

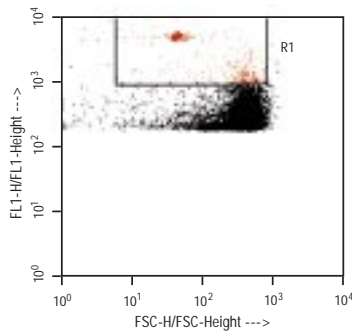
Application Note 11

Detection of *Cryptosporidium* Oocysts and *Giardia* Cysts in Water

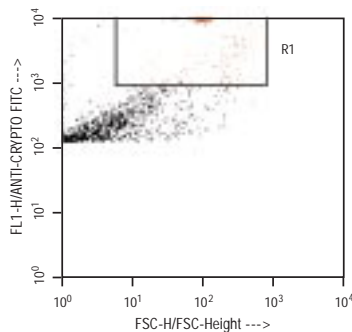
Introduction

The current detection technique for *Cryptosporidium* oocysts and *Giardia* cysts in water is labor intensive and inefficient.¹ A large volume of water (10 to 1000 L) is passed through a cartridge filter, the (oo)cysts are eluted from the filter material together with debris, concentrated by centrifugation, purified by flotation, and stained with FITC-labeled monoclonal antibodies specific to the (oo)cyst wall. During sample treatment significant loss of (oo)cysts occurs, resulting in low overall recoveries.^{2,3} The stained concentrates are examined by epifluorescence microscopy for particles with the morphological and fluorescence characteristics of (oo)cysts. Microscopic examination is time consuming and interpretation is difficult because of the presence of debris. Using a flow cytometer—such as a FACSort™* or FACSCalibur™ to rapidly analyze the light-scatter and fluorescence characteristics of particles, and to sort particles with defined characteristics, made it possible to purify the (oo)cysts from the debris. (Oo)cysts were sorted onto membrane filters. The FACSort produced highly purified, easily examined preparates. Moreover, with the FACSort, a higher percentage of samples was found positive for cysts and oocysts.

* For research use only.



1a



1b

Figure 1 FSC-FL1 dot plots with the sort region (R1):

1a: *Cryptosporidium* oocyst suspension.

1b: *Giardia* cyst suspension.

Materials and Methods

Sample collection

Sixteen river water samples (200 L) and nine samples from sewage treatment plants (sewage: 10 L, secondary effluent: 100 L) were collected using a petrol-driven centrifugal pump and filtered through wound polypropylene cartridge filters having a nominal porosity of 1 mm (Filterite, type DFT classic), at a rate of 3 to 4 L/min. Three samples of reservoir water (1000 to 2000 L) were collected from a pressurized tap.

Sample processing and staining

Filters were processed according to the method described by LeChevallier⁴ with minor modifications.⁵ A fraction of each sample concentrate was processed for direct staining and another fraction for FACSORT analysis. For FACSORT analysis, concentrates were finally siphoned down to approximately 300 mL after flotation and washing. Volumes of 100 mL of undiluted monoclonal antibodies specific to *Giardia* (*Giardia*Cel IF test, Cellabs Diagnostics, Australia) and *Cryptosporidium* (DetectIF *Cryptosporidium*, Shield Diagnostics, UK) were added to resuspended pellets, and incubated for 30 to 45 minutes at 37°C. After staining, suspensions were washed in phosphate-buffered saline (PBS) and filtered through 35-mm mesh filters to remove large particles that could block the FACSORT fluid system.

Flow cytometry and sorting

Samples were analyzed at a flow rate of 60 μ L/min on HI. FITC fluorescence was collected in the FL1 channel of the FACSORT flow cytometer. The instrument threshold was set to FL1. The FACSORT amplifiers were set with FSC and FL1 in log mode; SSC signals were not considered. Green FITC CaliBRITE™ beads were used for flow cytometer setup and performance verification. Instrument control settings were optimized so that stained (oo)cysts appeared in the top of the FSC-FL1 dot plot. Optimal settings were stored and recalled in subsequent analyses. In the FSC-FL1 dot plot a rectangular sort region was defined by analyzing pure suspensions of *Cryptosporidium* oocyst and *Giardia* cyst suspensions. The sort region included both cysts and oocysts. The sort mode was set to RECOVERY to sort all potential (oo)cysts. Sorted particles were collected using the FACSORT Cell Concentrator Module on 13- or 25-mm diameter polycarbonate membrane filters with a porosity of 1.2 mm (Millipore RTTP 01300 or 02500). To assure a smooth filter surface, an additional support filter with a porosity of 5.0 μ m was applied (Millipore SMWP 01300 or 02500). Filters were placed on microscope glass slides with cover slips, embedded in a drop of DABCO-glycerol mounting medium. Preparations were sealed with colorless nail polish, stored at 2° to 8°C in the dark and examined with a Zeiss Axioskop epifluorescence microscope (they were screened at magnification 250x; suspected (oo)cysts were analyzed in more detail at 1000x).

Results

With the FACSsort analysis incorporated in the detection method, a higher number of samples was positive for Giardia and Cryptosporidium and, on average, higher numbers of Giardia cysts and Cryptosporidium oocysts were detected in these samples, than with the method without FACSsort analysis (Table 1).

Table 1 Detection of Cryptosporidium oocysts and Giardia cysts in water samples with and without purification with the FACSsort flow cytometer.

	Giardia		Cryptosporidium	
	% positive samples	Geometric mean concentration per liter	% positive samples	Geometric mean concentration per liter
With FACSsort	96	8.6	74	0.95
Without FACSsort	86	4.1	64	0.38

Figure 1 shows the position of the Cryptosporidium oocysts and Giardia cysts in the sort region. The position of the oocysts in the sort region is clearly distinct from most of the debris particles, but it is obvious that a fraction of the debris is included in the sort region, making microscopic confirmation necessary. Because of their larger size, Giardia cysts give a higher forward scatter signal than Cryptosporidium oocysts. Also, the fluorescence signal of Giardia cysts is higher. Figure 2 shows (oo)cysts from a water sample with the characteristic green fluorescence of the (oo)cyst wall after sorting with the FACSsort flow cytometer. Note the difference with the direct microscopic preparation (Figure 3).

Discussion and Conclusions

Sorting (oo)cysts from water concentrates with the FACSsort on the basis of forward light scatter and specific immunofluorescence resulted in more positive water samples and higher numbers of (oo)cysts found than by direct examination with epifluorescence microscopy. These direct microscopic preparations were usually difficult to interpret: (oo)cysts may have been obscured by debris particles, and weakly fluorescing (oo)cysts were difficult to detect or may have been missed because of background fluorescence. When the FACSsort was used to sort (oo)cysts onto 25-mm diameter membrane filters, the preparations were cleaner and easier to interpret, thus giving more reliable counts. Microscope time was reduced to approximately 15 to 20 minutes by sorting onto 13-mm diameter membrane filters. An absolute discrimination of (oo)cysts from other particles present in environmental samples could not be achieved because of limited discriminative properties of the antibodies used in this study. Not the antibody specificity but rather the strength of the fluorescent staining in relation to the background of (auto)fluorescent debris (algae) causes contamination of the (oo)cyst sort region. Flow cytometric sorting should, therefore, be considered as an additional purification step. Microscopic confirmation of sorted particles

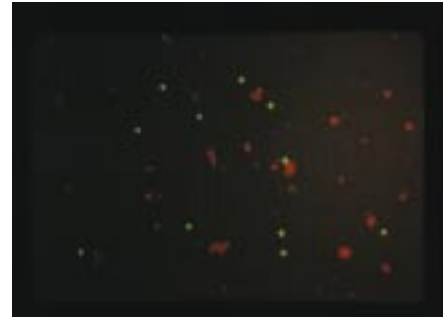


Figure 2 Detection of Cryptosporidium oocysts in a water sample after sorting on FSC and FL1 characteristics. Oocysts are stained with a mAb-FITC conjugate (Shield Diagnostics, UK) and are clearly visible. No debris is present and oocysts can be easily detected.

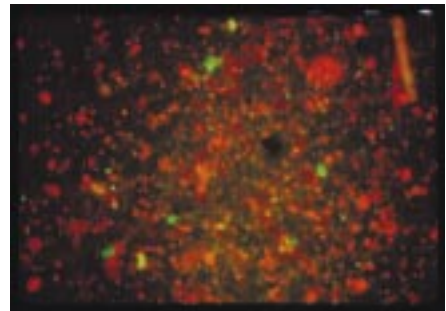


Figure 3 Detection of Cryptosporidium oocysts in a water sample by the standard method. Oocysts are stained with a mAb-FITC conjugate (Shield Diagnostics, UK) and are visible as round, apple-green fluorescing rings. Oocysts are enclosed by debris, which may hide oocysts in microscopic detection.

BDIS publishes this method

as a service to investigators.

Detailed support for non-flow

cytometric aspects of this

procedure may not be

available from BDIS.

is still necessary: in 10 river water samples we analyzed with the FACSsort flow cytometer, the average number of sorted particles was 5100, while microscopic confirmation showed that the average numbers of *Cryptosporidium* oocysts and *Giardia* cysts in these samples were 2 and 55 respectively. The purified state of the sample after sorting, however, makes microscopy easier, faster, and more reliable.

Hints

Commercially available monoclonal antibodies specific to *Cryptosporidium* oocysts vary in the fluorescence intensity they produce with oocysts. For optimal performance of this application on the FACSsort or FACSCalibur flow cytometer, it is desirable to use the monoclonal antibody that gives the most intense fluorescence of oocysts and a low background, to maximize differentiation from debris.

References

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