Highly Specific Vertical Flow-Based Point-of-Care For Rapid Diagnosis of Lupus

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Objective

To construct an in-house vertical flow-based point-of-care (POC) device for diagnosing Systemic Lupus Erythematosus (SLE) in serum by detecting ANA and anti-dsDNA biomarkers in the comparative BSA range of 50 µg/ml to 250 µg/ml.

Background

- Systemic Lupus Erythematosus (SLE) is an autoimmune disease that causes multiple organ system inflammation and tissue damage.
- The antibodies, anti-double stranded DNA (anti-dsDNA) and anti-nuclear antibodies (ANA), are used as biomarkers for SLE.
- The vertical flow assay (VFA) has antigens (Hep 2 lysate and dsDNA) bound to the nitrocellulose membrane (NCM).

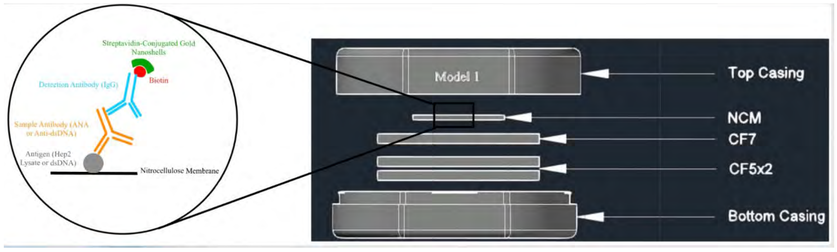

Figure 1. On the right, the main components of the device are shown. On the left, the magnified image of the membrane displays the binding complex that produces colorimetric signaling.

- Primary antibodies from a patient's serum sample bind onto their respective antigen on the membrane.
- The addition of a biotinylated detection antibody (IgG) will then bind onto the bound primary antibodies.
- Streptavidin-conjugated gold nanoshells (GNS) will then bind to IgG to produce colorimetric detection.

Methods

- Determine the ideal membrane through quality of colorimetric signal in all four zones.
- Adjusted the in-house fabricated assay diluent's Tween20 concentrations, pH, and presence of other reagents to develop an all-purpose assay diluent.
- Developed a standard curve by running the VFA system under 5 different concentrations (0, 0.5, 1, 5, and 10 IU/ml) of antigens and obtaining signal intensity measurements via the use of an image processing software (ImageJ)
- Evaluated the standard curve's validity in distinguishing healthy vs. active SLE levels of biomarkers by running healthy vs. active SLE patient samples

Results

Figure 2. The membraned applied with serum standard of concentration ranging from 0-10 IU/ml.

The membranes from **Figure 2** were analyzed in Image J to obtain a standard curve, shown in **Figure 3**. The standard curve established that the signal intensities increases as the concentration of biomarkers increased.

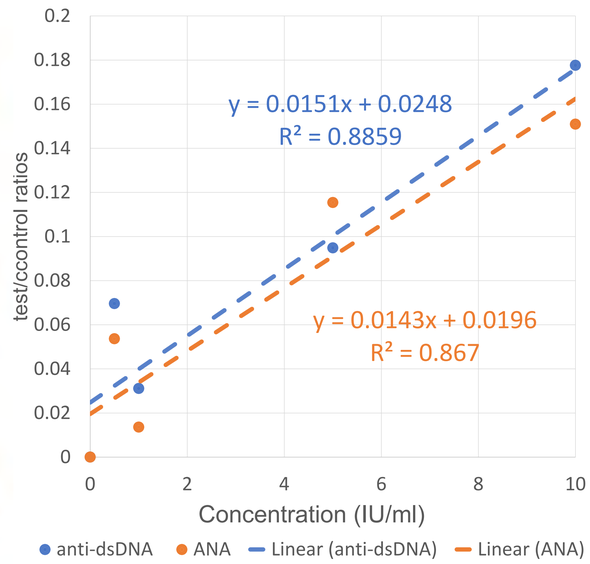

Figure 3. Standard curve for spiked serum for both ANA (orange) and anti-dsDNA (blue) from the in-house VFA system.

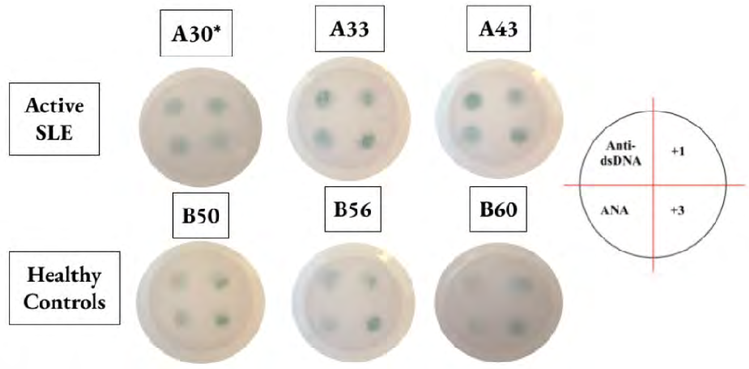

Figure 4. ANA and anti-dsDNA detection results in Active patients (A33, A43, A54) on the top row and in Heathy controls (B50, B51, B56) on the bottom row. Map on the right shows designated zones for patient serum samples. Bovine serum albumin (positive control) range: +1 = 50 µg/ml, +3 = 250 µg/ml. *+1 and +3 zones are reversed

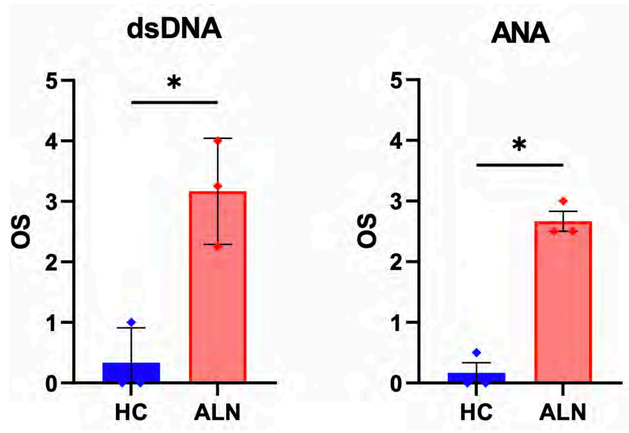

Figure 5. Average of observing scores (OS) graded from active Lupus nephritis (ALN) and healthy control (HC) patients from **Figure 4** to establish that healthy patients can be distinguished from active patients on this VFA system. *p<0.05

Conclusion

The in-house VFA system for diagnosing SLE produced successful results. The standard curve shows a signal gradient effect with increments in concentration of human serum standard. Based on the observing score (OS), patients and healthy samples were able to be distinguished.