A Smartphone Application for Quantitative Titration of Lentiviral Vectors

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Abstract

Lentiviruses are extremely versatile viral delivery tools used in a growing number of applications ranging from basic research to clinical applications. For nearly all of these applications it is essential to accurately determine the infectious titer of the vector stock, as it contributes to the transduction efficiency as well as the final integrated copy number within the transduced cells. However, titration protocols are laborious and time-consuming, with timelines ranging from approximately three hours to two weeks. Here we present a smartphone application for the analysis of a lateral flow assay specific for lentiviral p24 protein that can provide infectious unit values (IFU/ml) in just 10 minutes. The application functions on both iOS and Android devices. The assay consists of two steps: addition of lentiviral supernatant to the lateral flow cassette, followed by imaging of the developed bands with a smartphone camera. The application compares the band intensity of the sample to a pre-loaded, lot-specific standard curve and then produces a value that can be used to normalize virus stocks in a manner similar to using an ELISA titration assay. In addition, a reference virus with a known IFU/ml titer can be used to generate an IFU/ml titer for subsequent unknown samples. Using the software to analyze vectors made with several third- and fourth-generation packaging systems, we obtained accurate titer values across a broad range of dilutions demonstrating R^2 values of 0.99 and coefficients of variation of less than 30% when compared to GFP titers on HT1080 cells. Taken together, this quantitative, inexpensive, and highly convenient titration technology can decrease lentiviral vector titration time to approximately 10 minutes, reduce labor and material requirements, and expedite transduction experiments.

Conclusions

- Lenti-X™ GoStix™ are an easy method to verify the relative quantity of your lentiviral supernatants in just 10 minutes
- Lenti-X GoStix can be rendered quantitative through the use of image analysis software
- Lenti-X GoStix Plus titration results are in agreement with standard titration methods (IFU/ml)
- The smartphone app adds an easy, quantitative means for consistent, effective analysis of lentiviral titers via Lenti-X GoStix

1 Timelines for titration methods

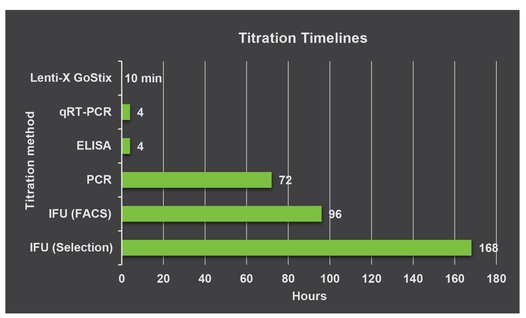

Figure 1. Most commonly used methods of lentiviral vector titration and their associated timelines (in hours). Lenti-X GoStix: a lateral-flow-based method for the detection of lentiviral p24 in supernatants. **qRT-PCR:** quantitation of viral RNA genomes by qRT-PCR. **ELISA:** measurement of the amount of p24 capsid protein. **PCR:** detection of integrated DNA proviruses by qPCR. **IFU (FACS):** determination of the percentage of infected cells via FACS analysis of RFP/GFP positive cells. **IFU (Selection):** infectious units determined by quantifying the number of drug resistant colonies in transduced populations.

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2 Rapid testing of lentiviral supernatants with Lenti-X GoStix

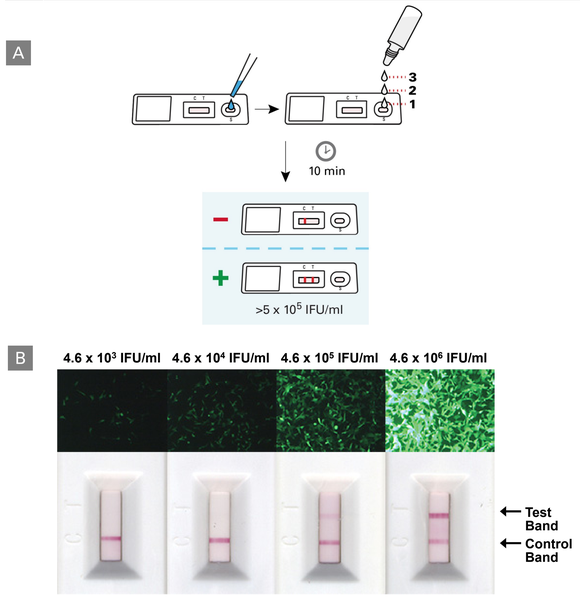
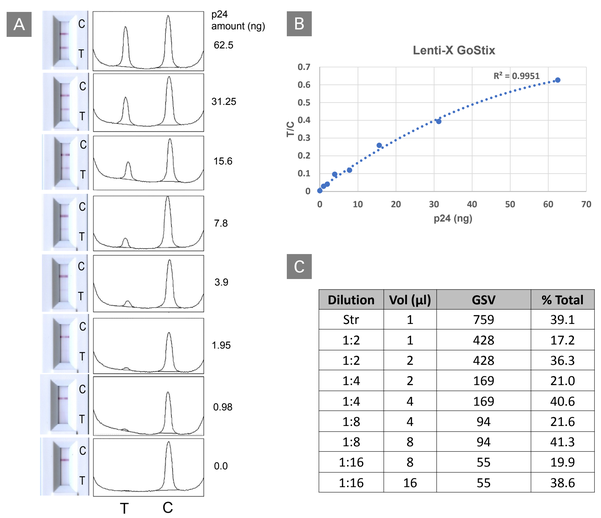


Figure 2. Rapid testing of lentiviral supernatants with Lenti-X GoStix. Panel A. This lateral flow assay detects lentiviral p24 in lentiviral supernatants simply by adding 20 µl of lentiviral supernatant and incubating at room temperature for 10 minutes. The presence of a band signals the presence of usable lentivirus. **Panel B.** In order to approximate the amount of lentivirus in a preparation, lentivirus encoding *ZsGreen1* was prepared using Lenti-X Packaging Single Shots (Cat. # 631275, 631276). Serial dilutions were made and then added to cultures of HT1080 cells for determination of transduction efficiency at 48 hours, as well as analyzed by the Lenti-X GoStix. A clear relationship was observed between the signal intensity on the Lenti-X GoStix and the percentage of cells transduced.

3 Densitometric analysis of Lenti-X GoStix correlates with transduction efficiency



C

Dilution	Vol (µl)	GSV	% Total
Str	1	759	39.1
1:2	1	428	17.2
1:2	2	428	36.3
1:4	2	169	21.0
1:4	4	169	40.6
1:8	4	94	21.6
1:8	8	94	41.3
1:16	8	55	19.9
1:16	16	55	38.6

Figure 3. Densitometric analysis of Lenti-X GoStix correlates with transduction efficiency. Panel A. A dilution series of recombinant p24 was analyzed on the Lenti-X GoStix. The Lenti-X GoStix were then scanned on a flatbed scanner at 300 dpi resolution. Test (T) and Control (C) values were determined by ImageJ-based densitometric analysis. **Panel B.** The T/C ratio was calculated and plotted against the p24 amount (ng) to generate a standard curve. **Panel C.** This standard curve was used to determine the GoStix Value (GSV) of a freshly prepared lentiviral vector stock. These values were then used to determine volumes applied to HT1080 cells. Transduced cells were analyzed by FACS 48 hours post-transduction.

4 p24 production is dependent upon the system used and harvest time

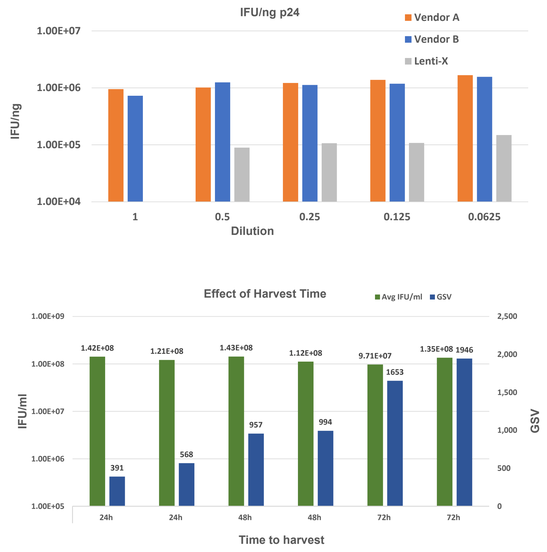


Figure 4. p24 production is dependent upon the system used and harvest time. Panel A. *ZsGreen1* lentivirus preparations were made in accordance with the manufacturer's instructions using Lenti-X Single Shots or one of two other commercially available (third-generation) lentiviral packaging systems. Serial dilutions were made and were applied to Lenti-X GoStix followed by densitometry. Virus dilutions were also titrated on HT1080 cells to determine IFU/ml. Subsequently, the IFU/ng p24 was then calculated for each system. **Panel B.** *ZsGreen1* lentivirus was made using Lenti-X Single Shots and harvested at the times indicated. Lentivirus preparations were applied to Lenti-X GoStix followed by densitometry. Virus dilutions were also titrated on HT1080 cells to determine IFU/ml.

5 Lenti-X GoStix-based titers correlate with transduction titers

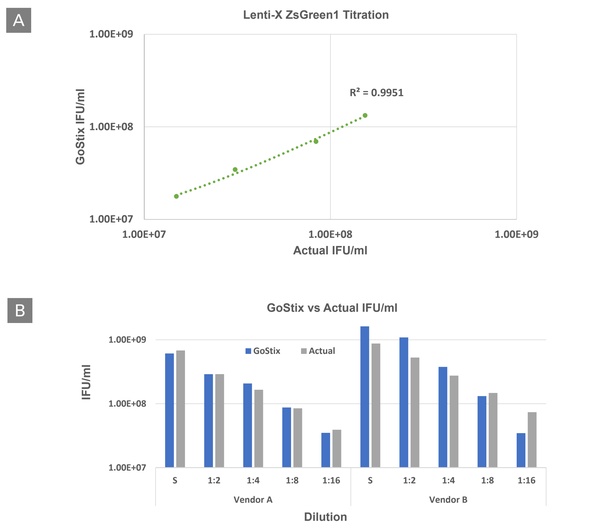


Figure 5. Lenti-X GoStix-based titers correlate with transduction titers. Lenti-X *ZsGreen1* lentivirus was prepared using either Lenti-X Packaging Single Shots (**Panel A**) or one of two other commercially available (third-generation) lentiviral packaging systems. (**Panel B**). Samples for all preparations were serially diluted and added to Lenti-X GoStix, and densitometric analysis was performed. In addition, each dilution was titrated on HT1080 cells to determine actual IFU/ml. The IFU/ml for the unknown samples was calculated using an IFU/ng ratio determined from a reference virus produced using the same packaging system and the same harvest time as the unknown samples. The IFU/ml calculated from the Lenti-X GoStix was then plotted against the actual IFU/ml.

6 Lenti-X GoStix Plus smartphone application provides easy analysis

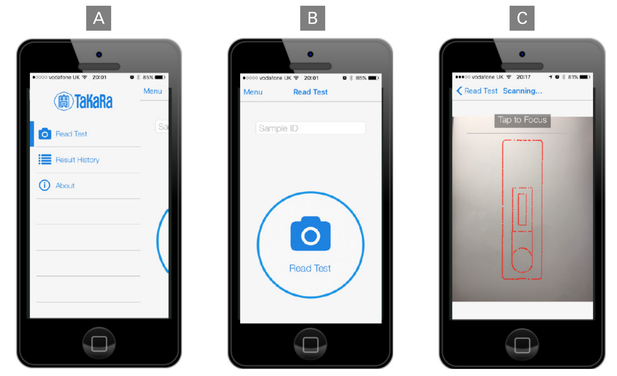


Figure 6. Lenti-X GoStix application pilot screen shots. From the home screen (**Panel A**), select "Read Test" to arrive at the sample acquisition screen (**Panel B**). Click on the "Read Test" camera icon to load the scanning window (**Panel C**). Tap the screen to focus and take a photo of the Lenti-X GoStix. Densitometric analysis of the photo and comparison to a lot-specific p24 standard curve will occur within the app and provide a Lenti-X GoStix Value (GSV).

7 Lenti-X GoStix Plus smartphone application gives results consistent with flatbed scanning

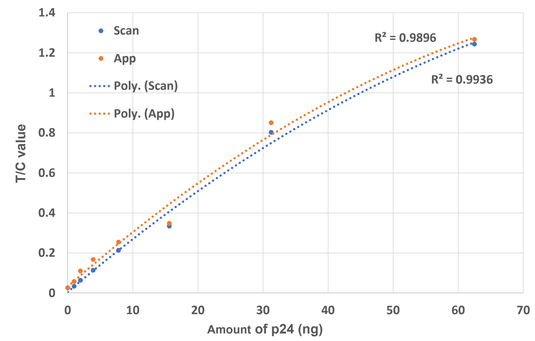


Figure 7. Lenti-X GoStix paired with its companion smartphone app yields results consistent with the flatbed scanning method. A dilution series of recombinant p24 was analyzed on the Lenti-X GoStix. The GoStix were then scanned on a flatbed scanner at 300 dpi resolution or using the Lenti-X GoStix Plus smartphone application. For the scanned samples, test (T) and control (C) values were determined by ImageJ-based densitometric analysis. For both methods, the T/C ratio was calculated and plotted against the p24 amount (ng).

8 Lenti-X GoStix sample

Figure 8. Sample images of Lenti-X GoStix with 3.9 and 31.25 ng of recombinant p24 protein.

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