BioReliance

Biologics Safety Testing Services

Detection of Replication Competent Lentivirus: Expansion of test methods for gene therapy vectors

Introduction

Lentiviruses comprise a genus of the Retroviridae family that includes both primate (e.g. HIV and SIV) and non-primate (e.g. EIAV, FIV) pathogens. Replication-incompetent vector particles derived from lentiviruses have been shown to mediate transfer and expression of heterologous genes (transgenes) into a variety of cells.

The major safety concerns regarding lentivirus vector manufacture and clinical use are listed below:

- Potential generation of replication competent lentivirus (RCL) during the production process
- In vivo recombination with endogenous lentiviral sequences
- Insertional mutagenesis of proviral DNA in, or close to, active genes which may trigger tumour initiation or promotion

Therefore, it is recommended that biosafety testing in support of gene therapy clinical products be performed at multiple stages during the production process.

BioReliance has provided full testing services in support of gene therapy vectors and products for the past 25 years. BioReliance continues to be at the forefront of biosafety testing.

	Pre-Clinical	Phase I	Phase II	Phase III	Licensed product
Raw Material Screening		■	■	■	■
Sterility Testing Mycoplasma Testing Specific & Adventitious Virus Assays					
cGMP Contract Manufacturing		■	■	■	■
Process Development Project Planning MCB & WCB Production cGMP Storage of Cell Banks & Clinical Material Clinical Lot Production					
Bulk and Final Product Testing / Lot Release		■	■	■	■
Purity Tests Analytical Testing Stability Testing					
Vector & Producer Cell Characterization		■	■	■	
Isoenzyme DNA Fingerprinting Genetic Stability DNA Sequencing					
Vector & Producer Cell Safety Testing		■	■	■	
Biodistribution Studies Sterility & Mycoplasma Tests for Species-specific Viruses Adventitious Virus Tests Modified Tests on Individual Viral Vectors Co-cultivation					
Clinical Trial Laboratory Services		■	■	■	
Virus Shedding Transgenic Expression Immune Response Screening					
Toxicology Services	■				
Summary Reports of Production		■	■	■	
Contract Regulatory Affairs	■	■	■	■	■

Sensitive test method for emergence of replication competent lentiviruses in gene therapy vector products

Assay supports BioReliance's existing biosafety testing methods for gene therapy products

Fully validated, regulatory compliant test method

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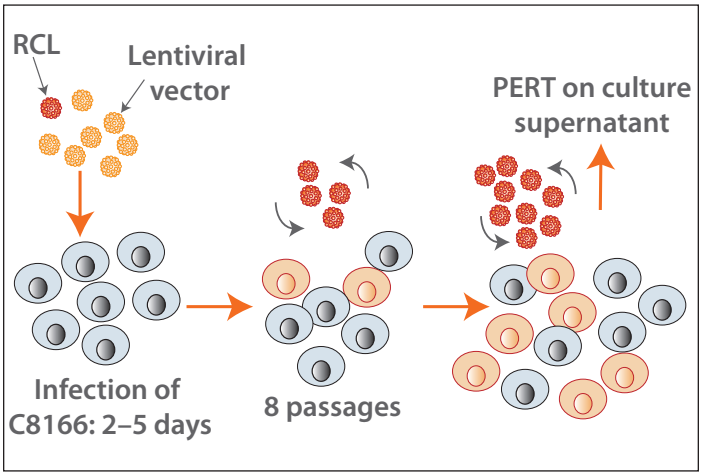
With our introduction of the RCL assay, BioReliance now offers the complete range of tests needed to meet Regulatory requirements (see below). The current recommendation is to test 1% of the total cells or 10^8 (whichever is less) pooled vector-producing cells by co-culture with a permissive cell line. It is recommended that vectors with a tropism for human cells are tested on a human cell line and that 5% of the clinical lot material is tested by inoculation onto a permissive cell line. Assays should be performed over a minimum of 5 passages in order to amplify any potential RCL present.

Assay method for detection of Replication Competent Lentivirus

RCL amplification is performed by culturing vector containing supernatant or vector-producing cells on a permissive cell line followed by sensitive detection methods. The non-adherent human cell line C8166 T cell line is able to amplify RCL, since these cells are highly susceptible to infection. The cultures are maintained over multiple passages in order to amplify any low level infectious virus and also to remove vector associated Reverse Transcriptase (RT) activity by dilution. The cell culture supernatant fluids from the later passages (passage 6 and passage 8) are harvested and the final passage supernatant is typically assayed for the presence of retrovirus by Product Enhanced Reverse Transcriptase (PERT) assay. Earlier passage supernatants may be analysed if required.

End point detection methods

The PERT assay is an extremely sensitive assay for detection of RT activity and has been reported to be up to 10^6 fold more sensitive than conventional RT assay.† The assay is an RT dependent polymerase chain reaction (PCR) and therefore combines the broad specificity of conventional RT assays with the high sensitivity of PCR. Like the conventional RT assay, this method is used to detect RT activity packaged into extracellular retroviral particles. The assay involves converting an RNA template to cDNA and amplifying the cDNA using a product specific primers. The enzymatic RT function which is detected using the PERT assay system allows detection of structurally distinct retroviruses.



RCL assay. The RCL-permissive cell line C8166 is exposed to the vector sample overnight and the inoculum removed the following day. The culture is then diluted regularly for 8 passages to allow RCL amplification. Samples of culture supernatant are collected following passage 6 and 8 and assayed for viral replication by measurement using PERT assay.

Ordering Information

Assay Description	UK Assay Number	US Assay Number	Regulatory Compliance	Sample Requirements
Infectivity assay for the detection of replication competent Lentivirus (RCL)	009130GMP.BUK	009130GMP.BSV	GMP	1% of total cells or 10^8 (whichever is less) pooled vector producing cells. 5% of total vector production volume

References

US FDA:

Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy 1998

Guidance for Industry: Supplemental guidance on testing for replication competent retrovirus in retroviral vector based gene therapy products and during follow-up of patients. 2006

Guidance for FDA reviewers and sponsors. Content of and review of chemistry, manufacturing and control information for human gene therapy IND applications 2008

EU:

The European Medicines Agency (EMA) Evaluation of medicines for Human Use. Committee for Medicinal Products for Human Use (CHMP). Guideline on development and manufacture of lentiviral vectors. EMEA/ CPMP/BWP/2458/03. 2005

Quality, preclinical and clinical aspects of gene transfer medicinal products. CHMP/GTWP/234523/09. 2009

† Arnold, B.A., Hepler, R.W., Keller, P.M., 1998. One step fluorescent probe product enhanced reverse transcriptase assay. Biotechniques 25, 98–106.

Heneine, W., Yamamoto, S., Switzer, W.M., Spira, T.J., Folks, T.M., 1995. Detection of reverse transcriptase by a highly sensitive assay in sera from persons infected with human immunodeficiency virus type 1. J. Infect. Dis. 171, 1210–1216.

Pyra, H., Böni, J., Schüpbach, J., 1994. Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement. Proc. Nat. Acad. Sci. USA 91, 1544–1548.

Robertson, J.S., Nicolson, C., Riley, A.M., Bentley, M., Dunn, G., Corcoran, T., Schild, G.C., Minor, P., 1997. Assessing the significance of reverse transcriptase activity in chick cell-derived vaccines. Biologicals 4, 403–414.

Silver, J., Maudru, T., Fujita, K., Repaske, R., 1993. A RT PCR assay for the enzyme activity of reverse transcriptase capable of detecting single virions. Nucleic Acids Res. 21, 3593–3594.

www.bioreliance.com

North America Toll Free: 800 553 5372 Tel: 301 738 1000

Europe & International Tel: +44 (0)141 946 9999 • Japan Tel: +81 (0)3 5796 7430

Email: info@bioreliance.com

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