



ABBOTT RAPID DIAGNOSTICS

COVID-19 Testing: What We Thought We Knew, What We Think We Know, & What We Don't Know

November 2020

Agenda

- ❑ **What We Thought We Knew In the Beginning**
- ❑ **SARS-CoV-2 and COVID-19 Clinical Disease**
- ❑ **Testing Recommendations & Guidance**
- ❑ **Immunological Technology Overview**
 - ❑ **Viral Antigen Tests**
 - ❑ **Antibody Tests**
- ❑ **Molecular Technology Overview**
 - ❑ **PCR and Isothermal Techniques**
 - ❑ **Infectivity and Ct values**

In the beginning...

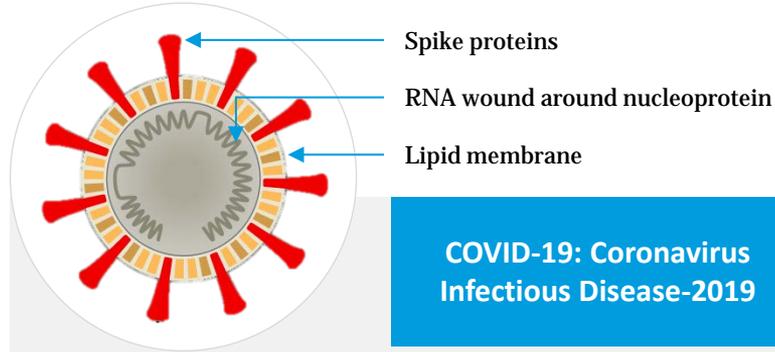
Initial assumptions on the types of diagnostic tests:

- **Antibody/Serology** – The test that everyone needs to get back to work
- **Molecular** – Make it as sensitive as humanly possible
- **Viral Antigen** – Are they helpful at all?

COVID-19 TESTING

SARS-CoV-2 & COVID-19 Clinical Disease Overview

SARS-CoV-2 and COVID-19



COVID-19: Coronavirus Infectious Disease-2019

- Caused by SARS-CoV-2¹
- Declared a pandemic by the World Health Organization (WHO) on 11-Mar-2020

Six other types of Coronavirus are known to infect humans²

- Some cause common cold
- Two caused previous outbreaks: SARS and MERS

Attributes

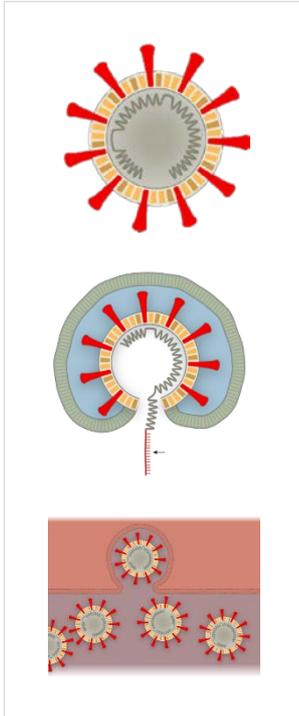
- Named after the crownlike spikes on the surface²
- Virus is enveloped in bubble of oily lipid molecules
- Oily membrane falls apart on contact with soap³

1. [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-\(covid-2019\)-and-the-virus-that-causes-it](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it)

2. <https://www.cdc.gov/coronavirus/types.html>

3. <https://www.nytimes.com/interactive/2020/03/11/science/how-coronavirus-hijacks-your-cells.html?>

Transmission and Infection



Respiratory droplets enter the body through the nose, mouth or eyes⁴

Attaches to cells in airway that express the ACE2 receptor protein³

- Virus infects a cell by fusing its membrane to the cell's membrane
- Hijacks the cell, assembles new copies of the virus and releases millions of copies

Clinical Presentation (& rate of occurrence)⁵



Mild to Moderate cases (81%)
Mild symptoms up to mild pneumonia



Severe cases (14%)
Dyspnea, hypoxia, or >50% lung involvement on imaging



Critical cases (5%)
respiratory failure, shock, or multiorgan system dysfunction

High Risk Patient Population⁵

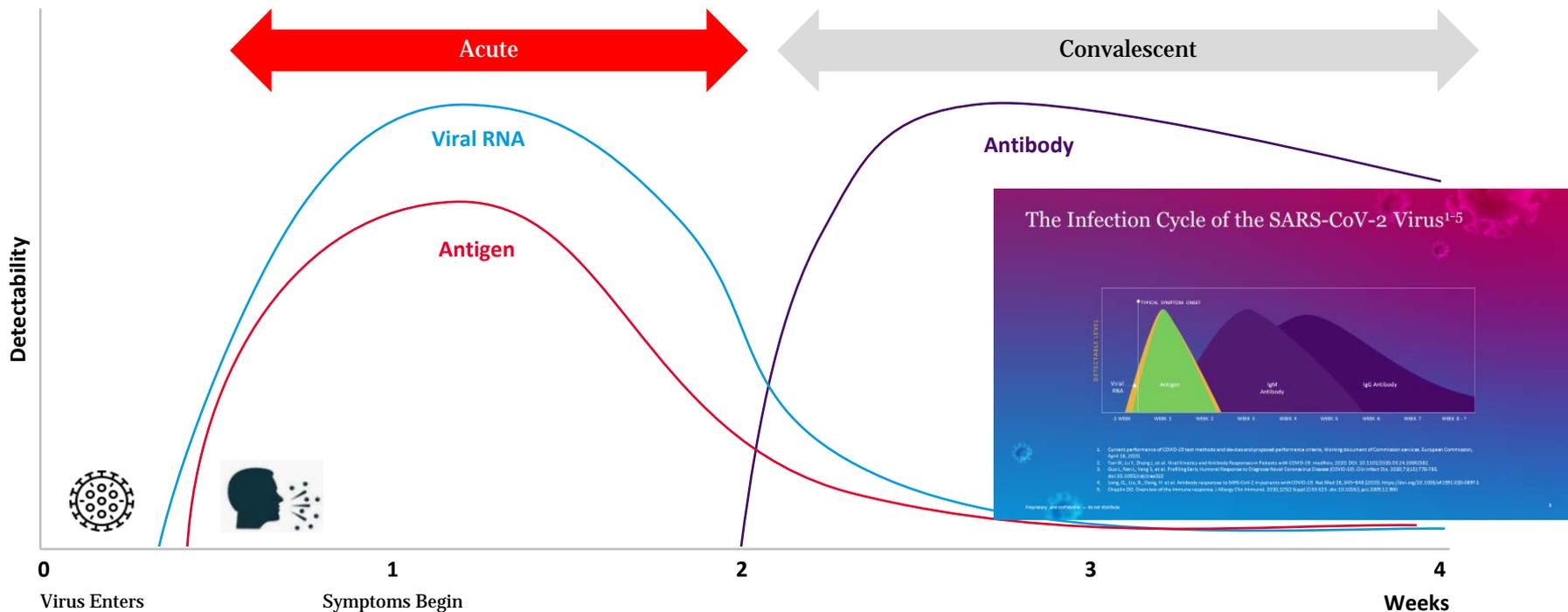
- Elderly, Cardiovascular Disease, Diabetes, Hypertension

3. <https://www.nytimes.com/interactive/2020/03/11/science/how-coronavirus-hijacks-your-cells.html?>

4. <https://www.cdc.gov/coronavirus/2019-ncov/prepare/transmission.html>

5. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html>

When to Test for COVID-19^{6,7,8}

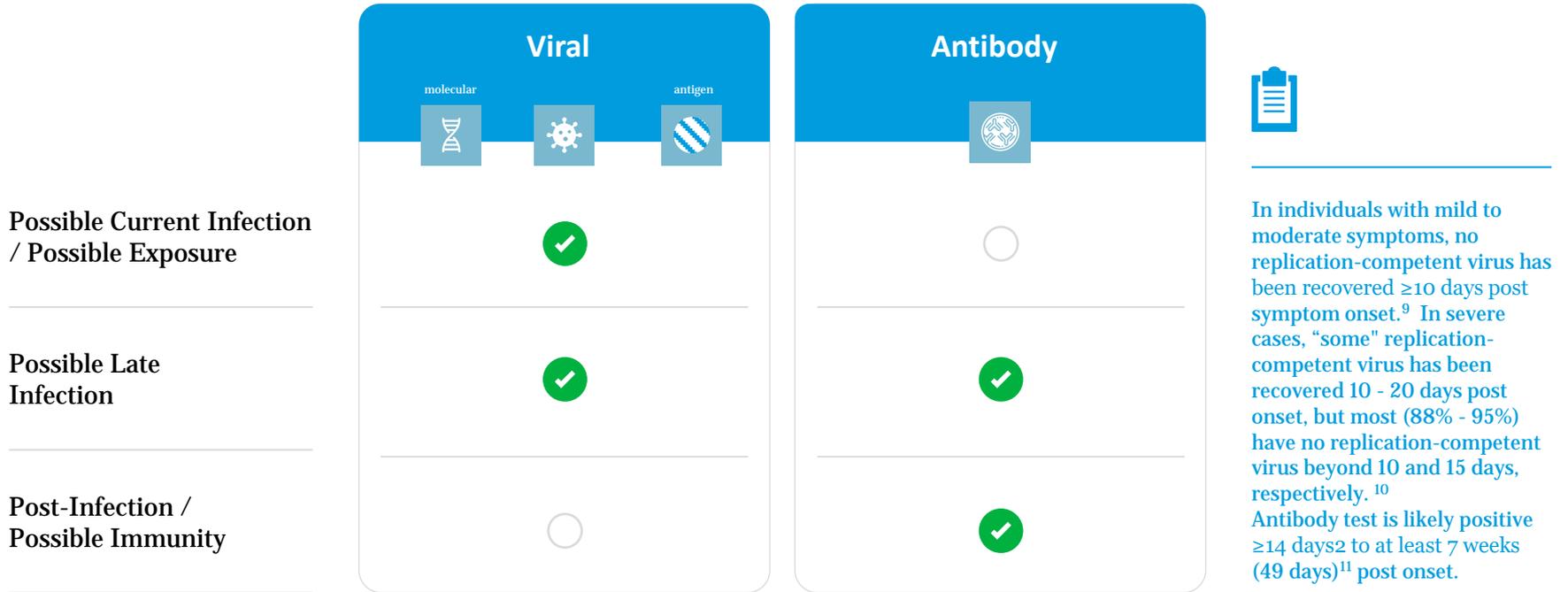


6. Sethuraman, N., Jeremiah, SS., Ryo, A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 6 May 2020. doi: 10.1001/jama.2020.8259

7. Theel ES, The role of antibody testing for SARS-CoV-2: is there one? J Clin Microbiol 58:e00797-20. 2020. <https://doi.org/10.1128/JCM.00797-20>.

8. CDC Symptom-Based Strategy to Discontinue Isolation for Persons with COVID-19. Updated Sept 2020. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html>

Test Utility By Disease Phase



9. CDC. Duration of Isolation and Precautions for Adults with COVID-19. Updated August 16, 2020. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html>, accessed August 20, 2020.

10. Guo L, et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). *Clin Infectious Diseases*, ciaa310.

11. Tan W, Lu Y, Zhang J, et al. Viral kinetics and antibody responses in patients with COVID-19. medRxiv March 26, 2020. Available as preprint <https://www.medrxiv.org/content/10.1101/2020.03.24.20042382v1>

Diagnostic Tests Types and Use

	Molecular Test	Immunoassay Tests		Non-disease Specific Test
		Antigen Test	Antibody Test	
How does it work?	Detects viral genetic material by amplifying RNA target sequence	Detects antigen through antibodies directed toward a target protein	Detects antibodies through laboratory or lateral flow test, for example	Detects symptoms or signs of disease through scans, imaging or observation
Where is the test performed?	Typically performed in lab or point of care, although samples may be taken outside of the lab.	May be performed in laboratory or point of care	May be performed in laboratory or point of care	In a hospital, clinic, or point of care depending on equipment required
What is it typically used for?	Testing suspected cases of COVID-19	Testing suspected cases of COVID-19 or candidates for further testing (like PCR)	Assessing infection and/or exposure rates in a community	Screening or triage for further testing
What does a positive result indicate?	Depending on the assay, confirms a case of SARS-CoV-2 infection	Depending on the assay, confirms a case of SARS-CoV-2 infection	Previous exposure to SARS-CoV-2 or late stage infection	Further testing is needed if results suggest possible SARS-CoV-2 infection
	Current infection		Past exposure to virus	

12. Kelly-Cirino, C. Special COVID-19 ECHO Session #2 Webinar from African Society of Laboratory Medicine. (06 May 2020). https://aslm.org/wp-content/uploads/2020/04/COVID-19_FIND_ECHO-session-30.03.2020-FINAL.pdf
13. Patel, R., Babady, E., Theel, ES., et. al. Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: Value of Diagnostic Testing for SARS-CoV-2/COVID-19. <https://mbio.asm.org/content/11/2/e00722-20>

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What is it typically used for?	Testing suspected cases of COVID-19	Testing suspected cases of COVID-19 or candidates for further testing (like PCR)	Assessing infection and/or exposure rates in a community	Screening or triage for further testing
What does a positive result indicate?	Depending on the assay, confirms a case of SARS-CoV-2 infection	Depending on the assay, confirms a case of SARS-CoV-2 infection	Previous exposure to SARS-CoV-2 or late stage infection	Further testing is needed if results suggest possible SARS-CoV-2 infection

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13. Patel, R., Babady, E., Theel, ES., et. al. Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: Value of Diagnostic Testing for SARS-CoV-2/COVID-19. <https://mbio.asm.org/content/11/2/e00722-20>

COVID-19 TESTING

Recommendations & Guidances

CDC Guidelines for COVID-19 Viral Testing

- **Viral testing are recommended to diagnose acute infection and to guide contact tracing, treatment options, and isolation requirements.**
 - Includes both molecular and antigen testing
 - For both symptomatic and asymptomatic individuals
 - Asymptomatic transmission of the virus has been established and specific people or groups may be advised to be tested for public health reasons
- **For all groups, if test results are positive, isolate for minimum of 10 days after symptom onset.**
 - Also recommend, 24 hours free from fever (without medication) and with improvement of other symptoms
 - A follow-up negative test is not needed to return to work/school if no hospitalization was needed AND the above conditions are met
 - Repeat testing is not needed for 3 months

CDC Guidelines for COVID-19 Viral Testing

- **For all groups, if test results are negative the following recommendations are emphasized:**
 1. Wear a mask
 2. Physically distance (>6 feet or around 2 meters)
 3. Avoid crowds and indoor crowded places
 4. Wash your hands frequently
 5. Monitor yourself for symptoms
- **Testing is advised if you are in a high SARS-CoV-2 transmission zone and attended a public or private gathering of more than 10 people (without universal mask wearing and/or physical distancing).**
- **Self-isolation at home is recommended until your test results are known**

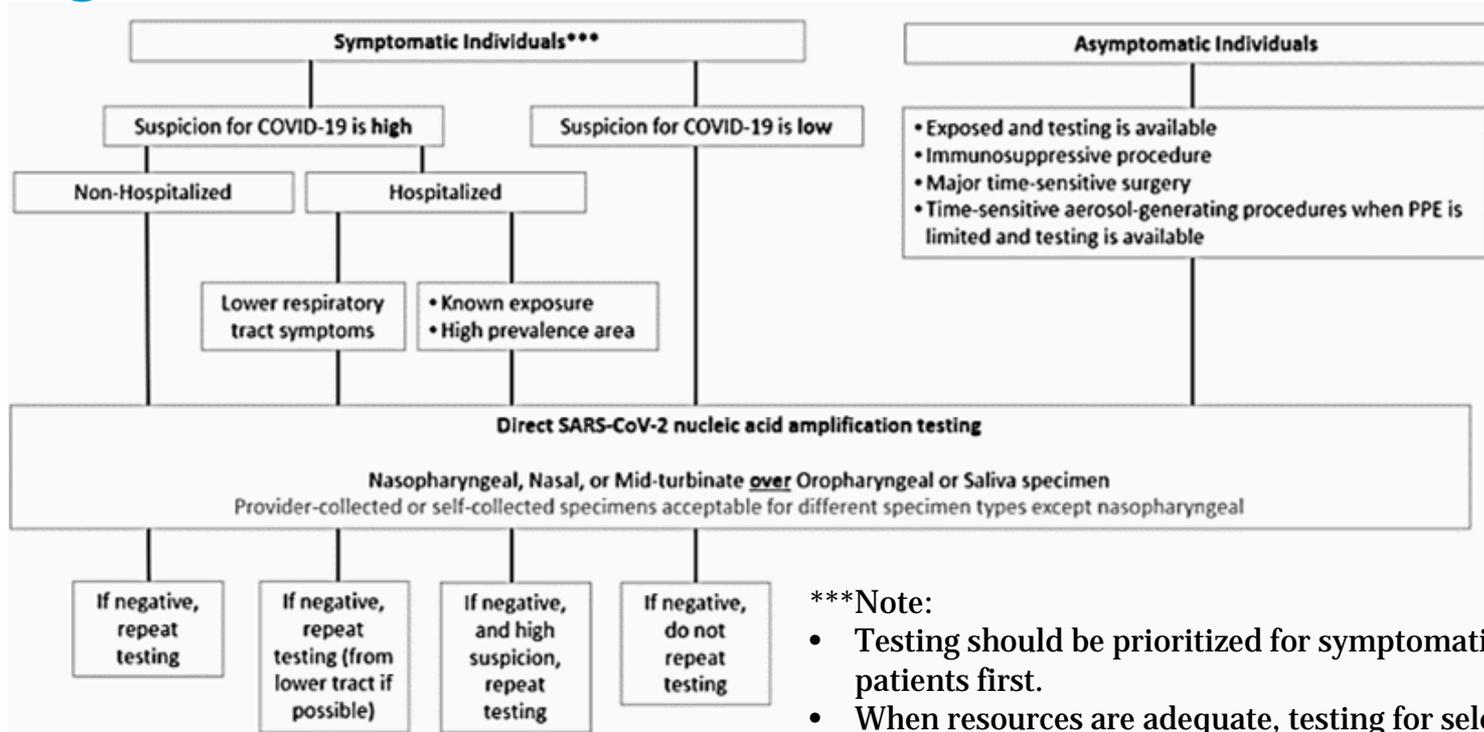
CDC Guidelines for COVID-19 Viral Testing

- **If you work, live in, or receive care in a nursing home test will be required for reopening.**
 - Those with symptoms must be tested and be isolated until test results are known
 - An outbreak in the facility will require regular repeated testing until it is over, for negative results
 - Any positive results should isolate for 10 days as indicated on the previous slide
- **First responders, healthcare workers, or critical infrastructure workers should test in accordance with guidelines set by employers.**
 - Positive results should follow the recommendations previously discussed. Those with negative results are still advise to wear a mask when unable to physically distance.

CDC Guidelines for COVID-19 Antibody Testing

- **Antibody testing is currently not recommended for determining acute infection. Instead they maybe used in combination with viral testing for patients presenting late in their illness.**
- **Important for surveillance and epidemiologic studies, such as understanding previous infection and the transmission dynamic of the virus.**

IDSA Guidelines¹⁵ recommendations where molecular testing can be used



***Note:

- Testing should be prioritized for symptomatic patients first.
- When resources are adequate, testing for selected asymptomatic individuals can also be considered.

15. Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19 <https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/>

15 DIAGNOSTIC RECOMMENDATIONS FOR NUCLEIC ACID TESTING

IDSA Guidelines on the Diagnosis of COVID-19

Recommendation 1: NAAT (Symptomatic)

Recommendation 1: The IDSA panel recommends a SARS-CoV-2 nucleic acid amplification test (NAAT) in symptomatic individuals in the community suspected of having COVID-19, even when the clinical suspicion for COVID-19 is low (strong recommendation, very low certainty of evidence)

- The panel considered symptomatic patients to have at least one of the most common symptoms compatible with COVID-19
- Clinical assessment alone is not accurate in predicting COVID-19 diagnosis
- The panel considered timeliness of SARS-CoV-2 NAAT results essential to impact individual care, healthcare institution, and public health decisions. In the outpatient setting, results within 48 hours of collection is preferable

Recommendation 2: NP, NMT, and NA vs. OP or saliva swabs (symptomatic)

Recommendation 2: The IDSA panel suggests collecting nasopharyngeal, or mid-turbinate or nasal swabs rather than oropharyngeal swabs or saliva alone for SARS-CoV-2 RNA testing in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19 (conditional recommendation, very low certainty of evidence)

- This recommendation does not address testing a combination of specimen types due to lack of evidence
- The panel considered symptomatic patients to have at least one of the most common symptoms compatible with COVID-19

Recommendation 3: Swab collection by patients or healthcare providers (symptomatic)

Recommendation 3. The IDSA panel suggests that nasal and mid-turbinate (MT) swab specimens may be collected for SARS-CoV-2 RNA testing by either patients or healthcare providers, in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19 (conditional recommendation, low certainty of evidence).

- Appropriate specimen collection and transport to the laboratory is critical. A clear, step-by-step protocol needs to be presented to patients attempting self-collection
- The majority of self-collection studies were performed in the presence of a healthcare worker
- The available evidence for nasal and MT swabs as alternatives to healthcare personnel collection is based on assessment of symptomatic patients
- The panel considered symptomatic patients to have at least one of the most common symptoms compatible with COVID-19

Recommendation 4: Upper vs. lower respiratory tract samples

Recommendation 4: The IDSA panel suggests a strategy of initially obtaining an upper respiratory tract sample (e.g., nasopharyngeal swab) rather than a lower respiratory sample for SARS-CoV-2 RNA testing in hospitalized patients with suspected COVID-19 lower respiratory tract infection. If the initial upper respiratory sample result is negative, and the suspicion for disease remains high, the IDSA panel suggests collecting a lower respiratory tract sample (e.g., sputum, bronchoalveolar lavage fluid, tracheal aspirate) rather than collecting another upper respiratory sample (conditional recommendations, very low certainty of evidence)

- The panel considered timeliness of SARS-CoV-2 NAAT results essential to impact individual care and isolation decisions. In the hospital setting, results within 24 hours of collection is preferable

Recommendation 5: Single vs. repeating RNA test (symptomatic)

Recommendation 5: The IDSA panel suggests performing a single viral RNA test and not repeating testing in symptomatic individuals with a low clinical suspicion of COVID-19 (conditional recommendation, low certainty of evidence)

- A low clinical suspicion should be informed by epidemiological information available in for the region coupled with clinical judgment
- The panel considered symptomatic patients to have at least one of the most common symptoms compatible with COVID-19

Recommendation 6: Single vs. repeating RNA test (symptomatic)

Recommendation 6: The IDSA panel suggests repeating viral RNA testing when the initial test is negative (*versus* performing a single test) in symptomatic individuals with an intermediate or high clinical suspicion of COVID-19 (conditional recommendation, low certainty of evidence)

- Intermediate/high clinical suspicion typically applies to the hospital setting and is based on the severity, numbers and timing of compatible clinical signs/symptoms
- Repeat testing should generally occur 24-48 hours after initial testing and once the initial NAAT result has returned as negative
- Another specimen type, preferably a lower respiratory tract specimen if the patient has signs/symptoms of LRTI, should be considered for repeat testing
- The panel considered symptomatic patients to have at least one of the most common symptoms compatible with COVID-19

Recommendation 7: Rapid vs. standard RNA testing (symptomatic)

Recommendation 7: The IDSA panel makes no recommendations for or against using rapid (i.e., test time \leq 1 hour) versus standard RNA testing in symptomatic individuals suspected of having COVID-19 (knowledge gap)

- Overall, there was inadequate information to compare the performance characteristics of the rapid and standard nucleic acid amplification tests in any symptomatic patient population, including outpatients and hospitalized patients. The panel does not recommend for or against rapid NAATs in symptomatic individuals suspected of having COVID-19 at this time due to the lack of quality evidence

Recommendation 8: RNA testing in exposed individuals (asymptomatic)

Recommendation 8: The IDSA panel suggests SARS-CoV-2 RNA testing in asymptomatic individuals who are either known or suspected to have been exposed to COVID-19 (conditional recommendation, very low certainty of evidence)

- Known exposure was defined as direct contact with a laboratory confirmed case of COVID-19
- Suspected exposure was defined as working or residing in a congregate setting (e.g., long-term care, correctional facility, cruise ship, factory, among others) experiencing a COVID-19 outbreak
- The risk of contracting SARS-CoV-2 may vary under different exposure conditions
- This recommendation assumes the exposed individual was not wearing appropriate PPE
- The decision to test asymptomatic patients will be dependent on the availability of testing resources

Recommendation 9: RNA testing in unexposed, hospitalized individuals (asymptomatic)

Recommendation 9: The IDSA panel suggests against SARS-CoV-2 RNA testing in asymptomatic individuals with no known contact with COVID-19 who are being hospitalized in areas with a low prevalence of COVID-19 in the community (conditional recommendation, very low certainty of evidence)

- Asymptomatic individuals are defined as those with no symptoms or signs of COVID-19
- A low prevalence of COVID-19 in the community was considered communities with a prevalence of <2%
- This recommendation does not apply to immunocompromised individuals
- This recommendation does not apply to individuals undergoing time-sensitive major surgery or aerosol generating procedures

Recommendation 10: RNA testing in unexposed, hospitalized individuals (asymptomatic)

Recommendation 10: The IDSA panel recommends direct SARS-CoV-2 RNA testing in asymptomatic individuals with no known contact with COVID-19 who are being hospitalized in areas with a high prevalence of COVID-19 in the community (i.e., hotspots) (conditional recommendation, very low certainty of evidence)

- Asymptomatic individuals are defined as those with no symptoms or signs of COVID-19.
- A high prevalence of COVID-19 in the community was considered communities with a prevalence of >10%.
- The decision to test asymptomatic patients (including when the prevalence is between 2 and 9%) will be dependent on the availability of testing resources

Recommendation 11: RNA testing in immunosuppressed individuals or before immunosuppressive procedures (asymptomatic)

Recommendation 11: The IDSA panel recommends for SARS-CoV-2 RNA testing in immunocompromised asymptomatic individuals who are being admitted to the hospital regardless of exposure to COVID-19 (strong recommendation, very low certainty of evidence)

- This recommendation defines immunosuppressive procedures as cytotoxic chemotherapy, solid organ or stem cell transplantation, long acting biologic therapy, cellular immunotherapy, or high-dose corticosteroids

Recommendation 12: RNA testing in immunosuppressed individuals or before immunosuppressive procedures (asymptomatic)

Recommendation 12: The IDSA panel recommends SARS-CoV-2 RNA testing (*versus* no testing) in asymptomatic individuals before immunosuppressive procedures regardless of a known exposure to COVID-19 (strong recommendation, very low certainty of evidence)

- This recommendation defines immunosuppressive procedures as cytotoxic chemotherapy, solid organ or stem cell transplantation, long acting biologic therapy, cellular immunotherapy, or high-dose corticosteroids
- Testing should ideally be performed as close to the planned treatment/procedure as possible (e.g. within 48-72 hours)
- Many of these patients require frequent, repeated or prolonged visits to receive treatment
- This recommendation does not address risks or strategies to deal with SARS-CoV-2 transmission in outpatient settings such as infusion centers

Recommendation 13: RNA testing for surgeries and aerosol-generating procedures (asymptomatic)

Recommendation 13: The IDSA panel suggests for SARS-COV-2 RNA testing in asymptomatic individuals (without known exposure to COVID-19) who are undergoing major time-sensitive surgeries (conditional recommendation, very low certainty of evidence)

- The panel defined time-sensitive surgery as medically necessary surgeries that need to be done within three months.
- Testing should ideally be performed as close to the planned surgery as possible (e.g., within 48-72 hours).
- To limit potential poor outcomes, deferring non-emergent surgeries should be considered for patients testing positive for SARS-CoV-2.
- Decisions about PPE use for the aerosol generating portions of these procedures may be dependent on test results when there is limited availability of PPE. However, there is a risk for false negative test results, so caution should be exercised by those who will be in close contact with/exposed to the upper respiratory tract (e.g., anesthesia personnel, ENT procedures).
- The decision to test asymptomatic patients will be dependent on the availability of testing resources.
- This recommendation does not address the need for repeat testing if patients are required to undergo multiple surgeries over time.

Recommendation 14: RNA testing for surgeries and aerosol-generating procedures (asymptomatic)

Recommendation 14: The IDSA panel suggests against SARS-CoV-2 RNA testing in asymptomatic individuals without a known exposure to COVID-19 who are undergoing a time-sensitive aerosol generating procedure (e.g., bronchoscopy) when PPE is available (conditional recommendation, very low certainty of evidence)

- The panel defined time-sensitive procedures as medically necessary procedures that need to be done within three months
- Procedures considered to be aerosol generating

Recommendation 15: RNA testing for surgeries and aerosol-generating procedures (asymptomatic)

Recommendation 15: The IDSA panel suggests SARS-CoV-2 RNA testing in asymptomatic individuals without a known exposure to COVID-19 who are undergoing a time-sensitive aerosol generating procedure (e.g., bronchoscopy) when PPE is limited, and testing is available (conditional recommendation, very low certainty of evidence)

- The panel defined time-sensitive procedures as medically necessary procedures that need to be done within three months
- Testing should be performed as close to the planned procedure as possible (e.g., within 48-72 hours)
- Decisions about PPE will be dependent on test results because of limited availability of PPE. However, there is a risk for false negative test results, so caution should be exercised for those who will be in close contact with/exposed to the patient's airways
- Procedures considered to be aerosol generating
- The decision to test asymptomatic patients will be dependent on the availability of testing resources
- This recommendation does not address the need for repeat testing if patients are required to undergo multiple procedures over time

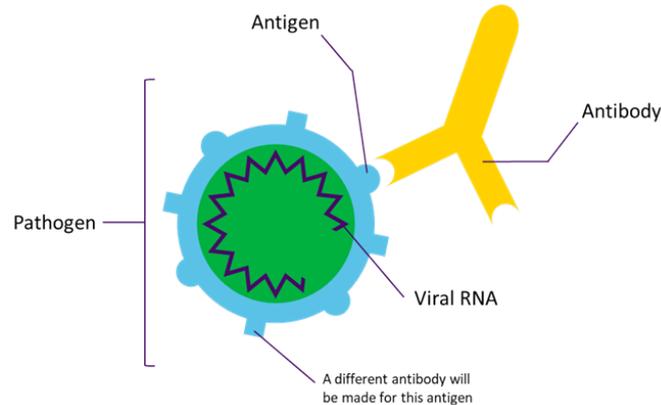
COVID-19 TESTING

Serology Testing

Immunological Testing

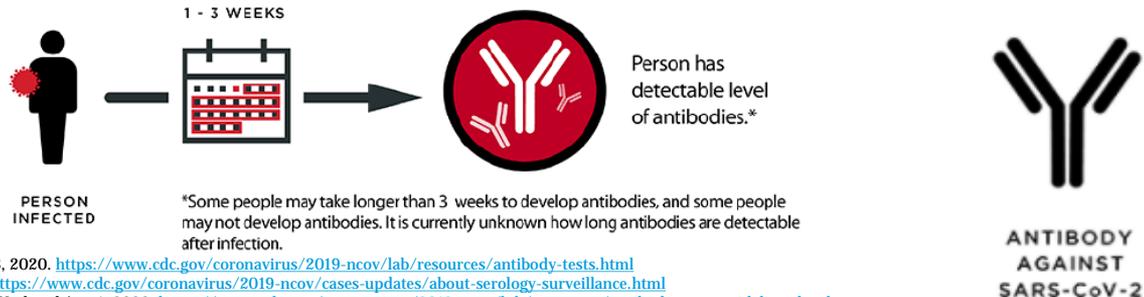
Antigen (Ag): A molecule on the surface of a cell recognized by an antibody

Antibody (Ab): a molecule made by the body to bind to an antigen



Antibody Testing

- Evidence suggests Abs develop 1-3 weeks after symptom onset, when infectiousness has decreased. Currently, there is no identified advantage to any of the available antibody assay (IgM, IgG, or total Ab).
- While it is believed that some amount of immunity develops, the US CDC stresses that more research is needed before serological test results should be used for modifying guidance or recommendations for public health.
- Antibody testing could be used for surveillance of SARS-CoV-2 outbreaks, but recommendations are that serology should not be used alone for acute cases in clinical care or for contact tracing.



*Some people may take longer than 3 weeks to develop antibodies, and some people may not develop antibodies. It is currently unknown how long antibodies are detectable after infection.

16. CDC. Using Antibody Tests for COVID-19. Updated May 28, 2020. <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests.html>

17. CDC. About Serology Surveillance. Updated July 6, 2020. <https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/about-serology-surveillance.html>

18. CDC. Interim Guidelines for COVID-19 Antibody Testing. Updated Aug 1, 2020. <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html>

19. CDC. What COVID-19 Seroprevalence Surveys Can Tell Us. Updated July 8, 2020. <https://www.cdc.gov/coronavirus/2019-ncov/covid-data/seroprevalence-surveys-tell-us.html>

CDC Key Points on COVID-19 Antibody Testing

- Positive antibody test presumes person has been infected in the past and does not mean currently infected
- The antibodies usually develop 1-3 weeks after infection.
 - Check package inserts for each specific test
- Not yet enough information to say if the person is protected from reinfection

²⁰<https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests.html>

CDC Key Points on COVID-19 Antibody Testing

- **Healthcare workers should know how the tests work and use caution when interpreting**
 - Current tests have had false positives and false negatives
 - Antibody tests shouldn't be used to diagnose a person with an active infection
- **Positive antibody tests without symptoms or if no recent exposure can continue normal activities**
- **If positive on antibody test and sick, should follow CDC guidelines on self care and protecting others**

²⁰<https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests.html>

COVID-19 TESTING

Molecular Technology Overview

Molecular Technology & Examples

Isothermal

NEAR

- Nicking Enzyme Amplification Reaction
- Abbott ID NOW™

LAMP

- Loop Mediated Isothermal Amplification
- Meridian Illumigene™

HDA

- Helicase Dependent Amplification
- Quidel Solana™

PCR

PCR

- Polymerase Chain Reaction
- Roche LIAT™
- Cepheid GeneXpert®

PNA FISH

- Peptide Nucleic Acid-Florescence In Situ Hybridization
- Biofire® FilmArray®



PCR
PNA FISH
Isothermal

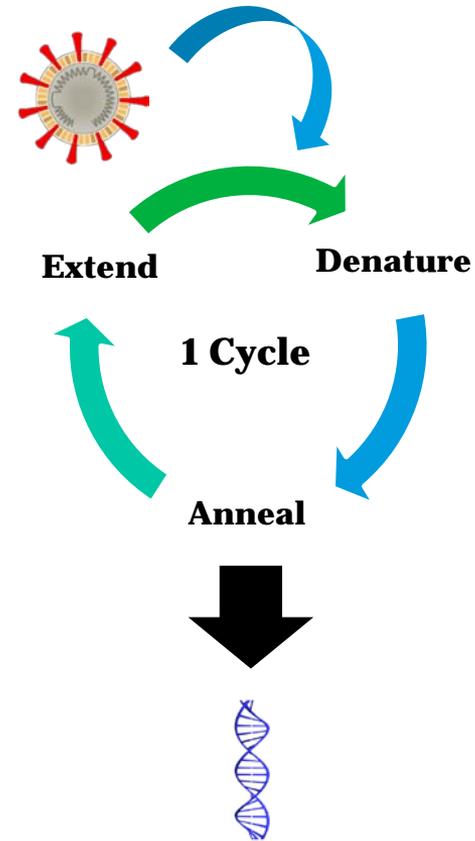
NEAR
LAMP
HDA

PCR Duplicates Viral RNA With Temperature “Cycles”

PCR is traditionally considered “gold standard” with high sensitivity

VIRUS PRESENT IN SAMPLE

- PCR uses thermocycling to amplify genetic material
- Each cycle typically includes steps of denaturing, annealing, and extension at different temperatures
- Ct = CYCLE THRESHOLD
- A Ct value represents the cycle number where DETECTABLE DNA is attained in the sample

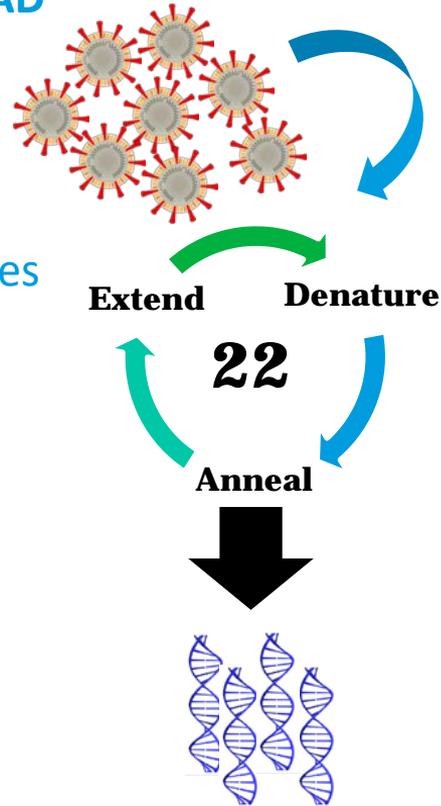


Cycle Times Are Representative of Viral Load

“HIGH” VIRAL LOAD

High viral loads require fewer cycles to generate detectable DNA

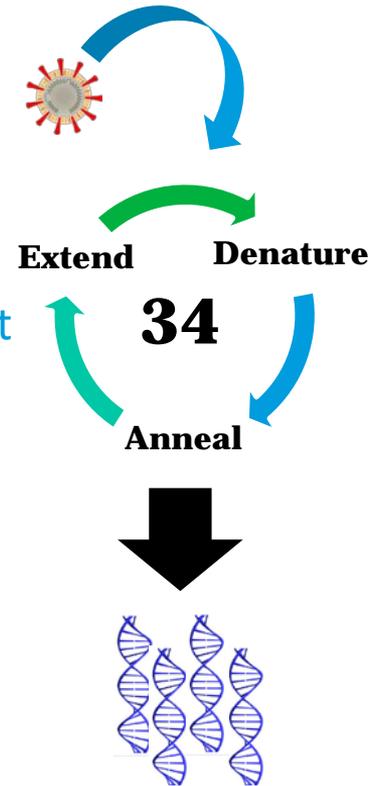
Cts are inversely lower.



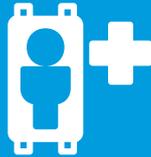
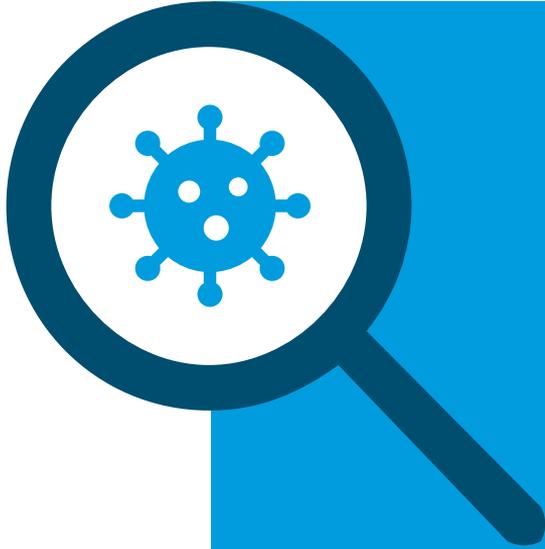
“LOW” VIRAL LOAD

Low viral loads require more cycles to generate sufficient detectable DNA

Cts are inversely higher.



Isothermal & PCR – It's All Molecular, but with a few differences



COVID-19 positive by PCR
does not always confirm
the patient is infectious



RNA can readily be detected from defective or inactivated non-infectious viruses at high Ct counts



If there is viable virus in these high Ct count samples, it is likely that the dose is too far below the infectiousness threshold (median TCID₅₀)



Molecular is a very useful tool, but mindfulness around the sensitivity of the assay versus the infectivity of the patient is crucial

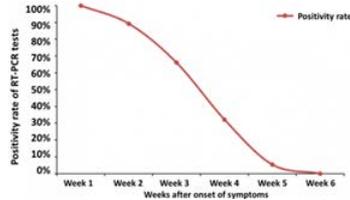
Evidence strongly indicates that PCR “positive” from samples with Ct >33 is from defective non-infectious virus

Time is also a key factor when considering active infections

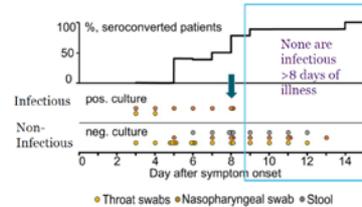


Findings

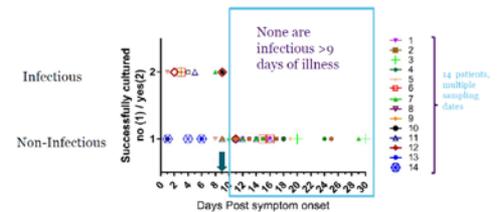
- Viral shedding peaks at time of onset of symptoms¹⁹, yet RT-PCR can remain positive for weeks²⁰
- 8-9 days past symptom onset, it is difficult to grow cultures (ref: Wolfel et al.)
- When culture positivity persists beyond 9 days, it is associated with high viral loads²¹



RT-PCR positivity over time, Lavezzo et al.**



Wolfel, R. et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 581, 465-469 (2020).



CDC: <https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html>

22. He, X. et al. Temporal dynamics in viral shedding and transmissibility of COVID-19 <https://www.nature.com/articles/s41591-020-0869-5>

23. Lavezzo, E. et al. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo', <https://www.nature.com/articles/s41586-020-2488-1>

24. Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants <https://www.medrxiv.org/content/10.1101/2020.06.08.20125310v1>

Targeted Molecular COVID-19 testing for virus in infectious phase which is what matters most



Findings

RT-PCR samples $Ct \leq 33$ the virus is likely present and infectious

This 0-7 days early phase is critical in terms of contagion and transmission

When previous studies are re-analyzed and the samples with $Ct > 33$ are eliminated, the sensitivity of the ID NOW™ COVID assay increases dramatically.



Inferences

Early detection not only yields faster treatment decisions but also helps break the transmission cycle

Cost effectiveness and deploy-ability at point of care can enable effective mass screening to help stop the pandemic

Do Molecular Tests Satisfy All Needs?

Depending on the type, it may not be timely

Depending on the availability, it may not be able to be purchased

Depending on the cost, it may not be affordable especially for repeat testing

COVID-19 TESTING

Antigen Testing

Mina – Rethinking COVID-19 Test Sensitivity²⁵

Four Factors to consider

- Targeted stage of the infectivity timeline
- Testing frequency
- Speed of results
- Testing frequency

Key Points

- The authors strongly encourage regulatory agencies such as the NIH, CDC, and FDA to encourage the development of “COVID-19 filters” to improve efforts to defeat the disease transmission with the evaluation of tests emphasizing the planned testing strategies.
- Focus must be fixed on the detection of infectiousness and away from test sensitivity (or LoD) in order to select appropriate tests to meet the demands for broad screening of COVID-19.
- Screening and clinical testing must be complementary, and each should target the appropriate stage of SARS-CoV-2 infectivity timeline to maintain usefulness.

Screening tests should be low cost and allow for rapid results with a high frequency of use, even if the test has lower sensitivity.

²⁵Mina, Michael J; Parker, Roy; Larremore, Daniel B Rethinking Covid-19 Test Sensitivity - A Strategy for Containment Journal Title: New England Journal of Medicine DOI: 10.1056/NEJMp2025631 PMID: 32997903

Viral Antigen Testing

- Most likely to perform well in patients with high viral loads (Ct values ≤ 25) early in the infectivity timeline.
- Could be implemented to reduce the number of molecular tests and to support rapid disease identification and management with minimum performance characteristics $>80\%$ sensitivity and 97-100% specificity.
- Patients who present more than 5-7 days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher.
- A negative result cannot completely rule out an active COVID-19 infection, and WHO recommends, repeat testing or preferably NAAT confirmatory testing performed whenever possible, particularly in symptomatic patients.

NOTE: WHO does not recommend Ag testing in low prevalence settings, including scenarios such as airport or border screenings. More research is needed to determine the utility of antigen rapid diagnostic tests (Ag-RDTs).

26. World Health Organization. (2020). Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: interim guidance, 11 September 2020. World Health Organization. <https://apps.who.int/iris/handle/10665/334253>. License: CC BY-NC-SA 3.0 IGO

Antigen Testing

The nucleoprotein of COVID-19 is conserved

- Does not cross-react with other coronaviruses

Testing is less expensive which means it can be done more frequently if test is approved for asymptomatic

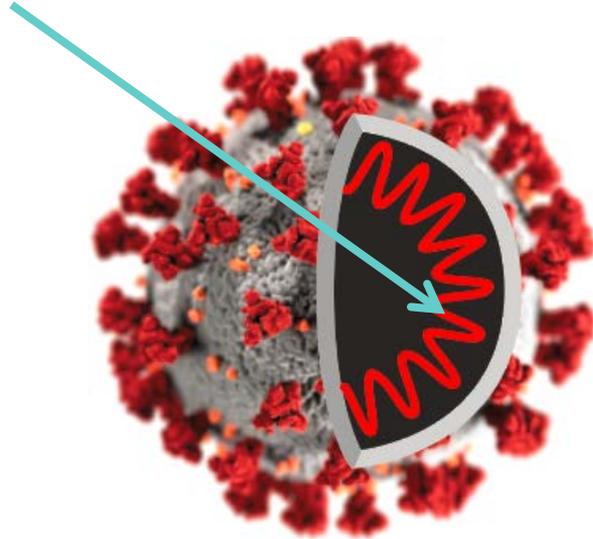
Results are fast

Sensitivity

- Less sensitive usually than molecular
- May be more sensitive than molecular with repeated testing

COVID-19 Ag Rapid Test Device

- *In vitro* diagnostic rapid test for qualitative detection of SARS-CoV-2 antigen (Ag)
- Detects nucleocapsid protein inside the SARS-CoV-2 virus



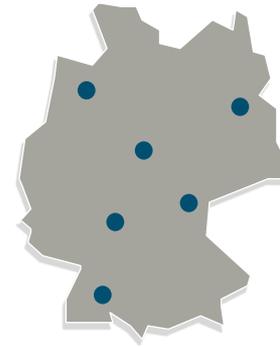
Deploy at Point of Care

- **Portable format allows fast setup of decentralized testing sites**
- **Deploy in lab and non-lab decentralized locations**
- **Samples are taken and directly applied to test at point of care**
- **No requirement to ship samples**

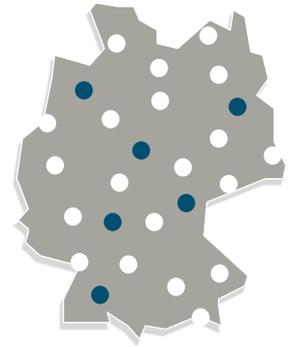


Extend Capacity of Lab Testing

- Relieve burden on busy labs
- Mass testing becomes achievable
- Extend geographic coverage
- Test can be run outside lab setting
- Deploy where lab PCR is unavailable/inconvenient



Lab Only



Lab + POC



Faster Answers for Patients

- Test is run at point of care
- Answers in 15-20 minutes, while patients wait
- Fewer follow up calls to patients
- Fast alternative if lab PCR unavailable
- Fewer bottlenecks on throughput
- Help reduce overall community transmission



Accessible Solution

- Less costly than molecular
- No capital equipment required
- No pre-installed instrumentation required
- Fewer infrastructure needs
- Simplified training



Discussion

More testing for COVID-19 is essential in helping control the pandemic

Different tests are more/less sensitive at different times of the infection

Rapid results allow a reduction in;

- **Additional unnecessary testing**
- **Confirmation that mild symptoms may not be COVID-19**
- **Congregation of potentially infectious people**
- **Testing in non-traditional sites**



Abbott