E.coli Performance Evaluation Report

1. 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. "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. Ducky Biotechnology 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 Binding\ washing\eulution three steps. The validation exercise successfully passed all criteria for linearity, LOQ, accuracy, specificity and precision.

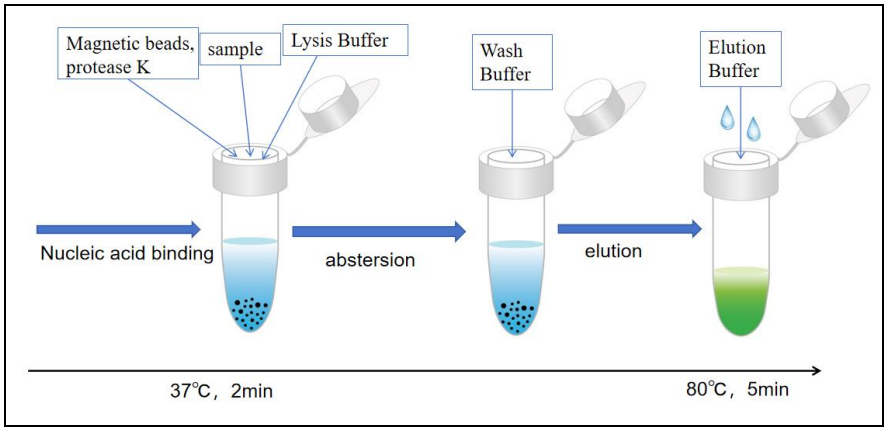
2. INTRUCTION

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. 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.

3. METHOD

3.1.1. Genomic DNA Extraction

Steps	Genomic DNA Extraction Steps
1	Binding DNA
2	Washing DNA
3	Eluting DNA
4	Quantitative PCR



3.1.2. qPCR Steps

Steps	Quantitative PCR Steps
1	Add qPCR MIX and extracted template.
2	Centrifuge the mutile well
3	7500 real time PCR thermal cycler program Using TaqMan chemistry
4	Check sensitivity, linearity and slope

4. RESULTS

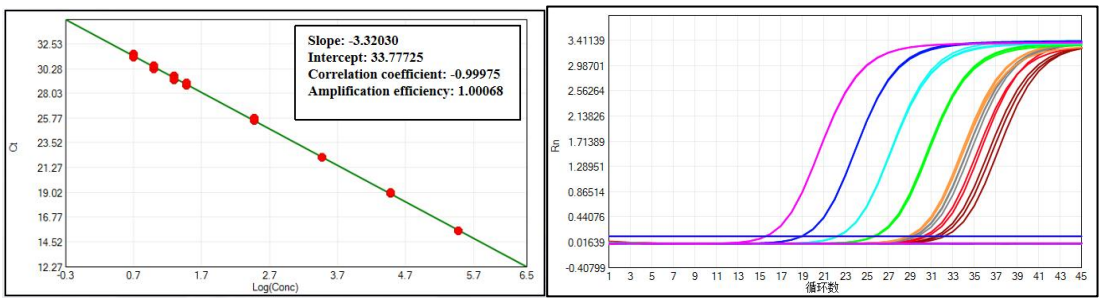
4.1. Summary

1	Linearity	Linear relationship should be observed between test Concentration log value vs. Ct with Slope -3.3 ± 0.50. Regression NLT 0.95. and amplification efficiency was 100% ±10%.	Slope was -3.32030，Regression coefficient associated with standard solutions was 0.99975, and amplification efficiency was 100.068%.
2	LOQ	Precision NMT 30%	the variation coefficient and accuracy deviation were within 30% for samples of 5fg/μL and above.
3	Accuracy	% Recovery within 50% to 150%	91.10%-104.45%
4	Specificity	The genomes of HEK293, MDCK, BHK21, Vero, CHO, Pichia Pastoris and common human genome cells in the environment were detected, and each cell or strain was detected three times.	No Amplification
5	Precision	Repeatability [lntra run %RSD]: NMT 30%	The intra run precision was within 30%.

		The total precision [day to day %RSD]: NMT 30%(Measurement Reproducibility: Three batches of the kit were used to detect precision sample S1-S4 in two working days with three experimentalists, and the precision of the kit was evaluated with 5 multiple wells per concentration per experiment.	The total precision was within 30%.

4.2. Linearity

Eight concentration samples of 5fg/μL, 10fg/μL, 20fg/μL, 30fg/μL, 300fg/μL, 3pg/μL, 30pg/μL, 300pg/μL were detected. Regression coefficient associated with standard solutions was 0.99975, and amplification efficiency was 100.068%.

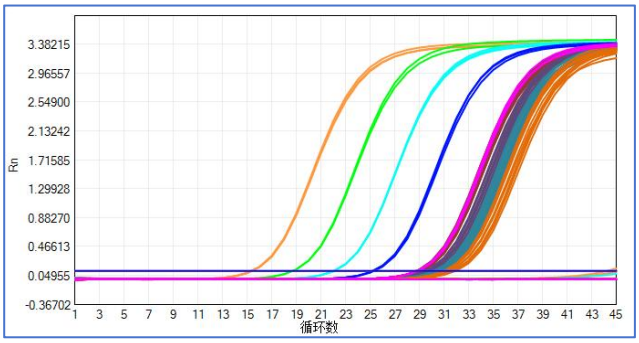


E.coli Linearity							
Standard	Ct1	Ct2	Ct3	Ct	Test Concentration	Theoretical Concentration	Accuracy deviation
ST1	28.96	28.79	28.98	28.91	2.88E+01	30	4.08%
ST2	25.62	25.58	25.74	25.65	2.72E+02	300	9.23%
ST3	22.21	22.2	22.24	22.22	2.89E+03	3000	3.65%
ST4	19.03	18.91	18.97	18.97	2.70E+04	30000	9.85%
ST5	15.55	15.53	15.49	15.52	2.90E+05	300000	3.21%

4.3. LOQ

4.3.1. Requirements: Precision NMT 30%

The E. coli DNA standard was diluted into 5 concentration samples of 0.5fg/μL, 1fg/μL, 5fg/μL, 10fg/μL, and 30fg/μL. 10 multiple pores were detected for each concentration sample, and the variation coefficient and accuracy deviation were within 30% for samples of 5fg/μL and above. It was proved that the limit of quantitation of this kit could reach 5fg/μL.

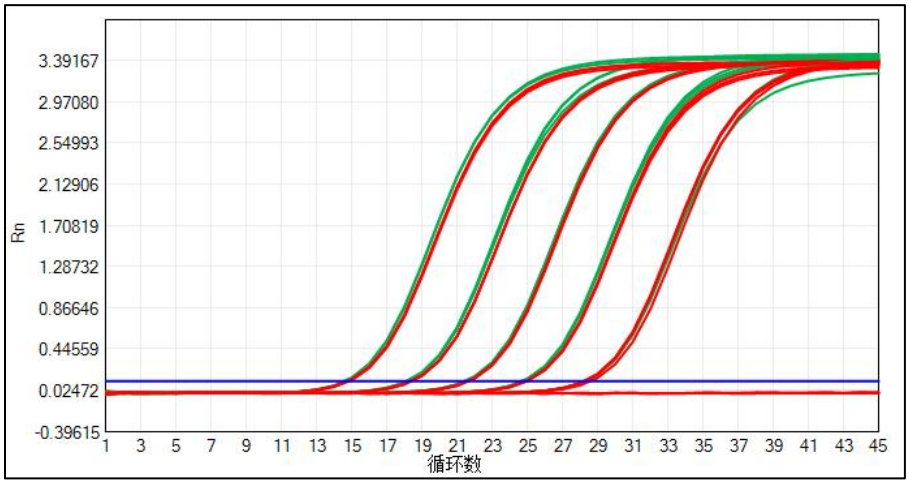


Limit of quantitation for E. coli				
Sample concentration	**5fg/μL**	**10fg/μL**	**20fg/μL**	**30fg/μL**
Detection concentration	6.35	8.54	15.55	26.24
	6.62	11.41	16.21	32.49
	3.98	7.87	15.23	24.67
	3.95	10.87	16.21	28.90
	6.53	9.67	13.18	27.92
	6.35	9.87	19.25	23.83
	5.39	9.22	16.78	27.73
	5.20	7.71	12.91	24.50
	4.32	12.14	15.03	25.36
	4.23	7.09	20.48	30.12
Average	5.29	9.44	16.08	27.18
CV	21.0%	17.8%	14.7%	10.2%
Accuracy deviation	5.88%	-5.61%	-19.58%	-9.42%

4.3.2. Recovery Requirements:within 50% to 150%

Five samples of 0.03pg/μL, 0.3pg/μL, 3pg/μL, 30pg/μL and 300pg/μL were prepared, purified by sample preparation kit and detected by amplification kit. The recoveries of all simulated samples were between 70% and 130%.

Recovery of E.coli samples with different concentrations						
Sample concentration（fg/μL）	**Test Concentration（fg/μL）**				**CV**	**Recovery**
30	3.36E+01	3.45E+01	3.38E+01	3.40E+01	1.17%	106.8%
300	3.85E+02	3.98E+02	3.85E+02	3.89E+02	1.62%	122.5%
3000	3.65E+03	3.60E+03	3.60E+03	3.61E+03	0.65%	113.6%
30000	3.34E+04	3.38E+04	3.43E+04	3.38E+04	1.12%	106.4%
300000	3.96E+05	3.86E+05	2.63E+05	3.43E+05	17.68%	107.7%

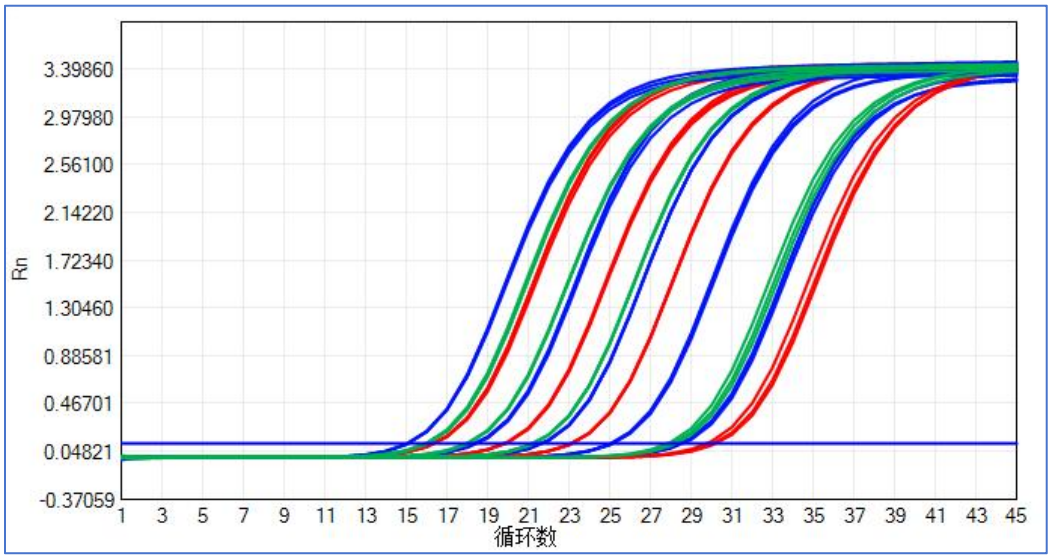


4.4. Accuracy

Requirements: Recovery within 50% to 150%

The 4 real samples were added with the corresponding concentration of E.coli DNA, purified by sample preparation kit and detected by amplification reagent, and the recovery rate was between 70% and 130%.

E. coli Recovery					
Sample	Sample concentration（pg/μL）	The amount of DNA added/pg	The concentration of a sample with added DNA（pg/μL）	Elution volume（μL)	Recovery
Sample-1	1.30E+02	3.18E+03	1.62E+02	100	99.29%
Sample-2	1.18E+01	3.18E+03	4.08E+01	100	91.10%
Sample-3	1.22E+00	3.18E+02	4.54E+00	100	104.45%
Sample-4	1. 09E-02	3. 18E+00	4. 20E-02	100	97.85%



4.5. Specificity

The genomes of HEK293, MDCK, BHK21, Vero, CHO, Pichia Pastoris and common human genome cells in the environment were detected, and each cell or strain was detected three times. The detected Ct values were all No Ct.

Add sample	Ct 1	Ct 2	Ct 3
HEK293	NoCt	NoCt	NoCt
MDCK	NoCt	NoCt	NoCt
BHK21	NoCt	NoCt	NoCt
Vero	NoCt	NoCt	NoCt
CHO	NoCt	NoCt	NoCt
Pichia pastoris	NoCt	NoCt	NoCt
Human	NoCt	NoCt	NoCt
NTC	NoCt	NoCt	NoCt

4.6. Precision

4.6.1. Requirements: [Repeatability, Intra run %RSD] NMT 30%

E.coli accuracy/precision sample S1-S4 was tested, and its in-batch precision was within 30%, which met the requirements.

E.coli Repeatability [Intra run %RSD]				
Sample	S4	S3	S2	S1
Ct	17.96	22.58	28.34	30.38
	17.97	22.57	28.37	31.03
	17.94	22.56	28.28	30.42
	17.96	22.64	28.38	30.54
	18.04	22.57	28.25	30.81
	17.98	22.63	28.16	30.88
	18.01	22.59	28.3	30.43
	18	22.62	28.03	30.79
	18.04	22.64	28.19	30.72
	17.99	22.61	28.56	30.93
Concentration	4.14E+04	1.68E+03	3.07E+01	7.45E+00
	4.11E+04	1.69E+03	3.01E+01	4.75E+00
	4.20E+04	1.70E+03	3.20E+01	7.25E+00
	4.14E+04	1.61E+03	2.99E+01	6.67E+00
	3.92E+04	1.69E+03	3.27E+01	5.53E+00
	4.08E+04	1.62E+03	3.48E+01	5.27E+00
	4.03E+04	1.63E+03	3.81E+01	5.61E+00
	3.92E+04	1.61E+03	3.41E+01	5.89E+00

	4.06E+04	1.64E+03	2.64E+01	5.09E+00
Mean value	4.06E+04	1.65E+03	3.20E+01	6.07E+00
CV	2%	2%	10%	16%

4.6.2. Measurement Reproducibility: day to day and analyst to analyst, NMT 30%

Measurement Reproducibility: Three batches of the kit were used to detect precision sample S1-S4 in two working days with three experimentalists, and the precision of the kit was evaluated with 5 multiple wells per concentration per experiment. The accuracy/precision of E.coli reference product S1-S4 was tested by integrating factors such as inter-instrument, inter-reagent batch, inter-personnel, inter-day, etc. The total precision was within 30%.

E.coli Intermediate Precision（day to day and analyst to analyst）						
operator	DATE	Sample	S1	S2	S3	S4
operator 1	Day1	Concentration 1	5.95E+00	3.11E+01	1.65E+03	4.09E+04
			7.26E+00	3.48E+01	1.68E+03	3.90E+04
			6.77E+00	3.62E+01	1.78E+03	4.06E+04
			5.71E+00	3.36E+01	1.62E+03	3.79E+04
			6.46E+00	3.52E+01	1.68E+03	4.03E+04
			5.08E+00	3.24E+01	1.62E+03	3.92E+04
operator 1	Day2-1	Concentration 2	5.23E+00	3.56E+01	1.69E+03	4.20E+04
			8.30E+00	3.79E+01	1.65E+03	3.97E+04
			4.81E+00	3.32E+01	1.65E+03	3.95E+04
			7.85E+00	3.41E+01	1.82E+03	3.97E+04
			5.34E+00	3.17E+01	1.74E+03	4.17E+04
			7.43E+00	3.00E+01	1.76E+03	4.00E+04
operator 2	Day2-2	Concentration 3	7.47E+00	2.73E+01	1.53E+03	4.09E+04
			5.06E+00	3.32E+01	1.61E+03	4.12E+04
			7.06E+00	3.41E+01	1.58E+03	4.42E+04
			4.46E+00	3.05E+01	1.75E+03	4.07E+04
			5.38E+00	3.32E+01	1.63E+03	3.95E+04
			4.72E+00	2.97E+01	1.59E+03	4.30E+04
operator 3	Day 3	Concentration 4	8.74E+00	3.02E+01	1.69E+03	4.11E+04
			5.60E+00	3.32E+01	1.63E+03	4.05E+04
			8.98E+00	3.63E+01	1.76E+03	4.14E+04
			5.45E+00	2.82E+01	1.65E+03	4.20E+04
			6.97E+00	3.13E+01	1.60E+03	4.05E+04
Mean value			6.35E+00	3.27E+01	1.67E+03	4.07E+04
CV			21.3%	8.2%	4.3%	3.4%