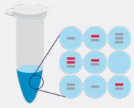
Streamline your adoptive cell therapy program with digital PCR

CellCarta

Our team has a unique expertise in digital and quantitative PCR to support you in ensuring your adoptive cell therapy (ACT) meets safety regulations relating to VCN and RCL/RCR levels, even in lymphodepleted samples. By detecting the smallest changes in VCN, dPCR is our method of choice for pharmacokinetics (PK) assays. As for replication competent viruses, they can easily be detected using qPCR, ensuring patient safety and regulatory compliance.

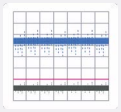
Understanding the value of digital PCR

Principle of digital PCR (dPCR)



Partitioning The PCR reaction mixture is partitioned into small reaction volumes (droplets or nanowells) with target and background DNA randomly distributed among the reactions

Amplification The target DNA is amplified by PCR using standard thermal cycling with fluorescent dyes or hydrolysis probes



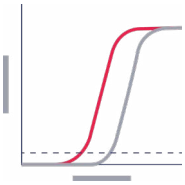
Detection Each reaction provides a fluorescent positive or negative signal indicating that target DNA was present or absent in the partition

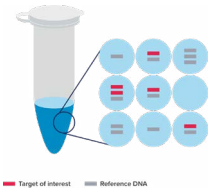
Calculation The fraction of positive droplets is used to calculate the target DNA concentration using Poisson statistics.

qPCR vs dPCR: a quick comparison

qPCR	dPCR

• Real-time detection
• Cq values providing relative quantities by comparison among samples
• Absolute quantities relying on calibration curves
• Variations in amplification efficiency (e.g. due to inhibitors) affect results
	• End-point detection
	• # positive partitions > Poisson > copies
	  • copies / µl
	  • ratio or fraction
	• Absolute quantities (copies) without a calibration curve
	• End point detection is not affected by changes in amplification efficiency





Clinical Application: Biodistribution of CAR constructs

Measure the accumulation/clearance of transduced cells by organs

qPCR offers a greater dynamic range and higher throughput

dPCR yields better data for very low concentrations of engineered cells

Clinical Application: VCN measurement

Determine the vector genomic integration number (vector copy number, VCN) in transduced cell preparations, tissue samples and/or blood samples. Pharmacokinetics (PK) assessment in different clinical samples allows to monitor the persistence of engineered cells.

qPCR does not accurately discriminate between larger copy numbers.

- qPCR can call apart a copy number 2 from 3 (Δ = 50%) but not 4 from 5 (Δ = 25%)

dPCR is preferred since it uses a robust count-based measurement and does not require a standard curve, facilitating long follow-up times

- dPCR has been demonstrated to differentiate small differences in copy number (e.g. 4 vs. 5)

Clinical Application: Detection of replication-competent viruses

Regulatory guidelines recommend the use of cell-based assays to detect the presence of replication competent retroviruses (RCR) or lentiviruses (RCL) at specific timepoints (pre-treatment, 3mo, 6mo, 12mo, long-term follow up). However, PCR-based assays are a quick alternative for initial assessment, providing results in 1 week vs. up to 6 weeks for cell-based assays.

qPCR is preferred over dPCR for RCR and RCL detection

De-risk adoptive cell therapy development using dPCR for PK studies

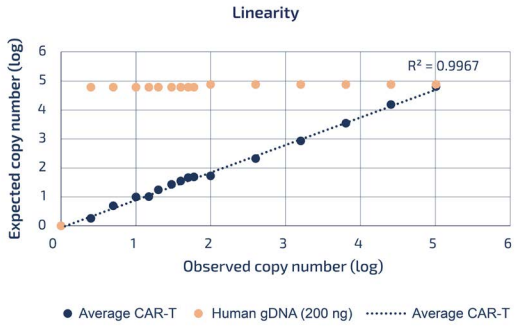
Accurately determine PK by vector copy number (VCN) assessment using dPCR

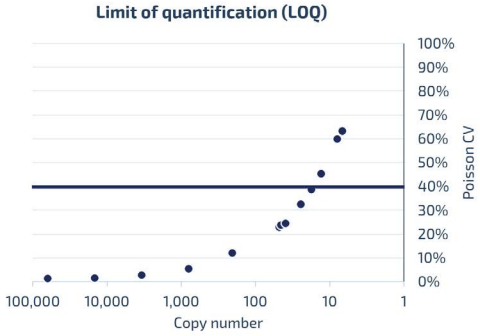
Data from a phase 1 clinical trial using whole blood and bone marrow samples

For the absolute quantification of CAR vector copy numbers, dPCR is favored over qPCR. dPCR assays specific to the vector sequences were developed and validated to monitor the CAR construct in DNA from clinical samples during the course of the trial, as per safety guidelines for approval of such therapy.

Linearity and limit of quantification (LOQ)

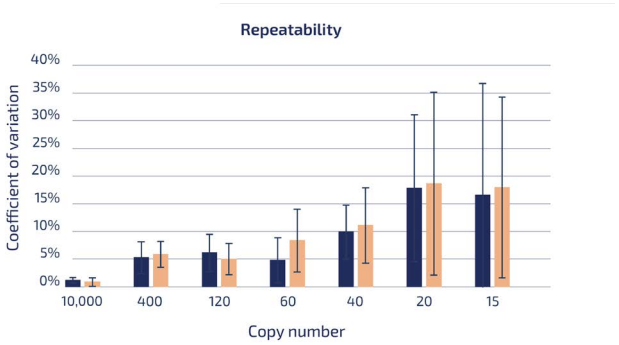
Linearity was evaluated using a large input of 200 ng of human genomic DNA with variable fractions of CAR-T spiked in. Despite the high background, linearity was good down to very small fractions of CAR-T-specific vector sequences. As expected, the precision decreased as the concentration decreases. The assay LOQ was ~18 molecules, based on a maximum upper limit of 40% for the precision CV.

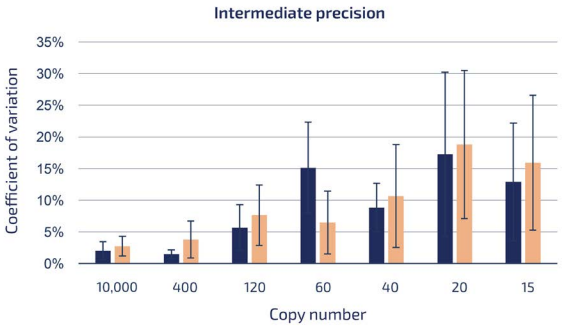




Repeatability and intermediate precision

Both of these parameters had good results at high and intermediate CAR-T concentrations but decreased at very low concentrations, in line with expectations.





replicate 1 replicate 2

SS_DigitalPCR_2025-02-13

CellCarta

ABOUT CELLCARTA

Leading provider of specialized precision medicine laboratory services to the biopharmaceutical industry. Leveraging its integrated analytical platforms in immunology, histopathology, proteomics and genomics, as well as related specimen collection and logistics services, CellCarta supports the entire drug development cycle, from discovery to late-stage clinical trials. The company operates globally with 9 facilities located in Canada, USA, Belgium, Australia and China.

www.cellcarta.com
info@cellcarta.com
Toll Free: + 1 877 776 3443

HEADQUARTERS

201 President Kennedy Ave., Suite 3900
Montreal, Quebec, Canada H2X 3Y7
Phone: + 1 514 360 3600