

TEST: BACTERIA QUANTITATION IN WOUND SPECIMENS BY AMPLIFICATION OF 16S RIBOSOMAL DNA USING REAL-TIME PCR

PRINCIPLE:

Patients with diabetes may develop ulcers in their lower extremities which, if not diagnosed early and treated appropriately, may lead to lower extremity amputation. It has been suggested that the bacterial flora of these wounds may play an adverse role in the healing process. Several different bacteria, either aerobic or anaerobic, have been identified in these wounds by traditional microbiological culture techniques. These cultures, though, are laborious and the turnaround time is long. In recent years there has been advancement in identifying bacteria by molecular biology techniques, following the need to rapidly identify bacteria in the wound specimens.

The purpose of this test is to quantitate bacteria in wound cultures by using real-time PCR technique. DNA is extracted from wound specimens and is amplified with primers specific for the bacterial 16S ribosomal RNA. By using real-time PCR, the total number of bacterial cell copies is measured and reported.

SPECIMEN COLLECTION:

Collect wound specimens (approximately 100 µl) via small curette or sterile swab from the advancing border of the wound and put it into a sterile vial containing 1 ml *RNAlater*TM stabilization reagent (Qiagen). Specimen will be stable in the stabilization reagent for 1 day at room temperature or for 7 days in the refrigerator. Keep the specimen in the -20°C freezer for longer storage.

REFERENCES:

Davies C et al. J Clin Microb. 2004;31:3549-3557

METHOD: Real-time Polymerase Chain Reaction

REFERENCE RANGE

The results will be reported as follows:

Bacterial DNA present

< 50,000 copies

50,000 – 5,000,000 copies

5,000,000 copies

Note: Data represents bacterial copies as a number of bacterial genomes present in approximately 100 µl of biopsy materials tested. Less than 50,000 copies is indicative of normal skin flora. Greater than 5,000,000 copies suggests infected materials. Between 50,000 and 5,000,000 copies suggests that patients be monitored carefully for signs of infection.

This test was developed by the Clinical Immunology Laboratory at the RFUMS/The Chicago School. The performance characteristics of this test were determined and monitored. However, the use of this test has not been cleared or approved by the US FDA.

Turnaround time: 3 business days