Biosafety testing of oncolytic viruses; overcoming challenges with adventitious agent testing and viral clearance

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Background

- Biosafety evaluation is a critical aspect of ensuring the development of safe and quality assured oncolytic vectors.
- Current biosafety testing principles for biologicals and gene therapies, as covered by regional regulations and guidelines, are also applied to oncolytic vectors. However, since the product is replication competent, there are technical challenges during biosafety testing, specifically for adventitious agent testing and viral clearance.
- Adventitious agent testing can be particularly challenging for oncolytic virotherapies due to their ability to replicate in *in vivo* and cell-based test systems typically used for detection of contaminating mycoplasmas and viruses, giving rise to false positive results and invalid assays. The use of antiserum to neutralize the oncolytic viruses prior to performing these tests has traditionally been used as a work-around but this can be costly, time-consuming and have variable success.
- Viral clearance can prove challenging for oncolytic virotherapies since the clearance methods used are designed to inactivate or remove adventitious viruses and can therefore have an impact on viability of the live virus product itself.
- Here we present a biosafety testing approach designed to meet current regulatory expectations that that provides solutions to these challenges.

Viral Clearance

Non-enveloped oncolytic viruses however, may be resistant to viral inactivation measures and there may also be removal steps that differentiate between the product and a non-enveloped adventitious virus.

We have evaluated the viral clearance potential of manufacturing processes for numerous non-enveloped viruses. Data from example cases are shown below.

Table 1. Examples of enveloped virus reduction for non-enveloped virus-like particle or viral vector products.

Process Step	Log_{10} Virus Reduction		
	BACV	VSV	WNV
Low pH Inactivation	3.48	1.16	3.44
Detergent Inactivation	≥5.15	≥4.41	ND
Chromatography 1	≥6.70	≥6.08	≥4.93
Chromatography 2	2.67	3.93	NR
Chromatography 3	1.82	3.84	ND
Virus Reduction Filtration (35nm)	≥4.41	≥4.83	ND

These data demonstrate that the manufacturing process for a non-enveloped virus can achieve effective reduction of enveloped viruses.

Low pH can achieve inactivation of enveloped viruses; however, the level of that reduction is dependent on the pH and the susceptibility of individual viruses to low pH.

Detergent can achieve effective inactivation of enveloped viruses.

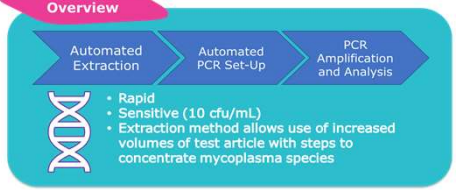
As with any product, the level of reduction achieved by a chromatography step is dependent on the chromatography resin, the conditions under which the chromatography step is run and the virus.

Using enveloped viral inactivation steps, large pore virus filters and even chromatography steps, the viral safety of oncolytic virus products can be enhanced.

Rapid Mycoplasma Testing
Real Time PCR

PCR offers a rapid and tractable solution for the detection of mycoplasma in oncolytic virus based therapies without the need for neutralization (to prevent replication within indicator cells).

Method Overview



The rapid mycoplasma assay couples automated DNA extraction with automated, real-time PCR. DNA is extracted from the test sample, followed by steps to concentrate Mycoplasma species before processing on a nucleic acid purification platform. The assay is validated and can detect mycoplasma species in a fraction of the time taken for the culture method.

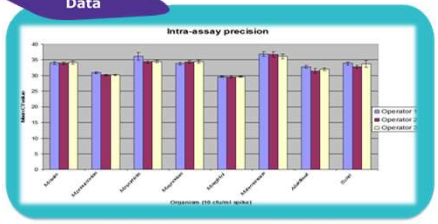
Culture vs. Rapid Method

Parameter	Pinnacle QPCR	Conventional Methods
Method	Real Time PCR	Agar & broth amplification; indicator detection system
Endpoint	Fluorescence	Mycoplasma colony count
Duration	1 day	28 days
Sensitivity	10 cfu/mL	10-100 cfu/mL
Specificity	High	High
Validation	Yes	Yes
Range of Species	102 species*	Viable

* Validated against *Mycoplasma orale, Mycoplasma hyorhinis, Mycoplasma synoviae, Mycoplasma fermentans, Mycoplasma arginine, Mycoplasma pneumoniae, Acholeplasma laidlawii, Spiroplasma citri.*

A comparability study was performed to demonstrate equivalence between the Pinnacle QPCR and the conventional detection methods. For more information, please see poster ID number P349 by Sarah Sheridan, Ph.D.

Validation Data

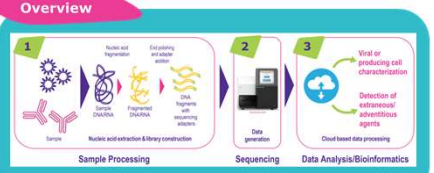


- The positive cut-off point of the test system was challenged with 8 mycoplasma strains over 3 independent operator runs
- Eight replicated samplings from each test strain were extracted, making a total of 192 independent extractions
- Each sample extract was subject to PCR in triplicate
- Results showed that the rapid test method has a high level of intra-assay precision for every organism tested and is able to routinely detect Mycoplasma at 10 cfu/ml of sample with suitable repeatability

Adventitious Virus Testing
Next Generation Sequencing

Next generation sequencing (NGS) affords a radically different approach to detecting known and unknown viruses in oncolytic virus based therapies without the need for neutralization (to prevent replication within the test system) and with pinpoint precision and accuracy.

Method Overview



NGS enables sequencing of millions to billions of DNA molecules rapidly & simultaneously. It is sequence-agnostic: nothing needs to be known about the sequence in order to gain information about it.

Case Study

Human embryonic kidney cell culture exhibited rapid (<24 hrs) cytopathic effect and cell death during Retrovirus testing.
- Seneca Valley Virus (SVV) identified; full genomic coverage found with ~95% identity
- Source of SVV was the test sample, a porcine-derived homogenate

Assay Performance

Unique mRNA to total mRNA total	Probability of obtaining at least one AA read (1 PTP)	Probability of obtaining at least one AA read (2 PTP)
1 in 1,000,000	63.21%	86.47%
1 in 800,000	71.35%	91.79%
1 in 600,000	81.11%	96.43%
1 in 500,000	86.47%	98.17%
1 in 400,000	91.79%	99.33%
1 in 300,000	96.43%	99.87%
1 in 200,000	99.33%	100.00%
1 in 100,000	100.00%	100.00%

Probability of adventitious agent (AA) detection by NGS. Confidence that AA low frequency events (column 1) would be detected using 1 or 2 plates (PTP) on the Roche 454 GS FLX® system.

Regulatory Expectations

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- **European Pharmacopoeia, 2.6.7 Mycoplasmas** Broth/agar and indicator cell assay described but allows PCR assay as long as it is of equivalent sensitivity and specificity
- **US FDA CBER Guidance for Industry:** Characterization and Qualification of Cell Substrates and Other Biological Starting Materials used in Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases, 2010

  "PCR-based assays may be used to detect mycoplasma, provided that such an assay can be shown to be comparable to the agar and broth procedure and the indicator cell culture procedure."
- Global regulatory authorities have approved a number of products, including monoclonals and gene therapies for which mycoplasma testing was performed using a nucleic acid test.

Regulatory Expectations

EP 5.2.14. Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines
- Novel, sensitive molecular techniques with broad detection capabilities are available, including deep sequencing or high throughput sequencing
- Use of broad molecular methods has highlighted the gaps with the existing testing strategy by identifying previously undetected viral contaminants

US FDA CBER Guidance for Industry (2010)
- Advances in science and technology are likely to yield additional information that could lead to modification or replacement of some described tests, and except where prohibited by regulation, manufacturers may use alternative test methods, where scientifically justified

Summary

Rapid molecular test methods can be used to overcome technical challenges during biosafety testing of oncolytic virus-based therapies, providing clear advantages over traditional methods:

- Equal or greater sensitivity
- Faster turnaround times
- Smaller test sample requirements

In addition, inactivation of enveloped viruses can be achieved to enhance the safety of non-enveloped oncolytic virus-based therapies.

These approaches are supported by the various biosafety testing regulations and guidance.