

Comparison of LFIRE™ to Commercial ELISA Diagnostic Kit For The Detection of Anti-RNP Autoantibodies

Summary

Label-free measurement of autoantibodies in patient sera for use in diagnosis of autoimmune diseases was compared with a commercial ELISA kit.

Background

The detection of Anti-Nuclear Antibodies (ANA's) has long been an important tool in the diagnosis of systemic autoimmune diseases. The antigens used in their detection are purified by the saline extraction of human or animal nuclei, this has led to them being termed Extractable Nuclear Antigens (ENA's). The most commonly measured ENA specifications are anti-SS-A/Ro, anti-SS-B/La, anti-Sm, anti-Sm/RNP, anti-Jo-1 and anti-Scl-70.

The intracellular antigen Sm/RNP is a complex of Sm and RNP components. While the Sm antigen can exist independently, the RNP antigen exists as part of the Sm/RNP complex. The Sm/RNP antigen complex is a target for immune response in many patients with Mixed Connective Tissue Disease (MCTD), Systemic Lupus Erythematosus (SLE), Primary Sjogren's Syndrome (SS) and Progressive Systemic Sclerosis (PSS). Studies have shown that anti-Sm/RNP antibodies occur in over 90% of MCTD cases, 35-45% of patients with SLE and approximately 30% of patients with SS. The Sm/RNP antigen is a ribonuclear protein and under Western Blot produces a band at approximately 68kD.

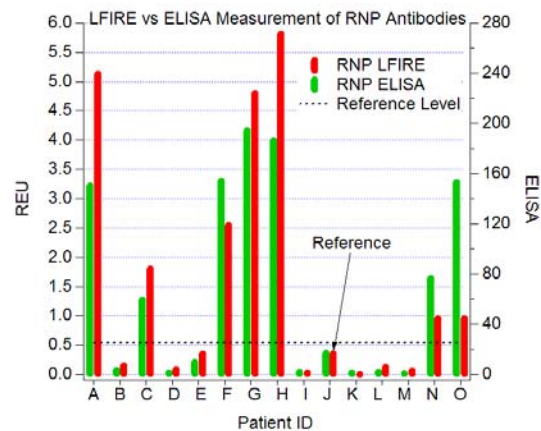
Method and Protocol

Microarray spots of purified RNP (calf thymus) were spotted on our high sensitivity LFIRE™ slides and a flow cell configuration was used to measure both end-point binding and kinetics. 15 patient sera were diluted in assay buffer and were

assayed using a commercially available multi-step ELISA kit and LFIRE™ single-step label-free detection for anti-RNP autoantibodies. All results were compared to the reference level used by the ELISA manufacturer and a constant scaling factor applied to the LFIRE signals.

Results

The following results were obtained in the figure below:



For this commercial ELISA, the positive cutoff was 25 units. In this experiment, 100% concordance was obtained for the 15 samples tested. Statistical analysis showed the signals to be highly rank correlated as well (Spearman $r = 0.01$, $p < .01$).

Using a single-step LFIRE™ assay, we were able to demonstrate significant concordance with a commercial ELISA diagnostic kit which utilizes multiple steps and secondary antibodies for enzyme-labeled detection.