Supporting information:

SERS-based sandwich immunoassays for multiplexed detection of Zika and dengue viral biomarkers

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S1. Determination of antibody coverage of nanotags

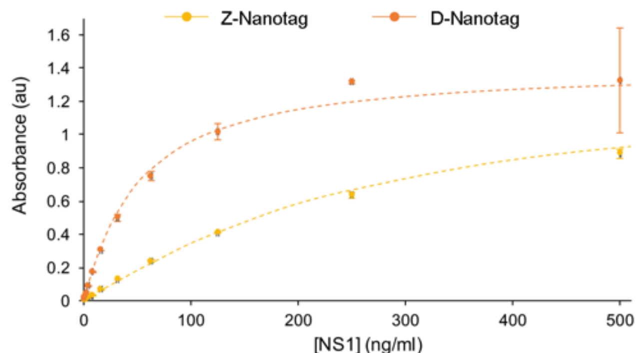

Figure S1. Determination of antibody coverage of nanotags by ELISA. Absorbance at 450 nm as a function of NS1 concentration. Ab coverage determined by fitting a sigmoidal curve (n=3; mean ± SD).

S2. Limit of Detection (LOD) calculation

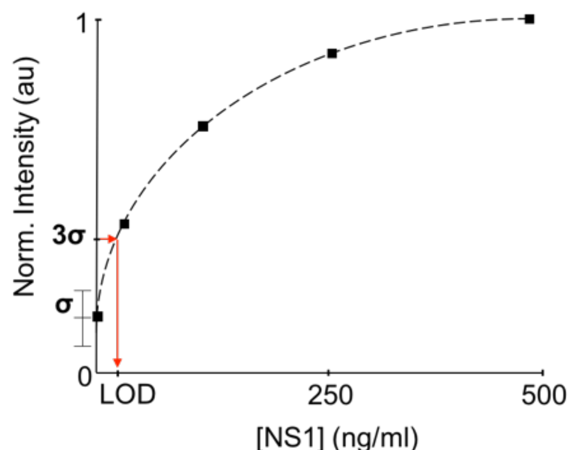

Figure S2. Schematic explanation of LOD calculation.

As explained in the Methods section, LODs of the single tests for both the colorimetric and SERS readouts were calculated following the standard definition used in the literature, which is the minimum concentration that yields an average test intensity that exceeds by 3 times the standard deviation of a blank (i.e., the test area intensity running sample without the antigen) over the average intensity of the blank[2,8].

Figure S2, shows and scheme of the LOD calculation, where the value of three times the blank's standard deviation (σ) is interpolated into the fitted Langmuir isotherm, rendering the minimum concentration of NS1 (ng/ml) detected by the technique.

The LOD values for the colorimetric immunoassay and the SERS immunoassay provided in this paper are calculated for the single-plex (individual) (Figure 4 and Figure5) assays for both

ZIKV and DENV, and the experiments were run at several concentrations plus the blank in triplicate to determine the standard deviation of the blank.

It can be observed that SERS signal for the blank in the single-plex tests (Figure 5 a, b) are within the same range as the blank in the multiplex tests (Figure 6 a, b, c), which suggests that multiplexing does not increase the LOD of the SERS-LFA, and thus the LOD calculated for single-plex tests can be utilized for multiplex. In the case of multiplexed assays, tests were run to determine whether or not the assay can distinguish one biomarker from the other, so they were run only at one concentration, so the LOD was not calculated for these.

S3. SERS background signal

It was observed that in the case of SERS, signal from the reporter can appear in areas outside of the test line due to the ability of SERS to pick up even small amounts of nanotag non-specific adsorption. This background signal on the nitrocellulose (outside of the test area) is already "contained" in the blank signal (within the test area), so it does not modify the LOD values presented. Typically, this is not observed for colorimetric readouts (i.e., the strip is usually white outside of the test area), and is a problem unique to SERS because of its high sensitivity. While this issue is not prohibitive, it is something that others should take into account when designing immunoassays for SERS readout.

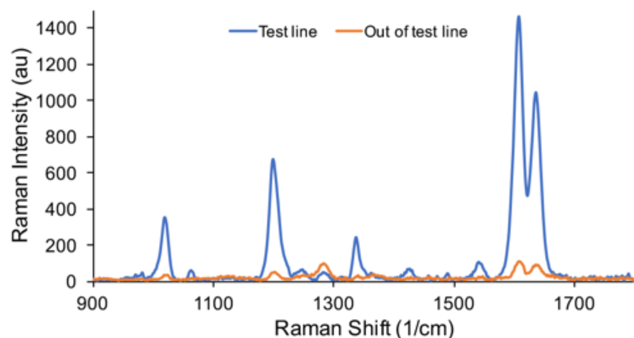

Figure S3. SERS background signal in individual tests. SERS spectra in the test line (blue) and out of the test line (orange) in an individual positive test for Zika. Averaged spectra of 10 measurements for each spot.

S4. SERS individual assays

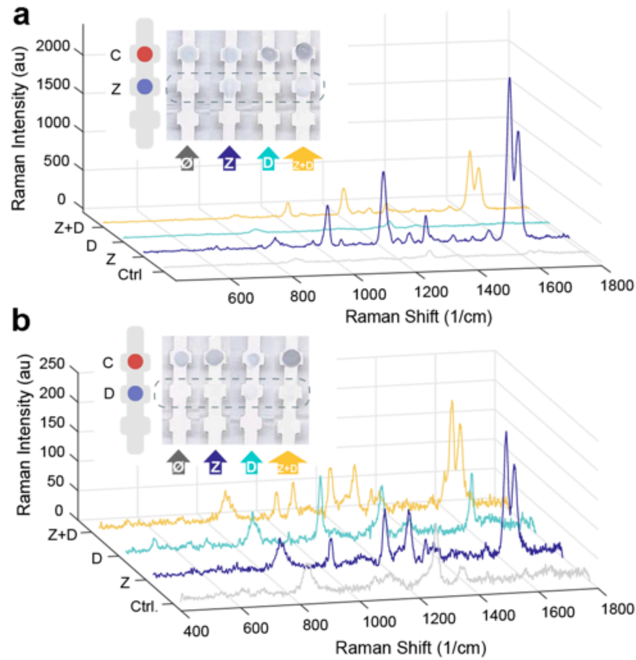

Figure S4. SERS individual assay. Individual assay with Zika and dengue test line painted in different strips. SERS spectra of Zika (a) and dengue (b) test lines. Samples that are run are control (0 ng/ml NS1) (gray), ZIKV NS1 (blue), DENV NS1 (cyan) and a mixture of ZIKV and DENV NS1 (yellow). Averaged spectra of 10 measurements for each spot.