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# Enhancing *Cryptosporidium parvum* recovery rates for improved water monitoring

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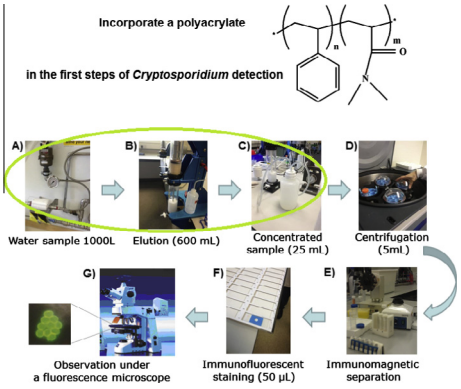
## HIGHLIGHTS

- Proposed modified detection method of *Cryptosporidium* with improved recovery rates.
- By using polymer coated filters more oocysts were detected compared to standard.
- Polyacrylates were incorporated in the detection method of *Cryptosporidium*.
- The elution buffer had also an effect on the recovery rates of the filters.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Water monitoring is essential to ensure safe drinking water for consumers. However existing methods have several drawbacks, particularly with regard to the poor recovery of *Cryptosporidium* due to the inability to efficiently elute *Cryptosporidium* oocysts during the established detection process used by water utilities. Thus the development of new inexpensive materials that could be incorporated into the concentration and release stage that would control *Cryptosporidium* oocysts adhesion would be beneficial. Here we describe improved filter performance following dip-coating of the filters with a "bioactive" polyacrylate. Specifically 69% more oocysts were eluted from the filter which had been coated with a polymer than on the naked filter alone.


## 1. Introduction

*Cryptosporidium* is a protozoan parasite which is a major cause of gastroenteritis outbreaks worldwide. In 2010 a waterborne cryptosporidiosis outbreak took place in Sweden and affected at least 27 000 inhabitants of Östersund (Widerström et al., 2014). In the Milwaukee outbreak in 1993 403 000 residents became ill (Corso et al., 2003). Studies revealed that among the residents of Milwaukee, and its vicinity, cryptosporidiosis-associated death increased after the waterborne outbreak (Hoxie et al., 1997). Additionally, medical costs and lost productivity exceeded $96 million (Corso et al., 2003). Waterborne cryptosporidiosis infections have also been detected in Spain (Fuentes et al., 2014), Australia

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(Ng-Hublin et al., 2014), Ireland (Pelly et al., 2007; Glaberman et al., 2002) and France (Dalle et al., 2003). Overall, during the period 2004–2010, 60% of worldwide waterborne parasitic protozoan outbreaks were caused by *Cryptosporidium* spp. (Baldursson and Karanis, 2011).

Water sources in the developed countries can be contaminated by animals, sewage discharge and distribution system failures (Barrett, 2014; Ercumen et al., 2014). *Cryptosporidium* has a very low infectious dose compared with other waterborne pathogens, which can be as low as 10 oocysts (King and Monis, 2007; Okhuysen et al., 1999). The symptoms of *Cryptosporidium* infection depend on the nutritional and immune status of the host and the site of infection which is mainly the small intestine although sometimes the infection may be spread throughout the gastrointestinal tract and extra-intestinal sites (Chalmers and Davies, 2010). Generally the symptoms are diarrhea and abdominal pain nausea, vomiting and low-grade fever, while they are accompanied occasionally with nonspecific symptoms such as myalgia, weakness, malaise, headache, and anorexia (Bouzid et al., 2013). Furthermore cryptosporidiosis can be fatal for children (Huang et al., 2004) and immunocompromised adults. Particularly people with low CD4 counts are at increased risk (Sorvillo et al., 1998, 1994; Hunter and Nichols, 2002). Furthermore there is no fully effective treatment or vaccine for the groups that are vulnerable to the life-threatening cryptosporidiosis while treatments exist for other waterborne pathogens such as *Shigella*, *Escherichia coli* (Striepen, 2013) and *Vibrio cholerae* (Leibovici-Weissman et al., 2014). The increased number of cryptosporidiosis outbreaks worldwide are attributed to *Cryptorporidium* resistance to standard chlorine disinfection (Campbell et al., 1982) and other disinfectants commonly used in laboratories and hospitals (Tzipori, 1983). Furthermore according to the World Health Organization *Cryptosporidium* is among the parasites of primary concern in order to supply safe water to consumers. For the above mentioned reasons regular monitoring of the water supply for the presence of *Cryptosporidium* is undertaken by water utilities. French authorities require the monitoring of *Cryptosporidium* oocysts in drinking waters and generally in water supplies (NF T90-455, AFNOR, 2001; Mons et al., 2009). In 1996, the United States Environmental Protection Agency (USEPA) started the process of acquisition and application of the newest technologies for the detection and identification of *Cryptosporidium* and *Giardia* (U.S. Environmental Protection Agency, 2001). These USEPA1622 and 1623 methods were prepared to give an estimation of the health risk caused by drinking water polluted with the protozoans such as *Cryptosporidium* and *Giardia* (Skotarczak, 2009).

The most commonly used method for the detection of *Cryptosporidium parvum* are the USEPA 1623 (DiGiorgio et al., 2002; Francy et al., 2004; Ongerth and Saaed, 2013) and since 2012 1623.1 (U.S. Environmental Protection Agency, 2012), and ISO 15553 "Water Quality – isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts from water". In the UK the procedure is similar to this method, starting from 1000 L of water with UK companies using the IDEXX Filta-Max filters. Due to the pore size of the cartridge filter all the *Cryptosporidium* oocysts are captured. Afterwards the oocysts are eluted from the IDEXX Filta-Max filter and concentrated on top of a membrane. Then the oocysts are eluted from the membrane, further concentrated by centrifugation and purified by immunomagnetic separation. Finally the oocysts are dissociated from the immunomagnetic beads, stained and enumerated under a fluorescence microscope (Fig. 1).

However current detection procedures, e.g. USEPA 1623, have less than 100% recovery rates. Recovery rate is the efficiency of the process in concentrating all of the pathogens of interest. For example a recovery rate of 100% means that all the pathogens of interest in the original sample are detected after the process. Recovery is particularly important for pathogens present in low numbers, as if the rate is low any pathogen in the sample might be lost during the sample processing stage. This is assumed to be due to the inability of controlling the elution of *Cryptosporidium* oocysts during the concentration stage of the process, with a wide range of average recovery efficiencies reported in the concentration stage, with one study finding average values of about 22%, while the average recovery rates for the next stages are above 60% (Rony, 2010). However 38% recovery is considered acceptable within the USEPA method 1623.1 guidelines (U.S. Environmental Protection Agency, 2012). According to Hu et al. (2004) the recovery rate of *C. parvum* oocysts was 18% when including the filtration step. Taking into account the infectious dose of *Cryptosporidium* it is obvious that the achieved recovery rates are low. Therefore modification of the concentration stage will lead to more accurate results. Thus modifications to USEPA 1623 have been proposed in the literature (Kimble et al., 2013). The recovery rates are impacted by the type of filter, the elution buffer, the water type and operator skill (Hill et al., 2005; Holowecky et al., 2009; Polaczyk et al., 2008). The recovery efficiency of the filter is influenced by its material but comparisons in water applications of different materials are difficult as the filter design itself varies.

Here we provide the first investigation of how the material influences the recovery rate of *Cryptosporidium* filters, using coated commercial filters in order to compare the performance of two different materials. An ideal material would have very good stability and long term durability. They will not be toxic or irritating to people who are handling/using them and they will be insoluble in water. In this study it is shown that the elution/recovery of *C. parvum* oocysts is enhanced by using commercially available filters which have been dip-coated with a specific polyacrylate that prevents *Cryptosporidium* adhesion, the polymer having been identified from a recent study (Wu et al., 2012).

## 2. Materials and methods

### 2.1. Materials

The foam discs and the membrane filters were obtained from IDEXX. The polymers PA6 and PA531 were synthesized via radical polymerization on a mmol scale as previously reported (Wu et al., 2012; Patent WO 2013/079938). The solvents tetrahydrofuran, toluene, hexane, diethyl ether, acetone and acetic acid were all obtained from Sigma–Aldrich. Crypto-a-Glo™ was obtained from Waterborne Inc. Oocysts were obtained from Scottish Water via Creative Science Company (oocysts were the Moredun isolate) and enumerated using FACS.

### 2.2. Filter preparation

Solvent Selection: Initially the goal was to find a solvent for the polymers that would not dissolve the filters. Thus commercially available filters were immersed for 5 min into three different potential coating solvents specifically tetrahydrofuran, acetone and acetic acid and the weight of each filter recorded before and after immersion.

Filter Coating: From the above experiment tetrahydrofuran was selected as the best solvent. Thus the filters were coated by dip-coating for 5 min into the polymer dissolved in tetrahydrofuran at concentrations of up to 1% (w/v). After the dip-coating the filters were dried in a fume hood for 24 h and the weight recorded to give coating efficiencies. The polymer loading is the percentage increase in the weight in comparison to the initial weight.

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