

## **Validation and Use of Point-of-Care Lateral Flow Chromatographic Immunoassays for Early Diagnostic Support During the COVID-19 Pandemic**

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In Delaware, the first case of coronavirus disease 2019 (COVID-19) was identified on March 11, 2020 and the first death attributed to COVID-19 occurred on March 26, 2020. The Delaware Public Health Laboratory (DPHL) was the only laboratory in the state that had testing capability for COVID-19. As of May 28, 2020, 9,171 cases were diagnosed and 345 Delawareans died due to complications associated with COVID-19. Since the first case was announced, Delaware moved rapidly to institute statewide mitigation and suppression strategies to limit effects on the populace and health infrastructure. However, the need for testing quickly overwhelmed supply chains and laboratory capacity for molecular testing with reverse transcriptase polymerase chain reaction (rt-PCR).<sup>1</sup> Consistent with FDA guidance, docket FDA-2020-D-0987, the Delaware Department of Health and Social Services, Division of Public Health (DPH) identified point-of-care lateral flow chromatographic immunoassays (“rapid tests”) as useful diagnostic adjuncts in a “PCR-sparing” testing strategy, given initial limitations in molecular testing capacity.<sup>2</sup>

In an effort to identify reliable rapid tests for implementation, DPH performed verification studies of point-of-care devices by various manufacturers. Extensive validation of the Pinnacle Biolabs COVID-19 Novel Coronavirus IgM/IgG Rapid Test was subsequently performed by the Delaware Public Health Laboratory.

The Pinnacle Biolabs COVID-19 Novel Coronavirus IgM/IgG Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy-colored conjugate pad containing recombinant COVID-19 antigen conjugated with colloid gold (COVID-19 conjugates) and quality control antibody gold conjugates; 2) a nitrocellulose membrane strip containing two test bands (T1 and T2 bands); and 3) a control band (C band). The T1 band is pre-coated with monoclonal anti-human IgG for the detection of anti-COVID-19 IgG, the T2 band is pre-coated with reagents for the detection of anti-COVID-19 IgM, and the C band is pre-coated with quality control antibody.

When an adequate volume of specimen (blood) is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. Anti-COVID-19 Ig antibodies—if present in the specimen—will bind to the COVID-19 conjugates. The immunocomplex is then captured on the membrane that is pre-coated with anti-human Ig antibodies. When a burgundy T1 or T2 band appears, it indicates an anti-COVID-19 IgG or IgM positive test result. Absence of both test bands suggests a negative result. Regardless of the presence of or absence of a detection band, the red quality control band C should appear; if it does not appear, the test result is invalid.

## Results

### Cross Reactivity/Analytical Specificity

A panel of 101 negative specimens was obtained, including 80 serum samples (frozen serum samples stored pre-pandemic), one sample (EDTA whole blood) from an individual confirmed to be negative for SARS-CoV-2 via rt-PCR, and 10 fresh fingerstick samples (capillary blood) from individuals confirmed to be negative for SARS-CoV-2 via rt-PCR. All samples were drawn from a population with a high prevalence of vaccination against influenza, hepatitis B virus, *Haemophilus influenzae*, and paramyxoviridae. In addition, five stored serum samples known to contain anti-RSV IgM and IgG as well as five stored serum samples known to contain anti-nuclear antibody (ANA) from individuals were tested.<sup>3</sup> Testing of the samples was performed in accordance with the manufacturer-supplied package insert. Of these samples, 100/101 showed no T1 or T2 bands, indicating a negative result (99% overall specificity), and 1/101 showed a T1 band only, indicating a negative result for IgM and positive result for IgG. Sample 1/101 was compared against chemiluminescent microparticle immunoassay, verifying a false positive for SARS-CoV-2 IgG.<sup>4</sup>

### Analytical Sensitivity

A total of 46 known-positive specimens (whole blood, EDTA) were obtained from consenting hospitalized patients confirmed to be infected with SARS-CoV-2 via rt-PCR. Immune status of the individuals or length of active infection was not known or collected. Testing of the samples was performed in accordance with the manufacturer-supplied package insert. Of the 45 specimens, 35 demonstrated a positive IgM and IgG band on rapid test, with one additional specimen demonstrating a positive IgM without IgG (80% sensitivity).

### Small-Scale Implementation and Prospective Verification

Following validation, DPH deployed rapid tests as part of outbreak investigations in areas with suspected or documented high prevalence of COVID-19 disease, principally within post-acute care facilities. Serological surveys can aid investigation of an ongoing outbreak and extent of an outbreak.<sup>5</sup> Tests were administered by licensed registered nurses or physicians, following training performed in-person or via instructional video. Specimens were collected in accordance with the manufacturer-supplied package insert. Specimens were collected simultaneously with nasopharyngeal swabs and compared to rt-PCR results. DPH monitored samples as part of a prospective observational effort to ensure satisfactory performance of rapid tests in a real-world setting. Institutional Review Board approval was not required, as testing was performed under executive authority consistent with the Eleventh Modification of the Declaration of a State of Emergency for the State of Delaware Due to a Public Health Threat.

Of these specimens, high specificity was maintained with no false positives identified by rt-PCR. Most specimens were identified to manifest both IgM and IgG, with some specimens showing IgM only and few showing IgG only. Multiple patients known to have remote infection with SARS-CoV-2 via positive rt-PCR testing manifested both IgM and IgG and were found to have repeat rt-PCR threshold cycle values ranging from low (17) to high (34).

## Large-Scale Implementation

Having gained confidence in the high specificity and strong negative predictive value of rapid tests, DPH opted to utilize these assays as part of a PCR-sparing strategy in a universal, community-wide outbreak investigation.<sup>6</sup> In late April of 2020, epidemiologic surveillance data and hospital indicators sparked concern for a high level of community prevalence of COVID-19 within sub-populations in Sussex County, Delaware. Focused molecular testing efforts consistently returned high rates of positive rt-PCR tests, with 40-50% of tests positive even among asymptomatic individuals. Subsequently, DPH partnered with hospital systems and community organizations in a directed effort to expand testing and provide education, social services, and wrap-around health services within affected communities. Partners implemented a multi-modal testing strategy harnessing the high specificity of rapid tests.

In late April and throughout May 2020, approximately 10,000 total tests (using both rapid and PCR tests) for COVID-19 were performed through community-based testing sites in Sussex County, including walk-up, drive-through, and test-in-place evolutions throughout Milford, Georgetown, Seaford, and the surrounding areas. Testing schema included progression to empiric isolation for those identified to have a positive IgM with rapid tests, out of concerns for a high-risk of SARS-CoV-2 infection, as well as secondary rt-PCR screening for those with negative antibody testing, effectively exploiting the high specificity of rapid tests while buttressing sensitivity via rt-PCR. Recognizing the inherent lag time of antibody response in the setting of acute infection, symptomatic individuals were referred directly to rt-PCR.

rt-PCR and serology results were concordant across all testing evolutions. Side-by-side ongoing validation and random quality assurance comparing serology and PCR results on individuals indicated continued high specificity of serology. DPH monitored disease incidence within communities and witnessed steady decline associated with intensity of testing and increase in social services. As COVID-19 incidence fell and molecular testing availability increased, serology was discontinued. By the conclusion of May 2020, COVID-19 incidence fell from a high of 60% to less than 5% at community testing sites.

## Conclusion

Following extensive validation and ongoing verification, DPH successfully deployed rapid antibody testing in a PCR-sparing strategy to greatly increase access to testing within high-prevalence communities. During early phases of the COVID-19 pandemic, identification of IgM served as a reasonable surrogate to identify high probability of infection with COVID-19, sparing the need for follow-up rt-PCR and permitting recommendations for empiric isolation. Falling COVID-19 incidence (and thus lower pre-test probability) coincided with significant improvements in molecular testing availability, allowing the discontinuation of rapid testing. Concentrated testing within Sussex County – facilitated by a PCR-sparing strategy utilizing rapid antibody testing and partnered with social services, community education, and wrap-around health services -- was associated with a significant decrease in COVID-19 incidence.

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