



## AEMTEK REPORT

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### *E. coli* Challenge Study Project No. 1507920

#### Summary:

The inactivation of *E. coli* by four different reFresh™ product formulations was evaluated in an *in vitro* system. A multi strain cocktail of *E. coli* was cultured to a concentration of 10<sup>5</sup> CFU/mL (5 logs). The reFresh products were added to separate tubes containing 5 logs of *E. coli* at the upper-labeled usage level, as indicated on the product packaging. The tube was mixed well and held for 5 minutes. After 5 minutes, an aliquot of the test solution was removed, diluted to proper levels and plated in replicates of 10 on 3M™ Petrifilm™ EC. The plates were incubated for 48 hours at 35°C and enumerated. The *E. coli* concentration was also enumerated in order to determine the exact concentration used in the study, which was used to calculate log reduction of the reFresh treatments. The results demonstrate that the reFresh™ Concentrate product was the most effective at reducing the initial bacterial concentration. There was a complete reduction in the 5 logs of *E. coli*, after only a 5 minute exposure. The other three products demonstrated a 0.5 log reduction or less with the reFresh™ F/P having the largest reduction, followed by reFresh™ F-Vegetable and reFresh™ F-Berry.

#### Materials:

The test organism was a multi strain cocktail of *E. coli* (ATCC 25922, 35218, 11229 and 8739). The strains were grown through successive passes in Buffered Peptone Water (BPW) and enumerated with dilutions in phosphate buffered saline (PBS) prior to inoculation. Sierra Natural Sciences provided four reFresh product formulations, which included, reFresh™ C, reFresh™ F-Vegetable, reFresh™ F-Berry, and reFresh™ F/P.



## Methods:

An overnight stock culture of the *E. coli* cocktail was prepared with the stock culture enumerated one day prior to study initiation. Serial dilutions were performed in PBS and plated onto 3M™ Petrifilm™ EC. The *E. coli* was added at a target concentration of 5 logs to Di Water in a 50mL sterile conical tube. The reFresh products were each added at their labeled usage rate to separate tubes each containing 5 logs of *E. coli*, mixed thoroughly and allowed to stand for 5 minutes. The usage rates for the reFresh products were as follows:

reFresh™ F-Vegetable:	3 oz. / 1 gallon water
reFresh™ F-Berry:	2 oz. / 1 gallon water
reFresh™ FP:	32 oz. / 50 gallons water
reFresh™ C:	1 oz. / 1 gallon water

After 5 minutes an aliquot was removed, diluted in PBS if necessary and plated onto 3M™ Petrifilm™ EC. Plating was performed in replicates of 10 for each of the reFresh products. Petrifilm plates were incubated at 35°C for 48 hours.

## Data:

After a 5-minute exposure of the *E. coli* cocktail to the reFresh™ products, *E. coli* counts indicated that a reduction was observed for all products, at varying levels of reduction. The data below demonstrate that the reduction in bacteria was the greatest for the reFresh™ Concentrate product followed by reFresh™ F/P, reFresh™ F-Vegetable and reFresh™ F-Berry.

reFresh™ Product Formulations Log reduction of <i>E. coli</i> after 5 minute exposure			
	CFU/g	Log <sub>10</sub>	Log <sub>10</sub> Reduction
<b><i>E. coli</i> Control</b>	1.45E+05	5.16	---
<b>reFresh™ F-Berry</b>	1.32E+05	5.12	0.04
<b>reFresh™ F-Vegetable</b>	1.00E+05	5.00	0.16
<b>reFresh™ F/P</b>	4.30E+04	4.63	0.53
<b>reFresh™ Concentrate</b>	0.00E+00	0.00	5.16



## Conclusion:

The results of the *E. coli* inactivation study demonstrate a reduction from the control *E. coli* culture for all of the four reFresh™ product formulations. The log reduction was greatest for the reFresh™ Concentrate, which had a complete reduction in the 5 logs of *E. coli* after only 5 minutes. This was followed by the reFresh™ F/P, F-Vegetable and F-Berry formulations, all of which had around a 0.5 log or less reduction. Additional trials at higher levels of initial bacteria concentration could be conducted in order to determine the full extent of the reFresh™ Concentrate's activity against *E. coli* or other bacteria.

Best Regards,

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