Efficacy, Duration and Residual Effects of Antimicrobials in Biomedical Research Surgical Facilities

LJ Wineinger, GK Moore and MR Talcott

Division of Comparative Medicine, Washington University School of Medicine, St. Louis, Missouri

Introduction

The objective of this study is to evaluate and compare multiple disinfectants for their immediate efficacy and the duration of their residual effect on the surfaces of facilities in which research surgeries are performed. Little information is available regarding standard disinfection protocols for biomedical research operating rooms and procedure areas. The ultimate goal is to establish an experimentally based, standardized protocol for effective sanitation of surgical areas commonly found in research settings. The first phase of the study seeks to determine the efficacy of multiple disinfectants. Samples to evaluate microbial growth will be taken from surgical procedure facilities prior to and after application of a treatment. The facilities will receive either the control agent or one treatment chemical and will be reassessed for the presence of microbes. To assess the duration of residual antimicrobial properties of the agents, RODAC plates will be used weekly for four weeks. We expect high level disinfectants to be more efficacious and possess a longer duration of sustained antimicrobial activity.

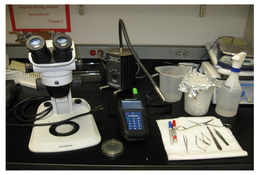

Figure 1. Photograph of a typical rodent surgical procedure area containing dissection scope, light source and associated instruments.

Materials and methods

Treatments include sterile water as the control, Nolvasan®, Roccal-D®, 200ppm bleach solution and 70% Isopropyl Alcohol. All chemical solutions were prepared according to manufacturers instructions and used within 24 hrs. Areas were first tested then thoroughly wetted with the randomly assigned treatment After being allowed to sit for ten minutes, the surface was wiped dry with sterile gauze then retested.

Samples were collected using both RODAC plates and the Charm® Luminometer. RODAC plates allowed the quantification of Colony Forming Units (CFU) read 48±2 hrs after collection. Colonies were identified morphologically. The luminometer provides results within 5 sec by measuring photons released by enzymatic activity of luciferase on the ATP produced by bacteria on the testing surface. Presence of ATP is proportional to the amount of light released which can be indicative of amount of bacteria present.

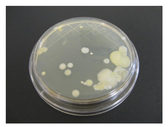

Figure 2. Image of a RODAC plate, Charm® Luminometer sample collection swab and novaLUM measuring unit: used in combination to quantify and identify microbial growth on procedure surfaces.

Results-Immediate Efficacy

Immediate Efficacy was tested in rodent surgical procedure areas. The luminometer measures photon production in Relative Light Units (RLU). Sampling revealed wide variation in pre-treatment luminometer measurements, ranging from two-thousand to 2.5 million RLU. This is partially due to time elapsed since last procedure performed and whether the surface was cleaned after the last procedure or is only disinfected prior to the next procedure.

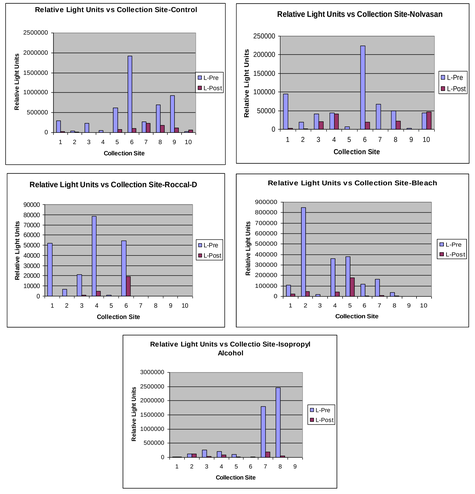

Figure 3. Each bar graph above represents the results obtained by application of one of the five randomly assigned treatments to rodent surgical procedure areas. Up to ten sites (data collection is still in progress) for each treatment were tested, both prior to ("Pre") and after ("Post") application of the treatment

For each testing location, the difference between the pre- and post-treatment RLU readings was calculated to indicate immediate efficacy in reduction of bacterial load. The difference was converted into percent in order to normalize the wide variation between locations. The percent reductions within each group were then averaged to provide an overall measurement of efficacy for each treatment.

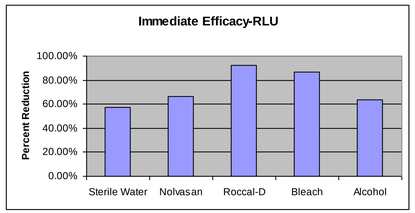

Figure 4. For each location, the difference between the pre- and post-treatment RLU readings was calculated to indicate immediate efficacy in reduction of bacterial load. The difference was converted into percent in order to normalize for the wide variation between locations. Within treatment groups, the differences were averaged to generate the above graph.

Results-Residual Effects

Figure 5. Large Animal Operating Room testing locations. Painted wall, Metal light fixture, Stainless steel horizontal surface.

Residual effects of the treatment chemicals were measured with RODAC plates via quantification of colony forming units (CFU). Samples were taken of demarcated, assigned areas prior to treatment, immediately after treatment and weekly thereafter for four weeks on three surface types (Wall, Horizontal Surface and Fixture).

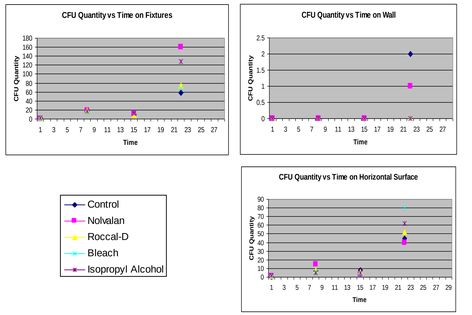

Figure 5. Grouped by location. Initial, Week 1, Week 2, Week 3 Data (collection is still in progress). CFU counts increased with time.

Identification

Colonies were identified as either Gram negative (-) or Gram positive (+) and as either bacilli (B) or cocci (C). Preliminary findings suggest that in the rodent procedure areas (+) C are the most prevalent at 49% of all colonies cultured followed by (-) B at 31%. (-) C and (+) B were in the minority, combined to represent only 20 % of all colonies.

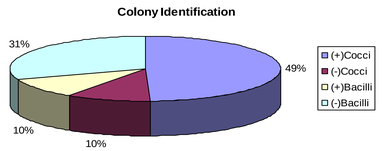

Figure 6. Relative distribution of colony morphology.

Discussion

Experimental design dictated that products sit in contact with surfaces for ten minutes. The RODAC plate requirements necessitated that the testing surface be wiped dry. Sterile water was chosen as the control because the mechanical action of wetting a surface for ten minutes and wiping it dry would be responsible for removal of some base level of bacterial removal.

The Charm® Luminometer measures ATP, which is present in both living organisms and organic material, thus it is possible to detect ATP that is not the result of viable bacterial organisms and can impact the overall results.

Data collection for the Immediate Efficacy phase has not yet been completed. The end result will be ten testing sites for each of the treatments and the control. We considered Roccal-D® and Nolvasan to be high level disinfectants, bleach and isopropyl alcohol to be low level disinfectants and sterile water as the control. In support of our hypothesis, preliminary data has indicated that a high level disinfectant, Roccal-D®, provides the most complete killing of bacterial populations, and that sterile water performed the worst, with decreases attributed to physical removal. We were surprised, however, to discover that Nolvasan did not surpass the disinfection capacity of 70% Isopropyl Alcohol and it was similarly unexpected to discover that a Bleach dilution of 200ppm appears to be a better disinfectant that Nolvasan.

Data collection for the Residual Effects phase is also incomplete. At this stage, CFU's have shown gradual increase as expected, however the superiority of one treatment's sustained ability to maintain low bacterial counts over that of another remains inconclusive. These data could have been complicated by non-uniform contamination of specific testing surfaces in the large animal operating room in association with use for scheduled surgeries.

It was significant that a large portion (31%) of bacterial colonies isolated are Gram negative bacilli due to their pathogenic propensity in veterinary medicine.

Further Investigations

- Statistical analysis was unavailable at the time of printing. However, all data will be evaluated for statistical significance upon completion of data collection.
- The data from the Immediate Efficacy Phase collected on RODAC plates was not included in this presentation but revealed results similar to that of the luminometer regarding the disinfection capacity of the treatments. Results from both RODAC and the luminometer will be evaluated for correlation.
- We will look at the different morphologically-identified groups of bacteria to determine if relative susceptibility of groups can be established.

Acknowledgments

Washington University in St. Louis School of Medicine

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College of Veterinary Medicine University of Missouri