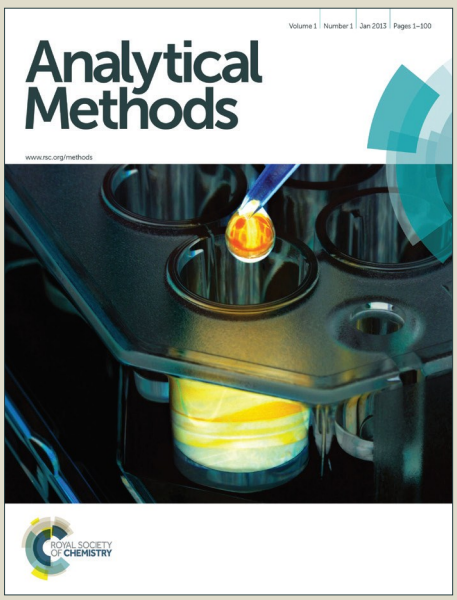
Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Journal Name

ARTICLE

Nanogold based lateral flow assay for *Salmonella typhi* detection in environmental water samples

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Jyoti Singh[1], Shivesh Sharma[1] and Seema Nara[1]*

Background: *Salmonella* infection is one of the leading cause of water and food borne diseases causing approximately 3.5-18 million deaths per year worldwide. The severity of infection demands early detection of bacteria in drinking water before its consumption. The present work, therefore, attempts to develop a rapid lateral flow assay for detecting *Salmonella typhi* in Ganges water using gold nanoparticles as label.

Methodology: Gold nanoparticles were synthesized using sodium citrate reduction and characterized using various techniques like UV-visible spectrometry, florescence spectrophotometer and TEM. An optimized amount of anti-*Salmonella* monoclonal antibodies was conjugated with synthesized gold nanoparticles. An optimum amount of anti-*Salmonella* polyclonal antibodies and anti mice antibodies were coated on

[1]*Department of Biotechnology, Motilal Nehru National Institute of Technology, Allahabad, Uttar Pradesh, India -211004*
Corresponding author.
Tel.: +91 0532 2271245. E-mail addresses: seemanara@mnnit.ac.in, seemanara@gmail.com

nitrocellulose membrane as test line and control line respectively. The strip detected *Salmonella* in sandwich assay format. The developed assay was tested with water samples collected from five different ghats of Ganges river water and the results were correlated with conventional culture based methods.

Results: Statistical validation of the assay results showed that the diagnostic and analytical sensitivity was 100% and 86.7% respectively. Specificity for LFIA strip was found to be 100%. Accuracy for *Salmonella* LFIA strip was found to be 93.1%. Positive predictive value and negative predictive value for LFIA strip was found to be 1 and 0.875 respectively.

Key Words: *Salmonella;* Gold nanoparticles; lateral flow immunoassay strip; sensitivity; Specificity

1. Introduction

Salmonella is one of the major causes of food and waterborne infections worldwide. *Salmonella* infection can result in manifestation of typhoid and paratyphoid fever, endocarditic and enteritis due to favourable high temperature of human body and environment. Waterborne *Salmonella* infections are a growing concern that needs attention towards deteriorating biological quality of drinking water. There have been reports of typhoid causalities by *Salmonella typhi* often associated with contaminated drinking water in the Indian subcontinent and countries of south–east Asia **[1].** A total of 164,044 *Salmonella* infections (approximately 32,000 annually) were reported from 1998-2002 by the National *Salmonella* Surveillance System **[2-6],** which is a passive, public health laboratory-based system. In 1997 Tajikistan was presented with an epidemic by multiresistant strains of *Salmonella* which spread through contaminated drinking water **[7,8].** In water borne human infections, increased frequency of antibiotics resistant *Salmonella* strains have been found to be implicated, which is another major health concern in developing countries **[9,10,11,12]**. Contaminated irrigation waters were often associated in related *Salmonella* outbreaks **[13].**

Ganges river is considered as lifeline of Indian subcontinent and its microbial load is continuously

increasing. **Singh and Saxena 2010 [14]** have isolated thirty three isolates of *Salmonella* belonging to eight serovars from the Ganges water, India. Studies also showed that addition of untreated sewage waste in river bodies has increased the prevalence of fecal origin *Salmonella* in different stretches of Ganges river water which is used majorly for drinking and domestic work **[15, 16, 17].** The ongoing depleting quality of drinking water resources demands for the detection methods which can be employed for timely biomonitoring of these resources.

Several physiological and biochemical methods have been developed for detection of *Salmonella* in water, but because of their limitations of selective plating, biochemical screening and serological confirmation they are considered to be time consuming **[18]**. To overcome these limitations PCR based methods become very popular like **Waage et al. 1999,[19]** used nested PCR to detect small number of *Salmonella* from water, **Moganedi et al. 2007 [20]** optimised PCR-invA primers for the detection of *Salmonella* in drinking and surface waters following a pre-cultivation step, **Kapley et al. 2001[21]** and **Saxena et al. 2013 [17]** showed the presence of *Salmonella* in Ganges water by using PCR and multiplex PCR respectively**. Wolter et al. 2008 [22]** designed a chemiluminescence microarray readout system for detecting *Escherichia coli O157:H7, Salmonella typhimurium, and Legionella pneumophila* in water, **Fey et al. 2004 [23]** used real time PCR reaction for accurate quantification of bacterial RNA targets in water, using *Salmonella* as a model organism**, Dunbar et al. 2003 [24]** used Luminex LabMAP system as a rapid and flexible platform for the simultaneous, multiplexed detection of DNA or protein from common bacterial pathogens. The PCR based methods are although accurate and sensitive but at the same time they require high end instrumentation and skilled persons.

Gold nanoparticle (AuNPs) based lateral flow immunoassays (LFIA) offers an easy solution to these limitations as it is a rapid, user friendly technique, requiring less technical expertise. AuNPs-LFIA has been reported for detection of various bacterial species from food and feed **[25, 26, 27].** A recent review discusses AuNPs based LFIAs reported so far for detection of enterobacteriaceae members in food and water

[28]. It concludes that LFIAs have been developed for detecting *Salmonella* in food and feed samples but the reports of detecting *Salmonella* in water or environmental samples using LFIA are scarce.

Although LFIA is a well established technique, we have not come across reports describing nanogold based LFIA for detecting *Salmonella* species in drinking water resources. An assay developed for detecting *Salmonella* in food/feed samples can not be successfully applied on other matrices like drinking water until and unless it is thoroughly validated on a particular matrix, owing to the difference in the basic nature of two matrices.

The existing scenario of increased contamination of drinking water resources, the need to evaluate water quality to determine its potability and the lack of any rapid and efficient method for detecting bacterial presence in water is the driving force behind this study. Driven by this need, the present study was conceptualised to develop a LFIA for salmonella detection in water and then statistically evaluate it by monitoring real water samples using Ganges water which is one of the major rivers flowing in the Indian subcontinent and millions of people are dependent on it for their water uses **[16].**

2. Experimental

2.1. Materials and reagents

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4$) was purchased from Sigma-Aldrich St. Louis, MO, USA and used directly without further purification; Bovine serum albumin (BSA) and tween 20 were purchased from Sisco research laboratories (SRL) India. Rappaport-vassiliadis *Salmonella* enrichment broth, nutrient agar, nutrient broth and trisodium citrate were purchased from Himedia India. *Salmonella* mouse monoclonal antibody, anti *Salmonella* antibody polyclonal, Rabbit anti mouse IgG H & L secondary antibody were procured from Abcam, India. Nitrocellulose membranes, conjugate Pad, sample pad and absorbent pads were purchased from Advanced Microdevices Pvt. Ltd., Ambala cantt India. *Salmonella enteritica* serovar *typhi (MTCC733, Salmonella enteritica serovar typhi (MTCC3216), Salmonella enteritica serovar, Salmonella typhimurium (MTCC1254)* were obtained from MTCC Chandigarh.

2.2. Preparation of colloidal gold nanoparticles

Gold nanoparticles were prepared by citrate reduction. 0.8M of $HAuCl_4$ salt (45 ml) was reduced by 38.75 mM trisodium citrate (5 ml) with vigorous boiling producing a deep cherry red colour **[29]**. Gold nanoparticle solution was then cooled at room temperature and stored at 4^0C till use.

2.3. Conjugation of gold nanoparticles with *Salmonella* antibody

For stable conjugation of AuNPs with antibody, the pH of AuNPs was optimised by NaCl flocculation assay **[30].** Briefly, 30 µl of 2.5 M NaCl was added to 1ml of AuNPs solutions of different pH ranging from 6 to 10. At optimum pH, the concentration of anti-*Salmonella typhi* monoclonal antibody (2-20 µg/ml) required for stable conjugation with AuNps was determined using flocculation assay **[31].** The optimized amount of antibody was coupled on the surface of citrate stabilised AuNPs following the procedure of **Nara et al., 2010 [29].** In brief, a fixed amount of antibody was added in AuNPs colloidal solution and kept on shaking at 200rpm at room temperature for 1 hour. Tween 20 (1 µl) and 150 µM BSA (100µl) was added to AuNPs solution and stirred for 30 minutes. Gold nanoparticle-antibody (AuNPs-Ab) conjugate was then centrifuged at 13000 rpm for 20 minutes at 4^0C followed by washing and resuspended in PBS buffer (10 mM pH 7.4) and stored at 4^0C till use.

2.4. Preparation of heat killed standard *Salmonella typhi* solution

Salmonella typhi was grown in nutrient broth for 24 hours at 37^0C and fixed by 1.3 M formaldehyde followed by heat killing at 121^0C for 15 minutes. 1 ml of this solution having optical density 1 at 600nm was centrifuged at 10,000 rpm for 20 minutes at 4^0C. The pellet obtained was washed once to remove any traces of formaldehyde and resuspended in 1 ml of 10 mM PBS pH 7.4. 100µl of 1.5 mM BSA was added to control the flow rate on nitrocellulose membrane and prevent nonspecific binding. This *S. typhi* solution was used as standard to check the viability of developed LFIA strip.

2.5. LFIA Development

For LFIA development, two types of sample pads (GFB-R4 and GFB-R7L) and conjugation pads (PT-R5 and PT-R7) were tested. The LFIA strip was prepared by immobilizing polyclonal anti-salmonella antibody (Test line) and goat anti-mice antibody (Control line) on the surface of nitrocellulose membrane (NC) fixed on a plastic backing. The NC membrane was then kept at 37^0C for 20 minutes so that the antibodies get completely adsorbed on the NC membrane which helps in reducing their chances of sweeping out of the membrane during assay run. AuNPs-Ab conjugate solution in resuspension buffer was then dispensed on the reservoir matrix so that each pad gets 10 µl of AuNPs-Ab solution and was air dried. This is followed by assembling various components of LFIA strip i.e. NC membrane, reservoir matrix, sample and adsorbent pad as shown in Figure 1.

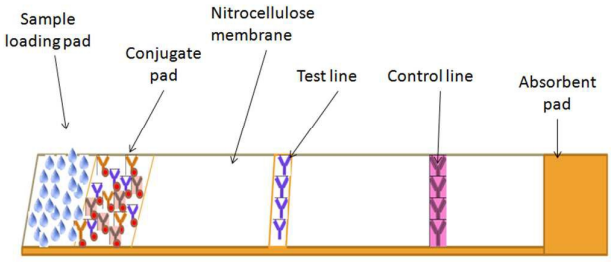


Figure 1: Schematic diagram of LFIA strip showing its various components.

The functioning and viability of the prepared strip was tested by running the standard *Salmonella typhi* solution as sample.

2.6. Water sample collection

Water samples were collected from five different ghats of Ganges river at Allahabad on the occasion of Kumbha mela namely Dashashmegha Ghat (S-1), Hanuman Ghat (S-2), Ram Ghat (S-3), Sangam Ghat (S-4), Arela Ghat (S-5) by immersion type of sampling method from 30 cm depth of water body in triplicates. Water samples were collected in plastic bottles and stored at 4^0 C till their analysis.

2.7. Testing of water sample with selective media

The Ganges water sample from Dashashmegha Ghat (S-1), Hanuman Ghat (S-2), Ram Ghat (S-3), Sangam Ghat (S-4), Arela Ghat (S-5) were enriched by inoculating 1 ml of water sample in 5ml of 1X lactose broth for 12 hours at 37^0C and further 100µl of it was transferred into rappaport-vassiliadis *Salmonella* enrichment broth for 16-20 hours. A loop full of each sample was streaked on Endo agar and *Salmonella-shigella.* The plates were incubated for 24 hours at 37ºC.

2.8. Testing of developed LFIA

The antibody coated, fully assembled and ready to run LFIA strips, were dipped into the sample solution which was allowed to move across the strip due to capillary action. The presence/absence of red color at test line indicates the presence or absence of *S. typhi* in the sample. There is always a red color at the control line. The strip takes 5-7 minutes for a single analysis. LFIA strips were tested with three standards strains of *Salmonella enteritica* serovar *typhi (MTCC733)*, *Salmonella enteritica* serovar *typhi (MTCC3216)* and *Salmonella enteritica* serovar *typhimurium (MTCC1254)* for determining its sensitivity. Lower limit of detection (LOD) of the strip was calculated by running the strips with decreasing concentrations (10^8cfu mL-1 to 10^1 cfu mL-1) of standard *S. typhi*. A total of 40 LFIA strips were run with standard *Salmonella typhi* (10^8cfu mL-1) to calculate diagnostic sensitivity through Simple Interactive Statistical Analysis (SISA) software based on following formula **[32].**

$$\text{Sensitivity} = \frac{true\ positive}{true\ positive + false\ negative}$$

Specificity of the strip was calculated with different bacterial strains listed in Table 1 as per below mentioned formula

$$\text{Specificity} = \frac{true\ negative}{false\ positive + true\ negative}$$

Other parameters that can be used to describe the quality and usefulness of LFIA strip are positive predictive value (PPV) negative predictive value (NPV), Kappa agreement measure and accuracy were calculated as:

$$\text{Positive predictive value} = \frac{true\ positive}{true\ positive + false\ positive}$$

$$\text{Negative predictive value} = \frac{true\ negative}{false\ negative + true\ negative}$$

$$\text{Accuracy} = \frac{true\ positive + true\ negative}{total\ no.of\ strips\ tested}$$

Positive predictive value of LFIA strip is the probability that a water sample is contaminated when a positive test result is observed while negative predictive value of LFIA strip is the probability that water sample is not contaminated when a negative test result is observed. Similarly, LFIA strip assay was also tested using environmental water samples from Ganges.

Microorganism	No. of LFIA strips tested (n)	True positive	True negative	False positive	False negative
Salmonella typhi (MTCC733)	15	15	-	-	-
Salmonella typhi (MTCC3216)	15	15	-	-	-
Salmonella typhimurium (MTCC1254)	10	10	-	-	-
E.Coli (ATCC 35218)	8	-	8	-	-
Staphylococcus aureus	4	-	4	-	-
Klebsiella pneumoniae	4	-	4	-	-
Staphylococcus epidermidis	4	-	4	-	-
Proteus mirabilis	4	-	4	-	-
Pseudomonas aeruginosa	4	-	4	-	-

Table 1: LFIA strips were run with different pre-cultured bacterial strains and the data was used to calculate diagnostic sensitivity and specificity.

A total of 30 LFIA strips were tested on five different water samples collected from Ganges after enrichment. Enrichment is an essential prerequisite for testing the water samples by LFIA to avoid the false negative results. Since, low count per milliliters of *Salmonella* in water samples is reported **[33]** therefore; there is a need for enrichment with a selective medium to increase the bacterial count per milliliters of water sample. However, even after enrichment, LFIA methods takes less time and technical expertise for analysis as compared to the total time taken by conventional culture methods and all PCR based methods (Table 2). The analytical sensitivity and specificity of the assay was also calculated using enriched water samples. These real samples were also analyzed on different days to calculate the accuracy of the developed assay.

S.No.	Steps in conventional culture method and time taken		Steps in PCR based methods	Steps in LFIA and total time taken	
1	Culture in nutrient broth	24 hrs	12hrs	Not required	-
2	Culture in rappaport-vassiliadis selective enrichment broth	12 hrs	Not required	Enrichment step done	12 hrs
3	Plating on differential media	12-24hr	Not required	Testing with strip	5-7 min
4	Further confirmation is required using biochemical tests which require more time, expertise and resources		Testing with specific primers and skilled man power expertise is required	No additional step required	

Table 2: Comparison of LFIA with conventional and PCR based methods for *Salmonella* detection

3. Results and Discussion

3.1. Characterisation of colloidal gold nanoparticles and probes

Gold nanoparticles (AuNPs) and gold nanoparticles conjugated with antibodies (AuNPs-Ab) were characterized for their conjugation, stability and original colour retention capacity using standard techniques like Transmission electron microscopy (TEM), Atomic force microscopy (AFM), UV-visible spectrophotometer, and florescence spectrophotometer.

A high optical density AuNPs were desired to be used in LFIA so as to achieve high visual signal. The stability of AuNPs at high optical density is affected by the nature and concentration of antibody added in the solution, characterisation gives us a clear picture of stability as well as conjugation.

The reduction of aurochloric acid was visibly evident from the colour changes associated with it (Figure 2a). The inset picture shows the colour changes before (1), and after the process of reduction (2). The aurochloric acid solution gradually underwent red shift with appearance of a sharp peak at 520 nm due to surface plasmon resonance (SPR) of nanoparticles which can be attributed to a narrow size distribution of the

particles formed in the solution. The AuNPs-Ab conjugate shows peak at 525 nm showing a peak shift of 5 nm (Fig. 2a). This shift reflects the binding of antibodies with the AuNPs surface. Florescence spectra of AuNPs and AuNPs-Ab was taken at excitation wavelength of 310nm show quenching of florescence of AuNPs-Ab solution as compared to AuNPs by 77 units, reflecting some interaction between antibody and AuNPs (Fig. 2b). AuNPs synthesized were also characterised by TEM, showing AuNPs to be spherical in shape, 20 nm in diameter and monodispersed (Fig. 3).

3.2. Flocculation assay

Exposure to high salt concentrations will change the surface plasmon resonance of the colloidal AuNPs which lead to the shift in the colour of AuNPs from cherry red to purple to grey and black precipitate **[30].**

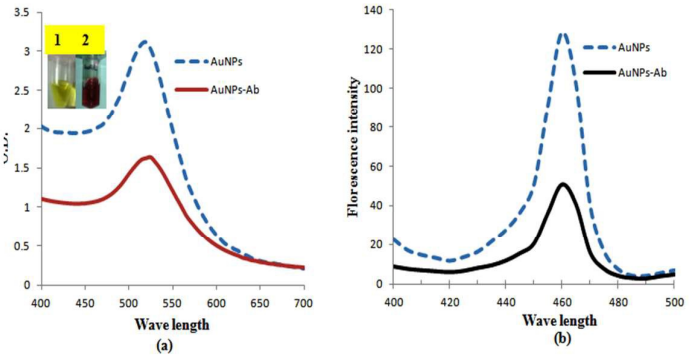


Figure 2 (a-b): (a) U.V. Visible Spectra of gold nanoparticles with and without conjugation with anti-S*almonella* antibody. Inset picture shows the color change in aurochloric acid before (1) and after reduction (2) with trisodium citrate, indicating the formation of AuNPs. (b) Florescence spectra of AuNPs and AuNPs-Ab measured at excitation wavelength 310nm.

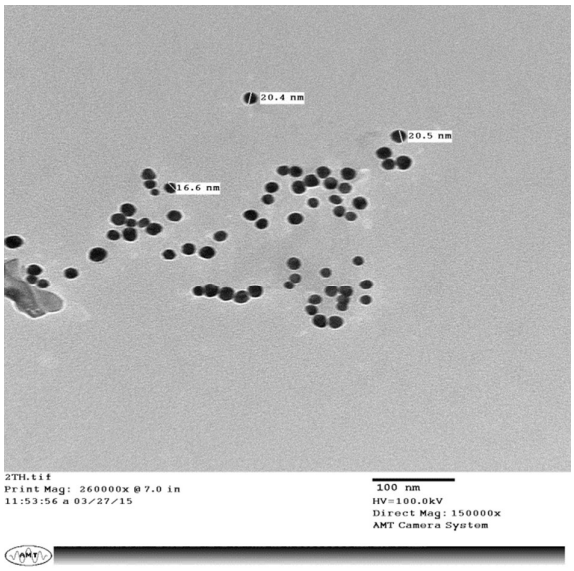


Figure 3: TEM micrograph of synthesized AuNPs.

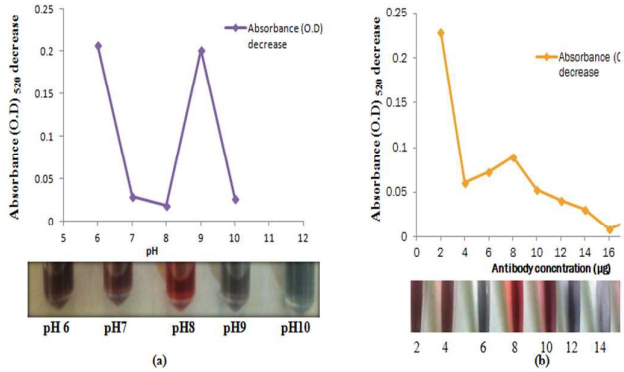


Figure 4 (a-b): Flocculation assay (a) for the optimization of pH and (b) for the optimization *S. typhi* antibody concentration for conjugation on AuNPs.

Optimum pH may confer some stability to AuNPs at high salt concentration by balancing the electrostatic charge distribution on the AuNps surface. With flocculation assay, AuNPs were tested at different pH for their stability (Fig. 4a). The results showed that at pH 8 AuNPs were more stable in an environment of high salt concentration. Similarly, flocculation assay showed 16µg as optimum concentration of antibody for stable conjugation with AuNPs (Fig. 4b).

3.3. Culture plate isolation of *Salmonella* from Ganges water Samples

All Ganges water samples collected from five different locations in Allahabad viz; Dashashmegha Ghat (S-1), Hanuman Ghat (S-2), Ram Ghat (S-3), Sangam Ghat (S-4), Arela Ghat (S-5) showed the presence of *Salmonella* by culture plate method.

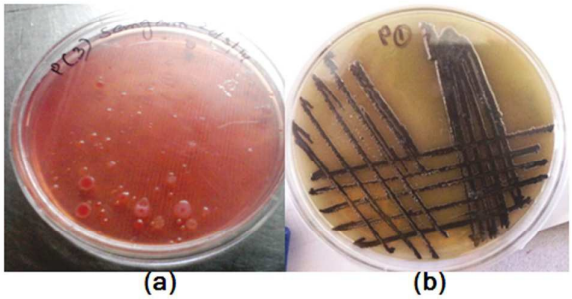


Figure 5: Confirmatory test of Ganges water samples for *Salmonella* by conventional culture methods (a) Endo agar and (b) *Salmonella*-shigella agar plates

Salmonella species formed white color faint colonies on endo agar and black colonies on Salmonella-Shigella agar respectively (Fig. 5).

3.4. LFIA

For LFIA development, two types of sample pads (GFB-R4 and GFB-R7L) and conjugation pads (PT-R5 and PT-R7) were tested. It was observed that GFB-R4 gave consistent results with visually darker test line as compared to GFB-R7L, it may be due to the presence of buffers and detergents in GFB-R7L which may be hampering the stability of AuNPs-Ab and its release form the matrix. For the conjugation pad, two options were tried, PT-R5

which has minimal additives and PT-R7 with buffers for uniform movement of gold. Both conjugate pads were found to produce equally good visual results on nitrocellulose membrane. Therefore, for developing the LFIA tests for *Salmonella*, GFB-R7L sample pad and PT-R7 conjugate pad was employed in further studies.

3.5. Diagnostic Sensitivity and Specificity of LFIA

The lower limit of detection of LFIA strip was determined by serially diluting standard *Salmonella typhi* solution (10^8-10^1 cfu mL-^{1}) in 10Mm PBS buffer pH 7.4 and tested with LFIA strips (Fig. 6). The LOD was found to be 10^4cfu mL-1 by naked eyes. To realize the full potential, and minimize the false negative events for developing a prototype

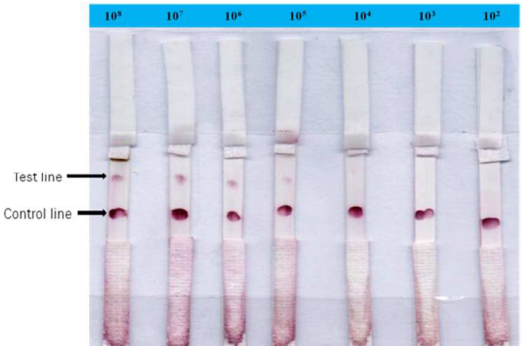


Figure 6: LFIA run for limit of detection using different *Salmonella* concentrations (10^8-10^1cfu/ml)

LFIA strip for *Salmonella* detection, diagnostic sensitivity shall be calculated using fully enriched *Salmonella* water samples (10^8cfu mL-^{1}). The diagnostic sensitivity of the developed strip was calculated to be 100%, no false negatives were obtained with standard *Salmonella* solution (10^8cfu mL-^{1}). The specificity of the developed assay was found to be 100% when tested with different closely related bacterial strains; no false positives were obtained (Table 1). For the developed LFIA, PPV and NPV was found to be 1 each. LFIA strip has kappa agreement measure of 1, which means that the test could correctly predicts the outcome 100% under ideal conditions.

3.6. Statistical analysis of LFIA strip for Analytical sensitivity and specificity

The diagnostic sensitivity and specificity is different from analytical sensitivity and specificity. The diagnostic sensitivity and specificity is determined by running the assay on standard solution of known concentrations, and defines the assay parameters under ideal conditions. Whereas, analytical sensitivity and specificity is calculated by running the assay on real samples **[30].** The

Ganges Sample	No. of LFIA strips tested (n)	True positive	False positive	False negative	True negative	Result of culture based method
Dashashmegha Ghat (S-1)	6	6	-	0	-	+
Hanuman Ghat (S-2)	6	4	-	2	-	+
Ram Ghat (S-3)	6	6	-	0	-	+
Sangam Ghat (S-4)	6	4	-	2	-	+
Arela Ghat (S-5)	6	6	-	0	-	+

Table 3: Data used for deducing analytical sensitivity of LFIA strip when run with different water samples from Ganges

LFIA strip was therefore evaluated with Ganges water samples after enrichment (Fig. 7). The water samples might contain *Salmonella* in low numbers in addition to a lot of other micro-organisms, and they may be injured. To minimize the risk of obtaining false negative results, a non-selective pre-enrichment of water samples followed by selective enrichment in rappaport-vassiliadis broth was done.

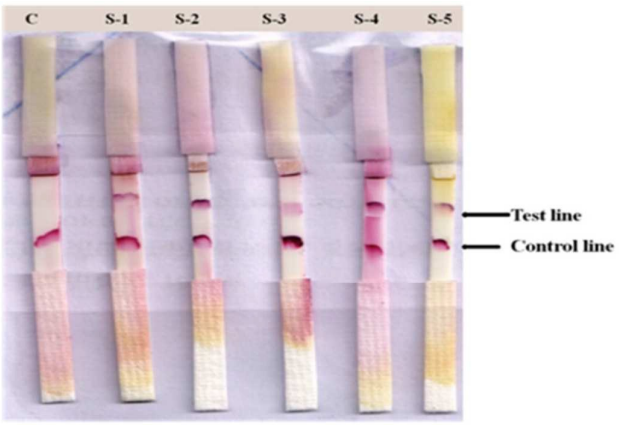


Figure 7: Testing of Ganges water samples after enrichment LFIA strip. Test line for the presence of *Salmonella* was formed in all IC strips for the water samples (C, S-1, S-2, S-3, S-4, S-5). C is the control sample with no antigen in test sample.

Table 3, depicts the results of Ganges water samples tested with developed LFIA and conventional culture based method. Based on these results, the analytical sensitivity was calculated to be 86.7%, whereas, the specificity was 100%. The accuracy of the developed strip was calculated by using the data in table 1 and table 3 and was found to be 93% which reflects the probability of LFIA strip to correctly depict the presence or absence of *Salmonella* in water samples. Kappa agreement measure was found to be 0.863, which means that

LFIA strip can correctly predict the outcome more than 86.3%. Also the positive predictive value and negative predictive value for *Salmonella* detection by LFIA strip was found to be 1 and 0.875 respectively which depicts the probability of LFIA strip to show a positive test when *Salmonella* is present and vice-versa.

LFIAs have been reported for detecting *Salmonella* spp. in various food matrices before or after enrichment **[28].** Variations in the LFIA formats such as modifications for signal amplification have also been demonstrated as proof of concept by taking *Salmonella* or any other bacterial species as a model organism. However, we have not come across any such report where LFIA has been actually used for detecting *Salmonella* spp. in water samples followed by presenting the statistical data. Water quality monitoring is equally important to prevent the outbreak/spread of *Salmonella* infections. Therefore, to do a reality check, we have developed a LFIA and used it for detecting *Salmonella typhi* in various water samples collected from different locations of Ganges river in Allahabad. The results were statistically analysed and it was observed that the assay was highly specific (100%). The diagnostic sensitivity which was determined using known concentration of pure *Salmonella typhi* was 100% indicating that the LFIA used in the study does not give any false negatives. However, the analytical sensitivity of this assay which was determined on real water samples after enrichment was observed to be 86.7%. This difference originates due to reporting of some false negatives with real water samples. Therefore, it is highly recommended to test the assay on as much number of real samples as possible so that accurate assessment can be done. Based upon the results with water samples, the assay was found to be highly accurate i.e. 93%. The positive predictive value and the negative predictive value of the assay was found to be 1 and 0.875 respectively.

Conclusion

In the present work, an AuNP based LFIA strip was developed and used for Salmonella *typhi* detection from Ganges water samples collected from five different locations in Allahabad, India. AuNPs were used in this study because of their surface plasmon resonance property, ease of synthesis and

conjugation and long shelf life at room temperature. The results showed developed LFIA to be statistically relevant (sensitivity, specificity, PPV and NPV) for detecting *Salmonella* in water samples. LFIA provide single handed information about the bacterial pathogen presence and absence while other techniques are not self sufficient to say about the presence of a particular bacteria in the sample. Most of the techniques relies upon more than one tests or parameters to reach a final conclusion. In comparision, LFIA need only enrichment of samples before detection, therefore it is comparatively rapid and require less technical expertise. The LFIA reported in this study is qualitative and sufficient enough to confirm about the water quality. Although quantitative data is always an advantage to obtain but in case of bacteria the main purpose of its development is solved with qualitative information for pathogen detection. Quanitative data is more relevant in case of antigens like hormone, toxins, pesticides, etc. The demand for rapid diagnostic methods is increasing and as per Hazard analysis and critical control points (HACCP), such assays will play an important role in healthcare management.

Acknowledgment

We acknowledge University Grants Commission (UGC) New Delhi, India for providing necessary financial support to conduct this study.

References

[1] B.Rowe, L.R.Ward, E.J. Threlfall, Multiresi stant Salmonella typhi– a world-wide epidemic, *Clin. Infect. Dis*. 1997, 24 (Suppl. 1) S106–S109.

[2] CDC. Salmonella surveillance: annual tabulation summary, 1998. Atlanta, GA: US Department of Health and Human Services, Public Health Service, CDC; 1994.

[3] CDC. Salmonella surveillance: annual tabulation summary, 1999. Atlanta, GA: US Department of Health and Human Services, Public Health Service, CDC; 1995.

[4] CDC. Salmonella surveillance: annual tabulation summary, 2000. Atlanta, GA: US Department of Health and Human Services, Public Health Service, CDC; 1996.

[5] CDC. Salmonella surveillance: annual tabulation summary, 2001. Atlanta, GA: US

Department of Health and Human Services, Public Health Service, CDC; 1997.

[6] CDC. Salmonella surveillance: annual tabulation summary, 2002. Atlanta, GA: US Department of Health and Human Services, Public Health Service, CDC; 1998.

[7] E. J. Threlfall, D. A. Murdoch, N. A. Banatvala, A. Bone, B. I. Shoismatulloev, L. R. Ward, Epidemic ciprofloxacin-resistant Salmonella typhi in Tajikistan. *The Lancet*, 1998, 351 (9099), 339.

[8] A. M. Musyoki, M. A. Suleiman, J. N. Mbithi, M. Maingi, Water-borne bacterial pathogens in surface waters of Nairobi river and health implication to communities downstream Athi river, *Life Science*, 2013, 3, 4-10.

[9] D. Lightfoot, Salmonella and other enteric organisms. In J. A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D. O. Cliver, G. F. Craun, R. Fayer, V. P. J., Gannon (Eds.), Water-borne zoonoses identification, causes, and control 2004, pp. 228–241, London: IWA Publishing, World Health Organization.

[10] K. Pond, Water recreation and disease infections: Plausibility of associated acute effects, sequelae and mortality. London: IWA Publishing, World Health Organization, 2005.

[11] M. F. Lynch, E. M. Blanton, S. Bulens, C. Polyak, J. Vojdani, J. Stevenson, E. D. Mintz, Typhoid fever in the United States, 1999–2006, *Journal of the American Medical Association*, 2009, 302(8), 852–869.

[12] J. O. Oluyege, A. C. Dada, A. T. Odeyemi, Incidence of multiple antibiotic resistant Gram-negative bacteria isolated from surface and underground water sources in south western region of Nigeria, *Water Science and Technology*, 2009, 59(10), 1929–1936.

[13] C. Levantesi, L. Bonadonna, R. Briancesco, E. Grohmann, S. Toze, V. Tandoi, Salmonella in surface and drinking water: occurrence and water-mediated transmission. *Food Research International*, 2012, 45(2), 587-602.

[14] R. Singh and M. Saxena, 16-23S rRNA Spacer Region Polymorphism in Gangetic River Water Isolates of Salmonella, *Journal of Water Resource and Protection,* 2010, 2, 756-761.

[15] A. Jyoti, P. Vajpayee, G. Singh, C. B. Patel, K. C. Gupta, R. Shanker, Identification of environmental reservoirs of nontyphoidal salmonellosis: aptamer-assisted bioconcentration and subsequent detection of Salmonella typhimurium by quantitative polymerase chain reaction, *Environmental science & technology,* 2011, 45, 8996-9002.

[16] J. Singh, S. Sharma, S. Nara, S. Devi, Harnessing Bacterial Indicators along with Physicochemical Parameters to Assess Pollution in the Ganges River, *Journal of Pure and Applied Microbiology*, 2013, 7, 1409-1415.

[17] M. K. Saxena, R. Singh, A. Yadav, S. Kiran, A. Saxena, B. D. Lakhchura, Isolation of Salmonella Typhimurium and Detection of Virulence Contributing Genes from Water of River Ganges, *Asian Journal of Water, Environment and Pollution,* 2013, 10, 141-147.

[18] P. K. Mandal, A. K. Biswas, K. Choi, U. K. Pal, Methods for rapid detection of foodborne pathogens: an overview, *American Journal Of Food Technology*, 2011, 6(2) 87-102.

[19] A. S. Waage, T. Vardund, V. Lund, G. Kapperud, Detection of low numbers of Salmonella in environmental water, sewage and food samples by a nested polymerase chain reaction assay, *Journal of applied microbiology*, 1999, 87(3), 418-428.

[20] K. L. M. Moganedi, E. M. A. Goyvaerts, S. N. Venter, M. M. Sibara, Optimisation of the PCR-invA primers for the detection of Salmonella in drinking and surface waters following a pre-cultivation step, *Water S,A* 33, 2007, 195-202.

[21] A. Kapley, K. Lampel, H. J. Purohit, Rapid detection of Salmonella in water samples by multiplex polymerase chain reaction, *Water environment research*, 2001, 73, 461-465.

[22] A. Wolter, R. Niessner, M. Seidel, Detection of Escherichia coli O157: H7, Salmonella typhimurium, and Legionella pneumophila in water using a flow-through chemiluminescence microarray readout system, *Analytical chemistry,* 2008, 80, 5854-5863.

[23] A. Fey, S. Eichler, S. Flavier, R.Christen, M. G.Höfle, C. A. Guzmán, Establishment of a real-time PCR-based approach for accurate quantification of bacterial RNA targets in water, using Salmonella as a model organism, *Applied and environmental microbiology,* 2004, 70, 3618-3623.

[24] S. A. Dunbar, C. A. Vander Zee, K. G. Oliver, K. L. Karem, J. W. Jacobson, Quantitative, multiplexed detection of bacterial pathogens: DNA and protein applications of the Luminex LabMAP™ system, *Journal of Microbiological Methods*, 2003, 53, 245-252.

[25] S. Xiulan, Z. Xiaolian, T. Jian, J. Zhou, F. S. Chu, Preparation of gold-labeled antibody probe and its use in immunochromatography assay for detection of aflatoxin B1, *International journal of food microbiology*, 2005, 99, 185-194.

[26] E. Torlak, I. M. Akan, M. Inal, Evaluation of RapidChek Select for the screening of Salmonella in meat and meat products, *Journal of microbiological methods,* 2012, 90, 217-219.

[27] C. C. Liu, C. Y. Yeung, P. H. Chen, M. K. Yeh, S. Y. Hou, Salmonella detection using 16S ribosomal DNA/RNA probe gold nanoparticles and lateral flow immunoassay, *Food Chemistry,* 2013, 141, 2526–2532.

[28] J. Singh, S. Sharma, S. Nara, Evaluation of gold nanoparticle based lateral flow assays for diagnosis of enterobacteriaceae members in food and water, *Food chemistry*, 2015, 170, 470-483.

[29] S. Nara, V. Tripathi, H. Singh, T. G. Shrivastav, Colloidal gold probe based rapid immunochromatographic strip assay for cortisol, *Analytica chimica acta*, 2010, 682(1), 66-71.

[30] C. Y. Yu, G. Y. Ang, A. L. Chua, E. H. Tan, S. Y. Lee, G. Falero-Diaz, M. Ravichandran, Dry-reagent gold nanoparticle-based lateral flow biosensor for the simultaneous detection of Vibrio cholerae serogroups O1 and O139, *Journal of microbiological methods*, 2011, 86(3), 277-282.

[31] N. Wangoo, K. K. Bhasin, S. K. Mehta, C. R. Suri, Synthesis and capping of water-dispersed gold nanoparticles by an amino acid: bioconjugation and binding studies, *Journal of colloid and interface science,* 2008, 323(2), 247-254.

[32] D. A. Grimes, K. F. Schulz, Uses and abuses of screening tests, The *Lancet*, 2002, 359(9309), 881-884.

[33] B. M. Hsu, K. H. Huang, S. W. Huang, K. C. Tseng, M. J. Su, W. C. Lin, D. D. Ji, F. C. Shih, J. L. Chen, P. M. Kao, Evaluation of different analysis and identification methods for Salmonella detection in surface drinking water sources, *Science of the Total Environment, 2011,* 409 4435-4441.