

Lab NO. 85515-85516  
Testing Laboratory:  
Sponsor:

Test: Time kill Test  
Accugen Laboratories, Inc.  
US Button

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## Final Report

Time Kill Test / Log Reduction Assay

### Test Materials:

Buttons

### Data Requirement:

Research Purpose

### Testing Laboratory:

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### Study Completion Date

07-09-09

## TEST SUMMARY

**Title:** Time Kill Test / Log Reduction Assay

**Purpose:** To determine antimicrobial activity of incorporated antimicrobial against microorganisms tested.

### Test Conditions:

Challenge Organisms:

Salmonella typhimurium ATCC # 13311  
E.coli ATCC # 8739  
Enterococcus faecalis ATCC # 51299

Test Material:

Antimicrobial agent incorporated Buttons and untreated buttons

Contact time:

0 hr, 6 hours, 24 hours

Contact temperature:

Room temperature (20±2C)

Media and reagents:

Soybean-casein digest agar (TSA)  
Tryptic Soy Broth  
*Diluent*, sterile water, 0.65 % saline or buffer  
Dey Engley (DE ) neutralizing broth  
Phosphate buffer

### Method:

Organism suspensions of 10<sup>6</sup> cfu/ml concentrations were inoculated onto the product for specified contact time and plate count test was carried out to enumerate the surviving organisms.

**RESULTS** Test Agent: Buttons


Microorganism	Organism Initial count(CFU/ml)	Control sample Contact time in hours			Test sample Contact time in hours		
		0	6	24	0	6	24
Salmonella typhimurium ATCC # 13311	3.5 x 10 <sup>6</sup>	3.5 X 10 <sup>6</sup>	3.6x 10 <sup>6</sup>	3.8x 10 <sup>6</sup>	3.5x 10 <sup>6</sup>	3.2 x 10 <sup>6</sup>	8.7 x 10 <sup>5</sup>
% Reduction	-	0%	0%	0%	0 %	9 %	75 %

Microorganism	Organism Initial count(CFU/ml)	Control sample Contact time in hours			Test sample Contact time in hours		
		0	6	24	0	6	24
E.coli ATCC # 8739	3.2 x 10 <sup>6</sup>	3.2 X 10 <sup>6</sup>	3.5x 10 <sup>6</sup>	3.4 x 10 <sup>6</sup>	3.2 x 10 <sup>6</sup>	2.09 x 10 <sup>6</sup>	9.9 x 10 <sup>5</sup>
% Reduction	-	0%	0%	0%	0%	34.7 %	69.1 %

Microorganism	Organism Initial count(CFU/ml)	Control sample Contact time in hours			Test sample Contact time in hours		
		0	6	24	0	6	24
Enterococcus Faecalis ATCC # 51299	2.2 x 10 <sup>6</sup>	2.2 X 10 <sup>6</sup>	6.9 x 10 <sup>6</sup>	2.3 x 10 <sup>6</sup>	2.2 x 10 <sup>6</sup>	2.03 x 10 <sup>5</sup>	2.52 x 10 <sup>5</sup>
% Reduction	-	0%	0%	0%	0%	8 %	88.5 %

**Conclusion:**

Buttons showed 75 % antimicrobial activity against Salmonella typhimurium, 69 % against E.coli and 88.5 % against Enterococcus faecalis at 24 hrs of exposure time.



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