Alameda County Health Care Services Agency
Public Health Department
www.acphd.org

Colleen Chawla, Director
Kimi Watkins-Tartt, Director
Nicholas Moss, MD, Interim Health Officer

Public Health Department: Main Line (510) 267-8000

COVID-19 Information: (510) 268-2101

Alameda County COVID-19 Laboratory Testing Guidance for Clinicians—September 2020

This guidance is intended to assist clinicians and health care facilities to use the most appropriate laboratory tests to identify current infection with SARS-CoV-2, the virus that causes COVID-19.

Available Test Types

Nucleic Acid Amplification Tests (NAATs), aka "Molecular" tests

The preferred and most common laboratory test for diagnosing active COVID-19 infections is a nucleic acid amplification test (NAAT), commonly referred to as a "molecular" test. There are several types of NAAT tests approved by the FDA Emergency Use Authorization (EUA) for COVID-19 testing – e.g., a real time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test, which is utilized by the CDC and UCSF Covid-19 assay; Transcription Mediated Amplification (TMA), utilized by the Hologic Panther system, etc. These are all molecular testing platforms that detect viral nucleic acids.

NAAT tests, run in laboratories that are designated by the Clinical Laboratory Improvement Amendments (CLIA) as "moderate or high complexity", whether they are RT-PCR or TMA-based tests, are still considered the gold standard for diagnosing SARS-CoV-2 infection in both symptomatic and asymptomatic patients. Sensitivity and specificity are comparable among many of the testing platforms run in moderate/high complexity CLIA laboratories. It is important to understand that most NAAT tests are reported as qualitative results only and may be sensitive enough to detect shedding of viral nucleic acids in patients who have recovered from active infection and are no longer infectious. This information forms part of the basis for the current public health recommendation NOT to use a test-based strategy for verifying clinical recovery and determining when to end isolation of infected patients. Refer to the Alameda County Public Health Department recommendation on releasing from isolation.

While specificity among commercial tests are high, sensitivity may vary depending on several factors, including the anatomic site, and timing of collection during the course of infection. [i] NAAT tests that are run in a CLIA-waived laboratory may have different sensitivity/specificity as compared to assays run in a CLIA moderate/high complexity laboratory. Thus, it will be important to fully assess the test prior to bringing it online in your facility. The two best known point-of- care molecular assays that can be run in a CLIA-waived laboratory such as a physician's office are the following:

- **Abbott ID NOW** is an isothermal NAAT. This is a CLIA-waived, point-of-care test (results available within 15 minutes) which is highly specific but considerably less sensitive in comparison to RT-PCR. As of June 19, 2020, several reports had been filed with the FDA regarding false negative results as compared with other molecular assays. One subsequently published study in the Journal of Clinical Virology, which directly compared results using the Abbott ID NOW with a non-point-of-care molecular assay, found the Abbott ID NOW to be 74% sensitive and 100% specific by comparison. For these reasons, negative results are considered "Presumptive Negative." Additional reports[ii] have shown that there may be the potential for false positive results if adequate cleaning of the instrument is not performed. Thus, re-testing of patients on another molecular testing platform *is recommended* for "presumptive negatives" as well as positive samples from asymptomatic persons without known exposures.

- **Cepheid GeneXpert® Xpress SARS-CoV-2** is a CLIA-waived, rapid point-of-care test using RT-PCR (results available within an hour). The sensitivity and specificity of this test are comparable with other RT-PCR assays offered in moderate/high complexity CLIA laboratories. Negative samples are considered negative.

Saliva Testing

On August 15, 2020, the FDA issued an EUA for SalivaDirect[1], an inexpensive, highly sensitive extraction-free RT-PCR assay which is performed on saliva, collected without preservatives in a sterile container. Studies by the developers at the Yale School of Public Health have demonstrated a high degree of concordance in testing outcomes when compared with RT-PCR on an NP swab (94.1% positive agreement and 90.9% negative agreement). Negative results should be confirmed by testing an alternative specimen type, if clinically indicated.

SalivaDirect testing is limited to high-complexity laboratories designated by the Yale School of Public Health. However, Yale intends to make the methodology available as an open-source protocol, noting that it "has been validated and authorized for use with different combinations of commonly used reagents and instruments, meaning the test could be used broadly in most high-complexity labs." Large scale studies of this testing methodology are still needed, but the low cost, ease of specimen collection, and

[1] FDA Issues Emergency Use Authorization to Yale School of Public Health for SalivaDirect. Accessed on September 14, 2020

reduction in need for supplies including reagents, swabs and PPE make this a promising new option when frequent and/or large-scale testing is needed.

Several additional molecular tests using saliva have received emergency use authorization by the FDA in the weeks following the approval of the SalivaDirect assay (scroll down to "Individual EUAs for Molecular Diagnostic Tests for SARS-CoV-2" and enter "saliva" in the search engine).

Antigen tests

Four rapid SARS CoV-2 antigen tests are now available[2] for point-of-care use, under FDA Emergency Use Authorizations (EUAs), and more antigen tests are in development. None of these tests is currently available for home use; all must be run in a healthcare setting with a CLIA certificate. The primary advantages of these tests are their low cost, portability, and rapid turnaround time – some yielding results in just 15 minutes.

Antigen tests qualitatively detect nucleocapsid protein from SARS-CoV-2. Although these tests are highly specific, their sensitivity is significantly lower than most molecular (NAAT) assays. Negative results using rapid antigen tests should be considered presumptive and should be confirmed using a non-point-of-care NAAT. For this reason, rapid antigen tests have received FDA emergency use authorization for use when pre-test probability is high – i.e., in symptomatic patients during the first few days of illness, in populations with a high prevalence of disease. Positive results may expedite clinical and infection control decisions.

It should be noted that samples for antigen testing should NOT be placed in viral transport medium, because the extra dilutional step may decrease the sensitivity of the assay and may carry the risk of cross-contamination resulting in false positive results. Instead, samples should remain dry and should be tested immediately or as soon as possible after collection.

[2] As of September 1, 2020

Summary of the characteristics of SARS-CoV-2 Antigen tests available as of September 1, 2020	
Test Name	Description
The **BD (Becton Dickinson) Veritor System for Rapid Detection of SARS-CoV-2**	This test should be run on nasal swabs ONLY and has a turnaround time of 15 minutes. Clinical studies run by the manufacturer documented 84% sensitivity and 100% specificity using this assay. The FDA EUA states that this test is indicated in "individuals who are suspected of COVID-19 by their healthcare provider within the first five days of the onset of symptoms."
The **Quidel Sofia 2 SARS Antigen FIA**	This test may be run on NP or nasal swabs and has a turnaround time of 15-30 minutes. Its sensitivity is estimated at 80%, with a specificity of 100%. The FDA EUA for this assay also states that the test is indicated for "individuals who are suspected of COVID19 by their healthcare provider within the first five days of the onset of symptoms." The Quidel Sofia 2 SARS Antigen FIA assay does not differentiate between SARS-CoV and SARS-CoV-2.
The **BinaxNOW COVID-19 Ag Card**	This test from Abbott Diagnostics is run on nasal swabs. The FDA EUA for this assay states that the test is indicated for "individuals suspected of COVID19 by their healthcare provider within the first seven days of symptom onset." In data submitted to the FDA from a clinical study conducted by Abbott, when used for this indication, the BinaxNOW COVID-19 Ag Card demonstrated sensitivity of 97.1% and specificity of 98.5%. This test can be used with a free mobile app called NAVICA that documents the results.
The **LumiraDx SARS-CoV-2 Ag Test**	This test is run on nasal swabs. The FDA EUA states that the test is indicated for "individuals who are suspected of COVID-19 by their healthcare provider within the first twelve days of the onset of symptoms". The manufacturer's instructions for use indicate sensitivity of 97.6% (confidence interval of 91.6-99.3%) and specificity of 96.6% (confidence interval 92.7-98.4%).

According to the Association of Public Health Laboratories (APHL) rapid SARS-CoV-2 antigen tests should generally NOT be used for screening asymptomatic individuals, including healthcare workers and emergency responders, and should not be the primary tests considered when expanding testing to underserved populations. However, the CDC has issued Interim Guidance suggesting the use of serial antigen testing in closed congregate settings, such as long-term care facilities or correctional facilities, because this strategy may allow rapid detection of persons with SARS-CoV-2 infection, enabling early implementation of measures to prevent transmission and reduce the risk of an outbreak. When antigen testing is deployed for screening testing in this manner, CDC recommends that POSITIVE tests be confirmed by RT-PCR if pre-test probability is low, and NEGATIVE tests be confirmed by RT-PCR when pretest probability is high. Persons should be isolated while awaiting confirmatory test results in either situation.

Modeling evidence suggests that similar use of rapid antigen testing for screening of asymptomatic cohorts, although an off label use of the technology, may reduce viral transmission and the risk of outbreaks in other settings if testing is deployed with sufficient frequency.

Serological testing

Serological testing (i.e. antibody testing) for surveillance and research purposes is beyond the scope of this guidance. Serological testing for diagnosis of acute COVID-19 infection is not currently recommended. Refer to the ACPHD Serological Testing for COVID-19 FAQ for more information on serological testing. Serological testing is recommended to support a diagnosis of Multisystem Inflammatory Syndrome in Children (MIS-C); see the ACPHD Health Alert for more information.

Guide to collecting specimens from the respiratory tract to detect SARS-CoV-2

The table below is designed to assist clinicians to understand and evaluate the different types of specimens that may be used to diagnose SARS-CoV-2 infection in patients.

For initial diagnostic testing for the presence of SARS-CoV-2, Alameda County recommends collecting and testing an upper respiratory specimen. If the initial upper respiratory sample result is negative and the suspicion remains high, a lower respiratory tract sample may be appropriate – for example, in patients who are intubated or who have a tracheostomy. Sputum induction to diagnose SARS-CoV-2 is NOT recommended, as this is an aerosol-generating procedure that may pose unnecessary risk to healthcare workers. In situations where patients have developed a productive cough, especially if they are hospitalized, clinicians should consider collecting and testing sputum from the lower-respiratory tract.

There are several options for collecting a swab from the **upper respiratory tract** that could be performed either **by health care personnel or supervised onsite self-collection**. Studies in the past have found NP swabs to be more sensitive than OP swabs for detecting other human coronaviruses, and a combination of NP and OP swabs to be more sensitive than either alone[iii]. However, current studies find that anterior nasal or nasal mid-turbinate specimens, even self- collected with supervision, may yield specimens which have sensitivities comparable to NP swabs.[iv] [v] When possible, for increased sensitivity, consider collecting swabs from two anatomical sites.

Respiratory specimen collection to detect SARS-CoV-2

Tract	Anatomic Site	Collection	Comments
Upper Respiratory Tract	Nasopharyngeal (NP) swab	Use only synthetic fiber swabs with plastic or wire shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.	The NP swab is collected by a health care provider from the posterior nasal pharynx, making this collection method the most likely to result in the patient sneezing or coughing, thus generating potentially infectious aerosols.
	Oropharyngeal (OP) swab		For increased sensitivity, consider collecting a swab from a second anatomical site.
	Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW)	Attach catheter to suction apparatus	Specimen and the non-bacteriostatic saline used to collect the specimen should be placed immediately into a sterile transport tube
	Nasal mid-turbinate swab	Use a flocked tapered swab	May be collected by patient when supervised by a health care worker
	Anterior nares (nasal swab)	Use a flocked or spun polyester swab, sample both nostrils with same swab	
Lower Respiratory Tract	Bronchoalveolar lavage	Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container	Most often used in hospitalized patients who are receiving invasive mechanical ventilation
	Endotracheal aspirate		

Specimen Collection

The CDC has provided guidelines for collecting, handling, and testing clinical specimens for COVID19, which include PPE requirements, techniques to prevent contamination of swabs, and techniques for specimen collection.

- **Specimen Collection Instructions**

Proper collection of specimens is the most important step in the laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to false negative test results. For more information, including illustrations and step-by-step guidance applicable to respiratory viruses in general, see the CDC Influenza Specimen Collection instructions. The New England Journal of Medicine's How to Obtain a Nasopharyngeal Swab Specimen guidance presents written visual, and video aids for collecting an NP swab.

- **Swab Type**

  Swab specimens for COVID19 testing should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.

- **Multiple sites**

  Consider collecting samples (no more than two) from multiple upper respiratory sites to increase sensitivity. If two swabs are collected, they should be combined in a single tube to maximize test sensitivity and limit use of testing resources.

- **Transport**

  Unless otherwise indicated, all swabs to be used for NAAT (molecular) tests should be placed immediately into a sterile transport tube containing 2-3mL of either viral transport medium (VTM), Amies transport medium, Universal Transport Medium (UTM), sterile saline, or a transport medium provided by the reference testing laboratory. Swabs for rapid antigen testing should NOT be placed in VTM, and delays between sample collection and testing should be minimized.

- **Self-collection at home**

  The FDA has issued a number of EUAs for **home collection kits**. These are NOT home-based tests, but rather kits that a person uses to collect a specimen from the anterior nares at home. The specimen is then packaged up and sent (e.g.,

via FedEx) to the appropriate lab for testing – generally using a molecular testing platform.

Providers may identify home collection kits with EUAs by navigating to In Vitro Diagnostics EUAs, then scrolling down to the section labeled "Individual EUAs for Molecular Diagnostic Tests for SARS-CoV-2" and typing "home collection" into the search box. Some of these kits include web-based instructional videos, and some require that a healthcare provider observe the collection of the sample by video in real time.

Home-based rapid antigen testing, using a lateral flow assay, may become available in the future but is not available at this time.

Laboratory Availability for Covid19 testing

Besides using the correct specimen collection method from the most appropriate anatomic site, work with your laboratory to understand their requirements for the type of specimens that are acceptable as well as the transport medium. For example, tracheal aspirate specimens may take longer to process than NP/OP swabs at some laboratories, and not all laboratories are properly validated to test these specimens.

Use the table below to identify laboratories that have the capacity to accommodate testing. Many new laboratories have established the capability and capacity for molecular Covid19 testing and can offer 24-48-hour turnaround time for results upon receipt of the specimen.

Available Testing Laboratories for Molecular Covid19 Testing		
Testing Lab	**Contact Information**	**Scope of Test Availability**
Laboratories Providing Testing for the General Population		
Laboratory Testing Facilities Across California	https://testing.covid19.ca.gov/ Select the "Labs with Testing Capacity" icon	
Alameda County Public Health Department sponsored free COVID-19 Testing Sites	https://covid-19.acgov.org/testing.page	
California Department of Public Health sponsored free INDIVIDUAL COVID-19 Testing Sites	https://testing.covid19.ca.gov/	
Avellino	Provides swabbing kits and transport media. Contact: Liz Puwal Liz.Puwal@avellino.com	Contacted ACPHD to report Turn Around Time for testing results within 72 hours from time of sample receipt
Biocept	Provides swabbing kits and transport media. Contact: Mary Nothum mnothum@biocept.com	Contacted ACPHD to report 24-48 hour Turn Around Time for Testing results from time of sample receipt
Exceltox	Provides swabbing kits and transport media. Contact: Jonathan Pittman jonathan@exceltox.com	Contacted ACPHD to report 24-48 hour Turn Around Time for Testing results from time of sample receipt
Fulgent	Provides swabbing kits and transport media. Contact: William Pirjamali wpirjamali@fulgentgenetics.com	Contacted ACPHD to report 24-48 hour Turn Around Time for Testing results from time of sample receipt
SDI	Provides swabbing kits and transport media Contact: Ozman Mohiuddin Ozman@sdilabsinc.com	Contacted ACPHD to report 24-48 hour Turn Around Time for Testing results from time of sample receipt

Available Testing Laboratories for Molecular Covid19 Testing		
Testing Lab	**Contact Information**	**Scope of Test Availability**
Quest Diagnostics	Provides swabbing kits and transport media Contact: 1866-697-8378	
LabCorp	Provides swabbing kits and transport media Contact: https://www.labcorp.com/provider-services	
Verily	Contracts with Quest/LabCorp for Testing. Contact Verily for more information. Contact: contact@projectbaseline.com	
Laboratories Offering Molecular Covid19 testing for Specific Populations		
Kaiser	Currently not accepting testing for Non-Kaiser member. If a Kaiser member, request testing through Kaiser doctor.	
Sutter	Call 866-961-2889 to inquire about whether your patient qualifies for testing.	
UC Berkeley (IGI)	Provides swabbing kits and transport media Contact: Lea Witkowsky@berkeley.edu lwitkowsky@berkeley.edu	Long Term Care Facilities, Hospitals, UC Berkeley
Stanford	Call 1877-717-3733 to inquire about whether your patient qualifies for testing.	
UCSF (Chan Zuckerberg)	Provides transport media, not swabs Contact: Robert Puccinelli Robert.puccinelli@czbiohub.org	Mainly supporting testing for Public Health Departments

Resources and Links

- Alameda County Department of Public Health Testing for COVID-19 Information
- Alameda County Department of Public Health Testing Sites
- CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19
- Alameda County COVID-19 Testing Supplies Survey- If your organization needs testing supplies specifically for the COVID-19 testing, please complete this survey.
- Nasal (Anterior Nasal) Specimen Collection for SARS-CoV-2 Diagnostic Testing (Nasal (Anterior Nasal) Specimen Collection for SARS-CoV-2 Diagnostic Testing Factsheet
- New England Journal of Medicine How to Obtain a Nasopharyngeal Swab Specimen

[i] Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 2020;323(22):2249-2251. doi:10.1001/jama.2020.8259

[ii] Pradhan R. Problems With Trump-Touted COVID-19 Test Pile Up. Kaiser Health News. https://www.thedailybeast.com/problems- with-trump-touted-abbott-rapid-covid-19-test-pile-up. Published June 19, 2020. Accessed June 19, 2020.

[iii] Lieberman D, Lieberman D, Shimoni A, Keren-Naus A, Steinberg R, Shemer-Avni Y. Identification of respiratory viruses in adults: nasopharyngeal versus oropharyngeal sampling. J Clin Microbiol. 2009;47(11):3439-3443. doi:10.1128/JCM.00886-09

[iv] Tu Y-P, Jennings R, Hart B, et al. Patient-collected tongue, nasal, and mid-turbinate swabs for SARS-CoV-2 yield equivalent sensitivity to health care worker collected nasopharyngeal swabs. medRxiv. Published online April 6, 2020:2020.04.01.20050005. doi:10.1101/2020.04.01.20050005

[v] Self-collection: an appropriate alternative during the SARS-CoV-2 pandemic - Abstract - Europe PMC. Accessed June 10, 2020. https://europepmc.org/article/ppr/ppr150237