Development and Comparative Assessment of a Rapid, High-Throughput Method for Accurate Quantification of Host Cell DNA Impurities in Final Drug Product and Process Intermediates

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INTRODUCTION

Biologics manufactured in cell lines carry inherent risk of incorporating host cell material in the drug product. In particular, elevated levels of residual host cell DNA (hcDNA) contaminants increases oncogenic risk and may lead to a systemic induction of the immune response. The World Health Organization and European Pharmacopoeia have established a guideline for the acceptable level of hcDNA (<10 ng per dose) in biologics. As such, regulatory agencies have focused on quantifying and characterizing hcDNA in gene therapy products as a critical parameter for product safety.

Therefore, a highly sensitive, accurate, and robust method of hcDNA quantitation provides valuable information during process development and is essential as a safety and quality metric of the final drug product. In this work, we report a robust, high throughput qPCR method capable of accurately quantifying total and encapsidated hcDNA in all process intermediates and final drug products within two hours.

METHODS AND MATERIALS

An in-house qPCR lab-developed test (LDT) was developed to quantitate hcDNA for in-process and final drug product samples. The LDT is designed to work in complex matrices to avoid necessitating DNA-purification/isolation steps that are typically required in many commercial kits. The performance of the LDT was compared against a leading commercial qPCR kit. Using a semi-automated pipetting workflow (Integra Assist Plus), samples were tested with and without nuclease pre-treatment allowing assessment of external DNA clearance during downstream purification and demonstrating consistency across sample preparations. Following heat-mediated de-capsidation, host cell DNA was quantified relatively via Taqman qPCR.

Using a hcDNA reference allowed for accurate and sensitive detection with an assay dynamic range of 900 pg-9 fg, a log greater range than the commercial kit (Figure 1). All standards, controls and test samples are assayed in triplicate on a 384-well plate using the QuantStudio 7 Real-Time PCR system.

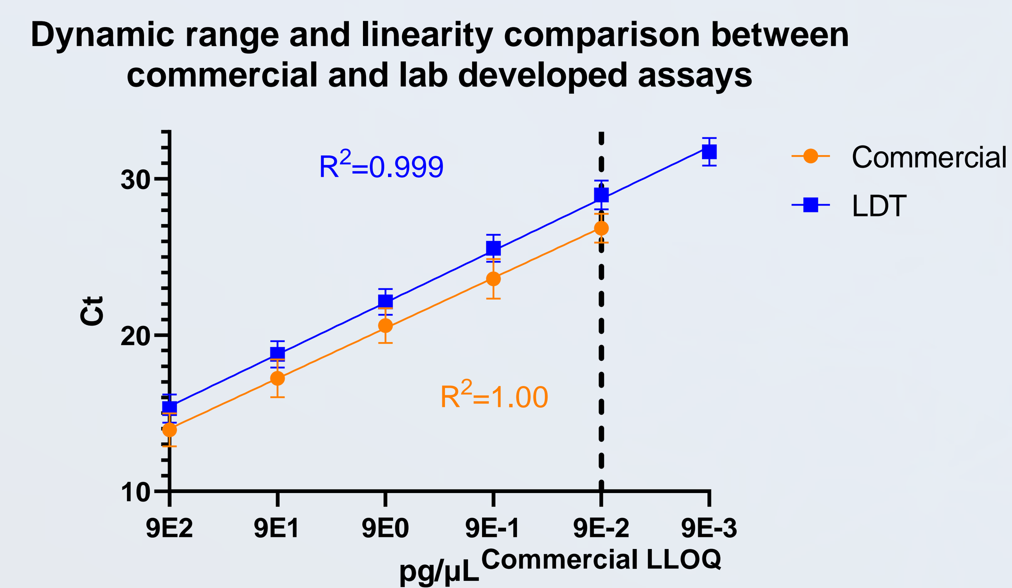

Figure 1. Cycle Threshold vs standard mass quantity. Due to background fluorescence, the commercial lab is limited to a 5-log dynamic range. Lower background fluorescence allows a 6-log dynamic range in the LDT.

RESULTS

Significant improvements in all assay parameters were observed with the LDT relative to the commercial kit (Table 1). Precision-repeatability was reduced to <10% and Intermediate Precision was reduced to <20%; compared to the commercial kit, this is a 10% decrease in %CV.

The LDT exhibited greater than 10-fold improvement in sensitivity. Specificity was improved in the presence of more complex matrices, including lysis reagents/inter-cellular material (0% vs 26% difference) and in a low pH process intermediate buffer with a high salt concentration (9% vs 14% difference).

Accuracy between the two quantitation methods was similar. Both assays exhibited ≥85% recovery of HEK293 DNA when diluted in final Drug Product buffer.

Serial dilution of a representative Drug Product sample showed ≤15% Relative Bias across both assays. However, compared to an orthogonal quantitation method (PacBio sequencing), the LDT produced a more analogous relative quantitation (-7% error) value compared to the commercial kit (+16% error).

Furthermore, linear regression analysis of the serially dilution produced R2 values >0.999 for both total and encapsidated hcDNA (Figure 2) with the LDT. In contrast, the commercial kit resulted in lower R2 values and statistically significantly different y-intercepts (Figure 3) between the total and encapsidated conditions.

Assay Comparison

Parameter		*Commercial*	*LDT*
Precision	Repeatability	>10%	<10%
	Intermediate precision	>30%	<20%
Accuracy	Spike/recovery	85%	96%
	Rel Bias	15% bias	4% bias
	% error relative to NGS	+16%	-7%
Sensitivity/ Specificity (Matrix effects)	LLOQ	~70 ng/mL	~3ng/mL
	% diff high salt low pH buffer	14%	9%
	% diff cell lysis	26%	0%

Table 1. Summary of prequalification results. Comparison of the LDT to the vendor-qualified commercial assay.

DISCUSSION

When compared to a validated, commercial residual hcDNA qPCR kit, the LDT exhibits improved performance across all metrics. By avoiding the DNA-isolation step needed with most commercial kits, the LDT allows for accurate quantitation of total and encapsidated hcDNA in a more time-efficient (<2 hours) and high-throughput (55+ samples) manner. Our robust assay accommodates multiple crude and in-process buffers without impacting accuracy.

CONCLUSIONS

We have shown that our lab developed residual hcDNA qPCR assay is suitable to robust and accurate quantitation of low levels of host cell impurities without extensive sample processing for intermediate and final drug product samples.

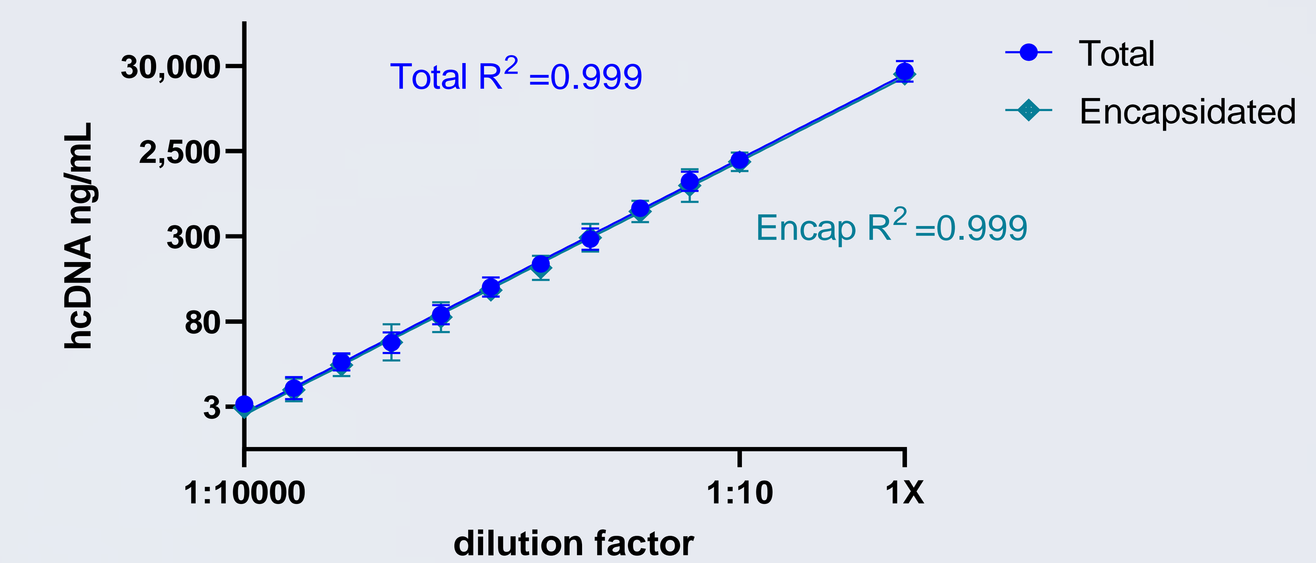

Figure 2. Plot of reportable mass quantitation vs dilution factor. The extended dynamic range allowed for more quantifiable results with statistically indistinguishable slopes and y-intercepts between total and encapsidated samples.

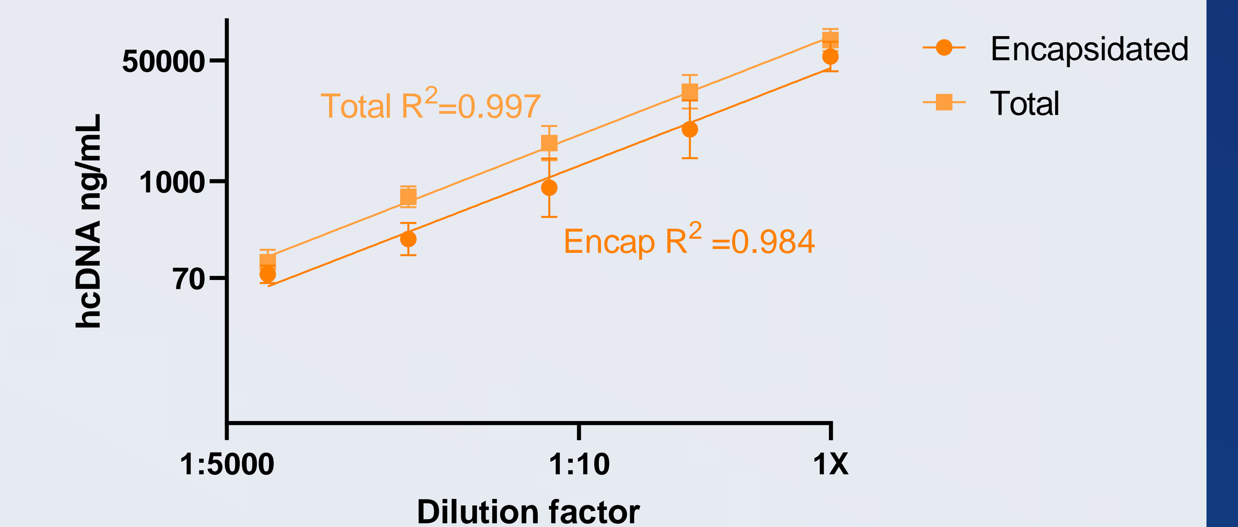

Figure 3. Plot of reportable mass quantitation vs dilution factor on the commercial assay. The limited dynamic range reduced the reportable results. The samples showed significant mass differences within the limited test range.

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