App Note

Accurate, one-step lentiviral titration with the RNA Module for Countable PCR

Soo-Mi Kweon[1], Emily Blair[2], Jeanette Mei[2], and Robert Lou[1]

Introduction

Accurate viral titration underpins every decision in cell and gene therapy — from vector design to process scale-up. Yet, conventional two-step RT workflows for titrating lentivirus can be unreliable, with high variability and cumbersome workflows — requiring separate steps for reverse transcription and amplification, multiple transfer steps, and variable enzyme efficiencies. This causes minor pipetting errors to accumulate and potentially shift viral counts by millions of particles, complicating downstream infections and process control.

> *The one-step kit really saves time and reduces operational chaos.*
>
> **Robert Lou**, *PhD*
> CTO, **Carrigent**

Highlights

- One-step RNA Module for Countable PCR matched the accuracy of Carrigent's established two-step RT workflow for lentiviral titration
- Highly reproducible lentiviral titers (<5%) were obtained from viral RNA supernatant
- Countable RT-PCR workflow reduced labor and enabled a faster turnaround
- The streamlined, GMP-ready workflow demonstrated suitability for process development and manufacturing

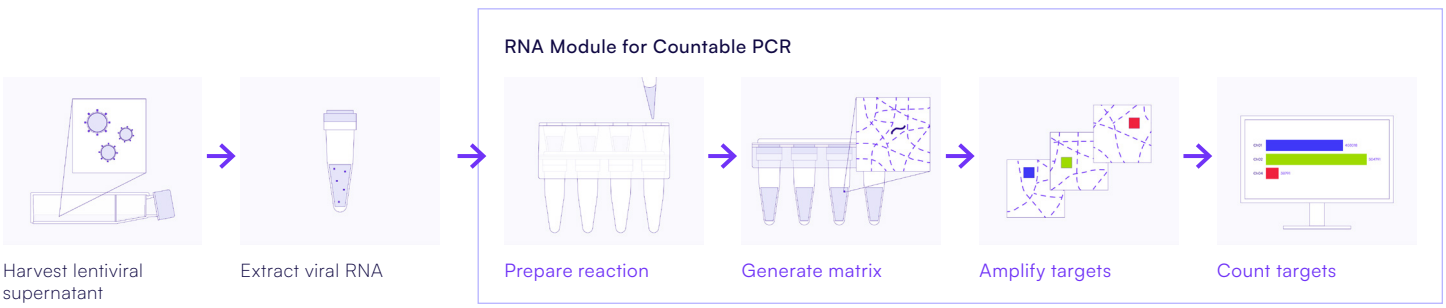

Figure 1. The workflow for lentiviral quantification includes viral RNA extraction from supernatant, Countable PCR setup with the RNA Module, isolation of single molecules in the matrix, and thermal cycling, culminating in true single-molecule counting on the Countable System.

[1] Carrigent, Covina, CA.
[2] Countable Labs, Palo Alto, CA.

Carrigent sought a faster, more reliable approach to quantify lentivirus from culture supernatant, without separate cDNA synthesis. Their goal was to maintain accuracy while reducing manual handling, enzyme variability, and time to result.

The RNA Module for Countable PCR was evaluated for its ability to:

- Streamline the workflow by setting up a single reaction for reverse transcription and amplification.
- Standardize RT efficiency through pre-validated enzyme chemistry.
- Deliver reproducible and reliable viral counts from purified lentiviral RNA or unpurified samples.
- Match or exceed the accuracy of Carrigent's established two-step assay.

By simplifying the workflow and reducing sources of variability, Carrigent aimed to establish a more reliable and standard procedure for viral titration, important for manufacturing and R&D in cell therapy.

Methods

Viral RNA was extracted from the lentiviral supernatant using a silica-membrane viral RNA extraction kit, then eluted in nuclease-free water for immediate use. The subsequent assay was performed using the *WPRE* hydrolysis probe-based chemistry available via the Countable Labs' Pre-designed Assays (**Figure 1**) (https://countablelabs.com/pre-designed-assays).

For the reaction setup, RNA, primers, probe, and one-step Countable RT-PCR master mix were combined per kit instructions (BK0003). Two different samples of the viral RNA were tested (S1, S2). A No-Template Control (NTC) and a No-Reverse-Transcriptase Control (NRC) were also included in duplicate to confirm robust assay performance. To achieve specific annealing, RNA and gene-specific primers were pre-incubated at 70°C for 5 minutes, then cooled on ice before proceeding.

RNA molecules were then compartmentalized in the Countable Matrix and amplified following the Countable PCR RNA Module protocol. Imaging and automatic data analysis were performed on the Countable System (Countable 4), which uses high-resolution 3D light sheet imaging to image the entire reaction tube and spatially identify amplified target molecules in each compartment.

Results

One-step RNA workflow enables high-quality, single-molecule counting

By isolating single viral RNA molecules and performing one-step RT and amplification of *WPRE* targets across 30 million individual compartments, the RNA Module for Countable PCR produced clean, high-quality amplification signal for all lentiviral samples within a single-tube workflow (**Figure 2**). The light sheet images show compartmentalization and amplification of single *WPRE* target molecules in each reaction, illustrating how the Countable System distinguishes positive compartments and directly counts single molecules.

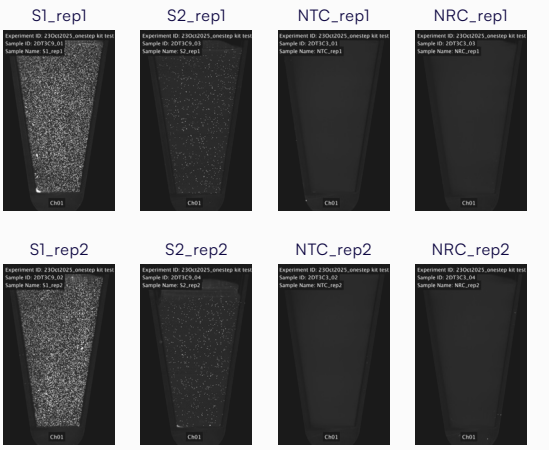

Figure 2. Countable PCR light sheet imaging enables single-molecule detection of *WPRE* target molecules across 30 million compartments in a single PCR tube, where each bright spot represents an amplified *WPRE* molecule. Shown are representative light sheet images from samples and negative controls, for two replicates each.

Both viral RNA samples (S1 and S2, each with two technical replicates each) showed a strong signal separation — confirming on-target amplification of *WPRE* from viral genomes (**Figure 3**). Countable PCR software automatically modeled amplification data, classified positives, and generated a digital QC record containing compartment data, imaging, and ID Scores stored in a 21 CFR Part 11—compliant format. The ID Score is Countable's built-in quality metric that measures signal separation and background noise across the entire reaction — providing an immediate and objective indicator of assay performance. Automated QC scoring assigned ID Scores between 93—98, reflecting excellent counting precision and reproducibility. No manual thresholding or curve fitting was required. This automated evaluation ensures reproducibility and data integrity aligned with GMP expectations.

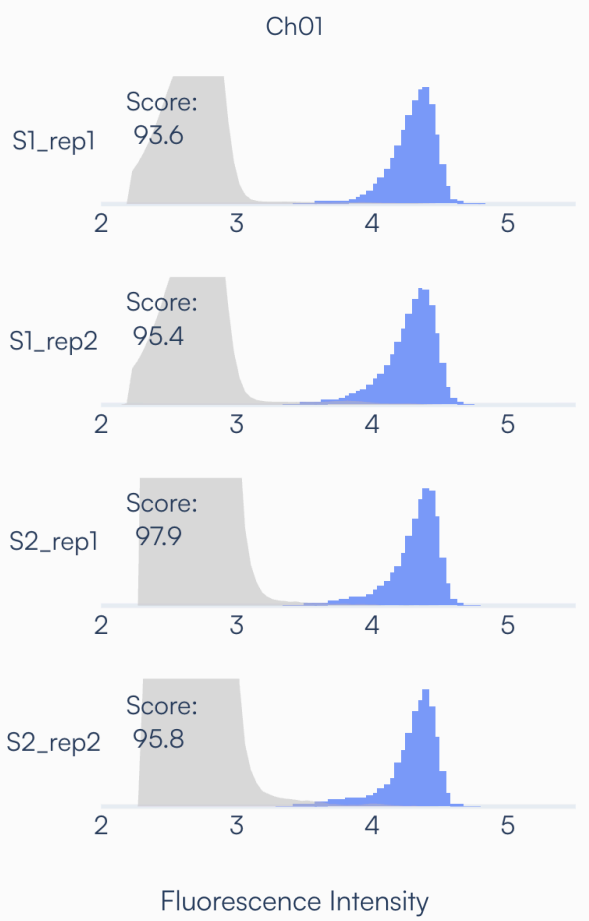

Figure 3. Fluorescence intensity histograms for *WPRE* assay from samples and technical replicates in the Countable PCR data summary. Each trace displays a distinct amplification profile with automatically generated ID Scores (93—98) confirming strong signal discrimination. Every Countable PCR run produces this standardized report with full plots and QC metrics stored in a 21 CFR Part 11—compliant format, supporting GMP-ready traceability.

Highly accurate and reproducible viral genome counts and minimal background

The Countable PCR software automatically quantifies positive signal across all compartments and outputs a direct count of target molecules. Across all samples, the RNA Module for Countable PCR delivered mean

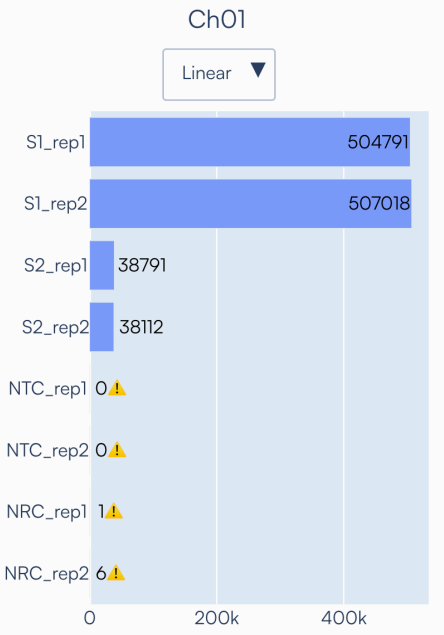

Figure 4. A quantification of ~500,000 and ~38,000 *WPRE* target molecule counts using the automated analysis on the Countable System from samples S1 and S2, respectively. Tight reproducibility (low %CV) and near-zero background in negative controls confirm high specificity. Yellow triangle icons automatically flag low-count samples, confirming the minimal background in this experiment's negative controls. Each Countable report includes visualization of counts, QC scores, and control flags for complete assay transparency.

viral genome titers of ~1 × 10^9 vg/mL, closely matching Carrigent's two-step benchmark. Replicates showed < 5 % CV, demonstrating excellent reproducibility (**Figure 4**). The controls validated assay specificity, with the NTC generating 0 positive counts and the NRT generating ≤ 6 positive events.

Conclusion

By using one-step Countable RT-PCR, Carrigent was able to streamline their lentiviral titration process while simultaneously:

- **Improving accuracy** by removing pipetting and RT variability.
- **Reducing cost and labor** — no separate RT kits or standard curves.
- **Providing traceable data** through automated QC and 21 CFR Part 11—compliant reporting.
- **Enabling rapid, pre-purification titer checks** for faster process decisions.

The RNA Module for Countable PCR delivers fast, accurate lentiviral titration, removing uncertainty from early-stage R&D to vector development and process optimization. By unifying the RT and PCR steps, the workflow reduces pipetting variability, eliminates enzyme-to-enzyme differences, and delivers accurate results in a fraction of the time — providing confidence in every viral count. The RNA Module for Countable PCR includes a pre-screened reverse transcriptase with the highest verified RNA-to-cDNA conversion efficiency, removing the need to source or optimize an RT enzyme. This standardized chemistry eliminates a key variable in conventional two-step assays and ensures consistent conversion yield across operators.

The *WPRE* hydrolysis-probe assay used in this evaluation is available to all researchers through Countable Labs' Pre-designed Assays, offering validated, ready-to-run panels for common vector elements and control targets.

For cell therapy programs, the RNA Module for Countable PCR transforms lentiviral titration from a variable, multi-step task into a reliable, one-step control point — even from crude material.

Learn how Countable PCR brings consistency to your viral vector workflow.

To talk to a Countable specialist about integrating one-step viral titering and Pre-designed Assays into your CGT program, visit countablelabs.com/contact.