

Water quality —

Part 1: Detection and enumeration of bacteriophages — Enumeration of F-specific RNA bacteriophages

The European Standard EN ISO 10705-1:2001 has the status of a
British Standard

ICS 07.100.20

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National foreword

This British Standard is the official English language version of EN ISO 10705-1:2001. It is identical with ISO 10705-1:1995.

The UK participation in its preparation was entrusted by Technical Committee EH/3, Water quality, to Subcommittee EH/3/4, Microbiology, which has the responsibility to:

- aid enquiries to understand the text;
- present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
- monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee and can be obtained on request to its secretary.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the BSI Standards Catalogue under the section entitled “International Standards Correspondence Index”, or by using the “Find” facility of the BSI Standards Electronic Catalogue.

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This British Standard, having been prepared under the direction of the Health and Environment Sector Board, was published under the authority of the Standards Board and comes into effect on 15 April 1996

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Summary of pages

This document comprises a front cover, an inside front cover, the EN ISO title page, the EN ISO foreword page, pages 1 to 11, the annex ZA page, an inside back cover and a back cover.

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

(ISO 10705-1:1995)

Qualité de l'eau — Détection et dénombrement des bactériophages —
Partie 1: Dénombrement des bactériophages
ARN F spécifiques
(ISO 10705-1:1995)

Wasserbeschaffenheit — Nachweis und Zählung von Bakteriophagen —
Teil 1: Zählung von F-spezifischen
RNA- Bakteriophagen
(ISO 10705-1:1995)

This European Standard was approved by CEN on 29 June 2001.

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Foreword

The text of the International Standard from Technical Committee ISO/TC 147, Water quality, of the International Organization for Standardization (ISO) has been taken over as a European Standard by Technical Committee CEN/TC 230, Water analysis, the Secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2002, and conflicting national standards shall be withdrawn at the latest by February 2002.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 10705-1:1995 has been approved by CEN as a European Standard without any modifications.

NOTE: Normative references to International Standards are listed in annex ZA (normative).

1 Scope

This part of ISO 10705 specifies a method for the detection and enumeration of F-specific ribonucleic acid (RNA) bacteriophages by incubating the sample with an appropriate host strain. The method can be applied to all kinds of water, sediments and sludges, where necessary after dilution. In the case of low numbers, a preconcentration step may be necessary for which a separate part of ISO 10705 will be developed. The method can also be applied to shellfish extracts. Depending on the relative abundance of F-specific RNA bacteriophages to background organisms, additional confirmatory tests may be necessary and are also specified in this part of ISO 10705.

The presence of F-specific RNA bacteriophages in a water sample generally indicates pollution by wastewater contaminated by human or animal faeces. Their survival in the environment, removal by widely used water treatment processes and concentration or retention by shellfish resembles that of foodborne and waterborne human enteric viruses, for example the enteroviruses, hepatitis A virus and rotaviruses.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 10705. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10705 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

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

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*.

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques*.

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples*.

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination*.

ISO 8199:1988, *Water quality — General guide to the enumeration of micro-organisms by culture*.

3 Definition

For the purposes of this part of ISO 10705, the following definition applies.

3.1

F-specific RNA bacteriophages

bacterial viruses which are capable of infecting a specified host strain with F-pili or sex-pili to produce visible plaques (clearance zones) on a confluent lawn grown under appropriate culture conditions, whereas the infectious process is inhibited in the presence of a concentration of 40 (occasionally 400) µg/ml of RNase in the plating medium

4 Principle

The sample is mixed with a small volume of semi-solid nutrient medium. A culture of host strain is added and plated on a solid nutrient medium. After this, incubation and reading of plates for visible plaques takes place. Where necessary, simultaneous examination of parallel plates with added RNase for confirmation by differential counts is carried out. The results are expressed as the number concentration of plaque-forming particles (C_{pfp}) per unit of volume.

5 Safety precautions

The host strain used is a *Salmonella typhimurium* mutant of low pathogenicity and should be handled in accordance with the appropriate national or international safety procedures for this bacterial species. F-specific RNA bacteriophages are non-pathogenic for man and animals, but are very resistant to drying. Appropriate precautions should therefore be taken to prevent cross-contamination of test materials, particularly when examining or handling cultures of high titre or when inoculating cultures of the host strains. Such procedures must be carried out in a biohazard cabinet or a separate area of the laboratory.

6 Diluent, culture media and reagents

6.1 Basic materials

Use ingredients of uniform quality and chemicals of analytical grade for the preparation of culture media and reagents, and follow the instructions given in Annex A. 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.

For the preparation of media, use glass-distilled water or deionized water free from substances which might inhibit bacterial growth under the conditions of the test, and in accordance with ISO 3696.

6.2 Diluent

For making sample dilutions, use peptone saline solution as indicated in A.8.

6.3 Reagents

6.3.1 *RNase from bovine pancreas*, specific activity approximately 50 units/mg (Kunitz).

6.3.2 *Antibiotic discs*, for susceptibility testing with nalidixic acid (130 µg; 9 mm) and kanamycin (100 µg; 9 mm).

6.3.3 *Glycerol*, 870 g/litre.

6.4 Microbiological reference cultures

Salmonella typhimurium strain WG49, phage type 3 Nal^r (F⁺ 42 *lac*::Tn5), NCTC 12484.

Bacteriophage MS2, NCTC 12487 or ATCC 15597-B1.

Escherichia coli K-12 Hfr from appropriate culture collection, e.g. NCTC 12486 or ATCC 23631.

NOTE 1 The NCTC strains are available from the National Collection of Type Cultures, 61 Colindale Avenue, London NW9 6HT, England. The ATCC strains are available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, U.S.A.

7 Apparatus and glassware

Usual microbiological laboratory equipment, including

7.1 *Hot-air oven for dry-heat sterilization and an autoclave*. Apart from apparatus supplied sterile, glassware and other equipment shall be sterilized according to the instructions given in ISO 8199.

7.2 *Incubator or water bath*, thermostatically controlled at 37 °C ± 1 °C.

7.3 *Incubator or water bath*, thermostatically controlled at 37 °C ± 1 °C and equipped with a rotary platform at 100 min⁻¹ ± 10 min⁻¹.

7.4 *Water bath*, thermostatically controlled at 45 °C ± 1 °C.

7.5 *Water bath or equivalent device*, for melting agar media.

7.6 *pH-meter*.

7.7 *Counting apparatus*, with indirect, oblique light.

7.8 *Deep freezer*, thermostatically controlled at -20 °C ± 5 °C.

7.9 *Deep freezer*, thermostatically controlled at -70 °C ± 10 °C.

7.10 *Spectrometer*, capable of holding 1 cm cuvettes or side-arm of nephelometric flasks (7.17) and equipped with a filter in the range 500 nm to 650 nm with a maximum bandwidth of ±10 nm.

Usual sterile, microbiological laboratory glassware or disposable plastics ware according to ISO 8199 and including the following.

7.11 *Petri dishes*, of diameter 9 cm or 15 cm.

7.12 *Graduated pipettes*, of capacities 1 ml, 5 ml and 10 ml.

7.13 *Glass bottles*, of suitable volumes.