Detection and Quantitation of Residual Host Cell DNA

White Paper

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Abstract

All biological drug products are required to be characterised for safety, quality and efficacy. ICH Q6B gives clear guidance on the requirements for product characterisation including the detection of product and process related impurities. This white paper discusses an approach to monitoring the levels of residual DNA derived from the production host for the drug including consideration of practical control of contamination as well as the MHRA's guidance on performing PCR analysis.

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Introduction

The expression of biological products using recombinant DNA technology has enabled the use of peptides and proteins for therapeutic use. One of the main concerns with this expression technology especially in immortal cell lines is the possibility of transference of the immortal trait to the end user of the medication. Thereby, potentially inducing cancer.

The World Health Organisation (WHO), has released guidance[1,2] requiring the monitoring throughout the manufacturing process of HCDNA to demonstrate reduction to safe levels, either by process validation or lot release testing.

The WHO go on to dictate that the levels of residual host cell DNA that should not exceed 10 ng/dose for parenteral administered drugs.

The WHO guidance for the potential risk of residual host cell DNA suggests these factors should be considered;

- The manufacturing processes ability to remove DNA
- The level of DNA fragmentation during processing
- Any DNA modifications during the processing that reduces its biological functions

The detection and quantification of residual host cell DNA is possible at very low levels using both fluorescence probes that bind to nucleic acid as well as more targeted analysis using polymerase chain reaction (PCR). Depending on the specific drug manufacturing process there are two main routes for dealing with residual host cell DNA control and monitoring.

Process control

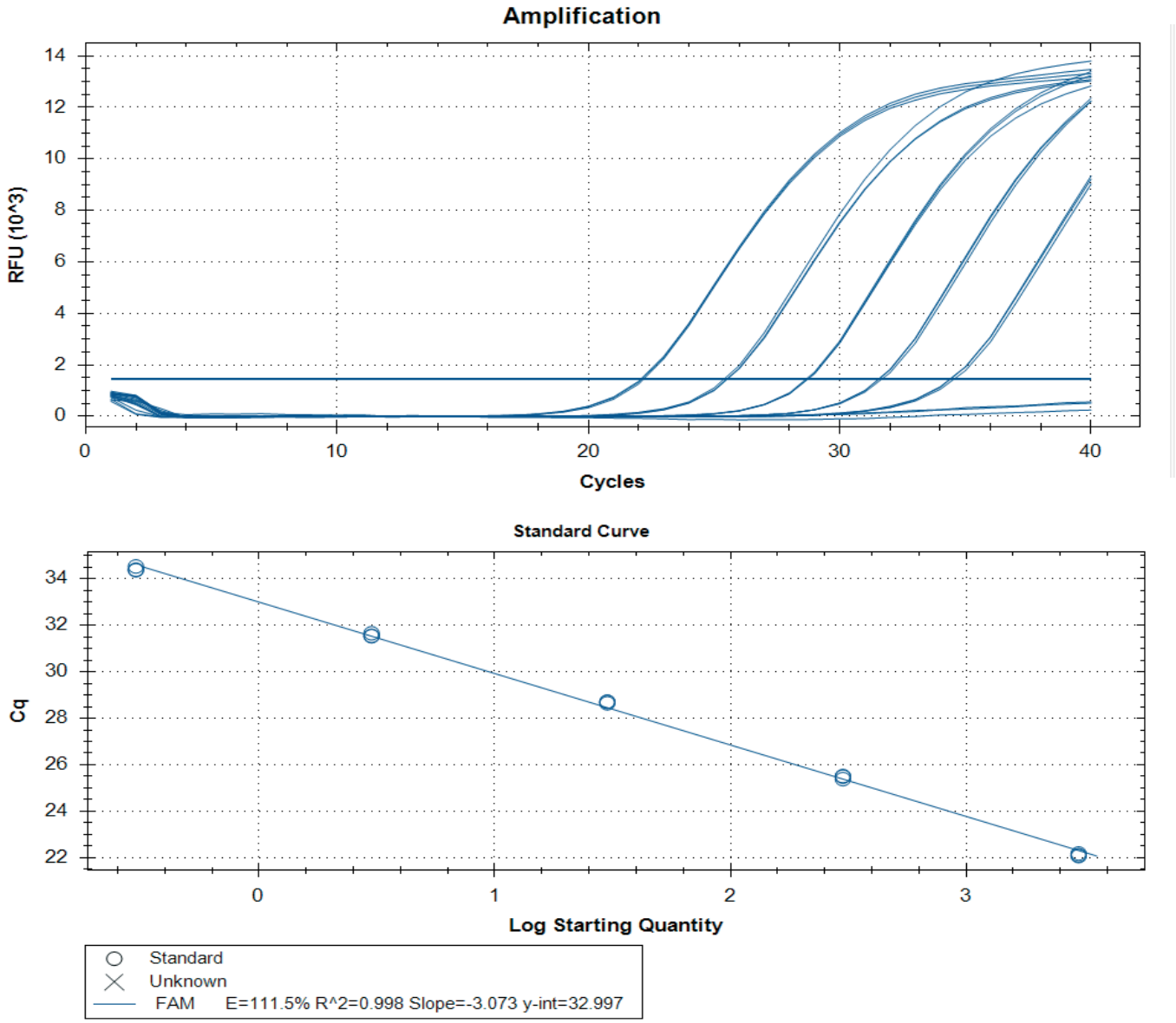
The demonstration to the regulatory authorities that the drug manufacturing process is able to routinely and consistently remove any possible residual DNA contamination leads to no further need for routine residual host cell DNA release testing of individual batches. This would typically involve the demonstration of clearance of intentionally added host cell DNA, of the size distribution expected in the drug manufacturing process, at greater than the expected concentration of residual DNA to steps in the process and quantifying the reduction in DNA. Data then needs to be collected from enough individual batches to prove that the residual DNA removal is robustly achieved by the process. Once a process has been validated for removal of residual DNA any change in the manufacturing process will necessitate a risk assessment to ensure that the process still meets the requirements of residual DNA clearance otherwise the validation will need to be repeated.

Batch monitoring

Where a process is known to produce an API that contains residual host cell DNA then there is a requirement to perform individual batch release for residual DNA. This data is used to monitor the manufacturing process to determine and control the batch to batch variation. This route would be more applicable to orally administer drugs which have a higher allowable tolerance for residual host cell DNA[3].

Quantitation of residual host cell nucleic acid

The use of nucleic acid probes that exhibit an increased fluorescence on binding to nucleic acid allows the sensitive detection of both DNA as well as RNA. Commercially available kits are readily available from a number of different suppliers. Currently RSSL use the Quant-iT Pico-Green dsDNA assay kit from Life technologies. This assay has sensitivity down to 5 ng/mL for dsDNA with a quantitation range up to 1000 ng/mL. The kit provides a double stranded DNA standard for the production of a standard curve against which the unknown samples can be measured. The advantages of using a general nucleic acid test include the detection of all forms of nucleic acid including smaller fragments and single stranded forms, off-the-shelf availability reducing the time from validation to an in-use assay. The differential response to RNA and single stranded RNA and DNA make quantitation of mixed populations of nucleic acid more difficult, which can be a disadvantage along with the detection of small fragments of nucleic acids or modified nucleic acids which may not have a high risk for the end user. These general nucleic acid assays are generally applied early in a drugs development before a more specific quantitative PCR assay is available.



The 'Gold Standard' for DNA analysis is quantitative or Real-Time PCR analysis (qPCR). Regions of the target DNA sequence are targeted using pairs of 'primers', small sequences of single stranded DNA, allowing these specific regions to be amplified (copied) repeatedly. Under optimal PCR conditions (i.e. non-limiting kinetics) the number of copies of an amplicon (region of target DNA defined by a pair of primers) will increase exponentially with each cycle of the PCR. Fluorescent dyes report the amount of DNA at the end of each cycle of the PCR allowing the progress of the amplification to be monitored. As the intensity of fluorescence signal is proportional to the concentration of DNA in the PCR then the cycle number producing a defined fluorescence signal (Cq/Ct value) can be used to generate a standard curve from which the level of residual host cell DNA in a sample can be determined. The process of DNA amplification makes analysis by PCR a very sensitive technique able to detect down to picogram per millilitre levels of residual host cell DNA.

The requirement for sample extraction

Whether using a general fluorescence based assay or PCR the nucleic acid is generally extracted from the sample as a first step to allow detection in the absence of inhibitory compounds that may be in the sample matrix. The technology most commonly used to purify nucleic acid from a sample is based on the reversible binding (ionic strength dependant) of nucleic acid to glass beads. Modified versions using magnetic beads can be performed with reduced processing times and have allowed the use of automation for the extraction process increasing throughput and improving precision.

In-order to determine the residual host cell DNA quantity an assessment needs to be made regarding the amount of nucleic acid lost during the extraction stage of the analysis. This requires the addition of a known amount of target DNA (the 'Spike') to each sample prior to the extraction process. Comparing the measured amount of DNA after extraction with the spiked amount allows for the calculation of the 'Extraction efficiency'. This value can then be applied to the measured amount of DNA in the sample to calculate the residual host cell DNA. The presence of inhibitory compounds extracted along with the nucleic acid is indicated by a reduction in the extraction efficiency.

Should a sample matrix result in an effect on the extraction efficiency then development of specific sample preparation procedures, usually dilution or buffer exchange, will be needed to increase the recovery of DNA prior to being possible to use the assay to measure the residual host cell DNA levels.

HCDNA testing at RSSL

Although residual host cell DNA analysis kits are available from numerous commercial suppliers. Currently RSSL are using the resDNASEQ kits (Life Technologies now sold under Thermo-Fisher brand). These kits are readily available for a range of host organisms:

- CHO (Chinese Hamster Ovary)
- Human
- MDCK (Canine Kidney)
- E.coli
- VERO (African Green Monkey)
- Pichia pastoris (Yeast)
- NS0 (Murine myeloma)

The advantage of these kits are that they are immediately validation ready, have multiple amplicons (sets of primers) making them more robust for detecting fragmented DNA and come complete with target host cell DNA standards and can be seamlessly integrated into a workflow that includes the DNA extraction.

Validation of residual host cell DNA analysis

The level of validation of the residual host cell DNA assay is going to be dependent on the stage in the drug lifecycle. Early stage development may not need a fully validated assay allowing the use of 'off-the-shelf' commercially available products. Later stage assays may well evolve to be targeted towards nucleic acid of greatest risk i.e. the elements of the host that make it immortal and the specific DNA sequences used to produce the biological product (Promoters, regulatory elements etc.). Validation of residual host cell DNA assays to the International Conference on Harmonisation tripartite guideline for an impurity assay should cover these aspects;

- Accuracy
- Precision
  - Repeatability
  - Intermediate precision
- Specificity
- Limit of quantitation (LOQ)
- Linearity
- Range
- Robustness

RSSL have validated several of the resDNASEQ kits as cGMP assays. The similarity between the commercial kit designs makes the process of validation streamlined with a typical kit validation being performed within 10 days.

Contamination control and assay validity

One of the major considerations when testing for residual host cell DNA is the presence of the host organism within the testing laboratory. The testing of samples derived from various organisms poses the risk of cross-contamination within the assay. This is particularly true for testing for human DNA as the analyst performing the assay can be a source of contamination. Assay contamination is monitored for using blank control samples run in parallel with the test samples. Contamination entering into the analysis will be indicated by a positive signal in the blank controls. In order to limit the number of failed assays due to contamination, control measures are used;

- Clean air environments during set up
  - Use of laminar flow cabinets
  - Use of robotic DNA extraction technology
- Segregation of activities
  - DNA extraction
  - Mastermix preparation
  - PCR set up
  - Running the PCR
- Use of a validated cleaning/decontamination program

Conclusion

Drug characterisation to ICH Q6B requires the accurate detection and quantitation of residual host cell DNA. The individual requirements of these assays will depend of the particular stage in the drug lifecycle, although high sensitivity is needed to meet the guidelines of the World Health Organization[1,2]. RSSL can support in both performing routine analysis as well as assay validation to ICH Q2 (R1) to support your drugs development regardless of its stage in the drug lifecycle. Using effective contamination control to ensure 'right first time' analysis with no false positive results caused by cross-contamination. This approach is designed to reduce validation and analytical time thereby reducing the costs of your residual host cell DNA analysis.

About the author

Phil Kuhlman, Biopharmaceutical Technical Specialist

Phil has seventeen years' industry experience, built on eight years of academic experience in protein-protein interaction and characterisation, having graduated in 1993 from Leicester University with a PhD in Biochemistry. He has gained a broad knowledge of the characterisation and use of proteins and nucleic acids through working in academia prior to moving into the biotechnology sector and then into a CRO. Phil's main expertise is in characterisation of biologics throughout the complete drug lifecycle with an emphasis on forced degradation studies for determining comparability between biosimilars and setting critical quality attributes (CQA's).

References

[1] Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology, World Health Organisation, 2013.
[2] Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. Annex 3 WHO Technical Report Series No. 978, 2013.
[3] Israel MA et al. Biological activity of polyoma viral DNA in mice and hamsters. Journal of Virology, 1979, 29:990-996.

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