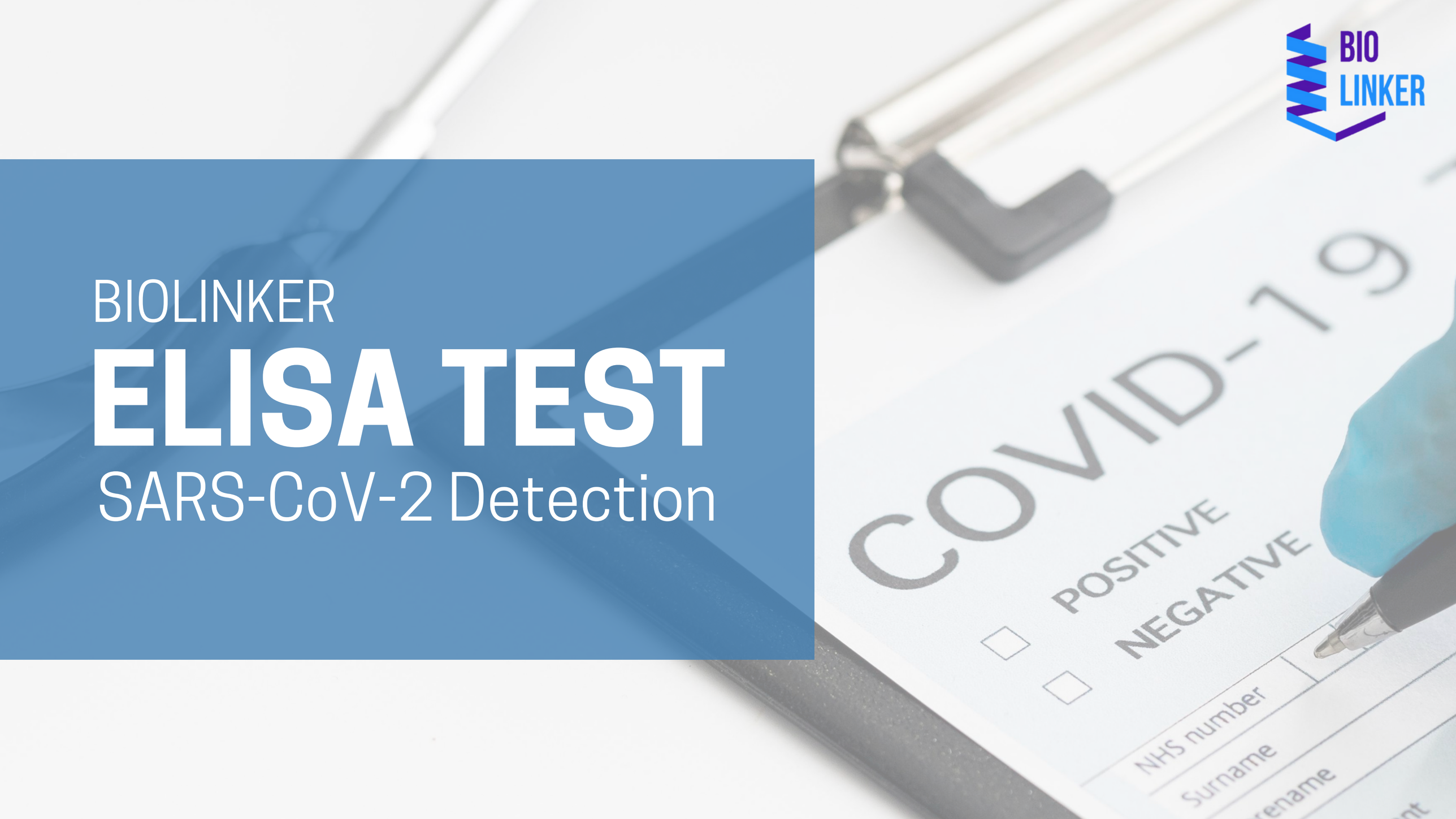
BIOLINKER

ELISA TEST

SARS-CoV-2 Detection



TEST PRINCIPLES

The Biolinker Enzyme Immunoassay Kit (ELISA) provides a semi-quantitative in vitro assay for human IgG class immunoglobulin antibodies to SARS-CoV-2 in serum or plasma with EDTA, heparin or citrate to support the diagnosis of SARS-CoV-2 infection and constitutes a complement for direct detection of the pathogen. Serology can be applied to epidemiological collection data. Combining technologies and national production, making the product with the best cost benefit on the market. The viral antigen used is a recombinant portion of the COVID19-specific nucleocapsid. This test is fast, sensitive and can be processed completely automatically.

Attention! This kit is for in vitro diagnosis use only

Antibody Against	Ig Class	Substrate
SRAS-CoV-2	IgG	Antigen-coated microplate wells

The kit has microplate strips, each with 8 disposable wells coated with a SARS-CoV-2 recombinant protein structure. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (and also IgA and IgM) will be bound to the antigen. To detect the bound antibodies, a second incubation is performed using the enzyme-coupled anti-human IgG (enzyme conjugate), catalyzing a color reaction.

Itens Included	Format
Microplate with Antigen Coated Wells: 12 strips of microplates containing 8 individual tear-off wells each, ready to use.	12 x 8
Calibrators (IgG, human), ready for use	1 x 2.0 mL
Positive control (IgG, human), ready for use	1 x 2.0 mL
Negative control (IgG, human), ready for use	1 x 2.0 mL
Enzymatic conjugate Peroxidase-coupled anti-human IgG; ready for use	1 x 12.0 mL
Sample buffer ready for use	1 x 100.0 mL
Wash buffer 10x concentrate	1 x 100.0 mL
Substrate/Chromogen Solution TMB/H2O2, ready for use	1 x 12.0 mL
Stop solution 0.5M hydrochloric acid, ready for use	1 x 12.0 mL
Plate protective pellicule	3 unity
Instructions (electronic or physical version)	1 flayer
Quality control certificate	1 protocol

Materials and tools required to perform the test that are not included in this kit:

- Automatic microplate washer: Recommended. Microplate washing can also be done manually
- Microplate reader: 450 nm wavelength, reference wavelength range from 620 nm to 650 nm Calibrated pipettes
- Repeater pipette: recommended for pipetting the enzymatic conjugate, substrate and wall solution
- Distilled or deionized water
- Incubator: for incubation of the microplate at +37°C
- Incubator or water bath: recommended to heat the wash buffer
- Stopwatch

Storage and stability conditions:

- All components come with proper temperature warning.
- Most of them must be stored between +2°C and +8°C. Do not freeze.
- Unopened, all kit components are stable until the expiration date.
- After opening, reagents are stable until the indicated expiration date if stored at +2°C to +8°C and protected from contamination.

Warnings:

- The product should only be used by laboratory employees, duly trained in a clinical or research laboratory;
- The microplate should not be used if the packaging is visibly damaged or liquids have leaked.
- Before using the product, read the instructions carefully. Use only the valid version provided with the product or electronically.
- Calibrators and controls are pre-diluted and ready to use, so do not dilute them.
- The pipetting volumes, incubation times, temperatures and preparation steps in the instructions must be respected.
- All patient samples, calibrator, controls and incubated microplate strips must be treated as infectious waste. All reagents must be disposed of in accordance with local regulations.
- Do not substitute or mix the BIOLINKER reagents with reagents from other manufacturers;
- Use your country's recommended Good Laboratory Practice (GLP) protocols and safety guidelines when using the kit.
- Avoid contact of samples and reagents with eyes and skin. In case of contact with eyes or skin, rinse with plenty of water, remove and wash contaminated clothing. In case of ingestion and seek medical attention.
- All reagents should be at room temperature (+18°C to +25°C) approximately 30 minutes before use.
- The Elisa incubator thermostat must be set to +37°C ± 1°C before use.

Sample preparation and stability:

- Human serum or plasma samples with EDTA or heparin can be used in the kit
- Patient specimens to be investigated can generally be stored for up to 14 days at a temperature between +2°C and +8°C.
- Diluted samples should be incubated the same day at a concentration of 1:10 in the sample buffer.

For example: dilute 100µl of serum in 900 uL in the sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing) . We use pipettes to homogenize.

Warning: Calibrators and controls are pre-diluted and ready to use, so do not dilute them.

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PERFORMING THE TEST MANUALLY

Incubation:

- Samples incubation (1st step): Transfer 100µl of the calibrators, positive and negative controls or diluted patient sample into the individual wells of the microplate according to the pipetting protocol.
- Incubate for 30 minutes at +37°C ± 1°C.
- For manual process, cover the finished plate with a protective film. When using an automated incubation processor, follow the equipment manufacturer's recommendations.

Washing:

- Manual Wash: Remove the protective film, empty the wells and then wash 5 times, using 300µl of ready wash buffer for each wash.
- Auto Wash: Remove the protective film and wash the reaction wells 5 times with 450µl of ready wash buffer (program setting: eg Tecan Columbus Washer “Overflow Mode).
- Leave a wash buffer in each well for 30 to 60 seconds per wash cycle, then empty the wells. After washing (manual and automated), carefully discard all liquid from the microplate by tapping it on absorbent paper with the openings facing down to remove all residual wash buffer.

Caution:

- Any residual liquid (>10µl) remaining in the reaction wells after washing can interfere with the substrate leading to falsely low absorbance values. Be sure to always dry your board well.
- Insufficient washes (less than 5 washes, insufficient volumes of wash buffer and short incubation times) can lead to falsely high absorbance values.
- Empty wells in the microplate strips must be filled with “blank” in the same way as the parameters to be investigated.

Conjugate incubation (2nd step):

- Pipette 100µl of the enzyme conjugate (Peroxidase-labeled Anti-Human IgG) into each microplate well. For manual performance, cover reagent wells with protective film.
- Incubate for 30 minutes at +37°C ± 1°C.

Substrate incubation:

- Pipette 100µl of substrate/chromogen into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to +25°C protect from direct light).

Stopping the reaction:

- Pipette 100µl of Stop Solution into each of the microplate wells in the same order and at the same rate as the Substrate/Chromogen Solution was introduced.

Measurement:

- Photometric measurement of color intensity should be performed at a wavelength of 450nm and reference value between 620 nm and 650 nm within 30 minutes after addition of the stop solution. Before measuring, gently swirl the microplate to ensure even distribution of the solution.

Conducting the test using automated analysis equipment:

- Sample dilution and testing are performed in a fully automated manner using an analysis device. The incubation settings programmed in the BIOLINKER Authorized Software may differ slightly from the ELISA specifications provided in the instruction for use. However, these conditions have been validated with respect to Analyzer I-2P.

Quality Control:

For each group of tests performed, the calibrator absorbance values and ratios determined for the negative and positive controls must be within the limits established for the relevant kit lot. A quality control certificate containing these reference values is included. If the specified values for the controls are not met, the test results may be incorrect and the test may be repeated.

Calculated the results:

The absorbance value of the calibrator defines the upper limit of the reference value for uninfected people (cut-off) recommended by BIOLINKER. Values above that indicated by the cutt-off are considered positive, those below are considered negative.

Semi-quantitative: Results can be evaluated semi-quantitatively by calculating the ratio of the absorbance value of the control or patient sample to the absorbance value of the calibrator.

The calculation must be done using the following formula:

Control or patient sample absorbance / Calibration Absorbance Ratio

BIOLINKER recommends the following interpretation of the result:

- **Ratio <0.8 ☒ Negative**
- **Ratio ≥0.8 a <1.1 ☒ Borderline**
- **Ratio ≥1.1 ☒ Positive**

For duplicate analyses, the two values should be averaged. If the values differ significantly from the other, the samples must be retested.

Test feature:

Calibration: As there are no international reference sera for antibodies against SARS-CoV-2, calibration is performed in relative units (UR).

For every group of tests performed, the calibrator absorbance values and the relative units and/or the ratios determined for the positive and negative controls must be within the limits established in the kit lot in question. A protocol containing the reference values is included. If the values specified for the controls do not agree, the results should not be considered and the test should be repeated.

The activity of the enzyme used is temperature dependent and values may vary if a thermostat is not used in all incubation steps. The higher the ambient temperature (+18°C to +25°C) during the incubation steps, the higher the values obtained. Corresponding variations are also applicable to the incubation time. However, the calibrators are subject to the same influences, which results in the compensation of most variations in the result calculation.

Antigen: The microplate wells are coated with recombinant structural protein (S1 domain) from SARS-CoV-2.

Measuring range:

White limit (LoB): ratio 0.13

Limit of detection (LoD): ratio 0.15

LoB and LoD were defined according to the requirements defined in the CLSI guideline EP17-A2 (Clinical and Laboratory Standards Institute, https://clsi.org/).

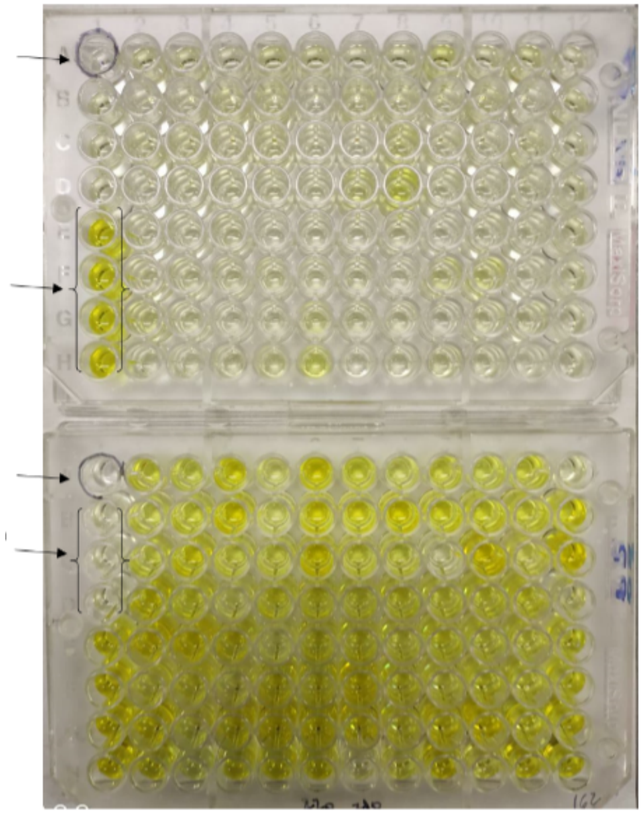
Test precision: Intra-laboratory precision studies were performed according to CLSI guideline EP05-A3. 4 samples (reactivity distributed over the entire measurement range) were measured. Precision is given as standard deviation (SD) and coefficient variation (CV).

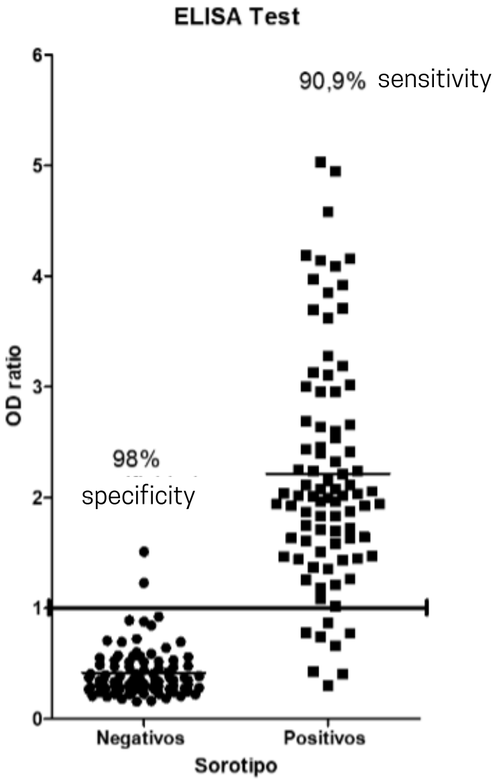
CLINICAL PERFORMANCE

SENSITIVITY VS SPECIFICITY

After the specificity analysis, we performed the sensitivity assay again with the same positive samples as in the previous assay of 200 samples, (n=100 positive and n=100 negative) and we could observe sensitivity and specificity results through these parameters.

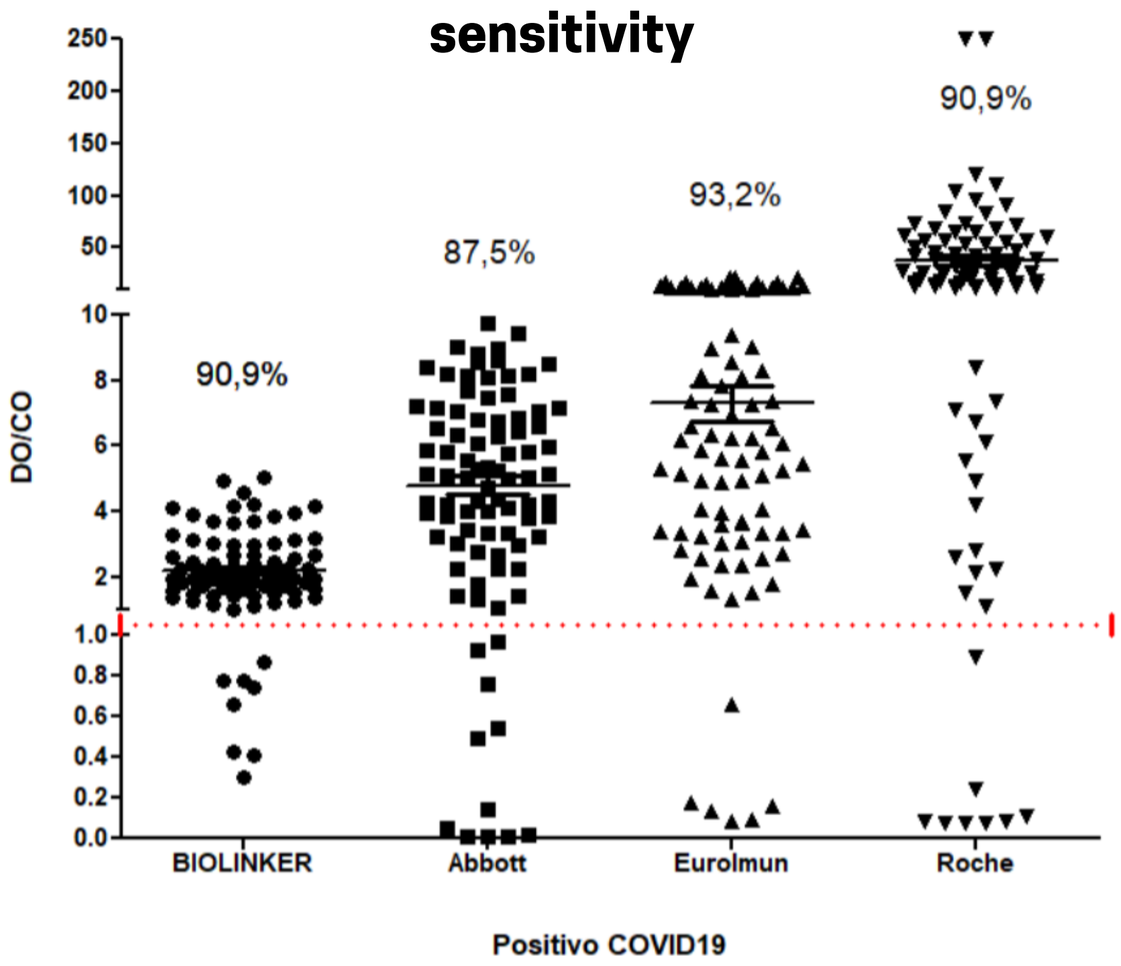
The sensitivity obtained using the chosen cutoff for this analyzed group was 90.9%.





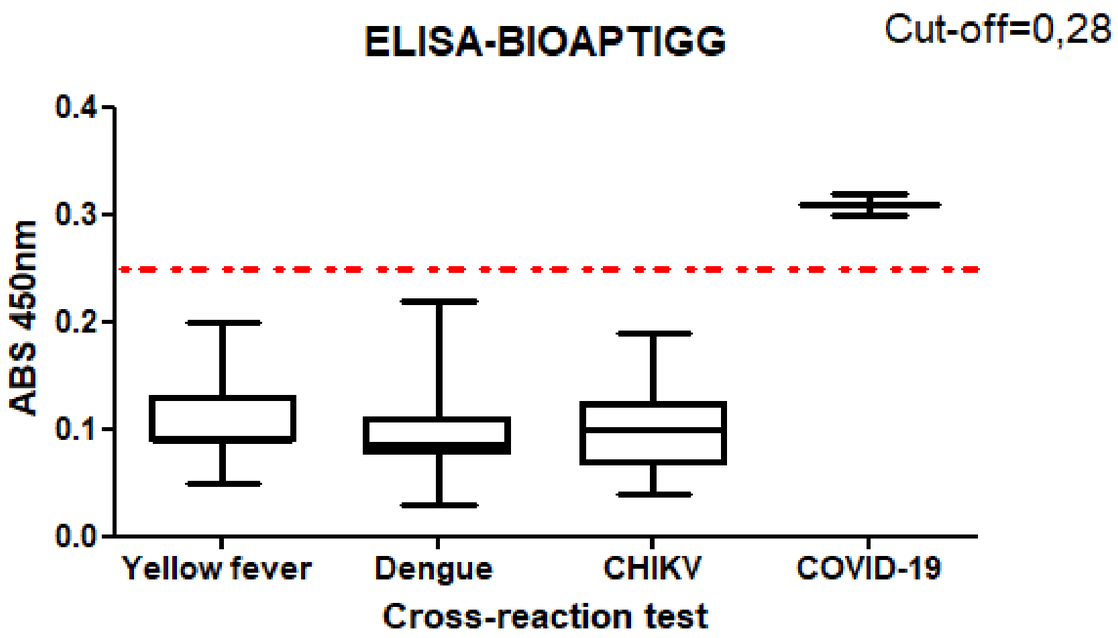
BIOLINKER VS OTHER MANUFACTURERS

When compared to other manufacturers, our kit obtained a higher value than several other manufacturers, when analyzed in a new comparative test using the same parameters and the same sample number.



Cross-reaction analysis

The same antigen coated plate were tested against to Yellow feverm Dengue, Chikunguya disease serum positeve patients. The cross-reaction activity test results show specificity and reactitivity only to COVID-19 positive patients.



Process limitations:

- For a medical diagnosis, the serological test result should always be interpreted together with the patient's clinical symptoms and other results, those of direct detection of the pathogen. A negative serological result does not exclude the presence of the disease.
- Performing correct sample collection and storage is crucial to test results.
- The test is only validated for the determination of anti-SARS-CoV-2 IgG in human serum or plasma.
- The binding activity of the antibodies and the activity of the enzyme used are temperature dependent. It is recommended to use the adjustable thermostat in the ELISA incubator at all stages of incubation. The higher the ambient temperature during the incubation phases, the higher the absorbance. The same variations also apply at times of hatching. However, calibrators are subject to the same influences, with the result that such variations will be largely compensated for in the result calculation.
- Insufficient washing (eg, less than 3 wash cycles, low wash buffer volumes, or very short retention times) can lead to falsely high absorbance readings.
- Residual liquid (>10µl) in reagent wells after washing can interfere with the substrate and lead to falsely low absorbance readings.
- Partial or complete adjustment of the test for the use of automated sample processing equipment or other liquid handling devices may result in differences between results obtained with automated processing and those obtained with manual procedures. It is the user's responsibility to validate the equipment used so that it gives test results within the reliable range.

Warning!

BIOLINKER guarantees this product performance from specifications until the expiration date indicated on the labels and in case the instructions for use are correctly followed.