

# Technical Note: Quantitation of Retrovirus-Like Particles (RVLPs) in CHO Derived Products

## Summary

Within the biopharmaceutical industry it is well recognized that Chinese Hamster Ovary (CHO) cells are the most commonly used production cell line for monoclonal antibodies and other recombinant proteins. Products derived from these cells have a very good safety record; there is no recorded incidence of patient infection from contaminants in a product manufactured from these cells. This is one of the reasons that CHO cells have been the cell substrate of choice for monoclonal antibody and other recombinant protein product development.

The Note for Guidance on Quality of Biotechnological Products: *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*<sup>1</sup>, states that a safety testing program is dependent on three critical aspects:

- testing of the starting materials, including the cell banks
- testing of the in-process intermediates, typically the bulk harvest
- evaluation of the viral inactivation and/or removal capacity of the downstream purification process

The package of recommended testing considers the history of the cell line, the origin of raw materials used during production, product dosage, therapeutic application, clinical stage, patient population, in addition to other factors which may impact the testing strategy.

During the production of therapeutic proteins, CHO cells express endogenous retrovirus like particles (RVLPs) which must be removed during product purification. Once the manufacturing process has been scaled up and ready for commercialization, it is recommended

that RVLPs be quantitated in the unprocessed bulk harvest material. Data from at least three lots of bulk harvest are required for submission to the regulatory authorities. This technical note will provide the scientific and regulatory landscape for key methods which are applied to ensure safety of such recombinant proteins, with a focus on alternate and superior methods to the standard transmission electron microscopy (TEM) method.

## Introduction

As part of its life cycle, a retrovirus integrates its own genome into its host cell genome. The genomes of many cell lines, including CHO cells, contain partial or complete retroviral sequences. These sequences can be expressed and result in defective retroviral-like particles (RVLPs). Before the advent of molecular biology, retroviruses were classified by the morphology of the virion, as observed by electron microscopy. Based on this classification, two types of RVLPs have been associated with CHO cells. Type A or intracisternal particles are immature capsid particles found in the cytoplasm, while type C particles assemble at and bud from the host cell's plasma membrane.

RVLPs have never been shown to have been transmitted to a patient through a CHO-derived monoclonal or other recombinant drug product nor have they been associated with a human disease. The regulatory agencies, however, expect that manufacturers of CHO-derived products quantitate the RVLPs in unprocessed bulk harvests.<sup>1,2</sup> The quantitative index of retroviral production enables monitoring of RVLP production over sequential lots, demonstrating the consistency of the upstream process. In addition, the number of RVLPs in the bulk harvest provides an expectation for the level of viral clearance in the

downstream manufacturing process; the manufacturing process should be demonstrated to have the capacity to remove or inactivate substantially more RVLPs than the number present in the bulk harvest.<sup>1</sup>

## Regulatory Expectations for RVLPs

ICH Q5A(R1) notes the importance of assessing the level of RVLPs in process intermediates from CHO-derived products:

- “Cells may have ... **endogenous retrovirus** which may be transmitted vertically from one cell generation to the next, ... Such viruses may be constitutively expressed or may **unexpectedly become expressed as an infectious virus**”.
- “...it is important to **estimate the amount of virus in the unprocessed bulk**”.
- “The amount of virus eliminated or inactivated by the production process **should be compared to the amount of virus which may be present in unprocessed bulk**.”
- “Cell lines derived from rodents usually contain endogenous retrovirus particles...The capacity of the manufacturing process to remove and/or inactivate rodent retroviruses from products obtained from such cells should be determined.”
- “...there is **excess capacity** for viral clearance built into the purification process to assure an appropriate level of safety for the final product.”

## Assays to Quantitate RVLPs in Unprocessed Bulk

Quantitative transmission electron microscopy (TEM) analysis has historically been the method of choice to quantitate retroviral particles in volumes of unprocessed bulk harvest (UPBH) supernatant. This method, which requires concentration of the sample to provide adequate assay sensitivity, is highly variable and in spite of the sample concentration, has low

sensitivity; the detection limit is typically  $1 \times 10^6$  particles/mL. Furthermore, the assay is very manual and time consuming; turn-around-times are at least 60 days. Because newer technologies have replaced many electron microscopy-based assays, experienced electron microscopists are difficult to find, and there is a long training curve for new microscopists.

New technologies have proven to be much more powerful than TEM for quantitation of RVLPs. Because type C RVLPs cannot be detected with an infectivity assay, current assays use molecular technologies. These methods are much more sensitive, more consistent and much faster than TEM. Two methods are detailed below.

1. RT-qPCR Assay for the Detection and Quantitation of Endogenous Type C Retrovirus-Like Particles in CHO Cells (305110GMP.BSV and 305110GMP.BUK) - the RT-qPCR assay was developed for the specific detection of type C RVLPs in CHO cells,<sup>3</sup> unlike the qPERT assay, which detects all retroviral reverse transcriptase. The qPCR method has a significant advantage over TEM analysis in its limit of detection and linearity of range of detection. Furthermore, a formal comparative analysis of this method with the TEM and PERT assays demonstrated the sensitivity of the molecular methods.<sup>4</sup> These data provide an appropriate degree of assurance of consistency of the qPCR results and positions this assay as relevant and tractable for the determination of C-type particles in UPBH from CHO cells (**Table 1**).

**Table 1. Comparison of classical TEM to molecular-based CHO particle qPCR methods.**

TEM	CHO Particle PCR
Historical Standard	Good Correlation with TEM
Highly Variable	Reproducible
Low Sensitivity (1E6 particles/mL)	Highly Sensitive
Requires Concentration of Sample	No Concentration Required
Results in Months	Results in Days

2. Quantitative Product Enhanced Reverse Transcriptase Assay for the Detection of Retrovirus in Biological Samples (105230GMP.BSV and 107325GMP.BUK) - this assay uses PCR to quantitatively detect the presence of reverse transcriptase, the enzyme that is present in the core and pre-integration complexes of all retroviruses. In this assay, the retroviral associated reverse transcriptase is used to make a cDNA copy of an RNA template. The sensitivity of the assay is enhanced by amplification of the cDNA using quantitative PCR, and detection of the PCR product is further enhanced using a fluorescent probe. The assay is reproducible and can be performed with relatively short turn-around times in a high throughput fashion. This assay detects the presence of reverse transcriptase, and not specifically reverse transcriptase that might be associated with a type C retroviral particle.

## Regulatory Acceptance of Molecular Methods

Regulatory agencies accept that as science progresses, assays that were once the standard may be replaced by more sensitive, accurate and reproducible assays. ICH Q5A notes that, "Numerous assays can be used for the detection of endogenous and adventitious viruses...They should be regarded as assay protocols recommended for the present, but the list is not all-inclusive or definitive. Since the most appropriate techniques may change with scientific progress, proposals for alternative techniques, when accompanied by adequate supporting data, may be acceptable."<sup>1</sup>

Quantitation of RVLPS by TEM or qPCR is acceptable to regulators as both assays provide sufficient accuracy and resolution to enable the sequential determination of retrovirus burden. The molecular methods are sufficiently validated and the sensitivity and linearity are demonstrably suitable for providing this quantitation.

The assay options described above are acceptable to regulators as they provide sufficient accuracy and resolution to enable the sequential determination of retrovirus burden. The qPCR assay (305110GMP.BSV) is validated and the reproducibility, sensitivity and linearity are demonstrably suitable for detection of type C RVLPS. When taken together with the rapid turn-around-times for the assay, it provides many advantages over the classical TEM assay.

## Conclusion

The qPCR method (305110GMP.BSV & 305110GMP.BUK) provides a number of advantages over the classical TEM assay. This method is acceptable to manufacturers and regulators, and has been used as supporting data in dossiers provided to support BLAs of biological products. The qPCR method can be used for pre-BLA products, and following risk assessment and change control procedures, for existing portfolio products.

## References

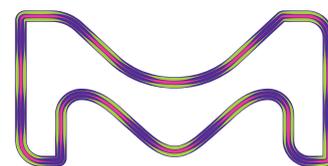
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