Electronic Supplementary Information

Nanobiosensing based on optically selected antibodies and superparamagnetic labels for rapid and

highly sensitive quantification of polyvalent hepatitis B surface antigen

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Optimization of migration conditions

To optimize the conditions for migration of serum samples containing magnetic nanoparticles (MP) across LF strip, serum diluents of different compositions (surfactants, proteins, etc.) were tested. Since normal human serum pH is 7.35 - 7.45, we used the solutions having similar pH.

Five serum diluents were tested for both HBsAg-negative (zero HBsAg) and positive (10 ng mL⁻¹ HBsAg) serum samples (50% of sample volume):

1) Phosphate buffered saline (PBS) (pH 7.4);

2) PBT buffer (PBS, 1 % BSA, 0.1 % Tween 20, pH 7.4);

- 3) PBT with casein concentrate (10%) buffer (pH 7.4);
- 4) Phosphate-based buffer (0.01 M NaH₂PO₄, 0.005 M NaOH, 0.05 % Tween 20, pH 7.2);
- 5) Commercially available calibrator concentrate (Cat. C212X, Xema medica, Russia).

It was found (Fig. S1) that the diluents 1 and 4 hindered migration of both negative and positive serum samples so that the majority of MP aggregated and stayed at the sample pad. With positive samples, no specific signal was formed at the test line. The diluents 2, 3, and 5 enhanced the MP migration, as well as their entrapment at the test line when using the positive samples. At the same time, the diluents 2 and 3 produced weaker magnetic signals in the positive serum samples than the diluent 5.

Thus, the best migration, highest magnetic signal in the positive serum samples, and lowest non-specific signal in the negative samples were achieved with the diluent 5, which was used in the followed experiments as the optimal one.



Fig. S1. Magnetic signals at the test line with negative (zero HBsAg) and positive (10 ng mL⁻¹ HBsAg) serum samples and various serum diluents. The data are shown as the means \pm standard deviations (n = 3). Statistical significance determined using the unpaired two-tailed Student's t-test is denoted by asterisks (*** - P < 0.001, **** - P < 0.0001); n.s. denotes no significance.