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Antibody Testing for COVID-19

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Version 3
May 13, 2020

It is now time for a discussion of antibody testing. Many people now want to know how many have been silently infected in the general population, how many are immune, and how this affects the fatality rate. This requires antibody testing and there is at least as much interest in this now, as there has been in the COVID-19 RT-PCR RNA testing that is used to declare someone infected.

Executive Summary

A positive RT-PCR test is used to tell people that they have COVID-19 RNA and are deemed infected and infectious, despite the technology's numerous flaws and known false positives. Antibody tests are now being used under the assumption that someone who is positive for antibodies for COVID-19 has previously been infected and, if they have recovered from symptoms, is now immune.

Antibodies are our body's immune system reaction to viral proteins, known as antigens. Antibody tests incorporate antigens, and a chemical that allows the intensity of the reaction to be measured using light. Ideally antigens would come from pure virus, but COVID-19 virus has never been purified, thus antigens are created artificially from proteins based on portions of the 30,000 base RNA genome that is believed to come from the virus.

The major antibody types that are looked for are IgM, believed to be a generic infection fighting antibody that arises about a week or so after infection, and IgG, believed to be more specific, and believed by some to take longer for the body to create. After the infection is resolved, IgM antibodies are believed to gradually disappear, while IgG remain, providing ongoing immunity.

Unfortunately, this idealized picture is not supported by the available evidence, either because the evidence does not exist, is insufficient, or because it directly contradicts the model.

Positive antibody tests should be impossible before the person is first infected (RNA positive). Yet, old blood samples (2019 or before) have tested positive in significant numbers. Almost 14% of saved blood from old donations tested positive in a Dutch study, and in the validation of the Cellex and Chembio tests, 4.4% and 3.6% of old samples were positive.

The idealized antibody model is based on the date of infection as the starting point, but this date is never known with certainty. Even when someone came into contact with a COVID-19 RNA positive person on a certain date that is not a guarantee that this was the date of infection, given that, prior to the lockdown, people could apparently be infected while playing in the park, eating at a restaurant, walking

down the street, attending a concert, or participating in any other now banned activity. When antibody surveys are performed, the vast majority of people who test positive had no idea that they had previously been infected, and cannot possibly be sure about the date. Thus, the incubation period for the virus is impossible to determine accurately, as well as the range of days after infection that IgM and IgG start to develop. This makes an accurate antibody model impossible to construct based on currently available data, despite numerous beautiful graphs showing this model in idealized form.

Simple models that illustrate the timing of antibodies show the quantity (titer) rising smoothly and, for IgM, eventually peaking and declining smoothly. Yet many studies have found negative tests throughout the symptomatic period. A test developed by the Wadsworth Centre in New York found 40% of samples negative for antibodies 11-15 days after symptoms started, and even more between 16-20 days. This indicates that antibodies may come and go randomly and not behave in a smooth and predictable fashion.

No test documentation, antibody surveys or scientific studies showed the disappearance of IgM antibodies, predicted by the model, perhaps because it does not happen, or it takes more than 30 days, the maximum examined. This might not be terribly important in practice, but it is another indication that the beautiful models shown in the form of graphs are simplistic, if not outright wrong.

Other problems with antibody tests include a significant number of samples testing antibody positive from people who were COVID-19 RNA negative (although some had 'COVID-like' symptoms), with no evidence that the person was ever infected. In one Chinese study the positive rate on presumably never infected people was 25%.

Antibody tests, like most infectious disease tests, are often reported as 'Positive' or 'Negative', but the results are really whether the intensity of a color change in the test kit was above or below an arbitrary number. The reliability of this was called into question, inadvertently, by one test manufacturer, who showed that continually diluting samples 50:50 did not result in a halving of the color change at each step. In some cases, less material resulted in significantly more intense color changes.

Researchers have tried to connect the antibody titer (in reality, this is just the color change intensity) with the severity of symptoms, but two Chinese papers that studied this had to admit that there was no difference between mildly and severely symptomatic people in the quantity of antibodies, nor between those with or without pre-existing conditions, nor in the duration of symptoms.

Test manufacturers always run their test on blood samples from people with unrelated medical conditions as a check. Even though only a small number of samples were examined, for a small number of conditions, different manufacturers found a significant percentage of samples positive for COVID-19 antibodies, that were known not to have COVID-19, but instead contained other viruses, bacteria or mycoplasma, or were from people with auto-immune conditions, indicating that the antibodies are not specific. For example, 10% of Hepatitis B samples were positive,

33% of Respiratory Synctitia Virus, 10% of auto-antibodies and 17% of Streptococcus.

A large number of population surveys have been compiled by Dean Beeler and they reveal a wide range of percentages of populations antibody positive, from less than 1% in many cases to 32% in a poor part of Boston. This is generally seen as an indication of how far through the population that the virus has rampaged. One flaw of most of these surveys is that the population is chosen non-randomly, and does not represent the general population. The group may be a household survey, volunteers, high school students and staff, health care workers, blood donors, or people going for blood tests at a lab.

But a far bigger problem is that the number produced is impossible to validate. When 1.5% of Santa Clara volunteers tested positive, it was assumed that that was truth. This 'truth' asserts that all of these people were RNA-positive at some point in the recent past. But there is absolutely no evidence for this. The 'truth' assumes that all the people were negative for COVID-19 antibodies prior to the assumed period of RNA-positivity. But there is absolutely no evidence for this.

It assumes that the 98.5% who tested negative were never RNA-positive. But there is absolutely no evidence for this. It assumes that the 98.5% never had the antibodies being looked for before. But there is absolutely no evidence for this.

I could assert that the real fraction positive in Santa Clara was 98.5%, not 1.5%, and there is no less evidence for my assertion than for the results from antibody testing.

These surveys often ask if people who tested antibody positive had 'COVID-like' symptoms in the last few weeks or months (and most say that they did not). But these symptoms (fever, cough, loss of smell or taste, fatigue) are so generic that they are absolutely not evidence that the people were previously COVID-19 RNA positive.

One solution would be a time series survey of a large number of people currently negative on both RNA and antibody tests (uninfected and never infected). Every few days these people would give a drop of blood and a nasal swab. Some would become RNA positive, and then could be examined more frequently for the exact pattern of antibody development, through to the disappearance of IgM antibodies. This experiment would be time consuming, intrusive, inefficient (as most people may never become infected) and expensive. But considering the vast sums of money spent on COVID-19 research, quarantining and treatment, and the even more tremendous sums of money lost by a hobbled economy, and the assertion of our politicians that they follow the science (not the head lemming), this would surely be worthwhile.

Antibody tests might be fatally flawed, but they can be used in highly destructive ways. If the number of people who are antibody positive remains below the level of 'herd immunity' (90% or so) it will be an excuse to promote or even mandate vaccination, after a vaccine is rushed onto the market. Antibody tests could also be used to indefinitely quarantine people who do not test positive, asserting that they are at danger of becoming infected, and then spreading it to others. They could be

used to separate families, arguing that the children must be put in foster homes because the parents are at risk of an infection at any time.

Faulty tests have been used to indefinitely quarantine Chinese citizens. But now, do we have more civil rights in the UK, United States, Canada or other modern, once democratic countries?

We have been here before. A BBC story from 2008, "Life Sentence", always makes me cry. Starting in 1907 nearly 50 women were locked in an asylum within the Long Grove insane asylum in Surrey because they were deemed carriers of typhoid. They were sane and healthy when they entered, but most were driven mad by the solitary confinement, by humiliations like toilets that flushed boiling water, warmly reminding them that even their excrement was a danger to the world, by the nurses wearing PPE. After they stopped imprisoning such women in the 1950s, the prisoners remained. In 1992, when the asylum closed for good, the three remaining women were deemed insane and relocated to other institutions, their entire lives destroyed by an infectious panic. Despite this, the UK Department of Health told the BBC that there never had been a policy of incarcerating people deemed carriers of an infectious disease [32].

This document is based on an examination of all antibody test documentation submitted to the US FDA (Food and Drug Administration) and a series of antibody surveys of groups of people from around the world.

A Little Background

COVID-19 is alleged to be an RNA virus, so the RNA will be in your body as soon as you are infected. RT-PCR is an ultra-sensitive test (capable of reliably detecting as few as five molecules of RNA in a sample, and possibly triggering on just one) and therefore should be positive almost immediately after infection.[1]

IgM antibodies are believed to be produced by the body as generic infection fighters, soon after infection. An infected person will not be IgM positive immediately, but within a few days at most. These antibodies persist for a while after the infection is resolved, but then fade away.

IgG antibodies are believed to be produced by the body as very specific fighters of a particular invader, such as COVID-19. Some scientists believer they take longer than IgM to be produced, but all agree that they persist long after the infection is resolved, possibly for a lifetime.

Antibodies and Antigens

Antibodies are believed to be generated by the immune system in response to a foreign protein, known as an antigen. In the case of COVID-19, an antigen would be a protein probably found on the outer shell of the virus (because the internal proteins

[1] Often only samples from some areas of the body are positive (e.g. nose but not throat or stool), leading to the belief that the virus, unlike blood borne viruses, only colonizes a small part of the respiratory tract. Samples from deep in the nose (nasopharyngeal) are believed to be most reliable for early detection [27].

are unlikely to stimulate an immune reaction). When an antibody binds to an antigen, it is a signal to the body to destroy the foreign object, such as a virus particle.

Antibody tests contain one of more of these antigens, that are bound to chemicals that produce some kind of color change or fluorescence when an antibody binds to them. The result of the antibody test is read as the intensity of this color change or fluorescence. This makes reading tests results easier to automate.

The antibody-antigen reaction is continuous, and not binary, not naturally 'negative' or 'positive'. Therefore, manufacturers recommend a particular intensity of color change or fluorescence as the division between 'negative' (antibodies not present) and 'positive' (antibodies present). Some manufacturers recommend an intermediate zone between negative and positive, and specimens in this zone may be re-tested, possibly immediately, or possibly in the future, when it is believed that, if the reaction is real, antibody levels will have increased to a clearly detectable level.

Since antigens are viral proteins the obvious place to obtain them would be from purified virus. However, since COVID-19 virus has never been purified, this is currently impossible.

In lieu of this, traditional, impure materials (e.g. nasal swab) would be added to a cell culture, and proteins that were believed to be viral would be purified and used as antigens. But in modern tests most antigen proteins are 'recombinant', produced artificially from the published 30,000 base RNA sequence believed to be COVID-19.

Sources of Data

This article is based on a review of all antibody tests approved under FDA Emergency Use Authorization [33], a list of surveys maintained by a third party [23] and several medical papers.

Status of Antibody Tests

The only jurisdiction with a formal structure for approval of antibody tests is the United States but, until very recently, it was just a charade, as the test manufacturers did not need to provide validation data. Now, validation data must be provided, but the FDA can only do a paper analysis [3].

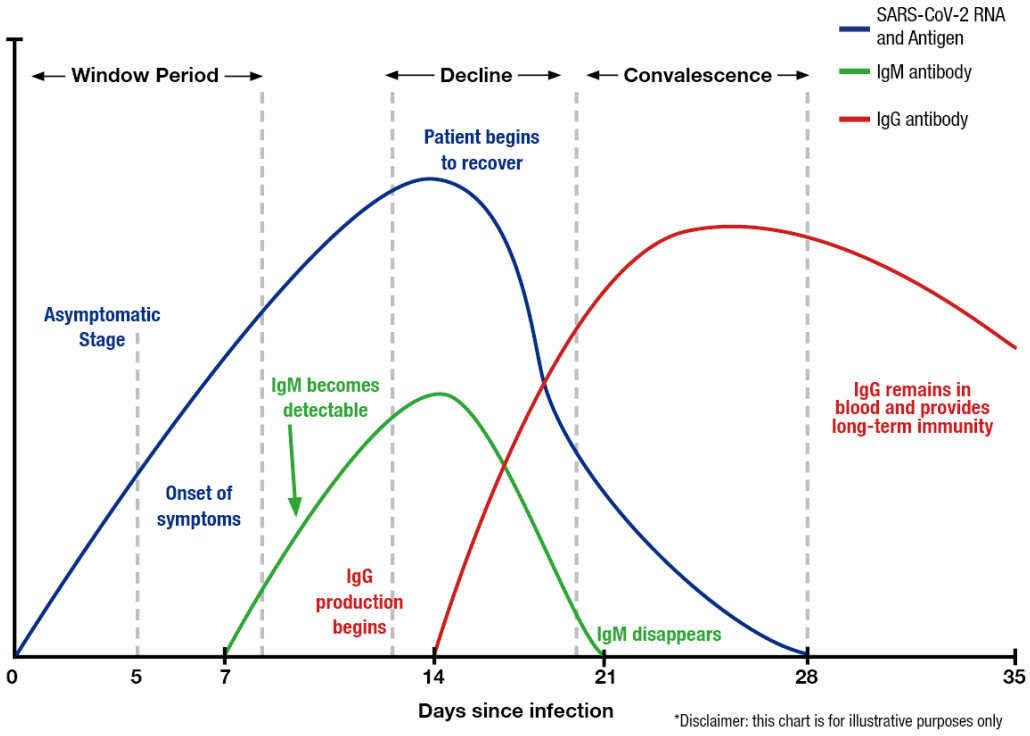
Imagine if auto-manufacturers had to build cars to certain EPA (US Environmental Protection Agency) fuel efficiency standards, but rather than sending a car to the EPA for testing, they could do the testing at their facilities, and just send the results in afterwards. Then, there would have been no need to write software to fake the fuel efficiency by running the engine differently under testing conditions.

A Theoretical Timeline

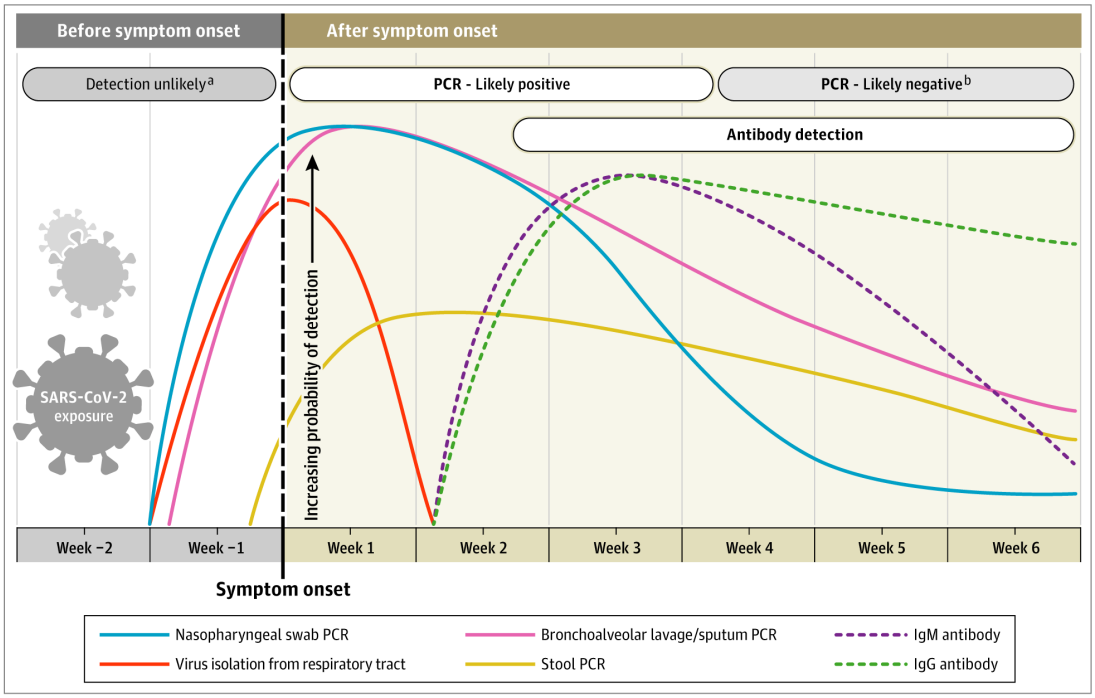
The theoretical timeline of an RNA virus disease is shown below:

Phase	Description	Exception
Pre-infection	No RNA, IgM or IgG	People will have antibodies to previous infections that may cross-react with COVID-19 antibodies.
Infection	RNA should be detectable by RT-PCR almost immediately.	
Incubation	During this period of a few days IgM antibodies should become detectable [1][2]. IgG may become detectable. It is believed that IgG antibodies develop at the same time or after IgM, but not before.	
Symptomatic resolution	If a person develops symptoms, they should have detectable RNA, IgM and IgG during this period	
Asymptomatic Resolution	Despite the lack of symptoms in many or most COVID-19 RNA positive people, people in this phase should similarly be positive for RNA, IgM and IgG.	
Cure	There is no functional virus left in the body so the person should be RNA negative. IgM and IgG will be positive.	RT-PCR tests may produce false positives results due to non-infectious RNA left over from the infection or other reasons.
Post-infection	At some point IgM antibodies wane and the person is left with just IgG antibodies, which provide immunity, possibly life-long.	

A graph from the test manufacturer Diazyme illustrates this belief, which indicates that the immune system is aware of the concept of a 7-day week (other similar graphs indicate that, for other viruses, multiples of 10 days are preferred) [26].



A paper from the Journal of the American Medical Association differs in showing IgM and IgG antibodies arising at the same time [27]:



This paper will show that COVID-19 antibody testing does not support this timeline.

Timeline in Practice

Pre-Infection: No Positive Test Results

Before people are infected with COVID-19 they should theoretically be negative for RNA and all types of antibodies.

In the following table, note that tests for only one antibody type will perform better as they only have one chance for a false positive, whereas tests for multiple antibody types could test positive for any type. The lowest value is shaded in blue, and the highest in red.

Test or Study	Antibodies	Pre-outbreak samples positive[2] (%)
Abbott test [6]	IgG	4/997 (0.4%)
Cellex test [4]	IgG or IgM	11/250 (4.4%)
Chembio test [5]	IgG or IgM	5/125 (3.6%)
DiaSorin test [9]	IgG	8/1090 (0.7%)
EuroImmun test [10]	IgG	30/1415 (2.1%)
Idaho survey [21]	IgG	1/1020 (0.1%)
Netherlands survey [24]	IgA, IgG, IgM	30/218 (13.8%)
Netherlands survey [24]	IgM	3/28 (10.7%)
Platelia serum [8]	Combined	3/687 (0.4%)
Roche test [13]	N gene	10/5272 (0.2%)
Wadsworth test [11]	IgA, IgG, IgM	1/256 (0.4%)

Infection: RNA-Positive Only

Theoretically, someone who has just been infected with COVID-19 will be rapidly positive for RNA (due to the sensitivity of the test) but it will take a few days for antibodies to develop. There is no data available at present, as it would require daily blood samples from a large number of people who were initially negative for all tests, so that the time series could be examined. This type of testing could validate all aspects of the theoretical timeline, but would also be very expensive, intrusive (daily swabs and blood tests) and would need a very large number of people

[2] Some tests had a 'borderline' or 'indeterminate' category and these were counted as positive.

because most may never have any positive tests and, ahead of time, it would be impossible to tell who would eventually become RNA-positive.

The best that can be done is to guess at the date of infection based on contact with someone who later tested RNA positive, but there is never proof that this was actually the date of infection, it is still just a supposition.

The time at which someone first develops symptoms or learns they are RNA-positive is not very useful because it may occur a variable number of days after they are infected.

Incubation: Antibodies Start to Develop

This part of the theoretical timeline has the same problem as the moment of infection and would also require time series with daily testing of a large number of people.

But perhaps we can sometimes be lucky and someone will be tested early enough that they will be RNA positive and the development of first IgM and then IgG antibodies.

In the Chembio test validation IgG antibodies were found in all four RNA positive samples collected within 6 days of the development of symptoms, but IgM antibodies only in one out of four [5]. It should have been the other way round if IgM occurs before IgG.

A study of 30 severely and mildly ill COVID-19 patients found that, "a higher proportion of patients…had earlier IgG than IgM seroconversion [first detection of antibodies]" [28].

Some tests and studies made it impossible to validate this theory because they used total antibodies, not distinguishing between IgM and IgG (Platelia [8]). Many other tests only reacted to IgG antibodies, so comparison with IgM was not possible.

There is limited information but it does not support the notion held by some that IgM antibodies develop before IgG.

This is consistent with the first SARS coronavirus in which IgG antibodies were found before IgM antibodies, calling into question the usefulness of IgM antibodies as an early warning system [25]. And, given that IgM antibodies disappear over time, they are not useful for determining later immunity either.

Symptomatic Resolution: RNA and Antibodies

Once symptoms are noticed, enough time should have passed for IgM antibodies to develop, so during the days or weeks of resolution of symptoms every patient should be positive for RNA, IgM and IgG.

During SARS, also blamed on a coronavirus, a small sample of isolated patients mostly developed IgG antibodies by 14 days after symptoms, and all by 30 days [25].

The Chembio test found IgG antibodies in 100% of RNA-positive samples from 0-21 days after first symptoms, except for 4/10 (40%) of samples collected between 7-10

days. The EuroImmun test had positive IgG results sporadically from the first day of symptoms through day 15, and then consistently through day 36, the last day tested, while negative tests were found from the day of symptoms through day 18. There were very small numbers of tests performed on each day (1-6) with an average of less than two tests per patient [10].

The Abbott IgG test had 0 positive results within 3 days of first symptoms, 25% positive within 3-7 days, 86% within 8-13 days and 100% after 14 days [6]. Similarly, Diasorin found 11/44 (25%) positive for IgG within 5 days of first symptoms, 44/49 (90%) between 6 and 14 days, and 40/41 (98) after 15 days [9].

The Ortho Vitrios test found 8% IgG negative to the 'N' gene within 5 days of the person testing RNA positive, but the fraction then went up, 11% on tests 6-15 days after RNA positivity, and 25% during the 16-22 day period [12]. They also tested people a known number of days after symptom, and again a significant fraction were negative: 8% 12-17 days after symptoms and more, 17%, 18-32 days after symptoms.

The Wadsworth test [11] simultaneously detects IgA, IgG and IgM antibodies, so cannot be used to distinguish the timing of different antibodies. However, it surprisingly had negative results on 40% of samples from people who were known to have been RNA positive for 11-15 days, 43% positive for 16-20 days, and 12% positive for more than 20 days. If indeterminate results are included with negative (since they are not clearly positive) the percentages are 69% (11-15 days), 51% (16-20 days) and 21% (over 20 days). Two additional studies with the Wadsworth test showed that, at least 25 days after symptom offset, 6% were antibody negative, and 12% were either antibody negative or indeterminate. In other words, negative results for IgA, IgG and IgM were found long after some antibodies should have developed.

Different tests give very different results, from Chembio, positive for IgG on all days after symptoms developed, to Abbott which had only 25% positive within 3 days of symptoms developing. This indicates that the tests are not all measuring the same thing, or not with the same level of sensitivity. Additionally, the relevant timing is from the date of infection, not symptoms, and that is unknown in almost every case.

A survey of 85 COVID-19 patients in Wuhan, China found that the majority of samples had detectable IgM antibodies from the first day measured to 30 days or beyond, but there was no time where all tests taken were positive (the maximum was 94% on day 19 after symptoms). IgG samples taken on day 30 or later were 100% positive, but only 14 out of 85 patients were tested during this period. Prior to 30 days all groups of samples had at least 9% negative, and some as much as 60%.

A large flaw in all the validations is that the people sampled at different times are not the same, so individual anomalies (such as the disappearance of IgG antibodies, and then reappearance) cannot be seen. Again, a time series could provide information that shows that the development of antibodies follows a predictable pattern in individuals.

The only thing approaching a timeline is found in the Abbott test documentation which shows two people who had two negative IgG tests followed by several positive tests. The two people, however, seroconverted at rather different times. One between days 6 and 7 after symptoms and the other between 10 and 11 [6]. As usual, the amount of time from infection to the development of antibodies was unknown. Since the Abbott test is IgG only, there was no information on IgM.

In summary, for much of the test documentation, there were a mixture of positive and negative IgG and IgM test results over much of the time tested, and for some tests, right up to the end of the period. This could be due to large variations in the development of antibodies in each person, false results from certain test kits, or both.

Asymptomatic Resolution: RNA and Antibody Positive

From a testing perspective the asymptomatic resolution of an infection should also be a time when people are positive for RNA, IgM and IgG. The problem is that the person affected is not sick, and much less likely to be tested. Again, a time series of many people could identify asymptomatic infections, and could test the hypothesis that these people would first become RNA positive, then IgM positive, then IgG positive before the resolution of the infection.

The information that is available on the appearance of antibodies in asymptomatic people who are RNA-positive is absent the date of infection, which is the only date that matters. Therefore, there is no useful information on this theoretical phase.

Post-Infection: Disappearance of IgM Antibodies

IgM antibodies should disappear after a person has eliminated the virus (becoming RNA negative).

The Chembio test validation obtained samples from 2 people at 21 days after symptoms, and both were IgM positive [5]. Similarly, a survey of 85 patients in Wuhan, China, followed patients for over 30 days and did not document the disappearance of IgM [29].

Additionally, the disappearance of IgM antibodies implies that they appeared in the first case, but even in people who are RNA positive with symptoms, there are still sometimes negative IgM tests. This is often masked by considering someone who is IgM OR IgG positive, to be antibody positive. The documentation available does not exclude the possibility that some people never generated IgM antibodies.

The data provided in this article does not support the notion that IgM antibodies eventually disappear, but it may just be because the patients were not followed long enough.

Performance Issues

Positive Results on Coronavirus Negative People

COVID-19 antibody tests should only very rarely be positive on people who tested RNA negative (who were likely tested multiple times, using samples from different areas of the body), even if they were hospitalized for symptoms that might have seemed 'COVID-like' but actually tested RNA negative. There is always the possibility that some of these people previously had a COVID-19 infection, probably asymptomatic (otherwise they would likely have been tested), but in none of these cases was there any evidence for this.

Test or Study	Antibodies	RNA negative, antibody positive (%)
Autobio test [7]	IgG or IgM	3/312 (1.0%)
Chembio test [5]	IgG or IgM	4/41 (10.3%)
Wadsworth [11]	IgA, IgG, IgM	1/30 (3%)
Wu [22]. Hospital patients.	IgG	39/380 (10.3%)
Wu [22]. Returning workers.	IgG	98/1021 (9.6%)
Xiang et al [29]	IgG	20/84 (24%)
Xiang et al [29]	IgM	21/84 (25%)

Antibody Measurement Performance

Antibodies are generally measured by a color change which can be monitored by reflectance, fluorescence or optical density. The color change should deepen, or the fluorescent glow should increase, with the quantity of virus in a predictable (preferably linear) fashion. In other words, if the blood is diluted 50% then the reflectance, fluorescence or optical density should drop by half.

In the Chembio validation, when blood samples were continuously diluted by half, they did not follow a pattern of optical reflectance that was related to the amount of dilution. With one sample, after reflectance dropped from 36 to 16 on the first dilution (close to half, as expected) the reflectance stayed between 11 and 16 until the fifth dilution where it rose to 24, which was almost considered a positive result (25 was the cutoff). On the second sample, the IgM reflectance almost doubled on the first dilution (as opposed to dropping). This was the only test validation that included a similar experiment, so there is no evidence that antibody testing results can be used to estimate the quantity of virus. It also calls into question the meaningfulness of a numeric cutoff in the first place, to distinguish positive from negative (and possibly borderline or indeterminate).

Disease Severity Predictive Value

The amount of antibody, measured by surrogates like reflectance or optical density, is often measured, with the implication that the level of antibodies reflects the severity of the disease. One survey of COVID-19 patients examined two types of IgM and IgG levels (anti-NP [internal nucleoprotein] and anti-RBD [surface spike protein receptor binding domain]) for a group of 7 severely ill patients and a group of mild case and concluded that, "Serum antibody levels were not correlated with disease severity" [28]. There was similarly no obvious pattern in the same study with patients with or without co-morbidities.

A paper from Shanghai studied antibody titers (levels) in 175 recovering COVID-19 patients, and found a weak correlation with age, but no correlation with people who never developed high levels of antibodies and the duration of disease [31].

Cross Reactions

Antibody tests are often subject to cross-reactions with other conditions. This could be because the medical condition produces similar antibodies, or because something related to that condition reacts with other test components.

Condition	Test or Study	Antibodies	Positive (%)
ANA (anti-nuclear antibodies)	Euroimmun [10]	IgG	1/29 (3.4%)
Auto-antibodies	Euroimmun [10]	IgG	1/10 (10%)
Chikungunya virus	Wadsworth [11]	IgA, IgG, IgM	2/5 (40%)
Chlamydophila	EuroImmun [10]	IgG	1/15 (7%)
Coronavirus 229E	Chembio [5]	IgG	1/1 (100%)
Cytomegalovirus (CMV)	Abbott [6]	IgG	1/5 (20%)
Hepatitis B positive	Diasorin [9]	IgG	1/10 (10%)
HIV	Wadsworth [11]	IgA, IgG, IgM	1/5 (20%)
Influenza A positive	Diasorin [9]	IgG	1/10 (10%)
Mycoplasma	EuroImmun [10]	IgG	1/15 (7%)
Respiratory Synctitia Virus (RSV)	EuroImmun [10]	IgG	1/3 (33%)
Rheumatoid factor	Diasorin [9]	IgG	1/10 (10%)

Streptococcus	EuroImmun [10]	IgG	2/12 (17%)
West Nile virus	Wadsworth [11]	IgA, IgG, IgM	1/5 (20%)

The choice of conditions to check for is completely under the control of the manufacturer and even when no cross reactions were found for a condition, the number of samples tested was so small that the possibility of a fairly high rate of false positive cross reactions still exists. For example, a sample of 10 cannot show that even a 10% false positive rate is unlikely.

General Criticisms of Tests

Even where test validation data conforms to the expectation about the behavior of antibodies, there are criticisms that can be made:

- Manufacturers are responsible for providing the data, and they know there is no point in submitting data with major red flags, meaning that they can spend time adjusting the samples they are using, and how they are analyzed to ensure that the submitted report makes their test looks good.
- There is no way to validate the manufacturer validation data.
- There is no consistent set of validation tests that need to be performed by all manufacturers.
- Time series from the time of infection through at least the decline of IgM antibodies are not provided in any case.
- When information is provided over time, it is not for the same people.
- Timing of antibody results is from the day of first symptoms, or the day of testing RNA-positive, not from the earlier date of infection.
- In many validation tests only tiny numbers of samples are tested. Sometimes a cross-reaction was searched for by testing only one sample. Yet, with even 1% cross reactions being important, well over 100 samples would be needed.
- Only a limited number of conditions were searched for cross-reactions.
- Since the tests were validated by the manufacturers in ideal environments, it can be predicted that performance will be lower when used in practice by purchasers of the tests.

These flaws in antibody tests are fatal. At present no antibody tests are properly validated, and the results cannot be relied upon, particularly not to make sweeping changes in society, such as mandatory vaccination and quarantine of people who do not have the ‘right’ antibody test results.

Population Surveys

Several surveys of local populations for antibodies have been undertaken. In many cases this is to estimate the penetration of COVID-19 into the general population, who have mostly been asymptomatic, or experienced only minor symptoms.

Population being Surveyed

It is very hard to compare these surveys because they use completely different samples of people. Some are random household surveys, although randomization may be reduced by allowing multiple household residents to participate. Others are surveys of blood donors, people who have given blood at a lab for reasons unrelated to COVID-19, volunteers recruited by facebook ads, or at a testing center in a public place. No survey can be taken as representative of the general population.

Validating the Fraction Positive

The result of a population survey that everyone is interested in is the percentage positive. This is generally much higher than expected by those who focus on the number of known cases, by dramatically expanding the number of likely cases. These surveys lead to the conclusion that the death rate from COVID-19 is greatly exaggerated (especially in two California surveys) and that herd immunity may occur naturally.

But there is no evidence that the fractions of the population that are antibody positive are meaningful, for several reasons:

- The presence of antibodies is taken to mean that the person was previously RNA positive with no symptoms, or minor symptoms. None of the surveys have proof that all the people, or even a majority, were previously RNA-positive (and presumed infected), and the time has obviously passed to obtain this information.
- The people were assumed to be antibody negative prior to becoming RNA positive. None of the surveys have evidence for this.
- The absence of antibodies is taken to mean that the person was never COVID-19 RNA positive. None of the surveys have evidence for this.
- It is assumed that the tests used would all give approximately the same result. Since there has been no cross-validation of tests, this is an unfounded assumption.

Virus purification cannot be used to validate antibody tests when the virus is believed to have been defeated and is no longer in the body. Only a time series could identify people who become RNA-positive, and then monitor their antibody development over time.

Summary of Fraction Positive

This section contains information from antibody surveys in a table maintained by Dean Bealer [23]. It shows the group being surveyed (cohort), the type of antibodies

looked for (not always provided), the percentage who were antibody positive and, in some cases, the percentage who were asymptomatic in the weeks before the test.

Acronyms used in the table: HCW = Health Care Workers; HS = High School; n/s = Not specified; THL = in-house antibody test.

Region	Cohort	Antibodies	Positive %	Asymptomatic
Boston (Chelsea)	RNA negative volunteers	n/s	32.0%	50.0%
Czech	Selected or random	n/s	0.4%	
Denmark	Blood donors 17-69	IgM	0.7%	
Denmark	Blood donors 17-69	IgG	0.7%	
Denmark	Blood donors 17-69	IgM+IgG	0.4%	
Geneva	Representative sample	IgG	5.5%	
Germany	405 households from random sample of 600	n/s	15.0%	
Helsinki	Gave blood sample at lab. Week 13	Rapid+THL	0.7%	
Helsinki	Gave blood sample at lab. Week 14	Rapid+THL	0.0%	
Helsinki	Gave blood sample at lab. Week 15	Rapid+THL	2.7%	
Idaho	General	IgG	1.8%	
Iran	196 households	IgM or IgG	22.2%	55.6%
Kobe	Outpatient blood tests	IgG	3.3%	
Los Angeles	Representative sample	n/s	4.1%	
Madrid	HCW	IgA, IgG, IgM	9.3%	
Miami-Dade	Random county residents	n/s	6.0%	50.0%
Moscow	First week of survey	n/s	3.0%	
Moscow	Second week of survey	n/s	9-10%	
Netherlands	RNA negative blood plasma donors	IgA, IgG, IgM	3.1%	
New York	Recruits at grocery stores and community centers	n/s	12.3%	
Oise, France	HS pupils, staff	IgG, anti-N gene	25.9%	17.0%
Oise, France	Blood donors	IgG, anti-N gene	3.0%	
Santa Clara	Facebook ads targeted at Santa Clara County	IgG or IgM	1.5%	
Scotland	Blood donors, March 17	n/s	0.0%	
Scotland	Blood donors, March 21-23	n/s	1.2%	
Slovenia	General	n/s	3.0%	95.1%
Stockholm	General	n/s	2.3%	
Sweden	HCW	n/s	20.0%	
Switzerland	Population representative survey, week 1	IgG	3.2%	

Switzerland	Population representative survey, week 2	IgG	5.3%	
Switzerland	Population representative survey, week 3	IgG	8.7%	
Switzerland	Population representative survey, 5-19 years old	IgG	6.1%	
Switzerland	Population representative survey, 20-49	IgG	8.4%	
Switzerland	Population representative survey, 50+	IgG	4.3%	
Switzerland	Population representative survey, Female	IgG	3.2%	
Switzerland	Population representative survey, Male	IgG	5.3%	
Wuhan	RNA-negative returning workers	IgG	9.6%	
Wuhan	RNA-negative returning workers	IgM	0.0%	
Wuhan	RNA-negative hospitalized patients	IgG	10.3%	
Wuhan	RNA-negative returning workers	IgM	0.0%	

At present this information is simply provided as a convenient summary. Drawing conclusions from it is difficult, except to say that if the antibody tests can be believed, in no area have the majority of people been infected. On the other hand, the results may not even be close to the number of people who actually did experience an infection as there is no way to validate an antibody test in the general population, without historical records of coronavirus 'infection' status (i.e. a time series documenting RT-PCR RNA positivity and the subsequent development of antibodies).

Where the fraction of people who had been asymptomatic in the weeks before the antibody test (not on the date of the test, as the infection has presumably been resolved some time ago), among a group of antibody positive people, was reported, the numbers were all over half, except for the study in Oise, France, in which participants were asked to report any respiratory symptoms over the last three months, which were mostly runny nose, cough, headache, tiredness, sore throat and fever. About half were listed as having "major" symptoms (so half were asymptomatic or had minor symptoms), but 'major' symptoms included fever, cough and loss of sensations of smell or taste. The bottom line is that if we define major symptoms by the need for hospitalization, 95% did not have major symptoms.

Antibodies and Air Pollution

An antibody survey of New York provided data for various regions of the state. There is an obviously higher rate of antibody positive people in the New York City area, and a dramatically lower rate in rural areas. This could be explained (and will be) by greater transmission in the city, but also could be due to greater air pollution in the city. There are already studies that show, for example, an association between air pollution and the frequency of RNA positive tests, and between air pollution and deaths blamed on COVID-19.

One study estimates, “An increase of 1 microgram per cubic meter of fine particulates in the air is associated with an 8% increase in the COVID-19 death rate in the United States” [16]. Another study found a similar correlation in China, Italy and the USA using satellite measures of particulate matter, Carbon Monoxide and Nitrogen Dioxide [18]. A study in England correlated COVID-19 lethality with Nitrogen Oxide, Nitrogen Dioxide and Ozone levels [19].

An Italian study showed a very high correlation between the number of times particulate matter limits were exceeded in an area and the number of infected (i.e. RNA-positive) people. Most of the polluted areas, by this measure, were in northern Italy [17]. A study in London, England showed a strong correlation between higher air pollution and higher numbers of RT-PCR RNA test rates [20].

Returning to the New York data, the highest fraction of people who tested antibody positive after volunteering for testing at grocery stores and community centers was in New York City (20%) followed by Westchester/Rockland (14%) and Long Island (11%). The regions with the lowest fraction testing positive were Southern Tier (2.4%), Capital District (2.2%) and Central NY (1.9%). Southern Tier is a hilly and agricultural area on the southern border of the state. The Capital District contains the city of Albany, and is dependent largely on government, healthcare and education employment. Central NY contains the city of Syracuse. While once industrial, most employment is now in education, research, health care and services.

This evidence is far from proof that false positive antibody tests can be induced by high levels of air pollution, but given that RNA positivity and COVID deaths are associated with air pollution, it is a hypothesis that should be considered.

Review of Timeline

Based on the findings in this paper we can review the evidence for the theoretical timeline.

Phase	**Description**	**Exception**
Pre-infection	No RNA, IgM or IgG.	A significant number of old blood samples had antibodies detected.
Infection	RNA should be detectable.	Outside the scope of this paper.
Incubation	IgM antibodies should become detectable [1][2]. IgG may become detectable.	Limited information as infection is usually only declared once symptoms or a positive RNA test is found. The date of infection is never known.

Symptomatic resolution	If a person develops symptoms, they should have detectable RNA, IgM and IgG during this period	There are several cases where samples are negative for all antibodies tested.
Asymptomatic Resolution	Despite the lack of symptoms in many or most COVID-19 RNA positive people, people in this phase should similarly be positive for RNA, IgM and IgG.	There is limited information since most asymptomatic people are not tested for RNA.
Cure	There is no functional virus left in the body so the person should be RNA negative. IgM and IgG will be positive.	A significant number of samples are negative for IgM and IgG even many days after first symptoms.
Post-infection	At some point IgM antibodies wane and the person is left with just IgG antibodies.	A significant number of samples are negative for IgG. There is no information on immunity from re-infection.

Conclusions

Positive COVID-19 antibody tests have only been found in a minority of people in the general population even where the virus is believed to have been circulating for months. These fractions are generally taken as truth, but one would expect a highly infectious virus to have spread much more widely. There is a lot riding on this data, if only a small minority of people have COVID-19 IgG antibodies, then it may be declared by vaccine proponents that natural immunity is not possible, and that a vaccine may still be necessary, even mandatory.

The faith in this data is hard to understand since there is no evidence that the vast majority of people in surveys were ever 'infected' (i.e. were ever RNA positive) and no evidence that the antibodies seen during the survey were not present in the past. On the other hand, there is also no evidence that the majority who test negative were truly never 'infected' (i.e. never were RNA positive).

Determining immunity is also virtually impossible. Obviously there would be ethical problems re-challenging people with a virus that is believed to be fatal in some people. There are, however, a significant number of people who test RNA positive after symptoms have resolved, and after testing RNA-negative. This could be used as evidence that re-infection is possible (strengthening the case for a vaccine) but given that these people are asymptomatic, may just indicate false positives [30].

There is no evidence currently that the presence of IgG antibodies prevents people from becoming RNA positive again or, conversely, that the absence of IgG antibodies makes people vulnerable to becoming RNA positive.

Proof that a group without COVID-19 IgG antibodies are more vulnerable could not just look at the re-occurrence of RNA, because that usually occurs without symptoms. Even if occurrence of RNA with symptoms is more common, one would have to show that the overall risk of serious illness and death was higher, after adjusting for baseline differences between the groups with and without IgG antibodies.

The one experiment that could show whether antibody tests are actually meaningful would be a time series of a large number of people who are currently negative on all tests. This experiment would be time consuming, inefficient (as many people would never become positive on any tests), intrusive (frequent nasal swabs and blood tests) and obviously very expensive. Those are practical considerations, but in the absence of such an experiment we are almost totally in the dark about COVID-19 antibody testing. Given the billions being spent on COVID and the trillions being lost by the economy, it surely is not impossible to do some worthwhile science.

Additionally, if virus was ever purified from people who were RNA positive and symptomatic, this could be used to expose animals, and could be used to detect antibodies that are definitely from COVID-19, and not just to proteins derived from the putative 30,000 base COVID-19 genome.

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Revision History

Revision	Major changes
3	Editorial revisions. Executive summary. Second paper showing no correlation between disease symptoms and antibody levels.