Smartphone-based titration of AAV vector preparations

Thomas P. Quinn*, Lily Lee, Mei Fong, Andre Dharmawan, Yi Zhao, Myn Uddin, Michael Haugwitz & Andrew Farmer

Takara Bio USA, Inc., San Jose, CA 95131 USA *Corresponding author: thomas_quinn@takarabio.com

Abstract

Adeno-associated virus (AAV)-based vectors have gained widespread recognition for their potential in gene therapy, thanks to their exceptional safety profile and ability to sustain long-term gene expression. With over 130 clinical trials and eight approved gene therapy products, AAV has emerged as one of the most reliable and versatile tools for delivering therapeutic DNA directly into living organisms. The AAV expression system offers several advantages, including efficient transduction, broad tropism, stable high yields, and compatibility with gene-editing components.

Recombinant AAV is typically produced through the co-transfection of HEK 293 cells with vectors containing an inverted terminal repeats (ITR)-flanked expression cassette, adenoviral helper sequences, and AAV *Rep* and *Cap* genes. Successful application of a recombinant AAV vector relies on knowing the titer of the AAV preparation, which permits calculation of the multiplicity of infection (MOI), which can influence the final expression level of the recombinant protein in transduced cells. Quantitative real-time polymerase chain reaction (ddPCR or qPCR), enzyme-linked immunosorbent assay (ELISA), cell-based infectious unit assays, and transmission electron microscopy (TEM) have all been used to determine AAV titers. However, these methods are time-consuming and labor-intensive, with time to results ranging anywhere from two hours to multiple days.

In this work, we present an iOS- and Android-compatible smartphone application (app) that analyzes a serotype-specific lateral flow assay and can deliver particle number values in 10 min when a reference virus with a known vector genome copy number or infectious titer is used. The simplicity of the assay facilitates easy monitoring and optimization of AAV production processes to ensure consistency and confidence in downstream applications. The two-step assay consists of adding a small amount (20 µl) of AAV-containing supernatant to the lateral flow device, followed by imaging and analysis of the results using a smartphone. Densitometric analysis of the observed bands is performed by the intuitive GoStix™ Plus software, which compares the results to an automatically downloaded, lot-specific standard curve (R^2 greater than 0.990). The result is a GoStix Value (GV) that, like an IFU or qPCR assay, can be used to normalize virus stocks before being used for transduction and expression of a recombinant protein.

To demonstrate the utility of GoStix Plus assays compared to ELISA, qPCR, and infectious unit assays, we used a ZsGreen1-expressing AAV2 vector. The tests demonstrated titer values similar to those generated by current titration methods, with coefficients of variation of less than 15%, indicating comparable precision to ELISA-based approaches. In addition, we were able to quantify AAV in both crude and purified preparations for titers ranging from 5×10^8 to 1×10^{10} particles/ml. In summary, this highly convenient, lateral flow-based titration technology can quantify AAV vector preparations in approximately 10 min, reduce expenses related to labor and materials, and accelerate AAV vector production and application.

1 Timelines for different AAV titration methods

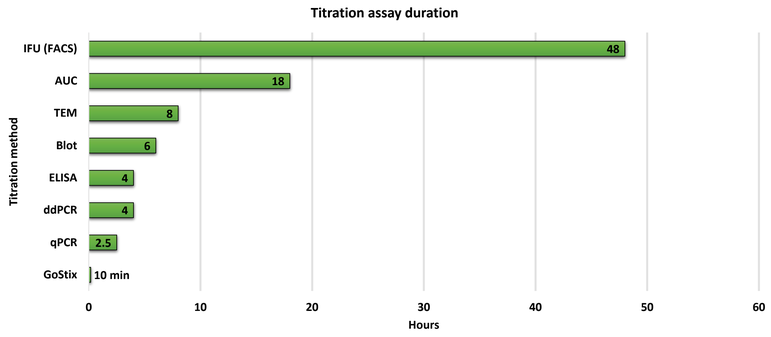

Figure 1. The timelines associated with most commonly used methods of AAV vector titration, as measured in hours. IFU: measurement of cells transduced by AAV particles followed by FACS analysis for gene expression. AUC: analytical ultracentrifugation. TEM: transmission electron microscopy of particles. Blot: quantitation by western blot for AAV capsid proteins. ELISA: measurement of AAV particles by serotype-specific ELISA. ddPCR: quantitation of viral genomes by ddPCR. qPCR: quantitation of viral DNA genomes by quantitative PCR. GoStix: quantitative detection of AAV capsid proteins using a lateral flow-based method.

2 Workflow for AAV titration using GoStix Plus software

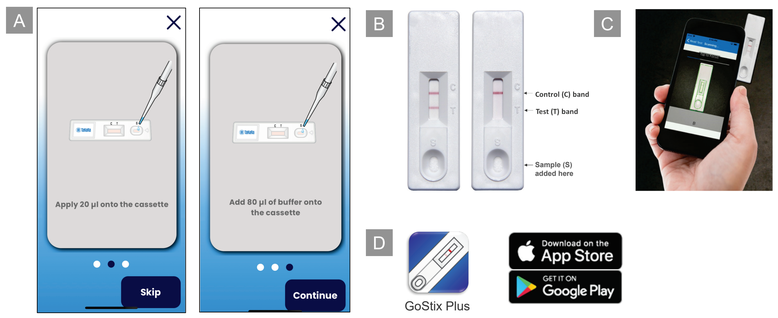

Figure 2. AAV titration is fast and easy with the GoStix Plus app. Panel A. To use this lateral flow assay to detect AAV particles present in samples, simply apply 20 µl of culture medium, followed by the addition of Chase Buffer and incubation at room temperature for 10 min. **Panel B.** Test and control bands develop during the 10-min incubation time. **Panel C.** Band intensities are captured, analyzed, and quantitated using the GoStix Plus smartphone app. **Panel D.** The GoStix Plus app can be downloaded free of change from the App Store (iOS) or Google Play (Android).

3 GoStix Plus app supports analysis of multiple analytes: IL-2, IFN-γ, TNF-α, IL-6, baculoviral gp64, AAV2/8/9, dsRNA & lentiviral p24

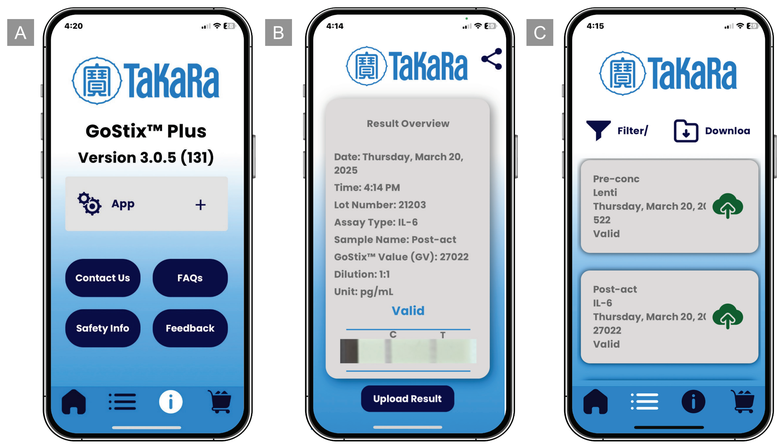

Figure 3. Screenshots from the GoStix Plus app. Panel A. The "Home screen" allows intuitive navigation of data and additional resources for integrating GoStix into your research. **Panel B.** The "Results overview" page displays the Test (T) and Control (C) band signals acquired automatically by the software, along with the GV generated through densitometric analysis. The software calculates the T/C ratio and compares it to a lot-specific control curve (see **Figure 4**, **Panel B**) to generate the GV. Samples are flagged as off-scale when the T/C ratio approaches 1, indicating the need for further dilution. The output includes a timestamp, control curve lot number, assay type, sample name, GV, sample dilution, and units of measurement. **Panel C.** The "Data registry" lists all current readings stored on the device. This data can be exported as a CSV file for formal recording and further analysis.

4 Lot-specific standard curves are used by GoStix app for titration

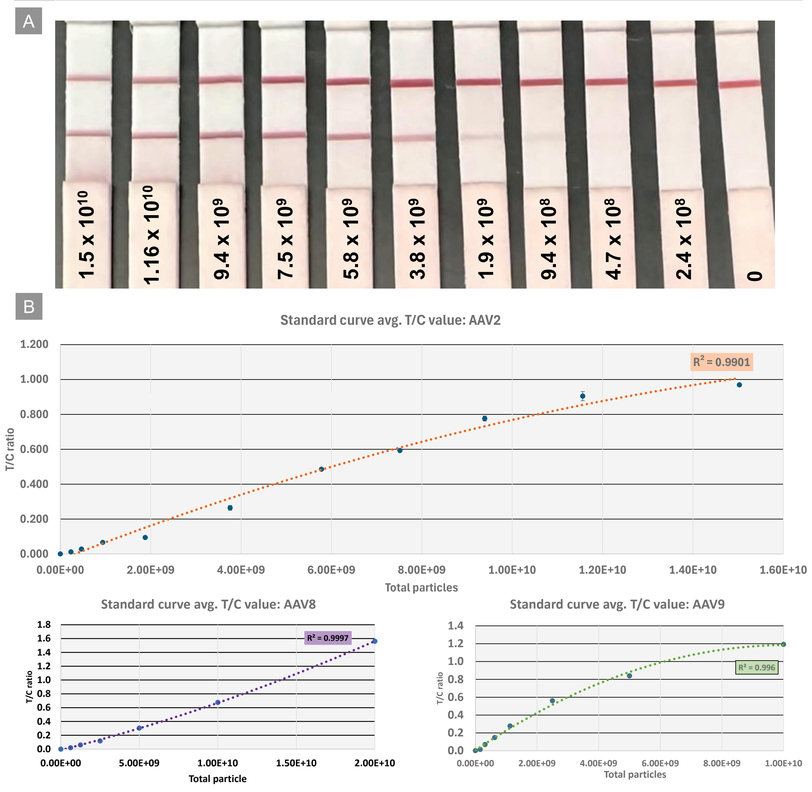

Figure 4. Generation of lot-specific standard curves. Panel A. For each AAV serotype (AAV2 is shown), a dilution series of viral particles is prepared in Chase Buffer and added to the GoStix Plus cassette in triplicate, developed for 10 min, and analyzed using the GoStix Plus app on an iOS device (iPhone 14 was used, Android version also available). **Panel B.** T/C ratios for each number of particles are then plotted to generate the standard curve. The results of the reads are then used to create the control curve (R^2 shown). This curve is downloaded to the application upon starting the app on any smartphone with an internet connection. Control curves for AAV2, AAV8 and AAV9 are shown. For all graphs, the dots represent the iOS T/C ratio, and the dotted line represents the polynomial regression line (best fit) from which the GV is calculated.

5 AAV GoStix Plus-based titers are comparable to qPCR- and ELISA-based titers

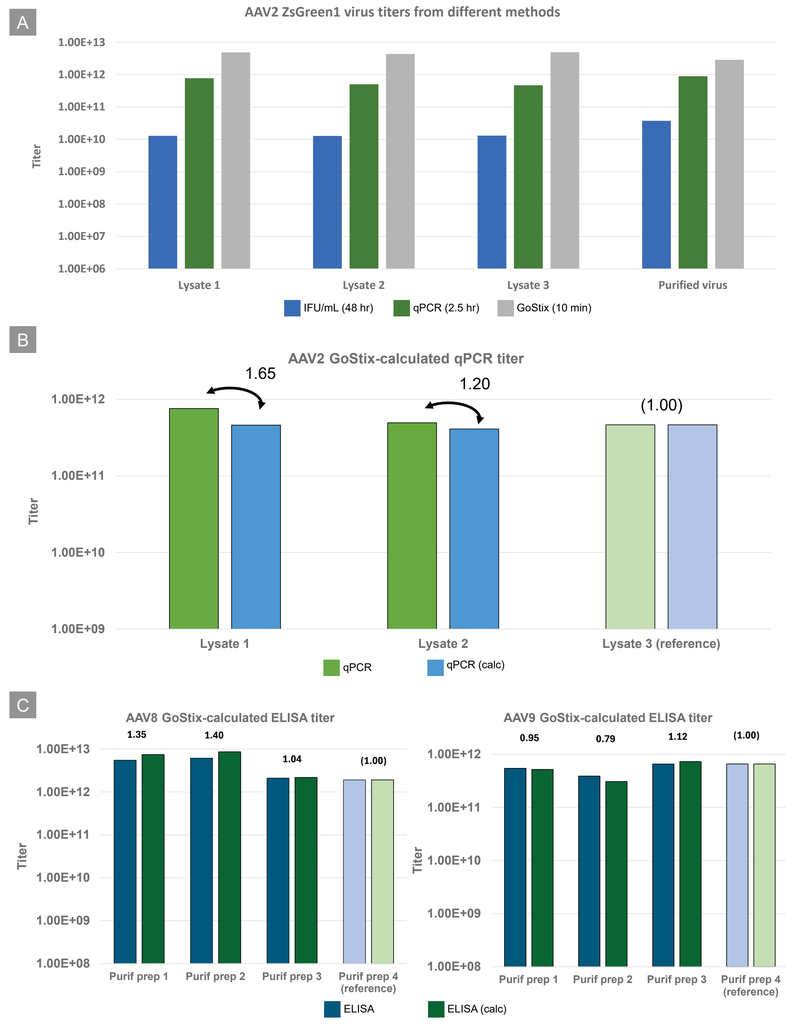

Figure 5. GoStix Plus generates titers comparable to those from established quantification methods. Panel A. Replicate preparations of AAV2 expressing ZsGreen1 were generated according to the manufacturer's instructions using the AAVpro® Helper Free System (AAV2) (Cat. # 6230). Cell lysates were prepared, and clarified supernatants were analyzed by qPCR (AAVpro Titration Kit (for Real Time PCR) Ver.2; Cat. # 6233), AAV2 GoStix Plus, and an IFU assay using HT1080 cells, assessed by FACS at 48 hr post-transduction. Representative titers obtained from each assay are shown**. Panel B.** Using lysate 3 as a reference, relative qPCR titers, labeled "qPCR (calc)," for lysates 1 and 2 were calculated based on the GoStix/qPCR ratio of lysate 3. The fold difference between actual qPCR titers, labeled "qPCR (calc)," for lysates and those estimated from GVs is shown. **Panel C.** Purified AAV8 and AAV9 preparations were titrated using both ELISA and GoStix Plus. Preparation 4 was used as a reference to determine the ELISA-equivalent value, labeled "ELISA (calc)," from GoStix Plus readings. The fold difference between actual ELISA titers and those estimated from GVs is shown. A strong correlation was observed between GoStix-calculated values and titers derived using qPCR (AAV2) or ELISA (AAV8 and 9).

Conclusions

- The GoStix Plus method provides fast and easy titration of AAV2, 8, and 9 preparations
- GoStix Plus results correlate well with those of ELISA, qPCR, and an infectious units (IFU) or assays, but are delivered in just 10 min
- The speed and ease-of-use of GoStix Plus assays permit real-time monitoring of AAV production and can serve as a complement to or replacement for more standardized, but labor-intensive methods
- The GoStix Plus app, with its updated user interface, yields titer measurements with high reproducibility across different mobile devices
- Additional GoStix Plus assays are available for measuring samples containing AAV, baculovirus, lentivirus, adenovirus, and cytokines.

Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999

800.662.2566
takarabio.com