# SARS-CoV-2 NeutraLISA

- **Surrogate virus neutralisation test (sVNT) for the determination of neutralising antibodies that inhibit the binding of SARS-CoV-2 S1/RBD to ACE2 receptors**
- **Supports the evaluation of the individual immune response following SARS-CoV-2 infection or vaccination with S1/RBD-based vaccines**
- **Established ELISA method – automatable and suitable for routine laboratory diagnostics, no BSL-3 laboratory required**

## Technical data

| | |
|---|---|
| **Antigen** | Solid phase: S1/RBD domain of the SARS-CoV-2 spike protein; liquid phase: human receptor protein ACE2 (angiotensin-converting enzyme 2); each presented recombinantly in human cells |
| **Test evaluation** | Semiquantitative, calculation of the inhibition in percent (%IH):<br>%IH = 100% - (extinction of patient sample x 100% / extinction of blank (mean))<br>Recommended upper threshold of the normal range (cut-off value): 25% IH |
| **Result interpretation** | EUROIMMUN recommends interpreting results as follows:<br>%IH <20: negative<br>%IH ≥20 to <35: borderline<br>%IH ≥35: positive |
| **Sample dilution** | Serum or plasma, 1:5 in working-strength sample buffer |
| **Reagents** | Enzyme conjugate and ACE2 dilution buffer can be used lot-indepent for the SARS-CoV-2 NeutraLISA; wash buffer, chromogen/substrate and stop solutions are interchangeable between EUROIMMUN ELISA lots and products |
| **Test procedure** | 60 min (37 °C) / 30 min (RT) / 15 min (RT) (sample/conjugate/substrate incubations), fully automated |
| **Measurement** | 450 nm, reference wavelength between 620 nm and 650 nm |
| **Test kit format** | 96 break-off wells; kit includes all necessary reagents and two controls |
| **Order number** | **EI 2606-9601-4** |

## Clinical significance

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) belongs to the coronavirus family, genus *Betacoronavirus,* and is the causative agent of COVID-19 (coronavirus disease 2019*).* SARS-CoV-2 is mainly transmitted by the respiratory uptake of virus-containing droplets and aerosols produced during speaking, breathing, coughing and sneezing. The incubation time of SARS-CoV-2 is three to seven, maximally 14 days. The infection can proceed asymptomatically or cause symptoms of a febrile diseases with irregular lung infiltrates. Some patients, especially elderly or chronically ill patients, develop acute respiratory distress syndrome (ARDS).

Suitable methods for the diagnosis of SARS-CoV-2 infections are the detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) or of virus protein by means of ELISA primarily in sample material from the upper (nasopharyngeal or oropharyngeal swab) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, etc.). The determination of antibodies enables confirmation of SARS-CoV-2 infection in patients with typical symptoms and in suspected cases. It also contributes to monitoring and outbreak control. The spike (S) and nucleocapsid (N) proteins of SARS-CoV-2 are highly immunogenic. To enter and thus infect new cells, the virus binds to the ACE2 receptor of the host cells using the receptor binding domain (RBD) of the spike protein. The RBD is the target antigen of over 90% of the neutralising antibodies in COVID-19 patients. Neutralising antibodies are associated with protective immunity against a second infection with SARS-CoV-2. The spike protein is therefore the target protein of almost all COVID-19 vaccines. Around 90% of SARS-CoV-2 patients develop specific antibodies up to day 10 after symptom onset. IgG, IgA and IgM against the spike protein often appear at the same time. For significant serological results, two patient samples should be investigated, one from the acute phase (week 1 of the illness) and one from the convalescent phase (3 to 4 weeks later).



EUROIMMUN Medizinische Labordiagnostika AG · Seekamp 31 · 23560 Lübeck (Germany) · Phone: +49 451 2032-0 · info@euroimmun.de · www.euroimmun.com

## Test principle

The test kit contains microplate strips each with 8 break-off reagent wells coated with recombinantly presented S1/RBD domain of the spike protein of SARS-CoV-2. In the first reaction step, the controls and samples are diluted with sample buffer containing soluble biotinylated ACE2 and then incubated in the reagent wells. If neutralising antibodies are present in the sample, they compete with the ACE2 receptor for the binding sites of the SARS-CoV-2 S1/RBD proteins. Unbound ACE2 is removed in a subsequent washing step. To detect the bound ACE2, a second incubation step with peroxidase-labelled streptavidin is performed, which catalyses a colour reaction in the third reaction step. The intensity of the formed colour is inversely proportional to the concentration of neutralising antibodies in the sample.

## Diagnostic sensitivity

The diagnostic sensitivity was determined by investigating 124 samples from convalescent COVID-19 patients, collected 15 days after symptom onset and positive in the neutralisation test ($PRNT_{50}$ or $NT_{50}$), using the EUROIMMUN SARS-CoV-2 NeutraLISA. The sensitivity amounted to 95.9 %.

| Days after symptom onset | EUROIMMUN SARS-CoV-2 NeutraLISA | | |
|---|---|---|---|
| | positive | negative | Sensitivity* |
| ≥ 15 | 118 | 5 | 95.9 % |

* Borderline results (n = 1) excluded

## Specificity

The specificity of the SARS-CoV-2 NeutraLISA was determined by analysing 159 samples that were positive for antibodies against other human pathogenic coronaviruses, other pathogens or against rheumatoid factors or that were negative in the neutralisation test ($NT_{50}$). Additionally, 600 samples collected from blood donors and children prior to the occurrence of SARS-CoV-2 (before January 2020) were investigated. The specificity of the SARS-CoV-2 NeutraLISA was 99.7 %.

| n | EUROIMMUN SARS-CoV-2 NeutraLISA | | |
|---|---|---|---|
| | positive | negative | Specificity* |
| 759 | 2 | 755 | 99.7 % |

* Borderline results (n = 2) excluded

## Method comparison

74 samples from patients with past confirmed SARS-CoV-2 infection were investigated using the EUROIMMUN SARS-CoV-2 NeutraLISA and a plaque reduction neutralisation test ($PRNT_{50}$ according to Wölfel et al. 2020). The agreement between the qualitative results of the tests was 98.6 %.

| n = 74 | | EUROIMMUN SARS-CoV-2 NeutraLISA | |
|---|---|---|---|
| | | positive | negative |
| SARS-CoV-2 $PRNT_{50}$ | positive | 71 | 0 |
| | negative | 1 | 2 |

52 samples from patients with past confirmed SARS-CoV-2 infection were investigated using the SARS-CoV-2 NeutraLISA from EUROIMMUN and a commercial surrogate neutralisation test (NT). The agreement between the qualitative results of the tests was 96.2 %.

| n = 52 | | EUROIMMUN SARS-CoV-2 NeutraLISA | |
|---|---|---|---|
| | | positive | negative |
| Commercial surrogate NT | positive | 50 | 2* |
| | negative | 0 | 0 |

* For this sample, no information on the clinical precharacterisation and no follow-up samples were available. Both samples were negative in the $PRNT_{50}$.

111 samples from patients with past confirmed SARS-CoV-2 infection were investigated using the SARS-CoV-2 NeutraLISA and the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) from EUROIMMUN. The agreement between the qualitative results of the tests was 99.1 %. Borderline results (n = 2) were excluded from the calculation.

| n = 111 | | EUROIMMUN SARS-CoV-2 NeutraLISA | | |
|---|---|---|---|---|
| | | positive | borderline | negative |
| EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG) | positive | 107 | 2 | 1 |
| | negative | 0 | 0 | 1 |

## Cross reactivity

55 samples positive for antibodies against at least one human pathogenic coronavirus (HCoV HKU1; HCoV OC43; HCoV NL63; HCoV 229-E) were analysed using the SARS-CoV-2 NeutraLISA. No cross reactions with antibodies against these endemic HCoV were observed in the analyses.



EUROIMMUN Medizinische Labordiagnostika AG · Seekamp 31 · 23560 Lübeck (Germany) · Phone: +49 451 2032-0 · info@euroimmun.de · www.euroimmun.com
EI_2606_D_UK_F02, 04/2021