

Replication Competent Retrovirus Testing

Scientific progress over the past decade has led to the development of gene therapy products that involve the genetic modification of a person's cells to treat or cure disease. Since gene therapy products contain genetic and other biological materials, many of the quality, efficacy and safety considerations that apply to recombinant DNA (rDNA) products and other biologicals manufactured by modern biotechnological methods will apply at certain stages in their manufacture.¹ A significant safety concern is the potential presence of Replication Competent Retrovirus (RCR) in retroviral or adenoviral vector-based gene therapy products. Although retrovirus vectors have a safe history of use in gene therapy protocols, knowledge of the long-term risks of retrovirus exposure is limited. It is essential that retrovirus-packaging cell lines and virus vectors are tested to confirm absence of RCR.

What Products Should be Tested?

Appropriate attention needs to be given to the quality of all reagents used in production of gene therapy products which are vector derived. Characterization of the final product is essential, as well as in-process control, a concept that has been highly effective in the quality control of bacterial and viral vaccines prepared by conventional methods and, more recently, of rDNA-derived products.¹

- Master cell bank
- Working cell bank
- End of production cells
- Vector containing supernatant
- Ex-Vivo Transduced T-Lymphocytes

When Should Testing be Performed?

RCR may develop at any step during manufacturing from development of the initial master cell bank through production of the retroviral vector supernatant. In addition, the growth of ex-vivo transduced cells provides the potential for amplification of any RCR contaminant, which may be below the level of detection in the retroviral vector supernatant. Therefore, current FDA testing recommendations include testing of material from multiple stages of product manufacture.²

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¹ CPMP Note for Guidance: **3AB6A**. *Gene Therapy Product Quality Aspects in the Production of Vectors and Genetically Modified Somatic Cells*. December 1994.

² FDA, CBER. *Guidance for Industry: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector-based Gene Therapy Products and During Follow-Up of Patients in Clinical Trials Using Retroviral Vectors*. 2000.

Study Design

Infectivity Assays

Inoculation (for bulk product) or co-cultivation (for cell suspensions) with *Mus dunni* cells or 293 cells and subsequent evaluation in the PG-4 S+L- assay.

Supernatant Testing Assays

Supernatant assays include culture of supernatant on a permissive cell line [*Mus Dunni*] for a minimum of 5 passages in order to amplify any potential RCR present. The amplified material is then detected in an appropriate indicator cell assay [e.g., PG-4 S+L- (1)]. All assays include relevant positive and negative controls to assess specificity, sensitivity and reproducibility of the detection method. Each lot of retroviral vector supernatant is tested for inhibitory effects on detection of RCR by using positive control samples that are diluted in vector supernatant.

Cell Testing Assays

Cell testing assays include 1% of the total cells or 108 (whichever is less) pooled vector-producing cells by co-culture with a permissive cell line will remain in place. Co-culture assays

should include culture with a permissive cell line [ex. *Mus dunni*] for a minimum of five passages in order to amplify any potential RCR present. The amplified material may then be detected in an appropriate indicator cell assay [e.g., PG-4 S+L- (1)]. All assays include relevant positive and negative controls to assess specificity, sensitivity and reproducibility of the detection method.

Assay Duration: Cell testing: 1 week without co-culture; Supernatant testing: 5 weeks with co-culture

Sample Requirement: Dependent on customer supplied product

Regulatory Compliance

- FDA/CBER Guidance for Human Somatic Cell Therapy and Gene Therapy (1998)
- FDA/CBER Supplemental Guidance on RCR testing (2000)
- EMEA/CPMP/BWP/3088/99

Final Report

MicroSafe Services from Millipore can provide each client with the following final reports:

- GLP report that includes all raw data, protocol methods and final result.
- GMP report that includes a certificate of results.

Custom Assays

We understand that no production plant is identical and that every process is unique. Using a combination of practical experience and a scientific approach, we will customize RCR testing methods to meet your specific requirements including scientifically sound data that will be accepted by regulatory authorities around the globe.

Your Partner in Microbiology

MicroSafe Services from Millipore delivers a complete range of critical testing and consultancy services for the development and production of biopharmaceuticals. Leaders in the field of microbiology including *in vitro* virology testing, our staff of accomplished scientists, engineers and microbiologists will help you meet your specific critical testing requirements throughout every phase of drug production. All testing is conducted under GMP and/or GLP certifications as well as our own internal quality system.

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