

Development of Lateral Flow Assay for the Detection of Tamm Horsfall Protein in Urine

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Abstract: Tamm Horsfall Protein (THP) is the most abundant urinary protein with concentrations of 20-200 mg/day and is known to be involved in a myriad of renal pathological processes such as renal stones and inflammation. THP is produced by renal epithelial cells and is the most abundant urinary protein, which tends to aggregate at higher concentrations. Therefore, our study was aimed to facilitate the detection of THP in urine by a rapid, efficient, and optimum detection using a lateral flow assay system. To develop the assay system, THP was isolated and purified from the urine of a healthy individual and used to generate antibodies in a white male New Zealand rabbit. These anti-THP antibodies were purified from the rabbit serum using the Protein-A column and further conjugated with gold nanoparticles. Gold nanoparticles (GNPs) of the size of 21 nm were synthesized by the chemical reduction method. Conjugation of gold nanoparticles and anti-THP antibodies was optimized by varying pH and concentrations. The developed lateral flow assay was tested with purified THP, and its sensitivity was calculated as 1 mg/ml of THP. This lateral flow immunoassay can detect THP in urine and can be beneficial for the detection of a various pathological or physiological phenomenon involving urinary Tamm Horsefall Protein secretions.

Keywords: Tamm Horsfall protein; lateral flow assay; gold nanoparticles; urinary protein; rapid detection.

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1. Introduction

In recent years, the emergence of Tamm Horsfall Protein (THP) as a prominent urinary protein has shown promising characteristics of being developed into a biomarker. THP is the most abundant urinary protein with concentrations of 20-200 mg/day and is known to be involved in the myriad of renal physiological processes such as renal stone suppression, inflammation, to name a few [1,2]. As per the literature, researchers have suggested that the level of THP tends to alter under the pathogenesis of AKI [3].

Many experiments focus on using THP as a biomarker for the diagnosis and prognosis of multiple diseases (diabetes, cardiovascular diseases). Serum level of THP in conditions of Tubular Atrophy (TA) and Interstitial Renal Fibrosis (IF) decreases even though eGFR remains normal in patients with glomerulopathies [4]. Another study by Wiromrat and co-workers showed lower levels of serum THP concentrations in teenagers with type 1 diabetes than non-diabetic juveniles [5].

However, there are studies where THP levels increase in certain disorders. Such as, a positive correlation was seen in the levels of THP with kidney volume and nephron mass in the population with Hypertension (SKIPOGH) [6]. Also, in recipients with a kidney transplant, an increase in THP concentration was observed depending upon the initial graft function in contrast to in healthy kidney donors, where serum levels were observed to be decreased after uninephrectomy [7,8].

In addition to discovering a potent biomarker, developing an efficient and optimum detection method becomes imperative. Considering the present day advancements, the feasibility lies in engrossing the benefits of lateral flow assay (LFA), which provides the benefit of being robust and point of care diagnostic [9]. LFA tests found their utility of paramount importance owing to their sensitivity, suitability for diagnosing various matters, and outcome in a shorter period. Additionally, the use of such devices has found its strong advantage in the geographical areas with poor resources, absence of clean laboratory facilities, and inaccessibility of medical care facilities for the patients. The working principle of LFA is based on the chromatographic technique where the test sample is dissolved in a solution, wades through various membranes, and interaction of test sample with presence or absence of analyte provides the result [10]. Additionally, gold nanoparticles (GNPs) are being used for colorimetric detection of the signal *via* conjugation with antibodies of interest [11,12].

However, no such kind of assay has been developed for easy and reliable detection of THP, with cost efficiency and fast outcome to diagnose renal physiology or pathology related to THP protein levels. Thereby, in the present research, we want to develop a rapid THP detection kit that could be used to diagnose diseases where THP level increases and in the prognosis of diseases where THP level decreases.

2. Materials and Methods

2.1. Experimental design.

Tamm Horsfall Protein was isolated from the urine of a single healthy individual. Further, IgG rabbit anti-human THP was raised by immunizing rabbit with isolated THP protein. On the other hand, Gold nanoparticles (GNPs) were synthesized with the chemical reduction method. The GNPs (gold colloids) were conjugated with the anti-human THP antibody previously raised in the rabbit. To identify the THP, a lateral flow assay (LFA) was developed. The development of the LFA consists of two parts: adsorption of gold conjugated anti-THP antibodies (purified in our laboratory) on the conjugate pad and coating of anti-human THP antibodies (Santa-Cruz) on the nitrocellulose membrane. The intensity of the bands was determined with the implementation of ImageJ software.

2.2. Chemicals required.

Anti-THP Antibody raised in rabbit and Goat anti-rabbit IgG-AP were procured from Santa Cruz Biotechnology (Pvt. Ltd.), Gold (III) Chloride hydrate ($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$) was acquired from Sigma Aldrich, Tri Sodium Citrate Dihydrate- Qualigens, 1-Step NBT/BCIP obtained from Thermo Scientific, Nitrocellulose membrane was procured from MDI. All the other chemicals used for the experiments were of analytical grade and had the highest purity.

2.3. *Animals.*

New Zealand White (NZW) male rabbits weighing 1.5–2 kg were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh, and the experiments were ethically approved by Panjab University Ethical Committee (PU/IAECS/S/15/108). The animals were kept in the Department of Biophysics, Chandigarh's animal house facility and cared for according to the guidelines of the University Ethics Committee.

2.4. *Isolation of human THP.*

The THP was isolated and purified by the process of Worcester et al. [13]. In the collected human urine of one individual, 0.58 M NaCl was added and allowed to precipitate overnight at 4°C. On the following day, the urine containing the salt was centrifuged at 10,410 g for 20 mins. After centrifugation, the supernatant was discarded, and the pellet was re-dissolved with triple distilled water (18 Ω resistivity). Again, the solution was centrifuged at 10,410 g for 5 mins. After discarding the pellet, 0.58 M NaCl was added to the supernatant and allowed to precipitate overnight at 4°C. The precipitation step was repeated twice more, except that 34,540 g was used. The final pellet was re-dissolved in triple distilled water. Desalting of isolated human THP was done by using centricone tube of pore size 10 kD.

2.5. *To determine the purity of isolated THP from urine using SDS-PAGE.*

50 µg of protein was loaded to 7.5 % of SDS-PAGE gel. The gel was run in the running buffer, and further, the bands were observed with the help of coomassie blue stain. Additionally, the presence of pure THP was confirmed using immunoblotting where transferred bands on nitrocellulose sheets were identified with the help of anti-THP raised in rabbit as the primary antibody and anti-THP rabbit labeled with AP as the secondary antibody. With the developing agent NBT/BCIP, the bands were visualized.

2.6. *Generation of antibody in the rabbit.*

Subcutaneous immunization was carried out with 1 mg/mL of conjugate mixture with an equal volume of complete Freund's adjuvant at four different rabbits sites. Further, for booster dose 1 mg/mL immunogen mixed with an equal volume of incomplete Freund's adjuvant was injected. These booster injections were given after the interval of 21 days, and the blood sample was collected from the ear vein of the rabbit after 7 days of a booster dose.

2.7. *Purification of antibody.*

2.7.1. *Serum collection.*

After three booster doses, 8 ml of blood was collected from the ear vein of the rabbit. The blood was kept overnight at 4°C. Serum was isolated from the blood sample by centrifugation at 3,000 rpm for 10min at 4°C, and isolated serum sample was stored at -20°C for further use.

2.7.2. *Affinity chromatography using protein A Column.*

IgG antibodies were purified from the serum sample by the procedure of Abcam [14]. Absorbance was measured at 280 nm, and the fractions with the highest absorbance were combined. The concentration of the antibodies was calculated with the help of $1 \text{ O.D.}_{280} = 0.73$

mg/ml IgG. However, the fractions/elutions were again characterized with Dot Blot to confirm the successful purification of antibodies.

2.8. Synthesis of gold nanoparticles.

The gold colloids are synthesized by the method by Frens [15] with some modifications. In a 50 ml flask, triple distilled water and 1 % gold chloride ($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$) were added with a magnetic bead and placed on a heating plate. The heating plate was allowed to reach the temperature of 100°C with continuous stirring. At the boiling point, 2.5 % sodium citrate was added immediately to the $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ solution. The solution was allowed to heat until the color changed from yellow to deep wine.

2.9. To standardize the pH at which antibody conjugates with GNPs.

The pH of the gold colloidal solution was varied with K_2CO_3 . In this solution of varied pH, $10 \mu\text{g}$ of the antibody (THP) was added with continuous stirring for 30 minutes at 4°C . After stirring the solution, absorbance (O.D.) was measured at 525 nm and 580 nm. Further, an equal volume of NaCl was added to the gold colloid solution, and again absorbance was measured at the same wavelength. The difference between the absorbance at wavelength 525 nm and 580 nm was calculated for analyzing the conjugation properties of GNPs with antibodies.

2.10. Development of lateral flow assay (LFA).

In LFA, two different antibodies were present with two distinct epitopes to bind the analyte molecule (THP). One of the antibodies (detection antibody) was labeled with a signal generator (GNPs) present on the test line, and the other antibody (capture antibody), which was immobilized onto the solid surface, was present on the control line.

The labeled antibodies were placed in a dehydrated state within the conjugate pad such that the binder can be instantaneously dissolved upon contact with an aqueous medium containing the analyte (THP); i.e., the substance to be measured. The antibody then participates in the binding reaction to form a complex with an analyte in the liquid phase. After binding, this complex moved forward continuously until it was eventually captured by the antibody immobilized on the surfaces of the nitrocellulose membrane. The membrane provides a uniform pore size, and immunocomplexes were formed. With this, the presence or absence of an analyte in a liquid sample can be visualized (Figure 1) [16,17].

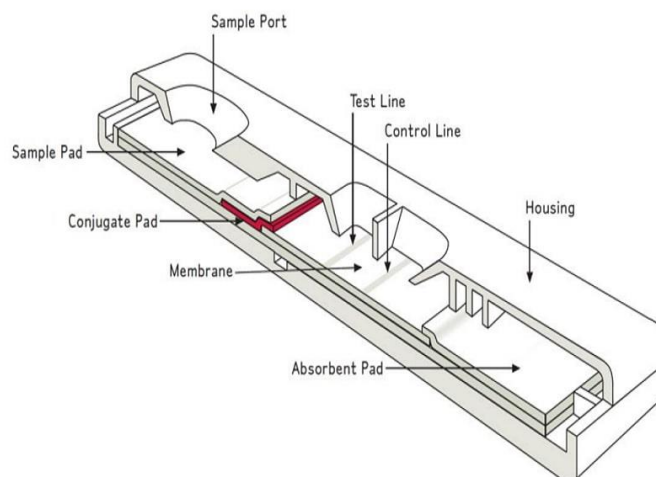


Figure 1. Setup of a commercially available LFA (Millipore, 2015).

2.10.1. Preparation of conjugate pad.

To adjust the pH range, 0.2 M K_2CO_3 was added to the colloidal gold solution while stirring. After 2 minutes, 10 μ g of THP antibody was added with continuous stirring for 5 mins. To stop the reaction, BSA was added after 2 minutes of stirring. The final solution was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and 0.1 ml of conjugate buffer was added to the pallet. Afterward, the sediment was collected and marked as THP-Gold conjugate. The conjugate pad was soaked in THP-Gold conjugate with 10 % conjugate buffer, and then it was kept for 72 hours at 37°C.

2.10.2. Preparation of test membrane.

On an MDI nitrocellulose membrane (NCM) of 15-micron pore size, 1.5 mm of control line was coated with 2.0 mg/ml of anti-rabbit IgG in 10 mM PB containing 2 % sucrose, and the 1.5 mm test line was coated with 1.5 mg/ml of anti-human THP raised in rabbit with the help of automatic reagent dispenser. Both the C-line and T-line were coated with 20 ml/hour of flow rate.

2.10.3. Assembly of the LFA strip.

On a backing card, NCM was attached and coated with T-line and C-line. This membrane was then dried for 2-3 hours. After that, the absorbent pad and conjugate pad were attached respectively to the NCM having a pore size of 15 μ m. The pads were attached so that they should not overlap with each other and were just in touch with the NCM. Afterward, a sample pad was attached. The final assembly of all the pads can be seen in Figure 2a. This set of membranes was then processed for cutting with dimensions 58 mm length and 4 mm breadth (Figure 2b). All the membranes were fixed in a plastic cassette to protect the test strip and easy sample application.

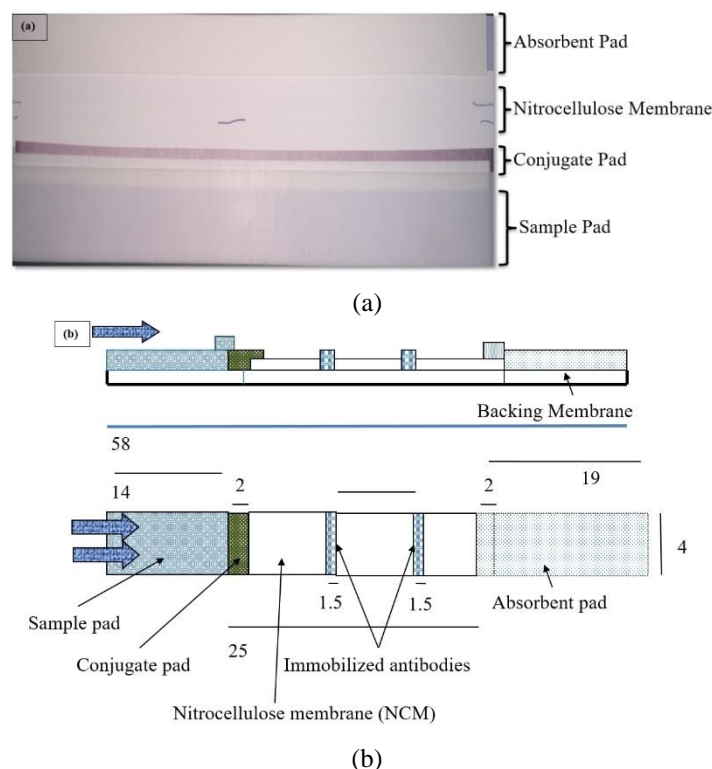


Figure 2. (a) Attachment of the different membranes to form a lateral flow assay; (b) Assembly of LFA strip (measurements in mm).

2.10.4. Testing of developed assembly of LFA.

50 μl of THP with a concentration of 6.15 $\mu\text{g}/\mu\text{l}$ dissolved in water was added directly onto the sample pad. To measure the intensity of bands, ImageJ software was implemented.

2.10.5. Shelf life and storage.

Store at 4°C for short term and -20°C for long term.

3. Results and Discussion

It has been documented that certain protein such as THP present in the urine plays a crucial role in many processes. It is produced by the epithelial cells in the kidney and secreted through proteolytic cleavage. Also, it has been implicated to alter under several diseases as suggested by reports describing its role in the progression of kidney injury [2]. The prevalence of renal diseases has been increasing at an alarming rate throughout the globe. The escalating trend in developing nations can be well attributed to the delay in detecting and diagnosing renal complications in due duration. Also, the lack of adequate medical facilities in the rural areas, especially the lack of testing kits, has contributed substantially towards the increasing epidemiological stats of renal diseases. By virtue of their nature, kidneys play a crucial role in regulating the body's functionality by maintaining blood pressure *via* regulation of salt and water balance, filtration, and excretion of toxic wastes through the urine. However, under certain derogatory conditions such as injury, the filtration process might get compromised, which poses a grave concern to normal renal functioning.

Untimely or delayed detecting renal injury can create an irresolvable condition; novel detection methods are critically needed. One such method available is to develop a lateral flow assay (LFA) [19]. Therefore, in the present study, LFA was developed, keeping THP as a novel biomarker that can be of utmost importance in providing point-of-diagnostics in the future to detect renal ailments.

Our study was designed to develop an immunoassay that can aid in the diagnosis of renal injury by taking a small sample quantity (urine) onto the sample pad of the dipstick. We proceeded to develop detection of THP protein using an in-house developed anti-THP antibody and another one commercially procured. To develop the assay, gold nanoparticles were used to provide colorimetric detection.

3.1. Isolation of human THP from urine.

The THP protein was purified from healthy human urine by the method of Worcester et al. [13]. To confirm the purity of THP by this method, the sample was run on Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis (SDS-PAGE) gel. The SDS_PAGE gel showed two bands, the first band was observed to be 150 kD, and the second band was ranged in between 100-75 kD. THP, a heavy protein of 90-100 kD, accumulates and aggregates, forming a heavier form. With the presence of beta-mercaptoethanol in the sample buffer of SDS-PAGE, a subunit of THP might have broken down, which might have shown a band of 100-75 kD (Figure 3a). On transferring these bands from SDS-PAGE gel for immunoblotting, the bands were confirmed to be THP protein (Figure 3b) [20].

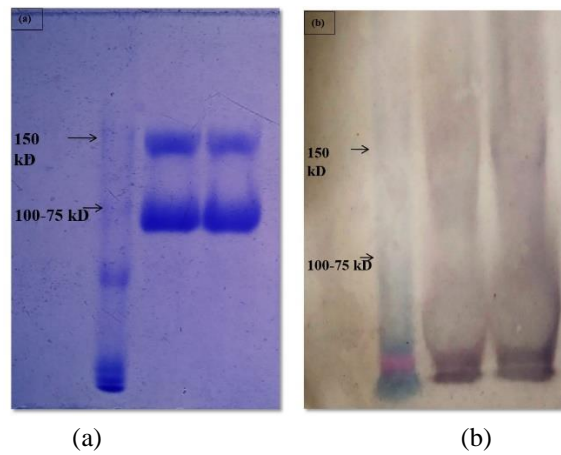


Figure 3. (a) SDS-PAGE gel for THP was run at 7.5 %, which results in two bands, one at 150 kD and the other between 100-75 kD; (b)Western Blotting of THP resulted in two bands of 150 kD and between 100-75 kD.

3.2. Purification of IgG antibodies with Protein-A column.

To generate the desired antibody, NZW male rabbits were procured and subcutaneously immunized with Freund’s adjuvant, followed by the booster doses to produce an anti-rabbit THP antibody. After that, the blood was collected from the ear veins of the rabbit, and serum was isolated. For IgG antibody purification, a serum sample was collected from THP immunized NZW rabbit, further processed using Protein-A column for extraction (Figure 4). The fractions were then assayed for protein (antibody) presence by measuring the absorbance at 280 nm. The fractions 1, 2, and 3 gave a positive absorbance of 0.105, 2.929, and 0.240, respectively. Other fractions gave negative absorbance; hence the antibodies were believed to be eluted in the first three fractions (Table 1).

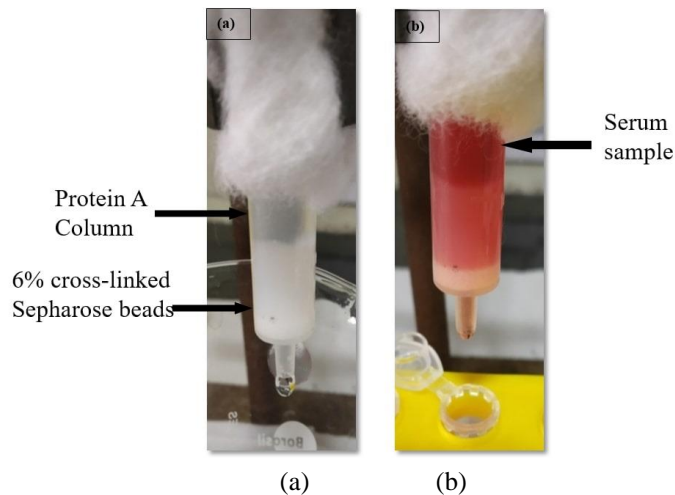


Figure 4. Protein A column: (a) Washing of the column with binding buffer; (b) Diluted serum sample with binding buffer (1:1 ratio).

Table 1. Absorption at 280 nm of eluted fractions containing IgG antibodies.

Elution	Protein	Unit	A280 (Abs)	260/280	Sample Type
1	0.105	mg/ml	0.105	0.64	1 Abs = 1 mg/mL
2	2.929	mg/ml	2.929	0.54	1 Abs = 1 mg/mL
3	0.240	mg/ml	0.240	0.58	1 Abs = 1 mg/mL
4	-0.019	mg/ml	-0.019	0.69	1 Abs = 1 mg/mL
5	-0.008	mg/ml	-0.008	-1.72	1 Abs = 1 mg/mL

3.3. Characterization of purified IgG.

The presence of purified IgG antibodies was confirmed with the dot blot method. Primary and secondary antibodies were used to bind to the particular target, resulting in color development at the site where the sample was present [21].

From the Protein A column, the eluted fractions were collected in which the first three elutions gave positive absorbance, and the rest of the fractions were negative. This was further verified with the dot blot using the antibodies procured from Santa Cruz Biotechnology (USA). The first blot was purified THP, the elution fraction no. 1 with absorbance 0.105 as shown in Figure 5 (pt.1), eluted fraction no. 2 of absorbance 2.929 shown in Figure 5 (pt.2), and fraction no. 3 with absorbance 0.240 depicted in Figure 5 (pt.3). However, the rest of the blots showed no color development, showing negative absorbance.

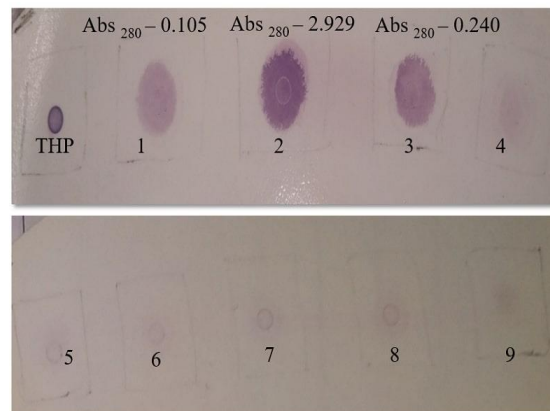


Figure 5. Dot Blot of the eluted solutions from Protein A column for purification of IgG antibody from a serum sample of rabbit.

3.4. Analysis and characterization of synthesized gold nanoparticles (GNPs).

For diagnostic purposes, GNPs are used as biomarkers and bio-delivery vehicles in medicine. In the current investigation, GNPs were synthesized by the chemical reduction method in which the Gold (III) Chloride hydrate was reduced with the addition of trisodium citrate. With the addition of citrate, the color changed from pale yellow to ruby red. The resultant size of GNP varies in the range of 20-40 nm in diameters. In the synthesis reaction, citrate acts as a stabilizing agent and reducing agent. The characterization of the colloidal gold depends on the size of the synthesized particle [12,22].

GNPs were synthesized by the chemical/reduction method using trisodium citrate. This procedure was developed by Turkevich [23] and advanced by G. Frens [15], whereby different concentrations of auric chloride and sodium citrate could be used to synthesize the desired size of GNPs. Similarly, in the current study, GNPs were synthesized via a chemical method with the Z-average size of the synthesized gold sample of 21 nm. The accurate conjugation of the gold nanoparticles with the antibody depends on the pH at which they bind together without any aggregation of gold colloids. GNP tends to aggregate and change the color from red to blue. In our study, pH was varied with K_2CO_3 from a range of pH 7-9 and an equal amount of sodium chloride which resulted in the aggregation of nanoparticles. The resulting alteration was observed with the optical density at 525 nm and 580 nm. At a pH of 8.5, the antibody-gold conjugation showed no aggregation of GNPs and was considered optimal for cytodagnostic purposes.

The characterization of GNPs was done with the help of Dynamic Light Scattering (DLS), Energy Dispersive X-ray spectroscopy (EDX), Transmission Electron Microscope (TEM), and Ultraviolet-Visible (UV-Vis) spectroscopy.

3.4.1. Dynamic light scattering (DLS).

DLS was performed to analyze the particle size and estimate the size distribution of the synthesized GNPs. Table 2 (a) and table 2 (b) show the size distribution of colloidal gold particles. DLS provides a Z-Average that gives the mean diameter of particles according to the intensity. The graph was plotted between the mean size and the percentage intensity as shown in Figure 6.

Z-Average (d.nm)	21.17
PdI	0.460
Intercept	0.740

(a)

	Size (d.nm)	% Intensity	St Dev (d.nm)
Peak 1	26.53	95.3	11.32
Peak 2	23.3	94.7	10.79
Peak 3	0.000	0.0	0.000

(b)

Table 2. (a) DLS average size of gold nanoparticles; (b) Size distribution of colloidal gold solution.

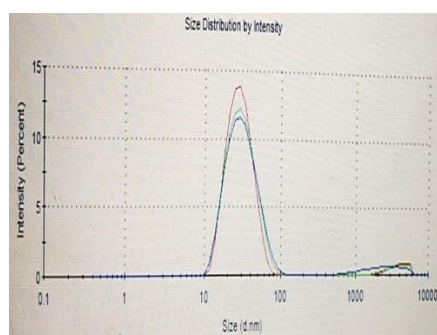


Figure 6. DLS of GNP with Z-average of 21.17 nm size.

It was found that 1 % of the gold sample had an average size of 21.17nm with peak 1 at 26.53 nm and intensity of 95 %, peak 2 showing 23.3 nm and intensity of 94 %. This indicated that most of the particles were observed to have an average diameter of 21 nm and were suitable for use.

3.4.2. Energy dispersive X- ray spectroscopy (EDX).

The current study's findings showed the elemental composition of GNPs by using EDX. The analysis provided the elemental composition of the desired sample as well as the proportion. EDX of gold colloids exhibited the peak intensity of elements that were present in GNPs w.r.t the energy (keV) of x-rays emitted by the solution. EDX confirmed the presence of Au, C, O, and Na, forming a part of the citrate layer adsorbed on the GNPs (Figure 7). Na⁺ ions played an important role in stabilizing the high capping of the gold surface by carboxylate anions. Similar results were observed with Au showing maximum and strong intensity with 2.2 keV, followed by oxygen (0.5 keV), sodium (1 keV), and carbon (0.2 keV). Hence, the energy of the components was found to be in a similar range as exhibited by [24].

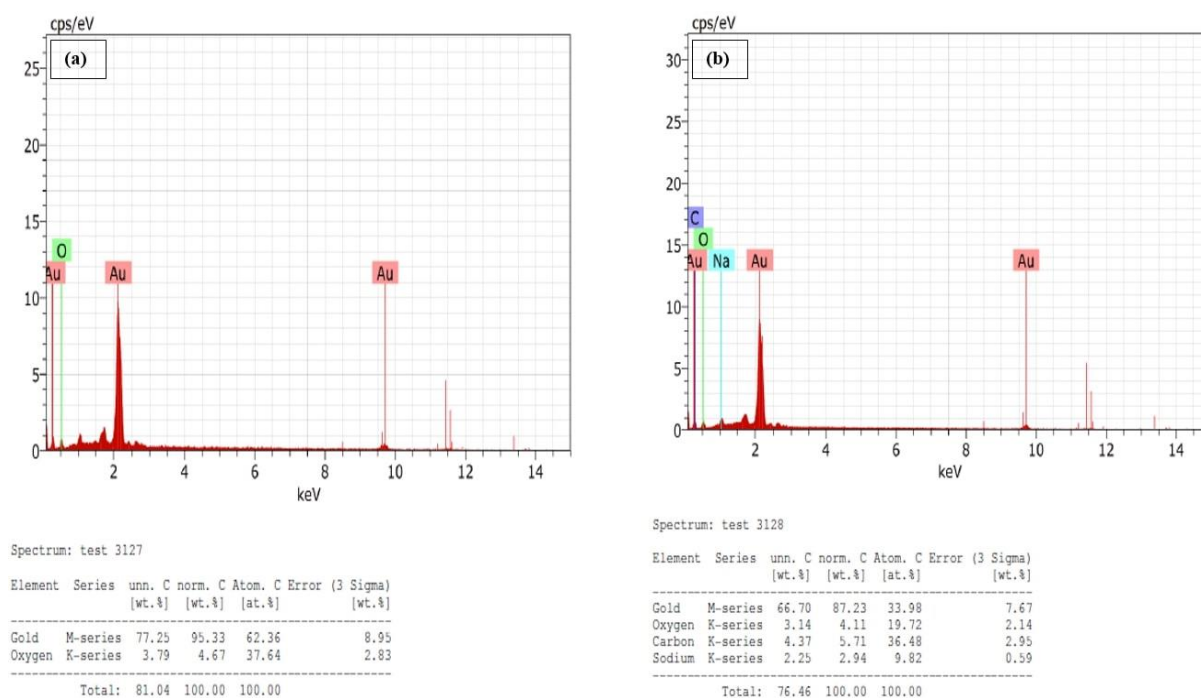


Figure 7. Energy-dispersive X-ray spectrometry (EDS) spectra of gold colloidal suspension.

3.4.3. Transmission electron microscope (TEM).

TEM images exhibited versatility in showing particles size of both lower and higher ranges. The colloidal gold solution was centrifuged (at 5000 rpm) for 15 mins to obtain size uniformity. Figure 8 shows the TEM images of GNPs and the size distribution of well-dispersed suspensions. The size of the gold colloids was determined by measuring the diameter of whole particles on TEM images. The average diameter of colloidal gold was found to be in the range of 14.3 nm- 21.1nm. The TEM images showed that the gold colloid was in a monodispersed state, probably due to a negatively charged layer of citrate ions, which repel each other. Moreover, the TEM images showed that most gold nanospheres were round or spherical.

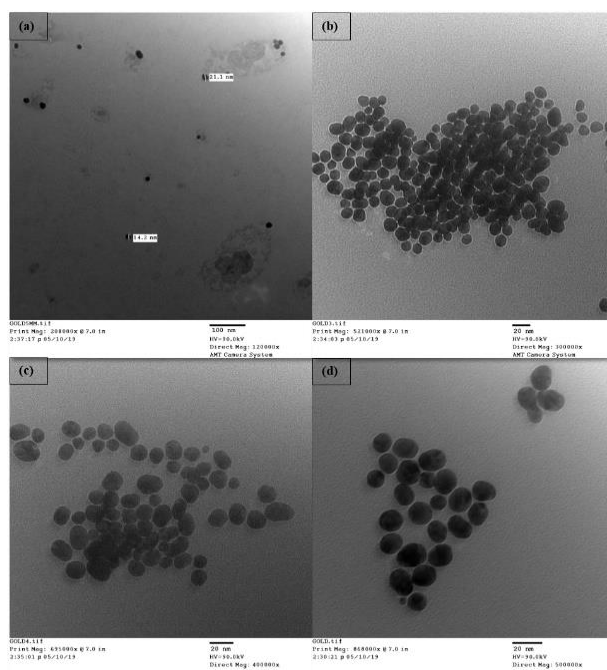


Figure 8. Transmission electron microscopy images of Gold Nanoparticles.

3.4.4. Ultraviolet-visible spectrum.

Spectrometry is an important aspect of the characterization of gold nanoparticles. With an increase in the particle size, the absorption peak shifted to a longer wavelength, and the width of absorption spectra was known to be correlated with the size distribution range. Generally, gold nanospheres display a single absorption peak in the visible range between 510-550 nm because of surface plasmon resonance. The maximum absorption was observed at 525 nm, which tends to impart a brilliant red color to GNPs and further varies according to their size. GNP absorption was measured in a single-beam spectrophotometer, and an absorption maximum was noted at 525 nm to be 1.621, as in Figure 9.

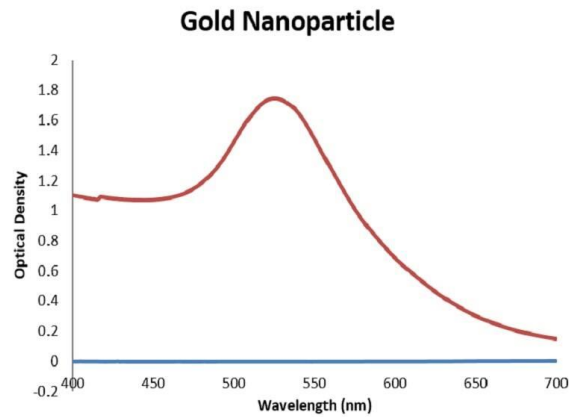


Figure 9. Absorption spectra of gold nanoparticles at λ max 525 nm.

3.5. Standardization of pH of conjugated GNPs.

For the conjugation of antibodies with the GNPs, standard pH was adjusted with K_2CO_3 . With the addition of NaCl, GNPs started to aggregate, thus changing the color of GNP from red to bluish purple. The pH was varied from 7.2 to 9 with K_2CO_3 . A further equal amount of 10 % NaCl was added to the wells to observe any changes in the solution. The difference in the optical density of the solution at two wavelengths (525 nm and 580 nm) was evaluated (Table 3).

Table 3. pH standardization of Gold Nanoparticles conjugated with THP antibody.

pH	O.D.	O.D.	O.D. 525-580	Added 10 % NaCl	O.D.	O.D.	O.D. 525-580
	525	580			525	580	
7.5	0.289	0.184	0.105		0.252	0.245	0.007
8	0.297	0.189	0.108		0.251	0.242	0.009
8.5	0.299	0.179	0.12		0.26	0.239	0.021
8.75	0.294	0.185	0.109		0.258	0.213	0.045
9	0.292	0.189	0.103		0.256	0.208	0.048

GNPs tended to aggregate, which could be distinguished by the color shift from ruby red to blue/purple, suggesting the accumulation of gold colloids. This effect was also dependent on the pH of the GNPs. With the increase in the pH, the O.D. tends to increase initially and then became constant, indicating that at pH 8 and 8.5 GNPs were not/ least aggregated, and at pH 8.7 and 9, maximum aggregation was observed (Figure 10a). The solution's minimal or no color change with pH 8.5 was observed (Figure 10b), which was further used in assay development found to be stable during the experimental purposes.

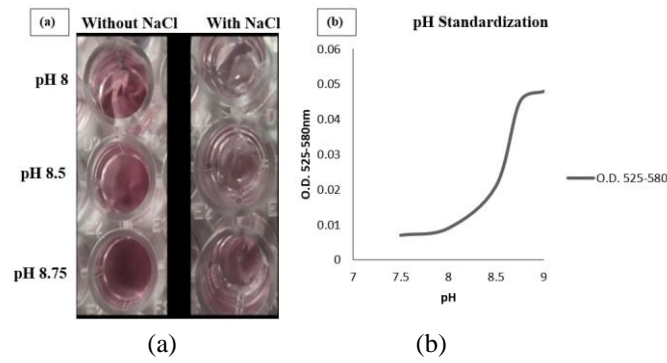


Figure 10. (a) After adding 10 % NaCl, a graph plotted between pH and the difference in the optical density of 525 nm and 580 nm (b) Colloidal gold nanoparticles conjugated with an antibody with varying pH from 7-9. On the left, solution without NaCl, and on the right, solution containing gold-antibody conjugate with NaCl.

3.6. Development of lateral flow assay (LFA).

LFA has become the most significant diagnostic device to detect diseases in the past few years. With crucial properties such as sensitivity, robust and swift outcome, effortless, cost-efficient, and *in-vitro* entails the need for such devices to be used in the remote areas experiencing limited resources, thereby providing a much needed point-of-care testing for the patients. LFA consists of a sample pad for the absorption process, a conjugate pad that stores the conjugated labels and antibodies. As the sample moves along the device, the nitrocellulose membrane will bind to protein at the test line. Further, after binding, a red-colored line will form. Finally, absorbing excess sample in absorbent pad [25, 26].

In the present study, the assay was developed with pH ranging from 7.5-9 and at two different protein concentrations, i.e., 1 mg/ml and 2 mg/ml, as seen in Figure 11a and Figure 11b. A control assay consisting of water as a sample was run in each pH range. This resulted in only the control line showing a negative result, i.e., no THP was detected in a water sample. Similarly, two test assays were developed and used to detect THP. THP as a sample was loaded onto the assembly for all the pH ranges. The findings exhibited a red line at the T-line, which verified the presence of THP (Figure 12). With the difference in pH, the intensity of the bands varies.

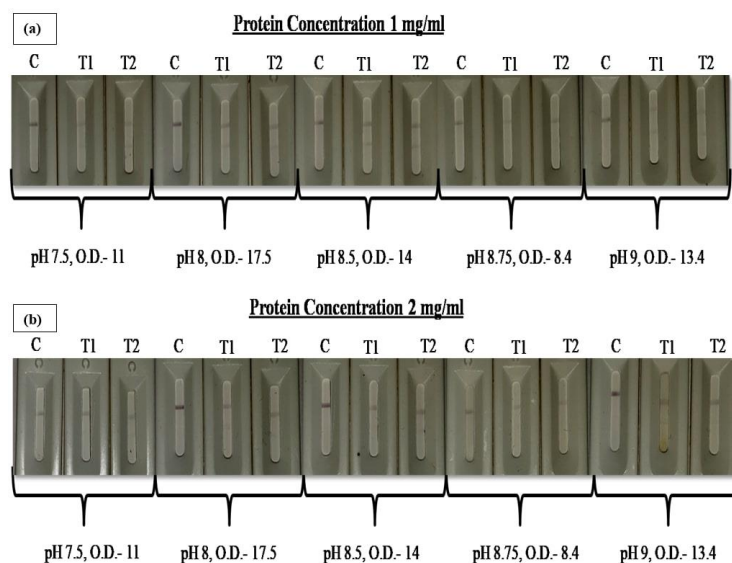
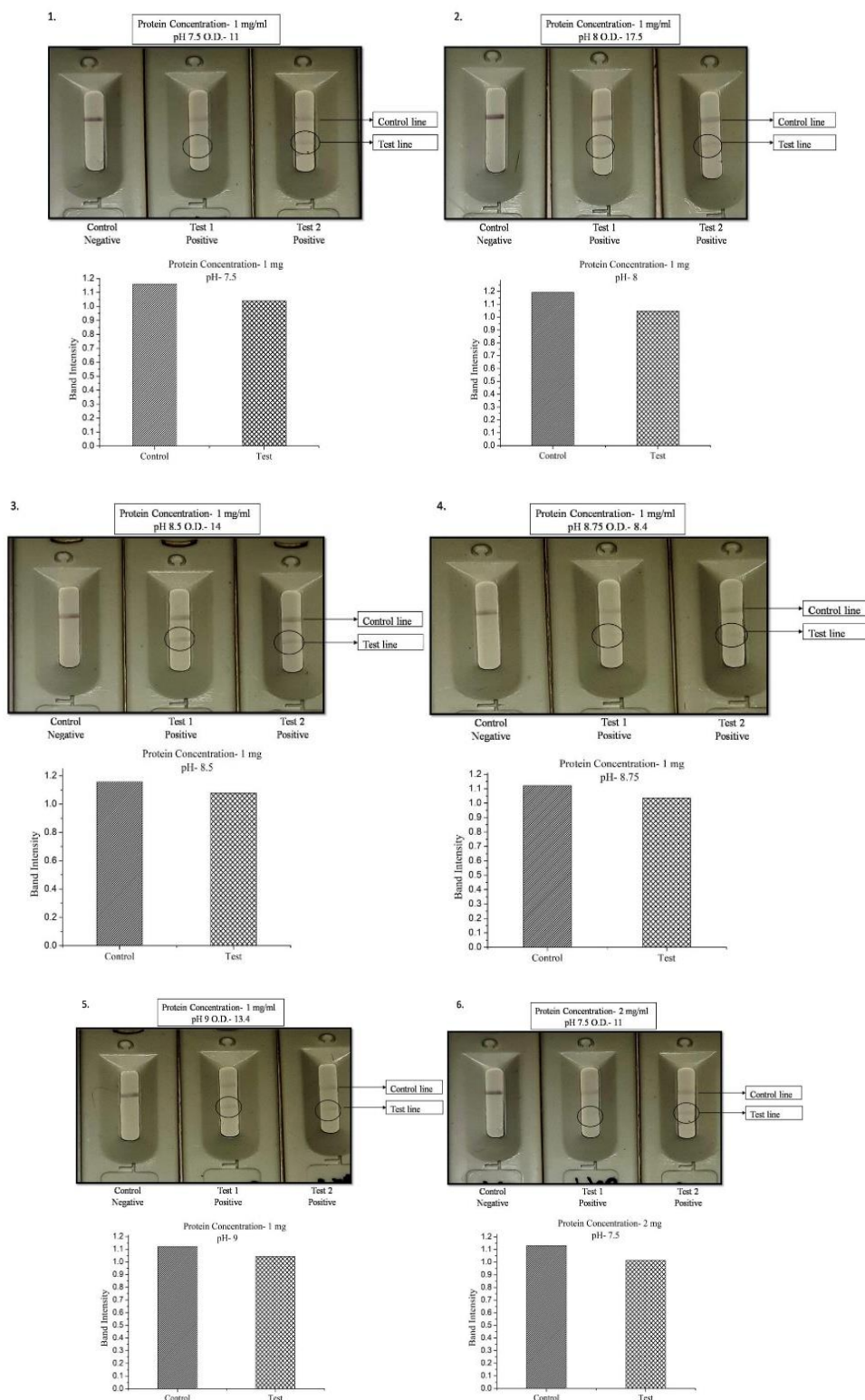


Figure 11. (a) Lateral Test assays of different pH having 1 mg/ml of protein concentration (b) Lateral Test assays of different pH having a protein concentration of 2 mg/ml. Each pH consists of three assays: a control with water as a sample and two test assays with THP as the sample. (C is Control. T1 is Test 1, and T2 is Test 2).

ImageJ software was used to measure the intensity of bands. The maximum intensity in all the test assays was observed in the control assay in which the sample tested was water which depicted a negative result. The intensity of the test sample was also observed in the assays with THP as the sample. The intensity of the control line was more as compared to the test line because the gold nanoparticles waded to the test line resulting in low intensity. The immunoassay test with a pH of 8.5 demonstrated the maximum band intensity with both the protein concentrations of 1 mg and 2 mg compared to the other test results, as shown in Fig. 12(3). This proves that the maximum binding of the GNPs and antibody is at pH 8.5, the standardized pH for the conjugation of gold colloids with the antibody.



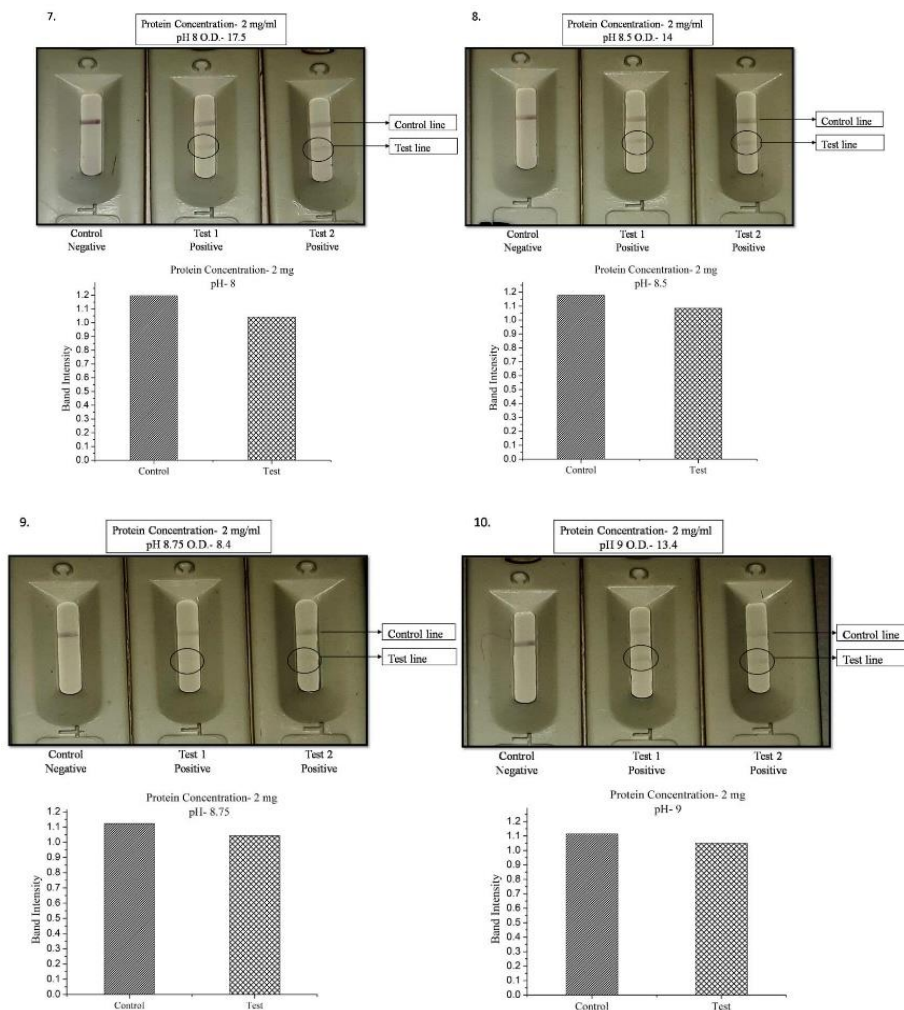


Figure 12. LFA test for detecting THP with protein concentrations of 1 mg and 2 mg and was developed. The first assay (Control assay) consisted of water as a sample that showed a negative result, i.e., no THP. To the other two assays (Test 1 assay and Test 2 assay), THP was loaded as a sample, verifying the presence of THP. The bar graph shows the band intensity of the control line and test line using ImageJ software. (1) pH 7.5 (2) pH 8 (3) pH 8.5 (4) pH 8.75 (5) pH 9 (6) pH 7.5 (7) pH 8 (8) pH 8.5 (9) pH 8.75 (10) pH 9.

4. Conclusions

A lateral flow immunoassay test was developed to detect Tamm Horsfall Protein. The said detection test can detect a minimum 1mg/ml THP in a sample. This test will have multiple uses in the diagnosis and prognosis of several ailments, and it can also help in studying renal physiology at the point of care.

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Conflicts of Interest

The authors declare no conflict of interest.

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