# Methods of Testing For *Giardia* in Water

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**ABSTRACT**

Since the 1960's when the first waterborne outbreaks of *Giardia* were reported in America, it has been recognised as a disease causing organism. From these outbreaks the USA Environmental Protection Agency (EPA) developed a method for testing large volumes of water for *Giardia* cysts, this was adapted into the $16^{th}$ edition of the Standard Methods.

To test the method cultured cysts were required for spiked trials. A published method of encystation by Schupp *et al* (1988) was investigated as a potential source of cysts. Morphologically correct cysts were gained in the greatest number at $37^{o}C$ over 72 hours at a bile concentration of 5g/l.

Using cultured cysts and cysts from animals and water, viability and the least number needed to iniate a culture were assessed. When 10 of the cysts produced *in vitro* were excysted it was possible to obtain a culture. For cysts from animal and water origins at levels up to 10,000 cysts, it was not possible to obtain cultures.

Variations of the Standard Method of water testing for *Giardia* had been reported by different laboratories. We investigated the sensitivity of this method using some of the reported variations such as staining on a membrane filter, the use of monoclonal antibody stains and methods of washing cysts free of the sampling core.

We found the method could detect to the $5 \times 10^{2}$cysts/500l of water, a recovery of 10%. The recoveries obtained over a range of cysts spiked was between 10-40%.

An alternative method to sampling and processing the sample was tangential filtration. Four tangential filtration units were compared to the concentration techiques of centrifugation and sedimentation (these were those used in the Standard Method). The tangential filtration units were found not to be as sensitive as centrifugation and sedimentation. They also

presented difficulties with particulate matter or sediment. When compared to the sampling method, the unit was unable to concentrate the 500l of tap water due to the sediment levels.

Staining methods were evaluated. Slide staining was compared to staining on a filter, the filter method was found to give a better recovery. Comparison between commercially available monoclonal antibody stain, a polyclonal antibody stain and Lugols iodine stain, found that the monoclonal and polyclonal antibody stains lead to easier identification by illuminating the cyst (it still had to be checked for internal morphology) than the iodine stain. The monoclonal antibody stains were found to be more specific than the polyclonal stain.

Methods of inactivating the antigens recognised by the monoclonal antibody stain persist so cross contamination between samples was investigated. Hypochlorite concentrations of 4% and higher over 20 minutes were found to inactivate the antigen recognised. Other chemicals were compared but none were found to inactivate the antigen.

A study of a family infected with *Giardia* was undertaken, to test methods used in the laboratory and study modes of transmission. *Giardia* cysts were found in the river that supplied the farm tank but not in the tank itself. The house tank also tested negative for *Giardia*. The family had young children attending school and playgroup, person to person transmission may also have been involved. Animals on the farm had positive tests for *Giardia*.

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