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Title: *Automating a Lentivirus Infectious Titer Assay (ITA)*

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Lentiviral vector (LVV) potency, as measured by transduction efficiency, is a Critical Quality Attribute (CQA) that must be monitored for every LVV sample. At Interius, the in vitro potency of LVV samples is assessed in an infectious titer assay via the transduction of SupT1, a suspension T cell line, that serve as model CD7+ target cells. Transduction of target cells by the LVV, encoding a CAR20 protein, is detected through antibody staining of SupT1 cells using an anti-idiotype monoclonal antibody targeted to the scFv domain of the CAR20 protein using flow cytometry. LVV containing samples are serially diluted and added to a fixed amount of target cells in a 96 well plate format. The transduced cells are incubated for 7 days, with intermittent medium refresh, followed by live cell staining for CD7+ and CAR+. The transducing units (TU) per mL are calculated by assessing the CAR+ cells amongst total live SupT1 cells assuming a Poisson distribution of single cell infections in the range of 2 – 20%.

The manual execution of the ITA method is a labor-intensive, multi-day process consisting of many discrete steps, followed by a long day of live cell staining and signal acquisition by flow cytometry. Standard 96 well plate reading flow cytometers are not well suited for high-throughput flow cytometry. To support the sample numbers required to develop multiple process development efforts simultaneously, a high throughput solution was required.

To this end, we have developed a fully automated, multi-step method covering sample dilution, SupT1 transduction, medium refresh, moving from 96-well to 384-well plates, 384-well live cell staining and HT flow cytometry on a Sartorius iQue, followed by automated data reduction. Each of these discrete steps is designed to reduce hands-on time and eliminate operator to operator variability while providing the added benefits of increased throughput and speed. Importantly, the same assay framework can be utilized for different products and target cells with minor modifications.