Proficiency - testing scheme for Staphyloccocal Enterotoxin detection in milk

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INTRODUCTION

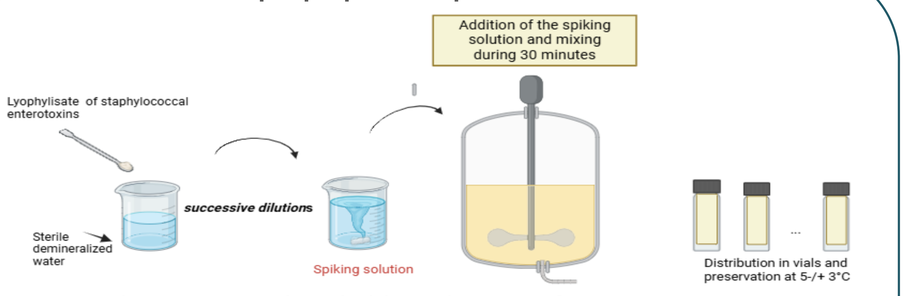
BIPEA is a European non-profit organization gathering more than 2900 member laboratories in the world throughout 130 countries. It offers more than 220 regular proficiency testing programs in various fields including microbiology.

Enterotoxins are toxic proteins produced by pathogenic bacteria such as *Staphylococcus aureus* and are responsible for foodborne illnesses that can cause severe gastrointestinal disorders. They are commonly found in products like milk and meat, which provide favorable conditions for the growth of Staphylococcus aureus and the production of its enterotoxins. Regulations set limits, require standard detection methods, and ensure the monitoring of sensitive foods.

However, because detecting these toxins remains a challenge, notably due to their low concentration levels in food, laboratories need to obtain recognition of their analytical procedures by customers and accreditation bodies according to the ISO 17025 standard [1]. To meet this demand, in 2021, BIPEA established an international Proficiency Testing Scheme (PTS) to evaluate laboratory performance in analyzing *Staphylococcal* enterotoxins (SE) in food. BIPEA is the only one PT organizer that propose a real food matrix spiked with SE concentrations comparable to those encountered in real-life. Thus, these samples have a big interest in accreditation procedure, training of analysts and method development and validation, This PT involves about 20 laboratories worldwide and is composed of two trials per year. The design is conducted in compliance with EN ISO 17043 [2], will be described, with a focus on the April 2024 trial. This process includes the preparation of spiked samples through to the statistical processing of participant results, following ISO 13528 [3].

METHODOLOGY

Sample preparation protocol



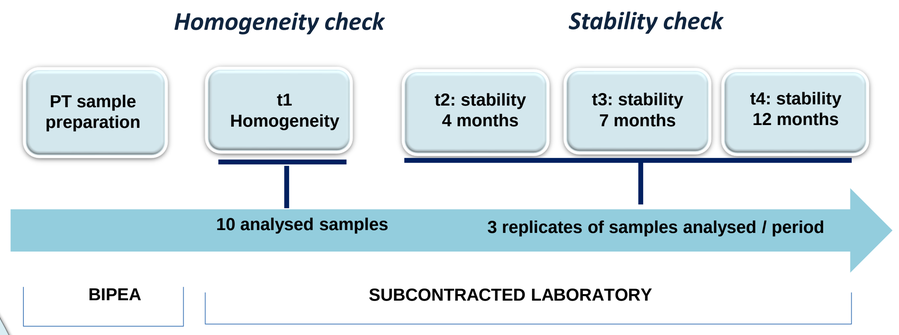
PT samples are stored and shipped at 5 ± 3°C.

For each session, Bipea sends 3 samples to each laboratory : 2 spiked samples (low and high contamination level) and 1 blank sample. Spiking is performed using SEA lyophilisate.

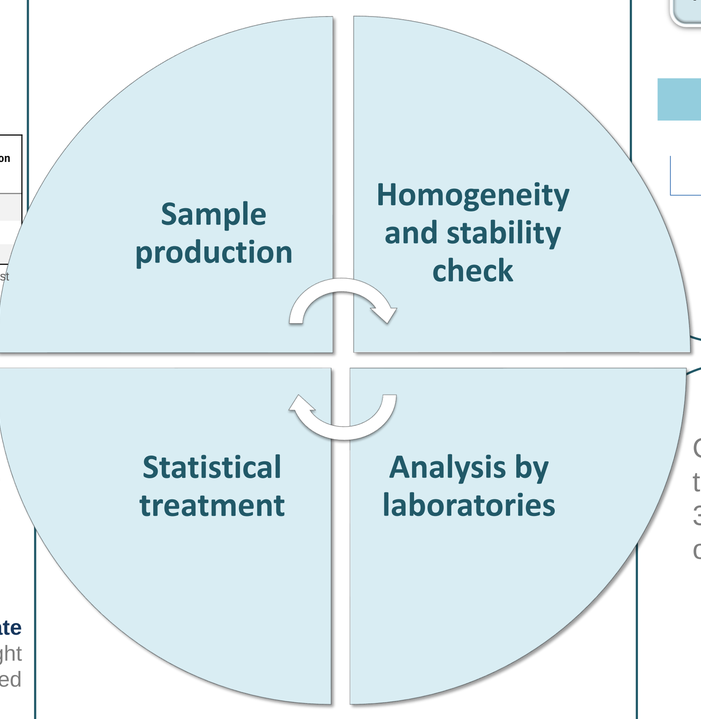
Sample	Contamination by SEA	Spiking concentration (ng/ml)
Sample 1	+ (High level)	0,06
Sample 2	- (Blank sample)	-
Sample 3	+ (Low level)	0,03

Table 1: Contamination diagram of April 2024 test

Design of homogeneity and stability studies



- Method: NF EN ISO 19020
- Kit vidas SET II used
- High level contamination

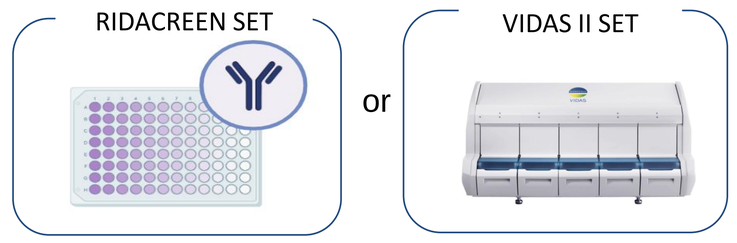


The participants are required to return their qualitative results via an online reply form. For statistical assesment: each qualitative result is analyzed individually against the expected result. The performance evaluation criteria for the entire test are established based on the following parameters:

- **Specificity rate** (r_{SP}): ability Ability to not detect SE in blank sample
- **Sensitivity rate** (r_{SE}): •Ability to detect SE in both spiked samples
- **Relative accuracy rate** (r_{AC}): abilty to find the right results for blank and spiked samples

Participant's performances are considered satisfactory if:
-Relative specificity = 100%
-Relative sensitivity ≥ 50%
-Relative accuracy ≥ 66%.

Once the homogeneity and stability checked, samples are shipped to participants who are invited to analyze the samples in a period of 3 weeks. In 2024, all participants implemented the ISO 19020 using one of the two validated kits:



RESULTS and DISCUSSION

HOMOGENEITY RESULTS

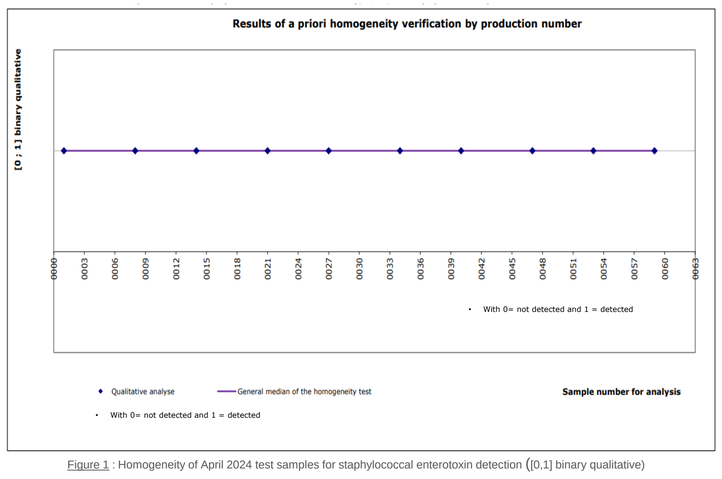


Figure 1 : Homogeneity of April 2024 test samples for staphylococcal enterotoxin detection ([0,1] binary qualitative)

All homogeneity samples were tested positive.
This result confirms the homogeneity of the batch (sample 1).

STABILITY RESULTS

	Sample number for analysis	Detection of staphyloccocal enterotoxins
t2 : 4 months	Replicate 1	detected
	Replicate 2	detected
	Replicate 3	detected
t3 :7 months	Replicate 1	detected
	Replicate 2	detected
	Replicate 3	detected
t4: 12 months	Replicate 1	detected
	Replicate 2	detected
	Replicate 3	detected

Table 2 : Results of a one-year study of enterotoxin stability in milk

The results of this stability study demonstrate that *Staphylococcal* enterotoxins are stable over a long period (at least one year), even under very low-temperature conditions.

TEST RESULTS

	Contamination			Specificity rate rSP (%)	Sensitivity rate rSE (%)	Relative Accuracy rate rAC (%)
Laboratory	Blank	low level	high level	-	-	-
1	not detected	detected	detected	100	100	100
2	not detected	detected	detected	100	100	100
3	not detected	detected	detected	100	100	100
4	not detected	detected	detected	100	100	100
5	not detected	detected	detected	100	100	100
6	not detected	detected	detected	100	100	100
7	not detected	detected	detected	100	100	100
8	not detected	detected	detected	100	100	100
9	not detected	detected	detected	100	100	100
10	not detected	detected	detected	100	100	100
11	not detected	detected	detected	100	100	100
12	not detected	detected	detected	100	100	100
13	not detected	detected	detected	100	100	100
14	not detected	detected	detected	100	100	100
15	not detected	detected	detected	100	100	100
16	not detected	not detected	detected	100	50	67
17	not detected	detected	detected	100	100	100
18	not detected	not detected	detected	100	50	67
19	not detected	detected	detected	100	100	100

Table 3: Overview results by laboratory of April 2024 test

The results of the Proficiency Test from April 2024, along with the evaluation of the 19 participating laboratories, are summarized in the table (left).

- The performance of the participating laboratories was generally satisfactory, particularly concerning relative specificity. No laboratory reported false-positive results for enterotoxin detection, indicating excellent reliability of the methods used to avoid false positives.
- Regarding relative sensitivity, the results of the laboratories were considered satisfactory, except for two laboratories that achieved relative sensitivity below 66% (r_{SE} < 66%). However, the false-negative results were observed on samples with low contamination levels.

↪ False-negative results remain a major concern for laboratories, particularly for low-contaminated samples.

↪ This issue is closely related to the detection limits of the methods used, which must be as low as possible to avoid missing contaminated batches.

A pie chart (right) also illustrates the distribution of methods used by laboratories during the test, highlighting the predominance of VIDAS SET II (75%) over RIDASCREEN (25%).

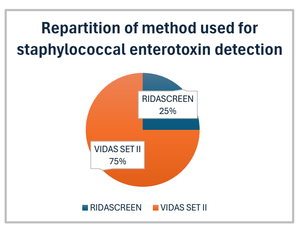


Figure 2 : Pie chart of method used by laboratory for SE detection (April 2024 test)

CONCLUSION

A PT program for detection of Staphylococcal Enterotoxins in milk, gathering about 20 laboratories around the world/year, was successfully implemented. These PT are interesting not only for laboratories, who can prove the reliability of their results to obtain recognition of their analytical performances by costumers and accreditation bodies, but also to provide a vision of the potential and limits of the performed method. In the future, it would be valuable for laboratories participating in the test to provide more detailed qualitative results by specifying the types of Staphylococcal Enterotoxins (SEA to SEE) detected. Additionally, the inclusion of quantitative data, such as the concentration of enterotoxins in milk, could be proposed.

Impact of the stability study: SEA is very stable in milk matrix (> one year). Big batch could be prepared for several PTs, Such stability would also allow the production of External Reference Materials (ERMs), providing additional support for laboratories seeking to retest the sample.

[1] **ISO 17025 -** General requirements for the competence of testing and calibration laboratories, [2] **ISO 13528** - Statistical methods for use in proficiency testing by interlaboratory comparison, [3] **ISO 17043** - Conformity assessment - General requirements for proficiency testing.