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Validation by interlaboratory trials of EN ISO 10272 - Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique

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ABSTRACT

The validation in an interlaboratory study of the International Standards Organization standard method for the enumeration of *Campylobacter* in foods (ISO 10272-2) was performed after preparation of the revised Standard based on scientifically sound and validated methods of analysis.

The matrices selected for testing in the collaborative trial were frozen spinach, minced meat, raw milk, chicken skin, and broiler caecal material. Each matrix was artificially inoculated with a different *Campylobacter* strain. Fifteen laboratories participated in the interlaboratory study.

As a general indication of repeatability limit (r), the following overall values can be used when testing chicken skin samples:

r = 0.98 (expressed as a difference between $\log_{10}$-transformed test results), or
r = 9.52 (expressed as a ratio between test results).

As a general indication of reproducibility limit (R), the following overall values can be used when testing chicken skin samples:

R = 1.31 (expressed as a difference between $\log_{10}$-transformed test results), or
R = 20.43 (expressed as a ratio between test results).

The validation data for all matrices were incorporated in the newly published ISO standard EN ISO 10272-2:2017 - Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* - Part 2: colony-count technique.

1. Introduction

In the last decade *Campylobacter* continued to be the most reported gastrointestinal bacterial pathogen in humans in the European Union (EFSA and ECDC, 2016). Broiler meat is considered the most common source of infection. In most of the cases only low numbers of *Campylobacter* are present in broiler meat products or other foods, requiring an enrichment method for the detection of *Campylobacter* as described in the International Standards Organization standard method ISO 10272-1 (Anon., 2006a). However, the increasing research using quantitative microbiological risk assessment to elaborate strategies to reduce numbers of *Campylobacter* also requires a method for enumeration as described in ISO/TS 10272-2 (Anon., 2006b; Nauta et al., 2012). Every five years an ISO Standard is subject to Systematic Review in order to determine whether it should be confirmed, revised or withdrawn. During the 5-year review of both parts of ISO 10272 the ISO Technical committee ISO/TC 34, *Food Products*, Subcommittee SC 9, *Microbiology* and the CEN Technical Committee CEN/TC 275 *Food analysis - Horizontal methods*, Working Group WG6 *Microbiology of the food chain*, concluded that a technical revision of the standard was considered necessary. The revision on both parts was elaborated by the assigned expert members of CEN Task Advisory Group (TAG) 19-*Campylobacter*. The following main changes in ISO 10272 part 2 on enumeration were applied:

- As *Campylobacter* can be found along the total food chain, testing of

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samples from the primary production stage was added to the scope. Caecal content samples are commonly used to confirm *Campylobacter* colonization of broiler flocks and may contain countable numbers of up to 10^8 cfu/g (Rodgers et al., 2012);
- Duplicate plating of serial dilutions was replaced by plating in single, to be in line with ISO 7218 (Anon., 2007, 2013);
- The confirmation tests on the study of microaerobic growth at 25 °C and aerobic growth at 41.5 °C were replaced by the study of aerobic growth at 25 °C as the former two growth tests incidentally give results that are difficult to interpret.
- Performance testing for the quality assurance of the culture media according to ISO 11133 (Anon., 2014) was added to the standard.

The revision and validation of the 2006 edition of ISO/TS 10272-2 (Anon., 2006b), resulted in a new version of this standard (Anon., 2017b) in which also the performance characteristics, established as described in this paper, have been included.

Within the framework of the European food hygiene legislation, the European Union (EU) aims at availability of standardised validated methods that are referred to in legislation in order to support the EU food policy. Commission Regulation No (EC) 2073/2005 on microbiological criteria for foodstuffs contains provisions requiring the use of analytical methods in the food chain (Anon., 2005). In this Regulation a reference method has been established for each microbiological criterion, e.g. the recently published process hygiene criterion for *Campylobacter* in broiler carcasses which aims at keeping under control contamination of carcasses during the slaughtering process (Anon., 2017e). Horizontal EN or EN ISO methods, when available, have received the status of reference methods in the Regulation and therefore should be standardised and validated. The validation of European standards is also of importance since it will lead to improved recognition of the standards by the international community, improved substantiation of the microbiological criteria and therefore improved consumer protection, and a better recognition of analytical results by courts, the European Food Safety Authority, other authorities and laboratory inspections at international level.

The European Union, represented by the European Commission, decided to award a grant for the elaboration of 15 published European Validated Standards (EN) in the field of food hygiene legislation under mandate SA/CEN/ENTR/381/2010-06 “Microbiology of Food and animals stuffs” (Mandate M/381) (Anon., 2010). The agreements were established by the European Commission and the European Committee for Standardization (CEN). The ‘Deutches Institut für Normung e. V’ (DIN) and the ‘Association Française de Normalisation (AFNOR)’ participated as members of CEN in this specific grant agreement. The elaboration of the standards was performed by sub-contractors under the mandate M/381, in which the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands) was the appointed subcontractor to perform validation work on the standard for enumeration of *Campylobacter* described in ISO 10272-2 (Anon., 2006b, 2017b) and the Netherlands Food and Consumer Product Safety Authority (NVWA, Wageningen/Utrecht, the Netherlands) was the appointed subcontractor to perform validation work on the standard for detection of *Campylobacter* described in ISO 10272-1 (Anon., 2006a, 2017a). The subcontractors were responsible for the collaborative validation studies, including preparation of protocols, data collection and evaluation. The institutes closely collaborated on the elaboration of the interlaboratory studies to validate both parts of ISO 10272.

In this manuscript we report the results of the collaborative study performed in June 2013 and the outcome of the statistical analysis to determine the precision data of the quantitative *Campylobacter* method as described in the revised standard ISO 10272-2 (Anon., 2017b).

2. Materials and methods

2.1. Design of the trial

The design of the trial was basically according to the description of the interlaboratory study for quantitative methods in ISO 16140:2003 (Anon., 2003). Five different matrices were selected to be tested in the collaborative trial, representing sample types that are usually tested for the prevalence or numbers of *Campylobacter* (Jacobs-Reitsma et al., 2008), and in order to validate the method horizontally. Identical to the interlaboratory study concerning part 1 of ISO 10272 on detection (Biesta-Peters et al., 2018), the matrices concerned frozen spinach, minced meat, raw milk, chicken skin, and broiler caecal material. Each matrix was artificially inoculated with a different *Campylobacter* strain: *C. jejuni* WDCM 00005 (frozen spinach), *C. coli* WDCM 00072 (minced meat), *C. jejuni* WDCM 00156 (raw milk), *C. coli* WDCM 00004 (chicken skin) and *C. jejuni* DSM 24306/CNET 076 (broiler caecal material). These strains are recommended for the quality control of culture media according to ISO 11133 (Anon., 2014) and according to the Handbook of Culture Media for Food and Water Microbiology (Corry and Atabay, 2012). Four levels of contamination per matrix were tested; L0 (blank), L1, L2 and L3 (Table 1), with two blind replicates per level, resulting in 40 samples to be tested per laboratory. Due to the vulnerable nature of the micro-organism, *Campylobacter* may not survive when distributing inoculated food samples. Therefore the matrices had to be artificially contaminated by the participants after receiving the samples, using the provided vials with pre-prepared *Campylobacter* cultures and levels. This approach has been successfully used in previous validation studies (Rosenquist et al., 2007) and is also commonly used by the European Reference Laboratory (EURL) for *Campylobacter* in their proficiency tests within the National Reference Laboratories (NRL) network (Ingrid Hansson, EURL-*Campylobacter*, personal communication). All test materials during the study were coded randomly in order to prevent biased analysis based on expected outcomes.

According to ISO 16140:2003 (Anon., 2003) a minimum of 8 valid data sets are needed for quantitative studies, but some more than just 8 participating laboratories would be preferred in order to be able to compensate for an unforeseen exclusions of data. The members of CEN/TAG 19-*Campylobacter*, as well as the network of EU NRLs-*Campylobacter* were contacted for participation in the study. The participants were informed about the time schedule and procedure of the validation process prior to the start of the study. Each participating laboratory was randomly assigned a unique participant code and was provided with a Standard Operating Procedure (SOP) of the interlaboratory study containing the detailed description of the enumeration method, a procedure for artificially inoculating the samples prior to investigation, a form to report the condition of the samples upon receipt and a form to report the materials used, incubation conditions and times, and final results (also see the SOP provided as a Supplementary data file). The test materials (matrix samples plus inoculation vials) were shipped under UN3373 conditions to the participants on June 10, 2013, using dry ice in polystyrene boxes and a courier service. All media and materials needed for analysis of the samples were provided by the participants. The participants had to start the analysis on June 12, 2013 and to report the results before July 1, 2013. The provided data were analyzed as described in Section 2.4.

2.2. Method for validation

The method was validated using test portions of 10 g for the matrices frozen spinach, minced meat, raw milk and chicken skin, and for a test portion of 1 g for the matrix broiler caecal material.

The diagram of the procedure for the *Campylobacter* enumeration method as used in the interlaboratory trial is shown in Fig. 1. The enumeration of *Campylobacter* was performed by making an initial suspension of the test portion in 90 ml, or 9 ml in the case of broiler

Table 1
Summary of the performance characteristics for the enumeration method for the matrices frozen spinach, minced meat, raw milk, chicken skin, and broiler caecal material

Matrix	Frozen spinach			Minced meat			Raw milk			Chicken skin			Broiler caecal material		
Strain used for inoculation	*C. jejuni* WDCM 00005			*C. coli* WDCM 00072			*C. jejuni* WDCM 00156			*C. coli* WDCM 00004			*C. jejuni* DSM24306/ CNET076		
Level of inoculation	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
Intended inoculation level (log_{10} cfu/g)	3.5	4.5	5.5	3.5	4.5	5.5	3.5	4.5	5.5	3.0	4.0	5.0	5.0	6.0	7.0
Number of participating laboratories	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Number of samples tested	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Number of samples excluded due to missing data[1]	2 (0)	4 (2)	2 (0)	5 (0)	4 (0)	4 (0)	4 (0)	5 (0)	2 (0)	3 (1)	2 (0)	2 (0)	3 (1)	7 (1)	7 (1)
Number of samples excluded due to less reliable results, based only on counts < 10 cfu per plate	5	4	0	7	5	1	3	3	1	6	3	2	0	5	0
Number of valid sample *pairs* used in the calculations	11	10	14	8	9	12	11	10	13	9	11	13	13	8	11
Mean value Σa (log_{10} cfu/g)	3.7	4.6	5.3	3.6	4.7	5.1	3.7	4.8	5.9	2.8	3.7	4.9	5.1	5.4	6.7
Repeatability standard deviation S_r (log_{10} cfu/g)	0.17	0.10	0.17	0.17	0.11	0.43	0.19	0.22	0.12	0.17	0.38	0.44	0.13	0.15	0.13
Repeatability limit *r*:															
As difference on log_{10} scale (log_{10} cfu/g)	0.47	0.28	0.48	0.49	0.32	1.20	0.53	0.62	0.34	0.48	1.06	1.23	0.36	0.42	0.35
Overall per matrix (log_{10} cfu/g)		0.42			0.77			0.51			0.98			0.38	
As ratio on normal scale (cfu/g)	3.0	1.9	3.0	3.1	2.1	15.7	3.4	4.2	2.2	3.0	11.5	17.1	2.3	2.6	2.2
Overall per matrix (cfu/g)		2.65			5.86			3.24			9.52			2.38	
Reproducibility standard deviation S_R (log_{10} cfu/g)	0.32	0.40	0.50	0.24	0.41	0.52	0.33	0.47	0.37	0.45	0.41	0.54	0.38	0.31	0.28
Reproducibility limit R:															
As difference on log_{10} scale (log_{10} cfu/g)	0.89	1.13	1.40	0.68	1.16	1.44	0.92	1.32	1.03	1.26	1.15	1.50	1.07	0.86	0.78
Overall per matrix (log_{10} cfu/g)		1.16			1.14			1.10			1.31			0.91	
As ratio on normal scale (cfu/g)	7.7	13.4	25.0	4.8	14.4	27.8	8.4	21.0	10.6	18.0	14.0	31.9	11.7	7.2	6.0
Overall per matrix (cfu/g)		14.3			13.7			12.6			20.4			8.14	

[1] Missing data due to data not reported, reported operator errors, etc (of which due to non-countable results due to swarming colonies on the plates).

caecal material, of peptone-salt solution. A 10-fold dilution series was made from the initial suspension and surface inoculation of modified Charcoal-Cefoperazone-Deoxycholate (mCCD) agar was performed in duplicate for both the initial suspension and the dilution series, using a known volume for inoculation. All plates were incubated at 41.5 °C for 44 h ± 4 h under microaerobic atmosphere. This was followed by enumeration of suspect colonies and confirmation of five characteristic colonies for one plate per countable dilution per sample. For confirmation the colonies were first purified on blood agar plates, followed by microscopy for characteristic morphology and motility, oxidase testing, and testing of absence of growth at 25 °C under aerobic conditions. The microaerobic atmosphere for incubation of *Campylobacter* was described to have an oxygen content of 5% ± 2%, carbon dioxide 10% ± 3%, optional hydrogen ≤10%, with the balance nitrogen. Participants were asked to use their customary microaerobic atmosphere and record this on the result form.

2.3. Preparation and stability testing of the test materials

Five different matrices were used for the collaborative trials. Fresh *Campylobacter*-free chicken carcasses for chicken skin sample preparations were imported from Sweden (Guldfågeln AB, Mörbylånga, Sweden). *Campylobacter*-negative broiler caecal material was kindly provided by Albert ter Laak (Wageningen Bioveterinary Research, Lelystad, The Netherlands). One batch of frozen spinach and one batch of minced meat (beef/pork) were obtained from a local supermarket. Raw cow's milk was directly obtained from a dairy farm near Zutphen (The Netherlands). Ten samples per matrix were tested for the absence of *Campylobacter* using ISO 10272-1:2006 before processing. All test portions were prepared at RIVM and the non-inoculated samples were stored at −20 °C before dispatch.

All samples had to be artificially contaminated by the participants just before the start of their analysis, using a dedicated vial per sample with (or without) *Campylobacter* cultures. All vials for inoculations were prepared by CHEK (nowadays: Biosisto, Assen, The Netherlands). Overnight cultures of the strains (*C. jejuni* WDCM 00005, *C. coli* WDCM 00072, *C. jejuni* WDCM 00156, *C. coli* WDCM 00004, *C. jejuni* DSM 24306) were prepared at the desired concentrations in blood-based medium with glycerol and stored at −80 °C before dispatching. Participants were instructed on the inoculation of the samples in the Standard Operating Procedure (SOP) for the collaborative study (see the SOP provided as a Supplementary data file).

Pre-tests were done at RIVM to check on the suitability of the matrix-inoculation vials combinations, to obtain the desired levels (L1, L2, L3, Table 1) of contamination of the various sample types.

The homogeneity of the samples was determined according ISO guide 34 (Anon., 2009). The homogeneity was determined for each batch of the matrix/inoculation vials combinations by determining the level of *Campylobacter* on mCCDA and on non-selective blood agar for 10 vials. If the obtained value was below the critical value, the batch of vials was considered homogeneous.

The stability was determined by analyzing the various batches of vials at different time intervals after production, as described for homogeneity. To determine the homogeneity and stability of the vials the between-groups variation and the within-groups variation was calculated, using ANOVA. If the obtained value was below the critical value, the batch of vials was considered homogeneous and stable.

2.4. Analysis of the data

Data have been excluded from the calculations only on the basis of clearly identified technical reasons (temperature or time abuse during transport of the samples; deviations to the SOP for the trial by participants). Enumeration results from the participants were included into the analysis of data according to ISO 7218 (Anon., 2007, 2013). Participants were asked to test the samples by duplicate plating, to gain

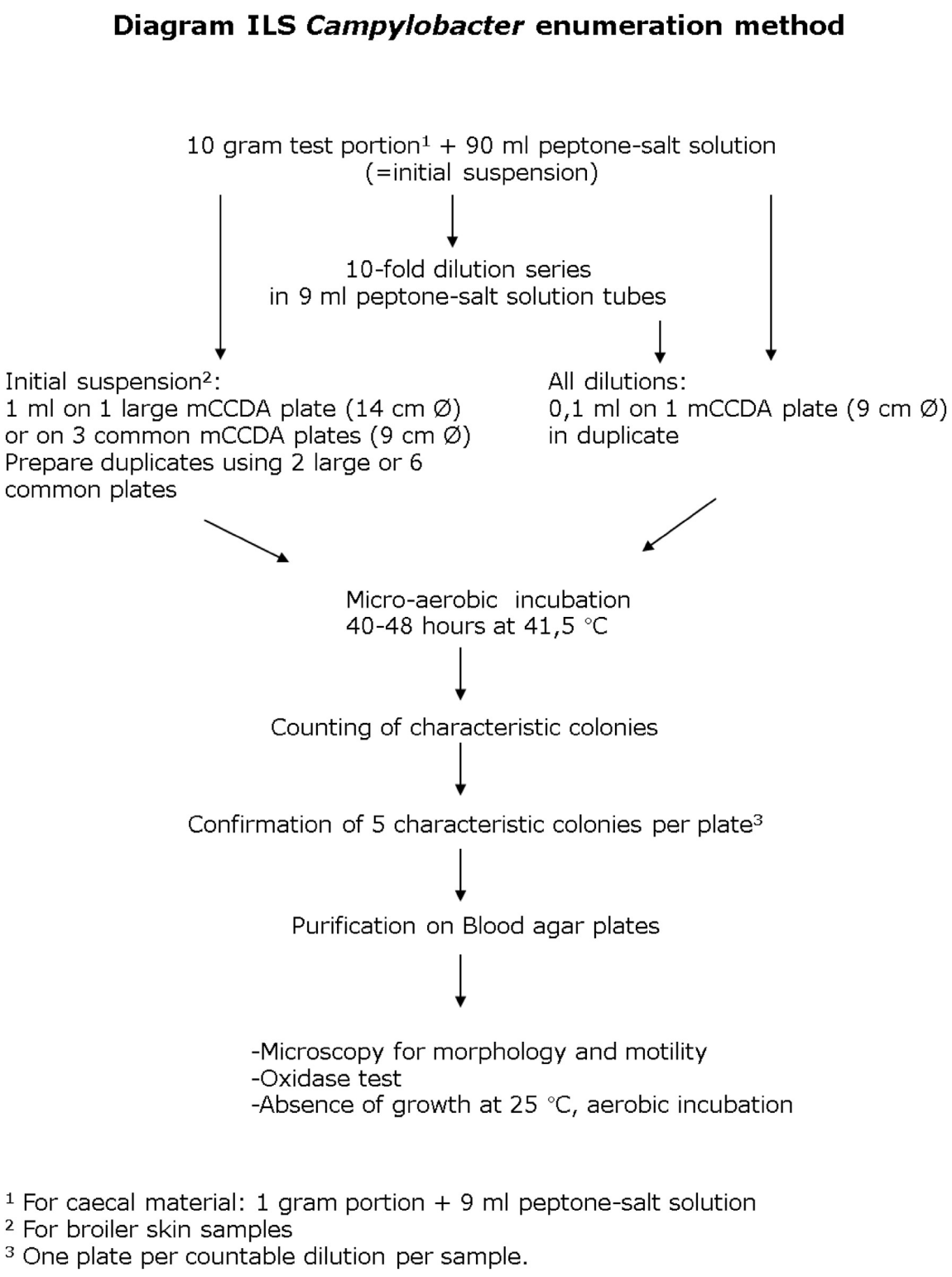

Fig. 1. Diagram of the procedure for enumeration of *Campylobacter*.

information on duplicate versus single plating at the same time. However, ISO 10272-2 is described to use single plating, and therefore only the first reported (single) result per sample was used for further analysis of the performance characteristics. The raw data were $\log_{10}$-transformed before performing calculations. Performance characteristics were then calculated in accordance with ISO 5725-2 (Anon., 1994) and ISO 16140 (Anon., 2003). The following parameters were calculated to derive the precision data for each of the matrices: the mean value ($\log_{10}$ cfu/g), repeatability standard deviation s_r ($\log_{10}$ cfu/g), and reproducibility standard deviation s_R ($\log_{10}$ cfu/g) (Anon., 1994).

The repeatability limit r was calculated from: $r = 2.8\ s_r$.
The reproducibility limit R was calculated from: $R = 2.8\ s_R$.

The constant $2.8 \sim z_\alpha\ 2$; with $z_\alpha = 1.960$ and 2-sided $\alpha = 0.05$ or 95% confidence level (Anon., 2003). Repeatability limit r and

reproducibility limit R can be expressed as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g) and as ratio on normal scale (cfu/g). Overall values per matrix for the repeatability limit r and the reproducibility limit R were derived from the mean of the variance estimates for all 3 levels per matrix.

3. Results

3.1. Homogeneity and stability of the test materials

The homogeneity and stability of the vials for artificial inoculation was tested and all batches were found to be homogeneous and stable. No problems were reported regarding the transport and receipt of the samples. All participants started the analysis within the given timeframe.

3.2. Results of the interlaboratory study

Fifteen laboratories from 11 different countries in Europe participated in the interlaboratory study. One laboratory was excluded from the dataset for all matrices, since only no growth or growth of < 10 colonies could be observed on the plates. A possible explanation for this result could be the mistakenly storage of the inoculation vials until analysis at −20 °C instead of at the requested −80 °C, which caused the *Campylobacter* strains to die, resulting in an inoculation level with no countable numbers. For the matrix minced meat one more laboratory was excluded from the dataset since no results were reported. Other data excluded from data analysis were merely single datapoints from laboratories, reported to be operator errors like a mistake in the inoculation of samples.

For all five matrices, results that were not-countable due to swarming of *Campylobacter* on the plates could not be taken into account. Also results based on counts of only < 10 colonies on a plate were excluded from the dataset, because these result only can be given as an estimate (ISO 7218, Anon., 2007, 2013). The final number of sample results retained after evaluation of the data and taken into account for calculation of the performance characteristics is shown in Table 1. Background information on the raw enumeration data per sample, both for the single plating as used in the calculations as well as for the duplicate plating, can be found in the Supplementary data file.

Eight laboratories used buffered peptone water as a diluent, whereas seven used peptone saline. In five cases ready to use medium was used, the other labs prepared their own medium. All labs used the compulsory mCCD agar for enumeration, of which 5 labs used ready to use plates. All laboratories dried their plates for a minimum of 10 min before use. The practice for drying of plates varied between laboratories. Mostly reported was drying for half an hour and the maximum reported drying time was 1 h. Most plates were dried at room temperature, but also temperatures of 30, 37 and 45 °C were reported. Four laboratories used microaerobic incubators for incubation of the plates, the others used jars of which the content was made microaerobic before storage in a general incubator. Four labs reported the gas content of the jars/incubator. These labs used the suggested gas composition without hydrogen gas. All laboratories respected the protocol for the confirmation of isolates. All blank samples were correctly reported to contain < 2.0 $\log_{10}$ cfu/g.

3.3. Performance assessment of the method

The overview of the calculated performance characteristics for the *Campylobacter* enumeration method for the matrices frozen spinach, minced meat, raw milk, chicken skin and broiler caecal material is given in Table 1.

3.3.1. Repeatability limit

The repeatability limit r is defined as the absolute difference between two independent single ($\log_{10}$-transformed) test results obtained using the same method on identical test material in the same laboratory by the same operator using the same apparatus within the shortest feasible time interval. The r value should not be exceeded in > 5% of the cases. If the difference between repeatability results within a laboratory exceeds r, the results may need further investigations.

As a general indication of repeatability limit (r), the following overall values (Table 1) can be used when testing *broiler caecal material samples*:

r = 0.38 (expressed as a difference between $\log_{10}$-transformed test results), or
r = 2.38 (expressed as a ratio between test results).

For example a test result of 1,000,000 or 1.0×10^6 or $\log_{10}$ 6.0 cfu per gram of broiler caecal material was observed in a given laboratory. Under repeatability conditions, the difference between $\log_{10}$-transformed results should not be greater than ± 0.38 $\log_{10}$ units. So the result from a second test of the same sample should be between 5.62 (6.0–0.38) and 6.38 (6.0 + 0.38) $\log_{10}$ units.

For non-log-transformed results, the ratio between the first test result and the second test result from the same sample should not be > 2.38. So the second test result should be between 420,000 (=1,000,000/2.38) and 2,400,000 (1,000,000 × 2.38) cfu per gram.

3.3.2. Reproducibility limit

The reproducibility limit R is defined as the absolute difference between two single ($\log_{10}$-transformed) test results obtained using the same method on identical test material in different laboratories with different operators using different equipment. The R value should not be exceeded in > 5% of the cases. If the difference between reproducibility results between laboratories exceeds R, the results may need further investigations.

As a general indication of reproducibility limit (R), the following overall values (Table 1) can be used when testing *broiler caecal material samples*:

R = 0.91 (expressed as a difference between $\log_{10}$-transformed test results), or
R = 8.14 (expressed as a ratio between test results).

The following overall values can be used when testing *chicken skin samples*:

R = 1.31 (expressed as a difference between $\log_{10}$-transformed test results), or
R = 20.43 (expressed as a ratio between test results).

As a first example; a test result of 1,000,000 or 1.0×10^6 or $\log_{10}$ 6.0 cfu per gram of broiler caecal material was observed in a first laboratory. Under reproducibility conditions, the difference between $\log_{10}$-transformed results should not be greater than ± 0.91 $\log_{10}$ units. So the result from a second laboratory should be between 5.09 (6.0–0.91) and 6.91 (6.0 + 0.91) $\log_{10}$ units.

For non-log-transformed results, the ratio between the test result from this first laboratory and a second laboratory should not be > 8.14. So the result from the second laboratory should be between 120,000 (=1,000,000/8.14) and 8,100,000 (1,000,000 × 8.14) cfu per gram.

As a second example; a laboratory wants to know the maximum value it may find for a poultry skin sample, which is still in compliance with a pre-set limit (e.g. a limit of 1000 or $\log_{10}$ 3). For this, the R value (on the log scale) has to be multiplied by a factor of 0.59.

The factor 0.59 reflects the fact that a test with a one-sided 95% interval is used to test whether the limit is exceeded; it is obtained from the following formula: $0.59 = 1.64 / (1.96 \times \sqrt{2})$.

The maximum value is 0.77 (1.31 × 0.59) as a difference between log10-transformed test results or 5.93 ($10^{0.77}$) as a ratio between test results. So results up to $\log_{10}$ 3.77 ($\log_{10}$ 3 + $\log_{10}$ 0.77) or 5900 (1000 × 5.93) do not indicate non-compliance with the limit.

4. Discussion

The requirement of a minimum of 8 valid data sets (16 paired sample results) per level and per matrix to calculate precision data was met, though it was a constraint that quite a number of data sets could not be used for the calculations due to only < 10 colonies per plate results for one or both samples per set.

The use of single or duplicate plating to establish the number of *Campylobacter* per sample did show some differences in results, within a maximum range of plus or minus 0.2 $\log_{10}$ cfu (see Supplementary data file). However, the overall effect on the mean, as well as on the repeatability and reproducibility standard deviations, and thereby on r and R, generally falls within the range of these parameters seen between the levels within a matrix or between the different matrices.

Following ISO 7218 (Anon., 2007, 2013) the performance characteristics for the ISO enumeration methods for e.g. *Listeria monocytogenes* (ISO 11290-2, Anon., 2017c) and for *Enterobacteriaceae* (ISO 21528-2, Anon., 2017d) were also established using single plating of the test samples. The standard deviations reported for these methods were mostly around or below 0.10 for repeatability and mostly around 0.30 or even below for reproducibility for the various matrices tested in the interlaboratory studies. As for *Campylobacter*, these studies were also performed under the CEN mandate M381, using the common approach. These precision data are generally lower than the data found in this study, but most likely this has to do with the more complicated nature of *Campylobacter* in culturing methods. This is confirmed by the comparison of the *Campylobacter* data from Rosenquist et al. (2007). They report a repeatability limit of 0.34 and a reproducibility limit of 1.46 for chicken minced fillet (*C. coli* strain inoculation), and 0.56 and 2.34 respectively for pasteurized whole milk (*C. jejuni* strain inoculation). Data were obtained from an interlaboratory study (11–12 participants), using mCCD agar as the enumeration medium. Habib et al. (2008) also report performance characteristics of mCCD agar and artificially contaminated chicken meat matrices, but based on intra-laboratory evaluations. Their data clearly show the impact of the differences between laboratories, as this intra-laboratory study design estimated values of 0.15 and 0.28 for repeatability and reproducibility in the chicken meat matrices.

The precision data found in our collaborative study provide evidence on the acceptability of the protocol, as described in the standard method ISO 10272-2, for the enumeration of *Campylobacter* in different matrices.

5. Conclusion

The method for enumeration of *Campylobacter* was validated for the matrices frozen spinach, minced meat, raw milk, chicken skin and broiler caecal material. The repeatability limit and the reproducibility limit were determined for the five selected matrices. The validation data were incorporated in the newly published ISO standard EN ISO 10272-2:2017 - Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* - Part 2: colony-count technique (Anon., 2017b).

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Appendix A. Supplementary data

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