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

Comment

The FDA has noted that unauthorized **fraudulent** COVID-19 test kits are being sold online. 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 Beware 2021). Consumers and health care professionals can help by reporting suspected fraud to the FDA's Health Fraud Program or the Office of Criminal Investigations.

On March 12 2021, the FDA issued a letter to clinical laboratories, point-of-care facilities, and health care providers warning that false positive results can occur with the Roche Molecular Systems, Inc, cobas SARS-CoV-2 & Influenza A/B Nucleic Acid Test for use on the cobas Liat System. Sporadic leaking of tubes can lead to erroneous results, particularly for Influenza B; abnormal PCR cycling may cause false positives with Influenza A, Influenza B and/or SARS-CoV-2 (FDA, Potential False Positives 2021).

Prior receipt of a COVID-19 vaccine will **not** affect viral testing for SARS-CoV-2.

Related Information

- COVID-19 Antigen, Upper Respiratory Specimen
- COVID-19, PCR, Respiratory Specimen
- COVID-19, PCR, Saliva
- Influenza and SARS-CoV-2 Antigen, Upper Respiratory Specimen
- Respiratory Panel, PCR, Nasopharyngeal

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 400 million infections have been confirmed globally in over 200 countries and territories with over 6 million deaths (WHO situation rept 2022).

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, or COVID-19**. The virus spreads by contact with respiratory fluids (droplets or aerosol) produced when an infected person exhales (eg, breathes, speaks, coughs, sneezes, or sings). These droplet/aerosol particles can be

- inhaled directly into lungs
- directly deposited on exposed mucous membranes (eyes, mouth, nose)

- transferred to mucous membranes by hands contaminated with virus-containing respiratory fluids or by indirectly touching surfaces with virus on them (CDC SARS-CoV-2 Transmission 2021)

COVID-19 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 2 to 3 percent (WHO situation rept 2021). The CDC currently estimates that about 30% of COVID-19 infections are asymptomatic, and 50% of transmission occurs prior to symptom onset (CDC Pandemic Planning 2021).

Nucleic acid amplification 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 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 flu season, the CDC developed a nucleic acid amplification test for the simultaneous detection and differentiation of SARS-CoV-2 and Influenza A/B in both upper and lower respiratory specimens. Several other nucleic acid amplification assays have received EUAs for SARS-CoV-2 and Influenza A/B detection/differentiation in nasopharyngeal and nasal specimens.

The FDA has also issued EUAs for COVID-19 and influenza (multianalyte) nasal swab home collection kits. These kits are available for individuals suspected of respiratory viral infection consistent with COVID-19 when home collection is determined to be appropriate by a health care provider. Specimens must be shipped to an approved laboratory for testing (FDA Exact Sciences 2021; FDA Quest Diagnostics 2020).

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 2021):

- Nasopharyngeal (NP) specimen collected by a trained health care professional. **Or**
- Oropharyngeal (OP) specimen collected by a trained health care professional. **Or**
- Nasal mid-turbine (NMT) specimen collected by a trained health care professional or by a supervised or unsupervised onsite self-collection (using flocked tapered swab), or self-collected at home following kit instructions. Both nares should be swabbed. **Or**

- Anterior nares (nasal swab; NS) specimen collected by a trained health care professional, or by a supervised or unsupervised onsite self-collection (using a flocked or spun polyester swab), or self-collected at home following kit instructions. Both nares should be swabbed using the same swab. **Or**
- Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) specimen collected by health care professional. **Or**

Lower Respiratory Tract 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 or oropharyngeal specimen: Use only synthetic fiber swab with plastic or wire shaft. Do not use calcium alginate swab or cotton swab with wooden shaft.
- Nasopharyngeal wash/aspirate or nasal wash/aspirate specimen: Sterile viral transport media tube

Volume / Minimum Volume

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

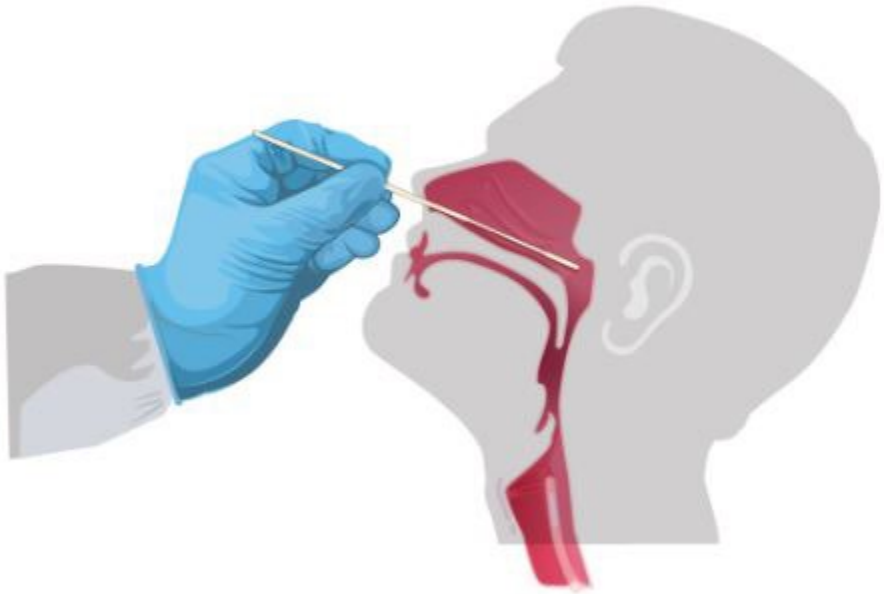
Collection

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. See CDC Nasopharyngeal Specimen Collection Steps.



CDC Interim Guidelines 2021

- **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. See CDC Nasal Mid-turbinate Specimen Collection Steps.

- **NS (anterior nares):** Using a flocked or spun polyester swab, insert the tip of swab 0.5 to 0.75 inch (1 to 1.5 cm) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab in a circular path against the nasal wall at least 4 times. Take approximately 15 seconds to collect the sample. Be sure to collect any nasal drainage that may be present on the swab. Sample both nostrils with same swab. Follow manufacturers instructions for swab storage and processing. See CDC How to collect Your Anterior Nasal Swab Sample for COVID-19 Testing.
- **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.

See CDC Influenza Specimen Collection instructions.

For more information see: Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing.

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, local, or reference laboratories, 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 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 caused 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).

Laboratory/Diagnostic Pearls

Childen tend to shed the influenza viruses (A and B) in larger amounts and for longer periods of time than adults (CDC Flu SC2 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: Guidance for Healthcare Workers about COVID-19 (SARS-CoV-2) Testing

See: Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing

See: Information for Laboratories

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

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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