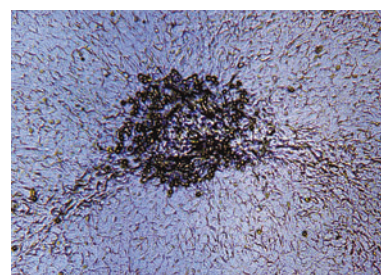




Xenotropic/Ecotropic Murine Leukemia Virus Testing

The risk of viral contamination is a feature common to all biotechnology products derived from cell lines. Murine cells used to produce biopharmaceutical products are known to harbor infectious retroviruses due to the breeding program (loss of a suppressor function on endogenous virus sequences) when used to produce the standard laboratory mouse. Retroviruses in these murine cells pose a particular problem in assessing the safety of cell substrates, as they may be transmitted in the germ line, since the viral genome persists within the cell. These endogenous retroviruses may be expressed without deleterious effects on the cells, and could be infectious to human cells.¹

The absence of murine retroviruses should be demonstrated using infectivity assays. Detection of murine retroviruses using the cytopathic effect (CPE) is a safety risk due to the low levels of the retrovirus that might be present and undetectable by cytopathology methods.²



What Products Should be Tested?

Selecting and testing cell lines and other raw material, including media components, for the absence of undesirable viruses is essential.

Production and Quality Control of Monoclonal Antibodies 3AB4a (75/318/EEC).

- Master cell bank
- End of production cells
- Unprocessed bulk

When Should Testing be Performed?

Murine retroviruses may develop at any step during manufacturing from development of the initial master cell bank through production of the retroviral vector supernatant. Both cells and supernatant from production of a MCB should be tested for murine retroviruses using a cell line permissive for the retrovirus, such as *Mus dunni*.³

Your
partner for
critical testing in
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1 ACTIP. *Safety Testing of Biotechnology Products Derived from Cell Lines of Human and Animal Origin*. (1998). Retrieved May 10, 2006, from The Animal Cell Technology Industrial Platform website: <http://www.actip.org/manuals/libraryfiles/safety.html>

2 CBER. *Points to Consider in the Characterization of Cell Lines Used to Produce Biologics*. Bethesda, MD: Office of Biologics Research and Review, FDA; 1993.

3 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

Detection of Ecotropic Murine Leukemia Virus in the XC-Plaque Assay

Culture method for the detection of infectious ecotropic murine leukemia virus. Test can be performed either directly or after amplification on sensitive mouse cells.

A double phase assay:

- First culture of specific cells that permit virus replication without CPE
- SC I cells are inactivated by UV irradiation
- Rat cells that harbor a defective retrovirus are the detection system
- Ecotrophic virus sequences restore the rat virus to competence

Detection of Xenotropic Murine Leukemia Virus in the S+L- Assay

Culture method for the detection of infectious retrovirus using either Mink S+L- assay or PG-4 S+L- assay. Tests can be performed either directly (quantitative) or after amplification on Mink lung cells.

- Assay cell line harbors a defective sarcoma virus that is restored to competence by co-infection with xenotrophic virus
- Results in uncontrolled cell proliferation

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

Sample Requirement: Dependent on customer supplied product

Regulatory Compliance

- European Pharmacopeia
- ICH Q5A/Q5B
- Center for Biologics Evaluation and Research, FDA, *Points to Consider in the Characterization of Cell Lines Used To Produce Biologicals* (1993)

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 xenotropic/ecotropic murine leukemia virus 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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Lit. No. SDS1703ENEU Rev. - 6/06 06-136 Printed in U.S.A.
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