BioReliance

Biologics Safety Testing Services

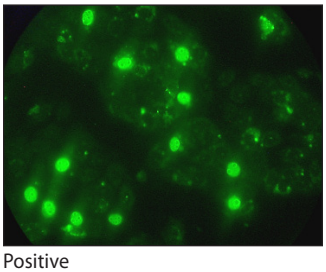
Assays for the Detection of Porcine Circovirus (PCV) in Biological Samples

Background on Circoviruses

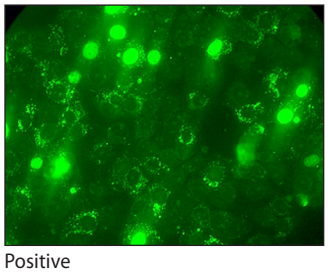
The first Circovirus was described in 1974 (I. Tischer) as a contaminant of porcine kidney cells in culture, replicating best in PK15 cells (ATCC - CCL 33). This virus was named *Porcine Circovirus*, or PCV. Since then, several similar viruses have been described that infect other species. As a group, these viruses are classified as the *Circoviridae*. The members of this family are small (17-22 nm) with a circular, negative sense, single-stranded DNA genome. They are icosahedral in shape and have no envelope. Two variants of the porcine virus have been described – PCV1 and PCV2. Members of the *Circoviridae* have also been identified in cattle, dogs and cats. PCV1 infection in swine is asymptomatic whereas PCV2 infection is linked to post-weaning multi-systemic wasting syndrome (PWMS) – a disease of livestock that can have significant commercial impact. PCV1 infection is thought to be ubiquitous in global livestock.

PCVs are a known contaminant of cell cultures and have been demonstrated to replicate in porcine cells as well as bovine, lamb, Vero and HeLa cells. Using a validated assay that detects both PCV1 and PCV2, BioReliance has shown that these sequences are a common contaminant of porcine trypsin. Studies have also shown that the virus is resistant to low pH and gamma irradiation treatment.[1]

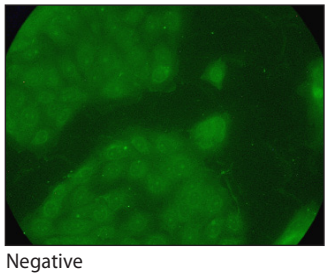
There are numerous routes through which a Circovirus may be introduced into cell cultures. Most are associated with the use of animal derived materials during the propagation and processing of the cells. Items such as porcine serum, trypsin, insulin, bovine and porcine plasma proteins, bovine origin attachment factors, tissue extracts and bovine or porcine derived cells can all potentially harbor PCV contamination. Risks can be minimized by testing cell banks and seed materials for the presence of viral sequences prior to development of clinical batches as well as by the reduction or elimination of animal derived raw materials used in the manufacturing process. Viral clearance studies can also be conducted to assess the ability of a downstream process to reduce or eliminate virus that may be present in a sample.



Positive



Positive



Negative

Porcine Circovirus: *Circoviridae.* Non enveloped; icosahedral capsids; 18-20nm diameter; circular single-stranded DNA. Possible contaminant of pig derived and Vero cell culture and products. Image displays indirect end-point immunofluorescence.

Circovirus contamination of cell lines and raw materials has raised concern among regulatory authorities.

BioReliance's fully validated Pinnacle Q-PCR™ assay can detect PCV 1 & 2 sequences in biological materials.

BioReliance's novel MP-Seq™ (massively parallel sequencing) service provides comprehensive safety assessments of cell substrates.

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BioReliance Offers Pinnacle Q-PCR™ Based Assays to Detect the Presence of Circovirus Specific Nucleic Acids

BioReliance has developed a fully validated quantitative polymerase chain reaction Pinnacle Q-PCR™ based assay that enables the rapid screening of biological samples for the detection of nucleic acids from Porcine Circovirus 1 & 2 (PCV1/2). This method is capable of detecting very low levels of potential contaminant (<20 copies/ml) due to the amplification of signal provided by the PCR method. The assay also includes all of the necessary internal controls to ensure assay accuracy, sensitivity and consistency.

Novel Technologies Offered by BioReliance Can Detect the Presence of Encapsidated Virus In Cell Culture Supernatant

BioReliance now offers the power of next generation sequencing technology – **MP-Seq™** (***m***assively ***p***arallel ***seq***uencing) – to provide an even greater sensitivity of detection to companies using animal derived materials and cell substrates in their manufacturing process. By utilizing this technology platform combined with novel bioinformatics algorithms, the presence of encapsidated viruses in cell culture supernatants can be detected with broad sensitivity (detecting all sequences – not only those targeted). This technology can also be used to detect latent and actively replicating virus in cells used for biomanufacturing – thus providing the highest level of assurance that materials being used for producing critical biological therapies are of the highest safety and quality.

PCV Infectivity Assay

BioReliance has designed a unique infectivity assay to detect the presence of intact PCV particles in a biological sample. The assay consists of a growth and amplification step utilizing a PCV permissive cell line (28 days in culture) followed by detection of related nucleic acids by PCR.

Ordering Information

Assay Number	Assay Description	Regulatory Compliance	Sample Requirements
300399GMP.BSV	Pinnacle Q-PCR™ Assay for the Detection of Porcine Circovirus (PCV) Types 1 and 2. (US Labs)	GMP	2 vials of 1×10^7 cells (preferably pelleted), frozen. 2×500 µl vials if not cells
107031GMP.BUK	Real time-PCR Assay for the Detection of Porcine Circovirus (UK Labs)	GMP	2 vials of 5×10^6 cells; or 2×500 µl vials if not cells
Custom	Amplicon MP-Seq™ Detection and characterization of encapsidated viruses in cell culture supernatant	GMP	Inquire
Custom	Transcriptome MP-Seq™ Detection and characterization of viral sequences in the genome of cells	GMP	Inquire
0339PCV.BSV	PCV Infectivity Assay with Q-PCR Endpoint	Non-GLP	12 ml of snap frozen cell lysate (10^7 cells/ml), or 100 ml of frozen bulk harvest or cell culture supernatant

[1] Plavsic, Z. Mark, Bolin, Steve. "Resistance of porcine circovirus to gamma irradiation." BioPharm International, April 2001.

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