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SCIENTIFIC REVIEW

SIMPLE WESTERN: NEW VACCINE TECHNOLOGY TO COMBAT OUTBREAKS

Simple Western emerged as a fast, automated and reproducible replacement of the traditional Western blot relatively recently, yet it has already impacted vaccine development to a wide extent. This is supported by the fact there are numerous publications by both academic and biopharma groups directly related to the study of vaccines.[1-22] This has enabled Simple Western to play an important role in developing vaccines against severe and deadly diseases, including Ebola and, more recently, COVID-19, which was featured in *Nature*.[1]

Simple Western is capable of separating proteins by either Size or Charge, both of which offer unique insight that has been leveraged in vaccine development. Unlike traditional Western blot, Simple Western is ideal for studying vaccines because it requires small sample sizes and has excellent sensitivity with a large linear range of detection. Furthermore, the Compass for Simple Western software provides easy translation of methods from development to manufacturing groups. Because regulatory agencies like the USDA and FDA typically require two methods to set release criteria, Simple Western makes a suitable orthogonal method in validation. Together with its automation, reproducibility, and quick time to results, Simple Western has proven to be instrumental in vaccine development, including bioprocess contaminant detection, and monitoring the humoral response. To elaborate on these advantages, this Scientific Review highlights selected publications that use Simple Western for vaccine development.

SIMPLE WESTERN IS POISED TO REPLACE WESTERN BLOT IN VACCINE DEVELOPMENT

Researchers at Merck were among the first to adopt Simple Western for vaccine development,[17] and they have since published many follow-up studies showing how Simple Western offers advantages at every stage of the vaccine development pipeline.[6,8,15,18,20,21] First, they showed that Simple Western has good reproducibility and intermediate precision with a CV of less than 10%.[17] Next, they showed that Simple Western can detect and quantify the common contaminant bovine serum albumin (BSA) in vaccine preparations to fulfill World Health Organization guidelines (50 ng per human dose).[20] For this application, they showed Simple Western has a linear range of two logs, high accuracy with >80% spike recovery, and a limit of quantitation of 5.2 ng/mL.[20]

More recently, researchers at Merck used Simple Western to develop a vaccine against human cytomegalovirus (HCMV), which is the leading cause of congenital viral infection. Here, they took advantage of both Simple Western's Charge and Size assays. With Simple Western Charge, they could determine the charge heterogeneity of the gH/gL/pUL128–131 pentameric complex, which mediates HCMV entry into endothelial and epithelial cells, and it is therefore a major target for neutralizing antibodies.[21] This charge heterogeneity profile gave important insight into the protein's glycosylation state and purity.

In a separate study, they used Simple Western Size to gain more information into how the neutralizing antibodies react with the complex under denaturing conditions, with the assumption that the denaturing conditions would disrupt the conformational epitope.[15] In line with this hypothesis, most antibodies did not react, and those antibodies that did were subsequently found to react with linear epitopes (FIGURE 1). Collectively, these studies demonstrate the utility of both Simple Western Charge and Size assays in vaccine development.

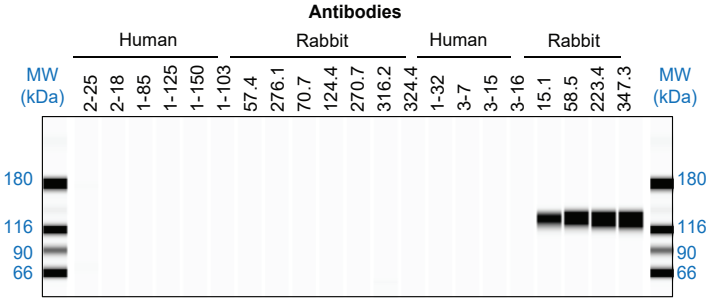

FIGURE 1. Simple Western was used to measure neutralizing antibodies binding against viral epitopes under denaturing and reducing conditions. The four antibodies that reacted in this experiment were subsequently found to interact with linear epitopes. The 17 antibodies that did not react were suggested to react with conformational epitopes. Adapted with permission from Ha *et al*.[15]

MEASURING THE HUMORAL RESPONSE IN HIGH-THROUGHPUT

The basis of successful vaccination is a humoral response of neutralizing antibodies that target and remove pathogens. Simple Western has been used to measure the humoral response to vaccines of several different pathogens, including Ebola,[4] rabies,[14] tuberculosis,[5] and *S. aureus*.[11] In one notable study

published in *Scientific Reports* in 2019, the authors heavily relied on the high-throughput and quantitative ability of Simple Western to evaluate serological immunoglobulin G (IgG) levels to develop new vaccine candidates against tuberculosis (TB).[5] With the high-throughput of Simple Western, they performed a large-scale screen of 219 antigens in 90 pulmonary TB patients to identify vaccine candidates. With the quantification of Simple Western, they determined the ratio of serum IgG expression level to the commercial antigen Rv0934 (**FIGURE 2**). Proteins with a ratio greater than 1 became candidate antigens. Such a large-scale screen would have been unmanageable with traditional Western blot, and that is not to mention that traditional Western blot is hardly quantitative.

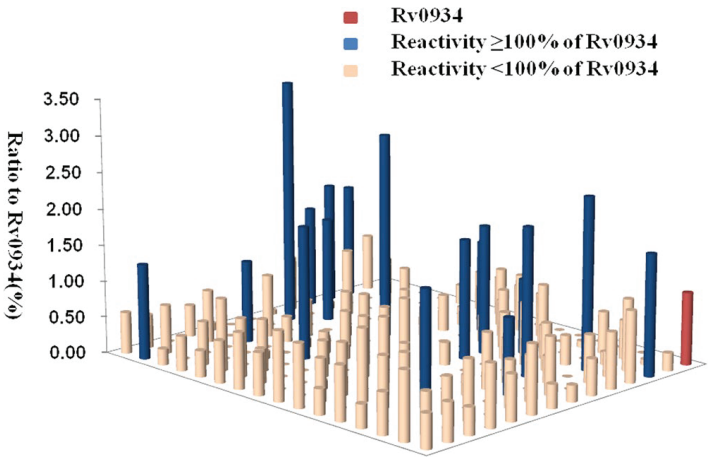

FIGURE 2. Serum IgG levels of 219 membrane proteins in 90 pulmonary TB patients determined by Simple Western. The heights and colors of the columns indicate the reactivity of each protein, as evaluated based on the ratio of reactivity between the tested protein and commercial Rv0934 protein. The red column represents the commercial Rv0934 protein, for which ratio was set to 1. The yellow columns represent proteins for which a response by serum obtained from active TB patients was obtained but with a reactivity ratio below 1. The blue columns represent proteins with a ratio above 1. Adapted with permission from Li *et al*.[5]

SIMPLE WESTERN CAN ANALYZE PRECIOUS SAMPLES LIKE EXOSOME-BASED VACCINES

Exosomes are rapidly gaining attention as new vaccine and drug delivery systems.[3] Exosomes are lipid nanovesicles naturally shed by cells, and they have several properties that make them promising candidates for vaccine and drug delivery. They are stable, safer than cells and viruses because they cannot propagate, and they can be engineered to carry custom biomolecules, including vaccine antigens and adjuvants. However, exosomes are difficult to isolate and sample sizes are extremely limited for proteomic analysis. Simple Western is the ideal solution for studying exosomes because it requires only 3 µL of sample and offers picogram-level sensitivity, reducing the number of exosomes typically required for traditional Western blot. For example, Codiak Biosciences has relied on Simple Western in their versatile platform to engineer exosomes as vaccines and other defined therapeutics.[3] For more information on characterizing exosomes with Simple Western, see our webpage at proteinsimple.com/extracellular-vesicle-protein-analysis-with-automated-western-blotting.html.

SIMPLE WESTERN CAN ANALYZE MULTIPLE, LARGE ANTIGENS SIMULTANEOUSLY

In addition to the advantages listed thus far, another major of advantage of Simple Western is its ability to analyze high molecular weight proteins, up to 440 kDa. This is well above what can be easily achieved with traditional Western blot, and Simple Western has been used for the analysis of large protein antigens like the 308 kDa TcdA toxin of the *C. difficile* vaccine.[2] It also has the multiplex capabilities known as superplex and RePlex to analyze multiple proteins simultaneously or perform total protein detection. Measuring multiple antigens of diverse molecular weights in a single immunoassay can be leveraged for monitoring the humoral response. For exactly this purpose, ProteinSimple offers a SARS-CoV-2 Serology Assay to detect and quantify the humoral response to five key SARS-CoV-2 antigens. Simple Western has also been leveraged for COVID-19 vaccine development at DIOSynVax.

The references below offer further reading on how Simple Western is used to study and develop vaccines:

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