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Cryptosporidium Infectivity by Cell Culture

Introduction

Following an extensive research and development project, ALS Melbourne now offers **NATA accredited *Cryptosporidium* Infectivity** assessment by cell culture from various water matrices including drinking, source/recreation and Class A recycled water. The accredited method follows APHA standard method 9711.

What is Cryptosporidiosis?

Cryptosporidiosis is a diarrheal illness infecting individuals with weakened immune systems as well as healthy humans. The environmental stage of the organism's life cycle, the oocyst, is excreted in the faeces of infected animals and humans and can contaminate sources of drinking water.

Water Industry Applications

An important element to accurately assess the exposure risk is to measure the prevalence of infectious oocysts in source/recreational, recycled and drinking waters. Determining the infectious status of *Cryptosporidium* oocysts present in a given water sample is particularly important for:

- Quality Microbial Risk Assessment studies
- Effective watershed management
- Efficacy assessment of disinfection processes
- HACCP planning.

Who Can Benefit?

Cryptosporidium Infectivity provides valuable data for any supplier of water destined for human consumption including catchment and water supply authorities, recycling scheme operators, disinfection/validation programs and local councils.

Method Information

ALS METHOD CODE

MM655

LIMITS OF REPORTING (LOR)

1 infectious oocyst per volume analysed

METHOD REFERENCE

Standard Methods for the Examination of Water and Wastewater, 22nd Ed. Section 9711, Pathogenic Protozoa, Part D, Infectivity of Cryptosporidium in Cell Culture.

Analysis of Cryptosporidium Infectivity

Infective *Cryptosporidium* oocysts are identified by ***in vitro*** culture on HCT-8 cells, which support the growth of multiple *Cryptosporidium* species including those that cause the majority of human infections. The developmental stages of the *Cryptosporidium* life cycle are identified in the infected HCT-8 cells by labelling with fluorescent markers and identifying fluorescent foci of infection by microscopy (Figure 1). The method is collectively known as the cell culture foci detection method (CC-FDM).

Advantage of Infectivity Testing

Conventional techniques to identify *Cryptosporidium* oocysts by microscopy do not provide any indication of whether the oocysts are infective or not and therefore cannot properly assess risk to human health. In general, less than 20% of *Cryptosporidium* oocysts excreted are infective. The analysis of infective *Cryptosporidium* oocysts by CC-FDM provides **a more accurate way to assess human health risk**.

Cryptosporidium Infectivity analysis enables:

- A potential reduction in the implementation of costly risk management strategies e.g. boil water notices, alternative water supplies;
- Accurate assessment of health based targets for microbial safety;
- Accurate assessment of the efficacy of chemical disinfection in the inactivation of oocysts.

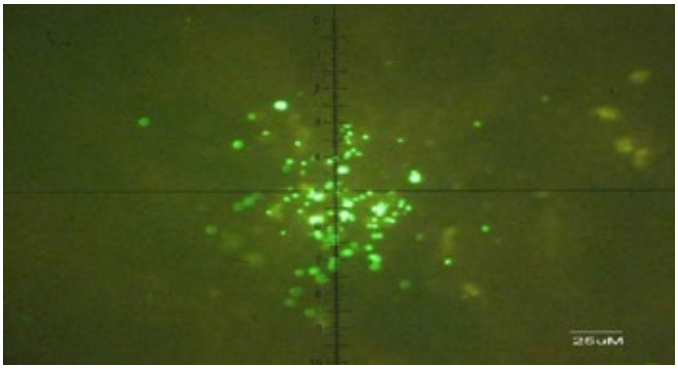

Figure 1: Cryptosporidium parvum infectious focus of detection (a.k.a foci) demonstrating various cell culture developmental stages under fluorescent (FITC) microscopy at 400x magnification.

Methodology

The 22nd Edition of Standard Methods for the Examination of Water and Wastewater was updated to include a ***Cryptosporidium in vitro*** cell culture assay (CC-FDM) (Section 9711, Part D). The published assay is the consensus of published methods using the HCT-8 cell line. The method outlines recovering indigenous oocysts from waste water, environmental water, recycled water or potable water by the USEPA Methods 1622 and 1623, which are then inoculated onto HCT-8 cells for ***in vitro*** growth (Figure 1) instead of being applied to a microscope slide for enumeration.

Proof of Concept

While multiple techniques (e.g. excystation, vital dye staining, reverse-transcriptase PCR, and ***in vitro*** cell culture with RT-PCR [CC-RTPCR]) are available to assess oocyst viability/infectivity, only the described ***in vitro*** cell culture (CC-FDM) assay has been demonstrated to be equivalent to the Gold Standard mouse infectivity model for measuring ***C. parvum*** infectivity. The assay was deemed to be the most suitable assay based on sensitivity (1 to 3 oocysts) and fewest false positives when compared to the CC-PCR and CC-RTPCR assays. In addition, HCT-8 cells support the growth of the three ***Cryptosporidium*** species responsible for 99% of human infection (***C. parvum, C. hominis*** and ***C. meleagridis***).

Sampling Requirements

Holding Time:	4 days (96 hours)
TAT:	5 – 7 days, upon sample receipt in Melbourne
Sample Shipping and Storage:	<20°C§
Sample Containers:	Clean plastic containers¥, 5L and 10L or field sampling filtration
Sample Volume:	Environmental Water: 10 – 50L, pending turbidity Swimming pools and water parks: 50 – 100L Treated Water: 50 – 100L Bore Water: 10 – 50L, pending turbidity Scat: 0.5g

§ In hot conditions and/or remote locations requiring overnight air-freight, ALS recommends that containers be immediately placed in an esky upon sampling and covered with sufficient ice (or ice bricks) to chill the sample.

¥ Chlorine decreases oocyst recovery and infectivity, therefore samples treated with chlorine (i.e. drinking water and swimming pools) need to be neutralised with 10% sodium thiosulphate.

For further information please contact the ALS Melbourne Client Services Team on (03) 8756 8000.

References

Johnson, A. M., G. D. Giovanni, and P. A. Rochelle. 2012. Comparison of assays for sensitive and reproducible detection of cell culture-infectious ***Cryptosporidium parvum*** and ***Cryptosporidium hominis*** in drinking water. Appl Environ Microbiol. 78:156-162.

Lalancette, C., G. D. Di Giovanni, and M. Prevost. 2010. Improved risk analysis by dual direct detection of total and infectious ***Cryptosporidium*** oocysts on cell culture in combination with immunofluorescence assay. Appl Environ Microbiol 76:566-577.

Rochelle, P. A., A. M. Johnson, R. De Leon, and G. D. Giovanni. 2012. Assessing the risk of infectious ***Cryptosporidium*** in drinking water. Jour. AWWA 104:79-80.

Rochelle, P. A., M. M. Marshall, J. R. Mead, A. M. Johnson, D. G. Korich, J. S. Rosen, and R. De Leon. 2002. Comparison of in vitro cell culture and a mouse assay for measuring infectivity of ***Cryptosporidium parvum***. Appl Environ Microbiol 68:3809-3817.

US EPA Method 1623 ***Cryptosporidium*** and ***Giardia*** in water by Filtration /IMS/FA.USEPA December 2005.EPA-815-R-05-002.Office of Water, Washington, DC 20460.