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Electronic Supplementary Information

Quantitation of lateral flow assays

Table S1: Relative intensity of the test line for six independent Lateral Flow Assays using functionalized M13 bacteriophage to detect MS2 virus. CC1-3: Reporter enzyme and antibody were covalently attached to M13. AV1-3: The antibody was attached through avidin:biotin linkage; the reporter enzyme was attached covalently.

Number of phage per strip	CC-1	CC-2	CC-3	AV-1	AV-2	AV-3
0	0	0	0	319	0	0
10	0	0	0	180	0	0
1.00E+02	0	0	0	325	233	318
1.00E+03	4613	5374	2100	2470	1541	3235
1.00E+04	7723	6944	3555	5498	3859	6387
1.00E+05	7679	9397	4662	5661	4514	5313
1.00E+06	10441	9004	3856	7818	4341	7486

Gold nanoparticle reporters

100 µl of 4 mM K_2CO_3 was added to 1 mL of 40 nm gold nanoparticles (10^{11}/ml, OD_{520}=1, DCN, Carlsbad, CA). Then 10 µg rabbit anti-MS2 antibodies (Tetracore Inc., Gaithersberg, MD) were adsorbed, for 20 min at 25°C on a rotator. BSA, 100 µl (10 % (w/v)) was added to block the nanoparticles. After 20 min, the functionalized nanoparticles were collected by centrifugation (5 min, 10000 x g). The particles were washed once with 1 mL of storage solution (PBS, pH 7.4; 1% (w/v) BSA; 10% (w/v) sucrose), thereafter suspended in 100 µL and stored at 4°C. To estimate the concentration of gold nanoparticles their absorbance at 520 nm was measured.

Gold nanoparticle lateral flow assay

Nitrocellulose membrane, Prima 60 (GE Healthcare Biosciences, Piscataway, NJ) was used. The detection line with anti-MS2 antibodies (1 µg/cm) and control line with anti-rabbit antibodies (0.25 µg/cm) were spotted using a Lateral Flow Reagent Dispenser (Claremont BioSolutions, Upland, CA) equipped with an external syringe pump (Chemyx, Stafford, TX). The strips were allowed to dry for 1 h at 37°C.

Assay strips (5 mm x 40 mm) were cut and 20 µl LFA buffer (100 mM Tris-HCl, pH 8; 0.3% (v/v) Tween-20; 0.2% (w/v) BSA; 0.1% (w/v) PEG 3350) was added at the bottom of the strip. Thereafter dilutions of MS2 viral particles in LFA buffer were added followed by 60 µl LFA buffer. Functionalized gold nanoparticle reporters ($2x10^9$) were added in 20 µl of LFA buffer, followed by another 60 µl LFA buffer. The strips were left to dry and thereafter scanned in a Perfection V600 flatbed color scanner (Epson, Long Beach, CA).

Electronic Supplementary Material (ESI) for Analyst
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The results of the traditional gold nanoparticle LFA detecting MS2 viral particles can be seen in figure S.1. 10^7 pfu of MS2 were clearly detected (10^6 pfu were weakly visible by eye, but not well in this scanned image) using 40 nm gold nanoparticles functionalized with anti-MS2 antibodies.

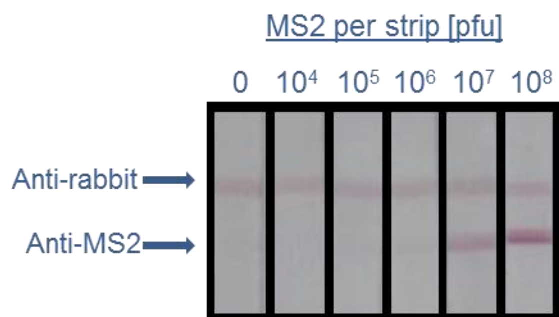

Fig. S.1. Immunochromatographic detection of bacteriophage MS2. Various numbers of MS2 phage were added to a nitrocellulose strip containing anti-MS2 and anti-rabbit antibodies. Gold nanoparticles with adsorbed rabbit anti-MS2 antibodies were captured on the test line by MS2, and on the control line by anti-rabbit antibodies