Influenza SARS-CoV-2 Multiplex Assay, CDC (Lab Tests and Diagnostic Procedures)

Comment

The FDA has noted that unauthorized **fraudulent** COVID-19 test kits are being sold online. Currently the only way to be tested for COVID-19 is through licensed health care providers. The FDA advises consumers and health professionals to be cautious of websites and stores selling products that claim to prevent, diagnose, treat, or cure COVID-19 (FDA 2020). Any concerns regarding fraudulent products, including tests, should be sent to: FDA-COVID-19-Fraudulent-Products@fda.hhs.gov.

Related Information

- Coronavirus Disease (COVID-19) Antigen, Upper Respiratory Specimen
- Coronavirus Disease (COVID-19), PCR, Respiratory Specimen
- Coronavirus Disease (COVID-19), PCR, Saliva

Overview

At the end of 2019, a novel coronavirus was identified as the cause of several cases of pneumonia in Wuhan City, Huber Province, China. Initially linked to a large seafood and animal market suggesting animal-to-human spread, person-to-person transmission was quickly confirmed (Li Q 2020). The virus spread rapidly worldwide and in January 2020, the World Health Organization (WHO) declared the outbreak a "public health emergency of international concern." On March 11, 2020, the WHO publicly characterized COVID-19 as a pandemic. Currently, more than 42 million infections have been confirmed globally in over 200 countries and territories with over 1.1 million deaths (WHO situation rept 2020).

The novel virus has been named **severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)** and the disease it causes has been named **coronavirus disease 2019 (COVID-19)**. The virus is thought to spread mainly by respiratory droplets produced when an infected person coughs or sneezes. The droplets can land in the mouths, noses and eyes of nearby people, or possibly be inhaled into the lungs. Touching a contaminated surface and transferring to eyes, nose or mouth is also thought to be a mode of transmission although not as dominant as by respiratory droplets. Symptoms typically appear within 2 to 14 days (median of 5 days) of exposure and include **fever, chills, fatigue, cough, shortness of breath, myalgia, recent loss of taste or smell, vomiting or diarrhea, and/or sore throat**. Sickness ranges from a mild respiratory illness to severe disease including respiratory failure, septic shock, or other organ failure. Most fatalities have occurred in patients with underlying comorbidities, with overall global fatalities around 3 percent (WHO situation rept 2020). Currently, there is no vaccine or specific treatment; care is supportive.

Molecular nucleic acid polymerase chain reaction testing has become the current gold standard method for diagnosis of SARS-CoV-2 infection (see COVID-19, Respiratory Specimen), and because COVID-19 infection symptoms can be similar to influenza and other respiratory infections caused by various pathogens, several panels have been developed for qualitative differentiation of nucleic acid from multiple organisms. In preparation for the upcoming flu season, the CDC has developed a nucleic acid

test for the simultaneous detection and differentiation of SARS-CoV-2 and Influenza A/B in both upper and lower respiratory specimens.

Use/Indications

Aid in the diagnosis of infection with SARS-CoV-2, Influenza A, and/or Influenza B; the detection of organism-specific viral RNA also provides epidemiological and surveillance information.

Test Includes

Rapid qualitative detection, differentiation, and identification of nucleic acid from SARS-CoV-2, influenza A and/or influenza B in upper and lower respiratory specimens

Specimen

Upper Respiratory Tract Specimen

For initial COVID-19 testing, the CDC recommends collecting an upper respiratory specimen. This includes (CDC, Interim Guidelines 2020):

- Nasopharyngeal (NP) specimen collected by a health care provider. **Or**
- Oropharyngeal (OP) specimen collected by a health care provider. **Or**
- Nasal Mid-turbine (NMT) specimen collected by a health care provider or by a supervised onsite self-collection (using flocked or tapered swab). Both nares should be swabbed. **Or**
- Anterior nares (nasal swab; NS) specimen collected by a health care provider or by onsite or home self-collection (using a flocked or spun polyester swab). Both nares should be swabbed. **Or**
- Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) specimen collected by health care provider.

Lower Respiratory Trace Specimen

- Bronchoalveolar lavage (BAL), tracheal aspirate, pleural fluid, lung biopsy
- Sputum - for patients who develop a productive cough; induction of sputum is **not** recommended.

Container(s)

- Confer with testing laboratory for proper specimen container and collection.
- Lower respiratory specimen: Sterile, screw-cap plastic container
- Nasal mid-turbinate (NMT) specimen: Flocked tapered swab
- Nasal swab or anterior nares (NS) specimen: Flocked or spun polyester swab
- Nasopharyngeal wash/aspirate or nasal wash/aspirate specimen: Sterile viral transport media tube

- NP or OP specimen: Use only synthetic fiber swab with plastic or wire shaft. Do not use calcium alginate swab or cotton swab with wooden shaft.

Volume / Minimum Volume

- 1 NP swab or 1 OP swab or 1 NS swab or 1 NMT swab
- Fluids: 2-3 mL

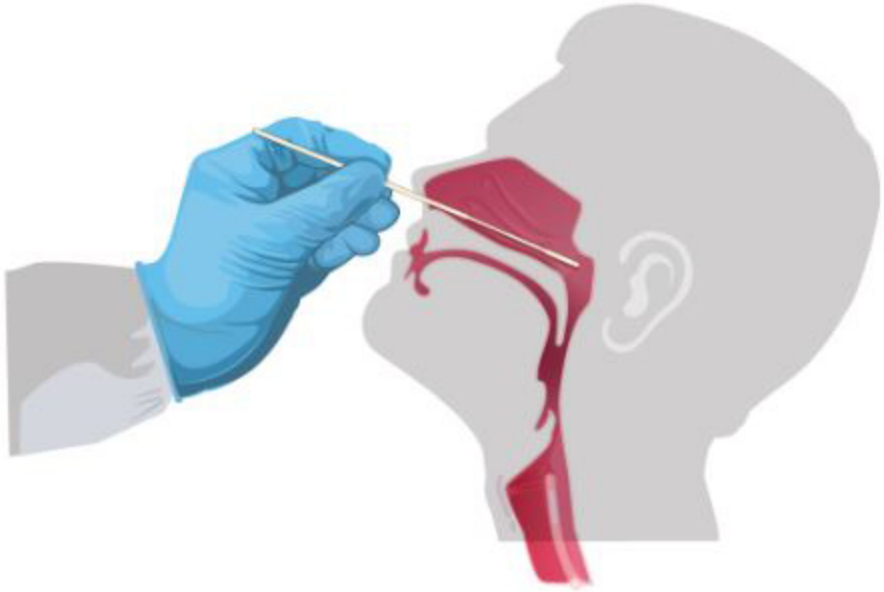
Collection

Prior to specimen collection, patient identity should be confirmed using two independent identifiers; use of a patient identification arm band or similar system is recommended. Specimen label(s) should include the two independent identifiers and the date of collection. There should be a method to identify the individual collecting the specimen. The specimen container(s) should be labeled in the presence of the patient after specimen is collected. Container(s) should **not** be prelabeled. Use computer-generated label(s), if available, to avoid transcription errors.

Specimen should be collected by trained health care professional using proper infection control techniques and wearing personal protective equipment, including an N95 respirator (or facemask if respirator is not available), eye protection, gloves, and a gown.

Upper Respiratory Tract Specimen

- **NP swab:** Insert a minitip swab with flexible (wire or plastic) shaft through the nostril parallel to the palate (not upwards) until resistance is detected or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Gently rub and roll swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Specimens can be collected from both sides using the same swab, but it is not necessary to collect from both sides if the minitip is saturated with fluid from the first collection. If a deviated septum or blockage create difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril. Place swab immediately into sterile tubes containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, or sterile saline. If both are collected, NP and OP specimens should be placed in the same vial at collection site.



CDC 2020

- **OP (throat) swab:** Avoiding the tongue, teeth, and gums, insert swab into the posterior of pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx. Place swab immediately into sterile tubes containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, or sterile saline. If both are collected, NP and OP specimens should be placed in the same vial at collection site.
- **NMT swab (deep nasal swab):** Use a flocked tapered swab. Tilt patient's head back 70 degrees. While gently rotating, insert swab less than one inch (about 2 cm) into nostril (until resistance is met at turbinates). Rotate the swab several times against nasal wall and repeat in other nostril using the same swab. Place swab immediately into sterile tubes containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, or sterile saline.
- **NS (anterior nares):** Using a flocked or spun polyester swab, insert the swab at least 0.5 inch (1 cm) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds. Sample both nares with same swab. Place swab immediately into sterile tubes containing 2 to 3 mL of viral transport media (VTM), Amies transport medium, or sterile saline.
- **NP wash/aspirate or nasal wash/aspirate (NW):** Sterile collection by health care provider. Attach catheter to suction apparatus. Have the patient sit with head tilted slightly backward. Instill 1 to 1.5 mL of nonbacteriostatic saline (pH 7.0) into one nostril. Insert the tubing into the nostril parallel to the palate (not upwards). Catheter should reach depth equal to distance from

nostrils to outer opening of ear. Begin gentle suction/aspiration and remove catheter while rotating it gently. Place specimen in a sterile viral transport media tube.

Lower Respiratory Tract Specimen

- **BAL, tracheal aspirate, pleural fluid, lung biopsy:** Sterile collection by health care provider. Collect 2 to 3 mL into sterile, leakproof, screw-cap container. Note, collection of lower respiratory specimen other than sputum may be limited to patients presenting with more severe disease (admitted to hospital and/or fatal cases).
- **Sputum:** Patient should be educated on difference between sputum and saliva. Patient should rinse mouth with water then expectorate deep cough sputum directly into sterile, leakproof screw-cap container. **Note:** Sputum induction is **not** recommended.

For more information see: CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19).

See Guidelines for submitting specimens to CDC for laboratory testing for SARS-CoV-2.

Processing and Storage

- For all specimens: Refrigerate at 2°C to 8°C and ship overnight on ice pack to CDC (or qualified laboratory). Specimens are stable 72 hours.
- For longer storage, freeze specimen(s) at -70°C.
- For state labs, follow specific shipping guidelines.

Methodology

Real time Reverse Transcriptase-Polymerase Chain Reaction (rRT-PCR)

Normal Values/Findings

Negative or not detected

Positive results are indicative of active infection

All positive SARS-CoV-2 results must be reported to local/state health departments.

Interpretative Information

Influenza A Result	**Influenza B Result**	**SARS-CoV-2 Result**	**Interpretation**
+	–	–	Influenza A RNA detected
–	+	–	Influenza B RNA detected
–	–	+	SARS-CoV-2 RNA detected
+	+	–	Influenza A RNA and Influenza B RNA detected

Influenza A Result	Influenza B Result	SARS-CoV-2 Result	Interpretation
+	–	+	Influenza A RNA and SARS-CoV-2 detected
–	+	+	Influenza B RNA and SARS-CoV-2 detected
+	+	+	Influenza A RNA, Influenza B RNA, and SARS-CoV-2 detected

- A positive result indicates the presence of organism-specific nucleic acid; results must be correlated with patient history and clinical symptoms. Positive results do not rule out infection with other pathogens (bacteria or coinfection with other viruses).
- A negative test result means that SARS-CoV-2 RNA and/or influenza was not present in the specimen above the limit of detection, thus a negative result does not rule out the possibility of either infection and should not be used as the sole basis for patient management decisions.

Limitations

- False negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also be the result of amplification inhibitor presence, the presence of organisms below the limit of assay detection, or mutations (of SARS-CoV-2, influenza A or B) in the rRT-PCR target region (CDC Flu SC2 2020).
- There is a decreased sensitivity for influenza A when a high titer of SARS-CoV-2 or influenza B is also present in patient specimen. If a negative result for influenza A is obtained and there is suspicion of coinfection, additional testing of specimen for Influenza A with an acceptable diagnostic test is recommended (CDC Flu SC2 2020).
- Individuals who received nasally administered influenza A vaccine may have positive influenza A test results for up to three days after vaccination.
- This assay cannot rule out infections caused by other bacterial or viral pathogens

Diagnostic Role

Similar to COVID-19 illness (and other respiratory infections cause by various pathogens), the most common symptoms of influenza are fever, cough, shortness of breath, fatigue, headache, myalgia, and arthralgia. Because treatment is available for some viral infections it is important to identify the correct pathogen and also investigate possible coinfection - which has been noted in some COVID-19 patients (Ding 2020; Kim 2020).

Additional Information

See Guidelines for submitting specimens to CDC for laboratory testing for SARS-CoV-2.

For more information, interim guidance has been issued by the United States CDC and the World Health Organization.

See: Flu Symptoms and Diagnosis

See: Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19).

See: Information for Laboratories

See: COVID-19 Testing and Reporting by Laboratories: Q & A

See: Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)

See: For Healthcare Professionals

See: FDA Emergency Use Authorizations

Index Terms

COVID-19, Influenza PCR; Flu SC2; Influenza A PCR; Influenza B PCR; SARS-CoV-2, Influenza PCR

Applies to

Pandemic; Person Under Investigation (PUI); Pneumonia of Unknown Etiology; Seasonal Influenza

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