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GUIDE FOR REFEREES within microbiology

Elaboration of analytical methods within NMKL

NORDIC COMMITTEE ON FOOD ANALYSIS
www.nmkl.org

NMKL

Nordic Committee on Food Analysis

http://www.nmkl.org

Foreword

This guide is issued to all persons who – based on their technical knowledge within a specific subject or field – are requested by NMKL to elaborate a method. Due to the fact that many such referees are persons who have no direct association with NMKL, it is necessary to explain both how NMKL works, and how the relationships between NMKL and the referees work, covering areas like the scope of the job, the time schedule, work processes, distribution of responsibility during the execution of the job, and any statistical treatment of the data produced through studies and comparisons.

The guide is divided into a general part containing a description of NMKL and its work methods and routines, and a specific part, which deals with all the tasks a referee can be presented with, as well a detailed description of each task type and how it should be carried out – both technically and "organisatorically".

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NMKL welcomes all comments, input and suggested changes/improvements offered by the readers of this guide.

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1. Glossary

COLLABORATIVE STUDY	Analysis based on a method draft, which is carried out in a number of laboratories, in order to determine the repeatability, reproducibility and ruggedness/robustness of the method. Collaborative studies are arranged by the referee.
COLLABORATIVE STUDY LABORATORY	A laboratory appointed by the National Committees which participates in the performance study of a method draft.
COLLABORATIVE STUDY PLAN	A plan on which the collaborative study of an approved method draft is based. Includes, among other things, time schedule, number of participating laboratories, matrices, concentration levels, and a description of which statistical model the referee is planning to use. Collaborative study plans are elaborated by the referee.
COLLABORATIVE STUDY REPORT	A report which summarizes and documents the work performed by the referee. The report forms the basis of NMKL´s evaluation of the method, and approval of the method draft. It also contains data and comments from any pre-studies which may have been performed. The collaborative study report is elaborated by the referee.
COMMISSORIUM / MOTIVATION	Task formulation which is issued by the General Annual Meeting, and which includes the referee's description of the scope of an assigned method performance study task. (In order to save time, the commissorium should as far as possible be formulated prior to the General Annual Meeting, so that the annual meeting only has to evaluate the commissorium.) The commissorium should contain all the parameters which the referee has to consider in his/her work. When the commissorium has been drawn up, it is supplemented with a time schedule.
COMPARATIVE STUDY	Analysis based on a method draft which is carried out in the laboratory of the referee, or another laboratory, with the purpose of determining the method's accuracy, sensitivity, specificity and ruggedness/robustness.
CONTACT PERSON	Person (co-worker) who has wide experience in the relevant topic, but who is not necessarily a member of the National Committee. The contact person will assist the referee in the relevant technical work.

CO-REFEREE	The co-referee shall support and guide the referee, especially when it comes to observing the rules in this guide (procedures and time schedules). The co-referee is jointly responsible for making sure that the delivered result complies with the procedures and time frames in this guide. It is not obligatory to appoint a co-referee, but it can be very useful, especially in cases where the referee is inexperienced or not a member of the National Committee. The co-referee and contact person may well be the same person.
EK-LIVS	The Nordic Committee of Senior Officials for Food Issues (appointed by the Nordic Council of Ministers).
EXECUTIVE COMMITTEE	Assembly of all the chairpersons of the National Committees, one of whom is appointed chairperson of NMKL. The Secretary General also participates. The Executive Committee meets once a year approx. six months before the General Annual Meeting. The Executive Committee deals primarily with administrative issues and technical topics which should not be postponed to the next General Annual Meeting.
GENERAL ANNUAL MEETING	The NMKL top decision-making body. Assembly of all the National Committees. A forum for discussion, revision and decisions concerning everything related to method development. All members of the National Committees, and the Secretary General, are delegates to the General Annual Meeting. The Nordic countries take turns in holding the General Annual Meeting over a course of 3-4 days at the end of August each year.
MATRICES	The relevant food or feed, with a given composition which contains the analyte in question. Examples of matrices is pork, fish, vegetables, cereals etc.
METHOD DRAFT	A method description on which NMKL bases its approval of an NMKL method. The method draft is elaborated by the referee.
NATIONAL COMMITTEE	Assembly of national experts within chemistry, microbiology and sensorial topics – primarily from public and private laboratories for the analysis of foods, and from institutions of education and research within the area of food analysis.
NMKL	Nordic Committee on Food Analysis.
NMKL METHOD	An analytical method for the determination of one or more parameters in foods or feeds (chemical, physical/chemical, sensorial or microbiological). NMKL methods have been reviewed/edited/developed and approved by NMKL. NMKL develops both methods that are validated collaboratively and methods which are not. The difference is designated in the

	following manner: *This NMKL method has not been validated in a collaborative study* and *This NMKL method has been validated in a collaborative study*.
PRE-STUDY	Performance study of a method draft in one or more laboratories with the purpose of gathering technical comments. Pre-studies are arranged by the referee.
REFEREE	An expert within a specific topic or area, who has been appointed by NMKL to execute an analysis job within the area of food analysis. The referee has the single responsibility for the referee work.
REVISION	Methods which have been validated in comparative method performance studies, are revised every 10 years. Methods which have not been validated in comparative method performance studies, are revised every 5 years.
SECRETARY GENERAL	An academically educated person, who coordinates the work of NMKL. The Secretary General is appointed by NMKL.
STATUS REPORT	Short report – only a few lines – written by the referee every 6 moths, and submitted to the Secretary General upon his/her request (form). The report describes the progress of the referee's work.
SUB COMMITTEE	Professional/technical division of NMKL members across the National Committees of each country. At the General Annual Meeting, the sub committees are forums for professional/technical discussions and decisions.
TIME SCHEDULE	Overview of the connection between expected professional effort, correspondence between the referee, contact person/co-referee etc., and the time frame. The time schedule can be revised upon request from both the referee and the National Committees.
VALIDATION	A validation includes a *comparative* study, i.e. an analysis of a method draft which is carried out in the laboratory of the referee or another laboratory, with the purpose of determining the method's accuracy, sensitivity, specificity and ruggedness, and a *collaborative* study with the purpose of determining the method's repeatability and reproducibility, as well at its ruggedness.
xNK	Abbreviation designating each of the National Committees: DNK (Denmark), FNK (Finland), INK (Iceland), NNK (Norway), and SNK (Sweden).

2. Description of NMKL, its work methods and routines

2.1 Organisation

Nordic Committee on Food Analysis (NMKL) is an independent Nordic cooperative body, which consists of experts within food analysis from the Nordic countries: Denmark, Finland, Iceland, Norway and Sweden. NMKL was founded in 1947, and is linked to the Nordic Committee of Senior Officials for Food Issues, "EK-Livs". NMKL's primary objective is to elaborate, validate, test and publish methods for the sampling and examination of foods with an aim to harmonise the methods and procedures that are used in public and private Nordic food control laboratories. NMKL also takes into consideration the private food laboratories' and the industry's need for validated and approved analytical methods.

NMKL finances its activities partly through funding from EK-Livs which supports the NMKL secretariat (in the form of part-time positions for the Secretary General and his/her secretary), and partly through the sale of NMKL methods and other publications, primarily reports and procedures, elaborated by NMKL.

Each member country has a National Committee, consisting of a chairperson, a secretary and a number of ordinary members. The individual National Committees are co-optive, and members are recruited from both private and public food laboratories, as well as other food analysis institutions or organisations. The constitution of the National Committees will at all times reflect the technical expertise needed by NMKL to be able complete its tasks. The respective countries' relevant food authority appoints NMKL members upon recommendation from each country's National Committee. Thus, NMKL consists of the members of the 5 National Committees and the general secretariat.

The work in NMKL's National Committees is unpaid. This also applies to referees, co-referees and contact persons. However, it is possible to apply for financial support from EK-Livs for specific projects.

The work of NMKL is lead by a chairperson, who is elected by the General Annual Meeting – from among the members of NMKL - for a period of 4 years. A general secretariat with a Secretary General and a secretary, is set up to coordinate the work of the 5 National Committees. The Secretary General is also elected at the General Annual meeting for a period of 4 years. In principle, this secretariat is rotated among the Nordic countries – right now, it is based in Norway. The Secretary General can be, but does not have to be, a member of a National Committee.

The organisation and work methods of the National Committees are defined internally in the respective countries. Topics are dealt with in meetings which are held in the individual National Committees 3-4 times a year. These meetings are used for reporting status on ongoing work, prioritising topics within the area of the National Committee, and discussing various reports from referees. New topics – tasks which different groups want NMKL to solve – are also discussed. New topics/tasks can be proposed either by a member of the National Committee, or by a person outside NMKL. The proposals must be substantiated and presented in written form. Proposals from persons

outside the NMKL organisation are submitted directly to the Secretary General, whereas suggestions from members of a National Committee, are not discussed until the next meeting in the relevant National Committee. If the National Committee decides to pursue the proposed topic, the proposal is forwarded to the Secretary General. The Secretary General sends all received proposals for new topics to all National Committees for evaluation and consideration. The evaluation from each individual National Committee is presented together at the next General Annual Meeting, which decides whether the proposal should be included on the NMKL agenda as a new topic. Provided the annual meeting has approved the topic, an expert – a referee – must be appointed to handle the topic. Each National Committee is asked to suggest a referee. Based on these suggestions, the Secretary General appoints a referee. The Secretary General is responsible for informing the referee of the exact purpose of the task, in the form of written terms of reference (commissorium), including a proposed time schedule for the implementation of the task.

The work at the General Annual Meeting is organised in 4 sub committees. Sub committee 1 deals with administrative and general political issues, sub committee 2 deals with microbiological topics, sub committee 3 deals with chemical topics, and sub committee 4 deals with sensorial topics. All members of the National Committees are attached to one of these sub committees, depending on their expertise and interest. Each sub committee is headed by a chairperson, who is elected among the sub committee members, and appointed by the General Annual Meeting. The chair person is elected for a period of 4 years.

Minutes are written from the National Committee meetings as well as from the General Annual Meetings. All NMKL members receive the minutes from the General Annual Meeting, in the same manner as the members of a National Committee receives the minutes from their own meetings.

2.2 Referee, co-referee and contact persons

NMKL attaches great importance to supporting and encouraging the referee in his/her work with solving the relevant task for NMKL.

If the referee is not a member of a National Committee, or is inexperienced, the National Committee who recommended the referee, may appoint a person – a *co-referee* – from within their own ranks, so that the referee at any time during the process can obtain NMKL relevant information and other forms of back-up from the co-referee. The other National Committees appoint a person with the adequate competence within the relevant topic – *a contact person*. The co-referee may also be the contact person. The name and address of the co-referee and contact person are submitted by the respective National Committees to the Secretary General, who in turn forwards this information to the referee.

The referee, co-referee and contact persons (co-workers) form the professional team which in close cooperation is responsible for the elaboration of the method as described in detail below. As responsible for the elaboration of the method, the referee's name and place of work will be stated in the final method text.

It is important that the referee includes the contact persons in the method work at an early stage.

This can be done through informal inquiries, in which the referee asks the contact persons to share their experience with the proposed method. If the referee is to revise an existing method, it is

recommended that he/she address the contact persons directly before the elaboration of a revised method draft, in order to get input for the revision. The contact persons do not necessarily have to await an initiative from the referee, but are free to offer his/her views for mutual benefit, shortly after being appointed.

The contact persons should offer his/her comments on the method text, and the referee must incorporate the comments in the draft. Subsequently, the draft is sent to the co-referee for evaluation of whether it is ready to be submitted to the local National Committee for approval. After the local National Committee has approved the draft, it is sent via the Secretary General to the other National Committees for approval.

Every six months (in December and May), the referee writes a status report and sends it to the Secretary General. If the referee does not submit a status report, it is the responsibility of the referee's National Committee to follow up on this.

The work of both the referee and the co-referee, is unpaid. However, it is possible to apply for financial support from EK-Livs for specific project work. If it is necessary for the referee, co-referee and contact persons to meet, NMKL may finance such a meeting. However, this must be agreed in advance with NMKL, by addressing the NMKL Secretary General. NMKL also invites a referee to present his/her work at the General Annual Meeting each year.

2.3 International cooperation

There is extensive cooperation and exchange of experience within the scientific world through international organisations as for instance AOAC INTERNATIONAL, International Organization for Standardization, ISO, the European standardisation organisation CEN, and the International Dairy Federation, IDF.

NMKL has a cooperation agreement with AOAC INTERNATIONAL and IDF concerning, among other things, information exchange and approval of each other's analytical methods. For NMKL´s methods to be internationally accepted, for instance by CEN (the European standardisation organisation), Codex Alimentarius or international sister organisations, it is important that the results of the method performance studies are published in English, and if possible in international periodicals, e.g. Journal of AOAC INTERNATIONAL. NMKL also has a cooperation agreement with NordVal.

3. Elaboration of analytical methods

3.1 Background

The food legislation in the Nordic countries and the EU may give rise to a need for the capability to analyse more parameters in more matrices than previously. For instance, the debate on pollution and various food additives may well trigger a need for new analytical methods. Furthermore, the knowledge of outbreaks of illness caused by the presence of pathogenic bacteria in foods, may also

bring about a need for new analytical methods.

These are circumstances which make it necessary for NMKL to implement method studies with the purpose of elaborating validated analytical methods, which can be used in food control throughout the Nordic countries and possibly internationally.

To this date – end of 2003 - NMKL has published approx. 170 analytical methods covering a variety of parameters and a number of different food groups and feeding stuffs, by means of traditional techniques and modern apparatus techniques, as well as biotechnology. It is especially the development of new techniques which makes it necessary to compare older, approved methods with the new options, in order to assess whether new techniques can replace or supplement approved analytical techniques.

For the most part, the analytical methods are used in connection with public food control within the Nordic countries, but they are also to a wide extent used by private laboratories. The methods are available for sale from the general secretariat. All methods are available in Swedish/Norwegian/Danish and English. Methods published after 1985, are also available in Finnish.

NMKL methods which have been validated in collaborative studies, are reviewed every 10 years – calculated from the date of approval – by NMKL with the purpose of assessing whether the method should remain unchanged, be revised (technically or layout-wise) or withdrawn. NMKL methods that have not been collaboratively studied, are reviewed every 5 years.

3.2 Purpose

The purpose of the task is to elaborate/develop/validate an analytical method, which uses modern analytical techniques, manual or fully or semi automatic, and which fulfils NMKL´s guidelines for the validation and collaborative study of analytical methods. The analytical method shall – when the elaboration, study and description has been completed – be included in the NMKL method collection. In addition, it is also an aim that the method gains international recognition in the relevant organisations.

3.3 Implementation

Before the work starts, the referee will receive the written commissorium from the Secretary General along with the corresponding time schedule and this guide. The nature of the referee's work depends on whether it concerns a method which is not collaboratively studied, and which will be published as such, or whether it is a method which is collaboratively studied and subsequently will be published with the label "This NMKL method has been validated in a collaborative study".

3.3.1 Elaborating a method which has not been collaboratively studied.

As mentioned under 2.2, the referee should as early as possible – as soon as the co-referee and contact persons have been named – approach these to gain from their experience and receive suggestions on choice of method, so that the technical team is as active as possible during the development of the method with the referee as the responsible coordinator.

Overview of literature and the referee's own examinations
The referee, which typically has extensive knowledge of the techniques that are used for the detection of the relevant microorganism or -isms, draws up an overview of relevant literature, including a description of possible suggested solutions. The overview also comprises any personal experiences the referee might have based on his/her own studies. These vary a lot.

Method draft
Furthermore, the referee prepares a detailed text for a method draft arranged according to the model given in NMKL report no. 19, 1998, *"Harmonisation of microbiological methods"* (this report is available in Danish and in Finnish). The method draft must include detailed descriptions so as to avoid the risk of misinterpretation. This applies especially to details concerning the composition of substrates, fermentation patterns etc. It is a good idea to let a colleague read through the text with a critical eye.
The method draft and a short report of the literature studies, as well as any personal examinations which may have been performed, are sent to the contact persons for comments. If the comments are not incorporated in the text, the reason for this must be given to the persons involved.

Submitting the report and method draft to local National Committee for approval
Provided the National Committee can approve the work, it will forward the report and draft to the Secretary General for further distribution to the other National Committees for approval or comments, with a suitable deadline.
The reply from the National Committee is forwarded to the referee via the Secretary General. If there are any comments on the method draft, the referee incorporates these in cooperation with the rest of the team. If any comments have not been incorporated, the reason for this must be stated.

NMKL's final approval of report and method draft
The method draft is now completed, and the referee sends it to the Secretary General for approval at the next General Annual Meeting or the next meeting in the Executive Committee. Due to the fact that this approval may, in the worst case, delay the approval with nearly half a year, the method draft can, depending on the circumstances, be pre-approved by the NMKL Executive Committee before subsequent formal approval at a General Annual Meeting.

An NMKL approved method draft will, as mentioned earlier, be published as a method with the following comment: *"This NMKL method has not been validated in a collaborative study"*. If there is a need for a collaborative study of the method, it is possible to apply for funding for this through EK-LIVS or NMKL.

3.3.2 Elaborating a collaboratively studied method

Number of participating laboratories
The number of laboratories participating in the study should generally be more than 8. There will always be some laboratories which fail to complete the study successfully, or something else goes wrong. There should always be valid data from at least 8 laboratories, to be included in the statistical calculations. However, 5 laboratories can be accepted if the method study involves highly specialised equipment or technical competence. The study should, however, not include more than

15 laboratories, as the extent of carrying out the study with more than 15 laboratories would be too great, both regarding the preparatory laboratory work, and the interpretation of data.
A pre-study of the method draft implemented with a small number of laboratories, can be useful to reveal any matters of dispute. Such a pre-study should be implemented by the referee.

Validation parameters
The method draft elaborated by the referee and approved by NMKL, forms the basis for the validation of the method through the collaborative study, which is a prerequisite for publishing the method with the following text; *"This NMKL method has been validated in acollaborative study"*.

A validation should basically include an examination or evaluation of the following characteristics:

Accuracy
Precision
Repeatability
Reproducibility
Sensitivity
Specificity
Ruggedness/Robustness

These concepts are described and defined in the documents that are listed under **4. References** (1, 4, 5, and 6).

It is important that the validation of the method also comprises such parameters as the stability of the samples or the ability of the bacteria to survive in the samples depending on time and storage temperature, the shelf-life of the used substrates, any light sensitivity of the substrates, the shelf-life of solutions and reagents etc.

Elaboration of collaborative study report and final method draft
After the validation work, the referee elaborates a collaborative study report which sums up and documents the work carried out by the referee in cooperation with the laboratories participating in the collaborative study. In the report, the above-mentioned validation parameters are commented on and evaluated. At the same time, the referee draws up a method draft in accordance with the harmonisation report (2). For methods which have been collaboratively studied, the method draft should also include a section about the precision of the method, although this is not stated in the harmonisation report. The following information may be included in this section: who has arranged the study, the number of participating laboratories and how many analyses each laboratory has performed. The exact results of the study are specified in a separate form, and submitted as an ANNEX to the method. An example of such an ANNEX is Appendix 4, *Presentation of results of quantitative collaborative method performance studies,* which pertains to the collaborative study of the method for the determination of *Bacillus cereus* in food, NMKL No. 67, 5th edition. 2003. Appendix 4 also contains an overview of the parameters which are supposed to be calculated.

Presentation of results of qualitative collaborative studies
The number of laboratories that participated in the study and which matrix was used in the examinations, can be specified in the section about the precision of the method in the method draft. This is also the place to specify that each sample was tested with two different contamination levels; one near the detection limit, and one approx. 10 times higher, as well as a negative control sample, provided, of course, that such a test has been carried out. If not, the results of the test

actually carried out, is stated. The obtained results are entered in a separate form which is enclosed with the method draft as an ANNEX in the same way as for quantitative studies.
Appendix 5, *Presentation of results of qualitative collaborative method performance studies,* includes an example of how to specify the results in such an annex, and which parameters should be calculated. The simple formulas that are used for calculation of specificity and sensitivity, are given in NordVal's Validation Protocol (6). The table in Appendix 5 is taken from ISO Standard 6579:2002 for the detection of *Salmonella* spp. In addition to dried egg powder, the study included fresh cheese coagulant and raw poultry meat.
The collaborative study report should be sent to the co-referee and contact persons for comments. If the contact persons or co-referee have any comments, these are forwarded to the referee, who incorporates them in the report. If any of the comments are not incorporated in the text, the reason for this must be given.
Subsequently, the referee sends the collaborative study report for approval in his/her National Committee, which in turns sends it on to all the National Committees via the Secretary General.
The final report should be sent to the Secretary General, and is archived here as documentation for the future NMKL method.

3.3.3 The collaborative method performance study – step by step

The Draft IDF/ISO Standard, version 2, March 2003 (5) provides useful guidelines on the details of the work.

A collaborative method performance study arranged by NMKL, encompasses the following operations:

- elaborating a collaborative study plan
- selecting laboratories
- sending a method draft and time schedule to participating laboratories
- procuring and preparing samples
- producing and sending samples
- analysing samples according to issued method text
- returning results to the referee
- statistical treatment of data
- reporting data and evaluating method

3.3.3.1 Elaborating the collaborative study plan

The referee shall elaborate a collaborative study plan which must be approved by the Secretary General, cf. 3.3.2. The plan should include the statistical model for the study – see below. The plan should be given the following name: *Collaborative study plan for --- (the name of the method draft)*, and, as a minimum, it should contain information on:

- statistical models
- number of matrices and parameter levels
- suggested time schedule for the study (sending out samples, analysing and returning results, as well as data processing and reporting)
- method draft

- sample coding forms
- references to previous reports

IMPORTANT: The collaborative study plan **shall not** at any time be sent to the participating laboratories.

3.3.3.2 Suggested time schedule for the study

In terms of a time schedule, a method performance study may be divided into 5 steps:

- selection, production and homogeneity testing of study samples
- dialogue with the appointed laboratories before the study
- analysis of the samples in the laboratories, and the returning of data
- collection of data and the statistical treatment of these
- elaboration of the collaborative study report

The collaborative study plan should include a suggested time schedule for the progress of the study. This should be divided into at least the 5 above-mentioned points. In this context, it is particularly important to specify when the referee wants the samples to be analysed in the participating laboratories, e.g. by entering "October-November" as a time frame. This information will be used in the selection of laboratories, so that the individual National Committees later will be able to find laboratories which also have available capacity in the relevant time period. The total length of the study including the reporting phase should, however, not exceed six months.

3.3.3.3 Method draft

The approved method draft **should** be quoted in the collaborative study plan. Furthermore, the method draft should – when the laboratories have been selected – be sent to these laboratories with the purpose of clarifying questions relating to the **understanding** the text, not as regards the **contents** of the text. The method draft should be drawn up in a Scandinavian language. However, the referee is also requested to prepare an English version. This should, in any case, be done when the method is published. The Secretary General can assist with translations.

3.3.3.4 Sample coding form

The collaborative study plan should include an overview of how the referee plans to code the samples. The coding can consist of number combinations and/or letter combinations. However, it must not be possible for the participating laboratories to reveal a system in the coding. The referee might wish to systemise the coding in order to facilitate the subsequent collection of data and the statistical treatment of these.

3.3.3.5 Approving the collaborative study plan

Which materials should be covered by the study, and their concentration levels, should be discussed with the contact person/co-referee. After this, the referee sends the collaborative study plan to the Secretary General. The Secretary General goes through the plan with the relevant sub committee chairperson, and subsequently approves the plan – sometimes after contacting the referee for clarification of questions, modification of text or similar. The approval is submitted in writing from the Secretary General to the referee. Following this, the study can be carried out according to plan.

3.3.3.6 Selecting laboratories

Each National Committee shall point out laboratories which have the relevant competence and routines for the relevant analytical technique – although not, of course, the actual method which is to be studied. The National Committees can use the report containing the method draft and time schedule for the relevant topic and method, as a reference when selecting laboratories. It lies upon the National Committees to ensure that the selected laboratories are prepared to perform the validated method EXACTLY as requested by the referee, and otherwise perform their part of the study with the utmost care and respect.

The National Committees forward the names of the collaborative study laboratories, and of persons to be contacted at the laboratories, directly to the referee, copying the Secretary General and the other National Committees.
The precision (repeatability and reproducibility) of an analytical method can only be assessed based on data from a sufficiently large number of valid data (data that do not include "incorrect data" such as dropped samples, defective equipment etc.).

In order to fulfil this requirement, the referee, as mentioned in 3.3.2, should try to include at least 10, or possibly 12-15, laboratories in the study. The reason for this, is that it is not uncommon that one or more laboratories which are signed up for the study, for some reason are unable to complete it. Also, quite often data from one or more participating laboratories for some reason have to be excluded before or after the statistical evaluation, e.g. if the laboratories have not adhered to the method text.

3.3.3.7 Sending the method draft and time schedule to participating laboratories

After approval of the collaborative study plan, the referee is able to determine the time frame more accurately.

REMEMBER: As mentioned before, the collaborative study plan **shall not** at any time be sent to the participating laboratories.

The method draft must be sent to the participating laboratories at least one month before the analyses are expected to take place. In this way, the laboratories are given an opportunity to familiarise themselves with the method in advance, and possibly try out the analytical method in practice before the actual study begins. This also allows the laboratories time to procure special chemicals and/or equipment they need to be able to participate in the study. The following information is sent out together with the method draft: When the study will actually take place, how many samples are included, when the analyses should be performed, and the deadline for returning the analysis results to the referee.

The laboratories should also be made aware of the fact that the purpose of the study is to test the performance of the method, and not of the laboratory itself. **The method must not be modified during the study!**

The referee should invite the participating laboratories to – before a set deadline – comment on the method draft if they need clarification or have any problems interpreting the text. If any relevant comments are received, the referee must draw up a new method draft, send it to the laboratories explicitly stating that the edition they first received, should be discarded. In certain cases, the

referee may apply for funds from NMKL to cover expenses in connection with meetings held with the collaborative study laboratories with the purpose of discussing the implementation of the study.

3.3.3.8 Producing and sending study samples

Method performance studies place great demands on the sample material, both liquid and solid materials. First of all, the material must be homogenous, to ensure that all the participating laboratories receive identical portions of the sample material. The collaborative study laboratory ensures that this is the case by performing homogeneity tests. Furthermore, the sample material should as far as possible be stable, such as freeze-dried cultures of microorganisms, so that it can withstand transport and storage under varying conditions. In method performance studies where it is known that the sample material will change with time, e.g. frozen or chilled samples, the logistics around sending and the time of analysis in the participating laboratories, should be strictly controlled in order to achieve a usable result of the study. Naturally, sending such samples requires special provisions when it comes to packaging (see Appendix 1).

The samples should as far as practicable be natural foods and feeds, as it is such matrices the method will eventually be used on. The section *Number of matrices and parameter levels* contains more details on this.

The referee must produce enough sample material to allow for homogeneity testing of the material, as well as sending additional sample material to the laboratories should this prove necessary. Samples can be ruined or lost during transport, more laboratories can be included in the study, or one or more study laboratories may request new or additional sample material. An amount of sample material equivalent to the double of the estimated potential need, should be sufficient.

The samples should be numbered and distributed as "blind" samples, i.e. with no reference to statistical model, content or identity. If it is critical that a certain amount of sample is weighed for analysis, or that the sample must be prepared in a special way, the referee can state a suitable concentration level and/or sample type (matrix). The amount of sample in each container should be adjusted according to the fact that the collaborative study laboratories will only be analysing the sample once, or exactly the number of times stipulated in the collaborative study plan (it should not be possible to repeat the analysis). 1 test sample is sent together with the samples for the actual study. The matrix and concentration of the test sample is made known to the laboratories.

The individual sample containers for each participating laboratory should be stored, packed and sent in a manner which ensures that all participants receive identical samples.

3.3.3.9 Number of matrices and parameter levels

Number of matrices In most cases, it is not possible to obtain an optimal validation of a method draft in accordance with the reference literature (1, 4, 5 and 6), due to a lack of resources. A method which applies horizontally to all foods and possibly also feeds, should ideally be validated by a large number of main matrices and several matrices from within the main groups, as detailed in the reference literature.
In many collaborative method performance studies performed by both NMKL and ISO over the last few years, 3 matrices, covering foods which have been relevant for the studied methods, have been used. This was also the case in the study of NMKL method no. 66, 2nd edition, 1999, concerning coagulase positive staphylococcus, and in ISO's international study of *General guidance for the enumeration of Bacillus cereus – Colony count technique at 30^0 C.* Under the section *Precision*

data, ISO indicates that the method has been studied for 3 types of foods contaminated at different levels, for which details are given in an annex to the method. It is here noted quite correctly and with due caution, that *The values derived from the interlaboratory test may not be applicable to other analyte concentration ranges and matrices than given in the annex.*
Parameter levels 3 contamination levels should be used in *qualitative* validations; one blank sample, one sample contaminated near the detection limit for the method provided that the homogeneity test ensures that the samples are positive, and finally a sample contaminated on a level approx. 10 times higher than the detection limit.
Quantitative validations should, in addition to blank samples, include high, medium and low contamination levels with a difference of approx. one logarithm unit in the number. Thus, 4 levels are studied (cf. 4).
The various options for sample contamination, the use of natural materials, mixing samples, or the use of artificially inoculated samples, are dealt with in ISO 16140, Annex C, page 34 (4).

3.3.3.10 Instructions for the laboratories

The sample shipments should be supplied with a note requesting the laboratories to examine the samples on arrival, and immediately notify the referee the sample(s) have been damaged or ruined during transport. The laboratories must also confirm in writing that they have received the samples. These two letters should be included in the sample shipment. The sample shipment should also contain information on how the samples should be stored until the time when they are analysed, and, if necessary, information on the time of analysis (day and/or week).

3.3.3.11 Analyses and result form

Along with the samples, the referee must send specific collaborative study instructions, which state any special analysis conditions, e.g. whether the samples should be analysed in a particular sequence. It is important that the laboratories are aware of the fact that the samples should be analysed under repeatable conditions (with the same apparatus by the same laboratory assistant, and within a short time frame).

Moreover, all participants should have a copy of appendix 2: *Instructions for participants in NMKL studies*.

The collaborative study laboratories should have a result form corresponding to the samples, in which it is stated how many samples the shipment consists of, their codes, and how many times each sample should be analysed. The result form should also specify the accuracy or number of decimals the results should be returned with. All data which are used to calculate the result, should also be entered in the result form, including the units the data are expressed in.

The participants should be requested to fill out the result form electronically, with a type writer or with extra distinct numbers to rule out the risk of incorrect entries. Finally, the form should give the participants the opportunity to enter comments. Appendix 3 contains a check list of which information a result form can or should contain.

The laboratories should also be informed that they must perform the analysis on the test sample before they start analysing the actual study samples. If the test sample gives a different result than that which was given by the referee, **the laboratory must not analyse the study samples** before

the error in the equipment, operation, preparation or other conditions have been found and documented to be rectified by means of analysing a new test sample. Should a laboratory experience other problems with the test samples, the referee must be contacted immediately. If the problems cannot be solved within the collaborative study time schedule, the laboratory should not participate in the study.

Finally, it must be firmly impressed upon the laboratories, that the participants under no circumstances must deviate from the issued method draft. If such deviations do occur, they must be stated on the result form together with a reason for the deviations. When sending the samples, the referee should notify the laboratory that if deviations from the method draft are discovered, it is very likely that the returned data will be excluded in the subsequent statistical treatment.

If it is necessary for the analyses to be are carried out on one specific day, the result form must also include fields for entering the analysis dates.

3.3.4 Elaboration of collaborative study report and statistical evaluation of data

When the referee has received the data from all the laboratories, the data have to be processed. In cases where the referee has received raw data from the laboratories, the results have to be statistically calculated.

In connection with *Microval'*s development of a protocol for alternative methods during an EU project, in 2003, ISO adopted a *Protocol for validation of alternative methods* ISO 16040 (4). The statistical principles used in this protocol are more robust in the sense that e.g. the exclusion of outliers is not included except in cases of obvious errors, e.g. relating to incorrect sample shipments. This is described in a little more detail for qualitative collaborative studies and equivalent quantitative studies, on page 15, under 5.2.1.3 and on page 26 under 6.3.2.3 *Outlier*.
Furthermore, it is possible to use an IDF/ISO draft from March 2003 concerning the determination of precision data in microbiological quantitative collaborative studies. (5).
Appendix 4, *Presentation of results of quantitative collaborative method performance studies*, contains an overview of the parameters that should be calculated. The equivalent information for qualitative studies is contained in appendix 5, *Presentation of results of qualitative collaborative method performance studies.*

If the referee does not have the necessary competence within statistics, it is recommended that he/she contacts a professional statistician.

The referee then makes a total assessment of the results in a summary report of the work. The report is sent to the contact persons/co-referee for comments, and these comments are incorporated in the report.

Unless the method description has already been translated into English in connection with the actual study, the referee is requested to do this now in cooperation with the Secretary General.

The report is sent to the Secretary General, who forwards it to all the National Committees. The National Committees discuss the report and submit their comments to the Secretary General, who presents the issue for approval at the next meeting in the Executive Committee or General Annual Meeting.

3.3.5 Preparation of final method draft

If the collaborative study report is approved, the referee is requested to elaborate the studied method in the scope and layout prescribed by NMKL, cf. Report no. 19 (1998) *Harmonisation of microbiological methods,* and include a section concerning the values for the *precision* of the method proven by the study, as well as a presentation of the results of the study in compliance with the setup in appendix 4. The final method draft should also list the names and work places of all the persons in the referee team.

The referee should write the text in Danish, Norwegian or Swedish, and English. If needed, the Secretary General can assist in completing the English text.

When this is completed, the referee's work is in principle over. The Secretary General takes over from here, and organises printing and further investigation of the method. However, the referee is expected to assist the Secretary General with proof reading and writing a short descriptive text in one of the Nordic languages, which will be presented together with the method in the NMKL Newsletter.

4. References

1. AOAC INTERNATIONAL – "Draft". AOAC International methods committee guidelines for validation of qualitative and quantitative microbiological official methods of analysis (Draft Microbiology Guidelines), AOAC International, June 15, 2001, page 1- 24.

2. Harmonisation of microbiological methods. NMKL report no. 19, 1998. (Available in Danish and Finish only).

3. Guide for referees within chemistry. Elaboration of analytical methods within NMKL. NMKL report no. 11, 2. edition, 2000. (Available in Danish only.)

4. ISO 16140. 2003. Microbiology of food and animal feeding stuffs – Protocol for validation of alternative methods; 74 pages.

5. Draft IDF/ISO Standard, March 2003. Microbiology of food and animal feeding stuffs. Protocol for the establishment of precision characteristics of quantitative methods by inter-laboratory studies; 12 pages.

6. NordVal Validation. Protocol for the validation of alternative microbiological methods. NV-DOC.D – 2002-10-22. Institute of Food Safety and Nutrition, Danish Veterinary and Food Administration, Mørkhøj Bygade 19, DK-2860 Søborg. Email: sq@fdir.dk.

5 Appendices

Appendix 1: Sending sample materials for method performance studies

Appendix 2: Instructions for participants in NMKL studies

Appendix 3: Check list for creating result forms

Appendix 4: Presentation of results of quantitative collaborative method performance studies

Appendix 5: Presentation of results of qualitative collaborative method performance studies

Appendix 1. Sending sample materials for method performance studies

Packaging It is very important to choose the right packaging. The packaging should enable easy placement and removal of the materials. Each individual sample should be doubly sealed to ensure that seepage from one sample does not compromise the other samples.

Sending When sending chilled or frozen samples, use insulating polystyrene packaging material which is large enough to cover cooler bricks/freezing elements in addition to the actual samples. If the samples are chilled, it is important to ensure that the cooler bricks are placed so that they do not cause one or more of the adjacent samples to freeze.
The ideal packaging for sending chilled/frozen samples contains temperature recording equipment. A blank sample should always be included so that it is possible to record the temperature upon arrival. The laboratories must be instructed to record temperature and time of arrival, as well as when the examination was started.
Analyse the samples as soon as possible after you have received them. If the samples are not used immediately, they must be placed in a refrigeration room, or be stored as described by the referee.
The actual shipment must take place in the fastest possible way as ”Courier” mail.
The collaborative study laboratory should test the mailing and distribution method by sending a set of samples to one of the participating laboratories, in order to be able to evaluate the impact of the transport both in terms of the microbiological stability of the samples, and in terms of security.

Extra sample material The collaborative study laboratory should always have extra sample material available, in case anything goes wrong in the sending process. If the packaging is leaking in any way, it should be possible to requisition new sample material.

Receiving laboratories The receiving laboratories should ensure that the samples can be received in compliance with applicable local regulations, and they are also responsible for reporting this to the collaborative study laboratory.
All necessary details of the reception of the samples should be recorded, such as the temperature in the samples, the time of arrival, the time when examinations were started etc. See also Appendix 3: Check list for creating result forms.

Appendix 2. Instructions for participants in NMKL studies

1. Read carefully through the method. Please contact the referee if anything is unclear.

2. Familiarise yourself with the method; carry out a test analysis with one or more test samples which the referee has sent you, or you produce or procure yourself according to instructions from the referee. If you encounter any problems with the test samples, please contact the referee. If the problems cannot be solved, please refrain from participating in the study.

3. When analysing the study samples, *follow the method exactly down to the smallest detail,* and do not modify anything even if you are convinced the modification will improve the method, or to make the method comply with the routines in your own laboratory. If you do make modifications, the results of the analyses may be discarded. If, for some reason, you are unable to follow the method exactly, please explain the reason for all deviations when returning the analysis results.

4. Try to carry out the analyses within the time frame set by the referee. If you experience any delays or other obstacles, please contact the referee.

5. Carry out only the number of determinations requested by the referee. (Do not carry out double determinations, and do not calculate the mean value).

6. Report all results unless the referee has instructed you to do otherwise.

7. Enter the results in the issued result form. If the results are not entered electronically, please write the numbers very clearly. Specify the results with the accuracy (number of figures/decimals) requested by the referee. Include comments, suggestions or criticism, and describe any encountered difficulties which you find to be important. If, during the study, you have tested modifications of the method, the referee would be interested to hear about them. Describe the modifications and corresponding results in a separate report.

Appendix 3. Check list for creating result forms

Take your time when creating the result forms. Think through which information you need to obtain from the participating laboratories.

The check list below gives *examples* of which information the referee may ask for in a result form:

1. Name and address of laboratory (telephone/telefax/e-mail).
2. Name of contact person.
3. Date and time the samples were received.
4. Temperature and condition of the samples upon reception.
5. Date and time when the analyses *should have been* carried out.
6. Date and time when the analyses *actually were* carried out.
7. The number of samples and their corresponding codes.
8. The number of determinations to be carried out on each sample.
9. Accuracy of the specification of results (number of figures/decimals).
10. Special equipment or apparatus used.
11. For microbiological substrates; brand, code number, batch number, expire date, pH.
12. Temperature of the thermostats, and the exact incubation times on the hour.
13. Name of the person(s) who actually carried out the analyses.

Enclose appendix 2: *Instructions for participants in NMKL studies*.

The result form must include a note to the laboratories requesting them to ensure that under no circumstances must the analysis deviate from the issued method text. See appendix 2 for details on this**.**

Appendix 4:Presentation of results of quantitative collaborative method performance studies

The table below contains the results of the studies of NMKL method no. 67, 5th edition, 2003, *Bacillus cereus*, determination in food.

Prøver *Samples*	*Rismel* / Rice flour				Grøt /*Porridge*				Ertesuppe/*Pea soup*				Cous-cous	
	Lavt nivå *Low level*		Høyt nivå High level		Lavt nivå *Low nivå*		Høyt nivå *High level*		Lavt nivå *Low nivå*		Høyt nivå *High level*			
Media; Blood agar (BA), Selective agar (SA)	BA	SA	BA	SA	BA	SA	BA	SA	BA	SA	BA	SA	BA	SA
Antall prøver: *No of Samples:*	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Antall laboratorier: *No of participating labs:*	13	12	13	10	13	11	12	10	13	11	13	10	13	9
Antall outliere: *Number of outliers:*	2	-	-	-	-	-	-	-	2	-	-	2	-	-
Ant. Aksepterte resultater: *No of acceptable results:*	24	24	26	20	26	22	24	20	24	22	26	18	24	18
Gjennomsnittsverdi/ *Mean* (x)($\log_{10}$ CFU/mL)	2.90	2.72	3.75	3.68	2.90	2.82	4.46	4.45	2.95	2.89	4.40	4.36	3.13	3.03
Repeterbarhet, *Repeatability,* (s_r) ($\log_{10}$ CFU/ml)	0.24	0.23	0.29	0.27	0.14	0.24	0.13	0.21	0.23	0.35	0.12	0.11	0.18	0.12
Repeterbarhet, relativ *Repeatability, relative* (RSD_r) (%)	8.3	8.5	7.7	7.2	4.7	8.6	2.9	4.6	7.8	12.3	2.8	2.5	5.7	3.9
Repeterbarhetsgrense *The limit of repeatability* (r) (2,8 x s_r) ($\log_{10}$ CFU/ml)	0.68	0.65	0.81	0.75	0.38	0.68	0.37	0.58	0.65	0.99	0.35	0.31	0.50	0.33
Reproduserbarhet, *Reproducibility,* (s_R) ($\log_{10}$ CFU/ml)	0.24	0.26	0.31	0.33	0.17	0.32	0.15	0.26	0.23	0.35	0.15	0.21	0.30	0.19
Reproduserbarhet, relativ *Reproducibility, relative* (RSD_R) (%)	8.3	9.6	8.2	9.0	5.9	11.5	3.4	5.8	7.8	12.3	3.5	4.8	9.5	6.3
Reproduserbarhets grense *Limit of reproducibility* (R) (2,8 x s_R) ($\log_{10}$ CFU/ml)	0.68	0.75	0.86	0.92	0.48	0.91	0.43	0.73	0.65	0.99	0.43	0.58	0.83	0.54

Appendix 5. Presentation of results of qualitative collaborative method performance studies [1)]

Table C.2 – Results of data analysis obtained with dried egg powder samples

	Dried egg powder (blank)	Dried egg powder (Low level contamination)	Dried egg powder (High level contamination)
Number of laboratories having returned results	26	26	26
Number of samples per laboratory	5	5	5
Number of excluded laboratories	5	5	5
Number of laboratories retained after exclusion	21	21	21
Number of accepted samples	105	105	104
Accuracy (specificity), 0/0	100	-	-
Accuracy (sensitivity), 0/0	-	98.1	99
Accordance, 0/0	100	96.2	98.1
Concordance, 0/0	100	96.2	98.1

[1)] This table specifies the results of one of the three matrices which was included in ISO's collaborative study of *Horizontal method for the detection of Salmonlla spp. ISO 6579:2002.*