Modification of AATCC-100-1993 to Evaluate Silver Ion Containing Wound Dressings with *Pseudomonas aeruginosa*

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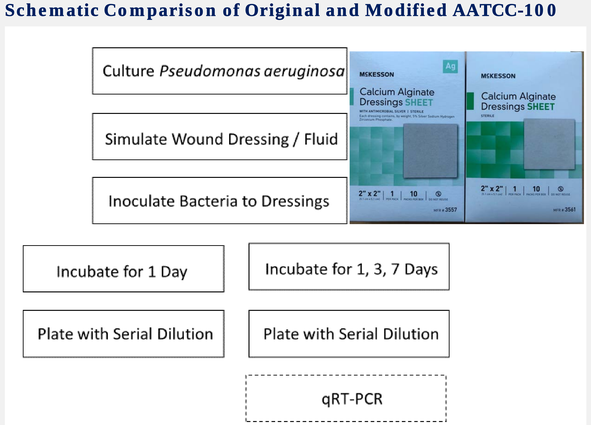
Abstract

Chronic wounds are a reoccurring healthcare problems in America and cost up to $50 billion annually. Improper wound care results in complications such as wound debridement, surgical amputation and increased morbidity/mortality due to opportunistic infections.1 FDA reviews antimicrobial data from wound dressings products submitted by sponsors. AATCC-100-1993 method is widely used to evaluate the efficacies of antimicrobial wound dressings with metal ions.2 However, this method may be limited due to testing methods that could miss viable but nonculturable (VBNC) bacteria. Therefore, this study proposes a new and efficient assay to measure time dependent efficacies of wound dressing using qRT-PCR. The test organism is *Pseudomonas aeruginosa* (an opportunistic pathogen; ATCC 15692) and compared with *Pseudomonas aeruginosa* (PA01). To mimic a wound dressing environment, samples of commercially available wound dressings with silver ion (positive treatment) and dressings without silver ion (negative treatment) are prepared under sterile conditions. One quadrant is used as a control to ensure sterility of sample preparation while the remaining three quadrants are used as biological replicates. These samples are saturated with 1.2mL of simulated wound fluid. Biological replicate samples are inoculated with 250uL of an overnight culture of *Pseudomonas aeruginosa*, 2.5x10^7 CFU/ml, respectively. Samples are incubated at 37˚C for 1, 3, 7 days. The wound dressing samples are vortexed for 30 seconds with 25mL of recovery buffer, three times. Aliquots are plated to measure CFU/mL and stored at -80˚C for molecular assays. Samples of harvested bacteria from each condition are re-inoculated into new wound dressing samples and incubated again for 1, 3, 7 days. Following, genomic extraction cDNA is to be synthesized from RNA samples and qRT-PCR analysis is to be used to track change in gene expression among a subset that are consistently expressed by viable bacteria. A panel of these genes expressed in viable bacteria from AATCC-100-1993 is to be compared to demonstrate microbial viability and reproducibility of the assay.

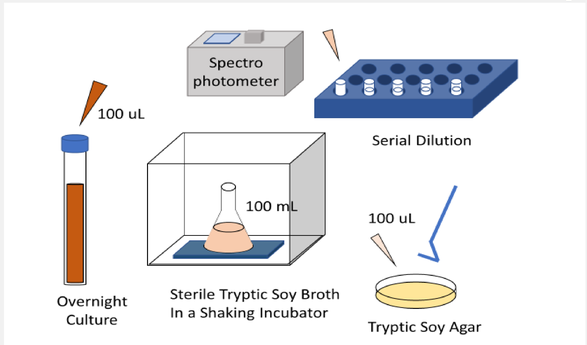
Materials and Methods

Schematic Comparison of Original and Modified AATCC-100

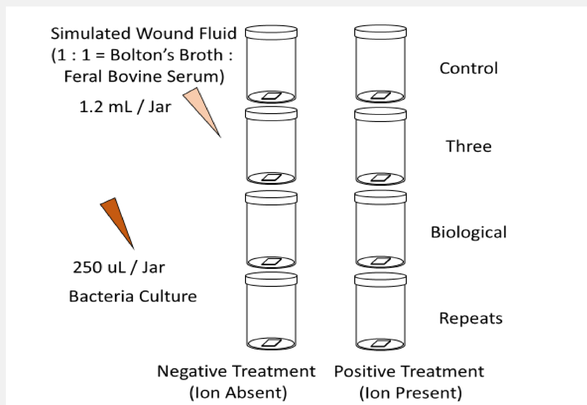


Materials and Methods

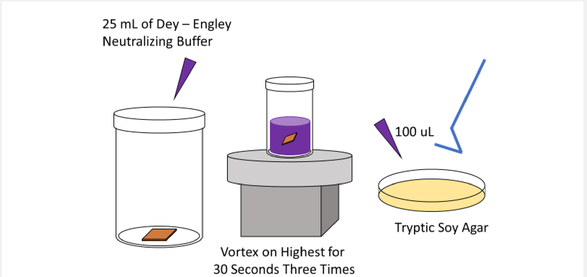
Generation of Calibration Curves and Initial Inoculation Sample



Day 1, 3, 7 Incubation Sample Preparation Layout

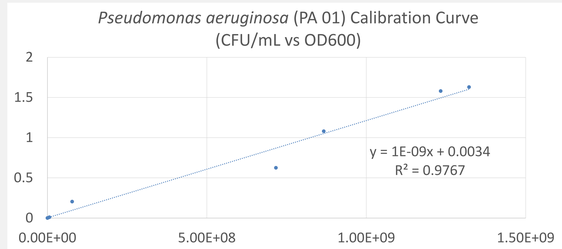


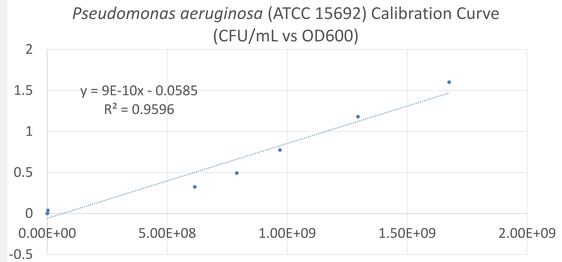
Harvesting Method Schematics



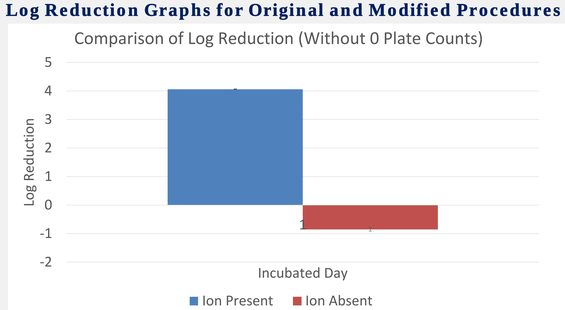
Results and Discussion

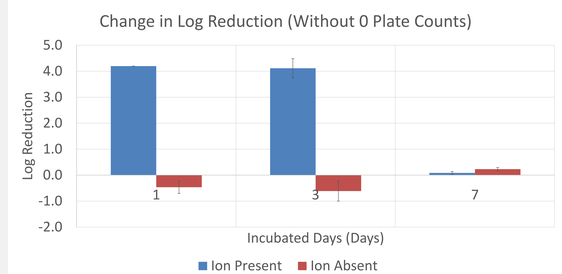
Calibration Curves





Log Reduction Graphs for Original and Modified Procedures





Results and Discussion

Randomness / Skewness Quantification with Kurtosis Approach

Collection of Randomness Analysis of Log Reduction (0 Counts = 0% Reduction)					
Condition	Day	Mean	Sample Standard Deviation	Sample Standard Error of the Mean	Kurtosis
Ag+	Day 1	1.372	2.068	0.689	-1.570
	Day 3	2.125	2.369	0.790	-2.322
	Day 7	0.029	0.051	0.017	2.448

Collection of Randomness Analysis of Log Reduction (0 Counts = 100% Reduction)					
Condition	Day	Mean	Sample Standard Deviation	Sample Standard Error of the Mean	Kurtosis
Ag+	Day 1	6.401	1.727	0.576	-1.493
	Day 3	5.521	2.364	0.788	0.424
	Day 7	5.203	3.839	1.280	-1.713

Collection of Randomness Analysis of Log Reduction					
Condition	Day	Mean	Sample Standard Deviation	Sample Standard Error of the Mean	Kurtosis
Ag-	Day 1	-0.180	0.459	0.153	4.273
	Day 3	-0.663	0.204	0.068	2.133
	Day 7	0.113	0.442	0.147	1.243

Both the original and modified AATCC-100-1993 methods with two strains of *Pseudomonas aeruginosa* show that silver ion containing wound dressings have log reduction results at 1 Day following incubation compared to those without silver. This value is also in agreement with the claimed efficacy by the manufacturer, which shows that the original AATCC-100-1993 is effective in measuring short-term antimicrobial properties. However, the modified method shows that antimicrobial property decrease as the incubation time lengthens. Kurtosis and skewness analysis shows that the original method is prone to high standard deviation and inconsistency for silver ion containing wound dressings due to frequent occurrences of '0' plate-counts. Furthermore, if the '0' plate-counts are considered as 100% reduction or 0% reduction, the skewness intensifies, which shows that solely depending on CFU/mL methods may lead to misinterpretation of data and the evaluation of antimicrobial wound dressing efficacy.

Conclusion

The modified AATCC-100-1993 is capable of tracking time dependent antimicrobial properties of wound dressings. However, solely depending on CFU/mL count techniques may result in high inconsistency and variability in experimental data. Therefore, further study into molecular assays such qRT-PCR is needed to evaluate the validity of microbial viability using current methods.

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