# Diagnosis and Tracking of Spinal *Staphylococcus aureus* Orthopaedic Implant Infections Using CHIP-based Arrayed Imaging Reflectometry (StaphAIR)

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**INTRODUCTION**: The majority of spinal infections are caused by *Staphylococcus aureus*[1], and spinal implant infections have a high rate of morbidity with a lethality of as much as 20% for infected patients[2]. Currently, infections are diagnosed and monitored using clinical indicators such as onset of back or neck pain, fever, or neurological symptoms, followed by blood work analysis for serum-based inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell counts (WBC)[1]. Although microbiological culture is the clinical gold standard for determining the infecting pathogen, its sensitivity is only 52.2%[1]. StaphAIR, an optical biosensor platform based on Arrayed Imaging Reflectometry (AIR), can be utilized to detect pathogen-specific antibodies during *S. aureus* infections[3]. In addition to serum fluid, a novel analytic fluid called MENSA was created from peripheral blood mononuclear cells of patient's whole blood and cultured to produce a "medium-enriched for newly synthesized anti-*S.aureus* antibodies"[4]. We hypothesize that serum and MENSA can be utilized along with the expanded StaphAIR array to provide a new avenue of diagnosis that overcomes the limitations of microbiological culture and allows for faster and more accurate diagnosis and tracking of *S. aureus* infections.

**METHODS**: ***StaphAIR antigen array:*** Besides the original 8 antigens used in the published study on StaphAIR[3], 13 additional antigens were chosen based on knowledge of *S. aureus* microbial pathogenesis and extensive literature searches. Antigens included secreted leukotoxins and enterotoxins, cell wall-modifying enzymes, and cell wall adhesins. 1410Å thick silicon/silicon dioxide chips were printed with antigens in 300–350 pL quantities, then blocked and stabilized as previously described[3]. The sensitivity of each antigen was tested using dilutions of pooled positive serum samples from *S. aureus* osteomyelitis. ***StaphAIR Immunoassay:*** Serum and MENSA samples were obtained from patients undergoing spinal implant revision surgery in an ongoing IRB-approved clinical study. Serum samples were diluted 1:250 with EAB20, MENSA samples were undiluted, and controls were EAB20 and RPMI + 10% FBS respectively. Chips were incubated in samples overnight at 4°C and underwent an amplification step in affinitive goat anti-human IgG. Chips were imaged at exposures from 6 to 1000 ms and optimal exposures for each antigen-antibody complex were individually controlled for overexposure. The reflectance of bound antibodies was quantified into thickness using the Adarza Biosystems ZIVA data analysis tool[3]. Controls were subtracted to obtain a final bound antibody thickness (expressed as Å) (**Fig. 1A**). ***Statistical analysis:*** Antibody thickness measurements were assessed for diagnostic ability using receiver operating characteristic (ROC) curve analysis, with overall accuracy summarized by the area under the ROC curve (AUC). p-value of <0.05 is considered significant.

**RESULTS**: Out of a total of 32 patient samples, 17 were confirmed to be uninfected (controls), 6 presented with *Cutibacterium acnes* infections, 1 with *Candida albicans,* 4 with *Staphylococcus epidermidis*, and 4 with *Staphylococcus aureus* (one of which was methicillin-resistant, MRSA) (**Fig. 1B**). As predicted, samples from *S. aureus*-infected patients 1008, 1009, 1020, and 1021 demonstrated higher antigen-specific IgG levels in serum samples. ROC curve analysis was performed for all 21 antigens to identify their individual diagnostic potential. IsdB, an iron-regulated surface determinant protein, showed the greatest diagnostic ability (AUC = 0.97, ***p<0.001) (**Fig. 1C**). A comparison of ongoing IgG responses in infected patients demonstrated that certain antigens are better predictors of ongoing infections (**Fig. 1D**).

**DISCUSSION**: In this ongoing clinical study, we demonstrate the strength of StaphAIR as a robust diagnostic tool for relatively non-invasively identifying patients with *S. aureus* spine hardware infections. Most importantly, our expansion of the antigen array from 8 to 21 antigens provides greater diagnostic accuracy, which is crucial when determining the next steps of treatment for patients with spinal infections. Ongoing studies involve analyzing anti-*S. aureus* antibodies longitudinally to track disease progression and treatment response post-surgery. Furthermore, our current efforts are aimed at increasing the sample size to 200 patients to robustly test this diagnostic tool.

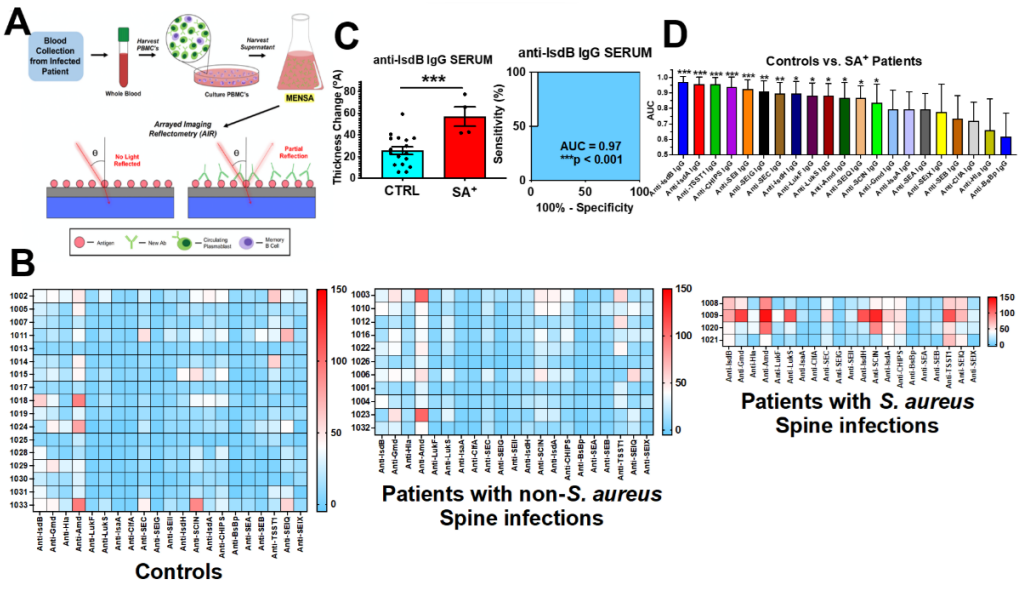


**Figure 1: StaphAIR immunodiagnostic assay for reliably identifying blood-based *S. aureus*-specific antibodies in patients undergoing spine hardware surgeries. A)** Schematic representation of sample sparing StaphAIR immunodiagnostic assay to detect anti-*S. aureus* antibodies. AIR microarray chips were coated with 21 different antigens (IsdB, IsdH, IsdA, Gmd, Amd, SCIN, CHIPS, Hla, LukF, LukS, SEA, SEB, SEC, TSST-1, SElG, SElQ, SElI, SElX, IsaA, ClfA, and SdrE (in sextuplicates) interspersed between internal assay controls (biotin-PNIPAM in triplicates). **B)** In an ongoing clinical study, multiplex StaphAIR immunoassays were performed on 32 patients undergoing spinal surgeries. Sample collection was performed at the time of spine surgery (time 0) and anti-*S. aureus* IgG responses were evaluated as the median thickness change (Å) for each of the 21 antigens. Expectedly, *S. aureus*-infected patients had higher serum antigen-specific IgG levels than controls and non-*S. aureus* infected patients. **C)** The primary anti-IsdB IgG levels in serum between *S. aureus* infected and uninfected control patients are depicted. This data was used to analyze the receiver operating characteristic (ROC) curve for IsdB antigen to identify their diagnostic potential. The resulting ROC curve is highly predictive of S. aureus spine hardware infections. **D)** The ROC curve analyses were performed for all 21 antigens and the resulting ROC curves are rank-ordered from highest to lowest AUC.

**SIGNIFICANCE**: Our clinical study supports the use of a specific panel of antigens for arrayed imaging reflectometry to relatively non-invasively diagnose *S. aureus* spine hardware infections in patients. Importantly, the sensitivity of the StaphAIR technique adds reliability that is currently not achievable with microbiological culture.

**REFERENCES**: [1] Tsantes AG, et al. Microorganisms. 2020. [2] Pendersen KM, et al. Spine J. 2015. [3] Klose AM, et al. Anal Chem. 2021. [4] Oh I, et al. Infection and Immunity. 2018.

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