Inactivation of Cryptosporidium Oocysts for Use in Immunological and Molecular Assay Applications

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Background
- Cryptosporidium spp. are obligate, intracellular parasites that can cause life-threatening diarrhea among children and immunocompromised adults [1, 2].
- Infection with the parasite is transmitted orally by thick-walled oocysts that can contaminate, persist, and resist disinfection in water and food [3].
- Previous studies reported the inactivation of oocysts by disinfectants such as sodium hypochlorite, peroxides, ozone, formaldehyde, and ammonia [4, 5]. The regular use of effective concentrations of these chemicals to produce inactivated oocysts in the laboratory is limited due to safety concerns and possible impact on the integrity of parasite antigens and nucleic acids.
- Molecular assays are replacing conventional methods for the detection of Cryptosporidium and other intestinal parasites in clinical and environmental samples [6, 7].
- There are limited studies on laboratory methods of oocyst inactivation that retain the properties of Cryptosporidium antigens and nucleic acids. Inactivated oocysts can be subsequently used in downstream research applications such as assay development performed under BSL1 conditions.

Objectives
- Evaluate simple methods of inactivation of Cryptosporidium oocysts that can be readily applied in the laboratory.
- Examine the loss of oocyst viability in vitro and determine the utility of non-viable oocysts stored for long periods of time in immunological and molecular assays.

Experimental Approach

**Inactivation of Cryptosporidium parvum oocysts**

**Assay Stability**

<table>
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<th>Oocyst strain</th>
<th>Inactivated Oocysts</th>
<th>Assessment of viability</th>
<th>In vitro infection assay</th>
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<td><em>C. parvum</em> Iowa strain</td>
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**Inactivation treatment**

- 1:10 negative control
- 2:heat (75°C, 10 min)
- 3: 0.05% H2O2
- 4: 0.05% H2O2 + 4°C

- Oocysts of *C. parvum* Iowa strain (5 x 10⁸, Sterile Labs, University of Arizona) were treated with increasing concentrations of ethanol or methanol (50-100%) for 30 min to 24 h at 4°C. Oocysts suspended in water or inactivated by heat (75°C, 10 min) were used as negative or positive controls of inactivation, respectively.

- Viability assays based on propidium iodide (PI) permeability, in vitro excystation of sporozoites, and infection of the Hct-8 cell line (ATCC® CCL-244™) were used to evaluate the effectiveness of the treatments.

- Inactivated oocysts recovered from the most effective treatments were tested in immunological (IFA, westerns) and molecular (qPCR) assays to determine their utility as reference reagents. The stability of inactivated oocysts in these assays was examined following long-term storage at 4°C.

**Results**

**Fig. 1. Inactivation of Cryptosporidium parvum oocysts**

**Results**

**Fig. 2. Propidium iodide incorporation into C. parvum oocysts**

**Fig. 3. Exocystation of *C. parvum* sporozoites following inactivation treatments**

**Fig. 4. Infectivity of *C. parvum* sporozoites following inactivation treatments**

**Fig. 5. Antigenicity of inactivated *C. parvum* oocysts**

**Fig. 6. Utility of inactivated *C. parvum* oocysts in qPCR**

**Summary**

- We present a convenient and simple method of inactivation of Cryptosporidium oocysts that can be readily used in the laboratory.
- Oocysts inactivated by 70% methanol for 24 h exhibit complete loss of viability in vitro and retain antigenicity, even after long term storage at 4°C.
- Oocysts inactivated by 70% methanol, or 100% ethanol are suitable for use as reference reagents in qPCR-based assays.

**References**