

mariAST[®] in-well culture methodology for direct, real-time and rapid phenotypic AST enables bacterial detection down to 1 CFU/well

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Introduction

mariAST[®] (ArcDia International Ltd) is the only published product concept that allows rapid and real-time phenotypic antimicrobial susceptibility testing (AST) directly from polymicrobial clinical samples. It is based on the same separation-free ArcDia[™] two-photon excitation (TPX) fluorescence technique for bioaffinity assays as mariPOC[®] (Figure 1). mariPOC[®] is a fully automated platform for rapid and multianalyte identification of pathogens (www.arcDia.com). When the detection of live bacteria with specific antibody reagents is combined with in-well culture, the theoretical detection sensitivity is 1 CFU/well. In few hours one CFU multiplies to bacterial concentration that exceeds the level needed for positive fluorescence readout. We studied how well the theoretical sensitivity can be reached empirically for *Staphylococcus aureus*.



Figure 1. Automated and multianalyte mariPOC[®] test system for point-of-care clinical diagnostics use.

Methods

A dilution series (0.1–102 CFU/well) from overnight cultured *S. aureus* was done into TSB medium. Immunoassay reagents in TSB medium and the bacterial dilutions in four replicates were combined in the wells of a 384-well plate (20 µl per well). The wells were sealed. Bacterial cultures were incubated inside a mariPOC[®] fluorometer (+32°C) and immunoassay signals were measured in 20 minute intervals up to 12 hours. Bacterial concentration of the original stock was determined on blood agar plates using standard plate culturing method.

Results

Analytical sensitivity for direct *S. aureus* detection without culture was 260 CFU/well. With the in-well culture assay one out of four replicates showed growth in *S. aureus* concentrations 0.1 and 0.4 CFU/well. Three out of four replicates from sample with initial concentration of 0.9 CFU/well showed growth at 7 hours (Figure 2).

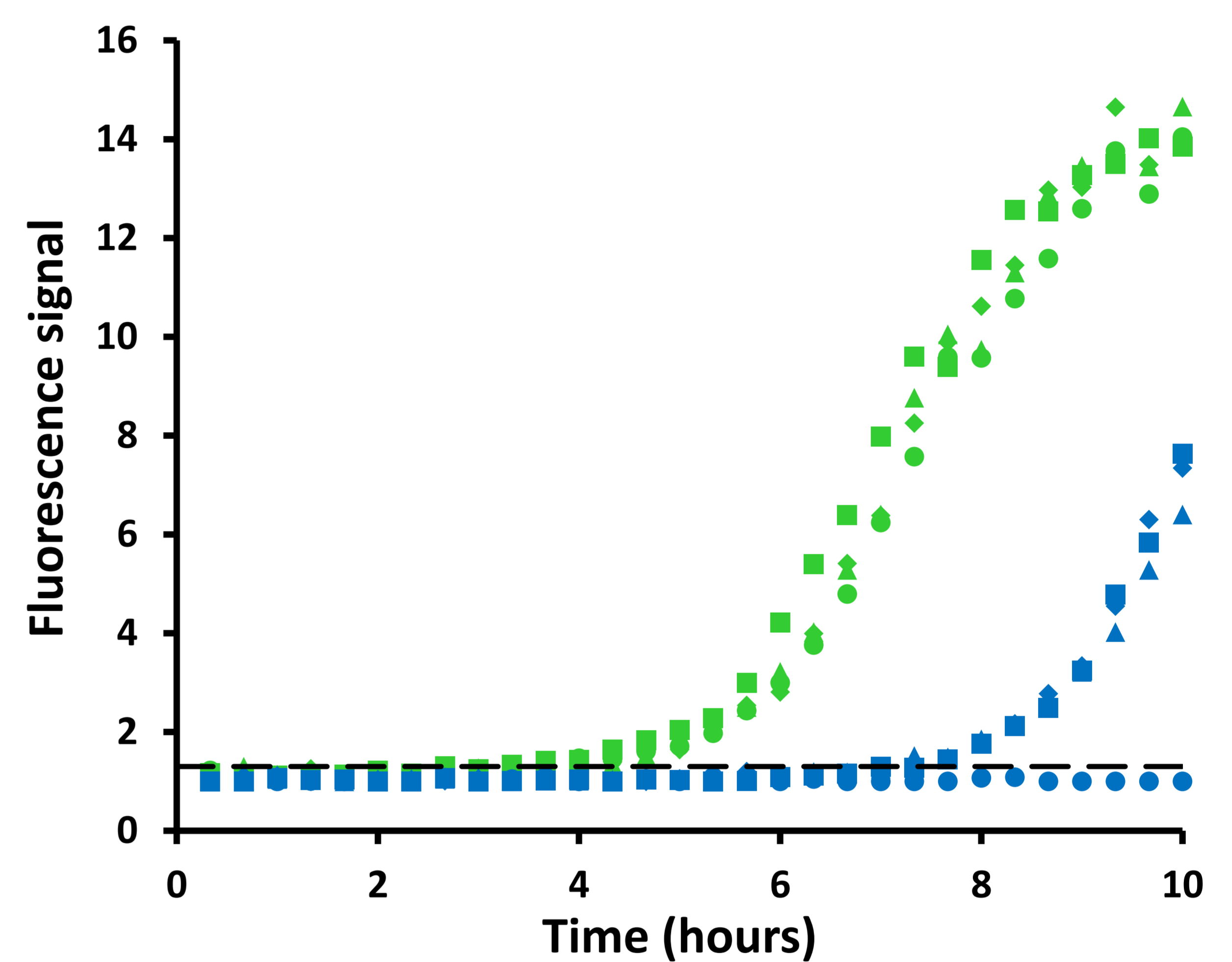


Figure 2. In-well culture of *S. aureus* combined with specific real-time immunoassay detection. Green and blue marks (unique symbols for replicates) show fluorescence levels of samples with initial *S. aureus* concentration of 30 and 0.9 CFU/well, respectively. Dashed line is the assay cut-off.

Conclusions

This study showed that even in suboptimal growth conditions (small well air volume, suboptimal temperature, non-breathable sealing tape) mariAST[®] assay methodology can reach the maximum sensitivity, one viable bacterium per reaction well, in 7–8 hours. In optimal growth conditions this time is expected to be about 4 hours. Sensitivity in CFU/mL units can be further improved by increasing the sample volume.