**Electronic Supplementary Material (ESI) for Analyst**

A 8-minute colorimetric paper-based reverse phase vertical flow serum microarray for screening of Hyper IgE syndrome

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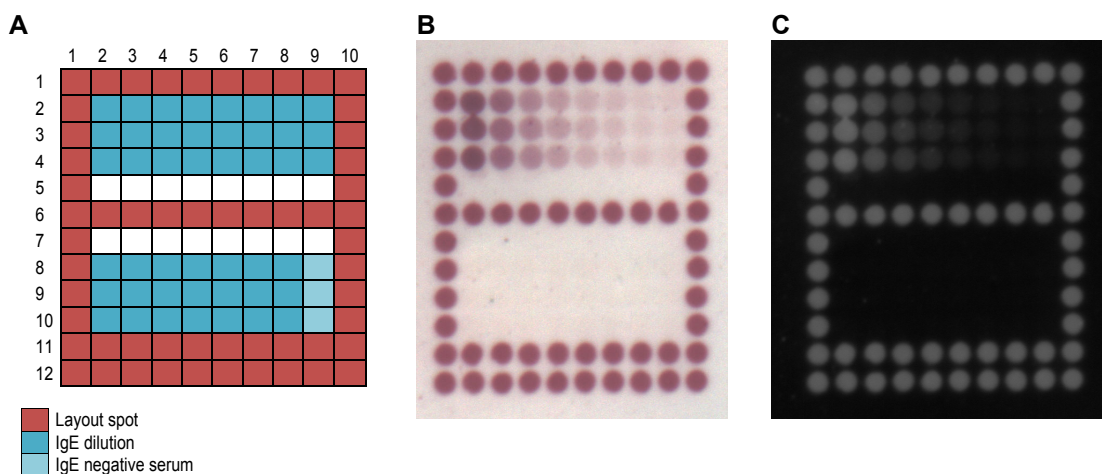
**Supplementary Figure 1. IgE dilution array**
1A) Layout of the IgE dilution array and sample orientation. Red spots are layout spots. Blue spots are serum samples spiked with IgE in dilution series and the light blue sample is IgE negative serum without spiked IgE. 1B) Color scan of the IgE dilution array of the same array at optimized conditions. 1C) Inverted image of the IgE serum array used for image analysis.
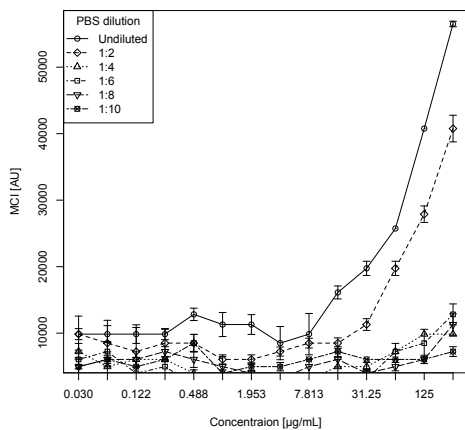
A

B
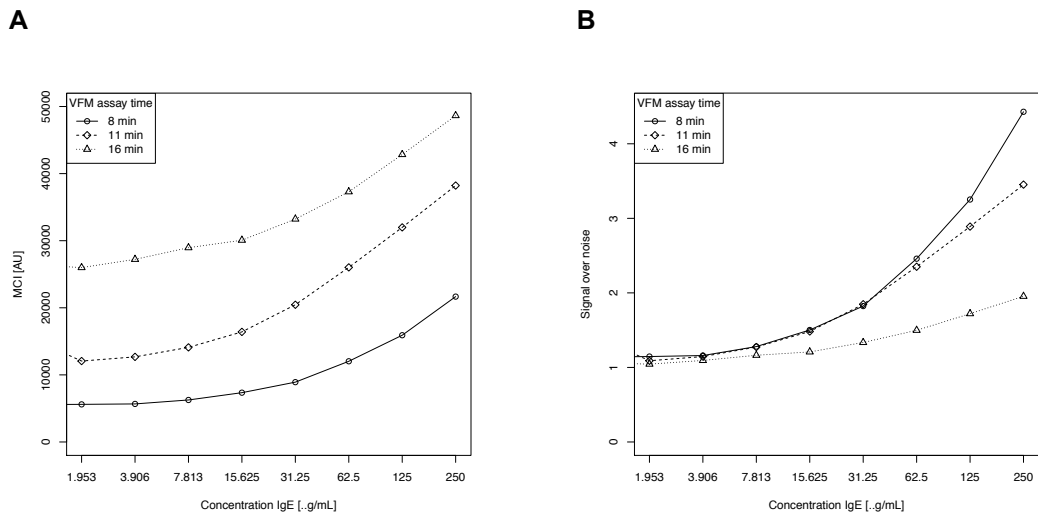

C

D

E

F
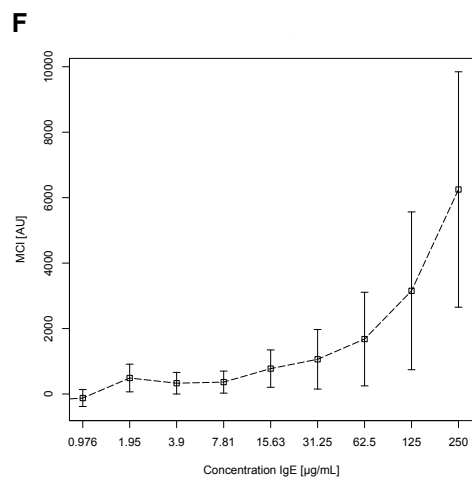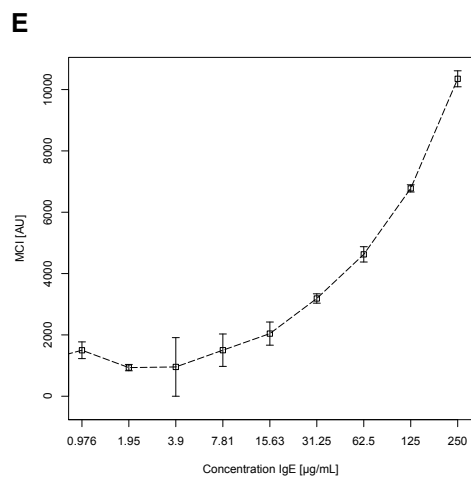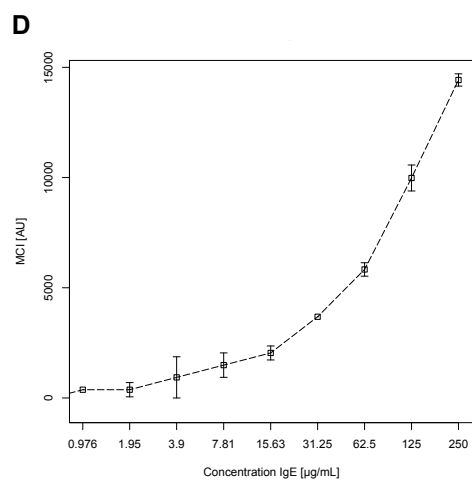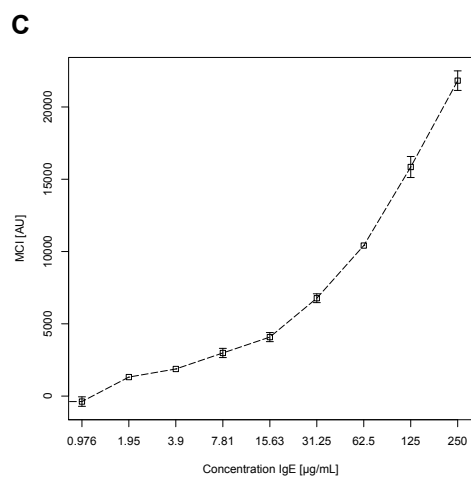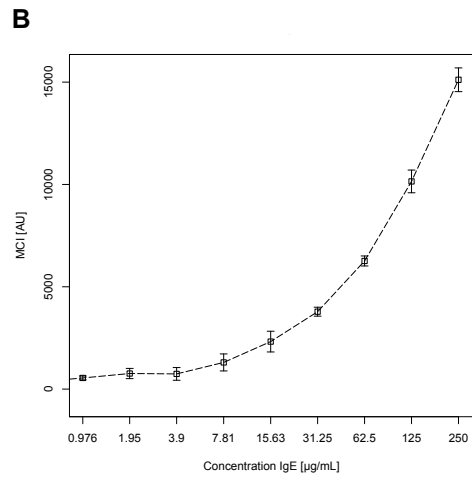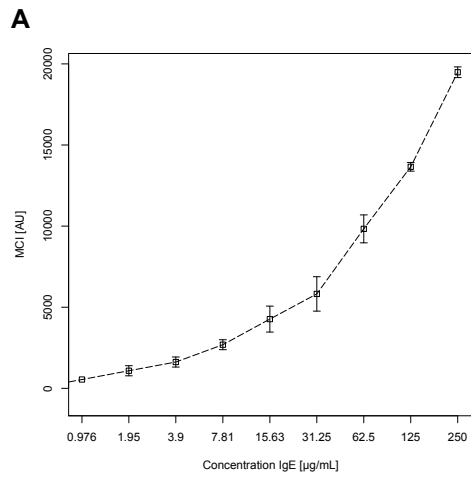
***Supplementary Figure 2. Variability gold nanoparticle dilution***
2A) Variability no gold nanoparticle dilution. 2B) Variability 1:2 nanoparticle dilution.
2C) Variability 1:4 gold nanoparticle dilution. 2D) Variability 1:6 gold nanoparticle dilution.
2E) Variability 1:8 gold nanoparticle dilution. 2F) Variability 1:10 gold nanoparticle dilution.
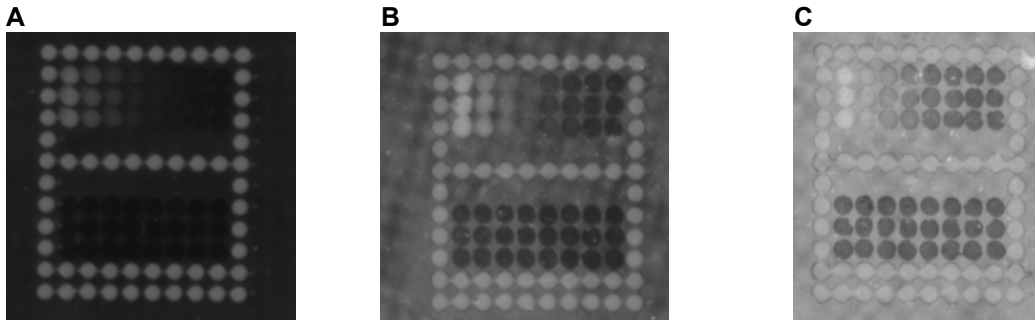
Supplementary Figure 3. IgE serum array with different assay times
Inverted colorimetric scan in black and white of the IgE array assay run. A) 2 minutes total assay time. B) 5 minutes total assay time. C) 10 minutes total assay time.


Supplementary Figure 4. Comparison of VFM assay with time
Intensity of the IgE dilution experiment run at 8, 11 and 16 minute total VFM assay time run at 1:4 dilution of gold nanoparticles and 1 mL/min flow rate. A) MCI for different assay times. B) S4B. Signal over noise for different assay times.

Supplementary Figure 5. IgE dilution in serum and PBS
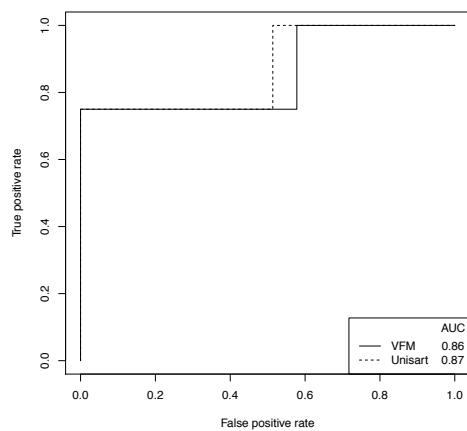MCI signal intensity of the IgE serum dilution in PBS.
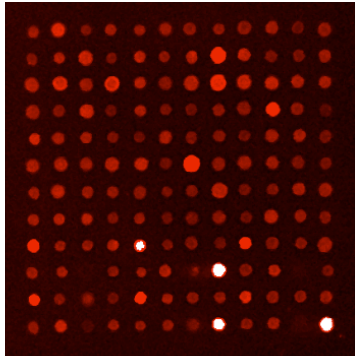
Supplementary Figure 6. Unisart array
Fluorescent scan of the Unisart serum array for the 113 serum samples including dilutions of
IgE and control spots.
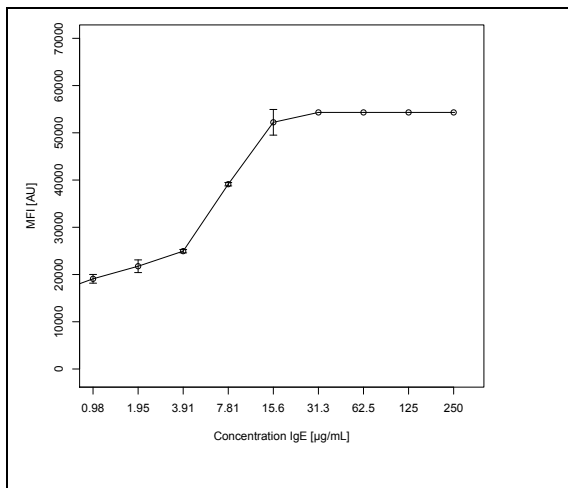
Supplementary Figure 7. MFI IgE dilution
Intensity of the IgE serum dilution serie from the Unisart reference array.

Supplementary Figure 8. ROC curves
ROC curves for VFM showed an AUC of 0.86 and the Unisart array showed an AUC of 0.87.