codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00153 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

ALINORM 10/33/23

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX ALIMENTARIUS COMMISSION

Thirty-third Session
Geneva, Switzerland, 5-9 July 2010

REPORT OF THE THIRTY-FIRST SESSION OF THE CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Budapest, Hungary
8-12 March 2010

Note: This document incorporates Codex Circular Letter CL 2010/6-MAS

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CX 4/50.2 **CL 2010/6-MAS**
March 2010

TO: - Codex Contact Points
- Interested International Organizations

FROM: - Secretariat, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, FAO, 00100 Rome, Italy

SUBJECT: **Distribution of the Report of the 31st Session of the Codex Committee on Methods of Analysis and Sampling (ALINORM 10/33/23)**

A. MATTERS FOR ADOPTION BY THE 33rd SESSION OF THE CODEX ALIMENTARIUS COMMISSION

Draft Guidelines at Step 5/8

1. Proposed Draft Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods (para. 33, Appendix III)

Methods of Analysis and Sampling

2. Methods of Analysis in Codex Standards at different steps, including methods of analysis for natural mineral waters (paras. 57-82, Appendix II)

Governments wishing to propose amendments or comments on items 1 and 2 above should do so in writing in conformity with the Guide to the Consideration of Standards at Step 8 (see Procedural Manual of the Codex Alimentarius Commission) to the above address **before 15 May 2010.**

Proposed Draft Guidelines at Step 5

3. Proposed Draft Revised Guidelines for Measurement Uncertainty (para. 56, Appendix IV)

Governments wishing to submit comments on the implications which the Proposed Draft Guidelines may have for their economic interests should do so in writing in conformity with the Procedure for the Elaboration of World-wide Standards at Step 5 to the Secretariat, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme at the above address **before 15 May 2010.**

SUMMARY AND CONCLUSIONS

The summary and conclusions of the 31st Session of the Codex Committee on Methods of Analysis and Sampling are as follows:

Matters for adoption by the 33rd Session of the Commission:

The Committee:

- advanced to Step 5/8 the Draft Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods (para. 33, Appendix III);
- advanced to Step 5 the Draft Revised Guidelines on Measurement Uncertainty (para. 56 Appendix IV);
- endorsed or updated the status of several methods of analysis in Codex standards, and proposed methods of analysis for natural mineral waters (paras. 57-82, Appendix II);

Other Matters of Interest to the Commission

The Committee:

- agreed to consider further at its next session procedures for conformity assessment and resolution of disputes, taking into account measurement uncertainty, sampling uncertainty and other relevant issues (para. 98).

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INTRODUCTION

1. The Codex Committee on Methods of Analysis and Sampling held its Thirty-First Session in Budapest, Hungary, from 8 to 12 March 2010, by courtesy of the Government of Hungary. The Session was chaired by Professor Árpád Ambrus, Deputy Director General, Hungarian Food Safety Office. Dr Béla Kovacs, Professor, University of Debrecen, acted as the Vice-Chairperson. The Session was attended by 162 delegates and observers representing 46 Member Countries, one Member Organisation (EU) and 15 international organizations

OPENING OF THE SESSION

2. The Session was opened by Dr Miklós Süth, State Secretary, Ministry of Agriculture and Rural Development who recalled the importance of the work of the Committee and its importance for Hungary which has hosted the Committee since 1972. The need for the Committee to agree on analytical methods and to provide uniform and clear guidance to countries, which serve as the basis for national legislation was highlighted. Dr Süth noted the full agenda of the Committee and stressed the importance of the work on development of criteria for methods for detection of foods derived from modern biotechnology as well the work on measurement and sampling uncertainty. He further informed the Committee about a world conference on sustainable food chains to be held in Hungary in August 2010 which would bring together all stakeholders and hoped that delegates would be able to also participate in this important event. In closing, Dr Süth wished delegates well in their deliberations and a successful meeting.

ADOPTION OF THE AGENDA (Agenda Item 1)[1]

3. The Committee agreed with the following proposals:

- to change the order of the agenda and to discuss Agenda Item 6 (Guidance on Uncertainty of Sampling) before Agenda Item 5 (Endorsement of Methods of Analysis Provisions in Codex Standards); and

- to discuss the elaboration of a discussion paper on the Guidelines for Settling Disputes on Analytical (Test) Results under Agenda Item 9, as proposed by the Delegation of Brazil.

4. The Committee adopted the Provisional Agenda as its Agenda for the Session with these amendments.

5. The Delegation of the European Union presented CRD 23 on the division of competence between the European Union and its Member States according to Rule of Procedure II.5 of the Rules of Procedure of the Codex Alimentarius Commission.

MATTERS REFERRED TO THE COMMITTEE BY THE CODEX ALIMENTARIUS COMMISSION AND OTHER COMMITTEES (Agenda Item 2)[2]

6. The Committee recalled that some concerns had been expressed by Brazil on the implications for exporting countries of the *Guidelines for Settling Disputes on Analytical (Test) Results*, adopted by the 32nd Session of the Commission.

7. The Chair recalled that the above *Guidelines* were technically sound but that some clarification may be needed on their application and that for this purpose he had prepared explanatory notes on the *Guidelines* after inviting contributions from interested delegations. He also pointed out that the *Guidelines* addressed only one aspect of dispute settlement and proposed to discuss this question further to decide if further work was necessary.

8. The Delegation of Brazil indicated that they had prepared a document highlighting their concerns and the implications of the *Guidelines* and that one of their experts would also make a

[1] CX/MAS 10/31/1

[2] CX/MAS 10/31/2, CX/MAS 10/31/2-Add.1, CRD 5 (comments of Philippines), CRD 7 (guidelines for settling disputes on analytical test results explanatory notes and issues)

presentation to delegates after the plenary session on the mathematical simulations presented in the discussion paper.

9. Some delegations pointed out that the issues raised about the Guidelines on Settling Disputes were relevant for compliance assessment purposes and would also be discussed in the framework of the revision of the *Guidelines on Measurement Uncertainty* and of the discussion paper on sampling uncertainty.

10. The Committee agreed to discuss the questions relating to the application of the Guidelines under Agenda Item 9. Other Business and Future Work, including the documents prepared by the Chair and by Brazil.

11. The Committee noted the clarification from the Committee on Nutrition and Foods for Special Dietary Uses on the calculation of energy and conversion factors for infant formula and on the use of microbioassay methods for the determination of Vitamin B_6.

12. It was agreed that the reply from the Committee on Processed Fruits and Vegetables to an earlier question on the *Standard for Preserved Tomatoes* and the correction to the *Standard for Cocoa Powders* would be considered under Agenda Item 5. Endorsement of Methods of Analysis.

PROPOSED DRAFT GUIDELINES ON CRITERIA FOR METHODS FOR DETECTION, IDENTIFICATION AND QUANTIFICATION OF SPECIFIC DNA SEQUENCES AND SPECIFIC PROTEINS, IN PARTICULAR IN FOODS DERIVED FROM MODERN BIOTECHNOLOGY (Agenda Item 3)[3]

13. The Committee recalled that the last session had agreed to return the text to Step 2 for redrafting by an electronic working group co-chaired by Argentina, Germany and the United Kingdom for circulation for comments and consideration by this Session.

14. The Delegation of Argentina introduced the report of the electronic Working Group and explained the process followed in the development of the text and of the success of using an internet platform, specially created to undertake the work of the Committee, which had greatly facilitated participation by large number of countries. This mechanism was available for use by other members and future Codex working groups. The Committee noted the unusually large number of active participants in developing the guidelines. This number clearly indicated the importance and relevance of the document.

15. Although the working group had taken into account the expansion of the scope as agreed by the last session, a final solution regarding the scope-related language could not be reached and in relation to this the working group had also proposed several options for the title. However, consensus had been reached on the majority of the remainder of the text.

16. The Committee expressed its appreciation to Argentina and the working group for the excellent work done.

17. The Committee agreed to first clarify the scope before continuing with further discussion on the proposed guidelines.

General Discussion (Scope)

18. Several delegations expressed their support for the broadening of the scope and thus their support for alternative paragraph 6:

[3] CX/MAS 10/31/3, CX/MAS 10/31/3-Add.1 (comments of Argentina, Canada, Iran, Japan, Kenya, New Zealand, Panama, USA, BIO, IFT, ILSI and ISO), CRD 3 (revised draft of the proposed draft Guidelines, prepared by Argentina), CRD 4 (comments of ISO), CRD 5 (comments of Philippines), CRD 11 (comments of Japan), CRD 13 (comments of the European Union), CRD 16 (comments of ILSI), CRD 17 (comments of AOCS), CRD 18 (comments of ICGMA), CRD 19 (comments of Croplife), CRD 20 (comments of USA/EU), CRD 27 (report of the in-session working group), CRD 28 (Table for inclusion in the guidelines as prepared by Japan).

"*These guidelines provide information criteria for the validation of food analysis methods involving the detection, identification and quantification of specific DNA sequences and specific proteins of interest that may be present in foods and that will be used by laboratories responsible for food analysis. These methods can provide molecular and immunological approaches for, including among other uses, tests for food authenticity, and biomarkers for foods containing material derived from recombinant- DNA organisms"*

and as a consequence alternative title 1:

"Proposed Draft Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods".

The Delegation of Argentina explained that alternative paragraph 6 was clearer and explicitly referred to biomarkers for foods derived from modern biotechnology while the original paragraph was not appropriate since the phrase "in foods derived from modern biotechnology" was used in a way that could be interpreted as being the matrix and not the analyte, which was not the original intention of the work.

19. The Delegation of Japan expressed the view that the document was comprehensive and informative, but contained too much detail and needed to focus on essential points. With regard to the scope, the Delegation reminded the Committee that the last session had had extensive discussion on the scope and that the discussion should not be re-opened and should rather focus on the proposed text. This view was supported by the Delegation of the Republic of Korea.

20. The Delegation of the European Union emphasized the importance of the work in view of the need for methods to identify genetically modified foods and while recognizing the decision to broaden the scope, expressed support for the original title which in its view would still be appropriate even with an expanded scope. The Delegation also recalled that the initial mandate to carry out this work was focused on methods to identify genetically modified foods, the need for which had on several occasions been underlined by both the Task Force on Foods Derived from Biotechnology and the Committee on Food Labelling. It therefore expressed the view that alternative paragraph 6 could be supported if it were amended to indicate that methods used could also be applied to foods derived from modern biotechnology.

21. To a proposal to use "recombinant DNA organism" rather than "modern biotechnology", it was clarified that the term "modern biotechnology" was widely understood and defined within Codex.

22. Following discussion, the Committee agreed to alternative paragraph 6 amended to indicate that foods derived from modern biotechnology were covered in the scope.

23. The Committee further agreed that an in-session working group, chaired by Argentina, would revise the body of the text taking into account the agreed scope and the written comments received.

TITLE

24. Several delegations expressed support for the alternative Title I which did not refer to foods derived from biotechnology, noting that there was no need to place specific emphasis on foods derived from modern biotechnology, as stated in the original proposal since this aspect was already covered by the scope and would be misleading to the user as these techniques were also used for authentication of foods and other purposes. Several other delegations expressed support for the original title stating that it reflected the agreed upon scope, was clear to users and in line with the original intent of the work to develop guidelines for methods for foods derived from modern biotechnology. Some delegations pointed out that the Commission had requested the Committee to consider expanding its scope, which it had done and that there was no need to repeat the scope in the title and that it should be kept short, simple and understandable.

25. The Committee considered several proposals to shorten the title by simply referring to "analysis" rather than to "detection, identification and quantification" and to indicate that the methods referred to in the guideline were applicable to not only identification of foods derived from modern biotechnology, but also for food authentication, food speciation and other purposes (e.g. identification of allergens, pathogens, etc) either in a footnote or directly in the title. Some concerns were raised

regarding the use of a footnote since users did not necessarily read footnotes and that footnotes did not appear in the titles of texts published on the Codex website and it would thus not be immediately clear to the user that the guidelines also applied to foods derived from modern biotechnology. It was pointed out that users of the described techniques would be familiar with its applications including that for foods derived from modern biotechnology.

26. After extensive discussion, the Committee agreed to the alternate Title I and inserted a footnote to indicate the application of the methods.

Body of the guidelines

27. The Committee considered the revised guidelines (CRD 27) as prepared by the in-session working group noting that the basis for discussion in the working group was CRD 3 which integrated all written comments received.

28. In addition to editorial corrections, improvement of text for purposes of clarity and updating of references, the Committee took the following decisions:

Section 4.1.4 – Unit of Measurement and reporting of results

29. The Committee considered the last section of paragraph 22 which had been square-bracketed due to lack of consensus in the working group. Following the explanation by the Delegation of Argentina that reference to "biological uncertainty" was not appropriate for this section; that its inclusion was misleading noting that "uncertainty" was related to method error distribution and not to other external factors; and that it was not relevant for food purposes, the Committee agreed to its deletion.

Methods acceptance criteria summary table

30. The Committee considered a proposal by the Delegation of Japan to insert a table summarizing the method acceptance criteria referred to in the Annexes of the Guidelines for better readability, as presented in CRD 28.

31. After discussion, the Committee agreed not to proceed with the insertion of the Table as criteria were clearly specified in the Annexes and it was difficult to summarize the information from the Annexes in one table.

32. In recognition of the extensive discussion and agreement reached, the Committee agreed to advance the Guidelines to Step 5/8 for adoption.

Status of the Proposed Draft Guidelines

33. The Committee agreed to forward the Proposed Draft Guidelines to the 33rd Session of the Commission for adoption at Step 5/8 with the recommendation to omit Steps 6 and 7 (see Appendix III).

PROPOSED DRAFT REVISED GUIDELINES ON MEASUREMENT UNCERTAINTY (Agenda Item 4)[4]

34. The Committee recalled that its last session had agreed to return the Proposed Draft Guidelines for redrafting by an electronic working group led by the United Kingdom, comments at Step 3 and consideration at the next session.

35. The Delegation of the United Kingdom, while introducing the document, recalled that the revision of the Guidelines originated from the requests from several delegations for more detailed explanations and that the revision was intended to clarify the significance and implications of measurement uncertainty, especially in relation to compliance with standards. The Delegation stressed the importance of taking into account measurement uncertainty when setting specifications in view of

[4] CX/MAS 10/31/4, CX/MAS 10/31/4-Add.1 (comments of Argentina, Brazil, New Zealand, Panama and IDF), CRD 8 (comments of Japan), CRD 12 (comments of Hungary), CRD 14 (comments of the EU), CRD 25 (revised Section 8 and new section)

the implications for enforcement purposes and recalled that the revision addressed only measurement uncertainty, not including uncertainty of sampling, as agreed earlier by the Committee.

General discussion

36. The Delegation of New Zealand did not support the approach taken to measurement uncertainty for several reasons: Codex procedures are based on controlling the frequency or cost of wrong decisions, and it would not be appropriate to make a judgment about the compliance of a lot based on an individual sample; the application of the revised provisions for the purpose of export and import; would significantly increase the costs of compliance for exporters and producers. The Delegation also recalled its general position that sampling uncertainty should be considered in conjunction with measurement uncertainty and that guidelines integrating both aspects should be developed for that purpose. The Delegation expressed concern at the process used for the development of the documents as they had made substantial comments that had not been taken into account in the revision of the document

37. The Committee noted that the Committee on Milk and Milk Products had expressed the view that sampling plans should be based on valid statistical principles and that the approach to measurement uncertainty assessment should fully take into account the specificities of milk and milk products (see CX/MAS 10/31/2).

38. Several delegations, while supporting the purpose of the revision to consider only measurement uncertainty and to include explanatory notes, pointed out that some provisions went beyond the scope of the Guidelines and some recommendations were too prescriptive and referred to decisions that should be made by governments when assessing compliance with standards, especially in section 8. The Committee also noted some concerns with the reference to accreditation, which was not required under the current Codex Guidelines.

39. The Committee considered the document section by section and, in addition to editorial changes or corrections, made the amendments and comments described below.

Introduction

40. The Committee noted some comments on the text of the Introduction, but decided to delete the entire section as it repeated the provisions in the Procedural Manual. It was recalled that the explanatory notes were intended to interpret the Guidelines , which were directed to governments, and that any relevant provisions in the Manual could be rewritten for general application and use by governments.

Section 1

41. The Delegation of New Zealand recalled its earlier proposal and written comments to amend the range “a±2u” as it did not provide a 95% level of confidence. The Committee however noted that Section 1 was only repeated from the main text of the Guidelines and could not be amended in the notes without amending the Guidelines, and the section was retained without change.

Section 2

42. The Committee agreed that no reference should be made to accreditation as in the framework of Codex it is only required to comply with the provisions of ISO/IEC 17025:2005 and the text was amended accordingly.

Section 3

43. The title was amended to reflect that measurement uncertainty may arise from both sampling and analysis. The second sentence and part of the last sentence of the section were deleted as they were not relevant for the guidelines.

Section 4

44. The first sentence was reworded to make it clear that “it is the uncertainty of the test result which is one of the factors when judging compliance with standards”, a reference to quality control was added in the second sentence and the rest of the text was rearranged for clarification purposes.

Section 5

45. The last sentence of the first paragraph was deleted as it referred to accreditation, in view of the earlier decision to delete references to accreditation.

46. The Committee agreed to delete the section from the EURACHEM Guide and the list of references as all other texts should be included by reference in Section 10 at the end of the document. It was agreed that these references were useful for information purposes but that any confusion should be avoided as to their status and an introductory sentence was added to reflect that they were not endorsed by Codex except when specified in Codex Guidelines.

Section 6

47. The Committee recalled that laboratories should be in compliance with ISO/IEC 17025 and amended the text accordingly, deleting the reference to accreditation according to its earlier decision.

Section 7

48. The titles of the columns in the Table were amended to read Nominal Concentration, Typical Expanded Uncertainty and Expected Range of Results. As the last row does not refer to a specific figure but to a concentration below100 µg/kg, the range of expected results was corrected to reflect that it would vary according to the nominal concentration.

49. The third paragraph on microbiological analysis was deleted as it was not covered by the Guidelines

Section 8

50. Several delegations expressed the view that the provisions in section 8, especially as regards situations I to IV, contained instructions that were too prescriptive as the action to be taken and the interpretation of the result were the responsibility of competent authorities

51. Some delegations proposed to delete the entire section, while other delegations considered that the explanations would be useful but should not contain prescriptive text. It was also noted that the Guidelines on Estimation of Uncertainty of Results (CAC/GL59-2006) applying to pesticides provided useful explanations that could be used and adapted to the purpose of the present Guidelines. After some discussion, the Committee considered a revised section proposed by the Delegation of the United Kingdom (CRD 25).

52. In section 8.1, first paragraph, it was agreed that the decision to be taken is whether the sample meets the specification and the text was amended accordingly. As regards terminology, it was clarified that the specification refers to the provision and the maximum level is the upper limit of the provision, and the term “upper control limit” was replaced with “maximum level” throughout the text. The reference to food pathogens was deleted in the second sentence in order to avoid any confusion, and some editorial changes were made for clarification purposes. A new sentence was inserted to explain the example presented in the diagram.

53. After considering some proposals to reword the explanations for each situation in the diagram, these explanations were deleted as the situations were clearly described in the text. The Committee agreed with the redrafted text of Situations I to IV as proposed in CRD 25.

Section 9

54. Several delegations expressed the view that Section 9. Use of Measurement Uncertainty and Definitions of a Dispute Situations addressed the question of dispute settlement and could create confusion or duplication with the Guidelines for Settling Disputes on Analytical (Test) Results adopted in 2009. The Committee therefore agreed to delete section 9 as it was proposed to discuss the issues associated with dispute situations and uncertainty of sampling from a general perspective under Agenda Item 6.

55. The Committee considered an additional section proposed by the Delegation of New Zealand in CRD 25 concerning “requesting and reporting measurement uncertainty”, describing the conditions under which competent authorities might request information on measurement uncertainty. After some

discussion, the Committee however recalled that there was a general provision to that effect in the Guidelines and it was not necessary to include a new section.

Status of the Proposed Draft Revised Guidelines for Measurement Uncertainty

56. The Committee agreed to advance the Proposed Draft Guidelines, as amended at the present session, for adoption at Step 5 by the 33rd Session of the Codex Alimentarius Commission (see Appendix IV).

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS (Agenda Item5)[5]

57. The report of the Working Group was presented by its Chair, Dr Roger Wood (United Kingdom). The Committee considered the methods proposed for endorsement and in addition to editorial changes made the amendments and recommendations presented below.

Fish and Fishery Products

Draft Standard for Sturgeon Caviar

58. The Committee endorsed the method for salt determined as chloride and expressed as sodium chloride as Type I due to the empirical extraction procedure and proposed a consequential amendment to the type of the same method in the Standard for Salted Fish and Dried Salted Fish of the *Gadidae* Family (CODEX STAN 167-1989).

Milk and Milk Products

59. The Committee noted that many corrections to existing methods had been proposed by the Committee on Milk and Milk Products (CCMMP), as a result of the ongoing update of methods by IDF and ISO.

60. Some amendments were made to the Type proposed by the CCMMP for a number of methods. It was noted that the same method could be listed as Type IV or as Type I depending on its validation status for the matrix concerned.

61. Some AOAC methods proposed by the CCMMP were deleted, especially AOAC 989.05 for total fat, AOAC 927.05 for water, AOAC 926.08 and AOAC 933.05 for milk fat, AOAC 990.19 for milk solids-non-fat (MSNF). The AOAC methods that were equivalent to the joint IDF | ISO methods were retained as alternative methods when applicable.

62. For milk fat purity in butter, dairy fat spreads and milk fat products, it was agreed that the principle was "calculation from determination of triglycerides by gas chromatography" and the method was endorsed as Type I.

63. As regards salt in butter, the CCMMP had proposed the IDF | ISO method as Type III and AOAC 960.29 as Type IV. It was clarified in the working group that initially this was a joint AOAC, ISO and IDF method, which was subsequently revised by IDF and ISO, and that no precision characteristics were defined for the AOAC method. The Committee agreed to list them as a single Type III method.

64. The Committee endorsed the two methods for natamycin (Type II and III) as proposed by the CCMMP in reply to an earlier question of the CCMAS in 2008 on the use of these two methods.

65. As on of the methods for milk fat in cottage cheese applies only up to 5% lactose while the other applies to the whole range of products, reference was made to the lactose content in order to clarify the need for two Type I methods.

[5] CX/MAS 10/31/5, CX/MAS 10/31/5-Add.1, CRD 1 (Report of the Working Group on Endorsement of Methods of Analysis and Sampling), CRD 10 (comments of Switzerland), CRD 21 (revised list of methods for dietary fibre), CRD 24 (list of methods for natural mineral waters)

66. As the method for the determination of ash (including P_2O_5) in edible casein depends on the type of product (rennet casein or acid casein), a footnote was inserted, recommending that the analyst refer to the scope of the method.

67. It was agreed that the methods by calculation would be presented as a single Type I method, indicating the principle of each of the methods used, and that a similar presentation should be used throughout CODEX STAN 234.

68. The methods for processed cheese products were deleted as the standards applicable to processed cheese products were proposed for revocation (CODEX STAN 286-1978, 287-1978 and 285-1978).

69. The Committee endorsed the amendments to the methods of sampling for milk and milk products as proposed by the CCMMP

70. It was noted that the Committee on Milk and Milk Products had completed its work and had proposed to adjourn *sine die*, while work on methods of analysis and sampling for milk and milk products was ongoing in IDF and ISO. The Committee agreed that it would continue reviewing the methods applicable to milk and milk products following the adjournment of the Committee on Milk and Milk Products.

Nutrition and Foods for Special Dietary Uses

Methods for Dietary Fibre

71. Following the adoption of the provisions for dietary fibre in the Table of Conditions for Claims in the *Guidelines for Use of Nutrition and Health Claims*, the Committee on Nutrition and Foods for Special Dietary Uses finalised the methods of analysis for dietary fibre at its last session (2009).

72. The Committee noted that the working group had discussed extensively the methods proposed for the determination of dietary fibre but could not reach a conclusion on the suitability of several methods and on their type and had agreed that some interested delegations would prepare a revised list to clarify the commodities covered and the components of dietary fibre determined by the proposed methods.

73. The Delegation of the United States and the Observer from AOCS informed the Committee that the revised Table in CRD 21 took into account the characteristics of several AOAC and AAAC methods.

74. The Committee noted that most of these methods were empirical and that some of them might be overlapping, and therefore agreed that they could be endorsed as Type IV in order to make them available as Codex methods and asked the CCNFSDU to define their scope more precisely. It was agreed that further endorsement of these methods would be considered when such clarification became available, as some of them might be suitable as Type I methods.

75. The Committee also made some specific amendments and comments to the list of methods, as follows. It was proposed to amend the title of the first group of methods to reflect that they measure the higher molecular weight fraction of dietary fibre based on solubility and not on the number of monomeric units. However the current title was retained, taking into account the definition of dietary fibre.

76. For the first two methods in the list, it was agreed to clarify which component of dietary fibre was determined under the “provisions”. Although a proposal was made to amend the description of the commodity to refer to specific foods studied in collaborative studies, it was recalled that the definition of dietary fibre applied to all foods. The general term “all foods” was therefore retained, with an additional description of the products concerned in some cases. It was also recalled that according to footnote 1, users should consult the description of each method for the food matrices concerned.

77. The Committee discussed the proposal to delete the AOAC 2001.03 method as some delegations considered that it had been replaced by the more recently validated AOAC 2009.01, however the Committee could not come to a conclusion on this question and agreed to ask the CCNFSDU for clarification on the need for this method.

78. The Committee agreed to delete the AAAC Intl 32 06 01 or AOAC 992.16 method as it applies to the same analyte and matrices as AOAC 991.43 and double counts fibre, and the AAAC Intl 32-22-01 or AOAC 992.28 as they measure the same components as AOAC 995.16, they are not used and kits are no longer available.

Processed Fruits and Vegetables

79. The 28th Session of the CCMAS (2007) temporarily endorsed the ISO/UNIUN method for the determination of drained weight for crushed style tomatoes in the *Standard for Preserved Tomatoes* pending confirmation of the correct ISO reference. The 24th Session of the Committee on Processed Fruits and Vegetables (CCPFV) could not identify the correct ISO reference and requested the CCMAS to delete this method and to endorse AOAC 968.30 as a method for "crushed style" preserved tomato only, with the following footnote: "Use a No. 14 screen instead of '7/16' or No. 8."

80. The Committee agreed to endorse the method as Type I and requested clarification from the CCPFV as to the change in the size of the sieve, as compared with the original AOAC method.

Cocoa Products and Chocolate

81. The Committee agreed to delete the AOAC 934.07 method for the determination of lead (Section 7.4) in the Standard for Cocoa Powders as no provision for lead exists in the Standard.

82. The Committee expressed its appreciation to Dr Roger Wood and to the working group for their excellent work and agreed that the working group would be reconvened prior to the next session. The status of the endorsement of methods of analysis and sampling is presented in Appendix II.

GUIDANCE ON UNCERTAINTY OF SAMPLING (Agenda Item 6)[6]

83. The Committee recalled that the last session agreed that an electronic working group led by the United Kingdom would revise the discussion paper in the light of the comments received at that session and to develop basic principles applicable to sampling uncertainty in order to allow this session to discuss the issue further and to decide how to proceed.

General Discussion

84. The Chairperson of the Committee proposed to have a general discussion on the approach and objectives of the paper noting that this matter could be also considered within the context of the issue raised by Brazil concerning the Guidelines for Settling Disputes on Analytical (Test) Results (CRD 7) and which would be discussed in an informal presentation by Brazil during the session.

85. The Committee noted the position of the Delegation of Argentina that the views of several countries in the Latin America and Caribbean region present at the session were that, due to the short availability of the document, members had not had sufficient time to consider its contents and therefore proposed that this matter be deferred to the next session and also that uncertainty of sampling should be handled as a separate matter.

86. There were no other views expressed and the Committee therefore agreed with the proposal of the Chairperson to allow delegates to reflect on this matter within the context of the concerns relating to settling of disputes raised by Brazil (presented at an informal session) in order to decide on the way to proceed.

General comments on the Discussion Paper

87. The United Kingdom introduced the paper and informed the Committee that the paper tried to describe some issues raised in light of other international activities in the area of uncertainty of sampling; illustrated the problem and demonstrated where and how sampling was dealt with in Codex (e.g., in the *General Guidelines on Sampling* – CAC/GL 50-2004); and that there might be a need in

[6] CX/MAS 10/31/6, CRD 7 (guidelines for settling disputes on analytical test results: explanatory notes and issues), CRD 9 (comments of Mexico), CRD 12 (comments of Hungary), CRD 15 (comments of the European Union), CRD 25 (proposal for section 8 prepared by United Kingdom and New Zealand), CRD 26 (Proposal for a discussion paper on conformity assessment based on product testing),

future for development of a guideline document and explanatory notes along similar lines of the guidance on measurement uncertainty. The paper also presented an estimate of sampling uncertainties likely to arise in the food sector. The Delegation pointed out that uncertainty of sampling is an important element together with measurement uncertainty in accepting or rejecting a lot. However, in many food systems sampling uncertainty was very large and would not be practical to consider in the enforcement process.

88. The Committee expressed its appreciation to the working group for the development of the discussion paper. The Delegation of New Zealand however expressed concern with the operation of the working group and the manner in which comments were considered.

89. The general view of the Committee was that work on uncertainty of sampling was premature as the current state of knowledge was insufficient and that more data or information was needed for food products where uncertainty of sampling could play a role and that it was important to address this complex issue on a scientific basis.

90. The Chairperson drew the attention to the current practices of establishing Codex maximum limits described in CODEX STAN 193-1995 In case of food contaminants and pesticide residues in food the maximum levels are based on the average concentration of the measurand in composite samples of specified minimum mass and number of primary samples / sample increments, while veterinary drug maximum residue levels refer to the residue in a single unit in a lot (e.g., one piece of meat cut from a carcass or a single chicken).

91. Therefore when the sample is taken according to the relevant guideline its measurand content should meet the specification and consequently sampling uncertainty should not be taken into account. On the other hand, the heterogeneous distribution of the measurand within a lot results in variation of the content of the sample which is the source of sampling uncertainty, which should be considered together with the uncertainty of analysis when the compliance to the specification is tested before placing the product on the market.

92. Concerns were also raised on the fact that there was no clear definition for uncertainty of sampling; that there was insufficient explanation in the paper on how the numbers were derived in the table for the evaluation of these numbers; and that the approach could be burdensome to producers, amongst others.

93. The Delegation of New Zealand expressed the view that the approach suggested in the paper disregarded the Codex approach to consider the control of risk of making incorrect decisions and that it had specific concerns with the procedures described in the EURACHEM/EUROLAB/CITAC/Nordtest Guide. The Delegation, supported by a number of other delegations, noted that the problem lay more with conformity assessment, proposed to develop principles for conformity assessment which would take into account sampling and measurement uncertainty.

94. The Observer from EURACHEM explained that their guidance combined existing practices to establish variation of sampling and provided relatively economical methods to estimate the scale of the problem so that the user could decide how to manage the problem; that the guidance could be provided to quote uncertainty and this information could be used to select appropriate sampling plans from the General Guidelines on Sampling. The problem was how to interpret sampling uncertainty in a consistent manner.

95. The Observer further explained how the figures in the table were derived and that they were based on data from open literature.

96. In view of the above, the Committee therefore agreed to discontinue further development of the paper on uncertainty of sampling as a separate issue.

97. In relation to the concerns raised by Brazil with regard to dispute settlements, it was pointed out that the *Guideline for Settling Disputes on Analytical (Test) Results* (CAC/GL 70-2009) addressed a narrow field of possible sources of disputes and that further general guidance was needed for producers on how to verify compliance of the product with a Codex limit.

98. In noting the proposal for general guidance on settling of disputes other than those already covered by the aforementioned guidelines and the earlier proposal for principles for conformity assessment, the Committee agreed to establish an electronic working group led by Brazil, with assistance from New Zealand, working in English only with the following terms of reference:

- prepare a discussion paper that would consider procedures for conformity assessment and resolution of disputes and what further guidance was needed taking into account:
  - emerging issues in relation to conformity assessment and resolution of disputes based on product testing.
  - all documents and papers presented to the current session.
- collect additional information on uncertainty of sampling as necessary.
- consider conformity assessment in the context of principles and guidelines for inspection and certification of food developed by CCFICS.
- take into account the following list of issues:
  - principles of conformity assessment
  - measure uncertainty and sampling uncertainty
  - the concept of "fit for purpose"
  - production and process control procedures to achieve compliance with specifications in a more effective manner than end-product testing.
- conformity assessment based on test result (s), sampling plan and decision rules
  - take into account procedures already developed by this and other committees
  - consider means for practical application of the *General Guidelines on Sampling*
  - examine the risk of wrong decisions taken on compliance or non-compliance
  - consequences of non-conformity
  - nature and sources of disputes noting the sources of disputes mentioned in footnote 3 of CL 70.
  - resolution of disputes taking into account GL 70.

METHODS OF ANALYSIS FOR NATURAL MINERAL WATERS (Agenda Item 7)[7]

99. The Committee recalled that its last session had agreed to send a circular letter requesting information on the methods of analysis for the substances in section 3.2 of the Standard for Natural Mineral Waters, as requested by the 31st Session of the Commission (2008) following the adoption of the health related substances in the Standard for Natural Mineral Waters. As a large number of methods had been put forward, the working group had agreed to apply the criteria approach and to assess the methods proposed in the *Working Instructions for the Implementation of the Criteria Approach in Codex.*

100. In reply to a question as to whether heavy metals should be considered, it was noted that the circular letter asked for information on methods for the substances in Section 3.2, especially sections 3.17 to 3.20 but not exclusively on the latter sections and therefore it was possible for the Committee to consider heavy metals, taking into account that the Committee on Natural Mineral Waters was adjourned and that the CCMAS could also consider the update of methods.

[7] CX/MAS 10/31/7 (comments of Argentina, Lithuania, and Philippines) CRD 1 (Report of the Working Group on Endorsement of Methods of Analysis and Sampling), CRD 6 (comments of Kenya and Thailand), CRD 10 (comments of Switzerland), CRD 24 (list of methods for natural mineral waters)

101. The Committee considered the list of methods prepared by the Observers from NMKL and ISO in CRD 24, which included the numerical values for the minimum applicable range, LOD, LOQ, RSD_R and recovery corresponding to each maximum level, and a list of methods assessed against these criteria. The first part of the list included the substances for which a maximum level is defined in the Standard for Natural Mineral Waters (3.2.1 to 3.2.16).

102. As the ISO 11885:2007 method for antimony, arsenic, lead and selenium has no LOD, it was deleted from the list of analytical methods for these substances but retained for the determination of other substances when it met the criteria. The Observer from EFBW indicated that this method was currently used for the analysis of mineral waters and that its characteristics were adequate for the determination of all substances in the list. The Committee however agreed that if the criteria approach was applied and the LOD was above the maximum level, the method could not be accepted as the selection was based on the criteria. However this did not mean that the method could not be used in practice or was not adequate for its purpose where the Codex criteria can be met.

103. As the Standard for Natural Mineral Waters refers to "borate expressed as boron", it was clarified that ISO 9390:1990 determines borate while ISO 11885:2007 and ISO 17294-2:2003 determines total boron. A note was therefore inserted for clarification purposes and the three methods were retained.

104. The Committee considered the list of methods applying to substances for which no maximum level is defined, as the Standard indicates that they "shall be below the limit of quantification": surface active agents, mineral oil, PCB pesticides and PAH. The Committee agreed that the criteria applied were the applicable range, LOD, RSD_R, and recovery.

105. For surface active agents, it was clarified that ISO 7875-1:1996 applies to anionic surface agents and uses chloroform and that ISO 7875-2:1984 applies to non-ionic surface agents. Taking into account that the first method was not collaboratively tested and that no precision data were available for the second, these methods were deleted, although it was noted that they were commonly used for the analysis of natural mineral waters.

106. It was agreed to clarify that the ISO and AOAC methods proposed for pesticides apply to organochlorine pesticides and PCBs.

107. The Committee discussed the question of the type of methods and some delegations proposed to list these methods as Type III. However it was agreed that in the case of the criteria approach, no type should be specified as the list only indicates that methods meet the criteria.

108. Following a request for clarification on the status of the current methods for mineral waters listed in CODEX STAN 234, it was noted that some of these methods were outdated and the Committee agreed that the current methods for health related substances would be replaced with the methods reviewed at the current session, presented according to the format used in CRD 24.

109. The Committee agreed to submit the methods of analysis for the health related substances in Section 2 of the Standard for Natural Mineral Waters for adoption by the 33rd Session of the Commission (see Appendix II).

REPORT OF AN INTER-AGENCY MEETING ON METHODS OF ANALYSIS AND SAMPLING (Agenda Item 8)[8]

110. The Secretary of the Inter-Agency Meeting, Dr Richard Cantrill (AOCS), introduced the report of the 22nd meeting of international organisations working in the field of methods of analysis and sampling (IAM) held on 5 March 2010. In addition to the matters on the agenda of the Committee, the meeting had considered the activities of the organisations concerned , some of which are highlighted below.

111. The IAM had considered the criteria approach and how standard-setting organisations deal with the HorRat values in determining the acceptability of methods of analysis containing precision data

[8] CRD 2 (Report of the 22nd Meeting of the International Organisations working in the field of methods of analysis and sampling (Inter-Agency Meeting)

112. The Committee noted that the IAM/MoniQa workshop on Codex methods of analysis organised prior to the meeting had been very successful and attended by a large number of delegates, and participants were invited to make proposals for a future workshop which might be held in 2011.

113. Following the discussion at the last session of the Committee on proprietary methods, the IAM had prepared a first draft paper, presented in the Annex to CRD 2 for information. The paper noted that proprietary methods were not clearly defined, highlighted some concerns that could arise from their use: they might prevent further development of new and better techniques, distort competition between companies producing the reagents, and create difficulties for government authorities if particular reagents were not readily available for official methods. It was recalled that the R5 method for the determination of gluten illustrated some of these problems as the reagents were not generally available. Several approaches were proposed in CRD 2 to address this issue, including the use of the criteria approach in Codex.

114. The Delegation of New Zealand recalled that it had earlier proposed to put forward a new procedure for evaluation of methods, and offered to contribute to future discussions on this issue.

115. The Observer from AOECS recalled that the R5 method is the most accurate method from the scientific point of view for the time being, and that if different methods were allowed, it would create serious problems as to how to handle different results for the same food sample: if one method detects a gluten content higher than 20mg/kg gluten and another method detects a level below 20mg/kg, it cannot be determined whether the food can be labelled "gluten free" or not. Moreover, the Observer noted that a method which underestimates the gluten content in foods poses severe health risks for gluten intolerant- consumers.

116. It was noted that the IAM would proceed with its consideration of proprietary methods, invited wider contribution than only IAM members and would provide an update to the next session of the Committee.

117. The Committee was informed that ISO 5725 was being revised and that the future document would contain four parts, to be revised before publication, and that work was ongoing in ISO/TC34/SC 16 on "Horizontal Methods for Molecular Biomarker Analysis".

118. The Committee expressed its appreciation to the international organisations participating in the inter-agency meeting for their contribution to its work and the organisation of the IAM/MoniQa workshop, and the Hungarian Food Safety Office for hosting the IAM. It was noted that the next IAM meeting would be held prior to the 32nd Session of the Committee.

OTHER BUSINESS AND FUTURE WORK (Agenda Item 9)[9]

119. The Committee did not discuss the issue of the elaboration of a discussion paper on the Guidelines for Settling Disputes on Analytical (Test) Results (see Agenda Item 1) under this agenda item since it was more appropriately dealt with in the discussion on uncertainty of sampling (see Agenda Item 6).

DATE AND PLACE OF NEXT SESSION (Agenda Item 10)

120. The Committee was informed that the 32nd Session of the Committee was scheduled to be held in Hungary from 7 to 11 March 2011 and that the exact date and venue would be determined by the host country and Codex Secretariat.

[9] CRD 7 (guidelines for settling disputes on analytical test results: issues raised by Brazil concerning the document Guidelines for Settling Disputes on Analytical (Test) Results)

SUMMARY STATUS OF WORK

Subject Matter	**Step**	**Action by**	**Document Reference in ALINORM 10/32/23**
Draft Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods	5/8	Governments 33rd CAC	para. 33 Appendix III
Endorsement of methods of analysis in Codex Standards, including methods of analysis for natural mineral waters		Governments 33rd CAC	paras. 57-82 Appendix II
Proposed Draft Revised Guidelines on Measurement Uncertainty	5	Governments 32nd CCMAS	para. 56 Appendix IV
Guidance on to be considered in conjunction with procedures for conformity assessment and resolution of disputes, uncertainty from measurement and sampling an dother related issues		Brazil/New Zealand/ Governments 32nd CCMAS	para. 98

APPENDIX I

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APPENDIX II

STATUS OF ENDORSEMENT OF METHODS OF ANALYSIS AND SAMPLING

A. Codex Committee on Fish and Fishery Products
B. Codex Committee on Milk and Milk Products
C. Codex Committee on Nutrition and Foods for Special Dietary Uses
D. Natural Mineral Waters
E. Codex Committee on Processed Fruits and Vegetables

A. CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS[1]

Draft Standard for Sturgeon Caviar

COMMODITY	PROVISION	METHOD	PRINCIPLE	Type
Sturgeon Caviar	Salt content	As in CODEX STAN 167-1989 (see below)[2]	Titrimetry	I

1. Principle

The salt is extracted by water from the preweighed sample. After the precipitation of the proteins, the chloride concentration is determined by titration of an aliquot of the solution with a standardized silver nitrate solution (Mohr method) and calculated as sodium chloride.

2. Equipment and chemicals

- Brush
- Sharp knife or saw
- Balance, accurate to 0.01 g
- Calibrated volumetric flasks, 250 ml
- Erlenmeyer flasks
- Electric homogenizer
- Magnetic stirrer
- Folded paper filter, quick running
- Pipettes
- Funnel
- Burette
- Potassium hexacyano ferrate (II), K4Fe(CN)6·3H2O, 15% w/v (aq)
- Zinc sulphate, ZnSO4·6H2O, 30% w/v (aq)
- Sodium hydroxide, NaOH, 0.1 N, 0.41% w/v (aq)
- Silver nitrate, AgNO3, 0.1 N, 1.6987% w/v (aq), standardized
- Potassium chromate, K2CrO4 5% w/v (aq)
- Phenolphthalein, 1% in ethanol
- distilled or deionized water

3. Procedure

(i) Five gram of homogenized subsample is weighted into a 250 ml volumetric flask and vigorously shaken with approximately 100 ml water.

(ii) Five millilitre of potassium hexacyano-ferrate solution and 5 ml of zinc sulphate solution are added, the flask is shaken.

(iii) Water is added to the graduation mark.

(iv) After shaking again and allowing to stand for precipitation, the flask content is filtered through a folded paper filter.

[1] ALINORM 10/33/18, Appendix V
[2] In line with this decision, the method will be retyped as Type I in CODEX STAN 167-1989

(v) An aliquot of the clear filtrate is transferred into an Erlenmeyer flask and two drops of phenolphthalein are added. Sodium hydroxide is added dropwise until the aliquot takes on a faint red colour. The aliquot then diluted with water to approximately 100 ml.

(vi) After addition of approximately 1 ml potassium chromate solution, the diluted aliquot is titrated under constant stirring, with silver nitrate solution. Endpoint is indicated by a faint, but distinct, change in colour. This faint reddish-brown colour should persist after brisk shaking.

To recognize the colour change, it is advisable to carry out the titration against a white background.

(vii) Blank titration of reagents used should be done.

(viii) Endpoint determination can also be made by using instruments like potentiometer or colorimeter.

4. Calculation of results

In the equation of the calculation of results the following symbols are used:

A= volume of aliquot (ml)

C= concentration of silver nitrate solution in N

V= volume of silver nitrate solution in ml used to reach endpoint and corrected for blank value

W= sample weight (g)

The salt content in the sample is calculated by using the equation:

Salt concentration (%) = (V x C x 58.45 x 250 x 100) / (A x W x 1000)

Results should be reported with one figure after the decimal point.

Consequential Amendment to Endorsement Status

COMMODITY	PROVISION	METHOD	PRINCIPLE	Type
Salted Fish and Dried Salted Fish of the *Gadidae* Family	Salt content	Described in the Standard	Titrimetry	I

B. COMMITTEE ON MILK AND MILK PRODUCTS[3]

METHODS OF ANALYSIS

Products	Provisions	Method	Principle	Type
Milk products	Iron	NMKL 139 (1991) (Codex gene method) / AOAC 999.11	Atomic absorption spectrophotometry	II
Milk products	Iron	NMKL 161 (1998) / AOAC 999.10	Atomic absorption spectrophotometry	III
Milk products	Iron	AOAC 984.27	Inductively Coupled Plasma opti emission spectrophotometry	III
Milk products	Iron	IDF 103A:1986 / ISO 6732:1985	Photometry (bathophenanthroline)	IV
Blend of evaporated skimmed milk and vegetable fat	Total fat	ISO 1737| IDF 13:2008	Gravimetry (Röse-Gottlieb)	I
Blend of evaporated skimmed milk and vegetable fat	Milk solids-not- (MSNF)[4]	IDF 21B:1987/ISO 6731:1989 and ISO 1737| IDF 13:2008	Calculation from total solids content a fat content Gravimetry (Röse-Gottlieb)	I
Blend of evaporated skimmed milk and vegetable fat	Milk protein in MSN	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	IV
Reduced fat blend of evaporated skimmed mi and vegetable fat	Total fat	ISO 1737| IDF 13:2008	Gravimetry (Röse-Gottlieb)	I

[3] ALINORM 10/33/11, para. 45-62, APPENDIX III

[4] Milk total solids and MSNF content include water of crystallization of lactose

Products	Provisions	Method	Principle	Type
Reduced fat blend of evaporated skimmed mi and vegetable fat	MSNF[4]	IDF 21B:1987 / ISO 6731:1989 and ISO 1737| IDF 13:2008	Calculation from total solids content a fat content Gravimetry (Röse-Gottlieb)	I
Reduced fat blend of Evaporated skimmed mi and vegetable fat	Milk protein in MSN	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	IV
Blend of skimmed milk and vegetable fat in powdered form	Total fat	ISO 1736|IDF 9:2008	Gravimetry (Röse-Gottlieb)	I
Blend of skimmed milk and vegetable fat in powdered form	Water[5]	ISO 5537|IDF 26:2004	Gravimetry, drying at 87 °C	I
Blend of skimmed milk and vegetable fat in powdered form	Milk protein in MSN	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	IV
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Total fat	ISO 1736|IDF 9:2008	Gravimetry (Röse-Gottlieb)	I
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Water[5]	ISO 5537|IDF 26:2004	Gravimetry, drying at 87 °C	I
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Milk protein in MSN	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	IV
Blend of sweetened condensed skimmed milk and vegetable fat	Total fat	ISO 1737| IDF 13:2008	Gravimetry (Röse-Gottlieb)	I
Blend of sweetened condensed skimmed milk and vegetable fat	Sucrose	ISO 2911|IDF 35:2004	Polarimetry	IV
Blend of sweetened condensed skimmed milk and vegetable fat	Milk solids-not- (MSNF)[4]	IDF 15B:1991 / ISO 6734:1989	Calculation from total solids content, content and sugar content	IV
Blend of sweetened condensed skimmed milk and vegetable fat	Milk protein in MSN	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	IV
Reduced fat blend of sweetened	Total fat	ISO 1737| IDF 13:2008	Gravimetry (Röse-Gottlieb)	I

[5] Water content excluding the crystallized water bound to lactose (generally known as “moisture content”)

Products	Provisions	Method	Principle	Type
condensed skimmed milk and vegetable fat				
Reduced fat blend of sweetened condensed skimmed milk and vegetable fat	MSNF[4]	IDF 15B:1991 / ISO 6734:1989	Calculation from total solids content, content and sugar content	IV
Reduced fat blend of sweetened condensed skimmed milk and vegetable fat	Milk protein in MSN	ISO 8968-1/2| IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	IV
Butter	Salt	ISO 1738|IDF 12:2004 / AOAC 960.29	Titrimetry (Mohr: determination of chloride, expressed as sodium chloride)	III
Butter	Milk fat purity	ISO 17678|IDF 202:2010	Calculation from determination triglycerides by gas chromatography	I
Cheese (and cheese rind)	Natamycin	ISO 9233-1|IDF 140-1:2007	Molecular absorption spectrophotometry	III
		ISO 9233-2|IDF 140-2:2007	HPLC	II
Cheese	Sodium chloride	ISO 5943|IDF 88:2006	Potentiometry (determination of chlori expressed as sodium chloride)	II
Cottage cheese	Fat-free dry matter		Calculation from dry matter content and content	I
		ISO 5534|IDF 4:2004 and ISO 1735|IDF 5:2004	Gravimetry, drying at 102 °C Gravimetry (Schmid-Bondzynski-Ratzlaf	
Cottage cheese	Milk fat	ISO 1735|IDF 5:2004	Gravimetry (Schmid-Bondzynski-Ratzla (for samples containing lactose up to 5%)	I
		ISO 8262-3|IDF 124-3:2005	Gravimetry (Weibull-Berntrop) (f samples containing lactose over 5%)	I
Cheese, unripened including fresh cheese	Protein	ISO 8968-1/2|IDF 2 1/2:2001/AOAC 991.20 and 991.2	Titrimetry (Kjeldahl)	I
Cream and prepared creams	Milk protein	ISO 8968-1/2|IDF 2 1/2:2001/AOAC 991.20	Titrimetry (Kjeldahl)	I
Cream	Milk fat	ISO 2450|IDF 16:2008	Gravimetry (Röse-Gottlieb)	I
Creams lowered in milk fat content	Milk fat	ISO 2450|IDF 16:2008 /AO/ 995.19	Gravimetry (Röse-Gottlieb)	I
Cream cheese	Dry matter	ISO 5534|IDF 4:2004	Gravimetry drying at 102 °C (forced	I

Products	Provisions	Method	Principle	Type
			oven)	
Cream cheese	Moisture on fat fr basis		Calculation from fat content and moistu content	I
		ISO 5534|IDF 4:2004	Gravimetry drying at 102°C (forced oven)	
		ISO 1735|IDF 5:2004	Gravimetry (Schmid-Bondzynski-Ratzlaf	
Dairy fat spreads	Milk fat purity	ISO 17678|IDF 202:2010	Calculation from determination triglycerides by gas chromatography	I
Edible casein products	Acids, free	ISO 5547|IDF 91:2008	Titrimetry (aqueous extract)	IV
Edible casein products	Ash (including P_2O_5)	ISO 5545|IDF 90:2008 or[6]	Gravimetry (ashing at 825 °C)	
Edible casein products		ISO 5544|IDF 89:2008		I
Edible casein products	Water[5]	ISO 5550|IDF 78:2006	Gravimetry (drying at 102 °C)	I
Edible casein products	Lead	NMKL 139 (199 (Codex general method) AOAC 999.11	Atomic absorption spectrophotometry	II
Edible casein products	Lead	NMKL 161 (1998) / AOAC 999.10	Atomic absorption spectrophotometry	III
Evaporated milks	Milk fat	ISO 1737| IDF 13:2008	Gravimetry (Röse-Gottlieb)	I
Evaporated milks	Protein	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 945.48H / AOAC 991.20	Titrimetry (Kjeldahl)	I
Fermented milks	Protein	ISO 8968-1/2|IDF 20-1/2:2001/ AOAC 991.20	Titrimetry (Kjeldahl)	I

[6] refer to scope of methods

Fermented milks	Milk fat	ISO 1211|IDF 1:2010 / AOA 989.05	Gravimetry (Röse-Gottlieb)	I
Fermented milks – Yoghurt and yogh products	*Lactobacillus delbrueckii* subs *bulgaricus* *Streptococcus thermophilus*	ISO 7889|IDF 117:2003	Colony count at 37 °C	I
Fermented milks	Lactic acid (to acidity expressed as lac acid)	IDF 150:1991 / ISO 11869:1997	Potentiometry, titration to pH 8.30 Spectrophotometry	IV
Fermented milks	Microorganisms constituting the starter culture	ISO 27205|IDF 149:2010 (Ann A)	Colony count at 25 °C, 30 °C, 37 °C a 45 °C according to the starter organism question	IV
Fermented milks	*Lactobacillus acidophilus*	ISO 20128|IDF 192:2006	Colony count at 37 °C	I
Fermented milks	Colony-forming ur of yeasts and/or moul	ISO 6611|IDF 94:2004	Colony-count at 25 °C	IV
Milk powders and cream powders	Milk fat	ISO 1736|IDF 9:2008	Gravimetry (Röse-Gottlieb)	I
Milk powders and cream powders	Protein (in MSNF[4])	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl digestion)	I
Milk powders and cream powders	Solubility Index	ISO 8156|IDF 129:2005	Centrifugation	I
Milk powders and cream powders	Water[5]	ISO 5537|IDF 26:2004[7]	Gravimetry (drying at 87°C)	I
Milk fat products	Milk fat	IDF 24:1964	Gravimetry (calculation from solids-not-content and water content)	IV
Milk fat products	Milk fat purity	ISO 17678|IDF 202:2010	Calculation from determination triglycerides by gas chromatography	I
Milk fat products	Water	ISO 5536|IDF 23:2009	Titrimetry (Karl Fischer)	II

[7] The method has only been validated for milk powders, not for cream powders

Milk products obtained from fermented milks heat-treated after fermentation	Protein	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	I
Mozzarella	Milk fat in dry matte with high moisture	ISO 1735|IDF 5:2004	Gravimetry after solvent extraction	I
Mozzarella	Milk fat in dry matte with low moisture	ISO 1735|IDF 5:2004	Gravimetry after solvent extraction	I
Sweetened condensed milk	Milk fat	ISO 1737| IDF 13:2008	Gravimetry (Röse-Gottlieb)	I
Sweetened Condensed Milks	Protein	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 945.48H / AOAC 991.20	Titrimetry (Kjeldahl)	I
Whey cheeses by concentration	Milk fat	ISO 1854|IDF 59:2008	Gravimetry (Röse Gottlieb)	I
Whey cheeses by concentration	Milk fat in dry matte		Calculation from fat content and dry mat content	I
		ISO 1854|IDF 59:2008 and	Gravimetry (Röse Gottlieb)	
		ISO 2920|IDF 58:2004	Gravimetry, drying at 88 °C	
Whey powders	Ash	ISO 5545|IDF 90:2008	Gravimetry (ashing at 825 °C)	IV
Whey powders	Milk fat	ISO 1736|IDF 9:2008	Gravimetry (Röse-Gottlieb)	I
Whey powders	Milk protein (total N x 6.38)	ISO 8968-1/2|IDF 20-1/2:2001 AOAC 991.20	Titrimetry (Kjeldahl)	I
Whey powders	Water[5]	ISO 5537|IDF 26:2004	Gravimetry (drying at 87 °C)	I

METHODS OF SAMPLING

Commodity Standard	Method of Sampling	Notes
Milk and Milk products		
Milk products	ISO 707|IDF 50:2008	General instructions for obtaining a samp from a bulk
Milk products	ISO 5538|IDF 113:2004	Inspection by attributes
Milk products	IDF 136A:1992 ISO 8197:1988	Inspection by variables

C. COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES[8]

Methods of analysis for dietary fibre: Guidelines for Use of Nutrition and Health Claims: Table of Conditions for Claims

Commodity	Provisions	Method	Principle	Type proposed
General methods that do not measure the lower molecular weight fraction (i.e. monomeric units<=9) (2)				
All foods (1)	Dietary fibre based on precipitation in 4 parts alcohol and part water. Resistant insoluble and soluble polysaccharid lignin, and plant cell wall. (4) (Total dietary fibre)	AOAC 985.29 AACC Intl 32-05.01 (1991,1999	Enzymatic gravimetric	IV
All foods (1)	Dietary fibre based on precipitation in 4 parts alcohol and part water. Resistant insoluble and soluble polysaccharid lignin, and plant cell wall (4). (Can determine total, but also determines soluble a insoluble dietary fibre)	AOAC 991.43 AACC Intl 32-07.01 (1999, 199 NMKL 129, 2003	Enzymatic gravimetric	IV
All foods (1)	Dietary fibre in food and food products with less than 2 starch (Foods with >10% TDF and < 2% starch (fruits)) (4)	AOAC 993.21	gravimetric	IV
All foods (1)	Dietary fibre based on precipitation in 4 parts alcohol and part water, quantitated as component neutral sugars, uron acids, plus Klason lignin. (4)	AOAC 994.13 AACC Intl 32-25.01 (1999, 1994) NMKL 162, 1998	Enzymatic gravimetric Colorimetric	IV

[8] ALINORM 10/33/26, Appendix II

Commodity	Provisions	Method	Principle	Type proposed
	(Determine sugars, useful for commodity where fibre a sugar are both necessary)			
General methods that measure both the higher (monomeric units > 9) and the lower molecular weight fraction (monomeric units <=9) [(2)]				
All foods [(1)]	Dietary fibre based on precipitation in 4 parts alcohol and part water. Resistant insoluble and soluble polysaccharid Resistant malto-dextrins, lignin, and plant cell wall. [(3)]	AOAC 2001.03 AACC Intl 32-41.01 (2002)	Enzymatic gravimetric and Liquid chromatography	IV
All foods [(1)]	Dietary fibre (Soluble + insoluble polysaccharides + lignir resistant starch + oligosaccharides).	AOAC 2009.01 AACC Intl 32-45.01 (2009)	Enzymatic-Gravimetric-High Pressure Liqu Chromatography Method	IV
Methods that measure individual specific components (monomeric units: the whole range for each type of components is covered) [(2)]				
All foods [(1)]	Insoluble dietary fibres in food and food products	AACC Intl 32-20.01 (1999, 198 AOAC 991.42 (Specific for insoluble fibre)	Enzymatic gravimetric	IV
All foods [(1)]	Soluble dietary fibres in food and food products	AOAC 993.19 (Specific for soluble fibre)	Enzymatic gravimetric	IV
All foods [(1)]	(1→3)(1→4) *Beta*-D-Glucans	AOAC 995.16 AACC Intl 32-23.01 (1999, 199	Enzymatic Colorimetric	IV
All foods [(1)]	Fructans (oligofructoses, inulin, hydrolyzed inul polyfructoses, fructooligosaccharides) (applicable to add fructans)	AOAC 997.08 AACC Intl 32-31.01 (2001)	Enzymatic HPAEC-PAD	IV
All foods [(1)]	Fructans (oligofructoses, inulin, hydrolyzed inul polyfructoses, fructooligosaccharides) (not applicable highly depolymerised fructans)	AOAC 999.03 AACC Intl 32-32.01 (2001)	Enzymatic colorimetric	IV
All foods [(1)]	Polydextrose	AOAC 2000.11 AACC Intl 32-28.01 (2001)	HPAEC-PAD	IV
All foods [(1)]	Trans-galacto-oligo saccharides	AOAC 2001.02 AACC Intl 32-33.01 (2001)	HPAEC-PAD	IV

Commodity	Provisions	Method	Principle	Type proposed
All foods [1]	Resistant starch (Recommended for RS2 & RS3)	AOAC 2002.02 AACC Intl 32-40.01 (2002)	Enzymatic Colorimetric	IV
Other methods[2]				
All foods	Insoluble glucans and mannans of yeast cell wall (for ye cell wall only)	Eurasyp (European association f specialty yeast product) – LM Bonanno. Biospringer- 2004 – online version : http://www.eurasyp.org/public.te hnique.home.screen.	Chemical & HPAE PAD	IV
All foods	Fructo-oligosaccharides (monomeric units<5)	Ouarné et al. 1999 in *Complex Carbohydrates in Foods*. Edited by S. Sungsoo, L. Prosky & M. Dreher. Marcel Dekker Inc, New York	HPAEC-PAD	IV
All foods	Non-starch polysaccharides (NSP) [3]	Englyst H.N, Quigley M.E., Hudson G. (1994) Determinatior of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic high performar liquid chromatographic or spectrophotometric measuremen of constituent sugars – Analyst 119, 1497-1509	Enzymatic G Liquid Chromatography	IV

[1] Users should consult the description of each method for the food matrices that were the subject of interlaboratory study in the Official methods of Analysis of AOAC International.

[2] Two issues are left for national authorities: to include monomeric units 3-9 and which isolated or synthetic compounds have physiological benefit. (Refer to GL 2-1985)

[3] Quantitation lost for resistant starch. Refer to specific methods.

[4] Quantitation lost for inulin, resistant starch, polydextrose and resistant maltodextrins. Refer to specific methods.

D. NATURAL MINERAL WATERS
Standard for Natural Mineral Waters (CODEX STAN 108-1981)

Provision	ML (mg/L)	Min. applicabl range (mg/L)	LOD (mg/L)	LOQ (mg/L)	Precision RSDR (%) No more than	Recovery (%)	Suggested methods meeting the criteria	Principle
Antimony	0.005	0.0028	0.001	0.002	44	80-110	ISO 17294-2:2003 ISO 15586:2003	ICP-MS GF-AAS
Arsenic	0.01	0.0056	0.002	0.004	44	90-107	ISO 17294-2:2003 ISO 15586:2003 ISO 11969:1996	ICP-MS GF-AAS AAS –hydride
Barium	0.7	0.35	0.07	0.14	34	95-105	ISO 11885:2007 ISO 17294-2:2003	ICP-OES ICP-MS
Borate	5	3.1	0.5	1	25	97-103	ISO 9390:1990 ISO 11885:2007 ISO 17294-2:2003	Spectrophotometry ICP-MS[9] ICP-MS
Cadmium	0.003	0.0017	0.0006	0.0012	44	80-110	ISO 11885:2007 ISO 17294-2:2003 ISO 15586:2003 ISO 5961:1994	ICP-OES ICP-MS GF-AAS AAS (section 3)
Chromium	0.05	0.028	0.01	0.02	44	90-107	ISO 11885:2007 ISO 17294-2:2003 ISO 15586:2003 ISO 18412:2005 (Cr VI) ISO 23913:2006 (Cr VI) ISO 9174:1998	ICP-OES ICP-MS GF-AAS Photometric CIA, spectrophotometry AAS (Section 4)

[9] Total Boron is determined

Provision	ML (mg/L)	Min. applicabl range (mg/L)	LOD (mg/L)	LOQ (mg/L)	Precision RSDR (%) No more than	Recovery (%)	Suggested methods meeting the criteria	Principle
Copper	1	0.52	0.1	0.2	32	97-103	ISO 11885:2007 ISO 17294-2:2003 ISO 15586:2003 ISO 8288:1986	ICP-OES ICP-MS GF-AAS Flame-AAS
Cyanide	0.07	0.039	0.014	0.028	44	90-107	ISO 14403:2002 ISO 6703-1:1998	CFA Photometric, trimetric
Fluoride	1.0	0.52	0.1	0.2	32	97-103	ISO 10304-1:2007 ISO 10359-1:19 (dissolved fluoride) ISO 10359-2:1994 (inorganic bound)	HPLC Electrochemical probe Digestion, distillation
Lead	0.01	0.0056	0.002	0.004	44	90-107	ISO 17294-2:2003 ISO 15586:2003 ISO 8288:1986	ICP-MS GF-AAS Method C (III)
Manganese	0.4	0.18	0.04	0.08	37	95-105	ISO 11885:2007 ISO 17294-2:2003 ISO 15586:2003	ICP-OES ICP-MS GF-AAS
Mercury	0.001	0.00056	0.0002	0.0004	44	80-110	EN 1483:2007 ISO 17852:2006 ISO 5666:1999 ISO 16590:2000	AAS Enrichment by amalgamation (I AFS AAS after tin(II) chlori reduction Enrichment by amalgamation (I
Nickel	0.02	0.011	0.004	0.008	44	90-107	ISO 17294-2:2003 ISO 15586:2003	ICP-MS GF-AAS
Nitrate	50	37	5	10	18	98-102	ISO 10304-1:2007 ISO 13395:1996 ISO 7890-3:1988	HPLC CFA, FIA, Spectrophotometry Spectrophotometry
Nitrite	0.1	0.03	0.01	0.02	44	95-105	ISO 10304-1:2007 ISO 13395:1996	HPLC CFA, FIA, Spectrophotometry

Provision	ML (mg/L)	Min. applicabl range (mg/L)	LOD (mg/L)	LOQ (mg/L)	Precision RSDR (%) No more than	Recovery (%)	Suggested methods meeting the criteria	Principle
							ISO 6777:1984	Spectrophotometry
Selenium	0.01	0.0056	0.002	0.004	44	90-107	ISO 17294-2:2003 ISO 15586:2003 ISO 9965:1993	ICP-MS GF-AAS AAS (Hydride)

Provision	ML	Applicable rang from:	LOD		RSDR (%)	Recovery	Suggested methods	Principle
Surface active agents	-	0.1 -5.0 mg/L 0.25-0.8 mg/L 0.05 – 5.0 mg/L	0.05 mg/		19 10 < 44	70-100	ISO 16265:2009	CFA
Mineral oil (hydrocarb index)	-	>0.1 mg/L			< 41	71-102	ISO 9377-2:2000	GC
PCB	-	> 10 ng/L >15 ng/L			27-79 <20	40-142 70-130	ISO 6468:1996 AOAC 990.16	GC ECD GC ECD
Pesticide (organochlorine)	-	> 10 ng/L > 15 ng/ L			27-79 <20	40-142 70-130	ISO 6468:1996 AOAC 990.16	GC ECD GC ECD
PAH	-	0.005 µg/L 0.04 µg/L 0.005 µg/L			<10 <18 <19	80-110 80-110 80-100	ISO 17993:2004 ISO 7981-1:2005 ISO 7981-2:2005	HPLC FD TLC HPLC

E. CODEX COMMITTEE ON PROCESSED FRUITS AND VEGETABLES[10]

Draft Standard for Preserved Tomatoes

Provision	Method	Principle	Type
Minimum Drained Weight	AOAC 968.30	Gravimetry (sieving) note: Use a No. 14 screen instead of '7/16' or No. 8	I

[10] ALINORM 09/32/27, para.14

ALINORM 10/33/23
APPENDIX III

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CRITERIA AND VALIDATION OF METHODS FOR DETECTION, IDENTIFICATION AND QUANTIFICATION OF SPECIFIC DNA SEQUENCES AND SPECIFIC PROTEINS IN FOODS*

(At Step 5/8 of the Procedure)

SECTION 1 – INTRODUCTION

1. Molecular and immunological analytical methods are currently the recognized tools for determination of DNA and protein analytes in foods. However, in order for the results obtained by such methods from different laboratories to gain wide acceptability and confidence as reliable, there is need for the analytical methods to satisfy certain quality criteria.

2. These guidelines provide appropriate criteria to validate the performance of methods developed to detect specific DNA sequences or specific proteins in foods.

3. Information relating to general considerations for the validation of methods for the analysis of specific DNA sequences and specific protein is given in the first part of these Guidelines. Specific annexes are provided that contain information on validation of quantitative Polymerase Chain Reaction (PCR) methods, validation of qualitative PCR methods and validation of protein-based methods**.**

SECTION 1.1 – PURPOSE AND OBJECTIVES

4. The goal of this document is to support the establishment of molecular and immunological methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, which produce results with comparable reproducibility when performed at different laboratories

5. The guidelines are aimed at providing guidance on how to establish methods to detect and identify specific DNA sequences and proteins in food by defining appropriate validation criteria, and whether or not a method complies with these criteria based on the performance characteristics of a method.

The guidelines specify the relevant criteria and give explanations on how to consider these criteria, i.e.:

-by providing the rationale for the most relevant criteria and

-by showing how to find out whether or not a method fulfils the given criteria requirements.

SECTION 1.2 SCOPE

6. These guidelines provide information on criteria for the validation of food analysis methods involving the detection, identification and quantification of specific DNA sequences and specific proteins of interest that may be present in foods, including those foods containing materials derived from modern biotechnology. These molecular and immunological methods are applicable to a wide range of uses such as tests for biomarkers in foods, including those derived from modern biotechnology and food authentication, and may be used by laboratories responsible for food analysis.

SECTION 2 – METHOD VALIDATION

7. The Codex Alimentarius Commission places an emphasis on the acceptance of methods of analysis which have been validated through a collaborative trial conforming to an internationally accepted protocol according to ISO 5725:1994 or the AOAC/IUPAC Harmonized Protocol. In this area there may be a need to adopt a formal single-laboratory validation as an interim measure in the absence of collaborative trial data.

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* for applications such as food derived from modern biotechnology, food authentication, food speciation and other purposes

However, methods used for the analysis of DNA sequences and proteins, must be capable of being performed in many laboratories.

Section 2.1 – Criteria Approach

8. These guidelines apply the "criteria approach".

Section 2.2 – General Method Criteria

9. The general criteria for the selection of methods of analysis have been adopted in the Codex Procedural Manual. Such criteria are applied in this guideline. Additional criteria are described in the appropriate annexes.

Section 2.3 – Validation Process

10. Method validation is a process to establish the performance characteristics and limitations of an analytical method. The results of a validation process describe which analytes can be determined in what kind of matrices in the presence of which interference. The validation exercise results in precision and trueness values of a certain analytical method under the examined conditions.

11. Formal validation of a method is the conclusion of a long process, which includes the following main steps:

- ***Pre-validation of the method***. Pre-validation should be performed on a case-by case as needed. Pre-validation should ensure that a method performs in a manner, which allows a successful conclusion of the validation study, i.e. it should provide evidence about the suitability of the method for its intended purpose. Pre-validation should preferably be carried out by involving 2 - 4 laboratories. Statistical analyses (e.g. of "repeatability" and "reproducibility") should be made according to the validation procedure to be subsequently used.
- ***Validation of the method.*** Validation through a collaborative trial is expensive to undertake and usually follows only after the method has shown acceptable performance both in a single-laboratory and a pre-validation study.

SECTION 3 – SPECIFIC CONSIDERATION FOR THE VALIDATION OF METHODS FOR THE DETECTION, IDENTIFICATION AND QUANTIFICATION OF DNA SEQUENCES AND PROTEINS

Section 3.1 – Method Development to Formal Validation

12. Common methodologies for DNA-based analysis are PCR-based methods used to detect a specific (targeted) DNA sequence. Common approaches for protein utilize Enzyme-Linked Immuno-Sorbent Assay (ELISA) and lateral flow devices. For DNA-based analysis, the PCR approach is presently most widely applied, although other DNA-based methods that achieve the same objective may be employed if properly validated. Both DNA and protein-based approaches are considered here.

Section 3.1.1 – Method Acceptance Criteria (Required condition for validation)

13. In order to evaluate a method prior to validation, information concerning both the method and the method testing is required, as detailed in Annex I.

14. The method evaluation should verify that the principle preconditions for using the method for Codex purposes are fulfilled. This section describes the method acceptance criteria, which have to be fulfilled by the method in order to conduct a pre-validation and full collaborative trial.

Section 3.1.2 – Applicability of the Method

15. Applicability of the methods could be determined by confirming whether the methods may be used in the intended foods with the required performance and it should be clearly stated. Especially, in analysis of the DNA sequences and protein, some methods that can be applied to a single raw matrix may not be necessarily applicable to complex matrices and/or processed food, since the DNA and protein may be altered.

16. In principle the method should be applicable to the matrix of concern. In the case of "general purpose" methods to identify and quantify DNA sequences and proteins in a range of food matrices, at least one extraction method applicable to a general food matrix should be available.

Section 3.1.3 – Principle condition

17. DNA-based methods should detect, identify and may quantify the levels of specific DNA sequence(s). Protein-based methods should detect, identify and may quantify the level of a specific protein in the product.

18. Currently, the DNA-based detection method typically consists of PCR methodology and includes:

- a protocol describing an extraction method which is applicable to a relevant matrix;
- a protocol describing the conditions, including the apparatus used, under which PCR can be used to detect the target DNA sequence;
- a description of the oligonucleotide primer sequences which uniquely amplify the target DNA sequence;
- If applicable, a description of the fluorescent oligonucleotide probe sequence which uniquely identifies the target DNA sequence.
- a description of oligonucleotide primer sequences, which amplify a taxon-specific DNA sequence that should be present in the conventional food matrix irrespective of the presence of the specific analyte, in order to differentiate a negative result from failed extraction/amplification processes, and to quantify the amount of target DNA relative to the taxon-specific DNA.
- if applicable, a description of the fluorescent oligonucleotide probe sequence which uniquely identifies the taxon-specific DNA sequence.
- a description of the method used to detect the DNA
- appropriate control samples and standards.
- descriptions of calculations used to derive the result.

19. Protein-based methods typically consist of a quantitative or qualitative method. These are usually immuno-sorbent analysis systems, and consist of the following:

- a protocol describing an extraction method which is applicable to a relevant matrix;
- a protocol describing the conditions, including the apparatus used, under which immuno-sorbent analysis can be used to detect the target protein;
- an antibody-coated support,
- an enzyme-conjugated secondary antibody,
- an enzyme substrate for colour development, and
- washing buffer and sample extraction buffer.
- a description of the method used to detect the protein
- appropriate control samples and standards.
- descriptions of calculations used to derive the result.

20. The method should fulfil the requirements below:

- Protein-based methods should allow for unequivocal detection, identification and/or quantification of a specific antigen or epitope.
- DNA-based screening methods are used to detect a target DNA present in multiple organisms. For instance, screening methods that are used to detect multiple transformation events should allow for detection of a target DNA sequence which is common to a number of transformation events.
- DNA-based specific methods that are used for unequivocal detection, identification and/or quantification of a specific organism which could be mixed with similar organisms should allow for the unequivocal detection, identification and/or quantification of a DNA sequence

that is unique or specific to that organism. For instance, target-specific methods that are used for detection of a single transformation event should allow for unequivocal detection, identification and/or quantification of a DNA sequence that is unique or specific to that transformation event. For food authentication, the specific target sequence/s should uniquely define the taxon as required.

- DNA-based taxon-specific methods that are used for detection or relative quantification of target DNA should allow for unequivocal detection, identification and quantification of a DNA sequence that is unique or specific to that taxon
- For target and taxon-specific methods used in relative quantification, identification of the amplified fragment, by e.g. probe hybridization or any appropriate equivalent method, is recommended.

Section 3.1.4 – Unit of Measurement and reporting of results

21. Appropriate units of measurement (e.g. target copy numbers or molar equivalents), performance and data reporting criteria should be specified for each method prior to their use. For qualitative analysis, the results can be provided as present or not detected and for this reason there is no unit of measurement.

22. Measurements may be explicitly expressed as weight/weight or by relative percentage. However, none of the current methods (DNA or protein based) are able to measure them directly.

Section 3.1.5 – Measurement Uncertainty

23. As mentioned in the Codex Guideline on Measurement Uncertainty (CAC/GL 54-2004), laboratories are required to estimate the uncertainty of their quantitative measurements. Sample preparation and analytical methods are two significant sources for error that should be considered when evaluating an analytical measurement. Analysts using methods which have been validated according to these guidelines should have sufficient information to allow them to estimate the uncertainty of their result.

24. For details, refer to the Codex Guideline on Measurement Uncertainty (CAC/GL 54-2004), the section entitled "*The Use of Analytical Results: Sampling Plans, Relationship between the Analytical Results, the Measurement Uncertainty, Recovery Factors and Provisions in Codex Standard*" from the Codex Procedural Manual.

Section 3.1.6 – Modular Approach to Method Validation

25. The "method" refers to all the experimental procedures needed to estimate the measurand in a particular matrix. For a particular material this may include the processes for DNA or protein extraction and the final quantification in a PCR or Immuno-sorbent assay system, or a determination of the presence or absence of the analyte via a qualitative method. In such a case, the whole chain from extraction up to the analytical step constitutes a method. However, it may be possible to use the same sample preparation (e.g. grinding) method in combination with the same DNA or protein isolation process for several different subsequent analyses to achieve economic efficiencies as long as the validated method processes remain the same.

26. It would be inappropriate to substitute alternative processes, such as a different DNA or protein isolation process, into a validated method without conducting additional studies to show that the substitution does not affect the performance of the method.

Section 3.2 – Collaborative Trial Requirements

Section 3.2.1 – General Information

27. The purpose of a collaborative trial is to validate the data provided by previous testing in a pre-validation or a single laboratory exercise and to determine methodological precision in terms of repeatability and reproducibility.

28. The values of any performance parameters reported from validation studies should be interpreted and compared with care. The exact values and their interpretation may depend – besides the performance of the method - on the extent of the method.

29. If a collaborative trial has been conducted according to the ISO 5725:1994 or the AOAC/IUPAC Harmonized Protocol, then this information can be used to assess the acceptability of the method.

Section 3.2.2 – Minimum Performance Requirements

30. In a collaborative trial, the method performance should comply with the relevant parts of the method acceptance criteria and fulfil the method performance requirements specifically set below for the collaborative trial. In particular, the compliance with the criteria for sensitivity and repeatability/reproducibility standard deviations and trueness should be assessed.

31. In addition to the method acceptance criteria, at least the method performance requirements listed in Annex I should be evaluated from the experimental data of a collaborative trial.

32. The methods and their associated validation data will be revised on a regular basis as the scientific knowledge and experience gained in validation and collaborative trials evolve. These Guidelines are complemented with practical information about the operational steps of the validation process.

Section 3.2.3 – Collaborative Trial Test Materials

33. In principle, the method should be applicable to and tested on the matrix of concern (i.e. on which any specification has been made).

34. The effects of materials/matrices on the extraction step in a protocol are important to any analysis. When the results of a validation study are reported, it is important that the report includes details of which matrix was analyzed and whether a purified protein or DNA was used as the target for the analysis.

Section 3.2.4 – Specific Information on the Validation of Methods

35. Specific information on the validation of quantitative and qualitative PCR methods is given in Annexes II and III respectively.

36. Specific information on the validation of quantitative and qualitative protein-based methods is given in Annex IV.

SECTION 4 – QUALITY CONTROL REQUIREMENTS

Section 4.1 – Laboratory Quality

37. CAC/GL 27 provides guidance for laboratories involved in the import and export of foods. This guidance is based on compliance with ISO/IEC Standard 17025, proficiency testing and internal quality control as well as the use of methods of analysis validated according to Codex requirements.

Section 4.2 – Reference Material

38. A suitable reference material is generally required for the validation of a method. There are a number of matrices that can be used to develop reference materials or working standards for methods of detection of DNA sequences and proteins. Each has its own advantages and drawbacks for particular purposes. The physical form of the reference material determines its suitability for use with any given method. For ground materials, differences in particle size distribution between reference materials and routine samples may affect extraction efficiency of the target protein or DNA and method reproducibility due to sampling error.

39. Reference material for DNA based methods can be a matrix containing the analyte, DNA extracted from matrix containing the analyte, a plasmid containing the specific DNA, or if certified reference materials are not available, control sample materials, for example from proficiency testing schemes. Use of plasmid or amplicon DNA requires careful consideration of the choice to be incorporated into the plasmid or amplicon to ensure that the plasmid or amplicon DNA will be fit for the required purpose.

40. Reference materials for protein-based methods can be e.g. the protein itself purified from recombinant microbes (such as *E. coli*), a ground plant matrix (typically leaf or grain), or a processed food fraction.

SECTION 5 – TECHNICAL AND METHODOLOGICAL INFORMATION

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ANNEX I: REQUIRED INFORMATION WHEN METHODS ARE TO BE CONSIDERED FOR VALIDATION

DESCRIPTION OF THE METHOD

1. A complete and detailed description of all the components of the method should be provided. The use of multiple plates for PCR and protein methods, as an example, should be explicitly addressed. The description should also include information on the scope of the method, and the unit of measurement should be clearly indicated, as well as the following:

Purpose and relevance of the method

2. The purpose of the method should be indicated in the method. The method should be fit for purpose for the intended use.

Scientific basis

3. An overview of the scientific principles on which the method is based (e.g., the molecular biology underlying the use of a real-time PCR method) should be provided.

Specification of the prediction model/mathematical model needed for the method

4. The DNA and protein-based techniques used to detect and quantify DNA sequences and proteins are based on different principles. In PCR the targeted DNA is amplified in an exponential manner. Moreover, the quantification by real-time PCR is often based on two independent PCR assays: one for the target DNA and one for the taxon specific DNA sequence. In contrast to PCR, immuno-sorbent assays involve binding one or more layers of antibodies to each initial target molecule, and amplification of the signal is proportional to the number of reporter molecules and, if applicable, the enzymatic reaction time.

5. If the derivation of the results relies upon a mathematical relationship this should be outlined and recorded (e.g., $\Delta\Delta$Ct method or a regression line or calibration curve obtained by other means). Instructions for the correct application of the model should be provided. These may include, depending on the method, a recommended number and range of levels to be analyzed, minimum number of replicates and/or dilutions to be included for routine analyses or the means and confidence intervals to evaluate the goodness-of-fit.

SPECIFIC INFORMATION REQUIRED FOR DNA-BASED METHODS

6. For DNA-based procedures, the following additional information should be supplied in particular:

Primer pairs

7. General methods have to provide the defined primer pairs and the sequence they target. Recommendations as to the efficiency/use of primer set have to be clearly stated, including if the primers are suitable for screening and/or quantification.

- ***Amplicon length***

8. Food processing will generally lead to a degradation of target DNA. The length of the amplified product may influence the PCR performance. Therefore the selection of shorter amplicon sizes (within reason) will increase the possibility to get a positive signal in the analysis of highly processed foodstuffs. In general the length of the amplified fragment for the taxon-specific DNA sequence and the target sequence should be in a similar size range.

- ***whether the method is instrument or chemistry specific***

9. At the moment a number of different types of real-time instruments and chemistries are available. These instruments and chemistries may have different performance such as stability of reagents, heating and cooling characteristics, which affects ramp rates and affects the time necessary for a whole PCR run.

10. Beside the differences in the heating and cooling system there are differences in the technique and software used to induce and subsequently to record the fluorescence. The detection and quantification of the fluorescence could also vary according to the recording instruments and software used. Qualitative methods generally tend to be less instrument-specific than quantitative methods.

11. The methods are generally instrument and chemistries dependent and cannot be transferred to other equipment and chemistries without evaluation and/or modification.

- ***whether single- or multi-plex PCR amplifications are undertaken***

12. Using more than one primer set in a single reaction is called multi-plex PCR.

13. The information provided should demonstrate the robustness of the method for inter-laboratory transferability. This means that the method should have been tested by at least one other laboratory besides the laboratory which has developed the method. This is an important pre-condition for the success of the validation of the method.

SPECIFIC INFORMATION REQUIRED FOR PROTEIN-BASED METHODS

14. The following additional information should be supplied for protein-based procedures:

Assay applicability

15. Food processing will generally lead to degradation or denaturation of the target protein, which may result in a substantial change in immunoreactivity. Immunoassays should be evaluated for applicability to the target in processed products. Empirical results from testing the method for applicability for target in processed foods should be provided.

Hook Effect

16. In an antibody-based lateral flow device and plate format assay, a hook (saturation) effect could lead to a false negative result. A thorough demonstration that the working concentration range comfortably covers the practical need of target analytical samples is necessary. Therefore, empirical results from testing for a hook effect in target matrices should be provided.

Confirmatory method

17. For immunoassays**,** antibodies may cross-react with other proteins present in the matrix; thus, it is necessary to demonstrate the selectivity of assays. Another method may be used as a confirmatory method. Empirical results from testing both methods with aliquots of the same analytical samples of known concentration may be provided.

INFORMATION ABOUT THE METHOD PERFORMANCE.

Selectivity testing

18. The method has to be clear on the use of appropriate negative controls, such as animal and plant-derived material, different strains or target DNA sequence which should be used with this purpose, if those have been defined.

19. Empirical results from testing the method with DNA from non-target species/varieties and DNA from the reference species/variety material should be provided. This testing should include closely related materials and cases where the limits of the sensitivity are truly tested. In addition it might be appropriate, particularly for taxon-specific DNA sequence, to test other sources of similar foods to reduce the potential for obtaining a false positive.

20. Similarly, for protein methods, empirical results from testing the method with proteins from non-target and closely relevant species/varieties/traits, and purified target protein and/or reference positive control materials should be provided.

Stability testing

21. Empirical results from testing the methods (to detect both reference and target DNA sequences, or proteins) with different species, subspecies, varieties, cultivars, animal lines, or microbial strains as appropriate, may be provided in order to demonstrate, for instance, the stability of the copy number and sequence conservation of the taxon-specific gene DNA, or the stability of expression of the protein.

22. For protein methods, empirical results from testing the methods with target material and its derived/processed products, as appropriate, should be provided to demonstrate the stability of the immunoreactive form of the protein.

Sensitivity testing

23. Empirical results from testing the method at different concentrations in order to test the sensitivity of the method should be provided. Limits of detection (LOD) may be defined using samples comprising of single ingredients only. For food products made up of multiple ingredients, the actual sensitivity will be reduced, as total extracted DNA will be derived from more than one ingredient so that the starting amount of the actual measurand will be decreased.

24. LOD should be determined for each method and matrix, if necessary.

Robustness testing

25. Empirical results from testing the method against small but deliberate variations in method parameters should be provided.

Extraction efficiency

26. Empirical results from testing the method for its extraction efficiency in each matrix should be provided to demonstrate the extraction is sufficient and reproducible. For quantitative detection, the method of calibration for incomplete extraction may need to be provided.

PRACTICAL APPLICATION OF THE METHOD

Applicability

27. Indication of the matrix (e.g., processed food, raw materials, etc.), the type of samples and the range to which the method can be applied should be given. Relevant limitations of the method should also be addressed (e.g. interference by other analytes or inapplicability to certain situations). Limitations may also include, as far as possible, possible restrictions due to the costs, equipment or specific and non-specific risks implied for either the operator and/or the environment.

Operational characteristics and practicability of the method

28. The required equipment for the application of the method should be clearly stated, with regards to the analysis *per se* and the sample preparation. Information on costs, practical difficulties, and on any other factor that could be of importance for the operators should be also provided.

Experimental design

29. The experimental design, including the details about the number of runs, samples, replicates, dilutions etc. should be stated.

Operator skills requirements

30. A description of the practical skills necessary to properly apply the proposed method should be provided.

ANALYTICAL CONTROLS

31. The proper use of controls when applying the method should be indicated, when available. Controls should be clearly specified and their interpretation recorded. These may include positive and negative controls, their detailed contents, the extent into which they should be used and the interpretation of the obtained values.

32. The following should be stated:

- Types of analytical controls used:

  i. Positive and negative controls

  ii. Internal control used if applicable (competitive or non competitive).

  iii. Other types of controls like matrix control (to confirm sample was added to PCR) or extraction processing.

- Control samples.
- Reference materials used.

METHOD PERFORMANCE

33. Data on the criteria referred to in Section 2.2, “General Method Criteria” should be provided, as well as a general assessment that the method is fit for its intended purpose.

ANNEX II: VALIDATION OF A QUANTITATIVE PCR METHOD

INTRODUCTION

1. DNA-based analysis is commonly performed using PCR. This technique amplifies a specific segment of DNA to the extent that its quantity can be measured instrumentally (e.g. using fluorometric means). Food processing operations (e.g. due to heat, enzymes and mechanical shearing), can result in degradation or reduction in the total amount of DNA. Methods should preferably be designed to amplify relatively short target- or taxon-specific DNA sequences.

2. Quantitative determinations are often expressed in terms of percent of a target-specific DNA sequence relative to a taxon-specific DNA sequence. In such a relative quantitative test, this measurement actually involves two PCR-based determinations – that of the target-specific DNA sequence and that of the endogenous, or taxon-specific sequence. Each of these determinations has its own uncertainties, and the two are likely to have different measurement characteristics. In most applications, the target DNA sequence will be present at low concentrations, and the taxon-specific DNA sequence will be present at concentrations 10 to 1000 times higher. It is thus important that both measurements are properly validated. In cases where the measurement is expressed directly as a percentage, these factors should be considered when validating the method. The results can be reported in other measure units such as copy numbers.

3. The consequence is that the analysis of DNA, especially in processed foods, aims at detecting a very small amount of target-specific DNA, often in the nanogram/gram range or lower. The result of a quantitative PCR analysis is often expressed in % as the relative amount of target DNA relative to the total amount of DNA of the comparator taxon/species DNA in a specific food matrix. The food matrix may also contain significant amounts of DNA from many other species/taxons.

4. Validation of methods consists of two phases. The first is an in-house validation of all of the parameters above except reproducibility. The second is a collaborative trial, the main outcome of which is a measure of the repeatability and reproducibility together with detailed information on the transferability of methods between laboratories. It is strongly recommended that a small-scale collaborative trial be performed to test the general robustness of a particular method before the expense of organizing a large-scale trial is incurred. In case any improvement of the method or the method description is needed, only limited expenses are incurred through the pre-trial, while a failure of a full interlaboratory method validation due to ambiguous method description is a very costly failure. Additionally, it may be pointed out that the implementation of an already validated method in a laboratory needs to include necessary experiments to confirm that the implemented method performs as well under local conditions as it did in the interlaboratory method validation. It is important to note that a method should be validated using the conditions under which it will be performed.

VALIDATION

5. A quantitative PCR assay should be validated for the intended use or application. The ISO 5725:1996 or AOAC/IUPAC Harmonized Protocol were developed for chemical analytical methods. These define the procedures necessary to validate a method. It is important to emphasize that all the principles and rules of the harmonized protocol are applicable to quantitative PCR methods.

6. A number of the parameters involved in validation of the performance of a quantitative PCR assay will be discussed in detail. These are scope, LOD and LOQ, trueness, precision, sensitivity and robustness. Other important factors are acceptance criteria and interpretation of results, and the issue of the units in which results are expressed.

7. There is a general scientific discussion about the interpretation of the percentage values. It is recognised that so far there is no reliable weight to copy number relationship because of uncertainty in the correlation of weight of ingredient to number of molecules of DNA. Both the weight to weight ratio and copy number to copy number ratio calculations are acceptable provided this is clearly stated when reporting results.

8. All parameters listed below, including selectivity and sensitivity, have to be assessed individually for each of the assays involved, including both reference and target specific PCR assays**.** These are given alphabetically, not necessarily in order of importance.

Applicability

9. The analytes, matrices and concentrations for which a method of analysis may be used should be stated.

10. It is required from an extraction method, independent of matrix to which it is to be applied, that it yields DNA of sufficient quantity, structural integrity and purity to allow a proper evaluation of the performance of the subsequent method steps (e.g. adequate amplification of DNA during the PCR step) to be undertaken.

11. In real-time PCR analysis, Ct-values can be used to estimate the efficiency of PCR. The efficiency can be tested, for example, by setting up a dilution series of the template DNA and determining the Ct-value (The threshold number of cycles at which the measured fluorescence signal crosses a user-defined threshold value) for each dilution. In the ideal situation, when amplification efficiency is 100%, a two-fold reduction in quantity of template DNA added to the PCR will result in an increase in the Ct value of one. Therefore, if DNA is diluted 10X, the theoretical difference in Ct values between the diluted and undiluted DNA should be approx 3.32. Theoretical numbers may not be achieved in real situations. Significant deviations from this relationship may indicate that the extracted DNA contains PCR inhibitors, that the DNA solution is not homogenous or the DNA quantity so low that stochastic variation in the amount of DNA in the reactions yield unreliable quantitative estimates. This is also the case for end-point PCR reactions carried out using fluorescent probes.

Dynamic Range - Range Of Quantification

12. The scope of the methods defines the concentration range over which the analyte will be reliably determined. The relative amount of taxon-specific DNA to total DNA in the DNA extract will vary depending on whether the DNA was extracted from a single ingredient or a complex food matrix. This desired concentration range defines the standard curves and a sufficient number of standards should be used, when applicable e.g. with calibration curves, to adequately define the relationship between concentration and response. The relationship between response and concentration should be demonstrated to be continuous, reproducible and should be linear after suitable transformation.

13. The range of a quantitative target-specific method can be designed to be from near zero to 100 percent relative to the taxon-specific DNA (w/w). However, it is common to validate a method for a range of concentrations that is relevant to the scope of the application. If a method is validated for a given range of values, the range may not be extended without further validation. For certain applications (e.g. food or grain analysis) the use of genomic DNA for the preparation of the standard curve (see discussion on the use of plasmid DNA below) may be considered. While it is easy to establish a nominal 100% standard it is difficult to reliably produce standard solutions below 0.1%. Additionally, the number of target sites (DNA sequence to be amplified) becomes so small that stochastic errors will begin to dominate and less reliable analysis is possible.

14. The DNA used as calibrator should be traced back (in its metrological meaning) to a reference of highest metrological order, e.g. a certified reference material. The range will be established by confirming that the PCR procedure provides an acceptable degree of linearity and trueness when applied to samples containing amounts of analyte within or at the extremes of the specified range of the procedure.

15. The unique characteristics of quantitative PCR impose particular restrictions on the low end of the dynamic range of a quantitative PCR. This is due to the difficulty in determining LOD and LOQ values due to the non-normal distribution of values in this range.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

16. If the validation of the quantitative PCR assay shows that the assay can measure DNA at (for example) 0.1% with acceptable trueness and precision, then it is often not necessary to determine the LOD and LOQ, as the method is only being applied above the range where these are relevant. However, if the method is being used at concentrations close to the LOD and LOQ (typically 0.01-0.05%), then the assessment of the LOD and LOQ will become part of the validation procedure.

17. In quantitative PCR, the distribution of measurement values for blanks is not Gaussian and typically follows a Poisson distribution. If the LOD is required, it should be experimentally determined. For quantitative methods the LOD is the amount of analyte at which the analytical method detects the presence of the analyte at least 95% of the time (≤5% false negative results)

18. For a quantitative method, it is important to know whether the LOQ for a particular matrix is close to the values to be measured. The LOQ needs to be experimentally determined, since the distribution measurement for quantitative PCR is not normally distributed.

19. In practice, two procedures have been employed to determine the LOQ. The first approach is to assay a number of conventional samples that have been supplemented (spiked) with known amounts of analyte. The LOQ is then the level at which the variability of the result meets certain preset criteria (such as +/- 2 SD from the lowest calibration data point, etc.). DNA extraction, however, may be difficult from some matrices, e.g. starches or ketchup, and lower extraction efficiencies may have to be accepted. When extraction efficiencies are low, this should be stated in the validation data and in the analytical report. A more complete approach is to test the method using a number of samples that contain known amounts of analyte. This is more complicated as it requires access to significant quantities of reference materials that contain a known range of concentrations of the DNA sequences of interest.

Practicability

20. The practicability of the method should be assessed by considering parameters such as: the quantity of samples that can be processed within a given time, estimated fixed costs to implement the method and the approximate cost per sample, practical difficulties on daily use or under particular conditions, as well as other factors that could be of importance for the operators.

Repeatability standard deviation (RSD_r)

21. The relative repeatability standard deviation for the PCR step should be ≤25% over the whole dynamic range of the method.

Reproducibility standard deviation (RSD_R)

22. The relative reproducibility standard deviation for the PCR step should be below 35% over the majority of the dynamic range, except at the limit of quantification, where the RSD_R could be higher.

Robustness

23. Robustness is a measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Examples of such variations include: reaction volumes (e.g., 29 vs. 30µl), annealing temperature (e.g., +/- 1°C) and/or other relevant variations. The experiments need to be performed at least in triplicate. The response of an assay with respect to these small changes should not deviate more than ±35% in reproducibility experiments from the response obtained under the original conditions.

24. The adequacy of the robustness testing needs to be demonstrated on a method-by-method basis. For instance, for a real-time PCR method, the following factors and their origin / source should ideally be taken into account: different thermal cycler models, DNA polymerase, uracyl-n-glycosylase, magnesium chloride concentration, primer forward and reverse concentration, probe concentration, temperature profile, time profile, dNTP (including dUTP, if applicable) concentrations.

Sensitivity

25. For a quantitative PCR method, a linear relationship of the Ct as a function of the logarithm of the template concentration should be obtained across the range of the method. The correlation coefficient, y-intercept and slope of the regression line should be reported. The % of residual for each of the calibrators should preferably be ≤30%.

26. Besides reporting the curve parameters, it is suggested to define which range of slope values is acceptable in order to conduct the quantification as it is also important to calculate the reaction efficiency. (Eg. -2.9 to -3.3 for DNA detection or the corresponding optimal values which indicate amplification efficiency close to 100%).

27. In cases where the ΔCt-method is employed by a laboratory instead of a calibration based quantitative method, it will be the responsibility of the analyst to ensure that the overall amount of DNA is well within the range for which the assay was validated.

Selectivity

28. The selectivity of the method should be demonstrated by providing experimental evidence. This demonstration should include analysis of samples containing a mixture of target DNA and non-target DNA where the limits of the detection (if appropriate to the dynamic range) are truly tested. As the method should be selective for the target DNA, it should only give a positive result with a food matrix containing the target DNA.

29. Primers and probes should have been checked against pertinent sequence databases for possible homologies with other sequences potentially present in the expected matrices, according to the intended use After such an assessment, selectivity should then be demonstrated experimentally.

30. For assays selective for the target DNA. Experimental evidence of selectivity for the target DNA should include:

- Assays of at least ten samples from different lots or batches of foods or ingredients lacking target DNA sequences, although the samples should contain taxon-specific DNA. All of these assays should have a negative result. For example, if the target DNA corresponds to a specific recombinant-DNA plant transformation event, samples could be derived from other (non-target) transformation events, as well as non-recombinant-DNA plants belonging to the same plant species.
- An appropriate number of DNA samples from each source should be tested.
- Two replicates should be analyzed for each DNA sample, which shall give results within a Ct-value of 0.5.

31. Test results should clearly indicate that no significant instrument reading or chemistry effects are observed.

32. For assays on taxon-specific DNA sequences. Experimental evidence of taxon selectivity should include:

- Assays of at least ten samples from different lots or batches of foods or ingredients derived from organisms belonging to the taxon of interest, but classified in different sub-taxon categories. All of these assays should have a positive result. For instance, if the taxon specificity supposedly corresponds to a plant species such as maize, the samples could correspond to maize varieties with different genetic origins.
- Assays of at least ten samples from different lots or batches of similar foods or ingredients derived from organisms not belonging to the taxon of interest, which may be present in the relevant food matrixes. All of these assays should have a negative result. For instance (and continuing with the earlier example) if the first ten assays were applied to different maize flours, in the second group of assays it could be appropriate to assay wheat/soy/rice flour.
- An appropriate number of DNA samples from each source should be tested.
- Two replicates should be analyzed for each DNA sample, which shall give results within a Ct-value of 0.5.

33. Test results shall clearly indicate that no significant instrument reading or chemistry effects are observed.

Trueness

34. As for any method, the trueness of a method should be determined by comparing results obtained from analysis of a reference material with the known or assigned value for that reference material. The impact of sample matrix effects, particularly when the sample matrix differs from that of the reference material, should be considered.

35. A trueness value of ± 25%, in regards to the PCR step, should be acceptable over the whole dynamic range.

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ANNEX III: VALIDATION OF A QUALITATIVE PCR METHOD

Introduction

1. A qualitative PCR should be validated as much as possible in the same way as it is intended to be used for routine analyses – that means the sensitivity of the method should be shown to be such that it can reliably detect a positive sample, and does not give rise to a significant number of false positives.

2. By their very nature, qualitative test results refer to the identification above/below a detection limit. Like the limit of detection for quantitative methods, the limit of detection for a qualitative method can be defined as the concentration at which a positive sample yields a positive result at least 95% of the time. This results in a rate of false negative results of 5% or less. This is also expressed as a ratio or percentage.

False Positive Rate

3. This is the probability that a known negative test sample has been classified as positive by the method. For convenience this rate can be expressed as percentage:

$$\% \text{ false positive results} = 100 \times \frac{\text{number of misclassified known negative samples}}{\text{total number of known negative samples}}$$

False Negative Rate

4. This is the probability that a known positive test sample has been classified as negative by the method. For convenience this rate can be expressed as percentage:

$$\% \text{ false negative results} = 100 \times \frac{\text{number of misclassified known positive samples}}{\text{total number of known positive samples}}$$

Note: since there are different definitions in use for the false positive and false negative rates, the validation report should clarify which one has been used.

5. In order to demonstrate the false negative rate for qualitative assay, a series of samples with a constant, known concentration of positive material in a pool of negative material have to be analysed and the results evaluated. It is important to note that the concept of confidence intervals and statistical uncertainty needs to be applied to the risk of false positive and/or false negative results as well. The desired level of confidence determines the size and number of pools that need to be tested.

Robustness

6. As with any validated method, reasonable efforts should be made to demonstrate the robustness of the assay. This involves careful optimisation and investigation of the impact of small modifications made to the method due to technical reasons, as described in the annex for quantitative PCR.

ANNEX IV: VALIDATION OF A PROTEIN-BASED METHOD

QUANTITATIVE TESTING

1. The following description of the procedure is only one of several possibilities to carry out an immunological detection assay for proteins of interest.

2. For example, in typical ELISA for proteins, the amount of the reporter substance from an enzymatic reaction is measured. The standard curve is generated by plotting the optical density (OD) on the y-axis against the concentration of the standards on the x-axis, obtaining a dose response curve using quadratic equation or other required curve fit model from the method. To obtain an accurate quantitative value, the OD for the sample solutions must pertain to the linear portion of the calibration curve. If the OD is too high, the sample solution must be diluted until the OD falls within the quantification range of the assay. The concentration of the protein analyte in the original sample is calculated by correcting for any dilution factor that was introduced in preparing the sample for application to the micro plate. The initial weight of the sample and the volume of extraction liquid, as well as any subsequent dilutions are used to calculate the dilution factor.

3. Various assay controls can be employed to demonstrate the performance of the assay. A blank sample such as an empty well or buffered solution can be run in parallel to determine any background response which shall be subtracted from sample and calibration responses if desired. A negative control sample (i.e. matrix extract solution known to contain no analyte) shall be used to demonstrate any non-specific response or matrix interference effects occurring in the assay. A positive control or matrix extract spiked with a known amount of the analyte can be run to demonstrate the accuracy of the test. Standards and samples can be run in an appropriate number of replicates to appreciate the precision of the test. Blanks, negative controls, positive controls, reference materials, and replicates can be run on each microplate to control for plate-plate variation.

REFERENCE MATERIALS

4. When applicable, the reference material consists of the same matrix as the target analytical sample to be tested. It typically includes negative control and positive reference materials. For example, if the matrix to be tested is soybean flour the standardized positive reference material would be soybean flour containing a known proportion of protein of interest. Alternatively, a pure sample or extract of the protein of interest may be used, providing the use of such protein reference materials has been validated against the matrix in question. In some cases the reference matrix, may be unavailable. Access to reference materials is important during the development, validation, and use of immunoassays for analysis of proteins in food matrix. The best available reference material should be used in order to comply with regulations and testing requirements.

5. Where food or food ingredients with and without the analyte are available, it is fairly straightforward to prepare a control sample with a known proportion of the target material. In other cases, generating control samples for certain matrices and analytes can be difficult. Stability and uniformity are important considerations. For example, if the matrix to be tested consists of a mixture of materials, the operator will need to combine materials in such a way as to achieve a homogeneous control sample with a known amount of the protein. The stability of these materials would need to be evaluated under storage and test conditions.

VALIDATION OF A QUANTITATIVE PROTEIN-BASED METHOD

6. The principles of method validation defined in the harmonized ISO/IUPAC/AOAC standard apply to protein methods.

7. Quantitative method validation parameters include accuracy/trueness, selectivity, extraction efficiency, sensitivity, range of quantification, precision, robustness, applicability and practicability.

8. Accuracy is demonstrated by measuring the recovery of analyte from spiked samples and is reported as the mean recovery at several levels across the quantitative range.

9. The recovery of proteins of interest should be determined by comparing results obtained from analysis of a reference material with the known or assigned value for that reference material. The impact of sample matrix effects, particularly when the sample matrix differs from that of the reference material, should be considered. The recovery should be between 70 and 120%.

10. Extraction efficiency is a measure of how efficient a given extraction method is at separating the protein analyte from the matrix. It is expressed as percent analyte recovered from the sample. It can be difficult to truly demonstrate efficiency of the extraction procedure. There may not be an alternate detection method against which to compare the immunoassay results. One approach to addressing extraction efficiency is to demonstrate the recovery of the target protein analyte from each type of food fraction by exhaustive extraction, i.e. repeatedly extracting the sample until no more of the protein is detected.

11. The intra-assay precision describes how much variation occurs within an assay. It can be evaluated by determining the variation between replicates (% Coefficient of Variation) assayed at various concentrations on the standard curve and on the pooled variation (RSD_r) derived from absorbance values in standards from independent assays performed on different days. Inter-assay precision describes how much variation occurs between separate assays and can be measured by analysis of quality control samples on every microplate. The quality control samples required would consist of two pools of extracts, one extract from target analyte-containing samples and one from the control samples. If the protein is stable in extract, it can be stored frozen and a portion would be thawed and assayed on every microplate. Inter-assay precision can be evaluated over time and expressed as % Coefficient of Variation.

12. The relative repeatability standard deviation (RSD_r) should be ≤25% over the whole dynamic range of the method.

13. The relative reproducibility standard deviation (RSD_R) should be below 35% at the target concentration and over the majority of the dynamic range, excepting at the limit of quantification, where it could be greater.

14. Dilution agreement or linearity is used to evaluate that the assay is capable of giving equivalent results regardless of where in the quantitative range of the standard curve the sample OD interpolates. To conduct these experiments, samples that are positive for the target protein are ideally diluted such that at least three of the dilutions result in values that span the quantitative range of the curve. The Coefficient of Variation of the adjusted results from several dilutions of a single sample extract should ideally be ≤ 20%.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

15. It is worth noting that if the LOD or LOQ is established to be much lower than the range in which the method is intended to be used, a precise determination is not necessary. This would be the case, for example, when the LOD is in the range of 1 ng/kg, while the range of the method validation extends only for concentrations ranging in µg/kg.

16. It is common practice when estimating the LOD to assume that it is the signal strength of a blank increased by three times the standard deviation of the blank. This method gives at best an estimate, and relies on normal Gaussian distribution of the blank measurements around zero. This can generally be assumed for methods such as ELISA, but the LOD is best determined experimentally. Alternatively the LOD is commonly defined as a concentration equal to the lowest standard used in the assay, should a positive value be consistently obtained with that standard.

17. For a quantitative method, it is important to know whether the LOQ for a particular matrix is close to the values to be measured.

Cross-reactivity

18. The cross-reactivity is the degree to which analogs or other molecules can bind to the detection antibodies and therefore should be characterized and described in the method. The absence of cross-reactivity should be assessed using experimental results from testing the method with proteins or molecules from non-target and closely related taxa, purified target protein or reference positive control materials. The potential for interferences from reagents and labware can be evaluated by assaying extracts from analyte-free material.

Matrix effects

19. If the response of the method is affected by a substance in the final extract other than the specific protein analyte, the non-specific response is referred to as a matrix effect. One way to manage matrix effects is to demonstrate that the analytical method gives similar results with or without sample matrix present in the extract. In this approach, freedom from matrix effects would have to be demonstrated in all matrices for

which the assay is to be used. Another approach (although less desirable) to managing matrix effects would be to prepare the standard solutions in extracts from analyte-free matrix. This would ensure that any matrix effects are consistent between the standards and the samples.

Robustness

20. Robustness is a measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Examples of such variations include: reaction volumes, incubation temperature (e.g. +/- 1°C for oven incubations and +/- 4°C for incubations at "room temperature") and/or other relevant variations. The experiments need to be performed at least in triplicate and the recovery needs to be calculated. The response of an assay with respect to these small changes should not deviate more than ±30% from the response obtained under the original conditions.

QUALITATIVE TESTING

21. Lateral flow devices are useful tools for on-site or field testing, although other immuno-sorbent assays such as traditional ELISA methods can also be used for qualitative testing. In order to ensure reliable results, assays should be validated and a description of the performance characteristics should include sensitivity, selectivity, applicability, limit of detection, robustness, matrix effects, and, if applicable, hook-effect.

VALIDATION OF A QUALITATIVE PROTEIN-BASED METHOD

22. The same principles apply to qualitative protein-based testing as to qualitative PCR testing. These approaches, including calculation of false positive and false negative rates, can therefore be applied to protein-based methods. In general, due to the reliable nature of protein-based lateral flow strip methods, they are not performed in duplicate on each sample. However, in ELISA testing (due to its quantitative nature), duplicate wells are typically used.

Applicability

23. The analytes, matrices and concentrations for which a method of analysis may be used should be stated.

24. Protein extraction can be a key factor in the performance of a protein method, and the buffers used can also affect the performance of the detection step. Thus careful optimization is required to ensure that protein detection methods are reliable. The criteria for determination of the LOD should be established for the method. For confirming the LOD of qualitative assays, fortification levels near to the LOD may be used, as long as one of the levels used meets the criteria of being above but close to the LOD. While such procedures can give an indication of the performance of the method, incurred samples with well known characteristics (if available) are the best matrix on which to establish the applicability of a method.

Practicability

25. The practicability of the method should be assessed by considering parameters such as: the quantity of samples that can be processed within a given time, estimated fixed costs to implement the method and the approximate cost per sample, practical difficulties on daily use or under particular conditions, as well as other factors that could be of importance for the operators.

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ANNEX V ANALYTICAL CONTROL ACCEPTANCE CRITERIA AND INTERPRETATION OF RESULTS FOR QUANTITATIVE PCR METHODS

1. At a minimum, the following acceptance criteria are common to all quantitative PCR methods and applicable to each PCR run:

- The mean of the replicates of the positive DNA target control at a relevant concentration deviates less than 3 standard deviations from the assigned value. When applicable, a target DNA control is defined as reference DNA or DNA extracted from a certified reference material or known to be a positive sample representative of the sequence or organism under study. The control is intended to demonstrate what the result of analyses of test samples containing the target sequence should be.
- The amplification reagent control shall not result in an amplification signal above the background noise. The amplification reagent control is defined as control containing all the reagents, except extracted test sample template DNA. Instead of the template DNA, a corresponding volume of nucleic acid free reagent (such as water or buffer) is added to the reaction.

2. To accept the result of an unknown sample, the relative standard deviation of the sample replicates should be ≤35 %.

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APPENDIX IV

PROPOSED DRAFT REVISED GUIDELINES ON MEASUREMENT UNCERTAINTY

EXPLANATORY NOTES TO THE CODEX GUIDELINES ON MEASUREMENT UNCERTAINTY

(To be included as an Annex to the Guidelines on Measurement Uncertainty (CAC/GL 54-2004)

(At Step 5 of the Procedure)

1 What is Measurement Uncertainty?

It is not always appreciated that analytical results are variable, and just how large that variability may be, particularly when low concentrations of a measurand (i.e. ppb levels) are being determined. As stated in the Guidelines, "most quantitative analytical results take the form of "a ± 2u" or "a ± U" where "a" is the best estimate of the true value of the concentration of the measurand (the analytical result) and "u" is the standard uncertainty to 68% level of confidence and "U" (equal to 2u) is the expanded uncertainty to 95% level of confidence.. The range "a ± 2u" represents a 95% level of confidence in which the true value would be found. The value of "U" or "2u" is the value which is normally used and reported by analysts, normally referred to as "measurement uncertainty" and may be estimated in a number of different ways. "

In food analysis it is the (approximately) 95% probability (i.e. 2u) which is used to calculate the expanded uncertainty. Other sectors may specify a different probability.

Thus measurement uncertainty can be regarded as the variability around the reported results which is quantified as the value "U" when considering the expanded uncertainty and within which the "true" result may be expected to lie.

2 Does the Measurement Uncertainty have to be Estimated in Codex?

Yes, one of the requirements of the ISO/IEC 17025:2005 Standard that Codex has adopted by reference is that the measurement uncertainty of a result must be estimated and then made available if requested or when the uncertainty affects compliance to a specification limit, for example a Codex Standard (the Codex Alimentarius Commission has developed Guidelines, CAC/GL 27-1997) which require laboratories involved in the import/export of foods to comply with general criteria in ISO/IEC 17025). As Codex is concerned with goods moving in international trade it would be anticipated that the request will be made.

3 Does Measurement Uncertainty Arise From both Sampling and Analysis?

Measurement uncertainty applies to the whole measurement process. However, this guidance only considers analytical measurement uncertainty.

4 What is the Relationship between Measurement Uncertainty, the Analytical Result and the Method Used to Obtain the Result?

It is the uncertainty of test results which is one of the factors when judging compliance with standards. Measurement uncertainty is not associated with a method, but the values that are obtained in the validation and/or in quality control of a method may be used to estimate the uncertainty of a result in some situations. The differentiation between measurement uncertainty associated with the result and precision obtained during the validation of the method is frequently not appreciated. As a consequence precision demonstrated for a validated method (the repeatability or reproducibility standard deviation) cannot be used as the sole estimate of the measurement uncertainty without qualification. In particular additional factors such as uncertainty associated with bias, matrix effect, and competence of laboratory must be considered.

5 Procedures for Estimating Measurement Uncertainty

There are many procedures available for estimating the measurement uncertainty of a result. The Codex guidelines do not recommend any particular approach, but it is important that whatever approach is used, the procedure is scientifically credible. No one approach may be said to be better than any other provided the procedure used is appropriate and credible - i.e. there is no "hierarchy" of the recognised procedures. All such procedures may be considered to be equally valid.

In general, procedures are based on a component-by-component ("bottom-up") approach or on a "top-down" approach using data from collaborative trials, proficiency studies, validation studies or intra-laboratory quality control samples, or a combination of such data..

In the *Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Foods* (CAC/GL 27-1997) there is a requirement to use validated methods and so it is usually more cost-efficient to use data from the method validation studies rather than using another approach (i.e. the component-by-component approach).

Users of validation data should note that sources of uncertainty that are not covered by validation studies include:

- Sampling
- Pre-treatment
- Method bias
- Variation in conditions
- Changes in sample matrix

For methods operating within their defined scope, when the reconciliation stage shows that all the identified sources have been included in the validation study or when the contributions from any remaining sources have been shown to be negligible, then the reproducibility standard deviation s_R, adjusted for concentration if necessary, may be used as the combined standard uncertainty."

It is recognised that further procedures for the estimation of measurement uncertainty are being developed, and that, in this evolving situation, further recommendations will be made as to acceptable procedures. It is anticipated that procedures based on results obtained from participation in proficiency testing schemes, as an example, will be developed.

6 Considerations when Estimating Measurement Uncertainty within the Context of Codex

It is important that the requirement to estimate measurement uncertainty does not impose any unnecessary additional workloads on laboratories.

When deciding on which procedure is to be used when estimating measurement uncertainty within the Codex context it is important to recognise that Codex has adopted a number of formal quality assurance measures which have to be implemented by control laboratories. In particular, such laboratories should:

- be in compliance with an Internationally recognised Standard (now with ISO/IEC 17025:2005 Standard); such compliance is aided by the use of internal quality control procedures,
- participate in proficiency schemes, and
- use validated methods.

It is essential that the information provided as a result of these requirements being implemented is used by laboratories when estimating their measurement uncertainties in order to avoid unnecessary work being carried out by laboratories. In Codex, where there is a high emphasis being placed on the use of "validated" methods of analysis, i.e. methods which have been validated through collaborative trials, information obtained from such trials can be used in many situations.

In addition information derived from internal quality control procedures may also be used to estimate uncertainties in some situations.

This section re-emphasises that for the analyst it is important that no unnecessary duplication of existing work is undertaken.

7 Values of Measurement Uncertainty Estimations

Stipulating information on the anticipated values of measurement uncertainty estimations is frequently not supported by analysts. The users of analytical data and the customers of the laboratories producing such data frequently ask for such information regarding the level of uncertainty that may be expected for test results. They have concerns that some laboratories underestimate the size of their uncertainties and so report unrealistically small uncertainties to their customers.

For chemical analyses, using the values of s_R from collaborative trials, it would not be unreasonable to anticipate that the (expanded) uncertainties reported by laboratories would be of the following orders:

Nominal Concentration	Typical Expanded Uncertainty	Expected Range of Results*
100g/100g	4%	96 to 104g/100g
10g/100g	5%	9.5 to 10.5g/100g
1g/100g	8%	0.92 to 1.08g/100g
1g/kg	11%	0.89 to 1.11g/kg
100mg/kg	16%	84 to 116mg/kg
10mg/kg	22%	7.8 to 12.2mg/kg
1mg/kg	32%	0.68 to 1.32mg/kg
< 100µg/kg	44%	0.56 x concentration to 1.44 x concentration µg/kg

* this effectively means that values falling within these ranges may be regarded as being of the same analytical population.

It would be expected that the reported measurement uncertainties by all laboratories would not significantly exceed the value estimated from the s_R at the concentration of interest if the laboratory is in "analytical control". Very experienced laboratories carrying out any particular analysis on a regular basis would be expected to obtain values less that the values given above.

8. Relationship between analytical results, measurement uncertainty and recovery factors

This section attempts to explain the significance of analytical results and their associated measurement uncertainty and recovery.

8.1 Measurement Uncertainty

It is important that measurement uncertainty is considered when deciding whether or not a sample meets the specification. This consideration may not apply when a direct health hazard is concerned. The significance of this can be illustrated by an example shown in the diagram below, which shows the simplest case when decisions are made based on a single test sample.

The example shown here is one where the test result is compared against the specification consisting of a maximum level.

Situation I

The analytical result together with the measurement uncertainty exceeds the maximum level. The result indicates that the measured analyte in the sampled lot is above the specification.

Situation II

The analytical result exceeds the maximum level by less than the measurement uncertainty but with the lower endpoint of the measurement uncertainty less than the maximum level.

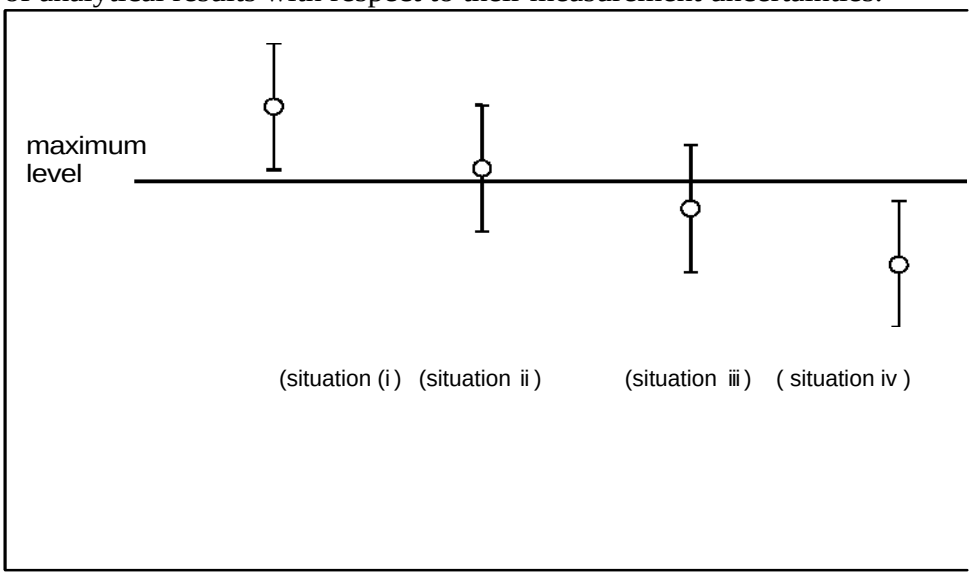
Situation III

The analytical result is less than the maximum level but with the upper endpoint of the measurement uncertainty being greater than the level.

Situation IV

The analytical result bounded by the expanded measurement uncertainty is less than the maximum level.

This diagram demonstrates the importance of defining clear guidelines to allow unambiguous interpretation of analytical results with respect to their measurement uncertainties.



82 Recovery

The Codex Alimentarius Commission has adopted the IUPAC Guidelines on the use of recovery information by reference (see CAC/GL 37-2001).

Analytical results should be expressed on a recovery corrected basis where appropriate and relevant, and when corrected it has to be stated.

If a result has been corrected for recovery, the method by which the recovery was taken into account should also be stated. The recovery rate is to be quoted wherever possible.

When laying down provisions for standards, it will be necessary to state whether the result obtained by a method used for analysis within conformity checks is expressed on an recovery-corrected basis or not.

9 Useful References

These references are not endorsed by Codex unless specified in other Codex guidelines.

Guides for the Estimation of Measurement Uncertainty

Guide 98, Guide to the Expression of Uncertainty in Measurement (GUM) ISO, Geneva (1995).

EURACHEM/CITAC Guide Quantifying Uncertainty In Analytical Measurement (Second Edition), EURACHEM Secretariat, BAM, Berlin, 2000. This is available as a free download from http://www.eurachem.ul.pt/

Analytical Methods Committee of the Royal Society of Chemistry "Uncertainty of Measurement - Implications of its use in *Analytical Science", Analyst, 1995, **120 (9)**, 2303-2308.*

ISO/TS 2 1748:2004 Guidance for the Use of Repeatability, Reproducibility and Trueness estimates in Measurement Uncertainty Estimation, ISO, Geneva (2004).

NIST Technical note 1297 (1994 Edition): "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results"

NMKL Procedure No. 5, 2nd edition (2003): "Estimation and Expression of Measurement Uncertainty in Chemical Analysis"

UKAS (United Kingdom Accreditation Service) 2000 The Expression of Uncertainty in Testing Edition 1, UKAS Publication ref: LAB 12

Eurolab technical Report No. 1/2007. Measurement Uncertainty Revisited: Alternative Approaches to Uncertainty Evaluation. Available as a free download from www.eurolab.org

Nordtest report TR 537. Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories. Available as free downloads from www.nordtest.org (although this handbook is directed towards environmental analyses, the approaches and examples described are applicable to the results from tests on foods and feeds)

Procedures for the Validation of Analytical Methods and Method Performance

"Precision of Test Methods", Geneva, 1994, ISO 5725, Previous editions were issued in 1981 and 1986. (not adopted by Codex).

"Protocol for the Design, Conduct and Interpretation of Method Performance Studies", ed. W. Horwitz, *Pure Appl. Chem.*, 1995, 67, 33 1-343. (adopted by Codex).

European Commission Decision 2002/657/EC implementing directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, Off J Eur Comm, L22 1 (2002) 8-36.

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ISO/IEC 17025:2005, General Requirements for the Competence of Testing and Calibration Laboratories, ISO, Geneva (2005).

EURACHEM Guidance Document No. 1/WELAC Guidance No. WGD 2: "Accreditation for Chemical Laboratories: Guidance on the Interpretation of the EN 45000 series of Standards and ISO/IEC Guide 25"

Z., Ben-David, H., Mates, A. 2001 Proficiency testing as tool for ISO 17025 implementation in National Public Health Laboratory: a mean for improving efficiency. Accreditation & Quality Assurance, **6**: 190-194

NMKL Procedure no. 3 (1996) "Control charts and control samples in the internal quality control in chemical food laboratories"
Örnemark, U., Boley, N., Saeed, K., van Berkel, P.M., Schmidt, R., Noble, M., Mäkinen, I., Keinänen, M., Uldall, A., Steensland, H., Van der Veen, A., Tholen, D. W., Golze, M., Christensen, J.M., De Bièvre, P., De Leer, W. B (ed). 2001

Proficiency testing in analytical chemistry, microbiology, and laboratory medicine – working group discussions on current status, problems, and future directions. Accreditation & Quality Assurance, **6**: 140-146.

Compliance

EURACHEM/CITAC Guide on the Use of uncertainty information in compliance assessment EURACHEM Secretariat, BAM, Berlin, 2007. This is available as a free download from http://www.eurachem.ul.pt/

Terminology

ISO (2nd ed., 1993) VIM "International Vocabulary of Basic and General Terms in Metrology". Geneva.

ISO Guide 99, International Vocabulary of Basic and General Terms in Metrology, 3rd Ed., VIM3, ISO, Geneva (2008).