Isolate® cryptosporidium

Immunomagnetic Separation (IMS) of *Cryptosporidium* Oocysts in Environmental Water Samples

Product No. ZAICS100: 100 test kit
Product No. ZAICS25: 25 test kit

INTENDED USE

Isolate is an *in vitro* procedure for the separation of *Cryptosporidium* oocysts from environmental water samples, in particular, with concentrates of drinking water supplies. Oocysts in the concentrate are captured by anti-*Cryptosporidium* oocyst wall-specific monoclonal antibodies conjugated to magnetic beads and separated from environmental debris using a powerful magnet. Oocysts are released from beads via acid dissociation, and beads are separated from oocysts by magnetic action. Detection of these captured and separated oocysts can then be performed by addition of fluorescein isothiocyanate (FITC)-conjugated anti-*Cryptosporidium* oocyst wall-specific monoclonal antibodies, followed by visualization of the labeled oocysts using a fluorescence microscope.

BACKGROUND

Cryptosporidium is a genus of coccidian protozoan parasite that contains several species, the most important of which are *Cryptosporidium parvum* and *Cryptosporidium hominis*. Both organisms cause mild to severe gastroenteritis and diarrhoea in humans, and can be life-threatening in immunocompromised persons. *Cryptosporidium* lives part of its life cycle as an oocyst, which survives in the environment for prolonged periods of time, and is unaffected by routine drinking water chlorination [1].

Cryptosporidium hominis is almost exclusively a parasite of humans. *Cryptosporidium parvum* is infectious for virtually all mammals, and is widespread in its distribution[2]. Drinking water supplies, and particularly surface water sources, are at risk of harbouring infectious oocysts and numerous outbreaks have been recorded[3,4,5,6]. Importantly, there is a poor correlation between particle counts and oocyst occurrence. Thus, while particle counting and turbidity can be used to monitor the overall functioning of a treatment plant, this data should not be used to predict the presence or absence of oocyst contamination, or as a replacement for a reliable and accurate oocyst monitoring method and program.

The importance of *Cryptosporidium* as a serious waterborne threat has prompted various governmental agencies to adopt programs to monitor for its presence. In 1996, the U.S. Environmental Protection Agency (USEPA) implemented the Information Collection Rule (ICR), which mandated 18 months of testing by the largest water utilities in the United States[8]. Unfortunately, the method adopted in the ICR for *Cryptosporidium* testing is generally acknowledged to be inaccurate, unreliable, and very labour intensive[9]. Since *Cryptosporidium* oocysts are very small and their abundance in water is generally very low (but as few as 30 oocysts can cause infection[10]), detection requires concentration of significant volumes of water through small pore filters. Subsequent identification of oocysts in these concentrates is difficult due to the presence of debris, algae, and other organisms. Conventional centrifugation and density gradient techniques used in the separation of oocysts from such environmental debris are very inefficient, and large losses are common[11]. Immunomagnetic separation (IMS) is a technique used frequently in blood separation applications and has been recently adapted for environmental microbiology applications[12, 13, 14, 15]. The USEPA adopted IMS as a procedure (Method 1622) that enables more accurate, reliable, and user-friendly determination of the presence of *Cryptosporidium* in water samples[8, 16]. IMS was also the method prescribed for oocyst isolation in the United Kingdom by the Drinking Water Inspectorate under the Water Supply (Water Quality) (Amendment) Regulations 1999[17].

MATERIALS PROVIDED

Crypto Magnetic Beads. Concentrated magnetic bead suspension conjugated to oocyst wall-specific anti-*Cryptosporidium* mouse monoclonal IgG in a buffer containing protein stabiliser and detergent.

Reagent A (sample diluent). Concentrated buffer solution used to reduce the interference of inorganic and organic debris.

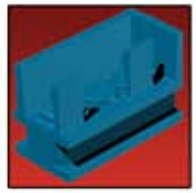
Reagent B (blocking buffer). Concentrated solution of protein used to reduce the binding of debris and organisms other than *Cryptosporidium* to Crypto Magnetic Beads.

Note: The preservative sodium azide is added to the above reagents. See warning note.

ADDITIONAL MATERIALS SUPPLIED BY TCS BIOSCIENCES LTD

- Variable speed rotator with angle of tilt set at 20° from horizontal (ZAICS1)
- ***Isolate*** Rotator Disc and Hub (ZAICS2)
- Incu-Clip (ZAICS3), Magna-Clip (ZAICS4), Micro-Clip (ZAICS5)
- Dissociation Block (ZAICS8)
- Glass Leighton tubes (ZAICS6), or equivalent.

Isolate Rotator ZAICS1 with Rotator Disc and Hub ZAICS2



Isolate Incu-Clip (Blue) ZAICS3

Isolate Magna-Clip (White) ZAICS4

Isolate Micro-Clip (White) ZAICS5

OTHER MATERIALS REQUIRED

- Hydrophobic adhesion slides, 9 mm single well, blue coated, available from Hendley, Essex, UK, Part No. PH246
- Vortex mixer
- Polypropylene microcentrifuge tubes, 1.5 ml
- Micropipettes (capable of delivering 5-1000 µl)
- Transfer pipettes
- Aspiration device (vacuum source equipped with liquid trap and pipettor tip, or equivalent)
- 0.1 N HCl, reagent grade
- 1.0 N NaOH, reagent grade
- Purified water (distilled, deionised laboratory grade water, or reverse osmosis water)

PRECAUTIONS

The laboratory must be appropriately equipped for testing for and disposing of *Cryptosporidium*.

Only qualified laboratory personnel with experience in microbiological techniques should perform and interpret this test.

All reagents are for *in vitro* use only.

Reagents must not be used beyond the expiration date indicated on the kit label.

Reagents must be brought to room temperature before use.

All reagents should be gently agitated prior to use. Use caution to avoid foaming. Crypto Magnetic Beads should be gently, but thoroughly mixed **immediately prior** to sampling, as the beads settle rapidly.

WARNING: Sodium azide is used as a preservative in all of the provided reagents, at a concentration of 0.05%. Sodium azide is a skin irritant. Avoid skin contact with reagents. Do not mix with acids as this may result in the formation of hydrazoic acid, an extremely toxic gas. Disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. This can be avoided by flushing with a large volume of water during such disposal.

The Magna-Clip, Micro-Clip and Dissociation Block must be used for the magnetic separation of beads from the test sample. Magnetic devices from other manufacturers may not be suitable for this IMS test.

CAUTION: The glass Leighton tubes are fragile and caution must be used when placing them in or removing them from the Clips.

Each time the reagents are used, the components should be visually inspected for signs of contamination, and discarded if contamination is evident.

LIMITATIONS OF THE PROCEDURE

Isolate IMS aids in the identification of oocysts of the genus *Cryptosporidium*. Many *Cryptosporidium*-specific monoclonal antibodies react with multiple species of *Cryptosporidium*.

All IMS techniques have been shown to be highly dependent on the quality of the specific source water (dissolved substances, quantity and type of suspended solids, mineral content, algal species present, etc.) If difficulty is encountered with a particular sample, contact TCS Biosciences Ltd.

SHELF LIFE AND STORAGE

The expiration date of the kit is indicated on the kit label.

The kit should be stored at 2-8°C and should be returned to the refrigerator after use.
CAUTION: DO NOT FREEZE.

PERFORMANCE CHARACTERISTICS

Research has demonstrated that *Isolate* can recover greater than 85% of oocysts seeded into water samples. Recovery will vary with water quality and turbidity. Generally, extremely turbid concentrates (>5000 NTU) containing large amounts of organic matter and algae are associated with lower recoveries, compared to concentrates with relatively more inorganics and lower turbidities (<5000 NTU).

REAGENT PREPARATION

1. The contents of the kit should be brought to room temperature prior to use. If crystals have formed in Reagent A upon 2-8°C storage, warm the bottle to room temperature with end over end mixing to redissolve all the precipitate before using. Providing that Reagent A will then be used within one month, it can be stored at room temperature.

2. Prepare 1.0 ml diluted IMS Reagent A for each sample to be tested by diluting the IMS Reagent A 1:10 in deionised water. The diluted IMS Reagent A can be stored at 2-8°C.

SAMPLE PREPARATION

Samples should be prepared by procedures described in government regulatory documents, or by other standard methods. ***Isolate*** has been designed to separate oocysts in water samples containing packed pellets of 2.0 ml or less (20%). After centrifugation, remove sample supernatant and add reagent water to give a final packed pellet volume of 2.0 ml or less (per 10 ml sample). If the final volume is greater than 10 ml, divide the sample into equal portions that will contain less than 2.0 ml packed pellet per 10 ml, and add reagent water to give 10 ml samples for analysis. See specific instructions in regulatory methods for details.

PROCEDURE

NOTE: Various governmental regulations may prescribe additional steps in the IMS, especially regarding the transfer of samples. If performing these analyses under such regulations, please consult the specific published procedure.

A. Oocyst Capture

1. Place 1 ml Reagent A and 1 ml Reagent B into a Leighton tube. Add to the Leighton tube the sample to be tested. Use 1 ml of purified water to rinse out the centrifuge tube, and add this also to the Leighton tube. Label the tube with the sample number.

2. Mix the Crypto Magnetic Beads thoroughly, avoiding frothing. Ensure that the beads are fully mixed by inverting the bottle to make sure that there is no residual pellet in the bottom. Add 100 µl of Crypto Magnetic Beads to the Leighton tube.

   NOTE: Ensure that Leighton tube cap has a sealing wad in place, and is properly tightened.

3. Incubate the sample by placing the Leighton tube into a BLUE Incu-Clip with the flat side facing down (A). Slide the Incu-Clip and Leighton tube onto the ***Isolate*** Rotator until it clicks into position, locating the cap of the tube in the ***Isolate*** Rotator Hub (B). Rotate at 20 rpm for at least 60 minutes at room temperature (C). **IMPORTANT**: Ensure that the Rotator angle is set at 20° from horizontal.

4. Carefully remove the Incu-Clip containing the Leighton tube from the ***Isolate*** Rotator. Remove the tube from the Incu-Clip (D) and place it into a WHITE Magna-Clip with the flat side facing down against the magnets (E). Slot the Magna-Clip onto the ***Isolate*** Rotator disc until it clicks into position, locating the cap of the tube in the ***Isolate*** Rotator Hub (F).

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5. Set the ***Isolate*** Rotator at 20 rpm and operate for at least 5 minutes. If the pellet was greater than 0.5 ml, operate for at least 10 minutes.

6. Switch off the ***Isolate*** Rotator when the Leighton tube is at the lowest point of the cycle, with its cap uppermost.

7. Remove the Magna-Clip (with the Leighton tube still in place) from the ***Isolate*** Rotator. If there are other samples on the ***Isolate*** Rotator, switch it back on to continue rotating them.

8. Immediately remove the cap and, with the flat side of the tube downwards, pour off the supernatant. Without disturbing the bead pellet, aspirate any remaining supernatant with a pipette.

9. Remove the tube from the Magna-Clip. Using 0.8 ml of Reagent A diluted 1:10 with reagent water, rinse the bead pellet from the side of the tube. Pipette the solution over the beads repeatedly to ensure ALL beads are rinsed from the tube.

10. Transfer the beads to a clean labeled 1.5 ml microcentrifuge tube. Rinse the Leighton tube with a further 0.2 ml of diluted Reagent A and add this to the microcentrifuge tube.

11. Cap the microcentrifuge tube and place in the Micro-Clip. Slot the Micro-Clip onto the ***Isolate*** Rotator and operate at 20 rpm for at least 2 mins. If the pellet was greater than 0.5 ml operate for at least 4 minutes.

12. Remove the Micro-Clip (with the tube still inside) from the ***Isolate*** Rotator. If there are other samples on the ***Isolate*** Rotator, switch it back on to continue rotating them.

13. Immediately remove supernatant from the tube and from the cap using a suitable pipette. Take care not to disturb the beads on the tube wall.

14. With samples containing a large amount of particulate material, it may be advantageous to wash the beads. Remove the tube from the Micro-Clip, add a further 1 ml of diluted Reagent A, cap the tube, gently mix the beads and repeat stages 11 - 13.

B. Dissociation of the beads/oocyst complex

15. Remove the microcentrifuge tube from the Micro-Clip and add 50µl 0.1N hydrochloric acid (HCl). Vortex the tube thoroughly for 10 seconds.

16. Stand the tube at room temperature for 5 minutes and then vortex the tube again for 10 seconds.

17. Place the tube into Dissociation Block and lay the Block flat on the workbench so that the tube is horizontal with the magnet below it. Leave for at least 30 seconds to allow the beads to separate.

18. Label a clean 9 mm well slide.

19. Add 5 µl 1.0 N sodium hydroxide (NaOH) directly to the sample well of the slide.

20. Carefully return the Dissociation Block to the upright position and transfer the supernatant from the tube to the well of the slide without disturbing the beads at the back wall of the tube.

21. Gently mix the sample with the NaOH using the transfer pipette.

22. Perform a second acid treatment to ensure complete release of oocysts by repeating steps 15 to 17 and 19, and place this onto the same slide well by repeating steps 20 and 21.

REFERENCES

1. Fayer R. (1995) Effect of sodium hypochlorite exposure on infectivity of *Cryptosporidium* parvum oocysts for neonatal BALB/c mice. *Applied Environmental Microbiology* **61**, 844-846.
2. O'Donoghue P. (1995) *Cryptosporidium* and Cryptosporidiosis in man and animals. *International Journal for Parasitology* **25**, 139-195.
3. Lisle JT, Rose JB. (1995) *Cryptosporidium* contamination of water in the USA and UK: a mini-review. *Journal of Water SRT - Aqua* **44**, 103-177.
4. MacKenzie W, Hoxie N, Proctor M, Gradus M, Blair K, Peterson D, Kazmierczak J, Addiss D, Fox K, Rose J, Davis J. (1994) A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. The *New England Journal of Medicine* **331**, 161-167.
5. Fricker CR, Crabb JH. (1998) Waterborne Cryptosporidiosis: Detection Methods and Treatment Options. *Advances in Parasitology* **40**, 241-278.
6. McTigue N, LeChevallier M, Clancy J, Fredricksen D. (1996) Assessment of particle removals by conventional filtration. American Water Works Association Annual Conference, June 23, 1996, Toronto, Canada.
7. USEPA (1994) Enhanced surface water treatment requirements. *Federal Register* **59,** 38832-38858.
8. USEPA (1996) Monitoring requirements for Public Drinking Supplies: *Cryptosporidium, Giardia,* viruses, disinfection by-products, water treatment plant data and other information requirements. *Federal Register* **61**, 24353-24388.
9. Clancy JL, Gollnitz WD, Tabib Z. (1994) Commercial labs: how accurate are they? *Journal of the American Water Works Association* **86**, 89-97.
10. Dupont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. (1995) The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine* **332**, 855-859.
11. LeChevallier MW, Norton WD, Siegel JE, Abbaszadegan M (1995) Evaluation of the immunofluorescence procedure for detection of *Giardia* cysts and *Cryptosporidium* oocysts in water. *Applied and Environmental Microbiology* **61**, 690-697.
12. Vartdal F, Gaudernack G, Funderud S, Bratlie A, Lea T, Ugelstad J, Thorsby E. (1986) HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation--a fast and reliable technique. *Tissue Antigens* **28**, 301.
13. Bifulco JM, Shaefer FW. (1993) Antibody-magnetite method for selective concentration of *Giardia lamblia* cysts from water samples. *Applied and Environmental Microbiology* **59**, 772-776.
14. Fricker CR, Turner NB, Rolchigo PM, Margolin AB, Crabb JH.(1995) Improved recovery of *Cryptosporidium* oocysts from drinking water using vortex-flow filtration combined with immunomagnetic separation. *The American Journal of Tropical Medicine and Hygiene*. Suppl. **53**, 285.
15. Jonas A, Crabb J, Murrin K. Slade JS, Fricker CR. (1997) Simultaneous concentration and separation of protozoan parasites and viruses using vortex flow filtration and immunomagnetic separation. Proceedings of the 1996 Water Quality Technology Conference. American Water Works Association. (9/8/98)
16. USEPA (1999) Method 1622: *Cryptosporidium* in water by filtration/IMS/IFA . Office of Water. Washington, DC.
17. Drinking Water Inspectorate, Department of the Environment, Transport and the Regions, United Kingdom (1999) Standard Operating Protocol for the Monitoring of *Cryptosporidium* Oocysts in Treated Water Supplies to Satisfy Water Supply (Water Quality) (Amendment) Regulations 1999, SI No. 1524.

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