USP Reference Standards for Residual DNA Testing of Recombinant Biotherapeutics

Shubrata Khedkar[1], Michael Huang[2], Maura Kibbey[2], Fouad Atouf[2], Mitali Samaddar[1] and Ranjan Chakrabarti[1]
[1]United State Pharmacopeia Pvt. Ltd., Hyderabad, India and [2]United State Pharmacopeia, Rockville, USA

ABSTRACT

Therapeutic recombinant proteins and monoclonal antibodies have emerged as important and growing classes of biologics products used in treatment and management of several diseases for current unmet medical needs. Biologics products manufactured using recombinant DNA technology has a risk of containing residual impurities originating from the host cells. One of the residual impurities is the host cell DNA which when present at high levels has a potential risk of being oncogenic or immunogenic. The FDA requires manufacturers to report how much residual DNA from the host cell remains in the drug substance after purification. Hence, it is critical that the host cell DNA present in recombinant therapeutic products be detected, measured and controlled. The standards-setting work of the United States Pharmacopeial Convention (USP) can provide industry with best practices or validated procedures that enable companies to develop their own assays, or, in the latter case, verify that the USP procedure is suitable for their purpose. USP has developed Reference Standards for Chinese hamster ovary (CHO) Genomic DNA and *Escherichia coli (E. coli*) Genomic DNA associated with the proposed USP chapter <509>, "Residual DNA Testing," that can be used by manufacturers of recombinant biotherapeutics to check the process performance and to measure the residual DNA, specifically in products produced in *E. coli* or CHO cell lines. USP India biologics lab evaluated quantitative PCR(qPCR) and validated the extraction procedure. TaqMan based qPCR was successfully evaluated. Validation was performed as per USP <1225> and ICH guidelines. The genomic DNA extraction procedure includes a proteinase K digestion step combined with a chaotropic salt (NaI) extraction and ethanol precipitation. The validation exercise successfully passed all criteria for specificity, linearity, accuracy, repeatability, intermediate precision, range and LOQ.

INTRODUCTION

Residual host-cell DNA is a process impurity in recombinant biotherapeutic products. Levels of host cell DNA needs to be monitored during process development and validation. There is a need for standardised procedures and physical reference standards that can be used by the manufacturers. Proposed USP chapter <509>, "Residual DNA Testing," (PF 42 (5)) with associated CHO and *E. coli* reference standards, can be used by manufacturers of recombinant biotherapeutics to check the process performance of the residual DNA testing and to measure the residual DNA, specifically in products produced in *E. coli* or CHO cell lines. Chapter includes an validated qPCR method; and also provides a genomic DNA extraction procedure; however, the end user may decide to use an alternate procedure. qPCR was included because it is by far the most commonly used technique in the industry due to its high sensitivity, specificity, dynamic range, high-throughput capability, the availability of standardized best practices, and regulatory familiarity. Primer and probe sequences and cycling conditions are specified and in addition requirements for system suitability (negative control solution, sensitivity, and linearity), spike recovery of extracted samples, and precision of sample replicates are covered.

METHOD

Table 1: Genomic DNA Extraction

Steps	Genomic DNA Extraction Steps
1	Sample digestion with Proteinase K
2	Sodium iodide treatment in presence of detergent and linear polyacrylamide
3	Ethanol precipitation and pellet resuspension
4	Quantitative PCR

Table 2: qPCR steps

Steps	Quantitative PCR Steps
1	Add extracted template, specific forward and reverse primers and probe
2	7500 real time PCR thermal cycler program Using TaqMan chemistry
3	Check sensitivity, linearity and slope

RESULTS

1. Validation of Genomic DNA Extraction Procedure - Robustness

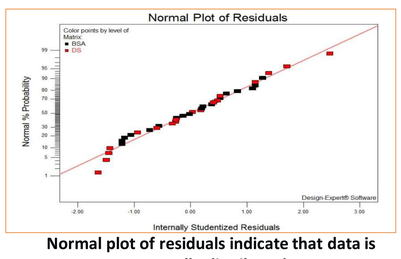
Figure 1: Critical factors and study design

Screen shot of the software used to design the robustness study - 2 Level factorial Design

	Name	Units	Type	Low	High
A [Numeric]	NaI Incubation	minutes	Numeric	13.5	16.5
B [Numeric]	Centrifugation	minutes	Numeric	13.5	16.5
C [Categoric]	Matrix		Categoric	BSA	DS

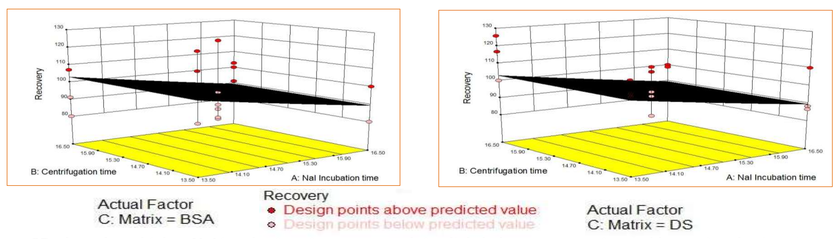
Robustness data from *E. coli*

Figure 2: Normal residual Plot



Normal plot of residuals indicate that data is normally distributed

Figure 3: 3D Plots



3D plots show higher NaI incubation time may result in slightly lower recoveries and hence incubation time should be monitored carefully

2. Validation of Genomic DNA Extraction Procedure Accuracy, Precision, Linearity Range and LOQ

Table 3: Validation criteria and results

S. No.	Parameter	Validation Target	Validation Result		Result Pass/ Fail
			E. coli	CHO	
1	Specificity	Ct if any, NLT lowest concentration of standard solution for Unrelated and Negative control solution	Ct of unrelated sample-39 and Negative control-38	No Amplification	PASS
2	Linearity	Linear relationship should be observed between Estimated vs. Expected concentration with Slope 1.00 ± 0.20. Regression NLT 0.95 and intercept- Significantly not different from zero. The plot of Residuals vs. Estimated concentration should show a random distribution about zero	Slope - 1.00, Regression - 0.99, Intercept -0.08, Residual plot showed random distribution about zero	Slope - 0.98, Regression - 0.99, Intercept - -0.01, Residual plot showed random distribution about zero	PASS
3	Precision	Repeatability [Intra run %RSD]: NMT 30%	1 - 17%	2 - 23%	PASS
		Intermediate Precision [day to day %RSD]: NMT 30%	5 - 16%	11 - 22%	PASS
		Intermediate Precision [analyst to analyst %RSD]: NMT 30%	13 - 16%	11 - 13%	PASS
4	Accuracy	% Recovery within 50% to 150%	68 - 112%	65 - 131%	PASS
5	Range	Should meet the set acceptance criteria for Precision, Accuracy and Linearity	50 - 0.01pg/µL	50 - 0.01pg/µL	PASS
6	LOQ	% Recovery within 50 -150 %	102 - 148%	109 - 141%	LOQ is 0.002 pg/µL
		Precision NMT 30%	7 - 14%	8 - 19%	

RESULTS

3. qPCR measurement

Representative Data

Figure 4: CHO

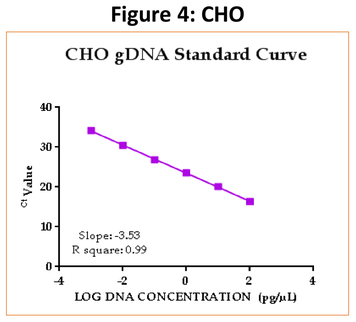


Figure 5: *E. coli*

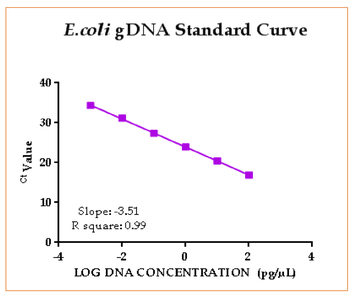


Figure 6: Amplification Plot

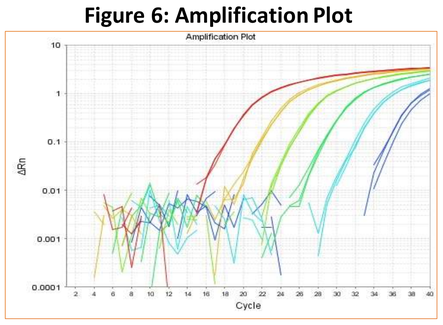


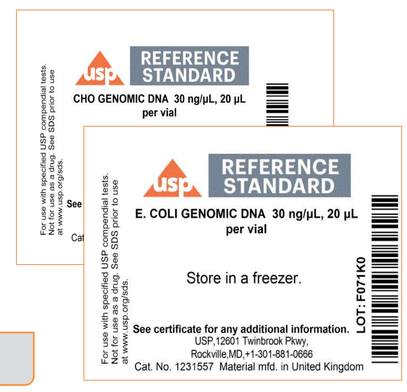
Table 4: System suitability criteria and results

S. No.	System suitability criteria	Result	
		CHO	*E. coli*
1	Negative Control--Ct NLT Ct of lowest concentration of standard	PASS	PASS
2	Sensitivity--Ct corresponding to lowest concentration of Standard solution is NLT 39	PASS	PASS
3	Linearity--Regression coefficient associated with standard solutions NLT 0.98	PASS	PASS
4	Slope --Slope is between -3.1 to -3.8	PASS	PASS

4. Reference Standard Concentration Assignment

A study was designed to verify the assigned concentration of genomic DNA by qPCR testing per Chapter <509>, in the labs of six collaborators, with each lab performing three assays and each assay using samples from separate candidate vials. Results from the study, supported the assigned concentration of 30 ng /µL CHO and 30 ng /µL *E. coli* Genomic DNA for the candidate reference standard lot. Reference Standard for CHO and *E. coli* genomic DNA are now available in USP reference standard catalog.

Figure 7: USP Reference Standard



Conclusions

USP India biologics lab performed validation of genomic DNA extraction procedure. The validation exercise successfully passed all criteria for specificity, linearity, accuracy, repeatability, intermediate precision, range and LOQ. Lab also successfully evaluated qPCR.

Residual DNA impurities must be controlled to acceptable levels in biotherapeutics to avoid potential safety risks such as immunogenicity and oncogenic. Hence, availability of USP Reference Standards for CHO and *E. coli* genomic DNA has a major impact in assuring safety of biotherapeutics products through the product lifecycle.

CHO genomic DNA and *E. coli* genomic DNA Reference Standards for residual host cell DNA are highly characterised materials associated with Chapter <509> with following benefits:

- Highly sensitive and specific residual genomic DNA measurement method
- Optional protein extraction procedure to minimize sample matrix effects
- Primer and probe sequences provided
- Flexibility in labeling oligonucleotides

This effort is a step forward towards our commitment to ensure quality and safety of medicines globally.

References

- **USP General Chapter , Residual DNA Testing <509>, Pharmacopeial Forum 42(5)**
- Genetic Engineering & Biotechnology News, Vol. 33, No. 9, May. 1, 2013
- Managing Residual Impurities During Downstream Processing, Jun 01, 201, BioPharm International, Volume 30, Issue 6, pg 26–28