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Supplementary Information

Specific detection of DNA through coupling of a TaqMan assay with surface enhanced Raman scattering (SERS)

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Experimental

Colloid Preparation. Silver citrate-reduced nanoparticles were prepared by heating 500 mL of distilled water to 45 °C and adding silver nitrate (90 mg). Heating was continued until 98 °C then sodium citrate (10 mL of 1% aqueous solution) was added. The temperature was maintained at 98 °C for 90 minutes with continuous stirring throughout. The resulting colloid was analysed by UV-vis spectroscopy and the λ_{max} was found to be 401 nm with a full width half-height (FWHH) measured to be 87 nm. The nanoparticles were characterised to be approximately spherical with a diameter of 36 nm and the concentration of the colloid was calculated to be 1.3 x 10^{-10} mol dm^{-3}.

Oligonucleotides. The DNA sequences in the assay (Table S1) were chosen to correspond to the *mec*A gene of *Staphylococcus aureus.* All oligonucleotides used within this study were purchased from MWG Operon (UK) on a 0.01 μM scale with HPLC purification, except the biotinylated TAMRA labelled probe which had been purchased from Integrated DNA Technologies (IDT) Inc. (Belgium) 250 nM scale with HPLC purification. The genomic DNA used within the study was EMRSA NCTC strain 13142 and SA NCTC strain 8325. The strains were obtained from the national health protection agency culture collections (HPACC Salisbury, UK) and cultured in sterile Tryptone Soy Broth (Oxoid). The cells were centrifuged at 16.110g for 10 min at 4 °C. The supernatant was then removed and 1 mL of Lysostaphin was added. The suspension was incubated for 30 min at 37 °C to lyse the cells. DNA was extracted according to the manufacture's protocol from the lysate using a QIAamp DNA minikit (Qiagen, UK).

Table S1: Oligonucleotide sequences and modifications

Name	*Sequence (5'- 3')*	*5' Modification*	*3'Modification*
TaqSERS probe	TGGAAGTTAGATTGGGATCATAGCGTCAT	Biotin	HEG-10A-TAMRA
MRSA Template	TGGTCTTTCTGCATTCCTGGAATAATGACGCTATGATCCC AATCTAACTTCCACATACCATCTTCTTTAACAAAATTAAA TTGAACGTTGCGATCAATG	-	-
Forward Primer	CATTGATCGCAACGTTCAATTT	-	-
Reverse Primer	TGGTCTTTCTGCATTCCTGGA	-	-

PCR Preparation. PCR products for *mecA* were prepared using a 50 μL reaction volume. Each reaction contained 5 μL of 10x PCR reaction buffer, 2 μL of 25 mM $MgCl_2$, 1 μL of 2.5 U μL^{-1} HotStar Taq DNA polymerase, all reagents were purchased from Qiagen, UK. 0.8 μL of 10 mM deoxynucleoside triphosphates (dNTPs) were also added (Merck,UK). 2 μL of 10 μM forward and reverse primer and 1.4 μL of 18 μM labelled probe were added. 33.8 μL of DEPC treated water and either 99 bases synthetic or genomic target DNA (2 μL, 6 ng μL^{-1}) were added to give a final volume of 50 μL. QPCR samples were

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also prepared which contained SYBR Green (3 µL, 10,000x dilution) and 32.2 µL DEPC water, no probe was added to these samples. The mixture underwent PCR thermal cycling using a Stratagene Mx3000P QPCR system. The cycling profile used was: 10 mins at 95°C; 30s at 95 °C, 60 s at 60 °C, 60 s at 72 °C repeated for 30 cycles, final extension at 72 °C for 10 mins. Throughout PCR the fluorescence emitted by samples containing SYBR green was monitored using a 516 nm emission wavelength filter set.

Bead Separation. Streptavidin coated magnetic beads (New England Biolabs, UK) were washed immediately prior to use. Streptavidin beads were prepared by washing 3 times in binding wash buffer, which contained Tris (20 mM), NaCl (300 mM) and $MgCl_2$ (5 mM). An excess of beads was added based upon the binding capacity of beads which was 500 pmol mg^{-1} for a biotinylated oligonucleotide, thus 1 mL of beads will bind to 2 nmol of biotinylated DNA. The beads were added directly to the PCR product. The samples were left to incubate at room temperature with the beads for 30 mins with gentle shaking. The beads were then separated from the supernatant by the application of a magnet and the supernatant was removed prior to SERS analysis.

Fluorescence Analysis. The fluorescence for the PCR product (10 µL) was analysed using a Stratagene MX3000P QPCR system. After the bead wash, 5 replicates of the PCR product were analysed 5 times using a TAMRA filter set, with an excitation wavelength of 556 nm and an emission wavelength 580 nm.

SERS. The PCR product (10 µL) was added to a disposable cuvette. To this, an aqueous solution of spermine hydrochloride (10 µL, 0.1 M), distilled water (250 µL) and silver citrate-reduced nanoparticles (250 µL) were added. The sample was thoroughly mixed and the SERS spectra were obtained within 5 minutes of colloid addition. The spectra were recorded on a Renishaw InVia Raman with an argon ion laser with an excitation wavelength of 514.5 nm using a 1 x 1s accumulation time and a 20x obj. The spectra were baseline corrected using Grams software. 5 aliquots of 4 replicate samples, a total of 20 SERS measurements per condition, were analysed.

Gel electrophoresis. Capillary gel electrophoresis was carried out using an Agilent Bioanalyzer and DNA1000 kit including microfluidic chips and reagents (Agilent Technologies, UK). Procedures were carried out following the manufacture's protocol.

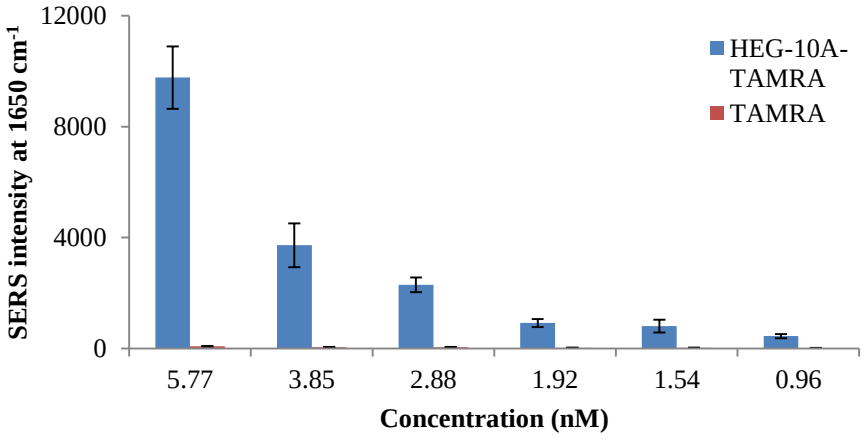

Figure S1: Comparison of SERS intensity at 1650 cm^{-1} of HEG-10A-TAMRA and TAMRA dye

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Dye labelled probe and dye were analysed directly using the SERS conditions described above. No SERS peaks were obtained from the unattached TAMRA dye, whereas strong SERS signals were obtained from the TAMRA dye through attachment of an oligonucleotide tail.

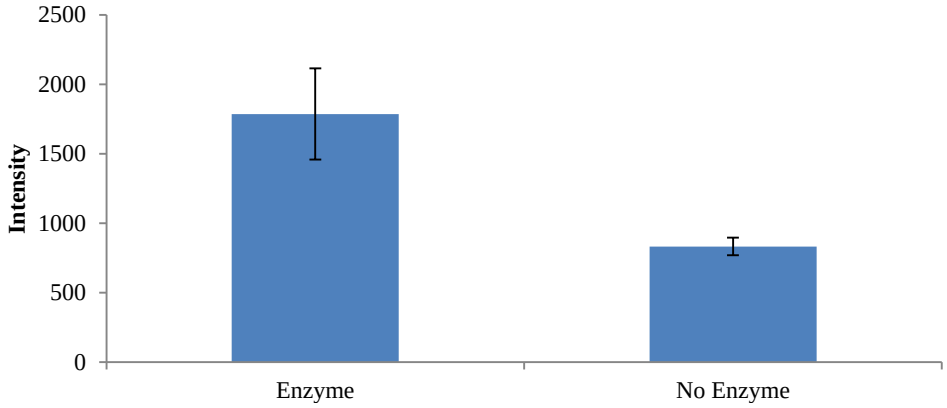


Figure S2: Comparison of enzyme digest with and without enzyme present.

Enzyme digestion of the TaqSERS probe was carried out by diluting the TaqSERS probe (0.5 µM) in 10x Qiagen PCR reaction buffer and adding 1 µL of 2.5 U μL^{-1} Qiagen HotStar Taq DNA polymerase. A control was also prepared which contain no enzyme. The samples were initially heated at 95 °C for 10 mins to activate the hot start enzyme and then the samples were heated at 37 °C for 2 hours. After heating samples were washed in a 3x excess of streptavidin magnetic beads and analysed by SERS. Both procedures as described above.

Significantly higher signal intensity was obtained from samples containing the polymerase enzyme, indicating the probe is being digested in the absence of hybridisation to the target gene. The small signal intensity obtained for the no enzyme control also indicates that the bead removal step is not 100 % efficient.

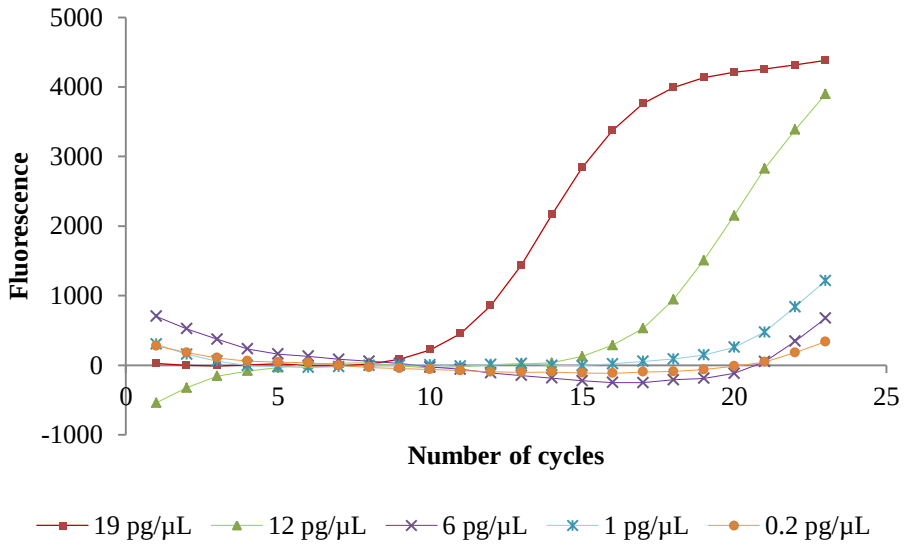
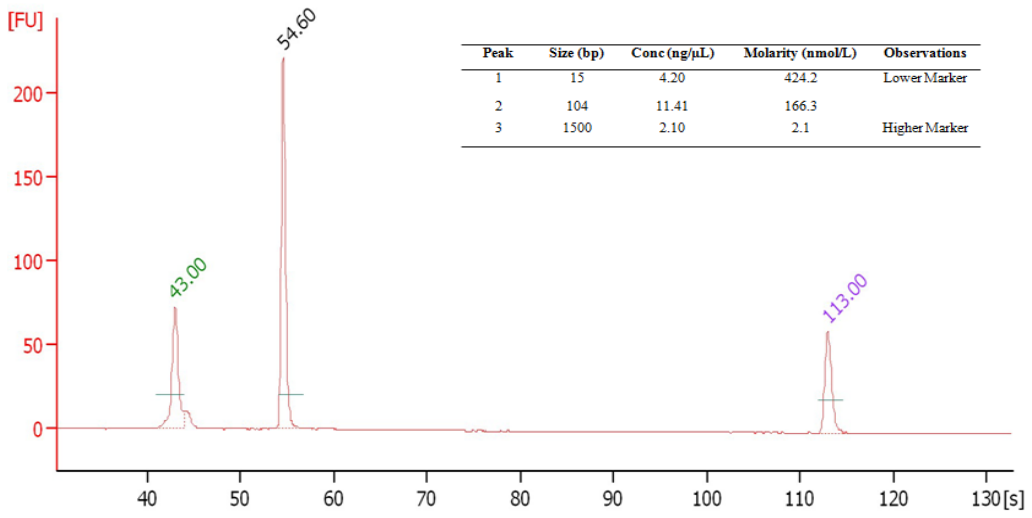
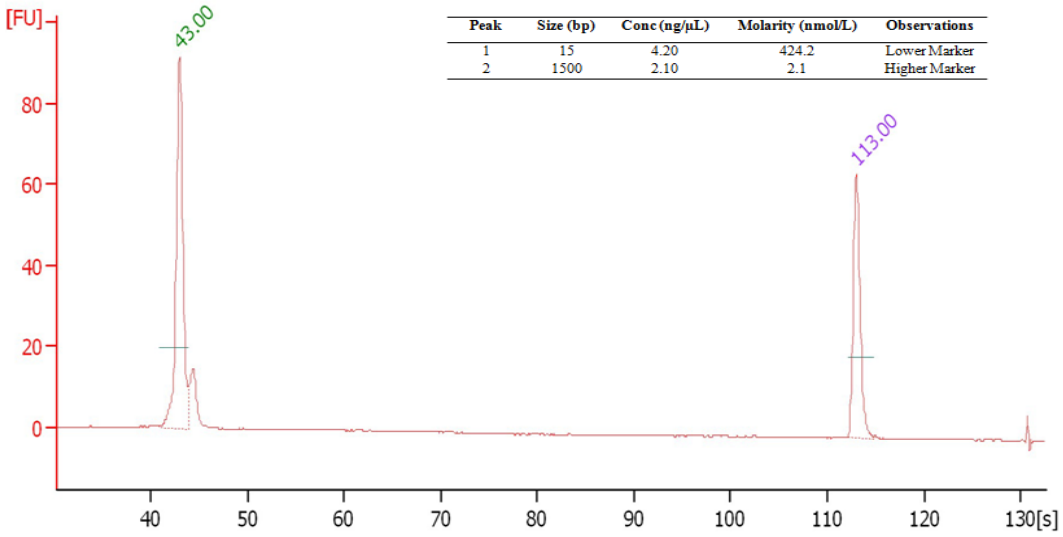


Figure S3: PCR amplification of different concentrations of synthetic MRSA template over 23 cycles.



Peak	Size (bp)	Conc (ng/µL)	Molarity (nmol/L)	Observations
1	15	4.20	424.2	Lower Marker
2	104	11.41	166.3	
3	1500	2.10	2.1	Higher Marker

Figure S4: Electrophorogram and quantitative data of MRSA PCR product



Peak	Size (bp)	Conc (ng/µL)	Molarity (nmol/L)	Observations
1	15	4.20	424.2	Lower Marker
2	1500	2.10	2.1	Higher Marker

Figure S5: Electrophorogram and quantitative data of SA PCR product

Intense peak present within MRSA electrophorogram shows specific amplification from the mecA gene. Whereas, no product peaks were obtained from the SA control and no amplification is observed.