Validation Guidance for Residual Host Cell DNA Testing

Components of a method validation per ICH Q2(R1) in the context of a qPCR assay for sensitive, accurate, and rapid quantitation of residual host cell DNA

Introduction

Development of a manufacturing process for a biotherapeutic that ensures consistent quality, purity and safety is a critical step in successfully developing the drug, getting it approved by regulatory agencies, and bringing it to the market.

Most biological drugs, such as antibodies, recombinant proteins, and vaccines, are produced in bacterial, yeast, or animal cell lines and may still contain trace amounts of DNA from the host cells, even after a rigorous purification process. The presence of residual host cell DNA molecules in a biotherapeutic could potentially cause an adverse event or present a safety issue.

Because of these potential risks to patient safety, regulatory authorities have set requirements to demonstrate that the levels of host-cell DNA in products manufactured in cell cultures are below certain limits established globally.

Guidance from Regulatory Agencies

Quantitation of residual host cell DNA requires a method with a level of sensitivity that enables meeting or exceeding the regulatory specifications for acceptable levels of host cell DNA in a dose of the product. Over the past decade, methods using real-time quantitative polymerase chain reaction (qPCR) have become a staple in quantitatively measuring nucleic acid-based impurities (e.g., residual host cell DNA). The US Pharmacopeia chapter 509 provides guidance on use of qPCR as the preferred method for highly sensitive, specific, and accurate quantitation of host-cell DNA in biopharma manufacturing.

Quality control (QC) methods using these assays require validation per International Conference on Harmonization guidelines, ICH Q2(R1), 'Validation of Analytical Procedures', to ensure that the analytical procedure is suitable for the intended use. Following the ICH guidance, manufacturers typically develop their own standard operating procedures (SOP) for executing analytical tests for parameters such as specificity, sensitivity, or limit of quantitation.[1,2]

"The testing is generally done during the purification process for two reasons: 1) to develop a thorough understanding of the capability of the purification process to remove the host-cell DNA; and 2) to understand the level of DNA that's present at the end of the purification process," says Mike Brewer, Director and Global Principal Consultant (Regulatory) for the BioProduction Division at Thermo Fisher Scientific.

How to design validation studies with a comprehensive commercial testing kit

When it comes to validating an assay developed in-house, the process is very similar to validating a commercially available assay, says Brewer, but generally, the development and qualification process prior to validation of an internally developed method is very time and labor intensive. "There is a lot of experimentation and documentation that goes into developing a method in-house, including selection of critical reagents, assay optimization, and development of SOPs, etc.," says Brewer. "With a commercially available assay, you're buying something that's essentially ready to use, so the process is much more streamlined."

The user guide that comes with a commercially available assay helps researchers develop their own SOPs and quickly qualify the performance of the method for use with customer-specific samples. Such guided qualification can significantly shorten the timeline from initial evaluation to a validated analytical method, adds Brewer.

resDNASEQ System for Residual DNA Testing

Thermo Fisher's resDNASEQ is a fully integrated real-time qPCR-based system for measuring residual host cell DNA. It combines a sample preparation method that enables quantitative DNA recovery with high precision from a variety of sample matrices, and specific qPCR-based assays for the host cell line used in the process being tested. This includes CHO (hamster), Human, *E. coli* (bacterial), Vero (monkey), NS0 (mouse tumor), Pichia pastoris (fungal), MDCK (canine kidney), HEK293 (human embryonic kidney) and Sf9 (insect)/Baculovirus host cell lines.

The following list highlights some of the key differentiators of the resDNASEQ system:

(1) Sample preparation

resDNASEQ employs Applied Biosystems' PrepSEQ – a system that uses magnetic bead-based sample preparation chemistry. First the proteins in the sample, product and host cell, are enzymatically digested, a solution is added to drive association of the DNA with the magnetic beads, and then a DNA binding step is performed. Following binding, impurities or potential qPCR inhibitors are removed via two wash steps, and then the DNA is eluted with a buffer that's compatible with qPCR.

"One of the unique things about this chemistry is that you get quantitative or near-quantitative recovery of the DNA from the test samples," Brewer says, claiming that minute quantities of DNA (think picogram or femtogram levels) can be accurately recovered from challenging sample matrices with high levels of protein (10-100 mg/mL of protein), low pH, and significant levels of salt and buffers typical of biopharmaceutical purification processes.

While a manual protocol is available for lower throughput applications, sample preparation can be automated with the KingFisher Flex instruments that allow high-throughput purification of DNA from 24-96 samples per run. Brewer notes that, although there are other methods that give a similar level of performance, most cannot be adapted to an automated platform. Additionally, methods that rely on alcohol precipitation to concentrate the DNA sometimes have issues with qPCR inhibitors co-precipitating and preventing accurate quantitation of the DNA.

(2) High specificity, sensitivity, and efficiency

According to Brewer, resDNASEQ qPCR primer/probe sets were developed with the help of advanced informatics to design an assay that is both specific to the species and highly sensitive. "The use of sophisticated informatics allowed us to select a high-copy, repetitive element dispersed across the genomes of the species that we're targeting, so the assay is designed to be highly specific and as sensitive as possible," he remarks. The sensitivity of resDNASEQ is reflected in its limit of quantitation (LOQ): 1.5 pg DNA/mL test sample for most mammalian species and 15 pg DNA/mL test sample for *E. coli*.

(3) AccuSEQ software for data analysis

The inclusion of purpose-built software by Applied Biosystems marks another differentiator of the resDNASEQ system. Instead of manually carrying out calculations, researchers can rely on the software to perform the calculations required for accurately reporting test results, including the mean quantity of DNA in the sample tested, the standard deviation, and the percent coefficient of variation for the sample replicates typically tested with a quantitative method.

Also, the AccuSEQ software enables compliance with the guidance provided in the US Food and Drug Administration's (FDA) 21 CFR Part 11 - Electronic Records. These regulations are set around data security and data integrity related to electronic documentation and electronic signatures.

(4) Highly-characterized DNA reference standards

Quantitation of the DNA present in the test samples are achieved by comparing the amount of DNA in the unknown sample to the standard curve generated from qPCR analysis of known amounts of host-cell standard DNA. But some commercially available qPCR assays do not include DNA reference standards, leaving researchers to develop their own method to purify the standard DNA.

The resDNASEQ assay kit comes with a highly-characterized DNA reference standard. Brewer notes that this component is particularly important because the regulatory expectation for host cell DNA testing is to use a quantitative assay, not a detection assay (a positive-negative readout). "Detection assays are validated differently than a quantitative test for impurities," he clarifies.

Establishing acceptance criteria

Once the analytical method is developed, to establish acceptance criteria, a round of pre-validation tests should be conducted. This type of qualification involves running multiple samples through experiments that would be typical of the actual validation.

"The point of the pre-validation is to get a feeling for the performance of the method – you can look at various metrics like percent recovery, coefficient of variation, etc. to figure out what you might assign for the assay acceptance criteria," says Brewer. "Analyzing the results from the pre-validation, perhaps with the help of a statistician, will help you confidently set appropriate acceptance criteria for the actual validation."

The criteria can be set in an acceptable range to allow for variation, but they must comply with regulatory guidance. For example, USP Chapter 509 states that percent recovery should be between 50-150% and percent coefficient of variation (%CV) should be 30% or less.

Method Validation Case Study

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. As mentioned above, analytical method validation is performed following the guidance provided in ICH Q2 (R1) Validation of Analytical Procedures.

To demonstrate a typical validation with resDNASEQ, a case study is presented below. This study was executed by Vitrology Ltd. (now part of SGS) using surrogate test sample matrices that are typical of a monoclonal antibody purification process. (Figure 1) The validation parameters tested in this case study include assay range, linearity, specificity, accuracy, precision, and limit of quantitation.

Specificity Assessment

The definition of specificity is the ability to unequivocally assess the analyte in the presence of expected components in a test sample, such as proteins, salts, buffers, degradants, impurities, etc.

Matrix		Buffers	IgG
M1	Ion Exchange	0.8 M NaCl 20 mM NaPO4 (pH 7.5)	10 mg/ml
M2	Hydrophobic Interaction	0.75M Ammonium sulfate 50 mM NaPO4 (pH 6)	10 mg/ml
M3	Ion Exchange	0.7 M KPO4 (pH 6)	10 mg/ml
M4	Protein A	100 mM Sodium citrate pH 3.0	10 mg/ml
M5	Bulk Drug Substance	3% Mannitol 2% Sucrose 10 mM L-Arginine 0.01% Tween 20	50 mg/ml

Figure 1. These matrices are designed to represent samples from ion exchange, hydrophobic interaction, Protein A affinity chromatography, and bulk drug substance. Added buffers and IgG represent the levels of protein typically present in a monoclonal antibody purification process.

Why this is necessary: To ensure the TaqMan assay does not detect off-target DNA that could be present in a test sample, and that the DNA in a test sample can be accurately quantitated following recovery from the test sample types included in the scope of the test method

Study design

Part 1 – Three samples with 3ng human DNA (off-target DNA) in dilution buffer were tested. The reason human DNA was used for specificity testing is due to the sensitivity of the assay and the fact that the analyst running the test could shed a small number of cells with measurable human DNA.

Part 2 – Four sample matrices were tested; 3 samples each (triplicate to calculate mean, standard deviation, and %CV), 10 pg CHO DNA spiked per sample.

Acceptance criteria: Validated assay should not recognize human DNA and it must accurately quantitate target DNA recovered from the test samples evaluated. The quantity measured should be between 50-150% of expected DNA.

Results: The assay does not detect human DNA, and the amounts of DNA measured in all sample matrices were between 50-150% of the expected CHO DNA.

Linearity Assessment

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the test sample (linearity is typically a hallmark of a qPCR assay).

Why this is necessary: To verify that the measured DNA in a test sample is calculated from the linear region of the standard curve, when comparing test sample to standard curve.

Study design: Four independent curves, prepared from a 10-fold dilution series of CHO standard DNA, were analyzed. Then the linearity (R^2 value) of the four different standard curves was assessed.

Acceptance criteria: R^2 must be greater than 0.98 for the four independent standard curves.

Results: R^2 of >0.98 was achieved for all four standard curves.

Range Assessment

The range of an analytical procedure is the interval between the upper and lower concentrations of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

Why this is necessary: To verify the range of the assay across the DNA concentrations analyzed to generate the standard curve.

Study design: Four independent curves, prepared from a 10-fold dilution series of CHO standard DNA with a concentration range of 3 ng to 30 fg, were analyzed.

Acceptance criteria: Assay quantitative range should be consistent in a minimum of four independent standard curves.

Results: The assay quantitative range was consistent in the four independent standard curves and it was determined to be 0.03-3000 pg for CHO DNA.

Accuracy Assessment

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted, either as a conventional true value or an accepted reference standard value, and the value found.

Why this is necessary: It determines how close the test results are for a specific analyte compared to the true measure, which is calculated by the amount of standard DNA spiked into test samples.

Study design: Three samples were tested at four different DNA spike levels: 100, 10, 1, and 0.1 pg.

Acceptance criteria: Accuracy is acceptable when the quantity of spike DNA levels are found within a range of ± 2 to 4-fold of the comparative standard value. Alternatively, it could be 50-150% of the comparative standard value with a %CV less than 25%.

Results: The assay was able to quantitate 100 pg, 10 pg, 1 pg, and 0.1 pg of DNA from the sample test matrix within the acceptance range of ± 2 to 4-fold of the comparative standard value.

Additionally, an internal validation study of the KingFisher Flex automated sample preparation system using the CHO, *E. coli*, and Vero assays, performed with two different instruments, demonstrated highly efficient recovery with accurate and precise quantitation (very low %CV) of DNA recovered from multiple test sample matrices. "This shows the robustness of both the resDNASEQ assays and the PrepSEQ sample prep process using the KingFisher Flex automated magnetic bead handler," remarks Brewer.

Quantitation Limit Assessment

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Quantitation limit

is a parameter of quantitative tests for low level compounds and sample matrices and it is used particularly for the determination of impurities and/or degradation products.

Why this is necessary: To verify accurate quantitation at the Limit of Quantitation (LOQ) of the assay.

Study design: Six samples of the surrogate bulk drug substance, each spiked with 30 fg of DNA, were tested. (Note: The overall method LOQ with spiking at this level is 1.5 pg DNA/mL test sample) The reason for using the bulk drug substance surrogate matrix for this test is because it represents a sample near the end of a purification where, typically, the specification of acceptable levels of host cell DNA would be applied.

Acceptance criteria: DNA recovery at LOQ must be between 50-150%.

Results: The samples met the acceptance criteria – percent recovery was between 50-150% for the six samples evaluated.

Precision Assessment

The precision of an analytical procedure expresses the closeness of agreement, or degree of scatter, between a series of measurements obtained from multiple sampling of the same homogeneous solution under the prescribed conditions. Precision maybe considered at three levels: repeatability, intermediate precision, and reproducibility.

Why this is necessary: To ensure precision under the same operating conditions over time (repeatability or intra-assay precision), and to ensure precision under laboratory variations, including over different days, different equipment, different analysis (intermediate precision).

Study design:

Part 1 – Six replicates of the surrogate bulk drug substance were spiked with 10 pg DNA/test sample and tested.

Part 2 – Two analysts ran the same assay in Part 1, twice (3 samples), on different days.

Acceptance criteria: For Part 1, %CV must be less than 25% for all samples tested. For Part 2, the mean %CV for the average amount of DNA measured in the 15 test samples must be less than 25%.

Results: The %CV and the mean %CV were both below the acceptance criteria of 25%.

Conclusion

Method validation of analytical methods that are used to test quality and safety of licensed products is a regulatory requirement. Residual host-cell DNA impurities, measured in the final dosage form, must follow regulatory guidelines established by agencies such as the US FDA, European Medicines Agency (EMA), the World Health Organization (WHO), and other regional regulatory jurisdictions. As the case study above demonstrates, resDNASEQ assays combined with PrepSEQ sample prep, perform in a manner that enables meeting or exceeding these expectations.

"The complete resDNASEQ solution includes all components required for quantitation of host cell DNA, from the sample prep to the quantitation method, instrumentation, the application specific software that enables compliance with CFR 21 Part 11 guidance, and even technical support – meaning a field application scientist can come to your lab and train people on how to run the assay and how to get valid results with it," mentions Brewer. "It is really an end-to-end solution."

To learn more about the resDNASEQ system, please visit: **thermofisher.com/resdnaseq**

References

1. Apostal, I., Kelner, D.N. "Managing the Analytical Lifecycle for Biotechnology Products – Part One." BioProcess Int, Sept. 2008. https://bioprocessintl.com/analytical/downstream-validation/managing-the-analytical-lifecycle-for-biotechnology-products-183144/
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