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Improved In Vitro Excystation Procedure for *Giardia lamblia* Cysts

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Giardia lamblia cysts obtained from human symptomatic and asymptomatic donors were excysted in vitro. Excystation averaged 87% for cysts from symptomatic donors and 70% for cysts from asymptomatic donors.

Giardiasis has become one of the most frequently occurring parasitic infections in the United States (4, 6). The etiological agent, *Giardia lamblia*, is an intestinal protozoan which is transmitted in the cyst stage. The increase in the incidence of this disease has prompted the need for a reliable bioassay system which offers a method for evaluating the percentage of living cysts in a given cyst population. Attempts to excyst *G. lamblia* cysts on a routine basis by using the Bingham-Meyer procedure (2) have been unsuccessful in our hands. The excystation procedure presented in this study represents an improvement over the Bingham-Meyer method and allows for the excystation of cysts isolated from both symptomatic and asymptomatic donors. The distinctions between symptomatic and asymptomatic carriers described by Wolfe (6) were used in our work.

G. lamblia cysts, present in fecal specimens obtained from two symptomatic donors and three asymptomatic donors, were separated from the fecal material by flotation on 1 M sucrose in a modification of the procedure of Sheffield and Bjorvatn (5). Two separate batches of cysts were obtained from each of the asymptomatic donors. Cysts were stored in distilled water at 5°C for a minimum of 1 week to allow for the cyst maturation period described by Bingham et al. (1). The maximum storage time for the cysts was 21 days. Cyst densities were determined by hemacytometer counts.

The excystation procedure comprised two steps: a low-pH induction step utilizing three separate solutions, and an excystment step utilizing an excystment medium. The procedure was conducted in 15-ml plastic conical screw-cap centrifuge tubes containing 0.5 ml of a cyst suspension with a total cyst density of 5×10^5 cysts. In the induction step, 5 ml of aqueous hydrochloric acid, pH 2.0, 2.5 ml of 1× Hanks balanced salt solution supplemented with 29 mM L-cysteine hydrochloride and 67 mM glutathione, and 2.5 ml of 0.1 M sodium bicarbonate were added to the cyst suspension in that order. After the last addition was made, the tube was immediately capped tightly, and the contents were blended. The acid and Hanks solution were prewarmed to 37°C, whereas the bicarbonate solution was hydrated just before use. The induction step was completed by incubating the mixture for 30 min in a 37°C water bath. At the conclusion of the initial incubation, the cysts were sedimented by centrifugation at 1,000 × *g* for 2 min, and the supernatant was discarded.

The medium used in the excystment step was composed of 0.5% (wt/vol) trypsin (1:100) dissolved in 1× Tyrode solution. To prepare the excystment medium, trypsin was dissolved in the Tyrode solution by vigorous mixing for 30 min. The undissolved trypsin was removed by centrifugation at 21,000 × *g* for 10 min. The supernatant was removed and filtered through a 0.45-μm membrane filter by positive pressure. The medium was adjusted to pH 8.0 with 7.5% (wt/vol) sodium bicarbonate and prewarmed to 37°C. The excystment step was initiated by suspending the cysts in 10 ml of the excystment medium. The cysts were again sedimented by centrifugation at 1,000 × *g* for 2 min, and the supernatant was discarded. The cysts were then resuspended in 0.5 ml of fresh excystment medium, and depression slides were filled with the suspension. The slides were covered with a glass cover slip, sealed with Vaseline-paraffin, and incubated in the inverted position for 1 h at 37°C in a warm-air incubator. The number of cysts which excysted was determined by microscopic count after the method of Bingham et al. (1).

TABLE 1. *Excystation of G. lamblia cysts from symptomatic and asymptomatic carriers*

Donor	Batch	No. of trials	Mean % excystation[a] ± standard error	% Range
Symptomatic I		6	90 ± 2.2	79–95
Symptomatic II		4	83 ± 2.4	75–87
Asymptomatic I	A	4	69 ± 2.6	60–73
	B	3	56 ± 2.7	50–61
Asymptomatic II	A	3	42 ± 1.5	40–46
	B	5	92 ± 1.1	88–95
Asymptomatic III		3	77 ± 2.1	73–82

[a] Excystation percentages were based on counting 800 to 1,000 cysts in each trial.

The results of 28 excystations are shown in Table 1. The mean percent excystation was 87 for the symptomatic donors and 70 for the asymptomatic donors. With the exception of cysts from batch B of asymptomatic donor II, the asymptomatic donors consistently yielded lower excystation percentages than the symptomatic carriers. The lowest percentage of excystation for cysts from symptomatic donors in this study was 10% higher than the maximum excystation percentage reported by Jarroll et al. (3), using the Bingham-Meyer procedure. Fifty attempts in our laboratory to excyst cysts, isolated from asymptomatic donor I, by using the Bingham-Meyer method resulted in only a 2 to 3% excystment. The present procedure represents a marked improvement for the in vitro excystation of *G. lamblia* cysts, but further research is required to more clearly delineate the various factors which promote optimal excystation.

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