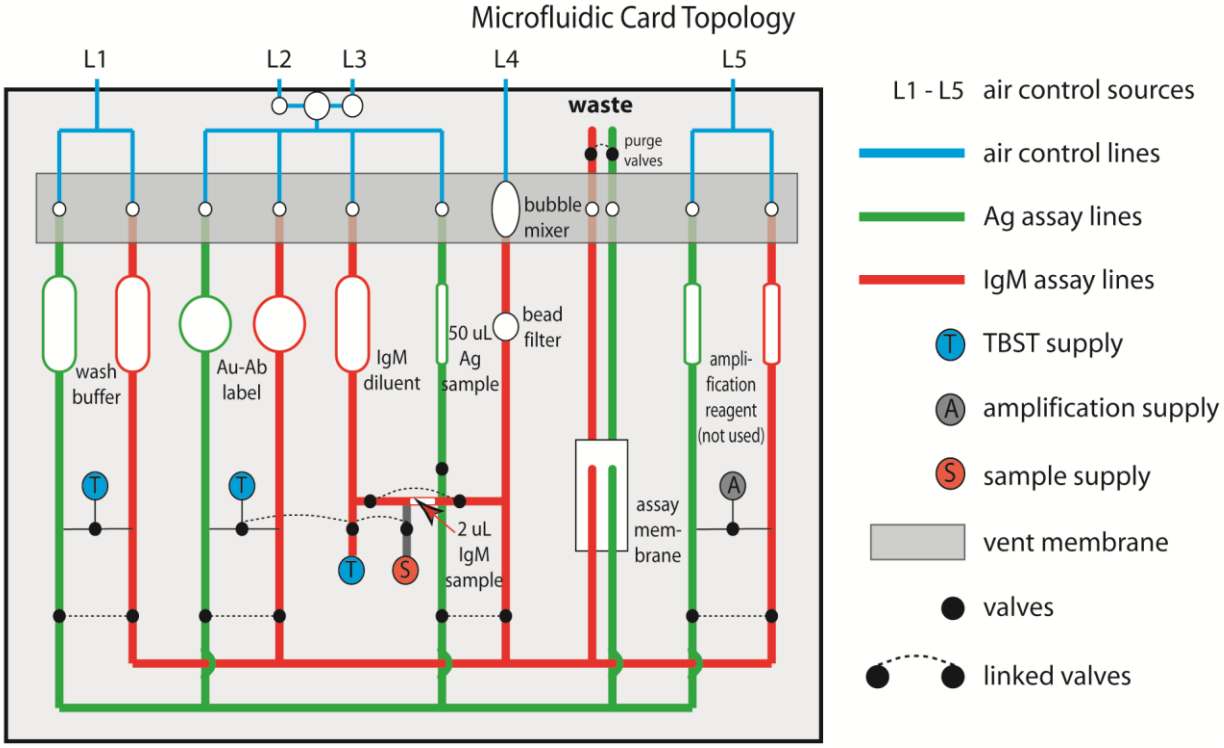
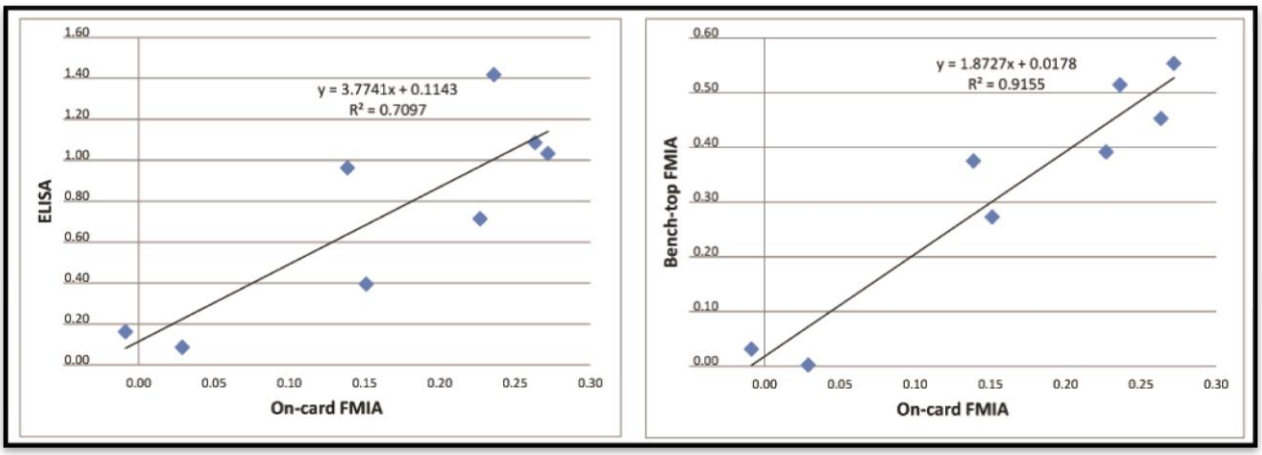


Supplementary Fig. 1 Topology of the DxBox microfluidic cards. The buffer supplies were branched off a single source from the control box so the only fluid added for operation was a blood or plasma sample into the sample supply port. The sample was split to aliquot samples for the Ag assay (50 μL) and the IgM assay (2 μL). The IgM sample was diluted with buffer (200 μL) in the bubble mixer, where the diluted sample was mixed by bubble action with dried protein G coated beads to remove IgG from the sample. From this point forward, the Ag assay (green lines) and IgM assay (red lines) were performed in parallel to multiplex as many valve control lines as possible. Each reagent was delivered to the assay membrane in a timed sequence by opening the corresponding linked valve and applying pressure to the appropriate control line. Between each fluid delivery, a small amount of liquid was cleared from the assay vent membrane by opening the purge valves. Chambers aliquoting an amplification reagent were designed into the card but not utilized.

Typhoid Results Correlation Analysis



Supplementary Fig 2. Correlation plots of Typhi-IgM data comparing the method used in this publication, on-card FMIA, against the two comparator methods, bench-top FMIA and ELISA. These plots show the equations used for linear scaling as well as the r^2 correlation coefficients.

To compare the assay results directly, we normalized the ELISA optical densities and bench-top FMIA assay signals to the same scale as the DxBox FMIA assay signal. First we plotted the DxBox FMIA signal versus the comparison method and found the best fit slope, m, and y-intercept, b. Then the scaled comparison results were defined as,

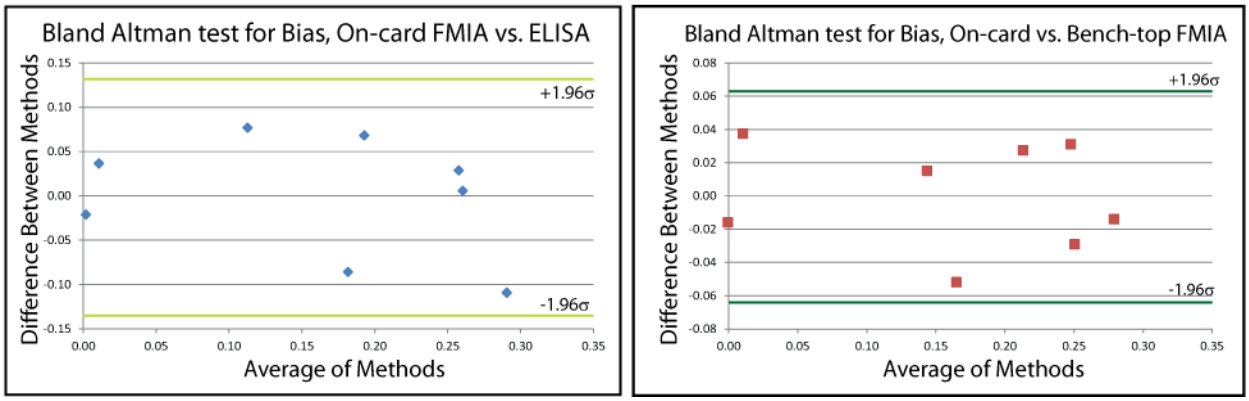
$$Y_{scaled} = \frac{(Y_o - b)}{m}.$$

Once the results were all on the On-card FMIA scale, we calculated the population standard deviation,

$$S_n = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(Y_i^{On-card} - Y_i^{Reference})^2}.$$

For small populations dividing (N-1) yields an overestimation of error rather than a potential underestimation possible with a divisor of 1/N. The value of S_n for the on-card FMIA compared to ELISA as the standard was 0.07, equal to 25% of the maximum on-card sample. Comparing the on-card FMIA to the bench-top FMIA gave an S_n of 0.03, 12% of the maximum on-card sample.

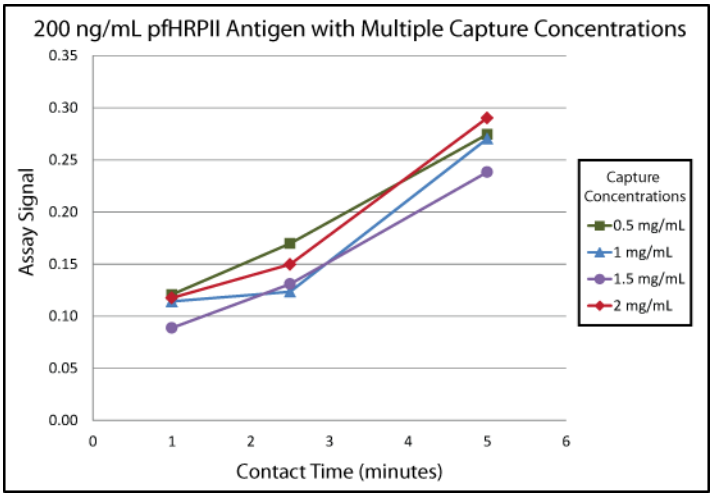
We prepared Bland Altman plots[1] of the on-card FMIA compared to both reference methods to look for bias in the measurements. Each patient sample is compared between the test method (on-card FMIA) and the reference method. The points were well scattered and all points fell within the 95% confidence interval, between +1.96*sigma and -1.96 sigma, suggesting acceptably low systematic errors in the on-card FMIA.



Supplementary Fig. 3. Bland Altman analysis to look for bias in the test method (on-card FMIA), as compared to the reference method. All points for both reference methods are well scattered and fall within the 95% confidence interval, suggesting no systematic bias in the on-card FMIA method.

pfHRPII antigen kinetics tested in bench-top FMIA

We saw sensitivity of signal intensity with contact time in the on-card DxBox FMIA format. To better explore that we used the bench-top FMIA format to test a range of concentrations of capture antibody, and a range of contact times. Across the full range of capture concentrations the same trend is seen, even slightly lowered contact time has a dramatic effect on assay signal. Thus, the conditions chosen for the on-card assays (target sample contact time of 5 minutes) are expected to be sensitive to variations in sample contact time created by bubbles or other errors that affect flow rate.



Supplementary Fig 3. Antigen assay signal change with contact time. A constant concentration of pfHRPII was tested varying both contact time and capture concentration. Results were collected in spiked FBS using the bench-top FMIA format.

1. J. M. Bland and D. G. Altman, *Int. J. Nurs. Stud.*, **47**, 931-936.