Electronic Supplementary Material (ESI) for RSC Advances.


Supporting information for *"Magnetic Paper - Based ELISA for IgM-dengue detection"* by G.A. Ortega, S. Pérez and E. Reguera

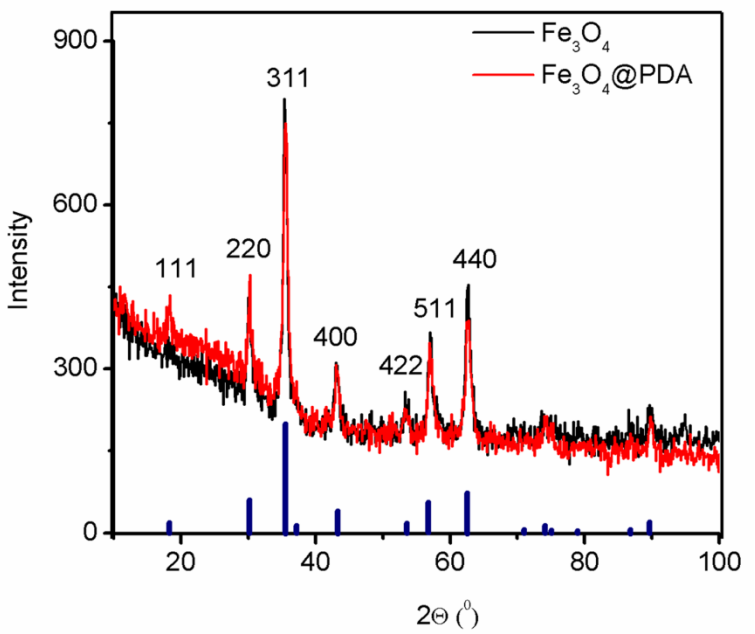


**Figure S1:** DRX patterns of $Fe_3O_4$ and $Fe_3O_4$@PDA nanoparticles.

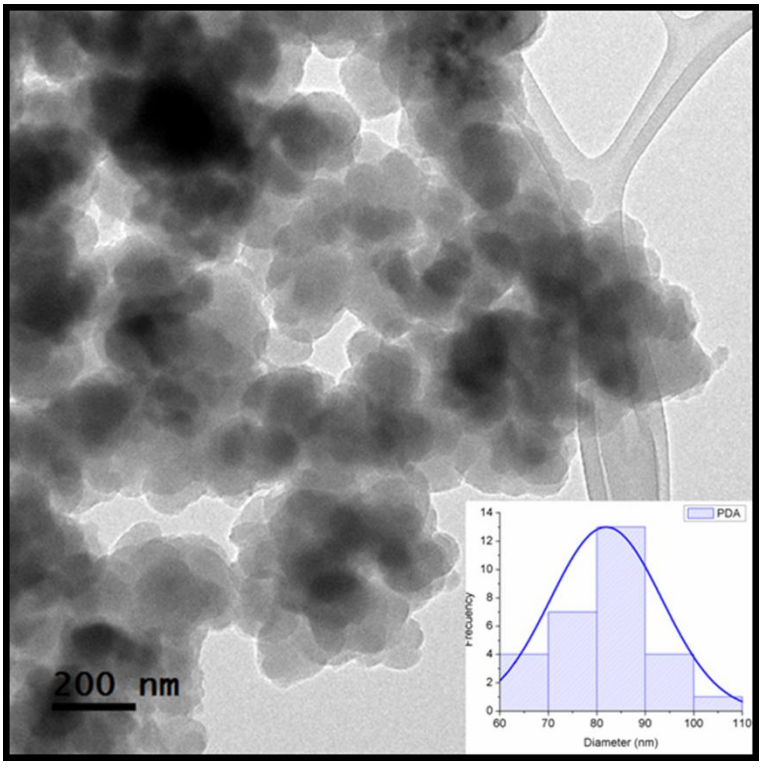


**Figure S2**: TEM micrographs of PDA nanoparticles with size distribution at the bottom.

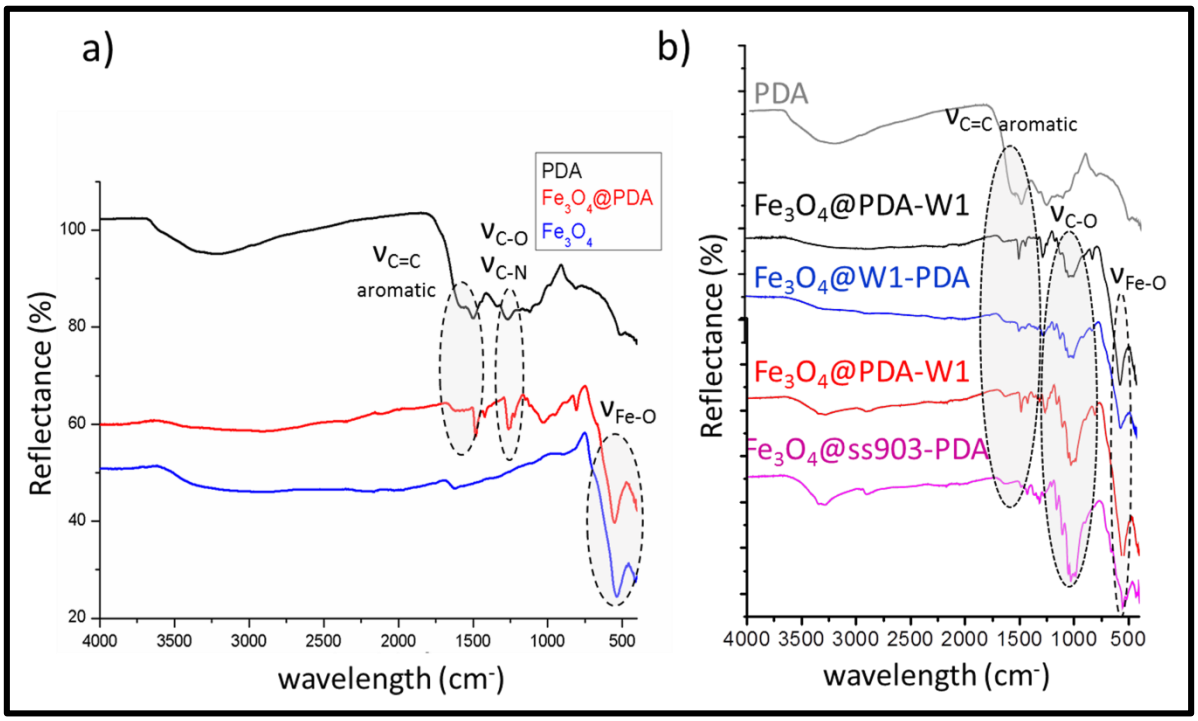


**Figure S3:** ATR-FT-IR spectrum of a) $Fe_3O_4$ and $Fe_3O_4$@PDA and b) the different capped paper: $Fe_3O_4$@W1-PDA, $Fe_3O_4$@ss903-PDA, $Fe_3O_4$@PDA-W1 and $Fe_3O_4$@PDA-ss903.

**Table S1:** TGA data of polydopamine, core-shell $Fe_3O_4$@PDA, pristine W1 and ss903 and coated papers.

| | **Residue (%)** | **Thermal degradation ($^0$C)** | **Mass of $Fe_3O_4$ (mg) in 5 mm of coated paper** |
|---|---|---|---|
| PDA | 16.3 | 373 | - |
| $Fe_3O_4$@PDA | 85.5 | 269 | - |
| W1 | 0 | 347 | - |
| ss903 | 0 | 353 | - |
| $Fe_3O_4$@W1-PDA | 16,5 | 336 | 0,13 |
| $Fe_3O_4$@PDA-W1 | 29,8 | 338 | 0,25 |
| $Fe_3O_4$@ss903-PDA | 21,8 | 320 | 0,35 |
| $Fe_3O_4$@PDA-ss903 | 10,7 | 337 | 0,17 |

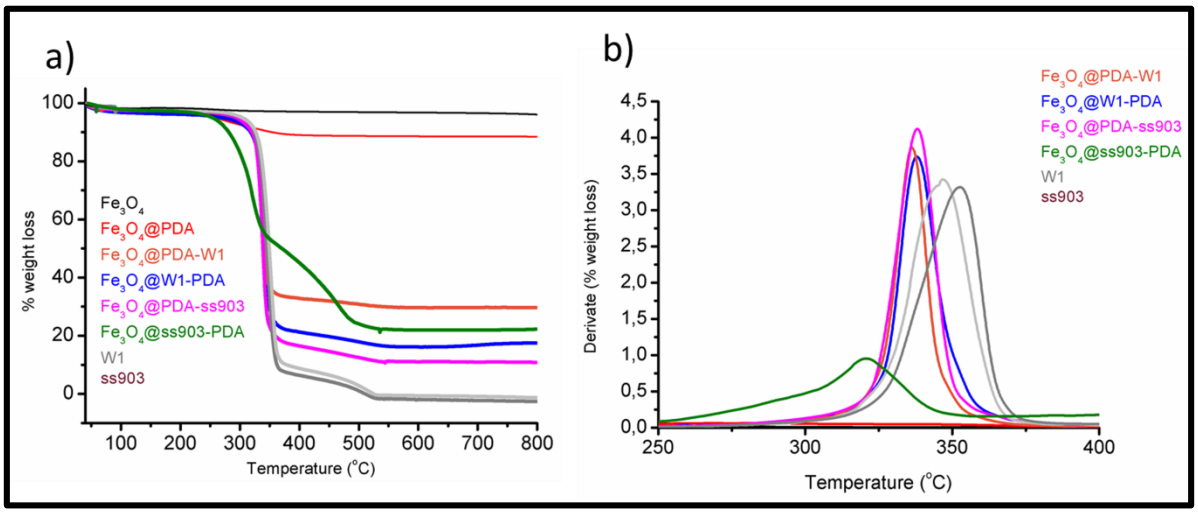


**Figure S4:** a) TGA analysis of different paper samples depicting variation in weight losses that were used to calculate the amount of deposited magnetite on each sample. b) Derivative weight loss curve.

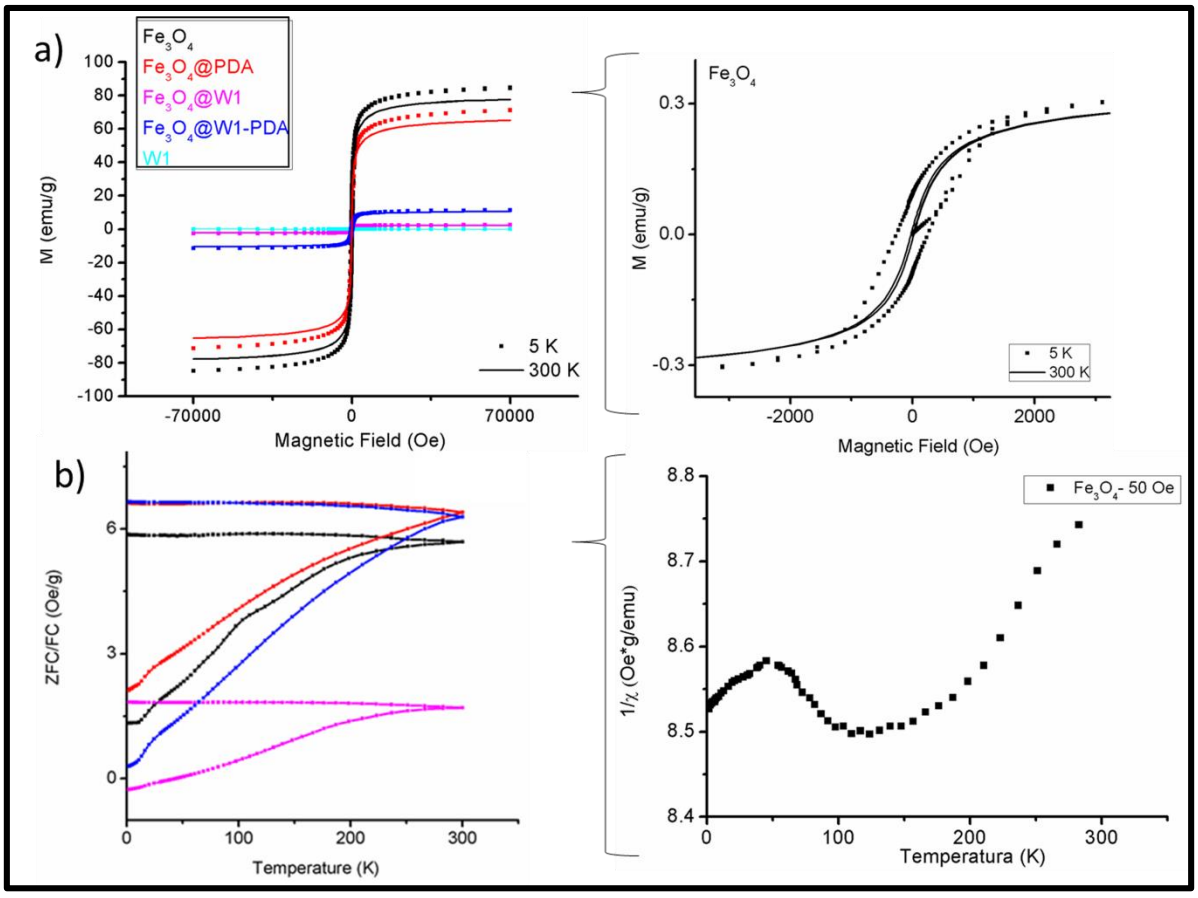


**Figure S5:** a) Mass magnetization versus applied field at 5 K and 300 K for $Fe_3O_4$ (zooming on the right side), $Fe_3O_4$@PDA, $Fe_3O_4$@W1 and $Fe_3O_4$@W1-PDA. b) ZFC/FC curves for the above samples and (on the right side), inverse susceptibility versus temperature curve for $Fe_3O_4$.

**S6: SDS-PAGE technique and Bradford assay for the study of amount of conjugated antibodies.**

According to SDS-PAGE result, for core-shell $Fe_3O_4$@PDA-AntiIgM, the absence of stains indicates that AntiIgM coupled on magnetite surface endured reductive treatment. In addition, the electrophoretic mobility of the AntiIgM antibodies linked $Fe_3O_4$@PDA is different to the pristine ones and they are accumulated in the well due to their greater size. Therefore, reductive SDS-PAGE technique is unable to quantify antibodies covalent coupled on magnetite-polydopamine surface. In that sense, the presence of AntiIgM fragments in the acrylamide gel lanes corresponding to different magnetite-paper systems is due to antibody absorption through physical associations into cellulose channels. In such cases, the antibodies are allowed to be reductive under the above-mentioned treatment. In the case of $Fe_3O_4$@PDA-AntiIgM-IgM, the heavy chain of IgM-dengue is not observed because it is tightly held to $Fe_3O_4$@PDA-AntiIgM through antigen-antibody interaction. The quantification for the light and J chain was not possible because the corresponding intensity is not within the calibration curve. Additionally, in the case of IgM-dengue electrophoresis gel, the stains for heavy and light chain corresponding to magnetite-paper systems have the contribution of the fragments of AntiIgM and IgM-dengue whose weights (position of the stains) match. Besides, J-chains stains are more intense than calibration curve intensity range, impeding the quantification. For these reasons, was impossible quantify the amount of IgM-dengue physically or covalent attachment on magnetite-paper by using SDS-PAGE.

In this regard, another method to determine the amount of antibodies coupled on magnetite nanoparticles was necessary employed. In this sense, samples of supernatants after every immobilization process were withdrawn and measured using Bradford assay. Therefore, the decrease in the supernatant concentration can be directly correlated to the total amount of the immobilized antibody considering covalent coupling, by physical association and antigen-antibody interactions.

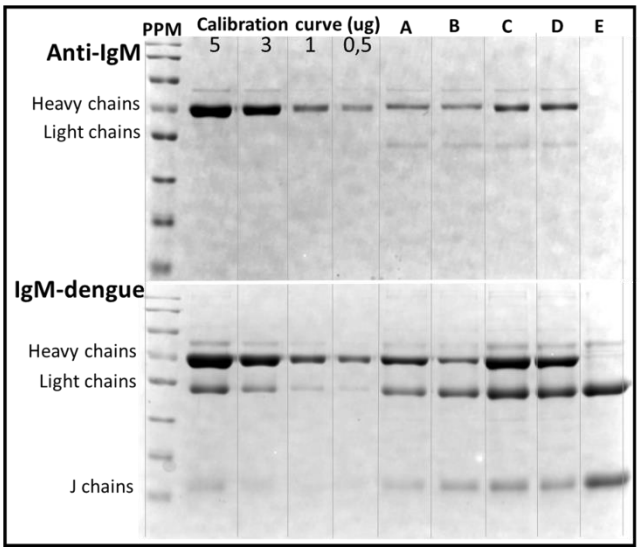


**Figure S6:** SDS-PAGE of AntiIgM (at the top) and IgM-dengue (at the bottom) for antibodies-conjugated systems: A. $Fe_3O_4$@W1-PDA, B. $Fe_3O_4$@PDA-W1, C. $Fe_3O_4$@ss903-PDA, D. $Fe_3O_4$@PDA-ss903 and E. $Fe_3O_4$@PDA.

**Table S2:** Amount of AntiIgM and IgM-dengue antibodies conjugated on core-shell $Fe_3O_4$@PDA PDA in suspension (NPs) and magnetite-paper surfaces (5 mm diameter).

| | Samples (5 mm of paper) | Ab (ug) | | | | Covalent coupling yield (%) | Total coupling yield (%) | Total coupling yield (%) per mg of magnetite |
|---|---|---|---|---|---|---|---|---|
| | | Method | | Covalent coupled | Covalent (AntiIgM ) or total (IgM) coupled per mg of magnetite | | | |
| | | SDS-PAGE | Bradford | | | | | |
| **AntiIgM** | $Fe_3O_4$@W1-PDA | 0.12 | 2.3 | 2.18 | 17.0 | 94.5 | 21.4 | 100 |
| | $Fe_3O_4$@PDA-W1 | 0.06 | 2.6 | 2.54 | 10.2 | 97.4 | 24.3 | 100 |
| | $Fe_3O_4$@ss903-PDA | 0.37 | 4.2 | 3.83 | 10.4 | 91.1 | 39.2 | 100 |
| | $Fe_3O_4$@PDA-ss903 | 0.34 | 4.1 | 3.76 | 22.1 | 91.5 | 38.3 | 100 |
| | $Fe_3O_4$@PDA (Nps) | 0 | 10.8 | 10.8 | 10.8 | 100 | 100 | 100 |
| **IgM** | $Fe_3O_4$@W1-PDA | - | 5.4 | - | 41.2 | - | 6.0 | 45.4 |
| | $Fe_3O_4$@PDA-W1 | - | 4.3 | - | 17.3 | - | 4.7 | 18.7 |
| | $Fe_3O_4$@ss903-PDA | - | 12.0 | - | 32.3 | - | 13.3 | 35.6 |
| | $Fe_3O_4$@PDA-ss903 | - | 13.3 | - | 78.1 | - | 14.6 | 86.1 |
| | $Fe_3O_4$@PDA (Nps) | - | 17.3 | - | 17.3 | - | 19 | 19 |

**S7: Preparation of AgNP-Based SERS nanoparticles.**

Silver nanoparticles (AgNP) are obtained through a typical reaction similar to the well-known Turkevich method. In these sense, 0.5 mL of $AgNO_3$ (0.1 mM) is added in 100 mL of water at refluxing condition. Sodium citrate solution 2 mL (w/w 5%) is added dropwise to the silver nitrate solution as soon as the boiling commenced. The color of the solution slowly turned yellow. Heating is continued for an additional 15 min, and then the solution was cooled to room temperature before employing for further experimentation.

Then, 1 mL (25 mM dissolved in 0.1 mM of sodium citrate solution) of 5,5′-dithio-bis(2-nitro-benzoic acid) (DTNB) is added dropwise to 25 mL of AgNP previously synthesized under continuous stirring, at room temperature overnight. After, AgNP-DTNB was washed via centrifugation at 12000 rpm for 15 min followed by resuspension of the pellet in 1 mL of ultrapure water.

For AntiIgM antibodies coupling the silver nanoparticles are previous activated with 50 mM of N-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDC) and 10 mM of N-hydroxysuccinimide (NHS) for 1 hour at room temperature. Then, the dispersion is separated by centrifugation (10 000 rpm, 15 minutes) and the sediment is re-dispersed in 300 uL of AntiIgM (1 mg/mL in PBS-Tween 1X, pH=7.4) under vigorous stirring for 3 hours at room temperature. After washing by centrifugation the AgNP-DTNB-AntiIgM is blocked by using dry milk 5% in PBS pH=7,4 for 2 hours and storage at $4^oC$ until use.

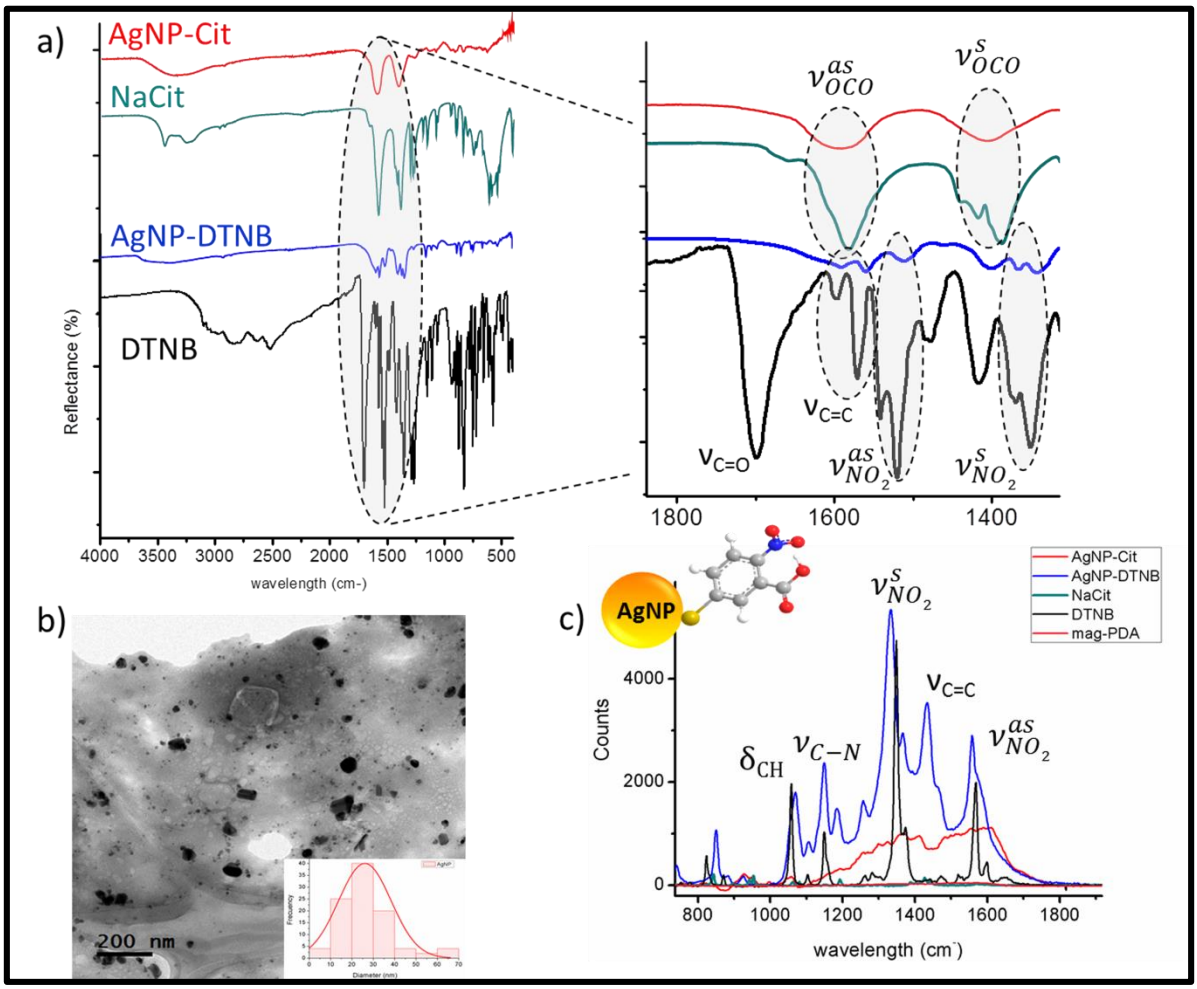


**Figure S7:** a) ATR-FT-IR spectrum of AgNP-Cit and AgNP-DTNB. b) TEM micrographs of AgNP-DTNB with size distribution at the bottom. c) Raman spectrum for AgNP-DTNB: 1070 ($\delta_{CH}$), 1148 ($\upsilon_{C-N}$), 1333 ($\upsilon^{s}_{NO_2}$), 1434 ($\upsilon_{C=C}$) and 1559 ($\upsilon^{as}_{NO_2}$).

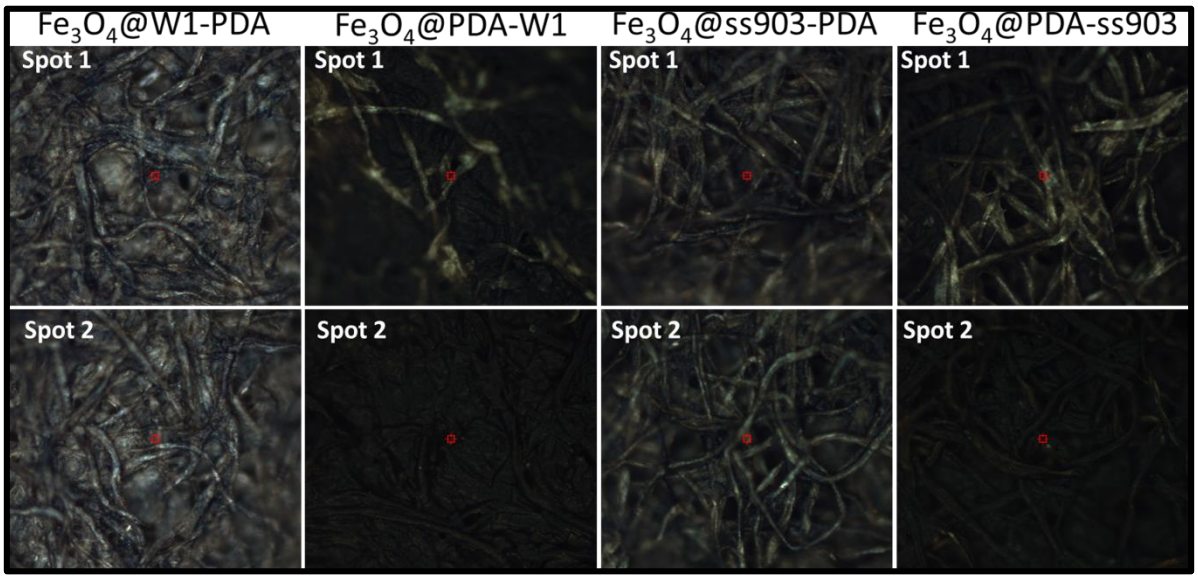


Figure S8: Images of microscope coupled with Raman spectrometer for capped papers.

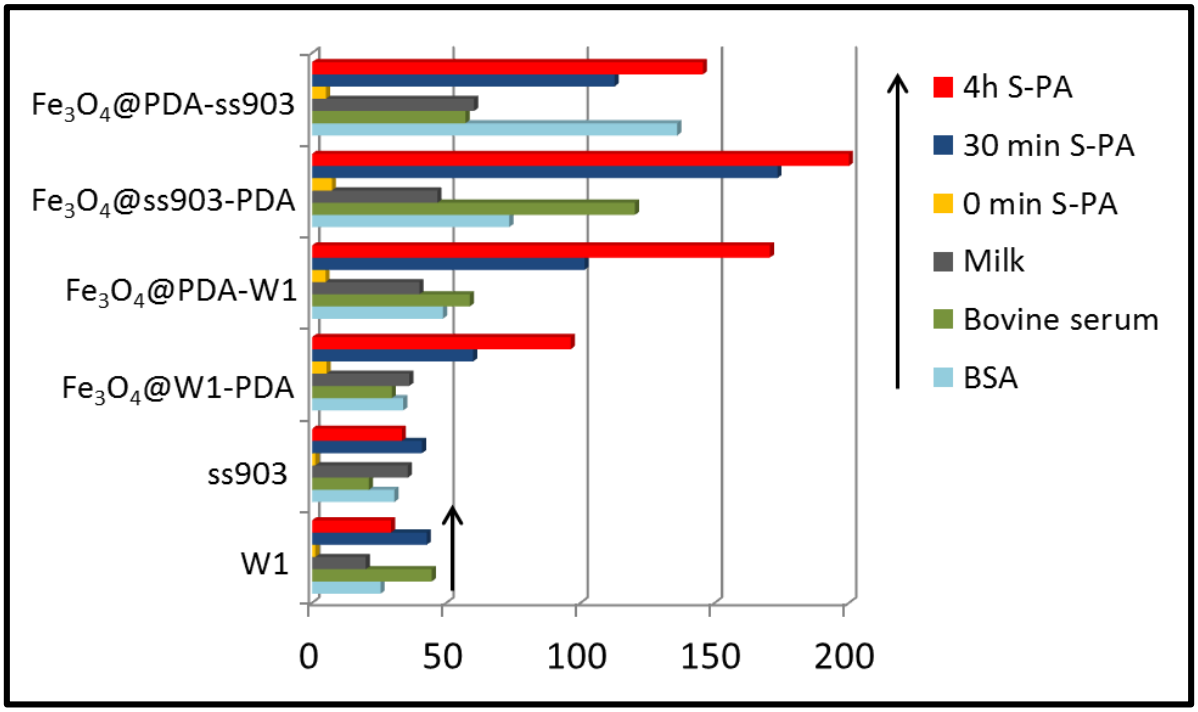


Figure S9: Study of different blockers for magnetite PDA coated papers.

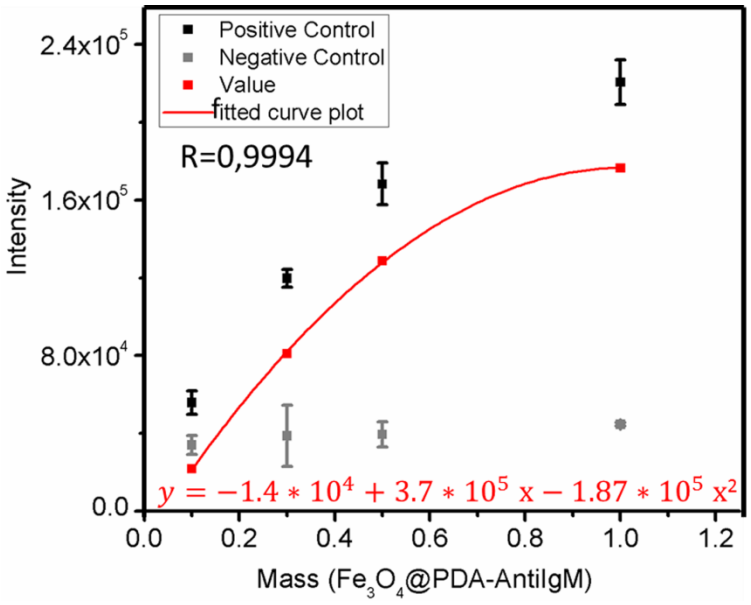


Figure S10: Titration in estimating the effective amount of $Fe_3O_4$@PDA-AntiIgM for capture as many antigens. Aliquots of $Fe_3O_4$@PDA-AntiIgM (10 mg/mL of inorganic core) corresponding to 0.1, 0.3, 0.5 and 1 mg.

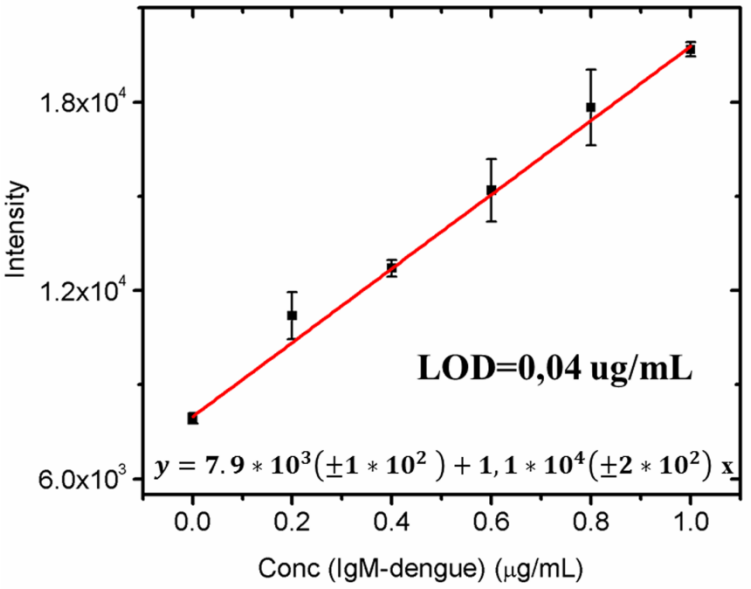


Figure S11: Results of the quantitative detection of IgM-dengue spiked in human serum samples by Magnetic Paper - Based ELISA method with $Fe_3O_4$@W1-PDA as solid support.