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EC MICROBIOLOGY
Research Article

Surface Microbiology: Implementation and Results of an Interlaboratory Proficiency Test

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Received: June 01, 2021; **Published:** April 28, 2022

Abstract

Microbiological surface control is an important component of monitoring and control of the production environment in the food industry. An ineffective assessment of surface hygiene can result in the non-detection of pathogenic or not microorganisms yet present, and thus in potential harmful effects in terms of quality, hygiene and public health. This implies that the laboratories carrying out this kind of analyses could demonstrate the reliability of their results. For this reason, BIPEA (Bureau Interprofessionnel d'Études Analytiques) introduced two new proficiency testing schemes for the detection of *Listeria monocytogenes* and *Salmonella* spp. in wipes for surface sampling, allowing the laboratories to assess their performance regarding such analyses. A proficiency test involves the analysis by each laboratory of the same sample for the determination of given analytical criteria. The implementation of these proficiency tests has three main steps: the preparation of homogeneous and stable samples, their analysis by the participating laboratories according to the reference method or an alternative method, and data processing with an assessment of laboratory performance.

These proficiency tests were successfully implemented, with very satisfactory participants results on homogeneous and stable samples. The participants performance was assessed by calculating their relative specificity, relative sensibility and relative accuracy. The two proficiency testing schemes are now proposed regularly by BIPEA, with two rounds per year.

Laboratories now have a means of demonstrating their competence in the detection of *Listeria monocytogenes* and *Salmonella* spp. as part of the microbiological surface control.

Keywords: *Proficiency Testing; Surface Microbiology; Hygiene; Salmonella; Listeria monocytogenes*

Introduction

The control of surface hygiene is a major issue in the food industry in order to protect food products from contamination by microorganisms in general and pathogenic germs in particular.

The assessment of microbiological contamination of surfaces is a part of the monitoring plan of the production environment. Its objectives are to detect or quantify any contamination of equipment, work surfaces and utensils and to verify the correct application of cleaning and disinfection procedures. Different techniques of surface sampling include contact boxes, swabs, sponges and wipes.

ISO 18593 [1] specifies the different methods for these sampling techniques, as for the detection or enumeration of the microorganism, the determination of contamination is carried out on the sample by a specific standard.

An ineffective assessment of surface hygiene can result in the non-detection of pathogenic or not microorganisms yet present, and therefore in potential harmful effects in terms of quality, hygiene and public health. This implies that laboratories performing microbiological surface control must assess their analytical performance as part of quality assurance.

To address this need, BIPEA developed and implemented two new interlaboratory proficiency tests in November 2020: the first one implementing wipes contaminated with a strain of *Listeria monocytogenes* (program 101b) and the second one implementing wipes contaminated with a strain of *Salmonella* spp. (program 101c).

The objective of these proficiency tests is to enable laboratories to demonstrate the reliability of their analytical results and to compare analytical data and protocols applied for the detection of *Salmonella* and *Listeria monocytogenes* for surface sampling with wipes.

Methodology

A proficiency test involves the analysis by each laboratory of the same sample for the determination of given analytical criteria. Generally speaking, the design of a proficiency test can be summarized in three main steps: the preparation of test items (samples), the analysis by the participating laboratories and the statistical processing of the data with the evaluation of the performance of the laboratories.

Preparation of samples

One of the critical steps when organizing a proficiency test is samples preparation. These must be sufficiently homogeneous and stable according to the requirements of ISO 13528 [2] to avoid considering sample-related deviations as deviations related to laboratory competence.

Samples were prepared by contamination of sterile wipes (300 x 300 mm) with a *Listeria monocytogenes* (wild-type strain) suspension in program 101b and with a suspension of *Salmonella enteritidis* (wild-type strain) under program 101c, each suspension being adjusted in terms of number of microorganisms. The contamination of the wipes with the target microorganisms was carried out according to a predefined contamination scheme unknown to the participants (cf. table 1 and 3).

For each target microorganism – *Salmonella* spp. and *Listeria monocytogenes*, the stability of the samples was evaluated over a 14-day period on three samples stored at (5 ± 3)°C. The homogeneity of the contaminated samples was also verified for each target microorganism by a study on 10 samples from a 36-sample batch, using a defined step. Detection analyses of *Salmonella* spp. and those of *Listeria monocytogenes* were carried out according to the reference method ISO 6579-1 [3] and an alternative method (AFNOR certificate No. AES 10/03-09/00) respectively.

Analyses by laboratories

For each test, three wipes were shipped to each participating laboratory in refrigerated package with a reply form.

The laboratories analysed the samples either by a reference method or by an alternative method.

For *Listeria monocytogenes*, the reference method is ISO 11290-1 [4] and for *Salmonella* spp., the reference method is ISO 6579-1.

Participants submitted their analyses results via online reply forms. They also had the option of indicating the date of analysis, the method and the culture medium used.

Given the unstable nature of the samples, the laboratories were asked to take over the wipes upon receipt and to submit their results within two weeks.

Statistical processing of data

As the data are qualitative (detected/undetected) results, they were evaluated as follows:

- If the target microorganism is detected when the sample was contaminated with the strain, then the result is considered satisfactory,
- If the target microorganism is not detected when the sample was not contaminated with the strain, then the result is considered satisfactory,
- In the case of a false positive or a false negative, the result is considered as incoherent.

In addition, laboratory performance was evaluated by calculating relative specificity (r_{SP}), relative sensitivity (r_{SE}) and relative accuracy (r_{AC}) according to the following equations:

$$r_{SP}(\%) = \frac{TN}{TN+FP} x\ 100 \qquad \text{(Eq. 1)}$$

$$r_{SE}(\%) = \frac{TP}{VP+FN} x\ 100 \qquad \text{(Eq. 2)}$$

$$r_{AC}(\%) = \frac{TN+TP}{TN+TP+FN+FP} x\ 100 \qquad \text{(Eq. 3)}$$

with:

TN the number of true negatives, FN the number of false negatives, TP the number of true positives and FP the number of false positives.

Thus the relative specificity allows to assess the laboratory's ability to find the negative samples of the test, the relative sensitivity allows to assess its ability to find the positive samples of the test and finally, the relative accuracy allows to assess the laboratory's ability to conclude correctly on the presence or absence of the strain sought. Thus, the results of each participant are considered satisfactory if r_{SP} = 100%, r_{SE} = 100% and r_{AC} = 100% and as unsatisfactory if r_{SP} < 100%, r_{SE} < 100% and r_{AC} < 100%.

Results and Discussion

In the stability study, the target microorganisms, *Salmonella* spp. and *Listeria monocytogenes*, were detected in the wipes after 14 days of storage at (5 ± 3)°C, leading to a conclusion of sufficient stability to meet the needs of the test and the requirements of ISO 17043 [5]. Similarly, as part of the homogeneity study, the target microorganisms were detected in the ten wipes, which led to the conclusion that there was sufficient homogeneity to meet the needs of the test and the requirements of ISO 17043.

The results of the two tests presented below were communicated to participants via reports.

Test results for *Listeria monocytogenes*

In November 2020, the test for *Listeria monocytogenes* detection included 6 participants: 4 laboratories used the reference method and 2 laboratories used an alternative method. The test results are given in table 1. For each sample, the results obtained by the participants are consistent with those expected.

	Sample 1	Sample 2	Sample 3
Theoretical contamination	Contamined	Contamined	Not contamined
Laboratories results	Detected: 6 Not detected: 0	Detected: 6 Not detected: 0	Detected: 0 Not detected: 6

Table 1: *Test results for Listeria monocytogenes.*

Based on the results of each laboratory for the 3 samples, the relative specificity r_{SP}, the relative sensitivity r_{SE} and the relative accuracy r_{AC} were calculated and are presented in table 2.

Laboratory No.	Relative specificity r_{SP} (%)	Relative sensitivity r_{SE} (%)	Relative accuracy r_{AC} (%)
1	100	100	100
2	100	100	100
3	100	100	100
4	100	100	100
5	100	100	100
6	100	100	100

Table 2: *Performance evaluation for Listeria monocytogenes.*

Laboratory results are considered satisfactory, with r_{SP}, r_{SE} and r_{AC} all equal to 100%. The overall performance during this test is therefore very satisfactory.

Test results for *Salmonella* spp

In November 2020, the test for *Salmonella* spp. Detection included 4 participants: 2 laboratories used the reference method and 2 laboratories used an alternative method.

The test results are given in table 3. For each sample, the results obtained by the participants are consistent with those expected.

	Sample 1	Sample 2	Sample 3
Theoretical contamination	Contamined	Not contamined	Contamined
Laboratories results	Detected: 4 Not detected: 0	Detected: 0 Not detected: 4	Detected: 4 Not detected: 0

Table 3: *Test results for Salmonella spp.*

Based on the results of each laboratory for the 3 samples, the relative specificity r_{SP}, the relative sensitivity r_{SE} and the relative accuracy r_{AC} were calculated and are presented in table 4. Laboratory results are considered satisfactory, with r_{SP}, r_{SE} and r_{AC} all equal to 100%. The overall performance during this test is therefore very satisfactory.

Laboratory No.	Relative specificity r_{SP} (%)	Relative sensitivity r_{SE} (%)	Relative accuracy r_{AC} (%)
1	100	100	100
2	100	100	100
3	100	100	100
4	100	100	100

Table 4: *Performance evaluation for Salmonella spp.*

Conclusion

These two interlaboratory proficiency tests enabled laboratories carrying out microbiological surface control to assess their performance regarding the detection of *Listeria monocytogenes* and *Salmonella* spp. in wipes. The results for each sample and the performance of each laboratory evaluated using relative specificity r_{SP}, relative sensitivity r_{SE} and relative accuracy r_{AC} are satisfactory.

On the basis of these results and the stability and homogeneity studies, these proficiency tests were successfully implemented.

These two tests were renewed in March 2021 and brought together more participants (12 for the *Listeria monocytogenes* detection test and 10 for the *Salmonella* spp. detection test) with satisfactory results as well. Thus, these two proficiency tests are now offered regularly with two rounds per year.

Laboratories now have a means of verifying the reliability of their results and of having recognised by the competent accreditation bodies, their analytical procedures for the detection of *Listeria monocytogenes* and *Salmonella* spp. as part of microbiological surface control.

Bibliography

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Volume 18 Issue 5 May 2022