R&D REPORT

NO. 19

An Evaluation of the Oxoid Listeria Rapid Test (Incorporating Clearview) for the Detection of *Listeria* from foods

November 1995

Campden & Chorleywood
Food Research Association

Campden & Chorleywood
Food Research Association

Director-General
Prof. C. Dennis
BSc, PhD, FIFST

Chipping Campden
Gloucestershire
GL55 6LD UK
Tel: +44 (0) 1386 842000
Fax: +44 (0) 1386 842100

R & D Report No. 19
Project No. 19593

An Evaluation of the Oxoid Listeria Rapid Test (Incorporating Clearview) for the Detection of *Listeria* from Foods

K.L. Jones, S. MacPhee, A.J. Turner and R.P. Betts

November 1995

Information emanating from this Research Association is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but is provided without liability in its application and use.

The information contained in this publication must not be reproduced without permission from the Director-General of the Association.

© CCFRA - Campden & Chorleywood Food Research Association

SUMMARY

The Oxoid Listeria Rapid Test, developed by Unipath, consists of a two step enrichment system followed by an immunoassay with a Clearview Listeria Test unit to give a presumptive result within 43h of sample receipt. Clearview Listeria is based on "dipstick technology" using an immunochromatographic technique. A monoclonal antibody raised against flagella antigen B, common to all *Listeria* species except *L.grayi* subsp. *grayi* and *L.grayi* subsp. *murrayi*, is used to identify positive samples. A visual result is obtained within 20 min after the addition of treated enriched sample to the test unit with no further manipulations required.

The Oxoid Listeria Rapid Test, incorporating Clearview Listeria, was evaluated with respect to its sensitivity, specificity and ability to detect *Listeria* from a range of inoculated foods and potentially naturally contaminated foods. All foods tested were set up in parallel with a cultural method based on the USDA enrichment procedure.

The sensitivity of the Clearview Listeria appeared to be dependent upon the serotype and strain tested. The minimum detection level of the *Listeria* strains tested ranged from 3.9×10^4 cfu/ml to 1.3×10^5 cfu/ml with the exception of *L.ivanovii* and *L.grayi* species.

The minimum detection level for *L.ivanovii* when tested with the Clearview Listeria was 1.1×10^7 cfu/ml. Work has since demonstrated that flagella formation in laboratory stock of cultures of this species can be poor or absent, thus reducing the amount of flagella antigen B available for the immunoassay. The significance of this in the detection of this species from food is uncertain.

The ten non-*Listeria* organisms tested with the Clearview Listeria did not give a positive detection, further demonstrating the specificity of the test.

With the inoculated foods tested, the Oxoid Listeria Rapid Test was comparable to the USDA based conventional method in terms of *Listeria* detection with a few exceptions; Clearview Listeria failed to detect a *L.monocytogenes* inoculated into beef and two *L.ivanovii* strains inoculated into brie, beef and pâté. The organisms were isolated from the enrichments used to inoculate the Clearview units.

When uninoculated foods were tested using the Oxoid Listeria Rapid Test, 19 of the 33 samples were found to be naturally contaminated with *Listeria.* A false negative result was obtained from one sample after *Listeria* was isolated from the enrichment used to inoculate the Clearview unit. When tested in parallel with the conventional method, *Listeria* was isolated from 22 of the 33 samples.

In operation, the Oxoid Listeria Rapid Test was found to be a simple and easy to use test for the detection of *Listeria* from foods.

CONTENTS

	Page No.
INTRODUCTION	1
MATERIALS AND METHODS	3
RESULTS AND DISCUSSION	8
CONCLUSIONS	20
REFERENCES	21

INTRODUCTION

Listeria species are widely distributed in nature, being found for example in soil, sewage, silage, healthy humans, animals and insects. They are also found in a variety of foods.

Six species of *Listeria* are recognised; *L.monocytogenes, L.innocua, L. seeligeri, L.ivanovii, L.welshimeri,* and *L.grayi* subspp. *grayi* and *murrayi*. *L.monocytogenes* is the principal pathogen in humans and animals (Lund, 1990). *L.ivanovii* is a pathogen of animals although it has been occasionally associated with human disease. *L.seeligeri* and *L.grayi* are usually considered to be avirulent, but each has been reported to cause human infection.

Listeria monocytogenes is a psychrotrophic organism that can tolerate high osmotic pressures and survive drying and freezing, and can thus survive for long periods in the environment (Lovett, 1989). Raw meats have been reported to be contaminated with high numbers of *L.monocytogenes*, as have ready to eat products, such as soft cheese and pâté (Lund, 1990). The increasing awareness of *L.monocytogenes* in foods has led to greater testing for the organism in foods, particularly in final products. Conventional cultural methods of detection, however, can take up to five days to obtain a presumptive result.

The complexity, subjectivity and long analysis time of conventional methods, combined with a requirement to analyse large numbers of samples, has led to the development of a number of rapid methods. One of the more commonly used rapid methods is the immunoassay, in particular, the enzyme linked immunosorbant assay (ELISA). ELISAs generally offer the advantages of being sensitive, specific and reducing the testing time to within 48 h for a presumptive result. Such assays, however, take approximately 2.5 h to complete due to several incubation periods, and they involve a number of washing steps. The majority of ELISAs are in microtitre plate format which means that multiple assays have to be performed at the same time.

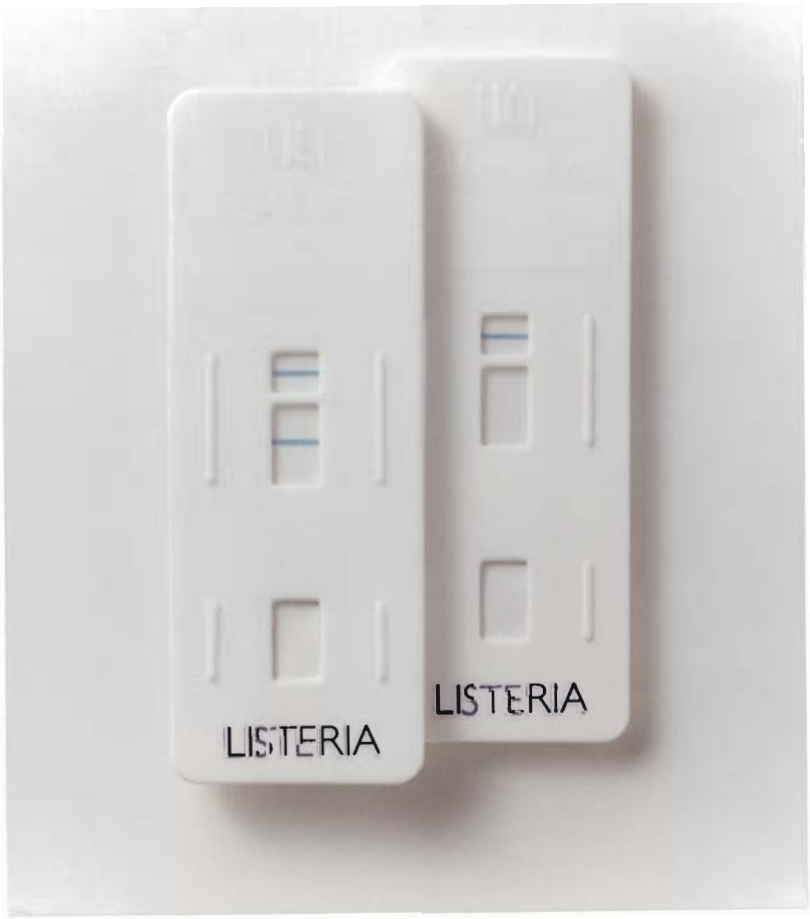
Unipath have developed the Oxoid Listeria Rapid Test incorporating the Clearview Listeria test unit which overcomes some of the limitations of traditional immunoassays. The Oxoid Listeria Rapid Test consists of a two step enrichment system followed by an immunoassay with a Clearview Listeria test unit to give a presumptive result within 43 h of sample receipt. The Clearview Listeria test unit is based on dipstick technology using immunochromatographic techniques (Figure 1). Each Clearview unit consists of a membrane strip on which a line of antibody specific to *Listeria* flagella antigen B, which is common to all species except *L.grayi* subspp. *grayi* and *murrayi*, is immobilised. More *Listeria*-specific antibody, bound to coloured latex particles, is run up the test strip by capillary action in the presence of the test sample. If *Listeria* antigen is present in the

sample, it forms a sandwich with the latex-labelled and immobilised antibodies, resulting in a blue line which is clearly visible as a positive result within 20 min. A second line of non-specific antibody is immobilised further up the test strip. This binds excess latex-labelled antibody, whether the antigen is present or not, and thus provides a control for the Clearview Listeria test unit.

The Oxoid Listeria Rapid Test, incorporating Clearview Listeria test unit, was evaluated with respect to its sensitivity, specificity and ability to detect *Listeria* from a range of inoculated foods and potentially naturally contaminated foods. All foods tested were set up in parallel with a cultural method based on the USDA enrichment procedure.

FIGURE 1

CLEARVIEW LISTERIA UNITS
(Left hand side, positive detection; right-hand side
Listeria not detected)



MATERIALS AND METHODS

Microorganisms

The organisms were obtained from the CCFRA Culture Collection.

Media and Test Kits

The Clearview Listeria test kits were supplied by Unipath. Media and reagents used in conjunction with the Oxoid Listeria Rapid Test were prepared following instructions detailed in the Listeria Rapid Test protocol.

Foods

All foods were either purchased from local retail outlets or obtained from CCFRA stocks and stored at 4°C pending analysis.

Test Protocols

i. *Listeria* Rapid Test

The enrichment and test procedure used was that recommended by Unipath for the Oxoid Listeria Rapid Test.

Food samples (25g) were enriched in 225ml Fraser Broth (Oxoid, CM895) with half strength supplement (SR 166M) (1/2 FB) and incubated at 30°C for 21-24h (Figure 2). After incubation, 0.1ml pre-enrichment broth was transferred into 10ml Buffered *Listeria* Enrichment Broth (BLEB) (Oxoid, CM897 + SR141E) and the selective broth incubated at 30°C for 21-24h. BLEB (2ml) was then transferred to a glass bottle and placed in a water bath at 80°C for 20 min. After cooling to room temperature, 135μl of the broth was added to the test window of the Clearview Listeria unit. The Clearview unit was incubated at room temperature for 20 min to allow colour development. Positive and negative samples were confirmed by sub-culture of the BLEB on modified *Listeria* selective medium (Oxford formulation) (Oxoid CM565+SR140). Plates were incubated at 30°C for 24h and 48h and presumptive positive colonies confirmed.

ii. Conventional Procedure

The enrichment procedure detailed in the USDA method was followed (Figure 3). Samples (25g) were enriched in 225ml UVM I (Oxoid, CM863 + SR142) and incubated at 30°C for 20-24h. After enrichment, 0.1ml of UVM I was transferred into 10ml Fraser Broth (FB, Oxoid CM 895 + SR156) and incubated at 35°C for 24h + 48h. FB cultures were streaked onto Oxford agar and incubated at 30°C for 24h + 48h. Presumptive positive colonies were taken for confirmation.

Sensitivity

Twelve *Listeria* spp. were inoculated into BLEB and incubated at 30°C for 21h-24h (Table 1). The cultures were serially diluted in BLEB to levels between 10^2 to 10^9 cfu/ml (as determined by plate counts on Tryptone Soya Agar (TSA, Oxoid CM131) and heated in a water bath at 80°C for 20 min. All the dilutions were tested with Clearview Listeria units following the manufacturer's instructions.

Exclusivity

Ten potentially cross reacting or competitive microorganisms were tested using the Clearview Listeria (Table 2). All the organisms were inoculated into BLEB (no supplement) and incubated at 30°C for 21-24h. Each culture was diluted 1:10 in BLEB (to a level greater than 10^7 cfu/ml) and heated in a waterbath at 80°C for 20 min. All the diluted BLEB cultures were tested with Clearview Listeria units following the manufacturer's instructions.

Inoculated Foods

Two strains of each *Listeria* spp. (except *L.grayi*) were inoculated individually into three food types (brie, beef steak and pâté) at a low level (<50 cfu/25g). Each inoculated food sample was enriched using the procedure recommended by Unipath as detailed in Figure 2 and tested with Clearview Listeria units. All foods were set up in parallel with the conventional method based on the USDA enrichment procedure as shown in Figure 3.

Characteristic colonies on Oxford plates were streaked onto TSA and further tested by a Gram stain, a catalase test, an oxidase test and motility.

Potentially Naturally Contaminated Foods

Foods likely to be naturally contaminated, or previously shown to contain *Listeria*, were tested by the Oxoid Rapid Listeria Test and by the conventional procedure. Thirty three samples, listed in Table 4, were set up according to the enrichment procedures detailed in Figures 2 and 3 and analysed.

Presumptive positive results on Oxford agar from both methods were confirmed by Gram stain, motility test, haemolysis test and biochemical reactions (Micro ID, Organon Teknika).

FIGURE 2

OXOID LISTERIA RAPID TEST PROTOCOL

25g sample + 225ml ½ FB

or

25g sample (containing <50 cfu *Listeria*/25g) + 225ml ½ FB

|

↓ 30°C/21-24h

0.1ml ½ FB into 10ml BLEB

|

↓ 30°C/21-24h

2ml BLEB 80°C/20min

|

↓

Clearview Listeria

½ FB = Fraser Broth (+ half strength supplement)

BLEB = Buffered *Listeria* Enrichment Broth (+ supplement)

FIGURE 3

USDA ENRICHMENT PROCEDURE

25g sample + 225ml UVM I

or

25g sample (containing <50 *Listeria*/25g) + 225ml UVM I

↓ 30°C/20-24h

0.1ml UVM I into 10ml FB

↓ 35°C/24h + 48h

Streak onto Oxford agar

↓ 30°C/24h-48h

Confirmatory tests on characteristic colonies

UVM I = *Listeria* Enrichment Broth Base (+ supplement)
FB = Fraser Broth (+ supplement)

RESULTS AND DISCUSSION

Sensitivity

The sensitivity of the Clearview Listeria test was determined by testing at cell concentrations between 10^2 - 10^9 cfu/ml. The minimum detection level appeared to be dependent on the serotype and strain tested. The sensitivity of the six strains of *L.monocytogenes* tested ranged from 3.9 x 10^4 cfu/ml to 1.3 x 10^5 cfu/ml (Table 1). For *L.innocua*, the sensitivity was found to be 1.2 x 10^5 cfu/ml, whilst for *L.welshimeri* the sensitivity was 1.1 x 10^5 cfu/ml.

With *L.ivanovii*, the minimum number of cells needed to give a positive result with the Clearview Listeria test unit was 1.1 x 10^7 cfu/ml. In tests with a second strain of *L.ivanovii*, the Clearview unit failed to detect the presence of the organism despite it being confirmed from the BLEB enrichment. Thus, *L.ivanovii* was not detected, even at a level of 6.6 x 10^8 cfu/ml. Work carried out by Unipath on these two strains of *Listeria* has since demonstrated that flagella formation is poor in cells grown in both BLEB base and BLEB at 30°C, thus reducing the amount of B flagella antigen available for the Clearview immunoassay. The significance of this in the detection of the species from food is uncertain. *Listeria grayi* subspp. *grayi* and *murrayi* were not detected by Clearview Listeria test at a level of 1.1 x 10^7 cfu/ml and 1.2 x 10^9 cfu/ml respectively due to the absence of the target flagella antigen, as discussed in the kit instructions.

The Clearview Listeria test thus demonstrated a wide spectrum of sensitivities ranging from 1.1 x 10^7 cfu/ml down to 3.9 x 10^4 cfu/ml. Generally, the sensitivity of the Clearview for the strains tested was comparable to sensitivities reported for other commercial 'rapid' methods such as the ELISA (Betts, 1992).

Exclusivity

Ten non-*Listeria* strains were tested using the Clearview Listeria to test if the device was able to detect other microorganisms that might be expected to survive the enrichment system (Table 2). None of the organisms tested, at a level above 10^7 cfu/ml, cross reacted with the Clearview Listeria test unit, demonstrating the specificity of the test.

Inoculated Foods

In order to test the combined enrichment and detection system, a range of foods were inoculated with low levels of *Listeria* (<50 cfu/25g). Eleven strains of *Listeria* were individually inoculated into brie, beef and pâté, enriched using the Oxoid Listeria Rapid

TABLE 1

SENSITIVITY OF THE CLEARVIEW LISTERIA TEST

Organism (CRA Code)	Viable Count (cfu/ml)	Clearview Result
Listeria monocytogenes 1/2a (1100)	7.2×10^7	+
	7.2×10^6	+
	7.2×10^5	+
	7.2×10^4	+
	7.2×10^3	-
	7.2×10^2	-
L.monocytogenes 1/2a (1101)	1.3×10^7	+
	1.3×10^6	+
	1.3×10^5	+
	1.3×10^4	-
	1.3×10^3	-
	1.3×10^2	-
L.monocytogenes 1/2b (1107)	3.9×10^7	+
	3.9×10^6	+
	3.9×10^5	+
	3.9×10^4	+
	3.9×10^3	-
	3.9×10^2	-
L.monocytogenes 1/2b (1108)	1.1×10^7	+
	1.1×10^6	+
	1.1×10^5	+
	1.1×10^4	-
	1.1×10^3	-
	1.1×10^2	-
L.monocytogenes 4b (1179)	4.6×10^7	+
	4.6×10^6	+
	4.6×10^5	+
	4.6×10^4	+
	4.6×10^3	-
	4.6×10^2	-

TABLE 1 (continued)

Organism (CRA Code)	Viable Count (cfu/ml)	Clearview Result
L.monocytogenes 4b (1180)	9.1×10^7	+
	9.1×10^6	+
	9.1×10^5	+
	9.1×10^4	+
	9.1×10^3	-
	9.1×10^2	-
L.innocua (1110)	1.2×10^7	+
	1.2×10^6	+
	1.2×10^5	+
	1.2×10^4	-
	1.2×10^3	-
	1.2×10^2	-
L.ivanovii (1120)	1.1×10^7	+
	1.1×10^6	-
	1.1×10^5	-
	1.1×10^4	-
	1.1×10^3	-
	1.1×10^2	-
L.ivanovii (426)	6.6×10^8	-
	6.6×10^7	-
	6.6×10^6	-
	6.6×10^5	-
	6.6×10^4	-
	6.6×10^3	-
L.welshimeri (1137)	1.1×10^7	+
	1.1×10^6	+
	1.1×10^5	+
	1.1×10^4	-
	1.1×10^3	-
	1.1×10^2	-
L.grayi subsp. *grayi* (1617)	1.3×10^7	-
	1.3×10^6	-
	1.3×10^5	-
	1.3×10^4	-
	1.3×10^3	-
	1.3×10^2	-
L.grayi subsp. *murrayi* (1611)	1.2×10^9	-
	1.2×10^8	-
	1.2×10^7	-
	1.2×10^6	-
	1.2×10^5	-
	1.2×10^4	-

TABLE 2

EXCLUSIVITY OF THE CLEARVIEW LISTERIA TEST

Organisms (CRA Code)	Viable Count (cfu/ml)	Clearview Result
Bacillus cereus (666)	1.6×10^7	-
B.subtilis (4112)	1.8×10^7	-
Staphylococcus aureus (4105)	8.0×10^8	-
Enterococcus faecalis (4113)	1.1×10^9	-
Brochothrix thermosphacta (1612)	5.3×10^7	-
Kurthia zopfii (3186)	8.0×10^7	-
Erysipelothrix rhusiopathiae (2069)	2.1×10^8	-
Escherichia coli (2074)	7.4×10^8	-
Salmonella typhimurium (4976)	4.2×10^8	-
Enterobacter aerogenes (4187)	1.2×10^9	-

Test protocol (Figure 1) and tested using the Clearview Listeria (Table 3). Samples were set up in parallel with the conventional procedure outlined in Figure 2.

Three strains of *L.innocua*, two strains of *L.welshimeri* and two strains of *L.seeligeri* inoculated into brie, beef and pâté were all detected using the Clearview Listeria test unit after enrichment following the Oxoid *Listeria* Rapid Test protocol. The presence of the organism was confirmed from the BLEB selective broth. *Listeria* was isolated from all of the corresponding inoculated samples enriched following the USDA based procedure.

L.monocytogenes 4b inoculated into brie and pâté at a level of 14 cfu/25g were both detected using the Clearview Listeria test unit, although the positive detection from the brie was weak. *Listeria* was isolated from the Oxford plates after selective enrichment, confirming the Clearview results. *Listeria* was also isolated after enrichment following the USDA conventional procedure. *Listeria* was not detected by the Clearview Listeria test unit in beef inoculated with 20 cfu *L.monocytogenes* 4b per 25g and enriched using the Oxoid Listeria Rapid Test protocol. The organism was, however, confirmed as being present in the BLEB selective enrichment, and therefore the Clearview Listeria test unit gave a false negative result. *Listeria* was isolated by the USDA method when *L.monocytogenes* 4b was inoculated into beef.

Listeria monocytogenes 1/2a inoculated into brie and pâté at a level of 13 cfu/25g was detected by the Clearview Listeria test unit device after enrichment and the organism was confirmed as being present in the BLEB. *Listeria* was also isolated from the FB after enrichment based on the USDA procedure. *L.monocytogenes* 1/2a inoculated into beef, however, was not detected by the Clearview Listeria test unit. The organism was not isolated from the BLEB enrichment, confirming the negative result by the Clearview Listeria test unit. When the inoculated beef was set up using the USDA enrichment procedure, *Listeria* was isolated and thus a positive detection by the conventional method occurred compared to a negative detection using the Oxoid Listeria Rapid Test procedure. It is worth noting that the two results are not directly comparable since the different enrichment systems require different samples of the food to be inoculated and enriched. When the samples are inoculated with very low cell concentrations, it cannot be guaranteed that each sample will contain the same number of cells.

For the two strains of *L.ivanovii* inoculated into brie, beef & pâté, the Clearview Listeria test units did not detect *Listeria* in any of the foods despite the *Listeria* being confirmed from the BLEB selective enrichment broths and thus false negative detections occurred. The minimum detection levels of *L.ivanovii* (CRA 1120), from the sensitivity work, was

TABLE 3

DETECTION OF *LISTERIA* FROM INOCULATED FOODS

Organism	Food Type	Inoculum Level (cfu/25g)	Clearview Result	Confirmation of Clearview Result	USDA
L.monocytogenes 4b (1179)	Brie	14	+ (w)	+	+
L.monocytogenes 4b (1179)	Beef	20	-	+	+
L.monocytogenes 4b (1179)	Pâté	14	+	+	+
L.monocytogenes 1/2a(1100)	Brie	13	+	+	+
L.monocytogenes 1/2a(1100)	Beef	20	-	-	+
L.monocytogenes 1/2a(1100)	Pâté	13	+	+	+
L.innocua (1110)	Brie	19	+	+	+
L.innocua (1110)	Beef	20	+	+	+
L.innocua (1110)	Pâté	19	+	+	+
L.innocua (1599)	Brie	20	+	+	+
L.innocua (1599)	Beef	16	+	+	+
L.innocua (1599)	Pâté	20	+	+	+
L.innocua (3538)	Brie	8	+	+	+
L.innocua (3538)	Beef	8	+	+	+
L.innocua (3538)	Pâte	8	+	+	+
L.ivanovii (1120)	Brie	17	-	+	-
L.ivanovii (1120)	Beef	18	-	+	+
L.ivanovii (1120)	Pâté	17	-	+	+
L.ivanovii (426)	Brie	30	-	+	-
L.ivanovii (426)	Beef	34	-	+	+
L.ivanovii (426)	Pâté	20	-	+	-
L.ivanovii (426)	Pâté(repeat)	29	-	+	-
L.welshimeri (1135)	Brie	14	+	+	+
L.welshimeri (1135)	Beef	20	+	+	+
L.welshimeri (1135)	Pâté	14	+	+	+
L.welshimeri (1137)	Brie	19	+	+	+
L.welshimeri (1137)	Beef	18	+	+	+
L.welshimeri (1137)	Pâté	19	+	+	+

TABLE 3 (continued)

Organism	Food Type	Inoculum Level (cfu/25g)	Clearview Result	Confirmation of Clearview Result	USDA
L.seeligeri (1148)	Brie	20	+	+	+
L.seeligeri (1148)	Beef	22	+	+	+
L.seeligeri (1148)	Pâte	20	+	+	+
L.seeligeri (1147)	Brie	18	+	+	+
L.seeligeri (1147)	Beef	15	+	+	+
L.seeligeri (1147)	Pâte	18	+	+	+
L.murrayi (1611)	Brie	7	-	-	-
L.murrayi (1611)	Beef	14	-	+	+
L.murrayi (1611)	Pâte	7	-	-	-
Control	Brie	0	-	-	-
Control	Beef	0	-	-	-
Control	Pâte	0	-	-	-
Control	Brie (repeat)	0	-	-	-
Control	Pâte (repeat)	0	-	-	-

shown to be 1.1 x 10^7 cfu/ml (Table 1). It is possible, therefore, that either the organism was at too low a level or flagella production was not sufficient to give a positive detection by the Clearview Listeria. Due to the false negative results obtained by the Clearview test with foods inoculated with *L.ivanovii* (CRA 426), the sensitivity of the organism was investigated. It was found that the Clearview Listeria device did not give a positive detection, despite being tested with a cell concentration of up to 6.6 x 10^8 cfu/ml (Table 1). In the corresponding samples enriched by the USDA based procedure, *Listeria* was isolated in all but three inoculated samples (strain 1120 in brie, strain 426 in brie, strain 426 in pâté). *Listeria ivanovii* CRA 426 inoculated into pâté was repeated but the organism was again not detected by the USDA conventional method.

With *L.grayi* subsp. *murrayi*, inoculated into beef and tested using the Oxoid Listeria Rapid Test protocol, the Clearview Listeria test unit did not give a positive detection as expected. The organism was detected in the inoculated beef enriched using the conventional procedure.

Naturally Contaminated Foods

A total of 33 potentially naturally contaminated foods were tested by the Oxoid Rapid Listeria Test, incorporating Clearview Listeria, in parallel with the conventional method based on the USDA enrichment method. The foods tested included cheese, raw meat, processed meat, sandwiches, a cooked ready meal, cake and salad (Table 4).

Of the four cheese samples tested, *Listeria* was not detected by the Clearview *Listeria* test unit after enrichment and the organism was not isolated from the selective broth. When set up using the USDA based method, *L.innocua* was isolated from one of the four samples. The most likely explanation for difference between the two methods is the distribution of the organisms with respect to sampling. Different samples of the food product are used for the two methods and so it is possible for the sample used in one method to be contaminated, to give a positive result, whilst the other is not and will give a negative one. The difference could also be attributed to improved isolation using the enrichment based on the USDA protocol.

Three of the eleven ham samples tested were negative using Clearview Listeria, and the organism was not isolated from the BLEB selective enrichment. *Listeria* was not isolated from the corresponding samples tested using the conventional method.

TABLE 4

DETECTION OF *LISTERIA* FROM POTENTIALLY NATURALLY CONTAMINATED FOODS

Food Type	Clearview Result	Confirmation of Clearview Result	USDA
Double Gloucester Cheese	-	-	*L.innocua*
Double Gloucester Cheese	-	-	-
Stilton Cheese	-	-	-
Brie	-	-	-
Ham	+	*L.seeligeri*	*L.monocytogenes*
Ham	+	*L.seeligeri*	*L.seeligeri*
Ham	+	*L.mono/seeligeri*	*L.seeligeri*
Ham	-	-	-
Ham	+	*L.seeligeri*	*L.seeligeri*
Ham	+	*L.seeligeri*	*L.seeligeri*
Ham	+	*L.seeligeri*	*L.seeligeri*
Ham	-	-	-
Ham	-	-	-
Ham	+	*L.seeligeri*	*L.seeligeri*
Ham	+	*L.seeligeri*	*L.seeligeri*
Ham Sandwich	-	-	-
Ham Sandwich	-	-	*L.innocua*
Minced Beef	+	*L.innocua*	*L.innocua*
Minced Beef	+	*L.innocua*	*L.innocua*
Minced Beef	+	*L.innocua*	*L.grayi/murrayi*
Minced Beef	+	*L.innocua*	*L.innocua*
Minced Beef	+	*L.monocytogenes*	*L.innocua*
Minced Beef	+	*L.innocua*	*L.innocua*
Minced Beef	+	*L.monocytogenes*	*L.innocua*
Minced Beef	+	*L.innocua*	*L.innɔcua*
Minced Beef	+	*L.innocua*	*L.innocua*

TABLE 4 (continued)

Food Type	Clearview Result	Confirmation of Clearview Result	USDA
Cooked Chicken	-	*L.monocytogenes*	*L.monocytogenes*
Cooked Chicken	+	*L.monocytogenes*	*L.monocytogenes*
Cannelloni	+	*L.monocytogenes*	*L.monocytogenes*
Cream Cake	-	-	-
Coleslaw	-	-	-
Salad	-	-	-
Tortellini Salad	-	-	-

In the remaining eight ham samples tested, the Clearview devices gave a positive detection. When sub-cultured from the selective broth, *L.seeligeri* was isolated from six of the samples. *Listeria seeligeri* was also isolated from the six corresponding samples set up by the USDA enrichment procedure.

In one ham sample, the Clearview Listeria test unit detected the presence of *Listeria* and the organism was subsequently confirmed and identified from the BLEB as either *L.monocytogenes* or *L.seeligeri*. *L.seeligeri* was isolated from the corresponding sample set up by the conventional method. In the remaining ham sample, the Clearview Listeria test unit gave a positive detection after testing using the Oxoid Listeria Rapid Test protocol and the organism was isolated and confirmed as *L.seeligeri*. When the ham sample was set up using the method based on the USDA enrichment procedure, *L.monocytogenes* was isolated from the ham. Thus, there were at least two species of *Listeria* present in the sample.

Clearview Listeria test units did not detect *Listeria* in the two ham sandwich samples tested and the organism was not detected in the selective broth. Using the USDA based enrichment method, however, *L.innocua* was isolated from one of the samples. The discrepancy between the Oxoid Listeria Rapid Test and the conventional procedure could have been due to sample distribution or the differences in enrichments as previously discussed.

Clearview Listeria test units detected nine minced beef samples positive for *Listeria* after enrichment using the recommended protocol. Seven of these samples were confirmed as *L.innocua* from the BLEB enrichment. Of the corresponding seven samples set up by the USDA enrichment procedure, *L.innocua* was isolated from six of the minced beef samples and *L.grayi* subspp. *grayi* or *murrayi* was isolated from the seventh sample showing that more than one *Listeria* species was present in the sample.

In the remaining two positive minced beef samples set up following the Oxoid Listeria Rapid Test protocol and detected by Clearview Listeria test units, *L.monocytogenes* was identified from the BLEB enrichments. In the corresponding samples set up following the USDA enrichment procedure, however, *L.innocua* was identified.

A further positive detection was obtained from one of the two cooked chicken samples enriched following the Oxoid Listeria Rapid Test procedure and tested using the Clearview Listeria test units. When the two sample BLEB enrichments were plated onto Oxford agar, however, *L.monocytogenes* was isolated from both samples. Thus the Clearview Listeria test unit gave a negative detection despite *Listeria* being isolated from the sample

enrichment. *L.monocytogenes* was isolated from the corresponding cooked chicken sample set up by the conventional method.

A cannelloni sample tested with the Clearview Listeria test unit after enrichment was positive for the *Listeria* and *L.monocytogenes* was isolated from the BLEB selective enrichment, confirming the positive result. *L.monocytogenes* was also isolated and identified from the cannelloni sample enriched following the USDA enrichment procedure.

Listeria was not detected from the remaining four samples (a cream cake, coleslaw, salad and tortellini salad) set up and tested using the Clearview Listeria test unit. The organism was not isolated from the selective enrichment used prior to testing with the Clearview device or from samples set up by the conventional procedure.

CONCLUSION

The work carried out using pure cultures of *Listeria* demonstrated the sensitivity of the Clearview Listeria test unit. The minimum detection level appeared to be dependent upon the strain tested. The minimum detection level for the *Listeria* strains tested ranged from 3.9×10^4 cfu/ml to $1,3 \times 10^5$ cfu/ml with the exception of *L.ivanovii* and *L.grayi* subspp. *grayi* or *murrayi*

The Clearview Listeria test unit did not give a positive result with the ten non-*Listeria* strains tested further demonstrating the specificity of the test.

With the inoculated foods tested, the Oxoid Listeria Rapid Test was comparable to the USDA based method in terms of *Listeria* detection, with a few exceptions. The Clearview Listeria test unit failed to detect one *L.monocytogenes* inoculated into beef and two *L.ivanovii* inoculated into brie, beef and pâté, despite *Listeria* being isolated from the BLEB enrichment used to inoculate the device. The non-detections using the Clearview Listeria test unit with these samples could have been due to the *Listeria* not being at high enough levels to give a positive detection. In the case of *L.ivanovii*, flagella formation may have been poor or absent, thus reducing the amount of flagella antigen B available for the immunoassay.

When tested using naturally contaminated foods, the Oxoid Listeria Rapid Test was comparable to the USDA method in terms of *Listeria* detection. The Oxoid method detected 19 samples positive for *Listeria*. An additional sample was found to contain *Listeria*, however, when the Oxoid Rapid Listeria Test selective enrichments were plated for confirmation. It is likely that the organism, identified as *L.monocytogenes*, was not at

a level high enough to give a positive detection when tested with the Clearview test unit. When tested in parallel to the conventional method, *Listeria* spp. were isolated from 22 samples. The difference between the methods is presumed to be due to the sample variation as mentioned previously.

In operation, the Oxoid Listeria Rapid Test was found to be a simple and easy to use test for the detection of *Listeria* from foods. The Clearview device gave a definitive result in just twenty minutes after inoculation with the sample enrichment broth.

REFERENCES

Betts, R.P. (1992). Conventional and rapid analytical microbiology. In "Chilled Foods: A Comprehensive Guide". C. Dennis and M.F. Stringer (eds). Ellis-Horwood, 197-228.

Lovett, J. (1989) *Listeria monocytogenes.* In 'Foodborne Bacterial Pathogens' M.P. Doyle (Ed). Marcel Dekker Inc., 284-306.

Lund, B.M. (1990). The prevention of foodborne listeriosis. British Food Journal 92 (4) 13-22.