Professional paper - Stručni rad UDK: 637.112.2

Validation and measurement uncertainty estimation in food microbiology: differences between quantitative and qualitative methods

Vesna Režić Dereani[1], *Marijana Matek Sarić*[2]*

[1]SMS Prehrambeno razvojni centar, Kurtovici bb, Klis
[2]Sveučilište u Zadru, Odjel za zdravstvene studije, Trg kneza Višeslava, Zadar

Prispjelo - Received: 20.03.2010.
Prihvaćeno - Accepted: 18.08.2010.

Summary

The aim of this research is to describe quality control procedures, procedures for validation and measurement uncertainty (MU) determination as an important element of quality assurance in food microbiology laboratory for qualitative and quantitative type of analysis. Accreditation is conducted according to the standard ISO 17025:2007. General requirements for the competence of testing and calibration laboratories, which guarantees the compliance with standard operating procedures and the technical competence of the staff involved in the tests, recently are widely introduced in food microbiology laboratories in Croatia. In addition to quality manual introduction, and a lot of general documents, some of the most demanding procedures in routine microbiology laboratories are measurement uncertainty (MU) procedures and validation experiment design establishment. Those procedures are not standardized yet even at international level, and they require practical microbiological knowledge, altogether with statistical competence. Differences between validation experiments design for quantitative and qualitative food microbiology analysis are discussed in this research, and practical solutions are shortly described. MU for quantitative determinations is more demanding issue than qualitative MU calculation. MU calculations are based on external proficiency testing data and internal validation data. In this paper, practical schematic descriptions for both procedures are shown.

Key words: measurement uncertainty, validation, food microbiology laboratory, qualitative and quantitative measurement

Introduction

Accreditation according to the HR EN ISO/IEC:17025:2007 is applicable to all organizations performing tests and/or calibrations. These include, first-, second- and third-party laboratories, and laboratories where testing and/or calibration forms a part of inspection and product certification (HR EN ISO/IEC:17025, 2007). Accreditation of laboratories according to HR EN ISO/IEC:17025:2007 or other internationally recognized schemes is an instrument which contributes to confidence in international trade, to consumer's and official bodies confidence, and is of concern of all participants in food chain.

Well-established written quality control procedures, validation and procedures for measurement uncertainty (MU) determination are prerequisite for accreditation in food microbiology laboratory. The participation in proficiency testing schemes, external quality control is absolutely obligatory for an accreditation laboratory. Reference materials have proved far more difficult to produce in a stable form for microbiological examinations than for chemical analyses. For microorganism enumerations (quantitative methods), the testing performance of laboratories is assessed through the trueness and the precision which, respectively, express the agreement

*Corresponding authorDopisni autor/: E-mail: marsaric@unizd.hr

between the average contamination obtained by a laboratory and the accepted reference contamination and the agreement between the counts obtained by a laboratory on the same samples (ISO 5725-1,1994; Augustin and Carlier, 2006). For microorganisms detection (qualitative methods), the testing performance is assessed through the laboratory sensitivity (no false-negative results) and specificity (no false-positive results). The proficiency testing program represents a privileged observation post to study the evolutions of the analytical methods used by the laboratories, to assess the impact of these methods on analytical results, and to assess the measurement uncertainty of bacterial counts (Augustin and Carlier, 2006). Their considerations are very useful for situation in Croatian laboratories, where usually only ISO methods are being accredited, due to legislative requirements or misunderstanding of this, which are not practical for routine laboratories. Augustin and Carlier (2006) have stated that, depending on the method, 50 or more % of participating laboratories use alternative validated methods instead of ISO methods and its number is increasing during recent years.

Differences between validation and MU concepts of quantitative and qualitative microbiological analysis are discussed and schematically shown in this paper.

Accreditation and quality assurance in food microbiology laboratory

Accreditation procedures according to the HR EN ISO/IEC:17025:2007. are one of well known ways of quality assurance in laboratories in general and also in food microbiology laboratory and they include elements shown in Picture 1.

Many factors determine the correctness and reliability of tests and/or calibrations performed by a laboratory. These factors include contributions from: human factors, accommodation and environmental conditions, test and calibration methods and method validation, equipment, measurement traceability, sampling and sample flow, handling of test and calibration items, culture media and reagents, waste treatment and disposal and so on (HR EN ISO/IEC:17025, 2007; Gašljević, 2004). According to author's experience, it is practical to compile all factors of influence and procedures, altogether with related timetable, on the same schema. This example is based on the experience of authors (Figure 1) and represents only one of many possible ways of presentation, but each laboratory can create schematic view of its own, according to their needs. In concentric circle closest to the centre, main elements of quality assurance are displayed. Further concentric circles describe each of the items in more details.

Thorough discussion on factors of influence specific for food microbiology analysis is provided by Corry et al. (2007). The authors recommend precautions needed to minimize uncertainty due to personnel, equipment, diluents and media, incubation, sampling, examining cultures, recording data and quality monitoring.

Validation of microbiological analysis

The final goal of a method validation in microbiology is to ensure that every future measurement in routine analysis will be close enough to the unknown true value (Hubert et al. 2003; Gonzalez and Herrador, 2007). Accordingly, the objectives of validation are not simply to obtain estimates of trueness or bias and precision but also to evaluate those risks that can be expressed by the measurement uncertainty associated with the result (Gonzales et al., 2005; Gonzalez and Herrador, 2007).

Different authors consider standard and reference methods as laborious, expensive and time-consuming. The alternative methods must be validated as fit for purpose, and ISO 16140 (2003.) has been developed to address this need for both quantitative and qualitative methods. Harmonisation of validation and acceptable protocols is also desirable, but is proving difficult to achieve amongst the diverse bodies formulating microbiological methods. Performance data for quantitative methods are now becoming available (Corry et al., 2007).

As Feinberg and Laurentie (2006) and Gonzales and Herrador (2007) pointed out, method validation together with uncertainty measurement or accuracy profile estimation, can provide a way to check whether a method is correctly fit for the purpose of meeting legal requirements. Fitness for purpose is the extent to which the performance of a method matches the criteria that have been agreed between the analyst and the end-user of the data or the consumer and that describe their needs (Gonzales and Herrador, 2007).

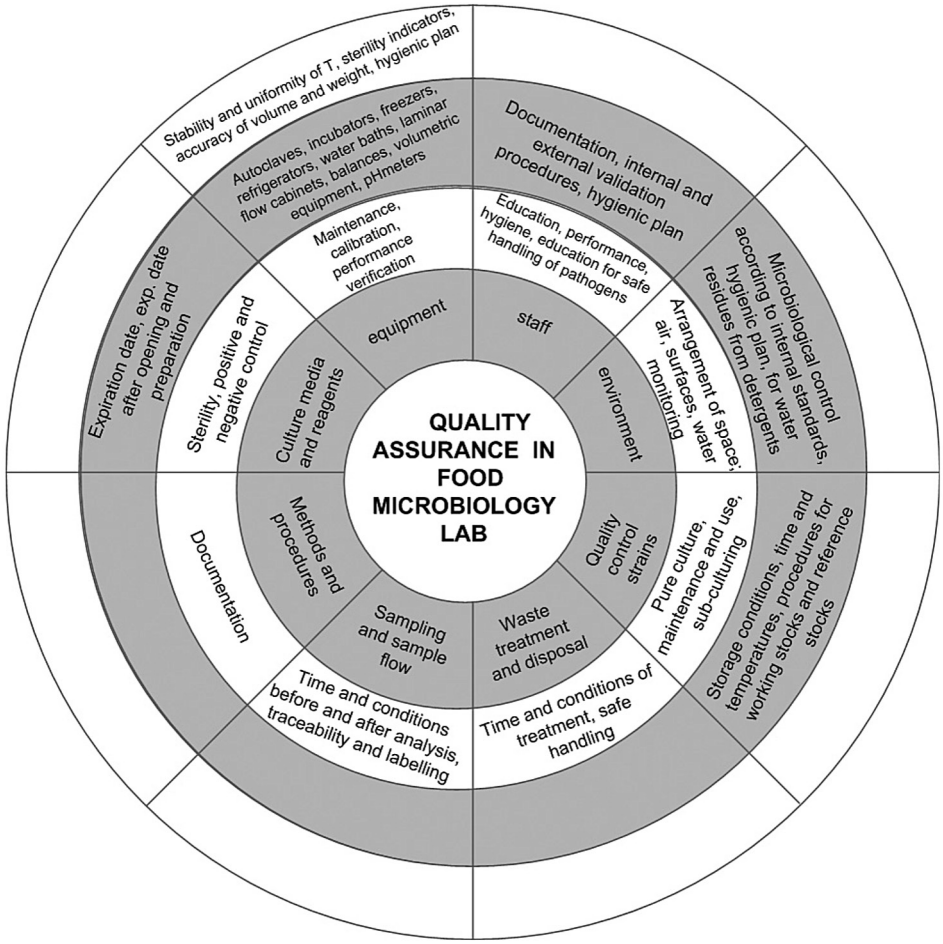

Figure 1. The most important elements of quality assurance in food microbiology laboratory
Slika 1. Najvažniji elementi za kontrolu kvalitete u laboratoriju za mikrobiologiju namirnica

Microbiological methods are not 100 % sensitive and they will therefore underestimate the level of target organisms to varying degrees. Similarly, many methods are not 100 % specific and this can lead to over-estimation of the target population by including false-positive results.

Internal control of methods is usually achieved by regular incorporation of internal quality control samples into routine work with a frequency depending on the degree of assurance desired (Corry et al., 2007). Internal validation of quantitative and qualitative microbiological analysis is based on experiments with spiked samples with known number of target micro-organism and occasionally with other microorganisms. Repeatability provides a measure of the variability between analyses conducted on identical test materials by the same technician in the same laboratory, under conditions as similar as possible (e.g. by using the same apparatus and reagents within the shortest possible interval of time), whilst the reproducibility measures the variability when the analyses are conducted by different technicians at different laboratories. The internal (or intermediate) estimation of reproducibility is obtained by analysis of data derived by different analysts operating within a single laboratory. Strains of microorganisms for validation are isolated and maintained at low temperature, under defined circumstances. Source of strains could be reference culture collections, proficiency testing or laboratory's own isolates. Each technician is tested for proper procedures performing, and results of each are validated, as well as common result for laboratory.

External validation is also based on spiked samples with known number of target microorganism and possibly with competitive microorganisms. It is performed by independent institutes or companies who send samples to participants. After trials, results of all laboratories are analysed and reports are sent to participants with statements of quality of analyses for all participants (z-score for quantitative analysis, sensitivity, specificity and accordance for qualitative methods).

Validation of qualitative microbiological analysis

In qualitative food microbiology, the usual measures of repeatability (r) and reproducibility (R) are inapplicable. Analysis is based on the probability of finding the same test results for identical test materials within and between laboratories, respectively. Good indicators for both external and internal validation of qualitative microbiological analysis are accuracy (specificity and sensitivity) and accordance. Specificity is (for blank samples) percentage of samples correctly identified as being negative. Sensitivity is the percentage of samples correctly identified as positives. Accordance is a new measure for compliance of results within the same laboratory, i.e. percentage of chance that two identical samples analysed by the same laboratory under standard repeatability conditions will give the same result - both found positive or both found negative (Langton et al., 2002). For qualitative microbiological analyses it is common to perform validation of method with blank samples, samples with low level of contamination and samples with high level of contamination (Ellison and Fearn, 2005). During the internal spiking sample experiment design, the best practice is to follow literature recommendations which are usually based on collaborative trials (Leuschner et al., 2004). Different types of matrices are used depending on availability and preferences of laboratory (Health Protection Agency, 2005).

Validation for quantitative microbiological analysis

The concepts of repeatability and reproducibility are widely used in the data analysis from collaborative trials in quantitative microbiology. The precision of standard enumeration methods has been derived from collaborative trials to validate methods for international use. Published methods include repeatability and reproducibility estimations (Corry et al., 2007).

Good indicators of quantitative methods acceptability are:

1. Selectivity/specificity (usually determined by quality of culture media)
2. Accuracy (determined by z-score from interlaboratory trials)
3. Precision (repeatability - parallel analysis of the same technician; and reproducibility - analysis of the same sample by different technicians and counting the same plates)
4. Limit of quantification

Measurement uncertainty for qualitative microbiological analysis

Measurement of uncertainty has been a commonplace requirement in physical and chemical analyses for many years but it is only recently that the subject has been addressed by microbiologists. Whilst the accepted concept is the measurement of the "level of uncertainty" associated with a microbiological test, the recipient really wants to know the "level of confidence" which the microbiologist can put on the particular result. Laboratory accreditation procedures, and both national and international definition and standardisation of laboratory methods seek to define the level of uncertainty which can be ascribed to a series of tests. The British Standards Institute, the International Standards Organisation (ISO), Codex Alimentarius, the International Dairy Federation (IDF), the Nordic Committee for Microbiological Standardisation (NMKL) and AOAC International are but a few of the organisations currently seeking to define and to provide measurements of uncertainty associated with methods used for the examination of foods for pathogenic and other microorganisms (Corry et al., 2007).

"Presence or absence" data are not easily amenable to statistical analysis to express reproducibility or repeatability. Classical MU determination for qualitative analysis does not apply, but laboratories have care that their false positive/false negative results ratio does not exceed published recommendations (literature or manufacturer specification) (CAEAL policy, 2003). It should be treated as a non-conformance with causes identified for corrective action. Specificity is percentage of known negative test materials that are correctly

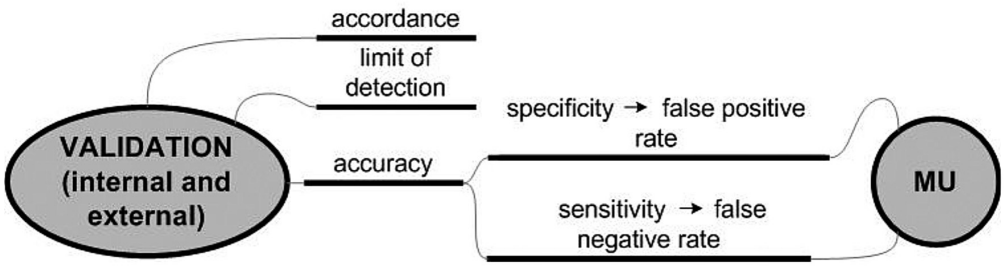

Figure 2. Schematic presentation of relationship between factors for validation and measurement uncertainty determination of qualitative methods in food microbiology laboratory
Slika 2. Shematski prikaz odnosa između faktora validacije i određivanja mjerne nesigurnosti kvalitativnih metoda u laboratoriju za mikrobiologiju hrane

identified as negatives (specificity = no. negative samples x 100/no. of blank samples). False positive percent is then calculated as: 100-specificity. Sensitivity is percentage of known positive test materials that are correctly identified as positive (sensitivity = no. positive samples x 100/ no. of truly positive samples).

False negative percentage is then calculated as: 100- sensitivity. Percentage of false negative and false positive results can be established by internal validation and/or from external proficiency testing. Literature reference for published specification could be ISO standard methods, for example HRN EN ISO 6579:2003 for Salmonella detection (HRN EN ISO 6579:2003). Other possible reference sources are scientific reports on interlaboratory trials (Leuschner et al., 2004).

In the case of qualitative methods validation is based on the calculation of accordance, limit of detection and accuracy (specificity and sensitivity) that are obtained from external and/or internal proficiency testing. MU (in fact - false positive and false negative rate) is then calculated from validation data - from sensitivity and specificity (schematically shown by authors in Figure 2).

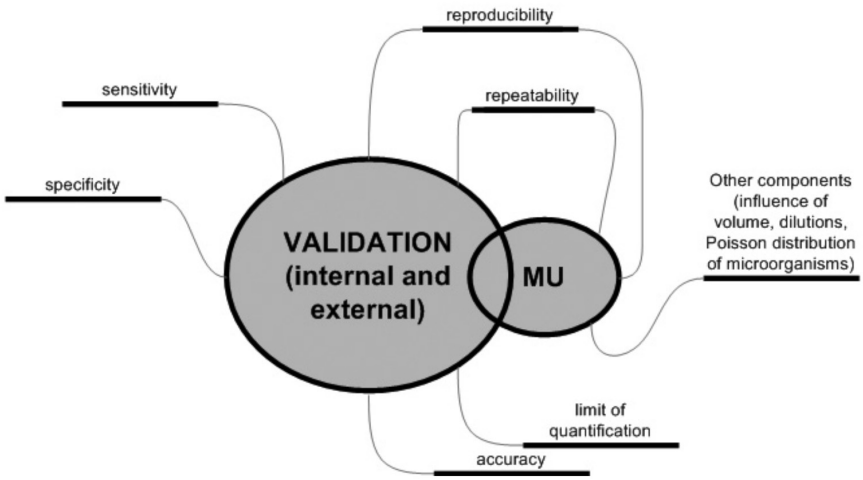

Figure 3. Schematic presentation of relations between factors for validation and measurement uncertainty determination of quantitative methods in food microbiology laboratory
Slika 3. Shematski prikaz odnosa između faktora validacije i procjene mjerne nesigurnosti kvantitativnih metoda u laboratoriju za mikrobiologiju hrane

Measurement uncertainty for quantitative microbiological analysis

Estimations of uncertainty relate to the data produced, using a specific method of analysis, but not to the method per se (Corry et al., 2007).

Combined standard MU determination (also known as Type B evaluation) according to Niemela, 2002 is usually used in Croatian laboratories. It considers all relevant factors which contribute to MU for particular analysis. Combined MU may be composed of:

1. Laboratory MU of counting
2. MU of volume
3. MU due to Poisson scattering of microorganisms
4. MU due to dilutions
5. MU of confirmed counts, etc.

In the Figure 3 authors of this article have tried to explain relations of factors for validation and MU determination in quantitative microbiological methods. Some factors (sensitivity, specificity, limit of quantification, accuracy) are determined and used as validation data only. Reproducibility and repeatability calculation is part of both validation and MU procedures. In addition, MU determination comprises other influences - volume uncertainties, dilutions, Poisson distribution of microorganisms etc.)

Conclusions

Crucial evidence for fulfilling accreditation requirements is regular and successful conduction of internal and/or external validation of methods in use. Credibility of measurement data has never caught the public's eye more than today. The key principle for quality and reliability of results is comparability between laboratories on a wider, international basis. In order to be comparable, results must be reported with a statement of *measurement uncertainty* (MU). Each laboratory has to conduct validation procedures defined by itself, but its revision has to be provided by an independent accreditation body. Validation measures for qualitative microbiological analysis are not standardized yet. Measurement uncertainty determination can be conducted based on validation data, and is also important part of quality control in food microbiology laboratory. In the case of qualitative methods, MU (false positive and false negative rate) is based and calculated from validation data. For quantitative methods, some of factors (reproducibility and repeatability) are common for validation procedures and MU determination and, in that case, validation and MU determination should be planned and performed jointly. Different approaches are possible, according to literature data, in each laboratory. This research describes only one of the possible solutions, and is based on practical experience of authors in fulfilling accreditation requirements.

Validacija i procjena mjerne nesigurnosti u mikrobiologiji hrane: razlika između kvantitativnih i kvalitativnih metoda

Sažetak

Cilj ovog rada je opisati postupke kontrole kvalitete te postupke validacije i određivanja mjerne nesigurnosti koji su važan element u osiguranju kvalitete rada laboratorija za mikrobiologiju namirnica, za kvalitativne kao i za kvantitativne metode određivanja. Akreditacija prema normi HRN EN ISO/IEC 17025:2007. Opći zahtjevi za osposobljenost ispitnih i umjernih laboratorija, koja jamči poštivanje standardnih operativnih postupaka i tehničku kompetentnost osoblja koje je uključeno u provođenje ovakvih ispitivanja, u posljednje vrijeme se na velika vrata uvodi u sve veći broj laboratorija za mikrobiologiju hrane u Hrvatskoj. Osim izrade priručnika kvalitete, te niza opće dokumentacije, jedna od najzahtjevnijih zadaća za rutinske mikrobiološke laboratorije prema zahtjevu HRN EN ISO/IEC 17025:2007 je izrada postupaka validacije i mjerne nesigurnosti (MU) ispitnih metoda. Ti postupci nisu još uvijek standardizirani ni na međunarodnoj razini, stoga je potrebno mnogo praktičnog mikrobiološkog znanja, ali i statističkih kompetencija za uspostavu ovih postupaka. Postoji razlika između provjere valjanosti kvantitativnih i kvalitativnih mikrobioloških metoda analize hrane i u ovom radu su one obrađene te opisana njihova praktična rješenja. Određivanje mjerne nesigurnosti kod kvantitativnih analiza zahtjevnije je nego kod kvalitativnih analiza. Kod kvantitativnih mikrobioloških metoda za proračun MU koriste se pojmovi ponovljivost (r) i reproducibilnost

(R) upravo iz validacijskih postupaka. Kod kvalitativnih mikrobioloških analiza uobičajene mjere r i R su neprimjenjive. Analiza se temelji na vjerojatnosti pronalaženja istog rezultata u identičnom test materijalu (uzorku) unutar i između laboratorija. Dakle, izračun MU temelji se na podacima interkalibracije i internim provjerama valjanosti podataka. U ovom radu prikazan je shematski prikaz za oba postupaka određivanja.

Ključne riječi: mjerna nesigurnost, validacija, laboratorij za mikrobiologiju namirnica, kvalitativna i kvantitativna mjerenja

References

1. Augustin, J.C., Carlier V. (2006): Lessons from the organization 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 23*, 1-38.
2. CAEAL Policy on the Estimation of Uncertainty Measurement in Environmental Testing (2003), http://www.caeal.ca/P19_CAEAL_Unce_Pol.pdf.
3. Corry, J.E.L., Jarvis, B., Passmore, S., Hedges, A. (2007): A critical review of measurement uncertainty in the enumeration of food micro-organisms. *Food Microbiology 24*, 230-253.
4. Ellison, S.L.R., Fearn, T. (2005): Characterising the performance of qualitative analytical methods: Statistics and terminology. *Trends in Analytical Chemistry 24* (6), 241-267.
5. Feinberg, M., Laurentie, M. (2006): A global approach to method validation and measurement uncertainty. *Accred. Qual. Assur 11*, 3-9.
6. Gonza´lez, A.G., Herrador, M.A., Asuero, A.G. (2005): The expression of uncertainty and confidence in measurement. *Talanta 65*, 1022-1030.
7. Gonzalez, A.G., Herrador, M.A. (2007) A Practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. *Trends in Analytical Chemistry 26* (3), 227-238.
8. Gašljević, V. (2004.): Validacija mikrobioloških metoda. Seminar Hrvatskog mjeriteljskog društva, Materijali, 1. izdanje 05/2004.
9. Health Protection Agency (2005), Uncertainty of measurement in testing. National standard method QSOP 4 Issue 5. http://www.hpa-standardmethods.org.uk/pdf sops.asp.
10. HRN EN ISO 6579:2003 Microbiology of the food and animal feeding stuffs-Horizontal method for the detection of *Salmonella* spp.
11. HRN EN ISO/IEC: 17 025:2007 Opći zahtjevi za osposobljenost ispitnih i umjernih laboratorija (Uključujući ispravak AC:2006).
12. Hubert, P.H., Nguyen-Huu, J.J., Boulanger, B., Chapuzet, E., Chiap, P., Cohen, N., Compagnon, P.A., Dewe, W., Feinberg, M., Lallier, M., Laurentie, M., Mercier, N., Muzard, G., Nivet, C., Valat, L. (2003): *STP Pharma Pratiques 13* (3), 101.
13. ISO 5725-1:1994 Accuracy (Trueness and Precision) of Measurement Methods and Results-Part 1: General Principles and Definitions. International Organization for Standardization, Geneva, Switzerland.
14. ISO 16140:2003 Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods, Geneva, Switzerland.
15. Leuschner, R.G.K., Bew, J., Boughtflower, M. P. (2004): A collaborative study to evaluate qualitatively powdered baby food validation samples artificially contaminated with *Salmonella anatum*. *International Journal of Food Microbiology 97*, 43-51.
16. Langton, S.D., Chevennement, R., Nagelkerke, N., Lombard, B. (2002): Analysing collaborative trials for qualitative microbiological methods: accordance and concordance. *International Journal of Food Microbiology 79*, 175-181.
17. Niemela, S.I. (2002): Uncertainty of quantitative determinations derived by cultivation microorganisms. Centre for metrology and accreditation, Publication J3/2002.