Proficiency-testing scheme for detection and enumeration of Campylobacter in poultry meat

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INTRODUCTION

Campylobacter is considered to be the most common bacterial cause of human gastroenteritis worldwide. Laboratories performing microbiological analyses on food stuffs therefore have a key role to play and must ensure their performance through regular proficiency-testing schemes (PTSs).

This work describes the design and the implementation of PTS for the analyses of poultry meat samples spiked with two different strains of Campylobacter : *Campylobacter jejuni* or *Campylobacter coli*.

The goal of this PTS is to allow laboratories to demonstrate the reliability of their results and to compare each other analytical data and protocols used for the detection and the enumeration of Campylobacter in poultry meat sample.

METHODOLOGY

The setting up of a proficiency test can be schematized by 3 main steps: preparation of the samples, analyses by the laboratories and statistical treatment of the data.

SAMPLES PRODUCTION

One of the fundamental aspects for the implementation of a PT is the preparation of homogenous and stable samples.
For this PT, samples were prepared by spiking individually each sample of poultry meat with a suspension of Campylobacter in well controlled proportions.
According to the requirements of the ISO 13528 standard [1], homogeneity of the samples was verified by experimental studies on 10 samples *in duplo* taken randomly across a batch of samples (the results of the February 2019 test homogeneity check are summarized graphically in Figure 1).
Stability of the samples was proved by analyzing 3 samples after 2, 4, 9 and 15 days (the results of the stability check are summarized in Table 1).

ANALYSES

Four samples were shipped at -(24±6) °C to the laboratories participating to the test: three samples for the detection and one sample for the enumeration.
Four reply forms were made available to allow the laboratories to return their analysis results.
Moreover, participants were invited to enter in the reply form some complementary information such as the date of the beginning of the analysis, method used, growth medium used and supplier of the used media.
Given the stability of the product, the participants were invited to analyze the samples as soon as possible after the reception.

STATISCAL TREATMENT

The statistical treatments of the quantitative results were conducted according to ISO 13528 standard [1]. The assigned values (x_{pt}) were estimated using the robust means of the results transformed in log(CFU/g). The proficiencies of each laboratory were evaluated thanks to tolerance values (TV) of twice the standard deviations.
The results (x) could be evaluated and classified through z-scores :
$z \leq |2|$: satisfactory, $|2| \leq z < |3|$: questionable and
$z \geq |3|$: unsatisfactory

Where : $z = \frac{x - x_{pt}}{\left(\frac{TV}{2}\right)}$

RESULTS and DISCUSSION

The first PT of Campylobacter analysis was set up in June 2018 and gathered ten laboratories around the world. The strain used was *Campylobacter jejuni*. The second PT was organized in February 2019 and gathered twenty-two laboratories. The strain used was *Campylobacter coli.* Nine to twenty laboratories gave their results together with useful information for the interpretation of the data. The main statistical parameters of these tests are summarized in Table 2 (detection) and Table 3 (enumeration).
The results of the homogeneity check (test of February 2019) are summarized graphically in Figure 1. These data show that the samples are homogenous enough to meet the requirements of the test, with a gap between the minimal and maximal values lower than 0,5 CFU/g in log.
The analyses results of the stability checks showed a satisfactory recovery rate considering the expected concentration after storing the samples at -(24±6) °C for 15 days (see Table 1). The variability of the performed method can explain the difference between the results collected from D2 to D15.
The laboratories' quantitative results are shown graphically on Figures 2 and 3. On the histograms, assigned value and tolerance interval are indicated in the x-axis and the results of the laboratories are shown in different colours as a function of the performed method. For the test of June 2018 (figure 2), 4 laboratories used ISO 10272-2 [2] method (in purple), 5 laboratories used another method (in orange). For the test of February 2019 (figure 3), 7 laboratories used ISO 10272-2 [2] method (in purple) and 13 laboratories used another method (in orange). However, no tendency was highlighted as a function of the method used.
The laboratories' qualitative results are shown in table 2. All the laboratories obtained results in line with those expected except for one laboratory which reported a false negative result for the test of February 2019.

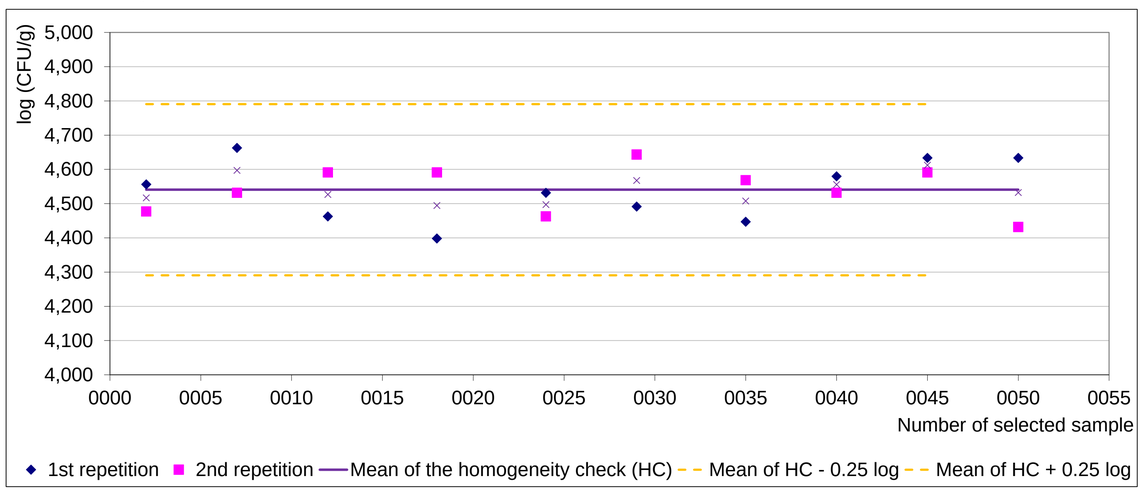

Figure 1. Results of the homogeneity check of the samples as a function of the production order for the test of February 2019

Statistical parameter \ Analytical parameter	June 2018 *Campylobacter jejuni*	February 2019 *Campylobacter coli*
Number of returned results (p_{ca})	10	22
Assigned values for proficiency testing (x_{pt})	4.098	4.488
Standard uncertainty of the assigned value (u_{xpt})	0.296	0.123
Robust standard deviation of the results (x_{pt}), from all the results which participated to the estimation of the assigned values	0.670	0.311
Number of results taken into account for the estimation of the assigned value (p_{xpt})	8	10
Coefficient of variation (%) (CV x_{pt})	16	7
Standard deviation for proficiency assessment σ_{pt}	0.670	0.311
Tolerance value (TV)	1.340	0.622
Number of results out of the tolerance interval p_D	0	7

Table 3. Summary of the statistical treatment of the data, log (CFU/g)

log(CFU/g) \ Day of analysis	D_2	D_4	D_9	D_{15}
Campylobacter jejuni	5.78	5.75	5.42	5.29
Campylobacter coli	5.80	-	5.55	5.37

Table 1. Average results of the enumeration of Campylobacter in poultry meat sample after 15 days at -(24±6) °C

		Sample 1	Sample 2	Sample 3
June 2018 *Campylobacter jejuni*	**Expected results**	+	-	-
	Laboratory results	Detected : 9 Not detected :0	Detected : 0 Not detected : 9	Detected : 0 Not detected : 9
February 2019 *Campylobacter coli*	**Expected results**	-	-	+
	Laboratory results	Detected : 17 Not detected : 0	Detected : 17 Not detected : 0	Detected : 18 Not detected : 1

Table 2. Summary of the qualitative results

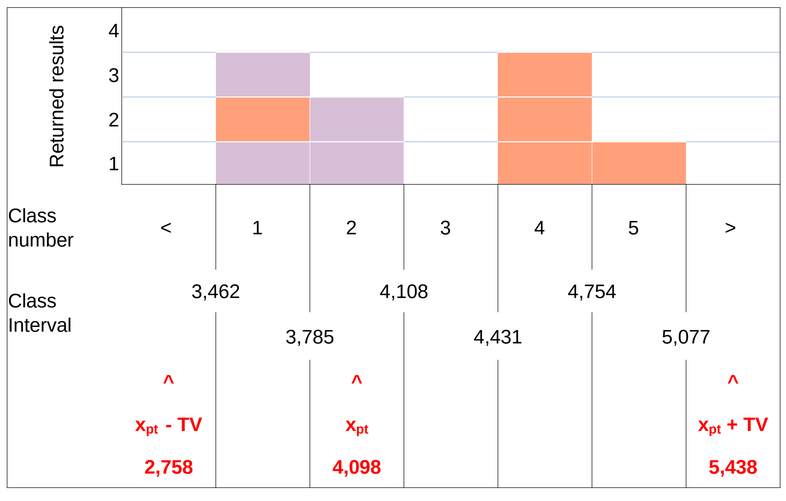

Figure 2. Distribution of the June 2018 test's results

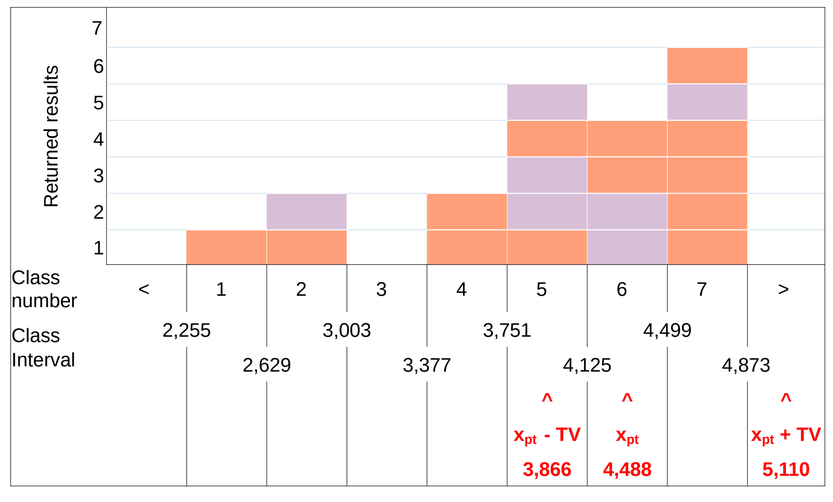

Figure 3. Distribution of the February 2019 test's results

CONCLUSION

Two PT for detection and enumeration of Campylobacter in poultry meat were successfully implemented and the results were published in interlaboratory comparison reports distributed to the participants. These first two PTs were transformed into a regular PTS, including two tests per year. Laboratories can now monitor punctually and/or continuously through time the reliability of their results for Campylobacter analysis.

REFERENCES

(1) **ISO 13528:2015** - Statistical methods for use in proficiency testing by interlaboratory comparisons
(2) **ISO 10272-2** - Microbiology of the food chain -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 2: Colony-count technique