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# Laboratory Testing of Severe Acute Respiratory Virus Coronavirus 2

## A New York Institutional Experience



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### KEYWORDS

• Laboratory testing • Severe acute respiratory virus coronavirus 2 • COVID-19 • Pandemic

### KEY POINTS

- Severe acute respiratory virus coronavirus 2 virus traumatized New York in March and April of 2020 and the coronavirus disease 19 (COVID-19) pandemic left an indelible mark on the majority of individuals particularly, in New York City.
- There are 2 major categories of tests used to detect current or past viral infection: molecular and serologic assays.
- Molecular assays are designed to determine whether a patient is actively infected with a pathogen of interest.
- Serologic assays determine a patient's exposure history.

## INTRODUCTION

Throughout March, April, and May 2020, the severe acute respiratory virus coronavirus 2 (SARS-CoV-2) virus traumatized New York and the coronavirus disease 19 (COVID-19) pandemic has affected almost everyone, irrespective of title, status, or ethnicity. It has left an indelible mark on how people regard and conduct everyday life in the midst of the crisis. Clinical molecular laboratory scientists have been frustrated, exhausted, and perplexed at the implementation of diagnostic assays for the detection of SARS-CoV-2 and tests that measure the consequences of infection. Test management has deviated from routine operations under the auspices of regulatory bodies such as the Clinical Laboratory Improvement Amendment (CLIA), US Food and Drug Administration (FDA), College of American Pathologists, and Centers for Medicare & Medicaid Services (CMS). The implication of test validation and

approval has received a new meaning under Emergency Use Authorization (EUA). Perhaps the most noteworthy outcome is that this scenario has made laboratory professionals more visible and respected and induced a deeper sense of ownership of the profession. This brief article provides an overview of the types of testing available for SARS-CoV-2 patient management, as well how testing has affected the situation in New York City.

SARS-CoV-2 first emerged in Wuhan City, Hubei Province, China in December 2019. This novel coronavirus was subsequently isolated and sequenced [1] and has since spread worldwide causing severe disease, termed COVID-19. The World Health Organization (WHO) declared it a pandemic on March 11, 2020 [2]. Since the beginning of the outbreak, clinical laboratories have been developing various assays to aid in detecting SARS-CoV-2 and managing patients with COVID-19, although delays in deploying high-volume

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diagnostic testing, especially in the United States, have impeded public health containment strategies.

## LABORATORY TESTS FOR DETECTION OF SEVERE ACUTE RESPIRATORY VIRUS CORONAVIRUS 2

Clinicians rely on laboratory testing to provide clinically relevant, actionable results that can direct both inpatient and outpatient care. There are 2 main categories of tests used to detect current or past viral infection: molecular and serologic assays. Antigen-detection assays have also been used historically for diagnostic purposes. Molecular assays are designed to determine whether a patient is actively infected with a pathogen of interest, whereas the purpose of serologic testing is to determine prior exposure. The most widely used assays for detection of SARS-CoV-2 use reverse transcriptase polymerase chain reaction (RT-PCR). This technique is already commonly used in microbiology laboratories to detect RNA specific to respiratory viral pathogens, such as influenza and respiratory syncytial virus [3]. The WHO developed the first quantitative RT-PCR test for detecting SARS-CoV-2 and subsequently the US Centers for Disease Control and Prevention (CDC) began shipping its own RT-PCR test kits after receiving EUA by the FDA on February 4, 2020. However, there were complications that became apparent during the validation process that caused a setback in deploying the assay to the diagnostic community [4]. On February 29, 2020 the Wadsworth Center of the New York State Department of Public Health's RT-PCR assay was the second test to receive EUA. However, this assay was not designed for high-throughput testing, and it analyzed approximately 50 to 60 specimens per day per platform with a turnaround time of 4 to 6 hours from sample to answer. Consequently, testing remained at a minimum until mid-March 2020, when commercially available, fully automated SARS-CoV-2 real-time assays began receiving EUA. These high-throughput automated assays include, but are not limited to, the cobas SARS-CoV-2 Test run on the Roche COBAS 6800/8800 platform and the Abbott RealTime SARS-CoV-2 assay with the m2000 platform. Rapid point-of-care (POC) tests such as Xpert Xpress SARS-CoV-2 (Cepheid) and ID NOW COVID-19 (Abbott), which test single specimens, also became available. These molecular assays detect various viral targets, including SARS-CoV-2-specific targets such as ORF1 a/b, a nonstructural region and N2, a nucleocapsid recombinant protein as well pan-Sarbecovirus targets such as the envelope E-gene.

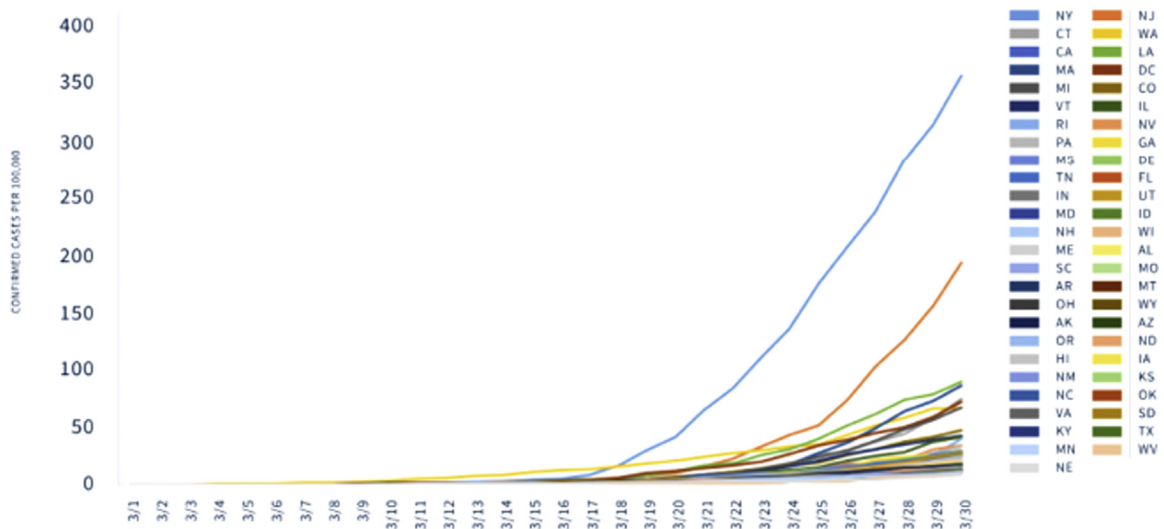
The ability to batch samples greatly increased testing capabilities in New York City. However, because of significant shortages of testing reagents, positive controls, collection swabs, transport media, and personal protective equipment, only the most critically ill patients presenting to the hospital were being tested. As a result, the biased positive rate of patients tested in New York State was around 50% and New York City was more than 70%. This crucial shortage in testing capacity significantly affected the public health response's ability to contain the virus. The number of SARS-CoV-2-positive cases increased exponentially in New York and adjoining states such as New Jersey, making this region the epicenter of the pandemic (Fig. 1).

With the increase in the number of assays that were verified in several hospitals and laboratories within New York, testing was gradually expanded in April 2020 beyond individuals with a very high pretest probability, to include all symptomatic individuals and people with exposure to known SARS-CoV-2. With this increase in the overall number of tests performed, the overall positive test rate decreased to approximately 20%, a more accurate reflection of the incidence of patients with COVID-19 (Fig. 2). With practicing of social distancing and contact precautions, in addition to expanded testing, the positive rate within the New York community has remained steady since early May 2020, at about 5% to 7%. The overall statistics for New York from early March until May 26th, 2020 can be seen in Fig. 2. Briefly, since the start of the pandemic, more than 2 million tests have been performed with an overall positive rate of approximately 20%. In terms of demographics, not only was incidence higher in men but they also had a much higher fatality rate (58.2%) compared with women (41.8%). Communities of color and lower socioeconomic status also were more seriously affected with higher rates of infection and mortality [5].

### Preanalytical Variables of Severe Acute Respiratory Virus Coronavirus 2 Diagnostics

To date there are more than 80 commercial laboratories and/or test kit manufacturers that have received approval for emergency use by the FDA for SARS-CoV-2 testing, with most being molecular assays [6]. Various reports document success with different specimen types ranging from nasopharyngeal (NP), oropharyngeal (OP), anterior nasal, and midturbinate nasal swabs to nasal washes and saliva [7]. In addition, the FDA recently granted EUA for an RT-PCR laboratory-developed test (LDT) for qualitative detection of SARS-CoV-2 in saliva specimens and a test that uses a

## CUMULATIVE CASES PER 100,000: ALL STATES



**FIG. 1** The number of positive cases statewide in the United States. (From March 31 White House briefing presentation. Available at: <https://assets.documentcloud.org/documents/6823042/0331-Briefing-BIRX-Final.pdf>.)

home collection kit with nasal swabs [6] (for details see <https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization>). A recent report showed comparable detection of respiratory viruses by RT-PCR with saliva and NP specimens [8]. Saliva as a specimen type, is appealing for its reduced risk posed at the time of collection; however, larger studies comparing saliva with other validated specimen types are essential for documenting the reliability of this specimen type. In an effort to expand testing capabilities, manufacturers and laboratories have adopted self-collection devices using predominantly anterior nares and midturbinate for sample collection [9]. However, the wide range of specimen types and their varied collection times during the course of COVID-19 infection could contribute to the false-negative rates seen in the RT-PCR assays. A recent study showed that the false-negative rate for SARS-CoV-2 RT-PCR testing can be as high as 67% in individuals tested up to 5 days after exposure and 21% in cases tested 8 days after exposure [10].

Acceptable NP and OP swabs are made with materials such as Dacron and rayon, because they do not inhibit the PCR reaction. Although specimens collected

with NP and OP swabs differ in tip size and flexibility, both have been used to successfully collect specimens for identification of SARS-CoV-2 [11]. Other specimens validated by different laboratories include nasal swabs, NP or nasal washes/aspirates, sputum, saliva, and bronchoalveolar lavage [12]. Because each of the specimen types examines different anatomic areas with variable levels of viral inoculum, the possibility of false-negative results should be ruled out for optimal patient management. The NP swab remains the gold-standard specimen source.

Transport media for swabs are reagents that retain virus viability in the specimen and minimize bacterial overgrowth for the time necessary to transport it to the clinical laboratory. Evaluation of different types of transport media, including but not limited to viral transport media and universal transport media, showed that specimens consistently yielded amplifiable RNA with mean cycle threshold differences of less than 3 over the various conditions assayed, thus supporting the use and transport of alternative collection media [13]. For SARS-CoV-2, the FDA has strongly recommended that viral culture not be performed. Thus, alternatives to classic viral transport media have been validated in light of media shortages. These alternatives

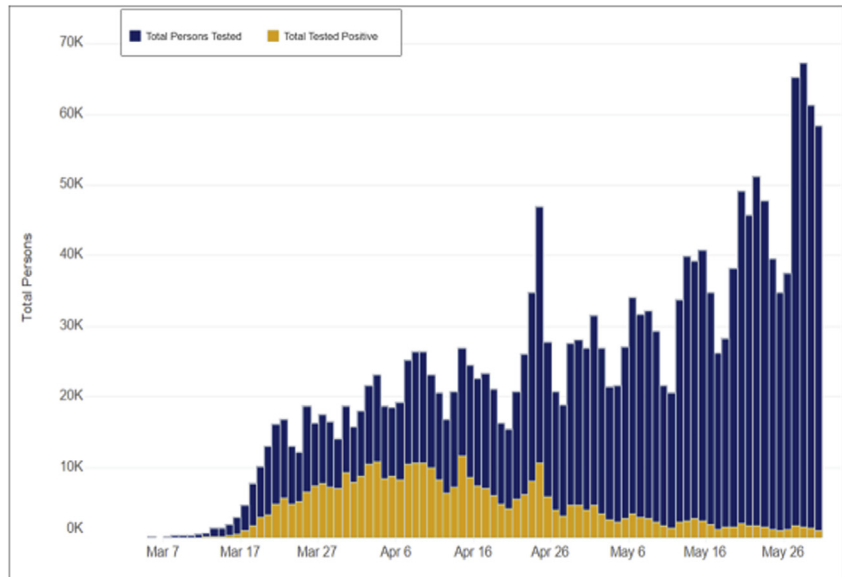


Total Persons Tested  
2,063,825

Total Tested Positive  
370,770

Sex Distribution  
Female 48.4%  
Male 51.0%  
Unknown 0.6%

## Total Persons Tested and Total Positives



**FIG. 2** Number of individuals tested and number of positive cases from March to May 26, 2020. (Data from New York State Department of Health. NYSDOH COVID-19 tracker. Available at: <https://covid19tracker.health.ny.gov/views/NYS-COVID19-Tracker/NYSDOHCOVID-19Tracker-DailyTracker?%3Aembed=yes&%3Atoolbar=no&%3Atabs=n>.)

include normal saline, Amies transport media, and Hanks balanced salt solution.

A caveat to interpreting molecular results is that it can be difficult to ascertain whether a patient has an active infection or was previously infected. Molecular assays can detect viral RNA both when patients are actively shedding the virus (current infection) and when there is residual viral RNA present. Therefore, these assays are most useful in acute settings to detect patients with SARS-CoV-2, where the results can optimize potential therapy and isolation protocols to ensure that appropriate personal protective equipment protocols are used for containment of the virus.

In addition to RT-PCR, reverse transcription loop-mediated isothermal amplification (RT-LAMP) technologies with increased levels of sensitivity have shown utility in resource-limited settings [14]. Notably, the first test using CRISPR (clustered regularly interspaced short palindromic repeats)-Cas12-based technology for SARS-CoV-2 detection was recently granted EUA (Sherlock Biosciences). The test has a limit of detection of 100 viral copies and involves a 2-step process, where SARS-CoV-2 RNA undergoes RT-LAMP followed by transcription of the amplified DNA, which activates

CRISPR cleavage of reporter genes resulting in a fluorescent readout. The entire process can be completed in an hour [15]. Tests that use high-throughput sequencing of the SARS-CoV-2 genome are also being used in a research setting. These tests give additional information on viral mutations and can trace the global evolution of the pandemic.

## RAPID POINT-OF-CARE MOLECULAR ASSAYS

POC testing is beginning to be available for SARS-CoV-2. POC testing refers to a broad category of diagnostic tests that can be performed where patient care occurs. Functionally, these tests have a rapid turnaround time and can potentially be performed by select nonlaboratory clinical personnel. However, at this current time, most clinical laboratories prefer to have all specimens set up by medical technologists in a biosafety cabinet rather than a POC setting. The ID NOW COVID-19 molecular POC test (Abbott) uses isothermal nucleic acid amplification (a technique similar to PCR) to detect SARS-CoV-2 in about 15 minutes. However, because of evidence that samples collected in transport media

may be below the assay's limit of detection [16–19], the EUA for this test was modified for testing only from direct swabs. Preliminary data indicates that, despite this modification, the Abbott ID NOW COVID-19 had a significant false-negative rate when using dry nasal swabs [20]. Some additional rapid assays that are commonly used are the Xpert Xpress SARS-CoV2 (Cepheid) and the BIOFIRE COVID-19 test.

## SEROLOGIC ASSAYS FOR CORONAVIRUS DISEASE 19

The other major type of diagnostic assay is serologic. These assays determine a patient's exposure history. At this time, it is unknown whether antibody detection equates to immunity. These assays detect the presence of antibodies against SARS-CoV-2 antigens in a patient's serum. There is a delay between the initial viral infection and the production of antibodies by the immune system. During this likely asymptomatic time, termed the window period, a patient who is infected with SARS-CoV-2, but has not yet produced antibodies, would test negative on such an assay. As the immune system mounts a response against the virus, immunoglobulin (Ig) M antibodies are initially produced, which are short lived, followed by a more durable IgG antibody response (Fig. 3). Therefore, serologic tests may be unique to 1 class of immunoglobulins or detect multiple and can typically be completed in 1 to 2 hours.

At present, there are at least 12 EUA serology assays, some of which are automated [6]. Most commercial serologic SARS-CoV-2 assays use a lateral flow assay technique and format, and for many of these there are unsubstantiated, or even false, claims about test performance [21]. The estimated median seroconversion time is 7 to 12 days, with virtually all patients with COVID-19 producing detectable antibodies approximately 15 days after onset of symptoms [22–24]. Therefore, these assays are most helpful in determining an individual's exposure status and perhaps in assessing the individual's immune response to SARS-CoV-2. Going forward, these assays can be particularly helpful in identifying SARS-CoV-2 in individuals who may have had symptoms consistent with SARS-CoV-2 but were never tested with an RT-PCR assay, as well as individuals who may have had asymptomatic infection. Given that ~80% of SARS-CoV-2 cases are mild to moderate in severity [24,25], and that molecular testing has predominantly been restricted to the most severely ill patients, the true number of SARS-CoV-2 cases is likely to be vastly greater than that available from molecularly confirmed case counts. Thus, serologic testing will help

identify the number of past infections, which can help epidemiologists better understand the true burden of disease to model viral dynamics.

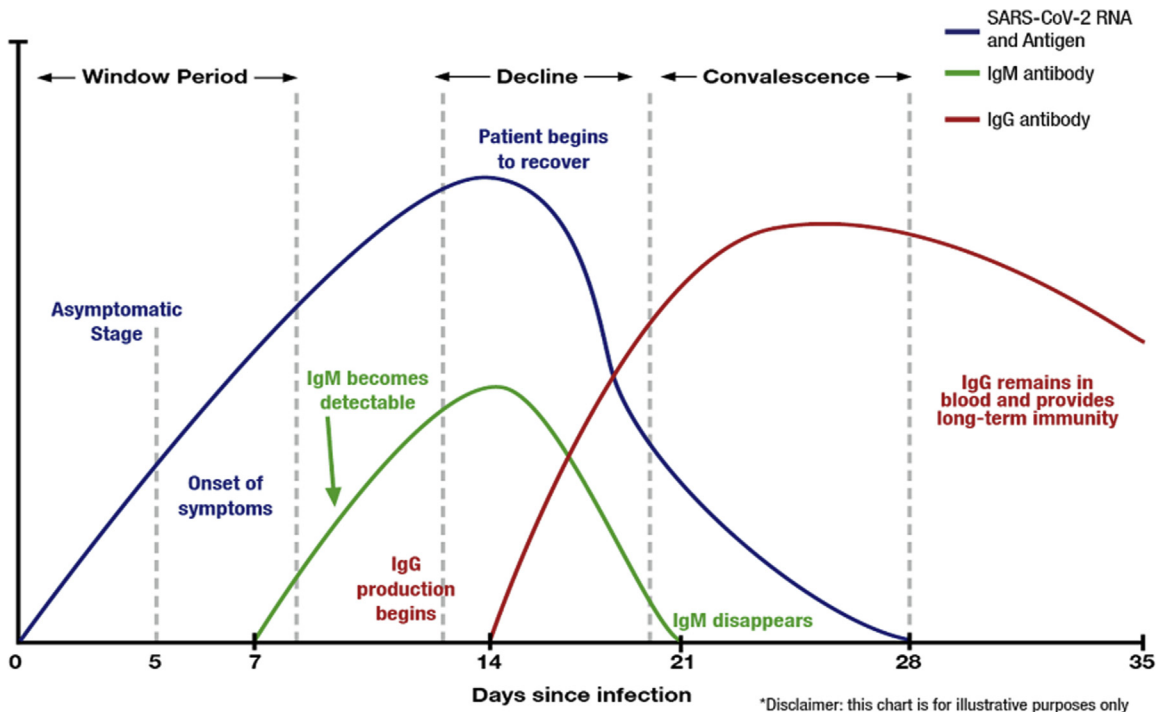
SARS-CoV-2 testing is also important for identifying potential convalescent plasma donors for clinical trials. Studies are currently underway where patients who have recovered from SARS-CoV-2 and have detectable antibodies against SARS-CoV-2 can donate plasma, which can then be transfused to patients who are currently critically ill with COVID-19. Theoretically, the neutralizing antibodies against SARS-CoV-2 present in the plasma will help patients currently infected overcome the illness. Serologic testing to identify anti-SARS-CoV-2 antibodies is now part of the donor work-up to determine eligibility for clinical trials. At some institutions in New York City, potential donors also require RT-PCR testing to determine whether they are still actively shedding virus and, therefore, contagious.

There is now 1 antigen-detection assay available from Quidel that uses a lateral flow CLIA of SARS-CoV and SARS-CoV-2. Information provided by the manufacturer in the package insert indicates an 80% concordance compared with PCR. Historically, antigen-detection kits for viruses have not performed well, so the utility for SARS-CoV-2 remains to be determined. Such tests are used routinely for other viruses: human immunodeficiency virus (HIV) p24 antigen as part of fourth-generation and fifth-generation HIV tests, and also for hepatitis B surface antigen [26].

## Considerations for Laboratory Testing During Unprecedented Times of Community Infections

Laboratories regulated by CLIA were able to get EUA for LDTs either directly from the FDA or from the Wadsworth Center of the New York State Department of Health, as in the case of several laboratories in New York. The EUA route permitted the laboratories to implement the LDTs for routine clinical diagnostics. However, in spite of these sanctions, the inability to provide broad diagnostic testing was widely seen as a failing effort to contain the virus. This setback of optimal testing in a crisis was largely caused by the lack of a national laboratory testing strategic plan that brings together the major players in diagnostic testing, including public health, clinical/hospital-based, and commercial laboratories. Clinical hospital-based laboratories play a major role in identification and containment of infectious threats, and a coordinated laboratory network would likely be more effective at damage control earlier in pandemics such as the current one [27].





**FIG. 3** The antibody response to SARS-CoV-2. (From Diazyme Laboratories. Why do we need antibody tests for COVID-19? Available at: <https://www.diazyme.com/covid-19-antibody-tests>; with permission.)

In summary, testing has been critical to understanding and managing the SARS-CoV-2 pandemic. Although both molecular and serologic tests provide meaningful data for treating patients with SARS-CoV-2, each methodology has a different clinical utility. Moving forward, clinical laboratories will continue to be on the forefront of combating this pandemic by developing new assays and implementing increased testing capabilities to meet the high-volume demands necessitated by this pandemic. A concerted rather than isolated effort may be the best approach to accomplish mass-scale testing.

## DISCLOSURE

The authors have nothing to disclose.

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