



# Point-of-Care VFA for Rapid Diagnostic Testing of Lupus Nephritis Using Saliva

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## Objective

Develop and optimize a vertical flow assay (VFA) able to rapidly diagnose and distinguish between healthy and active lupus nephritis (LN) saliva samples utilizing dsDNA and ANA antibodies.

## Background

- The global prevalence of Systemic Lupus Erythematosus (SLE) is estimated to be 43.7 per 100,000 people. When lupus begins to affect the kidney and cause renal failure it is known as LN. LN typically occurs within 5 years of the initial onset of SLE.
- Current methods for the diagnosis (including blood withdrawal and kidney biopsies) of LN tend to be invasive.
- A point-of-care assay presents a more convenient method of diagnosis being more accessible to low-resource areas.
- The VFA's capability for rapid diagnosis would increase patient retention, improving patient health outcomes.
- Saliva is also a more convenient body fluid to test than blood or urine as it is easier to obtain from patients.

## Methods

- Build the VFA cartridge as shown in Figure 1.
- Dilute stock samples of detection, gold nanoparticles, HEP-2, pooled saliva, and IU to desired concentrations.
- Develop a serum and saliva standard curve by running the VFA experiment using 7 different concentrations (0, 1.25, 2.5, 5, 10, 15, and 20 IU). Analyze results using ImageJ, developing an imaging score (IS).
- Use the procedure and results from serum and salivary standard curve to begin patient saliva testing for LN using the VFA and develop an observance score (OS).

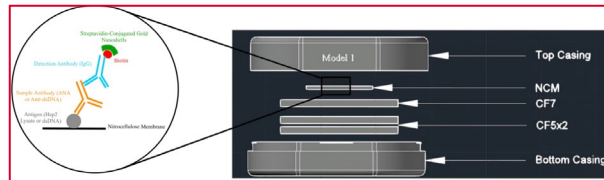


Figure 1: Schematic outlining the components of the VFA with an illustration displaying the molecular mechanisms that allow for diagnostic signals.

## Results

- It was determined that concentrations below 5 IU indicated a healthy sample, between 5 IU and 10 IU indicates possible autoimmune disease, and above 10 IU indicated active LN.
- Further statistical analysis validated the significance of the results.

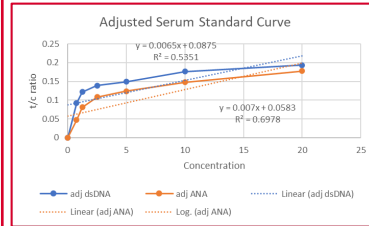


Figure 2a: Serum standard curve displaying the correlation between antibody concentration in serum to VFA signal intensity; extracted using ImageJ.

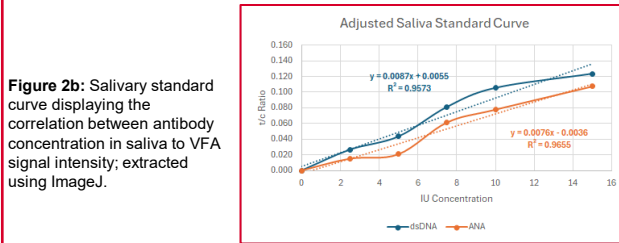


Figure 2b: Salivary standard curve displaying the correlation between antibody concentration in saliva to VFA signal intensity; extracted using ImageJ.

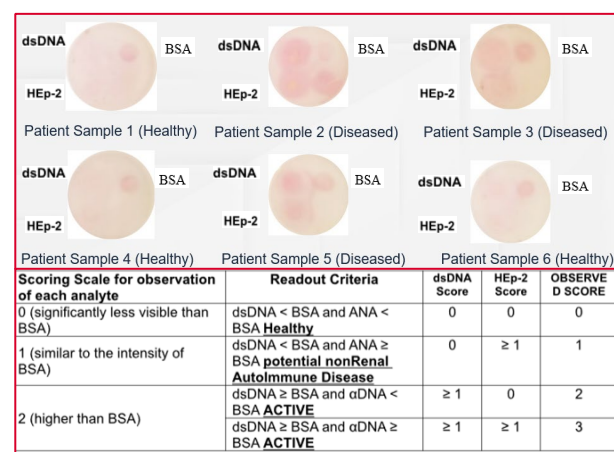


Figure 3: The top image displays the results of six preliminary patient samples (dsDNA is spotted TL, ANA is spotted BL, and BSA is spotted TR) while the table details the observing scoring (OS) system used to evaluate the signals. Note: Ignore spot in the bottom right quadrant of Patient Sample 2.

## Conclusion & Future Directions

- A point-of-care VFA was developed and optimized to be able to diagnose LN in under 30 minutes.
- There is a demonstrable link between the concentration of dsDNA antibodies in saliva and the intensity of the signals in the VFA.
- A salivary standard curve was able to be quantitatively extracted using ImageJ, validating a relationship between concentration and signal strength.
- Further optimization of the VFA is necessary to provide more distinguishable results with patient samples including considerations regarding sample preparation and antibody concentration.