# Positive internal witnesses for screening virus BVD, by real-time RT-PCR

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As the name suggests, bovine viral diarrhea (BVD) is caused by a virus (RNA simple-stranded) which affects cattle. It can be detected by real-time RT-PCR (Reverse Transcription and Polymerase Chain Reaction).

## Introduction

PCR is an in vitro enzymatic method that leads to an amplification of a specific nucleotide sequence, by the elongation of 2 DNA primers, which hybridize specifically with a DNA sequence. Some viruses replicate genetic information in the form of RNA (single-stranded), which requires reverse transcription in to transcribe to cDNA. After obtaining cDNA, PCR contains three main steps repeated over several cycles: denaturation of the cDNA strands, hybridization of specific primers and Taqman probe, and primer elongation. Nucleic acids extracts, called internal positive witnesses were extracted from positive BVD samples. The Ct value (Threshold Cycle) was ascertained using real-time RT-PCR, before samples were frozen.

## Method

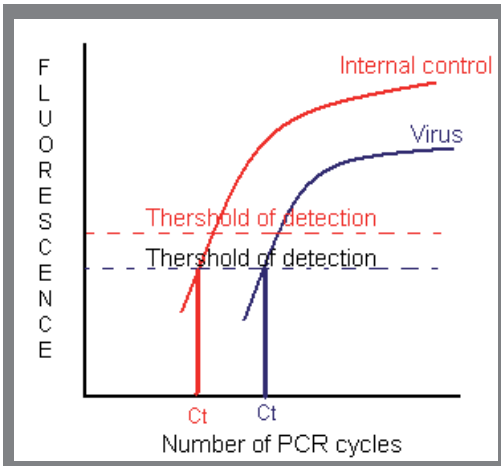
Nucleic acids of the sample were extracted following a protocol supplied with a kit that screened for BVD and a positive sample field. The total RNA (RNA viral included) and DNA were extracted with this method. There were 2 pairs of primers within the extraction kit: a specific set for viral nucleic sequence and another set for an internal control. This internal control was bovine GAPDH RNA (glyceraldehyde 3-phosphate deshydrogenase). It helped to validate extraction and amplification as well as negative samples.

## Results

The term amplification was used if there was an observed fluorescence curve with a linear increasing slope followed by a stabilisation at the end (Figure 1). The sample was positive, if there was an amplification event for both the internal control as well as the viral RNA. After amplification of the positive internal control, 2 curves of amplification were observed. Therefore samples were aliquoted into microvials (5µL) and frozen at -20°C to be later used in PCR experiments in further BVD research. The manufacture of these internal positive standards allowed for the establishment of a control card, a tool later used for validation and quality control of testing.

## Conclusion

The control card of internal positive standards (Figure 2) is a graphical tool, with five horizontal lines, which are: the upper critical limit, the upper limit of surveillance, the average, the lower monitoring limit, and the lower critical limit. The average was calculated from the first 10 Ct values for a determined sample. Then during the following analyses, the average was used to define a value of Ct standard for the control card. 3 cases were possible: the value observed lied outside the critical limits (the result was non-compliant and the technician had to repeat the analysis), the value observed lied between the critical limit and the limit of surveillance (the result was acceptable, and did not infer the need for a new analysis; however a follow up confirmation analysis was needed to analyze for repeatability), the value observed lied between the limits of surveillance: the result was in line. The upper and lower limits were defined by two or three standard deviations.



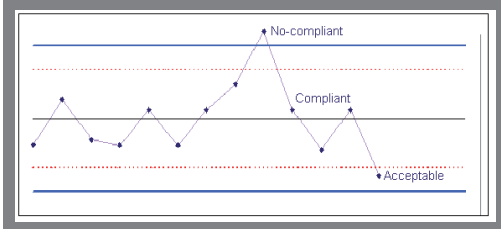




Figure 1 : Chart obtained after real-time RT-PCR of the positive sample of ground.

Figure 2 : Control card of positive internal witness: upper and lower critical limits, upper and lower monitoring limits, average and values of Ct.

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