PROFICIENCY-TESTING SCHEME FOR HEPATITIS A, NOROVIRUS GI AND NOROVIRUS GII IN MINERAL WATER

Caterina MAZZONI, Roman LE NEVE, Anne TIRARD, Abdelkader BOUBETRA, Caroline LAURENT
Bureau Interprofessionnel d'Études Analytiques (BIPEA) - 189 rue d'Aubervilliers, 75018 PARIS – France. Tel .+33 1 40 05 26 30
Corresponding author: cmazzoni@bipea.org

INTRODUCTION

Norovirus GI, Norovirus GII and Hepatitis A are the leading causative agents of foodborne disease outbreaks worldwide. The number of laboratories detecting viruses has gradually increased in recent years to answer the growing demand of food routine control. However, due to their low infectious doses and low concentrations in food samples, these analyses are a challenge for the laboratories, that have few means at their disposal to check their analytical results even if an erroneous outcome can have significant consequences both from a public health and an economic point of view. One of the most effective tools of monitoring the laboratory performances is taking part in Proficiency Tests (PT), participation in which is a mandatory requirement of ISO 17025:2017 standard [1].
Nonetheless, the setting up of PT for the detection of these viruses is a challenge, above all to prepare stable and homogeneous samples. Since June 2018, Bipea has been organizing proficiency-testing schemes (PTS) for the analysis of viruses in mineral water. Real samples are produced by spiking water with a suspension of Noroviruses GI and GII and Hepatitis A at 4 different levels of concentration (without, low, medium, and high spiking). This work describes the setting up and the results of a PT on mineral water contaminated with Norovirus GI, Norovirus GII and Hepatitis A.

MATERIALS & METHODS

Sample production and shipment
A batch of mineral water is first analyzed to detect the possible presence of viruses and then divided into **4 series of samples**. Three of these series are individually contaminated with suspensions of Norovirus GI, Norovirus GII and Hepatitis A at different concentrations. Homogeneity and stability of the samples are checked through the analysis of the target viruses according to ISO 15216-2:2019 standard [2]. Homogeneity is assessed by comparing the results obtained on 10 samples. All samples of contaminated series must be positive for Norovirus GI, Norovirus GII and HAV so that the production is considered homogeneous. Stability is assessed on 3 spiked samples stored at (5±3) °C by comparing the results of the tests for all viruses over the test period. Samples are considered stable if the presence of the target viruses is confirmed in all samples.

Analyses by laboratories
Laboratories are invited to analyze these samples using the technique or method they practice routinely, for instance, either the standards (qualitative detection) or through alternative methods. Participants then submit their analysis results, in which they can also provide additional information about the method, recovery rates, quantification and detection limits and the date of analysis.

Statistical treatments
Qualitative results returned by laboratories are collected and analyzed statistically. A global relative overview to find negative or positive samples and, in general, to give a right conclusion is defined for each laboratory, according to the following parameters:

- **Specificity rate** (r_{SP}): Number of negative results found by the laboratory divided by the total expected negative samples, expressed in percentage.
- **Sensitivity rate** (r_{SE}): Number of positive results found by the laboratory divided by the total expected positive samples, expressed in percentage.
- **Relative accuracy rate** (r_{AC}): Number of true negative and positive results found by the laboratory, divided by the total analyzed samples, expressed in percentage.

Participants' performances are considered as satisfactory if:
- Relative specificity = 100%
- Relative sensitivity > 66%
- Relative accuracy ≥ 75%.

RESULTS

Results obtained on the PT of January 2022 are examined in detail. Contamination ranges of samples proposed for this PT are detailed in Table 1.
Table 2 summarizes the results obtained by the 9 laboratories participating to this PT on contaminated samples. Laboratories performances for each analyzed virus are detailed in Table 3. Participants' results were satisfactory, especially for relative specificity, with only one laboratory that found false positive results for Norovirus GI, Norovirus GII and Hepatitis A virus. Concerning the relative sensitivity, results of all laboratories were satisfying for hepatitis A virus and only one has r_{SE} < 66% for Noroviruses GI and GII. In general, the greater number of false negative results were observed on samples with low contaminations. These data are reassuring, as scores of relative sensitivity mix up results obtained on all positive samples, but a laboratory that does not find a positive result for low levels is less problematic than the one that does not find positive a sample spiked at high levels. However, false negative results remain a warning for laboratories even for low spiked samples, as closely related to the detection limits which should be the lowest possible to avoid the non-detection of contaminated batches, which could lead to a harmful impact on consumers health.

Contamination diagram	Norovirus GI (UG/500 mL)	Norovirus GII (UG/500 mL)	Hepatitis A (UG/500 mL)
Negative sample	-	-	-
Low contamination	8230	8000	8640
Mid contamination	24700	24400	25900
High contamination	55600	55500	58300

Table 1. Viruses spiking concentrations of the 4 samples proposed for the PT of January 2022

Laboratory	Norovirus GI			Norovirus GII			Hepatitis A		
Contamination	Low	Mid	High	Low	Mid	High	Low	Mid	High
1	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected
2	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected
3	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected
4	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected
5	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected
6	Not detected	Detected	Detected	Not detected	Detected	Detected	Not detected	Detected	Detected
7	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Detected	Detected	Not detected
8	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected
9	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected

Table 2. Results obtained by each laboratory on detection of Noroviruses GI, GII and Hepatitis A virus in positive samples

Laboratory	Relative specificity r_{SP} (%)			Relative sensitivity r_{SE} (%)			Relative accuracy r_{AC} (%)		
	Norovirus GI	Norovirus GII	Hepatitis A	Norovirus GI	Norovirus GII	Hepatitis A	Norovirus GI	Norovirus GII	Hepatitis A
1	100	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100	100
4	100	100	100	100	100	100	100	100	100
5	100	100	100	100	100	100	100	100	100
6	0	0	0	67	67	67	50	50	50
7	100	100	100	0	0	67	25	25	75
8	100	100	100	100	100	100	100	100	100
9	100	100	100	100	100	100	100	100	100

Table 3. Relative specificity, relative sensitivity and relative accuracy obtained by each laboratory on detection of Norovirus GI, Norovirus GII and Hepatitis A virus.

CONCLUSION

A PTS for detection of Norovirus GI, Norovirus GII and Hepatitis A in mineral water, gathering about 9 laboratories around the world, was successfully implemented. Laboratories' results are satisfactory, negative samples are correctly detected for all viruses by most of the participants. Sensitivity defects have been observed for some laboratories with a greater number of false negative results for the samples slightly contaminated. 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 have a state of the art of potentialities and limits of the performed method.

REFERENCES

1. **ISO/IEC 17025:2017** - General requirements for the competence of testing and calibration laboratories.
2. **ISO/TS 15216-2:2019** - Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 2: Method for qualitative detection.