Evaluating laboratory performance using rapid methods as compared to reference methods for the detection of pathogens in food

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INTRODUCTION

The contamination of food products by microorganisms is a major concern and can have enormous consequences, both economically and for consumer health. Two of the major food-borne pathogens are *Salmonella* spp. and *Listeria monocytogenes*, outbreaks of which are responsible for hundreds of thousands of deaths worldwide. It is therefore crucial for laboratories to be able to detect the contamination of food by these and other pathogens. The ISO 6579-1 and ISO 11290-1 standards describe, respectively, reference methods for the detection of *Salmonella* spp. and *Listeria monocytogenes* and *Listeria* spp. in food products, but the significant waiting times associated with these culture-based methods can limit their practical use [1, 2]. However, rapid methods have also been developed, including molecular methods based on polymerase chain reaction (PCR) techniques and methods involving enzyme immunoassays, which allow quicker results and can therefore provide a significant benefit to health systems.

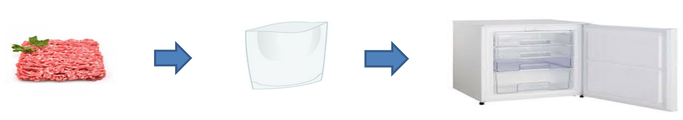
Through examination of Proficiency Testing (PT) results from BIPEA (Bureau Interprofessionnel d'Études Analytiques) over a five-year period from 2020 to 2025, organized according to the ISO 17043 standard, results can be compared between the reference methods and rapid methods with different principles [3]. The rapid methods studied here performed at least as well as the culture-based methods over this timespan.

DESIGN and IMPLEMENTATION

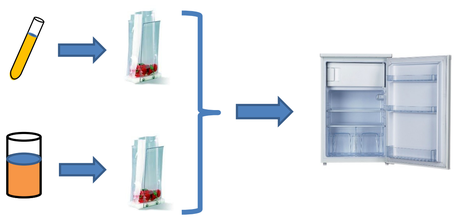
PT implementation can be summarized in 3 main steps: sample preparation with homogeneity/stability checks, analysis by laboratories, and statistical treatment of results

Production of Samples

To prepare for sample production, minced beef free of target microorganisms was separated into 24g test portions in individual stomacher bags, before freezing for storage.



Positive samples were then spiked with calibrated solutions of the target and non-target microorganisms, while negative samples were not spiked. All samples were refrigerated until shipment.



- Homogeneity testing was performed on 10 positive samples of each batch.
- Stability testing additionally confirmed the stability of the samples for 10 days at 5 ± 3 °C.

Analysis of Samples

Each laboratory received 3 samples, either **contaminated** or **uncontaminated**, according to an unknown scheme.

Participating laboratories were then requested to analyze each of the 3 samples to identify the **presence** or **absence** of the target microorganism. For this analysis, they were asked to note the method used:

- Reference method
  ISO 6579-1 *or*
  ISO 11290-1
- Alternative method: Growth medium
- Alternative method: PCR
- Alternative method: Enzyme immunoassay
- Other methods

Evaluation of Results

As the results for each sample were qualitative (detected/not detected), they were evaluated as follows:

✓ Detection of the bacteria in a **positive** sample
✓ Non-detection of the bacteria in a **negative** sample
✗ False negative or false positive

Each laboratory's ability to correctly identify positive and negative samples was assessed by calculation of relative specificity (r_{SP}), sensitivity (r_{SE}), and accuracy (r_{AC}):

$$r_{SP}(\%) = \frac{TN}{TN + FP} x\ 100$$

$$r_{SE}(\%) = \frac{TP}{VP + FN} x\ 100$$

$$r_{AC}(\%) = \frac{TN + TP}{TN + TP + FN + FP} x\ 100$$

TN: True negatives
FN: False negatives
TP: True positives
FP: False positives

RESULTS and DISCUSSION

- For this study, the results from 2 PT programs were examined: a program for the detection of *Salmonella* spp. in minced beef and a program for the detection of *Listeria monocytogenes* in the same matrix.
- Results were studied over a period of 5 years (2020-2025), with 3 rounds per year and 3 samples per round per participating laboratory, for a total of 45 sets of samples analyzed over this timespan.
- For the detection of *Salmonella* spp., the number of participating laboratories increased from about 50 to about 85 from 2020-2025. For the detection of *Listeria monocytogenes*, participating laboratories increased from about 40 to about 70 over the same period.
- The graphs below show the total number of results received for each proposed method over this period, as well as the rate of those results that were unacceptable (false negatives or false positives).

Fig. 1 Participation and rates of unacceptable results, per method, for the detection of *Salmonella* spp. in minced beef in BIPEA PTs, 2020-2025

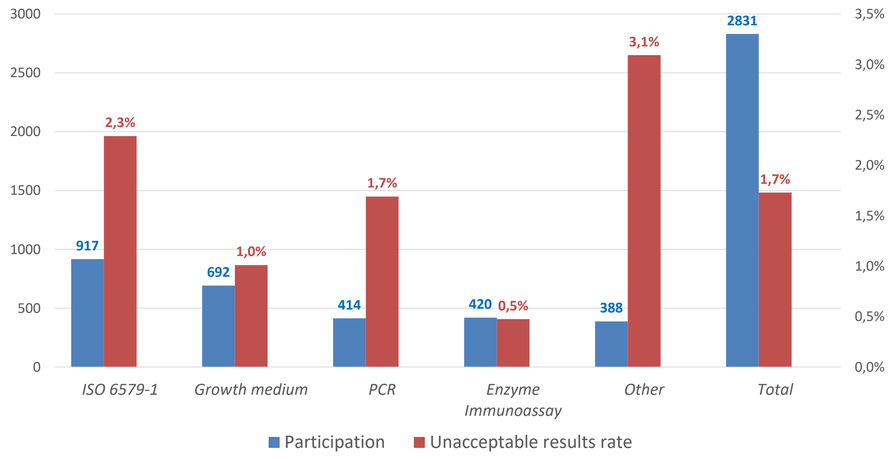
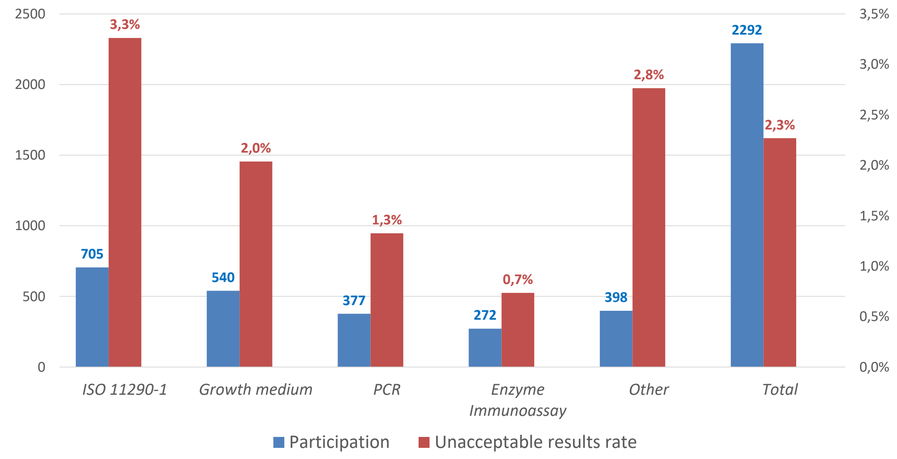


Fig. 2 Participation and rates of unacceptable results, per method, for the detection of *Listeria monocytogenes* in minced beef in BIPEA PTs, 2020-2025



By examining the results in Figures 1 and 2, several important conclusions can be observed:

- For both *Salmonella* spp. and *Listeria monocytogenes*, both **PCR** and **enzyme immunoassay** methods yielded lower rates of unacceptable results than the reference ISO methods.
- For both microorganisms, **enzyme immunoassays** were the most effective method, with less than 1% of false negatives or false positives observed over several hundred analyzed samples.

It is important to note that, for these two programs, results and performances were good for all methods, with less than 3% of unacceptable results overall, so the statistical differences described here are minimal. However, this data makes it clear that, in the case of these 2 microorganisms in the matrix minced beef, the rapid methods perform as well or better than the culture-based reference methods.
Further studies should be conducted to determine if this conclusion is applicable to other matrix types and other microorganisms.

CONCLUSION

In conclusion, the results of the trials studied here suggest that methods for the detection of pathogens based on PCR and enzyme immunoassay techniques can be a useful alternative for laboratories that improve the long waiting times associated with the culture-based reference methods without sacrificing performance. Over five years of proficiency tests, participants using rapid methods based on these principles recorded lower rates of unacceptable results for the detection of *Salmonella* spp. and *Listeria monocytogenes* in minced beef than participants performing the reference methods.
In future work, it would be interesting to compare results obtained using different methods for the analysis of additional microorganisms and categories of food matrices, to determine whether the equivalent performances of rapid methods observed here can be generalized.

REFERENCES

[1] **ISO 6579–1:2017** - Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.
[2] **ISO 11290–1:2017** - Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 1: Detection method
[3] **ISO/IEC 17043:2023** - Conformity assessment - General requirements for the competence of proficiency testing providers