Accurate Quantitation of Proteins Involved In Autoimmune Disease Using Simple Western

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Abstract

Why do researchers use Western blots? Since the technique was developed thirty years ago it's become a trusted method for confirming the presence or absence of a protein but lacks the quantitative rigor for accurate measurement of the amount of protein present. The process requires many steps which introduces multiple variables. While portions of the technique have been automated to improve consistency, until now there has been no major leap in the technology that propels this method of protein analysis from qualitative to quantitative.

Simple Western is the modern evolution of traditional immunoassay techniques. Wes, the latest addition to the Simple Western platform, is an easy to use, fully automated system that removes the variability seen with traditional Westerns for more reproducible results run to run and between users. Researchers can now identify their protein and achieve reliable quantitation of their target proteins.

To demonstrate the precision and data reliability of Wes we look at proteins indicative of autoimmune disease in human samples from either normal individuals or individuals suffering from the disease. Wes not only reduces the hands on time and the time to results, but generates highly reproducible data that gives researchers a high degree of confidence in the differences detected between the two disease states. Accurate quantitation of these proteins can lead to the identification of predictive biomarkers for either diagnosis or response to treatment.

Assay Principles

Wes is a bench top instrument capable of running 25 samples size-based Simple Western assays simultaneously in approximately 3 hours (Figure 1). Assay kit includes a prefilled plate that contains all the reagents except samples, and antibodies (Figure 2). Samples for Wes are separated by size in a self-contained capillary cartridge and immobilized to the capillary wall via a proprietary UV capture method. Target proteins are immunoprobed with an antibody followed by HRP chemiluminescent detection (Figure 3). Wes allows for the automation of the entire Western blot procedure, which results in increased reproducibility and significant time savings.

FIGURE 1. Wes

FIGURE 2. Capillary cartridge and pre-filled plate

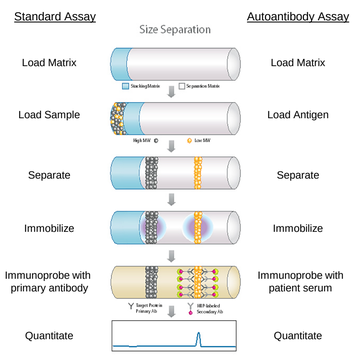

FIGURE 3. Wes Assay Steps

Patient Serum Information

Patient	Gender	Age	Medications	Diagnosis	SLEDAI Score
Normal	Male	58	N/A	N/A	N/A
Systemic Lupus Erythematosus (SLE)	Male	30	Prednisone, Lisinpril, Vicodin HP, Clonidine HCL, Furosemide, Cyclobenzaprine	SLE, Nephritis and nephropathy, HTN, Lupus Nephritis	10

FIGURE 5. Information about patient samples tested provided by Bioreclamation IVT. The SLEDAI score indicates severity of the SLE flare. A SLEDAI score >3 points indicates a mild to moderate flare and SLEDAI score >12 points indicates a severe flare

Antigens Tested

Antigen	Prevalence in System Lupus Erythematosus (SLE)	Antigen Indicated in Other Autoimmune Disease
PCNA	<10%	N/A
Ro/SS-A	25-50%	Sjögren's syndrome, Neonatal lupus syndrome
U1-snRNP	30-40%	Mixed connective tissue disease
U1-snRNP A	30-40%	Mixed connective tissue disease

FIGURE 6. The purified antigens tested. All antigen are recombinant proteins with the exception of RNP/Sm that was isolated from bovine tissue (Diarect).

Simple Western Detects Autoantibodies In Patient Serum

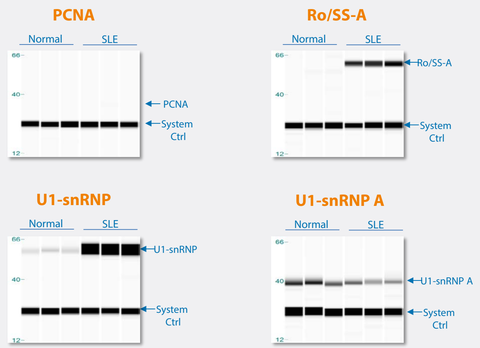

FIGURE 7. Representative lane view images of the data collected. The antigen (0.1 µg/µL) was run in triplicate and immunoprobed with patient serum diluted 1:150 in Protein Simple Antibody Diluent II. Both samples did not contain autoantibodies to PCNA while SLE patient sample contained significantly more Ro/SS-A and U1-snRNP autoantibodies compared to the normal patient sample. Low levels of U1-snRNP A were detected in both normal and SLE patient samples. A system control protein was included to normalize the data.

Precise Data Quantitation

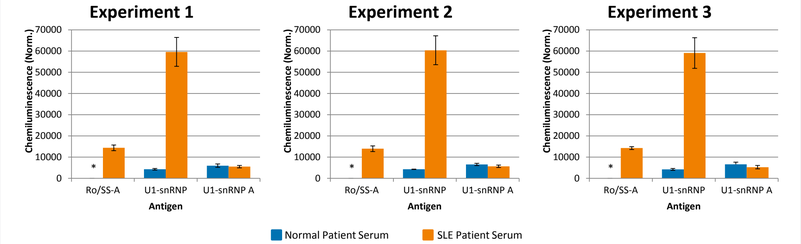

FIGURE 8. The same experiment was run three times on Simple Western. Compass software automatically determines peak areas, normalizes the data to the system control, and performs statistical analysis of the data. The data distinctly demonstrates the increased levels of anti-Ro/SS-A and anti-U1-snRNP in the SLE patient samples. PCNA was not detected in either sample. (*) indicates no peak was detected.

Antigen	Experiment 1 CVs (n=3)		Experiment 2 CVs (n=3)		Experiment 3 CVs (n=3)		3 Experiment Inter-assay CVs (n=9)	
	Normal	Patient	Normal	Patient	Normal	Patient	Normal	Patient
Ro/SS-A	ND	9.3%	ND	6.4%	ND	4.8%	ND	6.3%
U1-snRNP	8.9%	11.4%	1.9%	2.8%	10.7%	12.3%	7.0%	9.9%
U1-snRNP A	13.3%	10.4%	7.8%	9.6%	15.9%	15.3%	12.0%	10.8%

FIGURE 9. Compass software used the normalized peak areas to calculate the inter-assay CVs for each antigen in each experiment to demonstrate data precision. The same experiment was run 3 times and the data generated was used to determine the intra-assay CV. The results clearly demonstrates the precision offered by Simple Western since CVs were all ≤20%.

Conclusion

- Simple Western generates highly reproducible and quantitative assessment of autoantibodies in patient serum samples
- Increased levels of Ro/SS-A and U1-snRNP autoantibodies detected in SLE patient serum samples compared to normal patient serum samples
- Wes, a Simple Western family member, streamlines the total time to results to less than 3 hours in a walk-away mode with a total hands-on time of less than 30 minutes
- The data for all three experiments were collected and analyzed in 1 day.