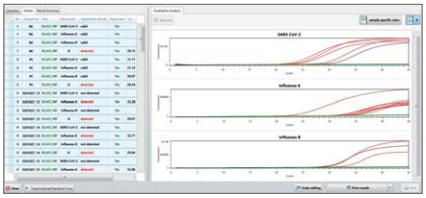
EURORealTime SARS-CoV-2/Influenza A/B



- **Reliable PCR test for specific detection of SARS-CoV-2, including all currently relevant variants, as well as of influenza virus types A and B in one sample**
- **Quick and simple pathogen detection by means of reverse transcription and real-time PCR in one step**
- **For differential diagnostic clarification of symptoms that can be associated with COVID-19 as well as influenza**

Technical data

Test principle	Reverse transcription of the genomes of SARS-CoV-2 and influenza virus types A and B, followed by PCR amplification and real-time detection using specific primers and probes
Test procedure	Reverse transcription and real-time PCR in one test (approx. 90 min), software-aided evaluation
Sample material	Nasopharyngeal swab
Reagents	Ready for use
Sample material	RNA from nasopharyngeal swabs
Controls	Internal inhibition and extraction control (RNA), SARS-CoV-2/influenza-A/B-positive control (RNA), negative control
CE-IVD mark	Test system validated for automated nucleic acid extraction with the chemagic 360-D (Revvity); test system validated for the following real-time PCR cyclers: Eonis Q96 (Revvity), LightCycler 480 II (Roche), 7500 Fast Real-Time PCR Instrument (Applied Biosystems), CFX96 Touch (Bio-Rad), qTower^3 (Analytik Jena); other instruments must be validated by the user
Test kit format	100, 200 or 1000 reactions
Order number	**MP 2606-0100-20, -0200-20, -1000-20;** not available in Brazil, China and the USA

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*. At the end of 2019, SARS-CoV-2 was identified as the causative pathogen of clustered cases of pneumonia of unclear origin. It caused an infection wave that has spread rapidly worldwide and was declared a pandemic by the WHO at the beginning of 2020. 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). Influenza viruses (flu viruses) belong to the family of orthomyxoviruses. They are classified into types A to D. Other than virus types C and D, virus types A and B cause seasonal epidemics. Pandemics are caused by influenza A viruses of zoonotic origin. Beside humans, they also circulate in farm animals, such as pigs, horses and poultry, as well as in wild birds and are divided into 18 haemagglutinin subtypes (H1–18) and 11 neuraminidase subtypes (N1–11). By contrast, influenza B viruses, with two genetically different lines circulating worldwide (Yamagata and Victoria), are only found in humans.

Seasonal flu outbreaks, which usually occur during the winter months, spread rapidly. Every year, 5% to 10% of adults and 20% to 30% of children worldwide become infected. In the early disease stage, COVID-19 and influenza cannot be distinguished based on the symptoms. In the same way, infections with influenza virus types A and B cannot be clinically delimited. Diagnosis is made after identification of the pathogen in samples from the upper respiratory tract by means of nucleic-acid or antigen detection.

	SARS-CoV-2	Seasonal influenza virus
Transmission	Droplets, aerosols and contact infection	
Highest infectiousness	Generally shortly before symptom onset	After symptom onset
Incubation period	4 to 6 days	1 to 4 days
Risk factors for a severe course	Risk increases with age; obesity, high blood pressure, chronic diseases	Younger than two years and older than 65 years of age; immunosuppression, pregnancy, obesity, chronic diseases
Most frequent disease symptoms	Fever, chills, headache, muscle pain, dry cough, shortness of breath, fatigue, olfactory loss, gastrointestinal complaints	Fever, chills, headache, muscle pain, cough, sputum, stuffed nose, sore throat, fatigue
Disease peak	2nd to 3rd week	Within 3 to 7 days

Clinical characteristics of infections with SARS-CoV-2 and influenza virus types A and B

Coinfections with influenza virus types A and B are unusual and primarily nosocomial. Coinfections with influenza viruses were found in only 0.7% of patients infected with SARS-CoV-2.

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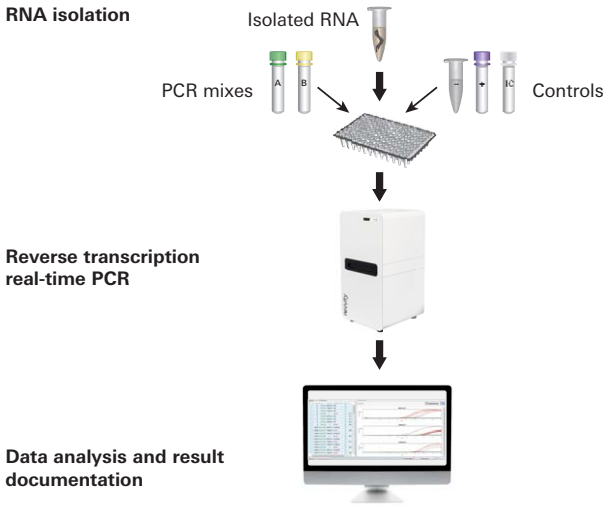
Test principle

The test system is based on a one-tube reaction using reverse transcription (RT) to convert 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) and of one defined section each in the genomes of influenza virus types A and B. Reverse transcription, amplification and detection of the cDNA of SARS-CoV-2 and influenza virus types A and B 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/influenza A/B-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, error-free test procedure.



Analytical sensitivity

The primers and probes used in the test system were developed based on the following sequences, which are registered in the National Center for Biotechnology Information (NCBI): NC_045512.2 (SARS-CoV-2), NC_007367.1 (influenza virus A subtype H3N2), NC_026431.1 (influenza virus A subtype H1N1), NC_002211.1 (influenza virus type B). The limit of detection (LoD) was determined using quantified SARS-CoV-2- and influenza virus A/B-specific RNA. 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 was determined to be 1.5 cp/µl (SARS-CoV-2 and influenza virus A subtypes H3N2 and H1N1) and 3 cp/µl (influenza virus type B) 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 SARS-CoV-2: 13 Feb 20, status influenza viruses: 17 Sep 20) were taken into account (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Additionally, nucleic acids of pathogens that may occur in the respiratory tract or are closely related to SARS-CoV-2 or influenza viruses were investigated using the EURORealTime SARS-CoV-2/Influenza A/B. No cross reactions were detected. To exclude cross reactivity with human genomic DNA or RNA, 100 ng of each was used per reaction. No cross reactions were detected.

Evaluation

It was evaluated whether the results obtained for a clinical sample panel using the EURORealTime SARS-CoV-2/Influenza A/B agreed with those obtained with other reference tests for SARS-CoV-2, influenza virus A and influenza virus B.

SARS-CoV-2:

132 samples (nasopharyngeal swabs)		External precharacterisation using SARS-CoV-2 real-time PCR reference test	
		positive	negative
EURORealTime SARS-CoV-2/ Influenza A/B	positive	55	0
	negative	1*	76

* very weakly positive as precharacterised

Positive agreement: 98.2 %
Negative agreement: 100 %

Influenza virus type A:

175 samples (nasopharyngeal swabs)		External precharacterisation using influenza real-time PCR reference test	
		positive	negative
EURORealTime SARS-CoV-2/ Influenza A/B	positive	50	0
	negative	3**	122

** weakly or very weakly positive as precharacterised

Positive agreement: 94.3 %
Negative agreement: 100 %

Influenza virus type B:

175 samples (nasopharyngeal swabs)		External precharacterisation using influenza real-time PCR reference test	
		positive	negative
EURORealTime SARS-CoV-2/ Influenza A/B	positive	47	1***
	negativ	0	127

*** very weakly positive according to the test result

Positive agreement: 100 %
Negative agreement: 99.2 %

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