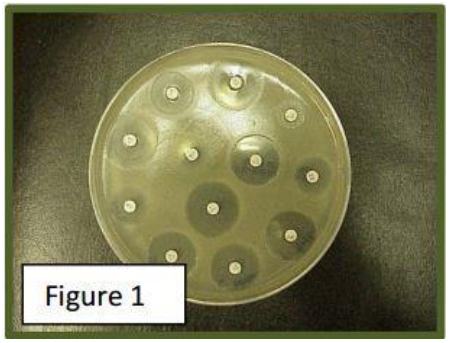
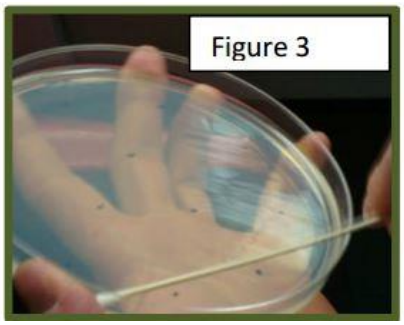
**Antibiotic Susceptibility Test (AST)**
**Clinical Microbiology Laboratory of Atlanta Medical Center**
**March 6, 2010**

**Objective: Determination of antibacterial properties of BerbereX Wound Cleanser**

**Background** - Antibiotic susceptibility testing (AST) is one method to assess which antibiotic will be most successful in treating a bacterial infection in vivo but also can be used to demonstrate concentrations of bacteria and compare the effectiveness or release pattern of the antibiotic by the Kirby-Bauer method. Wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear zone of inhibition is seen around the wafer indicating poor growth. This test offers a rapid interpretation. Two key antibiotics routinely used in orthopaedic surgery have laboratory standards; Tobramycin (10ug/ml) and Vancomycin (30ug/ml), are used to define resistance and sensitivity. Standardized discs with known concentrations are laid upon a lawn of bacteria. The bacteria are allowed to grow on enriched agar, and the zones of inhibition are measured (Figure 1). Sensitivity is assessed by reading the zone of inhibition in a plate grown to confluence:




| **Tobramycin Sensitivity Assessment** | | **Vancomycin Sensitivity Assessment** | |
|---|---|---|---|
| > 15mm | sensitivity less than 4ug/ml | > 17mm | sensitivity of less than 4 ug/ml |
| > 13-14 mm | intermediate resist. at 8ug/ml | 15-16 mm | intermediate resist. 8-16 ug/ml |
| < 12 mm | resistance at < 16 ug/ml | < 14mm | resistance < 32 ug/ml |



**Preparation of Bacterial Standards**
Standard suspensions of Pseudomonas aeruginosa (27853) for tobramycin, and Enterococcae faecalis (29212) for Vancomycin, were prepared in saline at 0.5 McFarland units. Four sites were selected and marked on the culture dish to identify and sustain position for plating and subsequently tracking reaction (Figure 2). Mueller-Hinton plates were swabbed to inoculate the entire 50-mm plate surface (Figure 3). At each of the 4 locations indicated, 0.05 ml of BerbereX solution was placed and cultures were incubated overnight. Zones of inhibition were photographed for the 4 sites (Figure 4). Tests for Enterococcus faecalis were carried out simultaneously but are not shown. Average diameter of inhibition on the P. aerguinosa plate was 21 millimeters, and that plated on E. faecalis was 12.5 millimeters.

**Summary**
BerbereX demonstrates antibiotic properties that inhibit bacterial growth when used as a topical solution on standard bacterial cultures. Inhibition was more pronounced for gram-negative rods than for gram-positive cocci at the end of overnight exposure.

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