FINAL RESEARCH REPORT
Antibacterial Susceptibility Test Results for the Alka-Hydroxy® Product
July 17, 2006

The **purpose** of this series of experiments was to test the susceptibility of both Gram-positive and Gram-negative bacteria to the modified liquid silicon product Alka-Hydroxy®.

Six **organisms** were tested. The Gram-negative bacteria were *Escherichia coli* K-12, *Escherichia coli* O157:H7 (ATCC 35150), and *Salmonella enterica* serovar Typhimurium LT2. The Gram-positive bacteria were *Enterococcus faecalis* (ATCC 19433), ***Staphylococcus aureus*** (ATCC 12600), and *Streptococcus pyogenes* (ATCC 19615).

The **method** used was the broth microdilution method for susceptibility testing of antibacterial agents as recommended by the Clinical and Laboratory Standards Institute (CLSI). The detailed procedure is as follows.

1. A culture of the test organism was grown at 35°C with aeration in sterile Cation-adjusted Mueller-Hinton broth (Becton-Dickinson, Sparks, MD) until slightly turbid, and the turbidity was then adjusted to a 0.5 McFarland standard [equivalent to approximately 10^8 colony-forming units (cfu) per ml]. To improve cell yield in the *Enterococcus faecalis* and *Streptococcus pyogenes* cultures, the Cation-adjusted Mueller-Hinton broth was supplemented with 2.5 mg per mL of yeast extract (Becton-Dickinson).

2. Appropriate dilutions of the Alka-Hydroxy® product were prepared in sterile Cation-adjusted Mueller-Hinton broth such that the final concentrations of the product were 0.5%, 1.0%, 2.0%, 3.0%, and 5.0%. A 0% (no-product) control was also included.

3. Appropriate volumes of the standardized cell suspension were added to the dilutions of the product to achieve a final cell density of 5×10^5 cfu per mL. The dilutions were then aliquoted into the wells of a 96-well microtiter plate (with a volume of 0.2 mL per well).

4. The first two wells in each column of the microtiter plate were reserved for a no-cell control, and the remaining six wells were used for the cell-inoculated dilutions of the product. Columns 1–6 represented the 0%, 0.5%, 1.0%, 2.0%, 3.0%, and 5.0% concentrations, respectively.

5. The microtiter plates were incubated at 35°C for 16–20 hours. The culture turbidity was measured by visible-light spectroscopy (i.e., the absorbance of the culture at a wavelength of 590 nm) using a microtiter plate reader, and the results were recorded automatically.

6. The baseline absorbance of the wells containing the uninoculated dilutions (no-cell controls) was subtracted from the absorbances of the wells containing the cell-inoculated dilutions of the product. For each set of six replicate wells, the average (mean) absorbance, % inhibition, and standard deviation were calculated from the collected data.

The **results** of the experiments are summarized in the appended data tables. The minimum inhibitory concentration (MIC) of the product was the 1.0% concentration for all three of the Gram-negative bacteria (*E. coli* K-12, *E. coli* O157:H7, and *S. enterica*). However, the three Gram-positive bacteria (*E. faecalis*, ***S. aureus***, and *S. pyogenes*) showed greater resistance. The MIC of the product was the 2.0% for *S. aureus* and 3.0% for *E. faecalis* and *S. pyogenes*, although partial inhibition was seen at lower concentrations for all three of these organisms.

The **conclusions** drawn from the experimental results are that the product completely inhibits the growth of all bacterial strains under the test conditions at a concentration of 3.0%. Depending on the specific organism, complete inhibition can occur at a concentration as low as 1.0%.

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Escherichia coli K-12

	Alka-Hydroxy® Percent Concentration					
Sample	**0.0%**	**0.5%**	**1.0%**	**2.0%**	**3.0%**	**5.0%**
1	0.940	0.492	0.000	0.000	-0.001	-0.001
2	0.944	0.489	0.000	0.000	-0.001	0.000
3	0.934	0.481	0.000	0.000	-0.001	-0.001
4	0.984	0.501	0.000	0.000	0.000	0.000
5	0.971	0.494	0.001	0.000	0.000	0.001
6	1.000	0.490	0.001	0.000	0.000	0.006
AVERAGE	**0.962**	**0.491**	**0.000**	**0.000**	**-0.001**	**0.001**
STD DEV	0.027	0.007	0.001	0.000	0.001	0.003
% Inhibition	**0.0%**	**49.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Escherichia coli O157:H7

	Alka-Hydroxy® Percent Concentration					
Sample	**0.0%**	**0.5%**	**1.0%**	**2.0%**	**3.0%**	**5.0%**
1	0.387	0.205	0.000	0.000	-0.001	-0.001
2	0.359	0.203	0.000	-0.001	-0.001	0.000
3	0.370	0.196	0.001	0.000	0.000	0.001
4	0.365	0.200	-0.001	-0.001	-0.002	-0.001
5	0.368	0.200	-0.001	0.000	-0.001	0.001
6	0.345	0.219	0.000	0.001	0.000	0.000
AVERAGE	**0.366**	**0.204**	**0.000**	**0.000**	**-0.001**	**0.000**
STD DEV	0.014	0.008	0.001	0.001	0.001	0.001
% Inhibition	**0.0%**	**44.3%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Salmonella enterica

	Alka-Hydroxy® Percent Concentration					
Sample	**0.0%**	**0.5%**	**1.0%**	**2.0%**	**3.0%**	**5.0%**
1	0.293	0.143	0.000	0.001	-0.001	0.001
2	0.301	0.143	-0.001	-0.001	-0.001	0.000
3	0.294	0.138	-0.001	0.000	0.000	0.001
4	0.278	0.145	-0.001	0.000	-0.001	0.001
5	0.274	0.144	-0.001	0.002	-0.001	0.001
6	0.261	0.151	-0.002	0.001	-0.001	0.002
AVERAGE	**0.284**	**0.144**	**-0.001**	**0.001**	**-0.001**	**0.001**
STD DEV	0.015	0.004	0.001	0.001	0.000	0.001
% Inhibition	**0.0%**	**49.3%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Enterococcus faecalis

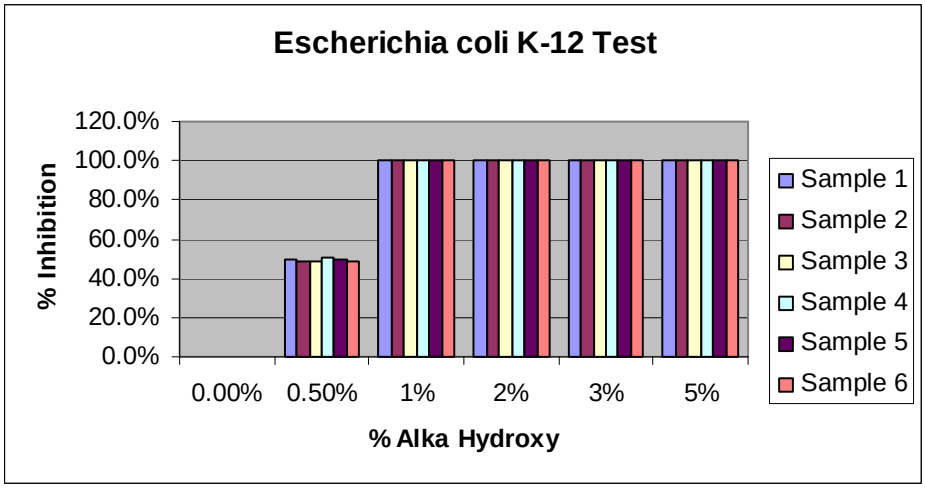
	Alka-Hydroxy® Percent Concentration					
Sample	**0.0%**	**0.5%**	**1.0%**	**2.0%**	**3.0%**	**5.0%**
1	0.262	0.267	0.058	0.020	0.000	-0.001
2	0.261	0.266	0.057	0.031	-0.001	-0.002
3	0.265	0.267	0.064	0.040	-0.001	-0.001
4	0.262	0.277	0.067	0.044	-0.001	-0.002
5	0.274	0.276	0.079	0.052	0.000	0.000
6	0.261	0.271	0.120	0.073	0.000	0.001
AVERAGE	**0.264**	**0.271**	**0.074**	**0.043**	**-0.001**	**-0.001**
STD DEV	0.005	0.005	0.024	0.018	0.001	0.001
% Inhibition	**0.0%**	**0.0%**	**72.0%**	**83.7%**	**100.0%**	**100.0%**

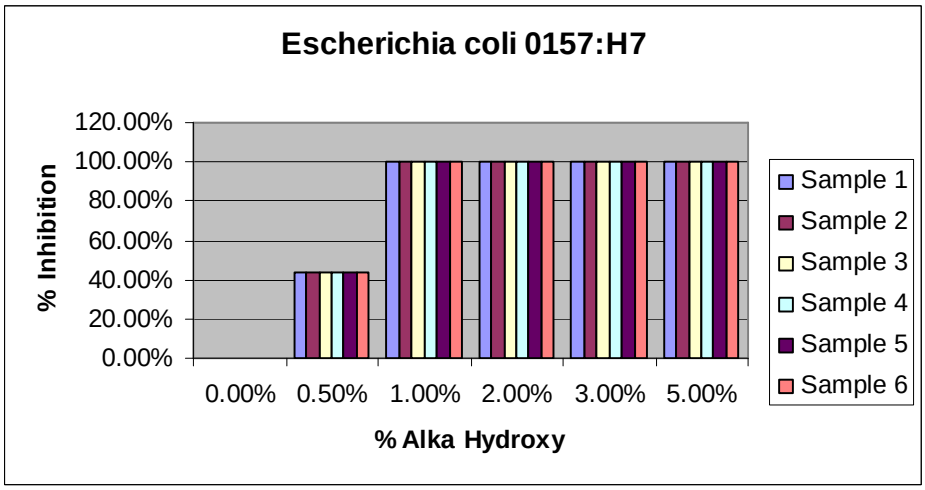
Staphylococcus aureus

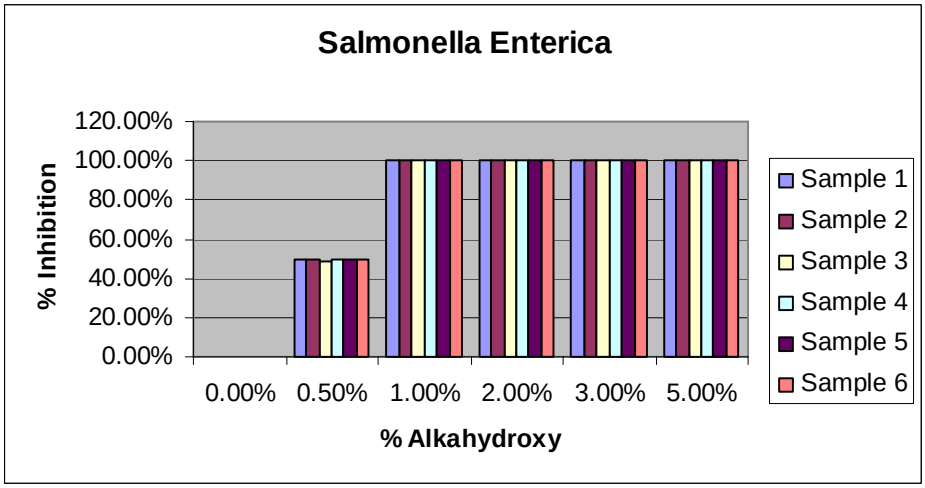
	Alka-Hydroxy® Percent Concentration					
Sample	**0.0%**	**0.5%**	**1.0%**	**2.0%**	**3.0%**	**5.0%**
1	0.164	0.113	0.090	0.000	0.000	-0.001
2	0.172	0.114	0.053	0.000	-0.002	-0.002
3	0.178	0.105	0.099	0.001	-0.002	-0.003
4	0.196	0.109	0.090	0.002	-0.001	-0.003
5	0.222	0.117	0.091	0.004	-0.002	0.000
6	0.276	0.166	0.128	0.004	-0.002	-0.002
AVERAGE	**0.201**	**0.121**	**0.092**	**0.002**	**-0.002**	**-0.002**
STD DEV	0.042	0.023	0.024	0.002	0.001	0.001
% Inhibition	**0.0%**	**39.8%**	**54.2%**	**99.0%**	**100.0%**	**100.0%**

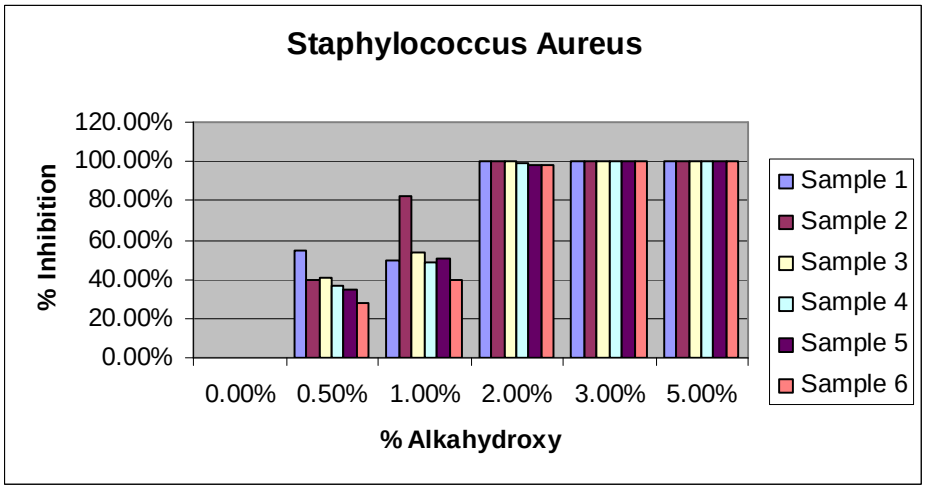
Streptococcus pyogenes

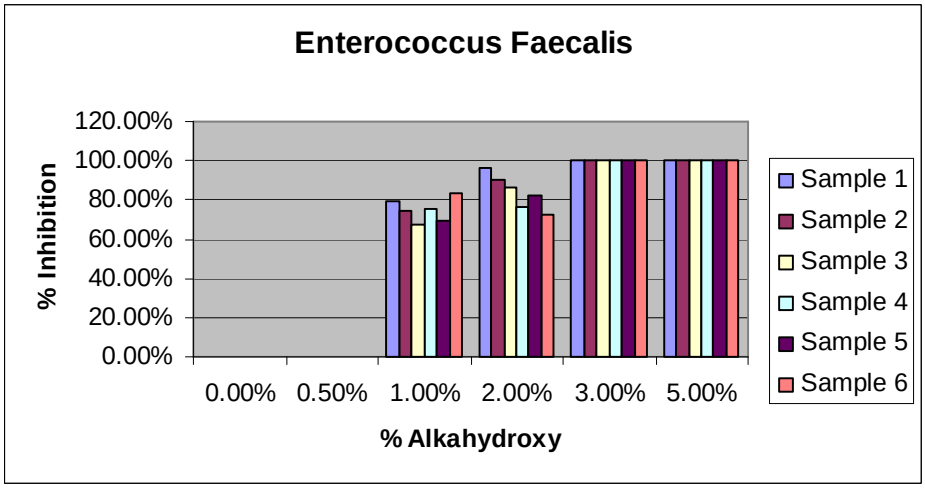
	Alka-Hydroxy® Percent Concentration					
Sample	**0.0%**	**0.5%**	**1.0%**	**2.0%**	**3.0%**	**5.0%**
1	0.123	0.004	0.001	0.000	-0.006	-0.006
2	0.142	0.003	0.003	0.007	-0.009	-0.007
3	0.123	0.005	0.004	0.013	-0.004	-0.011
4	0.146	0.002	0.006	0.016	-0.003	-0.010
5	0.128	0.006	0.010	0.028	-0.002	-0.008
6	0.104	0.007	0.010	0.030	-0.003	-0.003
AVERAGE	**0.128**	**0.005**	**0.006**	**0.016**	**-0.005**	**-0.008**
STD DEV	0.015	0.002	0.004	0.012	0.003	0.003
% Inhibition	**0.0%**	**96.1%**	**95.3%**	**87.5%**	**100.0%**	**100.0%**

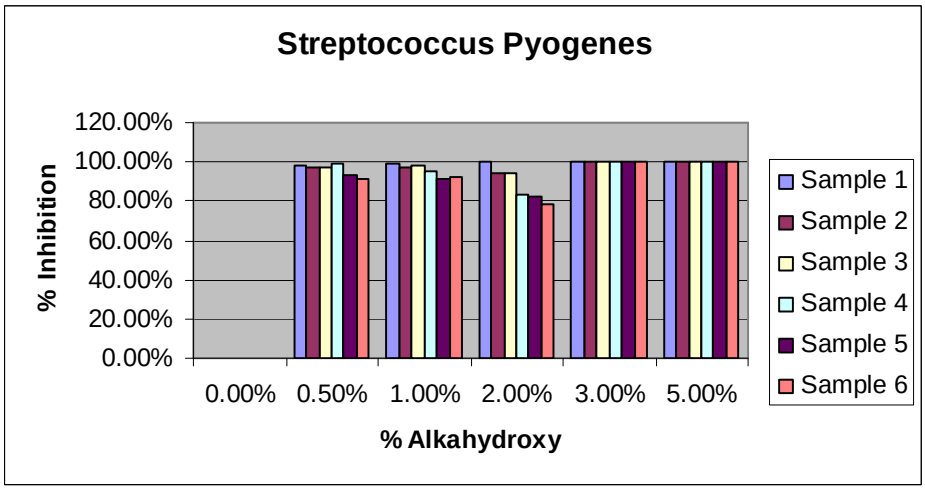

Escherichia coli K-12 Test
% Inhibition
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
0.00%
0.50%
1%
2%
3%
5%
% Alka Hydroxy
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6


Escherichia coli 0157:H7
% Inhibition
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
0.00%
0.50%
1.00%
2.00%
3.00%
5.00%
% Alka Hydroxy
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6


Salmonella Enterica
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
% Inhibition
0.00%
0.50%
1.00%
2.00%
3.00%
5.00%
% Alkahydroxy
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6


Staphylococcus Aureus
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
% Inhibition
0.00%
0.50%
1.00%
2.00%
3.00%
5.00%
% Alkahydroxy
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6


Enterococcus Faecalis
% Inhibition
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
0.00%
0.50%
1.00%
2.00%
3.00%
5.00%
% Alkahydroxy
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6


Streptococcus Pyogenes
% Inhibition
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
0.00%
0.50%
1.00%
2.00%
3.00%
5.00%
% Alkahydroxy
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6