SARS-CoV-2 UTAB FS*

Order Information

Cat. No.	Kit size			
1 7508 99 10 935	R1 2 x 15 mL	+	R2	1 x 10 mL
1 7501 99 10 021	SARS-CoV-2 UTAB Sample Dilution Matrix			6 x 25 mL

Intended Use

Immunoturbidimetric reagent for quantitative in vitro determination of SARS-CoV-2 total antibodies (IgG and IgM) in serum on photometric systems. The test is intended to characterize a vaccine-induced immune response and as an aid to identify individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection, dating back at least 14 days.

Summary

In 2019, a novel corona virus first emerged in Wuhan, China. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) quickly spread across the globe, causing a pandemic. SARS-CoV-2 is mainly transmitted through droplets and aerosols. Symptomatic, but as well pre-symptomatic and asymptomatic SARS-CoV-2 carriers can be potential sources for viral transmission. About 20% of patients develop severe disease, requiring intensive care unit treatment. The current gold standard for diagnosing COVID-19 is the real-time reverse transcription polymerase chain reaction (rRT PCR). Specimen like nasopharyngeal swab and oropharyngeal swab are commonly used for PCR testing. PCR-based methods inform about the presence of the virus at the time of sampling, but they do not provide any information about past infections or the presence of anti-viral antibodies. Seroprevalence studies are of utmost importance to assess the proportion of a population that has developed antibodies against a new virus and could therefore potentially exhibit immunologic protection against subsequent infection. They provide a more accurate picture of the further development of the pandemic and assist the development of new vaccines. Based on the currently available data, IgM and IgG antibodies to SARS-CoV-2 develop several days post disease onset; IgM antibodies start to be detectable around 5–10 days post disease onset and rise rapidly. IgG antibody concentrations follow the IgM response shortly. Significant interindividual differences are observed for time of occurrence of antibodies and their concentration; the median seroconversion for COVID-19 immunoglobulin G and M (IgG and IgM) is 14 days after infection. It is currently unknown how long IgM and IgG antibodies remain detectable after an infection.

Coronaviruses (CoVs) are enveloped viruses with a positive-sense, single-stranded ribonucleic acid (RNA). The genome encodes four important structural proteins, which are required to produce a structurally complete virus particle: the spike protein (S), the nucleocapsid protein (N), the membrane protein (M) and the envelope protein (E). The S protein on the surface of the virus is the most important structural component since it is involved in infection. Spike proteins are large membrane-anchored proteins that assemble to form trimers on the surface of the virus (form the crown-like appearance). Each spike monomer contains a receptor-binding domain (RBD) in the N-terminal S1 subunit which is responsible for binding to the ACE2 receptor (angiotensin-converting enzyme 2) on the host cell. Interactions between the receptor-binding domain (RBD) in subunit S1 and the ACE2 receptor lead to a large-scale structural rearrangement of the spike protein, which is essential for virus entry. It is believed, that neutralizing antibodies (nAB) targeting the S protein may induce protective immunity against viral infection. The protective effect of neutralizing antibodies is primarily mediated by blocking the interaction between the virus and its host cell, thereby inhibiting viral entry into the cell and preventing viral infection. The nucleocapsid protein (N-protein) is the most abundant protein in SARS-CoV-2, but antibodies to the viral N-protein decline faster than those to the receptor-binding domain or to the entire spike protein and, therefore, may substantially underestimate the proportion of SARS-CoV-2 exposed individuals. [1-11]

Method

Particle enhanced immunoturbidimetric test.

Photometric determination of SARS-CoV-2 total antibody concentration present in the sample by measurement of antigen antibody reaction between human SARS-CoV-2 total antibodies and the SARS-CoV-2 receptor-binding domain in the spike protein S1.

Reagents

Components and Concentrations

R1:	TRIS	pH 6.5	0.1 mol/L
R2:	TRIS	pH 9.0	0.1 mol/L
	SARS-CoV-2 protein S-RBD recombinant, covalently bound to polystyrene particles.		

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents and protect them from light.

Warnings and Precautions

1. The reagents contain sodium azide (0.9 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [12].
4. To our knowledge, all currently available vaccines are designed to induce a specific immune response by using the spike protein resp. the receptor binding domain. Please note, that we have not tested the immune response for all these vaccines. However, if any other viral protein has been used for vaccine development, our test will not react accordingly!
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only.

Waste Management

Refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Serum

Stability:

3 days	at	15 – 25°C
42 days [13]	at	4°C
3 months	at	–20°C

Only freeze once. Discard contaminated specimens.

Assay Procedure

Applications for automated systems are available on request.

Basic parameter for BioMajesty® JCA-6010/C

Wavelength	658 nm
Temperature	37°C
Measurement	2-point assay
Sample/calibrator	10 µL
Reagent 1	90 µL
Reagent 2	30 µL
Addition Reagent 2	Cycle 21 (~300 s)
Absorbance 1	Cycle 25-24 (~350 s)
Absorbance 2	Cycle 42-41 (~600 s)
Calibration	Spline

Note: For adapted procedures, calculate volumes of sample, calibrator and reagents appropriately and keep exactly to timing.

Interpretation of Results

The SARS-CoV-2 total antibody concentration of unknown samples is derived from the calibration curve using an appropriate mathematical model such as spline. The calibration curve is obtained with four calibrators at different levels, including a matrix-based zero-value.

Stability of calibration: 2 weeks

Calibrators and Controls

DiaSys TruCal SARS-CoV-2 is recommended for calibration. TruCal SARS-CoV-2 values have been made traceable on BioMajesty® JCA-BM6010/C in a method comparison of SARS-CoV-2 UTAB FS versus a commercially available test. Use DiaSys TruLab SARS-CoV-2 for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal SARS-CoV-2	1 7500 99 10 058	4 x 1 mL
TruLab SARS-CoV-2 Level 1	5 1750 99 10 046	3 x 1 mL
TruLab SARS-CoV-2 Level 2	5 1760 99 10 046	3 x 1 mL

Performance Characteristics

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range from 1.5 up to 150 AU/mL. In the case of a vaccine-induced immune response, antibody titers may rise to extremely high levels. Samples after vaccination should be diluted with SARS-CoV-2 UTAB Sample Dilution Matrix 1 + 20 and the result multiplied by 21. If the result obtained is <150 AU/mL, the sample has to be measured undiluted.	
Limit of detection	1.5 AU/mL
No prozone effect up to 1000 AU/mL.	

Interfering substance	**Interferences ≤ 20% up to**
Bilirubin (conjugated)	60 mg/dL
Bilirubin (unconjugated)	60 mg/dL
Hemoglobin	1000 mg/dL
Lipemia (Triglycerides)	1000 mg/dL
Rheumatoid factor	1000 IU/mL

Cross reactivity
DiaSys SARS-CoV-2 UTAB FS was tested for potential cross reactivity using samples containing antibodies to other pathogens and other diseases/states of disease. No false positive results were observed with below mentioned potential cross-reactants.
Chlamydia pneumonia
Epstein Barr virus (EBV)
Respiratory syncytial virus (RSV)
Influenza A
Human coronavirus OC43 (HCoV-OC43)
Human coronavirus NL63 (HCoV-NL63)
Human coronavirus HKU1 (HCoV-HKU1)
Measles
Varicella zoster virus (VZV)
Cytomegalovirus IgG (CMV G)
Cytomegalovirus (CMV)
Herpes simplex virus (HSV)
EBV viral capsid antigen (VCA)
Epstein-Barr Virus-Specific IgG antibody (EBNA)
Hepatitis E IgG (HEV)
Toxoplasma gondii

Precision		
Within run (n=20)	**Sample 1**	**Sample 2**
Mean [AU/mL]	31.3	50.2
CV [%]	1.60	3.20
Between day (n=20)	**Sample 1**	**Sample 2**
Mean [AU/mL]	40.1	48.6
CV [%]	5	4

Specificity = 98.7% (CI 95%, 95.3 – 99.8%)

Sensitivity = 98.0% (CI 95%, 89.6 – 100%)

Clinical Performance
Negative predictive value (NPV): A total of 151 samples, obtained prior to the COVID-19 outbreak, were included in the study. 2 false positive sample were detected. The resulting overall negative predictive value (NPV) in the internal study was 99.3% (CI 95%, 95.5 – 99.9%).
Positive predictive value (PPV): A total of 51 samples from patients tested positive for SARS-CoV-2 were measured with the SARS-CoV-2 UTAB FS assay. 1 false negative sample was detected. The overall resulting positive predictive value (PPV) in the internal study was 96.2% (CI 95%, 86.3 – 99.0%).

Reference Range

≤30 AU/mL negative for anti- SARS- CoV- 2 antibodies

>30 AU/mL positive for anti- SARS- CoV- 2 antibodies

A negative test result does not completely exclude the possibility of infection with SARS-CoV-2. A positive antibody test result indicates that a person has been infected with SARS-CoV-2 [12], but does not necessarily show immunity. Serum samples from the very early phase, the so-called pre-seroconversion phase, can yield negative results. For this reason, the test cannot be used to diagnose acute infection. Furthermore, it cannot be excluded that titers decrease over time and eventually lead to a negative result. The results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Literature

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