	Positive	Positive	Positive
E. coli E0026	University Surrey	Food (unknown)	Positive	Negative	Positive	Positive
E. coli E0025[g]	University Surrey	Food (unknown)	Negative	Positive	Positive	Positive
E. coli	Oxoid	ATCC 13216	Positive	Positive	Positive	Positive
E. coli E0033	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli[h]	Oxoid	ATCC 35218	Negative	Negative	Positive	Positive
E. coli E0039	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli E0034	University Surrey	Food (unknown)	Positive	Positive	Positive	Positive
E. coli	Oxoid	ATCC 11775	Positive	Positive	Positive	Positive

[a] Detection of approximately 10–100 CFU/mL using Coliform Detection Devices.

[b] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 800 CFU/mL.

[c] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 100 CFU/mL.

[d] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 100 CFU/mL.

[e] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 1000 CFU/mL.

[f] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 110 CFU/mL.

[g] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 770 CFU/mL.

[h] Positive at CFU/mL between 100–1000 at 6 h. Lowest level detected at 6 h was 100 CFU/mL.

luciferase reagent in the bulb portion of the device. The substrate is manufactured to have the recognition part (or sugar in these cases) for the enzyme under test, linked by a cleavable bond to luciferin. On successful cleavage of the substrate the luciferin is released, which in the presence of luciferase and other factors emits light in relation to the amount of enzyme present in the growing culture. At the specific time point of 6 h, the concentration of enzyme is directly related to the starting concentration of bacteria. This 6 h quantification point is true for both coliforms and *E. coli*. The time point when all bacteria, independent of starting inoculum, are detected is 8 h; hence, even levels of less than 10 coliforms will have produced enough enzyme by 8 h to be determined as positive in the assay.

General Information

The use of coliforms for general hygiene status of food has been around for many years. The use of coliforms and easy use of growth and discrimination have made them a good selected target for the measurement of possible fecal contamination of food and raw ingredients. The measurement is then used to discriminate against the food if it is highly contaminated with coliforms. This can mean that the food is also contaminated with a more serious or pathological pathogen, which may cause more serious morbidity than the coliforms alone. The term coliforms is unique to a group of organism's that share a similar biochemical profile and can be associated with either

Table 4. Exclusivity for noncoliforms at 6 and 8 h incubation in the luminometers SS Plus and Pi102[a]

			Micro-Snap Coliform		Micro-Snap *E. coli*	
Organism	Source	Origin	SS Plus	Pi 102	SS Plus	Pi 102
MRSA 1	Surrey Hospital	Clinical	Negative	Negative	Negative	Negative
S. aureus 6538	Oxoid	Unknown	Negative	Negative	Negative	Negative
Salmonella Virchow	Campden CRA1011	Food (unknown)	Negative	Negative	Negative	Negative
Salmonella Seftenberg	Campden CRA9281	Food (unknown)	Negative	Negative	Negative	Negative
Salmonella Infantis	Campden CRA1038	Food (unknown)	Negative	Negative	Negative	Negative
Salmonella Nottingham	Campden CRA1004	Food (unknown)	Negative	Negative	Negative	Negative
Serratia flexneri	Campden CRA325	Food (unknown)	Negative	Negative	Negative	Negative
Serratia boydii	Campden CRA324	Food (unknown)	Negative	Negative	Negative	Negative
Serratia marcescens	Campden CRA1521	Food (unknown)	Negative	Negative	Negative	Negative
Serratia proteomaculans	Camp CRA16483	Food (unknown)	Negative	Negative	Negative	Negative
Serratia liquifaciens	Campden CRA1491	Food (unknown)	Negative	Negative	Negative	Negative
Edwardsiella tarda	Campden CRA8392	Food (unknown)	Negative	Negative	Negative	Negative
Providencia rettgeri	Campden CRA8386	Food (unknown)	Negative	Negative	Negative	Negative
Yersinia enterocolitica	Campden CRA4103	Food (unknown)	Negative	Negative	Negative	Negative
E. faecalis ATCC 10100	University Surrey	Water	Negative	Negative	Negative	Negative
Proteus vulgaris	Campden CRA1581	Unknown	Negative	Negative	Negative	Negative
Salmonella Jena NCTC 5765	Oxoid	Unknown	Negative	Negative	Negative	Negative
Salmonella Dublin NCTC 9676	Oxoid	Unknown	Negative	Negative	Negative	Negative
S. typhimurium 14028	Oxoid	Unknown	Negative	Negative	Negative	Negative
Candida albicans 10231	Oxoid	Unknown	Negative	Negative	Negative	Negative
P. aeruginosa 10145	Oxoid	Unknown	Negative	Negative	Negative	Negative
Bacillus subtilis 6633	Oxoid	Unknown	Negative	Negative	Negative	Negative
L. monocytogenes NCR 5214	University Surrey	Salad	Negative	Negative	Negative	Negative
L. innocua ATCC 33090	Oxoid	Unknown	Negative	Negative	Negative	Negative
Bacillus cereus ATCC 11778	Oxoid	Unknown	Negative	Negative	Negative	Negative
P. mirabilis ATCC 43071	Oxoid	Unknown	Negative	Negative	Negative	Negative
B. cepacia ATCC 25608	Oxoid	Unknown	Negative	Negative	Negative	Negative
S. Enteritidis ATCC 13076	Oxoid	Unknown	Negative	Negative	Negative	Negative
P. fluorescens ATCC 13525	Oxoid	Unknown	Negative	Negative	Negative	Negative
Listeria innocua ATCC 33090	Oxoid	Unknown	Negative	Negative	Negative	Negative

[a] Detection of approximately 10^8 CFU/mL using Coliform Detection Devices.

human or animal fecal matter. These bacteria can grow quickly and be easily measured by conventional tests in a short time, usually 24 h or less. Coliforms can ferment lactose to produce energy, and the resultant reaction produces several enzymes for their survival. One of these enzymes is β-galactosidase, used to convert lactose into glucose which is then used by the bacteria to produce energy for growth.

Test Kit Information

(**a**) *Kit name*.—MicroSnap–Rapid Determination of Coliform and *E. coli*.

(**b**) *Cat. Nos*.—ES-1000 (Enrichment Swab); MS-Coliform; and MS-ECOLI.

Ordering Information

(**a**) *USA*.—Hygiena, 941 Avenida Acaso, Camarillo, CA 93012, Tel: +1 805.388.8007 x300.

(**b**) *Europe*.—Hygiena International, Unit 11 WENTA Business Centre, Colne Way, Watford, Hertfordshire, WD24 7 ND UK, Tel: +44 (0)1923 818821.

(**c**) *Test kit reagents:*

Collection device.—Contains 1.2 mL proprietary enrichment media Tryptone soya broth (TSB), inducers, and growth promoters).

Table 5. Enumeration of coliforms using Micro-Snap Coliform Detection Device versus reference method–Pi102 luminometer

Run	Target level[a]	Food	Ref. mean[b]	Ref. s_r[c]	Micro-Snap mean	Micro-Snap s_r	Mean difference	*P*-value[d]	95% LCL[e]	95% UCL[f]
1	10 K	Beef	4.08	0.09	4.30	0.15	–0.21	0.09	–0.48	–0.06
1	1 K	Naturally contaminated	3.23	0.15	3.19	0.26	–0.08	0.57	–0.29	0.12
1	100		2.97	0.21	2.70	0.06	0.18	0.07	–0.02	0.38
2	10 K	Beef	4.78	0.27	4.91	0.23	0.13	0.96	–0.44	0.46
2	1 K	*E. cloacae* (E0002)	3.92	0.16	4.33	0.27	–0.41	0.08	–0.89	–0.07
2	100	*E. coli* ATCC 8739	2.92	0.31	3.45	0.60	–0.44	0.10	–1.02	0.14
3	10 K	Beef[g]	5.04	0.00	5.12	0.11	–0.08	0.30	–0.25	0.10
3	1 K	Naturally contaminated	3.66	0.00	3.73	0.10	–0.07	0.31	–0.24	0.10
3	100		2.38	0.00	2.59	0.18	–0.21	0.13	–0.49	0.09
1	10 K	BLT	4.72	0.11	4.99	0.07	–0.27	0.05	0.03	0.48
1	1 K	*K. pneumoniae* ATCC 700603	3.63	0.34	3.80	0.12	–0.17	0.20	–0.49	0.14
1	100	*E. coli* NCTC 13216	2.70	0.33	2.71	0.07	–0.01	0.96	–0.45	0.43
1	10 K	Cod	4.91	0.31	4.23	0.11	0.68	0.00	0.43	0.93
1	1 K	Naturally contaminated	4.59	0.20	4.46	0.08	0.12	0.14	–0.06	0.31
1	100		3.37	0.22	3.87	0.40	–0.50	0.13	–1.29	0.25
2	10 K	Cod	3.74	0.27	3.83	0.16	–0.08	0.25	–0.26	0.09
2	1 K	Naturally contaminated	2.82	0.34	3.20	0.34	–0.29	0.23	–0.86	0.28
2	100		1.89	0.19	2.38	0.11	–0.49	0.00	–0.71	–0.27
1	10 K	Cooked chicken	3.65	0.25	4.39	0.13	–0.74	0.00	–1.00	–0.48
1	1 K	*E. aerogenes* ATCC 10006	3.06	0.07	3.03	0.03	0.03	0.24	–0.03	0.08
1	100	*E. coli* ATCC 25922	1.96	0.05	1.69	0.07	0.26	0.07	0.18	0.34
1	10 K	Lettuce	4.68	0.29	4.71	0.25	–0.04	0.57	–0.20	0.13
1	1 K	*K. pneumoniae* (ESBL 13)	3.92	0.22	4.71	0.29	–0.79	0.06	–0.11	1.62
1	100	*E. coli* (EC 64)	3.04	0.00	2.97	0.10	0.07	0.16	–0.04	0.19
1	10 k	Milk	4.82	0.03	4.88	0.07	–0.06	0.14	–0.15	0.03
1	1 k	*K. oxytoca* (K0005)	3.82	0.03	4.18	0.69	–0.35	0.31	–1.19	0.49
1	100	*E. coli* (EC54)	2.82	0.03	2.98	0.19	–0.15	0.14	–0.38	0.08
2	10 k	Milk	4.59	0.04	5.09	0.72	–0.49	0.00	–0.59	–0.39
2	1k	*K. oxytoca* (K0005)	3.61	0.09	5.03	0.06	–1.49	0.00	–1.60	–1.39
2	100	*E. coli* (EC54)	2.62	0.08	2.67	0.03	–0.06	0.23	–0.19	0.06
3	1 k	Milk[g]	3.86	0.00	4.09	0.28	–0.23	0.14	–0.50	0.12
3	100	*K. oxytoca* (K0005)	2.90	0.00	2.20	0.05	0.70	0.00	0.63	0.78
3	10	*E. coli* (EC54)	2.06	0.00	1.77	0.21	0.29	0.05	0.01	0.47
1	10 k	Raw chicken	4.14	0.14	3.98	0.15	0.16	0.06	–0.01	0.33
1	1 k	Naturally contaminated	3.04	0.00	2.94	0.12	0.09	0.14	–0.05	0.24
1	100		2.37	0.35	2.16	0.17	0.21	0.06	–0.02	0.43
2	10 k	Raw chicken	4.06	0.18	4.25	0.19	–0.19	0.05	–0.36	–0.02
2	1 k	Naturally contaminated	2.97	0.17	2.82	0.38	0.15	0.24	–0.15	0.43
2	100		2.29	0.35	2.15	0.19	0.14	0.15	–0.32	0.25
1	10 k	RTE ham	4.50	0.13	4.12	0.02	0.38	0.07	0.22	0.50
1	1 k	*C. diversus* (C0011)	3.34	0.07	3.31	0.07	0.03	0.50	–0.09	0.16
1	100	*E. coli* (EC 67)	2.59	0.39	2.70	0.26	–0.11	0.44	–0.46	0.24
1	10 k	Prawn	3.61	0.13	3.47	0.23	0.13	0.25	–0.14	0.40
1	1 k	Naturally contaminated	2.61	0.13	2.57	0.27	0.05	0.79	–0.40	0.49
1	100		1.74	0.20	1.30	0.09	0.44	0.05	0.13	0.75
1	1000	Mineral	3.29	0.14	3.33	0.35	–0.04	0.75	–0.35	0.28
1	100	*C. freundii* (C0012)	2.31	0.09	2.17	0.61	0.14	0.57	–0.50	0.78
1	10	*E. coli* (EC 19)	1.27	0.13	1.53	0.39	–0.26	0.30	–0.89	0.36

[a] Target levels in CFU/g.

[b] Mean result for the reference method relevant to each food type.

[c] Repeatability SD.

[d] *P*-value for a two-tail unmatched *t*-test, *P*-value < 0.05 indicates significance at the 95% confidence level.

[e] 95% Lower confidence limit for difference of means.

[f] 95% Upper confidence limit for difference of means.

[g] Test conducted at the independent laboratory.

Table 6. Enumeration of coliforms using Micro-Snap Coliform Detection Device versus reference method–EnSURE luminometer

Run	Target level[a]	Food	Ref. mean[b]	Ref. s_r[c]	Micro-Snap mean	Micro-Snap s_r	Mean difference	*P*-value[d]	95% LCL[e]	95% UCL[f]
1	10 K	Beef	4.08	0.09	4.23	0.10	–0.15	0.07	–0.31	0.02
1	1 K	Naturally contaminated	3.23	0.15	2.97	0.31	0.30	0.11	–0.12	0.49
1	100		2.97	0.21	2.46	0.12	0.35	0.12	–0.23	0.74
2	10 K	Beef	4.78	0.27	4.56	0.18	0.26	0.13	–0.12	0.45
2	1 K	*E. cloacae* (E0002)	3.92	0.16	4.23	0.23	–0.26	0.06	–0.51	0.03
2	100	*E. coli* ATCC 8739	2.92	0.31	3.45	0.26	–0.46	0.18	–0.38	0.61
3	10 K	Beef[g]	5.04	0.00	4.94	0.11	0.10	0.21	–0.09	0.30
3	1 K	Naturally contaminated	3.66	0.00	3.32	0.12	0.34	0.01	0.13	0.54
3	100		2.38	0.00	2.08	0.21	0.20	0.11	–0.07	0.47
1	10 K	BLT	4.72	0.11	4.27	0.06	0.46	0.05	–0.35	0.58
1	1 K	*K. pneumoniae* ATCC 700603	3.63	0.34	3.38	0.38	0.03	0.86	–0.48	0.48
1	100	*E. coli* NCTC 13216	2.70	0.33	2.40	0.04	0.30	0.12	–0.13	0.43
1	10 K	Cod	4.91	0.31	4.55	0.18	0.33	0.06	0.03	0.43
1	1 K	Naturally contaminated	4.59	0.20	4.79	0.25	–0.20	0.05	–0.37	–0.02
1	100		3.37	0.22	3.80	0.03	–0.32	0.10	–0.48	–0.15
2	10 K	Cod	3.74	0.27	3.94	0.25	–0.20	0.11	–0.47	0.08
2	1 K	Naturally contaminated	2.82	0.34	3.13	0.17	–0.14	0.37	–0.51	0.25
2	100		1.89	0.19	2.11	0.26	–0.28	0.09	–0.48	–0.18
1	10 K	Cooked chicken	3.65	0.25	3.34	0.08	0.16	0.13	–0.07	0.38
1	1 K	*E. aerogenes* ATCC 10006	3.06	0.07	3.00	0.06	–0.14	0.15	–0.65	0.15
1	100	*E. coli* ATCC 25922	1.96	0.05	1.78	0.21	0.13	0.38	–0.13	0.27
1	10 K	Lettuce	4.68	0.29	4.24	0.48	0.49	0.12	–0.78	0.20
1	1 K	*K. pneumoniae* (ESBL 13)	3.92	0.22	3.95	0.34	–0.12	0.43	–0.52	0.27
1	100	*E. coli* (EC 64)	3.04	0.00	3.09	0.11	–0.13	0.18	0.36	0.09
1	10 k	Milk	4.82	0.03	4.63	0.08	0.20	0.05	0.07	0.32
1	1 k	*K. oxytoca* (K0005)	3.82	0.03	3.94	0.50	–0.11	0.63	–0.50	0.49
1	100	*E. coli* (EC54)	2.82	0.03	3.11	0.24	-0.29	0.05	–0.56	0.01
2	10 k	Milk	4.59	0.04	4.84	0.26	–0.24	0.24	–0.72	0.24
2	1 k	*K. oxytoca* (K0005)	3.61	0.09	4.03	0.10	–0.31	0.05	–0.50	0.01
2	100	*E. coli* (EC54)	2.62	0.08	3.12	0.12	–0.51	0.00	–0.56	–0.30
3	1 k	Milk[g]	3.86	0.00	2.57	0.09	1.29	0.00	1.13	1.46
3	100	*K. oxytoca* (K0005)	2.90	0.00	1.79	0.07	1.11	0.00	0.99	1.24
3	10	*E. coli* (EC54)	2.06	0.00	2.09	0.06	–0.02	0.56	–0.13	0.09
1	10 k	Raw chicken	4.14	0.14	4.43	0.25	–0.24	0.12	–0.50	0.10
1	1 k	Naturally contaminated	3.04	0.00	3.98	0.25	0.78	0.03	–0.96	–0.60
1	100		2.37	0.35	2.07	0.16	0.22	0.08	–0.04	0.47
2	10 k	Raw chicken	4.06	0.18	4.54	0.51	–0.49	0.16	–0.28	0.30
2	1 k	Naturally contaminated	2.97	0.17	3.40	0.55	–0.43	0.07	–0.41	0.05
2	100		2.29	0.35	2.30	0.28	–0.11	0.63	–0.68	0.47
1	10k	RTE ham	4.50	0.13	4.78	0.13	–0.20	0.14	–0.50	0.10
1	1k	*C. diversus* (C0011)	3.34	0.07	3.67	0.26	–0.31	0.09	–0.48	0.07
1	100	*E. coli* (EC 67)	2.59	0.39	2.44	0.42	0.06	0.73	–0.41	0.50
1	10k	Prawn	3.61	0.13	3.53	0.40	0.07	0.76	–0.50	0.60
1	1k	Naturally contaminated	2.61	0.13	2.95	0.34	–0.34	0.15	–0.49	0.18
1	100		1.74	0.20	1.61	0.12	0.09	0.40	–0.18	0.37
1	1000	Mineral	3.29	0.14	3.20	0.40	0.09	0.52	–0.28	0.46
1	100	*C. freundii* (C0012)	2.31	0.09	1.90	0.47	0.35	0.21	–0.28	0.48
1	10	*E. coli* (EC 19)	1.27	0.13	0.90	0.21	0.37	0.06	–0.12	0.47

See Table 5 for footnotes a–g.

Table 7. Enumeration of coliforms using Micro-Snap Coliform Detection Device versus reference method–SS Plus luminometer

Run	Target level[a]	Food	Ref. mean[b]	Ref. s_r[c]	Micro-Snap mean	Micro-Snap s_r	Mean difference	*P*-value[d]	95% LCL[e]	95% UCL[f]
1	10 K	Beef	4.08	0.09	4.17	0.28	–0.09	0.45	–0.47	–0.11
1	1 K	Naturally contaminated	3.23	0.15	2.90	0.55	0.33	0.32	–0.46	0.50
1	100		2.97	0.21	2.03	0.25	0.85	0.00	0.49	1.21
2	10 K	Beef	4.78	0.27	4.71	0.20	0.12	0.53	–0.36	0.51
2	1 K	*E. cloacae* (E0002)	3.92	0.16	4.13	0.33	–0.22	0.33	–0.47	0.34
2	100	*E. coli* ATCC 8739	2.92	0.31	3.61	0.32	–0.62	0.00	–0.75	–0.49
3	10 K	Beef[g]	5.04	0.00	5.07	0.18	–0.03	0.85	–0.39	0.34
3	1 K	Naturally contaminated	3.66	0.00	3.40	0.13	0.26	0.05	0.03	0.51
3	100		2.38	0.00	2.48	0.10	–0.11	0.22	–0.32	0.10
1	10 K	BLT	4.72	0.11	4.62	0.10	0.11	0.08	–0.02	0.25
1	1 K	*K. pneumoniae* ATCC 700603	3.63	0.34	3.96	0.10	–0.18	0.12	–0.44	0.07
1	100	*E. coli* NCTC 13216	2.70	0.33	2.49	0.33	0.22	0.24	–0.22	0.67
1	10 K	Cod	4.91	0.31	4.45	0.17	0.46	0.04	0.04	0.87
1	1 K	Naturally contaminated	4.59	0.20	4.90	0.21	–0.32	0.06	–0.65	0.02
1	100		3.37	0.22	3.90	0.20	–0.53	0.08	–1.17	0.11
2	10 K	Cod	3.74	0.27	4.13	0.20	–0.29	0.12	–0.50	0.11
2	1 K	Naturally contaminated	2.82	0.34	3.48	0.41	–0.26	0.16	–0.61	0.16
2	100		1.89	0.19	2.14	0.12	–0.25	0.07	–0.48	0.39
1	10 K	Cooked chicken	3.65	0.25	3.17	0.07	0.42	0.04	0.17	0.86
1	1 K	*E. aerogenes* ATCC 10006	3.06	0.07	2.96	0.05	0.10	0.06	–0.01	0.19
1	100	*E. coli* ATCC 25922	1.96	0.05	1.76	0.07	0.09	0.19	–0.07	0.26
1	10 K	Lettuce	4.68	0.29	4.77	0.44	–0.04	0.52	–0.49	0.47
1	1 K	*K. pneumoniae* (ESBL 13)	3.92	0.22	4.27	0.59	–0.45	0.22	–0.32	0.42
1	100	*E. coli* (EC 64)	3.04	0.00	2.81	0.17	0.16	0.05	–0.01	0.32
1	10 k	Milk	4.82	0.03	4.17	0.09	0.65	0.00	0.52	0.79
1	1 k	*K. oxytoca* (K0005)	3.82	0.03	3.58	0.38	0.25	0.22	–0.22	0.41
1	100	*E. coli* (EC54)	2.82	0.03	2.76	0.13	0.06	0.44	–0.15	0.28
2	10 k	Milk	4.59	0.04	4.53	0.16	0.06	0.50	–0.16	0.28
2	1 k	*K. oxytoca* (K0005)	3.61	0.09	3.61	0.16	–0.00	0.90	–0.09	-0.09
2	100	*E. coli* (EC54)	2.62	0.08	2.36	0.36	0.26	0.17	–0.18	0.69
3	1 k	Milk[g]	3.86	0.00	2.75	0.10	1.12	0.00	0.90	1.33
3	100	*K. oxytoca* (K0005)	2.90	0.00	2.50	0.04	0.40	0.05	–0.12	0.49
3	10	*E. coli* (EC54)	2.06	0.00	2.63	0.03	–0.58	0.00	–0.63	–0.52
1	10 k	Raw chicken	4.14	0.14	4.45	0.17	–0.31	0.09	–0.51	0.09
1	1 k	Naturally contaminated	3.04	0.00	3.57	0.40	–0.41	0.38	–0.54	0.70
1	100		2.37	0.35	2.90	0.11	–0.62	0.05	–0.10	1.21
2	10 k	Raw chicken	4.06	0.18	4.14	0.36	–0.08	0.81	–0.67	0.51
2	1 k	Naturally contaminated	2.97	0.17	2.79	0.39	0.17	0.50	–0.42	0.43
2	100		2.29	0.35	2.23	0.13	0.05	0.76	–0.43	0.50
1	10 k	RTE ham	4.50	0.13	4.24	0.16	0.32	0.09	–0.08	0.52
1	1 k	*C. diversus* (C0011)	3.34	0.07	3.65	0.12	–0.33	0.06	–0.04	0.58
1	100	*E. coli* (EC 67)	2.59	0.39	2.78	0.26	–0.28	0.15	–0.42	0.16
1	10 k	Prawn	3.61	0.13	3.73	0.24	–0.13	0.69	–0.49	0.61
1	1 k	Naturally contaminated	2.61	0.13	2.69	0.11	–0.03	0.45	–0.50	0.56
1	100		1.74	0.20	2.11	0.10	–0.27	0.21	–0.45	0.21
1	1000	Mineral	3.29	0.14	3.27	0.32	0.01	0.90	–0.35	0.36
1	100	*C. freundii* (C0012)	2.31	0.09	1.83	0.37	0.45	0.13	–0.19	0.53
1	10	*E. coli* (EC 19)	1.27	0.13	1.21	0.10	0.06	0.51	–0.19	0.33

See Table 5 for footnotes a–g.

Table 8. Enumeration of *E. coli* using Micro-Snap *E. coli* Detection Device versus reference method–Pi 102 luminometer

Run	Target level[a]	Food	Ref. mean[b]	Ref. s_r[c]	Micro-Snap mean	Micro-Snap s_r	Mean difference	*P*-value[d]	95% LCL[e]	95% UCL[f]
1	10 K	Beef	3.71	0.24	3.90	0.32	–0.19	0.45	–0.50	0.44
1	1 K	Naturally contaminated	2.78	0.15	3.10	0.14	–0.32	0.05	–0.01	0.64
1	100		2.61	0.25	2.33	0.55	0.28	0.22	–0.26	0.48
2	10 K	Beef	4.90	0.15	5.05	0.14	–0.15	0.35	–0.54	0.25
2	1 K	*E. cloacae* (E0002)	3.62	0.42	3.85	0.24	–0.23	0.40	–0.41	0.44
2	100	*E. coli* ATCC 8739	2.63	0.27	2.83	0.12	–0.21	0.26	–0.53	0.21
3	10 K	Beef[g]	4.66	0.00	4.64	0.11	0.03	0.71	–0.15	0.20
3	1 K	Naturally contaminated	3.58	0.00	3.12	0.24	0.46	0.12	–0.60	0.28
3	100		0.79	0.00	0.87	0.48	–0.07	0.80	–0.46	0.49
1	10 K	BLT	4.18	0.14	3.97	0.10	0.20	0.06	–0.02	0.42
1	1 K	*K. pneumoniae* ATCC 700603	3.51	0.42	3.13	0.10	0.37	0.14	–0.20	0.94
1	100	*E. coli* NCTC 13216	2.11	0.20	2.31	0.19	–0.19	0.33	–0.66	0.28
1	10 K	Cod	4.50	0.31	4.47	0.05	0.02	0.87	–0.32	0.36
1	1 K	Naturally contaminated	3.85	0.19	3.79	0.19	0.06	0.70	–0.32	0.43
1	100		2.70	0.25	2.67	0.16	0.02	0.81	–0.18	0.22
2	10 K	Cod	3.42	0.31	3.58	0.06	–0.16	0.38	–0.60	0.29
2	1 K	Naturally contaminated	2.84	0.47	2.70	0.28	0.14	0.34	–0.23	0.51
2	100		1.94	0.22	2.42	0.39	–0.48	0.07	–0.07	0.61
1	10 K	Cooked chicken	3.66	0.26	4.25	0.33	–0.58	0.04	–1.13	–0.04
1	1 K	*E. aerogenes* ATCC 10006	3.04	0.49	2.93	0.35	0.11	0.61	–0.46	0.69
1	100	*E. coli* ATCC 25922	2.04	0.09	1.69	0.36	0.35	0.18	–0.24	0.52
1	10 K	Lettuce	4.03	0.24	4.11	0.07	–0.08	0.48	–0.36	0.20
1	1 K	*K. pneumoniae* (ESBL 13)	2.93	0.09	3.15	0.34	–0.22	0.35	–0.78	0.35
1	100	*E. coli* (EC 64)	2.13	0.55	2.10	0.52	–0.50	0.01	–0.77	–0.24
1	10 k	Milk	4.47	0.31	4.97	0.04	–0.50	0.02	–0.82	–0.14
1	1 k	*K. oxytoca* (K0005)	3.85	0.19	3.80	0.19	0.06	0.70	–0.32	0.43
1	100	*E. coli* (EC54)	2.70	0.25	2.68	0.16	0.02	0.81	–0.18	0.22
2	10 k	Milk	4.40	0.18	4.28	0.15	0.11	0.42	–0.24	0.47
2	1 k	*K. oxytoca* (K0005)	3.31	0.13	3.17	0.16	0.15	0.18	–0.11	0.42
2	100	*E. coli* (EC54)	2.34	0.20	2.29	0.19	0.05	0.65	–0.21	0.30
3	1 k	Milk[g]	4.04	0.00	4.21	0.31	–0.08	0.70	–0.60	0.44
3	100	*K. oxytoca* (K0005)	1.46	0.00	1.78	0.14	–0.29	0.01	–0.47	–0.12
3	10	*E. coli* (EC54)	1.63	0.00	1.60	0.17	–0.05	0.57	–0.26	0.16
1	10 k	Raw chicken	3.69	0.31	3.47	0.05	0.21	0.20	–0.17	0.59
1	1 k	Naturally contaminated	2.43	0.16	2.30	0.19	0.14	0.38	–0.24	0.51
1	100		1.66	0.14	1.12	0.16	0.54	0.03	0.10	0.86
2	10 k	Raw chicken	3.07	0.13	2.87	0.32	0.20	0.37	–0.34	0.51
2	1 k	Naturally contaminated	2.44	0.01	2.16	0.10	0.28	0.01	0.11	0.44
2	100		1.45	0.10	1.57	0.09	–0.12	0.16	–0.32	0.07
1	10 k	RTE Ham	3.61	0.33	3.61	0.10	0.01	0.98	–0.47	0.48
1	1 k	*C. diversus* (C0011)	3.22	0.33	3.51	0.40	–0.29	0.27	–0.50	0.33
1	100	*E. coli* (EC 67)	2.06	0.20	1.60	0.09	0.20	0.05	–0.48	0.41
1	10 k	Prawn	3.09	0.24	2.97	0.69	0.12	0.77	–0.48	0.51
1	1 k	Naturally contaminated	1.98	0.23	1.21	0.64	0.77	0.05	–0.03	1.52
1	100		1.05	0.23	1.10	0.32	–0.04	0.92	–0.53	0.54
1	1000	Mineral	3.71	0.24	3.90	0.32	–0.19	0.45	–0.50	0.44
1	100	*C. freundii* (C0012)	2.78	0.15	3.10	0.14	–0.32	0.05	–0.01	0.64
1	10	*E. coli* (EC 19)	2.61	0.25	2.33	0.55	0.28	0.22	–0.26	0.48

See Table 5 for footnotes a–g.

Table 9. Enumeration of *E. coli* using Micro-Snap *E. coli* Detection Device versus reference method–EnSURE luminometer

Run	Target level[a]	Food	Ref. mean[b]	Ref. s_r[c]	Micro-Snap mean	Micro-Snap s_r	Mean difference	*P*-value[d]	95% LCL[e]	95% UCL[f]
1	10 K	Beef	3.71	0.24	3.89	0.34	–0.17	0.58	–0.44	0.43
1	1 K	Naturally contaminated	2.78	0.15	2.76	0.17	–0.06	0.72	–0.51	0.39
1	100		2.61	0.25	2.22	0.13	0.39	0.09	–0.68	0.11
2	10 K	Beef	4.90	0.15	4.55	0.30	0.35	0.18	–0.25	0.95
2	1 K	*E. cloacae* (E0002)	3.62	0.42	4.04	0.20	–0.42	0.05	–0.57	0.08
2	100	*E. coli* ATCC 8739	2.63	0.27	3.25	0.10	–0.62	0.00	–0.86	-0.38
3	10 K	Beef[g]	4.66	0.00	4.24	0.07	0.42	0.05	-0.09	0.54
3	1 K	Naturally contaminated	3.58	0.00	2.80	0.24	0.78	0.00	0.42	1.14
3	100		0.79	0.00	2.34	0.07	–1.55	0.00	–1.66	–1.43
1	10 K	BLT	4.18	0.14	3.83	0.31	0.34	0.09	–0.07	0.77
1	1 K	*K. pneumoniae* ATCC 700603	3.51	0.42	3.06	0.30	0.45	0.17	–0.30	0.68
1	100	*E. coli* NCTC 13216	2.11	0.20	1.99	0.09	0.12	0.37	–0.21	0.45
1	10 K	Cod	4.50	0.31	4.34	0.08	0.16	0.26	–0.17	0.49
1	1K	Naturally contaminated	3.85	0.19	4.19	0.21	–0.33	0.09	–0.73	0.08
1	100		2.70	0.25	2.94	0.19	–0.24	0.04	–0.48	–0.01
2	10 K	Cod	3.42	0.31	3.51	0.05	–0.09	0.61	–0.54	0.36
2	1 K	Naturally contaminated	2.84	0.47	2.55	0.20	0.29	0.11	–0.11	0.60
2	100		1.94	0.22	2.34	0.19	–0.40	0.07	–0.05	0.61
1	10 K	Cooked chicken	3.66	0.26	3.32	0.08	0.28	0.13	–0.13	0.69
1	1 K	*E. aerogenes* ATCC 10006	3.04	0.49	2.33	0.06	0.07	0.74	–0.45	0.58
1	100	*E. coli* ATCC 25922	2.04	0.09	1.60	0.21	0.07	0.38	–-0.13	0.27
1	10 K	Lettuce	4.03	0.24	4.35	0.44	–0.39	0.06	–0.51	0.03
1	1 K	*K. pneumoniae* (ESBL 13)	2.93	0.09	3.01	0.23	–0.06	0.67	–0.45	0.32
1	100	*E. coli* (EC 64)	2.13	0.55	2.54	0.28	–0.41	0.30	–0.68	0.48
1	10 k	Milk	4.47	0.31	4.34	0.11	0.16	0.26	–0.17	0.49
1	1 k	*K. oxytoca* (K0005)	3.85	0.19	4.19	0.30	–0.33	0.09	–0.73	0.08
1	100	*E. coli* (EC54)	2.70	0.25	2.94	0.27	–0.23	0.05	–0.48	0.01
2	10 k	Milk	4.40	0.18	4.06	0.18	0.32	0.07	–0.04	0.52
2	1 k	*K. oxytoca* (K0005)	3.31	0.13	3.47	0.17	–0.15	0.05	–0.29	0.02
2	100	*E. coli* (EC54)	2.34	0.20	2.48	0.32	–0.14	0.44	–0.32	0.61
3	1 k	Milk[g]	4.04	0.00	3.00	0.35	1.04	0.00	0.54	2.91
3	100	*K. oxytoca* (K0005)	1.46	0.00	1.74	0.14	–0.28	0.02	0.02	028
3	10	*E. coli* (EC54)	1.63	0.00	1.20	0.13	0.43	0.29	–0.53	0.41
1	10 k	Raw chicken	3.69	0.31	3.34	0.08	0.35	0.06	–0.02	0.51
1	1 k	Naturally contaminated	2.43	0.16	3.07	0.21	–0.64	0.01	–0.96	–0.31
1	100		1.66	0.14	1.94	0.19	–0.29	0.15	–0.46	0.16
2	10 k	Raw chicken	3.07	0.13	2.97	0.38	0.07	0.75	–0.50	0.64
2	1 k	Naturally contaminated	2.44	0.01	3.10	0.20	–0.66	0.01	–0.61	–0.30
2	100		1.45	0.10	1.33	0.20	–0.09	0.71	–0.43	0.55
1	10 k	RTE Ham	3.61	0.33	3.78	0.44	–0.11	0.79	–0.53	0.49
1	1 k	*C. diversus* (C0011)	3.22	0.33	3.69	0.23	–0.45	0.07	–0.59	0.06
1	100	*E. coli* (EC 67)	2.06	0.20	2.67	0.13	–0.55	0.05	–0.56	0.01
1	10 k	Prawn	3.09	0.24	3.00	0.35	0.04	0.90	–0.44	0.52
1	1 k	Naturally contaminated	1.98	0.23	2.07	0.04	–0.02	0.86	–0.25	0.22
1	100		1.05	0.23	2.04	0.04	–0.99	0.00	–1.26	–0.73
1	1000	Mineral	3.71	0.24	3.89	0.34	–0.17	0.58	–0.44	0.43
1	100	*C. freundii* (C0012)	2.78	0.15	2.76	0.17	–0.06	0.72	–0.51	0.39
1	10	*E. coli* (EC 19)	2.61	0.25	2.22	0.13	0.39	0.09	–0.68	0.11

See Table 5 for footnotes a–g.

Table 10. Enumeration of *E. coli* using Micro-Snap *E. coli* Detection Device versus reference method–SS Plus luminometer

Run	Target level[a]	Food	Ref. mean[b]	Ref. s_r[c]	Micro-Snap mean	Micro-Snap s_r	Mean difference	*P*-value[d]	95% LCL[e]	95% UCL[f]
1	10 K	Beef	3.71	0.24	3.46	0.18	0.25	0.55	–0.38	0.50
1	1 K	Naturally contaminated	2.78	0.15	2.56	0.23	0.13	0.53	–0.42	0.49
1	100		2.61	0.25	2.18	0.11	0.43	0.00	0.19	0.67
2	10 K	Beef	4.90	0.15	4.47	0.25	0.43	0.01	0.35	0.51
2	1 K	*E. cloacae* (E0002)	3.62	0.42	4.15	0.21	–0.47	0.13	–0.43	0.23
2	100	*E. coli* ATCC 8739	2.63	0.27	2.87	0.18	–0.25	0.13	–0.60	0.11
3	10 K	Beef[g]	4.66	0.00	4.38	0.11	0.28	0.17	–0.18	0.44
3	1 K	Naturally contaminated	3.58	0.00	2.80	NA	1.78	NA	NA	NA
3	100		0.79	0.00	0.88	0.15	–0.08	0.49	–0.39	0.23
1	10 K	BLT	4.18	0.14	4.00	0.36	0.15	0.48	–0.38	0.67
1	1 K	*K. pneumoniae* ATCC 700603	3.51	0.42	3.33	0.20	0.06	0.73	–0.43	0.46
1	100	*E. coli* NCTC 13216	2.11	0.20	2.33	0.23	–0.18	0.17	–0.48	0.12
1	10 K	Cod	4.50	0.31	4.30	0.10	0.19	0.26	–0.22	0.61
1	1 K	Naturally contaminated	3.85	0.19	4.15	0.20	–0.30	0.14	–0.75	0.16
1	100		2.70	0.25	2.73	0.18	–0.04	0.60	–0.24	0.16
2	10 K	Cod	3.42	0.31	3.69	0.19	–0.27	0.23	–0.40	0.26
2	1 K	Naturally contaminated	2.84	0.47	2.73	0.15	0.11	0.53	–0.34	0.55
2	100		1.94	0.22	2.53	0.17	–0.59	0.01	–0.97	–0.22
1	10 K	Cooked chicken	3.66	0.26	3.66	0.48	–0.01	0.98	–0.50	0.61
1	1 K	*E. aerogenes* ATCC 10006	3.04	0.49	3.18	0.18	–0.12	0.64	–0.48	0.59
1	100	*E. coli* ATCC 25922	2.04	0.09	1.94	0.22	0.10	0.36	–0.17	0.38
1	10 K	Lettuce	4.03	0.24	3.80	0.21	0.23	0.25	–0.25	0.51
1	1 K	*K. pneumoniae* (ESBL 13)	2.93	0.09	2.96	0.44	–0.03	0.91	–0.41	0.35
1	100	*E. coli* (EC 64)	2.13	0.55	1.94	0.16	0.18	0.30	–0.25	0.41
1	10 k	Milk	4.47	0.31	4.30	0.10	0.19	0.26	–0.22	0.61
1	1k	*K. oxytoca* (K0005)	3.85	0.19	4.15	0.20	–0.30	0.14	–0.75	0.16
1	100	*E. coli* (EC54)	2.70	0.25	2.73	0.18	–0.04	0.60	–0.24	0.16
2	10 k	Milk	4.40	0.18	3.79	0.18	0.60	0.03	0.09	1.13
2	1 k	*K. oxytoca* (K0005)	3.31	0.13	3.28	0.26	0.03	0.78	–0.27	0.33
2	100	*E. coli* (EC54)	2.34	0.20	2.55	0.31	–0.35	0.03	–0.64	–0.05
3	1 k	Milk[g]	4.04	0.00	3.42	0.09	0.62	0.03	0.04	0.80
3	100	*K. oxytoca* (K0005)	1.46	0.00	1.60	0.15	–0.14	0.25	–0.43	0.15
3	10	*E. coli* (EC54)	1.63	0.00	1.78	0.11	–0.14	0.05	–0.25	0.03
1	10 k	Raw chicken	3.69	0.31	3.80	0.11	–0.11	0.46	–0.51	0.28
1	1 k	Naturally contaminated	2.43	0.16	2.94	0.25	–0.45	0.05	–0.92	0.01
1	100		1.66	0.14	2.14	0.19	–0.48	0.02	–0.71	–0.11
2	10 k	Raw chicken	3.07	0.13	3.20	0.17	–0.13	0.38	–0.50	0.23
2	1 k	Naturally contaminated	2.44	0.01	2.69	0.24	–0.26	0.11	–0.60	0.09
2	100		1.45	0.10	2.51	0.21	–1.07	0.00	–1.57	–0.58
1	10 k	RTE ham	3.61	0.33	3.66	0.25	–0.05	0.86	–0.53	0.46
1	1 k	*C. diversus* (C0011)	3.22	0.33	3.36	0.38	–0.11	0.66	–0.47	0.36
1	100	*E. coli* (EC 67)	2.06	0.20	2.35	0.12	–0.29	0.14	–0.42	0.15
1	10 k	Prawn	3.09	0.24	2.70	0.37	0.35	0.21	–0.32	0.62
1	1 k	Naturally contaminated	1.98	0.23	1.82	0.04	0.23	0.06	–0.01	0.46
1	100		1.05	0.23	1.63	0.14	–0.51	0.04	–0.61	0.01
1	1000	Mineral	3.71	0.24	3.46	0.18	0.25	0.55	–0.38	0.50
1	100	*C. freundii* (C0012)	2.78	0.15	2.56	0.23	0.13	0.53	–0.42	0.49
1	10	*E. coli* (EC 19)	2.61	0.25	2.18	0.11	0.43	0.00	0.19	0.67

See Table 5 for footnotes a–g.

Table 11. Detection of coliforms using Micro-Snap Coliform Detection Device versus reference method

					Hygiena Micro-Snap Coliform Detection Device							
					Presumptive		Confirmed			Reference Method[g]		
Matrix	Inoculating strains	MPN/portion[a]	Instrument	N[b]	X[c]	POD_{CP}[d]	x	POD_{CC}[e]	$dPOD_{CP}$[f]	x	POD_R[h]	$dPOD_C$[i]
Raw ground beef-1	Naturally contaminated		SS Plus	5	5	1.00 (0.84, 1.00)	5	1.00 (0.84, 1.00)	0.00 (–0.26, 0.26)	5	1.00 (0.84, 1.00)	0.00 (–0.26, 0.26)
		201 (124,281)	EnSURE	5	5	1.00 (0.84, 1.00)	5	1.00 (0.84, 1.00)	0.00 (–0.26, 0.26)	5	1.00 (0.84, 1.00)	0.00 (–0.26, 0.26)
			Pi102	5	5	1.00 (0.84, 1.00)	5	1.00 (0.84, 1.00)	0.00 (–0.26, 0.26)	5	1.00 (0.84, 1.00)	0.00 (–0.26, 0.26)
			SS Plus	20	19	0.95 (0.76, 1.00)	19	0.95 (0.76, 1.00)	0.00 (–0.19, 0.19)	20	1.00 (0.84, 1.00)	–0.05 (–0.24, 0.12)
		254 (185,320)	EnSURE	20	19	0.95 (0.76, 1.00)	19	0.95 (0.76, 1.00)	0.00 (–0.19, 0.19)	20	1.00 (0.84, 1.00)	–0.05 (–0.24, 0.12)
			Pi102	20	19	0.95 (0.76, 1.00)	19	0.95 (0.76, 1.00)	0.00 (–0.19, 0.19)	20	1.00 (0.84, 1.00)	–0.05 (–0.24, 0.12)
Raw ground beef-2			SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
	E. cloacae (E0002)	<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	10	0.50 (0.30, 0.70)	12	0.60 (0.39, 0.78)	–0.10 (–0.37, 0.19)	11	0.55 (0.34, 0.74)	0.05 (–0.24, 0.33)
	E. coli (ATCC 8739)	5.02 (3.39, 6.65)	EnSURE	20	12	0.60 (0.39, 0.78)	12	0.60 (0.39, 0.78)	0.00 (–0.28, 0.28)	11	0.55 (0.34, 0.74)	0.05 (–0.24, 0.33)
			Pi102	20	11	0.55 (0.34, 0.74)	12	0.60 (0.39, 0.78)	–0.05 (–0.33, 0.24)	11	0.55 (0.34, 0.74)	0.00 (–0.28, 0.28)
Raw ground beef[j]	Naturally contaminated	9.76 (5.98, 13.50)	SS Plus	20	12	0.60 (0.39, 0.78)	15	0.75 (0.53, 0.89)	–0.15 (–0.40, 0.13)	17	0.85 (0.64, 0.95)	–0.25 (–0.48, 0.03)
			EnSURE	20	12	0.60 (0.39, 0.78)	15	0.75 (0.53, 0.89)	–0.15 (–0.40, 0.13)	17	0.85 (0.64, 0.95)	–0.25 (–0.48, 0.03)
			Pi102	20	14	0.70 (0.48, 0.85)	15	0.75 (0.53, 0.89)	–0.05 (–0.31, 0.22)	17	0.85 (0.64, 0.95)	–0.15 (–0.39, 0.11)
Raw cod-1	Naturally contaminated		SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
		3.1	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			SS Plus	20	16	0.80 (0.58, 0.92)	16	0.80 (0.58, 0.92)	0.00 (–0.44, 0.44)	17	0.85 (0.64, 0.95)	–0.05 (–0.29, 0.19)
		8.61 (3.21-12.82)	EnSURE	20	19	0.95 (0.76, 1.00)	16	0.80 (0.58, 0.92)	0.15 (–0.07, 0.37)	17	0.85 (0.64, 0.95)	–0.05 (–0.29, 0.19)
			Pi102	20	19	0.95 (0.76, 1.00)	16	0.80 (0.58, 0.92)	0.15 (–0.07, 0.37)	17	0.85 (0.64, 0.95)	–0.05 (–0.29, 0.19)
Raw cod-2	Naturally contaminated		SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
		<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	15	0.75 (0.53, 0.89)	14	0.70 (0.48, 0.85)	0.05 (–0.22, 0.31)	11	0.55 (0.34, 0.74)	0.15 (–0.14, 0.41)
		3.36 (1.42, 4.78)	EnSURE	20	16	0.80 (0.58, 0.92)	14	0.70 (0.48, 0.85)	0.10 (–0.17, 0.35)	11	0.55 (0.34, 0.74)	0.15 (–0.14, 0.41)
			Pi102	20	14	0.70 (0.48, 0.85)	14	0.70 (0.48, 0.85)	0.00 (–0.27, 0.27)	11	0.55 (0.34, 0.74)	0.15 (–0.14, 0.41)
Cooked chicken			SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
	E. aerogenes (ATCC 10006)	<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	14	0.70 (0.48, 0.85)	16	0.80 (0.58, 0.92)	–0.10 (–0.35, 0.17)	14	0.70 (0.48, 0.85)	0.00 (–0.27, 0.27)
	E. coli (ATCC 25922)	6.72 (5.31, 8.1)	EnSURE	20	17	0.85 (0.64, 0.95)	16	0.80 (0.58, 0.92)	0.05 (–0.19, 0.29)	14	0.70 (0.48, 0.85)	0.10 (–0.17, 0.35)
			Pi102	20	17	0.85 (0.64, 0.95)	16	0.80 (0.58, 0.92)	0.05 (–0.19, 0.29)	14	0.70 (0.48, 0.85)	0.10 (–0.17, 0.35)

Table 11. (*continued*)

					Hygiena Micro-Snap Coliform Detection Device							
					Presumptive		Confirmed			Reference Method[g]		
Matrix	Inoculating strains	MPN/portion[a]	Instrument	N[b]	X[c]	POD_{CP}[d]	x	POD_{CC}[e]	$dPOD_{CP}$[f]	x	POD_R[h]	$dPOD_C$[i]
Milk-2			SS Plus	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
	K. oxytoca (K0005)	<3.0	EnSURE	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			Pi102	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			SS Plus	20	15	0.75 (0.53, 0.89)	15	0.75 (0.53, 0.89)	0.00 (–0.26, 0.26)	17	0.85 (0.64, 0.95)	–0.10 (–0.34, 0.15)
	E. coli (EC 54)	5.7 (2.4, 7.7)	EnSURE	20	15	0.75 (0.53, 0.89)	15	0.75 (0.53, 0.89)	0.00 (–0.26, 0.26)	17	0.85 (0.64, 0.95)	–0.10 (–0.34, 0.15)
			Pi102	20	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.05 (–0.21, 0.30)	17	0.85 (0.64, 0.95)	–0.10 (–0.34, 0.15)
Milk[j]			SS Plus	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
	K. oxytoca (K0005)	<10	EnSURE	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			Pi102	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			SS Plus	20	19	0.95 (0.76, 1.00)	15	0.75 (0.53, 0.89)	0.20 (–0.03, 0.42)	17	0.85 (0.64, 0.95)	–0.10 (–0.34, 0.15)
	E. coli (EC 54)	13.8 (6.12, 20.3)	EnSURE	20	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.05 (–0.21, 0.30)	17	0.85 (0.64, 0.95)	–0.10 (–0.34, 0.15)
			Pi102	20	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.05 (–0.21, 0.30)	17	0.85 (0.64, 0.95)	–0.10 (–0.34, 0.15)
Raw chicken-1	Naturally contaminated		SS Plus	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
		<3.0	EnSURE	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			Pi102	5	1	0.20 (0.08,0.42)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			SS Plus	20	15	0.75 (0.53, 0.89)	16	0.80 (0.58, 0.89)	–0.05 (–0.30, 0.21)	18	0.90 (0.76, 1.00)	–0.15 (–0.38, 0.09)
		4.43 (3.15, 5.71)	EnSURE	20	17	0.85 (0.64, 0.95)	16	0.80 (0.58, 0.89)	0.05 (–0.19, 0.29)	18	0.90 (0.76, 1.00)	–0.10 (–0.33, 0.13)
			Pi102	20	16	0.80 (0.58, 0.89)	16	0.80 (0.58, 0.89)	0.00 (–0.25, 0.25)	18	0.90 (0.76, 1.00)	–0.10 (–0.33, 0.13)
Raw chicken-2	Naturally contaminated		SS Plus	5	0	0.00 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
		<3.0	EnSURE	5	0	0.00 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			Pi102	5	1	0.20 (0.08, 0.42)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			SS Plus	20	9	0.45 (0.26, 0.66)	8	0.40 (0.22, 0.61)	0.05 (–0.24, 0.33)	6	0.30 (0.15, 0.52)	0.10 (–0.18, 0.36)
		1.35 (0.22, 2.48)	EnSURE	20	9	0.45 (0.26, 0.66)	8	0.40 (0.22, 0.61)	0.05 (–0.24, 0.33)	6	0.30 (0.15, 0.52)	0.10 (–0.18, 0.36)
			Pi102	20	9	0.45 (0.26, 0.66)	8	0.40 (0.22, 0.61)	0.05 (–0.24, 0.33)	6	0.30 (0.15, 0.52)	0.10 (–0.18, 0.36)
Raw prawn	Naturally contaminated		SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
		<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	19	0.95 (0.76, 1.00)	17	0.85 (0.64, 0.95)	0.10 (–0.11, 0.32)	15	0.75 (0.53, 0.89)	0.10 (–0.15, 0.34)
		5.46 (0.73, 9.19)	EnSURE	20	19	0.95 (0.76, 1.00)	17	0.85 (0.64, 0.95)	0.10 (–0.11, 0.32)	15	0.75 (0.53, 0.89)	0.10 (–0.15, 0.34)
			Pi102	20	17	0.95 (0.76, 1.00)	17	0.85 (0.64, 0.95)	0.00 (–0.44, 0.44)	15	0.75 (0.53, 0.89)	0.10 (–0.15, 0.34)

Table 11. (*continued*)

					Hygiena Micro-Snap Coliform Detection Device							
					Presumptive		Confirmed			Reference Method[g]		
Matrix	Inoculating strains	MPN/portion[a]	Instrument	N[b]	X[c]	POD_{CP}[d]	x	POD_{CC}[e]	$dPOD_{CP}$[f]	x	POD_R[h]	$dPOD_C$[i]
Mineral water			SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
	C. freundii (C0012)	<1.00	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	5	0.25 (0.11, 0.47)	9	0.45 (0.26, 0.66)	–0.20 (–0.45, 0.09)	9	0.45 (0.26, 0.66)	–0.20 (–0.45, 0.09)
	E. coli (EC 67)	1.77 (0.77, 2.77)	EnSURE	20	5	0.25 (0.11, 0.47)	9	0.45 (0.26, 0.66)	–0.20 (–0.45, 0.09)	9	0.45 (0.26, 0.66)	–0.20 (–0.45, 0.09)
			Pi102	20	9	0.45 (0.26, 0.66)	9	0.45 (0.26, 0.66)	0.00 (–0.28, 0.28)	9	0.45 (0.26, 0.66)	0.00 (–0.28, 0.28)

[a] MPN = Most probable number is based on the POD of reference method.

[b] N = Number of test potions.

[c] x = Number of positive test portions.

[d] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

[e] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

[f] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values. If the confidence interval of a dPOD does not contain zero, the difference is statistically significant at the 5% level.

[g] Reference method used was appropriate to the food type.

[h] POD_R = Reference method positive outcomes divided by the total number of trials.

[i] $dPOD_C$ = Difference between the candidate method confirmed result and the reference method result POD values. If the confidence interval of a dPOD does not contain zero, the difference is statistically significant at the 5% level.

[j] Study conducted at the independent laboratory.

Table 12. Detection of *E. coli* using Micro-Snap *E. coli* Detection Device versus reference method

					Hygiena Micro-Snap *E. coli* Detection Device							
					Presumptive		Confirmed			Reference Method[g]		
Matrix	Inoculating strains	MPN/portion[a]	Instrument	N[b]	X[c]	POD_{CP}[d]	x	POD_{CC}[e]	$dPOD_{CP}$[f]	x	POD_R[h]	$dPOD_C$[i]
Raw ground beef-1	Naturally contaminated		SS Plus	5	2	0.10 (0.03, 0.30)	1	0.05 (0.00, 0.24)	0.05 (–0.15, 0.26)	2	0.10 (0.03, 0.30)	–0.05 (–0.26, 0.15)
		1.1 (0.2, 2.3)	EnSURE	5	2	0.10 (0.03, 0.30)	1	0.05 (0.00, 0.24)	0.05 (–0.15, 0.26)	2	0.10 (0.03, 0.30)	–0.05 (–0.26, 0.15)
			Pi102	5	2	0.10 (0.03, 0.30)	1	0.05 (0.00, 0.24)	0.05 (–0.15, 0.26)	2	0.10 (0.03, 0.30))	–0.05 (–0.26, 0.15)
			SS Plus	20	13	0.65 (0.43, 0.82)	9	0.45 (0.26, 0.66)	0.20 (–0.10, 0.46)	5	0.25 (0.11, 0.47)	0.20 (–0.09, 0.45)
		49.5 (22.1, 67.2)	EnSURE	20	13	0.65 (0.43, 0.82)	9	0.45 (0.26, 0.66)	0.20 (–0.10, 0.46	5	0.25 (0.11, 0.47)	0.20 (–0.09, 0.45)
			Pi102	20	13	0.65 (0.43, 0.82)	9	0.45 (0.26, 0.66)	0.20 (–0.10, 0.46	5	0.25 (0.11, 0.47)	0.20 (–0.09, 0.45)
Raw ground beef[j]	Naturally contaminated	5.2 (3.6, 6.8)	SS Plus	20	12	0.60 (0.39, 0.78)	13	0.65 (0.43, 0.82)	–0.05 (–0.32, 0.23)	14	0.70 (0.48, 0.85)	–0.10 (–0.36, 0.18)
			EnSURE	20	11	0.55 (0.34, 0.74)	13	0.65 (0.43, 0.82)	–0.10 (–0.37, 0.19)	14	0.70 (0.48, 0.85)	–0.15 (–0.41, 0.14)
			Pi102	20	14	0.70 (0.48, 0.85)	13	0.65 (0.43, 0.82)	0.05 (–0.23, 0.32)	14	0.70 (0.48, 0.85)	–0.05 (–0.32, 0.23)
BLT sandwich			SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
	K. pneumoniae (ATCC 700603)	<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
		19.6 (13.9, 26.1)	SS Plus	20	15	0.75 (0.53, 0.89)	15	0.75 (0.53, 0.89)	0.00 (–0.44, 0.44)	19	0.95 (0.76, 1.00)	–0.20 (–0.42, 0.03)
	E. coli (NCTC 13216)	19.6 (13.9, 26.1)	EnSURE	20	15	0.75 (0.53, 0.89)	15	0.75 (0.53, 0.89)	0.00 (–0.44, 0.44)	19	0.95 (0.76, 1.00)	–0.20 (–0.42, 0.03)
			Pi102	20	15	0.75 (0.53, 0.89)	15	0.75 (0.53, 0.89)	0.00 (–0.44, 0.44)	19	0.95 (0.76, 1.00)	–0.20 (–0.42, 0.03)
Raw cod-1	Naturally contaminated		SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
		<10	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			SS Plus	20	17	0.85 (0.64, 0.95)	17	0.85 (0.64, 0.95)	0.00 (–0.23, 0.23)	20	1.00 (0.84, 1.00)	–0.15 (–0.36, 0.04)
		18.6 (9.9, 29.1)	EnSURE	20	17	0.85 (0.64, 0.95)	17	0.85 (0.64, 0.95)	0.00 (–0.23, 0.23)	20	1.00 (0.84, 1.00)	–0.15 (–0.36, 0.04)
			Pi102	20	17	0.85 (0.64, 0.95)	17	0.85 (0.64, 0.95)	0.00 (–0.23, 0.23)	20	1.00 (0.84, 1.00)	–0.15 (–0.36, 0.04)
Raw cod-2	Naturally contaminated		SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
		<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	8	0.40 (0.22, 0.61)	8	0.40 (0.22, 0.61)	0.00 (–0.28, 0.28)	7	0.35 (0.18, 0.57)	0.05 (–0.23, 0.32)
		3.3 (1.4, 5.2)	EnSURE	20	7	0.35 (0.18, 0.57)	8	0.40 (0.22, 0.61)	–0.05 (–0.32, 0.23)	7	0.35 (0.18, 0.57)	0.00 (–0.26, 0.26)
			Pi102	20	12	0.60 (0.39, 0.78)	8	0.40 (0.22, 0.61)	0.20 (–0.10, 0.46)	7	0.35 (0.18, 0.57)	0.05 (–0.23, 0.32)
Cooked chicken			SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
	E. aerogenes (ATCC 10006)	<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	15	0.75 (0.53, 0.89)	14	0.70 (0.48, 0.85)	0.05 (–0.22, 0.31)	18	0.90 (0.48, 0.85)	–0.20 (–0.43, 0.05)
	E. coli (ATCC 25922)	4.5 (3.4, 5.6)	EnSURE	20	15	0.75 (0.53, 0.89)	14	0.70 (0.48, 0.85)	0.05 (–0.22, 0.31)	18	0.90 (0.48, 0.85)	–0.20 (–0.43, 0.05)
			Pi102	20	16	0.80 (0.58, 0.92)	14	0.70 (0.48, 0.85)	0.10 (–0.17, 0.35)	18	0.90 (0.48, 0.85)	–0.20 (–0.43, 0.05)

Table 12. ***(continued)***

					Hygiena Micro-Snap *E. coli* Detection Device							
					Presumptive		Confirmed			Reference Method[g]		
Matrix	Inoculating strains	MPN/Portion[a]	Instrument	N[b]	X[c]	POD_{CP}[d]	x	POD_{CC}[e]	$dPOD_{CP}$[f]	x	POD_R[h]	$dPOD_C$[i]
Milk-2			SS Plus	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
	K. oxytoca (K0005)	<10	EnSURE	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			Pi102	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			SS Plus	20	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.05 (–0.21, 0.30)	13	0.65 (0.43, 0.82)	0.10 (–0.18, 0.36)
	E. coli (EC 54)	11.2 (5.5, 16.2)	EnSURE	20	16	0.80 (0.58, 0.92)	15	0.75 (0.53, 0.89)	0.00 (–0.26, 0.26)	13	0.65 (0.43, 0.82)	0.10 (–0.18, 0.36)
			Pi102	20	13	0.65 (0.43, 0.82)	15	0.75 (0.53, 0.89)	0.05 (–0.21, 0.30)	13	0.65 (0.43, 0.82)	0.00 (–0.28, 0.28)
Milk[j]			SS Plus	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
	K. oxytoca (K0005)	<10	EnSURE	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			Pi102	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			SS Plus	20	17	0.85 (0.64, 0.95)	14	0.70 (0.48, 0.85)	0.15 (–0.11, 0.39)	19	0.85 (0.64, 0.95)	–0.25 (–0.47, –0.01)
	E. coli (EC 54)	28.9 (13.1, 42.3)	EnSURE	20	17	0.85 (0.64, 0.95)	14	0.70 (0.48, 0.85)	0.15 (–0.11, 0.39)	19	0.85 (0.64, 0.95)	–0.25 (–0.47, –0.01)
			Pi102	20	18	0.90 (0.70, 0.97)	14	0.70 (0.48, 0.85)	0.20 (–0.05, 0.43)	19	0.85 (0.64, 0.95)	–0.25 (–0.47, –0.01)
Raw chicken-1	Naturally contaminated		SS Plus	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
		<3.0	EnSURE	5	0	0.00 (0.00, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)
			Pi102	5	1	0.20 (0.08,0.42)	0	0.00 (0.00, 0.44)	0.00 (–0.44, 0.44)	1	0.05 (0.00, 0.24)	–0.05 (–0.24, 0.12)
			SS Plus	20	13	0.65 (0.43, 0.82)	14	0.70 (0.48, 0.85)	–0.05 (–0.32, 0.23)	15	0.75 (0.53, 0.89)	–0.10 (–0.36, 0.18)
		4.1 (3.4, 4.6)	EnSURE	20	14	0.70 (0.48, 0.85)	14	0.70 (0.48, 0.85)	0.00 (–0.27, 0.27)	15	0.75 (0.53, 0.89)	–0.05 (–0.31, 0.22)
			Pi102	20	16	0.80 (0.58, 0.92)	14	0.70 (0.48, 0.85)	0.10 (–0.17, 0.35)	15	0.75 (0.53, 0.89)	–0.05 (–0.31, 0.22)
Raw prawn	Naturally contaminated		SS Plus	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
		<3.0	EnSURE	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			Pi102	5	0	0 (0.00, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)	0	0 (0.00, 0.44)	0 (–0.44, 0.44)
			SS Plus	20	17	0.85 (0.64, 0.95)	15	0.75 (0.53, 0.89)	0.10 (–0.15, 0.34)	18	0.90 (0.70, 0.97)	–0.15 (–0.38, 0.09)
		6.2 (4.1, 8.7)	EnSURE	20	17	0.85 (0.64, 0.95)	15	0.75 (0.53, 0.89)	0.10 (–0.15, 0.34)	18	0.90 (0.70, 0.97)	–0.15 (–0.38, 0.09)
			Pi102	20	17	0.85 (0.64, 0.95)	15	0.75 (0.53, 0.89)	0.10 (–0.15, 0.34)	18	0.90 (0.70, 0.97)	–0.15 (–0.38, 0.09)

[a] MPN = Most probable number is based on the POD of reference method.

[b] N = Number of test potions.

[c] x = Number of positive test portions.

[d] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

[e] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

[f] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values. If the confidence interval of a dPOD does not contain zero, the difference is statistically significant at the 5% level.

[g] Reference method used was appropriate to the food type.

[h] POD_R = Reference method positive outcomes divided by the total number of trials.

[i] $dPOD_C$ = Difference between the candidate method confirmed result and the reference method result POD values. If the confidence interval of a dPOD does not contain. zero, the difference is statistically significant at the 5% level.

[j] Study conducted at the independent laboratory.

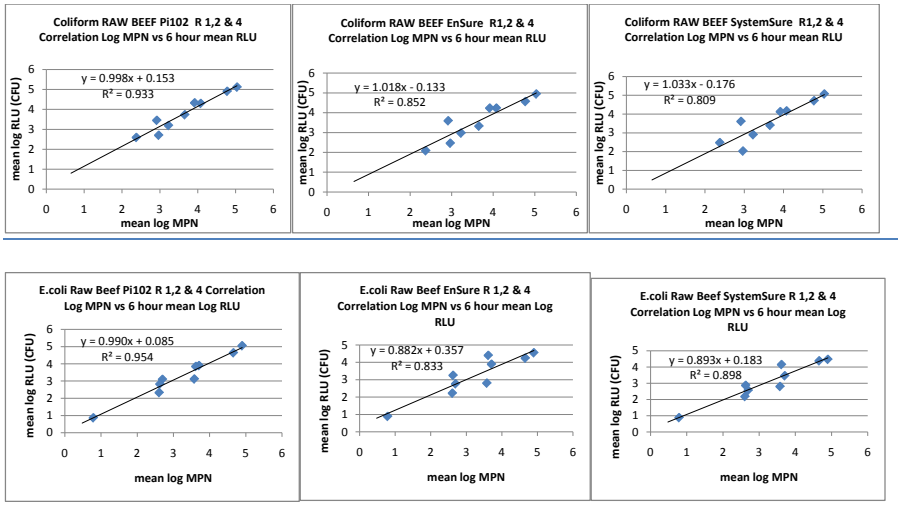

Figure 1. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from raw ground beef plotted against mean log-transformed MPN from all runs and all luminometers.

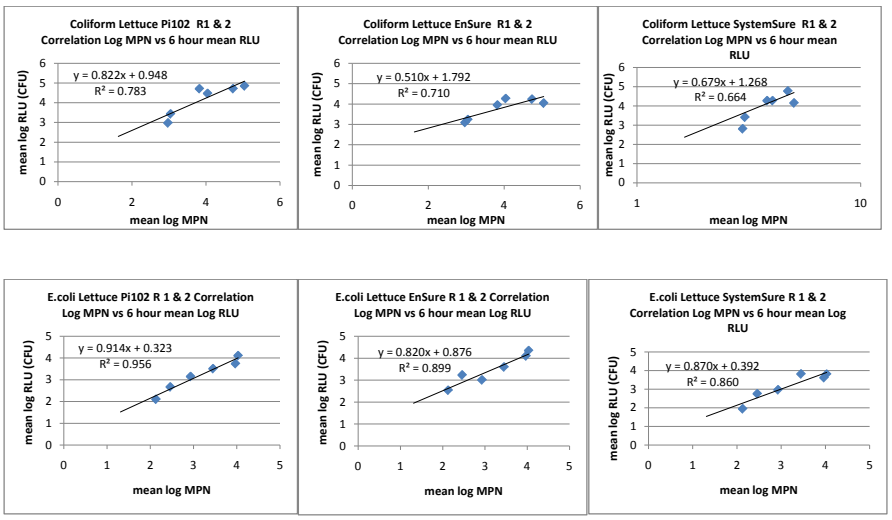

Figure 2. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from lettuce plotted against mean log-transformed MPN from all runs and all luminometers.

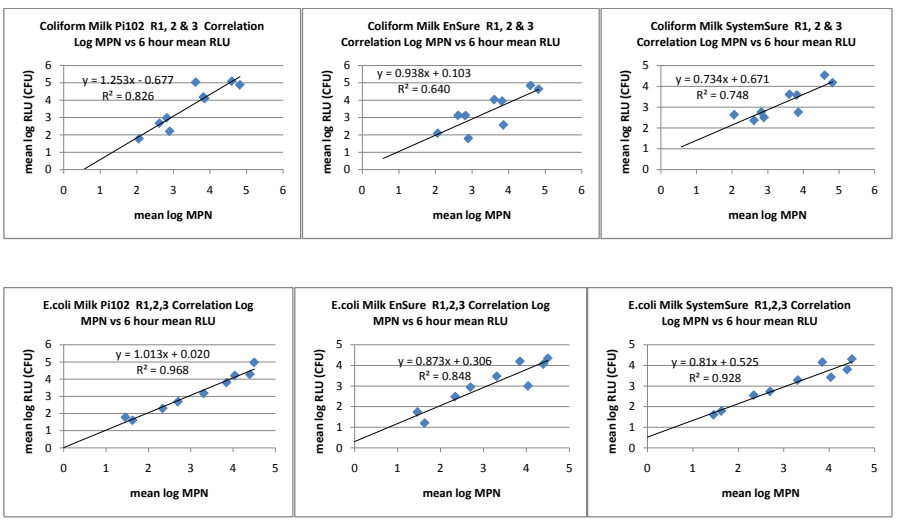

Figure 3. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from milk plotted against mean log-transformed MPN from all runs and all luminometers.

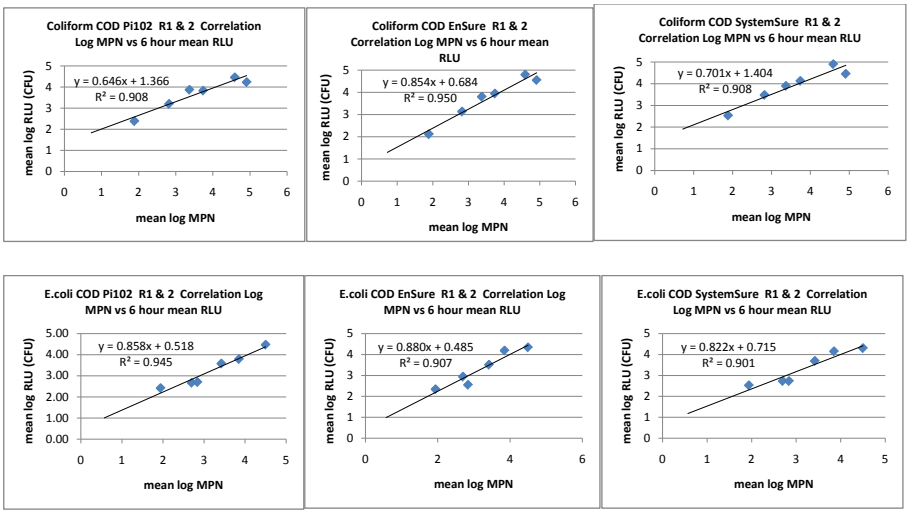

Figure 4. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from cod plotted against mean log-transformed MPN from all runs and all luminometers.

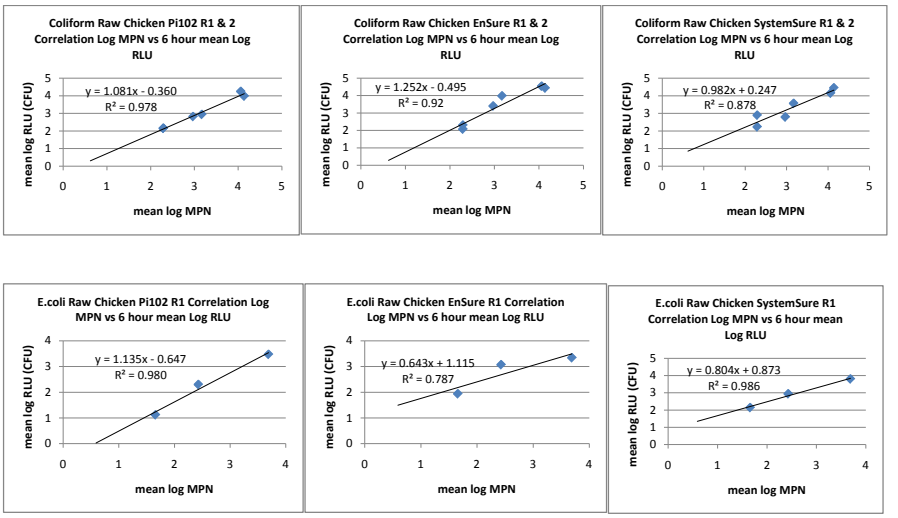

Figure 5. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from raw chicken plotted against mean log-transformed MPN from all runs and all luminometers.

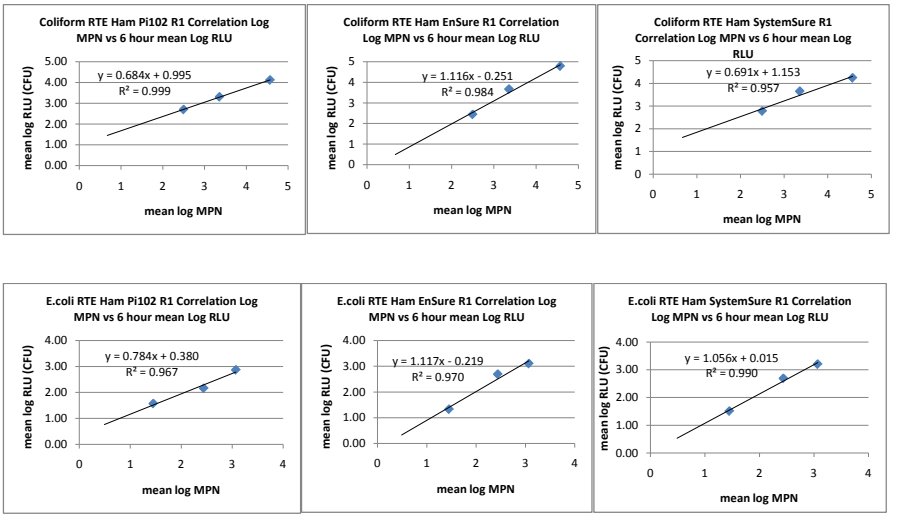

Figure 6. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from RTE ham plotted against mean log-transformed MPN from all runs and all luminometers.

Detection device.—Contains reagents for measuring β-galactosidase (substrate, luciferase, buffers, and lysis agent).

(**d**) *Additional supplies and reagents*.—Recommended diluents for product samples (e.g., buffered peptone water, maximum recovery diluent, and Butterfields buffered phosphate diluent); sterile 0.45 µm filters and filtration apparatus; pipets, P200 and P1000; pulsifier bags or equivalent.

Apparatus

(**a**) *Heating block*.—VWR (Leicestershire UK) Model No. 949313, or equivalent.

(**b**) *Temperature-controlled water bath*.—STS 50L Model, or equivalent.

(**c**) *Microbiological incubators*.—LTE Scientific Ltd (Lancashire, UK) Model IP100 and Gallencamp 110 or equivalent.

(**d**) *Microgen pulsifier* or equivalent stomacher.

(**e**) *Hygiena Pi102*.—Bench top model based on photon multiplier tube.

(**f**) *Hygiena EnSURE*.—Handheld instrument, multipurpose instrument based on photodiode technology.

(**g**) *Hygiena SystemSURE Plus*.—Handheld instrument, entry level instrument based on photodiode technology.

Standard Reference Materials

Bacteria used in the study were procured from several sources:

(**a**) Oxoid Ltd, Wade Rd, Basingstoke, Hampshire RG24 8PW, UK.

(**b**) Health Protection Agency, Manor Farm Rd, Porton Down, Salisbury, Wiltshire SP4 0JG, UK.

(**c**) University of Surrey, Food Microbiology Dept, Stag Hill, Guildford, Surrey GU2 7XH, UK.

(**d**) Royal Surrey Hospital, Egerton Road, Guildford, Surrey GU2 7XX, UK.

(**e**) Medical Microbiology Department, University of Aberdeen, Aberdeen AB25 2ZD, UK.

Safety Precautions

Components of MicroSnap Enrichment Swab devices do not pose any health risk when used correctly. Used devices that confirm positive may be biohazardous and should be disposed of safely in compliance with Good Laboratory Practices and Health and Safety regulations.

(**a**) Devices are designed for single use. Do not reuse.

(**b**) Do not use devices after expiration date.

(**c**) Sampling should be done aseptically to avoid cross-contamination.

(**d**) Ensure proper incubation temperature and time for the test application.

(**e**) When activating devices, ensure that the liquid in the bulb is transferred to the tube below.

Sample Preparation

(**a**) Prepare a 10% food homogenate using recommended diluents and standard microbiological procedures as indicated in the appropriate reference method for food.

(**b**) Beverage or water samples may be added directly to the MicroSnap Enrichment Swab Device.

Step 1: Enrichment

(**a**) Add 1 mL of sample to the MicroSnap Enrichment Swab (MS-ES-EC-100).

(**b**) Activate device by bending the bulb to break the snap valve.

(**c**) Squeeze bulb to release the enrichment broth into the swab tube by raising the bulb/swab assembly (about 1–2 in) and separating it from the swab tube to release the internal pressure. Ensure the enrichment broth is in the bottom of the swab tube. Replace bulb/swab assembly firmly to close the device.

(**d**) Shake tube gently to mix sample and enrichment broth.

(**e**) Incubate at 37 ± 0.5°C for 6 h.

(**f**) For large filterable liquids, collect sample up to 100 mL capacity and filter through 0.45 µm filter membrane (25 or 47 mm).

(**g**) Aseptically remove filter after filtration and place it in a sterile 47 mm Petri dish.

(**h**) Add 2 mL enrichment media from enrichment broth vial (MS-EB-EE-100) to the Petri dish.

(**i**) Incubate Petri dish at 37 ± 0.5°C for 6 h.

Step 2: Detection

(**a**) Transfer enriched sample to the MicroSnap Coliform Test (MS-CC-100). Aseptically remove an aliquot of the sample (0.1 mL, or 2–3 drops) from the MicroSnap Enrichment Swab and transfer to the MicroSnap Coliform Test. The Enrichment Swab can be used as a Pasteur pipet for convenience. Squeeze and release the bulb to mix and withdraw the sample. Remove the swab from the tube and carefully dispense 2–3 drops (about 0.1 mL) to the graduated fill line marked on the bottom of the MicroSnap Coliform Test device. The remaining enrichment broth can be returned to the Enrichment Swab for repeat testing, confirmation of *E. coli* using the MicroSnap *E. coli* Test (MS-EC-100), or for further testing and storage as required. For filtered samples, aseptically pipet 0.1 mL of the incubated broth from the Petri dish to the MicroSnap Coliform Test.

(**b**) Activate the MicroSnap Coliform Test. Bend the bulb to break the snap valve. Squeeze the bulb three times to release the reagent.

(**c**) Shake gently to mix.

(**d**) Incubate for 10 min at 37 ± 0.5°C for 10 ± 0.2 min.

(**e**) Insert the whole device into the luminometer, close the lid, and press the OK button to initiate measurement. Results will appear after 15 s.

(**f**) Read result as RLU from the display on the luminometer.

Further Testing

If a positive result is obtained using the MicroSnap Coliform Test, confirm the presence or absence of *E. coli* from the sample by retesting the same MicroSnap Enrichment using

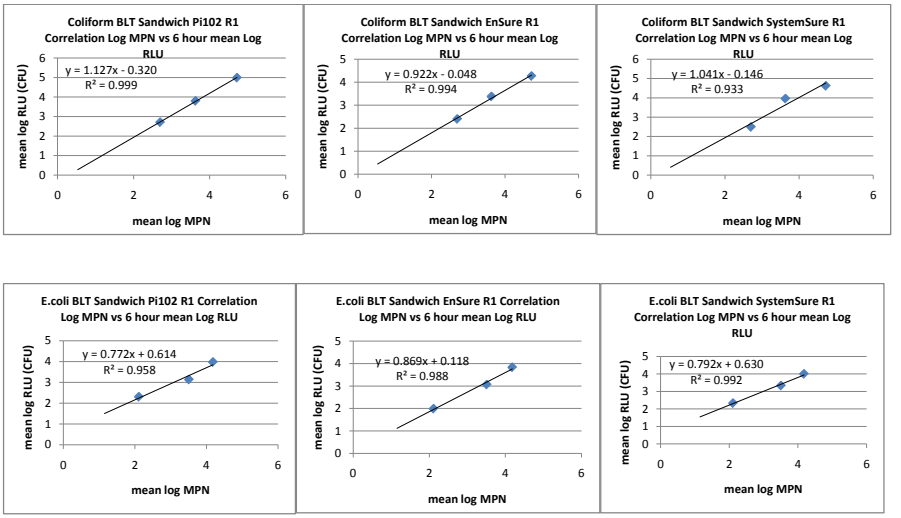

Figure 7. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from BLT sandwich plotted against mean log-transformed MPN from all runs and all luminometers.

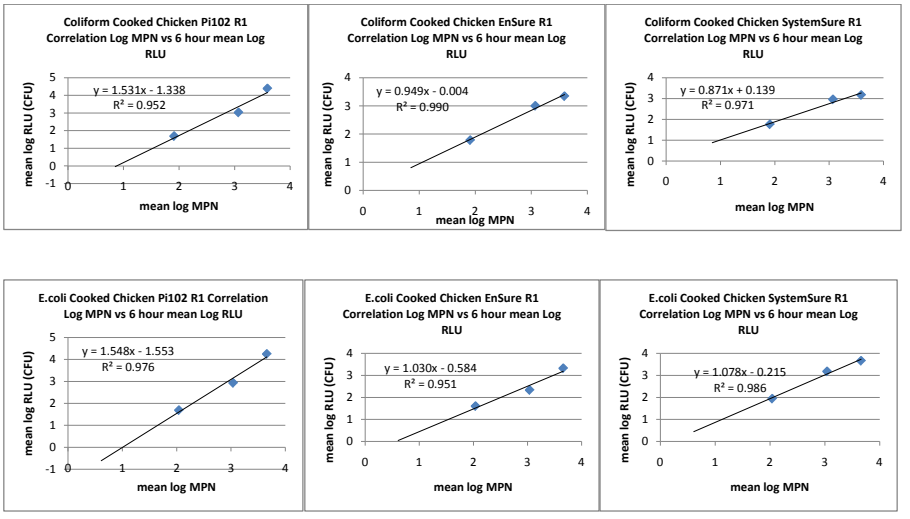

Figure 8. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from cooked chicken plotted against mean log-transformed MPN from all runs and all luminometers.

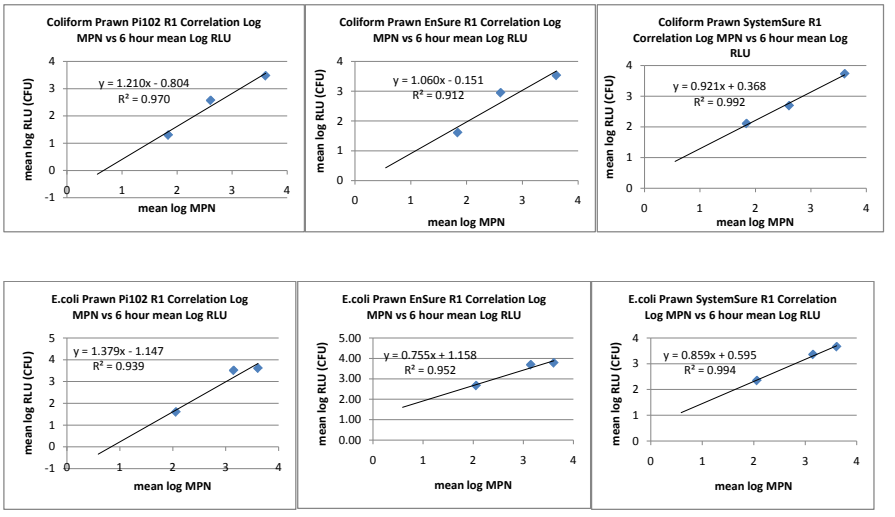

Figure 9. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from prawn plotted against mean log-transformed MPN from all runs and all luminometers.

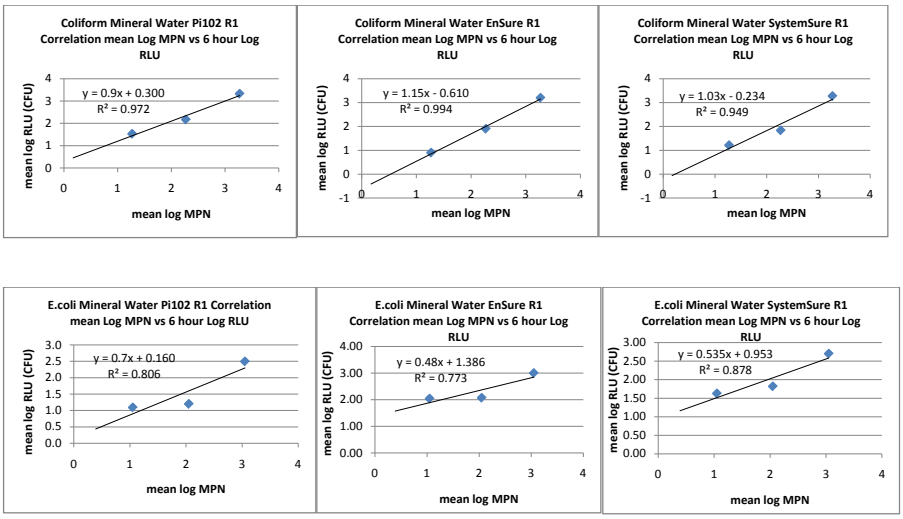

Figure 10. Mean log-transformed data for detection of *E. coli* and non-*E. coli* coliforms from mineral water plotted against mean log-transformed MPN from all runs and all luminometers.

the MicroSnap *E. coli* Test. If the *E. coli* test is run solely, the coliform test will confirm the presence of the organism.

Interpretation of Results

Results displayed on the luminometer are shown as RLU. Conversion tables provide equivalent CFU values for RLU measurements for each luminometer. The RLU output is compared to the appropriate table to obtain the quantitative measure of coliform and *E. coli* numbers present in the original sample.

Validation Study

The validation study was conducted according to the AOAC Research Institute *Performance Tested Methods*^{SM} program and the AOAC INTERNATIONAL guidelines for the validation of microbiological methods (5, 6). Method developer studies were conducted in the laboratories of Hygiena and included the inclusivity/exclusivity study, matrix studies for all claimed matrixes, product consistency and stability studies, and robustness testing. The independent laboratory study was conducted by Leatherhead Food Research and included a matrix study for two of the claimed food matrixes.

Inclusivity and Exclusivity Studies

MicroSnap Enrichment devices were inoculated with 1 mL of each of different strain of bacteria at several dilutions. The bacteria were initially grown overnight in a nonspecific media, TSB, and diluted in maximum recovery diluent to the selected dilution levels. The dilution series encompassed a range to include low levels around 1–10 to 100–1000 CFU/mL. These levels were included, ensuring that low and medium levels of bacteria were used to verify the detection of inclusivity at low levels of target bacteria. For noncoliforms, enrichment devices were inoculated with approximately 10^8 CFU/mL to stress the system with overproduction of enzymatic action similar to β-galactosidase and β-glucuronidase from these true negatives.

Twenty-five non-*E. coli* coliforms, 25 *E. coli* (50 total inclusivity organisms), and 30 noncoliforms were tested for production of β-galactosidase and β-glucuronidase at 6 and 8 h incubation at 37 ± 0.5°C for all levels of dilution. At 6 and 8 h, 100 µL was removed from each MicroSnap Enrichment device and added to a Coliform Detection Device or an *E. coli* Detection Device. These detection devices were then incubated at 37 ± 0.5°C for 10 ± 0.2 min and assayed in two luminometers, the SystemSURE Plus and the Pi102, which represent the most sensitive and least sensitive instruments in the study.

Results

Tables 1–3 demonstrate the inclusivity for coliforms and *E. coli* at both 6 and 8 h in both the SystemSURE plus and Pi102 luminometers. Table 4 demonstrates the exclusivity of the assay using a panel of noncoliform organisms from both Gram-negative and Gram-positive genera.

Table 1 shows two and three strains not detected after 6 h in the SystemSURE Plus and Pi102, respectively. The strains not detected were *Enterobacter aerogenes,* ATCC 10006 (Oxoid), *Klebsiella oxytoca* (Surrey University), and *Citrobacter freundii,* ATCC 8090 (Oxoid). These organisms appear to be late lactose fermenters and were below the LOD at 6 h. If a 10× higher inoculum is used for the three strains, all are detected as positive at 6 h. All strains were detected after 8 h.

Table 2 shows six and eight strains not detected after 6 h in the SystemSURE Plus and Pi102, respectively. After 8 h of incubation, only two strains were detected in the SystemSURE Plus. The strains not detected after 6 h were *E. coli* strains 64, 67, and 57 (Aberdeen University), *E. coli* strains E0018, E0023, and E0025 (Surrey University), and *E. coli* ATCC 13216 and ATCC 35218 (Oxoid). If a 10x higher inoculum is used for the eight strains, all are detected as positive at 6 h. After 8 h, the two strains not detected on the SystemSURE Plus were *E. coli* EC0025 (Surrey University) and *E. coli* ATCC 13216 (Oxoid). Both strains were positive in the more sensitive Pi102 luminometer.

Table 3 shows seven and five strains not detected after 6 h in the SystemSURE Plus and Pi102, respectively. The strains not

Table 13. Lot-to-lot variation–mean CFU of three MicroSnap batches in *E. coli* detection

Estimated CFU/mL	Organism	4 h			6 h		
		Lot A	Lot B	Lot C	Lot A	Lot B	Lot C
10000	*E. coli*	2	7	11	12365	18712	13184
100		2	1	1	1774	2642	821
10000	*C. freundii*	3	4	6	36231	31197	41407
100		1	1	1	23	23	15
10000	*S. typhimurium*	0	1	1	>3	>10	>10

Table 14. ANOVA comparison of three Micro-Snap batches in *E. coli* detection

CFU/mL	Organism	4 h	6 h
10000	*E. coli*	0.000	0.414
100		0.556	0.097
10000	*C. freundii*	0.005	0.859
100		0.917	0.032
10000	*S. typhimurium*	0.178	0.825

detected after 6 h were *E. coli* 64 (Aberdeen University), *E. coli* EC 10 (hospital), ATCC strains 25922 and 35218 (Oxoid), and *E. coli* strains EC0019, EC0023, EC0025, and EC0026 (Surrey University). If a 10x higher inoculum is used for the seven strains, all are detected as positive at 6 h. After 8 h of incubation, all strains were detected on both instruments.

Table 4 shows that none of the exclusivity organisms were detected in the Coliform and *E. coli* Detection Devices on either luminometer. All 30 species tested demonstrated no enzyme production at both 6 and 8 h incubation periods.

Matrix Study—Method Comparison

Ten different food and beverage matrixes were evaluated by both the MicroSnap method and the appropriate reference method.

(**a**) AOAC *Official Method* **966.24** for *E. coli* in all matrixes (except bottled water).

(**b**) U.S. FDA-BAM, Chapter 4, for *E. coli* in bottled water.

(**c**) AOAC **966.24** for coliforms in raw ground beef, cooked chicken, raw chicken, and RTE ham.

(**d**) The SMEDP, Chapter 7, for coliforms in raw liquid milk.

(**e**) BAM, Chapter 4, for coliforms in raw fish, raw prawns, prepackaged lettuce leaves, sandwiches [bacon, lettuce, and tomato (BLT)], and bottled water.

Each food was subjected to an initial investigation of an aerobic plate count, growth on violet red bile (VRB) lactose agar and Tryptone bile X-glucuronide agar to estimate total aerobic count, coliform, and *E. coli* levels. When the estimated coliform and *E. coli* counts were verified, a replicate sample of the food was used for spiking with a strain of both *E. coli* and coliform. Each approximately 500 g bulk portion of each food was spiked at different levels with a different strain of coliform and *E. coli* chosen at random from the inclusivity study (Tables 5–10). Inoculated foods were stored at 4°C for 48 h prior to analysis. The inoculated foods were then mixed with the naturally low-contaminated foodstuffs to create different levels of contamination. Four matrixes (beef, raw chicken, raw cod, and raw prawns) were naturally contaminated with coliforms and *E. coli* and therefore, did not require artificial contamination.

Five replicate 50 g portions were tested at the zero (if applicable) and higher contamination levels for each method. Twenty replicate 50 g portions were tested at the low fractional level. For the test method, 1 mL from each replicate portion from each contamination level of each food was inoculated into a separate MicroSnap Enrichment Device, except the mineral water sample, which was inoculated directly from the source, and incubated for 6 and 8 h at 37 ± 0.5°C in a dry heat block. At each time point, 100 µL was removed from each replicate and added to both a MicroSnap Coliform Detection Device and a MicroSnap *E. coli* Detection Device. The detection devices were then incubated at 37 ± 0.5°C in a dry heat block for 10 ± 0.2 min. The devices were then assayed in three separate luminometers in sequence, first in the SystemSURE Plus, then in the EnSURE, and finally in the Pi102. The total testing time for each device was 40 s. To maintain timing integrity, each device was inoculated in a staggered time protocol.

*AOAC Official Method **966.24**—Coliforms and E. coli*

A three-tube most probable number (MPN) series was performed in lauryl sulfate tryptose (LST) broth. Each 50 g test portion was blended in 450 mL Butterfield's phosphate-buffered water and blended for 2 min. Ten-fold dilutions were made (1:10, 1:100, 1:1000, 1:10 K, and 1:100 K), and 1 mL of each was added to triplicate tubes of LST. Tubes were incubated for 48 ± 2 h at 35 ± 0.5°C in a water bath. Tubes were examined

Table 15. Stability of Micro-Snap Coliform and *E. coli* Detection Devices over 10 months at 4°C

	E. coli				*C. freundii*				*S. typhimurium*	
	4 h		6 h		4 h		6 h		4 h	6 h
Date	10K	100	10K	100	10K	100	10K	100	10K	10k
13/10/2011	51	<10	6633	631	<10	<10	42499	<3	<10	<3
21/12/2011	51	<10	26263	443	<10	<10	9806	15	<10	<3
10/02/2012	29	<10	34807	485	<10	<10	19018	15	<10	<3
13/04/2012	15	<10	22907	487	<10	<10	12909	9	<10	<3
08/06/2012	59	<10	46684	508	<10	<10	14704	15	<10	3
10/08/2012	32	<10	8466	428	<10	<10	14525	9	<10	<3

for gas formation at 24 and 48 h. Tubes producing gas were transferred to brilliant green lactose bile (BGLB) broth and EC broth. BGLB tubes were incubated for 48 ± 2 h at 35°C. Gas production in BGLB within 48 h is a confirmed coliform test. EC broths were incubated for 48 ± 2 h at 45.5 ± 0.05°C in a covered water bath. Tubes were examined for gas formation at 24 and 48 h intervals. Gas-positive EC tubes were struck onto Levine's eosin methylene blue (EMB) agar plates, which were then incubated for 24 ± 2 h at 35°C. Typical *E. coli* colonies were transferred from EMB agar to plate count agar (PCA) slants for further testing. Slants were incubated for 48 h at 35 ± 0.5°C and then stored at 4°C until confirmation testing. Colonies were confirmed using the analytical profile index (API) 20E.

SMEDP—Coliforms

A 1 mL amount of each dilution was transferred in duplicate to VRB agar using the pour plate method. Plates were incubated for 24±2 h at 32±0.5°C in a microbiological air incubator. Typical coliform colonies were transferred to BGLB and incubated 48±3 h at 32±0.5°C. The presence of gas indicates a positive confirmed test. Coliforms were reported in a countable range (15–150).

BAM—Coliforms (Except Bottled Water)

A three-tube MPN series was performed as described in AOAC *Official Method* **966.24**. Ten-fold dilutions were made (1:10, 1:100, 1:1000, 1:10K, and 1:100K), and 1 mL of each was added to triplicate tubes of LST, which were incubated at 35°C. The tubes were examined for gas, and reactions were recorded at 24 ± 2 h. Gas-negative tubes were reincubated for an additional 24 h and examined and recorded again at 48±2 h. A 10 µL volume was transferred by loop from each positive tube to BGLB broth. The BGLB tubes were incubated at 35±0.5°C and examined for gas production at 48±2 h.

BAM—Coliforms and E. coli, Bottled Water

A 10-tube MPN test was performed by splitting 100 mL of sample and inoculating 10 tubes containing 10 mL of 2X LST broths with 10 mL of undiluted and diluted samples. Ten-fold dilution series were made (neat, 1:10, and 1:100). Tubes were incubated at 35 ± 0.5°C in a water bath and were examined regularly at 24 and 48 h for the presence of gas. Gas-positive tubes were transferred to BGLB and EC broth. BGLB tubes were incubated for 48 h at 35 ± 0.5°C and examined for gas production. EC broths were incubated for 48 h at 45.5 ± 0.05°C in a covered water bath. Gas-positive tubes were streaked onto EMB plates. Typical colonies were struck to PCA slants, and then confirmed using API 20E.

Membrane Filtration Method (Use for Detection, 0.01 CFU/mL)

Test samples (100 mL) were filtered through a 0.45 µm filter and transferred to M-Endo medium agar and incubated at 35 ± 0.5°C for 22–24 h. Typical colonies were confirmed by inoculating growth from a sheen colony into tubes of LST and incubated at 35 ± 0.5°C for 48 h. Gas-positive tubes were transferred to Bacterial Chips Bacterial Genes and EC broth for identification. Confirmation of coliforms and *E. coli* was performed as described above.

Results—Quantitative Analysis

Results of the quantitative analysis are presented in Tables 11 and 12. Each table represents one instrument (Pi102, EnSURE, and SS Plus) and one detection device (Coliform and *E. coli*). RLUs for the MicroSnap method were converted to CFU using tables provided in the method's directions for use. Data for the MicroSnap and reference methods were then $\log_{10}$-transformed and compared. The mean, repeatability SD (s_r), *P*-value, mean difference between methods, and 95% upper and lower confidence intervals on the mean difference were calculated for each level of each food type. Data were plotted for each food type, instrument, and detection device. The regression coefficients (R^2) are presented in Figures 1–10 for each instrument, detection device, and food type.

For each instrument and each detection device, repeatability was comparable to that of the reference method. For the coliform detection device, s_r averaged 0.21 across foods for the Pi102, ranging from 0.03 (cooked chicken) to 0.72 (milk); 0.23 across foods for the EnSURE, ranging from 0.03 (cod) to 0.55 (raw chicken); and 0.21 for the SS Plus across foods, ranging from 0.05 (cooked chicken) to 0.59 (lettuce). Repeatability averaged 0.14 across foods for the reference methods, ranging from 0.03 (milk) to 0.39 (RTE ham). For the *E. coli* detection

Table 16. Comparison of the effect of increasing enrichment incubation time and assay incubation time from 8 to 10 to 12 min on the endpoint RLU

Incubation time, h	Mean 8 min assay RLU	Mean 10 min assay RLU	Mean 12 min assay RLU
2	0	1	1
3	2	5	7
4	100	207	330
5	4488	4784	4976
6	4344	5048	6169
7	3097	3327	4231
8	1156	2003	2085
24	6468	6222	6251

Table 17. Comparison of the effect of increasing enrichment incubation time and increasing assay time from 8 to 10 to 12 min on the assay output

Incubation time, h	Mean 8 min assay %	Mean 10 min assay %	Mean 12 min assay %
2	0	100	200
3	44	100	141
4	48	100	159
5	94	100	104
6	86	100	122
7	93	100	127
8	58	100	104
24	104	100	100

device, s_r averaged 0.24 across foods for the Pi102, ranging from 0.05 (cod) to 0.69 (mineral water); 0.21 across foods for the EnSURE, ranging from 0.05 (cod) to 0.44 (lettuce); and 0.21 across foods for the SS Plus, ranging from 0.09 (RTE ham) to 0.68 (lettuce). Repeatability averaged 0.19 across foods for the reference methods, ranging from 0.01 (RTE ham) to 0.55 (lettuce).

For each instrument, detection device, and food type, an unpaired Student's *t*-test (assuming unequal variances) was performed on log mean differences to determine significance between the MicroSnap and reference methods (P-value ≤ 0.05) at the 95% confidence level. For the Coliform Detection Device, a significant difference was found in six of the 48 levels tested using the Pi102 (cod 1, cod 2, cooked chicken, milk 2, and milk–Independent Laboratory); five of the 48 levels tested using the EnSURE (beef—Independent Laboratory, milk 2, milk—Independent Laboratory, and raw chicken); and five of the 48 levels tested using the SS Plus (beef 1, beef 2, milk 1, and milk—Independent Laboratory). For the *E. coli* Detection Device, a significant difference was found in six of the 45 levels tested using the Pi102 (cooked chicken, lettuce 1, milk 1, milk—Independent Laboratory, raw chicken, and RTE ham), eight of the 48 levels tested using the EnSURE (beef 1, beef—Independent Laboratory, milk—Independent Laboratory, raw chicken, RTE ham, and mineral water), and nine of the 45 levels tested using the SS Plus (beef 1, beef 2, cod 2, milk 2, milk–Independent Laboratory, raw chicken, RTE ham, and mineral water). A mean difference between methods was typically greater than 0.5 when the P-value was ≤ 0.05. The mean difference between the MicroSnap and the reference methods was less than 0.5 in the majority of the levels tested for each food type, instrument, and detection device. Neither the MicroSnap nor any of the reference methods consistently estimated a higher level of coliforms or *E. coli* when compared to each other, and the methods varied around a mean difference of 0 to a similar extent.

The regression coefficient (R^2) was determined for each instrument, detection device, and food type. Foods with multiple runs were assessed together. The overall regression values for all food types for each instrument and detection device were also determined. For the Coliform Detection Device, the overall R^2 for the Pi102 was 0.854 (92.4% agreement) and ranged from 0.783 (lettuce) to 0.999 (BLT), the overall R^2 for the EnSURE was 0.825 (90.8%) and ranged from 0.641 (milk) to 0.994 (BLT), and the overall R^2 for the SS Plus was 0.782 (88.4%) and ranged from 0.665 (lettuce) to 0.992 (prawns). For the *E. coli* Detection Device, the overall R^2 for the Pi102 was 0.935 (96.7% agreement) and ranged from 0.807 (mineral water) to 0.980 (raw chicken), the overall R^2 for the EnSURE was 0.767 (87.6%) and ranged from 0.774 (milk) to 0.988 (BLT), and the overall R^2 for the SS Plus was 0.883 (93.9%) and ranged from 0.665 (lettuce) to 0.992 (prawns). The mean average slope for the Coliform Detection Device was 1.0256 for Pi102, 0.9772 for EnSURE, and 0.8687 for SS Plus. The mean average slope for the *E. coli* Detection Device was 1.0091 for Pi102, 0.8350 for EnSURE, and 0.8522 for SS Plus, which demonstrates a good range of agreement.

Results—Qualitative Analysis

The results of the qualitative analysis are presented in Tables 11 and 12. Table 11 represents the results for the Coliform Detection Device for each matrix on each instrument; Table 12 represents the results for the *E. coli* Detection Device for each matrix on each instrument. The POD statistical model was used to compare the results of the MicroSnap system to the results of the appropriate reference method. Differences in the dPOD values, with confidence intervals, were calculated for each method. Differences between methods are considered statistically significant at the 5% level if the confidence interval of a dPOD does not contain a zero.

Achieving fractional recovery for both coliforms and *E. coli* in the matrixes was a challenge. Multiple runs were necessary in some matrixes (raw ground beef, raw cod, and raw chicken) to achieve the appropriate levels. Fractional recovery was never achieved for RTE ham, BLT, and lettuce (coliforms), and lettuce and mineral water (*E. coli*); these matrixes are not included in the qualitative claim in the MicroSnap method package insert. Hygiena will continue to test these and newer matrixes for inclusion in the document.

For both the Coliform and *E. coli* Detection Devices, some test portions gave positive results with the MicroSnap system that did not confirm as positive (false positives). The number of unconfirmed results varied with the matrix, instrument, and detection device. For the Coliform Detection Device, the results were as follows: eight unconfirmed for the SystemSURE Plus (one in raw cod 2, four in milk—Independent Laboratory, one in raw chicken 2, and two in raw prawn); 11 unconfirmed for the EnSURE (three in raw cod 1, two in raw cod 2, one in cooked chicken, one in milk—Independent Laboratory, one in raw chicken 1, one in raw chicken 2, and two in raw prawns); and nine unconfirmed for the Pi102 (three in raw cod 1, one in cooked chicken, one in milk 2, one in milk—Independent Laboratory, one in raw chicken 1, and two in raw chicken 2). For the *E. coli* Detection Device, the results were as follows: 12 unconfirmed for the SystemSURE Plus (five in raw ground beef 1, one in cooked chicken, one in milk 2, three in milk—Independent Laboratory, two in raw prawns); 12 unconfirmed for the EnSURE (five in raw ground beef 1, one in cooked chicken, one in milk 2, one in milk—Independent Laboratory, two in raw prawns); and 20 unconfirmed for the Pi102 (5 in raw ground beef 1, one in raw ground beef—Independent Laboratory, four in raw cod 2, two in cooked chicken, four in milk—Independent Laboratory, three in raw chicken 1, and two in raw prawns).

For both the Coliform and *E. coli* Detection Devices, some test portions gave negative results with the MicroSnap system where the presence of coliforms or *E. coli* was confirmed in the portion (false negative). The number of negative results that confirmed positive varied with the matrix, instrument, and detection device. For the Coliform Detection Device, the results were as follows: 14 negative portions confirmed positive for the SystemSURE Plus (two in raw ground beef 1, three in raw ground beef—Independent Laboratory, two in cooked chicken, two in lettuce, one in raw chicken 1, and four in mineral water); nine negative portions confirmed positive for the EnSURE (three in raw ground beef—Independent Laboratory, two in lettuce, and four in mineral water); and two negative portions confirmed positive in the Pi102 (one in raw ground beef 1 and one in raw ground beef—Independent Laboratory). For the *E. coli* Detection Device, the results were as follows: two negative portions confirmed positive for the SystemSURE Plus (one in raw ground beef—Independent Laboratory and one in

raw chicken); three negative portions confirmed positive for the EnSURE (two in raw ground beef—Independent Laboratory and one in raw cod), and two negative portions confirmed positive for the Pi102 (two in milk 2).

Based on the POD analysis, no statistically significant differences were found between the presumptive and confirmed results for each detection device, nor were any statistically significant differences found between the MicroSnap system and the reference method results. Because the MicroSnap test relies on a threshold, this can cause false positives that are close to the threshold to fall below or above the threshold. Overall, the MicroSnap system is effective for coliform and *E. coli* detection after 8 h of incubation, as compared to the reference methods, which give results in 2–4 days.

Lot-to-Lot Variation

To assess the lot-to-lot variation of MicroSnap *E. coli* and Coliform Devices, three different detection and enrichment batches were used. *E. coli* (ATCC 8739) and *C. freundii* (ATCC 8090) were used as positive tests for MicroSnap *E. coli* and MicroSnap Coliform, respectively. *Salmonella* Typhimurium (ATCC 14208) was used as a negative control for both devices. Organisms were tested at 10 000 colonies/g and 100 colonies/g at 4 and 6 h incubation time points.

Results

The three batches tested (Table 13 and 14) demonstrated some significant differences when the results from the 4 h incubation were analyzed, but at 6 h, when the cultures are more mature, there were no significant differences. Table 14 shows the *P*-values calculated. Overall, no batch of detection devices was more or less sensitive than other batches.

Stability

To assess the stability of the devices, a single MicroSnap batch was used on the same three organisms as previously used: *E. coli* ATCC 8739, *C. freundii* ATCC 8090, and *S.* Typhimurium ATCC 14208 (Table 15). Detection was tested at 2 month intervals with organism concentrations the same as previously used (10 000 colonies/g and 100 colonies/g).

The stability criteria were set as follows: (*1*) positive *E. coli* detection at 4 h of 10 000 bacteria; (*2*) positive *E. coli* detection at 6 h of approximately 100 bacteria; (*3*) positive *C. freundii* detection of 10 000 bacteria at 7 h; (*4*) negative *Salmonella* detection at all levels and time points; and (*5*) clear differential and semi-quantitation of 100 and 10 000 *E. coli* at 6 h.

Results

The overall stability of the batches of detection devices was excellent (Table 15). At no point during the 12 month stability trial was any deterioration in the cultures observed. The use of real cultures throughout the 12 months does lead to some inherent variation but overall, all targets for stability were met. The detection of low levels of *E. coli* at 6 h and the detection of high levels of *E. coli* and the late lactose-fermenter *Citrobacter* were achieved with good reproducibility.

Robustness

The robustness of the detection device was assessed using *E. coli* ATCC 8739 at 100 000 colonies/g. Results were taken at time points from 2 to 24 h. At each time point, the RLU measurement was taken 8, 10, and 12 min after device activation.

Results

Due to the use of enzyme kinetics at fixed time points, the development of the signal increases with time during the enzyme detection phase. It is thus critical to the correct functioning of the device and the estimation of the final counts that the timings used are accurate and standardized throughout the validation by customers and the final utilization of the test.

During the exponential phase of growth, the concentration of enzyme changes at a quicker rate; therefore, the enrichment time (6 h) has the greater effect on the absolute RLU, hence CFU, obtained. Concurrently, during the assay, incubation time of 10 min also has the greatest effect on the assay output (Tables 16 and 17). These two factors are those measured during the robustness in the testing of this system.

Discussion

The MicroSnap Coliform and *E. coli* detection system is primarily designed to give a rapid and semi-quantitative assessment of *E. coli* and coliforms in food samples. The product is sold as a system for quantitation and detection at low to medium levels of both *E. coli* and coliforms. The quantitation is designed to be an overall indicator of levels of bacteria in the samples being tested.

The MicroSnap coliform and *E. coli* tests are the first of a series of bioluminogenic assays designed to give the investigator the possibility to determine levels of contaminating organisms in 6 h due to the extreme sensitivity of these assays. The unique nature of the assay producing light in relation to the enzyme concentration means the system can detect low enzyme levels from low levels of bacteria that are actively growing in the sample under investigation.

The levels of quantitation required can be thought of as a traffic light type system for most investigators. The European Commission Regulation EC No. 2073/2005 of November 15, 2005 has set limits for most foods in the measurement of both pathogens (*Listeria* and *Salmonella*) and for indicator organisms, *E. coli*, coliforms, and *Enterobacteriaceae*. The regulations stipulate that certain levels need to be maintained for quality and safety. In regulation 2.1.6 governing minced meat, three levels are mentioned: <50 CFU/g, 50–500 CFU/g, and >500 CFU/g. The numbers of replicates run and the number of each replicates that are allowed at each level tested is prescribed. If all five replicates are <50 CFU/g, the meat is considered excellent; if two of the five replicates are between 50 and 500 CFU/g, it is considered adequate, and if any replicates are >500 CFU/g it is deemed not fit. These levels indicate low, medium, and high levels of contamination akin to a go, wait, and stop. Hence, the use of MicroSnap and other systems can be used to rapidly measure and track which level the sample best fits. The setting for these levels will be determined for each food by each investigator according to the regulations, or judged to be appropriate for each food by internal validation.

During the assessment of the AOAC Official Method, certain non-*E. coli* coliforms would not begin to grow in the first enrichment broth (LST) for the MPN, which led to the conclusion that some bacteria are incapable of being detected by the standard method. Both methods will never detect all bacteria being considered as potential targets. These exceptions could lead to both methods showing variations and nonsignificances.

Conclusions

The rapid method of detection of enzymes from bacterial species under investigation is a developing field; the use of bioluminogenic assays is beginning to show that earlier detection of these markers is possible, leading to rapid and more sophisticated assays than previously seen in chromogenic enzyme detection. Based on the results reported in this study, it is recommended that the MicroSnap Coliform and *E. coli* method be certified by the AOAC *Performance Tested Methods* Program for the detection and enumeration of coliforms and *E. coli* in a variety of foods. Some significant differences were observed in some dilutions of the food matrixes tested. The poorest sample performance in the quantitative study was milk, both in the mean differences/*P*-values and in absolute correlation coefficients. The lowest R^2 was 0.6407; this would be an agreement of 80%.

References

(1) AOAC Official Method **966.25**. http://www.eoma.aoac.org
(2) U.S. Food and Drug Administration-*Bacteriological Analytical Manual* (FDA-BAM), Chapter 4. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM064948
(3) *The Standard Methods for the Examination of Dairy Products* (SMEDP) (2004) Chapter 7, 17th Ed. http://www.apha.org/about/news/pressreleases/2004/04revised.htm
(4) Least Cost Formulations, Ltd (2011) *AOAC Binary Data Interlaboratory Study Workbook*. http://lcfltd.com/aoac/aoac-binary-v2-2.xls
(5) *Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* (2012) AOAC INTERNATIONAL, Gaithersburg, MD. http://www.aoac.org/vmeth/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf
(6) Feldsine, P.T., Kerr, D.E., Keung, S.C., Lienau, A.H., Miller, S.M., & Mui, L.A. (2002) *J. AOAC Int.* **85**, 1037–1044