Proficiency-testing scheme for Hepatitis A, Norovirus GI and Norovirus GII in strawberry

Caterina MAZZONI[1], Roman LE NEVE[1], Anne TIRARD[1], Abdelkader BOUBETRA[1]
[1] : 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 foodborne 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, which need to prove the reliability of their results to obtain recognition of their analytical procedures by customers and accreditation bodies. To meet this need, BIPEA organizes regular proficiency-testing schemes (PTS) for the analysis of viruses in food. This work describes the setting up of a PT conducted in July 2017 on strawberries contaminated with Norovirus GI, Norovirus GII and Hepatitis A.

OBJECTIVES

A proficiency test entails the analysis by different laboratories of the same analytical parameters on identical samples. The setting up of a proficiency test can be schematized by 4 main steps: test design, preparation of homogenous samples, analyses by the laboratories, statistical treatment of the data, with the estimation of an assigned value and evaluation of the laboratory performance.

For this PT, laboratories were invited to analyze strawberries' samples spiked with suspensions of Norovirus GI, Norovirus GII and Hepatitis A in well controlled proportions. The main goal of the test is to provide laboratories with means of objective assessment, to demonstrate the reliability of the data they produce and to compare results obtained with different analytical procedures for virus detection in samples very close to real ones.

MATERIALS & METHODS

One of the fundamental aspect for PT implementation is the preparation of homogenous and stable samples. For this PT, strawberries were first analysed to detect the possible presence of viruses, then spiked with calibrated suspensions of Norovirus GI, Norovirus GII and Hepatitis A at 3 different concentrations (between 50 and 2000 UG/g) and then frozen. Homogeneity of the samples was verified by experimental studies on 20 samples taken randomly across each batch of samples. The samples were considered homogenous enough if the measuring range of this check was less than 1 in log (UG/g). Stability of the spiked samples was proved by analysing 6 samples over a period of three weeks: 3 samples on the first day of the study (D0) and 3 samples on the last one (D1). Considering the nature of the tested viruses and the variability of the performed method, the stability of the samples was regarded as satisfactory if the maximum acceptable difference between the average results obtained at D0 and D1 was near 1 log (UG/g). For both studies, the analyses were performed according to ISO/TS 15216 standard. Once homogeneity and stability were demonstrated for each virus, the samples were shipped frozen to all the participants, along with a blank sample. Laboratories' results were collected via a reply form available online over a period of one month. Qualitative and/or quantitative results obtained according to ISO/TS 15216-1 [1], ISO/TS 15216-2 [2] or alternative methods must be filled in this form. Moreover, participants were invited to add some complementary information such as the identification number of the analysed samples, date of beginning of analyses, detection and quantification limits and extraction method and yields.

Statistical treatment of the collected data was conducted according to ISO 13528 [3]. The assigned (consensus) values (x_{pt}) were estimated using the robust means of all provided results from application of robust algorithm A. Performances of each laboratory were evaluated using a tolerance values (TV) of twice the standard deviations. These values were used to identify an interval around the assigned values: results out of these ranges were considered as a warning signal for the laboratories. Moreover, participants' results (x) were also evaluated through z-score (z):

$$z = \frac{x - x_{pt}}{\frac{TV}{2}}$$

Results of the laboratories which have a z-score ≤|2| were considered satisfactory and those with a z-score >|2| were classified as 'questionable' or 'unsatisfactory': if the laboratory z-score was |2| < z ≤ |3|, the result was considered questionable and >|3| unsatisfactory. The results were published in a specific interlaboratory comparison report distributed to all the participants who could then classify their results and implement some preventive and/or corrective actions if necessary.

RESULTS

Results of the homogeneity checks showed that the samples were homogenous enough to meet the requirements of the PT for each level of the researched viruses. Figure 1 summarizes graphically results of the homogeneity check of the samples as a function of the production order for the Norovirus I (high level). It can be noted that the gap between the minimum and maximum values is 0.933 UG/g in log (data are converted in log values) for this analytical parameter, which is in accordance with the results obtained for the other series of samples. The stability checks showed a satisfactory recovery rate considering the expected concentration after storing the samples at (-24±6) °C over a period of three weeks. The means of the results between the first day of analysis and the last one are described in Table 1 for the three viruses, for the samples at the highest concentration level. The variability of the performed method can explain the difference between the means of the results collected from the first day (D0) to the last day of analysis (3 weeks after).

27 laboratories returned results for Norovirus I, 26 for the Norovirus II and Hepatitis A detection. Most of the laboratories followed ISO/TS 15216-1:2013 and ISO/TS 15216-2:2013 methods, and only three laboratories indicated alternative methods. On average, 9 laboratories provided quantitative results. Concerning the qualitative data, seven laboratories obtained at least a false negative result on spiked samples and one laboratory returned false positive results for Noroviruses GI and GII on the blank sample. The extraction yields obtained by the laboratories vary widely, ranging from 3% to 90%. For each virus, assigned values (x_{pt}) were calculated from the robust means of the all quantitative results except those considered as incoherent. The main statistical parameters are summarized in Table 2. The results show a dispersion of the data that would be due to the extraction procedures and their extraction yields as well as to the detection method. Only a few laboratories obtained results out of the tolerance interval.

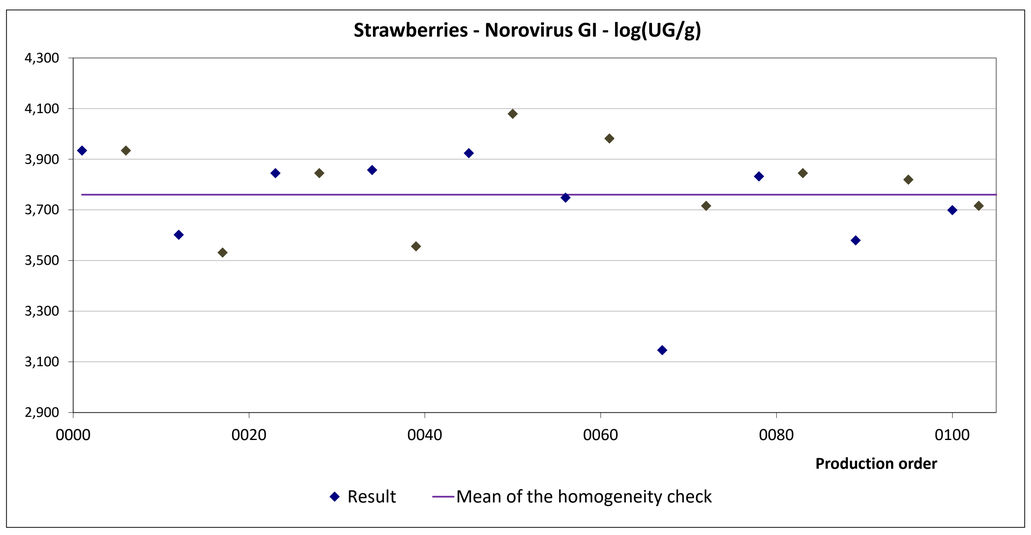

Figure 1. Results of the homogeneity check of the samples as a function of the production order for the Norovirus I, high level of concentration

Virus	D0	D1 (3 weeks after)
	Mean - log(UG/g)	
Norovirus GI	2.789	3.881
Norovirus GII	3.760	4.443
Hepatitis A	4.279	3.926

Table 1. Average results of the stability checks over a period of 3 weeks for the high level viruses concentration series of samples

Analytical parameters	Norovirus GI				Norovirus GII				Hepatitis A			
Sample	Blank	Level 1	Level 2	Level 3	Blank	Level 1	Level 2	Level 3	Blank	Level 1	Level 2	Level 3
p_{ca}[1]	27	26	26	26	27	26	26	26	27	26	26	26
Qualitative p_{ca}[2]	27	26	26	26	27	26	26	26	27	26	26	26
Quantitative p_{ca}[3]	6	10	8	9	6	10	8	9	6	10	9	9
x_{pt}[4]	-	1.968	2.197	2.608	-	2.788	2.702	3.220	-	1.869	2.138	2.086
$u(x_{pt})$[5]	-	0.245	0.215	0.464	-	0.310	0.365	0.351	-	0.105	0.230	0.176
$s(x_{pt})$[6]	-	0.392	0.345	0.908	-	0.608	0.772	0.794	-	0.167	0.487	0.398
$p(x_{pt})$[7]	-	4	4	6	-	6	7	8	-	4	7	8
$CV(x_{pt})$ - %[8]	-	20	16	35	-	22	29	25	-	9	23	19
σ_{pt}[9]	-	0.392	0.345	0.908	-	0.608	0.772	0.794	-	0.167	0.487	0.398
TV[10]	-	0.784	0.690	1.816	-	1.216	1.544	1.588	-	0.334	0.974	0.796
p_D[11]	-	3	3	1	-	1	0	0	-	3	1	0

1. P_{ca}: Number of returned results
2. Qualitative p_{ca}: Number of qualitative results
3. Quantitative p_{ca}: Number of quantitative results
4. x_{pt}: Assigned value (quantitative results)
5. $u(x_{pt})$: Standard uncertainty of the assigned value
6. $s(x_{pt})$: Robust standard deviation of the results
7. $p(x_{pt})$: Number of results taken into account for the estimation of the assigned value
8. $CV(x_{pt})$ - %: Coefficient of variation
9. σ_p: Standard deviation for proficiency assessment
10. TV: Tolerance value
11. p_D: Number of results out of the tolerance interval

Table 2. Summary of the statistical treatment of quantitative data

CONCLUSION

A PTS for detection and quantification of Norovirus GI, Norovirus GII and Hepatitis A in strawberries, gathering about 30 laboratories around the world, was successfully implemented. The statistical treatment of the data was performed according to ISO 13528 [3]. Assigned (consensus) values were calculated from the participants' results and the performances of the laboratories could then be evaluated individually and collectively according to ISO 17043 [4]. The results were published in an interlaboratory comparison report distributed to the participants who could draw up a general inventory of their analytical skills and have a very useful tool to verify the reliability of their results as well as to detect bias or non-compliant results for each tested concentration of viruses. New matrices will be tested in the future by BIPEA: water, raspberry and salad.

REFERENCES

1) **ISO/TS 15216-1:2013** - Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 1: Method for quantitative detection
2) **ISO/TS 15216-2:2013** - 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
3) **ISO 13528:2015** - Statistical methods for use in proficiency testing by interlaboratory comparisons
4) **ISO 17043:2010** - Conformity assessment - General requirements for proficiency testing.