## **Bacteria Test Results**

STUDY RUN BY: MICROBIOTEST, INC.

**STUDY TITLE:** Fruit and Vegetable Antibacterial Wash Test

STUDY SPONSOR: Tersano (International) SRL

**TEST AGENT NAME:** lotus Sanitizing System

**ACTIVE INGREDIENT: Ozone** 

CHALLENGE BACTERIA: Escherichia coli, ATCC 11229

Listeria monocytogenes, ATCC 19111 Salmonella choleraesuis, ATCC 10708

**EXPOSURE TIME**: 30 seconds and 2 minutes (For each fruit and vegetable type, per exposure time, three inoculated, dried carriers were added and the bowl adapter containing 2 L of sterile tap water.)

The system was activated and ozone was generated. The generation time for each batch of test material ranged from 3 minutes and 20 seconds to 5 minutes. The automated system prompted the user when ozone generation was complete. For each evaluation, the exposure time was initiated at the completion of each ozone generation.

**RESULTS**: All plates were examined for purity. The challenge microorganisms were confirmed by Gram stain and colony morphology to be consistent with *E. coli, L. monocytogenes* and *S. choleraesuis*. The sterility controls exhibited no growth. There were no bioburden counts recovered from any of the fruit and vegetables evaluated. Percent reduction and log10 reduction were calculated using the following equations:

Initial count (CFU/Carrier) - Test result (CFU/Carrier) Initial count (CFU/Carrier) X 100 = Percent Reduction

 $Log_{10}$  Initial Count (CFU/Carrier) –  $Log_{10}$  Test Results (CFU/Carrier) =  $Log_{10}$  Reduction

## Test Results (30 second contact time)

| Microorganism    | Carrier Type    | CFU/Carrier | Log <sub>10</sub><br>Reduction | Percent<br>Reduction |
|------------------|-----------------|-------------|--------------------------------|----------------------|
| E. coli          | Tomato          | 1.00E+01    | 4.98                           | 99.9989              |
|                  |                 | 1.00E+01    | 4.98                           | 99.9989              |
|                  |                 | 1.00E+01    | 4.98                           | 99.9989              |
|                  | Grape           | 1.30E+03    | 2.14                           | 99.2778              |
|                  |                 | 9.60E+02    | 2.27                           | 99.4667              |
|                  |                 | 1.90E+03    | 1.98                           | 98.9444              |
|                  | Snow Pea<br>Pod | 2.40E+03    | 3.00                           | 99.8400              |
|                  |                 | 1.20E+03    | 3.10                           | 99.9200              |
|                  |                 | 5.40E+03    | 2.44                           | 99.6400              |
|                  | Kumquat         | 2.30E+02    | 2.87                           | 99.8647              |
|                  |                 | 1.00E+01    | 4.23                           | 99.9941              |
|                  |                 | 1.00E+01    | 4.23                           | 99.9941              |
|                  |                 | 1.00E+01    | 5.28                           | 99.9995              |
|                  | Tomato          | 1.00E+01    | 5.28                           | 99.9995              |
|                  |                 | 1.00E+01    | 5.28                           | 99.9995              |
|                  |                 | 4.30E+04    | 2.02                           | 99.0444              |
| L. monocytogenes | Grape           | 2.80E+04    | 2.21                           | 99.3778              |
|                  |                 | 7.20E+04    | 1.80                           | 98.4000              |
|                  | Snow Pea<br>Pod | 1.30E+04    | 2.45                           | 99.6486              |
|                  |                 | 2.60E+03    | 3.15                           | 99.9297              |
|                  |                 | 2.10E+04    | 2.25                           | 99.4324              |
|                  | Kumquat         | 3.70E+04    | 1.61                           | 97.5333              |
|                  |                 | 2.30E+05    | 0.81                           | 84.6667              |
|                  |                 | 1.80E+05    | 0.92                           | 88.0000              |
| S. choleraesuis  | Tomato          | 1.00E+01    | 3.43                           | 99.9630              |
|                  |                 | 1.00E+01    | 3.43                           | 99.9630              |
|                  |                 | 1.00E+01    | 3.43                           | 99.9630              |
|                  | Grape           | 1.00E+01    | 4.04                           | 99.9909              |
|                  |                 | 1.00E+01    | 4.04                           | 99.9909              |
|                  |                 | 1.00E+01    | 4.04                           | 99.9909              |
|                  | Snow Pea<br>Pod | 1.70E+03    | 2.31                           | 99.5143              |
|                  |                 | 1.80E+02    | 3.29                           | 99.9486              |
|                  |                 | 4.60E+02    | 2.88                           | 99.8686              |
|                  | Kumquat         | 1.80E+02    | 2.14                           | 99.2800              |
|                  |                 | 1.00E+01    | 3.40                           | 99.9600              |
|                  |                 | 1.00E+01    | 3.40                           | 99.9600              |

## Test Results (2 minute contact time)

| Microorganism    | Carrier Type    | CFU/Carrier | Log <sub>10</sub> | Percent   |
|------------------|-----------------|-------------|-------------------|-----------|
|                  |                 |             | Reduction         | Reduction |
| E. coli          | Tomato          | 1.00E+01    | 4.98              | 99.9989   |
|                  |                 | 1.00E+01    | 4.98              | 99.9989   |
|                  |                 | 1.00E+01    | 4.98              | 99.9989   |
|                  | Grape           | 2.70E+02    | 2.82              | 99.8500   |
|                  |                 | 5.00E+01    | 3.56              | 99.9722   |
|                  |                 | 3.90E+02    | 2.66              | 99.7833   |
|                  | Snow Pea<br>Pod | 8.40E+02    | 3.00              | 99.9440   |
|                  |                 | 1.10E+03    | 3.13              | 99.9267   |
|                  |                 | 2.00E+03    | 2.88              | 99.8667   |
|                  | Kumquat         | 1.00E+01    | 4.23              | 99.9941   |
|                  |                 | 1.00E+01    | 4.23              | 99.9941   |
|                  |                 | 1.00E+01    | 4.23              | 99.9941   |
|                  |                 | 1.00E+01    | 5.28              | 99.9995   |
|                  | Tomato          | 1.00E+01    | 5.28              | 99.9995   |
|                  |                 | 1.00E+01    | 5.28              | 99.9995   |
|                  |                 | 2.20E+03    | 3.31              | 99.9511   |
| L. monocytogenes | Grape           | 7.20E+03    | 2.80              | 99.8400   |
|                  |                 | 4.00E+03    | 3.05              | 99.9111   |
|                  | Snow Pea<br>Pod | 2.50E+04    | 2.17              | 99.3243   |
|                  |                 | 2.70E+03    | 3.14              | 99.9270   |
|                  |                 | 1.60E+03    | 3.36              | 99.9568   |
|                  | Kumquat         | 1.10E+03    | 3.13              | 99.9267   |
|                  |                 | 5.40E+03    | 2.44              | 99.6400   |
|                  |                 | 9.20E+02    | 3.21              | 99.9387   |
| S. choleraesuis  | Tomato          | 1.00E+01    | 3.43              | 99.9630   |
|                  |                 | 1.00E+01    | 3.43              | 99.9630   |
|                  |                 | 1.00E+01    | 3.43              | 99.9630   |
|                  | Grape           | 1.00E+01    | 4.04              | 99.9909   |
|                  |                 | 1.00E+01    | 4.04              | 99.9909   |
|                  |                 | 1.00E+01    | 4.04              | 99.9909   |
|                  | Snow Pea<br>Pod | 1.20E+03    | 2.46              | 99.6571   |
|                  |                 | 1.30E+03    | 2.43              | 99.6286   |
|                  |                 | 1.40E+02    | 3.40              | 99.9600   |
|                  | Kumquat         | 1.00E+01    | 3.40              | 99.9600   |
|                  |                 | 1.00E+01    | 3.40              | 99.9600   |
|                  |                 | 1.00E+01    | 3.40              | 99.9600   |