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Research Article

Detection of Norovirus GI, Norovirus GII and Hepatitis A Virus in Strawberries: State of the Art through Proficiency-Testing Scheme Results

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Abstract

Hepatitis A virus and noroviruses are responsible for viral gastro-enteritis and hepatitis reported worldwide. As contaminated food and water are often the agents for transmitting these viruses, the request for analysis of Hepatitis A virus and Noroviruses in food has progressively increased in recent years. However, detection and/or quantification of low concentrations of these viruses in food matrices is usually complex, even if false negative or false positive results can have significant public health and economic consequences. This paper analyzes results obtained by laboratories participating in a proficiency test for the detection of Noroviruses GI and GII and Hepatitis A virus on artificially contaminated strawberries. Obtained data are encouraging as negative samples are correctly detected by the main participants and few false positive results were observed, above all on the samples slightly contaminated. The best results were observed on samples spiked with a high concentration of Hepatitis A. These data highlight that laboratories' specificity (the ability of the laboratory to find negative test samples in the absence of the analyte) is good but participants' sensitivity (the ability of the laboratory to find positive test samples in the presence of the analyte) is questionable.

Keywords: *Proficiency-Testing Schemes; Food Virology; Noroviruses; Hepatitis A; Quality Control; Laboratory Performance*

Abbreviations

NoV GI: Norovirus Genogroups I; NoV GII: Norovirus Genogroups II; HAV: Hepatitis A Virus; PT: Proficiency Tests

Introduction

Norovirus genogroups I (NoV GI) and II (NoV GII) and Hepatitis A virus (HAV) are the leading causative agents of foodborne disease outbreaks worldwide [1-4]. Frozen strawberries have been repeatedly identified as vehicles for these viruses' transmission causing large gastroenteritis outbreaks [5-7]. The number of laboratories detecting Noroviruses GI and GII and Hepatitis A virus has gradually increased in recent years to answer the growing demand of food routine controls. Most of them follow the requirements of ISO 15216-1:2017 and ISO 15216-2:2019 standards [8,9] that specify methods for quantification and detection of Noroviruses GI and GII and Hepatitis A virus, from test samples of foodstuffs or surfaces using real-time RT-PCR. Some laboratories have also developed or apply internal methods, mostly based on these cited standards.

Nevertheless, these analyses remain a challenge for laboratories due to the low concentrations in food samples and to the fact that virus detection, in food generally and berries particularly, is often hampered by the presence of RT-PCR-inhibiting substances. Moreover,

laboratories have few means at their disposal to check their analytical data even if an untrue result can have significant consequences both from a public health and an economic point of view. In this framework, analytical laboratories are subject to an increasing number of requirements regarding the reliability of the results they provide to test prescribers. Participation in proficiency tests (PT) allows laboratories to demonstrate and check the reliability of their results as they provide an independent assessment of the laboratory performance comparing its results to those of other laboratories. Furthermore, PT are interesting not only for laboratories, which can have a constant check of their performances, but also to have a state of the art for the potentialities and limits of the performed methods. The following paragraphs describe the design, the implementation and the results obtained for the analysis of viruses in frozen strawberries.

Materials and Methods

By their conception, proficiency tests entail the analysis by different laboratories of the same analytical parameters on identical samples. The setting up of a PT can be schematized by 3 main steps: preparation of homogeneous samples, analysis by the laboratories, and statistical treatment of the data, with evaluation of laboratory performances. The complexity of the matrix, strawberries, in terms of inhibition factors and stability of target viruses are non-negligible factors to be considered in setting up a PT, especially during the preparation of the test items.

Production and shipment of the samples

The most critical aspect for the implementation of a proficiency-test programme is the production of homogeneous and stable samples. For this PT, a batch of strawberries is first analysed to detect the possible presence of viruses and then divided into a series of samples. These samples are individually contaminated with calibrated suspensions of Norovirus GI, Norovirus GII and Hepatitis A at 4 different levels (without spiking, low, medium, and high concentration) and then frozen at -24 ± 6°C.

Homogeneity and stability of the samples are checked through the analysis of the target viruses according to the ISO 15216-2:2019 standard. The samples selected for the homogeneity and stability analyses are taken throughout the preparation process and are therefore representative of the entire production. These samples are analysed in a random order, independent of the order of production. Homogeneity is assessed by comparing the results obtained on 20 samples. For contaminated samples, the production is considered homogeneous if the presence of Norovirus GI, Norovirus GII and Hepatitis A virus is confirmed in all analysed samples. Stability is assessed on 6 samples stored at -24 ± 6°C by comparing the results obtained on the 3 target viruses over the proficiency test period. In detail, samples of spiked series are considered stable if the presence of the viruses is proved in all samples analysed within 4 weeks of the homogeneity control.

Analysis by laboratories

Laboratories are invited to analyse these samples using the technique or method they prefer, for instance either using 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 all laboratories are collected and analyzed statistically. A global relative overview to find negative or positive samples and, in general, to give a correct conclusion is defined for each laboratory, according to the following parameters [12]:

- **Relative specificity (r_{SP})**: Number of negative results found by the laboratory divided by the total expected negative samples, expressed as a percentage.
- **Relative sensitivity (r_{SE})**: Number of positive results found by the laboratory divided by the total expected positive samples, expressed as a percentage.

- **Relative accuracy (r_{AC})**: Number of true negative and positive results found by the laboratory, divided by the total analysed samples, expressed as a percentage.

Participants' performances are considered satisfactory if:

- The relative specificity is equal to 100%,
- The relative sensitivity is higher than 66%,
- And hence, the relative accuracy is higher than or equal to 75%.

All data are published in an interlaboratory comparison report, validated by both BIPEA and an external technical expert, that is distributed to the participants.

Results and Discussion

Results obtained on the PT of November 2021 are examined in detail. For this test, strawberries were artificially contaminated from 0 UG/g (non-spiked sample) to 2400 UG/g, according to the virus. Contamination ranges of spiked samples are detailed in table 1.

	Norovirus V GI	Norovirus V GII	Hepatitis A virus
Non-spiked sample	0	0	0
Low contamination, UG /g	200	29	200
Medium contamination, UG /g	800	120	800
High contamination, UG /g	2400	600	2400

Table 1: *Virus spiking concentrations of the 4 samples proposed for the PT of November 2021.*

Table 2 to 4 summarizes the results obtained by the 20 laboratories participating to this PT. Laboratories performances for each analysed virus are detailed in table 5. Participants' results were satisfactory, especially for relative specificity, with only one laboratory that found a false positive result for Norovirus GI, two for Norovirus GII and three for hepatitis A virus. Most laboratories had good performances on relative accuracy, except 4 laboratories that had a r_{AC} < 75% for all viruses and one on Norovirus GII only. Concerning spiked samples (relative sensitivity), 80% of laboratories detected the hepatitis A virus presence, 60% of laboratories the Norovirus GI and only 30% the Norovirus GII. This last result may be due to the spiking concentrations, as contamination ranges of Norovirus GII were lower than those of the other two viruses. In general, the greater number of false negative results were observed on samples with low and medium contaminations. These data are reassuring, as scores of relative sensitivity mix up results obtained on all spiked 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 they are close 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.

	Norovirus GI			
Laboratory	**Non-spiked sample**	**Low contamination**	**Medium contamination**	**High contamination**
1	Not detected	Detected	Detected	Detected
2	Not detected	Detected	Detected	Detected
3	Not detected	Detected	Detected	Detected
4	Not detected	Detected	Detected	Detected
5	Not detected	Detected	Detected	Detected
6	Detected	Not detected	Not detected	Not detected
7	Not detected	Detected	Detected	Detected
8	Not detected	Detected	Detected	Detected
9	Not detected	Detected	Detected	Detected
10	Not detected	Detected	Detected	Detected
11	Not detected	Not detected	Detected	Detected
12	Not detected	-	Detected	Detected
13	Not detected	Detected	Detected	Detected
14	Not detected	Detected	Detected	Detected
15	Not detected	Not detected	Not detected	Not detected
16	Not detected	Detected	Detected	Detected
17	Not detected	Detected	-	Detected
18	Not detected	Detected	Detected	Detected
19	Not detected	Not detected	Not detected	Not detected
20	Not detected	Not detected	Not detected	Not detected
Percentage of false negative/ False Positive results	5	26	21	20

Table 2: *Results obtained by each laboratory on detection of Norovirus GI (NoV GI).*

	Norovirus GII			
Laboratory	**Non-spiked sample**	**Low contamination**	**Medium contamination**	**High contamination**
1	Not detected	Not detected	Detected	Detected
2	Not detected	Not detected	Detected	Detected
3	Not detected	Detected	Detected	Detected
4	Not detected	Not detected	Detected	Detected
5	Not detected	Detected	Not detected	Detected
6	Detected	Not detected	Not detected	Detected
7	Not detected	Detected	Detected	Detected
8	Not detected	Detected	Detected	Detected
9	Not detected	Not detected	Not detected	Detected
10	Not detected	Detected	Detected	Detected

11	Not detected	Not detected	Detected	Detected
12	Not detected	-	Detected	Detected
13	Not detected	Detected	Detected	Detected
14	Not detected	Not detected	Detected	Detected
15	Not detected	Not detected	Not detected	Not detected
16	Not detected	Detected	Detected	Detected
17	Not detected	-	Detected	Detected
18	Not detected	Not detected	Detected	Detected
19	Detected	Detected	Not detected	Detected
20	Not detected	Not detected	Not detected	Not detected
Percentage of false negative / False Positive results	10	56	30	10

Table 3: *Results obtained by each laboratory on detection of Norovirus GII (NoV GII).*

	Hepatitis A virus			
Laboratory	**Non-spiked sample**	**Low contamination**	**Medium contamination**	**High contamination**
1	Not detected	Detected	Detected	Detected
2	Not detected	Detected	Detected	Detected
3	Not detected	Detected	Detected	Detected
4	Not detected	Detected	Detected	Detected
5	Not detected	Detected	Detected	Detected
6	Detected	Not detected	Not detected	Detected
7	Not detected	Detected	Detected	Detected
8	Not detected	Detected	Detected	Detected
9	Not detected	Detected	Detected	Detected
10	Not detected	Detected	Detected	Detected
11	Not detected	Detected	Detected	Detected
12	Not detected	Detected	Detected	Detected
13	Not detected	Detected	Detected	Detected
14	Not detected	Detected	Detected	Detected
15	Not detected	Not detected	Not detected	Not detected
16	Not detected	Detected	Detected	Detected
17	Not detected	Detected	Detected	Detected
18	Not detected	Detected	Detected	Detected
19	Detected	Detected	Not detected	Detected
20	Detected	Not detected	Not detected	Detected
Percentage of false negative / False Positive Results	15	15	20	5

Table 4: *Results obtained by each laboratory on detection of Hepatitis A virus (HAV).*

Laboratory	Relative specificity r_{SP} (%)			Relative sensitivity r_{SE} (%)			Relative accuracy r_{AC} (%)		
	NoV GI	NoV GII	HAV	NoV GI	NoV GII	HAV	NoV GI	NoV GII	HAV
1	100	100	100	100	67	100	100	75	100
2	100	100	100	100	67	100	100	75	100
3	100	100	100	100	100	100	100	100	100
4	100	100	100	100	67	100	100	75	100
5	100	100	100	100	67	100	100	75	100
6	0	0	0	0	33	33	0	25	25
7	100	100	100	100	100	100	100	100	100
8	100	100	100	100	100	100	100	100	100
9	100	100	100	100	33	100	100	50	100
10	100	100	100	100	100	100	100	100	100
11	100	100	100	67	67	100	75	75	100
12	100	100	100	67	67	100	100	100	100
13	100	100	100	100	100	100	100	100	100
14	100	100	100	100	67	100	100	75	100
15	100	100	100	0	0	0	25	25	25
16	100	100	100	100	100	100	100	100	100
17	100	100	100	67	67	100	100	100	100
18	100	100	100	100	67	100	100	75	100
19	100	0	0	0	67	67	25	50	50
20	100	100	0	0	0	33	25	25	25

Table 5: *Laboratories' performances: relative specificity, relative sensitivity and relative accuracy obtained by each laboratory on detection of Norovirus GI (NoV GI), Norovirus GII (NoV GII) and Hepatitis A virus (HAV).*

Conclusion

This study provides valuable information about the current state of the art on the detection of Norovirus GI, Norovirus GII and Hepatitis A virus in frozen strawberries. Non-spiked samples are correctly identified by most laboratories, with a relative specificity on Norovirus GI equal to 100% for all laboratories except one. Sensitivity defects have been observed for some laboratories with a greater number of false negative results for the samples slightly contaminated. The best results were observed on samples spiked with a high concentration of Hepatitis A virus, with only one laboratory that found a false negative result. The non-detection of contaminated samples, even at low concentrations, remains a potential risk to public health, especially for fragile and immunocompromised people. In this context, participation in interlaboratory tests remains the most efficient way for laboratories to check their detection limits and improve their analytical performances. Regular PT for virus detection in strawberries are organized every year to help laboratories to test their analytical skills, detect non-compliant results and to implement corrective and/or curative actions if necessary. Participating in PT is mandatory to obtain recognition of the analytical procedures by the accreditation bodies according to ISO 17025 [10] and to earn the confidence of customers and customer associations. These PT are accredited by COFRAC, the French accreditation body (Comité Français d'Accréditation) according to the ISO 17043 standard [13] and have been further developed to include frozen salads and water contaminated with Norovirus GI, Norovirus GII and Hepatitis A virus to allow laboratories to demonstrate their performances on these matrices too and to be consistent with the scope of ISO 15216 standard [8,9].

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