# Detection and Removal in Water Supply of Pathogenic Protozoa: *Giardia lamblia* and *Cryptosporidium parvum*

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*Giardia lamblia* and *Cryptosporidium parvum* are the major parasitic protozoa which are transmitted to humans in the form of (oo)cysts through untreated or treated water. The *Giardia* cysts are 10-16㎛ ovals and *Cryptosporidium* oocysts are 4 - 6 ㎛. Since their presence in water has led to frequent outbreaks of giardiasis and cryptosporidiosis in many countries, methodologies for monitoring and removing this protozoan (oo)cysts from water supply are of great concern for public health. In water treatment facilities, (oo)cysts are mostly removed from the water through chemical coagulation/precipitation and porous-media filtration. However, these protozoan (oo)cysts are resistant to common disinfectants like chlorine because they are covered with a well-defined rigid cell wall. This presentation summarizes the current status and limitations of the methodology for their detection and analysis and the removal tendency in water treatment procedure.

**Cultivation and *in vitro* Encystation of *Giardia lamblia***

The ingestion of *G. lamblia* cysts is followed by the excystation of the cysts to trophozoites and the subsequent colonization of the upper small intestine. The *in vitro* encystation conditions for *G. lamblia* were investigated to enhance the efficiency of cyst conversion and the resulting cyst density. The trophozoite of *G. lamblia* was cultivated to the late exponential growth phase, resulting in a high density over $6\times10^7$ cells/mL. The effects of pH, bile content, and induction time in the encystation medium on the *in vitro* cyst conversion from the trophozoites were examined. A cyst conversion of over 25 % and $10^7$ cysts/mL were routinely obtained using the optimized encystation conditions including a slightly alkaline pH, 10 to 15 mg/mL of bile concentration, and 48-50 hours of induction time.

**Detection and Analysis**

The procedure, suggested by The Method 1623 of US EPA to detect *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts from water sample, consists of filtration, elution, centrifugal concentration, immunomagnetic separation (IMS), and microscopic examination after staining with fluorescently labeled monoclonal antibody and 4',6-diamidino-2-phenylindole (Table 1). Even though the IMS technique greatly improved recovery efficiency for (oo)cysts compared to old methods, it is known that the overall recovery results for the Method 1623 only average less than 50%. We evaluated the extent of (oo)cyst loss in each step of the Method 1623 procedure, by comparing recovery yields for (i) IMS + microscopic examination, (ii) centrifugation + IMS + microscopic examination, and (iii) filtration/elution + centrifugation + IMS + microscopic examination.

Table 1. Procedure of detection and analysis for protozoan (oo)cysts

| Steps | Method |
|---|---|
| (1) Sampling | |
| (2) Filtration | 1 mm PES capsule filter |
| (3) Elution | Elution buffer, wrist action shaker |
| (4) Concentration | Centrifugation |
| (5) IMS | Immunomagnetic separation with antibody-coated paramagnetic microsphere |
| (6) Microscopic examination | ① IFA(immunofluorescence assay): FITC-mAb<br>② DAPI(4',6-diamidino-2-phenylindole) staining<br>③ DIC(differential interference contrast) analysis |

Fig. 1 showed that the overall (oo)cyst recovery was 39-43%. The (oo)cyst loss in IMS step was only 0-12% implying that IMS is a fairly reliable method of (oo)cyst purification. The steps for the centrifugation of eluted solution and collection of pellets before IMS lost 9-25% of (oo)cysts. The largest (oo)cyst loss was occurred at the filtration/elution step showing 35-50% of loss. The permeated loss of (oo)cysts was negligible during the filtration of water sample. These results implies that most of (oo)cyst loss is originating from the poor elution of (oo)cyst from the filter matrix and thus more improvement in the elution methodology is required to enhance recovery yield and reliability for (oo)cyst detection.

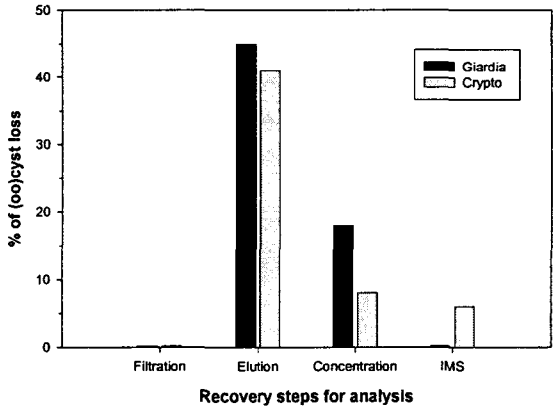


Fig. 1. (Oo)cyst loss during detection procedure.

**Application of PCR**

The polymerase chain reaction (PCR) is a complementary method for the confirmation of (oo)cyst analysis. For the specific detection of protozoan (oo)cysts in water sample, primers of 18S rRNA coding region and mRNA for heat shock protein *Hsp*70 are popularly used. Although PCR is a rapid

method to detect and identify target microorganisms compared to cultivation and infection methods, it is often critical for clinical and environmental samples to distinguish positive and negative results more quickly. When the result of positive or negative is urgently necessary, gel electrophoresis may be considered a time consuming step for such samples. Here we introduce a simple, one-step, homogeneous phase colorimetric detection method for the confirmation of DNA amplification in PCR. The principle of the current method is to detect and quantify the release of PPi from dNTPs, which occur stoichiometrically when DNA is synthesized. The decision for positive or negative result can be made by an absorbance change of visible wavelength or a color change with naked eyes.

**Water Treatment Plant**

We examined the trend and efficiency of pathogenic protozoa removal in a typical water treatment plant, and thus to collect cases of abnormal situation and diagnose causes and derive possible countermeasures to cope with such situations in the future. The typical series of water treatment is composed of coagulation/sedimentation, rapid filter and disinfection.

The protozoan (oo)cysts were found in raw water any time of investigation period. Approximately 90% of (oo)cysts were removed after sedimentation and 95% after sand filtration (Fig. 2). The overall removal efficiencies for protozoa, bacteria and algae were satisfactory in normal situation. The importance of proper backwashing schedule needs to be emphasized because the number of (oo)cysts in filter effluent was sometimes greater than the numbers in sedimentation effluent. It implies that the (oo)cysts caught inside filter media could be eluted with the flow of treated water.

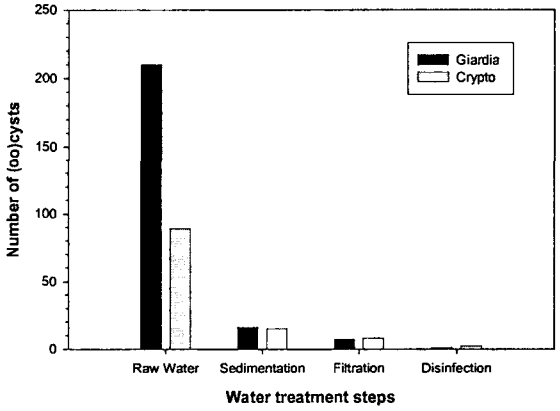


Fig. 2. Trend of (oo)cyst removal in water treatment plant