Er dagens deteksjonmetoder gode nok?

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Introduction

Waterborne outbreaks of cryptosporidiosis and giardiasis have led to the monitoring of water supplies, both raw and final, for the presence of these parasites. For three reasons, listed below, monitoring for *Cryptosporidium* oocysts has been given greater emphasis in recent years than monitoring for *Giardia* cysts.

1. *Cryptosporidium* oocysts are more environmentally robust than *Giardia* cysts and therefore less likely to be inactivated by water treatment.
2. *Cryptosporidium* oocysts are smaller than *Giardia* cysts and therefore less likely to be removed by filtration processes in water treatment. Also, because of the smaller size, *Cryptosporidium* oocysts are more difficult to detect in environmental samples.
3. Whilst both cryptosporidiosis and giardiasis are unpleasant diseases characterised by diarrhoea, as there are currently no chemotherapeutic or prophylactic drugs available against cryptosporidiosis it is more likely to be life-threatening.

The increased requirement for monitoring for these parasites has led to an increased need for assessing whether the analytical techniques available are sufficiently sensitive and reliable. The methods were first developed in the 1970s for analysing water samples for *Giardia* cysts (Jakubowski & Erickson 1979). Then, in the 1980s, these 'traditional' methods were then adopted for monitoring for *Cryptosporidium,* without addressing the failings within the method. These failings include the lengthy and tedious nature of the methodology and how easily its efficiency was affected by a variety of factors, such as turbidity and operator competence. Also, whilst, generally, the efficiency of the methodology was acceptable for analysis for *Giardia,* it was much less efficient for *Cryptosporidium.* Following an analysis of commercial laboratories in the US, Clancy et al (1994) reported a mean recovery of 2,7% of *Cryptosporidium* oocysts.

In recent years the need to assess the techniques for analysis of water samples for these parasites has been recognised and emphasis has been placed on developing new, faster, more efficient and reliable methodologies. In particular the Environmental Protection Agency (EPA) in USA has been developing and validating methodologies for analysis of water samples for *Cryptosporidium* (US EPA Method 1622). A similar approach is also being used in UK through the Department of Environment, Drinking Water Inspectorate.

The Analytical Method

The analytical method for both these parasites can be considered to consist of four main stages:

1) Collection
2) Concentration
3) Separation
4) Enumeration

1. Collection: The volume of water samples collected for analysis may range from as little as 10 L to as much as 10,000 litres. Choice of volume size will be determined by a number of factors including predicted probability of parasite detection.

Samples may be collected by filtration, or particularly when analysing small volumes for *Cryptosporidium*, chemical flocculation may be used.

Various types of filter have been used (see table 1 below) which have various merits and disadvantages. The US EPA Method 1622 mentions capsule, vortex flow and membrane filters.

Table 1: Types of filter used for collection

Filter	% Efficiency	Comments
Yarn wound	0,2% - 80%	simple, cheap, large volume, variable efficiency
Membrane	70% - 90%	simple, cheap, relatively small volume
Capsule	50% - 90%	simple, expensive
Vortex flow	50% - 90%	complex, expensive

Both calcium carbonate and aluminium sulphate have been used for chemical flocculation. Both are cheap, but are lengthy procedures with recovery efficiencies ranging from 30% to 80% for calcium carbonate (Fricker & Crabb 1998) and 50% to 80% for aluminium sulphate (C. Bissigger personal communication). Furthermore, calcium carbonate may inactivate *Crypto-*

sporidium oocysts (Campbell et al 1994); this may be important if the analysis is to include assessment of viability.

2. Concentration: Collection procedures, whether filtration or flocculation, typically result in a residual volume of between 50 ml to 4 litres. This is concentrated by centrifugation, usually at between 1000g to 1500g. Publications report a recovery efficiency of between 16% to 75% of this stage in the procedure (Watkins et al 1995). Typically, the recovery efficiency is between 70% to 80%, provided that the volume being centrifuged is small (250 ml) and that detergents are included during the procedure.

3. Separation: The parasites must be separated from the concentrated particulate matter before being enumerated. The 'traditional' method is by density flotation, either on sucrose or Percoll/sucrose. This method relies upon the buoyant density of the parasites being different from other matter in the water. This is a relatively inexpensive technique, but the recovery efficiency is variable and the skill of the operator is very important. The alternative method is immunomagnetic separation (IMS) which relies upon binding of the parasites to paramagnetic beads via antibodies, and collection of the beads and attached parasites by using a magnet. This is a simple technique with consistent recovery efficiencies of between 60% to 90% for the Dynal IMS (Campbell et al 1997). In table 2, data from seeding trials comparing recovery of *Cryptosporidium* oocysts using flotation techniques and Dynal IMS are described.

Table 2: Recovery of *Cryptosporidium* oocysts by flotation or IMS

	SUCROSE Mean % recovery (range)	PERCOLL/SUCROSE Mean % recovery (range)	IMS Mean % recovery (range)
Clean water	19,3 (13,9 - 26,7)	29,9 (19,4 - 33,7)	97,4 (93,6 - 101,7)
Turbid water	11,7 (8,7 - 17,3)	5,4 (4,1 - 6,9)	65,8 (60,5 - 72,8)

4. Enumeration: Following concentration and separation of the target organisms, they must be detected and enumerated. All the techniques routinely in use rely upon fluorescent antibody staining, followed by a microscope based detection technique. Even when more sophisticated techniques, such as flow cytometry, are used, fluorescence microscopy must be used for screening. In table 3 the various detection and enumeration techniques

available are listed and their efficiency, and merits and disadvantages described. US EPA Method 1622 recommends microscopy on a slide format.

Table 3: Techniques for enumeration of *Cryptosporidium* oocysts or *Giardia* cysts, based upon initial fluorescent antibody staining.

METHOD	% EFFICIENCY	COMMENTS
Microscopy on a slide	60% - 100%	simple, operator dependent
Microscopy on a membrane	50% - 100%	Variable, may hinder confirmation
Flow cytometry	30% - 100%	expensive
Laser scanning	70% - 100%	expensive

A further advantage of the IMS separation technique over flotation procedures is observed at the detection/ enumeration stage of the analytical procedure. Whereas flotation produces a relatively large volume of dirty concentrate (screening of 14 or more debris filled wells of a microscope slide is not uncommon), IMS gives only one well for screening, and obscuring debris is minimal.

Overall method efficiency

Whilst the efficiency of each of the four individual steps of the analytical technique outlined above may be optimised, the overall efficiency of the technique may still remain relatively low. For example, as demonstrated in table 4 below, even if the efficiency of each step is 80%, which could be considered satisfactory, the cumulative efficiency of the whole technique is only 40%.

Table 4: Effect of efficiency of individual steps on overall method efficiency

Method Step	Step Efficiency	Cumulative Efficiency
1. Collection	80%	
2. Concentration	80%	64%
3. Separation	80%	50%
4. Enumeration	80%	**40%**

Seeding trials on the efficiency of the whole method have been conducted in which 10 litre volumes have been seeded with both *Cryptosporidium oocysts* and *Giardia* cysts, collected by membrane filtration (step 1), concentrated by centrifugation (step 2), separated by Dynal IMS (step 3), and with enumeration by fluorescence microscopy on a slide (step 4). Results described in table 5 indicate that the efficiency of the individual steps in such a procedure must be satisfactory as the overall efficiency is relatively high. As *Giardia* cysts are considerably larger than *Cryptosporidium* oocysts, it is unsurprising that the recovery efficiency of the whole method is significantly higher for *Giardia* than *Cryptosporidium.*

Table 5: Recovery efficiency of whole method for both *Cryptosporidium* oocysts and *Giardia* cysts using membrane filtration and IMS

	Mean Percentage Recovery; (Range)
Cryptosporidium oocysts	39%; (22% - 52%)
Giardia cysts	67%; (52% - 89%)

Conclusions

Whilst there are always going to be room for improvements in the methodology, the following points can be made:

1) Compared to the 'traditional' method, the new method of membrane filtration and Dynal IMS provides consistent and high recovery efficiencies.
2) A lack of detection of parasites can be interpreted with greater confidence, rather than being considered to be a probable method failure.
3) The new methods are gradually being adopted by regulatory authorities world-wide (e.g. US EPA Method 1622).

References

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