Measurement Uncertainty According to ISO 19036:2019

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Abstract – ISO 19036 is an international standard that specifies and gives guidance for the estimation and expression of measurement uncertainty (MU) associated with quantitative results of microbial counts in foods. Standard describes top-down or global approach to MU, in which MU is calculated from experimental results with replication of the same analyses as part of the measurement process and expressed as a standard deviation of reproducibility of the final result. In a new revision from 2019 standard ISO 19036 considers three types of MU component: technical uncertainty, matrix uncertainty and distributional uncertainty. The aim of this study was to estimate all components of MU according to standard ISO 19036:2019. Technical uncertainty was estimated for quantitative enumeration tests for coagulasemonocytogenes, positive staphylococci, Listeria Escherichia coli, Enterobacteriaceae and aerobic mesophilic bacteria, while matrix uncertainty was estimated for ice cream, cheese, ready-to-eat vegetables, ready-to-eat food and cream cakes. Technical uncertainty was estimated from a reproducibility standard deviation of the final result of measurement process while the matrix uncertainty was estimated from within-sample variance by examination of multiple test portions from laboratory sample. Distributional uncertainty is estimated mathematically. The study showed that technical uncertainties of quantitative methods performed in our laboratory are the same magnitude and quite low. Matrix uncertainty was the largest contributor to combined uncertainty in composite food matrices. Our results are better than or in a close agreement with results from method validation interlaboratory studies and other published data.

Keywords – Measurement uncertainty; ISO 19036; Technical uncertainty; Matrix uncertainty; Distributional uncertainty

I. INTRODUCTION

ISO/IEC Guide 98-3 ("GUM") defines measurement uncertainty (MU) as a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand. It is used to express and quantify the lack of accuracy (trueness and precision) of the laboratory results or the other way around to indicate the degree of confidence that can be placed on results of measurement. The "parameter" associated with the result can be a standard deviation, a relative standard deviation, a confidence interval or a range [1].

GUM is a principal reference document on measurement uncertainty. According to this document the main approach for estimation of MU is to construct a mathematical measurement model that can quantitatively define all individual input quantities on which the quantity of measurand depends so that MU can be calculated from all the uncertainties of the input quantities. However, this model is not feasible in food microbiology since it is not possible to accurately quantify each input quantity because the analyte is a living microorganism with largely variable physiological states and many different strains/species/genera and for many input quantities their impact on measurand cannot be adequately quantified.

ISO 19036 is an international standard that specifies requirements and gives guidance for the estimation and expression of measurement uncertainty associated with quantitative results of microbial counts in foods. Standard describes top-down or global approach to MU, in which MU is calculated from experimental results with replication of the same analyses as part of the measurement process and it is expressed as a standard deviation of reproducibility of the final result. In this case standard deviation of most input quantities. In a new revision from 2019 standard ISO 19036 considers three distinct types of MU component: technical uncertainty, matrix uncertainty and distributional uncertainty [2].

Since reference values or assigned values are usually not available in food microbiology bias cannot be reliably estimated and is not included in the estimation of uncertainty according to standard ISO 19036:2019. Sampling uncertainty is also not covered by this document since it is not part of the uncertainty linked to measurement itself.

Standard ISO 19036:2019 also offers two options for estimating the MU. Option 1 includes all three components of uncertainty estimated individually and MU is reported as combined uncertainty (u_c) of technical, matrix and distributional uncertainty. Option 2 is based only on a technical uncertainty and reported as such, if that is consistent with laboratory protocols and client requirements [2].

A. Technical uncertainty (*u*_{tech})

Technical uncertainty arises from operational variability and is a characteristic of the method. It includes the variability of the taking, mixing, preparing of initial dilution and subsequent serial dilutions of the test portion from the laboratory sample, variability of inoculation, colony counting and confirmation, as well as the variability in incubation conditions and media content and quality. It is estimated from the standard deviation of reproducibility or intralaboratory reproducibility standard deviation (s_R) on the final result of the measurement. According to standard ISO 19036 technical uncertainty may be estimated on a single matrix since experimental protocol is designed to exclude contributions from the matrix.

B. Matrix uncertainty (umatrix)

In food microbiology test results can be significantly affected by matrix composition and microbial distribution. Matrix uncertainty however refers only to the effects of microbial distribution within a certain matrix and is a characteristic of that specific matrix. This uncertainty comes from the variations between results from different test portions of the same laboratory sample and it reflects the extent to which the individual test portions are not representative of the complete laboratory sample. Matrix uncertainty is considered as independent of the analytical method hence it can be applied to all quantitative tests in the same matrix. For homogenous or very well homogenized materials, such as liquids, powders, minced/chopped solids or fluids, matrix uncertainty is usually very small. On the other hand very heterogeneous and multi-component materials, such as cheeses, fresh-cut vegetables and ready-to-eat foods, can have very large matrix uncertainty. Matrix uncertainty is overestimate since it inevitably includes technical uncertainty that comes from operational variations between repeated analyses.

C. Distributional uncertainty (u_{Poisson})

Distributional uncertainty arises from intrinsic variability associated with the distribution of microorganisms in the sample and its dilutions. In microbiology intrinsic variability of microorganisms usually follows Poisson distribution [1]. Unlike technical and matrix uncertainty distributional uncertainty is calculated mathematically as Poisson standard uncertainty for each individual test result. In colony count methods minimum distributional uncertainty depends on the total number of counted colonies ($\sum C$).

ISO 19036:2019 also recognizes confirmation uncertainty in colony-count techniques that require confirmation of presumptive colonies and most probable number (MPN) uncertainty but these two uncertainties will not be discussed here.

The aim of this study was to estimate all components of MU according to standard ISO 19036:2019. Technical uncertainty was estimated for quantitative tests for coagulase-positive staphylococci (CPS), *Listeria monocytogenes* (LM), *Escherichia coli* colony count (EcCC), *Enterobacteriaceae* colony count (ECC) and aerobic colony count (ACC), while matrix uncertainty was estimated for ice cream, cheese, ready-to-eat (RTE) vegetables, ready-to-eat (RTE) food and cream cakes.

II. RELATED RESULTS IN THE LITERATURE

Besides respective ISO standards for microbiological methods [3-8], which include data obtained by method validation interlaboratory studies, the main source of related results is AFSSA/ISO Report of 2003/2004 ISO trials about uncertainty measurement [9]. The objective of these trials was to estimate the component of MU linked to taking of test portion and the preparation of initial suspension from laboratory (test) sample of different types of matrices. 13 microorganisms were enumerated in 91 various matrices, including aerobic mesophilic count, coliforms, *Escherichia coli* β-glucuronidase positive, Staphylococcus coagulase positive, Enterobacteriaceae and Listeria monocytogenes in meat and meat products, dairy products, fruits and vegetables, seafood, miscellaneous and composite foods. From obtained results standard deviations were calculated: between initial suspensions (s_{IS}) , between conditions (s_{cond}) , residual standard deviation between repetitions (sres) and total standard deviation (s_{tot}) , which is combined standard deviation of all three. Trials showed that standard deviation between initial suspensions (matrix specific) was the largest contributor to total standard deviation with mean value of 0.19 \log_{10} cfu/g (range 0.01 - 0.74 \log_{10} cfu/g, median 0.15 log₁₀ cfu/g). Standard deviation between conditions (intralaboratory reproducibility) was estimated lower, with mean value of 0.10 log₁₀ cfu/g $(range 0.01 - 0.58 \log_{10} cfu/g, median 0.07)$. Residual standard deviation had even lower range $(0.03 - 0.33 \log_{10})$ cfu/g, median 0.10) but mean value was 0.11 \log_{10} cfu/g. Total standard deviation ranged between $0.04 - 0.78 \log_{10}$ cfu/g, with median 0.23 \log_{10} cfu/g and mean value 0.26 \log_{10} cfu/g. In general high s_{tot} was due to high s_{IS} results.

Strong s_{IS} was explained by high heterogeneity of the distribution of microorganisms in the matrix and/or by a flora difficult to count. Trials also determined that for homogeneous samples (liquids and powders) matrix uncertainty is 0.1 log₁₀ cfu/g [9]. This finding was accepted by ISO and implemented in the standard ISO 19036:2019 as matrix uncertainty for homogenous matrices and samples that can be homogenized before taking the test portion [2].

Work by Jarvis and Corry contributed greatly to measurement uncertainty in food microbiology. Their respective studies, articles and books are invaluable sources of (microbiological and statistical) knowledge and data on the subject. Both authors (together with S. Passmore and A. Hedges) collaborated on a critical review of uncertainty in the enumeration of food micro-organisms [10] where they reviewed published data on uncertainty in food microbiology in regards to causes and magnitude of variability. They also suggested statistical methods that can be used to assess variability and precautions to be taken in order to minimize uncertainty.

III. DESCRIPTION OF THE METHOD

A. Technical uncertainty

Technical uncertainty was estimated for quantitative for enumeration of coagulase-positive methods staphylococci, Listeria monocytogenes, Escherichia coli, Enterobacteriaceae and aerobic mesophilic bacteria following the protocol from the standard ISO 19036, shown in Figure 1. At least 10 laboratory samples (artificially contaminated) of same matrix type were analysed on different days so that as much variability as possible was included in estimation. Two parallel test portions (2 x 10 g) of each laboratory sample were analysed in reproducibility conditions as different as possible (different analysts, different culture media batches, different incubators and pipettes). Artificially contaminated samples were prepared with fresh cultures incubated in TSB broth at 37 °C/18-20 h, from which 1 McFarland (10⁸ cfu/mL) suspension was prepared and decimally diluted to ~ 10^5 cfu/mL. 1 mL of 10^5 cfu/mL suspension was inoculated in each initial suspension (see Fig. 1).

Analyses of each test portion were performed as in routine testing according to methods specific for target microorganisms [3-7]. Colony counts between 30 and 250-300 cfu/plate were deemed as acceptable results. Reliability of results was tested according to ISO 14461-1 [8] and non-reliable counts were excluded. Results (colony forming units per gram, cfu/g) were then log_{10} -transformed. Intralaboratory reproducibility standard deviation (*s_R*) was calculated according to equation (1).

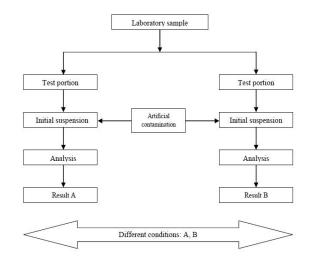


Figure 1. Experimental protocol for estimation of intralaboratory reproducibility (ISO 19036:2019)

$$s_{IR} = \sqrt{\frac{1}{2n} \sum_{i=1}^{n} (y_{iA} - y_{iB})^2}$$
 (1)

where *i* is the index of the sample, i = 1 to n ($n \ge 10$) and y_{iA} , y_{iB} are the log₁₀-transformed data, in log₁₀ cfu/g, from conditions A and B respectively. Microsoft[®] Excel[®] software was used in calculations.

B. Matrix uncertainty

Matrix uncertainty was estimated by testing multiple test portions of the same sample (matrix) in repeatability conditions (same analyst, same equipment and same culture media batches within short period of time) as shown in Figure 2. For this estimation naturally contaminated samples were used (cheese, ice-cream, ready-to-eat vegetables, cream cake and ready-to-eat food). 11 test portions (11 x 10 g) were analyzed from one laboratory sample (matrix) according to specific standards [3-8]. Colony counts between 30 and 250-300 cfu/plate were deemed as acceptable results. Reliability of results was tested according to ISO 14461-1 [8] and non-reliable counts were excluded.

Test results (cfu/g) were \log_{10} -transformed and withinsample repeatability standard deviation (s_r) was calculated according to equation (2). This is equivalent to standard deviation of \log_{10} -results. Microsoft[®] Excel[®] software was used in calculations.

$$s_r = \sqrt{\frac{\Sigma(y_i - \bar{y})^2}{n-1}}$$
 (2)

where n = 11, y_i is \log_{10} -transformed result of test portion *i* and \bar{y} is an average of results in \log_{10} cfu/g.

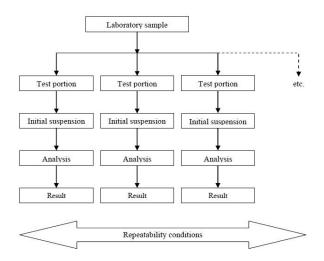


Figure 2. Experimental design for estimation of matrix uncertainty (ISO 19036:2019)

C. Distributional uncertainty

Poisson standard uncertainty $(u_{Poisson})$ in \log_{10} cfu/g is calculated according to formula (3).

$$u_{Poisson} = \frac{1/\ln(10)}{\sqrt{\Sigma C}} = \frac{0.4343}{\sqrt{\Sigma C}}$$
 (3)

where $\sum C$ is sum of all counted colonies. If $\sum C = 0$ (no colonies counted) $u_{Poisson} = 0.4343$.

D. Combined and expanded uncertainty

Combined uncertainty (u_c) is a combination of separately estimated technical standard uncertainty, matrix standard uncertainty and distributional standard uncertainty (equation (4)).

$$u_c(y) = \sqrt{u_{tech}^2 + u_{matrix}^2 + u_{Poisson}^2} \quad (4)$$

In a case when laboratory chooses option 2 for estimation of its uncertainty, combined uncertainty is equal to intralaboratory reproducibility standard deviation $(u_c = s_{IR})$.

Equation (5) is used for calculation of expanded uncertainty (U), with the coverage factor 2, which corresponds to a confidence level of 95%.

$$U = 2 u_c(y) \tag{5}$$

IV. RESULTS AND DISCUSSIONS

A. Technical uncertainty

Results of estimated technical uncertainty for quantitative tests for enumeration of coagulase-positive staphylococci (CPS), *Listeria monocytogenes* (LM), *Escherichia coli* colony count (EcCC), *Enterobacteriaceae* colony count (ECC) and aerobic colony count (ACC) are listed in Table 1, ranging from $0.04 \log_{10} \text{cfu/g to } 0.11 \text{ cfu/g}.$

 Table 1. Estimates of technical uncertainty by parameter and microbiological method.

Parameter/method	Matrix	$u_{tech} = s_{IR}$ (log ₁₀ cfu/g)
LM (ISO 11290-2)	Cheese	0.05
	Ice-cream	0.05
	Sliced ham	0.06
CPS (ISO 6888-1)	Cheese	0.06
	Ice-cream	0.07
	Minced meat	0.05
EcCC (ISO 16649-2)	Cheese	0.04
	Minced meat	0.07
	RTE vegetables	0.09
ECC (ISO 21528-2)	Ice-cream	0.11
	RTE vegetables	0.07
	Sliced ham	0.10
ACC (ISO 4833-1)	Ice-cream	0.08
	RTE food	0.09
	Sliced ham	0.09

Standard ISO 19036:2019 allows laboratories, whose precision (repeatability and reproducibility) is not greater than the corresponding values obtained in the interlaboratory study, to derive reproducibility from results of a method validation interlaboratory study. These reproducibility values are listed in specific standards [3-7] and can be used as general indications of the reproducibility limits $(R = 2.83 \cdot s_R)$ when testing food samples in general. Conversion of limits to standard deviations of reproducibility (s_R) gave the following results: aerobic mesophilic count/bacteria 0.16 log₁₀ cfu/g; Enterobacteriaceae 0.31 \log_{10} cfu/g; L. monocytogenes s_R = 0.15 \log_{10} cfu/g and coagulase-positive staphylococci s_R = $0.15 \log_{10}$ cfu/g. Standard ISO 16649-2 for *E. coli* does not include method validation data. Since our technical uncertainties range from 0.04 to 0.11 we can conclude that our reproducibility values are better than or in a close agreement with interlaboratory validation studies. Good precision makes us eligible for the use of these reproducibility values and we accepted them as benchmarking criteria (limits) in internal quality control. Our results are also in agreement with the data from ISO Trials where intralaboratory reproducibility was estimated $0.10 \log_{10} \text{ cfu/g}$ (range $0.01 - 0.58 \log_{10} \text{ cfu/g}$, median 0.07) [9].

Many studies of MU in microbiology reported high level of uncertainty. In study by Jarvis et al. [11] interlaboratory trials were performed to explore the uncertainty of data obtained by standardised microbiological methods for aerobic microorganisms, *Enterobacteriaceae* and *E. coli* when matrix (food) as source of variability had been reduced or removed. 19 different laboratories analyzed freeze-dried ampoules of standardised mixed culture. The study showed that reproducibility values ranged from 9.3 to 12.1% (corresponds to $0.58 - 0.77 \log_{10} \text{ cfu/g}$) for aerobic microorganisms, from 14.0 to 17.4% (corresponds to $0.72 - 0.88 \log_{10} \text{ cfu/g}$) for *Enterobacteriaceae* and from 21.1 to 30.9% (corresponds to $1.00 - 1.38 \log_{10} \text{ cfu/g}$) for *E. coli*. Major observation of this study was high level of uncertainty, which is much wider than would be expected from the commonly used microbiological rule that colony counts are reproducible within a range $\pm 0.5 \log_{10}$ of the estimated mean values. Authors concluded that uncertainty estimates are influenced by the test procedure itself, the choice of culture medium and the choice of statistical method for data analysis.

Similarly Corry et al. [12] reported in their study that relative percentage uncertainty of reproducibility for the aerobic counts ranged 5.5 to 10.5% (\pm 0.27 to \pm 0.60 log₁₀ cfu/g) of the mean counts, while for *Enterobacteriaceae* much higher values from 21.6 to 23.5% (\pm 0.74 to \pm 0.96 log₁₀ cfu/g). Authors preferred expressing the MU as relative standard deviation of reproducibility (percentage of the mean counts) because relative values are more useful in comparison with data obtained from other studies.

Study by Jarvis et al. [13] analysed MU from proficiency testing schemes, internal quality control monitoring and routine enforcement examination of foods. Authors found that proficiency test data showed extreme values of RSD_R up to $\pm 30\%$ depending upon the microorganism, the laboratory and the method of examination. RSD_R values of routine samples averaged around $\pm 12\%$ with maximum $\pm 41\%$, while internal quality control data for different microorganisms showed values up to $\pm 27\%$ depending on the microorganism and the examination procedure. Authors discussed that high levels of expanded uncertainty may be related to heterogeneous distribution of microorganisms both within- and betweensamples but also to other sampling and analytical factors.

Augustin and Carlier [14] examined data from French proficiency testing scheme RAEMA for the period 1999-2003. Their study showed that between-laboratory uncertainty varies with the enumeration method in use and this variability is relatively small (average $0.17 \log_{10} \text{cfu/g}$) for the enumerations without colony confirmation, i.e. for enumeration of aerobic microorganisms, the Enterobacteriaceae, E. coli and coagulase-positive staphylococci with the technique using the rabbit-plasma fibrinogen agar. However, in the case of enumeration methods with colony confirmation such as for coagulasepositive staphylococci with the Baird-Parker agar, enumeration of L. monocytogenes, enumeration of C. perfringens and enumeration of anaerobic sulfitoreducing bacteria the between-laboratory standard deviation is equal to $0.23 \log_{10} \text{cfu/g}$, $0.28 \log_{10} \text{cfu/g}$, 0.34 \log_{10} cfu/g and 0.47 \log_{10} cfu/g, respectively.

B. Matrix uncertainty

Estimates of matrix uncertainty are listed in Table 2. The highest matrix uncertainty was recorded for enumeration of *E. coli* in cheese $(0.23 \log_{10} \text{ cfu/g})$, enumeration of Enterobacteriaceae in ready-to-eat vegetables (0.21 $\log_{10} \text{cfu/g}$) and enumeration of aerobic mesophilic count in ready-to-eat food (0.20 log₁₀ cfu/g). All other matrix uncertainties were of the same magnitude as estimates of technical uncertainty. Our results are consistent with the findings published in Report of 2003/2004 ISO Trials about uncertainty measurement [9]. We presume that significant difference in matrix uncertainty for S. aureus and E. coli in cheese is mainly due to the level of contamination, although the presence of competitive flora and intrinsic factors of the matrix cannot be excluded. S. aureus was present at the level as much as 7x10⁶ cfu/g, ensuring more homogeneous distribution throughout the matrix in comparison to E. coli which was present at the level of 6.6x10³ cfu/g. Matrix uncertainty for ice-cream was determined as 0.1 log10 cfu/g for food category ii. in ISO Trials [2,9] but our results are below that value.

Table 2.	Estimates	of matrix	uncertainty	bv food	category.

Food category	Parameter/ method	u _{matrix} (log ₁₀ cfu/g)
Cheese	CPS	0.07
	EcCC	0.23
RTE vegetables	ECC	0.21
Ice-cream	ECC	0.09
	ACC	0.08
RTE food	ECC	0.05
	ACC	0.20
Cream cake	ECC	0.12
	ACC	0.06

Heterogeneous distribution of microorganisms in food matrices is a long known fact. Their spatial distribution in food may be random, even (regular, uniform) and/or contagious (aggregated or clumped). Even distribution and truly random distribution are rarely present in food, but contagious distribution of microorganisms in food is very often [14]. This means that distribution of microorganisms does not conform well to the normal distribution. In simple suspensions distribution of microorganisms conforms well to a random Poisson distribution, but that is not always the case. In solid and composite food the distribution is complex due to the presence of clumps and chains. Solid food such a cheese contain cells and clusters of microorganisms distributed within and between the original particles of food, which are generally not distributed randomly but as contagious distribution (clumps) [15]. Even adequately homogenized samples show variations in levels of contamination between different test portions, especially solid food matrices. These variations are "matrix uncertainty" [2].

C. Combined uncertainty (u_c)

Option 1: according to option 1 combined uncertainty is a combination of technical uncertainty, matrix uncertainty and distributional uncertainty. This option can be used if all component values are known.

	Option 1 (equation 4)			
Parameter/Food	$u_{tech} \ (\log_{10} \ cfu/g)$	u _{matrix} (log ₁₀ cfu/g)	u _{Poisson} (log ₁₀ cfu/g)	$u_c(y)$ (log ₁₀ cfu/g)
CPS/cheese	0.06	0.07	0.04	0.10
EcCC/cheese	0.07	0.23	0.04	0.24
ECC/RTE vegetables	0.09	0.21	0.04	0.23
ECC/Ice-cream	0.09	0.09	0.04	0.13
ACC/ Ice-cream	0.10	0.08	0.04	0.13
ECC/RTE food	0.09	0.05	0.04	0.11
ACC/RTE food	0.10	0.20	0.04	0.23
ECC/Cream cake	0.09	0.12	0.04	0.16
ACC/Cream cake	0.10	0.06	0.04	0.12
	u _{Poisson} according to equation (3)			

Table 3. Combined uncertainty according to option 1 of standard ISO 19036:2019 (example for $\sum C = 100$ cfu counts).

Table 4. Combined uncertainty according to option 2 of standard ISO 19036:2019.

	Option 2 (equation 1)		
Parameter/Food	s _{IR} (log ₁₀ cfu/g)	$u_c = s_{IR}^*$ (log ₁₀ cfu/g)	
LM/Cheese	0.05		
LM/Ice-cream	0.05	$\frac{0.05^2 + 0.05^2 + 0.06^2}{2} = 0.05$	
LM/Sliced ham	0.06	N 3	
CPS/Cheese	0.06		
CPS/Ice-cream	0.07	$\sqrt{\frac{0.06^2 + 0.07^2 + 0.05^2}{3}} = 0.06$	
CPS/Minced meat	0.05	N 3	
EcCC/Cheese	0.04		
EcCC/Minced meat	0.07	$\sqrt{\frac{0.04^2 + 0.07^2 + 0.09^2}{3}} = 0.07$	
EcCC/RTE vegetables	0.09	N 3	
ECC/Ice-cream	0.11		
ECC/RTE vegetables	0.07	$\int \frac{0.11^2 + 0.07^2 + 0.10^2}{2} = 0.09$	
ECC/Sliced ham	0.10	N 3	
ACC/Ice-cream	0.08		
ACC/RTE food	0.09	$\frac{0.08^2 + 0.09^2 + 0.09^2}{2} = 0.09$	
ACC/Sliced ham	0.09	N 3	
* $s_{IR} = \sqrt{\frac{s_1^2 + s_2^2 + s_3^2}{3}}$ (combined standard deviation of three s_{IR})			

Option 2: according to option 2 of standard ISO 19036:2019 combined uncertainty is equal to technical uncertainty if that is consistent with laboratory protocols and client requirements. In this option values of intralaboratory reproducibility (s_{IR}) are used as combined uncertainty for certain parameter, method and matrix. For example, for enumeration of *L. monocytogenes* in cheese combined uncertainty would be 0.05 log₁₀ cfu/g and expanded uncertainty 0.10 log₁₀ cfu/g. But for enumeration

of *L. monocytogenes* in ready-to-eat vegetables, for which we haven't experimentally determined its intralaboratory reproducibility, technical uncertainty would be derived as combined standard deviation of three s_{IR} determined for cheese, ice-cream and sliced ham (see Table 4.). Although standard ISO 19036:2019 states that technical uncertainty may be estimated on a single matrix we consider estimation on a few more matrices more reliable and realistic.

As can be seen combined uncertainty in option 1 differs from uncertainy in option 2 by the contribution from matrix. Also these values are overestimates because every component contains slight contribution from other components.

V. CONCLUSIONS AND OUTLOOK

Measurement uncertainty in food microbiology is very wide, much wider than in chemical analyses or physical measurements. Standard ISO 19036:2019 considers technical uncertainty as often the largest contribution to MU, although contribution from matrix uncertainty can be quite high(er). Our study showed different results. Technical uncertainties of quantitative methods performed in our laboratory are the same magnitude and quite low. Matrix uncertainty was the largest contributor to combined uncertainty in composite food matrices. Even the assigned uncertainty of 0.1 log₁₀ cfu/g for homogenous matrices is higher than most of our technical uncertainties. Our results are better than or in a close agreement with results from method validation interlaboratory studies and other published data. Matrix uncertainty can be minimized by good homogenization of sample before taking the test portions but this contributor is not completely under laboratory control. However, technical uncertainty is caused by variability in laboratory operations during analyses hence if controlled properly contribution from technical uncertainty will be very small.

REFERENCES

- [1] **ISO/IEC Guide 98-3:2008:** Uncertanty of measurement Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).
- [2] **ISO 19036:2019:** Microbiology of the food chain Estimation of measurement uncertainty for quantitative determinations
- [3] **ISO 6888-1:1999+Amd 1:2003:** Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Enumeration method
- [4] ISO 11290-2:2017: Microbiology of the food chain Horizontal method for the detection and enumeration of Listeria monocytogenes and Listeria spp. – Part 2: Technique using Baird-Parker agar medium
- [5] **ISO 4833-1:2013:** Microbiology of the food chain Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 °C by pour plate technique
- [6] **ISO 16649-2:2001:** Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of

beta-glucuronidase-positive Escherichia coli – Part 2: Colony-count technique at 44 $^\circ$ C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide

- [7] **ISO 21528-2:2017:** Microbiology of the food chain Horizontal method for the detection and enumeration of Enterobacteriaceae – Part 2: Colony-count technique
- [8] ISO 14461-2:2005: Milk and milk products Quality control in microbiological laboratories – Part 2: Determination of the reliability of colony counts of paralell plates nad subsequent dilution
- [9] Ah-Soon, C., Cornu, M.: Report of 2003/2004 ISO Trials about uncertainty measurement (June 2004). Available at: <u>http://standards.iso.org/iso/19036</u> (accessed 03 August 2020).
- [10] Corry, J. E. L., Basil, J., Passmore, S., Hedges, A.: A critical review of measurement uncertainty in the enumeration of food micro-organisms, *Food Microbiology*, 24, 2007, pp. 230-253.
- [11] Jarvis, B., Hedges, A. J., Corry, J. E. L.: Assessment of measurement uncertainty for quantitative methods of analysis: comparative assessment of the precision

(uncertainty) of bacterial colony counts, International Journal of Food Microbiology, 116, 2007, pp. 44-51.

- [12] Corry, J. E. L., Jarvis, B., Hedges, A. J.: Measurement uncertainty of the EU methods for microbiological examination of red meat, *Food Microbiology*, 24, 2007, pp.
- [13] Jarvis, B., Corry, J. E. L., Hedges, A. J.: Estimates of measurement uncertainty from proficiency testing schemes, internal laboratory quality monitoring and during routine enforcement examination of foods, *Journal of Applied Microbiology*, 103, 2007, pp. 462-467.
- [14] Augustin, J.-C., Carlier, V.: Lessons from the organisation of a proficiency testing program in food microbiology by interlaboratory comparison: analytical methods in use, impact of methods on bacterial counts and measurement uncertainty of bacterial counts, *Food Microbiology*, 26, 2006, pp. 1–38.
- [15] Jarvis, B.: The Distribution of Microorganisms in Foods in Relation to Sampling. In: *Statistical Aspects of the Microbiological Examination of Foods*, 3rd ed. Academic Press, London, 2016.