Anti-SARS-CoV-2 ELISA

Enzyme Immunoassay for Qualitative Detection of Antibodies against

SARS-CoV-2-S1 (RBD)

English

Europäische Union / European Union,
für In-vitro-Diagnostik / For In Vitro Diagnostic Use
IVD zum Gebrauch durch Fachpersonal / IVD for professional use!

Alle anderen Länder / All other countries:
Nur für Forschungszwecke. Nicht für diagnostische Anwendungen geeignet.
For Research Use Only. Not for use in diagnostic procedures.

CE IVD +2 +8 °C Σ 96 wells

REF **E111-IVD**

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1. INTENDED USE

Mediagnost Anti-SARS-CoV-2 ELISA E111-IVD is a highly specific enzyme immunoassay for the detection of IgG antibodies directed against SARS-CoV-2-S1 Receptor Binding Domain (RBD) in human blood.

The Anti-SARS-CoV-2 ELISA E111-IVD is For In Vitro Diagnostic Use.

CE Registration Code: DE/CA40/00809/30

The product is intended for use by professional persons only.

2. INTRODUCTION

In December 2019 a novel coronavirus SARS-CoV-2 was identified in Wuhan, China and was announced as the causative agent for COVID-19 disease. The incubation period of the disease is 2-14 days. Symptoms of COVID-19 include fever, fatigue and cough, shortness of breath, muscle pain and tiredness. Most of the patients have a good prognosis, some of severe cases may have pneumonia, severe acute respiratory distress or even succumb to the disease.

The entry process of SARS-CoV-2 to the host cell is mediated by the envelope-embedded surface-located spike glycoprotein S. The protein is cleaved by host proteases into the S1 and S2 subunits, which are responsible for receptor recognition and membrane fusion respectively[1].

As target antigen of the assay the recombinant Receptor Binding Domain (RBD) of SARS-CoV-2 S1 spike protein, which binds the ACE2 receptor, is used. The use of RBD increases the specificity of the assay since the domain is identical with SARS-CoV but not with MERS-CoV for example. Antibodies directed against the RBD neutralize both virus strains SARS-CoV and SARS-CoV-2[2].

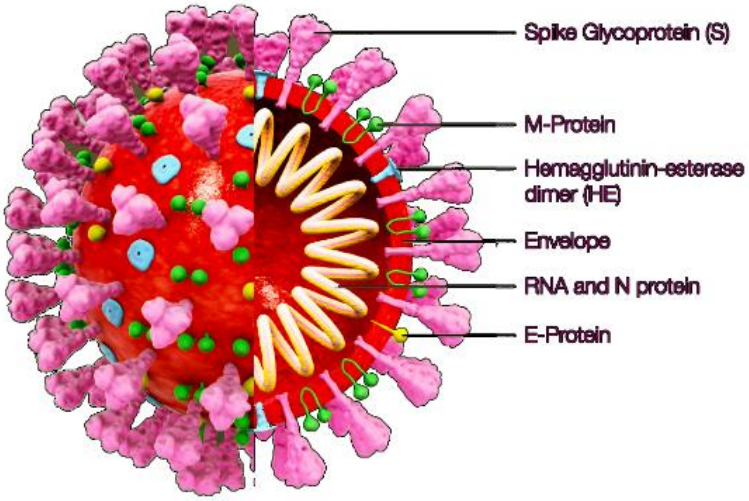


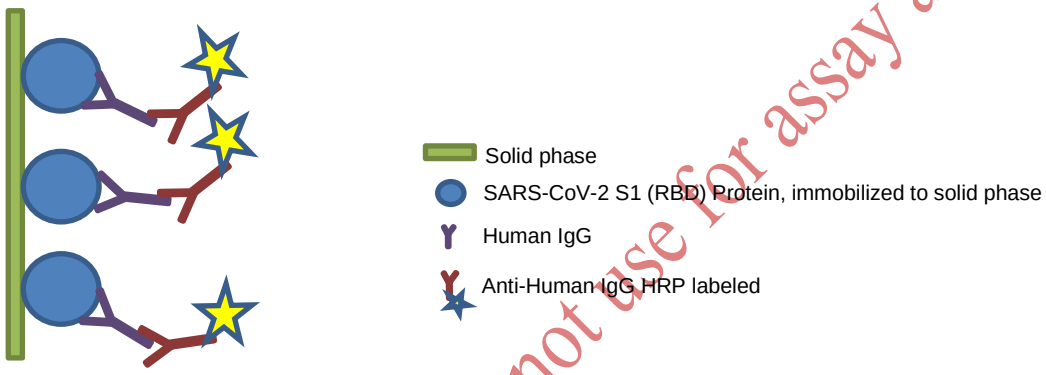
Fig.1: Structural features of Coronavirus[3]

Up to now there are seven coronaviruses described which can infect humans. Two of them are alpha coronaviruses (229E, NL63) and five are beta coronaviruses (OC43, HKU1, MERS-CoV, SARS-CoV, SARS-CoV-2)[4].

[1]Wang et al., Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2, Cell (2020), https://doi.org/10.1016/j.cell.2020.03.045
[2]Tai et al. https://doi.org/10.1038/s41423-020-0400-4
[3]Jean-Yves Sgro (2020): https://static-bcrf.biochem.wisc.edu/tutorials/booklets/SARS-COV-2_COVID-19_A_Coloring_Book-v1.0.pdf
[4]CDC: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases

3. ASSAY PRINCIPLE

Mediagnost Anti-SARS-CoV-2 ELISA E111-IVD is a two-step enzyme-linked immunosorbent assay. Wells of a 96-well microtiter plate are coated with recombinant SARS-CoV-2-S1 Receptor Binding Domain (RBD). After addition of human serum or plasma samples anti-S1 IgG antibodies from the sample bind to the immobilized antigen during a two hours incubation followed by several washing steps in order to remove unbound components. The bound anti-SARS-CoV-2 IgG antibodies are detected by incubation with HRP conjugated anti-human IgG for 30 minutes. Subsequently, a HRP substrate solution containing 3,3',5,5'-Tetramethylbenzidine (TMB) is added resulting in the formation of a blue colour. The reaction is terminated by the addition of 0.2 M H_2SO_4 changing the blue colour into yellow signals which are measured by an absorbance microtiter plate reader at 450 nm. The extinction increases with the amount of the captured antibodies directed against SARS-CoV-2-S1 (RBD) from the patient's sera.



4. KIT COMPONENTS

MTP	Microtiter Plate, coated with SARS-CoV-2 S1(RBD) protein, wells are separately breakable, ready for use	8 x 12 wells
DET	Antibody-HRP-Conjugate, goat anti-human IgG-antibody, ready for use	14 mL
PC	Positive Control: anti-SARS-CoV-2 positive control serum, human serum, ready for use	1 mL
NC	Negative Control: anti-SARS-CoV-2 negative control serum, human serum, ready for use	1 mL
DIL	Dilution Buffer, ready for use	100 mL
WP	Washing Buffer, 20-fold concentrated solution	50 mL
S	Substrate Solution (TMB), HRP substrate, ready for use	14 mL
SL	Stopping Solution, 0.2 M H_2SO_4, ready for use	14 mL
	Sealing Tape for covering microtiter plate	2
	Instructions for use	1

5. MATERIALS REQUIRED BUT NOT PROVIDED

Aqua dest. or deionized water for dilution of Washing Buffer WP
Precision pipettes with disposable plastic tips
Microtubes, buffer and reagent reservoirs
Incubator 37°C
Vortex-Mixer
Microtiter plate washer, alternatively manual washing
Microtiter plate reader capable of reading absorbency of 450 nm (reference filter ≥ 590 nm)

6. SAMPLES

Serum, EDTA- and Heparin Plasma
Multiple freeze-thaw cycles should be avoided.
The use of hemolytic, lipemic or icteric samples should be validated by the user.

7. STORAGE and STABILITY

Upon receipt store the kit at 2-8°C.
The shelf life of the components **after initial opening** is warranted for **4 weeks**, store the unused strips and microtiter wells **airtight** together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The 1:20 diluted Washing Buffer **WP** is stable at 2-8°C for 4 weeks.

8. PREPARATION of REAGENTS

Before use bring all reagents to room temperature 20-25°C.

8.1. Washing Buffer WP

Dilute WP 1:20 in Aqua dest., i.e. 50 mL WP + 950 mL Aqua dest.

8.2. Samples

Dilute samples 1:201 in Dilution Buffer DIL, e.g. 5 µL Sample + 1 mL DIL.

8 diluted samples were stored 3 h and 15 h at +4°C, at room temperature (20 -25°C) or frozen at -20°C: No influence on absorbance values was shown in comparison to the values measured directly after the dilution.

9. ASSAY PROCEDURE

Step 1	Addition of controls and samples
	Add 100 µL of each Blank, Positive Control (PC), Negative Control (NC) Blanks in double determination. Positive and Negative Controls in triple determination. Samples either in single or double determination (double as a general recommendation to indicate certain faults in individual assay runs) as indicated in the following pipetting scheme.

Pipetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S1										
B	Blank	S1										
C	PC	S2										
D	PC	S2										
E	PC	S3										
F	NC	S3										
G	NC	S4										
H	NC	S4										

Add 100 µL Dilution Buffer DIL as blank in wells A1 and B1
Add 100 µL Positive Control PC in wells C1, D1, and E1
Add 100 µL Negative Control NC in wells F1, G1 and H1
Add 100 µL of sample 1 (S1) diluted 1:201 in DIL in wells A2 and B2
Add 100 µL of sample 2 (S2) diluted 1:201 in DIL in wells C2 and D2

and so on.

Step 2	Incubation
	Cover the plate with sealing tape and incubate for 2 h at 37°C
Step 3	**Washing**
	Remove the sealing tape from the plate and aspirate the contents of the wells. Wash 3 x with 300 µL Washing Buffer WP per well
Step 4	**Addition of Conjugate and Incubation**
	Add 100 µL Antibody-HRP-Conjugate DET to each well, cover the plate with sealing tape and incubate for 30 min at 37°C
Step 5	**Washing**
	Remove the sealing tape from the plate and aspirate the contents of the wells. Wash 3 x with 300 µL Washing Buffer WP per well
Step 6	**Addition of Substrate and Incubation**
	Add 100 µL of Substrate Solution S to each well and incubate 10 min at 20-25°C in the dark
Step 7	**Addition of Stop Solution**
	Add 100 µL Stop Solution SL to each well
Step 8	**Measurement**
	Measure the absorbance within 30 min at 450 nm (reference filter ≥ 590 nm)
Step 9	**Evaluation of results**
	The test is valid if a P/N ratio of >5 is achieved
Step 10	**Cut-off determination**
	The cut-off is calculated 3 x and 5 x mean values of negative controls.
Step 11	**Interpretation of results**
	Values under 3 x cut-off are negative, Values above 5 x cut-off are positive Values in between both cut-offs are borderline.

10. TYPICAL RESULTS

Sample	OD 450 - ≥590 nm mean	Interpretation of results
Blank	0.008	should not exceed values of the Negative Control
Positive Control	3.007	positive
Negative Control	0.166	negative
Sample 1	2.806	positive
Sample 2	0.117	negative
Sample 3	0.586	borderline

EXAMPLE: CALCULATION P/N RATIO

$$\text{P/N Ratio:} \quad \frac{\text{mean Positive Control } 3.007}{\text{mean Negative Control } 0.166} = 18.11$$

EXAMPLE: CALCULATION CUT-OFF
cut-off: 3 x mean negative control = 0.498
cut-off: 5 x mean negative control = 0.830

The values shown in the table above result in the cut-off (3x) of 0.498 and cut-off (5x) value of 0.830.

Samples > 5 x cut-off
All samples of which signals are higher than OD 0.830 are positive, i.e. contain anti-SARS-CoV-2-S1 (RBD) antibodies.

Samples < 3 x cut-off
All samples of which signals are lower than OD 0.498 are negative, i.e. anti-SARS-CoV-2-S1 (RBD) antibodies are not detectable in the sample.

Samples in between 3 x cut-off and 5 x cut-off
All samples which show OD values in between are borderline. Since IgG antibodies to SARS-CoV-2 generally become detectable beginning 10-14 days following infection the borderline samples may indicate the beginning of seroconversion, i.e. the patient is possibly developing antibodies. Therefore, it is strongly recommended to repeat sample drawing and testing around 14 days after the first sample drawing.

Interpretation of results
The results should be interpreted in regard to anamnesis, further clinical observations and results of other diagnostic investigations.
Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.
Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E, or other courses.

11. LIMITATION OF PROCEDURE
The influence of the heterophilic antibodies, rheumatoid factors and anti-species antibodies is reduced, but cannot be completely excluded. Rheumatoid factors were inconspicuously up to 600 International Units/mL (measured concentrations between 10 to 600 IU/mL).

12. PERFORMANCE EVALUATION

Diagnostic Specificity and Sensitivity

	Anti-SARS-CoV-2 ELISA E111-IVD				
Serum samples of persons with positive PCR results and / or Covid-19 disease	**n**	**pos**	**border-line**	**neg**	**Sensitivity**
0 - 12 d*	36	23	2	11	69.4%
13 - 21 d*	42	34	4	4	90.5%
≥ 22 d*	85	70	11	4	95.3%
					Specificity
Blood donors	502	1**	6	495	98,6%
total	665	128	23	514	
Accuracy (no of correct assessments : 645) / (no. of all assessments 665) = 96,99%					

*days after onset of symptoms or positive PCR results.
**The blood donor sample derived from a pneumonia patient with heavy ARDS symptoms but with a negative PCR result for SARS-CoV-2.

To date there are no reference standard SARS-CoV-2 antigen or anti-SARS-CoV-2 antibodies available, accordingly absolute analytical sensitivity cannot be calculated.

Figure 3 shows the time course of antibody development of three clinically ill COVID-19 patients. The presence of IgG antibodies, following previously negative testing (days 10-13 after onset of symptoms) become detectable beginning days 17-20 and are increasing up to days 37-40 proving that the anti-SARS-CoV-2 ELISA E111-IVD is qualified to detect the entire existing antibody concentration range, even low levels at the onset of an immune response can be detected.

Analytical Sensitivity
Analytical Sensitivity defined as mean OD of the Negative Control NC plus 2 times the respective standard deviation. Analytical Sensitivity was here calculated by use of **Inter Assay measurements** of 6 different lots.

Mean Negative Control NC + 2SD: **0.224** = corresponds to Analytical Sensitivity of E111-IVD

Sample	Number of determinations	450 - ≥590 nm Mean value OD	Standard deviation OD	VC (%)	Analytical Sensitivity: Mean NC + 2SD OD
NC	26	0.144	0.04	28	0.224
(3fold NC):	26	0.433	Theoretical cut off Negative/ Borderline		
(5fold NC):	26	0.722	Theoretical cut off Borderline/ Positive		

Analytical Specificity
Up to now no serologically unique strains of SARS-CoV-2 have been described relative to the originally isolated virus.

Cross-reactivity of non-SARS-CoV-2 specific antibodies against SARS-CoV-2-S1 RBD protein in Anti-SARS-CoV-2 ELISA E111-IVD was examined using sera with known antibodies against confirmed past infections.

Antibody positive sera	Number of determinations	Anti-SARS-CoV-2 ELISA E111-IVD
Beta Corona HKU1*	1	Negative
SARS-CoV**	1	Negative
VZV	4	Negative
HCV	5	Negative
HAV	4	Negative
HBV	3	Negative
EBV	4	Negative
CMV	5	Negative
HSV	5	Negative

*The patient was tested PCR positive for Beta Corona HKU1 and PCR negative for SARS-CoV-2. Four weeks after PCR testing a serum sample was drawn from the patient and found to be negative in the Anti-SARS-CoV-2 ELISA E111-IVD.
**The patient was SARS-CoV infected in 2009.

Precision

Intra Assay Variance

3 Serum samples were measured 10-fold within one assay.
Mean variance was < 10%.

	Number of determinations	Mean value OD	Standard deviation OD	VC (%)
Sample 1	10	0.346	0.025	7.3
Sample 2	10	0.939	0.038	4.1
Sample 3	10	2.257	0.066	2.9

Inter Assay Variance

6 Serum samples were measured in independent assays, in 3 different lots.

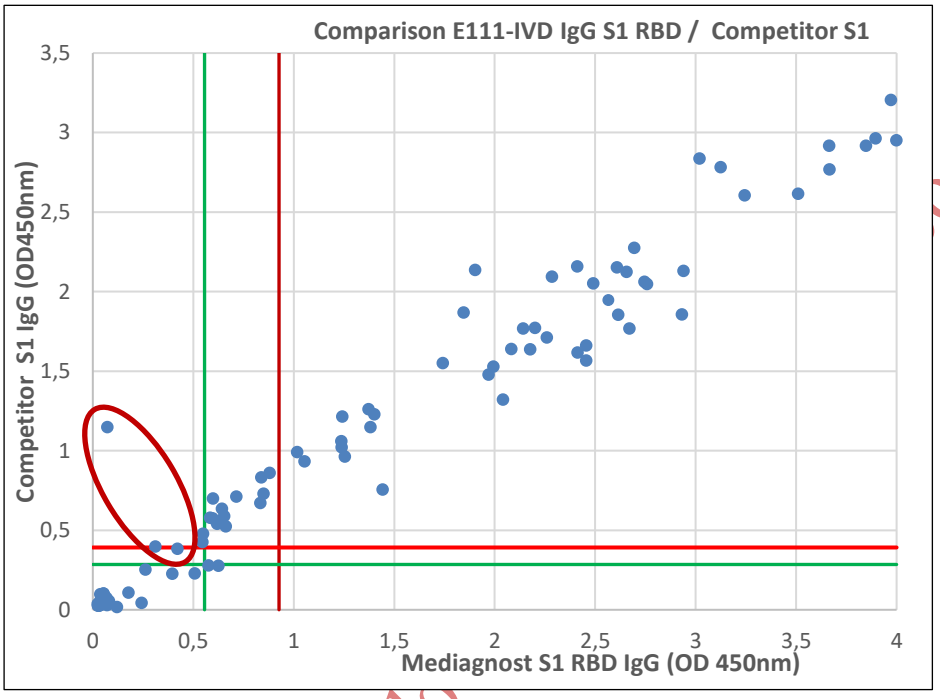
	Number of determinations	Mean Sample OD/PC OD	Standard deviation	VC (%)
Sample 1	9	1.05	0.0382	3.6
Sample 2	10	0.32	0.0369	11.7
Sample 3	10	0.74	0.0409	5.5
Sample 4	8	0.22	0.0254	11.7
Sample 5	10	0.21	0.0215	10.3
Sample 6	6	0.12	0.0141	11.6

Assay Comparison

Mediagnost E111-IVD is, in comparison with competitor assays, well suited to detect anti- SARS-CoV-2 IgG antibodies.
The chosen virus antigen S1 RBD, which serves as binding target for the antibodies, the S1 Receptor Binding Domain, seems to be superior to the complete Spike protein S1 or the Nucleoprotein NCP, both generating higher rates of false results as measured with the competitor assays.
Assays were performed and results calculated according to the manufacturer instructions.

In the figure, the respective **kit run specific cut offs**, with which the classification was carried out, are indicated either with a **red or green line**, respectively. For each assay, measured Optical Density (OD) values above the red line were judged as **positive**, below the green line as **negative**, and falling in between both line as **borderline**.

Fig. 2: Comparison of Mediagnost E111-IVD (uses the Receptor Binding Domain of Spike protein S1) with competitor anti-SARS-CoV-2 antibody IgG ELISA S1 (kit uses the complete Spike protein S1 of SARS-CoV-2 virus as antigen).



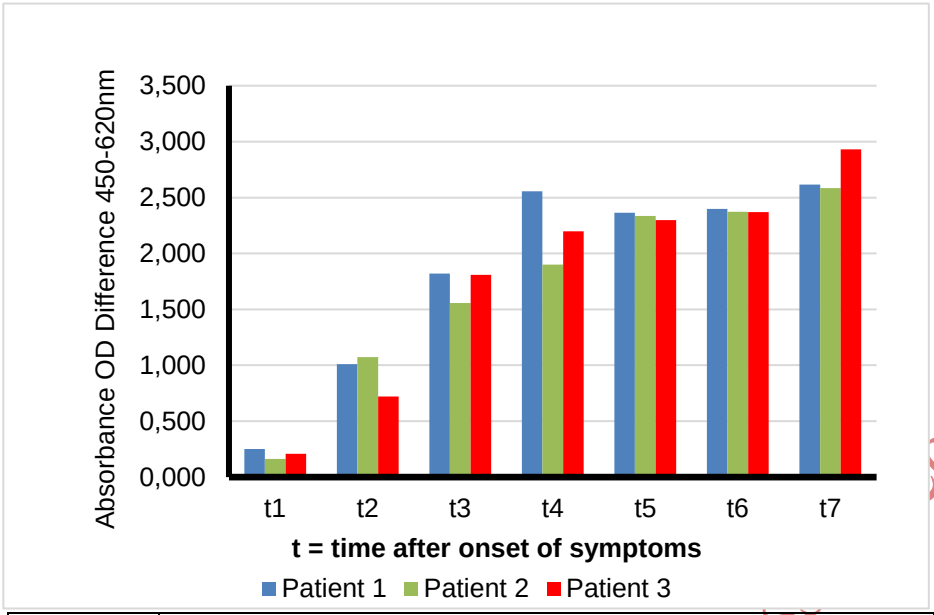
The correlation between both assays is relatively strong. Obvious is the higher degree of false positive samples with the competitor S1 assay, 3 instead of only one borderline with E111-IVD (marked with a red circle in the figure). The high false positive sample with competitor S1 for instance is a 4-year-old child with HHV 7 infection, however no SARS-CoV-2.

Samples

The influences of anti-coagulants on measurements were investigated in 6 corresponding serum, EDTA and Heparin plasma samples. In comparison to the ODs of the serum samples recovery was measured for Heparin and EDTA plasma samples on average 97.8% and 102.0 %, respectively.

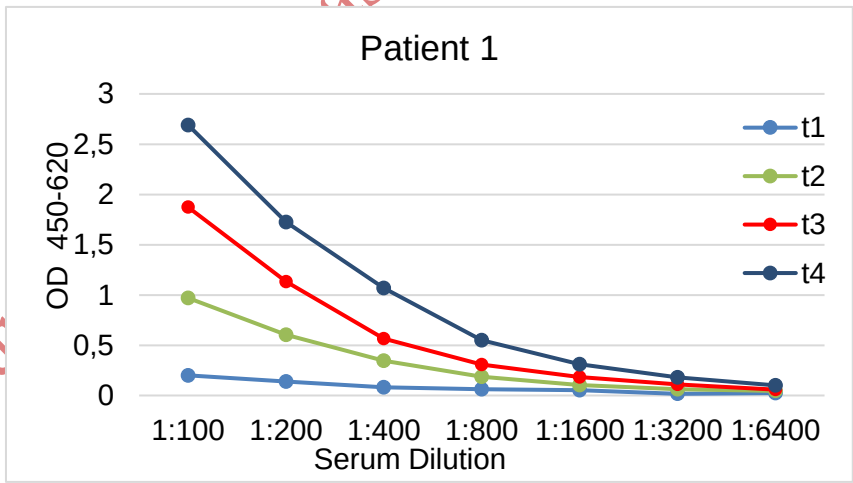
	Serum OD	**%**	**EDTA-Plasma OD**	**%**	**Heparin-Plasma OD**	**%**
Sample 1	3.601	100.0	3.677	102.1	3.496	97.1
Sample 2	0.410	100.0	0.385	93.9	0.389	94.9
Sample 3	2.946	100.0	2.918	99.0	3.084	104.7
Sample 4	0.501	100.0	0.458	91.4	0.517	103.2
Sample 5	1.242	100.0	1.218	98.1	1.284	103.4
Sample 6	0.129	100.0	0.132	102.3	0.14	108.5
mean	-	-	-	**97.8**	-	**102.0**

Fig. 3: Time course of IgG-antibody development against SARS-CoV-2-S1 (RBD) protein of three clinically ill patients



	Days after onset of symptoms						
Sampling at day	**t 1**	**t 2**	**t 3**	**t 4**	**t 5**	**t 6**	**t 7**
Patient 1	13	20	25	33	40	48	60
Patient 2	10	17	22	30	37	45	57
Patient 3	11	18	23	31	38s	46	58

Fig. 4: Titration of sera drawn from patient 1 at day 13 (t1), day 20 (t2), day 25 (t3) and day 33 (t4) after onset of symptoms.



13. PROTOCOL

Protocol at a glance

Add 100 µL of controls (2 x blank, 3 x PC, 3 x NC) in A1 to H1 wells

↓

Add 100 µL of samples 1:201 diluted in DIL in wells starting with A2

↓

Incubate at 37°C for 2 hours

↓

Aspirate well contents and wash each well 3 x with 300 µL 1 x WP

↓

Add 100 µL DET to each well

↓

Incubate at 37°C for 30 min

↓

Aspirate well contents and wash each well 3 x with 300 µL 1 x WP

↓

Add 100 µL S to each well

↓

Incubate for 10 min at 20-25°C in the dark

↓

Add 100 µL SL to each well

↓

Measure absorbance at 450 - ≥ 590 nm within 30 min

↓

Calculation and Interpretation

14. WARNINGS AND PRECAUTIONS

For in-vitro use only. For Professional use only. For In Vitro Diagnostic Use.

The Mediagnost kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Mediagnost will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore, all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents and samples. Follow the general practice of serology for sample storage and collection. The disposal of the kit components must be made according to the local regulations.

The test plate MTP is coated with recombinant Antigen.

Human Serum

Following components contain human material: **PC, NC**

Source human serum for the Control Sera provided in this kit was tested by recommended methods and found negative for Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known test methods can offer total assurance of the absence of infectious agents; therefore, all components and patient's specimens should be treated as potentially infectious.

Reagents: NC, PC, DIL, DET, WP

Contain as preservative a mixture of **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

Substrate Solution (S)

The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

Stopping Solution (SL)

The Stopping solution contains 0.2 M, Sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing, spread the eyelids.

Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

	Expiry Date	REF	Catalogue Number
	Consider instructions for use		Store at between
LOT	Lot-Batch Number	Σ	Contains sufficient for x tests
	Manufactured by		Keep away from sunlight!
HRP	Horseradish Peroxidase	POD	Peroxidase
IVD	In vitro Diagnostic Medical Device (for In Vitro Diagnostic Use)		