Smartphone-based titration of baculoviral and AAV vectors

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Abstract

Baculoviral expression systems are well-established in the manufacturing of diverse proteins, including viral vaccines and gene therapy vectors, such as AAV, in which yields can be as much as 10-fold higher than with traditional 293-based methods. The advantages of the baculoviral expression system include flexible product design, manufacturing speed scalability, and inherent safety. Recombinant baculovirus is constructed by first cloning the gene of interest (GOI) into a transfer plasmid. Subsequently, this transfer plasmid is co-transfected with a linearized parental AcMNPV genome into insect cells where homologous recombination takes place to generate a recombinant baculovirus. Successful expression of a recombinant protein relies on knowing the infectious titer of the baculovirus preparation, as it permits calculation of the multiplicity of infection (MOI) which can influence the final expression level of the recombinant protein. Plaque assays, endpoint dilution assays, quantitative real-time polymerase chain reaction, and flow cytometry, have all been used to determine baculovirus titers. However, these methods are time-consuming and labor-intensive, with time to results ranging anywhere from two hours to four days. In this work, we present an iOS and Android-compatible smartphone application that analyzes a gp64-specific lateral flow assay and can deliver plaque-forming unit values (PFU/ml) in 10 minutes when a reference virus with a known infectious titer is used. The simplicity of the assay facilitates easy monitoring and optimization of baculoviral production processes to ensure consistency and confidence in downstream applications. The two-step assay consists of adding a small amount (20µl) of baculoviral 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 that compares the results to an automatically downloaded, lot-specific standard curve. The result is a GoStix Value (GV) that, like a plaque or qPCR assay, can be used to normalize virus stocks before being used for the expression of a recombinant protein. Beginning with the construction and transfection of a ZsGreen1 expressing baculoviral vector, we were able to detect baculovirus as early as 4 days post-transfection (P0). In addition, we were able to demonstrate that these tests can be used to screen viral clones (plaques) for titer after one round of amplification (P1). The tests demonstrated titer values within 3-fold of current titration methods with coefficients of variation of less than 15%. Furthermore, to add to the utility of these lateral flow-based tests, we were also able to demonstrate the ability to detect and quantify baculovirus-produced AAV particles using an AAV capsid-specific antibody. This method will be the focus of our work moving forward. In summary, this highly convenient, lateral-flow-based titration technology can quantify both baculoviral and AAV vector preparations in approximately 10 minutes, reduce expenses related to labor and materials, and accelerate baculoviral vector production and protein expression workflows.

1 Timelines for different baculoviral titration methods

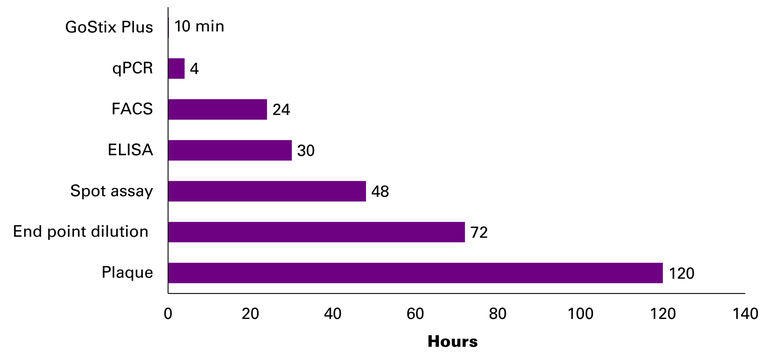

Figure 1. The timelines associated with most commonly used methods of baculoviral vector titration are measured in hours. Baculo GoStix: a lateral-flow-based method for the detection of baculoviral gp64. **qPCR:** quantitation of viral DNA genomes by quantitative PCR. **FACS:** measurement of gp64 by FACS analysis. **ELISA:** measurement of p35 protein from baculovirus-infected cells. **Spot Assay:** Antibody staining of gp64 in infected cells overlayed with agarose. **End Point Dilution:** measurement of TCID50 value. **Plaque:** Quantitation of the number of plaques observed in a monolayer of agarose-overlayed insect cells.

2 Workflow of baculovirus titration using GoStix Plus software

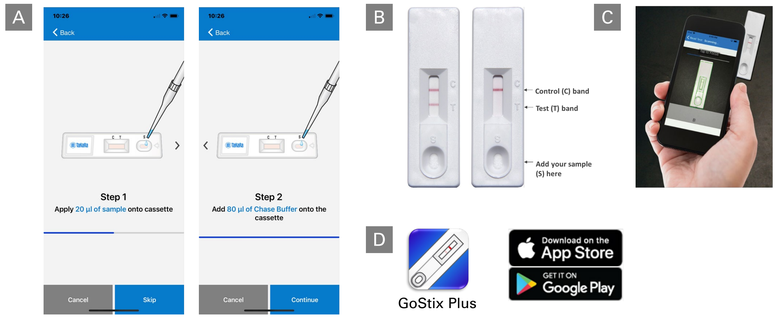

Figure 2. Baculovirus titration is fast and easy with the GoStix Plus app. Panel A. This lateral-flow assay detects gp64 present in samples by simply applying 20 µl of culture medium, followed by the addition of Chase Buffer and incubation at room temperature for 10 minutes. **Panel B.** Test and control bands develop during the 10-minute incubation time. **Panel C.** Band intensities are analyzed and quantitated using the GoStix Plus smartphone app. **Panel D.** The GoStix Plus App can be downloaded from the App Store or Google Play.

3 Establishment of control curve (iOS and Android)

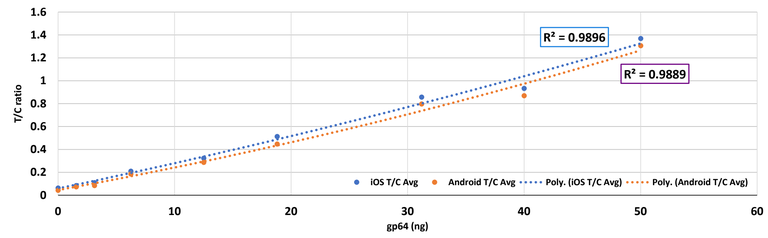

Figure 3. The standard curve was generated from average T/C values. For each lot, a dilution series of recombinant gp64 was prepared in chase buffer and added to GoStix Plus in triplicate, developed for 10 minutes, and analyzed using the GoStix Plus App on both iOS (iPod 7th Gen) and Android (Pixel 6A) devices. T/C ratios for different concentrations of gp64 were plotted to generate the standard curve. The combined result from the two devices was used to generate the final standard curve for the lot. This curve can be downloaded to the application on starting the app on a smartphone with an internet connection.

4 FACS analysis of isolated baculovirus plaques

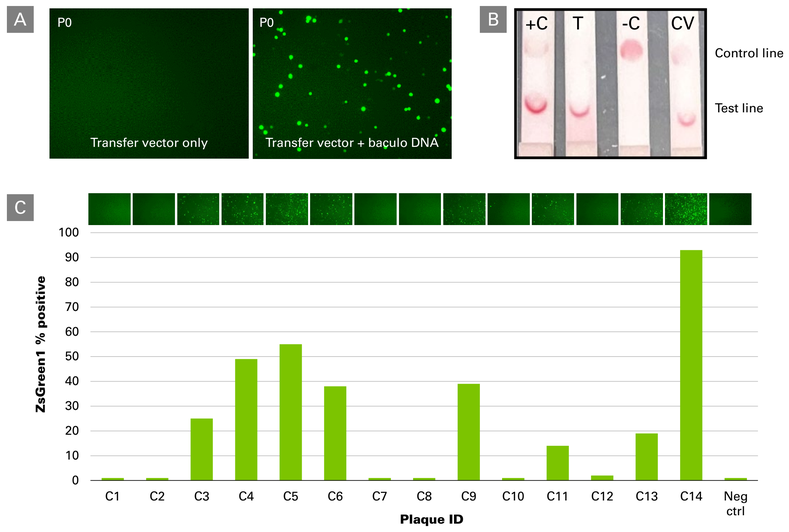

Figure 4. ZsGreen1-expressing clones identified by FACS. Panel A. ZsGreen1 was cloned into BacPAK8 vector using the In-Fusion® Snap Assembly Master Mix (Cat. #638947). The plasmid was co-transfected with pre-linearized BacPAK6 DNA into Sf21 cells using Bacfectin in the BacPAK™ Expression System (Cat. #631402). Fluorescent images taken prior to the Day 4 harvest showed a significant number of ZsGreen1-expressing cells in the co-transfected population. **Panel B.** Supernatant harvested from Day 4 (P0) culture was analyzed on lateral flow strips (spot test). A distinct signal was observed in the co-transfected cells. (+C: recombinant gp64 protein [20 ng]; T: transfected cell supernatant; -C: negative control, buffer only; CV: control virus [8.6 x 10^5 PFU]). **Panel C.** Fluorescent clones were isolated from the transfected plate (P0) 4 days post-transfection and seeded onto fresh Sf21 cells. At 72 hr, cells were imaged by fluorescence microscopy (100X magnification) and the corresponding wells were analyzed by FACS at 96 hr.

5 Comparison of gp64 detection by Baculo GoStix with other titration methods

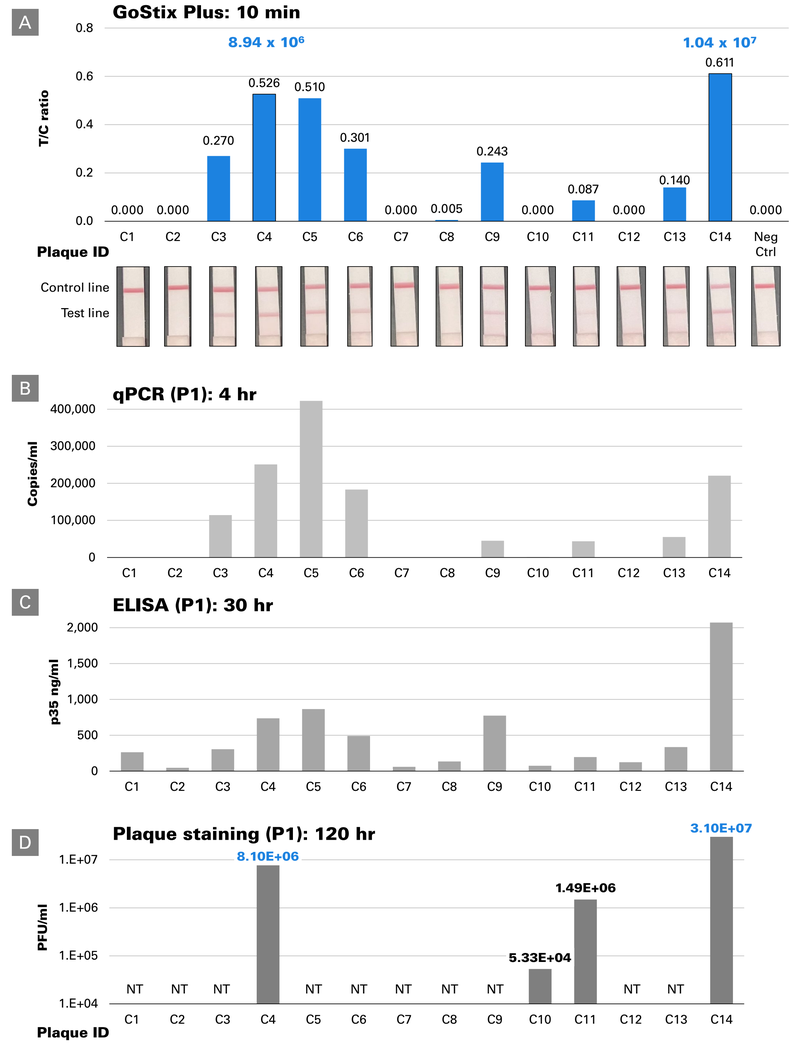

Figure 5. GoStix Plus titration is comparable to other methods. Panel A. Twenty microliters of baculovirus-containing media was added to the GoStix lateral flow tests, chased with 80µl of Chase Buffer, and incubated for 10 minutes. The strips were imaged and then scanned using the GoStix Plus software to determine the T and C line intensities. For Clones 4 and 14, PFU/ml values (**blue**) were calculated using Clone 11 as a reference. **Panel B.** Baculovirus supernatants were analyzed by qPCR for viral genomic DNA using the BacPAK qPCR Titration Kit (Cat. # 631414), which uses gp64-specific primers. **Panel C.** Analysis of ZsGreen1 clones by p35-specific ELISA following infection of Sf21 cells. **Panel D.** Four of the baculoviral supernatants were titered using the BacPAK Baculovirus Rapid Titer Kit (Cat. # 631406). NT: not tested. The assay stains infected Sf21 cells for the expression of gp64 post-infection. There is a good correlation between the physical titration methods (GoStix and qPCR) to the functional methods that require infection (ELISA and plaque staining).The time to obtain each result is shown at the top left of each graph.

6 Another analyte for lateral flow detection: AAV particles

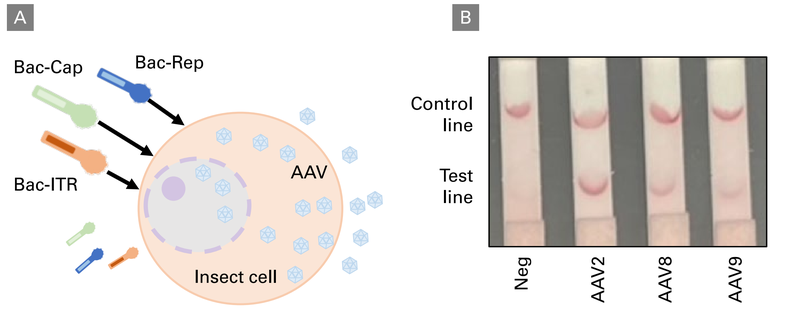

Figure 6. Lateral flow devices detected AAV particles produced in a baculovirus system. Panel A. AAV particles were produced in insect cells by co-infecting Sf9 cells with baculoviruses that express the Rep-, Cap-, and ITR-containing genome of AAV. AAV particles were then harvested and purified. **Panel B**. AAV particles of serotypes 2, 8, and 9 (~4 x 10^{11} vg equivalents) were run on lateral flow tests containing antibodies against AAV capsid protein. In each case, signals were observed for each of the 3 serotypes.

Conclusions

- The GoStix Plus method provides fast and easy titration of baculovirus preparations
- GoStix Plus results correlate well with those of ELISA, qPCR, and PFU assays but are delivered in just 10 minutes
- The speed and ease-of-use of GoStix Plus tests permit real-time monitoring of experiments and can serve as a complement or replacement for more standardized, but labor-intensive methods
- The GoStix Plus App, with its improved user interface, yields titer measurements with high reproducibility across different mobile devices
- The GoStix Plus assay is suitable for measuring samples containing baculovirus, lentivirus, adenovirus, and in the future, adeno-associated virus (AAV; Figure 6).