LABORATORY • DIAGNOSTICS

DRAFT

SARS-CoV-2 ViraChip® IgG Test Kit

Instructions for use

Emergency Use Authorization

Microarray based on an enzyme-immunoassay for the qualitative detection of **IgG** antibodies against specific **SARS-CoV-2** (*severe acute respiratory syndrome coronavirus 2*) antigens in human serum.

The **SARS-CoV-2 ViraChip® IgG Test Kit** uses the purified specific Spike antigens S1 and S2 and the Nucleocapsid antigen N of SARS-CoV-2 at defined positions on nitrocellulose.

The SARS-CoV-2 ViraChip® IgG Test Kit is manufactured according to the European guideline **98/79/EG** and a certified quality management system under MDSAP (Medical Device Single Audit Program including USA-specific requirements) only for use under Emergency Use Authorization.

Principle of the assay

A nitrocellulose membrane is fixed at the bottom of each well of a standard microtiter plate (MTP). The antigens are fixed on this membrane as analyte spots. The wells of these microarrays are single breakable and are stored in a holding frame with 96 positions. During the serum incubation step SARS-CoV-2-specific IgG antibodies bind to the immobilized antigens (spots) on the microarray. During the conjugate reaction, AP-conjugate binds to the antigen-antibody complex. The alkaline phosphatase converts the chromogen/substrate and thus, stains the antigen-antibody complex on the microarray purple. The washing procedures following serum, conjugate and chromogen/substrate incubation steps remove unbound antibodies and reagents.

In addition to the analyte spots, each microarray includes the following control spots: **serum controls**, **conjugate controls, calibrator controls** and a **negative control**. The analyte spots serve to detect antibodies against the SARS-CoV-2-specific antigens **S1, S2** and **N**.

For clear assignment, each well is coded by a color system: Therefore, the SARS-CoV-2 ViraChip® IgG is marked with a **full brown circle** on the rim of the well.

Order No.:	**V-COCGOK**	Order No.:	**V-COCGDK (Deca Kit)**
Kit size:	**1 MTP with 96 single breakable wells**	Kit size:	**10x 1 MTP with 96 single breakable wells**
Specimen:	**10 µl serum**	Specimen:	**10 µl serum**
Time for testing:	**approx. 130 minutes**	Time for testing:	**approx. 130 minutes**

Materials provided

1 or 10 MTP with 96 wells	**SARS-CoV-2 ViraChip® IgG Antigen Coated Wells** Single breakable wells with ViraChip® Microarrays, ready to use	(Prod. No.: V-COCGAC)
1x or 10x 12 ml	**ViraChip® AP-Anti-Human IgG Conjugate** Anti-human IgG Conjugate Solution for ViraChip® tests, from goat, with bovine alkaline phosphatase, ready-to-use	(Order No.: V-UVCGKI)
1x or 10x 100 ml	**ViraChip® / ViraStripe® / ViraBlot® Diluent / Wash Buffer** Wash Buffer Concentrate for ViraChip® tests, with detergents and salts to avoid unspecific binding, 10x	(Order No.: V-UVNUWP)
1x or 10x 12 ml	**ViraChip® Chromogen / Substrate Solution** Chromogen/Substrate Solution for ViraChip® tests, with BCIP/NBT, ready-to-use	(Order No.: V-UVCUCS)

Sample buffer required for sample dilution, is delivered separately

50 ml	**ViraChip® Sample Buffer** Sample Buffer for ViraChip® tests, with milk powder, detergents and salts to avoid unspecific binding, ready-to-use	(Order No.: V-UVCUPP)

Separately available

1 MTP strip with 8 wells	**SARS-CoV-2 ViraChip® IgG Antigen Coated Wells (8)** Single breakable wells with ViraChip® Microarrays, ready to use	(Order No.: V-COCGRT)
330 µl	**SARS-CoV-2 ViraChip® IgG Positive Control** Human, ready to use	(Order No.: V-COCGPK)
330 µl	**SARS-CoV-2 ViraChip® IgG,A,M Negative Control** IgG, IgA, IgM, human, ready to use	(Order No.: V-COCPNK)

Additionally required equipment

1. **ViraChip Software®**	for preparing, processing and evaluating the test run, from v1.3.0-1960	(Order No.: V-VCNUPR)
2. **2D-Barcode Scanner**	for reading of DataMatrix-Codes	
3. **Microtiter plate** with 96 empty wells	for completing partial strips of the microtiter plate in the holding frame	(Order No.: V-UVNMTP)
4. **Orbital shaker (750 rpm)** or **linear shaker (20 Hz)**	for mixing samples and reagents during processing	
5. **ViraChip® Scanner** or **ViraChip® Reader**	for digitalizing / measuring of developed ViraChip® tests, from ViraChip® Scanner v1.0 or ViraChip® Reader Rev.01	(Order No.: V-UVCSCA) (Order No.: V-UVCCAM)
6. Optional: **Fan**	for faster drying of the developed microtiter plates	

Preparation of reagents and patient samples

Bring all reagents and the packed microtiter plate to room temperature (RT: 20-23°C) prior to use. Mix all reagents thoroughly before use.

Wash Buffer Working Dilution:	Dilute **Wash Buffer Concentrate 1:10** with distilled or deionized water: 100 ml concentrate + 900 ml H_20.
Sample Buffer:	Ready to use.
Conjugate Solution:	Ready to use.
Chromogen / Substrate Solution:	Ready to use.

VIRAMED BIOTECH AG • Behringstraße 11 • D-82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
E-Mail: viramed@viramed.de • www.viramed.de • certified according to ISO 13485

MTP wells:	Carefully unpack the microtiter plate (MTP) and place the required number of wells in an empty holding frame (see section “Preparation and Processing of the test run”, step 3). Use wells directly after removing from packing. Return unused wells directly into the original packing, seal accurately and store at 2-8°C.
Patient samples:	Use 100 µl of a **1:76** dilution of patient serum, e.g. **10 µl of patient serum** + **750 µl Sample Buffer**.
Controls:	Optionally, use 100 µl of a **1:16** dilution of control serum, e.g. **10 µl of control serum** + **150 µl Sample Buffer**.

Figure of the SARS-CoV-2 ViraChip® IgG Microarray

Antigens:
Each SARS-CoV-2-specific antigen, **S1, S2** and **N**, is spotted three times with the same concentration as spot triplet. Each spot triplet corresponds to one band on a Western Blot / immunoblot.

Controls:
Each ViraChip® well contains the following integrated controls:
Two serum controls (sc), one negative control (nc), two IgG conjugate controls (ccG), two IgA conjugate controls (ccA) and six calibrator controls (cal).

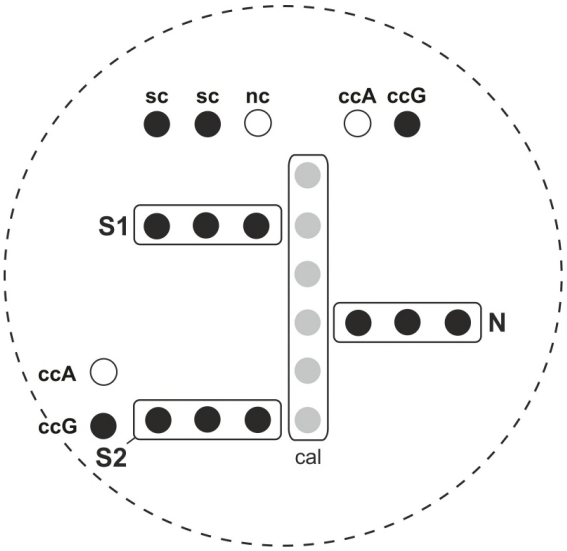

Figure 1: Schematic drawing of one well of the microtiter plate with the SARS-CoV-2 ViraChip® IgG Microarray (magnified). Spot layout for antigens and integrated controls.

Nomenclature and description of SARS-CoV-2 antigens from literature

Nomenclature:	Antigen:	Comment:
S1	Spike glycoprotein subunit S1	The S1 subunit of the SARS-CoV-2 spike protein contains the receptor binding domain (RBD), via which the virus binds to the surface of the host cell (1). Antibodies against S1 are considered to be specific and sensitive for SARS-CoV-2 (2).
S2	Spike glycoprotein subunit S2	The S2 subunit of the SARS-CoV-2 spike protein fuses host and virus membranes after the virus bound to the host cell, thereby allowing the virus genome to enter the host cell (1). The serological importance of antibodies against S2 has to be further studied.
N	Nucleocapsid protein	The nucleocapsid protein / nucleoprotein is necessary for the replication of coronaviruses. Through interactions with both the viral genome and with the membrane protein M, it is of fundamental importance in the assembly of the virion (3,5). Antibodies against the nucleocapsid protein have been described as specific and sensitive for SARS-CoV-2 (2).

VIRAMED BIOTECH AG • Behringstraße 11 • D-82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
E-Mail: viramed@viramed.de • www.viramed.de • certified according to ISO 13485

Preparation and Processing of the test run

Using automated processing, incubation times, volumes and the order of the work steps may differ from the procedure listed below. A detailed step-by-step guide for your individual automation solution and instructions for using the ViraChip® software will be provided during device set-up and training by Viramed Biotech AG. See also section "Notes on devices and software".

1. Assign
Test selection and transfer of the sample data in the assignment scheme of the ViraChip® Software.

2. Assemble
Scanning of the 2D bar codes from the label of the test kit and from the label of the microtiter plate to transfer the lot number and the lot specific factors.

3. Process
Perform all processing steps at room temperature (RT).

3.1 Preparation
- Place the number of wells as defined in the assignment into the holding frame.
- Fill free positions of a strip of the MTP in the holding frame with empty wells. The strips are labeled 1-12 on the microtiter plate.

Pay attention that no plastic particles fall into the wells while breaking the strips.

3.2 Preincubation
- Add 300 µl Wash Buffer Working Dilution to each well.
- Incubate by shaking for 5 minutes at RT.
- Aspirate liquid.

3.3 Serum incubation
- According to the assignment scheme add 100 µl of diluted patient serum or 100 µl of diluted control serum to the respective well.
- Incubate by shaking for 30 minutes at RT.
- Aspirate liquid.

3.4 3 x washing
- Add 300 µl Wash Buffer Working Dilution to each well.
- Incubate by shaking for 5 minutes at RT.
- Aspirate liquid.

3.5 Conjugate incubation
- Add 100 µl Conjugate Solution to each well.
- Incubate by shaking for 30 minutes at RT.
- Aspirate liquid.

Adjust aspiration needles or use pipettes in a way that the bottoms of the wells are not damaged.

The bottoms of the wells have to be covered completely with liquid during the incubation steps.

3.6 3 x washing
- Add 300 µl Wash Buffer Working Dilution to each well.
- Incubate by shaking for 5 minutes at RT.
- Aspirate liquid.

During the incubation and washing steps, use an orbital shaker with a shaking frequency of approx. 750 rpm or a linear shaker with a shaking frequency of approx. 20 Hz.

3.7 1 x washing
- Add 300 µl distilled or deionized water to each well.
- Incubate by shaking for 1 minutes at RT.
- Aspirate liquid.

3.8 Substrate incubation
- Add 100 µl Chromogen / Substrate Solution to each well.
- Incubate by shaking for 15 minutes at RT.
- Stop the reaction by aspirating the liquid.

3.9 3 x washing
- Add 300 µl Wash Buffer Working Dilution to each well.
- No incubation necessary.
- Aspirate liquid.

3.10 Dry wells.
Let the wells dry under continuous airflow for 20 minutes at 60 % humidity max.

At higher humidity levels, the drying time may be extended. Alternatively let wells dry protected from light for 12 hours at RT.

4. Scan
Measure ViraChip® Microarrays with the ViraChip® Scanner or with the ViraChip® Reader.

Measurement of spot intensities have to be performed within 24 hours after processing (store MTP in a dark place). Please refer to the device manual for a detailed procedure.

VIRAMED BIOTECH AG • Behringstraße 11 • D-82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
E-Mail: viramed@viramed.de • www.viramed.de • certified according to ISO 13485

5. Analyze

5.1 Verify the assignment of the antigen spot triplets and of the control spots (Technical validation)

Each spot is automatically assigned to the appropriate antigen spot triplet or to the appropriate control spot, based on its location, by the ViraChip® Software. The automatic assignment must be visually compared to the pattern in figure 1. Assignments that do not fit the illustrated pattern have to be classified as "invalid" in the field "QC". The corresponding sample must be repeated.

5.2 Validation

The validation is carried out automatically by the ViraChip® software.

A ViraChip® Microarray is valid, if the following control spots fulfill the requirements stored in the ViraChip® Software:
- **Serum controls (sc)** above threshold
- **Conjugate controls IgG** (ccG) above threshold

The Conjugate controls IgG have to react stronger than the controls specific for the other conjugate classes
- **Calibrator controls (cal)** above threshold
- **Negative control (nc)** below threshold

If these validation criteria are not fulfilled, the ViraChip® Microarray is classified as "invalid". ViraChip® Microarrays that are invalid must not be interpreted and must be repeated.

5.3 Evaluation of ViraChip® Microarrays

The evaluation of patient samples is carried out automatically by the ViraChip® software according to the interpretation criteria defined below:

The ViraChip® units of each spot triplets are calculated by the ViraChip® software, considering the lot-specific factors relative to the calibrator controls.

Interpretation criteria

ViraChip® units of the spot triplets	Result	Interpretation
At least **one** spot triplet **≥ 100 ViraChip®-units** out of: S1 or S2 or N	**Positive**	Specific IgG antibodies against **SARS-CoV-2** detectable.
At least **one** spot triplet **≥ 70 and < 100 ViraChip®-units** out of: S1 or S2 or N	**Equivocal**	Presence of specific IgG antibodies against **SARS-CoV-2** is questionable. In case of equivocal results the MiQ35a recommends to perform follow-up checks (4).
No spot triplet **≥ 70 ViraChip®-units** out of: S1 or S2 or N	**Negative**	Specific IgG antibodies against **SARS-CoV-2** are not detectable.

VIRAMED BIOTECH AG • Behringstraße 11 • D-82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
E-Mail: viramed@viramed.de • www.viramed.de • certified according to ISO 13485

Performance data

Sensitivity

To determine the sensitivity 170 sera from 90 hospitalized patients with clinically defined COVID-19 symptoms and positive SARS-CoV-2-PCR results were examined with the SARS-CoV-2 ViraChip® IgG. Blood was drawn between 2 and 59 days after onset of symptoms.

		SARS-CoV-2 ViraChip® IgG		
Blood sampling - days after onset of symptoms	Total n = 170	Negative n	Positive / Equivocal n	**Sensitivity (%)**
≤ 7	44	25	19	**43%**
8-17	82	17	65	**79%**
≥ 18	44	0	44	**100%**
In case of hospitalized COVID-19 patients the sensitivity amounts at 18 or more days after onset of symptoms to **100%**				

Table 1.

Specificity

To determine the specificity sera from healthy blood donors from Germany and the USA were examined with the SARS-CoV-2 ViraChip® IgG. Blood was drawn in Germany in 1995/96, before the SARS-CoV and MERS-CoV outbreaks, and in the USA in 2010.

	SARS-CoV-2 ViraChip® IgG		
Blood donors before the start of the COVID-19 pandemic	Negative n	Positive / Equivocal n	**Specificity (%)**
Sera from blood donors from Germany 1995/96, n = 104	104	0	**100%**

Table 2a.

	SARS-CoV-2 ViraChip® IgG		
Blood donors before the start of the COVID-19 pandemic	Negative n	Positive / Equivocal n	**Specificity (%)**
Sera from blood donors from the USA 2010, n = 94	93	1*	**99%**

Table 2b.

* One blood donor serum from the USA showed with the SARS-CoV-2 ViraChip® IgG an equivocal result.

Cross reactivity

A total of 74 samples were examined with the SARS-CoV-2 ViraChip® IgG, which on the one hand contain antibodies against other organisms, that can cause symptoms similar to COVID-19, and on the other hand can represent an atypical immunoactivity.

		SARS-CoV-2 ViraChip® IgG		
Collectives		Negative n	Positive / Equivocal n	**Specificity (%)**
HCoV	n=12	12	0	**100%**
MERS	n=7	7	0	**100%**
SARS-CoV-1	n=10	10	0	**100%**
EBV	n=8	8	0	**100%**
CMV	n=8	8	0	**100%**
Influenza A	n=7	7	0	**100%**
B. pertussis	n=6	6	0	**100%**
C. pneumonia	n=8	8	0	**100%**
ANA autoantibodies	n=8	8	0	**100%**

Table 3.

The ViraChip® Software 1.3.0, the ViraChip® Scanner v1.0 and the ViraChip® Reader Rev.03 were used to determine the performance data.

VIRAMED BIOTECH AG • Behringstraße 11 • D-82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
E-Mail: viramed@viramed.de • www.viramed.de • certified according to ISO 13485

Requirements on the user

1. This test is only to be carried out in a specialized laboratory which has the appropriate laboratory equipment (for example, according to GLP) and has any necessary regulatory approvals to handle the samples to be tested.
2. To ensure reliable results, follow carefully the Instructions for Use and "Good Laboratory Practice (GLP)".
3. This test is to be performed only by qualified personnel trained in the test procedure.

Storage and stability of reagents

1. ViraChip® Microarrays: In closed bags stable until the expiration date if stored at 2-8°C.
2. Wash Buffer Concentrate, 10x: Stable until the expiration date if stored at 2-8°C.
3. Wash Buffer Working Dilution: Stable for 2 weeks if stored at 2-8°C. For longer storage, aliquot and freeze at -20°C.
4. Sample Buffer: Stable until the expiration date if stored at 2-8°C.
5. Conjugate Solution: Stable until the expiration date if stored at 2-8°C.
6. Chromogen / Substrate Solution: Stable until the expiration date if stored at 2-8°C. Avoid exposure to light!
7. Once reagents and kit components have been opened for the first time, shelf life is usually maintained until the expiration date, if used in accordance with Good Laboratory Practice and in accordance with the conditions of storage stated on the label and if stored in the original packaging.

Warnings and precautions

1. All human serum components were tested for HCV, HIV1,2 antibodies and HBs antigens and found to be negative. Nevertheless, all human kit components as well as the patient samples should be considered as potentially infectious and handled according to safety precautions. While working with potentially infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be taken into account. This also applies to storage and disposal of chemicals and reagents being used.
2. While working with hazardous or toxic substances/ biological agents precautions have to be applied following national biosafety regulations. Precautions among others are:
- Do not pipette by mouth.
- Wear disposable gloves and safety glasses while working.
- Do not eat, drink or smoke in the working area.

3. Avoid contact of skin and mucous membranes with chromogen/substrate solution. In case of contact with skin and eyes wash immediately with large quantities of water.
4. Samples and all potentially contaminated materials must be decontaminated using validated laboratory techniques, e.g. by autoclaving 20 minutes at 121°C. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite. Incubate 30 min for complete disinfection.
5. Please refer to material safety data sheets for detailed information on potential risks, first aid guidelines, accidental release measures, handling and storage recommendations, personal protective equipment, directions for disposal and indications to toxicology.
6. Dust and other contaminations in the wells of the MTP have to be avoided, as this might lead to invalid results.

Collection, handling and storage of the patient sample

1. The **SARS-CoV-2 ViraChip® IgG Test Kit** must be used with human serum.
2. Sample collection should be performed by medical specialists according to applicable standards from whole blood (7).
3. Using heat-inactivated, hemolytic, icteric or lipemic specimens may lead to invalid results.
4. Specimens must not be microbially contaminated.
5. Normally, human serum can be stored up to 5 days at 2-8°C. Specimens may be stored at -20°C (or below) for long term storage.
6. Prior test processing, specimens should have reached room temperature. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.
7. Avoid multiple freeze and thaw cycles.

Limitations of the procedure

1. A positive test result of the respective patient sample is to be regarded as a symptom. Interpretation and assessment of test results may only be carried out by qualified medical personnel, evaluating all relevant data (6).
2. A negative result does not exclude a contact with the pathogen or the presence of a disease.
3. The detection of specific antibodies can vary within different assays from different manufacturers and can lead to different results due to different sensitivity, specificity and assay methodologies.
4. In immunosuppression, limited assessability applies (6).
5. Drugs and immunoglobulin doses can cause non-specific antibody responses (6).
6. *In vitro* diagnostics must not be used beyond expiration dates, as reliable results may not be possible.
7. Precise compliance with the IFU is essential for accurate test results. Insufficient washing can cause incorrect results.
8. The use of pooled samples in which several sera are examined in parallel is inadmissible, since the test sensitivity and specificity can be impaired with such approaches.

Notes to Equipment and Software

1. Automatized processing requires usage of processor type specific test procedures which are validated and programmed by Viramed Biotech AG.
2. Usage of processor specific consumables requires approval of the respective configurations according to manufacturer's instructions by Viramed Biotech AG.
3. The equipment and software configuration provided by Viramed Biotech AG must not be changed. Any alteration can lead to false results.
4. Only equipment specific software must be used. Changes of configuration files must be performed by Viramed Biotech AG.
5. Only measuring devices approved by Viramed Biotech AG are allowed to be used.
6. Assay interpretation of ViraChip® Microarrays have to be performed using the ViraChip® Software. A manual / visual interpretation is not possible.

VIRAMED BIOTECH AG • Behringstraße 11 • D-82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
E-Mail: viramed@viramed.de • www.viramed.de • certified according to ISO 13485

Literature

1. FANG, L: Structure, Function, and Evolution of Coronavirus Spike Proteins, Annu Rev Virol, 2016
2. OKBA, NMA et al.: SARS-CoV-2 specific antibody responses in COVID-19 patients, medRxiv, 2020
3. YU, IM et al.: Crystal Structure of the Severe Acute Respiratory Syndrome (SARS) Coronavirus Nucleocapsid Protein Dimerization Domain Reveals Evolutionary Linkage Between Corona- And Arteriviridae, J Biol Chem, 2006
4. PODBIELSKI , A et al.: MIQ Heft: 35a Infektionsimmunologische Methoden Teil 1, Elsevier Urban & Fischer, 2016
5. TOK, TT & TATAR, G: Structures and Functions of Coronavirus Proteins: Molecular Modeling of Viral Nucleoprotein, Int J Virol Infect Dis, 2017
6. THOMAS, L: Labor und Diagnose, Med Verlagsgesellschaft Marburg, 2012
7. TUCK, MK et al.: Standard Operating Procedures for Serum and Plasma Collection, J Proteome Res, 2009

Symbols used

	Manufacturer		Order Number
	Refer to Instructions for Use		Use by / Expiration Date
	*In-Vitro* Diagnostic Medical Device		Temperature Limitation (Storage)
	Lot Number		Positive control
	Sufficient for 96 tests		Negative control

VIRAMED BIOTECH AG • Behringstraße 11 • D-82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
E-Mail: viramed@viramed.de • www.viramed.de • certified according to ISO 13485