INTERNATIONAL STANDARD

ISO 10705-3

First edition
2003-10-01

Water quality — Detection and enumeration of bacteriophages —

Part 3: Validation of methods for concentration of bacteriophages from water

Qualité de l'eau — Détection et dénombrement des bactériophages —

Partie 3: Validation des méthodes de concentration des bactériophages dans l'eau

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6ba99564-5127-4c26-92af-f35b2cbc2be4/iso-10705-3-2003

Reference number
ISO 10705-3:2003(E)

© ISO 2003

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6ba99564-5127-4c26-92af-f35b2cbc2be4/iso-10705-3-2003

© ISO 2003

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword iv
1 Scope 1
2 Normative references 1
3 Terms and definitions 2
4 Principle 2
5 Reagents 2
6 Apparatus and glassware 3
7 Sampling 3
8 Preparation of sewage samples for spiking 3
9 Procedure 4
10 Calculation 5
11 Analytical quality control 6
12 Test report 7
Annex A (informative) Recommended methods for concentration of bacteriophages from water depending on the volume, turbidity and particle content 8
Annex B (informative) Example of a validation process of a concentration method 10
Bibliography 13

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6ba99564-5127-4c26-92af-f35b2cbc2be4/iso-10705-3-2003

© ISO 2003 — All rights reserved

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*.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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*

ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6ba99564-5127-4c26-92af-f35b2cbc2be4/iso-10705-3-2003

— *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

© ISO 2003 — All rights reserved

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]).

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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.

ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6be09564-5127-4e26-93af-f25b2abc2bcd/iso-10705-3-2003

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*

© ISO 2003 — All rights reserved

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.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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.

ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6ba99564-5127-4c26-92af-

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 (ρ = 870 g/l), autoclaved at (121 ± 3) ºC for 15 min and stored in the dark at room temperature for a period no longer than 1 year.

 © ISO 2003 — All rights reserved

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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 ± 5) °C or (−70 ± 10) °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:

ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6ba99564-5127-4c26-92af-f35b2cbc2be4/iso-10705-3-2003

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

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);

The number of degrees of freedom for T_1 is equal to $I(J-1)$ and

$$T_2 = \sum_{j=1}^{J}\left[\left(z_{i+} - \frac{z_{++}}{I}\right)^2 \Big/ \left(\frac{z_{++}}{I}\right)\right]$$

where

T_2 is Cochran's dispersion test statistic to determine the variation in pfp within different vials of one batch of reference material;

z_{++} is the total count of plaques for all vials and duplicates $z_{++} = \sum_{i=1}^{I}\left(\sum z_{ij}\right)$.

The number of degrees of freedom for T_2 is equal to $I-1$.

If the phages are randomly distributed within and between the vials, T_1 and T_2 follow approximately a χ^2 distribution with respectively 2 and 1 degrees of freedom. Accept the samples if $0,01 < T_1 < 5,99$ and $T_2 < 3,84$.

NOTE Somatic coliphages, F-specific RNA bacteriophages and bacteriophages infecting *Bacteroides fragilis* naturally occurring in raw sewage partially purified as indicated above, do not suffer significant inactivation when frozen with a volume fraction of 5 % glycerol and they can be preserved frozen below (–20 ± 5) °C and preferably at (–70 ± 10) °C without significant decrease in numbers for at least one year.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

9 Procedure

ISO 10705-3:2003
https://standards.iteh.ai/catalog/standards/sist/6ba99564-5127-4c26-92af-f35b2cbc2be4/iso-10705-3-2003

9.1 Preparation of spiked samples

9.1.1 Batch methods

Obtain samples from all types of water mentioned in the scope of the concentration procedure. Obtain the samples on different days, preferably representing different seasons and climatic conditions. Study a minimum of five samples for each sample water type. Let V_{max} be the maximum volume of sample to be treated by the concentration method under evaluation. The volume of sample to be obtained for the validation procedure shall then at least be $3 \times V_{max}$. Prepare containers with the following volumes of sample:

— $0,125 \times V_{max}$;

— $0,250 \times V_{max}$;

— $0,500 \times V_{max}$;

— V_{max}.

To each container, add 1 ml of spiking material (see Clause 8) pre-warmed at room temperature. Preserve the remainder of the spiking material on melting ice.

9.1.2 In-line concentration methods

Perform field studies for all types of water mentioned in the scope of the concentration procedure. Carry out the studies on different days, preferably representing different seasons and climatic conditions. Study a

© ISO 2003 — All rights reserved

minimum of five samples for each water type. Let V_{max} be the maximum volume of sample to be treated by the concentration method. Perform field studies with the following volumes of sample:

— $0,125 \times V_{max}$;

— $0,250 \times V_{max}$;

— $0,500 \times V_{max}$;

— V_{max}.

Treat each volume as described in the protocol of the concentration procedure. Add 1 ml of spiking material (see Clause 8) pre-warmed to room temperature to approximately 10 ml of diluent (5.2). Allow the concentration apparatus to operate under stable conditions. Inject then the total volume of diluent plus spiking material in the inflow to the concentration apparatus (e.g. by piercing the needle of a syringe through a hose) in four similar portions, each after passage of approximately one-fifth of the water volume to be treated.

9.2 Evaluation of recovery

Treat the spiked samples as described in the protocol of the test concentration method, including all sample transport and conservation steps, imitating as much as possible the sample transport steps of natural samples. Assay the total volume of the final concentrate in 1 ml portions, or fractions if the final volume of concentrate is not a whole number of millilitre. Any additional bacteriophages remaining on the concentration surfaces should be assayed when possible, e.g. phages retained in the filters.

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 10705-3:2003

In parallel, assay two 0,5-ml aliquots of spiking material. The values obtained shall be used to calculate the concentration efficiency, which will allow the determination of the number of phages introduced in the different volumes to be concentrated and will also allow the calculation of T_1 and T_2 of each one of the bottles with regard to other bottles. If more than 20 % (1 in 5) of the spiking material samples do not comply with the acceptable values of T_1 and/or T_2, discard the spiking material. If $\leqslant$ 20 % of the spiking material samples do not comply with T_1 and/or T_2, discard the results of this assay and perform a new assay.

Anomalous or extreme results are characteristic of microbiological measurement. Occasionally it is acceptable to discard a result on the basis of simple observation of the data. However, it is preferable to apply an appropriate statistical test. Use the Dixon test to discard extreme values.

Perform a minimum of five experiments with results that have not been rejected before data analysis.

If the method is evaluated using a natural water suspected of containing phages detected by the same bacterial host as the test bacteriophages, then determine the background counts of phages. Plaque an aliquot or concentrate V_{max} and count the concentrate. If the sample contains a number of phages > 20 % of the phages spiked into the sample, heat the water and keep it at 80 °C for 30 min and allow it to cool prior to use. If the number of naturally occurring bacteriophages is < 20 % of the added phages, then enumerate them and take them into account in the data analysis (Clause 10).

10 Calculation

Calculate the recovery, η, expressed as a percentage, as follows:

$$\eta = N_c / N_s \times 100\ \%$$

or in the case that the sample is contaminated with naturally occurring bacteriophages:

$$\eta = (N_c - N_{no}) / N_s \times 100\ \%$$

© ISO 2003 — All rights reserved