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# Project Summary

# Cytochemical Methods for Assessing *Giardia* Cysts Viability

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**Because of the high incidence, symptomology, and waterborne dissemination of *Giardia*, improved methods for determination of viability of cysts detected after isolation from water systems is necessary to assess potential danger to human health.**

**The viability of *Giardia* cysts has been assessed by eosin exclusion, *in vitro* excystation, and animal infectivity.**

**While each of these methods has its own value, none are both rapid and reliable.**

**The objective of this study was to develop a reliable, rapid, microscopically read method for determining the viability of *Giardia* cysts which is comparable to excystation and would provide a more practical method of estimating cyst viability.**

**Though methods dependent on cyst metabolism such as the activity of pyruvate: ferredoxin oxidoreductase (PFOR) and cell respiration gave promising results with trophozoites, they proved unreliable with cysts.**

**Hence, exclusion of the fluorescent dye, 3-(Dansylamido)-phenyl boronic acid (FluoroBora I) from *Giardia muris* cysts was compared with excystation as a measure of cyst viability. The effect of 22 different chemicals on excystation was determined and compared with the exclusion of the fluorescent dye FluoroBora I (FBI) under identical conditions. These data indicate that the dye exclusion method has potential for use as an alternative method to excystation as a measure of cyst viability.**

**Preliminary evidence suggests that the method, with some modifications, has the potential for use in the determination of the viability of *Giardia lamblia* cysts.**

***This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).***

## Introduction and Project Goals

*Giardia lamblia* is the most common human intestinal protozoan parasite reported in the United States and England. The organism exists in two morphologically distinct forms, the trophozoite and the cyst. The infective cyst form is transmitted via the fecal-oral route.

The high incidence, symptomology and waterborne dissemination of *Giardia* has resulted in improved methods for cyst detection in water systems. However a rapid, simple, reliable method for determining the viability of cysts, detected in water systems, and exposed to water treatment procedures is needed.

The viability of *Giardia* cysts has been assessed by eosin exclusion, *in vitro* excystation, and animal infectivity. While each of these methods has its own value, none are both rapid and reliable. Although eosin exclusion is a rapid method, it indicates higher cyst viability than can be demonstrated by excystation and shows little correlation with excystation. Excystation is the most frequently used method for determining cysts viability. It is tedious (2-3 h), and subjective. It cannot be used for determining the viability of individual or small numbers of cysts. The cost, time and large numbers of cysts required to perform animal infectivity studies make the method impractical for routine use.

The objective of this study was to develop a reliable, rapid, microscopically read method for determining the viability

of *Giardia* cysts which when compared to excystation would provide a more practical method of estimating cyst viability. The initial studies into developing an assay for viable cysts were dependent on various metabolic parameters such as cyst respiration and specific enzyme activities. The research was based on some preliminary data on cyst metabolism obtained in the author's laboratory. Cysts of *G. lamblia* respire in the presence of $O_2$ without added exogenous substrate at approximately 1/10 the rate of trophozoites (8 nmoles $O_2$ consumed $min^{-1}$ mg $protein^{-1}$ at 37°C). Cysts after activation at low pH and subsequent placement in nutrient medium increase their respiration five fold. The enzyme, pyruvate: ferredoxin oxidoreductase (PFOR)—a major enzyme in cell energy metabolism and an enzyme found only in anaerobes such as *Giardia*—is active in cyst homogenates. These data, though preliminary, were used in part in our attempts to develop a metabolic dependent direct microscopic measure of cyst viability.

Initially, attempts were made to assess viability with indicators of metabolic activity. These metabolic studies using PFOR and cell respiration were equivocal in that they were not effective with cysts, Triton treated cysts or induced cysts for measuring cyst viability. Unlike respiration, PFOR activity as measured by accumulation of nitroblue tetrazolium (appearance of a blue color throughout the cell) could be used for determining *G. lamblia* trophozoite viability. Neither method proved useful for determining cyst viability possibly because of cyst permeability.

In 1982 investigators published a method called boronic acid-dependent phase transfer, or "boradeption." In this method, specific fluorescent water-insoluble boronates are excluded by non-viable cells *in vitro*. When this technique was attempted with *Giardia*, the results using FBI with *Giardia lamblia* trophozoites, were similar to those found by previous investigators using CHO cells, i.e , non-viable cells excluded the dye. In contrast, viable *Giardia* cysts excluded the dye. This exclusion method using *G. muris* cysts, when compared with excystation, showed a high degree of association and a higher degree of reproducibility and precision as compared to excystation. Preliminary evidence suggests that the method, with some modifications, has the potential for use in the determination of the viability of *Giardia lamblia* cysts.

The goals of the project were the following:

1. to develop a simple and quick microscopically read method for determining *Giardia* cyst viability, useful to investigators with minimal training.

2. to correlate the new method with the currently accepted method of determining cyst viability, excystation.

3. to use the new method with *G. lamblia* as well as *G. muris* cysts.

4. to establish the reliability of the new method on low numbers of cysts (<50).

5. to assess the compatibility of the new method with immunological detection methods for *Giardia* cysts in water samples.

## Conclusions and Recommendations

a. Enzymatic and metabolic methods for determining viability of *Giardia* cysts proved unfeasible at the microscopic level.

b. Exclusion of the fluorescent dye 3-(Dansylamido)-phenyl boronic acid (FluoroBora I, FBI) from *Giardia muris* cysts shows good correlation (at cyst viability > 60%) with excystation as a method for determining cyst viability when viability of cysts was measured after contact with different disinfectants under identical conditions.

c. The FBI method is simple to perform, rapid (5 min) and can be microscopically read.

d. For untreated cysts, the FBI method shows a higher degree of precision between investigators than excystation at viability levels above 60%.

e. The FBI method is less subjective than excystation since (1) it relies on the observation of fluorescence or non-fluorescence as a measure of viability, whereas excystation requires differentiation between several stages of excysting organisms and (2) the excystation procedure is long and tedious (2-3 h) requiring control of a number of variables (temperature, pH, reducing conditions, etc.) not encountered in the FBI method.

f. The FBI method can be used to assess the efficacy of disinfectants in a rapid manner even if these compounds cause clumping. Clumping makes the excystation method not possible because of the difficulty in counting.

g. The FBI method has not yet been shown to be compatible with an immunofluorescence method for detecting *Giardia* cysts in water samples.

h. FBI estimates of viability are consistently higher than by excystation.

i. FBI can be used to estimate viability of *G. lamblia* cysts if the FBI method is compared with motility of trophozoites inside the cyst during excystation in contrast to excystation. This may point to the suggestion that low excystation rates of *G. lamblia* may be due to the excystation environment.

j. Based on the available data, the FBI method has the potential for use in the rapid assessment of *Giardia* cyst viability in the laboratory. The FBI method can be used for a quick assessment of disinfectants and chemotherapeutic agents in the laboratory.

k. Recommendations for further work include:

   1. comparison of optimized *G. lamblia* excystation method.

   2. comparison of excystation with FBI at viability 0-100 (esp < 50%).

   3. if no optimal *G. lamblia* excystation, in depth comparison of motility within the cyst and FBI under conditions of chemical treatment.

   4. improve compatibility of FBI with existing detection methods (conventional and immunofluorescence).

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*The complete report, entitled "Cytochemical Methods for Assessing* Giardia *Cysts Viability," (Order No. PB 88-124 748/AS; Cost: $14.95; subject to change) will be available only from:*

*National Technical Information Service*
*5285 Port Royal Road*
*Springfield, VA 22161*
*Telephone: 703-487-4650*

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