Supporting information

Near-digital amplification in paper improves sensitivity and speed in biplexed reactions

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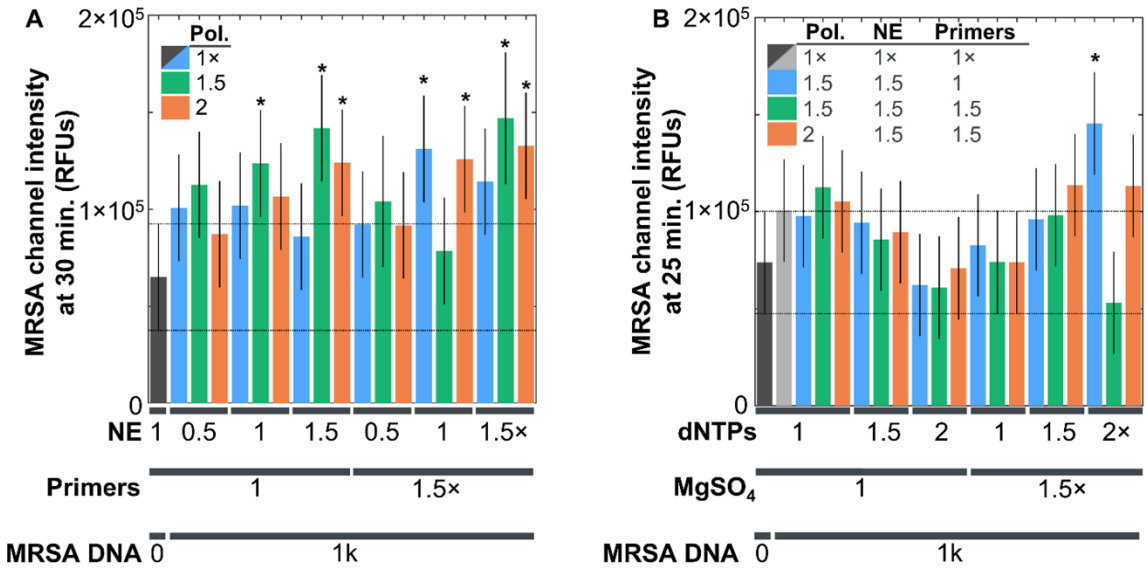

Figure S1. **Optimizing assay time to result** by varying the lyophilized master mix concentrations of polymerase (pol.), nicking enzyme (NE), primers, nucleotides (dNTPs), and magnesium sulfate ($MgSO_4$) relative to baseline (1×). All master mixes were lyophilized in glass fiber pads, rehydrated with MRSA DNA mixed with 100k copies of internal amplification control DNA, and run in a PMMA tray in a heated fluorescence plate reader. (**a**) Seven master mixes amplified by 30 minutes. (**b**) Three of the master mixes from Panel A were further tested with varying levels of nucleotides and magnesium sulfate. One master mix amplified by 25 minutes. In both panels, bars show mean and 95% confidence intervals for 3 replicates (* indicates $p<0.05$, one-way ANOVA relative to negative control).

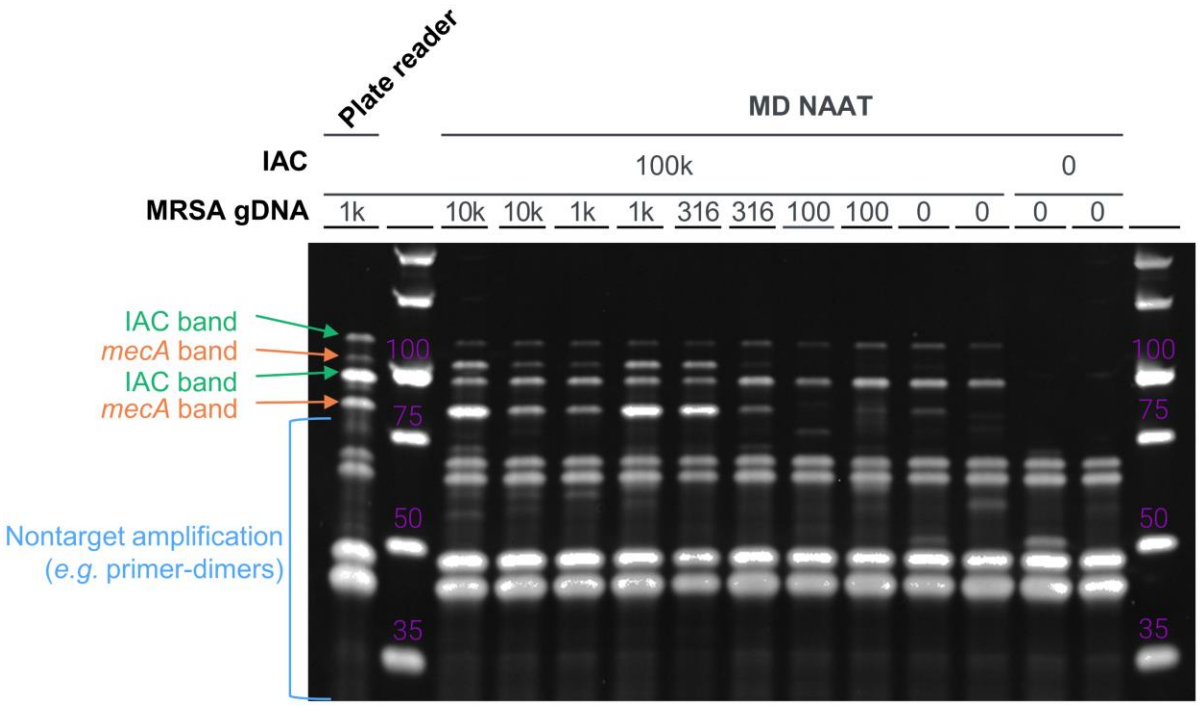

Figure S2. Gel electrophoresis of completed amplification reactions which were run in paper and centrifuged to isolate the liquid in the amplification pads corroborate real-time phone images. Amplification in glass fiber pads in the MD NAAT show amplification that was similar to a tube control run in a plate reader (far left). Bands corresponding to the *mecA* gene and internal amplification control (IAC) DNA were observed. The particular isothermal amplification strategy, isothermal strand displacement amplification, always shows bands from nontarget amplification (*e.g.* primer-dimers) such as those shown in the lower half of the gel. Truly negative reactions were indeed negative, all reactions containing IAC showed IAC-specific bands, and all MRSA-containing reactions showed *mecA*-specific bands at above 316 copies.