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Interlaboratory proficiency tests to assess the analytical competency of French official control laboratories for the analysis of *Listeria monocytogenes, Salmonella* spp. and coagulase-positive staphylococci in food

Léna Barre [a,*,1], Florence Guillier [b,*,1], Bertrand Lombard [c], Corinne Danan [a], Jacques-Antoine Hennekinne [b], Boris Constantin [d], Romain Le Neve [d], Sandrine Nguyen [d], Abdelkader Boubetra [d], Marianne Chemaly [e], Yacine Nia [b], Laetitia Bonifait [e,*]

[a] *ANSES, Laboratory for Food Safety, Salmonella and Listeria Unit, Maisons-Alfort, France*
[b] *ANSES, Laboratory for Food Safety, Staphylococcus, Bacillus and Clostridium Unit, Maisons-Alfort, France*
[c] *ANSES, Strategy and Programs Department, Research and Reference Division, Maisons-Alfort, France*
[d] *BIPEA, Proficiency Testing Provider, Paris, France*
[e] *ANSES, Ploufragan-Plouzané-Niort Laboratory, Hygiene and Quality of Poultry and Pig Products Unit, Ploufragan, France*

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ABSTRACT

A common interlaboratory proficiency testing scheme in food microbiology was organised yearly in France from 2019 to 2021 by the three National Reference Laboratories for *Listeria monocytogenes*, *Salmonella* spp. and coagulase-positive staphylococci (CPS). This proficiency testing scheme aimed to assess the performance of French official control laboratories for the detection and enumeration of these three major foodborne pathogenic bacteria covered by European regulations on microbiological criteria for foodstuffs. An average of 65 laboratories participated in the proficiency test (PT) each year, using either reference or validated alternative methods. For each PT, ten samples for detection and four samples for enumeration per pathogen were sent to each participant. Over the three years, the PT results demonstrated the ability of the laboratory network to detect L. *monocytogenes* and *Salmonella* spp., as well as to enumerate L. *monocytogenes* and CPS in food matrices. These PTs showcased the robustness of the official laboratory network and provided an opportunity to give scientific and technical advices in case of discrepancy results, representing an efficient tool for quality improvement of the analyses performed by the laboratory network.

1. Introduction

Ensuring the high-level protection of human health is a general objective that falls under the responsibilities of food business operators and competent authorities. In this framework, the management of foodborne zoonoses involves specific surveillance and control measures throughout the food chain to prevent outbreaks and intoxications in humans.

In Europe, the epidemiological situation of zoonoses reported by Member States is described every year in the joint EFSA/ECDC One Health zoonoses report (EFSA, 2024). This report indicates that salmonellosis, listeriosis and infections due to *S. aureus* are among the top ten reported foodborne zoonotic infections. Regarding foodborne outbreaks in 2022, in Europe, there were: 9210 confirmed human cases (1115 foodborne outbreaks) due to *Salmonella* spp.; 133 confirmed human cases (19 foodborne outbreaks) due to *Listeria monocytogenes* and 2268 confirmed human cases (207 foodborne outbreaks) due to *Staphylococcus aureus*.

With 65,208 confirmed human cases, salmonellosis was the second most commonly reported foodborne gastrointestinal infection in humans in the European Union (corresponding to a European Union notification rate of 15.3 cases per 100,000 population).

Listeriosis is one of the most serious foodborne diseases, under European Union surveillance, with the highest hospitalisation and case fatality rates (EFSA, 2024).

Coagulase positive staphylococci (CPS), in particular *S. aureus*, when

* Corresponding authors.
E-mail addresses: lena.barre@anses.fr (L. Barre), florence.guillier@anses.fr (F. Guillier), laetitia.bonifait@anses.fr (L. Bonifait).
[1] These authors contributed equally.

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they are present in food, can produce staphylococcal enterotoxins (SE). The ingestion of these SE has been reported as the cause of many food poisoning outbreaks (FPO) (Argudin et al., 2010; Denayer et al., 2017; Ercoli et al., 2017; Guillier et al., 2016; Romano et al., 2023). At European level for toxigenic bacteria, SE toxins were associated with the highest number of hospitalisations (EFSA, 2024).

These bacteria naturally contaminate various food matrices and thus represent a risk for consumers, especially in case of ready-to-eat or potentially undercooked food. Results from official controls indicate that food of meat or fish origin are the matrices most frequently contaminated by L. *monocytogenes* or meat products for *Salmonella* spp. For *S. aureus*, the most common food vehicles are milk and dairy products and composite foods (EFSA, 2024).

To ensure food safety, both food business operators and competent authorities are required to implement general and specific hygiene and monitoring measures, including microbiological analysis leading to search the presence and quantify these pathogens in foods.

In Europe, the Commission Regulation (EC) No 2073/2005 on microbiological criteria for food lays down the microbiological criteria for L. *monocytogenes*, *Salmonella* and CPS to be complied with by food business operators (Anonymous, 2005). These criteria define the foods to be tested, the analytical methods to be used and the limits tolerated regarding bacterial contamination. National competent authorities are responsible for checking that food business operators fulfil these requirements through their official controls. The proficiency of official laboratories performing analyses for competent authorities is essential for properly analysing food samples and detecting the presence of pathogens. By ensuring the reliability of the tests performed, PTs organised by NRLs contribute to ensure food safety at every stages of the food chain.

From 2019 to 2021, the French network of official laboratories was composed of:

- laboratories approved by the French General Directorate for Food (DGAL) overseen by the ministry in charge of agriculture, and
- laboratories belonging to the Joint Laboratory Service (SCL) of the General Directorate for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF) and the General Directorate of Customs and Excise (DGDDI) under the ministry in charge of consumer protection.

In this context, DGAL, the French competent authority, has designated the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) to supervise official laboratories, as National Reference Laboratories (NRL). Respectively, the Laboratory for Food Safety (*Salmonella* and *Listeria* Unit and *Staphylococcus*, *Bacillus* and *Clostridium* Unit) is NRL for L. *monocytogenes* and CPS and the Ploufragan-Plouzané-Niort Laboratory (Hygiene and Quality of Poultry and Pig Products Unit) for *Salmonella* spp. As such, these units harmonise and improve the methods of official laboratories and their use. NRLs are especially mandated, where appropriate, to organise regular inter-laboratory proficiency tests (PTs) in order to demonstrate the analytical competence of official laboratories (Anonymous, 2017a). The objectives of these PTs are i) to carry out initial and continuing assessments of the official laboratories' ability to implement the official analyses, ii) to provide technical advice in case of non-satisfactory results and iii) to provide scientific support to the competent authority in its decisions regarding approval of the control laboratories.

In comparison with commercial PT schemes dedicated to these analyses (Augustin and Carlier, 2002), the framework of these PTs scheme organised by the NRLs, and prescribed by the competent authority, presents specific requirements for approval of participating laboratories as official laboratories (Anonymous, 2017a). In particular, the choice of food matrices and the contamination levels must mimic as close as possible those found in everyday life in laboratories. The main commercial PT providers generally offer PTs with freeze-dried samples that are not representative of naturally contaminated food.

Between 2019 and 2021, French NRLs for L. *monocytogenes, Salmonella* spp. and CPS organised commonly three PTs in total, one each year, for official laboratories using fresh food samples spiked at different challenge levels. The aim was to monitor the ability of official laboratories to implement the detection and enumeration of L. *monocytogenes*, the detection of *Salmonella* spp. and the enumeration of CPS in food.

This paper describes the design, the implementation, the results and the follow-up of these PTs. It concludes on the robustness of the official analysis implemented for the microbiological control of food.

2. Materials and methods

PT development and implementation were carried out according to a plan defined and approved by the PT organisation team. This plan set up the PT according to the requirements of the NF EN ISO/IEC 17043 standard (Anonymous, 2010) and to the organisers' quality management system. All PTs have been organised according to the versions of the ISO standards at the time of PT organisation.

PT coordination, results processing as well as the drafting of the reports were jointly carried out by the three NRLs.

For these yearly PTs, sample preparation, homogeneity and stability testing, shipping of packages as well as collection of results were subcontracted to a commercial PT provider (BIPEA, France) accredited according to the NF EN ISO/IEC 17043 standard (Anonymous, 2010).

The session was launched by the three NRLs informing the participants of the objectives, purpose and basic design of the PT scheme, including relevant information such as the contact information of the PT provider, activities subcontracted, a detailed description of samples and statistical analysis, as well as the time schedule for the various steps of the PT.

2.1. Proficiency test design

Between 2019 and 2021, three PTs were organised by the french NRLs for L. *monocytogenes, Salmonella* spp. and CPS in food, one per year. To better assess the performance of the network during these three years, different types of samples were prepared for qualitative or quantitative determinations (Table 1).

These PTs included three main steps: preparation of homogeneous and stable samples, analyses by laboratories applying reference methods and/or alternative methods and statistical treatment of the data.

Food matrices used in these PTs were selected based on recent food recalls or FPO for the considered pathogens. They are regularly found in routine laboratory analyses. Prior to samples preparation, these matrices were tested to verify the absence of the target pathogens (Table 1). In addition, the subcontractor has been using these types of matrices for many years and rigorous control of the samples preparation process ensures they meet PT homogeneity and stability criteria. Moreover, these historical data could be used to demonstrate the suitability of the couple "matrix- bacteria". The weight of laboratory samples were 25 g for qualitative analysis and 10 g for quantitative analysis.

The selected strains used to contaminate the samples were representative of the contaminated samples routinely analysed by the French network of official laboratories. Strains came from the three NRL collections or from BIPEA's own collection. All strains were wild-type strains isolated from various matrices at the NRL laboratories, except for the *Staphylococcus epidermidis* strain (Table 2). All of them have been tested for the capacity of growth on the matrices prepared in the frame of PT organisation.

The PT criteria for the evaluation of performance were established according to the NF EN ISO 13528 standard (Anonymous, 2015).

The samples analyses had to be performed by the participants according to reference or alternative commercial methods, validated by a third party (e.g. AFNOR Certification, MicroVal, etc.), according to the NF EN ISO 16140-2 (Anonymous, 2016b) and the requirements of EC Regulation No 2073/2005 (article 5) (Anonymous, 2005).

Table 1
Overview of the PTs organised by the NRLs for L. *monocytogenes*, *Salmonella* spp. and CPS.

PT year	Target microorganism	Type of analysis	Food product	Number of replicates per type of sample	Pathogen Enumerated level in the samples			Annex flora Enumerated level
					Blank	Low	High	
2019	*L. monocytogenes*	Detection	Smoked salmon	2 blank 6 low level 1 high level 1 decoy*	Undetectable in 25 g	11 CFU/ 25 g	300 CFU/ 25 g	Presence of *Listeria innocua* • 200 CFU/25 g for blank • 5 CFU/25 g for low level • 120 CFU/25 g for high level Presence of *Shigella dysenteriae*
	Salmonella Enteritidis	Detection	Minced meat (beef)	2 blank 6 low level 1 high level 1 decoy	Undetectable in 25 g	11 CFU/ 25 g	270 CFU/ 25 g	• 24,000 CFU/25 g for blank • 25,000 CFU/25 g for low level • 19,000 CFU/25 g for high level Presence of *Staphylococcus epidermidis*
	Staphylococcus aureus	Enumeration	Milk	1 blank 1 low level 1 high level 1 decoy	< 10 CFU/mL	440 CFU/mL	15,000 CFU/mL	• 1000 CFU/mL for blank • 460 CFU/mL for low level • 28,000 CFU/mL for high level Presence of *Listeria innocua*
	L. monocytogenes	Enumeration	Smoked salmon	1 blank 1 low level 1 high level 1 decoy	< 10 CFU/g	250 CFU/g	9500 CFU/ g	• 760 CFU/g for blank • 16 CFU/g for low level • 830 CFU/g for high level Presence of *Proteus mirabilis*
2020	*Salmonella* Agona	Detection	Minced meat (beef)	2 blank 6 low level 1 high level 1 decoy	Undetectable in 25 g	4 CFU/ 25 g	190 CFU/ 25 g	• 1900 CFU/25 g for blank • 1900 CFU/25 g for low level • 1900 CFU/25 g for high level Presence of *Staphylococcus epidermidis*
	Staphylococcus aureus	Enumeration	Shrimp	1 blank 1 low level 1 high level 1 decoy	< 10 CFU/g	410 CFU/g	14,000 CFU/g	• 1200 CFU/g for blank • 400 CFU/g for low level • 15,000 CFU/g for high level Presence of *Listeria innocua*
2021	*L. monocytogenes*	Detection	Smoked salmon	2 blank 6 low level 1 high level 1 decoy	Undetectable in 25 g	10 CFU/ 25 g	400 CFU/ 25 g	• 120 CFU/25 g for blank • 90 CFU/25 g for high level Presence of *Proteus mirabilis*
	Salmonella Typhimurium monophasic variant	Detection	Dry sausage	2 blank 6 low level 1 high level 1 decoy	Undetectable in 25 g	3 CFU/ 25 g	240 CFU/ 25 g	

(*continued on next page*)

Table 1 (*continued*)

PT year	Target microorganism	Type of analysis	Food product	Number of replicates per type of sample	Pathogen Enumerated level in the samples			Annex flora Enumerated level
					Blank	Low	High	
								• 570 CFU/25 g for blank • 740 CFU/25 g for low level • 740 CFU/25 g for high level Presence of *Staphylococcus epidermidis*
	Staphylococcus aureus	Enumeration	Minced meat (beef)	1 blank 1 low level 1 high level 1 decoy	< 10 CFU/g	1900 CFU/g	15,000 CFU/g	• 530 CFU/g for blank • 230 CFU/g for low level • 3900 CFU/g for high level

* Decoy sample: i.e. a randomly chosen sample from the contaminated or blank samples.

Table 2
Bacterial strains used in each inter-laboratory proficiency test.

Bacterial strain	PT year(s)	PT provider code	PT strain status
Salmonella Enteritidis	2019	SAL1 – BM012	Target
Salmonella Typhimurium	2020	SAL12 – BM056	Target
Salmonella Agona	2021	SAL2 – BM044	Target
Shigella dysenteriae	2019	SHI1 – BM040	Non-target
Proteus mirabilis	2020–2021	PRO1 - BM042	Non-target
Listeria monocytogenes	2019–2021	LIS2 – BM014	Target
Listeria innocua	2019–2021	LIS1 – BM013	Non-target
Staphylococcus aureus	2019–2021	STA1 – BM016	Target
Staphylococcus epidermidis	2019–2021	STA2 – BM041 (ATCC 14990)	Non-target

For these PTs schemes, the reference methods were:

- the NF EN ISO 6579-1 (2017) method (Anonymous, 2017b) for the detection of *Salmonella* spp. in food,
- the NF EN ISO 11290-1 method (Anonymous, 2017c) for the detection of L. *monocytogenes* in food,
- the NF EN ISO 11290-2 method (Anonymous, 2017d) for the enumeration of L. *monocytogenes* in food and
- the NF EN ISO 6888 part 1 (Anonymous, 2021a) and/or part 2 (Anonymous, 2021b) methods for the enumeration of CPS.

2.2. Production of proficiency test items

Every year, all participants received the same number of samples per pathogen for analysis: ten samples for detection and four samples for enumeration. For both qualitative and quantitative analyses, three separate batches of samples were prepared: a batch of negative samples, a batch of low-contamination-level samples and a batch of high-contamination-level samples (Table 1). The contamination level of the different bacterial strains was set according to the type of pathogen and the method of analysis.

For qualitative analyses, the target concentrations for the low contamination level were planned so that participating laboratories would obtain in theory approximately 50 % positive samples, i.e. positive results should be observed for only a fraction of the samples. The purpose of this level was to evaluate the performance of each laboratory at a level near to the detection threshold of the reference methods, for L. *monocytogenes* and *Salmonella*. Laboratory performance was assessed by using an appropriate statistical analysis of the results with an expected proportion of positive replicated samples (Anonymous, 2019).

Over three years, samples consisted of solid matrices, such as minced meat, dry sausage, smoked salmon and shrimp, or milk for liquid matrix (Table 1).

Solid matrices were weighed in sterile stomacher bags and then frozen until the day of inoculation with the different bacterial strains. They were individually contaminated by adding 1 mL inoculum of bacterial suspension adjusted to the target concentration. A second weighing of the stomacher bag was carried out to check that the weight had been increased by spiking and to confirm that spiking was correctly implemented.

For the liquid matrix (milk), batches were prepared by contaminating a sufficient volume of milk, homogenised by shaking and then 30 mL were aliquoted in sterile vials using a gravimetric dilutor. The samples were stored at 3 ± 2 °C until the day of shipment.

Additionally, to better mimic real conditions, competitive flora were added to the samples (Table 2).

In each panel of ten samples for detection and four samples for enumeration, a decoy sample, randomly chosen from the contaminated or blank samples, was added to avoid any collusion between participants. Therefore, half of the participants received a negative sample and the other half a highly contaminated sample. Results obtained for the decoy samples were not included in the assessment of laboratory performance.

A specific labelling procedure was also used so that the batch number did not appear on the samples.

2.3. Homogeneity and stability

Several controls were carried out to check the contamination of the samples, their homogeneity and their stability. According to normative requirements (NF EN ISO/IEC 17043 and NF EN ISO 13528), the samples must be sufficiently homogeneous and stable to assess laboratory performance reliably (Anonymous, 2010, 2015).

Previous studies had been performed prior to the current PT to determine the feasibility of sample preparation, in terms of concentration, stability and homogeneity.

Production controls were carried out by BIPEA on the day of sample production, on five samples per contamination level (including the negative samples) using a reference method for each target pathogen. The purpose of these controls was to verify the sample contamination for qualitative analyses and the level of sample contamination for quantitative analyses.

Considering that the contaminated samples have a limited stability, the laboratories were asked to analyse samples on a fixed date. Homogeneity tests were performed on 20 samples, each analysed once, for

each contamination level, on the same day that samples were analysed by the participating laboratories.

This verification of homogeneity also helps to determine the stability of the samples, by comparison with the results obtained for the production controls. All of these homogeneity tests were carried out by an accredited NF EN ISO/IEC 17025 laboratory selected by BIPEA applying reference methods (Anonymous, 2017e).

2.4. Shipping

All panels were shipped in refrigerated packages to maintain a temperature of 5 ± 3 °C. A temperature logger was placed in each package to verify that the temperature was maintained throughout the transport to the participating laboratories and checked upon receipt.

2.5. Reporting and acceptability of results

At the end of the sample analysis period, participants submitted their results to the BIPEA platform. Then, BIPEA transmitted all the results with the blind coding removed to the NRL for assessment.

Prior to launching the statistical analyses, the reliability of the results was verified, to check any deviations:

- Analyses performed on a day different from the pre-determined date, incomplete results or results sent after the deadline,
- Storage temperature of samples prior to analysis different from that detailed in the instructions,
- Positive results obtained for negative samples (false positives).

If one of the deviations mentioned above was reported, none of the results of the corresponding laboratory were further assessed.

2.6. Evaluation of individual laboratory performance

2.6.1. Detection of Listeria monocytogenes and Salmonella spp.

For the qualitative methods in the PT, the results were reported in terms of detection or non-detection. According to the NF EN ISO 22117 standard (Anonymous, 2019) specific to PT organisation in food-chain microbiology, the interpretation of the participating laboratory's results was as follows:

- for negative samples: all samples should be found negative;
- for the high-contamination-level samples: all samples should be found positive;
- for the low-contamination-level samples: each laboratory was expected to find a proportion of positives among the replicated positive samples, calculated using the binomial distribution at a 95 % confidence level and the percentage of samples found positive by all participating laboratories. These results will depend on the consensus of positive findings obtained by all the laboratories. According to NF EN ISO 22117 part 8.4.2 (Anonymous, 2019), given a decision threshold of $p \leq 0.05$ and the probability of finding a certain number of positive samples according to the binomial distribution, an acceptability range) was defined to assess the laboratory's results which means that a number of samples out of the six samples of low-contamination-level are allowed to be not detected.

In addition, specificity and sensitivity rates per level of contamination (defined in NF EN ISO 16140-1) were calculated for each laboratory and for the results from all laboratories (Anonymous, 2016a).

2.6.2. Enumeration of Listeria monocytogenes and CPS

2.6.2.1. Determination of assigned value and standard deviation. According to the NF EN ISO 13528 standard (Anonymous, 2015), the consensus value from participants was used as one of the possibilities to determine the assigned value (x_{pt}). The standard deviation (σ_{pt}) used to assess laboratory proficiency was also derived from the results reported by the participating laboratories. This consensus approach is the preferred option when using empirical methods, where the result depends directly on the principle behind the method used, such as microbiological counting techniques.

For each contamination level or each combination batch/reference method (for CPS enumeration), the assigned value was the median of all participating laboratories (x^*) and the standard deviation was the robust standard deviation (s^*). x^* and s^* were calculated using Algorithm A (Annex C, NF EN ISO 13528, (Anonymous, 2015)). These robust estimators were chosen to avoid excluding statistical outliers, because robust statistics are less sensitive to extreme values than arithmetic statistics.

Calculations were carried out in an Excel spreadsheet developed and validated by ANSES.

The results of the negative control were not statistically evaluated.

2.6.2.2. Evaluation of individual laboratory performance. The individual z-score is one of the performance statistics recommended by NF EN ISO 13528 and is the most commonly used approach to assess individual laboratory performance in terms of trueness/bias (Anonymous, 2015).

For each laboratory i, an individual z-score was calculated as described in NF EN ISO 13528 (Anonymous, 2015):

$$z = \frac{(x_i - x_{pt})}{\sigma_{pt}} \quad (1)$$

where

x_i result for laboratory i,
x_{pt} consensus assigned value (x^*) and
σ_{pt} consensus standard deviation for proficiency assessment (s^*).

In case of heterogeneous samples, the between-sample standard deviation S_s was introduced in the calculation of the standard deviation for proficiency assessment (σ'_{pt}):

$$\sigma'_{pt} = \sqrt{\sigma_{pt}{}^2 + Ss^2} \quad (2)$$

where

σ_{pt} is the standard deviation for the proficiency assessment that does not include the heterogeneity of the samples and S_s is the between-sample standard deviation.

According to NF EN ISO 13528, the z-score is interpreted as follows (Anonymous, 2015):

- if $|z| \leq 2.0$ the result of the laboratory is acceptable;
- if $2.0 < |z| < 3.0$ the result of the laboratory gives a warning signal;
- if $|z| \geq 3.0$ the result of the laboratory is unacceptable (it gives an action signal).

At the end of the PT, depending on the results and in support of measures for continuous improvement, when laboratories do not obtain the expected results, NRL requested an internal performance review and a root-cause analysis to identify corrective actions.

3. Results

The capacity of the laboratories to detect L. *monocytogenes* and *Salmonella* spp. as well as to enumerate L. *monocytogenes* and CPS in food was assessed in different matrices previously incriminated in several FPOs in Europe: minced meat, dry sausage, smoked salmon, milk and shrimp (Table 1).

In the three PTs, a blank and two spiking levels (low and high) were used, each level being applied to the different matrices. Over the three years, all participants reported their results on time, thus all participants' results could be processed for the analysis. The results were

treated confidentially according to the NF EN ISO/IEC 17043 standard (Anonymous, 2010) and to the organisers' quality assurance management schemes.

A report including the interpretation of results was transmitted to all the participants. This report also included information on the analytical methods used by the laboratories (Fig. 1). Results showed a high percentage of laboratories (≈70 %) using the reference methods for the detection or enumeration of L. *monocytogenes* and for the detection of *Salmonella* spp. and almost all used (≈90 %) the reference methods for CPS enumeration.

For the homogeneity studies, analyses were conducted on the required day of analysis by participants.

- For the qualitative analysis (detection of L. *monocytogenes* and *Salmonella* spp.), the samples panels were considered as sufficiently homogeneous during the three PTs. L. *monocytogenes* and *Salmonella* were not detected in any of the blank samples, were detected in 100 % of the high contamination samples and were detected in ≥80 % of the low contamination samples.
- For the quantitative analysis (enumeration of *L. monocytogenes* and CPS), the samples were considered as sufficiently homogeneous except for L. *monocytogenes* in 2020 at low and high contamination levels and for CPS at low contamination levels in 2020 and 2021. In these three cases, as described in NF EN ISO 13528, the calculation of the standard deviation for proficiency assessment took this heterogeneity into account (Anonymous, 2015).

3.1. Results for Listeria monocytogenes.

3.1.1. 2019 and 2021 proficiency tests on the detection of Listeria monocytogenes

The qualitative results obtained, in 2019 and 2021, for *L. monocytogenes* are presented in Table 3. In 2019, for *L. monocytogenes* at the low contamination level, the laboratory results were considered acceptable if a positive result was obtained for at least one out of the six replicates.

In 2021, the laboratory results were considered acceptable if a positive result was obtained for at least three out of the six replicates.

The overall sensitivity for low level contamination was 62.1 % and 76.2 % for the detection of L. *monocytogenes* respectively in 2019 and 2021 (Table 3).

The overall sensitivity for high level contamination was 100 % and 98.5 % for the detection of *L. monocytogenes* respectively in 2019 and 2021.

The overall specificity for the blank samples was 99.3 % and 99.2 % for the detection of *L. monocytogenes* respectively in 2019 and 2021 (Table 3).

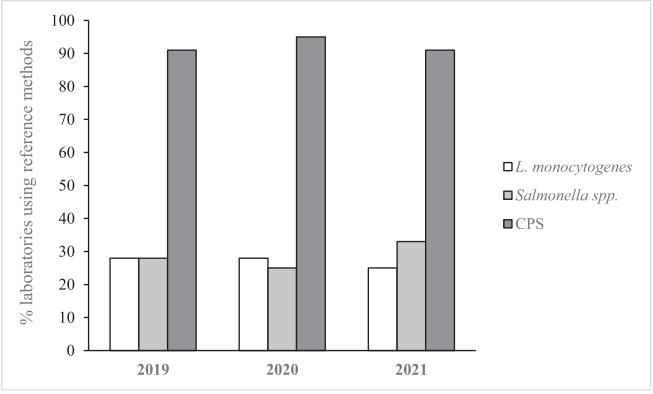

Fig. 1. Percentage of laboratories that used a reference method to carry out the analyses.

In 2019, among 69 participating laboratories, five laboratories obtained discrepancies, their results were considered as unacceptable by the NRL L. *monocytogenes*:

- Four laboratories did not detect any of the low contamination samples and
- One laboratory provided a positive result for a negative sample.

In 2021, among 65 participating laboratories, six laboratories obtained discrepancies and their results were considered as unacceptable by the NRL *L. monocytogenes*:

- One laboratory did not provide a result for a negative sample,
- Five laboratories did not detect L. *monocytogenes* at the low contamination level in at least three of the replicates (out of six replicates in total) and
- One laboratory did not detect L. *monocytogenes* at the high contamination level.

Laboratories with unacceptable results were contacted by the NRL L. *monocytogenes* and asked to implement corrective actions within their laboratory. To assess their appropriateness, these actions were evaluated by the NRL and deemed relevant for 100 % of laboratories.

3.1.2. 2020 proficiency test on the enumeration of Listeria monocytogenes

The quantitative results obtained, only in 2020, for L. *monocytogenes* are presented in Table 3 and Fig. 2. The assigned values (x_{pt}) and standard deviation for proficiency assessment (σ_{pt}) were calculated for each contamination level (Table 4).

Because the low and high contamination levels were not homogeneous, the heterogeneity of the samples was taken into account to determine the standard deviation for proficiency assessment.

For the high contamination level, the performance of four laboratories was not evaluated, because these laboratories reported their results as "> or < X CFU/g" and the organiser required a numerical value to calculate a *z*-score, according to the instructions to participants.

All evaluated laboratories demonstrated an acceptable performance in terms of trueness ($|z| < 3.0$).

The four non-evaluated laboratories were contacted by the organiser and had to identify and implement corrective actions within their laboratory. These actions were evaluated by the NRL and deemed relevant. After implementation of the corrective actions, 100 % of laboratories were considered to have reported acceptable results and their performance deemed satisfactory.

3.2. Results for Salmonella spp.

The qualitative results obtained for *Salmonella* spp. are presented in Table 5. According to NF EN ISO 22117, given a decision threshold of $p \leq 0.05$ and the probability of finding a number of replicated positive samples according to the binomial distribution, an acceptability interval was defined to assess participants' results (Anonymous, 2019). Therefore, for *Salmonella* detection at the low contamination level in the 2019, 2020 and 2021 PTs, based on the result of the consensus obtained by all the participants regarding the strains and the matrices used, a participant's result was considered acceptable when *Salmonella* spp. was detected at least in respectively five, three and one replicates out of the six replicates (Table 5). The sensitivity of these PTs for the detection of *Salmonella* spp. ranged from 46.3 % to 96.0 % for the low contamination samples and from 97.2 % to 100 % for the high contamination samples.

Overall, the success rate of satisfactory laboratories in these PTs for detecting *Salmonella* in food matrices exceeded 90 % over the period 2019–2021 whatever the strains and the matrices analysed. The addition of a competitive annex flora in the samples did not perturb the detection of *Salmonella* by the participating laboratories, whatever the contamination level.

Table 3
Summary of the evaluation of laboratory performance in the detection and enumeration of *Listeria monocytogenes*.

PT year	2019 (69 participants)			2020 (67 participants)			2021 (65 participants)		
Target strain	*L. monocytogenes*			*L. monocytogenes*			*L. monocytogenes*		
Contamination level	Blank	Low	High	Blank	Low	High	Blank	Low	High
Number of samples analysed	138	414	69	67	67	67	130	390	65
Number of acceptable results	137	257	69	NA	NA	NA	129	300	64
Specificity rate (%)	99.3	–	–	NA	NA	NA	99.2	–	–
Sensitivity rate (%)	–	62.1	100	NA	NA	NA	–	76.9	98.5
Number of correct replicates required to attain acceptable results	*2/2*	*1/6*	*1/1*	NA	NA	NA	*2/2*	*3/6*	*1/1*
Laboratories with acceptable results	*68/69*	*65/69*	*69/69*	NA	NA	NA	*64/65*	*60/65*	*64/65*
Laboratories with unacceptable results ($\|z\| \geq 3.0$)	NA	NA	NA	*0/67*	*0/67*	*0/63*	NA	NA	NA
Laboratories with warning results ($2.0 < \|z\| < 3.0$)	NA	NA	NA	*0/67*	*0/67*	*0/63*	NA	NA	NA
Laboratories with acceptable results ($\|z\| \leq 2.0$)	NA	NA	NA	*67/67*	*67/67*	*63/63*	NA	NA	NA
Number of satisfactory laboratories	*64/69 (93 %)*			*63/67 (94 %)*			*59/65 (91 %)*		

NA: Not applicable.

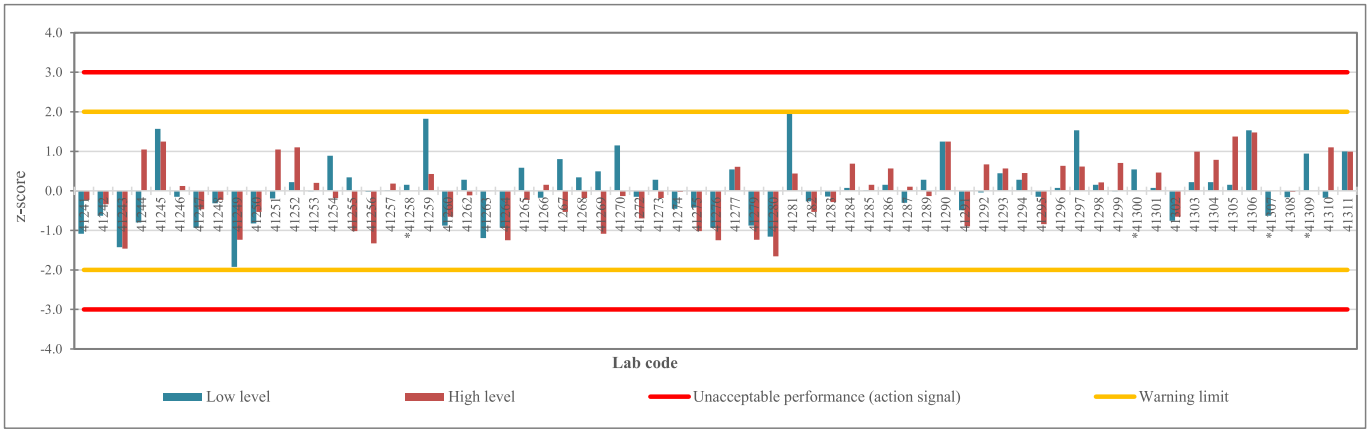

Fig. 2. *z*-scores for low and high contamination levels for L. *monocytogenes* enumeration (2020)
**: laboratory with data not assessed (partly or totally).*

Table 4
Statistical parameters to assess the performance for the PT for L. *monocytogenes* in 2020.

	2020		
Contamination level	n	x_{pt} (log CFU/g)	σ'_{pt}
Low	67	3.0	0.55
High	63	4.6	0.51

n: number of laboratories included in the calculations, per batch; x_{pt}: assigned value; σ'_{pt}: standard deviation for proficiency assessment (with heterogeneity of the contamination level taken into account meaning that S_s (between-sample standard deviation) was included in the calculation).

The overall specificity for the blank was at least 99.7 % for the detection of *Salmonella* spp. in these three PTs (Table 5).

In 2019, among 71 participating laboratories, five laboratories obtained discrepancies, their results were considered as unacceptable by the NRL *Salmonella*:

- Two laboratories did not detect *Salmonella Enteritidis* at the high contamination level and
- Three laboratories did not detect *Salmonella Enteritidis* at the low contamination level in at least two of the replicates (out of six replicates in total)

In 2020, among 69 participating laboratories, four laboratories

Table 5
Summary of the performance evaluation for *Salmonella* detection.

PT year	2019 (71 participants)			2020 (69 participants)			2021 (68 participants)		
Target strain	*S. enteritidis*			*S.* Agona			*S. typhimurium* monophasic variant		
Contamination level	Blank	Low	High	Blank	Low	High	Blank	Low	High
Number of samples analysed	142	426	71	138	414	69	136	408	68
Number of acceptable results	142	409	69	135	372	68	133	189	68
Specificity rate (%)	100	–	–	97.8	–	–	97.8	–	–
Sensitivity rate (%)	–	96.0	97.2	–	89.9	98.6	–	46.3	100
Number of correct replicates required to attain acceptable results	*2/2*	*5/6*	*1/1*	*2/2*	*3/6*	*1/1*	*2/2*	*1/6*	*1/1*
Laboratories with acceptable results	*71/71*	*68/71*	*69/71*	*66/69*	*69/69*	*68/69*	*65/68*	*65/68*	*68/68*
Number of satisfactory laboratories	*66/71 (93 %)*			*65/69 (94 %)*			*62/68 (91 %)*		

obtained discrepancies, their results were considered as unacceptable by the NRL *Salmonella*:

- Three laboratories provided a positive result for a negative sample and,
- One laboratory did not detect *Salmonella* Agona at the high contamination level

In 2021, among 68 participating laboratories, six laboratories obtained discrepancies, their results were considered as unacceptable by the NRL *Salmonella*:

- Three laboratories provided a positive result for a negative sample and,
- Three laboratories did not detect monophasic variant of *Salmonella Typhimurium* in any low contamination level

Laboratories with unacceptable results were contacted by the NRL *Salmonella* and asked to implement corrective actions within their laboratory. To assess their appropriateness, these actions were evaluated by the NRL and deemed relevant for 100 % of concerned laboratories.

3.3. Results for coagulase-positive staphylococci (CPS)

Regarding CPS enumeration, the quantitative results are presented in Table 6 and Fig. 3.

Table 7 gives the statistical parameters (assigned values (x_{pt}) and standard deviation for proficiency assessment (σ_{pt})) to assess the performance in the 2019 to 2021 PTs.

In 2020 and 2021, because the low contamination level was not sufficiently homogeneous, the heterogeneity of the samples was taken into account for the determination of the standard deviation for proficiency assessment.

In 2019 and 2020, the performance was assessed separately for the NF EN ISO 6888 part 1 and part 2 standard methods and the alternative commercial methods (TEMPO STA BIO 12/28–04/10, EASY STAPH BKR 23/10–12/15) (Anonymous, 2021a, 2021b). However, in 2021, performance was assessed overall, considering all the results whatever the method used.

In 2019, among 68 participating laboratories, seven laboratories showed discrepancies, their results were considered as unacceptable by the NRL for CPS (Table 6):

- One laboratory obtained a positive deviation (false positive): according to the rejection criterion, all the results of this laboratory were excluded from the proficiency assessment,
- One laboratory obtained a negative deviation (false negative) for the low contamination level and an unacceptable *z*-score ($|z| \geq 3.0$) for the high contamination level and
- Six laboratories obtained an unacceptable *z*-score ($|z| \geq 3.0$) for the low and/or high contamination levels.

All these laboratories were contacted by the NRL for CPS and received a deviation form to justify or explain their results. The proposed corrective actions were evaluated by the NRL and deemed appropriate. For information, one participant recognized a calculation mistake allowing to transform its results into satisfactory results for the competent authority.

Finally, after the treatment of deviations, only one laboratory and four laboratories obtained an unacceptable *z*-score for low and high contamination levels, respectively. After implementation of the corrective actions, 93 % of laboratories were considered to have reported acceptable results and their performance deemed satisfactory.

In 2020, among 67 participating laboratories, no laboratory obtained an unacceptable *z*-score ($|z| \geq 3.0$), but eight laboratories showed discrepancies:

- One laboratory reported a positive deviation (false positive) for the blank level: as a rejection criterion, all the results of this laboratory were excluded from the proficiency assessment and
- Seven laboratories reported a result as "< X CFU/g" for the low contamination level and their performance was not evaluated, because numerical values were requested by the organiser to calculate a *z*-score, according to the instructions to participants. The addition of a competitive annex flora in the samples and the low concentration of the target strain for the low contamination level appeared to perturb the enumeration of *S. aureus* in these participating laboratories.

All these laboratories received a deviation form to justify or explain such a result. However, after the implementation of corrective actions, the number of laboratories with acceptable results did not change (88 %).

In 2021, among 65 participating laboratories, two laboratories obtained an unacceptable *z*-score ($|z| \geq 3.0$) for the low or high contamination levels. There were no positive deviations (false positives), but one laboratory expressed results of negative sample as <1000 CFU/g. However, the results of this latter laboratory were not excluded from the analysis. Normally, the result for this level should be <10 or 100 CFU/g. The addition of a competitive annex flora in the samples appeared to perturb the enumeration of *S. aureus* for this participating laboratory. Nevertheless, it received a deviation form to justify or explain this result. Moreover, the references of laboratory code and sample codes for two laboratories were not in accordance with those expected. Their results were not taken into account to establish the assigned value and standard deviation for the proficiency assessment.

These laboratories were contacted by the NRL for CPS and asked to implement corrective actions within their laboratory.

Finally, after implementation of corrective actions, only one laboratory still obtained an unacceptable *z*-score for the low contamination level. The number of laboratories with acceptable results thus increased to 98 % (versus 92 % before corrective actions). In general, over the three years, most evaluated laboratories demonstrated acceptable performance in terms of trueness ($|z\text{- score}| < 3.0$), with >88 % of

Table 6
Summary of the performance evaluation for CPS enumeration.

PT Year	2019 (68 participants)			2020 (67 participants)			2021 (65 participants)		
Target strain	*S. aureus*			*S. aureus*			*S. aureus*		
Contamination level	Blank	Low	High	Blank	Low	High	Blank	Low	High
Number of samples analysed	68	68	68	67	67	67	65	65	65
Laboratories with unacceptable results ($\|z\| \geq 3.0$)	–	*2/66*	*5/67*	–	*0/59*	*0/66*	–	*1/62*	*1/63*
Laboratories with warning results ($2.0 < \|z\| < 3.0$)	–	*3/66*	*2/67*	–	*0/59*	*1/66*	–	*1/62*	*2/63*
Laboratories with acceptable results ($\|z\| \leq 2.0$)	–	*61/66*	*60/67*	–	*59/59*	*65/66*	–	*60/62*	*60/63*
Number of satisfactory laboratories	*61/68 (90 %)*			*59/67 (88 %)*			*60/65 (92 %)*		

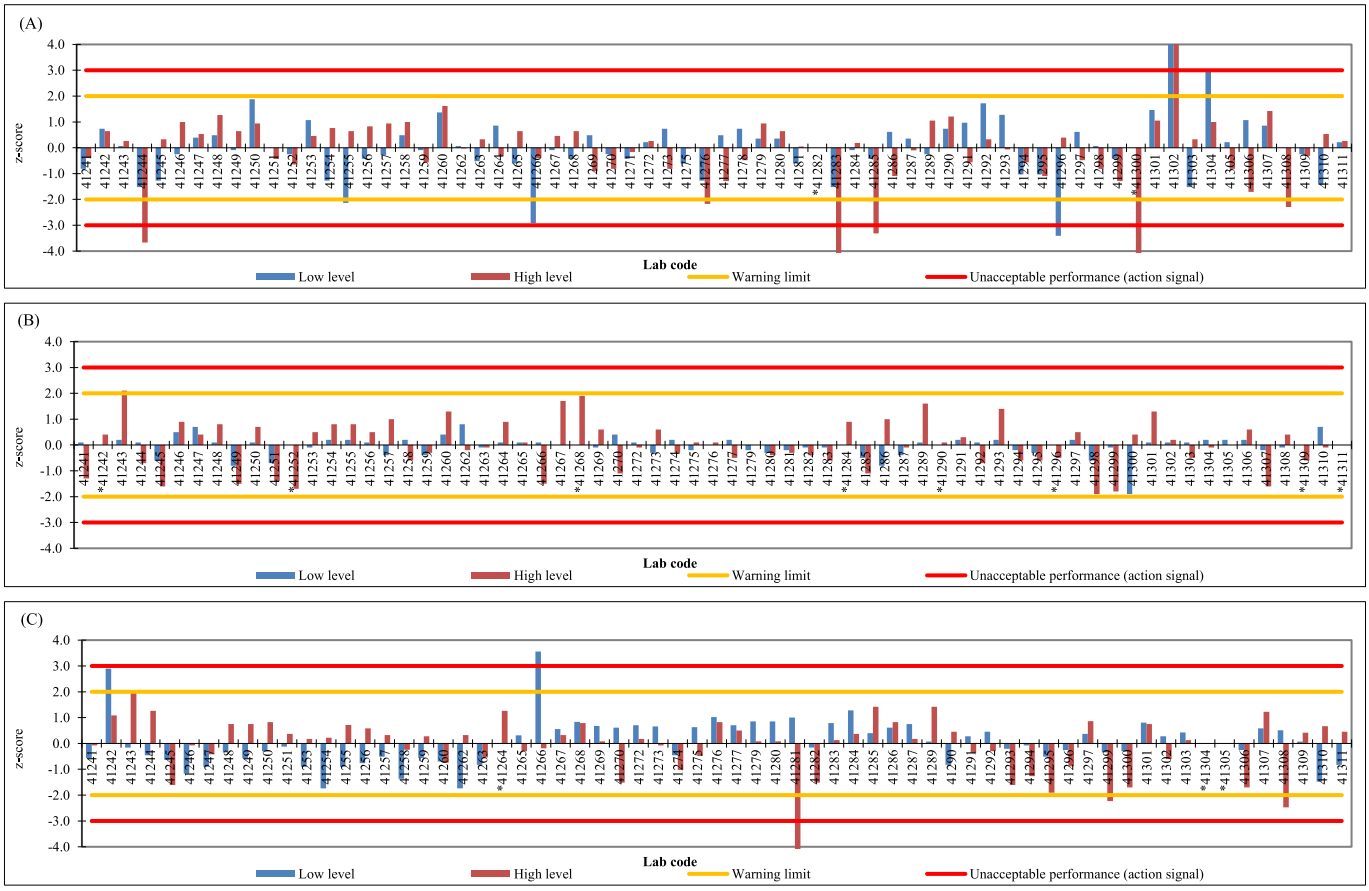

Fig. 3. *z*-scores for low and high contamination levels for CPS enumeration for the 2019 (A), 2020 (B) and 2021 (C) PT, regardless of the method used (NF EN ISO 6888-1, NF EN ISO 6888-2 and alternative methods (NF V08–057-1, TEMPO STA BIO 12/28–04/10, EASY STAPH BKR 23/10–12/15))
**: laboratory with data not assessed (partly or totally).*

Table 7
Statistical parameters to assess the performance for the PT for CPS in 2019, 2020 and 2021.

Contamination level	Method	2019			2020			2021		
		n	x_{pt} (log CFU/mL)	σ_{pt}	n	x_{pt} (log CFU/g)	σ_{pt}	n	x_{pt} (log CFU/g)	σ_{pt}
Low	NF EN ISO 6888-1[a]	6	2.25	0.18	9	2.23	0.63[d]	62	2.53	0.33[d]
	NF EN ISO 6888-2[b]	60	2.24	0.16	50[c]	2.05	0.55[d]			
High	NF EN ISO 6888-1[a]	6	3.95	0.10	9	3.77	0.19	63	3.73	0.16
	NF EN ISO 6888-2[b]	61	3.74	0.11	57	3.63	0.16			

n: number of laboratories included in the calculations, per batch; x_{pt}: assigned value; σ_{pt}: standard deviation for proficiency assessment.
[a] Alternative method NF V 08–057-1 was associated with the NF EN ISO 6888-1 standard.
[b] Alternative methods BKR 23/10–12/15 and BIO 12/28–04/10 were associated with the NF EN ISO 6888-2 standard.
[c] Results expressed as < xxx CFU/g were not taken into account: calculation of z-score is not possible.
[d] Corresponds to σ'_{pt} (with heterogeneity of contamination level taken into account meaning that s_s: (between-sample standard deviation) was included in the calculation).

satisfactory laboratories.

4. Discussion

To ensure food safety and quality, a network of laboratories that can reliably detect or enumerate foodborne pathogens is important for official controls, to prevent contaminated food products from being placed on the market and consumed (Chlebicz and Slizewska, 2018; Rodríguez-Lázaro et al., 2007). Thus, since 2019, the three NRLs for L. *monocytogenes*, *Salmonella* and CPS have organised annual PT and jointly evaluate the proficiency of almost 70 laboratories conducting analyses for official controls throughout the country, including overseas territories. These PTs represent around 4300 analysed samples for the three pathogens. Laboratory performance was evaluated for the first time through three common PT on a diverse panel of real food matrices previously involved in significant FPO, representative of routine laboratory analysis conditions and at contamination levels near the regulatory limits.

These PTs highlighted the satisfactory analytical capacity and the robustness of the French official laboratory network for the detection and enumeration of L. *monocytogenes*, the detection of *Salmonella* and the enumeration of CPS in foodstuffs. For the three annual PT, the results obtained using the detection and enumeration methods (reference or alternative methods) for these three pathogens were satisfactory.

One of the main advantages of these PTs schemes, for the participants and official control authorities, is that they were organised by the three NRLs (*L. monocytogenes*, *Salmonella* and CPS), using relevant fresh food matrices. Such matrices are rarely used for PTs organised by commercial providers (Augustin and Carlier, 2002, 2006). Moreover, these PTs provided the opportunity, when the same matrix was used, to select different strains for contaminating the samples.

Unlike commercial PT schemes, participating laboratories were invited to suggest corrective actions in response to the observations made by the PT organisers. In the frame of this follow-up, two types of deviations were communicated to the participants. The first type, which led to the cancellation of participation to the PT, included deviations on non-respect of the instructions to participants such as sample reception and/or non-respect of results submission deadline. The second type was related to the performance evaluation based on the results obtained such as false negative results. The initial step of the evaluation was to explain the deviation to the participants and request a primary corrective action. According to this proposal, the organisers decided whether the suggested corrective action was satisfactory or not. If needed, further exchanges could occurred between the organiser and the participant.

They were asked to determine potential areas of improvement for future testing. Good laboratory practices must be followed to maintain high-quality results. Corrective actions include operator training, using new or alternative testing methods or reagents (such as culture media), enhancing data quality control and upgrading, calibrating or replacing equipment.

These corrective actions were then assessed by the respective NRL to evaluate their effectiveness, adjustments could be made and their efficiency re-evaluated in the following year's PT. In the case of unsatisfactory results with insufficient corrective actions, in addition the NRL offered three options to the concerned laboratory: (i) scheduling a meeting to discuss and better understand the issues faced by the laboratory, with the goal of collaborating to find an appropriate solution; (ii) participating to a bilateral assay organised by the NRL or a commercial proficiency test, both providing to the laboratory a second opportunity to verify its ability to obtain satisfactory results in the frame of a global evaluation by the NRL; or (iii) participating in a dedicated training session, organised by the NRL, to help to strengthen knowledge and validate skills. Additionally, the laboratory was encouraged to propose preventive actions to improve the reliability of its results in the future.

NRLs focus not only on the outcome of the corrective actions, but also on the actions they can offer to their network. By consequences, in addition to technical training on detection, enumeration and identification methods for the three pathogens, NRLs also organise informative webinars for laboratories. At least every two years, the results of these PTs are presented at workshops organised for the network to review the different PT stages, from implementation to interpretation of the results. Difficulties encountered by laboratories are discussed, along with the corrective actions taken, to share information and experiences. These workshops are also an opportunity for the network to make suggestions, for example, different matrices of interest to test.

After this round of three annual PTs, the network of laboratories was considered as stable and efficient. For this reason, the frequency of this PT scheme has been reduced to a PT every two years. In the future, the three NRLs will continue to organise common PTs for their laboratory network, to maintain the high competency of the laboratories.

5. Conclusion

PTs were successfully implemented during a regular PT scheme and demonstrated the ability of French network of official laboratories to analyse L. *monocytogenes*, *Salmonella* spp. and CPS in real foodstuffs. By participating in these PTs, laboratories had the opportunity to demonstrate the robustness of their results and obtain recognition of their analytical performance through NF EN ISO 17025 accreditation. These PTs are a valuable tool for laboratories to improve and to carry out the necessary investigations and corrective actions if any unsatisfactory results were obtained in the analysis. The PTs also allow the laboratories to maintain their approval granted by the French ministry in charge of agriculture to perform official analyses.

CRediT authorship contribution statement

Léna Barre: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Formal analysis, Conceptualization. **Florence Guillier:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Formal analysis, Conceptualization. **Bertrand Lombard:** Writing – review & editing, Formal analysis. **Corinne Danan:** Writing – review & editing, Conceptualization. **Jacques-Antoine Hennekinne:** Writing – review & editing, Conceptualization. **Boris Constantin:** Writing – original draft, Resources, Conceptualization. **Romain Le Neve:** Writing – original draft, Resources, Methodology. **Sandrine Nguyen:** Resources. **Abdelkader Boubetra:** Writing – review & editing, Resources, Conceptualization. **Marianne Chemaly:** Writing – review & editing, Conceptualization. **Yacine Nia:** Writing – review & editing, Visualization, Validation, Supervision, Formal analysis, Conceptualization. **Laetitia Bonifait:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data availability

Data will be made available on request.

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