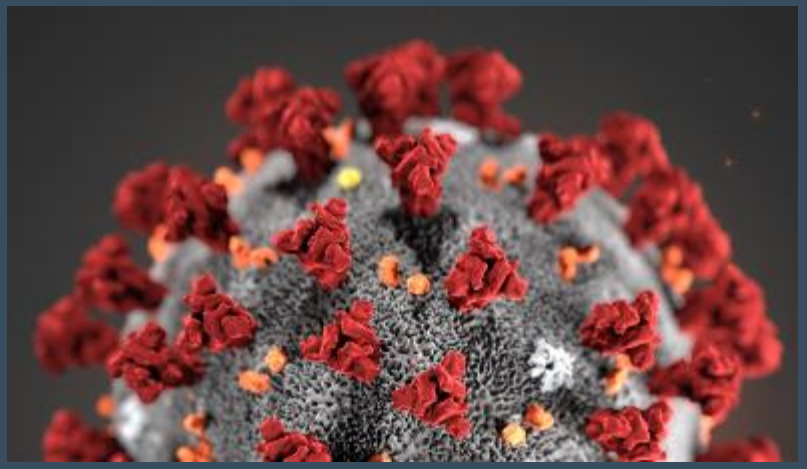
COVID-19 Serology



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May 11, 2020

Background

- Serological tests look for the presence of host antibodies developed in reaction to an infection, rather than directly detecting the pathogen itself (*e.g.*, real time RT-PCR)

- Once fine-tuned and fully-validated, serology may be used to look for surveillance purposes (very high specificity)

- It has not yet been determined whether the presence of antibodies correlates with immunity against re-infection

COVID-19 Serology

- Domains:
  - diagnosis
  - assessment of immune status
  - convalescent plasma for treatment/prophylaxis
  - vaccine immunogenicity trials
  - surveillance/epidemiology

Diagnosis: Acute or Recent Past

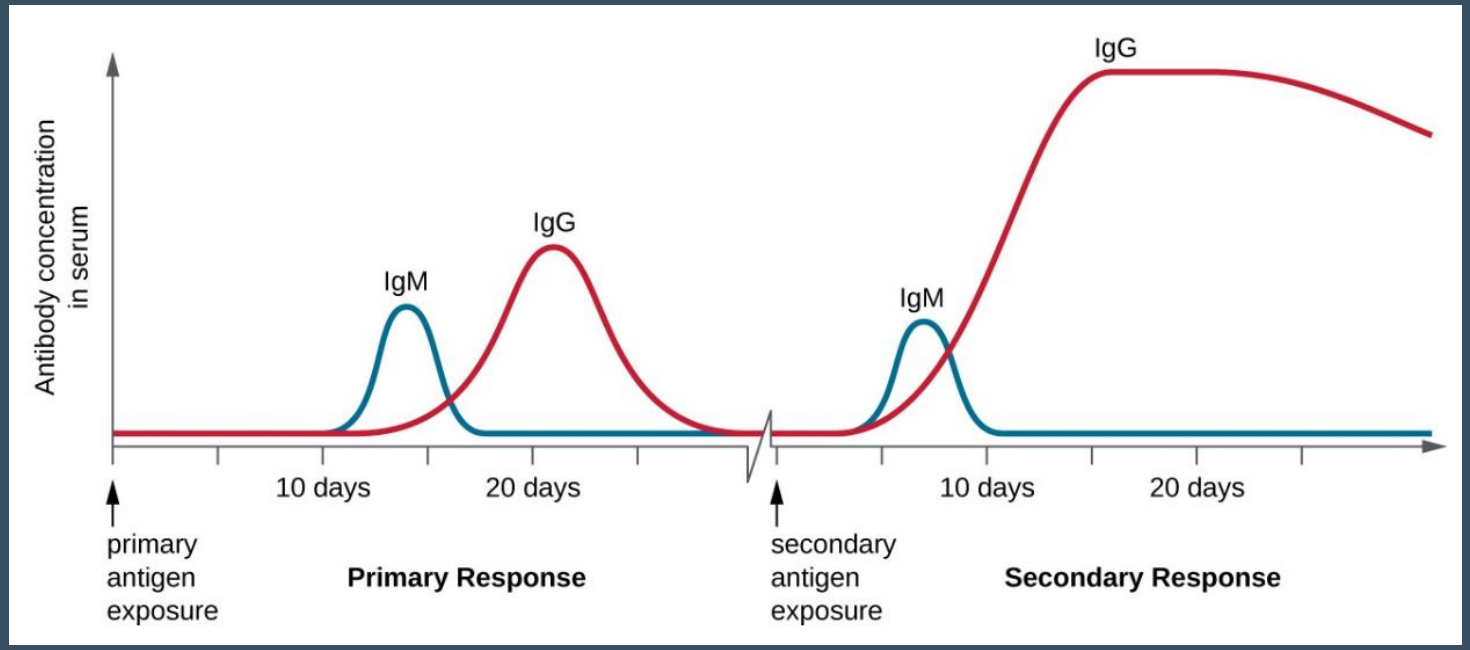
- Serology was never reliably employed routinely for diagnosing infections with “respiratory viruses” such as influenza, parainfluenza, RSV, common coronaviruses, *etc*.

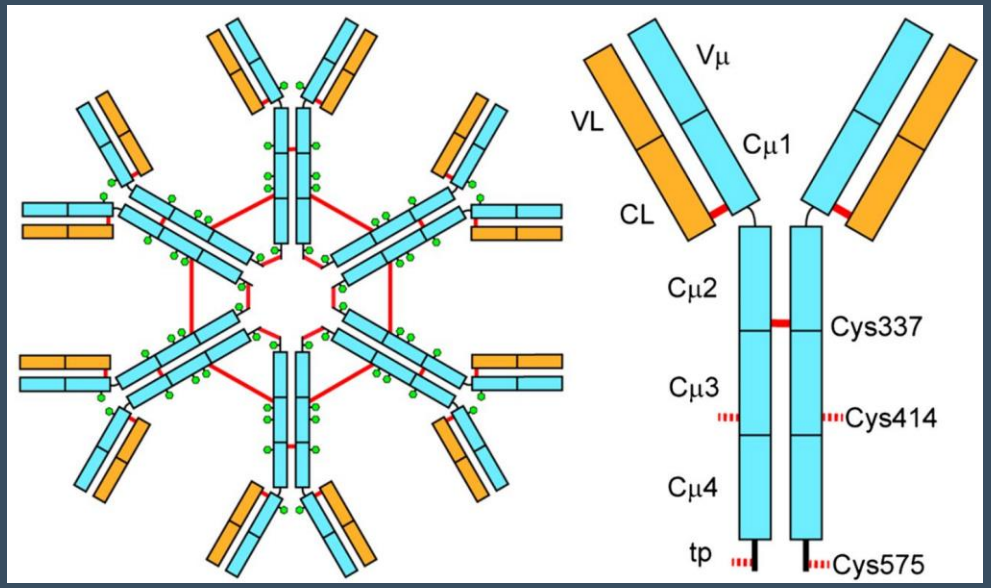
- (RT)-PCR (that detects viral nucleic acid) remains the only option for them

- Serology never routinely used for SARS or MERS either

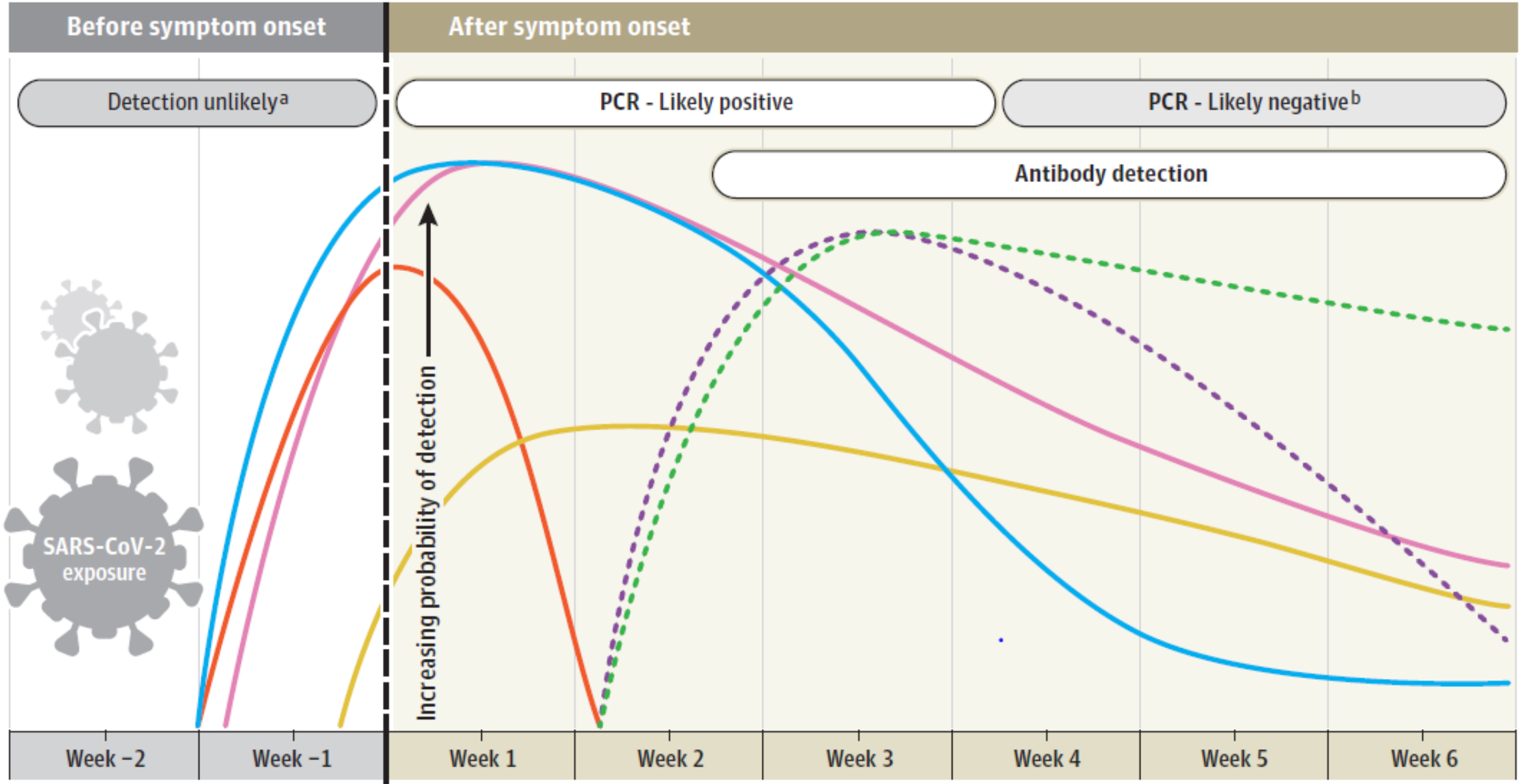
Basics of antibodies (aka, immunoglobulins)

- IgM appears first followed by IgG
- Antibody titers and avidity increases
- This may vary from one antigen/infectious disease to another





IgM **IgG**



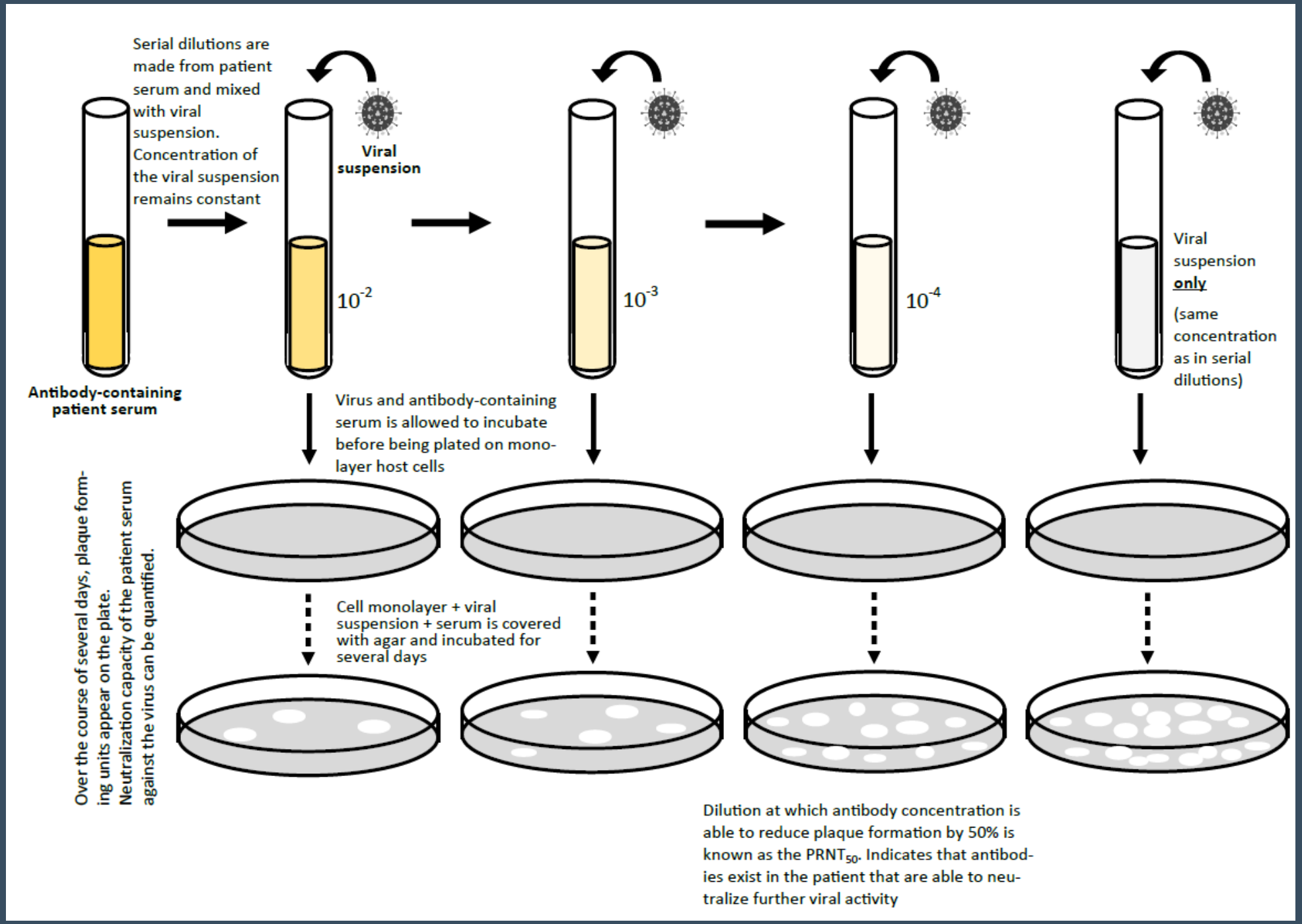
JAMA Published online May 6, 2020

Basics of serology

- **ELISA**: Enzyme-linked immunosorbent assay:
  - Typically measures antibodies in serum
  - It detects ALL antibodies, *i.e.*, directed against ALL epitopes (sites) on an antigen including both neutralizing and non-neutralizing antibodies (“ELISA antibodies”)

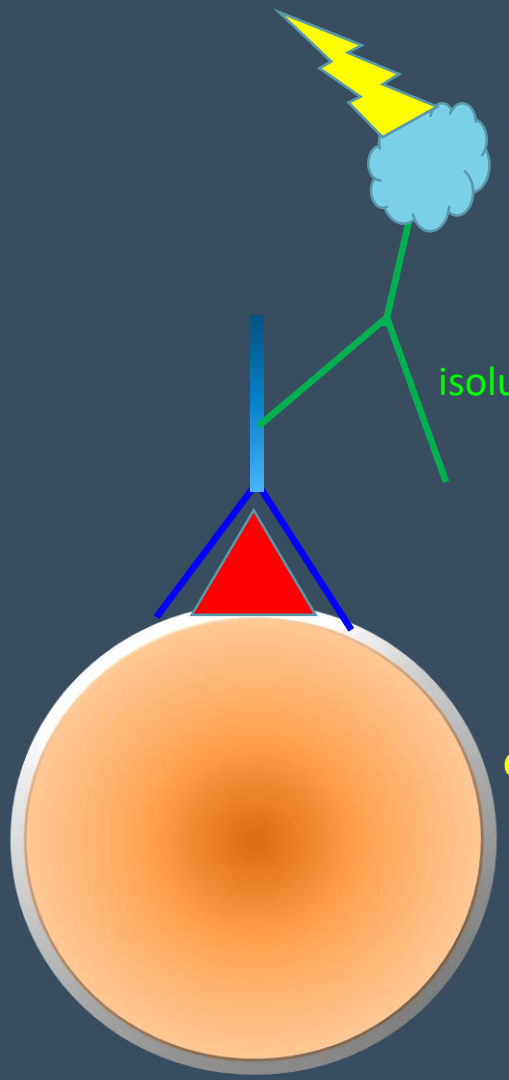
- **PRNT**: Plaque reduction neutralization test
  - Typically detects “neutralizing antibodies”, *i.e.*, those that usually block binding of virus to a host cell receptor (*e.g.*, ACE2 in case of SARS-CoV-2)
  - Requires culturing live virus, *i.e.*, it needs biosafety level-3 lab
  - Requires significant expertise; it is labor-intensive, not amenable to automation; BUT: it is usually the serological “gold standard”

PRNT


Serial dilutions are made from patient serum and mixed with viral suspension. Concentration of the viral suspension remains constant
Viral suspension
Antibody-containing patient serum
10^{-2}
10^{-3}
10^{-4}
Viral suspension only
(same concentration as in serial dilutions)
Virus and antibody-containing serum is allowed to incubate before being plated on mono-layer host cells
Cell monolayer + viral suspension + serum is covered with agar and incubated for several days
Over the course of several days, plaque form-ing units appear on the plate. Neutralization capacity of the patient serum against the virus can be quantified.
Dilution at which antibody concentration is able to reduce plaque formation by 50% is known as the $PRNT_{50}$. Indicates that antibod-ies exist in the patient that are able to neu-tralize further viral activity

Antigen: Spike or nucleocapsid

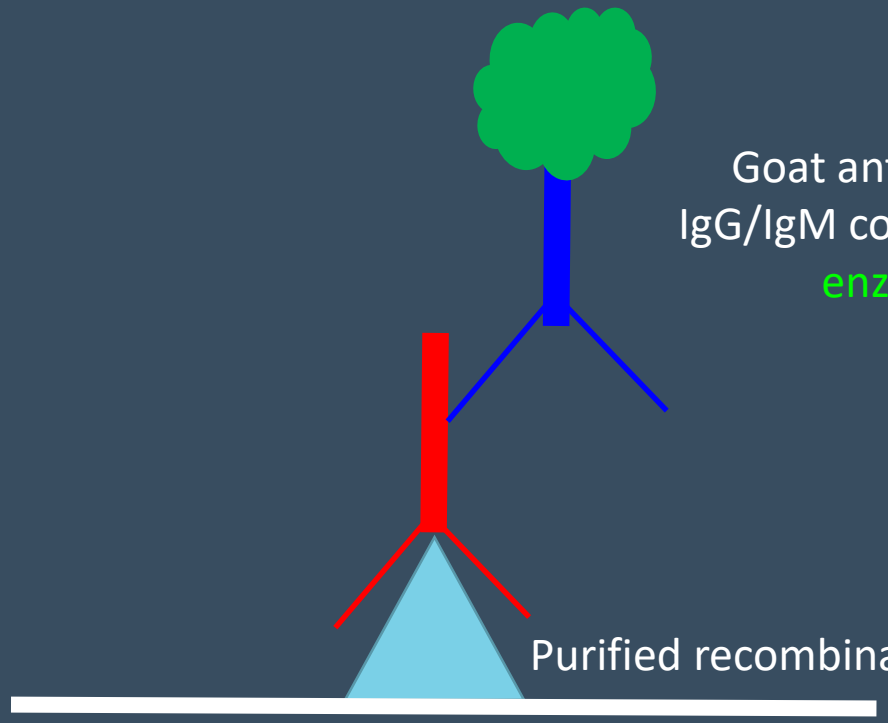
Chemiluminescent immunoassay



Murine anti-human
IgM and IgG mAb-
isoluminol/acridinium ester

**Ag coated
on mircoparticles**

Enzyme-linked immunosorbent assay (ELISA)



Goat anti-human
IgG/IgM conjugated to
enzyme

Purified recombinant Ags

Microtiter ELISA plate

Is IgM serology reliable for diagnosing acute symptomatic COVID-19?

- Short answer is: No…

  - Seroconversion is delayed even for IgM antibody
  - Overall abysmal **sensitivity as low as 4%** to ca. 50% in the first 10 days post "onset" of symptoms
  - False positivity can be observed in sera positive for:
    - Antinuclear antibodies (common >50 yrs of age)
    - Recent influenza infection and flu vaccination
    - Syphilis, Dengue, HSV-1, hMPV, Parvovirus, RF+, enterovirus, AND common CoVs (229E, OC43, NL63, HKU1)

Status of Currently-Available Testing

- Numerous commercial products on the market
  - Vast majority manufactured outside of the US
  - No peer-reviewed publications on their performance characteristics
- Relaxation of FDA review process in mid-March, now reversed…still…
- The FDA EUA letter clarifies under "***Criteria for Issuance of Authorization***":

> Based on the totality of scientific evidence available to FDA, it is reasonable to believe that your product may be effective in diagnosing recent or prior infection with SARS-CoV-2 by identifying individuals with an adaptive immune response to the virus that causes COVID-19, and that the known and potential benefits of your product when used for diagnosing recent or prior infection with SARS-CoV-2 by identifying individuals with an adaptive immune response to the virus that causes COVID-19, outweigh the known and potential risks of your product; and,

> There is no adequate, approved, and available alternative to the emergency use of your product.[5]

FDA U.S. FOOD & DRUG ADMINISTRATION

← Home / News & Events / FDA Newsroom / FDA Voices / Insight into FDA's Revised Policy on Antibody Tests: Prioritizing Access and Accuracy

Insight into FDA's Revised Policy on Antibody Tests: Prioritizing Access and Accuracy

Content current as of:
05/04/2020

AP AP News

US to rein in flood of virus blood tests after lax oversight

The New York Times

F.D.A. Orders Companies to Submit Antibody Test Data

Anand Shah, M.D.

At the time we issued our March 16 policy, a higher level of flexibility was appropriate for antibody tests than for molecular tests that detect the presence of the virus that causes COVID-19, since antibody tests are not meant for use to diagnose active SARS-CoV-2 infection. The flexibility in our March 16 policy allowed for early use of antibody tests to begin to answer critical population-level questions about the prevalence of COVID-19 infections in different communities, and whether the presence of antibodies conveys immunity, and, if so, for how long. Early availability of serology tests has helped generate important information that can inform the future use of serology tests.

Is IgG serology a reliable option for diagnosing acute or convalescent COVID-19?

- **Short answer is: No…**
- IgG seroconversion is much delayed post-onset of symptoms (>35 days in virtually 100% of cases) but typically occurs after 2-3 weeks post-onset
- It would require 2 blood draws → more exposure to healthcare environments
  - To avoid using IgM but use IgG only: one would need acute and convalescent draws
- Specificity is an issue so it requires confirmation using PRNT: by parallel testing with common coronaviruses: 229E, OC43, NL63, HKU1
- Manufacturers usually don't do this especially these days given the rush/intense competition….however it has been done and published by some researchers…

COVID-19 Serology for Immunity

- **Herd / Population Immunity**
  - Understanding the population at risk through sero-surveys
  - If the threshold of population has become immune (via infection or vaccination) then restrictions can be relaxed (ideally)
  - $R_T = sR_0$ → $R_0 = 2.2 – 5.7$ → Herd immunity cutoff: 55-87% (vaccine?)

- **Individual Immunity** (*e.g.*, Occupational Health)
  - Employees with a positive IgG may still be sick, shed the virus in their respiratory secretions and/or in stool
    - IgG ELISAs and PRNTs showed: despite seroconversion, respiratory samples may remain positive for RNA for a few weeks post onset
  - SARS (2002/2003): despite seroconversion by PRNT in >92% of patients, the virus still **grew** from upper resp. samples in 54% and 16% of cases, 2 and 3 weeks post onset of symptoms!

Is IgG serology a reliable option for COVID-19 immune status check?
Individual Immunity / Return to Work

- It has not yet been determined whether the presence of antibodies correlates with clinical immunity
  - The correlate of protection (COP) is not yet known
  - This has to be established via large and well-designed controlled trials
  - Common examples of COP: Hepatitis B surface Ab: 10 mIU/ml and Rubella IgG: 10 IU/ml → prevents infection

Quest Diagnostics Launches Consumer-Initiated COVID-19 Antibody Test Through QuestDirect™

Provides physician-guided insight into COVID-19 immune response without a visit to the doctor

Blood draws available with appointment at any of the company's 2,200 patient service centers

According to the FDA EUA letter:

your product may be effective in diagnosing recent or prior infection

Using kits from Abbott and Euroimmun companies

Quest Diagnostics™ CONTACT QUEST

QuestDirect™

...of past or recent exposure to COVID-19, a (this is not a test for an active infection).

OUR TESTS HOW IT WORKS

QUESTDIRECT / OUR TESTS / INFECTIOUS DISEASE / COVID-19 IMMUNE RESPONSE

COVID-19 Immune Response

Infectious Disease

$119.00

ADD TO CART

In addition to the test price, an additional $10.30 PWN Service Fee will be collected on behalf of PWNHealth and its affiliated professional entities for independent physician oversight of lab testing.

Is IgG serology reliable for COVID-19 convalescent donor screening?

- **The short answer is: no one knows (Jury's out…)**

- **Elisa vs. Neutralizing Antibodies**
  - We don't know if "neutralizing antibodies" are neutralizing enough!
  - *JAMA and PNAS*: neutralizing antibodies were present in both healthy recovered individuals as well as in critically-ill pre-transfusion!

- Overall literature summary:
  - Maybe more effective during the first 9 days (where virus still grew from resp. samples)
  - Antibody-dependent enhancement (ADE) → Immunopathology
  - ADE *may* have implications for both passive treatment and vaccination
  - Well-designed controlled trials will tell…

Current FDA language on convalescent plasma therapy

Recommendations for Investigational COVID-19 Convalescent Plasma

FDA U.S. FOOD & DRUG ADMINISTRATION

May 1, 2020

General caveat:

Although promising, convalescent plasma has not yet been shown to be safe and effective as a treatment for COVID-19. Therefore, it is important to study the safety and efficacy of COVID-19 convalescent plasma in clinical trials.

Recipient eligibility

- Laboratory confirmed COVID-19
- Severe or immediately life-threatening COVID-19,

Donor eligibility

- Defined SARS-CoV-2 neutralizing antibody titers
  - We recommend neutralizing antibody titers of at least 1:160. A titer of 1:80 may be considered acceptable if an alternative matched unit is not available.

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Cochrane Database of Systematic Reviews

Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a rapid review

Cochrane Systematic Review - **Intervention** | Version published: 14 May 2020

https://doi.org/10.1002/14651858.CD013600

We are very uncertain whether convalescent plasma is effective for people admitted to hospital with COVID-19 as studies reported results inconsistently, making it difficult to compare results and to draw conclusions. We identified very low-certainty evidence on the effectiveness and safety of convalescent plasma therapy for people with COVID-19; all studies were at high risk of bias and reporting quality was low.

Is IgG serology a reliable option for COVID-19 sero-surveys?

- The short answer is: it depends on the specificity of the assay
- *e.g.*, with 21,969 confirmed cases in OH these days
- If multiplied by 5 to roughly account to undiagnosed cases (total 109,845) (very generously calculated)
- OH population: 11,690,000
- Sero-prevalence: 0.94%
- With a sensitivity of 100%, and a specificity of 95% →
  - The positive predicative value would be around: **67%**
  - Meaning 33 out of 100 IgG positive results are false positive
  - They'll inflate the denominator and CFR will drop → undue relaxation

IDSA
Infectious Diseases Society of America

IDSA COVID-19 Antibody Testing Primer

Updated: May 4, 2020

As serological testing for SARS-CoV-2 advances, there are multiple issues that need to be addressed, from test quality to interpretation. Unlike molecular tests for COVID-19 (e.g., PCR), antibody tests may be better suited for public health surveillance and vaccine development than for diagnosis. The current antibody testing landscape is varied and clinically unverified, and these tests should not be used as the sole test for diagnostic decisions. Further, until more evidence about protective immunity is available, serology results should not be used to make staffing decisions or decisions regarding the need for personal protective equipment.

False negative risks if performed early in disease course, especially in mild disease;
False positive risks, particularly with tests for Immunoglobulin M (IgM) and potential cross-reactivity with common cold coronaviruses (e.g. HKU1, NL63, OC43, 229E).

https://www.idsociety.org/globalassets/idsa/public-health/covid-19/idsa-covid-19-antibody-testing-primer.pdf

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

COVID-19 Serology Surveillance Strategy

Print Page

Updated April 28, 2020

*Antibody test results **should not be used to diagnose someone with an active SARS-CoV-2 infection**. It typically takes 1 to 3 weeks after someone becomes infected with SARS-CoV-2 for their body to make antibodies; some people may take longer to develop antibodies.*

Questions CDC cannot answer through Serology Surveillance

- How much of the U.S. population is immune to COVID-19 and not able to get infected again?
- How many antibodies are needed to protect someone from COVID-19?
- How long will someone with antibodies be protected from COVID-19?
- Can you be re-infected with COVID-19?
- Can people with antibodies return to work?

World Health Organization

"Immunity passports" in the context of COVID-19

Scientific Brief

24 April 2020

At this point in the pandemic, there is not enough evidence about the effectiveness of antibody-mediated immunity to guarantee the accuracy of an "immunity passport" or "risk-free certificate." People who assume that they are immune to a second infection because they have received a positive test result may ignore public health advice. The use of such certificates may therefore increase the risks of continued transmission.

Take-home messages

- Serology is not an acceptable method for diagnosing acute cases
- Population and individual-level immunity following exposure to the virus is still debatable and being studied

- The role of antibody testing in particular populations needs to be defined through targeted serosurveys

- The role of serology in convalescent plasma therapy is not standardized yet
- Serology will play an important role in vaccine trials