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Estimation and comparison of interalaboratoarystandarad deviation (SIR) measurement uncertainty in Quantitative microbiological tests in different food items based on EN ISO:19036-2019

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ABSTRACT

The aim of study was estimated and compared intra-laboratory quantification deviation (SIR) or measurement uncertainty (MU) as a performance Charateristics in verification of the implementation step of quantitative test methods in food microbiology laboratories.The *aerobic mesophilic* colony counts of microorganisms (ACC) and *Enterobacteriaceae* colony count (ECC), as two important and common tests in the microbial evaluation of all type of food was selected and interlaboratoary standard deviation estimation of selected food items: minced meat, hamburger, soy powder, pasteurized liquid eggs, pasteurized and UHT milk, ice cream, Fruit juice, flour, cake and spice (PEPER) were calculated based on ISO-19036 standard method (2019).In this comparison, technical, matrix, distribution, confirmation and combined Uncertinity were calculated and reported.Calculation of the technical uncertainty of the ECC test in (pasteurized and ultra-heated milk, ice cream, fruit juice and pasteurized liquid eggs) by created the artificial contamination on three levels with the target organism (*Shigella felxseneri*) and in other food items it was natural contamination, in the ACC test was only natural contamination was calculated. The technical uncertainty results of the ECC test ranged from 0.487 to 0.07 and in the ACC test, from 0.390 to 0.105 log10 cfu/g. The highest values of technical and matrix uncertainty were observed in meat, cake, hamburger and cheese samples, which showed the heterogeneous foods (solid and semi-solid) and the lowest values were observed in liquid (homogeneous) samples. Evaluation of variability and followed the uncertainty is proposed as a way to standardize the expression of variability associated with data obtained in microbiological methods to highlight the causes and extent of several influencing factors.

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1. Introduction

Today, food contamination and spoilage has become a major global challenge. This means that ensuring food safety is as important as providing food. The number and types of microbes in food are important indicators of safety and quality. Microbiological analysis of food and food products is one of the most important factors in the production of healthy food, because pathogenic microorganisms in food products have dangerous effects on human health [1].

In quantitative microbial tests and in general all tests affecting the health of food, it is necessary to reach the real value of the results. In microbial tests, the test quantity (analyte/sample) is a living organism that is highly variable from a physiological point of view, because it has different genera, species, and strains. Also, although it is not impossible, some input quantities can be described with difficulty (such as the physiological state). The effects of many input quantities (such as temperature, water activity) on the results cannot be described quantitatively and accurately [2].

Measurement Uncertainty (MU) is used to indicate the inaccuracy (correctness and precision) associated with the analysis results. In the field of quantitative microbiology, measurement uncertainty represents the degree of confidence that can be used in the laboratory to calculate the number of microorganisms in food and/or other materials [3]. In performing microbiological tests, many causes of variability can be identified, for example: the ability of an isolate to produce typical reactions on a diagnostic medium, equipment and human errors in weighing, dispensing, dilution and other laboratory steps and the relative skill levels of technicians. [4]. The main approach in determining the measurement uncertainty is to design a mathematical measurement model that can quantitatively define all the individual input parameters on which the quantity of the test results depends, so that the measurement uncertainty can be calculated from all the parameter uncertainties. Calculated input[5].

In recent years, laboratories working in food microbiology have been trying hard to meet the requirements of the global standard EN ISO/IEC 17025[5], especially regarding the acceptance and validation of methods. Laboratories, in fact, must adopt appropriate methods and procedures for their use issued by the laws of national, European and/or international organizations. In addition, when laboratories agree to use internally developed methods or standard methods without validation data or outside their scope, they must evaluate and determine the reproducibility standard deviation based on the requirements of the ISO 19036 [6] series of standards. In-laboratory Standard (SIR) or Uncertainty (MU) measurements to confirm the implementation of quantitative microbial test methods. The use of valid reference methods is an essential part of any laboratory quality assurance program and has been accepted by all related centers. In addition, validation and certification of test methods is mandatory by ISO/IEC 17025 [5]. Finally, laboratories should ensure the quality of analytical data in a continuous process, and a useful way to meet this need is regular participation in skill testing projects in order to monitor the performance of methods and testers and estimate their accuracy [6].

Jarvis et al [7] estimates of reproducibility and reproducibility for an interlaboratory trial by three analysts in 19 laboratories of three International Standard Organization (ISO) colony count methods for aerobic organisms (ACC), Enterobacteriaceae (ECC) and Escherichia coli (EcCC).) and was used to calculate the measurement uncertainty parameters. The estimated values of uncertainty of reproducibility and reproducibility for ACC ranged from 9.3 to 12.1% and 2.0 to 3.9% of the mean log10 colony count, respectively, depending on the specific culture medium, the method of extracting the mean number and statistics. Reproducibility and reproducibility uncertainty estimates for ECC ranged from 14.0 to 17.4% and 4.1 to 6.7%, respectively.

Režić Dereani et al. [8] described quality control methods, validation methods and determination of measurement uncertainty (MU) as an important element in quality assurance in food microbiology laboratory for qualitative and quantitative type of analysis. The differences between the design of validation tests for quantitative and qualitative food microbiology analysis are discussed in this research and stated that MU calculations are based on external proficiency test data and internal validation data.

Bacteria of the Enterobacteriaceae family are used as an indicator of the sanitary conditions of production processes, because they are easily

deactivated by disinfectants, and if cleaning and hygiene conditions are not performed in factories, their amount increases in the product. Enterobacteriaceae are mostly mesophilic, and its psychrotrophic strains include Yersinia, Citrobacter, Enterobacter, Escherichia, Klebsiella, Serratia and Hafnia [9]. Quantitative microbial characteristics, the total count of mesophilic aerobic microbes and the count of Enterobacteriaceae bacteria as the most common microbial characteristics for different types of food in the authoritative references of international microbial characteristics [10] (ICMSF), regional European Union [11] and at the level National, such as the microbial characteristics in the Turkish Codex [12], the microbial characteristics of the Persian Gulf countries GFSA [13] and the microbial characteristics table of the Ministry of Health's Food and Drug Control Reference Laboratory M5 1400, [14] and in the standards of the national organization Iran ISIRI with the titles of standards of microbial characteristics of various food products, including microbial characteristics of dairy products 2406, confectionery and confectionery products 2395, characteristics of flour and grain products 2393 and 11603, characteristics of meat products 2303, 2304, characteristics 6037 types of spices, 15507 types of coffee products, 3307 types of cocoa products are presented [15], and the permissible limits of these indicators in these authoritative sources are in the range of 10 to 100ml or CFU/g for the ECC Enterobacteriaceae bacteria count test and 100 to 610ml or CFU/g. g, to test the overall count of mesophilic aerobic microorganisms ACC, in different types of the aforementioned food items.

The purpose of this article is to measure the standard deviation of the reproducibility of the intra-laboratory standard (uncertainty) based on the approach of the ISO 19036 international standard and based on the principles and requirements of the 16140-3 standard [16] and in order to confirm the implementation of the quantitative test method and for validation. (Guarantee the quality of test results) in food microbiology test methods and in order to continuously monitor the output quality of analytical data, it has been laboratory results. For this purpose, the steps of calculating SIR or uncertainty based on the instructions of the mentioned standards, based on the practical method and practical protocol, for each of the microbial quantitative test methods, including the number of Enterobacteriaceae bacteria colonies (ECC) and the number of aerobic colonies (ACC) in the items Pasteurized ice cream, pasteurized milk, high-temperature milk, juice, flour, cake, spices, minced meat and hamburger from ten different brands, estimated and calculated and finally the uncertainty values calculated among all types of food items based on the type of liquid nature (Homogeneous), semi-liquid and solid (heterogeneous) and the type of natural or artificial pollution have been compared.

2-Materials and methods

All culture media and reagents were purchased from Merck, Germany. Reference microorganism strain*Shigella flexneri* (ATCC) was prepared as a cryobank from Tav Biotechnology Company (Pasteur Institute of Iran). All the steps discussed in this section were carried out in the microbiology laboratory of FDCL, Mashhad, Iran.

Selection of types of food items based on Annexes A and B of the third part of ISO 16140 standard series 16140 [16], taking into account factors such as the characteristics of matrix types (food items) and different characteristics such as challenging (for example, high microbiota) , pH, spoilage microorganisms, composition and antimicrobial compounds have been carried out. To calculate the microbiological uncertainty, fresh ground meat (fresh meat), hamburger, pasteurized ice cream, wheat flour, soybean powder and spices (pepper) were selected from among the food items because these food items naturally contain microbial contamination (due to microbiota it) and different contamination levels cover the diversity required for this test method. Food items of pasteurized and ultra-warm milk and industrial fruit juice with a liquid and homogeneous nature were selected to measure uncertainty by creating artificial contamination. The selected cases were chosen for this study to cover the range of test methods and a wide range of usage of the food microbiology laboratory of Mashhad Food and Drug Deputy.

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Table 1 The selection chart of food items classification and suggested target combinations for verification studies

Food item	Food type	Food category
Minced meat	Fresh meats (unprocessed)	Raw meat and ready-to-cook meat products (except poultry)
Frozen burger patties (hamburgers)	Ready-to-cook (processed)	
Ultra-high temperature (UHT) milks	Sterilized or Ultra-high temperature dairy products	Heat-processed milk and dairy products
Pasteurized milks	Pasteurized milk-based products	
Soft cheeses		
ice creams	Pasteurized dairy products	
Spice(Black Pepper)	Seasoning	Dried cereals, fruits, nuts, seeds, and vegetables
Pasteurized whole liquid egg	Egg product (heat processed) without Additives	Eggs and egg products (derivatives)
Soybean powder	Plant origin ingredients	Pet food and animal feed
Cake	Dry and sugared low moisture(aw < 0,85)	Chocolate, bakery products and confectionary
Pasteurizedapple juice	Heat-processed fruit/vegetables juices	Processed fruits andvegetables
Wheat	Flours	Dried cereals, fruits, nuts, seeds and vegetables

2-1- Preparation of bacteria suspension

Cultivation of the target bacteria using a reference strain of bacteria*Shigella flexneri (12122*:ATCC) was performed in the food microbiology laboratory. The culture was taken from the target bacterial strain in sterile conditions and next to the flame with a sterile needle and cultured on plates containing BHI Agar medium. The plates were placed in a 37°C incubator for 24 hours. In order to prepare the suspension, all bacterial strains were cultured and activated in two steps in BHI Brotha sterile medium. After two stages of cultivation, after 18 hours of bacterial inoculation, the number of bacteria was determined using a spectrophotometer through turbidity measurement according to the McFarland method (the amount of light absorption equal to 0.13 to 0.08 at a wavelength of 600 nm). . In this turbidity number of 10^8 1.5 × bacteria were present per ml. To perform each stage of the experiment, the desired amount of the prepared bacterial suspension was taken and the bacterial suspension was used for inoculation with the number of 10^8 × 1.5 bacteria per milliliter was prepared [17].

2-2-How to create artificial contamination (Spiking) of test sections by inoculum:

Each 10-ml feed was artificially inoculated with 1 ml of three levels of cross-contamination with Shigella flexeni at three bacterial concentration levels determined at expected bacterial concentrations of 1000, 100, and 10. In addition, two experimental portions of Each of the food items that were not artificially contaminated and used as a negative control (blank). All samples were counted for Enterobacteriaceae colony count (ECC) according to the standard [18, 6].

2-3-Sample preparation

Saline peptone water and MRD buffered peptone water (SPW) as diluents (to avoid osmotic shock) and the initial dilution preparation step for 20 to 30 minutes at room temperature (18 to 27 °C) before homogenization. Done.

2-4- How to count colonies of aerobic mesophilic bacteria and enterobacteriaceae

Different dilutions of aerobic mesophilic bacteria were cultured by mixed culture method on plates containing Kant agar plate medium and for enumeration of Enterobacteriaceae colonies by mixed culture method on plates containing violet red bile lactose agar medium. Then the plates were kept in a greenhouse for 48 hours at a temperature of 30°C and after 48 hours the number of colonies was counted and recorded [19].

5-2-Technical uncertainty calculation method

Estimation and calculation of technical uncertainty for the quantitative test methods of

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enumeration of Enterobacteriaceae (ECC) and aerobic mesophilic bacteria (ACC) based on the ISO 19036 standard [6] were carried out. x 10 grams) of each laboratory sample in reproducible conditions by creating different diversity conditions including different laboratory equipment (dilution preparation process: gravimetric diluter with sterile filter bag, laboratory scale and sterile Erlenmeyer, different greenhouse, counterautomatic colony Scan 500 and countermanual colony and...), different materials (MRD, BPW, MRD, BPW diluents and culture media from different brands and numbers), different groups of examiners and different test times, in two test groups named A and B in order to create maximum variety of conditions (VARAIABLITY) was applied in a different laboratory in order to include as much diversity as possible in the estimation. In the samples without various natural contamination and less than 10 CF, such as ultra-heated milk, industrial fruit juice, artificial contamination in the range of the sample, the test quantity is counted in three levels of 10 , 100 and 1000CFU/TEST PORTION were created using the counted inoculum of the desired target bacteria including Shigella flexneri bacteria. For this purpose, artificially infected samples with fresh culture incubated in TSB broth at 37°C/18 to 20 hours, of which 1 McFarland suspension (10^8cfu/mL) was prepared and decimalized to 10^5cfu/mL were diluted and inoculated. The results of the test (cfu/g) were calculated with log10 transformation and the standard deviation of repeatability within the sample (sr) according to equation (2). This was equivalent to log10 standard deviation of the results. Microsoft Excel software was used in the calculations [21].

Equation 1:

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

Analysis of each test section was performed according to specific methods for the target microorganisms, as in routine tests. The number of colonies between 30 and 250-300 cfu/plate was considered as acceptable results. where i is the sample index, i = 1 to n ($n \geq 10$) and yiA, yiB are log10-transformed data in log10 cfu/g. Conditions A and B were used respectively, where condition A was the gravimetric diluter method and sterile filter bag, and condition B was the continuous laboratory method (Erlen Meyer and laboratory balance). Uncertainty estimation calculations were performed using Microsoft Excel software of ISO 19036 standard [21].

6-2-Matrix uncertainty calculation method

Matrix uncertainty was estimated by testing several experimental parts of a sample (matrix) in reproducibility conditions (identical tester and equipment and batches of culture medium and diluent in a short period of time). For this estimation, selected samples (11 x 10 grams) from a laboratory sample (matrix) were analyzed according to specific standards. Colony counts in plates with colonies between 30 and 250-300 cfu/g were considered as acceptable results. The reliability of the results was tested according to ISO 14461-1 [20] and unreliable counts were excluded. Constituent units per gram (cfu/g) were then log10-transformed. The standard deviation of intra-laboratory repeatability (sR) was calculated according to equation 1 [21].

Equation 2:

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

where n = 11, yi is the log10 transformation result of trial i and ȳ is the mean of the results in log10 cfu/g.

J. Uncertainty of distribution

The standard uncertainty of Poisson (UPoisson) in log10 cfu/g is calculated according to formula (3) [21].

Equation 3:

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

where ΣC is the total number of counted colonies. If 1ΣC= 0 (no colony count) UPoisson = 0.4343.

d. Compound and extended uncertainty (generalized or extended uncertainty)

Composite uncertainty (Uc) is a combination of separately estimated technical standard uncertainty, matrix standard uncertainty and distributed standard uncertainty (equation (4)) [20].

Equation 4:

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

In situations where the laboratory chooses Option 2 to estimate its uncertainty, the composite uncertainty is equal to the standard deviation of the within-laboratory repeatability (UC = SIR). Equation (5) is used to calculate the expanded uncertainty (2, coverage factor k). which corresponds to the confidence level of 95% [21].

Equation 5: U =2(y)

3- Discussion and conclusion

3-1-Technical uncertainty

In the calculation of technical uncertainty in the total count test in the tested foods, it is calculated based on natural contamination only, because in the total count test, a wide range of organisms including all aerobic mesophilic bacteria, as well as types of gram-positive and gram-negative bacteria, as well as types of fungi, including yeasts And molds can grow in the general culture environment, as a result, it is not possible to conduct tests and create artificial pollution due to the wide range of bacteria and fungi, and it is not possible to select the target organism and apply artificial pollution, so in all samples with natural pollution Technical uncertainty calculations were performed. In the case of samples of pasteurized milk, Farada and fruit juice from a variety of brands and even in some cases, rejected or expired samples were selected in order to vary the intensity of contamination, or in the case of pasteurized milk, different brands and types of normal pasteurized milk and shelf life. longer (ESL) and in the time span of different days, was used so that the variation of pollution intensity was possible.

Technical uncertainty in terms of the standard deviation of the reproducibility of the final results of the measurement process,*IN*_Or*S*_ Within a laboratory, either separate evaluation of individual factors contributing to operational or technical uncertainty (intra-laboratory) and combining them yields estimated technical uncertainty results for quantitative tests for enterobacteriaceae colony counts (ECC) and aerobic countable colonies (ACC) in Chart 1 are listed. The highest level of technical uncertainty for (ECC) and (ACC) was observed in the food items raw minced meat, hamburger, cake, wheat flour and pepper. In the calculation of technical uncertainty, the data are transformed into logarithms to reduce the effects of changes caused by different levels of contamination in different samples, and also to return the standard deviation of intra-laboratory reproducibility to normal. For the colony counting method, the total counting results of the selected plates should not be less than 30, and more than 300 or any maximum number determined in the specific standard. The test results were transformed into logarithms, for 10 laboratory samples using the experimental protocol and obtaining the results (*and*$_{to\ A}$And*and*$_{iB}$) for each sample, the estimated standard deviation of intra-laboratory reproducibility was calculated. The results of technical uncertainties vary from 0.06 to 0.8, it can be concluded that the reproducibility values are better than the inter-laboratory validation studies or match them. The good accuracy of the results shows that the use of these values is reproducible and they are acceptable as limit criteria in internal quality control. Our results are also consistent with the ISO test data, where the intra-laboratory reproducibility was 0.10_{10}logcfu/g (range $0.07–0.601_{10}$logcfu/g, average 0.201) was obtained.

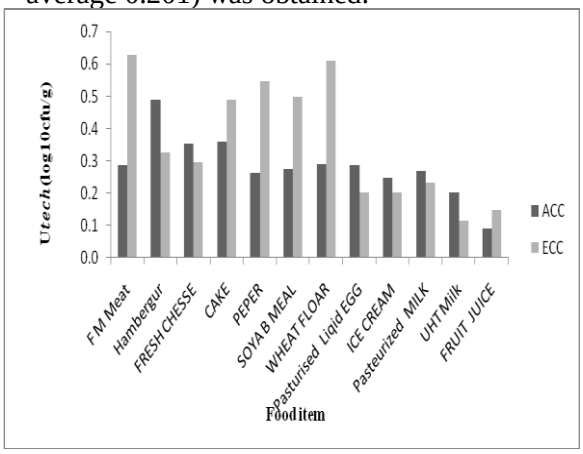

Fig 1 The technical uncertainty of microbiological test By creating artificial pollution at triple levels in calculating the technical uncertainty of the ECC

B: Uncertainty of the matrix

The uncertainty estimation of the matrix is given in Table 3. The highest matrix uncertainty for the count of Enterobacteriaceae in minced meat (0.524_{10}logcfu/g) and counting the number of aerobic mesophiles in fresh cheese (0.541_{10}logcfu/g). The results of this research are consistent with the findings published in the ISO 2005 test report, about the measurement of uncertainty [22], the differences were due to the difference in the type of selected matrices. The uncertainty of the matrix only deals with the microbial

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distribution in a certain matrix, that is, the changes between the results of several tests. It examines a laboratory sample and differs from the uncertainty of sampling, it is independent of the test and measurement method, and with a single estimate, it is always true for the same sample, and its amount is well mixed in liquid products, small in solids and Multi-component foods have a larger amount. The uncertainty of enterobacteriaceae and mesophilic bacteria in cheese is mainly due to its high level of contamination, although the presence of competitive flora and intrinsic factors of the matrix cannot be excluded. The uncertainty of the matrix has been calculated in the Enterobacteriaceae count test, but in the microbial test of counting aerobic mesophiles APC in the case of homogenizable matrices such as liquids and powders that have the ability to mix and homogenize and homogenize, by default the fixed value is 0.1 It is considered and it was calculated in heterogeneous foods such as cake, hamburger, minced meat and cheese, and the highest number of bacteria was obtained in the matrix of hamburger and cheese. According to the rules mentioned in the ISO 19036:2019 standard, the composite uncertainty is equal to the technical uncertainty if it is compatible with the laboratory protocols and customer requirements. In this option, intra-laboratory repeatability (SIR) values are used as the composite uncertainty for the parameter , a special method and matrix are used. The ISO 19036:2019 standard emphasizes that estimating technical uncertainty on a single matrix will be more reliable and realistic than multiple matrices, so in this study, using multiple matrices in calculating uncertainty Certainty has been used [6].

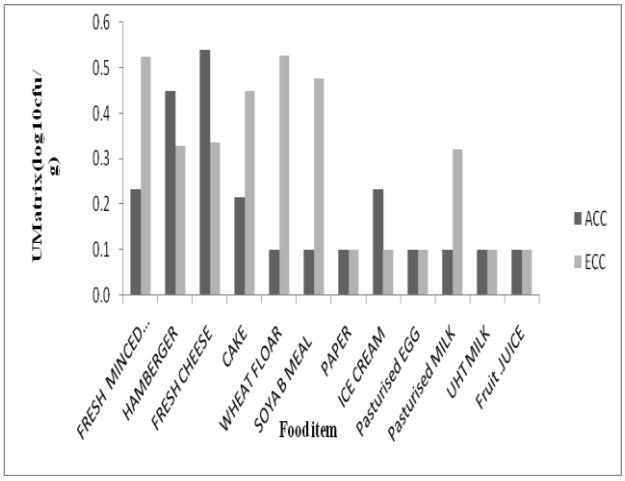

Fig 2 The matrix uncertainty of microbiological test

C: Compound and expanded uncertainty:

Table 2 shows the combination of technical uncertainty, matrix uncertainty and distributional uncertainty for Enterobacteriaceae bacteria. Examining the results of composite uncertainty for the count of Enterobacteriaceae showed that the highest amount was found in pepper, cake, hamburger and cheese, respectively, and the lowest amount was in wheat flour. These results show that reproducibility and reproducibility are more related to a simpler analytical method, which shows that uncertainty is definitely related to the amount of manual work and individual interpretation of results. The results confirm that reducing these sources of error reduces work and simplifies procedures and increases automation. It is important.

Table 2 The Uncertinity copmonents of Enterobacteriaceae)ECC(counttest

Parameter/Food	Utech (log10 cfu/g)	The matrix (log10 cfu/g)	**IN_{conf}** (log10 cfu/g)	Upoisson (log10 cfu/g)	Uc(y) (log10 cfu/g)	UExpanded (log10 cfu/g)
FRESH MINCED MEAT	0.628	0.524	0. 108	0.062	0.827	1.626
HAMBERGER	0.325	0.329	0.0686	0.120	0.477	0.954
FRESH CHEESE	0.297	0.336	0.052	0.082	0.468	0.937
CAKE	0.490	0.449	0.0526	0.064	0.676	1.353
WHEAT FLOAR	0.547	0.528	0.088	0.0.64	0.768	1.536
SOYA B MEAL	0.498	0.476	0.076	0.52	0.689	1.432
PAPER	0.610	0.1	0.0399	0.064	0.709	1.419
ICE CREAM	0.202	0.1	0.0642	0.194	0.343	0.686
Pasturised EGG	0.201	0.1	0.0453	0.099	0.307	0.614
Pasturised MILK	0.232	0.322	0.0584	0.386	0.406	0.813
UHT MILK	0.115	0.1	0.0526	0.058	0.245	0.490
Fruit .JUICE	0.147	0.1	0.0478	0.647	0.289	0.579

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In the ACC test, due to the presence of high microbial flora and the absence of a specific target microorganism, we do not perform a confirmation test, so we do not have UConfirmation. The results of examining the results of composite uncertainty for the counting of aerobic mesophilic bacteria showed that the highest amount was in pepper, cake and cheese, respectively, and the lowest amount was in juice. Uncertainty resultsPoisson For aerobic mesophilic bacteria in all food items, 0.079 was obtained because the uncertainty of the distribution depends on the number of counted colonies, in all items as the number of counted colonies is 30 colonies, constant ($\log^3$ 10), so it has become a fixed number.

Table 3 The Uncertinitycomponents ofACC test(for ∑C = 3log, 30000 cfu counts)

Parameter/Food	Utech (log10 cfu/g)	The matrix (log10 cfu/g)	Upoisson (log10 cfu/g)	IN*Combined*	
				in_c(and)	UExpanded (log10cfu/g)
F M Meat	0.286	0.233	0.079	0.377	0.755
Hamburger	0.490	0.449	0.079	1.341	0.670
FRESH CHESSE	0.354	0.541	0.079	1.302	0.651
CAKE	0.358	0.215	0.079	0.851	0.425
PEPPER	0.264	0.1	0.079	0.586	0.293
SOYA B MEAL	0.276	0.1	0.079	0.608	0.304
WHEAT FLOAR	0.289	0.1	0.079	0.317	0.635
Pasturised Liqid EGG	0.286	0.233	0.079	0.755	0.377
ICE CREAM	0.247	0.1	0.079	0.238	0.476
Pasteurized MILK	0.268	0.1	0.079	0.317	0.634
UHT Milk	0.201	0.1	0.079	0.278	0.556
FRUIT JUICE	0.090	0.1	0.079	0.357	0.715

In this study, the widely used quantitative microbiology tests are used to study the determination of the functional index of the standard deviation of intra-laboratory reproducibility or measurement uncertainty in order to determine the functional indices in the confirmation of the implementation of quantitative test methods, as well as the quality assurance criterion of microbial test results. and reporting the calculated values of these components and validating the results of microbial tests for the first time in the most widely used selected food items based on the application scope of the test methods and the work scope of the food laboratory based on the methods presented in ISO 19036 standards: 2020[6] was done for the first time in Iran. In this study, the uncertainty component of the measurement of MU related to the quantitative microbial test for aerobic mesophilic microorganisms and enterobacteriaceae in the testing and preparation section of the initial suspension from the laboratory sample (test) of different types of matrix (pasteurized ice cream, pasteurized milk, preheated milk, juice, flour, cake, spices, minced meat and hamburgers) were listed.

The results of this research showed that the heterogeneous distribution of microorganisms in food matrices is a well-known fact. Rohde et al. [23] pointed out that homogenization can be responsible for significant interlaboratory differences in pathogen detection in meat. Their spatial distribution in food may be random, uniform (regular, uniform) and/or spread or spread (collective or mass) model. Uniform distribution and random distribution rarely occur in food, but widespread distribution of microorganisms in food often exists [24]. This means that the distribution of microorganisms does not fit well with a normal distribution. In simple suspensions, the distribution of microorganisms conforms well to a random Poisson distribution, but this is not always the case. In solid and compound food, it is complicated due to the existence of lumps and distribution chain. Solid food, such as hamburger, contains cells and clusters of microorganisms distributed within and between the main food particles, which are generally not randomly distributed, but in contagion clusters [25]. Even adequately homogenized samples show variations in contamination levels between different parts of the test, especially solid food matrices. These changes are "matrix uncertainty".

These examples show that in heterogeneous foods, the variability between samples

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increases greatly, so the microbial mean for the constituents of heterogeneous foods must be shifted to a lower level, in order to achieve the same level of contamination for a heterogeneous food. reach the minimum possible amount and fulfill the rules related to FSO. Inter-sample variability in non-homogeneous foods may be reduced by taking larger samples or combining/homogenizing a set of sub-samples. If this is done, the number of sample distributions in step 1 will change and the uncertainty determination process must be repeated.

The series of ISO 16140 [16] standards helps user laboratories based on the principles and requirements of ISO 17025 [5] in the ability and capacity to implement and correctly implement reference test methods. This is possible by implementing the certification of the test method based on ISO 16140-3 [16]. In confirming the implementation of quantitative test methods, the functional index of standard deviation of intra-laboratory reproducibility (SIR) equivalent to uncertainty is calculated based on ISO 19036 standard [6]. have been calculated and compared to the acceptance limit. Other studies have been conducted in the field of implementing ISO 16140 standards [16].

Arienzo [26] investigated three different methods of microbiological analysis for the detection and quantification of total forms. Plate counting method, 3MPetrifilmTM plate counting method and MBS method. For all three methods, the contribution of R kg R is less than 10%, which indicates the ability to accurately evaluate the total concentration of coliforms in food samples. The results of the SIR investigation in all three methods of overall measurement of forms showed that a significant change is due to the interaction between the operators and the analyzed samples for the plate counting methods and the number of PetrifilmTM3M plates, while for the MBS method it has no significant effect on the measurement.

Savianoet al. [27] investigated the effect of data correlation on measurement uncertainty and consequently on the risk of compliance decisions. Rapid microbiological methods (RMM) were performed to determine the potency of cephalosporin antibiotics in pharmaceutical products using agar diffusion method. Conjoint analytical effects on inhibition resulted in data correlation that significantly reduced combined measurement uncertainties and thus the risk of making incorrect compliance decisions. According to the normal logarithm distribution of the power values, measurement uncertainties were reported as an uncertainty factor.

Many studies measuring uncertainty in microbiology reported a high level of uncertainty. In the study of Jarvis et al. [7], interlaboratory trials were conducted to investigate the uncertainty of data obtained by standard microbiological methods for aerobic microorganisms, Enterobacteriaceae and*E. coli*It was done in a simple matrix. This study showed that reproducibility values ranged from 9.3 to 12.1% (corresponding to 0.58 - 0.77 log10 cfu/g) for aerobic microorganisms, from 14.0 to 17.4% (corresponding to 0.72 - 0.88_{10}logcfu/g) and for*E. coli*. (1.00 - 1.38 $_{10}$logcfu/g). The results of this study showed a high level of uncertainty, which is much wider than expected from the common microbiological rule used in the colony.

Rezic DereaniMatek Sarić [8] reviewed quality control methods, validation methods and determination of measurement uncertainty (MU) as an important element of quality assurance in food microbiology laboratory for quantitative and qualitative type of analysis. The differences between the design of validation tests for quantitative and qualitative food microbiology analysis are discussed in this research and show that MU calculations are based on external proficiency test data and internal validation data.

Standardized methods are based on traditional microbiological culture standard methods that are widely used in food analysis laboratories, which have several problems such as subjectivity in the interpretation of some biochemical or morphological tests and the possible interference of matrices, especially when They provide high levels of pollution. In addition, the high cost of the tests is characterized both in terms of labor and resources, and most importantly, by the long time required to obtain definitive results (from 3 to 7 days). Evaluation of measurement uncertainty is an integral part of microbial testing, so that the measured results cannot be interpreted correctly without at least some knowledge of the associated uncertainty. To be sure of the measured value, it is necessary to identify the measurement errors and their possible effect on the result. To be estimated.

Evaluation of uncertainties is necessary for better quantification and quality improvement of microbial measurements. In particular, the reliable identification and counting of microorganisms in food can be considered as a relatively complex test in terms of the different conditions of the matrix test and human interpretation [28]. The results show that uncertainty is certainly related to the amount of manual work and individual interpretation of the results, suggesting that reducing these sources of error is important to obtain more reliable results.

4- The final conclusion

The results of this research showed that the uncertainty of measurement has an effect on the amount of uncertainty of measurement, and this effect can be very high. The technical uncertainties of the quantitative methods performed in the laboratory were small and insignificant. The uncertainty of the matrix has the greatest effect on heterogeneous food items, and the factor of mixed uncertainty was the factor of composite food matrices. Even the assigned uncertainty of 0.1 log10 cfu/g for homogeneous matrices exceeds many calculated technical uncertainties. Matrix uncertainty can be minimized by good homogenization of the sample before test sections are taken, but this factor is not completely under laboratory control. However, technical uncertainty arises from changes in laboratory operations during analysis, so if properly controlled, the contribution of technical uncertainty will be very small. The results of this test can be used by reference laboratories to prevent the use of multiple methods for a single target test, regulatory bodies and certification systems of specialized standards to guarantee the quality of laboratory results, validation and certification in the implementation phase of microbe reference standard methods. food science, to create coordination and facilitation. Based on the management principles of guaranteeing the quality of test results and validating the results of test laboratories, only using accredited and certified methods to achieve accurate and reliable test results and meet the needs of customers It is widely accepted to produce and present reliable results. These results can be a guide for managers to monitor the efficiency and performance of quality systems and evaluation and formal controls of supervisory systems, for testing experts, and in choosing the methods used by microbiological testing laboratories of food samples that are suitable for their purpose. facilitate these laboratories to verify the compliance of the obtained results with the performance characteristics defined in the framework of ISO standards guidelines. EN ISO 17025) and series of standards 16140-3 and 19036, for the validation of the implementation of reference standard test methods, they can be prepared to obtain valid national and international approvals in the evaluation and monitoring stages. This study is the objective process of gathering and estimating the components of Uncertainty in quantitative tests of food microbiology presents that it will be able to be used at the level of food microbiology laboratories. Observing these results show that reproducibility and reproducibility are more related to a simpler analytical method, indicating that uncertainty is definitely related to the amount of manual work and individual interpretation of results.

5- Resources

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مقاله علمی_پژوهشی

تخمین و مقایسه انحراف معیار تجدیدپذیری استاندارد SIR (عدم قطعیت) در آزمون های کمی میکروبی آیتم های مواد غذایی مختلف بر اساس استاندارد ایزو ۱۹۰۳٦ سال ۲۰۱۹

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چکیده

این مطالعه با هدف تخمین و مقایسه، انحراف معیار تجدید پذیری درون آزمایشگاهی (SIR) یا عدم قطعیت اندازه گیری (MU) بعنوان شاخص عملکردی در تصدیق انجام روش های آزمون کمی میکروب شناسی مواد غذایی، انجام شده است. آزمون های شمارش کلی میکروارگانیسم های هوازی مزوفیلACC و شمارش آنتر و باکتریاسهECC، بعنوان دو آزمون مهم در ارزیابی میکروبی مواد غذایی، انتخاب و محاسبات انحراف معیار تجدیدپذیری استاندارد درون آزمایشگاهی (عدم قطعیت) در آیتم های غذایی منتخب: گوشت چرخ کرده، همبرگر، پودر سویا، تخم مرغ مایع پاستوریزه، شیر پاستوریزه و فرادما، بستنی، آبمیوه، آرد، کیک و ادویه بر اساس استاندارد ایزو – ۱۹۰۳٦ سال ۲۰۱۹ انجام گردید. سایر مولفه های عدم قطعیت ماتریکس، توزیعی، مرکب وگسترده محاسبه و گزارش شدند. محاسبه عدم قطعیت فنی آزمون ECC در (شیر پاستوریزه و فرادما، بستنی، آب میوه و تخم مرغ مایع پاستوریزه) با ایجاد آلودگی مصنوعی در سه سطح توسط ارگانیسم هدف (Shigella felxseneri) و در سایر آیتم های غذایی آلودگی طبیعی بود، در آزمونACC صرفا آلودگی طبیعی محاسبه و نتایج، مورد مقایسه قرار گرفت. نتایج عدم قطعیت فنی آزمون ECC، از ۰.٤۸۷ تا ۰.۰۷و در آزمون ACC، از ۰/۳۹۰ تا ۰/۱۰۵ log10 cfu/g متغیر بود. بیشترین مقادیر عدم قطعیت فنی و ماتریکس در نمونه های گوشت، کیک، همبرگر و پنیر یعنی در مواد غذایی ناهمگن (جامد و نیمه جامد) و کمترین مقادیر در نمونه های مایع (همگن) مشاهده شد. ارزیابی تغییرپذیری و در نتیجه عدم قطعیت راهی برای استاندارد کردن بیان تنوع مرتبط با دادههای به دست آمده در روشهای میکروبیولوژیکی برای برجسته کردن علل و میزان چندین عامل متنوع موثر پیشنهاد می گردد.