Simple Western Analysis of Adeno-Associated Virus (AAV) Proteins for Cell and Gene Therapy

Nishant Pawa, Ph.D., Anusha Seneviratne, Ph.D. and Tony Bou Kheir, Ph.D., Cell and Gene Therapy Catapult, London, United Kingdom

Caroline Odenwald, Ph.D. and Katja Betts, EMSc, PROGEN, Heidelberg, Baden-Württemberg, Germany

Chris Heger, Ph.D., Yasef Khan, Charles Haitjema, Ph.D. and Daniela Ventro, Ph.D., ProteinSimple, San Jose, CA, USA

Introduction

In gene therapy, a therapeutic transgene is delivered to cells and patient tissues to treat an inherited or developed disease. This genetic reprogramming is typically achieved with viral vectors, the most promising of which is the adeno-associated viruses (AAV)[1]. As a safe and effective vector, AAV is the workhorse of *in vivo* gene therapy, with three approved products and many more currently in clinical trials[1,2]. An AAV particle is composed of 60 capsid protein molecules, with subunits of VP1, VP2 and VP3 at a ratio of 1:1:10[1]. The subunits are encoded by the *cap* gene and are created by alternative splicing and translation from different start codons.

During AAV manufacturing, critical quality attributes must be monitored, including the presence, identity and purity of viral vector proteins[3]. Traditionally, the identity of these proteins is monitored by Western blot using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). However, Western blotting is notoriously challenging; it's labor-intensive, suffers from poor reproducibility, and is only semi-quantitative. For this reason, the Cell and Gene Therapy Catapult (CGTC), an independent center of excellence at the forefront of technology and innovation for cell and gene therapy commercialization, is adopting next generation technologies to advance quality control (QC) of AAV-mediated gene therapy[3]. In this application note, you'll see how CGTC has used highly-specific antibodies exclusively manufactured by PROGEN with fully automated Simple Western™ assays on Wes™ to monitor and characterize AAV capsids during product purification.

How Simple Western Does AAV Protein Analysis Better

Simple Western assays are fully automated, capillary-based immunoassays that separate and analyze proteins by size from 2 kDa to 440 kDa. Unlike traditional Western blotting methods, this means Simple Western assays are reproducible and quantitative. They require only 3 µL of your sample to get pg-level sensitivity and automatically analyzed results within 3 hours! These are major advantages for the analysis of AAVs, which are difficult to manufacture and for which demand currently outpaces supply[4]. And, with 21 CFR 11-compliant Compass for Simple Western software, you'll be all set for adoption of Simple Western in a good manufacturing practice (GMP) environment. In a nutshell, Simple Western assays are easy-to-use, low-waste and single-use immunoassays that reduce the time and complexity of AAV manufacturing workflows!

Simple Western plugs and plays throughout the AAV product development pipeline, from measuring the expression of novel AAV capsids, to downstream manufacturing and QC, where accurately measuring product identity and purity are imperative[3]. Herein, AAV capsid proteins were clearly separated and identified via Simple Western assays on Wes. Total protein was also monitored to detect the presence of process-related protein impurities during purification, making Wes a valuable tool for the advancement of QC workflows for AAV-based gene therapies.

Materials & Methods

The default Wes sample preparation and assay conditions were followed using the 12–230 kDa Wes Separation Module (PN SM-W004) and the Anti-Mouse Detection Module (PN DM-002). For total protein detection, the instructions were followed in the Total Protein Detection Module for Wes (PN DM-TP01). Anti-AAV VP1/VP2/VP3 mouse monoclonal (PN 61058), anti-AAV2 VP1/VP2 mouse monoclonal (PN 61057) and anti-AAV2 VP1 mouse monoclonal (PN 61056) antibodies were obtained from PROGEN. AAV2 was purchased from Vigene Biosciences (custom order), or internally produced and purified at CGTC.

Identification of VP1/2/3 during AAV Purification

AAV2 was purified from whole HEK293 cell lysate using affinity chromatography, and the steps of the purification process (load, flow-through, wash and elution) were monitored on Wes. This analysis showed the presence of VP1, VP2 and VP3 proteins in the elution fraction using the immunoassay (**Figure 1A**) and total protein (**Figure 1B**). Less dilute samples were loaded for the immunoassay to detect low abundance immuno-reactive species. Closer analysis of the elution fraction revealed that the signal intensity of VP1 and VP2 were approximately equal, while VP3 had a significantly stronger signal (**Figure 2**).

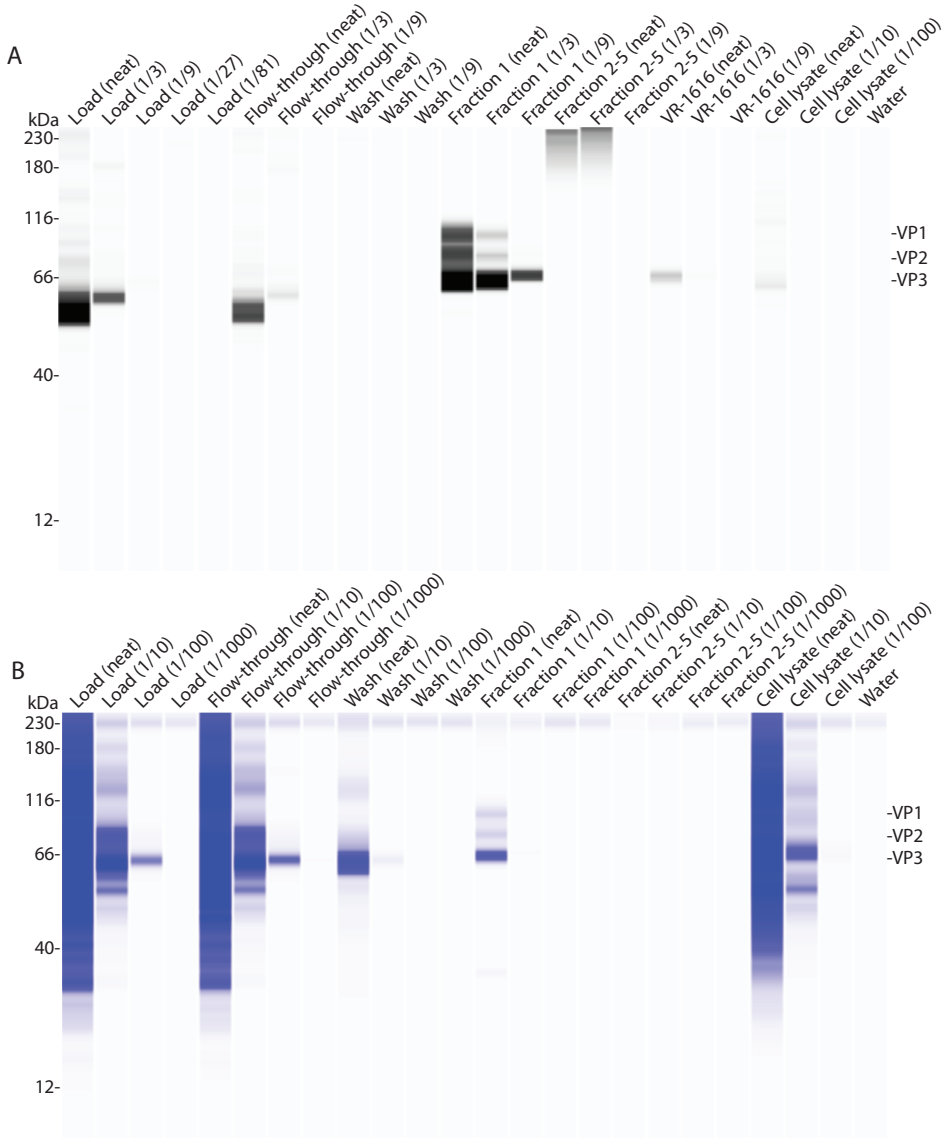

FIGURE 1. (A) Immunodetection of VP1, VP2 and VP3 proteins during purification from whole-cell lysate. Detection was performed with an anti-AAV VP1/VP2/VP3 mouse monoclonal antibody. (B) Total protein detection of each fraction. Load: input material loaded onto the columns; flow-through: material not bound on columns; wash: wash buffer from columns; fractions: eluate fractions from columns; VR1616: ATCC purified reference material loaded as a positive control (low titer); cell lysate: HEK293 cell lysate (without virus); water: negative control. Sample dilutions are shown in brackets.

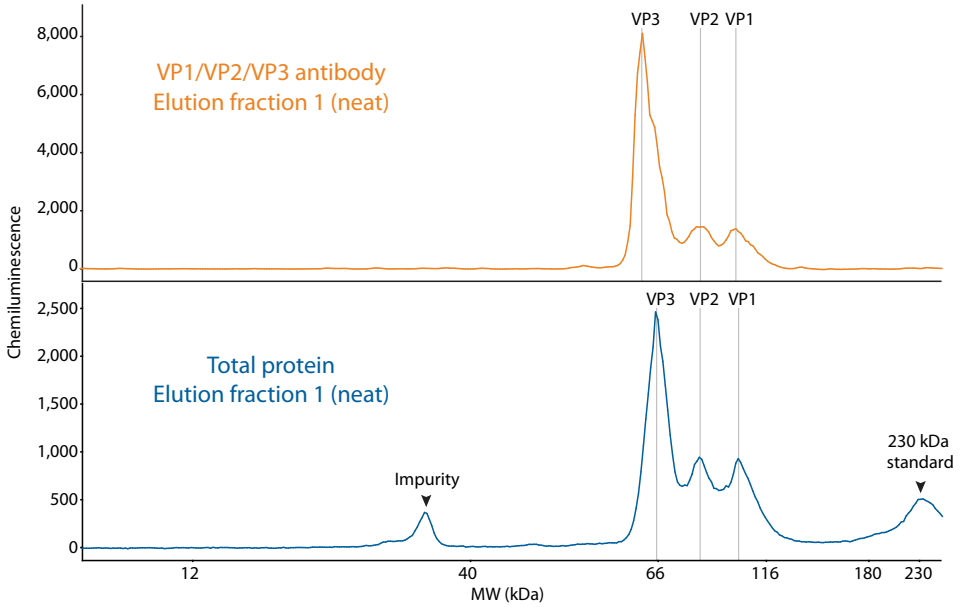

FIGURE 2. Electropherogram analysis of the elution fraction from the immunoassay (top panel) and total protein analysis (bottom panel). The immunoassay detection was performed with an anti-AAV VP1/VP2/VP3 mouse monoclonal antibody.

The total protein analysis of this fraction also showed an impurity of just under 40 kDa and the presence of the 230 kDa internal standard, whose presence is expected (**Figure 2**). The detection of impurities shows that Simple Western assays can also be used for bioprocess contaminant detection, including host cell proteins, and purification or media additives. Appropriate for these purposes, Simple Western assay sensitivity on Wes rivals traditional approaches, such as ELISA, and also provides information on molecular weight, degraded products, and oligomerization state, among other attributes.

The results presented in **Figure 1** and **Figure 2** leverage a single antibody (anti-AAV VP1/VP2/VP3 mouse monoclonal antibody) that detects all three AAV capsid subunits. Wes is an open platform amenable to any antibody, meaning other targets may be detected simply by swapping in different antibodies. For example, to detect specific capsid proteins, antibodies targeting AAV capsid proteins VP1/VP2/VP3, VP1/VP2 or VP1 were screened against commercially available AAV2 (**Figure 3**), enabling the identification of individual proteins in addition to all three. Because up to 25 different antibodies may be tested on a single Wes run, screening multiple antibodies is straightforward with Simple Western assays.

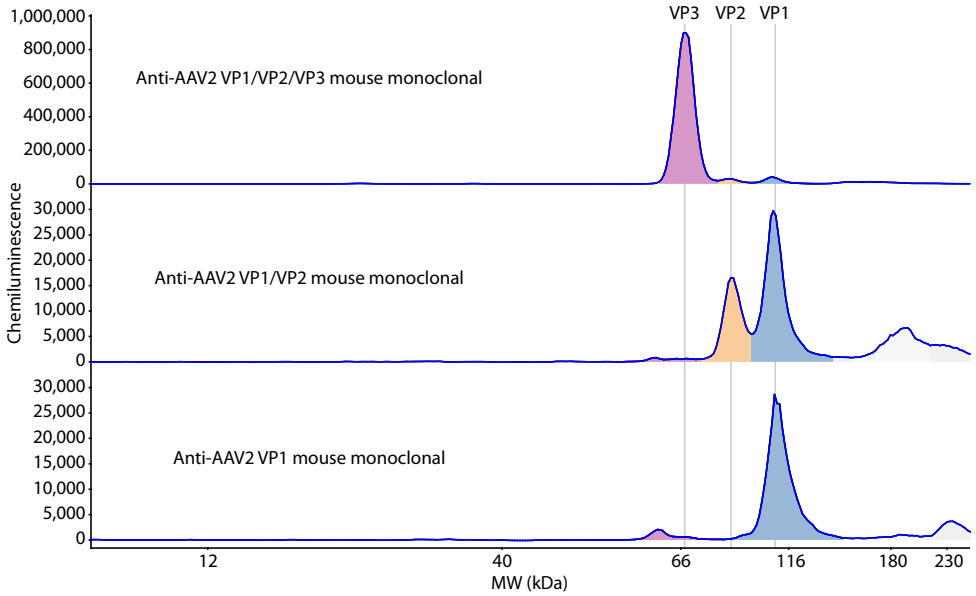

FIGURE 3. Antibody screen targeting VP1/VP2/VP3 proteins (top panel), VP1/VP2 proteins (middle panel) and VP1 only (bottom panel).

To determine the range of detection, commercially available AAV2 ($1x10^{13}$ GC/mL, 33.8 µg/mL) was subjected to a 2X dilution series from 1:8 to 1:256 and analyzed on Wes. The anti-AAV VP1/VP2/VP3 mouse monoclonal antibody was used to detect each capsid protein (**Figure 4A**). The total peak area of VP1/VP2/VP3 was calculated and plotted at each dilution factor (**Figure 4B**). This analysis indicated that detection of AAV proteins was possible right down to the lowest dilution tested of 1:256 (**Figure 4B**). Thus, Simple Western assays allow for sensitive detection of target proteins, as this corresponds to just 400 pg of protein loaded per well.

Conclusion

Simple Western assays are automated immunoassays also capable of total protein analysis, which greatly simplifies AAV product development workflows. In less than 3 hours, you can analyze up to 25 samples per run on Wes—all in a fully automated fashion! These advantages, combined with the small sample size requirement (3 µL), make Simple Western a powerful analytical tool throughout the AAV product development pipeline. For example, Wes can be applied for small scale testing and formulation in small bioreactors, allowing for process monitoring and optimization without sacrificing large amounts of precious sample. Here, AAV capsid proteins were monitored and quantitated on Wes during purification from whole-cell lysate. The VP1, VP2 and VP3 capsid proteins could be detected and measured either individually or simultaneously, depending on the AAV antibody used for detection. In addition to identity, other critical quality attributes to consider in AAV manufacturing include purity, potency, stability and safety. With additional antibodies, Simple Western assays can be extended to address these other attributes, including for measuring host cell proteins (purity), target protein expression (potency), presence over time (stability) and contaminants like Mycoplasma (safety).

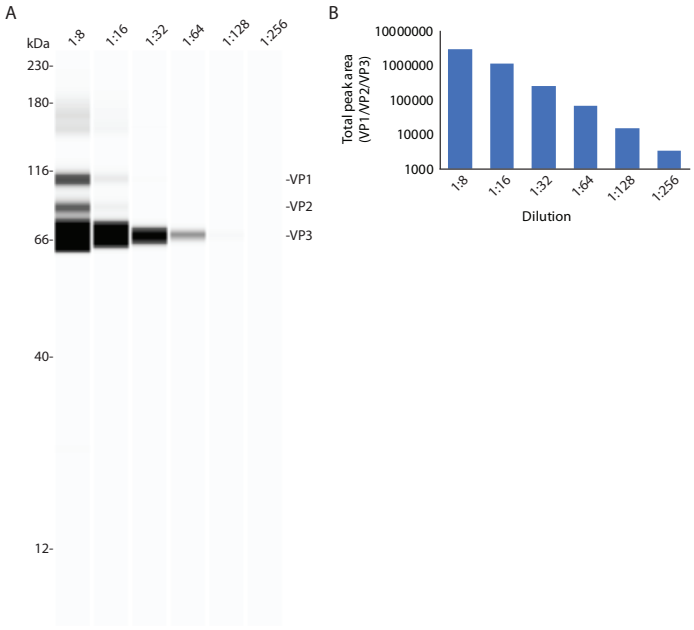

FIGURE 4. Range of detection of the anti-AAV VP1/VP2/VP3 mouse monoclonal antibody. (A) Lane view of VP1/VP2/VP3 detection of each dilution. (B) Total peak area of VP1/VP2/VP3 by dilution factor.

References

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Acknowledgment

This work was funded by Innovate UK, and performed in collaboration with Professor Farzin Farzaneh's group at Kings College London and Guys & St Thomas NHS Foundation Trust (GSTT).

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Toll-free: (888) 607-9692
Tel: (408) 510-5500
info@proteinsimple.com
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