Lösningar för egenkontroller

Listeria Swab
Hygiene control for *Listeria* on surfaces

web: www.egenkontroll.nu **e-mail:** haccp@egenkontroll.nu **tel:** 08-753 00 04 **fax:** 0709-66 78 96

besöksadress: Odengatan 40, 113 51 Stockholm **postadress:** Box 19087, 104 32 Stockholm

Table of contents

Features and Benefits

Concept

Listeria Swab is designed to minimize the risk of contamination of work areas or finished products by Listeria spp, in particular L. monocyto-genes. 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 List eria Swab uses a basic medium similar to the Oxford Listeria Selective Agar, and utilises 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 ausculin.

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
Listeria monocytogenes NCTC 11994	7	Black
Listeria monocytogenes NCTC 5214	19	Black
Listeria monocytogenes NCTC 7973	15	Black
Listeria ivanovii	10	Black
Listeria innocua NCTC 11288	9	Black
Listeria seeligeri	1 x 104	Black
Listeria Welshimeri NCTC 11857	1 x 103	Black
Listeria murrayi-	1 x 105	Black
Listeria grayii	1 x 105	Black
Staphylococcus aureus NTCT 6571	4 x 108	No change
Streptococcus faecalis NCTC 775	1.5 x 105	No change
Aeromonas hydrophi 1a NCTC 1767	1.5 x 107	No change
Bacillus subtilis NCTC 10400	4 x 107	No change
Bacillus pumilis NCTC 10327	1 x 107	No change
Bacillus cereus NCTC 10320	2 x 106	No change
Escherichia coli NCTC 9001	1 x 107	No change
Escherichia coli NCTC 10418	6 x 107	No change
Klebsiella pneumoniae	3 x 10^7	No change
Klebsiella aerogenes NCTC 7418	1 x 108	No change
Klebsiella aerogenes NCTC 11228	3 x 107	No change
Proteus vulgaris NCTC 1683	1 x 106	No change
Proteus mirabilis NCTC 841	5 x 108	No change
Citrobacter freundii	1.2 x 108	No change
Citrobacter diversus	3 x 10^6	No change
Enterobacter agglomerans	1.1 x 108	No change
Morganella morgani	1.4 x 108	No change
Serratia liquefacens	1 x 10^8	No change
Serratia marcescens	1 x 107	No change
Yersinia enterolytica	1 x 107	No change
Enterobacter cloacae	3 x 107	No change
Salmonella typhimurium NCTC 74	3 x 108	No change
Shigella flexenri	8 x 10^6	No change
Pseudomonas aeruginosa	9 x 107	No change
Pseudomonas putida NCTC 10936	1.5 x 107	No change
Pseudomonas flourescens NCTC 10038	1 x 107	No change
Pseudomonas maltophillia NCTC 102157	6 x 107	No change
Pseudomonas vesicularis NCTC 10900	4 x 106	No change
Pseudomonas cepacia NCTC 10743	3.5 x 107	No change
Pseudomonas putrefaciens NCTC 10735	3 x 106	No change
Candida albicans	2 x 106	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 methodo-logy, 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 organ-isms, and not exceed one dilution variation for the remainder. The battery of organisms includes 2 *Listeria monocytogenes,* 1 *Listeria inocua,* 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 con-centrations of 10^5-10^6, 10^3-10^4, and 10 -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-*List eria* samples.
The swab is inserted into the *List eria* 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 *List eria* 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 1.10^5 cfu for the non-*Listeria.*

Table 1: QC organisms

Species	Type
Listeria monocytogenes	NCTC 11994
Listeria monocytogenes	NCTC 5214
Listeria monocytogenes	NCTC 7973
Listeria innocua	NCTC 11288
Listeria grayii	NCTC 10815
Staphylococcus aureus	NCTC 6571
Staphylococcus aureus	
Lactobacillus acidophilus	
Klebsiella aero genes	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 mediumwith 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!