# Title of the method (Calibri Bold 16)

After the title, the first page should include a short description of the method, e.g, the same text as published about the method on the web page (Calibri 14).

The title should be precise and concise, starting with the analytes/agents, the principle, and matrices. Examples:

Bacterial count. Determination by direct epifluorescent filter technique (DEFT) in raw minced meat.

Tin (Sn). Determination in foods by inductively coupled plasma mass spectrometry (ICPMS).

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NMKL methods and procedures are available for purchase at [www.nmkl.org](http://www.nmkl.org).

NMKL invites all readers and users of the methods and procedures to submit comments and considerations on its contents to the NMKL secretariat at [post@nmkl.org](mailto:post@nmkl.org).

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**This NMKL method has been validated in an interlaboratory study.**

If the study has been conducted by another organisation than NMKL that should be stated here. The name(s) of the study organiser(s) and institute should be stated under Referee (section 14). (Calibri 14)

**This NMKL method has not been validated in an interlaboratory study.**

If an interlaboratory study has not been carried out, this section should state that and which validation that has been elaborated. (Calibri 14)

## 1. INTRODUCTION

(The text is to be written in Calibri font size 11)

The introduction is used to present additional information, such as comments concerning the technical content of the method or the reasons for its preparation. Background information should be included in this section.

## 2. ADDITIONS

Describe any major changes made in the method compared to the previous edition of the method. Furthermore, other information, which does not fit within any of the other sections, can be provided.

## 3. SCOPE AND FIELD OF APPLICATION

This section shall state:

* concisely what is analysed
* products/matrices to which the method applies
* possible limitations
* detection limit and/or quantification limit
* additional, useful information which could not be included in the title
* possible interferences

It shall also contain sufficient information to enable the user to judge quickly whether the method is applicable to the products being considered, or whether limitations exist.

It is sometimes necessary to provide several procedure descriptions for the determination of a given entity, depending, for example, on the composition of the product or on its differing contents for that entity, or the accuracy required, each procedure having its own scope and field of application. A clear distinction shall be drawn between the individual scopes of the procedures laid down if the method includes several procedures.

## 4. DEFINITION

1. **All caps, from the extended font menu (ctrl+D) (Calibri 12)**

Text (Calibri 11)

* 1. **Bold (Calibri 12)**

Text (Calibri 11)

* + 1. **Bold (Calibri 11)**

Text (Calibri 11)

This section shall give any definitions of terms used in the text that may be necessary for its complete understanding. It is recommended that reference be made to already existing definitions and/or terminology wherever possible. If numbering is required, please use a list or multilevel list following the formatting in the example above.

## 5. REFERENCES

Refer to the documents, indicating either the most recent edition or year or as undated; only stating the number. If the reference is specified without the year of publication, it might be noted that it refers to the latest version of the document, inc. possibly additions.

Regarding sampling, refer to NMKL Procedure No 12: Guide on sampling for analysis of foods.

The following documents should often be referred to in microbiological methods:

- NMKL Procedure No. 23: Guide on quality assurance in microbiological laboratories

- NMKL Method No. 91: Preparation of the test sample and initial suspension of food and animal feeding stuffs for quantitative microbiological examination

## 6. PRINCIPLE

This section indicates the essential steps in the method used, the basic principles and the properties of which use is made and, if appropriate, the reasons justifying the choice of certain procedures.

The text should be in accordance with the text of section 3, “Definitions” in terms of the properties tested.

Chemical reactions can be included in this section if they are considered necessary for the comprehension of the text or the calculations. These reactions shall, if appropriate, be expressed in ionic form. When titrations are involved, the reactions are particularly useful in indicating the number of equivalents in each mole of reactant.

## 7. SAFETY PRECAUTION

This section specifies general safety instructions.

## 8. REAGENTS AND PREPARATION OF SOLVENTS/ CULTURE MEDIA AND REAGENTS

The title of this section and subsections are selected based on what is appropriate for the respective method.

**FOR CHEMISTRY / SENSORY METHODS:**

Water shall meet the requirements specified in NMKL Procedure No 23. In the method description use the term "distilled or equivalent water”.

### 8.1 List of chemicals

The section shall begin, if applicable, with the following sentence or a suitably modified version of it: “Use reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.”

This section shall list all the reagents and materials used during the test, together with their essential characteristics (concentration, density, etc.), and shall specify, if necessary, their degree of purity. If they exist, Chemical Abstract Service Registry numbers (CAS numbers) should preferably be given. The list shall not include products used only in the preparation of a reagent unless the products are used several times.

The reagents and materials shall be identified by a (sequential) reference number. These numbers are referred to (in parentheses) in the procedure description. It is not necessary, however, to repeat the reference number every time if no ambiguity is introduced.

If necessary, the precautions to be taken for storing the reagents, and the time for which they may be stored, shall be specified.

It is desirable to list the substances/reagents in the following order:

* products used in their commercially available form
* aqueous solutions
* solutions of defined concentration
* standard reference solution (in mol/L)
* standard solution (in g/L(kg) or a submultiple thereof).

The expression “V1 +V2” means that the volume V1 of the solution in question is/has been added to the volume V2 of the solvent.

Expressions such as “V1:V2” or “V1/V2” which have different meanings in different countries shall not be used.

### 8.2 Preparation of solvents

### 8.3 Standards

### 8.4 Standard solutions

**FOR MICROBIOLOGY METHODS:**

Water shall meet the requirements specified in NMKL Procedure No 23. In the method description use the term "distilled or equivalent water”.

The possibility of using commercial substrates include, for example by reading "Commercial dehydrated culture media / materials are available and can be advantageously used. Using these, follow the manufacturer's instructions. "

Where relevant include safety precautions in connection with the preparation.

Sterilization conditions should be stated.

### 8.1 Diluent

Diluent and its composition must always be stated.

pH tolerances of diluent and substrates must be consistent with NMKL Procedure No. 23, i.e. ± 0.2, unless there is scientific justification for more stringent requirements.

The pH of the diluent and the substrates relates to the substrate ready for use, measured at 20 to 25 º C. Include in the description: "After sterilization (heat treatment), the pH of the ready for use medium (dilution) should be x.y ± 0.2 measured at 20 to 25 ºC." The time of the pH measurement is not conclusive and shall not be specified in the method.

The choice of diluent should be in accordance with NMKL Method No. 91, unless there are technical reasons for another composition.

### 8.2 Liquid enrichment broths, substrates and reagents for confirmation

**8.3.1 Name of broth, substrate or reagent**

The international name shall appear in the English text.

In the case of substrates with mixtures, the first sub-paragraph describes the composition and preparation of the basis substrate and the following sub point(s) describes the composition and preparation of the mixture(s).

All the ingredients of substrates are to be included, including the chemical formula, and any crystal water if relevant.

If it is essential for the quality of the substrate, the ingredients must be stated with the appropriate number of decimals, also for the whole gram and ml.

The water in substrates is given as the amount of water to be added. Where possible the composition of the substrate is given for the addition of 1000 ml of water. It should be clear stated if addition of water for dilutions, substrates are to a certain quantity, e.g. 1000 ml, or if an exact quantity is to be added (e.g. add 1000 ml).

### 8.3 Solid plating and confirmation media and reagents

**8.3.1 Name of medium or reagent**

The thickness of the plates or the amount of the substrates should be indicated, for example by the following text: "Pour the melted substrate in Petri dishes to a thickness of 3 mm. For a Petri dish with a diameter of 90 mm usually 18-20 ml agar required, or as specified in the method description. If the plates should be incubated beyond 48 hours or at temperatures above 40 °C, it is necessary with more agar."

**Requirements for equivalent substrates**

The general requirement of equivalent substrates is that they contain the same ingredients, both qualitatively and quantitatively as given in the method, and that physical properties such as pH are also identical.

A determination on whether substrates of different composition and/or other characteristics are equal must depend on a concrete assessment in each case.

## 9. APPARATUS AND EQUIPMENT

This section shall list the names and significant characteristics of all the apparatus and equipment, other than ordinary laboratory apparatus, to be used.

The items of apparatus shall be identified by a (sequential) reference number and referred to in the procedure description. It is not necessary, however, to repeat this reference number every time if no ambiguity is introduced.

## 10. SAMPLING

Refer as appropriate to specific sampling methods or to NMKL Procedure No. 12

The following terminology should be used.

* Laboratory sample
* Test sample
* Test portion

## 11. PROCEDURE

The contents in this section will differ depending on whether this is a microbiology method, a chemistry method or a sensory method and is outlined accordingly. The section may be divided into subsections as required, exemplified below.

**FOR CHEMISTRY METHODS:**

The procedure for the method developed according to NMKL protocols 1, 5 and 6 should be described (<https://www.nmkl.org/resources/method-development/>):

* NMKL Protocol No. 1: Referentvejledning for det kemiske område – Udarbejdelse af analysemetoder indenfor NMKL (Danish only)
* NMKL Protocol No. 5: Analytical Quality Control – Guidelines for the publication of analytical results of chemical analyses in foodstuffs. This protocol gives a guideline for publications on what that should to be included and reviewed when elaborating, review or issuing articles where chemical results are included.
* NMKL Protocol No. 6: Requirements for validation of chemical NMKL methods that are not to be validated collaboratively

### 11.1 Pre-treatment

This subsection shall give all the information necessary for the preparation of the test portion from the test sample or from the laboratory sample. It shall state the mass or volume of the test portion, the accuracy with which this must be measured and, if necessary, any other relevant characteristics.

### 11.2 “Name of the procedure to be performed”

**11.2.1 Name of specific sub-procedure or technique**

### 11.3 …

**FOR SENSORY METHODS:**

### 11.1 Pre-treatment

This subsection shall give all the information necessary for the preparation of the test portion from the test sample or from the laboratory sample. It shall state the mass or volume of the test portion, the accuracy with which this must be measured and, if necessary, any other relevant characteristics.

### 11.2 “Name of the procedure to be performed”

**11.2.1 Name of specific sub-procedure or technique**

### 11.3 …

**FOR MICROBIOLOGY METHODS**

The procedure for the method developed according to NMKL protocols 3 and 7 should be described (<https://www.nmkl.org/resources/method-development/>):

* NMKL Protocol No. 3: Guide for referees within microbiology. Elaboration of analytical methods in NMKL
* NMKL Protocol No. 7: Requirement to internal (inhouse/single laboratory) validation of presumptive NMKL microbiological methods

**For quantitative methods:**

### 11.1 Pre-treatment and dilution

This subsection shall give all the information necessary for the preparation of the test portion from the test sample or from the laboratory sample. It shall state the mass or volume of the test portion, the accuracy with which this must be measured and, if necessary, any other relevant characteristics.

Microbiological methods should refer to NMKL Method No. 91.

### 11.2 Plating

### 11.3 Incubation

Temperature tolerances: ± 1.0 °C for air incubators and ± 0.2 °C for water incubators, unless there are scientific reasons for stricter requirements. Temperature settings are given as: 37.0 ± 1.0 °C.

Generally, the following temperatures are applied: 20, 25, 30, 37, 42 and 44 °C. Other incubation temperatures are used only if scientifically reasoned.

Common incubation times:

* 24 hours ± 3 hours
* 48 hours ± 4 hours
* 72 hours ± 6 hours

### 11.3 Reading of plates

Perform the reading, calculations and the stating of the results in accordance with the NMKL Procedure No. 23.

When applicable, state a possible colony interval for reading.

For qualitative methods the results are given as detected / not detected in the amount of the sample analysed.

### 11.4 Confirmation

Use the following statement, unless otherwise scientifically reasoned: "Test a selection of suspect/ presumptive colonies - usually five of each type - if confirmation is necessary or desirable."

**For qualitative methods:**

### 11.1 Pre-treatment/ Test portion and initial suspension

This subsection shall give all the information necessary for the preparation of the test portion from the test sample or from the laboratory sample. It shall state the mass or volume of the test portion, the accuracy with which this must be measured and, if necessary, any other relevant characteristics.

Microbiological methods should refer to NMKL Method No. 91.

### 11.2 (pre-Enrichment)

### 11.2 (Selective) Enrichment

### 11.2 Plating

This title may vary and if treatments or plating steps before this title apply, they are presented in sequential order as separate titles.

### 11.3 Incubation

Temperature tolerances: ± 1.0 °C for air incubators and ± 0.2 °C for water incubators, unless there are scientific reasons for stricter requirements. Temperature settings are given as: 37.0 ± 1.0 °C.

Generally, the following temperatures are applied: 20, 25, 30, 37, 42 and 44 °C. Other incubation temperatures are used only if scientifically reasoned.

Common incubation times:

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Perform the reading, calculations and the stating of the results in accordance with the NMKL Procedure No. 23.

When applicable, state a possible colony interval for reading.

For qualitative methods the results are given as detected / not detected in the amount of the sample analysed.

### 11.4 Confirmation

Use the following statement, unless otherwise scientifically reasoned: "Test a selection of suspect/ presumptive colonies - usually five of each type - if confirmation is necessary or desirable."

## 12. CALCULATION OF RESULTS

Include how the calculations are to be carried out, the number of significant figures, as well as the unit(s) to be stated.

## 13. EXPRESSION OF THE RESULT

State how the results should be expressed, which unit etc.

## 14. RELIABILITY OF THE METHOD

This section specifies the year of the collaborative study, the matrixes and levels, the number of participating laboratories and the organiser of the study.

For qualitative methods, include the specificity and the sensitivity.

For quantitative methods include the precision (repeatability and reproducibility). The results from the study should be given in a schematic form, as an annex.

For methods not validated in interlaboratory study state: “This NMKL method has not been validated in an interlaboratory study.“

©NMKL. NMKL is responsible for the reliability of the method and questions should be directed to the NMKL secretariat: post@nmkl.org.

# ANNEX 1

Annexes can be included at the end of the method.

# ANNEX 2



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