Cell Culture Good Test of Crypto Infectivity

The drinking water profession is extremely concerned about the threat of Cryptosporidium and its effect on water supplies. Determining viability and infectivity for naturally occurring oocysts and those that have been exposed to disinfectants remain key issues. Although mouse models have generally been suggested as the “gold standard” for measuring oocyst infectivity, these assays have not met many of the criteria associated with a standard method. Points to consider include:

- A variety of mouse species has been used, including newborn Swiss-White, neonatal Balb-C, and outbred neonatal CD-1 (Balb-C and CD-1 being the most popular).
- Mouse infectivity has not been correlated with human infectivity.
- Mice do not support growth of the recently discovered human-specific genotype.
- Natural variation within mouse litters and susceptibility to infection by Cryptosporidium have not been reported.
- Determination of infection varies by laboratory protocol: (1) tissue sections can be evaluated using a variety of methods and (2) fecal samples can be evaluated using floatation techniques and microscopic evaluation.
- Enumeration may be performed by excretion rates, mathematical models using ID<sub>50</sub> calculations, or most-probable numbers.
- Round-robin testing has never been performed and thus far only one interlaboratory evaluation has been documented.

An alternative infectivity assay is available: cell culture. Cell culture has been used since the 1950s to study the infectivity of waterborne viruses and other coccidians and is a widely accepted tool for research. Cryptosporidium cell culture began in the 1970s and has been used extensively in the testing of pharmaceuticals. Water laboratories have already demonstrated through peer-reviewed literature the high sensitivity of cell culture to a single oocyst. ¹⁻⁶ Cell culture has also been used to detect naturally occurring oocysts. ⁷

With more than eight laboratories now using a common cell culture technique for viability and disinfection testing, round-robin testing can be achieved quickly and easily. The drinking water profession should move quickly to support round-robin testing and evaluate this powerful method for testing Cryptosporidium infectivity. When alternatives are available, it is no longer ethically acceptable to use animals.

Joan B. Rose, Theresa Slifko, and Debra Huffman
University of South Florida

References

1. Rochelle, P.A. et al. An Assay Combining Cell Culture With Reverse Transcriptase PCR to Detect and Determine the Infectivity of Waterborne Cryptosporidium parvum. Applied & Envir. Microbiol., 63:5:2029 (1997).
2. Slifko, T.R. et al. Unique Cultural Methods Used to Detect Viable Cryptosporidium parvum Oocysts in Environmental Samples. Water Sci. & Technol., 35:11:363 (1997).
3. Slifko, T.R. et al. An In-Vitro Method for Detection of Infectious Cryptosporidium Oocysts Using Cell Culture. Applied & Envir. Microbiol., 63:9:3669
4. Slifko, T.R. et al. Comparison of Four Cryptosporidium parvum Viability Assays: DAPI/PI, Excystation, Cell Culture, and Animal Infectivity. Proc. 1998 WQTC, San Diego.
5. Slifko, T.R. et al. A Most-Probable-Number Assay for the Enumeration of Infectious Cryptosporidium parvum Oocysts. Applied & Envir. Microbiol., (in press).
6. Slifko, T.R. et al. Impact of Purification Procedures on the Viability and Infectivity of Cryptosporidium parvum Oocysts. Proc. IAWQ Conf. On Waterborne Particles. Paris (1999).
7. DiGiovanni, G. et al. Detection of Infectious Cryptosporidium parvum in Surface and Filter Backwash Water Samples Using Immuno-magnetic Separation and Integrated Cell Culture–PCR. (Unpubl.).