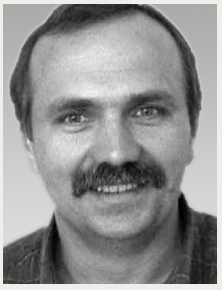
Detection of Classical Swine Fever with the LightCycler Instrument



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Present diagnostic tools for the control of Classical Swine Fever are characterized by occasionally insufficient specificity (ELISA techniques, conventional RT-PCR), the inability to perform early virus detection (ELISA) or an extended time required for detection (cell culture). In our experiments, real-time RT-PCR using a dually labeled CSFV probe in the LightCycler Instrument was evaluated by examining viral references and different blood sera. Real-time RT-PCR efficiently suppresses cross-reactions to related viruses, thus avoiding false-positive results in field samples. With respect to routine screening procedures, real-time RT-PCR combines the advantageous short timeframe of ELISA analysis with the specificity of cell cultivation, including immunofluorescence, and can therefore be considered a real breakthrough.

Introduction

Classical or European Swine Fever (CSF; Hog Cholera Disease) is a highly contagious viral infection of pigs, usually associated with dramatic mortality, especially in piglets. To reduce the economic impact, a very stringent and expensive eradication program was started within the European Community in the early nineties. It comprises comprehensive culling operations of infected and suspicious livestock, as well as monitoring programs.

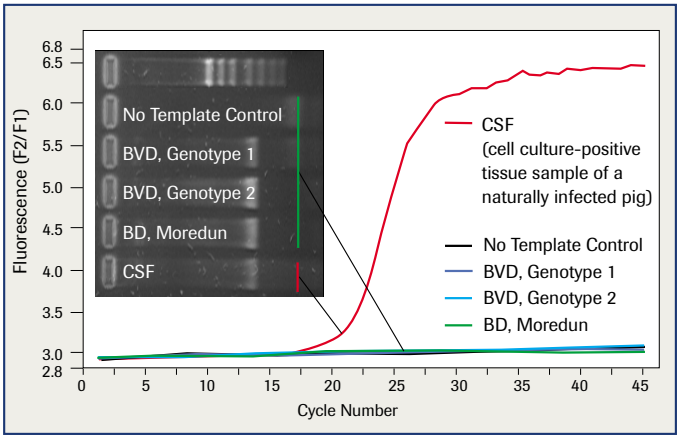

Figure 1: Specific detection of CSFV using real-time RT-PCR with pan-pesti primers and a CSFV probe. Gel analysis (2 % agarose) of PCR products shows signals also in related pestivirus samples.

The causative agent of CSF belongs to the genus *Pestiviridae* and is closely related to the bovine viral diarrhea virus (BVDV), also called bovine mucosal disease virus, and to the border disease virus (BDV) of sheep. Both BVDV and BDV are able to induce clinically inapparent infections of pigs accompanied by short viremic phases and antibody formation. In common monitoring programs, serological tests are used. For quarantine and control investigations, and to measure the spread after clinical outbreaks in domestic pigs or wild boar, the direct detection of the virus is preferred to guarantee virus detection in an early preclinical stage. At present, either antigen ELISAs or more time-consuming cell cultivation (as a "Gold Standard") are legally accepted by the EU administration for screening purposes or for the necessary confirmation of positive screening results, respectively. The main disadvantage of available antigen ELISAs is the high risk of false-positive results due to cross-reactivity with the related pestiviruses previously mentioned. Furthermore, the sensitivity is not sufficient to detect the viruses during the complete incubation time – a paradox with respect to the intended use.

The application of conventional reverse transcription-polymerase chain reaction (RT-PCR) has been limited due to the absence of significant advantages in specificity. Based on primers and a CSFV probe designed for an end-point 5' nuclease assay [1], protocols were adapted for single-tube RT-PCR in the LightCycler Instrument.

Materials and Methods

Sample material

For specificity tests, confirmed field samples and isolates of CSFV, BVDV genotype 1 and 2, and BDV were used. The sensitivity was examined using a ten-fold dilution series of sera from animals experimentally infected with different viral strains, kindly provided by the German Federal Research Center of Virus Diseases of Animals (Isle of Riems). Finally, field sera collected from healthy pigs (with positive or doubtful results in an antigen ELISA) were examined to directly compare these screening tests.

Isolation of viral nucleic acids

Viral RNA was isolated with the High Pure Viral RNA Kit, the High Pure Viral Nucleic Acid Kit, and the MagNA Pure LC Total Nucleic Acid Kit (only for dilution series).

LightCycler RT-PCR

One-step RT-PCR on the LightCycler Instrument was performed with LightCycler RNA Master Hybridization Probes, according to the manufacturer's instructions. Pestiviral primer A11, a CSFV-specific FAM/TAMRA-labeled probe [1], and the reverse primer A14 [2] were used. Final concentrations were 0.5 µM for each primer, and 0.1 µM for the probe. Amplification conditions were reverse transcription at 61 °C for 20 minutes, initial denaturation at 95 °C for 2 minutes and 45 cycles of denaturation (95 °C, 5 seconds), annealing including detection (58 °C, 15 seconds) and extension (72 °C, 15 seconds).

Results and Application

The application of the CSFV-specific dually labeled probe enables highly specific detection of CSFV. No cross-reactions to related pestiviruses were observed in fluorescence measurement, although the formation of amplicons of 220 bp occurred also with BVD and BD strains as shown in the agarose gel (Figure 1).

Positive RT-PCR results were found for all sera from experimentally infected pigs. For three of these sera, virus concentration was determined in cell culture by the Federal Research Center. Two sera revealed detection limits of RT-PCR in ten-fold dilution series of 10^{-6} compared to viral titre in cell culture of ~10^5 culture infectious doses (CID)/ml plasma. In one of the sera, the detection limit was reached at a dilution of only 10^{-1}. This corresponds to a lower viral titre of ~$10^{2.5}$ CID/ml plasma and a prolonged duration of clinical infection as an indicator for a lower susceptilbility of this animal (Figure 2).

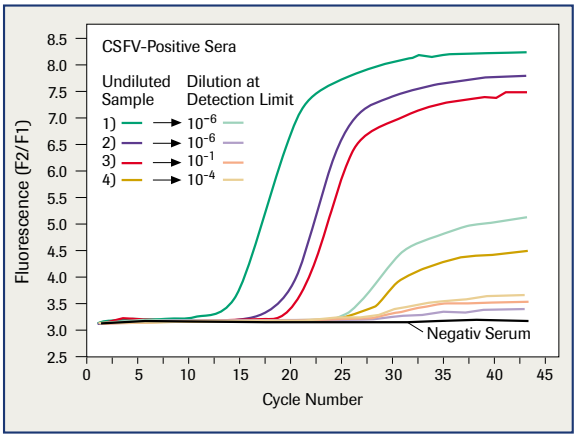

Figure 2: Detection limits of CSFV in blood of four experimentally infected pigs with clinical symptoms of CSF

None of 40 tested blood samples from clinically unsuspicious pigs with positive or doubtful results in routine antigen ELISA was positive for CSFV either in RT-PCR or in cell cultivation of blood leukocytes (Figure 3).

Summary

Real-time RT-PCR using a highly specific CSFV probe ensures sensitive and very specific detection of classical swine fever virus in a clinical specimen. The increase of specificity compared to conventional RT-PCR avoids false-positive results caused by cross-reactions with related pestiviruses or unknown "matrix effects". The false-positive results from the application of antigen ELISA tests in monitoring programs often give rise to temporary restrictions of farms leading to economic losses.

On the other hand, the safe detection of acute infections was also guaranteed in our experiments. In contrast to ELISA tests for the detection of CSFV antigen or CSFV antibodies, most RT-PCR protocols, as well as virus cultivation in cell cultures, enable virus detection in an early

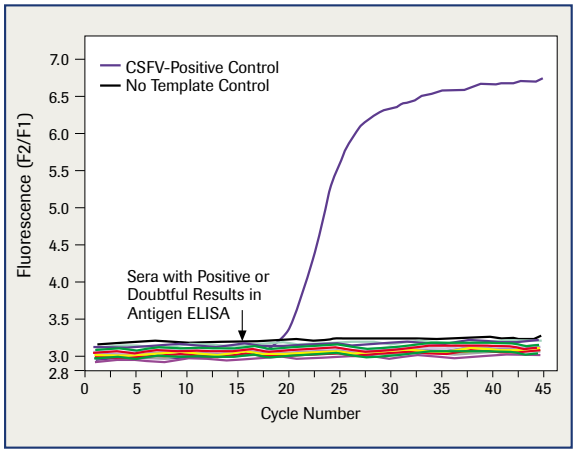

Figure 3: Absence of false-positive CSFV results in field samples of healthy pigs with previous positive or doubtful results in antigen ELISA and negative virus detection in cell culture

stage of infection. In addition, real-time RT-PCR shortens the time for analysis and reduces the contamination risk. Using the MagNA Pure LC System for the preparation of nucleic acids, the resulting uninterrupted sample processing helps to accomodate larger sample numbers.

Due to the high specificity and sensitivity, the short analysis period, and high-throughput capacity of automated systems, real-time RT-PCR can be considered the screening method of choice, if clinical signs or the epidemiological situation require direct virus detection.

References

1. McGoldrick A et al. (1998) J Virol Meth 72: 125–135
2. Drew TW et al. (1999) Vet Microbiol 64: 145–154

Order INFO

Product	Pack Size	Cat. No.
LightCycler – RNA Master Hybridization Probes	1 kit (96 reactions)	3 018 954
High Pure Viral RNA Kit	1 kit (100 purifications)	1 858 882
High Pure Viral Nucleic Acid Kit	1 kit (100 purifications)	1 858 874
MagNA Pure LC Total Nucleic Acid Isolation Kit	1 kit (192 isolations)	3 038 505

Development of a Quantitative LightCycler Assay for the Detection of Epstein-Barr Virus DNA in Research Samples

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Introduction

The Epstein-Barr Virus (EBV), one of the eight human herpes viruses (HHV4), is a double-stranded DNA virus of ubiquitous spread. The virus is transmitted by salivary contact and most often individuals become infected during their childhood. In these cases, primary infections are mostly asymptomatic or reveal only mild nonspecific symptoms. When infection with EBV occurs during adolescence or young adulthood, it causes infectious mononucleosis in up to 50 % of all cases, an illness associated with fever and swollen lymph glands. In some cases, EBV is described as the causative agent of chronic fatigue syndrome.

Worldwide, EBV has a prevalence of about 90 %. Once acquired, the virus establishes a lifelong latent infection and remains in epithelial cells of the throat and in B-lymphocytes. In very rare events, EBV can cause severe complications, such as Burkitt's lymphoma or nasopharyngeal carcinoma mainly known to occur in Africans or Asians.

Normally, EBV is under the control of T-cells, which prevent the outgrowth in healthy individuals. Under immunosuppressive therapy, serious complications may occur after organ or bone marrow transplants. EBV can be reactivated leading to a severe post transplant lymphoproliferative disorder (PTLD), which can have a fatal progression. The involvement of EBV and its epidemiology in all of these diseases is the subject of many research projects.

The current practice of EBV testing includes several serological assays which detect antibodies to the viral capsid antigen (VCA), the early antigen (EA), and the EBV nuclear antigen (EBNA) based on IgG or IgM measurement. The serological assays mainly used for the detection of EBV have some significant disadvantages: they are cumbersome and slow, they usually do not correlate to the quantitative viral load, and the individual antibody status can sometimes result in misinterpretation.

In this article, we describe the development of a highly sensitive and quantitative polymerase chain reaction (PCR) assay for EBV DNA with the LightCycler Instrument for research purposes. This PCR test directly measures the viral DNA load in either plasma or whole blood