# Understanding your IMMUNO-COV test results

The IMMUNO-COV test measures the level of protective SARS-CoV-2 virus neutralizing antibodies in your blood.

**Why measure your level of virus neutralizing antibodies (vnAbs)?**
Because vnAbs are the sentinels of immune defense that guard against COVID-19

***About COVID-19***

COVID-19 is a new disease caused by a virus (SARS-CoV-2). It spreads in the air and infects the upper airways, then spreads to the lower airways and lungs. The infection can be (i) asymptomatic, in which case you don't know you have it; (ii) symptomatic but mild in which case it is similar to a bad cold and you will probably recover quickly; or (iii) severe, requiring hospitalization, in which case you may need oxygen, you may get put on a ventilator and there is a chance you may die. Approximately 2% of people who get infected are killed by the virus infection. However, the percentage is much lower in children/young adults and much higher in the elderly(1). People with a weak immune system also have a much higher risk of death if they are infected with the virus(2).

***How the SARS-CoV-2 virus causes COVID-19***

Your body is made of cells, between 10 and 100 trillion of them. Compared to the size of a single cell, the SARS-CoV-2 virus is miniscule. Once it gets inside a cell in your body, it turns that cell into a virus factory that can manufacture up to 10 thousand new viruses in just one day. The new progeny viruses escape from the cell by killing it. Those "progeny" viruses are identical to the original virus. They infect other cells in your body, which in turn produce even more viruses. The rapid spread of the SARS-CoV-2 virus in your airways and lungs and the death of the infected cells is what makes you sick. If your immune system fails to eliminate it, the virus can eventually kill you.

***How the virus spreads from person to person***

The virus spreads to infect other people in droplets that are sprayed out into the air when you cough, sneeze or speak (we splutter when we speak, even if it doesn't seem like it). The droplets carry progeny viruses that have been released from infected cells lining the nose, mouth, throat and upper airways of people who have COVID-19. When those droplets get inhaled by someone else, they come into contact with the cells lining the nose, mouth, throat or upper airways of that person and can start a new infection. This is called virus transmission.

***What we know about the immune response to the SARS-CoV-2 virus***.

Thankfully, the immune system can fight back to stop the spread of the virus. If it couldn't, the virus would probably kill us all. At the heart of the immune response are B and T lymphocytes, specialized cells that recognize and fight the virus in different ways. **Cytotoxic T lymphocytes (CTL)** can destroy newly infected cells before they have a chance to release progeny viruses. CTL are important for controlling the spread of the infection after it has gained a foothold in your body. B lymphocytes make **antibodies** which attack virus particles and persist long after you have recovered. Antibodies are especially important for stoping you from getting infected a second time. M**emory T and B lymphocytes** are also formed during the initial virus infection. They can mediate a much faster (recall) immune response if you do get infected with the virus a second time.

In summary, CTL are ~~very~~ important for overcoming an infection, antibodies stop you from getting infected a second time, and memory lymphocytes ensure that you will rapidly fight the infection if you do get infected a second time.

***What does a COVID-19 vaccine do?***

The approved/EUA COVID-19 vaccines work is to trigger your body to make a small amount of the SARS-CoV-2 surface protein ("spike glycoprotein"). Your immune system then sees this spike protein and practices fighting it. Because it is a 'foreign' protein, the spike does not last very long in the body and is eliminated by the immune system.

The vaccines get your immune system to make both antibodies and memory lymphocytes against the SARS-CoV-2 virus before you ever see the virus. Then, if you do get exposed to the SARS-CoV-2 virus, the neutralizing antibodies you made in response to the vaccine will block it. And if the virus does manage to infect you, the memory lymphocytes you made in response to the vaccine will very rapidly give rise to a large army of antibodies and CTLs to fight the virus and stop its spread.

Stated another way, if you have been vaccinated you will have neutralizing antibodies as a first line of defense against the SARS-CoV-2 virus, and memory lymphocytes as a backup.

***What are antibodies?***

Antibodies are tiny proteins that circulate in the bloodstream and leach into the mucus in your mouth, nose and airways. They are much smaller than viruses. If you are vaccinated you make numerous different antibodies that recognize and bind to different sites all over the surface of the SARS-CoV-2 virus. But there is a very important small subset of antibodies, called virus neutralizing antibodies or vnAbs, that can bind the virus AND block it from infecting a new cell. vnAbs can therefore shut the infection down, either by blocking the virus from ever infecting you, or by preventing it from spreading in your body.

It is important to note that most of the antibodies you make when you are vaccinated against the SARS-CoV-2 virus, are not vnAbs. They bind to the virus, but cannot neutralize virus infectivity and therefore do not protect you from getting infected with SARS-CoV-2. Most rapid ELISA assays and serology tests measure total binding antibodies. **The IMMUNO-COV v2.0 assay specifically measures the level (titer) of the all-important vnAbs in your bloodstream**.

***What we know about vnAbs* and virus infections**

vnAbs are known to be the critical front line of defense that prevents us from getting a second "attack" of viral illnesses such as measles, mumps, rubella or chicken pox(3). COVID-19 is no exception. If there are sufficient vnAbs in the thick fluid (mucus) lining the nose, mouth and upper airways, the virus cannot even get a foothold in the body. It gets neutralized as soon as it lands on the mucus layer. This is sterilizing immunity. Alternatively, if the virus does manage to infect a few cells at those sites of entry, its early spread to other cells will be strongly inhibited by vnAbs that circulate in the bloodstream. Thus, the infection will be very quickly eradicated. CTL are the next line of defense that help to control the later spread of the virus if it is not immediately controlled by vnAbs.

The ways by which vnAbs neutralize viruses and block their ability to infect cells are varied. One way involves blocking the ability of the virus to bind to its receptor on the cell, which is needed for the virus to enter the cell. Another way is to block the ability of the virus to enter the target cell after it has bound the receptor. Tests like cPASS that only look at the binding of the SARS-CoV-2 spike to its receptor, miss vnAbs that function in different ways. IMMUNO-COV uses a live virus for testing and therefore measures all vnAbs more accurately.

Virus neutralization assays can be modified to measure neutralizing titers against the so-called "variants of concern". These variants are new versions of the SARS-CoV-2 virus that are less efficiently neutralized by vaccine-induced vnAbs. All currently approved vaccines were specifically designed to stimulate the immune system to recognize the spike glycoprotein from the original "Wuhan" strain of the virus. So the immune system does not neutralize the variants as effectively.

**What we know about vnAbs and COVID-19 from human therapy trials**

From human monoclonal vnAb antibody therapy trials we know that **vnAbs can prevent COVID-19**. If you have been exposed to a person with COVID-19, infusing vnAbs into your bloodstream will prevent you getting the illness (postexposure prophylaxis). This has been clinically proven using Regeneron's and AstraZeneca's monoclonal vnAb drug products to prevent COVID-19(4, 5).

We also know from human studies that **vnAbs can fight the early stages COVID-19 and clear the infection**. If you have developed symptoms of COVID-19 and even if you are sick enough to

go into hospital, infusing vnAbs into your bloodstream can help to cure you. This has been clinically proven using Regeneron's vnAb drug product(6).

Another lesson from human trials with convalescent plasma is that **vnAbs cannot fight the early stages of COVID-19 infection if the levels (titers) in your bloodstream are too low**. This is why convalescent plasma therapy does not work so well as a treatment in many cases(7). If the plasma you are given comes from a donor who has recovered from COVID-19 but does not have a very high antibody titer, the treatment just doesn't work.

**What we know about vnAb titers and COVID-19 risk from human vaccine studies**

Published data from many vaccine efficacy studies shows that vaccination offers protection against COVID-19. But no vaccine offers 100% protection (some offer only 50% protection) and **infections do occur in vaccinated people**(8, 9)**.** The risk of these breakthrough infections varies greatly between individuals. It depends on their age and health, the vaccine they received, how long ago they were vaccinated and whether they encountered a variant of the virus that is less susceptible to vnAbs.

Several studies have looked at the impact of vnAb levels on the risk of vaccinated people getting COVID-19 (9-12). There are two reasons for this(13). The first reason is to have a correlative test that can tell people something meaningful about their risk of getting COVID-19 and how that risk is changing over time. Knowing the risk of future infection is particularly important for people we classify as "vulnerable". Whether because they are very elderly, have a weakened immune system, etc., Vulnerable individuals are at high risk of severe illness and/or death if they get infected. The second reason is that we need a "mechanistic correlate of protection". That is, a lab test that can tell us whether a vaccine is working so that we do not have to wait to find out if the infection rate goes down in a large (about 30,000) group of vaccinated or boosted people, which can take a very long time.

There are now several published studies pointing to the conclusion that vnAb titer is the best correlate of protection available; both for proving vaccine efficacy and for determining individual risk of infection. The findings of these studies are summarized below:

First, a direct correlation was shown between vnAb titers and the efficacy of various approved vaccines (Pfizer, Moderna, Johnson and Johnson, AstraZeneca, Sinovac, Novavax and Sputnik) (10). The conclusion is that higher vnAb titers give better protection. Mathematical analysis of the data from these studies allowed the authors to correlate specific vnAb titers with defined levels of protection against both mild symptomatic COVID-19 and severe COVID-19 (requiring hospitalization). Analysis was done for both the original "Wuhan" strain of the SARS-CoV-2 virus and specific "variants of concern" with reduced susceptibility to vnAbs.

Second, both Astra Zeneca and Moderna recently published extensive analyses of their vaccine clinical trial data. These trials specifically looked at "correlates of protection" by comparing

people who got infected after they had been vaccinated with those who did not(11, 12). Both studies showed that the vnAb titer was the best correlate of protection, and was considerably better than other ELISA-based antibody tests that were used in these studies.

**What we know about vnAb titers and "Variants of Concern"**

Unfortunately, the SARS-CoV-2 virus is evolving all the time. Every time the virus infects a cell and multiplies, there is a chance that the progeny viruses will carry new mutations. Variants of concern (VOC) are new versions of the virus that become dominant over time because they spread faster in the population and/or resist neutralization by vnAbs. The delta variant is one such VOC. Delta spreads faster than the original "Wuhan" strain of the virus and is about five-fold less susceptible to neutralization by vnAbs(14). Detailed studies of several VOCs (alpha, beta, gamma, delta) show that their faster transmission and/or vnAb resistance are caused by small changes in their viral spike glycoproteins. It is expected that new VOCs that are even more strongly resistant to vnAbs will arise in the future because that is the only way the virus will be able to keep on infecting vaccinated people.

**What we know about vnAb titers and COVID-19 protection falling over time**.

Unfortunately, after getting vaccinated or recovering from COVID-19, your vnAb titer and your protection from COVID-19 do not stay high forever. Whether you recently got vaccinated or recovered from COVID-19, your vnAb titer reaches its maximum level within a month, and then steadily falls(10, 15-17). In many people it will drop to an undetectable level within a year and sometimes within just a few months. vnAb levels fall slightly faster after vaccination than after natural infection. But whether you have been vaccinated or infected, if you start off with a higher vnAb titer it will take longer to fall to a low level that is concerning. Conversely, if you start with a low vnAb titer, you may be ready for a booster shot after just a short time.

Falling antibody titers are a major problem for national and global vaccination programs. There are many examples, but perhaps the most informative is the experience in Israel. The Israeli government tried to achieve "herd immunity" by vaccinating the majority (78%) of the population very early after the Pfizer and Moderna vaccines were approved. Unfortunately, as vnAb levels in vaccinated Israelis fell, the level of population immunity fell. By July of 2021, there was an alarming increase in the number of COVID-19 cases in Israel, affecting both vaccinated and unvaccinated people. Alarmingly, on August 15 2021, 59% of patients in Israeli hospitals for severe COIVD-19had been fully vaccinated(18). This surge was due in part to waning immunity and in part to emergence of the delta variant that is less susceptible to vaccine-induced vnAbs. IOther countries that have experienced a delta surge have seen a spike in symptomatic cases but not in hospitalizations of vaccinated people. Thus, it has been argued that memory lymphocytes may be more important than vnAbs for protection against severe COVID-19 disease. However, it remains difficult to explain how so many vaccinated Israelisgot severe COVID-19 due to the delta variant. Perhaps the people in Israel were vaccinated so early they had lost their protective levels of vnAbs by the time of the delta surge. But people in other

countries that were vaccinated later still had protective vnAb levels at the time of the surge. Whatever the reasons behind these interesting national differences, the clear underlying message is that immunity levels are falling quite rapidly in vaccinated people.

**What don't we know about the immune correlates of protection from COVID-19?**

We don't yet know how much protection remains after your vnAb titer has fallen to a very low or undetectable level. As discussed above, vnAbs are the front line of defense in vaccinated people, serving to "nip the infection in the bud". Yet, the memory response provides an important second line of defense to accelerate the elimination of an established infection and diminish its severity. Several publications have shown that the memory response remains intact even after the vnAb titer has fallen to a nonprotective level(19,20). Thus, the memory response may be able to protect against severe COVID-19 even in vaccinated or previously infected people who have lost most of their vnAbs.

There is still some uncertainty over the relative durability of the vnAb and memory responses. It is likely that patients with a weak vnAb response also have a weaker memory response. Hence the peak vnAb titer measured early after vaccination (or back-calculated from a titer measured at a later time) could potentially be used to estimate the magnitude of the memory response.

**What other tests are available for assessing your level of protection from COVID-19?**

Numerous commercial tests have been developed for the detection (and in some cases quantification) of antibodies to SARS-CoV-2. There is also at least one commercially available test that measures blood levels of T lymphocytes. Tests that look at memory cells are complicated and are not yet offered commercially.

Of the numerous antibody tests you can get, most just tell you whether you have antibodies that can bind to SARS-CoV-2 virus. These tests are "ELISA" or "lateral flow" assays and should not be used to assess your protection against COVID-19. First, these assays do not use a live virus and so they cannot specifically detect functional vnAbs. Second, they typically give you a simple yes/no answer as to whether you have antibodies, and do not quantitate your level of antibodies. ELISA and lateral flow assays are useful if you want to know whether you have ANY antibodies at all. But they cannot tell you whether those antibodies are capable of neutralizing the virus, whether you have a dangerously low level, or whether your level is falling rapidly. Some of the available ELISA tests have been adapted to make them "semi-quantitative" and are reported as a titer rather than as a simple yes/no result. However, it is important to remember these assays are not measuring functional vnAbs titers which makes them unreliable for estimating COVID-19 risk.

**How many tests are there for measuring vnAb titers?**

There are several test formats that measure bona fide vnAbs. Typically, a very small volume of blood is added to a test virus which is then put onto cells in tissue culture. If there are vnAbs in the blood sample, the virus does not infect the cells. By using different dilutions of the blood sample and assessing whether they fully or just partially block the virus infection, the vnAbs can be quantified and the result can be reported as a titer.

If the virus used to assay vnAbs is an actual SARS-CoV-2 coronavirus,the test has to be done in a level 3 containment facility with the test operators wearing protective clothing. This is the so-called PRNT (plaque reduction neutralization titer) assay. It is the gold standard against which other vnAb tests (including IMMUNO-COV) are calibrated. However, because of the need for level 3 containment, the PRNT is not a practical test to use for individual risk assessment.

To facilitate the performance of vnAb tests outside of level 3 containment, an alternative format of vnAb assay has been developed. For these tests an alternative non-dangerous virus is given a SARS-CoV-2 coat of spike glycoprotein. This virus is called a pseudovirus or surrogate virus. It gets into and infects cells using the same machinery as the wild type SARS-CoV-2 virus. vnAbs block infection by wild type viruses and by pseudoviruses to the same degree.

Although pseudovirus tests are widely used in research laboratories, they are difficult to develop for large scale. Very large batches of pseudovirus are needed to get consistent test results time and again for many years, and these batches are difficult to produce. The Imanis team addressed this problem by generating a very large batch of their uniquely designed pseudovirus. Imanis generated enough of this pseudovirus to perform millions of tests, then used this virus to perfect and fully clinically validate their highly accurate and scalable vnAb assays. To our knowledge the IMMUNO-COV test is the only vnAb tests currently available for use clinically.

Because of the importance of vnAbs as an indicator of COVID-19 protection, there have been attempts to generate a high throughput assay to measure vnAbs without needing live virus. A commercial example is the cPASS assay, which detects and quantifies antibodies capable of preventing the SARS-CoV-2 spike glycoprotein from binding the cellular receptor. Although this assay is often called a neutralizing antibody assay, it is actually a receptor binding assay and does not measure virus neutralization. Our own experience is that titers obtained using the cPASS assay do not correlate closely with vnAb titers determined using the IMMUNO-COV assay.

**What the vnAb titer can tell you about your level of protection from COVID-19**

Your **risk** of getting COVID-19 is different from the **level of protection** you get from being vaccinated. Your level of protection is what we are measuring with the vnAb titer. For unvaccinated people the risk of getting sick and/or dying from COVID-19 varies enormously from person to person and depends on your age, general health, whether you are immune suppressed, etc. (1). When you are vaccinated that risk goes down by a certain percentage. That percentage of risk reduction is what the vnAb titer can help you to understand – it is what

we call the level of protection. 50% protection means you are 50% less likely to get sick or die from COVID-19 than if you had never been vaccinated. If you already had a very low risk of getting sick, the protection makes it lower still. But if you had a high risk, then 50% protection is not so reassuring and it would be much better if you had a higher vnAb titer and a protection level better than 90%.

Based on the published and unpublished information available at this time, vnAb titer provides a good measure of the level of protection your vaccine (or your previous COVID-19 illness) has given you from symptomatic COVID-19. If you have a high titer of vnAbs you probably won't get infected. If you have a low titer you are at higher risk. This is because the vnAbs function as an effective front line of defense against the virus and can stop it from getting foothold in the body.

To interpret actual numbers for the vnAb titer, it is important to think in terms of WHO (World health Organization) International Units (IU/mL). Reporting vnAb titers in WHO units is now standard practice at Imanis Life Sciences. Using these units allows you to compare your IMMUNO-COV titer with titers measured in other laboratories, or reported in publications. Until recently most laboratories were not using WHO units, making it very difficult to understand how results from one lab compared to another.

Based on published data(10) and our own studies calibrating the IMMUNO-COV titer with WHO International Units, we have estimated protection levels. A vnAb titer below 700 IU/mL means that, if you are exposed to the delta variant, your protection against mild symptomatic COVID-19 is only modestly reduced (up to 50%) compared to what it was before you got vaccinated. However, with that same vnAb titer of 700 IU/mL your level of protection from severe, life-threatening disease is excellent (90%), making it at least ten times less likely you will end up in the hospital. If your titer drops below 140 IU/ml your level of protection from severe, life-threatening disease will be much closer to that low 50% level.

You can use the IMMUNO-COV test to assess your level of protection. IMMUNO-COV can accurately measure your titer all the way down to 22 IU/mL. For the IMMUNO-COV test we need a blood sample collected by a phlebotomist. If you prefer to get the IMMUNO-COV test we can arrange for you to have done at a local blood collection facility.

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