Host Cell DNA Assay Re-Development and Qualification for a PEGylated Protein Drug Substance

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Biologic drugs must ensure that DNA from the host cell (hc) is below a recommended limit set by the FDA to be no higher than 100 pg/dose (or 10 ng/dose for high dose biologics). Residual hcDNA in a drug could have serious health implications for the patient, which is why the hcDNA assay is a critical release assay for biologic drugs in accordance with USP<509>. At Tanvex, two related biologic drugs, one PEGylated, and the other not, are produced from the same E. coli host cell bank. A feasibility study was conducted and showed that the already validated hcDNA method for the non-PEGylated protein could not be used for the PEGylated version of the drug. This poster covers the feasibility testing, method re-development and challenges faced working with a difficult PEGylated drug substance (DS). Various DNA extraction methods were tested, and one outperformed the others resulting in a fully qualified hcDNA assay which meets the USP<509> requirements.

Method Feasibility

Known concentrations of E.coli DNA were spiked into both non-PEGylated and PEGylated DS following the validated method used for the non-PEGylated DS prior to DNA extraction. The PEGylated DS failed to recover known amounts of DNA while the non-PEGylated DS passed all assay and sample criteria as expected, **Table 1**, demonstrating that we could not use the current test method to quantify the amount of hcDNA in PEGylated DS. Due to the possibility of matrix interference, additional sample dilutions 1:20, 1:100 and 1:1000 with spike concentrations of 100 or 500 pg/mL were tested but all tested conditions failed %Recovery of known spiked E.coli DNA concentrations, **Table 1**.

	Sample	Dilution	Avg Conc. [pg/mL]	Expected Conc. [pg/mL]	Recovery	Result
Initial Feasibility Testing	Non-PEGylated DS	2	85.3	100	85%	Pass
			83.1	100	83%	Pass
	PEGylated DS	2	1.3	100	1%	Fail
			2.4	100	2%	Fail
	PEGylated DS	2	1.4	100	1%	Fail
			1.2	100	1%	Fail
Additional Testing	PEGylated DS	20	2.8	100	3%	Fail
	PEGylated DS	100	34.9	100	35%	Fail
	Matrix	100	46.2	100	46%	Fail
	PEGylated DS	100	166.2	500	33%	Fail
	Matrix	100	202.5	500	41%	Fail
	PEGylated DS	1000	43.5	100	44%	Fail
	Matrix	1000	43.4	100	43%	Fail
	PEGylated DS	1000	276.4	500	55%	Fail
	Matrix	1000	169.6	500	34%	Fail

Table 1: Results of PEGylated and non-PEGylated Drug Substance using Validated method for non-PEGylated drug
*LLOQ is 2.7 pg/mL, % Recovery criterion = 70% – 130%

Samples from two different process steps were evaluated to test matrix interference including UFDF2 Pool (contains PEGylated protein) and CM Pool (contains non-PEGylated protein), **Figure 1**. Only the CM pool sample successfully recovered the known concentrations of spiked DNA, **Table 2**.

Process steps 1-10
CM Chromatography
PEGylation
Chromatography
UFDF2 Pool
PEGylated DS

Figure 1: Summary of process steps; non-PEGylated (Gray), PEGylated (Purple)

Sample	Dilution	Avg Conc. [pg/mL]	Expected Conc. [pg/mL]	Recovery	Result
CM Pool (Non-PEGylated Protein)	2	85.2	100	85%	Pass
	2	94.6	100	95%	Pass
UFDF2 Pool Matrix (No protein)	2	82.3	100	82%	Pass
	50	81.7	100	82%	Pass
	100	77.4	100	77%	Pass
	1000	72.0	100	72%	Pass
UFDF2 Pool (PEGylated Protein)	2	11.4	100	11%	Fail
	50	29.4	100	29%	Fail
	100	0.2	100	0%	Fail
	1000	0.03	100	0%	Fail

Table 2: Results of different process steps from drug production before and after PEGylation of drug. % Recovery criterion = 70% – 130%

- **Likely both interference from the presence of PEGylated protein and matrix interference from the DS interfere with assay.**

DNA Kit Comparison

Two additional Host Cell DNA kits were compared and tested for initial performance. The kit from Vendor A was tested during feasibility and determined to be non-compatible with the PEGylated protein DS. Method details comparing pros and cons as well as results are shown in **Table 3**.

DNA extraction method	qPCR Technology	Kit Pros	Kit Cons	Example qPCR Curves
Vendor A: Proprietary	SYBR	• In-house validated method for non-PEGylated DS • DNA standard and primers included	• Not compatible with Tanvex PEGylated DS • No flexibility to order primers separately	
Vendor B: Magnetic Beads with internal DNA control	Multiplex TaqMan	• User Friendly • Fully validated and FDA compliant software • USP<509> compliant • Multiplex TaqMan reaction with Internal control	• Expensive • Required Machine/ software updates • No flexibility to order primers separately	
Vendor C: Sodium Iodide/ glycogen as carrier molecules	TaqMan Primer/ Probe USP<509> Sequences	• Recognized in USP<509> • Flexibility in vendor purchasing for additional kit components	• Extraction kit requires several reagents to be purchased separately from a variety of vendors	

Table 3: Comparison of three Host Cell DNA assays

- **Vendor C = FujiFilm/Wako Extraction kit chosen for further development using qPCR primers and TaqMan probe sequences listed in USP<509>.**

Vendor C = FujiFilm/Wako Kit Optimization

The FujiFilm/Wako kit is a single tube extraction method and required investigation into various extraction tubes because they are vital to the assay performance. General assay procedure and equipment are shown in **Figure 2**.

USP <509> & <1130> were used to inform the optimization of this assay and to generate and set the system suitability. Optimization decisions are outlined in **Table 4**.

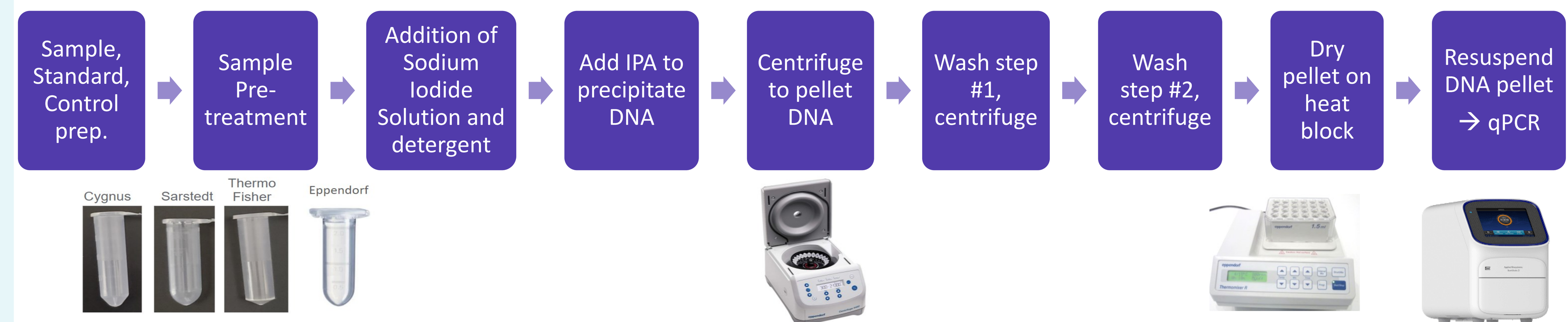

Figure 2: DNA extraction method summary

Step	Parameter	Testing Conditions	Result
DNA Extraction	Sample Pre-Dilution	Range from 1:2 – 1:20 dilution	1:3 pre-dilution of DS
	Sample Pre-Treatment	No Pretreatment Kit Option A: DTT, SDS, 55°C, 1hr Kit Option B: DTT, SDS, EDTA, Proteinase K, 55°C 1hr USP<509> variation: SDS, NaCl, Proteinase K, 55°C, 1hr	USP<509> variation: SDS, NaCl, Proteinase K, 55°C, 1hr
	E.coli DNA Spike Conc.	10 pg/mL, 100 pg/mL or 1000 pg/mL	100 pg/mL
	2 mL Extraction Tube Type	Cygnus Sarstedt Thermo Fisher Eppendorf	Sarstedt = primary tubes Thermo Fisher or Eppendorf = alternative
	Pellet Resuspension Volume	90 µL, 144 µL, 180 µL	Perform equally in qPCR
	Extracted DNA retention time	4°C and -20°C for up to 2 months	Up to 2 months at 4°C or -20°C. No impact on DNA quality.
	Pellet drying methods	Air, heat block attachments, drip upside down, pipette aspiration	Heat block 60-65°C for about 1hr in 1.5 mL block attachment
qPCR	Primer / TaqMan probe concentration	10 µM / 2 µM 25 µM / 2.5 µM 25 µM / 12.5 µM	No difference in assay performance.
	qPCR Assay Plate	MicroAmp Endura Plate MicroAmp Fast Optical Plate	Both plates are suitable.

Table 4: Developmental parameters for FujiFilm/Wako kit optimization

Method Qualification

Successful Qualification showed assay is **accurate, precise, linear and fit for use** for determining residual host cell DNA concentration in a PEGylated protein drug substance. Final assay criteria meet the USP<509> guidelines. Full summary of qualification parameters and results shown in **Table 5**. Assay linearity shown in **Figure 3.**

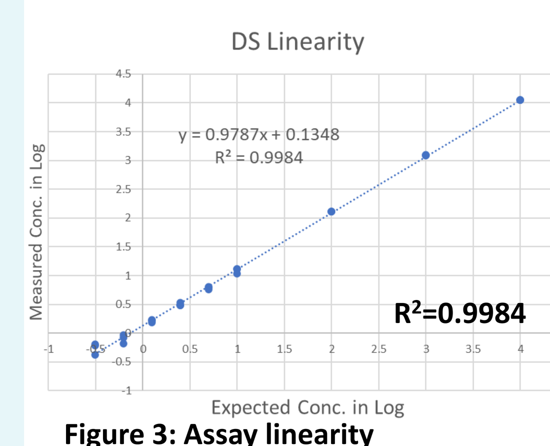

Figure 3: Assay linearity

Parameter	Quantity/ Range	Acceptance Criteria	Results
Assay Standard Curve	Concentrations: 0, 1, 10, 100, 1000, 10000 pg/mL	• Ct of NTC (if any) ≤ Ct of 1 pg/mL standard • Ct of 1 pg/mL standard ≤ 39 • $R^2 \geq 0.99$ • -3.8 ≤ Slope ≥ -3.1	Pass
Assay Controls	10 and 100 pg/mL	• RSD of controls at each conc. ≤25% • Recovery 70 - 130% of spike DNA level	Pass
Specificity	100 pg/mL CHO DNA	• No E. coli DNA detected	Pass
Linearity	9 spike levels: 0, 0.3125, 0.625, 1.25, 2.5, 5, 10, 100, 1,000, 10,000 pg/mL	• R^2 of spike-recovery curve ≥ 0.98 • Within assay range, spike recovery of E.coli DNA must be 70 - 130% of the expected • RSD at each test concentration within method range ≤25%	Pass
Sensitivity	Range: 0.3125, 0.625, 1.25, 2.5, 5, 10 pg/mL	• Determine method LOQ according to ICH guidelines	5 pg/mL
Range	Same as linearity	• Same as linearity	10,000 - 10 pg/mL
Accuracy and Precision	0, 100, 8000 pg/mL	• Accuracy: Recovery 70 - 130% of spike DNA level • Precision: RSD at each test concentration within method range ≤25%	Pass

Table 5: Qualification Results for E.coli hcDNA Assay

Discussion and Conclusion

Both PEGylated protein and matrix effects impacted the hcDNA assay performance. The validated method for a related non-PEGylated protein was not compatible with the PEGylated version of the drug even with various feasibility experiments. Two additional extraction kits were tested which utilize different DNA extraction technologies and one proved to have superior performance and is more cost effective. The final hcDNA assay proved to be accurate, precise, and fit for use for determining residual E.coli hcDNA in the PEGylated drug substance.

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