# A swab based test for *Listeria monocytogenes*

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*Listeria monocytogenes* is a life-threatening foodborne pathogen which is capable of multiplying under refrigeration. Many manufacturers are now required to test for *L. monocytogenes* on surfaces within food production facilities. Normally, samples are sent to an off-site laboratory and with conventional methods it may be several days before results are available. By this time the product may well have been sold and consumed.

The Listeria Isolation Transwab® (LIT) is a self-contained swab device consisting of a swab and a tube of semi-solid indicator medium. The swab is rubbed over the test surface, placed into the tube and incubated at 37°C. A portable incubator could be used. *L. monocytogenes* is indicated by the medium 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*.

In the real world, a detection test has to find Listeria among a population of competing organisms, so a further experiment was conducted to see how LIT would cope with mixed populations.

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.

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## Background

*Listeria monocytogenes* is a life-threatening foodborne pathogen [1] causing 500 deaths annually in the United States [2], 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 as to its sensitivity and specificity.

A three-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.
3. To assess the response of LIT when challenged with a mixture of organisms

### Organisms

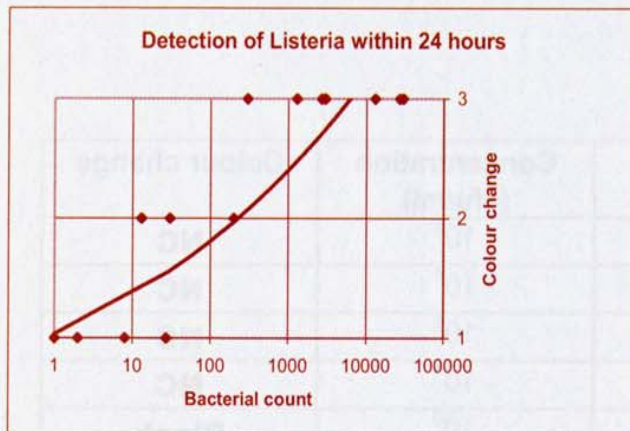
Serial dilutions of the following organisms were prepared: *L. monocytogenes* NCTC 5214, *L. innocua* NCTC 11288, *L. grayii* NCTC 10815, *S. aureus* NCTC 8532, *Lactobacillus delbruekii* 12712, *E. faecalis*., *E. coli* NCTC 9001

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 ($10^8$ cfu $ml^{-1}$) Serial dilutions were prepared by taking 1ml of suspension, adding to 10ml saline and mixing. Dilutions of $10^6$, $10^5$, $10^4$, $10^3$, $10^2$, and $10^1$ cfu $ml^{-1}$ were prepared in this way. All the dilutions were used for the sensitivity and specificity tests, while $10^5$ and $10^3$ cfu $ml^{-1}$ were used for the simulated contamination study, and $10^6$ and $10^4$ cfu $ml^{-1}$ for the multiple organism challenge.

# Results

## Sensitivity and Specificity

1. Serial dilutions of each bacterium are made, these are $10^{6}$ down to $10^{1}$ cfu $ml^{-1}$.
2. Replicate LIT swabs are inoculated for each dilution.
3. Each swab is inoculated with 100µl 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 37ºC 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.




**Colour Change categories**
1 = No change
2 = Black precipitate visible around swab bud
3 = Medium completely black

| ORGANISM | Results for matched swabs after 24 HOURS | | | | | |
|---|---|---|---|---|---|---|
| Dilution (cfu $ml^{-1}$) | $10^{6}$ | $10^{5}$ | $10^{4}$ | $10^{3}$ | $10^{2}$ | $10^{1}$ |
| *Listeria monocytogenes* N.C.T.C 5214 | 3 / 3 | 3 / 3 | 3 / 3 | 2 / 2 | 1 / 1 | 1 / 1 |
| *Listeria innocua* N.C.T.C 11288 | 3 / 3 | 3 / 3 | 1 / 1 | 2 / 2 | 1 / 1 | 1 / 1 |
| *Staphylococcus aureus* N.C.T.C 8532 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |
| *Lactobacillus delbruekii* N.C.T.C 12712 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |
| *Listeria Grayii* N.C.T.C 10815 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |
| *Enterococcus faecalis* | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |

| ORGANISM | Results for matched swabs after 48 HOURS | | | | | |
|---|---|---|---|---|---|---|
| Dilution (cfu $ml^{-1}$) | $10^{6}$ | $10^{5}$ | $10^{4}$ | $10^{3}$ | $10^{2}$ | $10^{1}$ |
| *Listeria monocytogenes* N.C.T.C 5214 | 3 / 3 | 3 / 3 | 3 / 3 | 3 / 3 | 3 / 3 | 1 / 1 |
| *Listeria innocua* N.C.T.C 11288 | 3 / 3 | 3 / 3 | 1 / 1 | 3 / 3 | 3 / 3 | 2 / 2 |
| *Staphylococcus aureus* N.C.T.C 8532 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |
| *Lactobacillus delbruekii* N.C.T.C 12712 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |
| *Listeria Grayii* N.C.T.C 10815 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |
| *Enterococcus faecalis* | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 | 1 / 1 |

## 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 $10^5$ cfu per ml and $10^3$ 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, before being inspected for a colour reaction.

## Results

| Dilution used (cfu $ml^{-1}$) | Inoculum (cfu $cm^{-2}$) | No. of swabs showing colour change |
|---|---|---|
| $10^5$ | 100 | 17 out of 18 (94.4%) |
| $10^3$ | 1 | 14 out of 18 (77.7%) |

## Challenge with multiple organisms

From suspensions of *Staphylococcus aureus, Eschericia coli,* and *Listeria monocytogenes*, dilutions containing $10^4$ or $10^6$ cfu $ml^{-1}$ were prepared. These dilutions were combined as follows:

*Mix A*
*S.aureus* $10^6$ cfu $ml^{-1}$ + *E. coli* $10^6$ cfu $ml^{-1}$ + *L. monocytogenes* $10^6$ cfu $ml^{-1}$.

*Mix B*
*S.aureus* $10^4$ cfu $ml^{-1}$ + E. coli $10^4$ cfu $ml^{-1}$ + *L. monocytogenes* $10^4$ cfu $ml^{-1}$

Triplicate LIT swabs were inoculated with 100 µl for each mix or single organism dilution. The swabs were inserted in the tubes of medium, incubated for 24 hours and any colour change recorded. Control plates were also made for each dilution or mix.

## Results

| Organism | Concentration (cfu/ml) | Colour change |
|---|---|---|
| *Staphylococcus aureus* | $10^6$ | **NC** |
| *Staphylococcus aureus* | $10^4$ | **NC** |
| *Eschericia coli* | $10^6$ | **NC** |
| *Eschericia coli* | $10^4$ | **NC** |
| *Listeria monocytogenes* | $10^6$ | **Black** |
| *Listeria monocytogenes* | $10^4$ | **Black** |
| Mix A | $10^6 + 10^6$ | **Black** |
| Mix B | $10^4 + 10^4$ | **Black** |

*The results were consistent for the three swabs tested for each mix or organism dilution*

## Discussion

The scope of this study has been limited, but it does demonstrate 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. An 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.

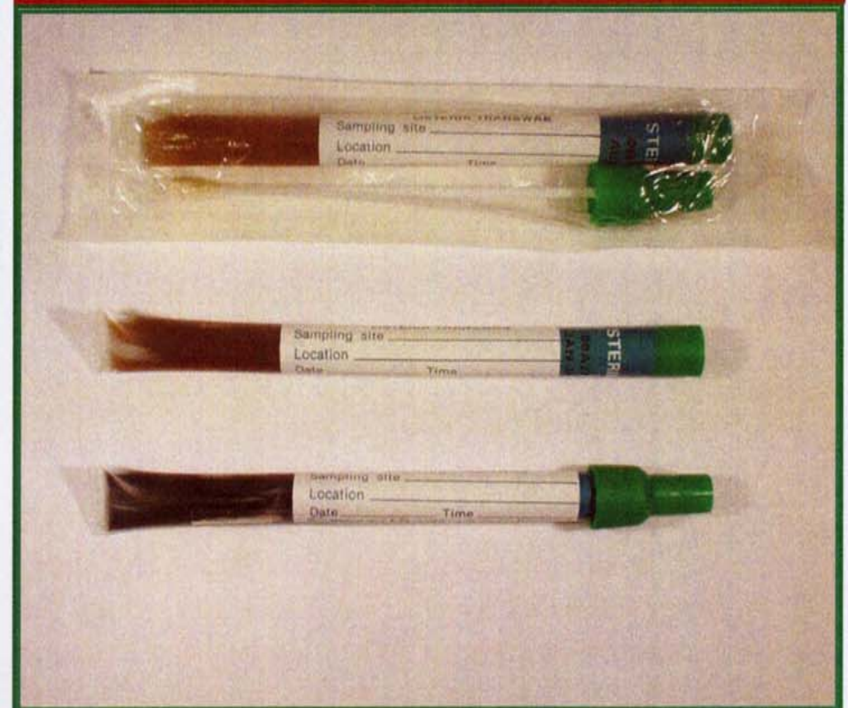
The tests using other organisms show that LIT is specific for *Listeria monocytogenes*. A more extensive study of 31 strains of various species of commonly encountered Gram-positive and Gram-negative organisms showed no false positive reactions with LIT [3].

In the final study LIT was challenged with a mixture of organisms and a positive reaction with *Listeria monocytogenes* was not impaired. It will be useful to extend this study with more complex populations of organisms to see if there are any situations which could result in false test results.

The Listeria Isolation Transwab has been used for a number of years in many countries in a variety of food processing operations and is known to be sensitive and selective. The studies reported here confirm that it does detect Listeria monocytogenes effectively, and should be a useful tool in the control of food production environments. Positive results should always be confirmed by further tests and investigated to establish their source and significance.

## 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.

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