Elecsys® Anti-SARS-CoV-2 Immunoassay

On May 2, 2020, *Roche Diagnostics received Emergency Use Authorization for the Elecsys® Anti-SARS-CoV-2 immunoassay.*

This in vitro test uses human serum or plasma (Li-Heparin, K2 EDTA, or K3 EDTA) and is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. Laboratories can run the test on Roche's ***cobas e*** *411,* ***e*** *601,* ***e*** *602, and* ***e*** *801 analyzers. These fully-automated systems provide SARS-CoV-2 antibody test results in approximately 18 minutes.*

Clinical data for this antibody test:

- *99.81% specificity achieved through the testing of 5,272 samples*
- *No cross reactivity found in 80 samples from individuals with past infection to common cold and other coronaviruses*
- *100% sensitivity ≥14 days after PCR confirmation*

Clinical utility of serological tests

Serological tests are blood tests that are used to detect the presence of antibodies (Abs) produced by the immune system in response to the infection. Once a correlation between the presence of antibodies and immunity is established, antibody assays may be useful for antibody prevalence screening and support of “return to work” strategies.

Antibody assays largely may be useful for:

1. The determination of seroprevalence in a given population to provide better clarity on virus circulation dynamics, case fatality rate, or the number of susceptible individuals. Continued surveillance of seroprevalence will also help determine ‘herd immunity’ under the assumption that if enough of the population develops (presumed) immunity, the risk of spreading the virus is dramatically decreased.
2. Identification of individuals who have been previously infected with SARS-CoV-2 and who may now be considered as potential convalescent plasma donors.
3. As a potential indicator of infection following a negative SARS-CoV-2 nucleic acid amplification test result in patients with symptoms of COVID-19 who present later in their illness.

For all applications, an antibody assay needs to reliably detect late or past infection and exhibit very high specificity for high affinity antibodies against SARS-CoV-2, without cross-reactivity to antibodies against related viruses. High clinical specificity is also critical as some populations have a low SARS-CoV-2 infection prevalence, which ultimately influences the positive predictive value (PPV) of the test. Serological immunoassays for SARS-CoV-2 maximized for specificity improve the confidence for providers to determine who has been exposed to SARS-CoV-2, especially in low prevalence populations.

Antibody properties

Antibody affinity

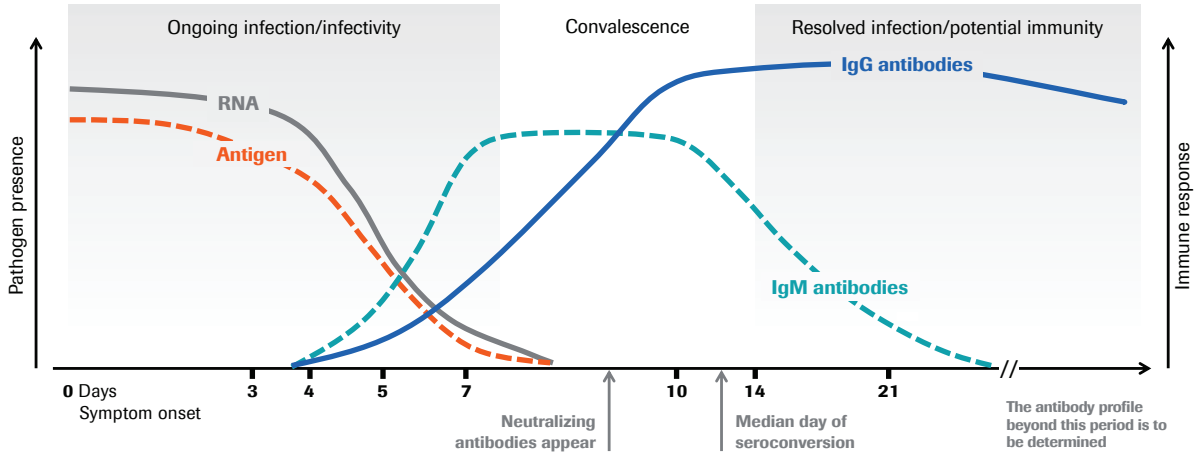
Antibody affinity is a measure of the strength of the interaction between an antigen’s epitope and an antibody's antigen-binding site. **High affinity antibodies bind to the antigen quickly and keep the strong bond even in difficult conditions,** such as when both the antibody and the antigen are in solution. In an immunoassay, this translates into exquisite specificity.

Affinity maturation

Affinity maturation is the process by which antibodies gain increased affinity, overall avidity, and anti-pathogen activity. The enhanced affinity is the result of somatic hypermutation (SHM) of immunoglobulin genes in B cells, coupled to selection for antigen binding. As successive generations of B cells mutate and are presented to the antigen, only those that recognize the antigen with high affinity will survive, while B cells producing antibodies with low affinity will be eliminated[1]. **Although IgG is traditionally thought to only appear late in immune maturation, there is evidence of low affinity IgGs appearing alongside IgM in SARS-CoV-2.[2,3]**

SARS CoV-2 infection

Illustrative course of molecular and serological biomarkers[2-14]



Assay designs of serological tests

Serological tests may be designed to detect one of the following:

1. A specific antibody class, such as IgA, IgM, or IgG
2. “Total antibodies” that detect both IgM and IgG but do not differentiate between them
3. **High-affinity antibodies, regardless of antibody class.** Note: this format differs from “total antibody” assays because the affinity of the immunoglobulin is a key factor in reactivity.

There are two approaches for reliable detection of late infection:

1. Design an IgG-specific assay, because onset of IgG is thought to be later than IgM
2. Design a high affinity (class independent) assay, because high affinity antibodies with potential neutralizing properties occur in later infection.

Assay design rationale

The Elecsys Anti-SARS-CoV-2 antibody test was designed to **maximize specificity** through detection of high-affinity antibodies.

Significant research and development drove the decisions for assay design and target antigen selection. The final assay was selected from more than 40 different combinations of four different assay formats and four different antigen targets including nucleocapsid, spike, envelope, and membrane epitopes. The antigens were selected based on the sequence of SARS-CoV-2 strains, the sequence of other coronaviruses, scalability, solubility, and information on immunogenicity. Parallel testing of the assay prototypes was performed with several hundred clinical pre-pandemic samples. The nucleocapsid antigen target was selected due to experimental data performance and manufacturability. This target exhibited the:

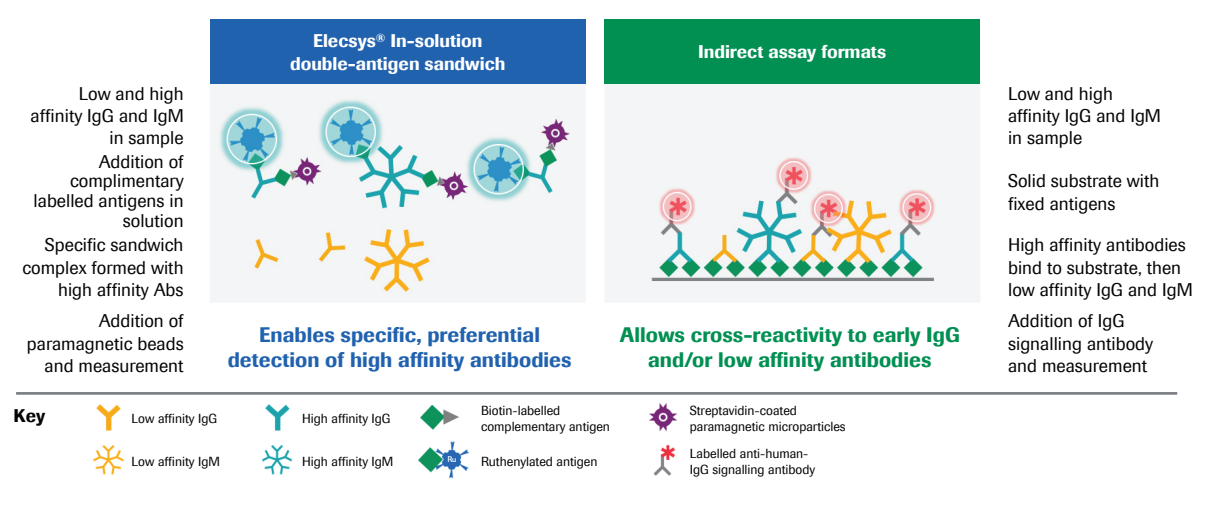
1. Highest specificity of all the tested antigen targets
2. Ability to be synthesized in soluble form
3. Reliability for large scale production

The Roche assay is unique among the currently commercially available immunoassays because it detects high-affinity antibodies to SARS-CoV-2. The assay relies on a double antigen sandwich (DAGS) format that enriches detection of higher affinity antibodies, which are more likely to be specific for SARS-CoV-2. Although this assay format is agnostic to the antibody isotype and in principle can detect high affinity antibodies of all isotypes, it preferentially detects IgG antibodies since these are most likely to evolve to become high affinity. The nucleocapsid antigen is abundantly expressed and is a useful target for sensitive detection of virus-specific antibodies. These features provide an optimal combination of high specificity and sensitivity for the detection of immune exposure to SARS-CoV-2.

In contrast, indirect IgG assays, which are selective for the IgG class, utilize the avidity of the antigen/antibody reaction. Because the antigens are immobilized on a solid surface and are presented in excess, there is a greater chance that a low affinity antibody will remain bound. Immunoassays prone to binding low affinity antibodies will demonstrate decreased specificity compared to those targeting high affinity antibodies.

Antibody assay formats

Direct vs Indirect[15-20]



Serology tests do not specifically discriminate between neutralizing and non-neutralizing antibodies. **Tests directed towards the detection of high affinity antibodies increase the likelihood of including neutralizing antibodies.** To date, investigators have shown neutralizing properties in anti-nucleocapsid antibodies, anti-spike antibodies, and anti-receptor binding domain antibodies present in samples from recovered SARS-CoV-2 infected patients[12,21-23]. At this time, there is no definitive evidence about the presence of neutralizing antibodies in SARS-CoV-2 exposed patients or whether the presence of an immune response confers protective immunity, but evidence continues to be generated.

Naming conventions

The Elecsys® Anti-SARS-CoV-2 uses a modified recombinant protein representing the nucleocapsid (N) antigen for the determination of antibodies against SARS-CoV-2. **For the greatest flexibility in offering multiple serology assays in a single Laboratory Information System, we recommend referring to this test as an Anti-SARS-CoV-2 antibody test.** For more specific information for providers, results can include a footnote or comment referring to the DAGS technology and a reference to the package insert.

Indications for testing[24]

The test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown how long antibodies persist following infection and whether the presence of antibodies confers protective immunity.

The Elecsys Anti-SARS-CoV-2 assay should not be used to diagnose acute SARS-CoV-2 infection. Results are for the detection of SARS-CoV-2 antibodies.

References

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24. Elecsys® Anti-SARS-CoV-2 method sheet (v2, May 2020)

Warning:

- Not for screening of donated blood
- This test has not been FDA cleared or approved
- This test has been authorized by FDA under an EUA for use by authorized laboratories
- This test has been authorized only for detecting the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb- 3(b)(1), unless the authorization is terminated or revoked sooner