

A day in the life of *Cryptosporidium*

Since 1989, a number of waterborne outbreaks of cryptosporidiosis have prompted the UK Government to review water testing procedures, with the result that continuous sampling and monitoring are now recommended at 300-400 source sites around the country. Similar concerns have surfaced in many other countries. The result is a greater burden of testing for water and environmental laboratories.

John Haigh, Olympus UK

UK Statutory Instruments 1999 No. 1524 [*The Water Supply (Water Quality) (Amendment) Regulations 1999*] identifies a standard operating protocol (SOP) for isolating and enumerating waterborne *Cryptosporidium parvum* oocysts. The only acceptable method for identifying *Cryptosporidium* oocysts is immuno-fluorescence staining and examination and identification under high magnification microscopy using a microscope equipped with both epifluorescence and differential interference contrast (DIC) optics. Much of the analysts' time is spent in screening samples which raises health and safety concerns. The BX2 Series microscopes from Olympus provide an ideal tool for this analysis by combining advanced fluorescence and DIC capability, with ergonomic design specifically for long term screening.

The parasite

Cryptosporidium parvum is an ubiquitous, protozoan parasite discovered by Tyzzer in 1912. Oocyst measure approximately 5µm in diameter and are the transmissive stage of the parasite's life cycle. *C. parvum* infects the small intestine of a broad range of mammals, including humans. Symptoms can range from self-limiting diarrhoea in immuno-competent individuals, to life threatening disease in severely immuno-compromised individuals.

C. parvum is frequently found in neonates, although it can also infect adults. The severity of cryptosporidiosis is dependent upon both host and parasite factors, particularly the immune status of the host, which explains why the disease can be more severe in neonates than in older animals. *C. parvum* has a one-host life cycle and the parasites live in the cells lining in the intestine (enterocytes). All reproductive (asexual and sexual) stages occur within these enterocytes, inside one host. The infection is passed from one susceptible host to another in an environmentally resistant stage called the oocyst. Susceptible humans can be infected at any time in their lives, but previous exposure to the parasite confers a partial immunity to challenge infections.

Once inside an enterocyte, development of *Cryptosporidium* occurs rapidly, each generation developing and maturing in as little as 12 – 24 hours. Due to the speed of reproduction, large numbers of parasites can colonise the intestinal tract within a week. The ileum soon becomes crowded and secondary sites, including the duodenum become infected.

In immunosuppressed patients, for example those with AIDS, the parasite can colonise the stomach, biliary, pancreatic duct and respiratory tract. It is in such patients that cryptosporidiosis can be fatal, with profuse intractable diarrhoea with severe dehydration, malabsorption and wasting being clinical signs of disease. Chronic infections are due to an inability to eliminate *Cryptosporidium* from the body. In the absence of effective anti-parasite specific drugs, the only natural, effective therapy for the parasite at present is a healthy, intact immune system.

Some sources of infection

A major route of transmission is person to person, although waterborne transmission is also well documented. Ineffective or inadequate treatment has occasionally resulted in community outbreaks of cryptosporidiosis.



Cryptosporidium shown with different contrast methods: DIC (1), immuno stained (2), overlay immuno stained + DAPI (3), Overlay of DIC, immuno stained + DAPI (4)

Currently, there are no typing methods that can help us determine the source of individual cases or outbreaks at present. Reported *Cryptosporidium* oocyst counts from public water supplies can be highly variable. Current concentration techniques for oocysts in environmental samples are poor and the antibody used for detection can cross-react with algae or other particles commonly found in water. One report demonstrated that different US laboratories performing occurrence testing on *Cryptosporidium* oocysts in water have widely varying degrees of accuracy.

Microscopic detection

Most microorganisms of relevance to the water industry can be detected from very small numbers, often only one organism, by growing them in artificial media or tissue culture until their numbers increase sufficiently for them to be detected reliably. This is not possible with *C. parvum*, as there is no reliable method available. Oocysts are concentrated from large volumes of water, separated from contaminating debris, using a commercially produced immunomagnetsable separation kit, originally developed at the Scottish Parasite Diagnostic Laboratory, detected, and, where necessary, tested for viability. Developing effective methods for sampling, concentration and identification for *Cryptosporidium* is presently the subject of considerable research, worldwide. Because microscope companies realise that much of an analyst's time is spent on microscopical identification, varying degrees of automation or semi-automation can be offered.

2 Olympus BX51 microscope



The UK Laboratory of the Government Chemist and the Scottish Parasite Diagnostic Laboratory have been granted the license to run the DWI approved External Quality Assurance (EQA) Scheme for *Cryptosporidium* detection. The SPDL has been training Water Industry personnel in *Cryptosporidium* analysis since 1989 and Olympus microscopes are used regularly to demonstrate oocyst morphology and morphometry, currently as an adjunct to 'in house' training. Many of the definitive procedures in this new regulation were developed at the SPDL using Olympus microscopes with funding from the UK Department of the Environment.

Microscope set up and oocyst identification is the most exacting of the procedures in this Regulation. Oocysts fluoresce apple green using a commercially available fluorescein isothiocyanate labelled anti-*Cryptosporidium* monoclonal antibody (FITC-mAb), which highlights the outer wall of the oocyst, and

sporozoite nuclei stain sky blue with the nuclear fluorogen 4'6-diamidino-2-phenyl indole (DAPI). The FITC-mAb binds to the outer oocyst wall, so that the fluorescence can be used not only to recognise the organism but also to measure its size. The presence of sky blue nuclei within an oocyst provides further supporting evidence.

The BX2 Series includes a high performance fluorescence illuminator ideal for such dual label fluorescence work. Interchangeable or removable field and aperture stops provide maximum flexibility for specialist imaging applications. Up to six fluorescence mirror units can be attached to the highly rigid illuminator, complemented by two six filter through sliders for further flexibility. Excitation balancers (EXBA) from Olympus can also be incorporated. The balancers can be used to equalise the emission intensities of different fluorochromes so that fluorescence detail can be better visualised. They are available for most common dual or multi-label stains.

Direct observation of *Cryptosporidium* oocysts is essential, since many algal species show some degree of fluorescence under the same procedure, and species such as *Navicula minima* (a widely distributed freshwater diatom) can fluoresce brightly, being oocysts in both shape and size. In order to minimise reporting false positives, high magnification (x400 – 1000) is necessary to observe and confirm the presence of oocysts.

Ergonomic requirements

A well-organised testing regime gives substantial benefits both for water utilities in terms of operational savings and improved manpower use, and for consumers through faster detection and control of potentially health threatening contamination of drinking water supplies. Significant advances in filtration and separation techniques, and in procedures for concentration prior to identification, will ease the pre-analytical bottleneck, but, in the absence of an effective alternative, microscopy is likely to remain the primary means of confirmation, and enumeration.

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Reference list

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Figures

- 1 Image courtesy of the above mentioned Professor H. Smith and Dr. A.M. Grimason, SPDL, Glasgow, UK

Information

Further information is available at:

www.olympus-europa.com/microscopy

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