

Listeria Swab Hygiene control for *Listeria* on surfaces

Table of contents

| | |
|----------------------------------------------|---|
| 1 . Features and benefits | 3 |
| Concept | 3 |
| Handling | 3 |
| The swab | 3 |
| The medium | 3 |
| 2 . Test performance | 3 |
| Analytical Sensitivity and Specificity | 3 |
| QC Procedures | 5 |
| 3 . Instructions for Use | 6 |
| Test procedure | 6 |
| Part components | 7 |
| Sterilization | 7 |
| Shelf Life / Expiry Date | 7 |
| Storage | 7 |

Concept

Listeria Swab is designed to minimize the risk of contamination of work areas or finished products by *Listeria* spp, in particular *L. monocytogenes*. The test is based upon an enhanced Esculin medium formation. The hydrolysis of Esculin by *Listeria* spp results in the formation of a distinctive black-colored precipitate.

Handling

1. Remove swab and sample designated area.
2. Remove the cap of the culture tube and discard.
3. Insert the swab all the way into the culture tube, closing the tube with the swab cap. Note details of the sample taken on the tube.
4. Incubate at 37°C for an initial 24 hours.



The Swab

The tip of the swab used in the test is composed of calcium alginate. The formulation allows the tip to dissolve completely when immersed in the medium, ensuring that all organisms collected during sampling will come into direct contact with the medium.



The medium

The *Listeria* Swab uses a basic medium similar to the Oxford *Listeria* Selective Agar, and utilizes aesculin hydrolysis as the indicator of the presence of *Listeria*. The media is made selective for *Listeria* with a cocktail of anti-microbial and anti-fungal reagents, similar to but more stable than those used in the Oxford media.

The medium gives the distinctive black-brown precipitation that is seen with the Oxford medium. It has been shown that, with few exceptions, the only strains to produce the black phenolic compounds are *Listeria* spp. Some Gram positive cocci survive in the medium but do not constitute a problem as they are slow growing, and do not hydrolyze aesculin.

Test Performance

Analytical Sensitivity and Specificity

The product has been developed in the laboratory by inoculating the swab tip with a large variety of organisms at 108, 105, 103 and 10 cfu per swab. These have been placed into the swab medium and grown for 72 hours to detect false positives and false negatives. The results found with this approach are given in the table 1 below:

Table 1: analytical sensitivity and specificity of the Listeria swab.

| Organism | CFU in Sample | Color Change |
|--------------------------------------------|-----------------------|--------------|
| <i>Listeria monocytogenes</i> NCTC 11994 | 7 | Black |
| <i>Listeria monocytogenes</i> NCTC 5214 | 19 | Black |
| <i>Listeria monocytogenes</i> NCTC 7973 | 15 | Black |
| <i>Listeria ivanovii</i> | 10 | Black |
| <i>Listeria innocua</i> NCTC 11288 | 9 | Black |
| <i>Listeria seeligeri</i> | 1 x 10 ⁴ | Black |
| <i>Listeria Welshimeri</i> NCTC 11857 | 1 x 10 ³ | Black |
| <i>Listeria murrayi</i> | 1 x 10 ⁵ | Black |
| <i>Listeria grayii</i> | 1 x 10 ⁵ | Black |
| <i>Staphylococcus aureus</i> NTCT 6571 | 4 x 10 ⁸ | No change |
| <i>Streptococcus faecalis</i> NCTC 775 | 1.5 x 10 ⁵ | No change |
| <i>Aeromonas hydrophi 1a</i> NCTC 1767 | 1.5 x 10 ⁷ | No change |
| <i>Bacillus subtilis</i> NCTC 10400 | 4 x 10 ⁷ | No change |
| <i>Bacillus pumilis</i> NCTC 10327 | 1 x 10 ⁷ | No change |
| <i>Bacillus cereus</i> NCTC 10320 | 2 x 10 ⁶ | No change |
| <i>Escherichia coli</i> NCTC 9001 | 1 x 10 ⁷ | No change |
| <i>Escherichia coli</i> NCTC 10418 | 6 x 10 ⁷ | No change |
| <i>Klebsiella pneumoniae</i> | 3 x 10 ⁷ | No change |
| <i>Klebsiella aerogenes</i> NCTC 7418 | 1 x 10 ⁸ | No change |
| <i>Klebsiella aerogenes</i> NCTC 11228 | 3 x 10 ⁷ | No change |
| <i>Proteus vulgaris</i> NCTC 1683 | 1 x 10 ⁶ | No change |
| <i>Proteus mirabilis</i> NCTC 841 | 5 x 10 ⁸ | No change |
| <i>Citrobacter freundii</i> | 1.2 x 10 ⁸ | No change |
| <i>Citrobacter diversus</i> | 3 x 10 ⁶ | No change |
| <i>Enterobacter agglomerans</i> | 1.1 x 10 ⁸ | No change |
| <i>Morganella morganii</i> | 1.4 x 10 ⁸ | No change |
| <i>Serratia liquefacens</i> | 1 x 10 ⁸ | No change |
| <i>Serratia marcescens</i> | 1 x 10 ⁷ | No change |
| <i>Yersinia enterocolitica</i> | 1 x 10 ⁷ | No change |
| <i>Enterobacter cloacae</i> | 3 x 10 ⁷ | No change |
| <i>Salmonella typhimurium</i> NCTC 74 | 3 x 10 ⁸ | No change |
| <i>Shigella flexneri</i> | 8 x 10 ⁶ | No change |
| <i>Pseudomonas aeruginosa</i> | 9 x 10 ⁷ | No change |
| <i>Pseudomonas putida</i> NCTC 10936 | 1.5 x 10 ⁷ | No change |
| <i>Pseudomonas fluorescens</i> NCTC 10038 | 1 x 10 ⁷ | No change |
| <i>Pseudomonas maltophilia</i> NCTC 102157 | 6 x 10 ⁷ | No change |
| <i>Pseudomonas vesicularis</i> NCTC 10900 | 4 x 10 ⁶ | No change |
| <i>Pseudomonas cepacia</i> NCTC 10743 | 3.5 x 10 ⁷ | No change |
| <i>Pseudomonas putrefaciens</i> NCTC 10735 | 3 x 10 ⁶ | No change |
| <i>Candida albicans</i> | 2 x 10 ⁶ | No change |

The same procedure was carried out using several dairy products as carriers of the inocula. The results of these tests paralleled those shown in Table 1.

Finally a variety of foodstuffs were tested with conventional methodology, including enrichment in UVM *Listeria* broths and culture onto Oxford *Listeria* selective plates. In comparison with the *Listeria* swab a 97.2% correlation of results was found based on 197 negative and 53 positive (mostly doctored) specimens.

QC Procedures

Selective agent testing

The selective agent cocktail is subjected to an MIC study of 36 organisms and the end point of no growth (MIC) must agree for 75% of the organisms, and not exceed one dilution variation for the remainder. The battery of organisms includes 2 *Listeria monocytogenes*, 1 *Listeria innocua*, 10 Gram-positive organisms, 20 Gram-negative organisms and 3 yeasts.

Performance of the finished product

The product is tested using the normal test procedure.

In the control of the finished product the test organisms below are suspended to an opacity that is equivalent to a 0.5 McFarland standard. This equates to a concentration of 10^8 - 10^9 cfu/ml. The suspensions of *Listeria* are diluted by 10^{-3} , 10^{-2} , and a further 10^{-2} , giving final concentrations of 10^5 - 10^6 , 10^3 - 10^4 , and 10^{-1} - 10^2 cfu/ml. Non-*Listeria* are suspended to the same opacity, but only diluted by 10^{-2} to give a final concentration of 10^6 - 10^7 cfu/ml.

Duplicate 0.2ml drops of each test organism at each dilution are placed onto a sterile plastic surface, and each drop is picked up with a swab from the *Listeria* swab. This gives a final content in each swab of 10^4 - 10^5 , 10^2 - 10^3 , and 1-10 cfu per swab tip for *Listeria*, and 10^5 - 10^6 for non-*Listeria* samples.

The swab is inserted into the *Listeria* medium and incubated at 37°C. The color after one day and two day incubation is recorded.

0.02 ml of the lowest dilution for the non-*Listeria*, and the two lowest dilutions of *Listeria* are inoculated onto a nutrient agar, and the number of cfu determined in the initial inoculum. This should indicate that *Listeria* tests were carried out with the 1-10 cfu at the lowest dilutions, and with circa $1 \cdot 10^5$ cfu for the non-*Listeria*.

Table 1: QC organisms

| Species | Type |
|----------------------------------|------------|
| <i>Listeria monocytogenes</i> | NCTC 11994 |
| <i>Listeria monocytogenes</i> | NCTC 5214 |
| <i>Listeria monocytogenes</i> | NCTC 7973 |
| <i>Listeria innocua</i> | NCTC 11288 |
| <i>Listeria grayii</i> | NCTC 10815 |
| <i>Staphylococcus aureus</i> | NCTC 6571 |
| <i>Staphylococcus aureus</i> | |
| <i>Lactobacillus acidophilus</i> | |
| <i>Klebsiella aerogenes</i> | NCTC 11228 |

All *L. monocytogenes*, *L. innocua*, and *L. ivanovii* tests should give a color change at all dilutions. *L. grayii* (not implicated with any human disease) will only show a positive result at higher concentrations, and the non-*Listeria* should give no positives at any concentration.

Abbreviations

| | |
|-------|-------------------------------------------|
| cfu: | colony forming units |
| NCTC: | National collection of Type Cultures (UK) |
| MIC: | Minimum inhibitory Concentration |
| UVM: | University of Vermont medium |

Instructions for Use

Test procedure

- Peel back wrapper to expose both caps. Remove swab and sample test site.
- Remove cap of culture tube with thumb and forefinger and discard.
- Insert swab in culture tube and push down firmly to immerse the swab completely.
- Fill in time, date and site details.
- Incubate at 37°C for up to 48 hours.
- Read results. A positive result is indicated by a color change from light brown agar to black / dark brown commencing around the bud, any color change being significant. Negative result shows no color change.
- Record results and dispose of the tube.



Part components

Each sterile, self-contained peel pouch contains:

- One green-capped plastic shaft with calcium alginate tip.
- One labelled and sealed tube containing selective culture medium with indicator.

Sterilization

Listeria swab is sterilized by irradiation.

Shelf life / Expiry date

The expiry date is 12 months from the month of manufacture and is printed onto the tube label and peel pouch.

Storage

Listeria swabs should be stored in a dry place between +5°C and +25°C.
Do not freeze!