An in vitro assay for detection of adventitious viruses of Ovine and Caprine origin in raw materials used in the production of biopharmaceuticals for use in humans

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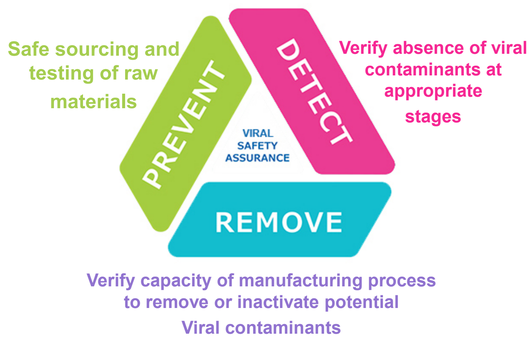
Introduction

A critical aspect of developing biotechnological medicinal products is biosafety evaluation of raw materials used in their manufacture. Biosafety testing includes the application of a wide variety of methods, test systems and technologies to detect adventitious agents and other contaminants at various stages of the biomanufacturing process from raw materials to drug product.

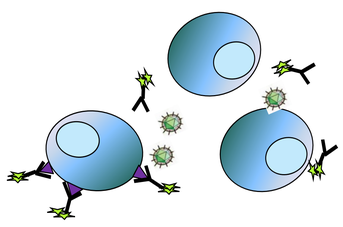


The use of in vitro test systems in biosafety testing is prescribed in safety testing regulations and guidelines globally, many of which focus on the detection of contaminants of human origin.

Risk of contamination of cell banks with ovine and caprine viruses

Cell banks used for the production of biopharmaceuticals are typically established from research cell banks (RCBs). The cells for these RCBs may initially be selected for suitability by fluorescence-activated cell sorting (FACS) using fluorescent antibodies directed to specific phenotypic markers.

These fluorescent antibodies may be derived from polyclonals raised in ovine or caprine donor species. The use of such polyclonals in this way introduces a risk of exposure of these selected research banks to viruses of ovine and caprine origin.



This risk must be addressed in the overall biosafety evaluation of raw materials intended for use in the manufacture of the biopharmaceutical product.

Methods

In vitro adventitious virus assays were challenged to provide evidence that a broad range of ovine and caprine viruses are detectable by classical reporting endpoints of cytopathic effect (CPE), hemadsorption (HAD) and immunofluorescence assay (IFA) using fetal lamb kidney (FLK), Madin Darby bovine kidney, (MDBK) and Vero C1008 detector cells.

Assay design was based on methods described in European regulatory guidance comprising a 28-day cultivation period with subculture or blind passage every 7 days followed by endpoint testing. Multiple replicates were used to challenge repeatability and intermediate precision and a range of virus doses were inoculated to determine detection limit.

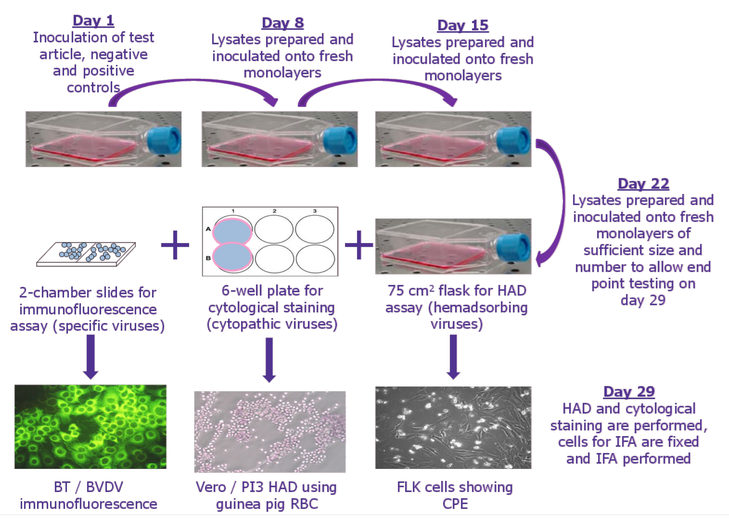


Figure 1. In vitro assay schematic.

Sensitivity of in vitro assay for detection of ovine and caprine viruses

Regulatory guidance

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). Quality of biotechnological products: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (ICH Q5A R1)

Appropriate species-specific tests should be performed, depending on the passage history of the cell line, to detect possible contaminating viruses

CPMP Note for Guidance on Production and Quality Control of Animal Immunoglobulins and Immunosera for Human Use (CPMP/BWP/3354/99)

A list of viruses of concern and which are classified as pathogenic for humans

Ph. Eur. Chapter 5.2.3 'Cell Substrates for the Production of Vaccines for Human Use'

Method for in vitro assays for cell lines used to produce human vaccines

Ph. Eur. Chapter 5.2.4 'Cell Cultures for the Production of Veterinary Vaccines'

Method for in vitro assays for cell lines used to produce veterinary vaccines

Results

Detection of ovine and caprine viruses by in vitro assay

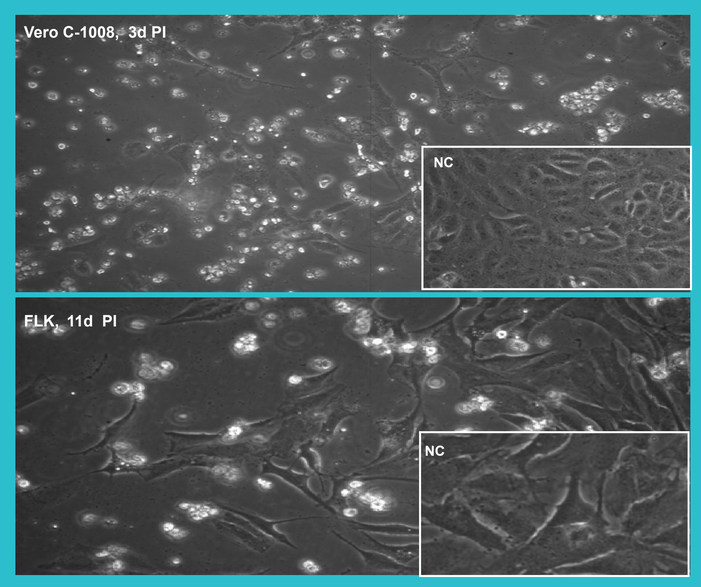


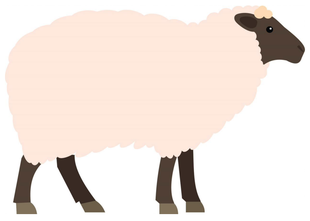
Figure 2. Cytopathic effect of SBV on Vero C1008 and FLK detector cell lines.
NC = Negative control

Sensitivity of in vitro assay for detection of ovine and caprine viruses

Detector cell line	Positive control virus	Cytopathic effect ($TCID_{50}$)	Hemadsorption ($HAID_{50}$)	Immuno-fluorescence ($FAID_{50}$)
Foetal lamb kidney	Schmallenberg virus	5	x	x
	Bovine Herpesvirus-1	10	x	x
	Border disease virus	x	x	10
	Louping ill virus	x	x	10
Madin Darby Bovine Kidney	Cytopthic Bovine viral diarrhoea virus	100	x	x
	Bovine Parainfluenza virus	x	10	x
	Bluetongue virus	x	x	1
	Non-cytopathic Bovine viral diarrhoea virus	x	x	1
Vero C1008	Bovine Parainfluenza virus	10	x	x
	Epizootic haemhorragic disease virus	x	x	1
	Akabane virus	x	x	10

Table 1. Detection limit of detector cell line / virus combinations using classical endpoint tests
x = Not applicable or not tested

PROCEDURES FOR CONTROL OF VIRUSES IN PRODUCTS OF OVINE ORIGIN



CLOSED FLOCKS

Routine health screening e.g. for:

- BDV
- Maedi-Visna
- Orf

- Sentinel animals maintained in flock for several years and screened for CNS pathology

BULK PRODUCTS:
Serum
Milk
Tissues

VIRUS DETECTION

Infectivity assays using ovine human and monkey cells

FINAL PRODUCT

- Validation of purification process. Scrapie validation may be necessary for some tissue but not usually serum
- Sterility, Mycoplasma
- Viral screening for certain viruses dependant on validation data

Summary

Ovine and Caprine viruses pose a risk of contamination of Research Cell Banks where antibodies of ovine or caprine origin are used for clone selection.

We demonstrate robust detection and report of 10 viruses of ruminant origin using standard in vitro assay cell lines and methods for detection of adventitious viruses.

We demonstrate detection limits between 1 to 100 TCID50, HAID50 or FAID50 of 10 viruses of ruminant origin.

The in vitro adventitious virus test with CPE, HAD and IFA endpoint testing provides sufficient and robust detection of Ovine and Caprine viruses that may be present in Research Cell Banks selected using antibodies of ovine or caprine origin.