

**Summary about the Disinfection Capacity of Toucan**  
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Test Organism	starting concentration [cfu/ml]	rest cfu after 0.5 min	1 min	2 min	5 min	10 min
			< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
methicillin-susceptible <i>Staphylococcus aureus</i>	1.5 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Methicillin-resistant <i>Staphylococcus aureus</i>	1.3 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Vacomycin-resistant <i>Enterococcus faecium</i>	1.1 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Enterococcus faecalis	1.2 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Escherichia coli	1.6 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Klebsiella pneumoniae	1.3 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Enterobacter cloacae	1.2 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Salmonella Typhi	1.4 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Salmonella Enteritidis	1.8 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Shigella sonnei	1.0 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Pseudomonas aeruginosa	1.2 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Acinetobacter baumannii	1.4 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Stenotrophomonas maltophilia	1.5 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Staphylococcus Aureus, ATCC 29213	1.2 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
E. coli, ATCC 25922	1.3 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Pseudomonas aeruginosa, ATCC 27853	1.5 x 10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Bacillus subtilis, ATCC 6633 (vegetative form)	5.3 x 10 <sup>7</sup>	< 10 <sup>7</sup>	< 10 <sup>3</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>

**Conclusion:**

The electrolyzed water produced from Toucan-Eco has strong disinfecting effect against general bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vacomycin-resistant *Enterococcus faecium* (VRE), *E. coli*, *Salmonella*, *Shigella*, and thus it is believed that it can be useful as a sterilizing/disinfecting agent for hygiene control to prevent the occurrence of food poisoning and for control against infectious diseases.



**Table 1. The bacterial disinfection efficacy of the disinfectant liquid generated by the provided Toucan-Eco unit. Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005)**

<b>Microorganism</b>	<b>Number of Sprayed Inoculated Slides</b>	<b>Number of Tubes Demonstrating Growth</b>	<b>Positive Control (un-sprayed slide)</b>	<b>Negative Control (un-inoculated slide)</b>
<b><i>Staphylococcus aureus (MRSA)</i></b>	<b>10</b>	<b>None</b>	<b>Growth</b>	<b>No-Growth</b>
<b><i>Salmonella enterica</i></b>	<b>10</b>	<b>None</b>	<b>Growth</b>	<b>No-Growth</b>
<b><i>Listeria monocytogenes</i></b>	<b>10</b>	<b>None</b>	<b>Growth</b>	<b>No-Growth</b>
<b><i>Pseudomonas aeruginosa</i></b>	<b>10</b>	<b>None</b>	<b>Growth</b>	<b>No-Growth</b>
<b><i>E. coli</i> O157:H7</b>	<b>10</b>	<b>None</b>	<b>Growth</b>	<b>No-Growth</b>

\* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the disinfectant and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and examined for growth as described in the methodology section.

**Table 2. The bacterial disinfection efficacy of the disinfectant liquid generated by the provided Toucan-Eco unit; Germicidal Spray Products as Disinfectants (2005)**

<b>Microorganism</b>	<b>Number of Sprayed Inoculated Slides (number of replicates tested)</b>	<b>Average microorganism cfu/ml inoculated per slide<sup>#</sup></b>	<b>Average cfu/ml recovered from each of slides sprayed*</b>	<b>Percent Reduction</b>	<b>Log<sub>10</sub> reduction</b>
<i>Staphylococcus aureus (MRSA)</i>	10	>1.0 x 10 <sup>5</sup>	<1.0	>99.999%	>5.0
<i>Salmonella enterica</i>	10	>1.0 x 10 <sup>5</sup>	<1.0	>99.999%	>5.0
<i>Listeria monocytogenes</i>	10	>1.0 x 10 <sup>5</sup>	<1.0	>99.999%	>5.0
<i>Pseudomonas aeruginosa</i>	10	>1.0 x 10 <sup>5</sup>	<1.0	>99.999%	>5.0
<i>E. coli</i> O157:H7	10	>1.0 x 10 <sup>5</sup>	<1.0	>99.999%	>5.0

<sup>#</sup> This number represents the average number of microorganisms recovered from glass slides inoculated, dried, and not exposed to disinfection treatment (positive control).

\* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the disinfectant and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and examined for growth as described in the methodology section.

**Table 3. The viral disinfection efficacy of the disinfectant liquid generated by the provided Toucan-Eco unit. Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005) and ASTM E1053 “Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces”**

<b>Microorganism</b>	<b>Number of Sprayed Inoculated Slides (number of replicates tested)</b>	<b>Average infectious particles (iu) /ml inoculated per slide<sup>#</sup></b>	<b>Average iu/ml recovered from each of slides sprayed*</b>	<b>Percent Reduction</b>	<b>Log<sub>10</sub> reduction</b>
<b>Murine Norovirus MNV-1 (Human Norovirus Surrogate)</b>	<b>5</b>	<b>4.6 x 10<sup>4</sup></b>	<b>&lt;0.5</b>	<b>&gt;99.999%</b>	<b>&gt;5.0</b>
<b>Poliovirus CHAT Lsc1</b>	<b>5</b>	<b>1.3 x 10<sup>5</sup></b>	<b>&lt;0.5</b>	<b>&gt;99.9999%</b>	<b>&gt;6.0</b>

<sup>#</sup> This number represents the average number of infectious virus particles recovered from glass slides inoculated, dried, and not exposed to disinfection treatment (positive control).

\* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the disinfectant and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and enumerated for infectious viral particles on respective cell monolayers as described in the methodology section.

**Table 4. Inactivation of *E. coli* O157:H7 and *Listeria monocytogenes* at the indicated time points following introduction to the disinfectant liquid produced by the Toucan-Eco unit.**

Microorganism	Bacterial cfu/ml at the indicated time points following study start <sup>1</sup>								
	0 (control)	10 seconds	30 seconds	60 seconds	90 seconds	120 seconds	180 seconds	5 minutes	Control (Final)
<i>E. coli</i> O157:H7	2.9 x 10 <sup>6</sup>	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	2.9 x 10 <sup>6</sup>
<i>Listeria monocytogenes</i>	1.1 x 10 <sup>6</sup>	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	<1.0 (>99.9999% or >6 Log <sub>10</sub> reduction)	8.9 x 10 <sup>5</sup>

<sup>1</sup> Aliquots of the above bacteria were added to 200 ml of Class I ASTM water (Control) and 200 ml of the liquid generated by the Toucan-Eco unit. The flasks containing the liquids were agitated on an orbital shaker at a medium speed. At each of the indicated time points following the start of the study, samples were removed, neutralized and assayed for the bacterial species by spread plating onto TSA and incubation at 36.5°C for 24-36 hours.