

Evaluation of self-contained test for *Listeria monocytogenes*

A. Jordan, V. Cutler & D. J. Shedden

IFT
**International Food Safety and Quality
Conference & Expo**

November 5-7
**2003-10-27 Orange County
Convention Centre**
Orlando FL, U.S.A.

Medical Wire & Equipment, Corsham,
Wiltshire SN13 9RT, England
E-mail: info@mwe.co.uk
Fax: +44 (0) 1225 810153



 **MW&E**

Abstract

Listeria monocytogenes is a life-threatening foodborne pathogen causing 500 deaths annually in the United States, many preventable by proper controls at production sites. It poses a particular threat in that it is capable of multiplying at low temperatures, and so refrigeration can never be a complete safeguard. Many manufacturers are now required to test for *L. monocytogenes*, not only in finished product, but on surfaces within food production facilities. Normally, samples are sent to a laboratory, often remote from the production facility, and with conventional methods it may be several days before results are available and any recalls or remedial action instigated. By this time the product may well have been sold and consumed.

The Listeria Isolation Transwab® (LIT) is a self-contained swab device for the detection of *L. monocytogenes*. The device consists of a swab and a tube of indicator gel. The swab is rubbed over the test surface, placed into the tube and incubated at 37°C. A portable incubator would be suitable. *L. monocytogenes* is indicated by the gel turning from its original straw colour to black after overnight incubation.

A study was designed to determine the detection limit for *L. monocytogenes* in a simulated food manufacturing situation, and to assess the specificity for *Listeria* spp. relative to other environmental organisms.

Food-grade stainless steel plates were inoculated with known numbers of *L. monocytogenes* organisms, and dried before sampling by LIT. The inoculated LIT tubes were incubated for up to 48 hours, and the presence or absence of detectable colour change recorded at 24 hours and 48 hours. At 24 hours, LIT showed a visible colour change for as few as 57 organisms in the initial inoculum, while at 48 hours, an inoculum of less than 10 organisms was detectable.

Specificity was tested by direct inoculation of LIT swabs with serial dilutions of various organisms. The results are presented showing LIT to be highly sensitive for *L. monocytogenes*, while also being sensitive to *L. ivanovii* and *L. innocua*, which, though less common, are also potential food pathogens. No colour change was detected at 24 hours or 48 hours for any of the other organisms, including *S. aureus*, *E. coli*, and *S. faecalis*.

The results show LIT to be sensitive and specific as a method of detecting contamination of food processing areas with *L. monocytogenes* and *Listeria* spp. at an early stage and preventing contaminated food reaching the consumer. It could thus be suitable as a component of a HACCP programme. Any positive swabs could be sent to the laboratory for further investigation, typing, etc.

Background

Listeria monocytogenes is a life-threatening foodborne pathogen¹ causing 500 deaths annually in the United States², many preventable by proper controls at production sites. It poses a particular threat in that it is capable of multiplying at low temperatures, and so refrigeration can never be a complete safeguard. Many manufacturers are now required to test for *L. monocytogenes*, not only in finished product, but on surfaces within food production facilities. Normally, samples are sent to a laboratory, often remote from the production facility, and with conventional methods it may be several days before results are available and any recalls or remedial action instigated. By this time the product may well have been sold and widely distributed among the population, presenting a real threat to the consumer. Recall would be required, but would be prohibitively expensive.

It would be helpful if manufacturers had access to an 'early warning system' which would give confidence that the product is safe, or allow potentially contaminated food to be withdrawn before sale. Such results could still be confirmed by the conventional but slower methods.

The Listeria Isolation Transwab® (LIT) is a self-contained swab device for the detection of *L. monocytogenes*. The device consists of a swab and a tube of indicator gel. The swab is rubbed over the test surface, placed into the tube and incubated at 37°C. *L. monocytogenes* is indicated by the gel turning from its original straw colour to black after incubation overnight or for up to 48 hours.

Methods

If such a test method is to be used, and acceptable to regulators and retailers, there requires to be confidence in its sensitivity and specificity.

A two-part study was designed :

1. To determine the sensitivity and specificity of LIT for *Listeria* spp. relative to other environmental organisms.
2. To assess its ability to detect *L. monocytogenes* in a simulated food manufacturing situation.

Organisms

Serial dilutions of the following organisms were prepared: *L. monocytogenes* NCTC 5214, *L. innocua* NCTC 11288, *L. grayii* NCTC 10815, *S. aureus* NCTC 8532, *Lactobacillus delbruekii* NCTC 12712, *E. faecalis*.

The organisms were first grown up on blood agar for 24 hours, collected by inoculating loop, and used to prepare an 0.5 Macfarland suspension in saline (10⁸ cfu ml⁻¹) Serial dilutions were prepared by taking 1ml of suspension, adding to 10ml saline and mixing. Dilutions of 10⁶, 10⁵, 10⁴, 10³, 10², and 10¹ cfu ml⁻¹ were prepared in this way. All the dilutions were used for the sensitivity and specificity tests, while only the 10⁵ and 10³ cfu ml⁻¹ were used for the simulated contamination study.

Sensitivity and Specificity

1. Serial dilutions of each bacterium are made, these are 10⁶ down to 10¹ cfu ml⁻¹.
2. Replicate LIT swabs are inoculated for each dilution.
3. Each swab is inoculated with 100ml of one of the above dilutions, and then pushed home into the gel in its transport tube.
4. Control plates are also set up for each dilution using a sterile swab inoculated with 100µl of each dilution and plating blood agar.
5. The LIT swabs and control plates are incubated at 37°C for 24hrs.
6. After this time the level of growth on the control plates is recorded.
7. The tubes are looked at and any colour change is recorded.
8. No change is designated by '1', a change from straw to black around the swab bud is designated '2', while the whole medium turning black is designated by '3'.
9. The LIT swabs are incubated for a further 24 hours, after which any further colour changes are noted as above.

Simulated Contamination

(Detection on Stainless Steel)

Four large sheets (100cm x 50cm) of food grade stainless steel were marked with 10 cm x 10 cm squares, with 18 squares on each sheet.

Suspensions of *Listeria monocytogenes* NCTC5214 were prepared as indicated. Dilutions containing 10⁵ cfu per ml and 10³ cfu per ml were selected for this experiment. The squares were inoculated by pipetting 0.1ml of the suspension onto the square, and spreading with an applicator. The maximum loads were thus 100cfu cm⁻² and 1 cfu cm⁻², representative of low level contamination.

The plates were allowed to dry in air before sampling with Listeria Isolation Transwab®. For each dilution, one swab was used for each square.

After sampling, the Listeria Isolation Transwabs® were incubated at 37°C for 48 hours, after which they were inspected for a colour reaction.

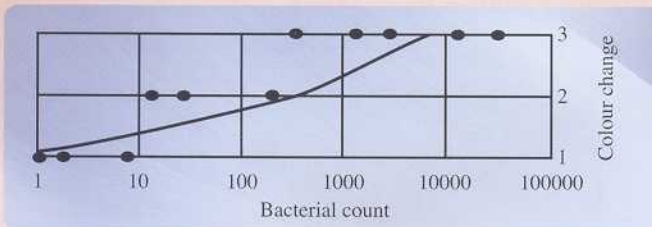
Results

Sensitivity and Specificity Colour Change Categories

- 1 = No change
- 2 = Black precipitate visible around swab bud
- 3 = Medium completely black



Detection of *Listeria* within 24 hours



Discussion

The scope of this study has been limited, but it can be seen that the Listeria Isolation Transwab® is capable of detecting low numbers of *Listeria monocytogenes*, and of recovering them even after drying on a stainless steel surface, such as could be encountered in many food processing environments. A regression analysis of the sensitivity results at 24 hours showed that as few as 57 organisms could be detectable at that stage. Further incubation to 36 or 48 hours as recommended by the manufacturer will result in even single figure numbers of cfu being detectable. It is important to detect even these low numbers because of listeria's special ability to multiply at low temperatures and become a threat within the normal shelf-life of many chilled foods.

The tests using other organisms show that LIT is specific for *Listeria monocytogenes*. A more extensive study of 31 strains of various species of commonly encountered Gram-positive and Gram-negative organisms showed no false positive reactions with LIT³. Other studies are currently ongoing into possible chemically caused false positives.

Conclusion

The results show LIT to be sensitive and specific for *Listeria monocytogenes*, and effective in detecting contamination with Listeria in food processing areas at an early stage and preventing contaminated food reaching the consumer. It is self-contained, and the incubation stage could be carried out in a small portable incubator. It could thus be suitable as a component of a HACCP programme. Any positive swabs could be sent to an outside laboratory for further investigation, typing, etc, and in the meantime the batch of food product could be quarantined.

References

- Anon. (1988), Foodborne Listeriosis. Report of a WHO Informal Working Group. *Bulletin of the World Health Organisation*, 66, 421-428
- FSIS (2000), Revised Action Plan for Control of *Listeria monocytogenes* for the Prevention of Foodborne Listeriosis.
- Turner, R. (1994), A study of MW570 Listeria Transwab, Personal Communication, Abtek Biologicals Ltd., Liverpool, UK

ORGANISM

24 HOURS

Dilution (cfu per ml)	10 ⁶	10 ⁵	10 ⁴	10 ³	10 ²	10 ¹
<i>Listeria monocytogenes</i> N.C.T.C 5214	3/3	3/3	3/3	2/2	1/1	1/1
<i>Listeria innocua</i> N.C.T.C 11288	3/3	3/3	1/1	2/2	1/1	1/1
<i>Staphylococcus aureus</i> N.C.T.C 8532	1/1	1/1	1/1	1/1	1/1	1/1
<i>Lactobacillus delbruekii</i> N.C.T.C 12712	1/1	1/1	1/1	1/1	1/1	1/1
<i>Listeria Grayii</i> N.C.T.C 10815	1/1	1/1	1/1	1/1	1/1	1/1
<i>Enterococcus faecalis</i>	1/1	1/1	1/1	1/1	1/1	1/1

ORGANISM

24 HOURS

Dilution (cfu per ml)	10 ⁶	10 ⁵	10 ⁴	10 ³	10 ²	10 ¹
<i>Listeria monocytogenes</i> N.C.T.C 5214	3/3	3/3	3/3	3/3	3/3	1/1
<i>Listeria innocua</i> N.C.T.C 11288	3/3	3/3	1/1	3/3	3/3	2/2
<i>Staphylococcus aureus</i> N.C.T.C 8532	1/1	1/1	1/1	1/1	1/1	1/1
<i>Lactobacillus delbruekii</i> N.C.T.C 12712	1/1	1/1	1/1	1/1	1/1	1/1
<i>Listeria Grayii</i> N.C.T.C 10815	1/1	1/1	1/1	1/1	1/1	1/1
<i>Enterococcus faecalis</i>	1/1	1/1	1/1	1/1	1/1	1/1

Simulated Contamination

Dilution used (cfu ml ⁻¹)	Inoculum (cfu cm ⁻²)	No. of swabs showing colour change
10 ²	100	17 out of 18 (94.4%)
10 ³	1	14 out of 18 (77.7%)





Medical Wire & Equipment

Corsham, Wiltshire, England SN13 9RT

Telephone: +44(0)1225 810361 Fax: +44(0)1225 810153

E-mail: info@mwe.co.uk Website: www.mwe.co.uk