Evaluation of self-contained Listeria test for the food, dairy and beverage industries

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ABSTRACT

A study was set up to assess the performance of a novel method for the detection of Listeria in food production facilities.

Listeria is a life-threatening foodborne pathogen causing 500 deaths annually in the United States, many preventable by proper controls at production sites. Many manufacturers are now required to test for listeria.

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.

The Listeria Isolation Transwab® (LIT) is a self-contained swab device for the detection of Listeria. 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 37C. A portable incubator would be suitable. Listeria is indicated by the gel turning from its original straw colour to black.

A study was designed to determine the detection limit for Listeria in a simulated food manufacturing situation.

Food-grade stainless steel plates inoculated with known numbers of listeria organisms, and dried were sampled by LIT. 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.

The results show LIT to be sufficiently sensitive as a method of detecting listeria contamination at an early stage and preventing contaminated food reaching the consumer.

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 *Listeria 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 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 *Listeria 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^{O}C. *Listeria monocytogenes* is indicated by the gel turning from its original straw colour to black after incubation overnight or for up to 48 hours.

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

A two-part study was designed :

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

Methods

Organisms

Serial dilutions of *Listeria monocytogenes* NCTC 5214 were prepared.
The organisms were first grown up on blood agar for 24 hours, collected by inoculating loop, and used to prepare a suspension in saline with a bacterial concentration of 10^8 colony forming units per millilitre (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 tests, while only the 10^5 and 10^3 cfu ml^{-1} were used for the simulated contamination study.

Sensitivity

This part of the study was designed to measure the optimum sensitivity of LIT in laboratory conditions.

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 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 100ml 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.

Simulated Contamination

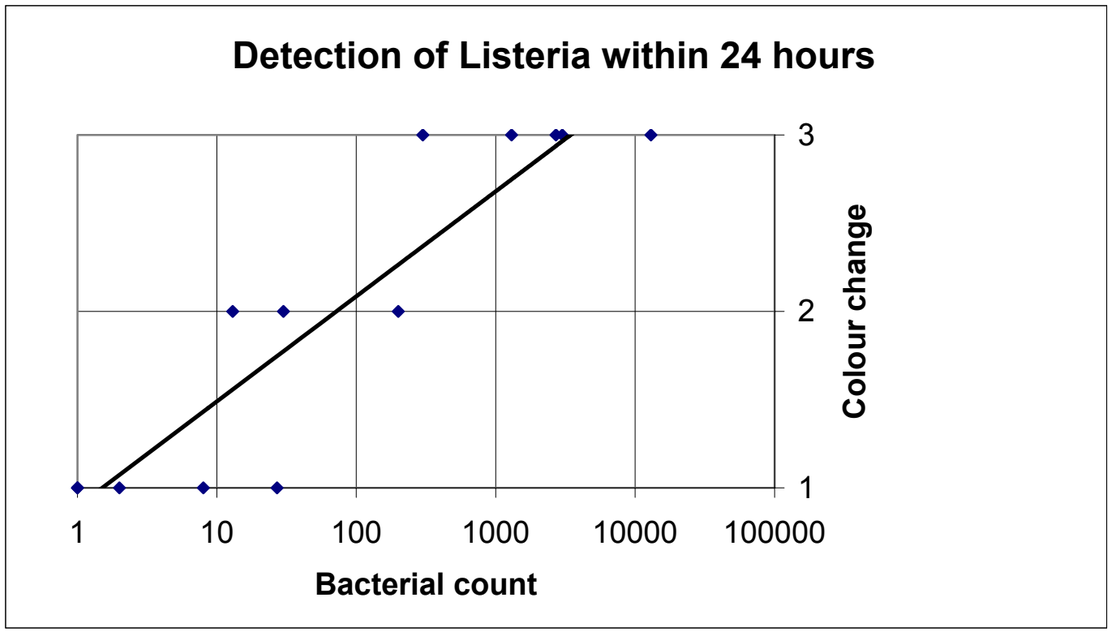
(Detection on Stainless Steel)

This part of the study was designed to assess the sensitivity of LIT in a manufacturing situation.

1. 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.
2. 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 100ml of the suspension onto the square, and spreading with an applicator.
3. The maximum loads were thus 100cfu cm^{-2} and 1 cfu cm^{-2}, representative of low level contamination.
4. The plates were allowed to dry in air before sampling with Listeria Isolation Transwab. For each dilution, one swab was used for each square.
5. After sampling, the Listeria Isolation Transwabs were incubated at 37C for 48 hours, after which they were inspected for a colour reaction.

Results

Sensitivity



Bacterial count

Number of bacterial cells inoculated onto the swab bud measured in colony forming units.

Colour change categories

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

Interpretation

Given that '2' represents the minimum visible level of colour development to indicate the presence of listeria, a regression analysis of the graph suggests that the minimum number of listeria organisms detectable at 24 hours is 57

Simulated Contamination

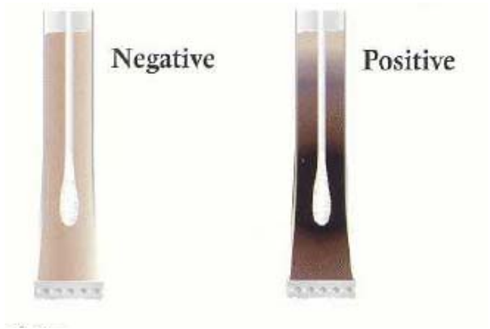
Dilution used (cfu ml^{-1})	Inoculum (cfu cm^{-2})	No. of swabs showing colour change
10^5	100	17 out of 18 (94.4%)
10^3	1	14 out of 18 (77.7%)

Dilution used

Bacterial concentration measured in colony forming units (cfu) per ml. One cfu means one organism which will be capable of multiplying to form a visuible colony on an appropriate agar medium.

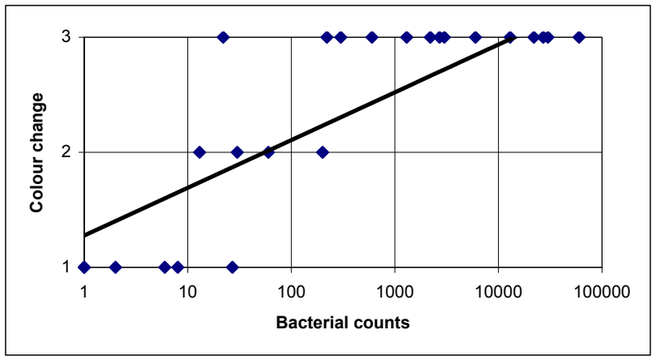
Inoculum

The effective number of organisms estimated to be present on the sampled area of stainless steel plate measured in cfu per square centimetre.



Discussion

This study has shown that the Listeria Isolation Transwab® is capable of detecting low numbers of *Listeria monocytogenes* within 24 hours, 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.



Since the study reported above was completed, it has been completed using a larger numbers of swabs and other strains of *Listeria monocytogenes*. The results obtained confirmed the above study with less than 60 organisms being detectable at 24 hours. (Strains used : NCTC 5214, and NCTC 7973).*See inset*

Other studies have been reported elsewhere using other organisms to show that LIT is specific for Listeria monocytogenes[3,4]. An 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].

The fact that the test is self-contained means that it can be used on premises remote from a laboratory. Positive results must always be properly investigated, and samples of product must still be sent for testing in the normal way, but LIT will allow management at the manufacturing site to react earlier to possible adverse incidents. Using LIT would certainly be a valuable contribution to any due diligence and/or HACCP programmes.

References

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Conclusion

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

Listeria Isolation Transwab®

Easy to use early warning test for Listeria contamination in food production premises.

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