Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Gold nanoparticles based immuno-bioprobe for detection of Vi capsular polysaccharide of *Salmonella enterica* serovar Typhi

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Supplementary text

Blood Culture Test

Samples of 5 ml blood were collected in ethylene diaminetetraacetic acid (EDTA) tubes from healthy persons. For the culture of *Salmonella enteric* serovars Typhi, 4 ml of healthy blood was spiked with serovar Typhi. EDTA blood spiked with serovar Typhi using 10^2 /10^1 cells mL^{-1} was inoculated into 40 mL of medium containing tryptone soya broth. The inoculated medium was incubated at 37 $^{\circ}$C and examined for bacterial growth over 0- 24 h periods. Cultures at different time period (0- 24 h) were sub-cultured onto MacConkey agar medium to count *Salmonella* serovar Typhi after 24 and 48 h incubation time respectively. Colonies of serovar Typhi were identified using anti Vi-specific antisera (Denka Seiken Co., Ltd Coventry, UK). It was also confirmed by ELISA and developed immunoblot assay (Fig. 9). In other set of experiment, seven different healthy blood samples were spiked with the same strain of serovar Typhi using 100 and 10 cells mL^{-1} and inoculated in soya broth media. The inoculated media were incubated at 37 $^{\circ}$C for 3 h. ELISA and immunodot blot was performed to check the expression of Vi antigen in spiked sample (ST1) .We observed the expression of Vi antigen was changed in different blood samples spiked with serovar Typhi which was confirmed by ELISA and developed immunoblot methods .The data indicate the variations of Vi antigens expression among different individuals (Result and discussion part, section no. 3.6, line 332-333 fig. SD 6).

Supplementary figures

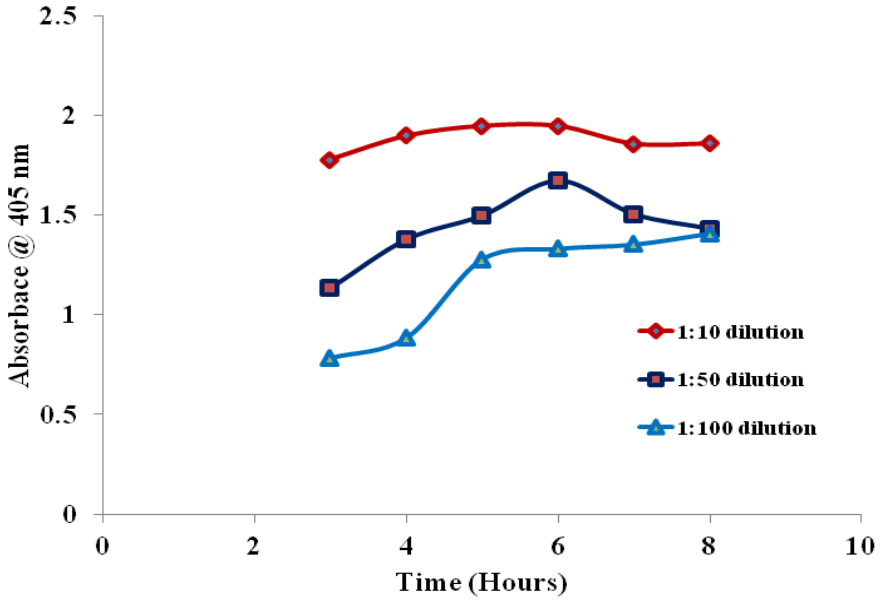

Fig. SD 1: Effect of time on Vi antigen expression by bact ELISA using different dilutions of anti-serum.

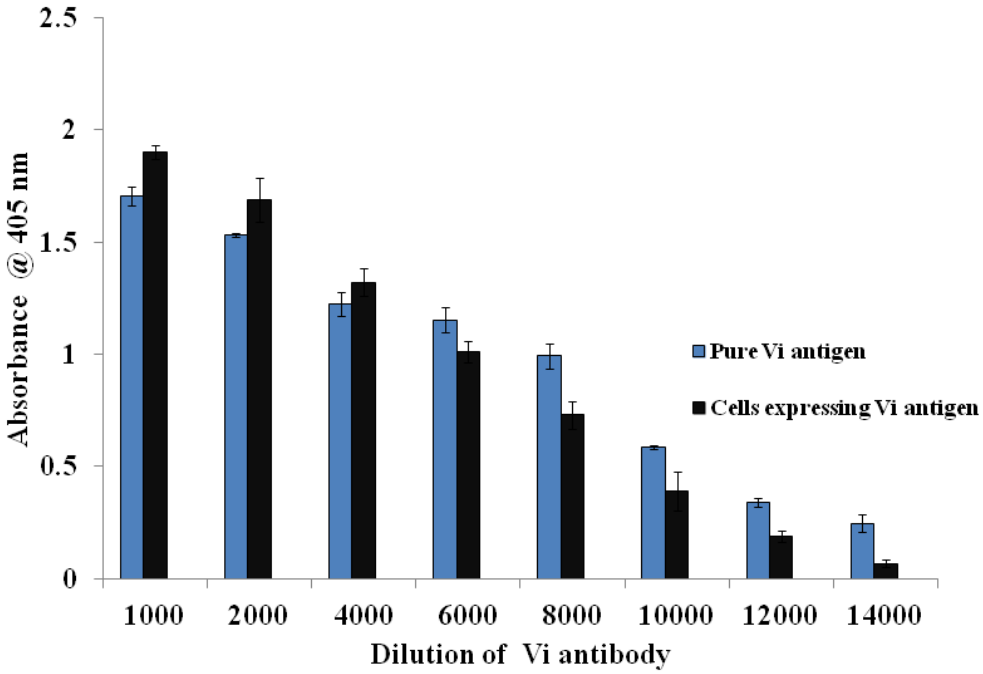

Fig. SD 2: Determination of IgG titre against Vi antigen by ELISA.

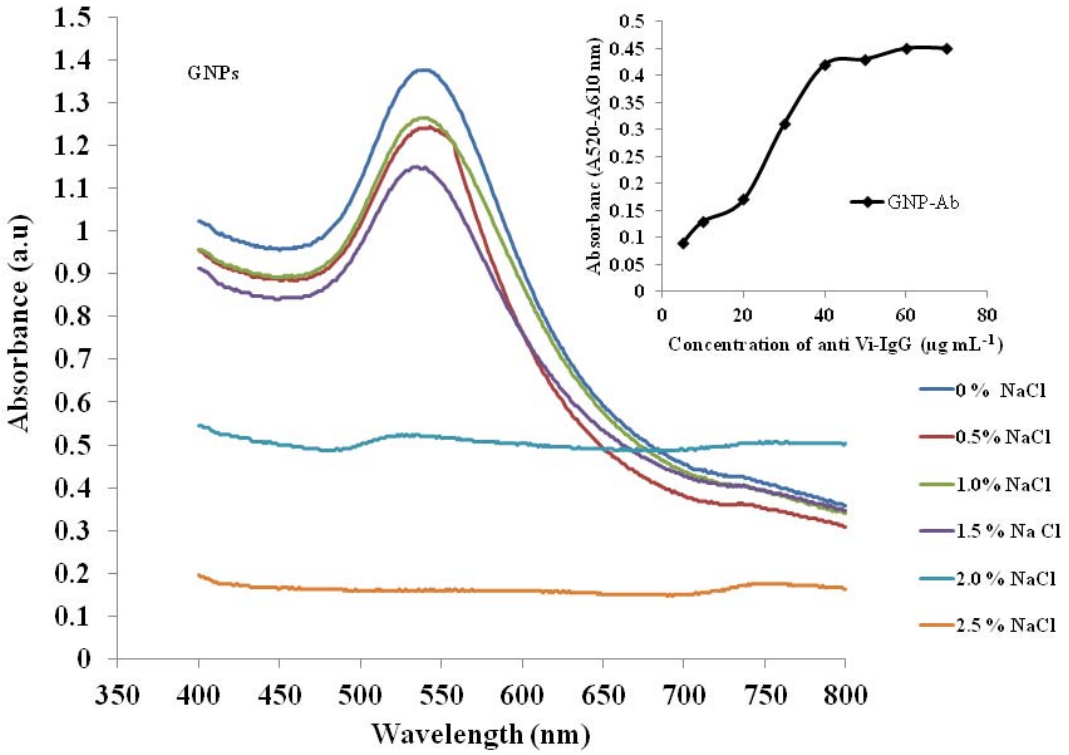

Fig. SD 3: Spectra analysis of GNPs (~30 nm) incubated with various concentrations of NaCl. Inset shows the flocculation assay determining the amount of antibody, required to obtain stable GNPs.

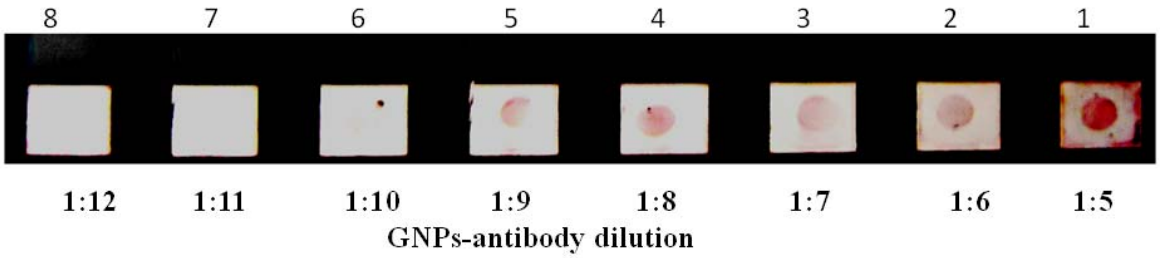

Fig. SD 4: The detection of Vi antigen by sandwich immuno-dot blot assay using different dilutions of GNPs-antibody conjugate.

Lane 1: Purified Vi antigen
Lane 2: Standard Vi antigen

Fig. SD 5: Analysis of Vi antigen, by polyacrylamide gel electrophoresis (10% SDS-PAGE) and band was visualised by silver staining. Lane1 – Purified Vi antigen, Lane 2- Standard Vi antigen.

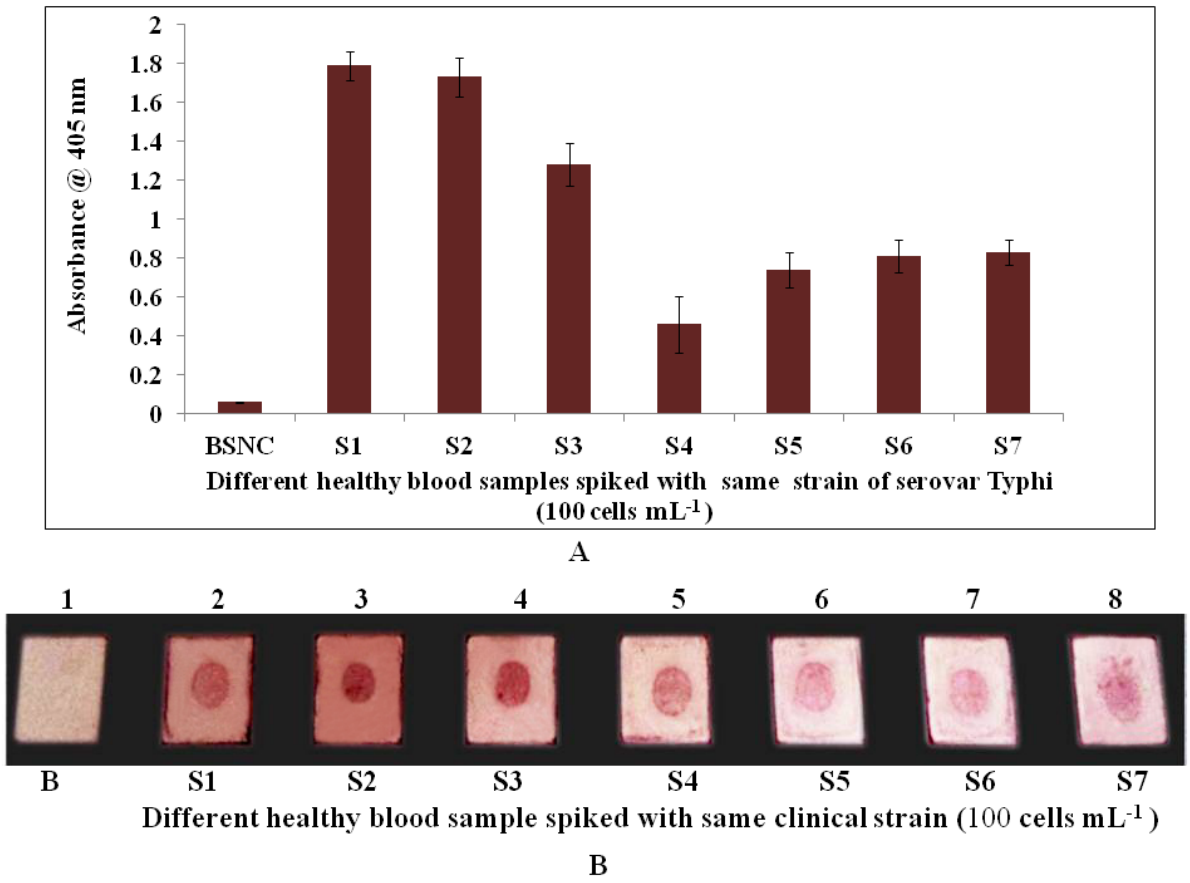

Fig. SD 6: Detection of Vi antigen in different healthy individuals spiked with cultured serovar Typhi using 100 cells mL^{-1} by ELISA and immunodot blot technique at different time intervals (0-3.30 h) respectively.

Supplementary table

ST 1: Comparative analysis of blood culture test spiked with cultured serovar Typhi by using different techniques.

Test		No. of bacteria cells mL^{-1} spiked in healthy blood	Time interval of blood culture (h)							
			0	**0.30**	**1.0**	**1.30**	**2.0**	**2.30**	**3.00**	**3.30**
Blood Culture	**24 h**	100	--	--	--	++	++	++	++	++
	48 h		--	--	++	++	++	++	++	++
Dot blot			--	--	++	++	++	++	++	++
ELISA			--	±	++	++	++	++	++	++
Blood Culture	**24 h**	10	--	--	--	--	++	++	++	++
	48 h		--	--	--	--	++	++	++	++
Dot Blot			--	--	--	--	±	++	++	++
ELISA			--	--	--	±	++	++	++	++

In case of blood culture test detection of serovar Typhi takes more than 24 hours.

- -- No detection
- ++ Detection
- ± Not prominent detection

In the blood culture test, samples were drawn at different time intervals (0-3.30 h) and cultures were sub-cultured onto MacConkey agar medium to count *Salmonella* serovar Typhi after 24 and 48 h respectively. However, in case of ELISA and dot blot, the detection of Vi antigen was observed after 1 and 1.30 h respectively. Indicating the format detection of serovar Typhi by earlier than the traditional blood culture test and also easy to perform in the clinical lab. Detection of Vi antigen was observed in the representative healthy blood sample spiked with cultured serovar Typhi at different intervals (0-3.30 h) using whole bacterial ELISA (Bact.ELISA) technique (as mentioned in manuscript section 2.6).