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A highly sensitive and specific gel-based multiplex RT-PCR assay for the simultaneous and differential diagnosis of African swine fever and Classical swine fever in clinical samples

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Abstract -
The development and standardisation of a novel, highly sensitive and specific one-step hot start multiplex RT-PCR assay is presented for the simultaneous and differential diagnosis of African swine fever (ASF) and Classical swine fever (CSF). The method uses two primer sets, each one specific for the corresponding virus, amplifying DNA fragments different in length, allowing a gel-based differential detection of the PCR products. Universal detection of ASF and CSF virus strains was achieved through selection of primers in conserved viral genome regions. The detection range was confirmed by analysis of a large collection of isolates of the two viruses. The high specificity of the assay was proven by testing related viruses, uninfected cell line cultures and healthy pig tissues. Additional confirmatory tests of the ASF and CSF virus amplicon specificity, based on restriction endonuclease analysis with BsmA I or Ban II, respectively, are also described. The analysis of whole blood and serum samples from experimentally infected animals proved the usefulness of the method for an early diagnosis of both diseases, even before the appearance of the first clinical signs. A study of 150 positive field samples from several ASF and CSF outbreaks showed the suitability of this method for a rapid (less than five hours), sensitive and specific differential diagnosis in clinical samples. In addition, a highly sensitive and specific uniplex RT-PCR for CSFV was also developed and standardised as a powerful tool for fast and early diagnosis of the disease.

Key words
: African swine fever virus / Classical swine fever virus / differential diagnosis / multiplex RT-PCR

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