

**Study to determine the sensitivity and specificity of the
Pathogenic *Listeria* Detection Kit (SwabSURE-Listeria)**

CONFIDENTIAL TO: Dr. Irina Barbolina
Technical Director
Technical Service Consultants Ltd.
The Ropewalk, Schofield Street
Heywood
Lancashire
OL10 1DS, UK

MB/INT/114444

BY: C.L. Baylis and R.A. Green

17th June 2009

This report shall not be reproduced except in full without written approval from Campden BRI.

Confidentiality notice: The information in this document is only intended for the individual or entity to whom it is addressed. It may contain privileged and confidential information that is exempt from disclosure by law and if you are not the intended recipient you must not copy distribute or take any action in reliance on it. If you have received this document in error please notify us immediately by telephone.

INTRODUCTION

Dr. Irina Barbolina, Technical Director, Technical Service Consultants Ltd., approached Campden BRI to evaluate the performance of the Pathogenic *Listeria* Detection Kit (SwabSURE-*Listeria*).

The protocol used to evaluate the SwabSURE-*Listeria* kit was provided by Technical Service Consultants Ltd. This study determined the specificity and sensitivity of the test.

MATERIALS AND METHODS

The SwabSURE-*Listeria* kit was used in accordance with the instructions provided by Technical Service Consultants Ltd. (see Appendix). Each test consisted of a foam swab to sample the chosen site (Appendix, Fig. 1). This swab is subsequently inserted into a second tube containing a selective diagnostic medium for the growth of *Listeria* species; antibiotics that restrict or inhibit the growth of non-target organisms, and a chromogenic compound for the detection of the two main pathogenic *Listeria* species (*L. monocytogenes* and *L. ivanovii*). The presence of *L. monocytogenes* and/or *L. ivanovii* at the concentration of ≥ 1 cell/sample results in colour change of the medium from straw-yellow to turquoise-blue (Appendix, Fig. 2). Each test was incubated at 37°C and results observed and recorded at 24h and 48h.

1) Specificity (inclusivity)

The specificity (inclusivity) of the SwabSURE-*Listeria* kit was determined using 51 strains of pathogenic *Listeria* spp. (40 *L. monocytogenes* and 11 *L. ivanovii*). The strains used are presented in Table 1. Each strain was grown in Tryptone Soya broth (TSB) and diluted to give different concentration of each strain between approximately 10 and 100 cfu/sample. Two levels (~1-50 and ~50-100 cfu/swab) to cover the range of concentrations requested by the client were tested. Duplicate swabs were inoculated with diluted culture to achieve the correct

concentration per swab. A swab inoculated with sterile medium only (negative control) was also tested. The swabs were processed in accordance with the manufacturer's instructions.

2) Specificity (exclusivity)

The specificity (exclusivity) of the SwabSURE-Listeria kit was determined using 50 strains of non-target organisms. The strains used are presented in Table 2. Each of the 50 strains of non-target organisms were cultured in appropriate media depending on their specific growth requirements. The media and incubation temperatures used were; TSB 37°C (all *Listeria* strains), Nutrient broth (NB) 30°C (*Bacillus* spp., *Micrococcus* spp. and *Pseudomonas* spp.), NB 37°C (*Staphylococcus* spp., *Enterococcus* spp. and *Rhodococcus equi*) and members of the Enterobacteriaceae (e.g. *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp. and *Salmonella*), NB 25°C *Brochothrix thermosphacta*, deMan Rogosa Sharpe broth (MRSB) *Lactobacillus* spp. (microaerophilic atmosphere) and *Pediococcus* spp. (aerobic atmosphere) and Malt Extract broth (yeast strains).

All of the strains were diluted to give a final concentration of approximately 100-1000 cfu per swab. Each strain was initially diluted to a concentration of ~1,000 cfu per swab which represented the highest level (worst case) specified by the client. Swabs were processed in accordance with the manufacturer's instructions. The results from this study were recorded along with any observations. Any strains giving positive results (false positive) at the high contamination level were repeated at a lower concentration of ~100 cfu per swab.

3) Sensitivity

The sensitivity of the SwabSURE-Listeria kit was determined using 10 strains of pathogenic *Listeria* spp. (6 *L. monocytogenes* and 4 *L. ivanovii*). The strains used are presented in Table 3. Each strain was grown in tryptone soya broth (TSB) and diluted to give different concentration of each strain between 1 and 100,000 cfu/swab. A total of 4 different levels were tested (1-10, 100-1000,

1000-10,000 and 10,000-100,000 cfu/swab) to cover the range of concentrations requested by the client. Duplicate swabs were inoculated with diluted culture to achieve the correct concentration per swab. A swab inoculated with sterile medium only (negative control) was also tested.

The swabs were processed in accordance with the manufacturer's instructions. The results (e.g. medium colour after 24h and 48h incubation) were recorded along with any other observations during the study.

Table 1
Target *Listeria* strains used to establish the specificity (inclusivity) of the SwabSURE-*Listeria* kit

Number	Organism	Campden code	Serogroup	Source	Origin
1.	<i>Listeria monocytogenes</i>	6600	4b	NCTC 11994	NCTC
2.	<i>Listeria monocytogenes</i>	6601	1/2b	NCTC 10887	NCTC
3.	<i>Listeria monocytogenes</i>	1100	1/2a	Stilton cheese	HPA
4.	<i>Listeria monocytogenes</i>	1101	1/2a	Coleslaw salad	HPA
5.	<i>Listeria monocytogenes</i>	1102	1/2a	Lettuce	HPA
6.	<i>Listeria monocytogenes</i>	1103	1/2a	Pate	HPA
7.	<i>Listeria monocytogenes</i>	1104	1/2a	Soft cheese	HPA
8.	<i>Listeria monocytogenes</i>	1105	1/2a	Raw milk	HPA
9.	<i>Listeria monocytogenes</i>	1108	1/2b	Pate	HPA
10.	<i>Listeria monocytogenes</i>	1149	1/2b	Pork liver pate	HPA
11.	<i>Listeria monocytogenes</i>	1150	1/2b	Fish fingers	HPA
12.	<i>Listeria monocytogenes</i>	1151	1/2c	Hard boiled eggs	HPA
13.	<i>Listeria monocytogenes</i>	1152	1/2c	Cooked turkey	HPA
14.	<i>Listeria monocytogenes</i>	1153	1/2c	Pate	HPA
15.	<i>Listeria monocytogenes</i>	1154	1/2c	Ice cream	HPA
16.	<i>Listeria monocytogenes</i>	1155	1/2c	Sliced ham	HPA
17.	<i>Listeria monocytogenes</i>	1157	3a	Cooked chicken	HPA
18.	<i>Listeria monocytogenes</i>	1158	3a	Cooked chicken	HPA
19.	<i>Listeria monocytogenes</i>	1160	3a	Pate	HPA
20.	<i>Listeria monocytogenes</i>	1161	3a	Boiled ham	HPA
21.	<i>Listeria monocytogenes</i>	1162	3a	Cooked turkey	HPA
22.	<i>Listeria monocytogenes</i>	1163	3b	Pepper quiche	HPA
23.	<i>Listeria monocytogenes</i>	1164	3b	Chicken	HPA
24.	<i>Listeria monocytogenes</i>	1165	3b	Chicken liver pate	HPA
25.	<i>Listeria monocytogenes</i>	1166	3b	Pate	HPA

Number	Organism	Campden code	Serogroup	Source	Origin
26.	<i>Listeria monocytogenes</i>	1168	3b	Cooked turkey	HPA
27.	<i>Listeria monocytogenes</i>	1170	3c	Cooked chicken	HPA
28.	<i>Listeria monocytogenes</i>	1171	3c	Cooked chicken	HPA
29.	<i>Listeria monocytogenes</i>	1172	3c	Cooked ham	HPA
30.	<i>Listeria monocytogenes</i>	1173	3c	Chicken & lettuce sandwich	HPA
31.	<i>Listeria monocytogenes</i>	1174	3c	Sandwich	HPA
32.	<i>Listeria monocytogenes</i>	1175	4b	Cooked turkey	HPA
33.	<i>Listeria monocytogenes</i>	1176	4b	Cheese	HPA
34.	<i>Listeria monocytogenes</i>	1178	4b	Chicken salad roll	HPA
35.	<i>Listeria monocytogenes</i>	1179	4b	Cheese	HPA
36.	<i>Listeria monocytogenes</i>	1180	4b	Soft cheese	HPA
37.	<i>Listeria monocytogenes</i>	1980	4b	Dairy environment	Food Producer
38.	<i>Listeria monocytogenes</i>	3630	1/2a	Stilton cheese	Food Producer
39.	<i>Listeria monocytogenes</i>	3650	4b	Dairy environment	Food Producer
40.	<i>Listeria monocytogenes</i>	2080	1/2a	Drain sample	Food Producer
41.	<i>Listeria ivanovii</i>	6599	N/A	NCTC 11007	NCTC
42.	<i>Listeria ivanovii</i>	1123	N/A	Soft cheese	HPA
43.	<i>Listeria ivanovii</i>	1120	N/A	Radish	HPA
44.	<i>Listeria ivanovii</i>	1121	N/A	Belgian salami	HPA
45.	<i>Listeria ivanovii</i>	1122	N/A	Salami	HPA
46.	<i>Listeria ivanovii</i>	1124	N/A	Unknown	HPA
47.	<i>Listeria ivanovii</i>	1125	N/A	Unknown	HPA
48.	<i>Listeria ivanovii</i>	1126	N/A	Unknown	HPA
49.	<i>Listeria ivanovii</i>	1127	N/A	Unknown	HPA
50.	<i>Listeria ivanovii</i>	1128	N/A	Unknown	HPA
51.	<i>Listeria ivanovii</i>	1129	N/A	Unknown	HPA

N/A = Not applicable

HPA = Health Protection Agency Centre for Infections, Colindale, London, United Kingdom.

NCTC = National Collection of Type Cultures, Colindale, London, United Kingdom.

Table 2
Non-target strains used to establish the specificity
(exclusivity) of the SwabSURE-Listeria kit

Number	Organism	Campden code	Source	Origin
1.	<i>Listeria innocua</i>	1110	Pate	HPA
2.	<i>Listeria innocua</i>	1111	Cheese	HPA
3.	<i>Listeria innocua</i>	1112	Lettuce	HPA
4.	<i>Listeria innocua</i>	1117	Salami	HPA
5.	<i>Listeria innocua</i>	6602	NCTC 11288	NCTC
6.	<i>Listeria welshimeri</i>	1130	Salami	HPA
7.	<i>Listeria welshimeri</i>	1132	Raw chicken	HPA
8.	<i>Listeria welshimeri</i>	1134	Chicken	HPA
9.	<i>Listeria welshimeri</i>	1135	Smoked mackerel	HPA
10.	<i>Listeria seeligeri</i>	1139	Lettuce	HPA
11.	<i>Listeria seeligeri</i>	1146	Pate	HPA
12.	<i>Listeria seeligeri</i>	6603	NCTC 11856	NCTC
13.	<i>Listeria grayi</i>	9298	NCTC 10815	NCTC
14.	<i>Listeria grayi</i>	12524 A	NCTC 10812	NCTC
15.	<i>Listeria grayi</i>	12526 A	NCTC 10813	NCTC
16.	<i>Bacillus cereus</i>	1761	Dairy product	Dairies Association
17.	<i>Bacillus cereus</i>	4110	ATCC 10876, NCTC 7464	NCTC
18.	<i>Bacillus pumilus</i>	16384	NCIMB 9369	NCIMB
19.	<i>Bacillus licheniformis</i>	8478	NCIMB 9375	NCIMB
20.	<i>Bacillus subtilis</i>	4112	ATCC 6633, NCTC 10400	NCTC
21.	<i>Brochothrix thermosphacta</i>	16019	NCTC 10822	NCTC
22.	<i>Citrobacter freundii</i>	40	NCTC 9750	NCTC
23.	<i>Enterobacter aerogenes</i>	15736	NCTC 10006, ATCC 13048	NCTC
24.	<i>Enterobacter cloacae</i>	1472	Dried milk	Campden
25.	<i>Enterococcus faecalis</i>	4113	NCTC 775	NCTC
26.	<i>Enterococcus faecalis</i>	16049	NCIMB 13280, ATCC 29212	NCIMB
27.	<i>Escherichia coli</i>	11017	NCTC 12241, ATCC 25922, NCIMB 12210	NCTC
28.	<i>Escherichia coli</i>	16041	Raw ground beef	Campden
29.	<i>Hafnia alvei</i>	4009	Sandwich	Food Producer
30.	<i>Klebsiella oxytoca</i>	8387	NCTC 8167	NCTC
31.	<i>Lactobacillus gasseri</i>	6804	NCIMB 13081	NCIMB

Number	Organism	Campden code	Source	Origin
32.	<i>Lactobacillus plantarum</i>	166	NCTC 6376	NCTC
33.	<i>Micrococcus luteus</i>	16258	NCTC 2665	NCTC
34.	<i>Pediococcus pentosaceus</i>	16030	Brine	Campden
35.	<i>Proteus mirabilis</i>	1588	Poultry	Campden
36.	<i>Proteus vulgaris</i>	1581	Poultry	Campden
37.	<i>Pseudomonas aeruginosa</i>	8299	NCIMB 10753	NCIMB
38.	<i>Pseudomonas fluorescens</i>	15937	NCIMB 10586	NCIMB
39.	<i>Pseudomonas fragi</i>	16050	NCTC 10689, ATCC 4973	NCTC
40.	<i>Rhodococcus equi</i>	4055	NCTC 1621	NCTC
41.	<i>Salmonella</i> Enteritidis	1004	Chicken	HPA
42.	<i>Salmonella</i> Typhimurium	1009	Milk	HPA
43.	<i>Staphylococcus aureus</i>	1216	NCTC 10655, ATCC 19095	NCTC
44.	<i>Staphylococcus aureus</i>	1224	Margarine	Campden
45.	<i>Staphylococcus aureus</i>	1227	Frozen cooked peeled prawns	HPA
46.	<i>Staphylococcus aureus</i>	4105	NCIMB 12702, ATCC 25923	NCIMB
47.	<i>Candida utilis</i>	16329	NCYC 321	NCYC
48.	<i>Saccharomyces cerevisiae</i>	15968	Spoilage: low fat natural yoghurt	Food Producer
49.	<i>Candida parapsilosis</i>	16160	Spoilage	Food Producer
50.	<i>Pichia anomala</i>	16175	Spoilage	Food Producer

ATCC = American Type Culture Collection, Manassas, USA.

HPA = Health Protection Agency Centre for Infections, Colindale, London, United Kingdom.

NCIMB = National Collection of Industrial, Marine and Food Bacteria, Aberdeen, Scotland, United Kingdom.

NCTC = National Collection of Type Cultures, Colindale, London, United Kingdom.

NCYC = National Collection of Yeast Cultures, Institute of Food Research, Colney, Norwich, United Kingdom.

Table 3
***Listeria* strains used to establish the sensitivity of the**
SwabSURE-*Listeria* kit

<i>Listeria monocytogenes</i> Campden code 6600	<i>Listeria monocytogenes</i> Campden code 1164
<i>Listeria monocytogenes</i> Campden code 6601	<i>Listeria ivanovii</i> Campden code 6599
<i>Listeria monocytogenes</i> Campden code 1100	<i>Listeria ivanovii</i> Campden code 1123
<i>Listeria monocytogenes</i> Campden code 1155	<i>Listeria ivanovii</i> Campden code 1120
<i>Listeria monocytogenes</i> Campden code 1158	<i>Listeria ivanovii</i> Campden code 1122

RESULTS

The specificity results obtained with the SwabSURE-*Listeria* kit are presented in Table 4 (inclusivity) and Table 5 (exclusivity). The test successfully detected all of the *L. monocytogenes* strains within 48h. Fifteen strains (38%) yielded an earlier (24h) positive result with at least one of the contamination levels used, although only one swab per duplicate was positive at 24h with three of these strains. The test can therefore provide an earlier indication of *Listeria monocytogenes* contamination if sufficient cells are present, although to confirm the presence of *L. monocytogenes* the test must always be incubated for the full 48h.

None of the *L. ivanovii* strains yielded positive results with the SwabSURE-*Listeria* kit after 24h. After 48h, incubation of 9 of the *L. ivanovii* strains gave positive results with the test. At the lowest contamination level 3 strains (1122, 1128 and 1129) gave a negative result with one of the duplicate swabs and one strain (1124) was negative with both swabs. Two strains of *ivanovii* (1121 and 1125) were negative at both contamination levels.

Table 4
Specificity results obtained using strains of
Listeria monocytogenes* and *Listeria ivanovii
with the SwabSURE-*Listeria* test

Number	Organism	Campden code	CFU/swab	SwabSURE- <i>Listeria</i>	
				24 h results	48 h results
1.	<i>Listeria monocytogenes</i>	6600	4.8E+02	+,+	+,+
			6.0E+01	-, -	+,+
2.	<i>Listeria monocytogenes</i>	6601	5.2E+02	+,+	+,+
			6.5E+01	-, -	+,+
3.	<i>Listeria monocytogenes</i>	1100	4.4E+02	-, -	+,+
			5.5E+01	-, -	+,+
4.	<i>Listeria monocytogenes</i>	1101	4.4E+02	+,+	+,+
			5.5E+01	-, -	+,+
5.	<i>Listeria monocytogenes</i>	1102	4.4E+02	+,+	+,+
			5.5E+01	-, -	+,+
6.	<i>Listeria monocytogenes</i>	1103	5.2E+02	+,+	+,+
			6.5E+01	-, -	+,+
7.	<i>Listeria monocytogenes</i>	1104	3.3E+01	-, -	+,+
			1.3E+01	-, -	+,+
8.	<i>Listeria monocytogenes</i>	1105	5.5E+01	+, -	+,+
			2.2E+01	-, -	+,+
9.	<i>Listeria monocytogenes</i>	1108	4.5E+01	-, -	+,+
			1.8E+01	-, -	+,+
10.	<i>Listeria monocytogenes</i>	1149	6.0E+01	-, -	+,+
			2.4E+01	-, -	+,+
11.	<i>Listeria monocytogenes</i>	1150	1.1E+01	-, -	+,+
			4.0E+00	-, -	+,+
12.	<i>Listeria monocytogenes</i>	1151	5.0E+01	-, -	+,+
			2.0E+01	-, -	+,+
13.	<i>Listeria monocytogenes</i>	1152	5.5E+01	-, -	+,+
			2.2E+01	-, -	+,+
14.	<i>Listeria monocytogenes</i>	1153	3.6E+01	+,+	+,+
			1.4E+01	-, -	+,+
15.	<i>Listeria monocytogenes</i>	1154	5.0E+01	-, -	+,+
			2.0E+01	-, -	+,+

Number	Organism	Campden code	CFU/swab	SwabSURE-Listeria	
				24 h results	48 h results
16.	<i>Listeria monocytogenes</i>	1155	4.4E+02	+,+	+,+
			5.5E+01	-,-	+,+
17.	<i>Listeria monocytogenes</i>	1157	6.0E+01	-,-	+,+
			2.4E+01	-,-	+,+
18.	<i>Listeria monocytogenes</i>	1158	2.2E+02	+,+	+,+
			2.8E+01	-,-	+,+
19.	<i>Listeria monocytogenes</i>	1160	4.6E+01	-,-	+,+
			1.8E+01	-,-	+,+
20.	<i>Listeria monocytogenes</i>	1161	8.0E+01	-,-	+,+
			3.2E+01	-,-	+,+
21.	<i>Listeria monocytogenes</i>	1162	7.0E+01	-,-	+,+
			2.8E+01	-,-	+,+
22.	<i>Listeria monocytogenes</i>	1163	4.7E+01	-,-	+,+
			1.9E+01	-,-	+,+
23.	<i>Listeria monocytogenes</i>	1164	5.2E+02	+,+	+,+
			6.5E+01	-,-	+,+
24.	<i>Listeria monocytogenes</i>	1165	7.0E+01	-,-	+,+
			2.8E+01	-,-	+,+
25.	<i>Listeria monocytogenes</i>	1166	5.5E+01	-,-	+,+
			2.2E+01	-,-	+,+
26.	<i>Listeria monocytogenes</i>	1168	2.7E+01	-,-	+,+
			1.1E+01	-,-	+,+
27.	<i>Listeria monocytogenes</i>	1170	6.5E+01	-,-	+,+
			2.6E+01	-,-	+,+
28.	<i>Listeria monocytogenes</i>	1171	4.0E+01	-,-	+,+
			1.6E+01	-,-	+,+
29.	<i>Listeria monocytogenes</i>	1172	5.0E+01	+,+	+,+
			2.0E+01	-,-	+,+
30.	<i>Listeria monocytogenes</i>	1173	6.0E+01	+, -	+,+
			2.4E+01	+, -	+,+
31.	<i>Listeria monocytogenes</i>	1174	6.5E+01	+,+	+,+
			2.6E+01	-,-	+,+
32.	<i>Listeria monocytogenes</i>	1175	7.5E+01	+,+	+,+
			3.0E+01	-,-	+,+
33.	<i>Listeria monocytogenes</i>	1176	6.5E+01	-,-	+,+
			2.6E+01	-,-	+,+
34.	<i>Listeria monocytogenes</i>	1178	6.5E+01	-,-	+,+
			2.6E+01	-,+	+,+
35.	<i>Listeria monocytogenes</i>	1179	2.5E+01	-,-	+,+
			1.0E+01	-,-	+,+

Number	Organism	Campden code	CFU/swab	SwabSURE-Listeria	
				24 h results	48 h results
36.	<i>Listeria monocytogenes</i>	1180	2.9E+01	-, -	+, +
			1.2E+01	-, -	+, +
37.	<i>Listeria monocytogenes</i>	1980	2.6E+01	-, -	+, +
			1.0 E+01	-, -	+, +
38.	<i>Listeria monocytogenes</i>	3630	3.7E+01	-, -	+, +
			1.5E+01	-, -	+, +
39.	<i>Listeria monocytogenes</i>	3650	3.4E+01	-, -	+, +
			1.3E+01	-, -	+, +
40.	<i>Listeria monocytogenes</i>	2080	4.1E+01	-, -	+, +
			1.6E+01	-, -	+, +
41.	<i>Listeria ivanovii</i>	6599	3.9E+02	-, -	+, +
			4.9E+01	-, -	+, +
42.	<i>Listeria ivanovii</i>	1123	6.5E+01	-, -	+, +
			2.6E+01	-, -	+, +
43.	<i>Listeria ivanovii</i>	1120	3.2E+01	-, -	+, +
			1.3E+01	-, -	+, +
44.	<i>Listeria ivanovii</i>	1121	5.0E+01	-, -	-, -
			2.0E+01	-, -	-, -
45.	<i>Listeria ivanovii</i>	1122	1.5E+01	-, -	+, +
			6.0E+00	-, -	-, +
46.	<i>Listeria ivanovii</i>	1124	8.0E+00	-, -	+, +
			1.0E+00	-, -	-, -
47.	<i>Listeria ivanovii</i>	1125	1.8E+01	-, -	-, -
			7.0E+00	-, -	-, -
48.	<i>Listeria ivanovii</i>	1126	4.0E+01	-, -	+, +
			1.6E+01	-, -	+, +
49.	<i>Listeria ivanovii</i>	1127	1.7E+01	-, -	+, +
			7.0E+00	-, -	+, +
50.	<i>Listeria ivanovii</i>	1128	6.0E+00	-, -	+, +
			1.0E+00	-, -	+, -
51.	<i>Listeria ivanovii</i>	1129	1.0E+00	-, -	+, -
			2.0E+00	-, -	+, -

+ = Positive (blue in colour)

- = Negative

During the assessment of the 50 non-target strains the majority (49/50), including non-pathogenic *Listeria* spp., gave negative results with the SwabSURE-Listeria kit (Table 5).

Table 5
Specificity results obtained using strains of non-target
organisms with the SwabSURE-Listeria test

Number	Organism	Campden code	CFU/swab	SwabSURE-Listeria	
				24 h results	48 h results
1.	<i>Listeria innocua</i>	1110	4.6E+03	-,-	-,-
2.	<i>Listeria innocua</i>	1111	1.8E+03	-,-	-,-
3.	<i>Listeria innocua</i>	1112	5.2E+03	-,-	-,-
4.	<i>Listeria innocua</i>	1117	3.8E+03	-,-	-,-
5.	<i>Listeria innocua</i>	6602	3.4E+03	-,-	-,-
6.	<i>Listeria welshimeri</i>	1130	2.6E+03	-,-	-,-
7.	<i>Listeria welshimeri</i>	1132	2.8E+03	-,-	-,-
8.	<i>Listeria welshimeri</i>	1134	1.3E+03	-,-	-,-
9.	<i>Listeria welshimeri</i>	1135	1.4E+03	-,-	-,-
10.	<i>Listeria seeligeri</i>	1139	1.2E+03	-,-	-,-
11.	<i>Listeria seeligeri</i>	1146	5.0E+02	-,-	-,-
12.	<i>Listeria seeligeri</i>	6603	1.3E+03	+,+	+,+
			1.3E+03	+,+	+,+
			1.3E+02	+,+	+,+
			1.3E+01	-,-	-,+
13.	<i>Listeria grayi</i>	9298	1.4E+03	-,-	-,-
14.	<i>Listeria grayi</i>	12524 A	1.1E+04	-,-	-,-
15.	<i>Listeria grayi</i>	12526 A	9.8E+02	-,-	-,-
16.	<i>Bacillus cereus</i>	1761	1.0E+02	-,-	-,-
17.	<i>Bacillus cereus</i>	4110	3.0E+02	-,-	-,-
18.	<i>Bacillus pumilus</i>	16384	7.0E+02	-,-	-,-
19.	<i>Bacillus licheniformis</i>	8478	7.0E+02	-,-	-,-
20.	<i>Bacillus subtilis</i>	4112	2.0E+02	-,-	-,-
21.	<i>Brochothrix thermosphacta</i>	16019	1.7E+04	-,-	-,-
22.	<i>Citrobacter freundii</i>	40	4.6E+03	-,-	-,-
23.	<i>Enterobacter aerogenes</i>	15736	5.0E+03	-,-	-,-
24.	<i>Enterobacter cloacae</i>	1472	8.6E+03	-,-	-,-
25.	<i>Enterococcus faecalis</i>	4113	1.5E+03	-,-	-,-
26.	<i>Enterococcus faecalis</i>	16049	1.3E+03	-,-	-,-
27.	<i>Escherichia coli</i>	11017	6.0E+03	-,-	-,-
28.	<i>Escherichia coli</i>	16041	6.6E+03	-,-	-,-
29.	<i>Hafnia alvei</i>	4009	4.0E+03	-,-	-,-
30.	<i>Klebsiella oxytoca</i>	8387	3.0E+03	-,-	-,-
31.	<i>Lactobacillus gasseri</i>	6804	1.3E+04	-,-	-,-
32.	<i>Lactobacillus plantarum</i>	166	9.0E+04	-,-	-,-
33.	<i>Micrococcus luteus</i>	16258	1.4E+04	-,-	-,-

Number	Organism	Campden code	CFU/swab	SwabSURE-Listeria	
				24 h results	48 h results
34.	<i>Pediococcus pentosaceus</i>	16030	2.6E+04	-, -	-, -
35.	<i>Proteus mirabilis</i>	1588	6.8E+03	-, -	-, -
36.	<i>Proteus vulgaris</i>	1581	9.0E+03	-, -	-, -
37.	<i>Pseudomonas aeruginosa</i>	8299	8.2E+04	-, -	-, -
38.	<i>Pseudomonas fluorescens</i>	15937	1.4E+05	-, -	-, -
39.	<i>Pseudomonas fragi</i>	16050	1.3E+05	-, -	-, -
40.	<i>Rhodococcus equi</i>	4055	2.0E+04	-, -	-, -
41.	<i>Salmonella</i> Enteritidis	1004	1.2E+04	-, -	-, -
42.	<i>Salmonella</i> Typhimurium	1009	7.0E+03	-, -	-, -
43.	<i>Staphylococcus aureus</i>	1216	5.4E+02	-, -	-, -
44.	<i>Staphylococcus aureus</i>	1224	1.5E+03	-, -	-, -
45.	<i>Staphylococcus aureus</i>	1227	1.5E+03	-, -	-, -
46.	<i>Staphylococcus aureus</i>	4105	5.2E+03	-, -	-, -
47.	<i>Candida utilis</i>	16329	3.6E+03	-, -	-, -
48.	<i>Saccharomyces cerevisiae</i>	15968	7.8E+03	-, -	-, -
49.	<i>Candida parapsilosis</i>	16160	1.8E+03	-, -	-, -
50.	<i>Pichia anomala</i>	16175	5.4E+03	-, -	-, -

+ = Positive (blue in colour)

- = Negative

The one strain from the exclusivity organisms which yielded a positive result with the kit was a strain of *L. seeligeri* (6603). Additional testing of strain 6603 was conducted using more dilute suspensions of the culture and even at the lowest concentration this strain still gave a positive result (Table 5). This result may not be consistent for all *L. seeligeri* strains as two (1139 and 1146) gave a negative result with the test.

Table 6
Sensitivity results obtained using the SwabSURE-Listeria test

Number	Organism	Campden code	CFU/swab	SwabSURE <i>Listeria</i>	
				24 h result	48 h result
1.	<i>Listeria monocytogenes</i>	6600	2.4E+05	+,+	+,+
			2.4E+04	+,+	+,+
			2.4E+03	+,+	+,+
			2.4E+01	-, -	+,+
2.	<i>Listeria monocytogenes</i>	6601	2.6E+05	+,+	+,+
			2.6E+04	+,+	+,+
			2.6E+03	+,+	+,+
			2.6E+01	-, -	+,+
3.	<i>Listeria monocytogenes</i>	1100	2.2E+05	+,+	+,+
			2.2E+04	+,+	+,+
			2.2E+03	-, -	+,+
			2.2E+01	-, -	+,+
4.	<i>Listeria monocytogenes</i>	1155	2.2E+05	+,+	+,+
			2.2E+04	+,+	+,+
			2.2E+03	+,+	+,+
			2.2E+01	-, -	+,+
5.	<i>Listeria monocytogenes</i>	1158	1.1E+05	+,+	+,+
			1.1E+04	+,+	+,+
			1.1E+03	+,+	+,+
			1.1E+01	-, -	+,+
6.	<i>Listeria monocytogenes</i>	1164	2.6E+05	+,+	+,+
			2.6E+04	+,+	+,+
			2.6E+03	+,+	+,+
			2.6E+01	-, -	+,+
7.	<i>Listeria ivanovii</i>	6599	1.9E+05	+,+	+,+
			1.9E+04	+,+	+,+
			1.9E+03	-, -	+,+
			1.9E+01	-, -	+, -
8.	<i>Listeria ivanovii</i>	1123	2.6E+04	+,+	+,+
			2.6E+03	+,+	+,+
			2.6E+02	-, -	+,+
			7.0E+00	-, -	+,+
9.	<i>Listeria ivanovii</i>	1120	1.3E+04	+,+	+,+
			1.3E+03	-, +	+,+
			1.3E+02	-, -	+,+
			3.0E+00	-, -	-, +
10.	<i>Listeria ivanovii</i>	1122	6.0E+03	+,+	+,+
			6.0E+02	-, -	+,+
			6.0E+01	-, -	+,+
			2.0E+00	-, -	-, -

+ = Positive (blue in colour)

- = Negative

The results of the sensitivity study are presented in Table 6. The test is able to yielded positive results after 48h even at the lowest contamination levels tested.

CONCLUSION

The SwabSURE-Listeria kit provides a convenient ready-to-use test to confirm the potential presence of *Listeria monocytogenes* and *L. ivanovii*. Although some strains of *Listeria monocytogenes* give a positive result with the test in 24h which could be used as an early indication of contamination, it should be checked after 48h which is when all of the strains tested gave positive results. Some strains of *L. ivanovii* may fail to give a positive result with this test and some strains of *L. seeligeri* can give rise to positive results. The test provides good sensitivity and enables contamination levels below 100 cells per swab to be detected. This study demonstrated the specificity and sensitivity of the SwabSURE-Listeria kit using pure cultures. It did not assess the affect of competing or mixed populations of microorganisms on the test or the affect of environmental debris/soil, chemical residues etc which may be encountered in food production environments.

APPENDIX

Product information and instructions provided by Technical Service Consultants Ltd.

Pathogenic *Listeria* detection kit (SwabSURE-*Listeria*)

Test system for detection of pathogenic *Listeria*
from food contact and environmental surfaces

Content:

- Foam-tip swab, pre-moistened in the neutralising buffer, polyester shaft with breakpoint at 65 mm. Swab in tube, packed in the peel pouch. Product Code: TS/5-63WP (Fig.1)
- *Listeria* detection chromogenic medium - 1 ml in tube (Fig. 2 – prototype samples).

Principle:

- Foam swab improves recovery of contaminant microorganisms from surfaces. Neutralising buffer provides inactivation of disinfectants and chemicals residue on surfaces, and it also maintains viability of sampled microorganisms.
- Detection medium contains nutrient substrates for enriched growth of *Listeria* species; antibiotics – for suppression of non-target organisms, and chromogenic compound – for detection of pathogenic *Listeria* species (*L. monocytogenes* and *L. ivanovii*). Presence of *L. monocytogenes* and/or *L. ivanovii* at the concentration of ≥ 1 cell/sample results in colour change of the medium from straw-yellow to turquoise-blue.

Target organisms: *L. monocytogenes*; *L. ivanovii*

Detection level: ≥ 1 CFU/swab (internal trials)

False positive: not detected

False negative: in the presence of biocides at very high concentration

Instruction for Use:

1. Identify the site or surface for sampling. Define sampling area (e.g. by using sterile template).
2. Peel open the pouch and remove the tube.
3. Use the blue cap as a holder, remove swab from the tube and sample defined area by rubbing the swab horizontally, vertically and then diagonally for about 20 seconds across the entire area rotating the swab between thumb and index finger to ensure that the entire surface of the swab is used.
4. When sampling is completed insert swab into the tube and close tightly using the swab cap. Ensure that tube is tightly closed.
5. Record sample details on the tube label.
7. Transport samples to the laboratory as soon as possible.
8. Holding blue cap remove sampled swab from the tube, place it into diagnostic medium tube and break it into the medium (break point on the swab makes this procedure very easy). Mix by inverting the tube or using Vortex mixer.

9. Place tubes with the sampled swabs in the rack and incubate them horizontally at 37°C for 48h. Check for colour change in 24 and 48h. Change of medium colour from straw-yellow to turquoise-blue indicates presence of *L. monocytogenes* or/and *L. ivanovii*.
10. Identity of organism could be confirmed by subculturing onto selective media and further biochemical tests.



Fig. 1. Foam swab for sampling of food-contact and environmental surfaces



Fig. 2. Chromogenic detection of pathogenic *Listeria*. Control – Abiotic control; 183-7 – 183-3 – *L. monocytogenes*, NCTC11994; concentration - 1-10000 CFU/swab; incubation – 48h 37°C).