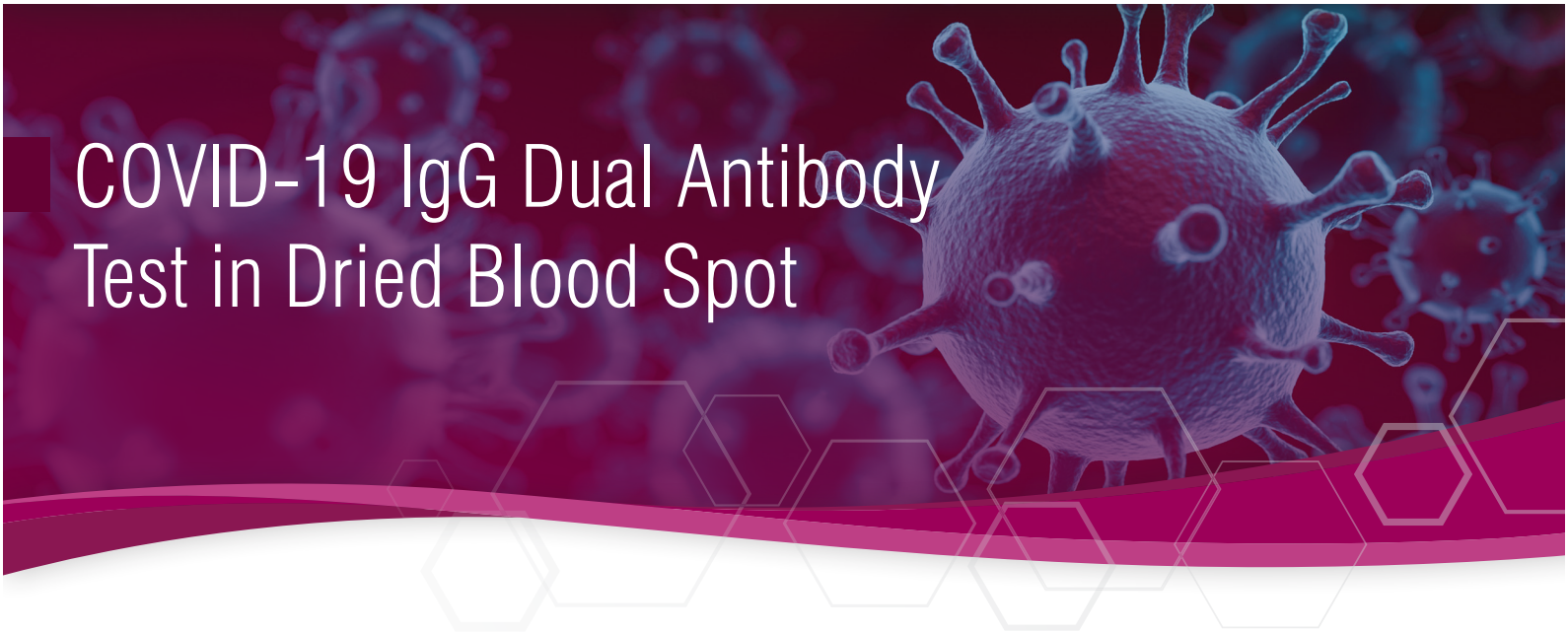
ZRTLABORATORY

PROVIDER DATA SHEET

COVID-19 IgG Dual Antibody Test in Dried Blood Spot



The rapid global spread of the respiratory coronavirus SARS-CoV-2 that manifests as the disease known as COVID-19 has in just a few short months wrought devastating consequences to the physical and economic health of society. The virus is believed to have originated in Hubei Provence, China, in 2019. The virus invades host cells through the interaction of a spike protein with the ACE2 receptor, present in many different cell types throughout the body (e.g., lung, vascular, heart, intestine, testes) that are vulnerable to COVID-19 infection. COVID-19 has an incubation period averaging 5 days[3] and can be spread by people before they develop symptoms of disease, resulting in widespread impacts on health care systems, the economy, and social interaction. The CDC publishes up-to-date statistics on the extent of the infection in the US on its website[4].

Testing blood for COVID-19 specific antibodies is key to identifying people who have been exposed to the virus and is essential for tracking exposure and controlling further viral spread and outbreaks that may occur. This is especially important for medical professionals, first responders and service workers who are at a high risk of contracting or spreading COVID-19 infections due to their direct contact with others.

The FDA cautions that a negative COVID-19 antibody test result cannot rule out exposure to the virus, especially in people with no symptoms, a short symptom duration, or those in the early stages of the disease (< 10 days from symptom onset). Antibodies may not develop at detectable levels until about 10 days from symptom onset[2,5,6]. It is also not yet known if the presence of antibodies confers immunity to a second infection[7].

According to the Infectious Diseases Society of America (IDSA)[8], antibody testing may be useful for:

- Patients with symptoms suggestive of COVID-19 who didn't receive molecular RT-PCR virus testing;
- Identification of convalescent plasma donors;
- Evaluation of vaccine responses;
- Studies of disease prevalence.

Who Should Test?

- Patients with a confirmed* COVID-19 infection who have had no symptoms for at least 7 days
- Patients without a confirmed* COVID-19 infection but had a flu-like illness and have had no symptoms for at least 7 days
- Patients without a confirmed* COVID-19 infection who had few or no symptoms but want to see if they have developed antibodies

Note: *Testing should be done at least 20 days after symptoms first started to allow enough time for IgG antibodies to be produced by the body (seroconversion)[1,2].*

* Confirmed by RT-PCR (molecular) nasal/mouth swab/saliva testing

Dried Blood Spot Testing
Minimally-invasive test kits

Serologic Tests for COVID-19 Antibodies

Detection of antibodies against SARS-CoV-2 is an indirect marker of COVID-19 infection. Blood is most commonly used to test for antibodies against SARS-CoV-2 viral antigens using lateral flow (LFA), enzyme linked (ELISA), or chemiluminescent (CLIA) immunoassays. The most common SARS-CoV-2 viral antigens to test against include the S1 spike protein (S1), nucleocapsid protein (NP), and the receptor binding domain (RBD). Antibody testing primarily involves Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies, but also can look at Immunoglobulin A (IgA). IgM and IgA antibodies are produced as a first-line defense against COVID-19, followed by specific long-term defense IgG antibodies. Antibody tests may look at total antibody levels (IgA/IgM/IgG), a combination of the two (IgM/IgG), or a single antibody. IgM and IgA antibody tests are less specific than IgG, meaning there is a higher probability of cross reactions with antibodies from viruses other than SARS-CoV-2 (false positives), such as SARS-CoV and MERS[5,9,10]. IgG antibodies are associated with viral neutralizing activity and may play a part in long term immune protection from COVID-19 re-infection[11,12].

The incubation period of a COVID-19 infection can be up to 14 days, where the viral load is increasing, but antibodies are not yet present[13] For COVID-19 infections specifically, mean seroconversion of IgM and IgG occurs around 13-15 days following symptoms, with the greatest chance of detecting IgG antibodies in all those infected at 25-27 days from the start of symptoms[14]. IgM and IgA antibodies begin to drop once infection ceases while IgG antibodies remain elevated for an indeterminate time, since SARS-CoV-2 has only been around since December 2019.

ZRT Laboratory's dried blood spot COVID-19 dual antibody test looks at IgG antibodies against the SARS-CoV-2 S1 spike and nucleocapsid proteins. Two separate results will be provided, which will help confirm true positives and reduce false negatives. The CDC has stated the importance of reflex testing to rule out false positives, and literature shows that the SARS-CoV-2 antibody response against different viral proteins can vary significantly and at different times[14,15,16]. Unfortunately, most reflex tests (if they are offered) only retest if the first result is positive, which means missed false negative results. ZRT Laboratory is the first laboratory to provide two separate IgG antibody results for every sample, as we believe this is important for confirmation purposes and due to the variation in antibody responses to the SARS-CoV-2 virus.

We recommend that patients test at least 20 days from when the symptoms first appeared, to be sure that the COVID-19 IgG antibodies are present in sufficient quantity for the ELISA assays used by ZRT Laboratory to detect them. It is possible in primarily asymptomatic or mild symptom patients for antibodies to drop below detectable levels as time passes.

Point of Care, Serum or Dried Blood Spot?

Some point-of-care (POC) lateral flow cassette IgM/IgG tests are showing lower sensitivity and specificity than is required for population studies[17]. While lateral flow cassettes are fast, convenient, and can use finger stick blood samples, they produce an unacceptable number of false positives and false negatives for population studies, and many have not worked as advertised. It is also possible to misinterpret the qualitative visual results or perform the procedure incorrectly. Serum/plasma testing for COVID-19 antibodies using ELISA or chemiluminescent immunoassays has high sensitivity and specificity and allows for simultaneous testing of positive and negative controls run in concert with the patient samples. Lateral flow cassettes do not offer positive and negative controls.

An ideal alternative to serum testing for COVID-19 antibodies is collection of whole blood from the finger with the use of a lancet. Blood drops are deposited on a filter card, dried, and then sent to the laboratory for testing at our CLIA-certified high-complexity laboratory. This combines the convenience of finger-stick collection with the high sensitivity and specificity of ELISA testing. ZRT has been performing hormone and other analyte

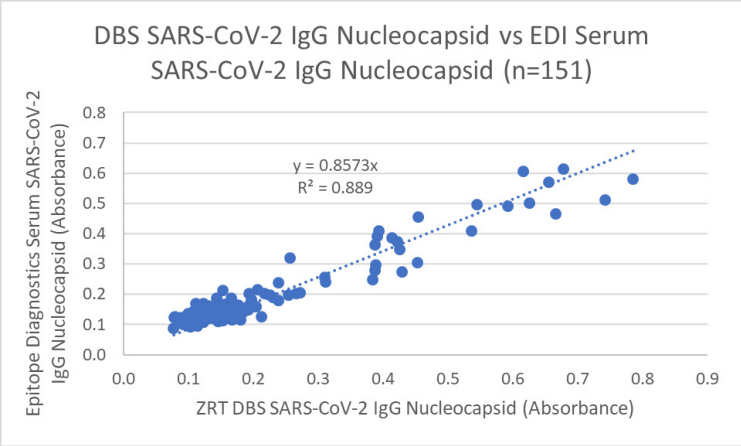
testing in finger stick dried blood spots (DBS) for over 15 years and has now applied this simple collection to COVID-19 antibody testing. Some of the more obvious advantages of DBS vs conventional serum testing are:

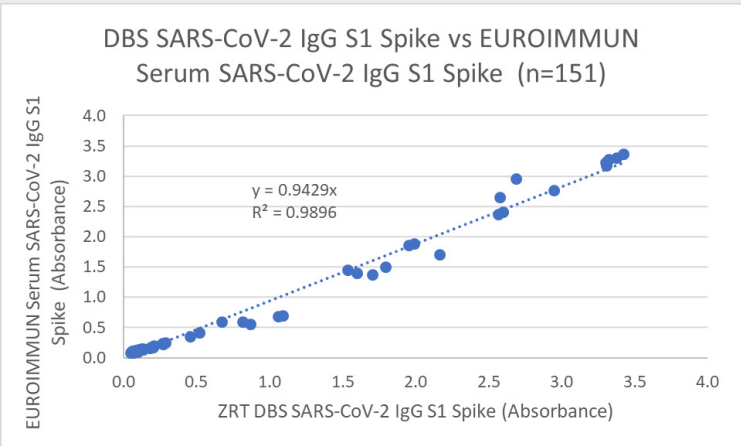
- Easy to collect – see our sample collection instructions and videos at www.zrtlab.com
- Safe – finger stick DBS collection has been safely performed for decades
- Stable – unlike serum there are no requirements for freezing or refrigerating samples for transportation; DBS samples being tested for SARS-CoV-2 IgG are stable for at-least 30 days across all expected shipping temperatures and conditions according to our validation work
- Quality and quantity of blood spot sample is visible on the filter card – unlike nasal or mouth swabs used for molecular (PCR) testing which have high false negative rates due to improperly collected samples, it is very easy to see blood on filter paper and determine the quality of collection
- Easy to ship – according to the CDC, DBS are exempt human specimens for shipping purposes.
- Low viral risk for sample handlers – most viruses are not viable once dried, making DBS safer to work with than liquid whole blood or serum, or respiratory or fecal samples[18]. Live virus doesn't last more than about 24 hours on cardboard, resulting in minimal risk to mail carriers and lab personnel receiving specimens for testing[19].

Sensitivity and Specificity of the Test

As with all diagnostic testing, screening for COVID-19 IgG antibodies is only of value if it is highly sensitive and specific. Specificity of a test means that a negative result correctly identifies people who do not have the condition being tested, while sensitivity means that positive results correctly identify people who do have the condition[20]. Tests with a high false positive rate (low specificity) will result in people believing they have been exposed to the virus when in fact they have not[21]. This can happen when the test can't properly distinguish between antibodies to SARS-CoV-2 and other past viral infections.

The dried blood spot test for COVID-19 IgG antibodies at ZRT is exceptionally sensitive and specific for SARS-CoV-2 IgG antibodies. ZRT is a CLIA-certified high complexity testing facility and all dried blood spot assays are stringently validated as laboratory developed tests (LDTs). Our own studies have shown strong concordance between serum and dried blood spot samples from the same individuals.





The graphs show excellent correlations between dried blood spot and a matching serum sample from the same individual, using ELISA assay kits manufactured by Epitope Diagnostics, Inc (EDI) and Euroimmun.

Positive and negative concordance are used to compare two assay methods. In this case, the ZRT DBS SARS-CoV-2 IgG S1 Spike assay was compared to the EUROIMMUN Serum SARS-CoV-2 IgG S1 Spike assay, and the ZRT DBS SARS-CoV-2 IgG Nucleocapsid assay was compared to the Epitope Diagnostics (EDI) Serum SARS-CoV-2 IgG Nucleocapsid assay. The serum assays were run according to manufacturer recommendations. Results can be seen below.

		EDI SARS-CoV-2 IgG Nucleocapsid – Serum		
		Positive	Negative	Total
ZRT SARS-CoV-2 IgG Nucleocapsid Results Dried Blood Spot	Positive	24	3	27
	Negative	0	124	124
	Total	24	127	N=151

ZRT's dried blood spot SARS-CoV-2 IgG Nucleocapsid results and matching EDI serum SARS-CoV-2 IgG Nucleocapsid results show 100% positive concordance and 97.6% negative concordance.

		EUROIMMUN SARS-CoV-2 IgG S1 Spike – Serum		
		Positive	Negative	Total
ZRT SARS-CoV-2 IgG S1 Spike Results Dried Blood Spot	Positive	24	1	25
	Negative	0	126	126
	Total	24	127	N=151

ZRT's dried blood spot SARS-CoV-2 IgG S1 Spike results and matching EUROIMMUN serum SARS-CoV-2 IgG S1 Spike results show 100% positive concordance and 99.2% negative concordance.

Sensitivity and specificity are used to describe the performance of a serology assay. Sensitivity is the assay's ability to correctly identify patients with antibodies (true positive rate). Specificity is the assay's ability to correctly identify patients without antibodies (true negative rate). PCR molecular testing is used to confirm a true positive/negative result, but it is possible for there to be false negatives with SARS-CoV-2 PCR testing, so results must be looked at with caution. Results can be seen below.

		PCR-Confirmed Results		
		Positive	Negative	Total
ZRT SARS-CoV-2 IgG Nucleocapsid Results Dried Blood Spot	Positive	19	1	20
	Negative	1	14	15
	Total	20	15	N=35

ZRT's dried blood spot test shows 95% sensitivity and 93.3% specificity for antibodies to the nucleocapsid protein.

		PCR-Confirmed Results		
		Positive	Negative	Total
ZRT SARS-CoV-2 IgG S1 Spike Results Dried Blood Spot	Positive	19	0	19
	Negative	1	15	16
	Total	20	15	N=35

ZRT's dried blood spot test shows 95% sensitivity and 100% specificity for antibodies to the S1 spike protein.

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