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Electronic Supplementary Information

Rapid and sensitive colorimetric detection of pathogens based on Silver-Urease interactions

We report a simple and economically feasible pathogen sensing strategy that exploits the surface properties of receptor coated AgNPs probe to preferentially bind bacterial cell surface than urease. The receptor coated AgNPs actively binds to bacteria thus rendering urease active in solution that generates a colorimetric shift from yellow to pink through phenol red. In the absence of pathogens, receptor coated AgNPs block urease by binding to its active site, thereby no change in color.

Experiments

S1 Chemicals and Materials

Urease from jack bean, silver nitrate ($AgNO_3$), tannic acid ($C_{76}H_{52}O_{46}$) and sodium citrate dihydrate ($HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$), urea AR grade 98% ($NH_2CONH_2$), Polyethylenimine~50% in H_2O, sodium phosphate dibasic (Na_2HPO_4), potassium phosphate monobasic (KH_2PO_4). GOPS (3-glycidyloxypropyl) trimethoxysilane ($C_9H_2O_5Si$) purchased from Sigma Aldrich (UK). Milli-Q water with 18 $M\Omega \cdot cm^{-1}$ resistivity was used for all aqueous solutions. Syringe filters (0.2 μm) were purchased from Merck Millipore, micro centrifuge tubes (1.5 ml) were procured from Tarsons.

S2 Techniques and instruments for characterization

All Ultraviolet-Visible (UV-Vis) spectroscopy measurements were performed using BioTek Synergy H1 spectrophotometer (UK). Transmission electron micrographs (TEM) were obtained using JEOL 2100 Transmission electron microscope (USA). AgNPs size and charge distribution analysis were carried out using a Zetasizer NanoZS (Malvern, UK). Gel based characterizations were done with BioRad gel assembly. The sedimentation experiments of

protein capped AgNPs were done on Beckman Coulter (AUC) Life Sciences, Proteome Lab XL-A/XL-I (US).

S3 Urease stock and substrate composition

Urease enzyme (1 mg/mL) stock was prepared in 10 mM phosphate buffer that was used in all the experiments. For practical applicability of assay and to maintain standard salt concentration for reproducibility of protocol, all the experiments were performed in artificial tap water prepared by recipe previously reported in literature.[1] Substrate stock solution consists of 16 mM urea, 10 mM HCl and 200 μM phenol red.

S4 Microbial strains and culture

Bacterial strains of Salmonella Typhimurium (MTCC 3232), *Lactobacillus plantarum* (MTCC 4464), *Micrococcus luteus* (MTCC 1538),*Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 740)were procured from Microbial Type Culture Collection, IMTECH in lyophilized form. The culture was revived in 0.85% normal saline and streaked on a nutrient agar plate. From the streaked plate, a single colony was inoculated in 100 mL nutrient broth and grown overnight at 37°C in an incubator shaker. 1 mL of primary culture was subcultured in nutrient broth and kept overnight at 37°C in shaking condition. Cells used for the experiment were of $O.D_{600}$=1 (10^9cells per mL) and further dilutions were made using the same.

S5 Synthesis of anionic AgNPs

Although in literature numerous methods have been reported for synthesis of AgNPs; the two most common are citrate based reduction[2] and sodium borohydride[3] based reduction method. Initially we exploited both methods for synthesis of nanostructures but as reported earlier in numerous reports the particles synthesized by using sodium citrate were of large size (Fig. S1) and those by sodium borohydride were highly polydisperse (Fig. S2). So, we utilized

tannic acid mediated synthesis approach to synthesize monodisperse anionic AgNPs of small size.[4]

Prior to experimentation, all glassware were treated with aqua regia solution for 2 hours to remove any residual metal ions and further treated with GOPS (3-Glycidyloxypropyl) trimethoxysilane for 1 hour for surface salinization. Monodisperse anionic AgNPs were synthesized via modified citrate based reduction method using sodium citrate and tannic acid. The experiment can be briefly described as following.

For synthesizing tannic acid capped anionic AgNPs of 20 nm, A freshly prepared 50 mL solution of 65 mg sodium citrate (5mM) and 2 mg tannic acid (0.025 mM) was boiled along with vigorous stirring, followed by addition of 1 mL of 4.2 mg per mL silver nitrate (25mM). The color of the solution immediately turns yellow indicating the initiation of nucleation process. The solution was further stirred for 20 min in order to complete the nucleation process and was then stopped from boiling. The size of AgNPs was controlled by varying tannic acid concentration (see Table S1). As-prepared anionic AgNPs were stored at 4°C for further experimentation. The size of the AgNPs was controlled by adjusting the concentration of tannic acid and characterized using UV-Vis spectroscopy (Table S1). As prepared AgNPs were stored at 4° C and used for experimental purposes.The as-prepared tannic acid capped anionic AgNPs were also characterized using DLS, ζ potential, TEM analysis and UV-Vis spectroscopy.

The concentration of AgNPs was measured using optical density method via beer lamberts law. Earlier reported work on AgNPs provides calculated charts of number of nanoparticles corresponding to optical density @ 400 nm.[5]

S6 Synthesis of cationic PEI capped cationic AgNPs

As prepared tannic acid capped anionic AgNPs were firstly washed to remove residual salts. The washing was done by centrifugation at 7500 rpm for 25 minutes. The optical density (400 nm) of washed anionic AgNPs was set to one (7×10^{10} particles per mL) and this solution was then used to prepare cationic AgNPs. To prepare PEI capped cationic AgNPs, 5

mL of anionic AgNPs solution was added dropwise in 5 ml of 3 mg per mL PEI solution under vigorous stirring for an hour at room temperature. The particles were again centrifuged to remove residual PEI and set to OD_{400}=1. This solution was then stored at 4°C and was used in further experiments.The as-prepared PEI capped cationic AgNPs were characterized using DLS, ζ potential, TEM analysis and UV-Vis spectroscopy.

S7 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE)

For SDS PAGE analysis, anionic AgNPs were conjugated with 50 μg/mL urease at 37˚C for 15-20 min. Then particles were washed twicewith deionized water to remove any unbound residual protein. The supernatantcontaining unbound urease was discarded and the pellet was treated with 1% SDS and incubated at 100 ˚C for 15 min to detach the bound urease for running onto the gel. The solution was again centrifuged to get the protein in supernatant which was suspended in loading buffer [62.5mM Tris-HCL pH 6.8, 2% (w/v) SDS, 10% glycerol, 0.01% (w/v) bromophenol blue and 40mM DTT] for 5 min in boiling condition followed by loading onto the 10% SDS-PAGE gel. After running the gel under constant voltage of 140V, it was stained with commassie blue.

S8 Agarose gel electrophoresis

The anionic AgNPs were capped with different concentrations of urease and run onto 0.1% agarose gel under a voltage of 80 volts for an hour. The difference in the mobility and retardation was observed at higher concentrations of urease used for capping AgNPs (Fig. S3).

S9 Density gradient centrifugation

After the anionic AgNPs were synthesized, the concentrated particles were subjected to density gradient centrifugation using sucrose solutions. 1mL of concentrated AgNPs were deposited on top of continuous sucrose solution gradient ranging from 2.5-20% w/v with a difference of 2.5% w/v. Aliquots of 1mL of each concentration of sucrose solution were

carefully layered one on the top of other in the order from the densest to the least dense in a 15 mL falcon tube, while avoiding mixing between the layers. The falcon was placed in a swinging bucket rotor and spun at 4000 rpm for 1 hour at 4˚C. Post centrifugation, the fractions of separated particles were separated and analyzed by DLS (Fig. S4b).

S10 Analytical ultracentrifugation (AUC)

For measurements of urease AgNPs interaction, 400 µg/ml of urease was incubated with the particles and left for overnight incubation at 4˚C. Unbound fraction of enzyme was removed and sample was prepared with a final 2 OD absorbance and bare AgNPs were also prepared with 1 OD absorbance at 400 nm (measured in AUC sapphire cell of pathlength 1.2 cm). AUC continuous distribution measurement was done at 6000 rpm with An50 Ti Rotor. Absorbance scans were recorded at 280 nm and 400 nm for protein and nanoparticles respectively. AUC data was analyzed using the program SEDFIT by selecting continuous distribution c(s) model (Fig. S4a).[6]

To ensure the consistency in measurement of urease conjugation over the particles, density gradient centrifugation using sucrose solution was performed that provided AgNPs of discrete size and shape. This technique aided in achieving homogenous solution of monodisperse particles separated as layers via sucrose gradient, which was further analysed by AUC.

S11 Theoretical calculation of urease immobilization on AgNP

A concentration of 400µg/mL of urease was incubated with tannic acid capped AgNPs in 10 mM phosphate buffer at 4˚C for overnight. Next day, unbound enzyme was removed by centrifugation and its concentration was recorded as 100µg/mL at 280 nm. Furthermore, in accordance to a recent study[7], calculations for the immobilization of urease enzyme onto AgNPs were performed as amount of capping was inferred in terms of efficiency of protein immobilization in a percentile value which is as follows:

$$\% \text{ Immobilization} = (A - B) * 100/A$$

Where: A = total protein used for conjugation,

B = protein content in supernatant of AgNP-urease conjugate.

In addition to the above, furthermore the number of molecules of urease adsorbed on the surface of AgNPs were also calculated by considering the urease as a rigid sphere moiety[8]. The theoretical diameter of urease as seen from its three dimensional structure in Pymol software is 10 nm (radius=5nm). Therefore, cross sectional area can be calculated by $\sigma = \pi r^2$ =78.5 nm^2. Simultaneously, when we calculate the area for the spherical AgNP, it comes out to be 2826 nm^2. Hence, maximum number of urease molecules corresponding to close arrangement of urease over the AgNPsis of 36 for a 30 nm size of AgNP.

S12 Transmission Electron Microscopy

TEM measurements were performed on a JEOL Model 2100 instrument operated at an accelerating voltage of 120 kV. Carbon coated TEM grids were used to prepare the samples by placing a drop of centrifuged, concentrated and resuspended AgNPs and AgNPs conjugated with urease enzyme on it. After drying the film and removal of extra solution, negative staining via 2% phosphotungstic acid (PTA) for 30 seconds for enzyme conjugated particles was done to visualize protein immobilized on the particle surface.

S13 Urease activity inhibition assay

In order to determine the inhibition efficiency, stock solution of AgNPs was prepared ($7*10^{10}$ particles per mL).Different volumes from stock solution were added to 15 µg/mL of optimized urease concentration followed by addition of 100 µL of substrate solution. A change in color was observed for 20 minutes followed by UV-Vis analysis. The minimum volume of AgNPs required for inhibiting the enzyme was optimized as 10 µL for detection studies (Fig. S6).

S14 Bacterial detection assay with PEI capped cationic AgNPs

Different dilutions of various microorganisms from 10^8 to 10 cells per mL, were incubated with cationic PEI capped AgNPs. After incubation for 10 minutes, an optimized concentration of 15 µg/mL of urease was added followed by the substrate solution. Change in color was seen and further characterization was done by UV-Vis spectroscopy.

S15 Specific detection of Salmonella Typhimurium with antibody functionalized AgNPs

Tannic acid capped anionic AgNPs were functionalized with antibodies generated in our laboratory against Salmonella. Initially, we enquired about the effect of tannic acid capping on antibody immobilized anionic AgNPs. Table S2 illustrates effect of capping agent (tannic acid on antibody conjugation) by shift in UV-Vis spectrum. The process involves the mechanism of physio-sorption at alkaline pH that coats 50µg/ml of bioreceptor by overnight incubation at 4°C. Antibody conjugated anionic AgNPs were first tested for blocking urease, to determine a lowest concentration required for enzyme inhibition (Fig. S5). Further, the assay was performed by adding the substrate solution and the change in color was observed along with the control for few minutes. For characterization, effect of capping agent (tannic acid on antibody conjugation) was studied and a shift was observed in UV-Vis spectrum. Zeta potential studies were also performed to validate antibody conjugation. A change in surface zeta potential from -28 mV to 21.75 mV was observed after antibody conjugation (Fig. S7).

S16 Tap water testing (Real time sampling)

The assay was also performed with tap water to test its efficacy for real samples. Tap water was centrifuged at 6000 rpm for 20 minutes to remove any particulate impurities. To decrease the concentration of salts, tap water was further diluted ten times of its original concentration. This step can easily be performed onsite and will help stabilize AgNPs preventing their salt driven aggregation that might give false positive results. Around 100 cells per mL of contaminated water sample were differentiated as seen via colorimetric

analysis and respective UV-absorption graph also showed an increase in the signal with the increasing contamination (Fig.S8 and S9).

S17 Test with anionic AgNPs

We also checked anionic AgNPs as controls for bacterial detections. Clearly anionic surface of AgNPs did not show affinity towards negative bacterial surface (Fig. S10A). Fig. S10B shows the broad range detection of PEI-AgNPs with other bacteria.

S18 Metal ion test

Permissible concentrations of the metal ions were mixed in accordance with 1 mL volume of the reaction along with optimized urease and the substrate solution. The color changed from yellow to pink indicating that none of the metal ions in the WHO permissible amounts inhibited the urease enzyme. Therefore, the assay is applicable to metal ion containing water sample in a certain allowed concentration. Fig. S11a shows a visual colorimetric signal of the metal ion assay with AgNP and graphical readout at 570nm shows permissible ionic concentration did not hamper urease activity (Fig. S11b).

S19 Apple juice and Lake Water sample preparation

Both the samples were prepared according to previously described protocols[9]. The lake water was directly used in the experiment, while the apple juice was first treated with 0.2M NaOH to adjust pH to 7.4. Initially, both the samples were spiked with 10^7 Salmonella Typhimurium cells per ml. The samples were then centrifuged at 13000 g for 5 min. The supernatant was removed and the pellet was re-suspended in artificial tap water. The sample was then tested with anti-Salmonella IgGAgNPs test described above.

S20 Arduino based device kit for point of care application

For point of care studies we have devised an arduino based test kit. Fig. S12 shows solid work drawings of the device. Arduino Mega and TCS3200 light to frequency convertor was used in device. Fig. S13 shows the images of the various levels of contamination detection via device.

Programming of the device

```
#include <SPFD5408_Adafruit_GFX.h>    // Core graphics library
#include <SPFD5408_Adafruit_TFTLCD.h> // Hardware-specific library
#include <SPFD5408_TouchScreen.h>     // Touch library

// Calibrates value
#define SENSIBILITY 300
#define MINPRESSURE 10
#define MAXPRESSURE 1000

//These are the pins for the shield!
#define YP A1
#define XM A2
#define YM 7
#define XP 6

short TS_MINX=150;
short TS_MINY=120;
short TS_MAXX=920;
short TS_MAXY=940;

// Init TouchScreen:

TouchScreen ts = TouchScreen(XP, YP, XM, YM, SENSIBILITY);

// LCD Pin

#define LCD_CS A3
#define LCD_CD A2
#define LCD_WR A1
#define LCD_RD A0
#define LCD_RESET A4 // Optional : otherwise connect to Arduino's reset pin


// Assign human-readable names to some common 16-bit color values:
#define WHITE   0xFFFF //
#define YELLOW    0xFFE0 //
#define CYAN    0x07FF  //
#define MAGENTA     0xF81F
#define RED     0xF800  //
#define GREEN  0x07E0
#define BLUE  0x001F  //
#define BLACK   0x0000 //
/*
#define BLACK   0xFFFF
#define BLUE    0xFFE0
#define RED     0x07FF
#define GREEN   0xF81F
#define CYAN    0xF800
#define MAGENTA 0x07E0
#define YELLOW  0x001F
```

Calling Libraries

Defining Pins

Defining color codes

```
  drawBorder();
  tft.setCursor (70, 20);
  tft.setTextSize (3);
  tft.setTextColor(RED);
  tft.println("SALMONELLA");
  tft.setCursor (80, 50);
  tft.println("DETECTION");
  tft.setCursor (90, 80);
  tft.println("TEST KIT");
  tft.setTextSize (2);
  tft.setCursor (40, 120);
  tft.println("PU,CHD & CSIR-IMTECH");
  tft.setCursor (30, 150);
  tft.println("Submitted By:");
  tft.setCursor (160, 175);
  tft.println("Pargat Singh");
  tft.setCursor (60,200);
  tft.setTextSize (2);
  tft.setTextColor(BLUE);
  tft.println("Touch to proceed");

  waitOneTouch();
  TSPoint p;
  p = waitOneTouch();
  p.x = mapXvalue(p);
  p.y = mapYvalue(p);


}
void loop()
{
    tft.fillScreen(CYAN);
    tft.setCursor (85, 20);
    tft.setTextSize (3);
    tft.setTextColor(RED);
    tft.println("TESTING");

    digitalWrite(S2,LOW);
    digitalWrite(S3,LOW);
    for(int i = 0; i<20;i++)
    {
    r_frequency = pulseIn(sensorOut, LOW);
    delay(250);
    tft.setCursor (80,200);
    tft.setTextSize (2);
    tft.setTextColor(BLUE);
    tft.println("Please wait");
    red_frequency += r_frequency;
    }
    red_frequency = red_frequency/20;
```

Device on screen

```
// Init LCD

Adafruit_TFTLCD tft(LCD_CS, LCD_CD, LCD_WR, LCD_RD, LCD_RESET);

// Dimensions

uint16_t width = 0;
uint16_t height = 0;

// Buttons
//#define BUTTONS 8
//Adafruit_GFX_Button buttons[BUTTONS];

uint16_t buttons_y = 0;


#define S0 30   //  yellow
#define S1 28   //  orange
#define S2 22   //  gray
#define S3 24   //  blue
#define sensorOut 45   //  purple
float r_frequency = 0;
float g_frequency = 0;
float b_frequency = 0;
float red_frequency = 0;
float green_frequency = 0;
float blue_frequency = 0;
float result_frequency = 0;
// green gnd
// red  vcc

void setup(void) {
    pinMode(S0, OUTPUT);
    pinMode(S1, OUTPUT);
    pinMode(S2, OUTPUT);
    pinMode(S3, OUTPUT);
    pinMode(sensorOut, INPUT);

    // Setting frequency-scaling to 20%
    digitalWrite(S0,HIGH);
    digitalWrite(S1,LOW);
//  Serial.begin(9600);
//  pinMode(10, OUTPUT);
  tft.reset();
  tft.begin(0x9341);
  tft.setRotation(3); // Need for the Mega, please changed for your choice or rotation initial

  width = tft.width() - 1;
  height = tft.height() - 1;
  drawBorder();
  tft.setCursor (70, 20);
```

Initializing LCD

Defining Sensor colors

Setting screen hieght

```
    }
    if(result_frequency >= 28.2)
    {
    tft.fillScreen(GREEN);
    tft.setCursor (65, 20);
    tft.setTextSize (3);
    tft.setTextColor(BLACK);
    tft.println("TEST REPORT");
   // tft.setCursor (95, 50);
   // tft.println(result_frequency);
    tft.setCursor (95, 70);
    tft.println("POTABLE");
    tft.setCursor (120,110);
    tft.println("AND");
    tft.setCursor (110, 150);
    tft.println("SAFE");
    tft.setCursor (80,200);
    tft.setTextSize (2);
    tft.setTextColor(GREEN);
    tft.println("Please Wait");

    }



    tft.setCursor (60,200);
    tft.setTextSize (2);
    tft.setTextColor(BLUE);
    tft.println("Touch to proceed");

    waitOneTouch();
    TSPoint p;
    p = waitOneTouch();
    p.x = mapXValue(p);
    p.y = mapYValue(p);

    red_frequency=0;
    green_frequency=0;
    blue_frequency=0;
    result_frequency=0;

}

TSPoint waitOneTouch() {
  TSPoint p;
  do {
    p= ts.getPoint();
    pinMode(XM, OUTPUT); //Pins configures again for TFT control
    pinMode(YP, OUTPUT);
  } while((p.z < MINPRESSURE )|| (p.z > MAXPRESSURE));
```

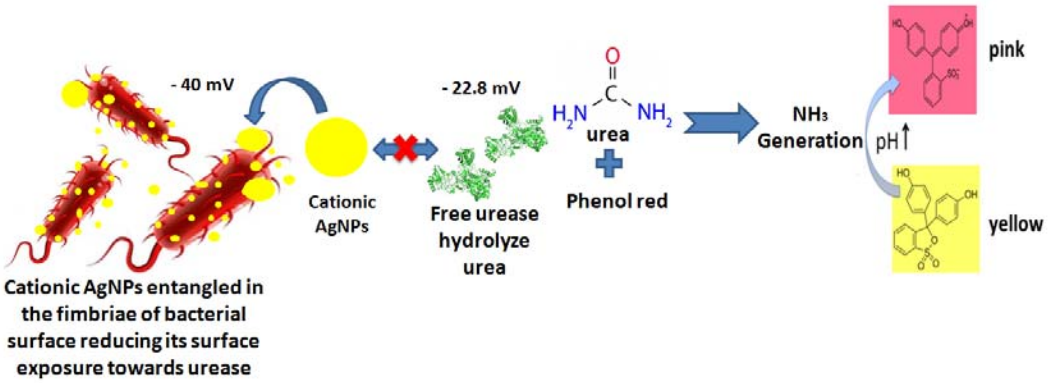
For Potable and safe samples

```
    result_frequency = red_frequency/(red_frequency+green_frequency+blue_frequency)*100;
/*
    tft.setCursor (95, 50);
    tft.println(red_frequency);
    tft.setCursor (95, 80);
    tft.println(green_frequency);
    tft.setCursor (95, 110);
    tft.println(blue_frequency);
    tft.setCursor (95, 140);
    tft.println(result_frequency);
*/


   // red_frequency= 90;
    if(result_frequency < 27)
    {
    tft.fillScreen(RED);
    tft.setCursor (65, 20);
    tft.setTextSize (3);
    tft.setTextColor(BLACK);
    tft.println("TEST REPORT");
  //  tft.setCursor (95, 50);
  //  tft.println(result_frequency);
    tft.setCursor (95, 70);
    tft.println("HIGHLY");
    tft.setCursor (45,110);
    tft.println("CONTAMINATED");
    tft.setCursor (95, 150);
    tft.println("WATER");
    tft.setCursor (80,200);
    tft.setTextSize (2);
    tft.setTextColor(RED);
    tft.println("Please Wait");

    }
    if(result_frequency >= 27 && result_frequency < 28.2)
    {
    tft.fillScreen(YELLOW);
    tft.setCursor (65, 20);
    tft.setTextSize (3);
    tft.setTextColor(BLACK);
    tft.println("TEST REPORT");
   // tft.setCursor (95, 50);
   // tft.println(result_frequency);
    tft.setCursor (45,110);
    tft.println("CONTAMINATED");
    tft.setCursor (95, 150);
    tft.println("WATER");
    tft.setCursor (80,200);
```

Resultant Frequency calculations

For Highly contaminated samples
(10^8 to 10^5 bacterials cells per mL)

For medium contaminated samples
(10^4 to 10^2 bacterials cells per mL)



Scheme S1. Illustration ofmolecular interactions and reaction mechanism in the bioassay.

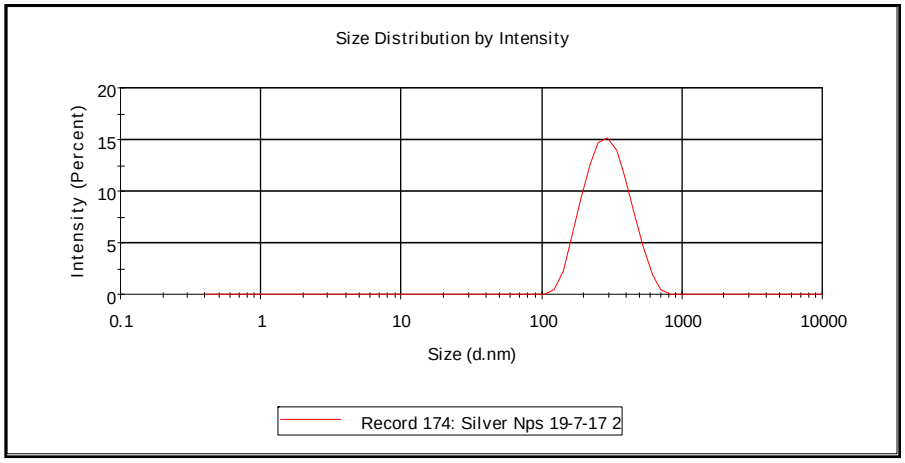


Fig. S1 DLS of AgNPs synthesized by citrate based reduction.

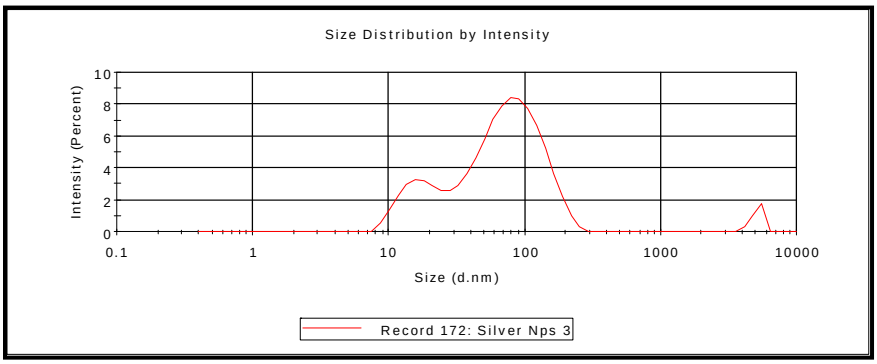


Fig. S2 DLS of AgNPs synthesized by sodium borohydride based reduction.

Electronic Supplementary Material (ESI) for ChemComm.

Table S1 Effect of various concentrations of tannic acid on AgNPs synthesis.

Tannic Acid Conc. (mM)	Z(average)	Quality report (DLS)	λ_{max}
0	-	-	-
0.001	62.91	Not good	-
0.005	22.41	Not good	403
0.01	22.52	Not good	402
0.025	23.28	Good	403
0.1	29.05	Good	410
0.5	38.15	Good	425
1	36.39	Good	422
5	34.23	Good	425

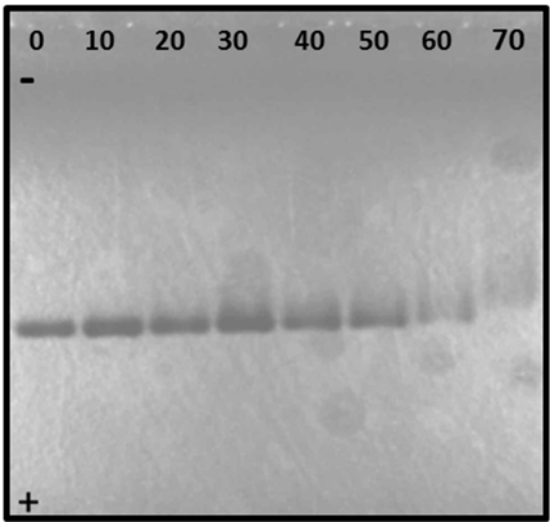


Fig. S3 Agarose gel electrophoresis of AgNPs showing retardation in band on increasing the concentration of urease (µg/mL) immobilization (left to right).

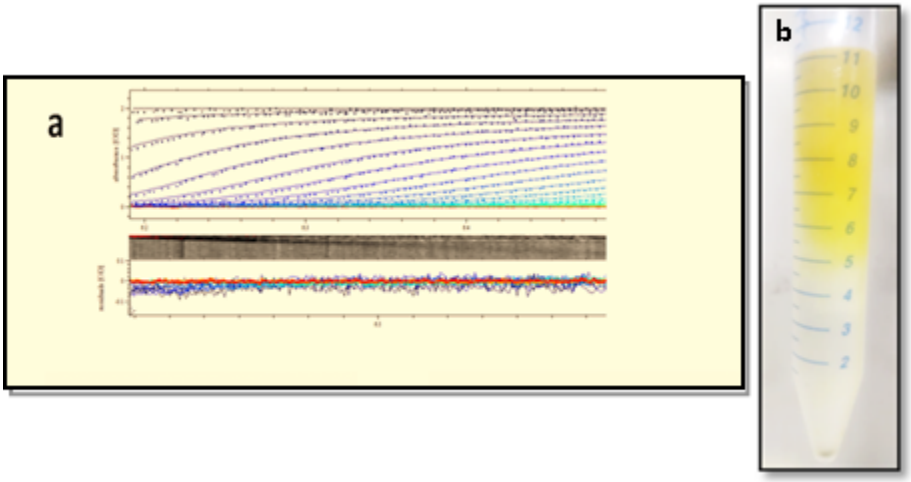


Fig. S4SV-AUC experiments(a) Lamm equation fitted sedimentation profile of Urease-AgNPs. (b) Density gradient centrifugation to get monodisperse AgNPs

.

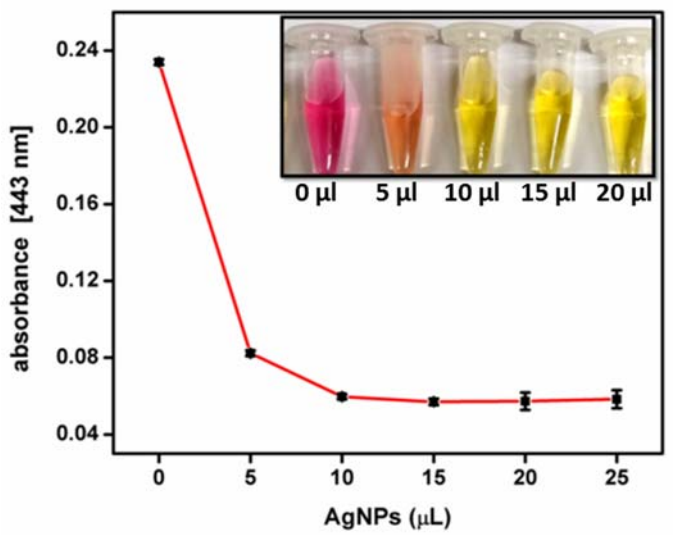


Fig. S5 Colorimetric optimization of cationic AgNPs (inset is the UV spectrophotometric analysis).

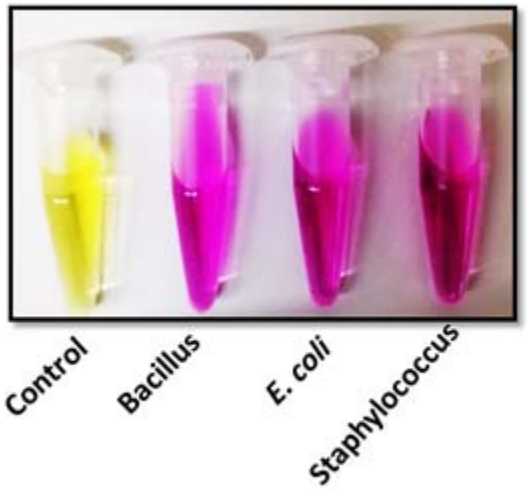


Fig.S6 Colorimetric detection of other bacterial (with cationic AgNPs) species along with control.

Table S2 Effect of tannic acid concentration on antibody conjugation as seen by UV-Vis studies.

Tannic Acid Conc. (mM)	λ_{max}(Before Conjugation)	λ_{max} (After Conjugation)
0.025	401	403
0.1	407	411
0.5	420	420
1	420	420

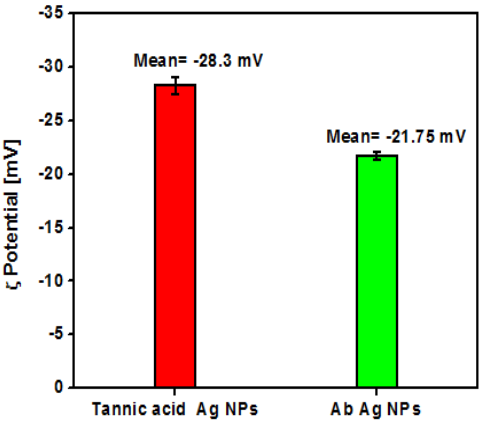


Fig.S7 Zeta potential change before and after antibody (IgG) conjugation.

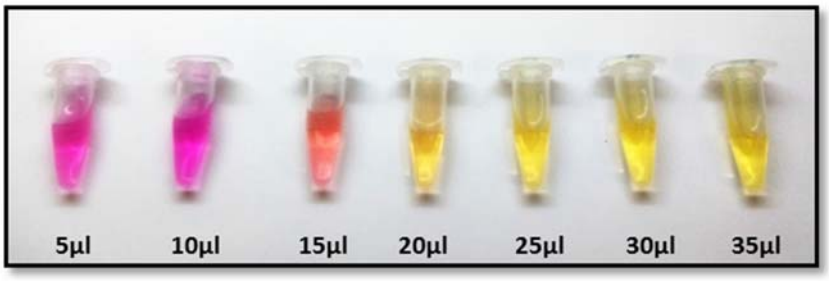


Fig. S8 Colorimetric optimization of anti-Salmonella IgG coated anionic AgNPs

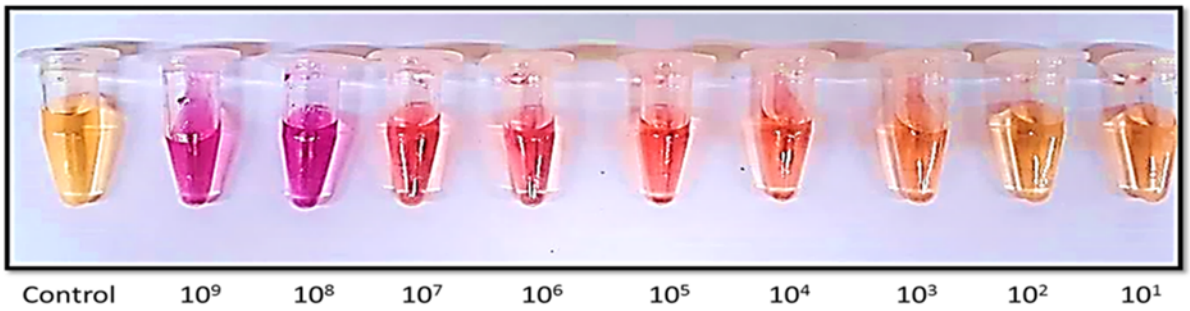


Fig.S9 Visual colorimetric signal of tap water spiked with Salmonella Typhimurium (IgG-AgNPs assay).

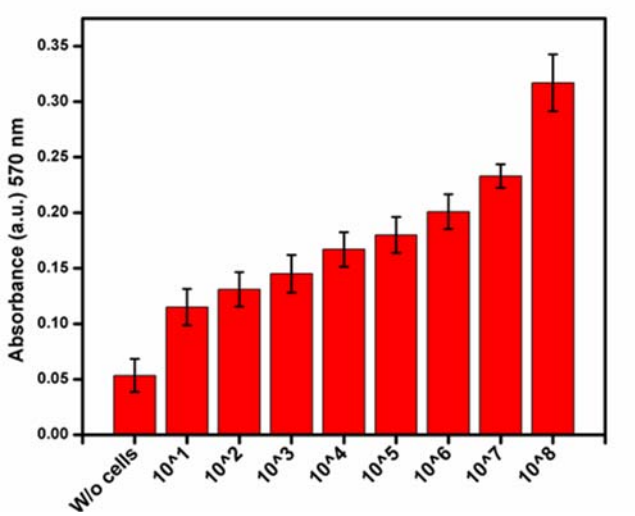


Fig.S10 Absorbance graph for tap sample based IgG-AgNP assay.

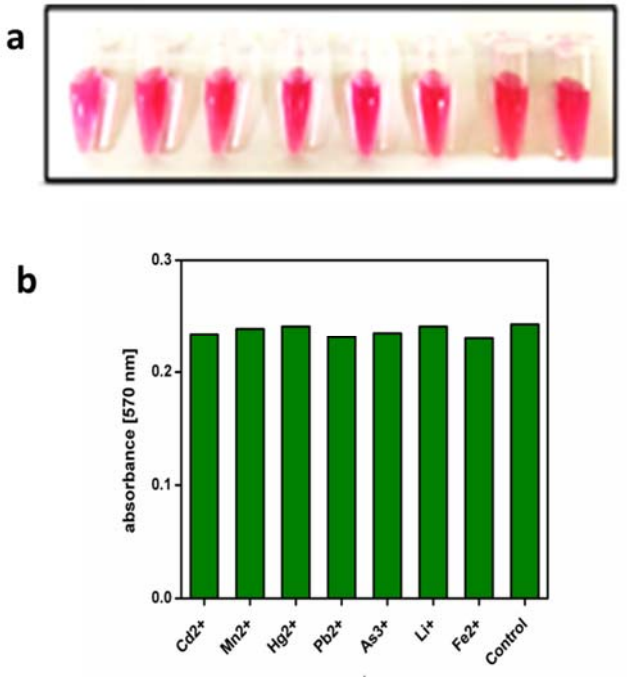


Fig. S11(a) Effect of various metal ions at WHO permissible concentration on ureaseactivity (b) Graphical representation of the absorbance of metal ion assay.

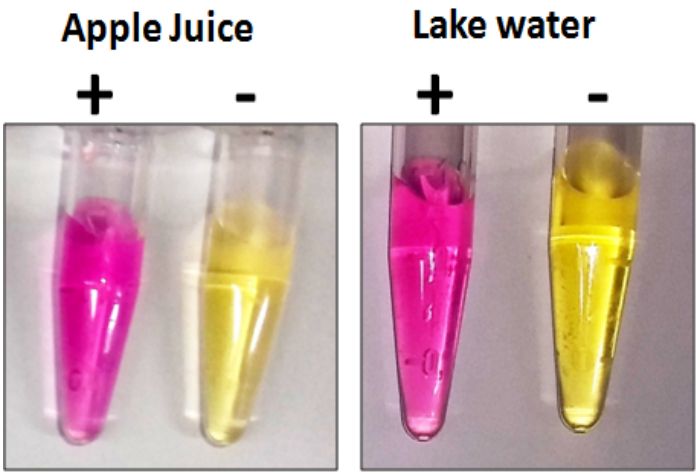


Fig. S12 Detection of Salmonella Typhimurium in complex samples represented by apple juice and lake water.

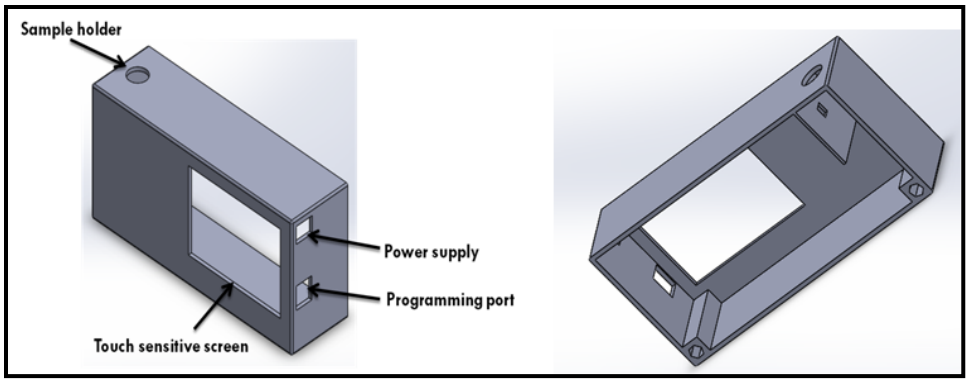


Fig. S13 Design and configuration of the arduino based device for bacterial contamination detection.

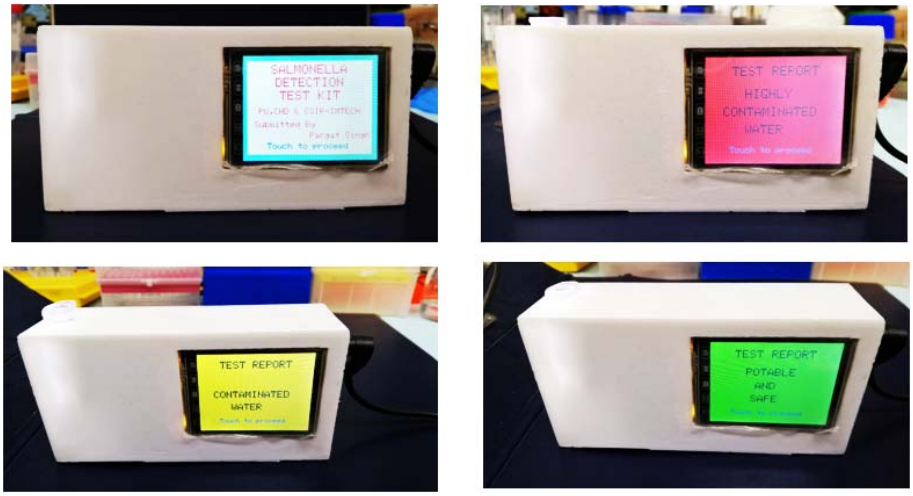


Fig. S14 Visual images of device showing different levels of contamination in the sample.

Table S3 Figure of merit of recently reported colorimetric based detection of bacteria

S. No.	Detection Probe	Detection strategy	Limit of Detection	Reference
1.	Bacteria specific RNA cleaving DNAzyme probe	Litmus based	5×10^5 cells.mL^{-1}	[9]
2.	Enzyme-nanoparticle conjugate system	Colorimetric	1×10^2 cells.mL^{-1}	[10]
3.	Dopamine capped Fe_3O_4 nanoparticles	Colorimetric	1×10^4 cells.mL^{-1}	[11]
4.	Gold-nanoparticle enzyme complex	Electrochemical	1×10^2 cells.mL^{-1}	[12]
5.	Inkjet-printed Enzymatic test strip	Colorimetric	1×10^2 cells.mL^{-1}	[13]
6.	Silver nanoparticle-Enzyme complex	Colorimetric	1×10^2 cells.mL^{-1}	This work

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