Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A Multicolor Multiplex Lateral Flow Assay for High-Sensitivity Analyte Detection Using Persistent Luminescent Nanophosphors

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Optimizing the Assay Buffer

Running the antibody-conjugated SAO and SBMSO in an LFA first requires a suitable assay buffer. Four different buffers generically labeled A, B, C, and D were each tested for the detection of hCG with anti-hCG conjugated SAO PLNPs. These buffers consist of different types of buffering agents (e.g., HEPES, Tris HCl), blockers such as BSA to reduce non-specific binding and surfactants such as Tween 20 to minimize particle aggregation. The compositions of the buffers are given in Table S-1. In these assays, anti-hCG conjugated SAO was added directly to the sample pad along with antigen hCG. 40 μL of buffer solution containing 0.1 mg/mL SAO and 10 ng/mL hCG was added onto the sample pad using a pipette. The strips were allowed to run for 20 minutes to ensure maximum binding. They were then imaged using an Alpha Innotech FluorChem gel documentation system (Figure S-1). In the ideal case, there should be two bands present, one at the test line (TL) and one at the control line (CL), for the positive tests and only one band present at the control line for the negative tests. According to Figure S-1a, buffer A does not produce bands at the test line for the positive tests even though it shows very faint bands at the control line. Also, the sample pad and the interface between the sample pad and the membrane are bright implying that the most of particles are stuck at the beginning of the strip. Buffer B (Figure S-1b) gives clear bands for binding at the test line and control line for the positive tests, but there is non-specific

binding at the test line for the negative tests. Buffer C (Figure S-1c) also shows clear binding on test and control lines for the positive tests, and there is a clear difference at the test line between positive and negative tests. However, there are also faint bands at the test line of the negative tests. Furthermore, in both Figure S-1b and S-1c, the sample pad is bright indicating that buffer B and C do not carry the particles properly towards the membrane. Finally, buffer D was formulated by adding BSA to buffer C to reduce non-specific binding (Figure S-1d). The need for BSA in the buffer suggests surface passivation may not be complete and could be further optimized. Nevertheless, it gives the brightest test line for the positive tests and zero non-specific binding for the negative tests. Also, there are less particles stuck at the sample pad compared to the other buffers. SBMSO particles were also tested with buffer D and they produced similar results (Figure S-1e) showing excellent binding for the positive test lines and minimal non-specific binding in the negative control tests. Therefore, buffer D was selected as the optimum buffer to run all LFA tests in this study.

Buffer A	Buffer B	Buffer C	Buffer D
100 mM NaCl 0.1% Tween 20 0.5% Sucrose 1% PEG 3750 0.1% SDS 0.01% BSA 1M HEPES (pH=7.5)	50 mM NaCl 0.1% Tween 20 10 mM Tris HCl 0.25% PVP-40 1M HEPES (pH=8)	50 mM NaCl 0.1% Tween 20 10 mM Tris HCl 0.25% PVP-40 (pH=8)	50 mM NaCl 0.1% Tween 20 10 mM Tris HCl 0.25% PVP-40 0.1% BSA (pH = 8)

Table S-1. The composition of buffer A, B, C, and D.

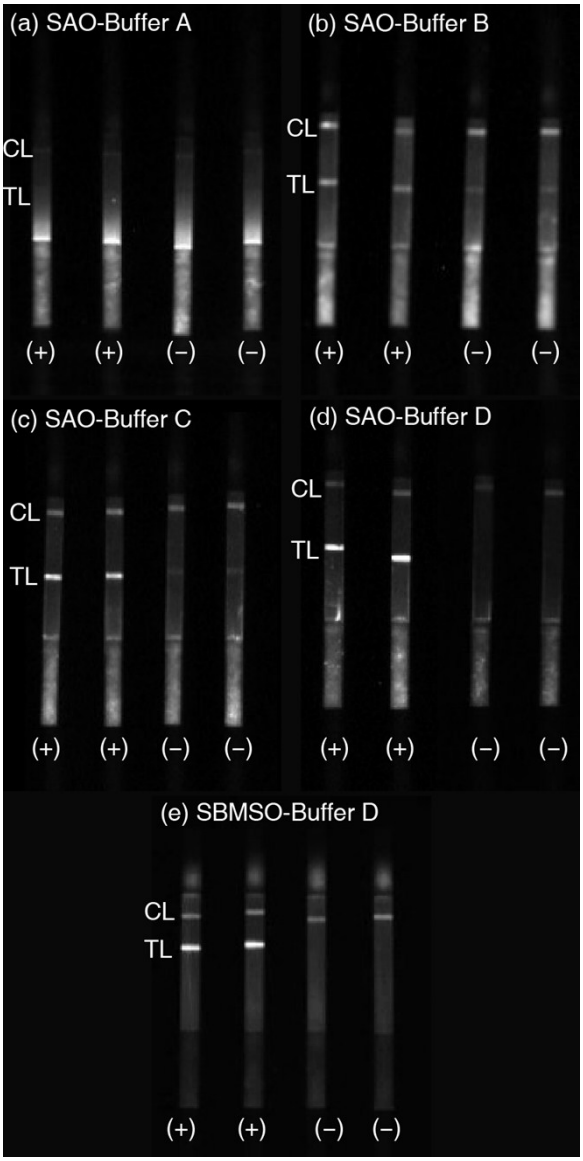
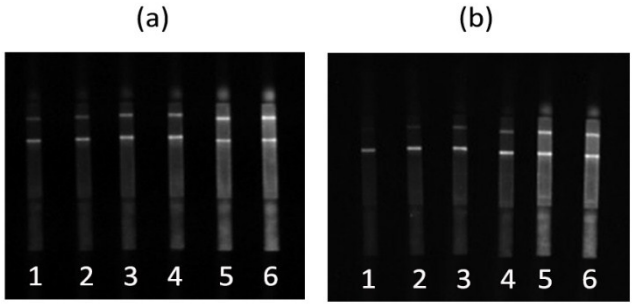

(a) SAO-Buffer A
CL
TL
(+) (+) (−) (−)
(b) SAO-Buffer B
CL
TL
(+) (+) (−) (−)
(c) SAO-Buffer C
CL
TL
(+) (+) (−) (−)
(d) SAO-Buffer D
CL
TL
(+) (+) (−) (−)
(e) SBMSO-Buffer D
CL
TL
(+) (+) (−) (−)

Figure S-1. Optimizing the assay buffer for SAO and SBMSO using anti-hCG conjugated PLNPs shows buffer D yields the best test results with a clear test and control line for the positive test (+) and minimal non-specific binding in the negative test (-).

Optimizing the Particle Concentration

To optimize the particle concentration for SAO and SBMSO, hCG assays were run using the same conditions as above, but with different PLNP concentration and the Fluorchem images of the strips are shown in Figure S-2. The optimum particle concentration was selected as the concentration that gives the brightest test line while still showing a clear control line. According to the plots in Figure S-3, 0.13 mg/mL is suitable for SAO whereas 1 mg/mL is optimal for SBMSO and as shown in Figure 4a and 4b, these particle concentrations did not show any non-specific binding. Therefore, these optimized concentrations were used to run all the LFAs in the study.



1 – 0.06 mg/mL	1 – 0.4 mg/mL
2 – 0.08 mg/mL	2 – 0.5 mg/mL
3 – 0.1 mg/mL	3 – 0.7 mg/mL
4 – 0.13 mg/mL	4 – 1 mg/mL
5 – 0.2 mg/mL	5 – 2 mg/mL
6 – 0.4 mg/mL	6 – 3 mg/mL

Figure S-2. Optimizing particle concentration for (a) SAO and (b) SBMSO.

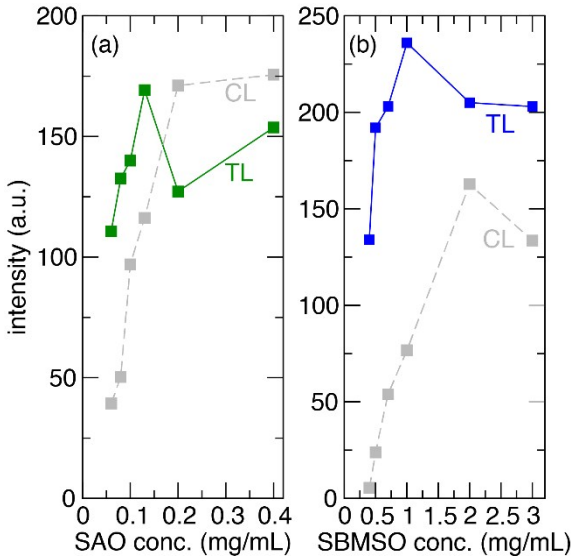


Figure S-3. Optimum particle concentration for (a) SAO and (b) SBMSO was determined by the maximum intensity of the test line (TL) detected by the FluorChem imaging system as a function of concentration. The FluorChem images used to measure the intensities of the test lines are shown in Figure S-2.

Detection Limits of Individual Assays on iPhone 5S

The iPhone images of SBMSO in trial 1, 2 and 3 that used to calculate the average intensity ratio of test line/control line at different hCG concentrations are shown in Figure S-4. A similar set of iPhone images of SAO were used to calculate the average intensity ratio of test line/control line at different concentrations of PSA.

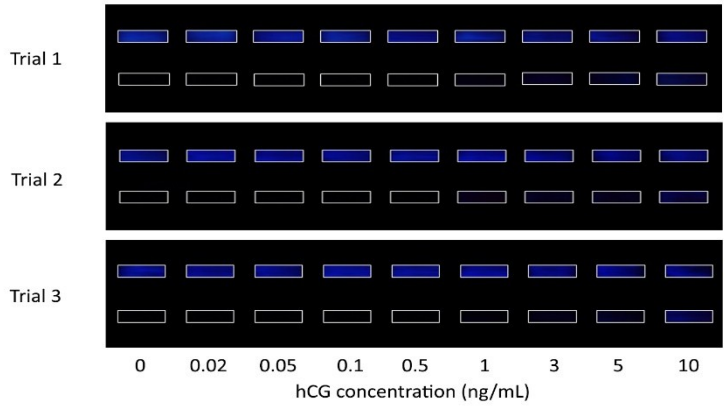


Figure S-4. iPhone images of SBMSO at different concentrations of hCG.

Detection Limits of Multiplex Assay on iPhone 5S

The iPhone images of SAO and SBMSO in the multiplex assay that used to calculate the intensity ratio of test region/control region at different concentrations of hCG, in the presence of a constant concentration of 0.1 ng/mL of PSA are shown in Figure S-5. A similar set of iPhone images were used to calculate the intensity ratio of test region/control region at different concentrations of PSA, in the presence of a constant concentration of 1 ng/mL of hCG.

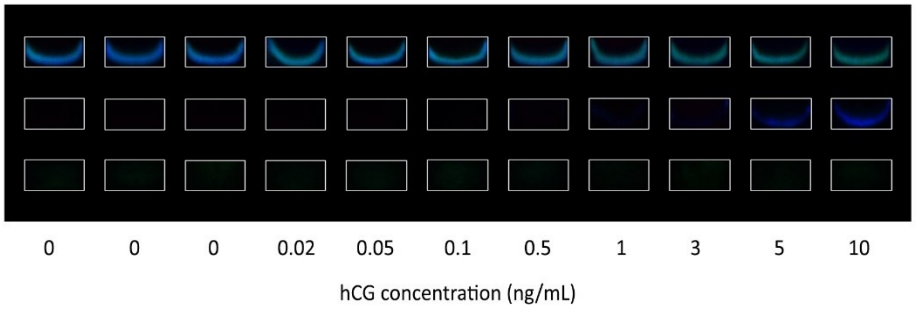


Figure S-5. iPhone images of SBMSO and SAO at different concentrations of hCG and a constant concentration of 0.1 ng/mL of PSA.