Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV) belongs to the family of coronaviruses and, like SARS-CoV, is classified in the genus Betacoronavirus. At the end of 2019, SARS-CoV-2 was identified as the causative pathogen of clustered cases of pneumonia of unclear origin. The virus caused an infection wave that has spread rapidly over the world and was declared a pandemic by the WHO at the beginning of 2020.

SARS-CoV-2 is mainly transmitted via aerosols during coughing or sneezing or at close contact with an infected person. Human-pathogenic coronaviruses originate from the animal kingdom, however, the reservoir for SARS-CoV-2 is unknown. The incubation time of SARS-CoV-2 is three to seven, maximally 14 days. The symptoms of SARS-CoV-2 infection are fever, coughing, breathing difficulties, fatigue and loss of smell or taste as well as gastrointestinal complaints. In most patients the infection manifests with symptoms of a mild febrile illness with irregular lung infiltrates. Some patients, especially elderly or chronically ill patients, develop acute respiratory distress syndrome (ARDS). In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO. Until the end of 2021, over 281 million COVID-19 cases with more than 5.4 million deaths were registered worldwide. In the course of the pandemic, several virus variants emerged that carry mutations that can affect immune escape, infectivity and disease progression (variants of concern, VOC).

Suitable methods for the diagnosis of SARS-CoV-2 infections include, in particular, the detection of viral RNA by RT-PCR (reverse transcription polymerase chain reaction) or of virus protein by means of ELISA or rapid test, primarily in sample material from the upper respiratory tract (nasopharyngeal or oropharyngeal swab, anterior nasal swab). The viral load is highest in the first week of the illness. Virus RNA can be detected for up to 14 to 17 days after the onset of symptoms. The detection of viral antigens is less sensitive than the RT-PCR test.
Test principle

The test system uses a one-tube reaction based on reverse transcription (RT) for conversion of viral RNA into complementary DNA (cDNA), followed by PCR amplification and fluorescence-based real-time detection of two defined sections in the SARS-CoV-2 genome (ORF1ab gene and N gene). Reverse transcription, amplification and detection of the SARS-CoV-2 cDNA are performed by means of specific primers and probes. The test contains an internal amplification control which serves as inhibition control and additionally as extraction control. A SARS-CoV-2 positive control provided with the test kit is analysed as an external control in every test run. The EURORealTime Analysis software supports the user in analysing and evaluating the measurement values from different real-time cyclers, including all controls. Furthermore, the software provides full guidance through the individual work steps, thus ensuring a simple and error-free test procedure.

Analytical sensitivity

The primers and probes used in the EURORealTime SARS-CoV-2 were developed based on the following sequence for SARS-CoV-2: NC_045512.2 (National Center for Biotechnology Information (NCBI)). The limit of detection (LoD) was determined using quantified SARS-CoV-2-specific RNA (in vitro transcripts (IVT)). The LoD was confirmed in three independent investigations using three independent lots with 21 replicates each in the presence of 200 ng of human nucleic acid in ≥ 95 % of the reactions. The LoD is the minimum detection limit and amounts to 1 cp/μl nucleic acid eluate. Usually, fewer copies (cp) of RNA are detected with the test system.

Analytical specificity

The analytical specificity of the test system is ensured by the primer and probe design and the PCR conditions given in the instructions for use. All primers and probes used in the test system were checked for potential homologies by means of sequence comparison analyses in order to exclude potential cross-reactivity. All available sequences in the "nr" database of the NCBI (status 13 February 2020) were taken into account (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Additionally, nucleic acid of pathogens that are found in the respiratory tract or are closely related to SARS-CoV-2 were investigated using the EURORealTime SARS-CoV-2. No cross reactions (CR) were detected (see table). To exclude cross reactivity with human genomic DNA or RNA, 100 ng of each per reaction was used. No cross reactions were detected.

Evaluation

In an evaluation study, the diagnostic sensitivity and specificity of the EURORealTime SARS-CoV-2 in comparison to a SARS-CoV-2 real-time PCR reference test were analysed. The analysis of 164 throat swabs showed 98.2 % agreement between positive results and 100 % agreement between negative results obtained with the two test systems.