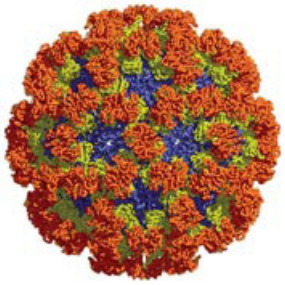
BioReliance
Biologics Safety Testing Services

Detection of Calicivirus 2117 by Real-Time RT-PCR



All biotechnology products derived from cell lines carry the risk of viral contamination by endogenous virus or from adventitious introduction during manufacturing processes. Regulatory authorities therefore recommend that therapeutic products and the raw materials used during manufacturing of therapies undergo rigorous biosafety testing.

Contamination during manufacturing of a therapeutic can arise from various sources including: (1) Contaminated reagents and raw materials (2) Adventitious introduction during manual processes or (3) Endogenous introduction from host cells. In addition to the threat of disease, cell culture contamination during the production process can have a significant negative financial impact to a pharmaceutical company due to a decline in cell productivity and interruptions in production for bioreactor sanitization.

In 2003, a calicivirus isolate of unknown origin (designated isolate 2117) was identified from a Chinese Hamster Ovary (CHO) cell culture exhibiting striking cytopathogenic changes. (Oehmig, A, Buttner, M., Weiland, F., Werz, W., Bergemann, K., and Pfaff, E. (2003) "Identification of a calicivirus isolate of unknown origin." *J. of Gen. Virology.* Vol. 84, pp.2837-2845.) The virus described showed significant similarity to members of the genus Vesivirus. The origin of the virus and the route of entry into cell culture was never determined.

In 2009, a major biotechnology company announced an interruption in manufacturing due to "Vesivirus 2117" contamination of CHO cell cultures, and confirmed that similar contamination events had also been noted at two separate facilities the previous year. Screening for this virus in both raw materials and subsequent downstream cultures/products is therefore considered of prime importance to the biopharmaceutical industry.

The virus family Caliciviridae is a heterogeneous group of non-enveloped viruses with positive-stranded RNA genomes. They are currently classified into five genera based on differences in genome organisation, coding strategies, host range and persistence. Viruses in the Vesivirus genus are widely distributed in a variety of mammalian species, where they cause systemic and frequently persistent infections, often with high morbidity and mortality.

Calicivirus image courtesy of Dr. B.V.V. Prasad, Baylor College of Medicine, USA.

Fully validated, GMP compliant assay

Excellent reproducibility and sensitivity

Important addition to the broadest line of virus detection assays available

BioReliance® by SAFC

Detection of Calicivirus 2117 by Real-Time RT-PCR (continued)

BioReliance has developed a fully validated real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay that enables rapid screening of biological samples for the detection of Calicivirus 2117. RT-PCR can selectively amplify fragments of nucleic acid by over a million-fold and is therefore a very useful technique for detecting low levels of potential contamination in test samples. Amplification of the virus target relies upon complementary binding of a specific primer pair and an internal probe. As amplification of the Calicivirus 2117 target proceeds (if present), an increase in fluorescence proportional to an increase in PCR product is observed (Figure 1). The BioReliance assay also includes all necessary internal controls to ensure assay sensitivity and consistency.

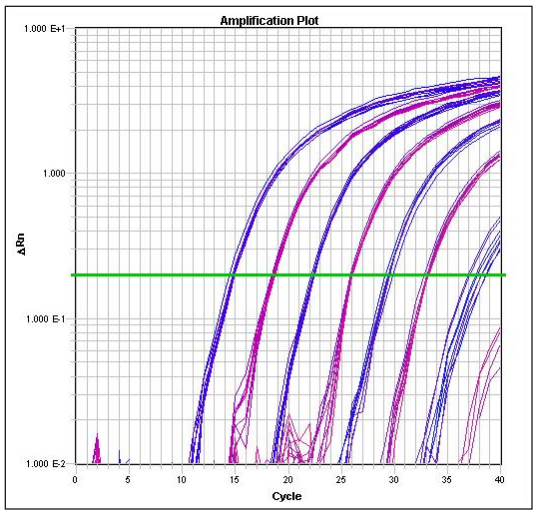


Figure 1. BioReliance's fully validated Calicivirus 2117 real-time RT-PCR assay demonstrates excellent sensitivity and reproducibility, as demonstrated from this read-out of fluorescent signal relative to the amount of control template present in the sample.

Ordering Information

Assay Number	Assay Description	Regulatory Compliance	Sample Requirements
107185GMP	Detection of Calicivirus 2117 (Vesivirus 2117) by Real-time RT-PCR	GMP ICHQ2(R1), EP (01/2008:20621) FDA PTC (1993)/ICH Q5A EMEA Draft 2006 FDA Draft Guidance CMC	Cell pellets/suspensions 2 x 10^7 cells (per ml if suspension)

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