SERION ELISA *agile* SARS-CoV-2 IgA/IgG

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Enzyme immunoassay for determination of human antibodies

For sale in the U.S. for Research Use Only. Not for diagnostic use.

SERION ELISA *agile* SARS-CoV-2 IgA	Order no. ESR400A
SERION ELISA *agile* SARS-CoV-2 IgG	Order No. ESR400G

1 INTENDED USE

The SERION ELISA *agile* SARS-CoV-2 IgA and IgG tests are qualitative and quantitative immunoassays for the detection of human antibodies in serum or plasma directed against SARS-CoV-2.

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2 BACKGROUND

The beta coronavirus SARS-CoV-2, which causes the disease COVID-19, has been responsible for a global pandemic since early 2020. Common symptoms of COVID-19 are similar to those of a cold or flu, such as fever, cough, difficulty breathing or pneumonia in both lungs. In severe cases, the infection can lead to death.

During the first week after the onset of symptoms, qRT-PCR is used as a reliable method for detecting SARS-Cov-2 infection, and, as the infection progresses, the combination of qRT-PCT and antibody tests is often used. The use of antibody detection is useful in the later stages of infection when the virus is no longer detectable and can also be used to identify individuals who have developed immunity after infection and/or who may be potential plasma donors for therapeutic purposes.

The SERION ELISA *agile* SARS-CoV-2 IgA test is based on a mixture of highly purified recombinant nucleocapsid and whole spike protein (S1/S2 ectodomain) of SARS-CoV-2 representing the most important immunogenic proteins of SARS-CoV-2 and allowing for sensitive detection of anti-SARS-CoV-2 IgA antibodies. For the SERION ELISA *agile* SARS-CoV-2 IgG test, the whole spike protein is used exclusively for specific antibody detection and for correlation with protective antibodies that mainly target the spike protein.

3 TEST PRINCIPLE SERION ELISA *agile*

The test strips of the SERION ELISA *agile* microtiter plates are coated with specific antigens of the pathogen of interest. Diluted samples are incubated in the coated wells. Specific antibodies present in positive samples bind to the antigens and are detected with alkaline phosphatase-labeled secondary antibodies. This enzyme catalyzes the conversion of the colorless substrate p-nitrophenyl phosphate into the colored product p-nitrophenol. The signal intensity of the reaction product is proportional to the antibody concentration in the sample and is measured photometrically.

4 KIT COMPONENTS, STORAGE AND STABILITY

Test Components	Pieces/ Volume	Storage	Stability
Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96) MTP, 1 frame. The coating material is inactivated.	12 pieces	Unopened After opening at 2-8°C in closed aluminum bag with desiccant	See expiry date 6 months
Standard serum (ready-to-use) STD, Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Amaranth O	2 x 1 ml	Unopened After opening at 2-8°C	See expiry date 6 months
Negative control serum (ready-to-use) NEG, Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Lissamin Green V	1 ml	Unopened After opening at 2-8°C	See expiry date 6 months
Anti-human IgA or IgG conjugate (ready-to-use) Conjugate Anti-human IgA or IgG polyclonal antibody, conjugated to alkaline phosphatase, stabilized with protein stabilization solution; Preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane	14ml	Unopened After opening at 2-8°C	See expiry date 6 months
Washing solution concentrate (sufficient for 1000ml WASH, Sodium chloride solution with Tween 20 and 30mM Tris-HCl, pH 7.4; Preservative: <0.1% sodium azide	33.3ml	Unopened/after opening at 2-8°C Working dilution at 2-8°C Working dilution at room temperature	See expiry date 2 weeks 1 week
Dilution buffer (ready-to-use) Diluent S5, Protein-containing phosphate buffer with Tween 20; Preservative: <0.1% sodium azide; coloring: 0.01g/l Bromphenol blue	2 x 55ml	Unopened After opening at 2-8°C	See expiry date 6 months
Stopping solution (ready-to-use) STOP, <0.1N sodium hydroxide, 40mM EDTA	15ml	Unopened After opening at 2-8°C	See expiry date 6 months
Substrate (ready-to-use) Substrate, Para-nitrophenylphosphate in solvent-free buffer; Preservative: <0.1% sodium azide	14ml	Unopened After opening at 2-8°C	See expiry date 6 months
Quality control certificate with standard curve and evaluation table INFO, (quantification of antibodies in U/ml)	2 pages	N/A	N/A

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- Common laboratory equipment
- Photometer for microtiter plates with filter, wavelength 405nm, recommended reference wavelength 620nm-690nm (e.g., 650nm)
- Microtiter plate washer
- Incubator 37°C
- Moist chamber
- Distilled water
- Click-Clips (Order No. VT120.1)
- Optional: SERION ELISA *control*

6 TEST PROCEDURE SERION ELISA *agile*

6.1 General information and statements of warnings

The SERION ELISA *agile* is designed for use by qualified personnel who are familiar with good laboratory practice.

All kit reagents and samples should be handled carefully using established good laboratory practice.

The test kit contains dilutions of human sera. Although all sera used have been tested and found negative for anti-HIV Ab, HBs-Ag (Hepatitis B Virus Surface Antigen) and anti-HCV Ab, they should be considered potentially infectious.

The instructions for use must be strictly followed.

Only use SERION ELISA *agile* reagents when using SERION ELISA *agile* immunoassays. They must not be exchanged with reagents of other manufacturers or components of the SERION ELISA *classic* line. Standard and control sera and conjugate of the SERION ELISA *agile* are lot-specific. Washing solution, dilution buffer, substrate, and stop solution can be used for all SERION ELISA *agile* independent of lot and kit.

The test reagents should be protected from strong light during storage and incubation.

The SERION ELISA *agile* is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

6.2 Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples.

6.2.1 Dilution of Samples

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

SERION ELISA *agile* SARS-CoV-2 IgA and IgG

$V_1 + V_2 = 1:100$	e.g. add each to	5ul 500ul	sample dilution buffer

After dilution and before pipetting into the microtiter plate, the samples must be mixed thoroughly to prepare a homogenous solution.

6.2.2 Sample Storage

Samples should not be stored more than 7 days at 2-8°C. Extended storage is possible at ≤-20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

6.3 Preparation of Kit Reagents

Bring all reagents to room temperature before use.

6.3.1 Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

6.3.2 Negative Control Sera / Standard Sera (ready-to-use)

Negative control and standard sera are ready-to-use. For each test run (independent of the number of microtiter test strips to be used) negative control and standard sera must be included. Standard sera should be set up in duplicate.

6.3.3 Anti-human IgA or IgG Conjugate (ready-to-use)

6.3.4 Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with distilled H_2O to a final volume of V_2. Bottles used for the working solution should be cleaned regularly. Discard cloudy solutions.

Example:

Buffer concentrate (V_1)	**Final volume (V_2)**
33.3ml	1000ml
1.0ml	30ml

6.3.5 Dilution Buffer for Samples (ready-to-use)

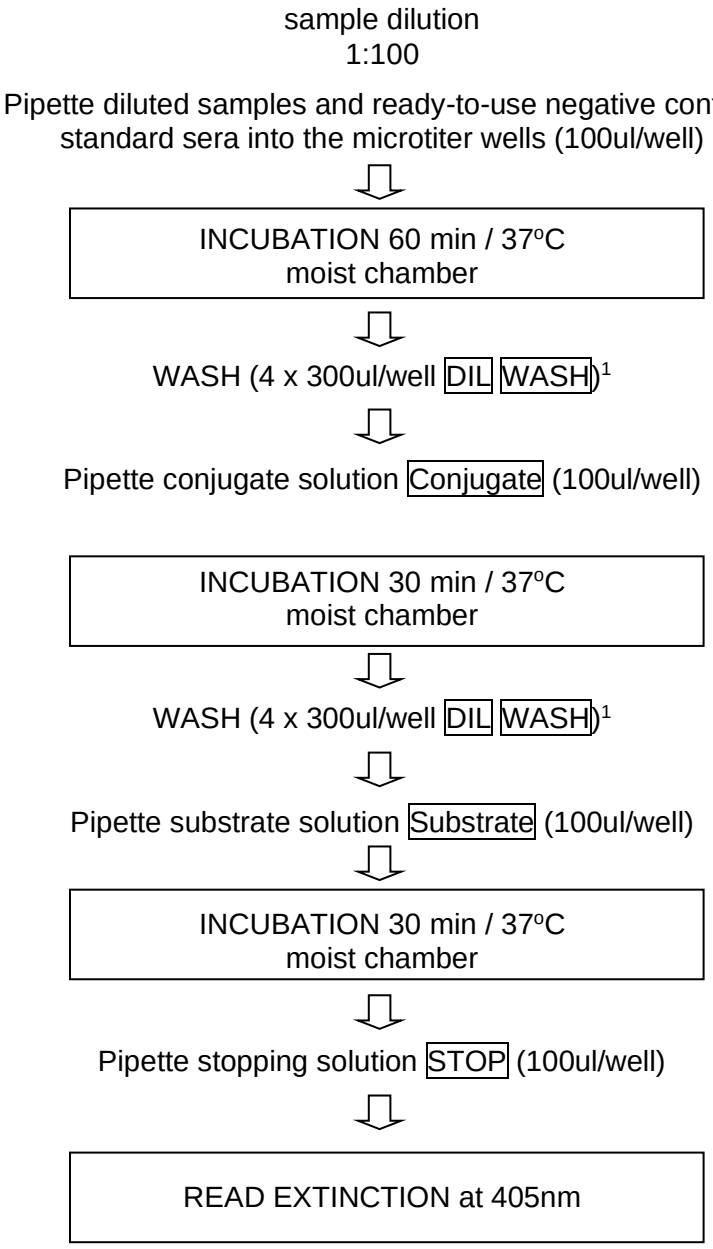
Discard cloudy solutions.

6.3.6 Substrate (ready-to-use)

Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product! Avoid contamination.

6.3.7 Stopping Solution (ready-to-use)

6.4 Overview – Test Procedure



[1]For manual use: tap plate at the end of the wash procedure on paper towel.

6.5 Manual Test Procedure

1. Place the required number of **wells in the frame** and prepare a protocol sheet.
2. Add each **100ul of diluted sample or ready-to-use negative control / standard sera** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

Well	Quantitative ELISA
A1	Substrate blank
B1	Negative control serum
C1	Standard serum
D1	Standard serum
E1	Sample 1 . . .
F1	Sample 2 . . .

3. **Sample incubation** for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber.
4. After incubation **wash** all wells with washing solution (by automated washer or manually):
    - aspirate or shake out the incubation solution
    - fill each well with 300ul washing solution
    - aspirate or shake out the washing solution
    - repeat the washing procedure 3 times (altogether 4 times!)
    - dry by tapping the microtiter plate on a paper towel
5. **Addition of conjugate**
   Add 100ul of the ready-to-use IgA/IgG conjugate to the appropriate wells (except substrate blank).
6. **Conjugate incubation** for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber.
7. After incubation **wash** all wells with washing solution (see above).
8. **Addition of substrate**
   Add 100ul of ready-to-use substrate solution to each well (including well for substrate blank!)
9. **Substrate incubation** for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber. Ensure incubation is in the dark.
10. **Stopping the reaction**
    Add 100ul of stopping solution to each well, shake microtiter plate gently to mix.
11. **Read extinction**
    Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

6.6 Automated Test Procedure

SERION ELISA *agile* are suited for use with Immunomat (using the following consumables: VT124, VT111, VT112) and suited for processing on similar analyzers. For processing on the Immunomat, the current software version including reagent check has to be used. Please note that under special working conditions, internal laboratory adaptations of the substrate incubation times may be necessary.

6.7 SERION ELISA *controls* (external Positive Control / Accuracy Control)

For the periodic verification of the test method, and in order to fulfill the requirements of laboratory internal quality management systems, we recommend using SERION ELISA *controls* to determine precision and accuracy of SERION ELISA *agile* test runs. SERION ELISA *controls* are separately available, and the usage is described in specific instruction manuals. SERION ELISA *controls* are not available in all countries, and the customer should consult the local distributor.

7 TEST EVALUATION

7.1 Qualitative Evaluation

For the SERION ELISA *agile* test evaluation, a lot-specific quality control certificate with standard curve and an evaluation table is included in the test kit so that the obtained OD values may be assigned to the corresponding antibody activities. The substrate blank must be subtracted from all OD values prior to evaluation. Mean OD value of the standard serum STD tested in duplicate has to be used.

Method 1:
In the first line of the evaluation table, several ranges of OD values for the standard serum are depicted covering the whole standard validity range. According to the measured mean OD value of the standard serum, the corresponding column can be chosen. That column contains the information of the upper and lower cut-off OD values to allow evaluation of the test sample. OD values below the lower cut-off are evaluated as negative for antibody and values above the upper-cut off are evaluated as positive for antibody. Implementation of the correction factor F is not necessary in the context of the evaluation table.

Method 2.
To fix the cut-off ranges, multiply the mean value of the measured standard OD with numerical data of the quality control certificate (see special case formulas), e.g.:

OD = 0.502 X MW (STD) with upper cut-off
OD = 0.352 X MS(STD) with lower cut-off

7.2 Quantitative Evaluation

The mathematical curve fitting for antibody quantification with SERION ELISA *agile* immunoassays is based on the 4-parameter logistic (4 PL) function

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve and are indicated on the quality control certificate of each individual SERION ELISA *agile* test.

The correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ STD\ OD\ value}$$

7.3 Automated Evaluation / Software

Institut Virion\Serion recommends the use of the SERION *easy*/ANALYZE software for the automated evaluation of optical measurement signals.

7.4 Borderline Range

The borderline ranges are specified on the quality control certificate and indicate the range of borderline test results. Values below this range indicate a negative result; values above the borderline range indicate a positive result.

7.5 Limits of Quantification

The limits of quantification are specified on the quality control certificate. If a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

7.6 Criteria of Validity

- The substrate blank must be <0.25 OD.
- The negative control must be negative.
- The mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which is given on the lot-specific quality control certificate.
- The variation of OD values of the standard serum must not be higher than 20%.

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

8.1 Cross-reactivities

SERION ELISA *agile* SARS-CoV-2 IgA
No cross-reactivity with sera positive for Epstein-Barr Virus VCA IgM, Adenovirus IgA, Influenza A Virus IgA, rheumatoid factor (RF), anti-nuclear antibodies (ANA), and other coronaviruses has been observed. Potential cross-reactivities not tested cannot be ruled out.

SERION ELISA *agile* SARS-CoV-2 IgG
No cross-reactivity with sera positive for Epstein-Barr Virus VCA IgG, Adenovirus IgG, Influenza A Virus IgG, rheumatoid factor (RF), anti-nuclear antibodies (ANA), and other coronaviruses has been observed. Potential cross-reactivities not tested cannot be ruled out.

8.2 Interfering substances

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To determine the influence of interfering substances, sera with different reactivities were analyzed with SERION ELISA *agile* SARS-CoV-2 IgA/IgG. No interferences have been detected for sera with concentrations up to 2.00g/L hemoglobin, 11.50g/L lipemia/triglyceride 0.,201g/L bilirubin (conjugated and unconjugated), 1.6mg/ml EDTA, 16 IU/ml heparin, or 0.106 mol/L citrate.

8.3 Disposal

Please observe the relevant statutory requirements!

9 REFERENCES

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