



SLOVENSKI STANDARD

SIST ISO 10705-3:2007

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Kakovost vode - Ugotavljanje prisotnosti in števila bakteriofagov - 3. del - Validacija metod za koncentriranje bakteriofagov iz vode

Water quality -- Detection and enumeration of bacteriophages -- Part 3: Validation of
methods for concentration of bacteriophages from water

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Qualité de l'eau -- Détection et dénombrement des bactériophages -- Partie 3: Validation
des méthodes de concentration des bactériophages dans l'eau

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INTERNATIONAL
STANDARD

ISO
10705-3

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**Water quality — Detection and
enumeration of bacteriophages —**

Part 3:

**Validation of methods for concentration
of bacteriophages from water**

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Qualité de l'eau — Détection et dénombrement des bactériophages —
(standards.itih.ai) Partie 3: Validation des méthodes de concentration des bactériophages
dans l'eau

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 10705-3 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

ISO 10705 consists of the following parts, under the general title *Water quality — Detection and enumeration of bacteriophages*:

- *Part 1: Enumeration of F-specific RNA bacteriophages*
- *Part 2: Enumeration of somatic coliphages*
- *Part 3: Validation of methods for concentration of bacteriophages from water*
- *Part 4: Enumeration of bacteriophages infecting Bacteroides fragilis*

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Water quality — Detection and enumeration of bacteriophages —

Part 3:

Validation of methods for concentration of bacteriophages from water

WARNING — Persons using this part of ISO 10705 should be familiar with normal laboratory practice. This part of ISO 10705 does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is imperative that personnel involved in validation of methods for concentration of bacteriophages from water have relevant experience with the methods of enumeration of bacteriophages (see ISO/TR 13843^[1]).

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1 Scope

This part of ISO 10705 specifies the general principles for assessing the performance of methods for the concentration of bacteriophages from water. Concentration is recommended for those water samples expected to contain < 3 pfp (plaque-forming particles) per millilitre. Concentration methods can be applied to all kinds of water provided that the amount and nature of suspended solids and/or dissolved matter do not interfere with the concentration procedure.

This part of ISO 10705 does not give specific details of concentration methods, but outlines the fundamental principles for evaluating the suitability of a particular method for a given type and volume of water. Annex A gives examples of methods that have been found satisfactory and their fields of application.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 8199, *Water quality — General guide to the enumeration of micro-organisms by culture*

ISO 10705-1, *Water quality — Detection and enumeration of bacteriophages — Part 1: Enumeration of F-specific RNA bacteriophages*

ISO 10705-2, *Water quality — Detection and enumeration of bacteriophages — Part 2: Enumeration of somatic coliphages*

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ISO 10705-4, *Water quality — Detection and enumeration of bacteriophages — Part 4: Enumeration of bacteriophages infecting *Bacteroides fragilis**

ISO/IEC Guide 2, *Standardization and related activities — General vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/IEC Guide 2 and the following apply:

3.1**bacteriophages**

bacterial viruses which are capable of infecting selected host strains

NOTE Bacteriophages produce visible plaques (clearance zones) in a confluent lawn of the host strain grown under appropriate culture conditions.

4 Principle

The sample is treated according to a method of choice, by which the bacteriophages are concentrated from a relatively large volume of sample (100 ml up to several litres) to a smaller volume (typically from a few to 20 ml). The concentrated sample is then analysed for bacteriophages according to an International Standard method or other suitable protocol.

The concentration method to be evaluated should be carefully described in a protocol, following ISO standard layout as much as possible. The description should include the target group(s) of bacteriophages and their detection method(s), the types of water and ranges of volumes to be analysed, as well as exceptions to the field of application, e.g. turbidity.

The method is validated according to principles laid down in this part of ISO 10705. The validation procedure consists of determining the recovery of bacteriophages from a series of samples, seeded with naturally polluted water (raw or treated sewage). The recovery is studied in a range of volumes, and particular attention is paid to its reproducibility.

5 Reagents

Use ingredients of uniform quality and chemicals of analytical grade for the preparation of culture media. For information on storage see ISO 8199, except where indicated in this part of ISO 10705. Alternatively, use dehydrated complete media and follow strictly the manufacturer's instructions.

Other grades of chemicals may be used provided they can be shown to lead to the same results.

5.1 Water, for the preparation of media, glass-distilled water or de-ionized water free from substances that might inhibit bacterial growth under the conditions of the test, and at least Grade 3 as specified in ISO 3696.

5.2 Diluent, for making dilutions, peptone-saline solution or another suitable diluent in accordance with ISO 6887-1 or ISO 8199.

5.3 Culture media and reference cultures, as specified in the corresponding standard method of ISO 10705-1, ISO 10705-2 and ISO 10705-4 for the phage assay.

5.4 Glycerol ($\rho = 870 \text{ g/l}$), autoclaved at $(121 \pm 3) \text{ }^\circ\text{C}$ for 15 min and stored in the dark at room temperature for a period no longer than 1 year.

6 Apparatus and glassware

SAFETY PRECAUTIONS — Field apparatus should be disinfected before use. Apply safety precautions appropriate to the disinfectant solution used. Some stages of the concentration process may involve the application of hydrostatic or pneumatic pressure. Observe relevant safety precautions.

Use usual microbiological laboratory equipment as specified in the method for the phage assay (Clause 8), and the protocol for the concentration method.

7 Sampling

Samples up to 10 l can conveniently be transported to the laboratory. Take the samples and deliver them to the laboratory as specified in ISO 8199 (see also ISO 19458^[2]). For larger samples, it is advisable to perform the first step of the concentration procedure in the field. This process may take up to several hours. If parallel examination for indicator bacteria or other micro-organisms is carried out, take a time-proportional sample for these analyses, preferably by filling a sample bottle with a side flow from the concentration apparatus. Filters, precipitates or other products from the first concentration step may be further treated in the field, or may be transported to the laboratory. Include the transport and storage conditions of intermediate stages of the process in the validation procedure.

8 Preparation of sewage samples for spiking

Obtain a sample of primary or secondary (biologically treated) sewage and centrifuge at 1 000 g for 20 min or filter through an 8 µm to 12 µm membrane filter. Store supernatant or filtrate on melting ice. Enumerate the target bacteriophages in 1 ml volumes according to the chosen method. If necessary, dilute the sample to obtain a concentration of 60 pfp to 200 pfp (plaque-forming particles) per millilitre. Add glycerol to obtain a final volume fraction of 5 %; mix well. Distribute 10-ml aliquots into glass or plastic bottles (or tubes, or vials) and freeze at $(-20 \pm 5) ^\circ\text{C}$ or $(-70 \pm 10) ^\circ\text{C}$. Thaw two bottles at room temperature. From each bottle, examine two 0,5-ml aliquots for the target bacteriophages. The average counts should be within the limits as specified above (i.e. 30 pfp to 100 pfp per plate). Analyse the counts for within and between bottle homogeneity as follows:

$$T_1 = \sum_{i=1}^I \sum_{j=1}^J \left[\left(z_{ij} - \frac{z_{i+}}{J} \right)^2 \right] / \left(\frac{z_{i+}}{J} \right)$$

where

T_1 is Cochran's dispersion test statistic to determine the variation in pfp within one vial of reference material;

z_{i+} is the total count of plaques of the duplicates of one vial.

$$z_{i+} = \sum_{j=1}^J z_{ij}$$

I is the number of vials (in this case 2);

J is the number of duplicates (in this case 2);