

Coliform and *E. coli* Levels in Water from Ponds & Other Water Reservoirs on Calvin's Campus

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INTRODUCTION

Water is used in all aspects of life: drinking, cooking, and recreational use just to name a few. The water we come into contact with needs to be sampled frequently to make sure it can be safely used. Water comes into contact with a number of things, any of which may cause pollutants as well as microorganisms to enter the water system.

The Environmental Protection Agency (EPA) has guidelines concerning the quality of water for drinking, partial body contact, as well as for total body contact. The EPA uses *E. coli* bacteria counts to determine if a body of water is safe for human contact; this is a widely accepted method for determining the amount of fecal matter contamination in water (1). While many coliforms are commonly found in decaying matter in soil, *E. coli* is only found in fecal matter from warm-blooded animals.

Because of the wildlife present on Calvin's campus, the ponds could be contaminated with *E. coli* from animal feces. This study was designed to assess the number of coliform and *E. coli* bacteria present in the various ponds and other water reservoirs on the Calvin College campus.

METHODS

- Water samples were collected in sterile tubes at the water's edge.
- Serial dilutions in water (1:10 and 1:100) were made for each sample.
- 1 mL and 0.1 mL undiluted sample, and 0.1 mL diluted samples were spread on Endo agar plates. This medium inhibits the growth of Gram-positive and endospore-forming bacteria. Coliform bacteria appear metallic red due to bacterial fermentation of lactose to acetaldehyde and subsequent reaction with dyes in the medium.
- Control samples of *S. epidermidis* (non-coliform bacterium), *E. coli*, drinking fountain water and sterile water were also collected.
- 2 mL of each sample was also mixed with Easygel® agar and allowed to harden. All coliforms produce the enzyme β-galactosidase, and in this media, a chromogenic substrate is cleaved by this enzyme to produce a pink/red product. This media also contains a chromogenic substrate for glucuronidase; a teal product results from glucuronidase activity. *E. coli* produces both β-galactosidase and glucuronidase enzymes, and the combination of these two colors produces a blue/purple product (2).
- All plates were incubated at 37 C for 48 hours. After incubation, colonies were counted to assess the presence of coliform and *E. coli* bacteria.

RESULTS

Table 1: Total Coliform Counts

Site	Endo (Bacteria/100 mL)	Easygel® (Bacteria/100 mL)
North Pond	6,400,000	188,000
South Pond A	80,000	11,100
South Pond B	20,000	63,200
Whiskey Pond	86,000	10,800
Buttonbush Pond	600,000	7,500
East Drainage	60,000	20,200
Central Drainage	230,000	5,100
West Drainage	174,000	16,400
Seminary Pond	50,000	88,400
Ravenswood Pond	18,000	32,500
Presidents Pond	31,000	36,700
Bunker Interpretive Center		
Toilet Effluent	23,600,000	20,600
Gray Water	1,370,000	TNTC
Solarium	250,000	TNTC
Controls		
Drinking Fountain	0	0
Sterile Water	0	0
<i>E. coli</i> Culture	151,000,000	TNTC
<i>S. epidermidis</i> Culture	0	0

TNTC = Too Numerous To Count

Table 2: *E. coli* Counts

Site	Easygel® (Bacteria/100 mL)
North Pond	100
South Pond A	300
South Pond B	0
Whiskey Pond	50
Buttonbush Pond	0
East Drainage	850
Central Drainage	450
West Drainage	50
Seminary Pond	250
Ravenswood Pond	2,000
Presidents Pond	250
Bunker Interpretive Center	
Toilet Effluent	14,600
Gray Water	TNTC
Solarium	TNTC
Controls	
Drinking Fountain	0
Sterile Water	0
<i>E. coli</i> Culture	3.1 x 10 ¹⁰
<i>S. epidermidis</i> Culture	0

TNTC = Too Numerous To Count

The Endo Agar data shows large numbers of coliforms in the North Pond, Buttonbush, Central and West Drainage Pond subsurface sampling sites, as well all sites sampled at the Bunker Interpretive Center. The data obtained by using Easygel® agar also indicates high levels of coliforms in the North Pond, Gray Water, and Solarium Sites.

E. coli was detected at North Pond, South Pond, all drainage ponds, the Seminary Pond, Ravenswood Pond, Presidents Pond, and at all Bunker Interpretive Center Sites.

The positive control, the *E. coli* culture, showed metallic red colony formation on the Endo agar and blue-purple coloration on Easygel® as expected. The negative controls, drinking fountain water, sterile water, and *S. epidermidis*, exhibited no growth on either Endo agar or the Easygel® agar.

DISCUSSION

The EPA uses *E. coli* bacteria counts as one method to determine if the water may be contaminated with fecal matter. The EPA's guidelines are 1000 *E. coli*/100 mL for partial body contact and 130 *E. coli*/100 mL for total body contact (1). Using these guidelines, it would not be deemed safe to swim or be thrown in the South Pond, East and Central Drainage Ponds, the Seminary Pond, Ravenswood Pond, or Presidents Pond. In addition, protective wear should be worn when touching the water in Ravenswood Pond.

Water from the Bunker Interpretive Center sites contained surprisingly high levels of *E. coli* bacteria; this suggests that this system may need additional maintenance.

Two methods were used to assess total coliform counts, Endo agar and Easygel® agar. For several samples the results obtained by the two methods were similar (31,000 cfu/100 mL vs. 36,700 cfu/100 mL in the Presidents Pond sample), but as the number of bacterial colonies increased, the discrepancy between the two methods also increased. This may be due to a difficulty in counting large numbers of colonies on the Easygel plates, since a single water sample volume was used for the more costly Easygel plates and serial dilutions were used for the Endo agar plates.

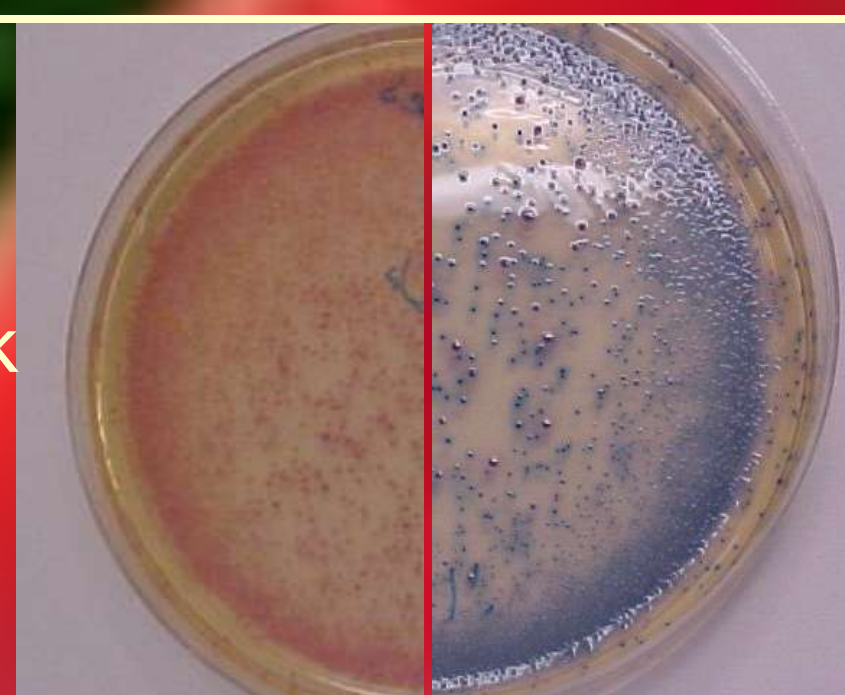
MAP OF CAMPUS SAMPLING SITES



REFERENCES

1. Water Quality Standards. http://www.epa.gov/waterscience/standards/wqslibrary/mi/mi_5_wqs.pdf (8 Nov 2005)
2. "Detection of Waterborne Coliforms and *E. coli* with Coliscan Easygel." Micrology Labs. 2005. Micrology Laboratories. <http://www.micrologylabs.com> (7 Nov 2005)

Coliscan EasyGel®:
E. coli are blue/purple
Other Coliform are pink
Non-Coliform are uncolored or teal-green



E. aerogenes *E. coli*
(other coliform)



Endo Agar
Coliform bacteria are metallic red

E. coli