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PUBLICATION SPOTLIGHT

SIMPLE WESTERN GUIDES PROCESS DEVELOPMENT OF AN EBOLA VACCINE

VACCINES: POWERFUL WEAPONS AGAINST INFECTIOUS DISEASES

As powerful weapons to prevent and eradicate infectious diseases, vaccines are in continual demand to battle new outbreaks that emerge worldwide. Among such outbreaks are those caused by the extremely deadly ebolavirus (**FIGURE 1**) that have struck Africa in recent years, which had mortality rates up to 90%.[1] Central to early vaccine development is the assessment of key protein antigen expression levels, while final product purity must be carefully monitored in late-stage development to avoid dangerous immunogenic side effects. Though Western blot has traditionally been used for these purposes, its laboriousness and irreproducibility often hamper the ability to bring vaccines to market in time to win the battle against new pathogens. Thus, a new solution is needed to streamline protein detection and quantification in vaccine process development.

SIMPLE WESTERN COVERS THE ENTIRE VACCINE DEVELOPMENT PIPELINE

With its automation, reproducibility, sensitivity, and quick time to results, Simple Western has proven to be an invaluable tool in replacement of traditional Western blot across the entire vaccine development pipeline.[2-5] For example, Simple Western can be used to measure protein in crude cell lysates in upstream samples,[2,3] as well as in samples further downstream like detecting impurites[4] and final product release, stability, and characterization.[5] Recently, a vaccine against the Zaire ebolavirus was demonstrated to be safe and effective in humans, resulting in approval by the FDA and EMEA in 2019. Because of its advantages of increased linear range, higher throughput, and enhanced reproducibility, Minsker and colleagues turned to Simple Western instead of manual Western blot to guide process development of the Ebola vaccine.[1] In this Publication Spotlight, we provide more detail on how Simple Western was used to characterize Ebola vaccine development, and how Simple Western applies to vaccine development in general.

HOW THE EBOLA VACCINE WORKS

The Ebola vaccine is based on a recombinant vesicular stomatitis virus vector carrying the Zaire ebolavirus glycoprotein (GP), the antigen that elicits an immune defense against the virus. The GP is synthesized as a single polypeptide and is cleaved by a protease to create a heterodimer consisting of GP1 and GP2 subunits linked together by a disulfide bond. Neutralizing antibodies against membrane-bound GP likely play an important role in protection against the lethal ebolavirus, while the cell-mediated immune response involves a robust T-cell response.[1]

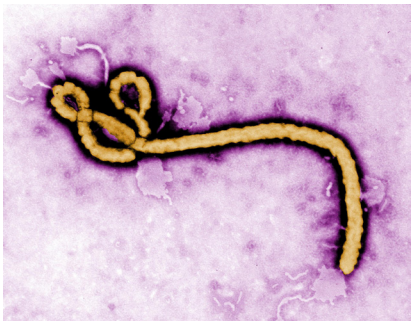

FIGURE 1. Colorized transmission electron micrograph of the ebolavirus by CDC microbiologist Frederick A. Murphy (CC BY 2.0).

SIMPLE WESTERN CHARACTERIZES EBOLA VACCINE DEVELOPMENT IN EACH PROCESS STEP

The biosynthesis of the Ebola vaccine occurs in Vero cells, from which it is purified, enzymatically digested, concentrated, and buffer-exchanged into a final drug product. From upstream viral harvest to downstream drug product formulation, all steps of this process are monitored by Simple Western using an anti-Zaire ebolavirus GP antibody (**FIGURE 2**).[1] Due to differences in molecular weights, the GP antigen and its variants, including the GP1 and GP2 subunits, are resolved and quantified automatically in a single run. Because Simple Western is a highly specific and sensitive immunoassay, these glycoproteins can be characterized in all purification steps, upstream or downstream and regardless of purity. This is in contrast to CE-SDS, which is not as sensitive or specific as Simple Western, requiring at least partially pure and concentrated material for detection.[3]

Because the electropherogram profiles are unique based on the corresponding process step, the profiles can be used for each process step as an identity signature to assess process performance during development.[1]

Minsker and his coworkers further used Simple Western to understand the types and quantities of GP variants generated across the process. For example, Simple Western was useful for characterizing the enzymatic digestion following viral harvest, monitoring changes across the downstream purification steps, and assessing process robustness across multiple lots. Finally, by performing a direct comparison with traditional Western blot, they showed that Simple Western provides a similar separation profile, but with improved reproducibility, quantification, and ease-of-use.[1]

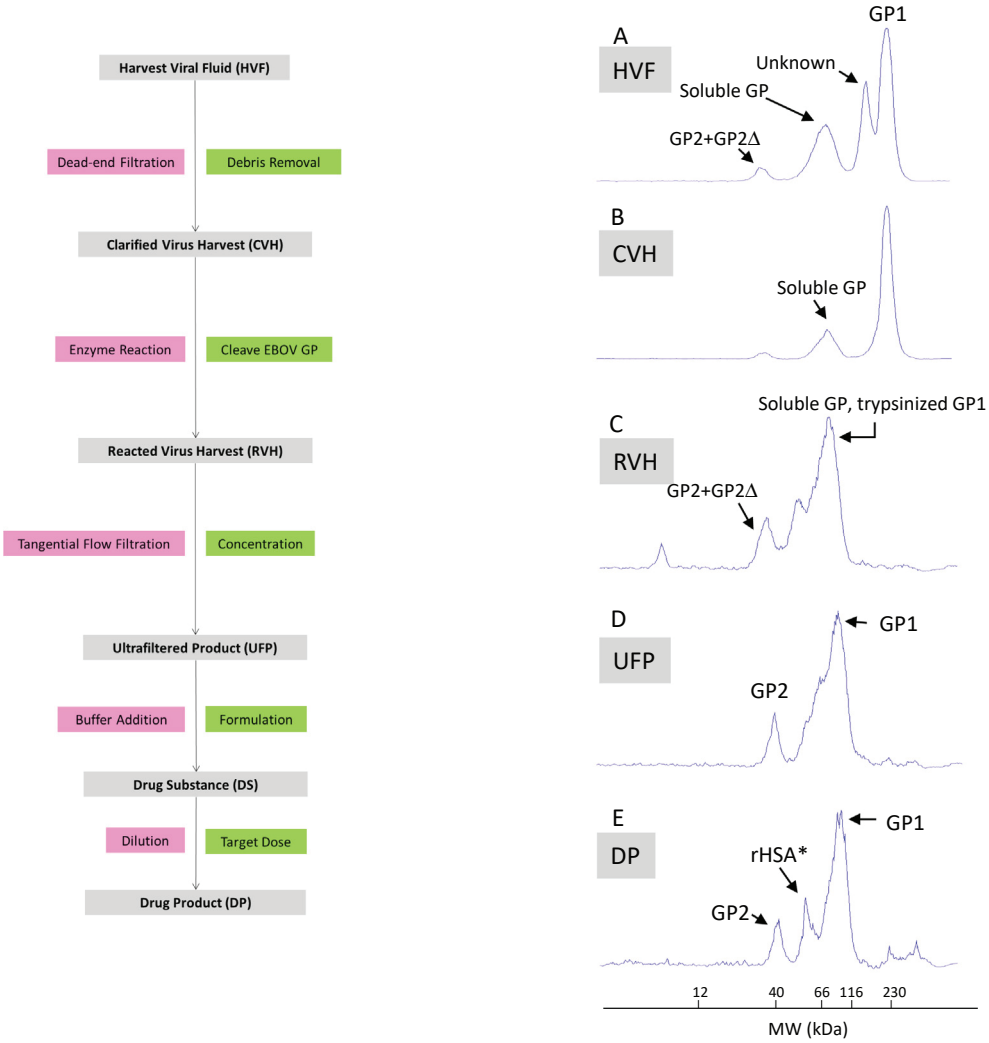

FIGURE 2. Process Step Electropherogram profiles obtained by Simple Western. (**A**) Harvest Virus Fluid (HVF) consists of GP1 (260 kDa), unknown (190 kDa), soluble GP (95 kDa), and GP2 + GP2D (40 kDa); (**B**) Clarified Viral Harvest (CVH) consists of GP1 (260 kDa), soluble GP (95 kDa), and GP2 + GP2D (40 kDa), the unknown 190 kDa peak is removed; (**C**) Reacted Viral Harvest (RVH) consists of trypsinized GP1, soluble GP (95 kDa), GP2 + GP2D (40 kDa), and lower MW proteins (<12 kDa) – likely GP1 fragments resulting from trypsin treatment; (**D**) Ultrafiltered Product (UFP) consists of GP1 (95 kDa) and GP2 (40 kDa), all soluble GP and shed GP are removed; (**E**) Drug Product (DP) consists of GP1 (95 kDa) and GP2 (40 kDa), an rHSA peak at ~ 60 kDa is observed in DP due to cross reactivity of rHSA with the primary antibody and the DP concentration is about 2-logs lower than in UFP. Adapted with permission from Minsker et al. *Vaccine*, 2020; 38(45):7166–7174.[1]

MOBILIZING A VACCINE-BASED DEFENSE AGAINST NEW PATHOGENS WITH SIMPLE WESTERN

Given the unpredictability of how and when new infectious diseases emerge and the urgency to fight back when outbreaks occur, the vaccine development and approval process must be as nimble and efficient as possible. Against this backdrop, manual Western blots are sorely outdated, creating bottlenecks that slow process development to bring a new vaccine to market. Major progress has been made with Simple Western assays, which can alleviate process development bottlenecks by delivering automated and reproducible protein quantification with a quick time to results. Simple Western also comes with a Total Protein Assay, allowing you to normalize your protein expression data and detect host cell proteins and other impurities. To maximize the amount of data generated per sample, Simple Western instruments like Jess offer RePlex, which efficiently removes antibodies after the first probing cycle for a second round of probing with fresh antibodies or total protein detection, all in an automated fasion. Taken together, Simple Western is a multi-attribute method, delivering identity with specific antibodies and purity with total protein detection. As Simple Western's role in developing a vaccine candidate against the COVID-19 pandemic further exemplifies,[6] the future of vaccine development is looking bright with Simple Western.

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