Willi Fox – COVID-19 IgM/ IgG Test

(SARS-CoV-2)

Instructions for use

(IFU)

Rapid test for the detection of anti-SARS-CoV-2
IgM und IgG Antibodies
in whole blood, serum or plasma

1. Intended use

The Willi Fox – COVID-19 IgM/ IgG rapid test is a qualitative, membrane based immunoassay for the detection of anti-SARS-CoV-2 IgM and IgG antibody in whole blood, serum or plasma through visual interpretation of color development.

COVID-19 is the disease associated with SARS-CoV-2, which was identified in China at the end of 2019. Coronaviruses cause respiratory and intestinal infections in animals and humans.

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals as camels, cattle, cats and bats or birds. Corona viruses cause respiratory, enteric, hepatic and neurologic diseases. Six coronavirus species are known to cause human disease. Four viruses strains HCoV-NL63, HCoV-229E, HCoV-OC43 und HKU1 are prevalent and typically cause only mild upper respiratory diseases in immunocompetent individuals. Although some of them can cause severe infections in infants, young children and elderly individuals.
The two other strains SARS-CoV and MERS-CoV are highly pathogenic, zoonotic and can cause severe respiratory syndrome.
The virus is transmitted mainly via respiratory droplets that people sneeze, cough or exhale.
The incubation period for SARS-CoV-2 virus is currently estimated between two and 14 days.
Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause severe pneumonia, acute

respiratory distress syndrome, kidney failure, sepsis and septic shock that can lead to the death of the patient. People with existing chronic conditions seem to be more vulnerable to severe illness. Detection of IgM indicates recent infection and can be used for early diagnosis of infection. While IgG antibodies gradually appear and increase in the late stage of infection.

The *Willi Fox* – Troponin I Test is a simple test that utilizes a combination of particle conjugated anti-cTnl antibodies and capture reagent to selectively detect cTnl in whole blood, serum or plasma. The minimum detection level is 0.5 ng/mL.

2. Test principle

The *Willi Fox* – COVID-19 IgM/IgG Rapid Test Device (Whole Blood/Serum/Plasma) is a qualitative, membrane based immunoassay for the detection of anti-SARS-CoV-2 IgM and IgG antibody in whole blood, serum or plasma through visual interpretation of color development.

Anti-human IgG and anti-human IgM are used to detect specific antibodies in the human whole blood, serum or plasma specimen. When specimen is added to the sample well, specific IgM and/or IgG antibodies, if present, will bind to the SARS-CoV-2 antigens conjugated to colored particles on the conjugate pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-human IgM and/or anti-human IgG antibodies immobilized on the test region(s). Excess colored particle are captured at the internal control region.
The presence of a red band(s) on the test region(s) indicates a positive result for the particular IgG and/or IgM antibodies, while its absence indicates a negative result. A red band at the control region (C) serves as a procedural control, indicating that membrane wicking is working.

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3. Material provided

• Individually packed test devices	*Kit with single-pouched test cassettes in foil pouches together with desiccant – The desiccant is not a test component, please give it to the waste!*
• Disposable pipettes	inside pouch
• 1 Buffer	
• Package insert	Information for user

4. Materials required but not provided

- Tubes for taking blood samples
- Lancets (only for whole blood from fingertip)
- Centrifuge (for plasma / serum)
- Heparinised capillaries and dispensary bulb (only for whole blood from fingertip)
- Timer

5. Storage and stability

- The *Willi Fox* – COVID-19 IgM/ IgG rapid test should be stored at 2-30°C and is stable until the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use.
- Do not freeze!
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

6. Precaution

- The *Willi Fox* – COVID-19 IgM/ IgG rapid test is intended for use with human whole blood, serum, or plasma specimens only.
- Dispose the used test device according to the local regulations.
- Do not use after the expiration date.
- The test device should remain in the sealed pouch until ready to use.
- Do not use test of pouch has been damaged.
- Humidity and high temperature can adversely affect results.
- All specimens might be potentially infectious. Proper handling and disposal methods should be established. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.

Ergänzen

7. Specimen collection and preparation

General comments:

- The *Willi Fox* – COVID-19 IgM/ IgG rapid test should be only used with human blood, whole blood serum or plasma..
- Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after specimen collection.
- Do not leave specimens at room temperature for prolonged periods.
- Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.

- Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens.
- Whole blood collected by fingertip should be tested immediately.
- Containers containing anticoagulants such as EDTA, citrate, or heparin should be used for whole blood storage.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Icteric, lipemic, hemolysed, heat treated and contaminated specimens may cause erroneous results.
- There is a slight possibility that some whole blood specimens with very high viscosity or which have been stored for more than 2 days may not run properly on the test device. Repeat the test with a serum or plasma specimen from the same patient using a new test device.

Specimen collection:

Whole blood from fingertip:

- Wash the hand of the patient with soap and warm water or clean the puncture site thoroughly with alcohol.
- Massage the hand in direction of the fingertip of the middle finger or ring finger without touching the puncture.
- Prick the fingertip with a sterile lancet. Wipe the first drop of blood.
- Rub the hand from the wrist to the palm and to the finger to form a round drop at puncture.

Collection of whole blood from fingertip using a capillary:

- Take a blood sample filling the capillary with 10 µl of the sample. Avoid air pockets.
- Put the dispensary bulb at the top of the capillary and press it to dribble blood sample in the specimen well (S) of the test device.

8. Test procedure and results

Bring tests, specimens, buffer, and/or controls to room temperature (15-30°C) before use.

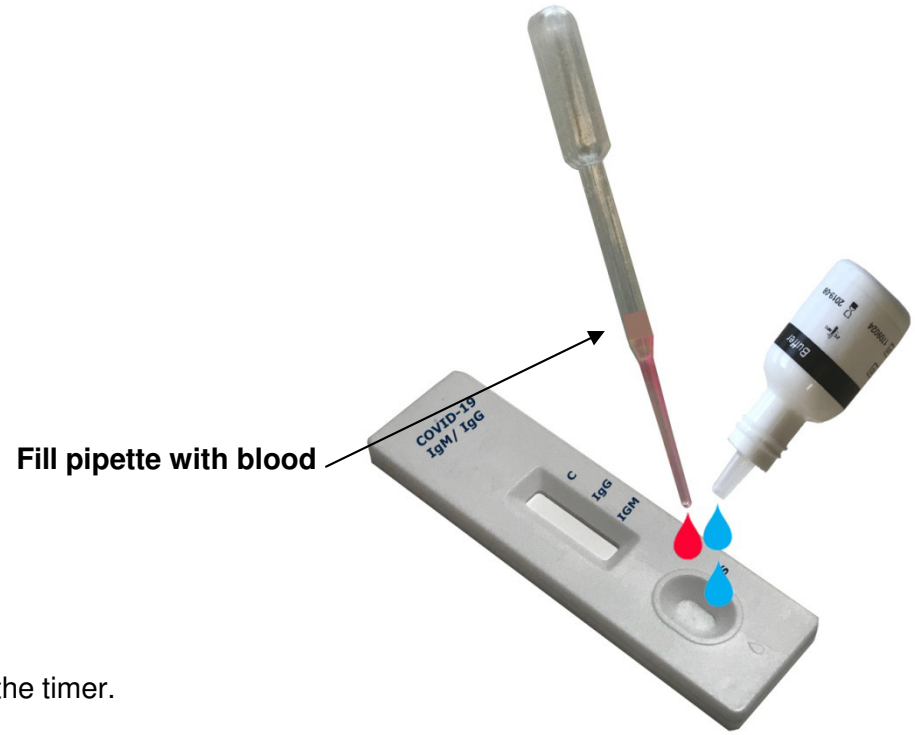
1. Remove the pouch and label the test device

- Remove the test device from the sealed pouch and use it as soon as possible.
- Best results will be obtained if the assay is performed within one hour.
- Place the *Willi Fox* – COVID-19 IgM/ IgG rapid test on a clean and level surface.

2. For whole blood (fingertip or venipuncture blood) serum or plasma

- Hold the provided pipette vertically and transfer **1 drops of whole blood, serum or plasma** (ca. 10 µl) into the round specimen well (S) of the *Willi Fox* – COVID-19 IgM/ IgG rapid test.

- Add **2 drop of buffer** (ca.80 µl)



- Start the timer.

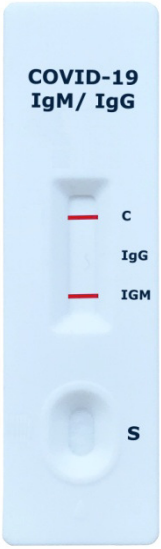
Attention: Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area.

3. Wait for the colored line(s) to appear.

- As the test begins to work, color will migrate across the membrane.
- The result should be read **at 15 minutes**.
- Do not read results after more than 30 minutes.

Do not read results after more than 30 minutes!

Interpretation of results

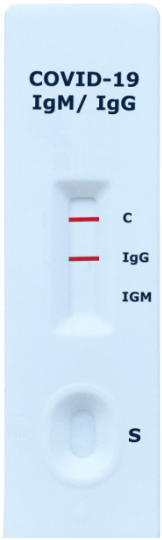


IgM positive:

Two red lines appear on the membrane.

One line appears in the control region (C) and another line appears in the IgM test region (IgM).

Attention: The intensity of the red colour in the test line region will vary depending on the concentration of antibodies present in the specimen. Therefore, also faint reddish test result lines should be considered positive.

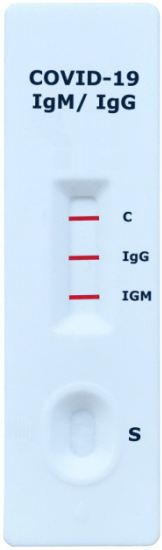


IgG positive:

Two red lines appear on the membrane.

One line appears in the control region (C) and another line appears in the IgG test region (IgG).

Attention: The intensity of the red colour in the test line region will vary depending on the concentration of antibodies present in the specimen. Therefore, also faint reddish test result lines should be considered positive.



IgM and IgG positive:

Three red lines appear on the membrane.

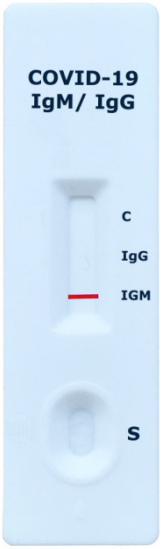
One line appears in the control region (C) and two lines appear in the IgM and IgG test region (IgM&IgG).

Attention: The intensity of the red colour in the test line region will vary depending on the concentration of antibodies present in the specimen. Therefore, also faint reddish test result lines should be considered positive.

IgM and IgG negative:

One red lines appears on the membrane in the control line region (C)..

No red lines appear in the test regions. No anti-SARS-CoV-2 antibodies have been detected.



Invalid:

No red line appears on the membrane in the control line region (C).

The test is not valid. Review the procedure and repeat with a new test.

- Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.
- If the problem persists, discontinue using the test kit immediately and contact your local distributor.

9. Quality control

Internal procedural control:

As internal procedural control the *Willi Fox* – COVID-19 IgM/ IgG rapid test includes the control line. It is only formed if sufficient specimen volume has been added and the chromatography has been finished successfully.

External procedural control:

Control standards are not supplied with this kit; yet, we recommend that positive and negative controls should be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

10. Limitations of the test

- The *Willi Fox* – COVID-19 IgM/ IgG rapid test is for professional in vitro diagnostic use, and should only be used for the qualitative detection of anti-SARS-CoV-2 IgM and anti-SARS-CoV-2 IgG.
- No meaning should be inferred from the colour intensity or width of any apparent lines.
- The *Willi Fox* – COVID-19 IgM/ IgG rapid test will only indicate the presence of anti-SARS-CoV-2 antibodies in the specimen and should not be used as the sole criteria for the diagnosis of COVID-19. A negative result at any time does not preclude the possibility of 2019-nCoV infection..
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
- The hematocrit level of the whole blood can affect the test results. Hematocrit level needs to be between 25% and 65% for accurate results.
- The test will show negative results under the following conditions: The titer of the novel coronavirus antibodies in the sample is lower than the minimum detection limit of the test, or the novel coronavirus antibody has not appeared at the time of sample collection (Asymptomatic stage).

12. Test performance

A. Analytical sensitivity and specifity

79 specimens were collected from patients exhibiting pneumonia or respiratory symptoms. 83 specimens were also collected from convalescent patients. 227 negative specimens were collected in the study.

Result for IgM detection:

Method		PCR+	PCR-	Total
Willi Fox - COVID-19 IgG/IgM Rapid Test	IgM+	74	2	76
	IgM-	5	225	230
Total		79	227	306

Relative sensitivity: 93.7% (86.0%-97.3%)*
Relative specificity: 99.1% (96.8%-99.8%)*
Overall agreement: 97.7% (95.4%-98.9%)*
*95% Confidence Interval

Result for IgG detection:

Method		PCR+	PCR-	Total
Willi Fox - COVID-19 IgG/IgM Rapid Test	IgG+	82	3	85
	IgG-	1	224	225
Total		83	227	310

Relative sensitivity: 98.8% (93.5%-99.8%)*
Relative specificity: 98.7% (96.2%-99.5%)*
Overall agreement: 98.7% (96.7%-99.5%)*
*95% Confidence Interval

B. Precision

Intra-Assay

One lot of *Willi Fox* - COVID-19 IgG/IgM rapid test was used in the intra-assay study. Specimens with anti-SARS-CoV-2 IgM and IgG negative, with low positive IgM, with high positive IgM, with low positive IgG and with high positive IgMG were tested in 10 replicates.
The specimens were correctly identified >99% of the time.

Inter-Assay

Three kit lots of *Willi Fox* - COVID-19 IgG/IgM rapid test from three independent production lots were tested to evaluate whether they are consistency in performance. Specimens with anti-SARS-CoV-2 IgM and IgG negative, with low positive IgM, with high positive IgM, with low positive IgG and with high positive IgMG were tested in 10 replicates.
The specimens were correctly identified >99% of the time.

C. Cross-Reactivity

Serum or plasma specimens from individuals with infections, which are common in regions where the *Willi Fox* - COVID-19 IgG/IgM rapid test would be used, were tested for interference. These infections included unrelated viral infections and other unrelated microbial infections. The following substances showed no interference with the *Willi Fox* - COVID-19 IgG/IgM rapid test:

Anti-HAV IgM+	EBV IgG+	Typhus IgM+
Anti-HEV IgM+	Anti-Dengue virus+	Lyme-Borreliosis+
HBsAg+	Anti-Yellow fever virus+	P.falciparum+
Anti-HCV+	Anti-Zika virus+	P. vivax+
Anti-HIV+	Anti-Chikungunya+	Toxoplasmosis
Anti-Rubella IgM+	Chagas IgG+	HAMA+
Anti-CMV IgM+	Anti-Syphilis+	RF+
Anti-HSV-1 IgM+	Anti-Clamydia+	ANA+
Anti-HSV-II IgM+	Anti-Tuberculosis	

None of the substances tested interfered in the assay.

D. Interfering Substances

Die folgenden Substanzen in den angegebenen Konzentrationen wurden mit dem *Willi Fox* – COVID-19 IgM/ IgG Test getestet. Keine von ihnen hat die Testleistung des *Willi Fox* – COVID-19 IgM/ IgG Tests beeinträchtigt:

Potentially interfering substances present in the blood, commonly used anticoagulants, mediations, some therapeutic drugs and commonly used consumables like coffee and alcohol were tested with and without SARS-COV-2 IgG or SARS-COV-2 IgM.

The following substances showed no interference with the *Willi Fox* - COVID-19 IgG/IgM rapid test:

Substances	**Concentration**
Blood analytes	
Albumin	5 g/dL
Bilirubin	5 mg/dL
Hemoglobin	20 g/dL
Triglycerides	500 mg/dL
Anticoagulants	
EDTA	3.4 µmol/L
Heparin	3000 U/L
Sodium citrate	5 mg/mL
Potassium oxalate	2 mg/mL
Abnormal blood sample	
Visual hemolysis	NA
Icteric	NA
Lipemic	NA
Common medicines	
Acetylsalicylic	3.62 mmol/L
Ascorbic acid (Vitamin C)	342 µmol/L
Amoxicillin	206 µmol/L
Aspirin	4.34 mmol/L
Fluconazole	245 µmol/L
Ibuprofen	2425 µmol/L
Loratadine	0.78 µmol/L
Nadolol	3.88 µmol/L
Naproxen	2170 µmol/L
Paroxetine	3.04 µmol/L
Anti-malarial medicines	
Quinine	148 µmol/L

Anti-tuberculosis medicines	
Rifampicin	78.1 µmol/L
Isoniazid	292 µmol/L
Ethambutol	58.7 µmol/L
Common consumables	
Coffee (caffeine)	308 µmol/L
Alcohol (ethanol)	86.8 mmol/L

13. Literature

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14. Symbols

REF	Article number		For single use only
LOT	Lot number		Expiry date
	Storage		Content
IVD	Only for in vitro diagnostics use		Instructions for use

CE

All *Willi Fox* – COVID-19 IgM/ IgG rapid tests are manufactured and distributed by:

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