Supporting Information

SERS Detection of Multiple Antimicrobial-Resistant Pathogens Using Nanosensors

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Experimental

Chemicals and Antibodies

Silver nitrate, sodium citrate, sodium chloride, iron(II)chloride tetrahydrate, iron(III)chloride hexahydrate, sodium hydroxide (anhydrous), glucose, calcium nitrate tetrahydrate, magnesium nitrate hydrate, 7-dimethylamino-4-methylcoumarin-3-isothiocyanate (DACITC), concanavalin A (ConA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide sodium salt (NHS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) powder, 2-(*N*-morpholino)ethanesulfonic acid (MES), phosphate buffer (PB), agarose, TRIS Borate EDTA buffer (TBE), gel loading buffer, Luria-Bertani broth with agar (LB Miller), Luria-Bertani (LB) broth and MacConkey agar and MacConkey agar were purchased from Sigma-Aldrich (Irvine, UK). Malachite green isothiocyanate (MGITC), heterobifunctional thiol/carboxy polyethylene glycol ($CTPEG_{635}$) were purchased from Thermo Fisher Scientific (Inchinnan, UK). Heterobifunctional thiol/carboxy polyethylene glycol ($CTPEG_{5000}$) was purchased from Nanocs (New York, USA) and 4-(1H-pyrazol-4-yl)-pyridine (PPY) was purchased from Fluorochem (Hadfield, UK). Doubly distilled and deionized water ($d.H_2O$) was prepared in-house.

Escherichia coli and *Staphylococcus aureus* antibodies were purchased from Fitzgerald Industries International (Acton, USA) while the *Salmonella typhimurium* antibodies were purchased from Solus Scientific (East Kilbride, UK).

Nanoparticle Synthesis

Synthesis of silver nanoparticles: Silver nanoparticles (AgNPs) were synthesized using a modified Lee and Meisel method.[1] Distilled water (500 mL) was added to a round bottom flask and heated to 45 °C using a Bunsen burner. Silver nitrate (90 mg in 10 mL $d.H_2O$) was added and heated rapidly to 98 °C with vigorous stirring. Sodium citrate was added (100 mg in 10 mL $d.H_2O$) and the solution was allowed to boil for 30 min with continuous stirring, then cooled to room temperature.

Synthesis of magnetic nanoparticles: Maghemite (γ-Fe_2O_3) magnetic nanoparticles (MNPs) were prepared by coprecipitation, following a method previously reported by Kumar *et al.*[2] Acidified iron salt solution was prepared by adding together iron(II)chloride tetrahydrate (1.98 g), iron(III)chloride hexahydrate (5.335 g) and concentrated hydrochloric acid (821 μL) and making the volume up to 25 mL with $d.H_2O$. Using a round-bottom flask, sodium hydroxide (15.1 g) was dissolved in $d.H_2O$ (250 mL) and heated to 50 °C on a heating mantle. The acidified iron salt solution was then added dropwise with vigorous stirring and a black precipitate formed immediately. Stirring was continued for a further 20 min at 50 °C, then the solution left to settle and cool. The black precipitate was washed twice with $d.H_2O$ (200 mL) and then nitric acid (125 mL; 0.1 M) was added and the solution heated to 95 °C with constant stirring, for 40 min. The resultant reddish-brown solution was centrifuged (3800*g* for 20 min) in triplicate and re-suspended in $d.H_2O$; this produced the stock maghemite MNPs.

Synthesis of silver coated magnetic nanoparticles: Ag@MNPs were prepared using a glucose-reduction method, reported by Donnelly *et al.*[3] Stock MNPs (1 mL) were added to a round bottom flask, along with glucose (0.25 g), silver nitrate (1%; 1.5 mL) and $d.H_2O$ (4 mL). The mixture was sonicated for 10 min and then heated at 90 °C for 30 min while rotating. The resultant Ag@MNPs were centrifuged (3800*g* for 20 min) three times and re-dispersed in sodium citrate (5 mM; 6 mL). Note that magnetic collection of a solution of the synthesized Ag@MNPs resulted in a clear

supernatant, indicating that no non-magnetic silver nanoparticles were formed during the silver nitrate reduction.

Preparation of Biomolecule-Nanoparticle Conjugates

SERS active antibody functionalized silver nanoparticle conjugates: On-nanoparticle coupling was adapted from a previous report by Simpson *et al.*[4] Raman reporters used were malachite green isothiocyanate (MGITC; 10 µL; 10 µM); 7-dimethylamino-4-methylcoumarin-3-isothiocyanate (DACITC; 5 µL; 0.1 mM) and 4-(1H-pyrazol-4-yl)-pyridine (PPY 10 µL; 0.1 mM) and these were used to detect *E. coli, S. typh* and MRSA respectively.

Raman reporter (volume and concentration as above) was added to AgNPs (1 mL, 0.4 nM total volume), shaking for 30 min and then centrifuged at 3800*g* for 20 min. The supernatant was removed and the resultant pellet re-suspended in d.H_2O (980 µL) and $CTPEG_{635}$ (20 µL; 1 mM). This mixture was then shaking for 3 h before being centrifuged at 1600*g* for 10 min. The pellet was kept and the supernatant removed and centrifuged at 4200*g* for 15 min. The supernatant from the second centrifugation step was subsequently removed and discarded and the pellets from each centrifugation step combined. The combined pellets were then re-suspended in 2-(*N*-morpholino)ethanesulfonic acid buffer (MES; 855 µL; pH 6.0) containing 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; 58 µL; 0.1 mg/mL) and *N*-hydroxysulfosuccinimide sodium salt (NHS; 87 µL; 0.1 mg/mL) and left to shake for 30 min at room temperature. Note the EDC and NHS solutions were mixed together before being added dropwise to the pellets. The conjugates were centrifuged as described previously and then re-suspended in phosphate buffer (PB; 995 µL; 10 mM; pH 7) containing 25 µg/mL antibody (Ab). The conjugates were left shaking for 2 h before being placed in the fridge overnight (4 ^{o}C). The following morning, the conjugates were centrifuged using the same conditions previously employed and the pellets re-suspended in PB (1 mL). Note, the antibodies were specific for one bacterial strain only. The number of antibodies which adsorb onto a nanoparticle surface can be determined using Fluorescence spectroscopy. Our previous publication provides full experimental details on how this can be achieved.[5] Briefly, a strong reducing agent such as dithiothreitol (DTT) can be used to displace the antibody from the nanoparticle surface and by monitoring the emission from the supernatant (containing the DTT displaced fluorescent dye modified antibody) and plotting a calibration graph the number of antibodies present in the sample can be calculated.

Lectin functionalized silver coated magnetic nanoparticle conjugates: $CTPEG_{5000}$ (10 µL; 1 mM) was added to Ag@MNP (400 µL) and d.H_2O (90 µL). The solution was mixed for 3 h before being centrifuged at 1600*g* for 10 min. The pellet was kept and the supernatant removed and centrifuged at 4200*g* for 15 min. The supernatant from the second centrifugation step was subsequently removed and discarded and the pellets from each centrifugation step combined. The combined pellets were then re-suspended in MES buffer (310 µL; pH 6.0) containing EDC (50 µL; 2 mg/mL) and NHS (120 µL; 2 mg/mL) and left to shake for 30 min at room temperature. Note the EDC and NHS solutions were mixed together before being added dropwise to pellets. The conjugates were centrifuged as described previously and then re-suspended in HEPES buffer (475 µL; 10 mM, pH 7.4) containing 5 µg/mL Con A. The conjugates were left shaking for 16 h at room temperature. Following mixing, the previous centrifugation steps were employed and the pellets re-suspended in HEPES buffer (480 µL; 10 mM, pH 7.4) containing calcium nitrate and magnesium nitrate (0.2 mM). It should be noted, that the PEGylated linker used for these conjugates was larger than the one previously reported by Simpson *et al.* Extra stability was required due to the increased size of the magnetic nanoparticles over the silver nanoparticles previously used.[4]

Bacterial Strains and Growth Conditions

Bacterial Strains: Escherichia coli ATCC 25922; *Staphylococcus aureus* (methicillin resistant) ATCC BAA-1766 and *Salmonella typhimurium* ATCC 14028 were used in this study. *E. coli* and MRSA were grown on LB Miller agar in an aerobic atmosphere for 24 h at 37 ^{o}C. *S. typh* was grown on MacConkey agar in an aerobic atmosphere for 24 h at 37 ^{o}C. Harvested cells were suspended in either LB or MacConkey broth to obtain an optical density ($OD_{600\ nm}$) of 0.6. Cells were then plated onto LB or MacConkey agar plates as triplicates and incubated for 24 h as described previously. All the strains were grown using the same batch of culturing plates, in order to reduce any potential unwanted phenotypic variation.

Bacterial Sample Preparation

Bacterial slurries were prepared by harvesting the biomass from the surface of each plate using sterile inoculating loops and re-suspending in physiological saline solution (1 mL; 0.9% NaCl). The prepared bacterial slurries were washed by centrifugation (*E. coli* - 650*g* for 5 min; *S. typh* and MRSA - 1600*g* for 5 min) and the pellets re-suspended in 1 mL saline. The wash step was repeated a further twice with the final re-suspension being in d.H_2O (1 mL). Note the $OD_{600\ nm}$ was recorded for all samples and bacterial concentrations were obtained by serial dilutions based on plate-counting results. All samples were stored at −80 °C until further analysis.

Characterization of Biomolecule-Nanoparticle Conjugates

Extinction spectroscopy, Dynamic light scattering and Zeta potential analysis: A Varian Cary 3000 Bio UV-Visible spectrophotometer with a wavelength range from 200-900 nm was used to acquire all the extinction spectra. Dynamic light scattering and zeta potential measurements were performed in plastic cuvettes using a Malvern Zetasizer nano ZS system. Note for the zeta potential measurements a dip cell was placed into the cuvette and analysis followed.

Gel Electrophoresis: Agarose (1 g) was dissolved in 1x Tris Borate EDTA buffer (10 mL of TBE + 90 mL d.H_2O) by heating. The gel solution was cooled, poured and allowed to set. The gel was added to the electrophoresis tank, the tank was filled and the gel was completely covered with 1x TBE buffer. AgNPs, PEG_{635} functionalized AgNPs and *E. coli* antibody conjugated AgNPs (1 mL each sample), were centrifuged at 6000 rpm for 10 min. The supernatant was removed and the pellet retained (20-50 µL). An aliquot (10 µL) of each sample was removed and mixed with 6x loading buffer (1 µL); the samples were then loaded into a well in the gel. An electric field of 160 mV and 20 A was applied to the gel to separate the samples based on mass to charge ratio. Hence the samples moved towards the positive electrode due to the citrate layer on the nanoparticles. The gel was run for ~30 min.

SERS Analysis: The samples were analyzed using a SnRI (Snowy Range Instruments, Laramie, USA) portable Raman spectrometer and a laser excitation wavelength of 532 nm. All the measurements had a 0.5-1 s acquisition time and a laser power operating at 50 mW. Each sample was prepared in triplicate and 5 scans of each replicate were recorded.

SERS Analysis for Detection Assay

For the detection assays, all samples were analyzed immediately after preparation using a Renishaw InVia Raman microscope (Renishaw plc, New Mills, UK). Specifically, the Raman spectra were acquired using a 532 nm laser excitation with a 20x long working distance objective lens focused into a 96 well plate containing 300 µL of the bacteria-NP conjugate solution. The laser power was adjusted each time to ~0.8 mW and an acquisition time of 1 s with a static scan being centered at 1300 cm^{-1} was employed. For all Raman spectra, data handling was carried out using GRAMS/AI software. The peak intensities were obtained by scanning 3 replicate samples 5 times and in all plots the error bars represent one standard deviation.

Chemometrics

All multivariate statistical analysis was carried out in MATLAB software (The MathWorks, Natick, MA). Prior to conducting principal component analysis (PCA) the data was baseline corrected using asymmetric least-squares (AsLS).[6] PCA was performed to assess the reproducibility and separation of individual features within the data, such that in PCA scores plots if replicates cluster together then the analytical approach can be considered robust. PCA reduces the dimensionality of the SERS data, making it easier to identify any variations in the spectra.[7] It was carried out on four different data sets, three consisting of spectra obtained from single pathogen detection experiments and one data set obtained for the multiplex detection of the three pathogens.

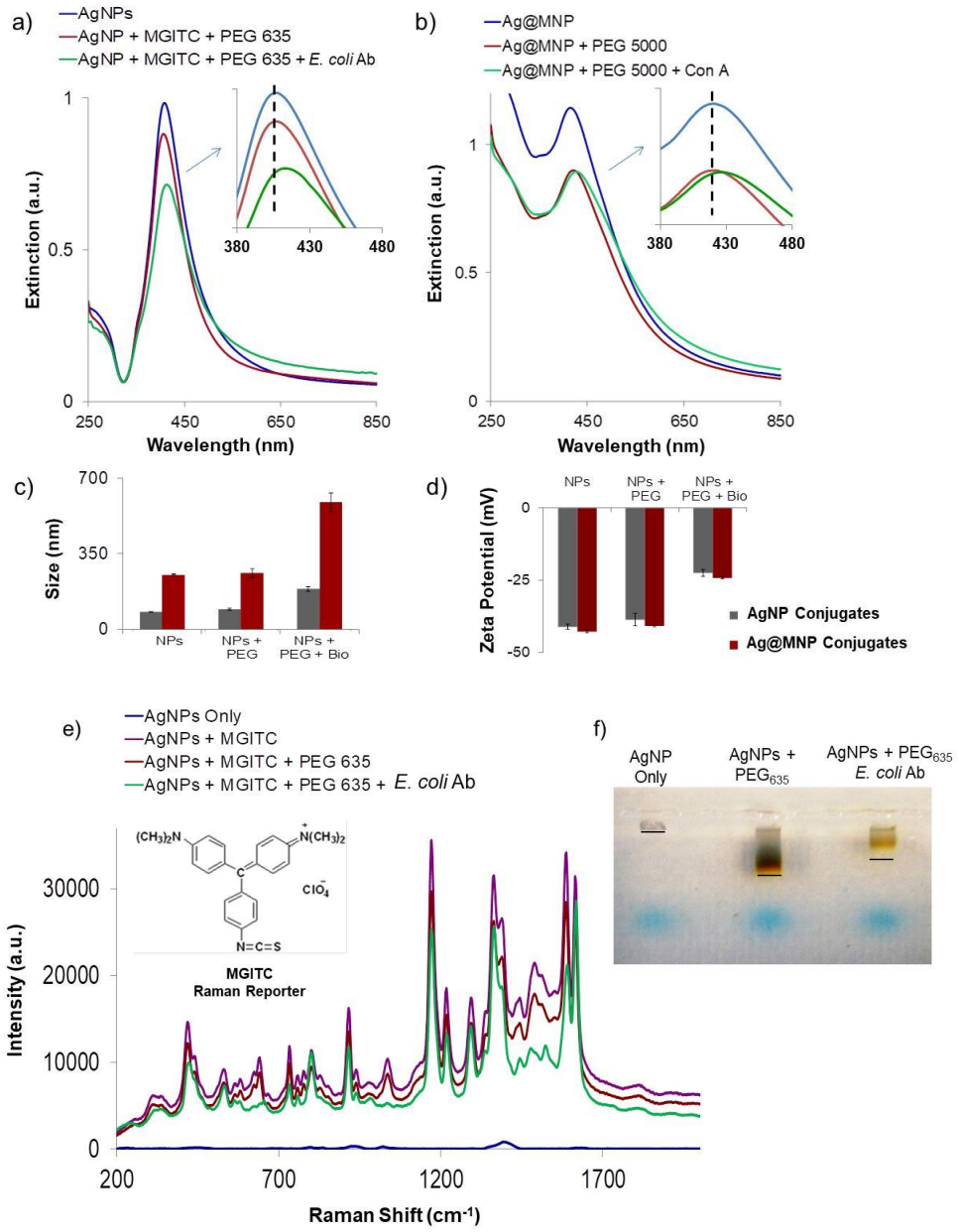

Figure S1. Characterization of biomolecule-NP conjugates. a) Extinction spectra showing the conjugation steps involved in the preparation of SERS active *E. coli* antibody functionalized silver nanoparticles; note, AgNPs were functionalized with MGITC (Raman reporter) and then conjugated to *E. coli* antibodies using a thiol PEG_{635} linker and EDC/NHS cross coupling chemistry. b) Extinction spectra showing the conjugation steps involved in the preparation of Con A functionalized silver coated magnetic nanoparticles; note, conjugation of Con A lectin to Ag@MNPs was achieved using a thiol PEG_{5000} linker and again cross coupling chemistry. The inserts in the extinction spectra further highlight the shifts associated with molecular adsorption of the biomolecules onto the NP surfaces. c) Dynamic light scattering and d) zeta potential for both conjugates at each stage of the conjugation. The mean of 3 replicate samples is shown along with standard deviation error bars. e) SERS spectra obtained with a 532 nm laser and 0.5-1 s acquisition time; showing the change in spectrum at each step of the conjugation process for *E. coli* antibody functionalized AgNPs only. Similar optimization and conjugation steps were conducted for *S. typh* and MRSA conjugates. Note the Raman reporters used were DACITC and PPY respectively. f) Gel electrophoresis for bare AgNPs and those functionalized with PEG_{635} and *E. coli* antibody.

Results and Discussion: Characterization of Biomolecule-NP Conjugates

Maghemite (γ-Fe_2O_3) magnetic nanoparticles were synthesized using a coprecipitation method, and then coated with a silver shell via a glucose reduction protocol previously described by Donnelly *et al.*[3] The addition of a silver shell renders the nanoparticles (NPs) SERS active and allows for ease of subsequent bio-functionalization. Silver coated magnetic nanoparticles were functionalized with a heterobifunctional thiol/carboxy PEGylated linker, in which the terminal mondentate thiol allowed for immobilization on the nanoparticle surface while the carboxyl group facilitates conjugation to amine-containing affinity ligands such as biomolecules, via carbodiimide cross-coupling chemistry.[8] Using this cross-linking approach, the lectin Concanvilin A (Con A) was attached to silver coated magnetic nanoparticles.[4] Con A was used as the lectin of choice as it has previously been reported to bind to terminal α-D-mannosyl and α-D-glucosyl groups expressed on the surface of bacteria.[9,10] Conjugating Con A to the surface of silver coated magnetic nanoparticles, in the presence of a magnet allowed for us to develop a unique and efficient way of capturing bacteria from a sample matrix.

Con A functionalized magnetic nanoparticles were prepared using modified methods previously described by Simpson *et al.* and Donnelly *et al.*[3,4] The magnetic nanoparticle conjugates were optimized and characterized using extinction spectroscopy, dynamic light scattering and zeta potential analysis. The data obtained at each stage of the conjugation process can be seen in Figure S1. In terms of extinction spectroscopy, it has previously been reported that when a shift in the localized surface plasmon resonance (LSPR) occurs and/or broadening of the peak is observed, it is indicative of a change in size, shape, aggregation and/or change to the surface environment of the nanoparticle.[11] It can be observed in Figure S1 (b), that upon addition of the PEGylated linker dampening of the extinction maxima was observed, following this a LSPR shift from 420 to 430 nm plus broadening of the peak was observed after the addition of the Con A lectin. As the peak changed at each stage of functionalization, it was likely that the size, shape and surface environment was changing and hence attachment of the lectin to the nanoparticle surface was successfully occurring. Aggregation of the nanoparticles was unlikely, as the role of the PEGylated linker is to maintain the stability of the nanoparticles whilst providing an attachment group for the biomolecule.[4]

The successful attachment of the lectin to the magnetic nanoparticles was further confirmed with an increase in size at each stage of the conjugation, with a decrease in the zeta potential (Figure S1 (c) and (d) respectively). A size increase was to be expected as both the PEG linker and Con A lectin are regarded as large molecules and the fact that an increase in size from 253.3 nm to 589.1 nm was observed, did indeed confirm that they were successfully attaching each time. Conversely, the zeta potential value decreased at each stage of the conjugation thus further confirming that the surface environment of the nanoparticle was changing each time. Zeta potential provides information regarding the charge on the nanoparticle surface and thus indicates the stability of colloidal suspensions.[12] As the nanoparticles possess a negatively charged citrate layer on the surface, the more negative the value the more stable the nanoparticles are said to be in solution. A colloidal solution with a value greater than ±25 mV is considered stable and since the Con A functionalized magnetic conjugates possessed values around this area they are deemed stable and thus suitable for use in the detection assay.[12]

It was also important to optimize and characterize each step of the conjugation process for the SERS active antibody functionalized silver nanoparticles. Silver nanoparticles were initially synthesized using a modified Lee and Miesel method.[1] Following this the SERS active conjugates were prepared by functionalizing the silver nanoparticles with a Raman reporter, PEGylated linker and antibodies that were bacterial strain specific. Again, carbodiimide cross-linking chemistry was used to

immobilize the antibodies onto the nanoparticle surface[4] and the conjugates characterized using the same techniques as those previously employed for the Con A functionalized magnetic nanoparticles. It should be noted that the three sets of antibody conjugates (*E. coli, S. typh* and MRSA) were optimized and characterized in the same way, with similar trends being observed in the data sets hence, only the data associated with the *E. coli* antibody is shown. It can be observed in Figure S1 (a), that dampening of the LSPR peak occurred after the addition of the Raman reporter and PEGylated linker and following this a slight LSPR shift from 407 to 413 nm plus broadening and further dampening of the peak was observed upon conjugating with the *E. coli* antibody. In addition, a size increase from 80.9 nm to 186.6 nm and decrease in zeta potential (Figure S1 (c and d)) were observed at each stage of the conjugation confirming the successful attachment of the biomolecule. As before, the zeta potential values at each stage of the functionalization remained below -25 mV, hence confirming the stability of the colloidal solutions and in particular indicating that the SERS active conjugates were stable. Moreover, the SERS results showed that after each addition was made (Raman reporter, PEG linker then antibody) a slight decrease in the signal was observed. However, the decrease was negligible as the SERS active biorecognition conjugates still produced excellent SERS signals with only a 1 s accumulation time and 532 nm laser excitation (Figure S1 (e)). For further clarification on the successful attachment of the *E. coli* antibody to the AgNPs and to confirm the stability of the colloidal solutions, gel electrophoresis was performed (Figure S1 (f)). This is a technique that enables isolation of mixtures of DNA, RNA and proteins based on their molecular size.[13] It involves the separation of molecules by means of an electric field which attracts and repels molecules through small pores of a gel. Moreover, the rate of molecule migration through the pores is inversely proportional to their mass to charge ratio, thus smaller molecules move the furthest.[13] This method is commonly employed to confirm antibody conjugation to nanoparticles as shown in Figure S1 (f). The bare AgNPs are not stable in the loading buffer; hence they aggregate and don't migrate out of the well as shown. However, the PEG_{635} functionalized AgNPs and *E. coli* antibody conjugated AgNPs, do migrate out of the well and through the small pores of the gel towards the positive electrode, thus confirming that additional stability is gained from adding the PEGylated linker. Further the PEG_{635} functionalized AgNPs which are smaller in size to the antibody conjugated AgNPs, migrate through the gel more easily and hence travel the furthest which is consistent with previous findings. Therefore the difference in migration through the gel confirms that the surface environment of the NPs has changed each time thus indicating that successful functionalization has been achieved. This was repeated for *S. typh* and MRSA antibody functionalized AgNPs and for lectin conjugated magnetic nanoparticles with similar results being obtained for each. Therefore, these results along with those observed for the Con A functionalized magnetic nanoparticles suggested that the functionalization conditions were optimal for use in the next stage of the studies which was developing the assay for single pathogen detection.

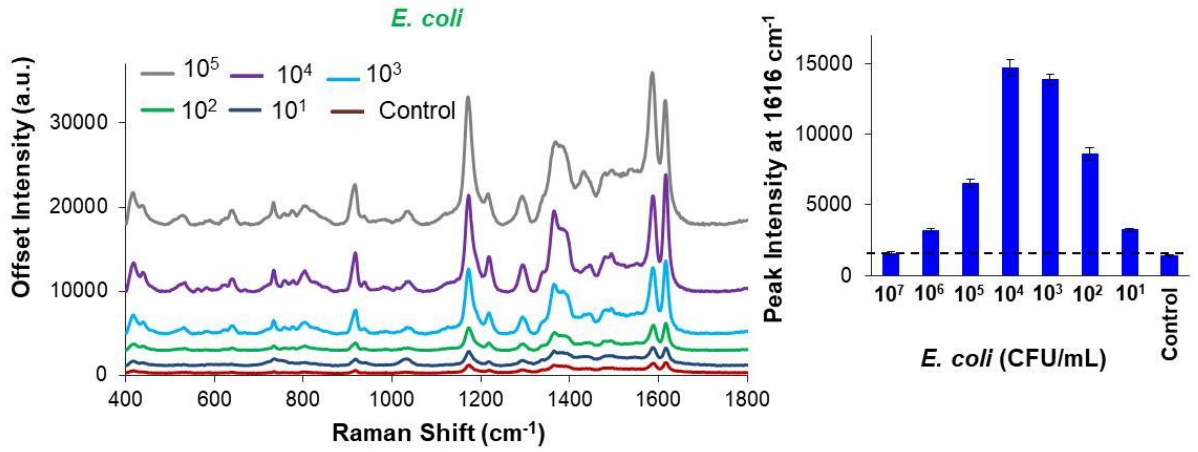

Figure S2. The assay was performed for *E. coli* bacterial detection over the bacteria concentration range 10^7 to 10^1 CFU/mL. The associated bar charts show the SERS peak intensities at each of the bacteria cell concentrations and these are compared to a control sample (no bacteria present). The characteristic peak at 1616 cm^{-1} was used to calculate the peak intensities. SERS spectra were recorded using a 532 nm laser excitation with an accumulation time of 1 s. Peak intensities were obtained by scanning 3 replicate samples 5 times and the error bars represent one standard deviation. The dashed line gives visual clarification of the SERS peak intensity of the control. It should be noted that if intense signals are to be obtained from highly concentrated bacterial samples then diluting the bacterial-complex in d.H_2O before analysis is required. This will bring the samples into the concentration dependent part of the calibration curve and obtain signals similar to those with bacteria concentrations of $\geq 10^4$ CFU/mL.

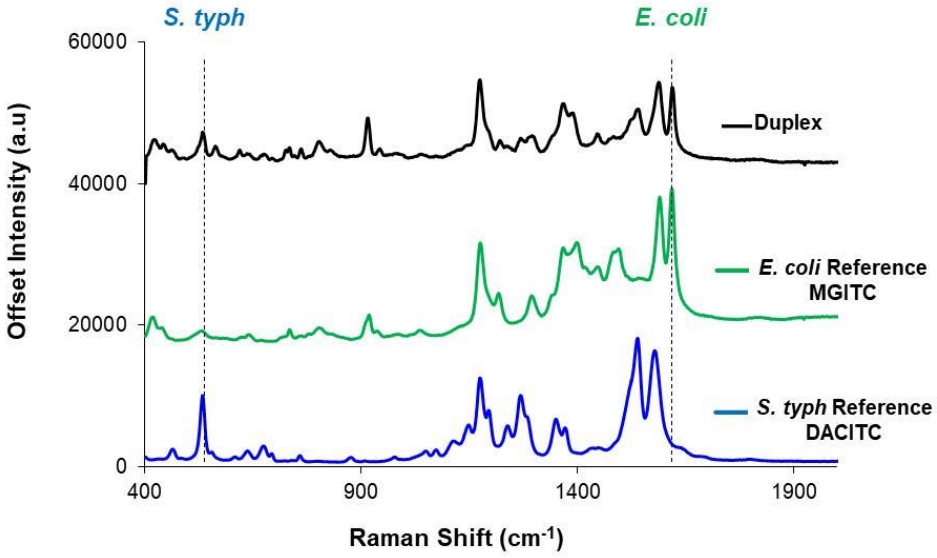

Figure S3. SERS spectra obtained from duplex pathogen detection using the SERS assay. a) Stacked SERS spectra showing the raw spectra obtained from the detection of two bacterial pathogens simultaneously using the detection assay plus the SERS spectra obtained from the detection of each pathogen separately. DACITC spectrum (blue) represents *S. typh*; MGITC spectrum (green) represents *E. coli*; and the black spectrum is the duplex. The black dotted lines shows peaks at 535 and 1616 cm^{-1} used to identify *S. typh* and *E.coli* bacteria respectively. The bacteria concentration used was 10^3 CFU/mL. SERS spectra were recorded using a 532 nm laser excitation with an accumulation time of 1 s.

References

(1) Lee, P.; Meisel, D. *J. Phys. Chem.,* **1982**, *86*, 3391-3395.

(2) Kumar, G. P.; Rangarajan, N.; Sonia, B.; Deepika, P.; Rohman, N.; Narayana, C. *Bull. Mater. Sci.,* **2011**, *34*, 207-216.

(3) Donnelly, T.; Smith, W. E.; Faulds, K.; Graham, D. *Chem. Commun.*, **2014**, *50*, 12907-1291

(4) Simpson, J.; Craig, D.; Faulds, K.; Graham, D. *Nanoscale Horiz.,* **2016**, *1*, 60-63.

(5) McKenzie, F.; Steven, V.; Ingram, A.; Graham, D. *Chem. Commun.,* **2009**, 2872-2874.

(6) Eilers, P. H. *Anal. Chem.,* **2004**, *76*, 404-411.

(7) Kim, D. H.; Jarvis, R. M.; Xu, Y.; Oliver, A. W.; Allwood, J. W.; Hampson, L.; Hampson, I. N.; Goodacre, R. *Analyst*, **2010**, *135*, 1235-1244.

(8) Zheng, M.; Davidson, F.; Huang, X. *J. Am. Chem. Soc.*, **2003**, *125*, 7790-7791.

(9) Shen, Z.; Huang, M.; Xiao, C.; Zhang, Y.; Zeng, X.; Wang, P. G. *Anal. Chem.,* **2007**, *79*, 2312.

(10) Firon, N.; Ofek, I.; Sharon, N. *Infect. Immun.*, **1984**, *43*, 1088-1090.

(11) Haiss, W.; Thanh, N. T. K.; Aveyard, J.; Fernig, D. G. *Anal. Chem.*, **2007**, *79*, 4215-4221.

(12) Instruments, M.: *Zetasizer Nano Series User Manual*, 2009.

(13) Scitable by Nature Education, 2014, Available from www.nature.com/scitable/definition/gel-electrophoresis-286, Acessed Sept 2017.