Vital Dye Staining of *Giardia* and *Cryptosporidium* [Project #160]

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**OBJECTIVES**
The main objective of this research was to develop vital dye staining methodology for testing the viability of *Giardia* spp. and *Cryptosporidium* spp. that can be incorporated into the existing immunofluorescence assay used for detection of protozoan parasites in drinking water.

**BACKGROUND**
An ongoing challenge in disinfection of *Giardia* and *Cryptosporidium* is the difficulty in defining the viability of parasites before and after disinfection. The ideal test for defining viability of the parasites is to determine their infectivity in susceptible hosts. However, animal infectivity assays are difficult and time consuming relative to the normal laboratory analyses in the water industry. Thus, vital dye staining assays needed to be developed as surrogates for animal infectivity assays.

**HIGHLIGHTS**
- A vital dye staining technology for *Giardia* and *Cryptosporidium* was developed that was stable in untreated, heat-inactivated, and chemically-treated parasites.
- When properly optimized, vital dye staining was not related to the in vitro excystation of cysts or oocysts but was related to animal infectivity.
- The combined immunofluorescence-vital dye assay developed in this study will be useful for the evaluation of the presence of parasites in water samples, as well as testing the inactivation of organisms after chemical disinfection.

**APPROACH**
The approach was to evaluate the staining of *Giardia* cysts and *Cryptosporidium* oocysts using dyes that differed in size, change, and staining characteristics. The staining results were then compared to animal infectivity and in vitro excystation using split samples of cysts and oocysts.

**RESULTS**
Among the dyes tested, syto-9 and syto-59 were found to stain parasites more consistently over a wide range of conditions including temperature, pH, chemical disinfection, and heat inactivation. These dyes were incorporated into the existing immunofluorescence assay. This assay will be an invaluable tool for detection of viable organisms in drinking water.

**IMPACT**
It is believed that the nucleic acid dye technology developed in this study will be invaluable for the assessment of the viability of protozoan parasites in drinking water and the assessment of the inactivation of organisms after chemical disinfection. Properly used and optimized, this vital dye technology may in the future replace existing viability determination technologies such as in vitro excystation and ultimately, animal infectivity analyses.
PARTICIPATING UTILITY

Aqualta Ltd., Edmonton, Alta., Canada