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Microbiological Analysis
Final Report – 48 hours

Report Release Date: 11/28/2014
Product: CM Armor Surface Cleaner

Experiment 1: Antibiotic Testing

Plate	Substance	Staphylococcus aureus (Gram positive)	Phosphate Buffered Solution (PBS) - control
MacConkey	CTEK	No Growth	No Growth
	PBS	No Growth	No Growth
Blood Agar	CTEK	16mm	No Growth
	PBS	0mm	No Growth

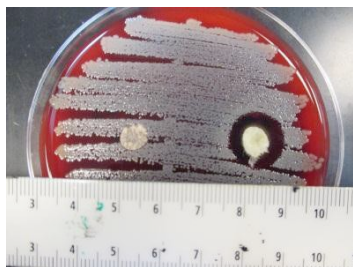Note: Hemolysis of red blood cells was noted around the red blood cells.

Figure 1: Blood Agar Plate with Staphylococcus aureus

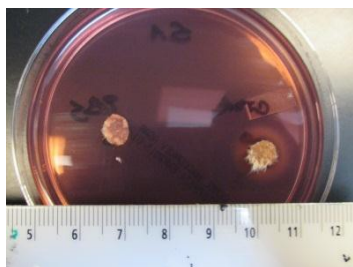**Figure 2: Blood Agar Plate with PBS (Contamination Control)**

Figure 3: MacConkey Plate with Staphylococcus aureus

Figure 4: MacConkey Plate with PBS (Contamination Control)

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Experiment 2: Surface Resistance

Table 2: Surface Growth Results					
Hours	Variables	Growth observed	Optical Density (OD) – 600m	Colony Forming Units (CFU)/mL**	1:10 CFU/mL**
24 hours	Untreated Slide/PBS	No	.000	0	0
	Untreated Slide/ Staphylococcus aureus	Yes	.001	>200	>200
	CTEK/ Staphylococcus aureus	No	.002	0	0
48 Hours	Untreated Slide/PBS	No	.079		
	Untreated Slide/ Staphylococcus aureus	Yes	.577		
	CTEK/ Staphylococcus aureus	Yes	.179		

**Sub-cultures were cultured at 100% and 1:10 dilution. Read 24 hours after inoculation of sub-culture.
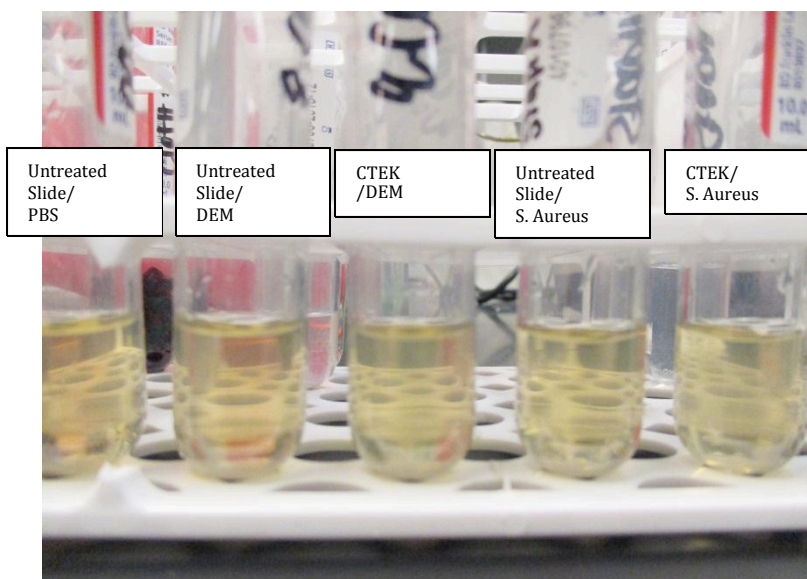

| Untreated Slide/ PBS | Untreated Slide/ DEM | CTEK /DEM | Untreated Slide/ S. Aureus | CTEK/ S. Aureus |

Figure 7: LB Broth immediately after inoculation with swab from described surface.

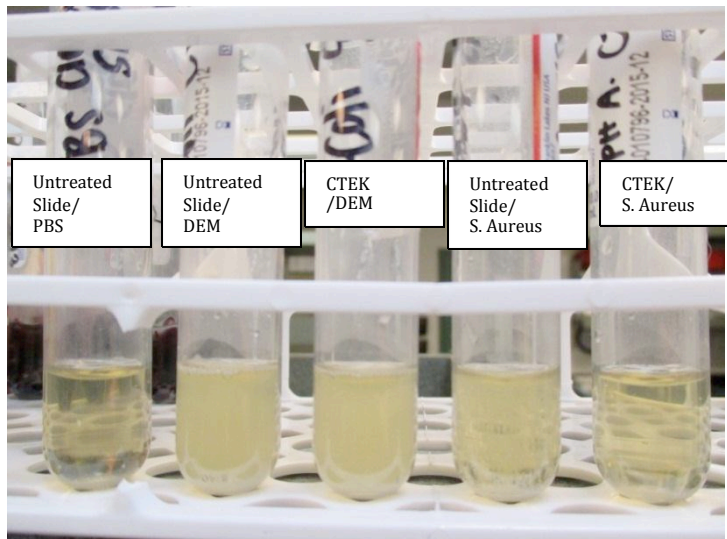| Untreated Slide/ PBS | Untreated Slide/ DEM | CTEK /DEM | Untreated Slide/ S. Aureus | CTEK/ S. Aureus |

Figure 8: LB Broth Incubated for 24 hours after inoculation with swab from described surface.

Figure 10: Blood Agar Plate inoculated from CTEK slide with Staphylococcus aureus

Figure 12: Blood Agar Plate inoculated from untreated slide with Staphylococcus

Microbiological Analysis
Preliminary Report – 24 hours
Report Release Date: 12/17/2014

Table 2: CM Armor Hand Sanitizer Result Table						
Hours	Bacteria	CM Armor added?	Incubation Time of Bacteria on Slide (minutes)	Growth observed	OD	CFU/mL**	1:10 CFU/mL**
24 hours	None	No	30	No	.010	0	0
	Staphylococcus aureus	No	30	Yes	.656	>100,000	>100,000
		Yes	10	No	.012	0	0
		Yes	30	No	.012	0	0
	Escherichia coli	No	30	Yes	.455	>100,000	>100,000
		Yes	10	No	.016	0	0
		Yes	30	No	.016	0	0
	Klebsiella pneumonia	No	30	Yes	.552	>100,000	>100,000
		Yes	10	No	.011	0	0
		Yes	30	Yes	.015	0	0

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Discussion

These experiments and this discussion is part of a research experiment performed by VantagePoint Laboratory Partners as purely research. These results should not be regarded for clinical purposes. For all experiments preformed, no replicates were run therefore all results could be statistical anomaly. For conclusive results, a much more extensive study is suggested. The results from these experiments may provide insight into what experiments and methods an extensive study could include. All methods used for these experiments were developed at VantagePoint Laboratory Partners and are described below in the Methods section.

Figure 1-6 illustrate that there is no growth of Staphylococcus aureus around CM Armor but grows uninhibited around the phosphate buffered solution (PBS). The contamination control was free of any growth. The slight discoloration around the CM Armor discs is caused by hemolysis of the blood cells in the blood agar plate. Experiment 1 results indicate that the CM Armor material has antibiotic properties toward Staphylococcus aureus (gram positive). Please note that in experiment 2, growth after exposure to nutrient rich environments imply that the CM Armor material may be bactericidal. The hemolysis caused by the CM Armor material may indicate toxicity to humans. Further experimentation should involve comparing it to other known positive antibiotic controls and toxicity testing. Previous testing from another lab indicate that the material is effective against Klebsiella pneumonia (gram negative) so should also be included in future experimentation.

In table 2, growth of Staphylococcus aureus was noted in the OD at 48 hours, the growth could not be confirmed by CFU because sub-cultures were not produced for 48 hours. Experiment 2 results indicate that the CM Armor material is effective in preventing the growth of Staphylococcus. Since Staphylococcus aureus grew once being re-introduced to a nutrient rich environment it is possible that the CM Armor material is bacteriostatic and not bactericidal.

Readings were halted after preliminary results after discussion with Alliance Clinical Research. Further experimental research requested by Alliance Clinical Research to be adapted to manufacturer's instructions of product use. According to the inventor a mechanical pressure is required for cell death. Experiment 4 will be commence pending client approval.

Method

This method was developed and run by VantagePoint Laboratory Partners. There are three experiments run in tandem. The first is culturing S.Aureus and determining if the material does kill and prevent growth of bacteria. The second is determining if using the solution as a surface cleaner would prevent bacterial growth. The cultures will be grown on MacConkey and Blood Agar, colony counts, and optical density (OD) will be taken of each culture.

I. Experiment 1 – Test for antibiotic properties
1. Take S. Aureus and inoculate 10mL of LB broth.
2. Incubate for 24 hours at 37°C.
3. Remove one milliliter and centrifuge at 4000rpm for 3 minutes.
4. The pellet should be resuspended immediately in 1mL phosphate buffered solution (PBS).
5. Take 300uL of the suspension and add to 10mL of PBS to achieve an optical density of 0.05 A600nm ($\sim 10^7$ colony forming units (CFU)/mL).
6. A Blood Agar plate and a MacConkey plate respectively will be seeded evenly with the PBS solution of $\sim 10^7$ colony forming units (CFU)/mL.
7. A separate Blood Agar and MacConkey plate will be inoculated with clean/plain PBS, these plates will act as the contamination controls.
8. All four plates will have a 5uL well of surface CTEK solution added to plate at a marked location. This mimics antibiotic disk resistance testing.
9. All four plates will also have a 5uL well of PBS added to the plate at a marked location. This is a control.
10. After 24 hours the plates will read. The width of any antibiotic zone will be measured and compared.

II. Experiment 2 – Test Surface Resistance to Antibiotics

1. The suspension that was created in Experiment 1 with ~10^7 CFU/mL will be be diluted with PBS in a 10-fold dilution to get a solution that is ~10^6 CFU/mL.
2. 25uL suspension of the bacteria will be added to a test surface (untreated microscope slide) and a microscope slide coated with the CTEK surface solution and then removed 5 minutes later using a cotton-tipped swab.
3. A third untreated microscope slide will have 25uL of PBS added to it and then removed 5 minutes later using a cotton-tipped swab (contamination control).
4. Each swab will be incolutated into a separate 1ml LB broth test tube.
5. The test tubes will be vortexed for 2 minutes
6. The test tubes will be incubated for 72 hours at 37℃.
7. At 24, 48 and 72 hours each solution will be sub-plated which will be incubated for 24 hours and then CFU will be counted. Plating 10-fold dilutions will be done when possible.
8. At 24, 48 and 72 hours each solution will be tested with spectroscopy to determine the OD at 600nm.

Quality Control
A. Acceptable Results:
Contamination Control (plates with no bacteria added) – should have no colony growth or OD
Culturing Control (plates with bacteria and no antibacterial substance) - PBS
If any of the controls have unexpected results, the experiment may have been exposed to contamination and could indicate unreliable data.

References
1. G. A. Pankey, L. D. Sabath. *Clinical Relevance of Bacteriostatic versus Bactericidal Mechanisms of Action in the Treatment of Gram-Positive Bacterial Infections.* Oxford Journals - Medicine & Health - Clinical Infectious Diseases Volume 38, Issue 6. Pp. 864-870.
2. Moore G, Griffith C., *A comparison of traditional and recently developed methods for monitoring surface hygiene within the food industry: an industry trial.* Int J Environ Health Res. 2002 Dec;12(4):317-29.
3. Michigan State University. *Examples of Antibiotic Sensitivity Testing Methods.* 11/18/14 http://amrls.cvm.msu.edu/microbiology/detecting-antimicrobial-resistance/test-methods/examples-of-antibiotic-sensitivity-tesing-methods
4. S Sutton. *Measurement of Cell Concentration in Suspension by Optical Density.* The Microbiology Network. 11/28/14. http://www.microbiol.org/resources/monographswhite-papers/measurement-of-cell-concentration-in-suspension-by-optical-density/