WASHOE COUNTY HEALTH DISTRICT
ENHANCING QUALITY OF LIFE

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EPI-NEWS

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COVID-19 OUTBREAK IN WASHOE COUNTY (6)

Viral Testing vs. Antibody Testing

Introduction

The Washoe County Health District (WCHD) reported the first case of COVID-19 on March 5, 2020. As of June 12, a total of 1,914 cases have been reported, which corresponds to an incidence rate of 406 cases per 100,000 population. See following table for an overview of morbidity, mortality, and testing of COVID-19 outbreak from the national, statewide, and local perspective.

Characterization	US	NV	Washoe
No. Cases	2,016,027	10,678	1,914
Cases per 100,000 population	614	347	406
Number of Deaths	113,914	462	69
Case Fatality Ratio (%)	5.65	4.33	3.61
Deaths per 100,000 population	34.71	15.00	14.63
Number of tests	22,517,262	229,273	38,965
Tests per 1000 persons	68.61	74.44	82.64

Data as of 6/12/2020

Daily epidemiology updates of COVID-19 can be found at https://covid19washoe.com. WCHD has published a series of Epi-News on COVID-19. Those issues can be found at www.TinyURL.com/WashoeEpiNews. One issue on the laboratory testing of COVID-19 was published on May 15. This newsletter will focus on serologic testing (i.e., antibody testing) on an in-depth level based on recently released interim guidelines published by CDC. [i]

Viral Testing

Viral detection methods such as molecular tests or antigen tests detect acutely infected persons. There are nearly 30 different molecular tests the FDA has cleared for emergency use authorization (EUA) - as of June 12, 2020. All testing for SARS-CoV-2 (the virus that causes COVID-19) should be conducted in consultation with a healthcare provider. Specimens should be collected around 2-3 days after symptom onset to pursue a viral testing. The type of specimen collection should be based on the specific type of test being used. Generally speaking, these specimens are frequently collected: nasopharyngeal (NP) swab, oropharyngeal (OP) swab, nasal midturbinate swab, or anterior nasal swab, NP wash/aspirate or nasal wash/aspirate, or sputum for patients with cough. So far, only one antigen test has been authorized for EUA use as of June 12, 2020. [ii]

The Nevada State Public Health Laboratory (NSPHL), major clinical labs, and commercial labs like LabCorp and Quest offer molecular testing for diagnosis of COVID-19. The turnaround time is relatively quick, i.e., 24-48 hours.

Antibody Testing

SARS-CoV-2 antibody tests help determine whether an individual was ever infected – even if that person never showed symptoms. Antibody tests do not typically replace viral tests which are primary tools for diagnosing an active infection, but they do have several important applications in monitoring and responding to the COVID-19 pandemic. As of June 12, 2020, only two serology IgG and two serology total antibody tests were authorized for EUA use by FDA. [iii] LabCorp offers both tests. Quest has the IgG test only. It is important to note that all currently authorized tests are qualitative rather than quantitative.

To simply put, antibody tests **CAN BE** used for

1) Assessing a proportion of population previously infected with SARS-CoV-2;
2) Assessing rates of herd immunity by combining demographic and geographic patterns;
3) Identifying persons potentially infected with SARS-CoV-2 and determining who may qualify to donate blood that can be used to manufacture convalescent plasma as a possible treatment for severely ill persons.
4) Supporting diagnosis of acute COVID-19 illness for persons who present 9-14 days after illness onset, in addition to viral direct detection tests.

5) Helping establish a diagnosis when patients present with late complications of COVID-19 illness, such as multisystem inflammatory syndrome in children.

However, antibody tests **SHOULD NOT BE USED** for

1) Determining if an individual is immune to reinfection at this time;
2) Deciding about grouping persons residing in or being admitted to congregate settings, such as schools, dormitories, or correctional facilities;
3) Deciding about returning persons to the workplace.

Development of Antibodies and Immunity

Nearly all immune competent individuals will develop IgM, IgG, IgA antibodies following SARS-CoV-2 infection. For the time being, little is known about IgA response in the blood. IgM and IgG antibodies arise nearly simultaneously in serum within 2 to 3 weeks after illness onset. Thus, detection of IgM without IgG is uncommon. How long IgM and IgG antibodies remain detectable following infection is not known. Development of neutralizing antibodies can also be assessed. Neutralizing antibodies inhibit viral replication in vitro. Their presence correlates with immunity to future infection, at least temporarily. Studies suggest that the presence of antibodies may decrease a person's infectiousness and offer some level of protection from reinfection. However, definitive data are lacking, and it remains uncertain whether individuals with antibodies (neutralizing or total) are protected against reinfection with SARS-CoV-2, and if so, what concentration of antibodies is needed to confer protection.

Antigenic Targets

Understanding antigenic targets is helpful for interpreting test results. Spike glycoprotein (S) and nucleocapsid phosphoprotein (N) are two major antigenic targets of SARS-CoV-2 virus against which antibodies are detected. Multiple forms of S protein- full-length (S1+S2) or partial (S1 domain or receptor binding domain (RBD)) – are used as antigens. The protein target determines cross-reactivity and specificity because N is more conserved across coronaviruses than S, and within S, RBD is more conserved than S1 or full-length S.

Types of Antibody Testing

Antibody tests can be broadly classified to detect either binding or neutralizing antibodies. The former determines the immune response, the latter determines the functionality of antibodies.

Binding antibody detection: These tests use purified proteins of SARS-CoV-2, not live virus, and can be performed in lower biosafety level labs (e.g., BSL-2). Tests fall into two broad categories: **Point-of-care (POC)** tests and **laboratory tests. POC** tests generally are lateral flow devices that detect IgG or IgG and IgM, or total antibody in serum, plasma, whole blood, and/or saliva. An advantage is that it is quick by fingerstick rather than venipuncture. **Laboratory tests** use Enzyme-Linked Immunosorbent Assay (ELISA) or chemiluminescent immunoassay (CIA) methods for antibody detection. Based on the reagents, IgG, IgM, and IgA can be detected separately or combined as total antibody.

Neutralizing antibody detection: FDA has not yet authorized the use of neutralization tests for SARS-CoV-2. Neutralization tests determine the functional ability of antibodies to prevent infection of virus in vitro. Testing will require BSL-3 or BSL-2 labs, depending on what form of the SARS-CoV-2 virus is used. If virus neutralization tests (VNT) such as the plaque-reduction neutralization test (PRNT) and microneutralization are used, testing requires BSL-3 labs. If pseudovirus neutralization tests (pVNT) is used, testing can be performed in BSL-2 labs.

Test Performance

One previous issue on the laboratory testing of COVID-19 published on May 15 illustrated the test performance. How well a test performs depends on the sensitivity and specificity of this test. Sensitivity and specificity are determined by using a defined set of negative and positive samples. Another key factor is the prevalence of a disease or condition in the tested population. Three factors together determine positive predictive value (PPV) and negative predictive value (NPV). PPV is the probability that individuals with positive test results are truly antibody positive. NPV is the probability that individuals with negative test results are truly antibody negative. For example, in a population where the prevalence is 5%, a test with 90% sensitivity and 95% specificity will yield a PPV of 49%. In other words, less than half of those testing positive will truly have antibodies.

Testing Strategies

Three strategies can be used to improve PPV.

1. Choosing a test with a very high specificity, perhaps 99.5% or greater, will yield a high positive predictive value in populations tested with prevalence ≥ 5%. The serological test for IgG antibody against SARS-CoV-2 at the Nevada State Public Health Laboratory (NSPHL) has good sensitivity and specificity at 100% and 99.6%, respectively. If testing is done in a population where the prevalence is 2%, the PPV is 84% and NPV is 100%.
2. Focus testing on persons with a high probability of having SARS-CoV-2 antibodies, such as persons with a history of COVID-19-like illness.
3. A third approach is to employ an orthogonal testing algorithm in which persons who initially test positive are tested with a second test. See Table 1 below.

Table 1: PPV Using one test or two orthogonal tests, by the background prevalence in the population tested.

Prevalence	PPV for one test	PPV for 2 Orthogonal tests
2%	26.9%	86.9%
5%	48.6%	94.5%
10%	66.7%	97.3%
30%	88.5%	99.3%

Sensitivity=90%, Specificity=95%

Limitations of Serological Tests

Several limitations of serological tests:

1. At present, the immunologic correlates of immunity from SARS-CoV-2 infection are not well defined. Representatives from governmental agencies, research institutions, academia, and medical community are working together to determine whether positive serologic tests are indicative of protective immunity against SARS-CoV-2. This work includes:
    a. Assessing the level of antibodies required for protection from reinfection, the duration of that protection, and the factors associated with development of a protective antibody response;
    b. the kinetics of antibody response;
    c. longevity of antibodies;
    d. the ability of antibodies to protect from repeat infection;
    e. the protective titer of neutralizing antibody;
    f. the correlation f binding antibody titers to neutralization ability are yet to be determined.
2. Cross-reactivity with other coronaviruses, such as those that cause the common cold. This could result in false-positive test results.
3. Some persons may not develop detectable antibodies after coronavirus infection.
4. It is possible that antibody levels could wane over time to undetectable levels.
5. IgM and IgG antibodies are not present early in infection. Thus, serologic test results do not indicate with certainty the presence or absence of current of previous infection with SARS-CoV-2. This is why serologic tests are not recommended for diagnosis purpose.

Recommendations for persons who test positive for anti-SARS-CoV-2 antibodies

1. It cannot be assumed that individuals with truly positive antibody test results are protected from future infection.
2. Asymptomatic persons who test positive for antibody and who are without recent history of a COVID-19 compatible illness, have a low likelihood of active infection and should follow WCHD's guidance to prevent infection with SARS-CoV-2 and otherwise continue with normal activities, including work.
3. Persons who have had a COVID-19-compatible or confirmed illness should follow WCHD's guidance regarding resumption of normal activities, including work.
4. There should be no change in clinical practice or use of personal protective equipment (PPE) by healthcare workers and first responders who test positive for SARS-CoV-2 antibodies.

[i] https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html

[ii] https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

[iii] https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd