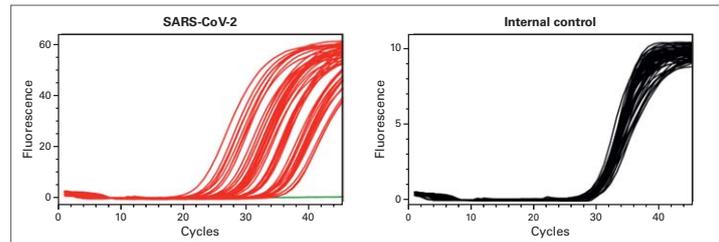




EURORealTime SARS-CoV-2



- PCR test for specific detection of SARS-CoV-2 (CE-marked and FDA-approved under the Emergency Use Authorization (EUA))
- Quick and simple pathogen detection by means of reverse transcription and real-time PCR in one step
- High sensitivity due to simultaneous detection of two SARS-CoV-2 target sequences
- Only one reaction per sample

Technical data

Test principle	Reverse transcription of the SARS-CoV-2 genome followed by PCR amplification and real-time detection using primers and probes
Test procedure	Reverse transcription and real-time PCR in one test (approx. 90 min)
Reagents	Ready for use
Controls	Internal inhibition and extraction control (RNA), SARS-CoV-2 positive control (RNA), negative control
CE-IVD mark	Complete system validated for the following real-time PCR cyclers: LightCycler® 480 (Roche), 7500 Fast Real-Time PCR Instrument (Applied Biosystems), CFX 96 Touch (Bio-Rad). Other devices should be validated individually by the user.
Test kit format	25, 50, 100 or 200 reactions
Order number	MP 2606-0125, -0225, -0425, -0100, -0200

Clinical significance

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*. The new coronavirus originated in China, in the city of Wuhan, Hubei province. It caused an infection wave that has spread rapidly over the country and worldwide. Just a few days after the first report about patients with pneumonia of unclear origin, SARS-CoV-2 was identified as the causative pathogen.

SARS-CoV-2 is mainly transmitted via aerosols during coughing or sneezing or at close contact with an infected person. Health care personnel and family members are among the high-risk populations. The zoonotic reservoir of the virus appears to be bats. 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 and fatigue. 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). The disease is fatal in up to 3% of cases. In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO.

Suitable methods for diagnosis of SARS-CoV-2 infections are direct detection of the virus by reverse transcriptase polymerase chain reaction (RT-PCR) primarily in sample material from the upper (nasopharyngeal or oropharyngeal swab) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, etc.) and the detection of antibodies against SARS-CoV-2 in blood. The determination of antibodies enables confirmation of SARS-CoV-2 infection in patients with typical symptoms and in suspected cases without symptoms. It also contributes to monitoring and outbreak control. For significant serological results, two patient samples should be investigated, one from the acute phase (week 1 of the illness) and one from the convalescent phase (3 to 4 weeks later).

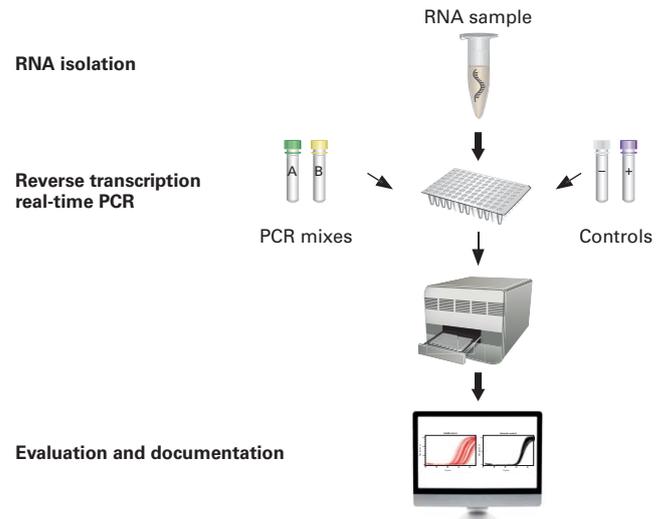
Cross reactions with antibodies within the genus *Betacoronavirus* are known. Currently, there is no medication or vaccine available against infection with this new virus.

For the diagnosis of SARS-CoV-2 infections, direct detection of the pathogen by means of nucleic acid amplification is the method of choice. It allows pathogen detection even in subclinical or asymptomatic cases within a few days after virus contact up to approx. 14 days after the onset of symptoms (Liu et al., 2020). However, with the start of the immune response and the associated reduction in the viral load the sensitivity of the direct detection decreases. The pathogen cannot be detected in all patients any more.



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 ORF1ab and N genes of the SARS-CoV-2 genome. 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 an inhibition control and can additionally be used as an extraction control. A SARS-CoV-2 positive control provided with the test kit is analysed as an external control in every test run. Evaluation and documentation are carried out by the evaluation software of the real-time PCR cyclers used.



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_04512.2 (*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 $\geq 95\%$ of the reactions. The LoD is the minimum detection limit and amounts to 1 cp/ μ l nucleic acid eluate. Usually, fewer copies (cp) of RNA are detected with the test system.

Analytical specificity

The specificity of the test system is ensured by the primer and probe design and the PCR conditions given in the test instruction. 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 were (CR) 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.

Pathogen nucleic acid (1 ng nucleic acid/reaction)	CR	Pathogen nucleic acid (< 1 ng nucleic acid/reaction)	CR
Parainfluenza virus 1–3	0%	Adenovirus 5	0%
<i>Bordetella pertussis</i>	0%	Enterovirus 71	0%
<i>Chlamydia pneumoniae</i>	0%	Influenza virus B	0%
Coronavirus HKU1	0%	<i>Mycobacterium tuberculosis</i>	0%
Coronavirus MERS	0%	Parainfluenza virus 4	0%
Coronavirus NL63	0%	<i>Pneumocystis jirovecii</i>	0%
Coronavirus OC43	0%	Respiratory syncytial virus B	0%
Coronavirus SARS HKU39849	0%		
Coronavirus 229E	0%		
<i>Haemophilus influenzae</i>	0%		
Influenza virus A	0%		
<i>Legionella pneumophila</i>	0%		
<i>Mycoplasma pneumoniae</i>	0%		
Parainfluenza virus 1–3	0%		

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 correlation of positive and negative results obtained with the two test systems in the analysis of 164 throat swabs was 98.2% and 100%, respectively.

164 samples (throat swabs)		External precharacterisation using SARS-CoV-2 real-time PCR reference test*	
		positive	negative
EUROIMMUN EURORealTime SARS-CoV-2	positive	107	0
	negative	2	55

*Test kit from TIB MOLBIOL