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Kakovost vode - Izolacija in identifikacija oocist Cryptosporidium in cist Giardia

Water quality - Isolation and identification of Cryptosporidium oocysts and Giardia cysts from water

Qualité de l'eau - Isolement et identification des oocystes de Cryptosporidium et des kystes de Giardia

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Water quality — Isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts from water

Qualité de l'eau — Isolement et identification des oocystes de Cryptosporidium *et des kystes de Giardia*

Reference number
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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
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Foreword

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ISO 15553 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Introduction

Cryptosporidium and *Giardia* are protozoan parasites that can cause enteric illness in humans. Both organisms are characterized by an ability to survive in the aquatic environment. *Cryptosporidium* in particular is resistant to chlorine at the concentrations used in the treatment of drinking and swimming pool waters. Consequently the absence of vegetative bacteria as indicators of faecal contamination does not necessarily indicate the absence of *Cryptosporidium* oocysts or *Giardia* cysts. The methods described in this document may be used to determine whether *Cryptosporidium* and/or *Giardia* are present in water supplies. The techniques have been selected on the basis of method development and peer review publication of the data thus obtained. They are further selected to give comparable recoveries of the methods or reagents used in the isolation of the organisms.

Water quality — Isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts from water

1 Scope

This International Standard specifies a method that is applicable for the detection and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts in water. It is applicable for the examination of surface and ground waters, treated waters, mineral waters, swimming pool and recreational waters.

This method does not allow identification to species level, the host species of origin or the determination of viability or infectivity of any *Cryptosporidium* oocyst or *Giardia* cyst which may be present. These procedures are for use by experienced analysts who have successfully completed competency tests prior to commencing analysis. In addition, such analysts should continue to demonstrate competency by examining seeded samples at regular intervals and taking part in external quality assurance schemes.

NOTE Bodies resembling *Cryptosporidium* or *Giardia* in morphology can be present and these may be mistaken for oocysts or cysts. Results should be interpreted with care. Where there is doubt about the identity of oocysts or cysts or where an unusually high result is obtained, it is advisable to have the slides examined by experts from other laboratories to confirm or refute the findings.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
Cryptosporidium
protozoan parasite, concentrated and selected from water samples with the methods described, which reacts with specific anti-*Cryptosporidium* antibodies and exhibits the typical morphological characteristics described in 7.4 of this International Standard

NOTE A more complete definition of the parasite and the different genotypes and species is given in Annex G.

2.2
Giardia
protozoan parasite, concentrated and selected from water samples with the methods described, which reacts with specific anti-*Giardia* antibodies and exhibits the typical morphological characteristics described in 7.4 of this International Standard

NOTE A more complete definition of the parasite and the different species is given in Annex G.

3 Principle

3.1 Concentration from water

The isolation of *Cryptosporidium* and *Giardia* from water requires the use of a procedure which allows the volume of the sample to be reduced whilst retaining any oocysts and cysts. The concentration procedure used however, is dependent upon the water type which is to be analysed, the volume of sample and the amount of particulate material in the sample. This document describes the use of two concentration techniques for varying volumes of water using cartridge filtration and elution followed by low speed centrifugation (7.1). Additional methods for the recovery of oocysts and cysts from small volumes of water or very turbid waters are given in Annex B. Some examples of recovery data for these techniques are given in Annex E.

Table 1 — Membrane filters/filtration systems used for the concentration of parasites from water samples

Membrane filter/filtration system	Application
Pall Envirochek™ STD [a]	Concentration of 10-litre to 200-litre (or more) samples of water
Pall Envirochek™ HV	Concentration of 10-litre to 1 000-litre samples of water
IDEXX Filta-Max®	Concentration of 10-litre to 1 000-litre samples of water

[a] It has been shown by some laboratories that this technique may be used successfully for larger volumes of water although the manufacturers' instructions may only include volumes up to 200 litres.

3.2 Purification and further concentration

After concentration of particulate material from filter eluates, oocysts and cysts are isolated using immunomagnetic separation (IMS) (7.2). Oocysts and cysts are attached to para-magnetic beads coated with specific antibody, the beads are separated from the unwanted particulate material using a magnet and then the oocysts and cysts are dissociated from the beads using acid and neutralized using alkali before immunostaining.

3.3 Detection of *Cryptosporidium* and *Giardia*

After IMS, organisms are labelled with monoclonal antibody (mAb) conjugated to a fluorochrome, usually fluoroscein isothiocyanate (FITC). In addition, any nuclear material is labelled with a nucleic acid stain to aid identification (7.3). Each sample is then examined for the presence of labelled *Cryptosporidium* oocysts and *Giardia* cysts using epifluorescence and differential interference contrast (DIC) microscopy (7.4).

4 Reagents

4.1 Reagents required for eluting Pall Envirochek™ STD capsule filters [1)]

4.1.1 Deionized water, 0,2 µm filtered at the point of use.

4.1.2 Laureth 12 detergent.

4.1.3 Tris buffer, pH 7,4 (A.1.1).

4.1.4 EDTA solution, 0,5 mol/l, pH 8,0 (A.1.2).

4.1.5 Antifoam A.

4.1.6 Elution buffer (A.1.3).

4.2 Reagents required for eluting Pall Envirochek™ HV capsule filters [1)]

4.2.1 Deionized water, 0,2 µm filtered at point of use.

4.2.2 Pre-treatment buffer (A.1.4).

4.2.3 Laureth 12 detergent

1) All products and reagents are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

4.2.4 Tris buffer, pH 7,4 (A.1.1).

4.2.5 EDTA solution, 0,5 mol/l, pH 8,0 (A.1.2).

4.2.6 Antifoam A.

4.2.7 Elution buffer (A.1.3).

4.3 Reagents required for eluting IDEXX Filta-Max® filters [1)]

4.3.1 Phosphate buffered saline (PBS) (A.2.1).

4.3.2 Polyoxyethylene(20)sorbitan monolaurate (Tween 20).

Store at room temperature (20 ± 5) °C. Expiry date one year.

4.3.3 Elution buffer (A.2.2).

4.4 Concentration and detection reagents

4.4.1 Methanol, analytical grade.

4.4.2 Magnetic beads, for the detection of *Cryptosporidium* and *Giardia.*

Expiry date printed by the manufacturer.

NOTE See Annex H for a list of suitable suppliers.

4.4.3 Fluorescently labelled monoclonal antibodies (mAbs) against *Cryptosporidium* and *Giardia*.

Store at (5 ± 3) °C. Expiry date as stated by the manufacturer. When stains are prepared from concentrated material using a diluent supplied by the manufacturer, the prepared solution is stored at (5 ± 3) °C for no longer than 6 months.

NOTE See Annex H for a list of suitable suppliers.

4.4.4 Immunofluorescence mounting medium (A.3.1).

NOTE See Annex H for a list of suitable suppliers.

4.4.5 4′,6′-Diamidino-2-phenylindole dihydrochloride dihydrate (DAPI) freeze dried reagent.

Store according to the manufacturer's instructions.

Expiry date printed by the manufacturer on each vial.

4.4.6 DAPI stock solution (A.3.2).

4.4.7 DAPI working solution (A.3.3).

4.4.8 Phosphate buffered saline (PBS) (A.2.1).

4.4.9 Non-fluorescing immersion oil.

Store at room temperature (20 ± 5) °C.

4.4.10 Stock suspensions of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts.

Store at (5 ± 3) °C, never allow the suspension to freeze and check quality regularly. Ideally, suspensions of oocysts and cysts should be no more than 3 months old. Stock suspensions should be checked microscopically to confirm that they are monodispersed and discarded if clumps or aggregates are detected. In addition, if mAb and DAPI staining become weak and oocysts become deformed, they should also be discarded.

4.4.11 Parasite storage medium (A.3.4).

5 Apparatus

Use usual laboratory equipment and, in particular, the following.

5.1 Scientific apparatus, required for concentration using Pall Envirochek™ STD or HV.[2)]

5.1.1 Sampling capsule, Envirochek™ STD or HV (Pall).

5.1.2 Peristaltic pump, capable of a flow rate of 2 l/min.

5.1.3 Silicon tubing, for use with the peristaltic pump.

5.1.4 Seeding container, 10 l, if seeding filters is required.

5.1.5 Wrist-action shaker, with arms for the agitation of the Envirochek™ STD or HV sample capsules.

5.1.6 Centrifuge, capable of a minimum of 1 100 *g*.

5.1.7 Centrifuge tubes, conical, plastic, screwtop, 250 ml capacity.

5.1.8 Centrifuge tubes, conical, plastic, screwtop, 50 ml capacity.

NOTE A flow meter and flow restrictor are required for taking water samples with the filter.

5.2 Specific apparatus, required for concentration using IDEXX Filta-Max®.[2)]

5.2.1 Sampling housing, Idexx Filta-Max®.

5.2.2 Sampling module, Idexx Filta-Max®.

5.2.3 Filter membranes, Idexx Filta-Max®.

5.2.4 Laboratory pump, capable of supplying 500 kPa (5 bar) pressure.

5.2.5 Peristaltic pump, capable of flow rate of 4 l/min.

5.2.6 Silicon tubing, for use with peristaltic pump.

5.2.7 Seeding container, 10 l, if seeding filters is required.

5.2.8 Wash station, automatic or manual, and wash station clamp set, Idexx Filta-Max®.

5.2.9 Vacuum set, includes plastic hand pump, waste bottle, tubing and magnetic stirring bar. Idexx Filta-Max®.

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2) All apparatus are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

5.2.10 Tubing set, includes elution tube, and middle section, concentrator tube and base, with line tap and steel rod Idexx Filta-Max®.

5.2.11 Membrane, for tubing set.

5.2.12 Plastic bag, for washing membrane.

5.2.13 Centrifuge, capable of 1 100 *g*.

5.2.14 Centrifuge tubes, conical, plastic, 50 ml capacity.

5.2.15 Forceps.

NOTE A flow meter and flow restrictor are required for taking water samples with the filter.

5.3 General apparatus [2].

5.3.1 Incubator, at (36 ± 2) °C.

5.3.2 Refrigerator, at (5 ± 3) °C.

5.3.3 Magnetic stirrer, and magnetic stirring bars.

5.3.4 Vortex mixer.

5.3.5 Wash bottles, polypropylene, 250 ml.

5.3.6 Calibrated micropipettes, adjustable: 1 µl to 10 µl with 1 µl to 10 µl tips; 20 µl to 200 µl with 10 µl to 200 µl tips; 200 µl to 1 000 µl with 100 µl to 1 000 µl tips.

5.3.7 pH meter.

5.3.8 Magnetic particle concentrators, with suitable tubes.

5.3.9 Well microscope slides, with special hydrophobic coating and coverslips.

5.3.10 Epifluorescence microscope, with a UV filter (350 nm excitation, 450 nm emission), FITC filter (480 nm excitation, 520 nm emission) filters,™ differential interference contrast (DIC) optics and an eye piece graticule. Total magnification 1 000 ×.

5.3.11 Microscope stage micrometer, 1 mm, ruled in 100 units.

5.3.12 Eyepiece graticule, ruled in 100 units.

5.3.13 Humidity chamber, e.g. consisting of a tightly sealed plastic container containing damp paper towels on which the slides are placed.

5.3.14 10 l containers, graduated in 1 l.

5.3.15 Neubauer haemocytometer slide.