

## Clinical Application of Immunoassay for Identification of *Staphylococcus aureus* and Monitoring Treatment Response in Diabetic Foot Infection

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**Introduction/Purpose:** Currently, there is no sensitive and specific diagnostic tool for identifying the active pathogen in a polymicrobial environment such as diabetic foot infection (DFI). In addition, monitoring the success of antibiotic treatment is limited to clinical signs and nonspecific inflammatory markers. Consequently, surgeons are often forced to make interventional decisions without adequate prognostic information. While DFI are generally polymicrobial, about 50% prominently include *Staphylococcus aureus* (SA). We investigated applicability of utilizing a novel diagnostic immunoassay that measures the patient's current production of anti-SA antibodies (IgG) to accurately diagnose SA and monitor its pathogenic activity. We hypothesize that 1) compared to standard culture, the immunoassay has a higher sensitivity to detect SA and 2) able monitor changes in pathogenic activity of SA in DFI.

**Methods:** From July 2015 to August 2016, we enrolled 20 diabetic patients with DFU who displayed clinical symptoms and signs of infection which necessitated hospitalization and undertook initial foot salvage therapy (FST): irrigation and debridement followed by wet-to-dry dressings and 6 weeks of intravenous antibiotic treatment. At weeks 0, 4, 8 and 12, the infected DFUs samples were obtained for standard culture and 16S rRNA microbiome analysis. Whole blood and serum samples were collected to measure the abundance of anti-SA IgG in the serum and in the in vitro secretions of antibody-secreting cells harvested from whole blood in "medium enriched for newly synthesized antibody". Sensitivity and specificity for detection of SA were compared against the standard culture and microbiome analysis. Preliminary analyses compare the ability of the SA immunoassay to track therapy and its concordance with changes in the microbiome.

**Results:** Of the 20 enrolled patients, 18 were available for at least partial follow-up and only four completed the entire sampling protocol. At the enrollment, 12 patients (60%) were identified positive for SA infection by at least one diagnostic method, while only 8 were diagnosed by standard culture. Six out of 10 SA-positive patients showed polymicrobial growth. The concordance rate for the presence or absence of SA was 85% between the immunoassay and microbiome, 70% between immunoassay and standard culture and 75% between microbiome and standard culture. Comparison of serial samples from the 7 subjects who were SA-positive by both the immunoassay and microbiome analysis demonstrated trends that the two novel assays provide complementary measures of therapeutic success. (Figure 1)

**Conclusion:** Measurement of anti-SA antibodies showed higher sensitivity than standard culture and was able to monitor changes in pathogenic activity of SA in DFI undertaking salvage treatment. This novel immunoassay may serve as an important diagnostic and prognostic tools for monitoring SA infection in polymicrobial DFI. It provides important information for counseling patients of treatment response, prognosis, and determining to pursue further foot salvage versus amputation. Future study will include expanding immunoassay measure other commonly found organisms in DFI.

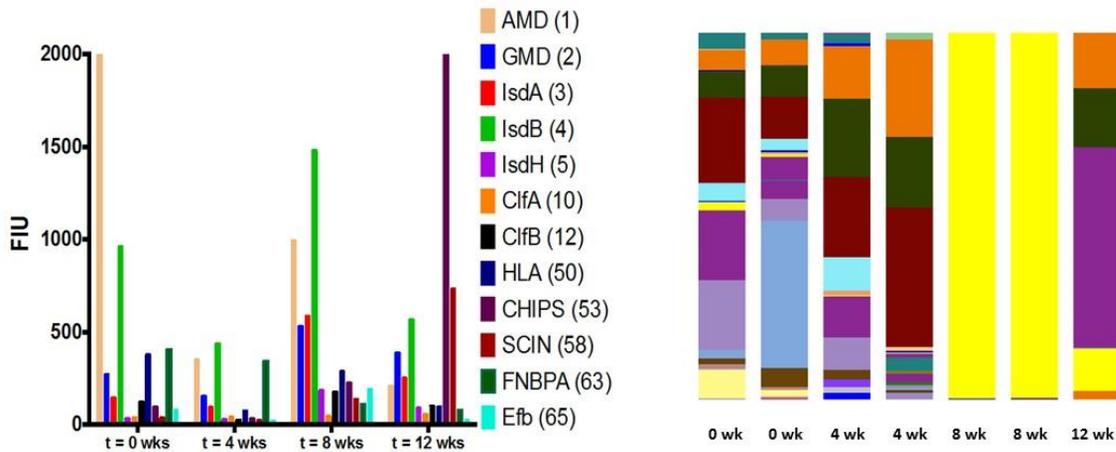


Figure 1. Patient is *S. aureus* positive by microbiome and immunoassay at enrollment, but negative by standard culture. Immunoassay showed decreased activity at 4 weeks, which was during the 6 weeks course of intravenous antibiotics treatment period. Resurging *S. aureus* activity was noted at 8 weeks with further increment by 12 weeks with antigen shift. The corresponding microbiome analysis shows relatively large *S. aureus* abundance (purple) at initial presentation. Overall microbial diversity is decreased by 8 weeks absence of *S. aureus*, then resurgence of *S. aureus* is noted at 12 weeks.