

## **UK WATER INDUSTRY RESEARCH LIMITED**

### **INTERNATIONAL COLLABORATIVE CELL CULTURE AND U/V STUDIES**

**Report Ref. No. 08/DW/06/21**

#### **Executive Summary**

In 2001 an agreement was entered into by representatives of the Drinking Water Inspectorate (DWI), the United Kingdom Water Industry Research (UKWIR), the American Water Works Association Research Foundation (AWWARF), the United States Environmental Protection Agency (US EPA), Kiwa and the Water Services Association of Australia (WSAA) to collaborate to implement a research programme into “*Cryptosporidium* Cell Culture and UV Treatment”. The aims of the programme were set out as:

- a) to identify an optimum cell culture technique that is suitable for routine use in disinfection studies and general risk assessment. This Phase would include proficiency trials on up to seven alternative cell culture assays.
- b) to investigate technology transfer and performance testing of the selected protocol(s). This will involve a Workshop that allows representatives of Phase 2 participants to receive training in the scientific basis and practical aspects of the assays. They will also be informed of the requirements for their subsequent performance testing trials.
- c) to apply the cell culture technique in UV treatment studies at pilot scale or plant scale.

A Management Group comprising representatives of UKWIR, AWWARF, WSAA, DWI, Kiwa and US EPA was established to manage the project, with support from Expert Advisory Groups that were to be established for each phase of the project.

The first phase of the project was put out to tender on two separate occasions without eliciting sufficient response to undertake proficiency trials for assessing infectivity of cryptosporidial oocysts amongst a number of laboratories. Having been unable to let a contract to compare a number of available tissue culture infectivity technologies, an optimized cell culture-immunofluorescence (IFA) procedure, using the HCT-8 cell line, was evaluated in ‘blind’ trials. Flow cytometry sorted suspensions consisting of between 0-100% viable oocysts were prepared, shipped to the testing laboratory and analyzed ‘blindly’ by cell culture-IFA. Excystation of oocysts was also assessed. Data indicated the control (100% live) oocyst suspensions yielded statistically similar results to a cell culture dose response curve data developed previously at the testing laboratory. For test samples containing oocyst suspensions of unknown infectivity, cell culture-IFA analyses indicated a high degree of correlation with the infectivity of the samples as prepared. Cell culture infectivity has been shown to correlate well with neonatal mouse infectivity assays and these ‘blind’ validation trials provide credibility for the cell culture-IFA procedure as a cost-effective and expedient alternative to mouse infectivity assays for determining *in vitro* infectivity of *C. parvum* oocysts.

Attempts by the Moredun Research Institute, Edinburgh to implement the tissue culture/RT-PCR infectivity assay for *Cryptosporidium parvum*, developed by Metropolitan Water Company of Southern California at the time of the study, were unsuccessful. Moredun found the method insufficiently sensitive and reproducible to allow its routine use. At best, the assay proved significantly more sensitive than neonatal mouse infectivity, but such performance was relatively infrequent, while reproducibility could vary considerably day to day.

The preliminary testing of a novel low pressure UV device for disinfection of *Cryptosporidium parvum* oocysts in a lowland river water demonstrated that disinfection could occur in turbidities up to 5 ntu, using *in vitro* excystation as a demonstration of oocyst viability.