Electronic Supplementary Material (ESI) for Analytical Methods.
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Supplementary Information

A proof-of-concept of lateral flow based luteinizing hormone detection in urine for ovulation prediction in the buffaloes

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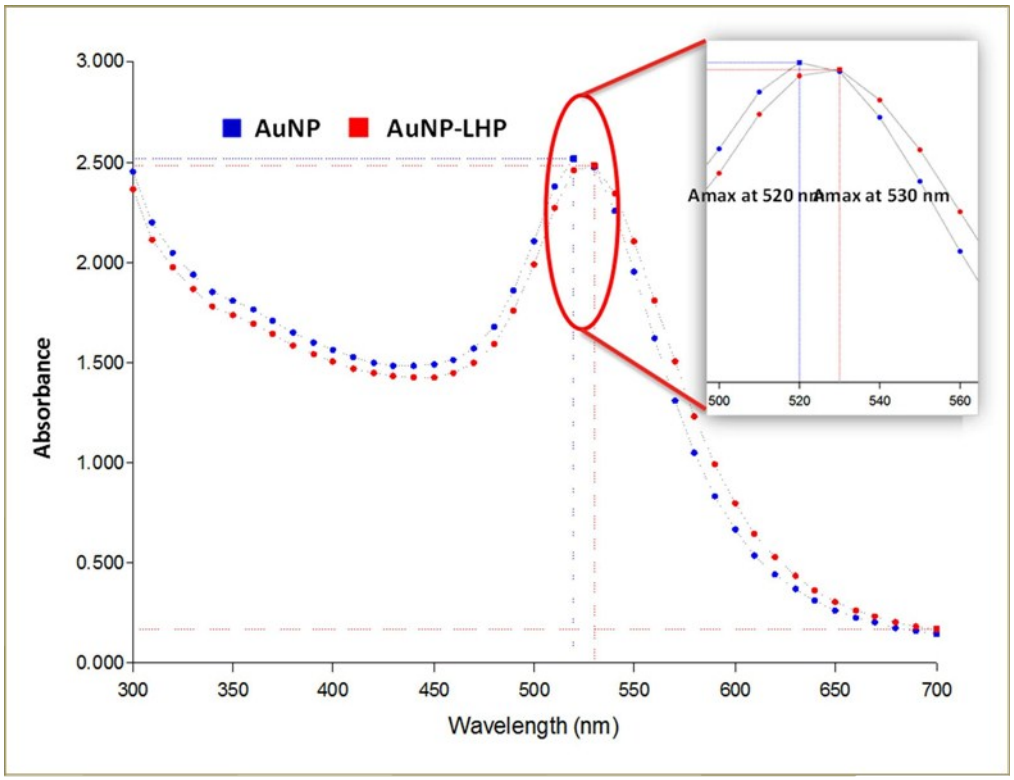
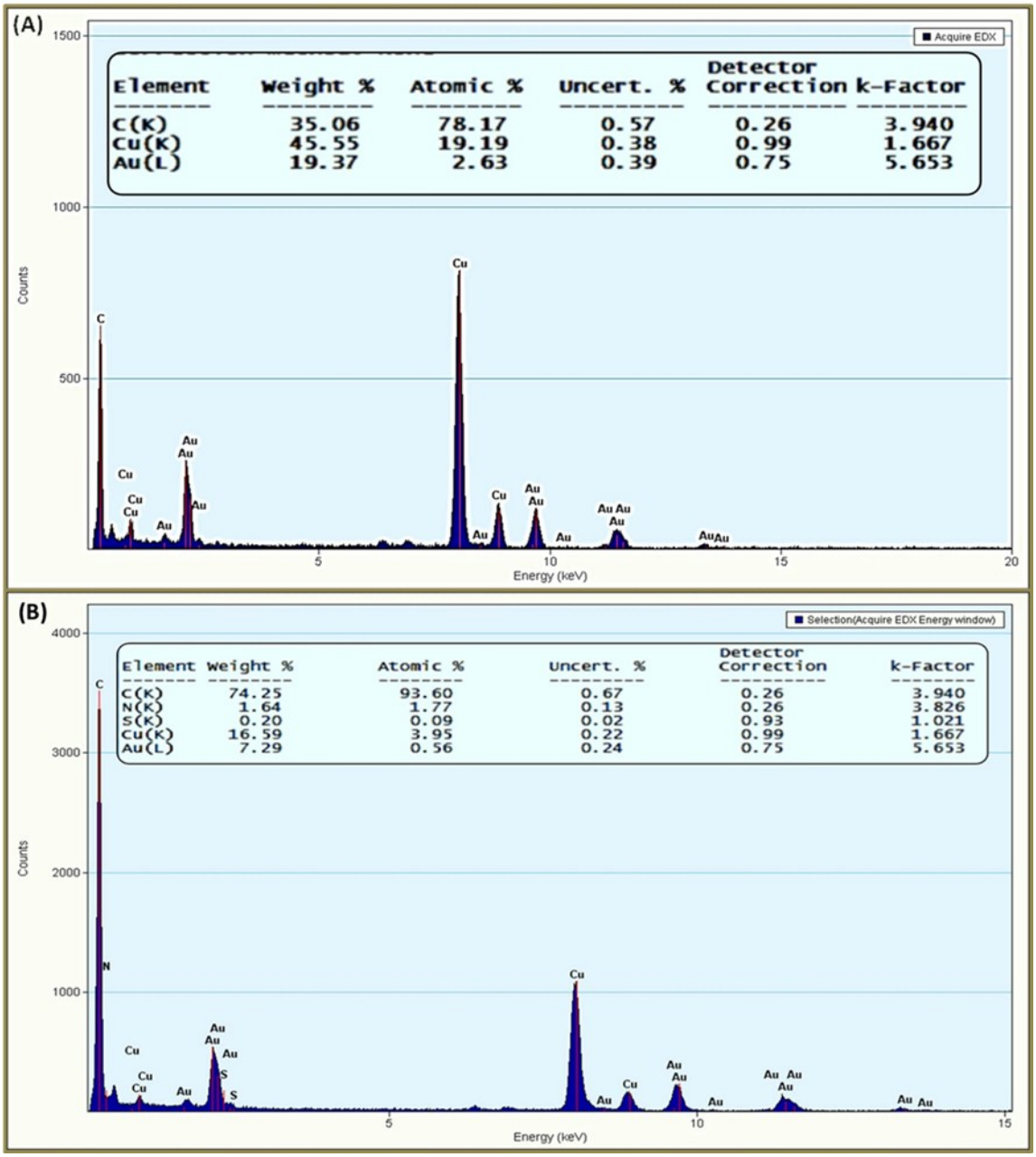
The Supplementary Information provides the detailed procedures and results on in silico identification and peptide and comparison between two approaches.

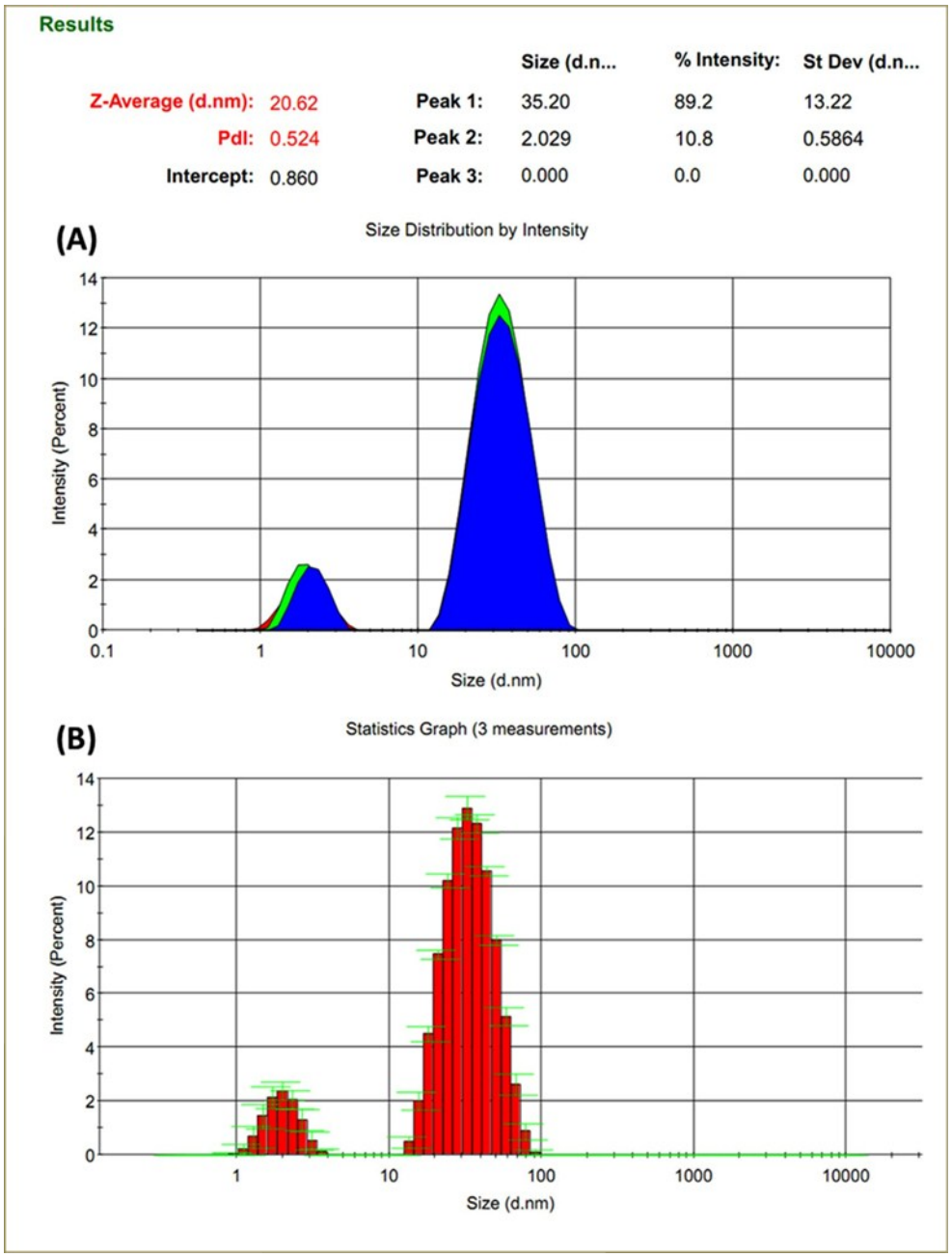
Supplementary Figures



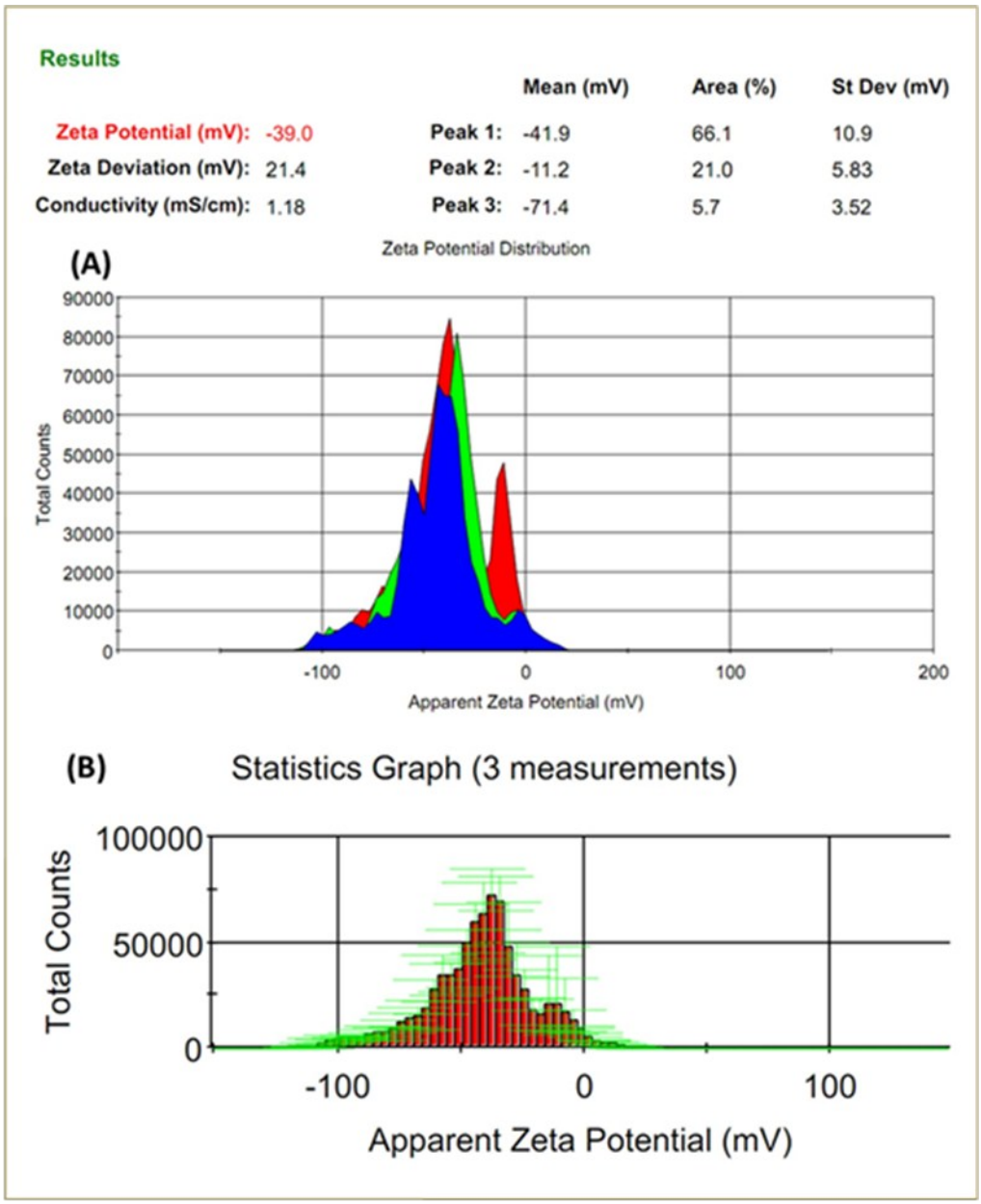
Supplementary Figure S1: **UV-Vis absorption spectrum for gold nanoparticles and gold nanoparticles-LHP conjugate (AuNP-LHP).** The figure represents the UV- Vis absorption spectrum of gold nanoparticles and gold nanoparticles-luteinizing hormone peptide. There is rightward shift in plasmon resonance band in AuNP-LHP conjugate compared to AuNP alone.



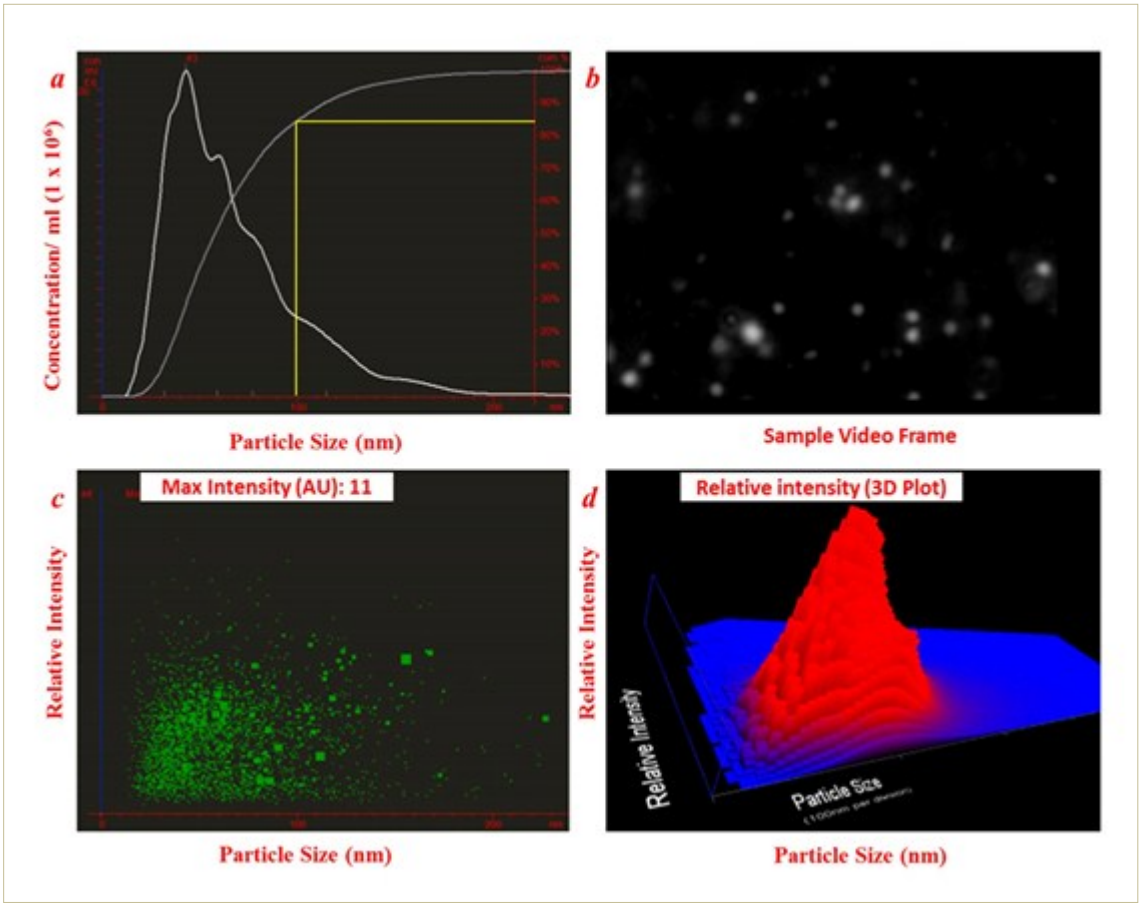
Supplementary Figure S2: **Energy dispersive X-ray spectroscopy (EDS/ EDX) Studies**: (A) elemental composition of AuNP by energy dispersive X-ray spectroscopy (EDS/ EDX), (B) elemental composition of AuNP-LHP by energy dispersive X-ray spectroscopy (EDS/ EDX).



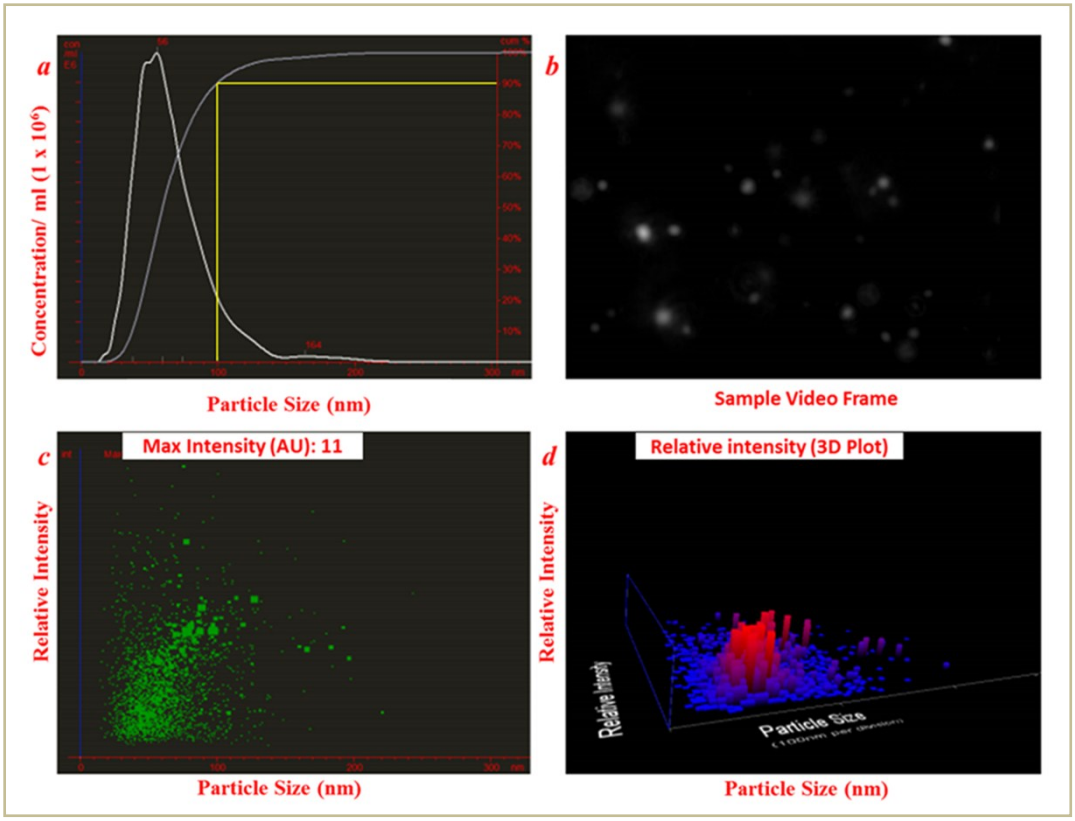
Supplementary Figure S3: **Dynamic light scattering (DLS) measurements for hydrodynamic diameter of colloidal gold nanoparticles.** (A) Represents the results from three readings, each coloured differently (B) Statistical graph of hydrodynamic diameter for three measurements.



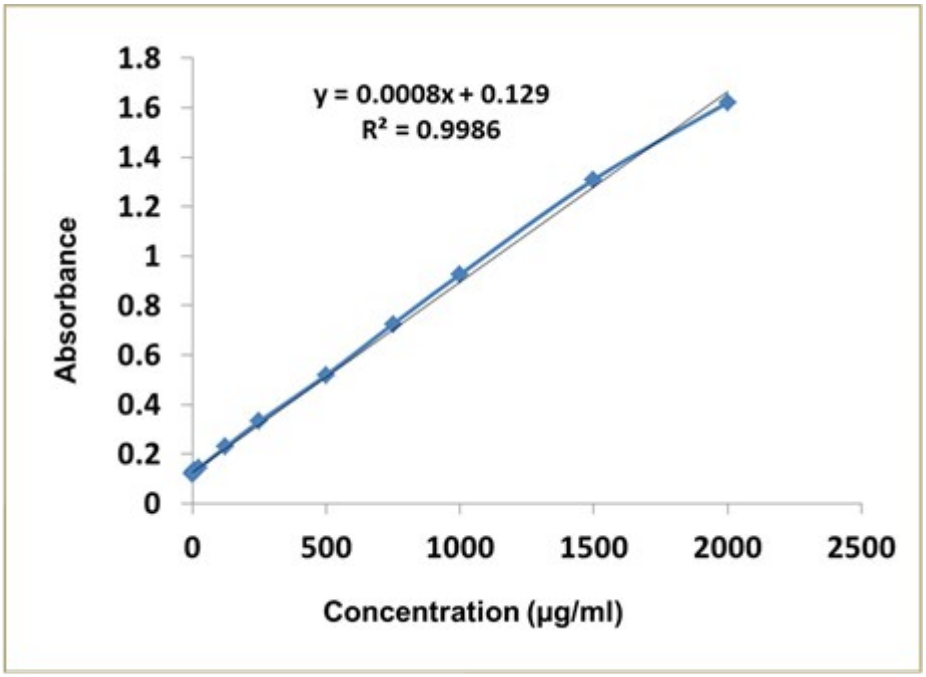
Supplementary Figure S4: **Dynamic light scattering (DLS) measurements for zeta potential of colloidal gold nanoparticles.** (A) Represents the results from three readings, each coloured differently (B) Statistical graph of hydrodynamic diameter for three measurements.



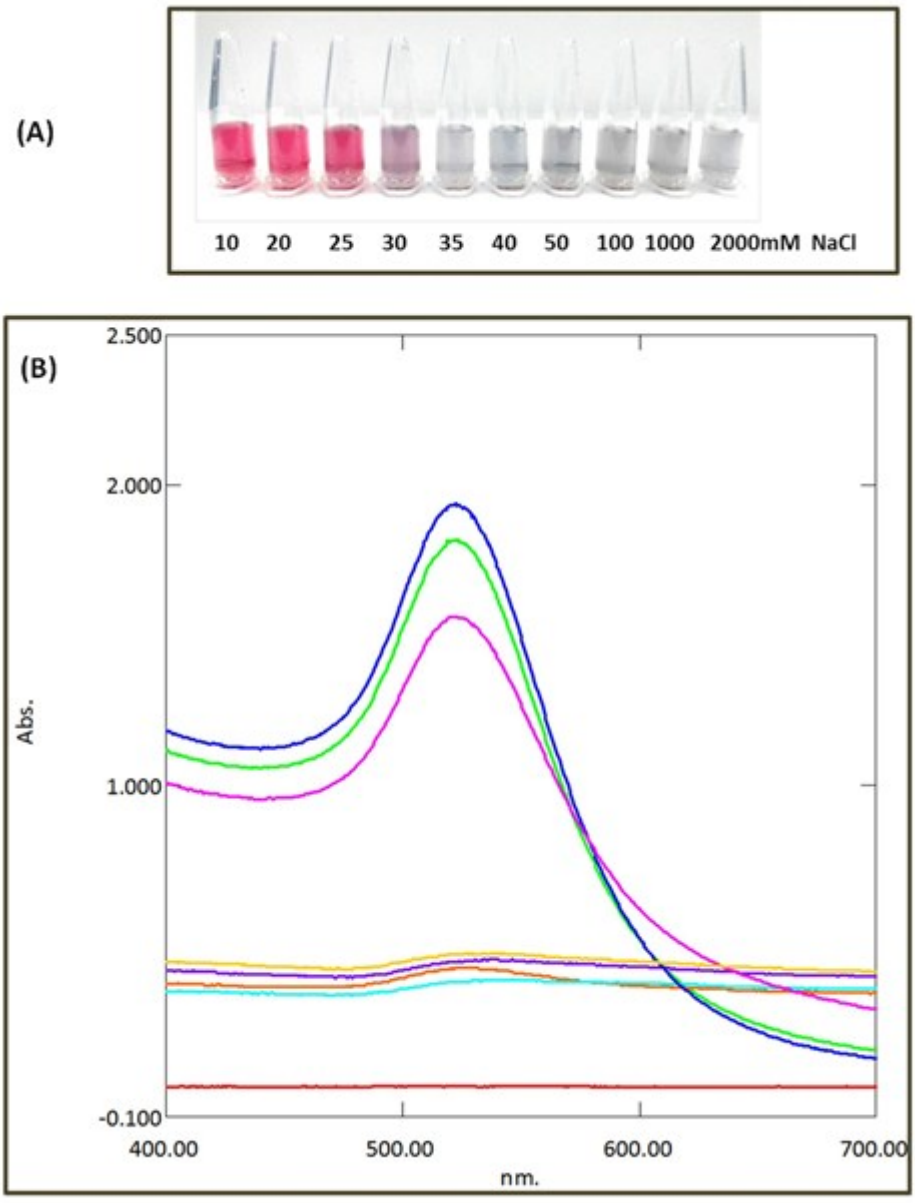
Supplementary Figure S5: **Summary of NTA measurements for total concentration and size distribution of the AuNP prepared by "Wet Chemical Method".**



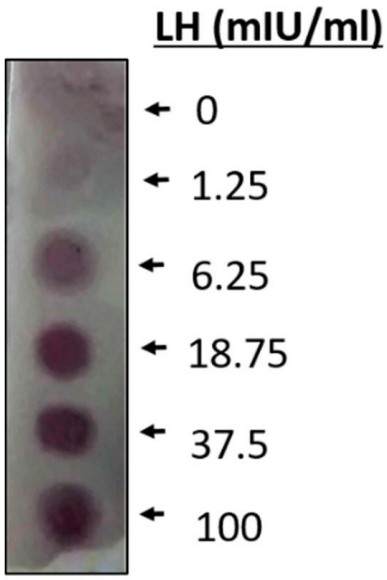
Supplementary Figure S6: **Summary of NTA measurements for total concentration and size distribution of the gold nanoparticles-luteinizing hormone peptide (AuNP-LHP) conjugate.**



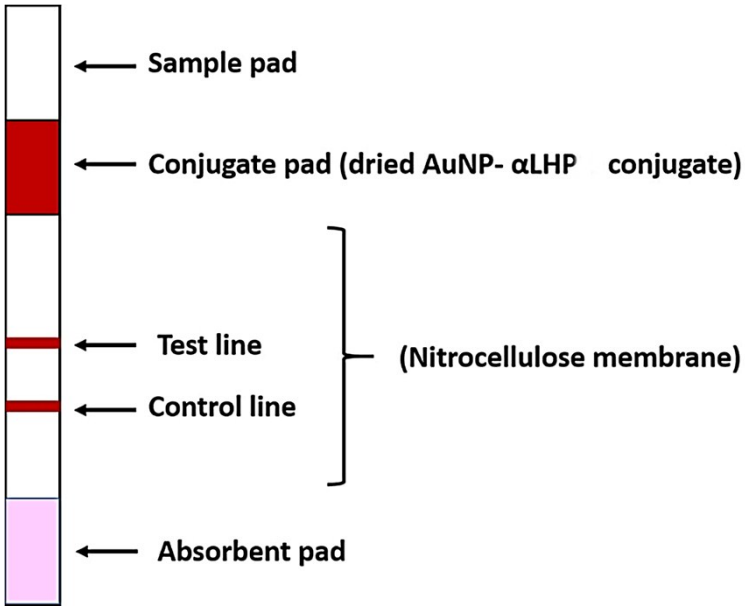
Supplementary Figure S7: **Quantification of LHP bound in the gold nanoparticles-luteinizing hormone peptide (AuNP-LHP) conjugate by BCA (bicinchoninic acid) assay or Smith assay.** The figure represents the standard curve using the peptide standards.



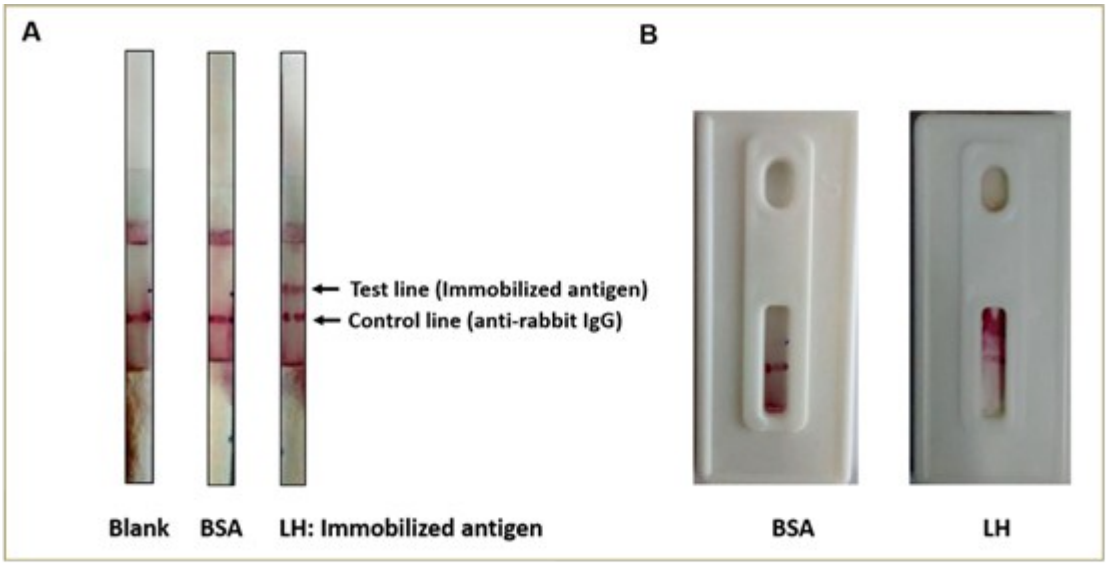
Supplementary Figure S8: In vitro **stability studies with gold nanoparticles (AuNP) for determining the critical coagulation concentration (CCC).**



Supplementary Figure S9: **Immunostrip assay of LH with AuNP-anti LHP (αLHP) conjugate.** LH was spotted on a nitrocellulose membrane at five different concentrations 1.25, 6.25, 18.75, 37.5 and 100 mIU/ml and was detected using AuNP-anti LHP conjugate. The color developed in nearly 3 minutes in all LH spots.



Supplementary Figure S10: **Schematic diagram of the lateral flow assay (LFA) test strip.**



Supplementary Figure S11: **Immunostrip studies to optimize the performance of lateral flow assay (LFA) test strip**. (A) Blank, BSA or LH was immobilized on test line of LFA strips. The assay was run upon applying borate buffer (pH 7.5) on sample pad of all strips. (B) LFA results when assay was done using strips caged in cassettes. The colour developed within 5-10 min.

Supplementary Tables

Supplementary Table S1: **Strengths and weaknesses of the AuNP-LHP strategy**

Sl. No.	AuNP-LHP Strategy	
	Strengths	**Weaknesses**
1.	Able to detect LH and have applications at point of care	Without any lateral flow of fluid through the membrane
2.	Traditional dipstick assay utilizing the direct immunoblotting technique	Not the direct format since visualization of colour is negatively correlated to concentration of LH in the samples
3.	Use the competitive format and the visualization of colour is negatively correlated to concentration of LH in the samples	Not easier to perform
4.	Does not require the necessity of LFA assembly	Less sensitive
5.	No skill and instrumentation required for making the LFA assembly	Require refrigeration temperature and shelf life not optimal at room temperature
6.	Can be used in resource-scarce laboratory settings	Preparation of larger batches difficult
7.	Does not require the washing step as in the ELISA	Problem of high background colour because washing is not done

Supplementary Table S2: **Strengths and weaknesses of the AuNP-anti LHP strategy**

Sl. No.	AuNP-anti LHP strategy	
	Strengths	**Weaknesses**
1.	Able to detect LH and have applications at point of care	Require the necessity of LFA assembly
2.	Lateral flow (XXXmmune) assay (LFA/LFIA) and utilizes the immunoblotting principle together with the lateral flow of fluid	More skill and instrumentation required for making the LFA assembly
3.	Use the competitive format and the visualization of colour is negatively correlated to concentration of LH in the samples	Not the direct format since visualization of colour is negatively correlated to concentration of LH in the samples
4.	Easier to perform, practical and flexible format more sensitive	
5.	Greater shelf life	
6.	Does not necessarily require refrigeration temperature	
7.	Large batches can be prepared	
8.	Does not require the washing step as in the ELISA and less problem of background colour	