TAIEX workshop
Ankara, 4-5 March 2013

Food and Consumer Product Safety Authority
Ministry of Economic Affairs, Agriculture and Innovation

Guidance document Regulation (EC) 882/2004

Microbiological sampling and testing of foodstuffs

II. Analysis

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Sample testing at laboratories

In the course of sampling and preparation of laboratory samples, precautions should be taken to avoid any changes, which might affect the contents, adversely affect the analytical determination or make the samples unrepresentative

On arrival at the laboratory, the following should be checked:

- the samples are accompanied by a sampling report (delivery note), containing necessary information
- the sample labelling agrees with the report; if not, the samples should be discarded
- the samples have been correctly transported (if necessary, control the temperature)
- the sample containers are intact

Requirements for official laboratories

Regulation (EC) 882/2004

The competent authority shall designate laboratories that may carry out the analysis of samples taken during official controls

Competent authorities may only designate laboratories that operate and are assessed and accredited in accordance with the following European standards:

(a) EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'

(b) EN 45002 on 'General criteria for the assessment of testing laboratories'

(c) EN 45003 on 'Calibration and testing laboratory accreditation system – General requirements for operation and recognition'

Requirements for official laboratories

Regulation (EC) 2076/2005

By way of derogation from Regulation (EC) No 882/2004, the competent authority may designate a laboratory not accredited, provided that the laboratory:

(a) demonstrates that it has initiated and is pursuing the necessary accreditation procedures in accordance with Regulation (EC) No 882/2004;

(b) provides the competent authority with satisfactory guarantees that quality control schemes for the analyses it conducts for the purpose of official controls are in place by 1 January 2006.

The derogation applies until 31 December 2009 after which all laboratories carrying out official controls must be accredited according to the standard EN ISO 17025

Requirements for official laboratories

Storage of isolated pathogenic micro-organisms

Presence/absence testing and enumeration of target micro-organisms during microbiological analysis yield bacterial isolates.

Bacterial isolates of pathogenic micro-organisms should be maintained and preserved for reference purposes according to general microbiological procedures.

Where possible and needed the isolates should be sent to appropriate reference laboratories for typing and identification. In outbreak situations typing and identification may be an immediate requirement.

The isolated pathogenic strains should be kept at the laboratory at least until deemed necessary by the competent authority.

Methods of analysis

CEN and ISO standards

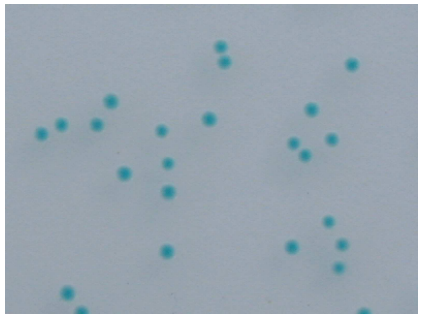
In the absence of analytical methods under EU law, methods standardised by CEN and/or ISO, when available, should be used for testing samples of official controls

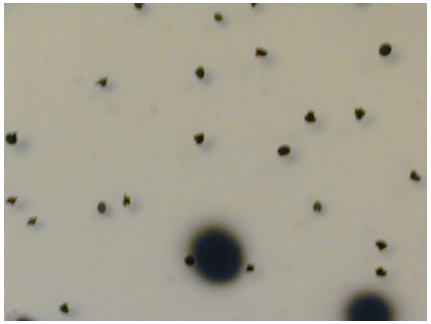
While CEN or ISO methods are not available for all hazards included in Regulation (EC) No 2073/2005, the use of other reference methods given in this Regulation are highly recommended

Methods of analysis

Basic ISO and CEN standards for Microbiology of Food and Feed

- ISO 7218 –General requirements and guidance for microbiological examinations
- ISO 6887 - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination
- ISO/TS 11133 - General guidelines on quality assurance for the preparation of culture media in the laboratory





Methods of analysis

ISO 7218:2007 – Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations

- ensure the validity of food microbiology examinations

- assist in ensuring that the general techniques used for conducting these examinations are the same in all laboratories

- contribute towards the safety of the laboratory personel by preventing risks of infection

Methods of analysis

ISO 6887

Preparation of test samples, initial suspension and decimal dilutions for microbiological examination

Part 1 General rules

Part 2 Specific rules for meat and meat products

Part 3 Specific rules for fish and fishery products

Part 4 Specific rules for other foods

Part 5 Specific rules for milk and milk products

Part 6 Specific rules for samples from the primary production

Methods of analysis

ISO/TS 11133-1:2009

General guidelines on quality assurance for the preparation of culture media in the laboratory

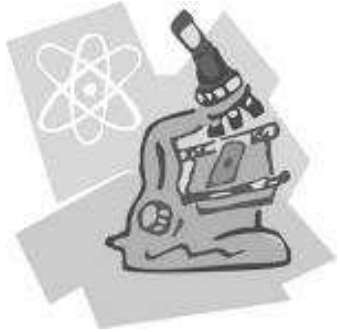
ISO/TS 11133-2:2003

Practical guidelines on performance testing of culture media

ISO/TS 11133-2:2003/Amd.1:2011

Test microorganisms for commonly used culture media

Methods of analysis



Methods in Regulation (EC) No 2073/2005

• *Salmonella*	EN/ISO 6579
• *Listeria monocytogenes*	EN/ISO 11290-1+2
• *Staphylococcus aureus*	EN/ISO 6888-2
• *S. aureus* enterotoxins	method EURL (Hennekinne)
• *Cronobacter*	ISO/TS 22964
• *Escherichia coli*	ISO 16649-1+2+3
• *Bacillus cereus*	EN/ISO 7932
• *Enterobacteriaceae*	ISO 21528-1+2
• Aerobic colony count	ISO 4833
• Histamine	HPLC method (Malle et al)

Methods of analysis

Other methods

If other methods are used they should provide at least equivalent results compared to the reference method

As regards the validation of microbiological methods, the procedure in EN ISO 16140, including an intralaboratory and an inter-laboratory study (collaborative study) is highly recommended

EN ISO 16140:2003 (under revision)

- General principles for the validation and the certification of alternative methods
- Qualitative methods - Technical protocol for their validation
- Quantitative methods -Technical protocol for their validation

Methods of analysis

Vocabulary (EN ISO DIS 16140-1)

validation - establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled

verification - demonstration that a validated method functions in the user's hands according to the method's specifications determined in the validation study and is fit for purpose

Methods of analysis

Vocabulary (EN ISO DIS 16140-1)

alternative method - the method submitted for validation

a method of analysis that detects or quantifies, for a given category of products, the same analyte as is detected or quantified using the corresponding reference method

proprietary method - method with a trademark/brand name, which is owned and generally marketed by a commercial company

Methods of analysis

Vocabulary (EN ISO DIS 16140-1)

interlaboratory study - study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative method performance parameters

method comparison study - a study, performed by the organizing laboratory to compare the alternative method with the reference method

Methods of analysis

Method comparison study – qualitative methods

Table 1 - Paired results of the reference and alternative method

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	+/+ positive agreement (PA)	-/+ positive deviation (PD) (R-/A+)
Alternative method negative (A-)	+/- negative deviation (ND) (A-/R+)	-/- negative agreement (NA)

— **Relative accuracy:** $AC = \frac{(PA + NA)}{N} \times 100\ \%$;

— **Relative specificity:** $SP = \frac{NA}{N_-} \times 100\ \%$;

— **Relative sensitivity:** $SE = \frac{PA}{N_+} \times 100\%$

Methods of analysis

Regulation (EC) 2073/2005 – Article 5

The use of alternative analytical methods is acceptable when the methods are validated against the reference method in Annex I and if a proprietary method, certified by a third party in accordance with the protocol set out in EN ISO standard 16140 or other internationally accepted similar protocols, is used.

If the food business operator wishes to use analytical methods other than those validated and certified as described in paragraph 3 (previous slide) the methods shall be validated according to internationally accepted protocols and their use authorised by the competent authority.

Methods of analysis

Other methods

Methods developed for use in special situations (e.g. outbreak investigation) should preferably be characterized for their performance by the following characteristics:

Qualitative methods	Quantitative methods
Limit of detection	Limit of quantification
Inclusivity & exclusivity	Inclusivity & exclusivity
Sensitivity	Linearity
Specificity	

As defined in Standard EN ISO 16140

Interpretation of microbiological analytical results

Microbiological criteria set down in Community legislation

Annex I to Regulation (EC) No 2073/2005 sets down limits for specific microbes, their toxins and metabolites in specific food categories at specific points of the food chain

Absence of Community criteria

Regulation (EC) No 178/2002

1. Food shall not be placed on the market if is unsafe

2. Food shall be deemed to be unsafe if it is considered to be:

(a) injurious to health; (b) unfit for human consumption.

3. In determining whether food is unsafe, regard shall be had:

(a)to the normal conditions of use of the food by the consumer

(b)to the information provided to the consumer, including information on the label

Interpretation of microbiological analytical results

Uncertainties related to analytical results are always present

The uncertainty of the analysis may be included in the result

The uncertainty related to the sampling is probably much greater compared to the uncertainty obtained on analysis

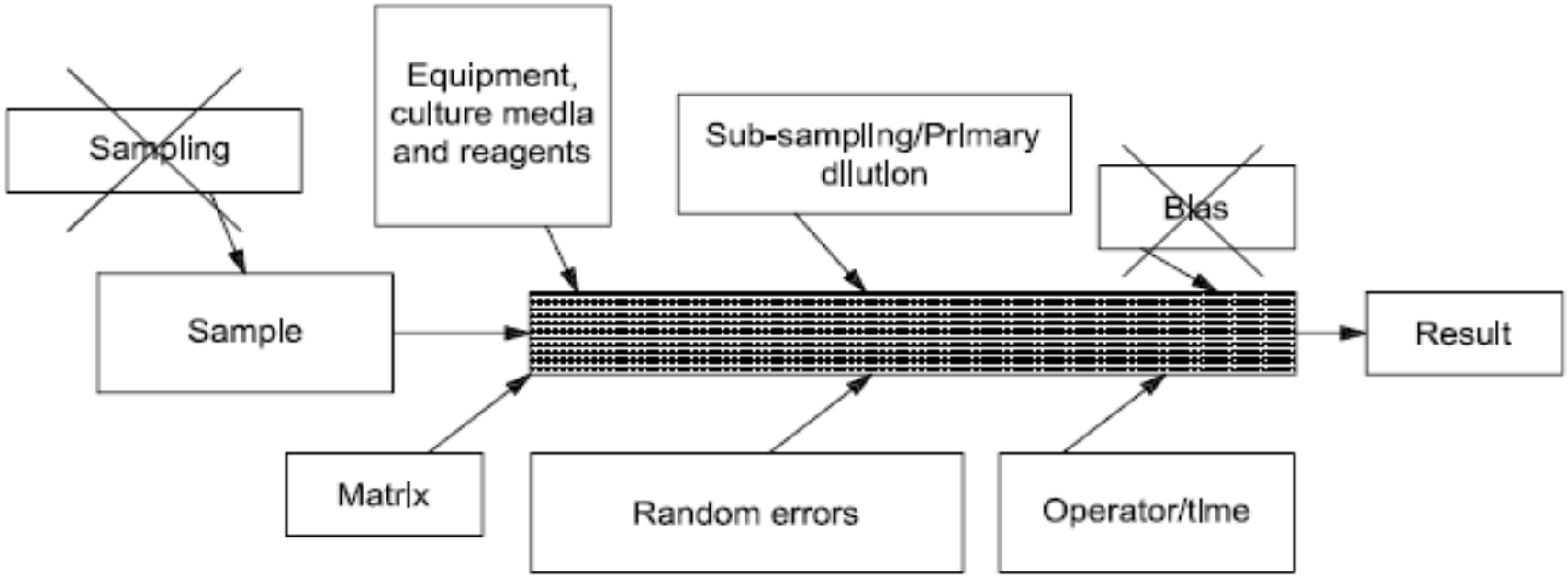
However, estimation of the uncertainty of sampling is more comprehensive and complicated

Measurement uncertainty (MU) linked to microbiological analyses is one of the factors affecting the test result;

MUs in microbiological analyses tend to be high, quite often of the order of 0.5-1.0 log units

Measurement uncertainty

Main sources of uncertainty of analysis



- Sampling can be significant part of total error, but not related to method
- True value not known, so bias is difficult to determine

Measurement uncertainty

At Community level, no implementing measures have been established on how measurement uncertainty (MU) should be taken into account when microbiological analyses results of foodstuffs are interpreted

Food business operators should always regard all test results above the limits as unacceptable regardless of the MU involved, whereas in the official controls the MU could be taken into account in order to be sure beyond reasonable doubt that the batch in question does not comply with the criterion

Measurement uncertainty

In the context of official controls it is recommended the following principles are taken into account:

- As regards food-borne pathogens the highest acceptable result including MU should still be low enough to ensure a high level of human health protection

- Indicators are used to determine the acceptable functioning of a production process. Therefore, the rules for interpretation of results of these indicators related to process hygiene criteria in Regulation (EC) No 2073/2005 need not be as strict as in the context of food safety criteria

Measurement uncertainty

Each accredited laboratory must calculate the MU in relation to each quantitative microbiological determination and if requested by the competent authority to attach it in the test report

ISO/TS 19036: Microbiology of food and animal feeding stuffs – Guide on estimation of measurement uncertainty for quantitative determinations

The estimation of MU is based on a standard deviation of reproducibility of the final result of the measurement process.

MU is based on experimental results with duplication of the same analysis in the laboratory under reproducibility conditions

Samples for a supplementary opinion

The competent authority must establish a procedure, which ensures that during the official sampling the food business operator or his representative is always aware of his right to obtain samples for a supplementary expert opinion. This right of the food business operator should always be respected

The competent authority should inform the food business operator about the limitations of supplementary sampling for microbiological analysis

For microbiological analysis the results obtained from samples for a supplementary opinion may be of limited value as the distribution of micro-organisms within a food is often not homogenous and bacteria may not survive or may even multiply during storage of the sample affecting the results of samples for a supplementary opinion

Thank you for your attention