FINAL REPORT

Citrobio Incorporated

PROTOCOL
ASTM E2315

ORDER Number
091212379

PREPARED FOR:

Citrobio Inc.
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Sarasota, FL 34243

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Certificate of Analysis

Client: Citrobio, Inc.

Contact: Richard Maguire
Project: Bacteria Kill Testing for Citrobio, Inc.

Product : Produce Wash
EMSL NO: 091212379

Sample received: 09/28/2012
Start date: 10/11/2012
Report date: 10/15/2012

Challenge Bacteria:
Escherichia coli ATCC 25922
Listeria monocytogenes ATCC 15313
Salmonella choleraesuis ATCC 1078

Experimental Summary:

The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, Citrobio Incorporated. The testing procedure is based on ASTM E2315 testing guidelines, with the testing conducted with a 20% produce wash solution, to demonstrate its effectiveness at killing *Escherichia coli, Listeria monocytogenes* and *Salmonella choleraesuis* on produce (grapes) matrices. The testing was conducted in our San Leandro Microbiology Laboratory.

Procedure:

The testing was done to determine the effectiveness of a 20% Produce Wash Solution (provided by Citrobio Incorporated) against *Escherichia coli, Listeria monocytogenes* and *Salmonella choleraesuis* at a 5-minute exposure time.

Culture Preparation:
Escherichia coli, Listeria monocytogenes and *Salmonella choleraesuis* were plated onto Tryptic Soy Agar with 5% sheep blood (TSAB), and incubated at 35°C for 24-h. Then a single isolated colony was taken and inoculated in 100 mL of Tryptic Soy Broth at 35°C for 24-h before testing was conducted.

Quantitative Test:
Cultures were prepared at two different time intervals to determine the log reduction and percent reduction of *Escherichia coli, Listeria monocytogenes* and *Salmonella choleraesuis*. An overnight culture was plated on TSAB to confirm a

population minimum of 1.0 x 10^6 CFU/mL. Bacterial solution of 1.0 x 10^6 CFU/mL was applied per gram of matrix. Next, the bacterial-matrix suspension was treated with an equal volume of 20% produce wash for 5-minutes. Treated and untreated cultures were then taken and serially diluted, and plated onto TSAB to determine the starting population after 0-min and 5-min treatments. TSAB plates were incubated at 35°C for 48-h and all tests were performed in duplicate.

Experimental Results:

Table 4. Efficacy of Citrobio 20% Produce Wash against *Esherichia coli* (in Grapes) at 5-min.

Viability control	Positive/Negative	Sterility Control	Positive/Negative
1	+	1	-
2	+	2	-
Sample	**CFU**	**Log Reduction**	**%Reduction**
Citrobio 5% Produce Wash	1.00 x 10^0	7.72	100.00%
Control-untreated water	5.24 x 10^7		

Table 5. Efficacy of Citrobio 20% Produce Wash against *Listeria monocytogenes* (in Grapes) at 5-min.

Viability control	Positive/Negative	Sterility Control	Positive/Negative
1	+	1	-
2	+	2	-
Sample	**CFU**	**Log Reduction**	**%Reduction**
Citrobio 5% Produce Wash	1.00 x 10^0	7.78	100.00%
Control-untreated water	5.98 x 10^7		

Table 6. Efficacy of Citrobio 20% Produce Wash against *Salmonella choleraesuis* (in Grapes) at 5-min.

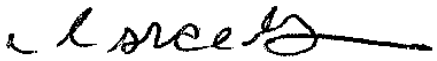
Viability control	Positive/Negative	Sterility Control	Positive/Negative
1	+	1	-
2	+	2	-
Sample	**CFU**	**Log Reduction**	**%Reduction**
Citrobio 5% Produce Wash	1.00 x 10^0	7.84	100.00%
Control-untreated water	6.96 x 10^7		

Conclusions/Observations:

A 20% Produce Wash, provided by Citrobio, was tested using an ASTM E2315 based protocol. Tables 4 through 6 show that the Citrobio 20% Produce Wash solution produced detectable log reductions of *E. coli*, *Listeria sp.* and *Salmonella sp.,* in grape matrices, after 5-min of exposure.

Tables 4 through 6 also indicate that at 5-min treatments, the Citrobio 20% Produce Wash respectively killed 100.00% of *E. coli*, *Listeria sp.* and *Salmonella sp.* in grapes.

In conclusion, the Citrobio 20% Produce Wash unit was able to produce detectable kill-rates for *E. coli*, *Listeria sp.* and *Salmonella sp.* in grapes after 5-min treatments.



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