VIROTECH SARS-CoV-2 IgG ELISA
(SARS-CoV-2 IgG ELISA)

Order No.: EC123G00

VIROTECH SARS-CoV-2 IgM ELISA
(SARS-CoV-2 IgM ELISA)

Order No.: EC123M00

VIROTECH SARS-CoV-2 IgA ELISA
(SARS-CoV-2 IgA ELISA)

Order No.: EC123A00

Colour code: none

FOR IN VITRO DIAGNOSTICS ONLY

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1 Intended Use

Intended use	Detection of IgG/IgM/IgA antibodies against SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) In view of the current COVID-19 outbreak situation, the prevalence of the population to be tested can be highly variable. To maximize the predictive values in every situation the VIROTECH SARS-CoV-2 IgG ELISA is validated with two different Cut off settings. *Low prevalence Cut off* Intended use: Screening for antibodies in a population with a low SARS-CoV-2 prevalence, e.g. for epidemiological studies with random selection of persons to be tested. *High prevalence Cut off* Intended use: Testing of patients within a suspected higher prevalence population/with a higher pre-test probability • Testing of patients with suspected previous SARS-CoV-2 infection (COVID-19 related symptoms, potential contact to SARS-CoV-2 infected patients or diagnosed SARS-COV-2 infection) • Testing in a current outbreak situation
Function	Detection of infection, aid to diagnosis
Type of test	Qualitative
Type of sample	Human Serum and Plasma (EDTA, Citrate, Heparin, CPD)
Specific Information	
Physiological or pathological state	The test is recommended for the detection of acute infections, for confirmation of pathogen contact and for assessment of the immune status.
Automation	Automated processing possible (see chapter 8)
Testing population	Patients with suspected infection with SARS-CoV-2 or persons with overcome (asymptomatic) SARS-CoV-2 infection
Further information	
Listing of the antigens	Recombinant Nucleocapsid protein of SARS-CoV-2
Intended user	Laboratory professional use

2 Summary and Explanation

Coronavirus Disease (COVID-19) is a new infectious disease that was first detected at the end of 2019 in Hubei province, China and has been designated a pandemic by the World Health Organization (WHO). The causative agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has demonstrated the capability to spread rapidly and has resulted in thousands of confirmed human cases of asymptomatic infection, mild illness, severe illness, and deaths.

Coronaviruses are enveloped, positive single-stranded large RNA viruses that infect humans, but also a wide range of animals. The common human Coronaviruses NL63, 229E, OC43 and HKU1 are widespread especially throughout the winter months and are responsible for 5 to 30% of all acute respiratory diseases, typically with mild symptoms (common cold). The immunity from previous infections last only for a short period of time and reinfections with the same pathogen is possible just after one year. Special interest was raised by two novel beta-coronaviruses that caused severe respiratory syndromes of the lower respiratory tract: SARS coronavirus that caused a worldwide epidemic in 2003 and MERS coronavirus that caused several hundred infections in 2012, especially in the middle east.

Four subfamilies, namely alpha-, beta-, gamma- and delta-coronaviruses exist. While alpha- and beta-coronaviruses apparently originate from mammals, in particular from bats, gamma- and delta-viruses originate from pigs, birds, and aquatic animals. The novel coronavirus SARS-CoV-2 belongs to the B lineage of the beta-coronaviruses and is closely related to the SARS-CoV virus. SARS-CoV-2 apparently succeeded in making its transition from animals to humans on the Huanan seafood market in Wuhan, China.

The initial clinical sign of the SARS-CoV-2-related disease COVID-19 which allowed case detection was pneumonia. However, it turned out that the course of the disease is non-specific, varied and varies widely, from asymptomatic courses to severe pneumonia with lung failure and death. Based on current knowledge, around 80% of the illnesses are mild to moderate.

The VIROTECH Diagnostics SARS-CoV-2 IgG/IgM/IgA ELISA Test is intended for the qualitative detection of IgG, IgM and/or IgA antibodies to SARS-CoV-2 in human serum to aid in the diagnosis of COVID-19 and is to be used in conjunction with clinical findings.

The utility of diagnostic testing is highly dependent on the prevalence of the disease in question in the test population that, together with the sensitivity and specificity of the diagnostic tests used, defines the positive and negative predictive values. They define the probability that a patient with a positive/negative test result is truly antibody positive/negative. The current SARS-CoV-2 pandemic is connected to very variable prevalence depending on the tested population, e.g. can be very low (0-5%) in low affected areas or significantly higher in a current outbreak, a clinical setting, or in patients with a higher pre-test probability. To guarantee the best diagnostic value in each setting, the VIROTECH SARS-CoV-2 IgG ELISA is validated with two different Cut off values. The *low prevalence Cut off* is optimized for a maximum specificity important for a high positive predictive value in test populations with a low SARS-CoV-2 prevalence, e.g. in epidemiological studies or mass testing of people without suspected previous SARS-CoV-2 infection. The *high prevalence Cut off* is optimized for an increased sensitivity important for testing of patients with a higher pre-test probability, e.g. because of a suspected previous SARS-CoV-2 infection based on symptoms, diagnostic testing, contact to infected patients or in a current outbreak situation with a significantly higher SARS-CoV-2 prevalence. For the best diagnostic value, the VIROTECH SARS-CoV-2 IgG ELISA should be used with the Cut off variant that is most suitable for the test population.

3 Test Principle

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunological detection method that makes use of the specific interaction between antibodies and antigens. For this purpose, a microplate is coated with specific antigens from an infectious agent. If the added human serum/plasma contains the appropriate antibody, it forms an immune complex with the antigen fixed on the microplate. Unbound antibodies are removed by the following washing process. Then, an added enzyme conjugate binds to the complex. Unbound conjugate is removed by the following washing process. After addition of the substrate (TMB), enzyme activity (peroxidase) produces a blue dye, which changes to yellow in the last step by adding the stopping solution.

4 List of materials provided

The following components are parameter and batch independent: PBS-Dilution Buffer, PBS-Washing Solution, TMB-Substrate, Citrate Stopping Solution
The following components are parameter and batch dependent: positive Control, Calibrator Control, negative Control, Conjugates.
The appropriate batch of plates is included in the Quality Control Certificate.

For processing an IgM ELISA (EC123M00) the product VIROTECH RF-SorboTech is additionally required (see 4.2).

4.1 Package Contents ELISA

IgG Kit	IgM Kit	IgA Kit	Plate and ready-to-use controls	Abbreviation
1	1	1	Microplate 1x96 antigen coated, breakable wells, lyophilized	**MTP**
1,3 ml	1,3 ml	1,3 ml	SARS-CoV-2 negative Control / neg. Ctrl, human serum/plasma with protein-stabilizer and preservative, ready-to-use	**NEG**
1,3 ml	1,3 ml	1,3 ml	SARS-CoV-2 Calibrator Control / Calibrator Ctrl human serum/plasma with protein-stabilizer and preservative, ready-to-use	**CAL**
1,3 ml	1,3 ml	1,3 ml	SARS-CoV-2 positive Control / pos. Ctrl human serum/plasma with protein-stabilizer and preservative, ready-to-use	**POS**

IgG Kit	IgM Kit	IgA Kit	Reagents	Abbreviation
2 x 50 ml	2 x 50 ml	2 x 50 ml	PBS-Dilution Buffer (blue), with preservative and Tween 20, ready-to-use / pH 7,2	**DILBUF**
50 ml	50 ml	50 ml	PBS-Washing Solution, 20x concentrated, with preservative and Tween 20, to be diluted / pH 7,2	**WASHBUF**
11 ml	11 ml	11 ml	Conjugate (anti-human), with protein stabilizer and preservative, ready-to-use	**CONJ**
11 ml	11 ml	11 ml	TMB-Substrate (3,3´,5,5´ Tetramethylbenzidine), ready-to-use / pH 5-5,1	**SUBS**
6 ml	6 ml	6 ml	Citrate Stopping Solution, ready-to-use / pH <1,2 (20°C)	**STOP**

IgG Kit	IgM Kit	IgA Kit	Documents	Abbreviation
1	1	1	Documents: short instructions, Hazard information ELISA, test scheme Quality Control Certificate with information about the target value	**N/A** **CERT ZW**
1	1	1	Homepage: www.virotechdiagnostics.com Material Safety Data Sheet	**N/A** **SDB**

All components are ready-to-use (RTU) except for the PBS-Washing Solution.

4.2 Additionally required product for processing the IgM test kit

RF-SorboTech (VIROTECH Absorbent)

Reagent for the pre-absorption of high IgG titres and IgM rheumatoid factors in serum, plasma and CSF samples.

Component ready-to-use	**Volume**	**Content**	**Order-No.**	**Abbreviation**
RF-SorboTech – (40 tests)	2,0 ml	Anti-human IgG, <0,1% sodium azide as preservative	161101	**RFSORBO**
RF-SorboTech - (80 tests)	2 x 2,0 ml 50 ml	Anti-human IgG, <0,1% sodium azide as preservative PBS-Washing Solution	B/300.00	**RFSORBO** **WASHBUF**
RF-SorboTech - (200 tests)	10,0 ml	Anti-human IgG, <0,1% sodium azide as preservative	161102	**RFSORBO**
Instructions for use				N/A

5 List of materials required but not provided

1.	Aqua dest/demin. Water	10.	ELISA hand washer or automatic washing device for microtiter plates
2.	Multichannel pipettes (50µl, 100µl)	11.	ELISA plate spectrophotometer for microplates (450/620nm filter, reference wavelength 620-690nm)
3.	Micropipettes (10µl, 100µl,1000µl)	12.	Incubator 37°C
4.	Disposable pipette tips	13.	Stopwatch
5.	Test tubes and test tube racks	14.	Disposable protective gloves (according to DIN EN 455)
6.	Cellulose wipes	15.	Safety goggles ((according to DIN EN166)
7.	Cover for ELISA plates	16.	ELISA-Processor (optional)
8.	Waste container for infectious material	17.	Evaluation software (optional, see chapter 8)
9.	Suitable reaction tubes e.g. HDPE (High Density Polyethylene), LLDPE (Linear Low Density Polyethylene), LDPE (Low Density Polyethylene), PP (Polypropylene), High-Purity Polypropylene		

6 Storage and Stability of the kit, set and ready-to-use components

1. Store all components at 2-8°C.
2. The shelf life of the individual components is indicated on the respective label.
3. The shelf life of the kit is stated on the kit box and on the Quality Control Certificate.
4. Do not freeze the components of the test kit and sets and protect them from excessive heat during storage.
5. Remove the required individual cavities/strips and store the rest in a sealed bag with desiccant at 2-8°C.
6. Store all reagents at 2-8°C immediately after use.
7. Store the ready-to-use Conjugate and the TMB-Substrate in the dark.
8. When the substrate develops colour, discard it.
9. Take only the amount of the ready-to-use Conjugate and the TMB-Substrate needed for the preparation.
10. Too much Conjugate or TMB Substrate must not be returned to the bottle but must be discarded.

Component	**Status**	**Storage**	**Shelf life**
Controls	After opening	+2 to +8°C	3 months
Microplate	After opening	+2 to +8°C (store in the supplied bag with desiccant)	3 months
PBS- Dilution Buffer (blue)	After opening	+2 to +8°C	3 months
RF-SorboTech	Undiluted, after opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After opening	+2 to +8°C (protect from light)	3 months
TMB-Substrate	After opening	+2 to +8°C (protect from light)	3 months
Citrate Stopping Solution	After opening	+2 to +8°C	3 months
PBS-Washing Solution	After opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

7 Warnings and Precautions

1. Only samples tested and found negative for HIV1-AK, HIV2-AK, HCV-AK and hepatitis B SURFACE antigen are used as control serum/plasma. Nevertheless, all materials should be considered as potentially infectious material and should be treated accordingly.
2. Ensure that the current laboratory guidelines are observed DGUV Information 213-850: "Working Safely in Laboratories, Basic Principles and Guidelines".
3. When performing the VIROTECH Diagnostics ELISA, the established precautions for microbiological hazards must be observed and standard procedures for the proper disposal of samples must be followed.
4. The reagents must not be mixed or exchanged with those of other manufacturers.
5. The components must not be used after the expiry date indicated on the label.
6. During storage and incubation, the reagents should be protected from excessive heat and direct sunlight.
7. Carefully shake the reagents before use.
8. A careful washing process is essential for the accuracy of the test. Ensure that all washing steps are carried out according to the regulations.

9. To avoid microbial contamination, only the required reagent volume should be taken, and no residue should be returned to the original bottle after the test.
10. Safety data sheets (MSDS) are available for all components of this ELISA. The relevant MSDS should be consulted before implementation. The corresponding MSDS can be found on the VIROTECH Diagnostics homepage: www.virotechdiagnostics.com.
11. It is recommended to wear laboratory coats, disposable gloves (according to DIN EN 455) and safety glasses (according to DIN EN 166) when performing the ELISA test and handling patient samples and ELISA components. Avoid contact between hands and eyes or with the mucous membranes. If contact occurs despite recommended protective measures, consult the appropriate MSDS for appropriate treatment.
12. The disposal of the materials used should be carried out according to the specific guidelines of the country.

8 ELISA Processors and Software

All VIROTECH Diagnostics ELISA can be processed by ELISA processors. The user is responsible for evaluating the test on the respective device. The user is obliged to carry out a regular device inspection and maintenance.

VIROTECH Diagnostics recommends the following procedure.

1. If the ELISA Processor is manufactured or major repairs are made to the ELISA Processor, the maintenance of the device must be carried out according to the specifications of the device manufacturer and then the ELISA Processor must be checked with the VIROTECH Validation/Validation ELISA; order number: EC250.00.
2. Verification with the VIROTECH Validation/Validation ELISA should generally be carried out once every six months.

For each test run, the release criteria of the batch-specific Quality Control Certificate for the product must be fulfilled.
This procedure guarantees the proper function of the ELISA Processor in combination with the VIROTECH ELISA and serves the quality assurance of the laboratory.

9 Test Procedure

Following the VIROTECH Diagnostics test procedure is important to ensure correct results.

9.1 Test material: serum and plasma samples

Serum or plasma (EDTA, heparin, CPD, citrate) can be used as test material.

RECOMMENDATION:

1. Store patient sample at 2-8°C for no longer than 7 days. For longer storage, freeze the samples at -20°C or lower; avoid repeated thawing.
2. Prepare fresh patient sample dilutions; shelf life maximum 6h at 2-8°C.
3. Do not use inactivated, icteric, haemolytic, lipaemic or turbid samples as they may give false positive or false negative results.

9.2 Handling and preparation of the samples and the reagents

GENERAL PREPARATIONS

1. Set incubator to 37°C; check that temperature is reached before starting incubation.
2. Set the photometer to an absorbance of 450/620 nm (reference wavelength 620-690 nm). Adjust the photometer so that the measured blank value is subtracted from all other absorbances
3. Bring the reagents to room temperature, then open the package of the microplate and take out the required number of wells, if necessary, remove the test frame. Close the package with the remaining wells.
4. Mix liquid components well before use.
5. Fill up PBS-Washing solution concentrate (50ml) to 1 l with aqua dest./demin. to the final dilution of 1:20 (1+19). If the concentrate crystallizes, bring it to room temperature before diluting and only then dilute it. Mix the ready-to-use Wash Solution thoroughly before use.

PREPARATION OF THE SERUM AND PLASMA SAMPLES

6. Working dilution of patient samples for IgG/IgA: 1:101 (1+100); e.g. 10µl Probe + 1ml PBS-Dilution Buffer.

Only for IgM test:

High IgG titres or rheumatoid factors can interfere with the specific detection of IgM antibodies and lead to false positive or false negative results. For a correct IgM detection, it is therefore necessary to pre-treat the patient samples with RF-SorboTech (VIROTECH Absorbent, see 4.2.1). For the ready-to-use controls the pre-treatment is not necessary.

Working dilution of patient samples for IgM:	Dilute RF-SorboTech 1:10 (1+9) in a suitable test tube (see chapter 5) with PBS-dilution buffer (VP). Dilute the patient sample 1:101 (1+100) with this dilution preparation. This corresponds to the working dilution. Incubate for 15 minutes at room temperature.

Example: Pipette 1 drop of RF-SorboTech (approx. 50µl) + 450µl PBS-Dilution Buffer 1:10 (1+9). Add 5µl serum to this preparation (500µl). This corresponds to a 1:101 (1+100) serum dilution. Incubate for 15 minutes at room temperature.

VIROTECH ELISA TEST PROCEDURE (Test procedure scheme, see separate document)

9.3 Serum and Plasma samples

1. Insert the required number of wells for the patient samples, the blank and all controls into the test frame.
2. Pipette 100µl of the ready-to-use Dilution Buffer as the blank value, 100µl of the controls and 100µ of the diluted patient samples for each test portion.
3. A duplicate measurement is recommended for blanks, controls and patient samples. For the Calibrator Control a duplicate preparation is mandatory.
4. After pipetting the patient samples, incubate with cover for 30 min at 37°C.
5. Finish the incubation by washing 4 times with 350-400µl ready-to-use washing solution (see 9.2.5) per well. The ready-to-use washing solution is filled into the cavities with a pipette or washing device and emptied again. After emptying during the last washing step, the residues of the ready-to-use washing solution are not left in the cavities, but the last liquid residues are removed by tapping on a cellulose base.
6. Pipette 100µl of the ready-to-use Conjugate into all wells.
7. Incubate the Conjugate with cover for 30 min at 37°C.
8. End incubation by washing 4 times (see 5.).
9. Pipette 100µl of the ready-to-use TMB Substrate into each well.
10. Incubate the TMB-Substrate with cover for 30 min. at 37°C. Place the plate in a dark place.
11. Stop the substrate reaction by pipetting 50µl citrate stop solution into each well. Then gently and carefully tap the plate until the liquids are completely mixed and a uniform yellow colour is visible.
12. Measure absorbance at 450/620nm (reference wavelength 620-690nm). The photometric measurement should be performed within 1h after addition of the citrate stop solution.

10 Interpretation of Results

10.1 Samples and controls

If a control or sample is determined more than once, the mean value of the OD values (OD = Optical Density) is calculated and used.

10.2 Test function check

The OD values of the blank, negative, positive and calibrator controls must meet the requirements of the Quality Control Certificate.

The value of the calculated VIROTECH units (see 10.3) of the positive Control must be within the range specified in the Quality Control Certificate.

If any of the above requirements for OD values or VE values are not met, the test must be repeated.

10.3 Calculation of VIROTECH units (VE)

The OD value of the blank value (LW) must be subtracted from all other OD values before calculation.

Correction Factor: To differentiate between positive and negative samples VIROTECH Diagnostics has defined a Cut off value. This Cut off value is correlated with the Calibrator Control via a Correction Factor. The correction factor is determined specifically for each batch and can be found on the Quality Control Certificate.

For the VIROTECH SARS-CoV-2 IgG ELISA, two different Cut off values are validated to guarantee maximum predictive values depended on the intended use of the assay. Please see chapter 1 – Intended Use for a description which Cut off and associated correction factor should be used in each situation. A separate Quality Control Certificate for each Cut off is provided.

The OD values are converted into VIROTECH units (VE) as follows.

1. Calculation of the Cut off value

Cut off value = average OD Calibrator Control x Correction Factor

2. Calculation of VIROTECH units (VE)

$$\text{VE (positive Control)} = \frac{\text{OD (positive Control)}}{\text{OD (Cut off value)}} \times 10$$

$$\text{VE (Patient Serum)} = \frac{\text{OD (Patient Serum)}}{\text{OD (Cut off value)}} \times 10$$

The VIROTECH units (VE) of the calibrator are predefined as stated in the Quality Control Certificate. The VE of the Calibrator can be calculated with the following formula: VE Calibrator = 10 / Correction Factor

10.4 Evaluation scheme of patient samples for IgG, IgM and IgA

result (VE)	Interpretation
< 9.0	negative
9.0 – 11.0	borderline
> 11.0	positive

1. If the VE of the sample is below the borderline range, the sample is considered negative.
2. If the VE of the sample is within the borderline range, the sample is considered borderline.
3. If the VE of the sample is above the borderline range, the sample is considered positive.

11 Limitations of the test

1. The test is an aid to diagnosis and should be used in conjunction with clinical findings. The results should not be used as the sole basis for the diagnosis or exclusion of SARS-CoV-2 infection or for information on infection status. The interpretation should always include the clinical picture, epidemiological data and any other available laboratory findings.
2. Negative results do not rule out SARS-CoV-2 infection, especially in persons who have been in contact with the virus. Further tests, e.g. for direct pathogen detection, should be considered in order to rule out infection in these persons. Serological evidence is best obtained by testing paired acute and convalescent samples collected at intervals of several weeks.
3. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera may give erroneous results and should not be used.

12 Performance Characteristics

12.1 Analytical Performance

12.1.1 Samples

Serum or plasma (EDTA, heparin, CPD, citrate) can be used as test material.
Information concerning storage and stability of samples are in chapter 6.

12.1.2 Accuracy

a) Trueness

For the VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA there is no WHO standard or comparable reference value by which the accuracy could be determined.

b) Precision

A precision sample panel consisting of a negative sample, a high negative sample, a low positive sample, and a moderate positive sample were tested in a total of 11 independent runs over a period of 4 days. Test were performed individually by three different persons. Within each run, each sample was tested in triplicate. Repeatability (Intra-Assay-Coefficient of variation) & Reproducibility (Inter-Assay-Coefficient of variation) were calculated.

Conclusion
Repeatability: Within the diagnostically significant assay range around the Cut off, the repeatability is below 10%. For low negative samples the standard deviation is below 0,2 VE.

Reproducibility: Within the diagnostically significant assay range around the Cut off, the reproducibility is below 15%. For low negative samples the standard deviation is below 0,2 VE.

1. **VIROTECH SARS-CoV-2 IgG ELISA**

		Repeatability		Reproducibility	
	Mean VE	**SD**	**%CV**	**SD**	**%CV**
Sample 1	0,5	0,1	17,8	0,11	21,6
Sample 2	9,3	0,5	5,9	0,5	5,7
Sample 3	16,1	0,8	5,2	0,6	3,5
Sample 4	23,1	1,4	5,9	0,7	3,1

2. **VIROTECH SARS-CoV-2 IgM ELISA**

		Repeatability		Reproducibility	
	Mean VE	**SD**	**%CV**	**SD**	**%CV**
Sample 1	0,9	0,1	14,1	0,17	20,0
Sample 2	5,0	0,5	9,5	0,6	13,0
Sample 3	16,9	1,0	5,9	1,5	8,5
Sample 4	25,3	1,2	4,8	1,7	6,8

3. **VIROTECH SARS-CoV-2 IgA ELISA**

		Repeatability		Reproducibility	
	Mean VE	**SD**	**%CV**	**SD**	**%CV**
Sample 1	0,3	0,1	44,7	0,15	50,6
Sample 2	4,3	0,3	7,3	0,5	10,4
Sample 3	11,6	0,7	6,0	1,4	12,4
Sample 4	16,9	1,0	5,7	1,8	10,4

12.1.3 Analytical sensitivity

N/A

12.1.4 Analytical specificity

a) Interferences

The VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA was evaluated for interferences according to guideline EP07-A3 (“Interference Testing in Clinical Chemistry” from the Clinical and Laboratory Standards Institute). Three samples, a negative, a low positive and a moderate positive were spiked with high levels of interferents and were tested along with serum without spiked interferents. The following table shows the tested substances added to patient samples at the indicated concentrations. These correspond to the recommendations in the CLSI guideline to represent pathological elevated concentrations in patient samples.

Interferent	**Test concentration**
Albumin	60 mg/ml
Bilirubin	0,4 mg/ml
Cholesterol	4 mg/ml
Hemoglobin	10 mg/ml
Triglycerides	15 mg/ml

No clinically significant interference effect was found for all tested substances in the VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA.

b) Cross-reactivity

1. **VIROTECH SARS-CoV-2 IgG ELISA**

Low prevalence Cut off

145 samples were tested on the VIROTECH SARS-CoV-2 IgG ELISA to evaluate the cross reactivity of the assay. Each sample was tested positive for one of the respective markers by a legally marketed device.

		VIROTECH SARS-CoV-2 IgG ELISA		
Samples positive for	**n**	**Positive**	**Equivocal**	**Negative**
Adenovirus	10	0	0	10
Parainfluenza	9	0	0	9
Candida albicans	10	0	0	10
Bordetella pertussis	10	0	0	10
Influenza A	10	0	0	10
Influenza B	10	0	0	10
Enterovirus	10	0	0	10
Respiratory-Syncytial-Virus	10	1	0	9
Chlamydia pneumoniae	10	0	0	10
Legionella pneumophila	14	0	0	14
Mycoplasma pneumoniae	10	0	0	10
Haemophilus influenza	10	0	0	10
Picornavirus	12	0	0	12
Streptococcus pneumoniae	10	0	0	10

To evaluate the cross reactivity to human coronaviruses other than SARS-CoV-2, samples with antibodies to different human coronaviruses were tested on the GSD SARS-CoV-2 IgG ELISA.

		VIROTECH SARS-CoV-2 IgG ELISA	
No	**Sample positive for**	**Units**	**Result**
1	HCoV-OC43	1,7	Neg
2	HCoV-OC43	1,6	Neg
3	HCoV-NL63	1,9	Neg
4	HCoV-NL63	1,8	Neg
5	HCoV-229E	2,3	Neg
6	HCoV-229E	2,1	Neg
7	HCoV-OC43 and HCoV 229E	2,3	Neg
8	HCoV-OC43/HCoV-HKU1	2,2	Neg

High prevalence Cut off

145 samples were tested on the VIROTECH SARS-CoV-2 IgG ELISA to evaluate the cross reactivity of the assay. Each sample was tested positive for one of the respective markers by a legally marketed device

		VIROTECH SARS-CoV-2 IgG ELISA		
Samples positive for	**n**	**Positive**	**Equivocal**	**Negative**
Adenovirus	10	0	0	10
Parainfluenza	9	0	0	9
Candida albicans	10	0	0	10
Bordetella pertussis	10	0	0	10
Influenza A	10	0	0	10
Influenza B	10	0	0	10
Enterovirus	10	0	0	10
Respiratory-Syncytial-Virus	10	1	0	9
Chlamydia pneumoniae	10	1	0	9
Legionella pneumophila	14	0	0	14
Myoplasma pneumoniae	10	0	0	10
Haemophilus influenza	10	0	0	10
Picornavirus	12	0	1	11
Streptococcus pneumoniae	10	0	1	9

To evaluate the cross reactivity to human coronaviruses other than SARS-CoV-2, samples with antibodies to different human coronaviruses were tested on the GSD SARS-CoV-2 IgG ELISA.

		VIROTECH SARS-CoV-2 IgG ELISA	
No	**Sample positive for**	**Units**	**Result**
1	HCoV-OC43	2,6	Neg
2	HCoV-OC43	2,5	Neg
3	HCoV-NL63	3,0	Neg
4	HCoV-NL63	2,7	Neg
5	HCoV-229E	3,6	Neg
6	HCoV-229E	3,2	Neg
7	HCoV-OC43 and HCoV 229E	3,6	Neg
8	HCoV-OC43/HCoV-HKU1	3,4	Neg

2. **VIROTECH SARS-CoV-2 IgM ELISA**

145 samples were tested on the VIROTECH SARS-CoV-2 IgM ELISA to evaluate the cross reactivity of the assay. Each sample was tested positive for one of the respective markers by a legally marketed device.

		VIROTECH SARS-CoV-2 IgM ELISA		
Samples positive for	**n**	**Positive**	**Equivocal**	**Negative**
Adenovirus	10	0	0	10
Parainfluenza	9	0	0	9
Candida albicans	10	0	0	10
Bordetella pertussis	10	0	0	10
Influenza A	10	0	0	10
Influenza B	10	1	1	8
Enterovirus	10	0	0	10
Respiratory-Syncytial-Virus	10	4	1	5
Chlamydia pneumoniae	10	0	0	10
Legionella pneumophila	14	0	0	14
Myoplasma pneumoniae	10	0	1	9
Haemophilus influenza	10	1	0	9
Picornavirus	12	0	0	12
Streptococcus pneumoniae	10	0	0	10

To evaluate the cross reactivity to human coronaviruses other than SARS-CoV-2, samples with antibodies to different human coronaviruses were tested on the GSD SARS-CoV-2 IgM ELISA.

		VIROTECH SARS-CoV-2 IgM ELISA	
No	**Sample positive for**	**Units**	**Result**
1	HCoV-OC43	1,4	Neg
2	HCoV-OC43	1,7	Neg
3	HCoV-NL63	1,5	Neg
4	HCoV-NL63	1,4	Neg
5	HCoV-229E	1,5	Neg
6	HCoV-229E	0,9	Neg
7	HCoV-OC43 and HCoV 229E	1,3	Neg
8	HCoV-OC43/HCoV-HKU1	1,4	Neg

3. **VIROTECH SARS-CoV-2 IgA ELISA**

145 samples were tested on the VIROTECH SARS-CoV-2 IgA ELISA to evaluate the cross reactivity of the assay. Each sample was tested positive for one of the respective markers by a legally marketed device

		VIROTECH SARS-CoV-2 IgA ELISA		
Samples positive for	**n**	**Positive**	**Equivocal**	**Negative**
Adenovirus	10	0	0	10
Parainfluenza	9	0	0	9
Candida albicans	10	0	0	10
Bordetella pertussis	10	0	0	10
Influenza A	10	0	0	10
Influenza B	10	0	0	10
Enterovirus	10	0	0	10
Respiratory-Syncytial-Virus	10	0	0	10
Chlamydia pneumoniae	10	0	0	10
Legionella pneumophila	14	0	0	14
Myoplasma pneumoniae	10	0	0	10
Haemophilus influenza	10	0	0	10
Picornavirus	12	0	0	12
Streptococcus pneumoniae	10	0	0	10

To evaluate the cross reactivity to human coronaviruses other than SARS-CoV-2, samples with antibodies to different human coronaviruses were tested on the GSD SARS-CoV-2 IgA ELISA.

		VIROTECH SARS-CoV-2 IgA ELISA	
No	**Sample positive for**	**Units**	**Result**
1	HCoV-OC43	1,2	Neg
2	HCoV-OC43	1,2	Neg
3	HCoV-NL63	1,5	Neg
4	HCoV-NL63	0,6	Neg
5	HCoV-229E	0,6	Neg
6	HCoV-229E	2,0	Neg
7	HCoV-OC43 and HCoV 229E	2,0	Neg
8	HCoV-OC43/HCoV-HKU1	0,6	Neg

c) Class specificity

1. **VIROTECH SARS-CoV-2 IgG ELISA**

Samples tested positive for IgG antibodies by the VIROTECH SARS-CoV-2 IgG ELISA were treated with RF-SorboTech to remove IgG class antibodies. Treated samples were measured together with untreated samples to demonstrate the class specificity.

	Untreated		Treated		
Sample	**VE**	**Result**	**VE**	**Result**	**Difference**
1	22,2	Pos	0	Neg	-100%
2	25,7	Pos	0	Neg	-100%
3	25,6	Pos	0,4	Neg	-98%
4	48,1	Pos	0,7	Neg	-99%

2. **VIROTECH SARS-CoV-2 IgM ELISA**

Samples tested positive or equivocal for IgM antibodies by the VIROTECH SARS-CoV-2 IgM ELISA were treated with dithiothreitol to inactivate IgM class antibodies. Treated samples were measured together with untreated samples to demonstrate the class specificity.

	Untreated		Treated		
Sample	**VE**	**Result**	**VE**	**Result**	**Difference**
1	10,9	Eq	0	Neg	-100%
2	58,6	Pos	2	Neg	-97%
3	70,7	Pos	1,4	Neg	-98%
4	34,5	Pos	4,6	Neg	-87%

3. **VIROTECH SARS-CoV-2 IgA ELISA**

Samples tested positive for IgM or IgG antibodies by the VIROTECH SARS-CoV-2 IgM or IgG ELISA were tested on the VIROTECH SARS-CoV-2 IgA ELISA. Negative IgA results demonstrate the class specificity.

	VIROTECH SARS-CoV-2 IgG ELISA		VIROTECH SARS-CoV-2 IgM ELISA		VIROTECH SARS-CoV-2 IgA ELISA	
Sample	**VE**	**Result**	**VE**	**Result**	**VE**	**Result**
1	9,8	**Eq**	0,4	Neg	2,8	Neg
2	9,5	**Eq**	0,9	Neg	4,2	Neg
3	30,7	**Pos**	15,7	**Pos**	6,6	Neg
4	32,9	**Pos**	6,3	Neg	8,3	Neg
5	15,7	**Pos**	42,3	**Pos**	6,7	Neg
6	21,9	**Pos**	14,9	**Pos**	4,3	Neg
7	16,2	**Pos**	8	Neg	8,5	Neg
8	15,5	**Pos**	3,8	Neg	0,4	Neg
9	2,2	Neg	9,2	**Eq**	3,3	Neg
10	13	**Pos**	31,7	**Pos**	2,6	Neg
11	2,2	Neg	14,2	**Pos**	1,3	Neg
12	2,4	Neg	35	**Pos**	2,2	Neg
13	3	Neg	10,4	**Eq**	1	Neg
14	1,7	Neg	9,3	**Eq**	1,3	Neg
15	3,4	Neg	18,5	**Pos**	1,3	Neg
16	1,9	Neg	37,3	**Pos**	1	Neg
17	0,4	Neg	51,2	**Pos**	0,3	Neg
18	3	Neg	24,1	**Pos**	2,1	Neg

12.1.5 Metrological traceability

There is no WHO standard or comparable reference value for the VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA.

12.1.6 Definition of the Cut off of the assay

More than 250 samples representing the healthy population, patients tested positive for SARS-CoV-2 by RT-PCR, and patients positive for other diseases that could be mistaken for COVID-19 were tested on the VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA. The assay Cut off was adjusted to obtain the optimal trade-off between sensitivity and specificity.

12.2 Clinical Performance Characteristics

12.2.1 Diagnostic Sensitivity

1. **VIROTECH SARS-CoV-2 IgG ELISA**

A) Hospitalized patients

A sensitivity study was performed with 61 blood samples from 42 patients hospitalized for COVID-19 related symptoms and tested positive for SARS-CoV-2 by RT-PCR testing were tested on the VIROTECH SARS-CoV-2 IgG ELISA. To determine the diagnostic sensitivity, the samples were sorted by the timing post symptom onset.

Low prevalence Cut off

Days post symptom onset	n	VIROTECH SARS-CoV-2 IgG ELISA			Sensitivity*
		Positive	Equivocal	Negative	
Day 0-5	13	1	0	12	7,7%
Day 6-8	14	4	1	9	28,6%
Day 9-11	17	8	2	7	47,1%
Day >=12	17	17	0	0	100,0%

*Equivocal counted as negative

High prevalence Cut off

Days post symptom onset	n	VIROTECH SARS-CoV-2 IgG ELISA			Sensitivity*
		Positive	Equivocal	Negative	
Day 0-5	13	1	0	12	7,7%
Day 6-8	14	5	1	8	35,7%
Day 9-11	17	10	0	7	58,8%
Day >=12	17	17	0	0	100,0%

*Equivocal counted as negative

B) Asymptomatic patients and patients with mild symptoms

A sensitivity study was performed with 70 blood samples from patients who were treated on an outpatient basis experiencing only mild to no symptoms but tested positive for SARS-CoV-2 by RT-PCR were tested on the VIROTECH SARS-CoV-2 IgG ELISA. All samples were taken at least 18 days after positive PCR result

Low prevalence Cut off

Samples tested	VIROTECH SARS-CoV-2 IgG ELISA			Sensitivity*
	Positive	Equivocal	Negative	
70	54	4	12	77,1%

*Equivocal counted as negative

High prevalence Cut off

Samples tested	VIROTECH SARS-CoV-2 IgG ELISA			Sensitivity*
	Positive	Equivocal	Negative	
70	64	1	5	91,4%

*Equivocal counted as negative

2. VIROTECH SARS-CoV-2 IgM ELISA

A sensitivity study was performed with 61 blood samples from 42 patients hospitalized for COVID-19 related symptoms and tested positive for SARS-CoV-2 by RT-PCR testing were tested on the VIROTECH SARS-CoV-2 IgM ELISA. To determine the diagnostic sensitivity, the samples were sorted by the timing post symptom onset.

Days post symptom onset	n	VIROTECH SARS-CoV-2 IgM ELISA			Sensitivity*
		Positive	Equivocal	Negative	
Day 0-5	13	0	0	13	0,0%
Day 6-8	14	6	1	7	42,9%
Day 9-11	17	7	1	9	41,2%
Day >=12	17	12	2	3	70,6%

*Equivocal counted as negative

3. VIROTECH SARS-CoV-2 IgA ELISA

A sensitivity study was performed with 61 blood samples from 42 patients hospitalized for COVID-19 related symptoms and tested positive for SARS-CoV-2 by RT-PCR testing were tested on the VIROTECH SARS-CoV-2 IgA ELISA. To determine the diagnostic sensitivity, the samples were sorted by the timing post symptom onset.

		VIROTECH SARS-CoV-2 IgA ELISA			
Days post symptom onset	**n**	**Positive**	**Equivocal**	**Negative**	**Sensitivity***
Day 0-5	13	1	0	12	7,7%
Day 6-8	14	7	0	7	50,0%
Day 9-11	17	11	0	6	64,7%
Day >=12	17	13	0	4	76,5%

*Equivocal counted as negative

4. VIROTECH SARS-CoV-2 IgG ELISA + VIROTECH SARS-CoV-2 IgA ELISA

A sensitivity study was performed with 61 blood samples from 42 patients hospitalized for COVID-19 related symptoms and tested positive for SARS-CoV-2 by RT-PCR testing were tested on the VIROTECH SARS-CoV-2 IgG ELISA and the VIROTECH SARS-CoV-2 IgA ELISA. To determine the diagnostic sensitivity, the samples were sorted by the timing post symptom onset.

Identical result for low & high prevalence cutoff (IgG ELISA)

		VIROTECH SARS-CoV-2 IgA ELISA + VIROTECH SARS-CoV-2 IgG ELISA			
Days post symptom onset	**n**	**Positive**	**Equivocal**	**Negative**	**Sensitivity***
Day 0-5	13	1	0	12	7,7%
Day 6-8	14	7	0	7	50,0%
Day 9-11	17	12	0	5	70,6%
Day >=12	17	17	0	0	100,0%

*Equivocal counted as negative

5. VIROTECH SARS-CoV-2 IgG ELISA + VIROTECH SARS-CoV-2 IgM ELISA

A sensitivity study was performed with 61 blood samples from 42 patients hospitalized for COVID-19 related symptoms and tested positive for SARS-CoV-2 by RT-PCR testing were tested on the VIROTECH SARS-CoV-2 IgG ELISA and the VIROTECH SARS-CoV-2 IgM ELISA. To determine the diagnostic sensitivity, the samples were sorted by the timing post symptom onset.

Identical result for low & high prevalence Cut off (IgG ELISA)

		VIROTECH SARS-CoV-2 IgA ELISA + VIROTECH SARS-CoV-2 IgG ELISA			
Days post symptom onset	**n**	**Positive**	**Equivocal**	**Negative**	**Sensitivity***
Day 0-5	13	1	0	12	7,7%
Day 6-8	14	8	0	6	57,1%
Day 9-11	17	10	0	7	58,8%
Day >=12	17	17	0	0	100,0%

*Equivocal counted as negative

12.2.2 Diagnostic Specificity

The diagnostic specificity VIROTECH SARS-CoV-2 IgG ELISA was determined by testing 399 samples from asymptomatic individuals without a history of SARS-CoV-2 infection either confirmed by a negative PCR result or based on the time of sampling before emergence of SARS-CoV-2. In addition, 41 samples from individuals without symptoms or history of SARS-CoV-2 infection that were sampled in Mai 2020 in Germany were tested.

Low prevalence Cut off

Sample panel	n	Positive on VIROTECH SARS-CoV-2 IgG ELISA	Specificity*			
Blood donors US (sampled before 2019)	138	0	100%			
Blood donors Germany (sampled before 2019)	118	0	100%			
Confirmed SARS-CoV-2 PCR negative patients, routine medical check-up (sampled after March 2020)	93	0	100%			
Children (Germany, sampled before 2019, gastrointestinal complaints)	50	0	100%			
Healthy patients, routine check-up (Mai 2020)	41	0	100%	**95% confidence interval**		
Total	**440**	**0**	**100%**	**99,1%**	**to**	**100%**

*Equivocal counted as negative

High prevalence Cut off

Sample panel	n	Positive on VIROTECH SARS-CoV-2 IgG ELISA	Specificity*			
Blood donors US (sampled before 2019)	138	8	94,2%			
Blood donors Germany (sampled before 2019)	118	1	99,2%			
Confirmed SARS-CoV-2 PCR negative patients, routine medical check-up (sampled after March 2020)	93	0	100,0%			
Children (Germany, sampled before 2019, gastrointestinal complaints)	50	0	100,0%			
Healthy patients, routine check-up (Mai 2020)	41	0	100,0%	**95% confidence interval**		
Total	**440**	**9**	**98,0%**	**96,2%**	**to**	**98,9%**
Total (without US)	**302**	**1**	**99,7%**	**98%**	**to**	**100%**

*Equivocal counted as negative

2. VIROTECH SARS-CoV-2 IgM ELISA

The diagnostic specificity VIROTECH SARS-CoV-2 IgM ELISA was determined by testing 125 samples from asymptomatic individuals from the US and Germany. Sample were taken before the emergence of SARS-CoV-2.

Sample panel	n	Positive on VIROTECH SARS-CoV-2 IgM ELISA	Specificity*			
Blood donors US (before 2019)	50	0	100%			
Blood donors Germany (before 2019)	75	0	100%	**95% confidence interval**		
Total	**125**	**0**	**100%**	**97%**	**to**	**100%**

*Equivocal counted as negative

3. VIROTECH SARS-CoV-2 IgA ELISA

The diagnostic specificity VIROTECH SARS-CoV-2 IgA ELISA was determined by testing 125 samples from asymptomatic individuals from the US and Germany. Sample were taken before the emergence of SARS-CoV-2.

Sample panel	n	Positive on VIROTECH SARS-CoV-2 IgA ELISA	Specificity*			
Blood donors US (before 2019)	50	0	100%			
Blood donors Germany (before 2019)	75	0	100%	**95% confidence interval**		
Total	**125**	**0**	**100%**	**97%**	**to**	**100%**

*Equivocal counted as negative

12.2.3 Comparison of methods

N/A

12.2.4 Prevalence

No exact information on the prevalence of SARS-CoV-2 is possible in the current situation and is rapidly varying over time and from region to region.

13. Literature

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