Protozoan Method Development Workshop

EXECUTIVE SUMMARY

October 20-22, 1997
Arlington, VA

As part of the EPA's ongoing effort to improve the methodology for *Cryptosporidium* and *Giardia*, the Agency held a public meeting on October 20-22, 1997, in Arlington, VA, to discuss the latest research developments. The purpose of the three-day workshop was to exchange information related to method development and to discuss possible criteria for method performance, within regulatory context, so that the EPA can better assess the state of method development for *Cryptosporidium* and address the needs associated with developing a method within the next two years.

Experts presented research findings that have been recently completed or are underway in the following areas: oocysts/cysts concentration, purification, quantification, and viability and speciation determination. In addition, EPA gave a status report on the development and validation of Method 1622, a new method currently being validated to improve measurement performance for *Cryptosporidium* and *Giardia*.

Background

Cryptosporidium oocysts and *Giardia* cysts are ubiquitous in the environment. The standard method for detecting these protozoa in water samples is the indirect fluorescent antibody (IFA) procedure as specified by the Information Collection Rule (ICR). The ICR method has been heavily scrutinized by scientists which led them to conclude that the method has low capture and recovery efficiencies; the results are widely variable both within and among laboratories; it is difficult to perform and requires a skilled microscopist; and it can determine neither viability nor speciation of oocysts and cysts.

EPA is considering requiring systems to monitor their source water for *Giardia* and *Cryptosporidium* to determine appropriate levels of treatment as a future regulatory option. To establish the level of treatment, it is necessary to be able to measure reliably the occurrence of the organism in the source water. An improved analytical method that meets certain acceptability criteria (e.g., acceptable recovery efficiencies, precision, and accuracy) is a precondition for implementation of the proportional treatment approach or the watershed-based approach (described below).

Summary

As part of the workshop, EPA described three rule options that are currently being considered for future regulations. These include: 1) fixed treatment approach (i.e., all systems would be required to provide at least the same level of treatment); 2) proportional treatment approach (i.e., level of treatment required to achieve a desired risk level would be based on the density of *Giardia* and *Cryptosporidium* in source water during the reasonably worst case occurrence period); and 3) watershed-based approach (i.e., systems would be required to monitor the source water and provide a level of treatment based on a combination of factors that indicates the level of vulnerability of the source water to pathogen contamination). The proportional treatment approach and watershed-based approach require source water monitoring. Therefore, an accurate and precise analytical method is essential to these two options.

EPA also described the approach that is being considered for defining method performance criteria. This approach is based on analysis of source waters with low pathogen occurrence levels (typical of systems with protected watersheds), assumed probability distributions of protozoan occurrence, and the assumption that there would be possible ranges of acceptable misclassification rates. Under the EPA's approach, systems could minimize the misclassification rate if they voluntarily increased their sampling

frequency above the minimum required. Misclassification might lead to an overestimation or an underestimation of the treatment removal presumed to be necessary to comply with a regulation. An overestimation would impose excess compliance cost, while an underestimation would lead to less treatment than intended and, therefore, a lower compliance cost, higher microbial risk, and possibly lower disinfection by-product levels.

EPA's derivation of the method performance criteria is as follows: (1) for a 15% or less chance of misclassifying the 90th percentile by 0.25 logs, 75% or greater recovery is needed if sampling includes 18 or 24 samples (sample volume of 25 liters or 10 liters, respectively) -or- 50% or greater recovery is acceptable with respective sample volumes of 50 liters or 25 liters; (2) for a 5% or less chance of misclassifying the 90th percentile by 0.5 logs, 25% or greater recovery is needed if sampling includes 18 or 24 samples (sample volume of 10 liters). The desired method performance criteria will probably be at least as good as (2) but depending upon significant factors (i.e., national distribution of level of treatment in place, national distribution of site-specific probability distributions of oocyst concentrations, and other water quality factors, basis of source water concentration levels: total oocyst count vs. viable oocyst count vs. infectious oocyst count, and the defined acceptable risk level, 10^{-4} or 10^{-3} level) the desired method performance criteria may approach (1).

The following include some of the method components presented by workshop experts: hollow fiber filtration, blood cell separator, flow cytometry, immunomagnetic separation, cell culture techniques, fluorochrome viability techniques, molecular probes and signature lipids. All of these have varying degrees of promise depending on site-specific water matrix factors, sensitivity desired for analysis, and cost considerations. Of the components presented, the Gelman capsule filter, flow cytometry, and use of fluorescent *in situ* hybridization (FISH) probes to determine speciation appeared most promising (yielding higher recovery efficiencies, low detection limits and reasonable cost per sample) as for being available by 1999. Under a proportional treatment technique or a watershed-based technique approach, where source water monitoring for protozoa would be required, EPA might allow systems to use a variety of methods that met the EPA's specified minimum performance criteria. Depending upon the source water characteristics, different method components may be more appropriate than others for meeting minimum method performance criteria.

After the presentations, the workgroup discussed the feasibility of incorporating the method components into an analytical method for *Cryptosporidium*. Critical issues raised concerning the methods presented, as well as the EPA's method performance criteria approach, were: 1) the need for subjecting the various method components and complete methods to different water matrices; 2) developing several methods for use with different water matrices and defining performance criteria for each type of water matrix (level of organic and inorganic matter, type and level of potentially interfering microbes, etc.); 3) defining threshold detection levels for the various method components; 4) need to develop criteria for establishing efficacy of any antibodies used in the method; 5) need for a reliable, readily available source of oocysts for research and to establish method performance; 6) identifying age and condition of oocysts used during method development research; and 7) need for the EPA to explain how it intends to use viable versus nonviable cysts/oocysts for regulatory purposes.

One workshop expert gave a presentation that demonstrated that to assure that the development of new methods proceeds as quickly and reliably as possible, it is essential that scientists document materials (i.e., quality of oocysts) and methods (i.e., enumeration techniques) when performing studies with *Cryptosporidium* oocysts. Such documentation of information would allow the study to be repeated by others and allow investigators to properly compare the accuracy and precision of the method with other analytical methods.

The workgroup agreed that future information exchange meetings would be beneficial. It was suggested that the workgroup reconvene to discuss research at annual conferences such as the American Society for Microbiology (ASM) and the American Water Works Association's Water Quality Technology Conference (WQTC).

Next steps

There will be a meeting on Tuesday, December 16, 1997 at RESOLVE, 1255 23rd Street, Suite 275, Washington, D.C., to summarize the findings and other information discussed during the workshop for stakeholders. EPA will present its programmatic and regulatory needs, describe the method performance criteria approach presented at the workshop, present available and near-term *Cryptosporidium* analytical methods, and discuss possible methods that might be appropriate for future regulations. The proceedings from this workshop will be available by March 1998.

For further information about this workshop or future *Cryptosporidium* method development meetings, contact Crystal Rodgers at (202) 260-0676, 401 M Street, SW (MC 4607), Washington, D.C. 20460, or by e-mail at rodgers.crystal@epamail.epa.gov. To register for the December 16 stakeholder meeting, please contact Eddie Scher of RESOLVE at (202) 965-6203 or Escher@RESOLV.org.