Factors that affect the performance of and modifications to Method 1623 in detecting *Cryptosporidium* oocysts and G*iardia* cysts in stream water

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Introduction and problem

To improve monitoring for *Cryptosporidium* oocysts and *Giardia* cysts in water, the U.S. Environmental Protection Agency (USEPA) developed Method 1623, which consists of filtration, concentration, immunomagnetic separation, fluorescent antibody staining, and microscopic evaluation. Although the performance of the method has been adequately validated for reagent-grade water and a limited number of source waters, there is a paucity of published data on how the method performs on raw surface waters. In addition, there is little published literature examining the relations between detections of oocysts and cysts and concentrations of indicator organisms (*Escherichia coli*, *Clostridium perfringens*, and coliphage).

Work on improving and modifying Method 1623 is ongoing in the scientific community. One possible improvement is the use of ColorSeed (Biotechnology Frontiers, North Ryde, NSW, Australia), a new product that enables the analyst to determine percent recovery and number of oocysts and cysts in the same water sample. Another promising modification is to use the ultrafilter unit----a low-cost, hollow fiber filtration unit, shown to be effective for concentrating *Cryptosporidium* oocysts from environmental water samples (Otto D. Simmons and others, Univ. of North Carolina—Chapel Hill, written commun., 2000) In addition, methods are being developed that determine the infectious state of the parasites without tedious microscopic examination. Currently, Method 1623 relies on microscopic observation to determine the presence or absence of *Cryptosporidium* and *Giardia* in water concentrates and gives no definitive information on the viability or infectivity of the parasites found. All of these modifications need to be field tested so as to gain USEPA approval for their inclusion in Method 1623.

Factors that affect the performance of and modifications to Method 1623 need to be tested on environmental-water samples collected in a nationally consistent manner. The national network design and objectives of the USGS National Water Quality Assessment (NAWQA) Program are well suited for the addition of microbiological sampling as a component of water-quality.

Goals and objectives

The goal of the study is to further the development and improvement of USEPA Method 1623 for detection of *Cryptosporidium* oocysts and *Giardia* cysts in environmental-water samples. Specific objectives are to

- compare the use of ColorSeed to traditional spiking procedures,
- further validate the performance of the hollow-fiber ultrafilter system for *Cryptosporidium* and *Giardia* recovery from stream-water samples, using Method 1623,
- determine the occurrence of the fecal indicators, *Escherichia coli (E. coli)*, *Clostridium perfringens* (*C. perfringens*), and somatic and F-specific coliphage as they relate to the presence of *Cryptosporidium* and *Giardia* in surface waters,

- identify the water-quality factors that affect recoveries of oocysts and cysts, and
- identify and quantify infectious *Cryptosporidium* oocysts in water samples concentrated by Method 1623, using a cell-culture method.

Approach

Twelve to fifteen NAWQA stream-water sites will be selected to include broad geographic coverage of the United States and a range of climates, land-use practices, and turbidities and will include sites with a high probability of detecting target organisms. The USGS will collect samples during two sampling rounds at each site from July 2001 through July 2002—one during low or medium flow and one during high flow. USGS field crews will add sampling for *Cryptosporidium* and *Giardia*, *C. perfringens*, coliphage, and *E. coli* to their regular water-quality activities.

During each sampling round, four replicate 10-L samples are collected for *Cryptosporidium* and *Giardia*. The replicates will be analyzed as follows:

- regular sample (no spike) analyzed by use of Method 1623,
- regular sample analyzed by use of the cell culture method,
- sample spiked with oocysts and cysts by the traditional method, and
- sample spiked with ColorSeed oocysts and cysts.