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COVID-19 (SARS-CoV-2) IgG ELISA



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 **96**



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CONTENTS

1. INTENDED USE 3

2. PRINCIPLE OF THE ASSAY 3

3. MATERIALS 3

4. STABILITY AND STORAGE 4

5. REAGENT PREPARATION 4

6. SAMPLE COLLECTION AND PREPARATION 4

7. ASSAY PROCEDURE..... 5

8. RESULTS 6

9. SPECIFIC PERFORMANCE CHARACTERISTICS 6

10. LIMITATIONS OF THE PROCEDURE 8

11. PRECAUTIONS AND WARNINGS 9

ABBREVIATIONS..... 10

SYMBOLS USED WITH DEMEDITEC ASSAYS 12

1. INTENDED USE

The COVID-19 (SARS-CoV-2) IgG ELISA is intended for the qualitative determination of IgG class antibodies against SARS-CoV-2 in human serum or plasma (citrate, heparin) to support the diagnosis of COVID-19 disease and constitutes a supplement to direct pathogen detection. In addition, serology can be used to collect epidemiological information on the prevalence of SARS-CoV-2.

2. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

3. MATERIALS

3.1. Reagents supplied

- SORB** **MT** **Microtiterplate**: 12 break-apart 8-well snap-off strips coated with SARS-CoV-2 nucleocapsid antigens; in resealable aluminium foil.
- SAM** **DIL** **IgG Sample Dilution Buffer**: 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
- STOP** **SOLN** **Stop Solution**: 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- WASH** **SOLN** **20x** **Washing Buffer (20x conc.)**: 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- ENZ** **CONJ** **Conjugate**: 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- SUB** **TMB** **TMB Substrate Solution**: 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- CAL** **C** **Positive Control**: 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02 % (v/v) MIT.
- CAL** **B** **Cut-off Control**: 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02 % (v/v) MIT.
- CAL** **A** **Negative Control**: 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015 % (v/v) CMIT/ MIT (3:1).

.As the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human), NIBSC code: 20/136, of the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK, does not yet provide an established cut-off for a qualitative result in a nucleocapsid based assay, Controls are currently adjusted using internal predefined quality control specimens.

For hazard and precautionary statements see 11.1

3.2. Materials supplied

- 1 Cover foil
- 1 Instructions for use (IFU)

3.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

4. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them before starting the test run!

5.1. Microtiterplate

The break-apart snap-off strips are coated with SARS-CoV-2 antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

5.2. WASH SOLN 20x

Dilute WASH SOLN 20x 1 + 19; e. g. 10 mL WASH SOLN 20x + 190 mL distilled water. The diluted buffer (WASH SOLN 1x) is stable for 5 days at room temperature (20...25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

5.3. SUB TMB

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. SUB TMB should be colourless or could have a slight blue tinge. If SUB TMB turns into blue, it may have become contaminated and should be thrown away.

6. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with SAM DIL. Dispense 10 µL sample and 1 mL SAM DIL into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

7. ASSAY PROCEDURE

Please read the instructions for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instructions for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of **WASH SOLN 1x** from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 11. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour \pm 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of **WASH SOLN 1x**. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL **SUB TMB** into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL **STOP SOLN** into all wells in the same order and at the same rate as for the **SUB TMB**, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the **STOP SOLN**.

7.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample. Bichromatic measurement using a reference wavelength of 620 nm is recommended. Where applicable calculate the **mean absorbance values** of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

In order for an assay run to be considered valid, these instructions for use have to be strictly followed and the following criteria must be met:

- **Substrate Blank:** Absorbance value < **0.100**
- **Negative Control:** Absorbance value < **0.200** and < **Cut-off**
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value > **Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

8.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

8.2.1. Results in Units [U]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Units} = \text{U}]$$

Example: $\frac{1.591 \times 10}{0.43} = 37 \text{ U}$

8.3. Interpretation of Results

Cut-off	10 U	-
Positive	> 11 U	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 U	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
Negative	< 9 U	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

8.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgG	Follows IgM production Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

9. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

9.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.815	4.06
#2	24	0.782	4.28
#3	24	0.362	8.71
Interassay	n	Mean (U)	CV (%)
#1	12	16.43	7.37
#2	12	12.76	4.11
#3	12	7.05	8.65

9.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. SARS-CoV-2 infections emerged in December 2019 in Wuhan, China. The expected prevalence values for German and US blood donor panels from before December 2019 therefore amount to 0 %. Additional panels were obtained before the outbreak of the pandemic from Scandinavian stroke patients (age >70 years) and from Scandinavian healthy patients (without previous and current symptoms) early during the pandemic.

The determined positive results correspond to a specificity of 99.5 % (95 %-confidence interval: 98.21 % - 99.94 %).

Sample Panel	Number of samples (n)	Positive	Equivocal	Negative	Specificity (Eqv. excluded)	95 % CI
Blood donors Germany	83	0	1	82	100 %	
Blood donors US	50	1	0	49	98.0 %	
Stroke patients	90	1	0	89	98.9 %	
Healthy Scandinavians	178	0	0	178	100 %	
Total	401	2	1	398	99.5 %	98.21 % - 99.94 %

9.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

42 samples from 25 patients tested positive for SARS-CoV-2 RNA by RT-PCR were tested.

Days post symptom onset	Number of samples (n)	Positive	Equivocal	Negative	Sensitivity (Eqv excluded)
0-5	13	1	0	12	7.69 %
6-8	10	4	0	6	40.00 %
9-11	10	4	0	6	40.00 %
≥ 12	9	9	0	0	100 %

Only with increasing duration of infection the antibody production starts to rise to a detectable level. Individually, this can vary from a few days up to 2 weeks. At the beginning of an infection a negative test result is therefore not a criterion for exclusion of an acute SARS-CoV-2 infection.

9.4. Interferences

Three clinical samples exhibiting differing reactivities were tested for interference with each substance listed in the Table below: a positive, a negative, and an equivocal sample. All samples exhibited a change of signal less than 15 % when tested with each potential interferant.

Interferant	Concentration tested
Albumin	60 mg/mL
Bilirubin conjugated	0.4 mg/mL
Bilirubin unconjugated	0.4 mg/mL
Cholesterol	4 mg/mL
Hemoglobin	10 mg/mL
Triglycerides	15 mg/mL

9.5. Cross Reactivity

131 samples with antibody activities to potentially cross reacting parameters (including antibodies to several respiratory pathogens) were tested to evaluate the cross reactivity of the assay.

Samples positive for antibodies to	Number of samples (n)	Positive	Equivocal	Negative
Adenovirus	10	0	0	10
Parainfluenzavirus	9	0	0	9
Candida albicans	8	0	0	8
Bordetella pertussis	9	0	0	9
Influenzavirus A	9	0	0	9
Influenzavirus B	10	0	0	10
Enterovirus	10	0	0	10
Respiratory syncytial virus	10	1	0	9
Chlamydia pneumoniae	9	0	0	9
Legionella pneumophila	8	0	0	8
Mycoplasma pneumoniae	9	0	0	9
Haemophilus influenzae	3	0	0	3
Coronavirus, other than SARS-CoV-2	1	0	0	1
Rheumatoid Factor positive	26	0	0	26

Cross reactions with antibodies to respiratory syncytial virus cannot be excluded. Cross reactivity with other human coronaviruses should be considered for result interpretation.

10. LIMITATIONS OF THE PROCEDURE


Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS


- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control, each laboratory should use additional known samples that comply with applicable national standards/laws.

11.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 3.1).
Therefore, the following hazard and precautionary statements apply.

	Warning	H317	May cause an allergic skin reaction.
		P261	Avoid breathing spray
		P280	Wear protective gloves/protective clothing.
		P302+P352	IF ON SKIN: Wash with plenty of soap and water.
		P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
		P362+P364	Take off contaminated clothing and wash it before reuse.

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to 3.1).
Therefore, the following hazard and precautionary statements apply.

	Warning	H315	Causes skin irritation.
		H319	Causes serious eye irritation
		P280	Wear protective gloves/ protective clothing.
		P302+P352	IF ON SKIN: Wash with plenty of soap and water.
		P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
		P337+P313	If eye irritation persists: Get medical advice/attention.

Further information can be found in the safety data sheet.

11.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

ABBREVIATIONS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one














SUMMARY OF TEST PROCEDURE**SCHEME OF THE ASSAY**
COVID-19 (SARS-CoV-2) IgG**Test Preparation**

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls. Select the required number of Microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit Incubate for 1 h at 37±1 °C Wash each well three times with 300 µL of WASH SOLN 1x					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of WASH SOLN 1x					
SUB TMB	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
STOP SOLN	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta y advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta