

COLIFAST

COLIFAST SYSTEMS ASA

MEMO

ATT: Susan Schulz

DATE: September 2, 1998

NO. OF PAGES: 2

SUBJECT: Modeling ingestion of *pH Cleanse and inactivation of *E. Coli* at low pH

Objective

The objective of the study was to test the effects of different doses of *pH Cleanse in a low pH environment on *E. Coli*. The *pH Cleanse was mixed at various dilutions with diluted lemon juice, as an example of an edible organic acid. The pH of the solution was 3.0, approaching the acidic conditions in the gastric environment. The source of *E. Coli* was the natural population occurring in raw sewage as a model of fecal contamination. The source was selected in order to simulate the physiologically robust native target organism as a model of a contamination episode, as opposed to laboratory strains of the same enteric organism. Also, the organic matrix provided a chemical demand for the oxidation potential in the mixture to compete for the active ingredient.

Study Design

Sewage was diluted in each of three trials to different levels ranging from 400 to 220000 Colony Forming Units per milliliter (CFU/ml), mixed with acidified *pH Cleanse and contacted for 30 sec to 5 minutes. In every case the *Ph Cleanse solution had been acidified for 1 minute prior to mixing with the diluted sewage.

Subsamples were then taken at the appropriate contact time, neutralized and plated on 3M Petrifilm, the incubated for 24 hours at 35 C. Both total coliforms and *E. Coli* were then enumerated.

<http://www.restandrepair.tv/>

Results

The results of the three trials are summarized and shown in the table below. Significant inactivation occurs even at very high dilutions and the relatively short contact times. There is also continued inactivation over time at least up to the 5 minutes tested in trial no. 3, indicating continued activity of the active ingredient in this complex organic matrix which would compete for the oxidation potential in the product.

<u>Trial no.</u>	<u>Dilution</u>	<u>Initial CFU/ml</u>	<u>Contact time</u>	<u>% Inactivation</u>
1	1:20	220000	1 min	>99.9999
	1:50		1 min	>99.9999
2	1:1000	9200	30 sec	74
	1:10000		30 sec	44
3	1:10000	400	30 sec	78
	1:10000		1 min	73
	1:10000		2 min	82
	1:10000		5 min	87

It should be noted that this bench study is a conservative model insofar as the anticipated extent of inactivation in at least three regards: 1) the pH 3 used in the trials is higher than in the gut (1-2), hence there may be less extensive activation of the active ingredient, which is inversely pH dependent; 2) the contact time is probably significantly less than the residence time in the low pH region in the gut; 3) the trials represent a single dose in time.

<http://www.restandrepair.tv/>