Testing Supply Substitution Strategies

This resource is intended for labs performing COVID-19 tests that are authorized. This resource includes validated supply alternatives that labs can use to continue performing testing when there is a supply issue with some components of a test.

The information in this resource is not intended to alter any already issued EUA for a COVID-19 diagnostic test nor is it intended to speak to any specific FDA regulatory requirement. Rather, the information is being provided to help address availability concerns regarding certain critical components of COVID-19 diagnostic tests during this pandemic.

START: Go to the Main Menu
Real-time RT Polymerase Chain Reaction (PCR) Component Substitution Strategies

Select a button to learn more about a topic

 Specimen Collection
Learn about specimen collection, swabs, and media

 Intro to PCR
Learn about RT-PCR: what it is and what it’s used for

 System Types
Compare use of Open Mix and Match Systems vs. Closed Systems, including instruments, supplies, and reagents

 PCR Testing Process
Learn about the steps in the PCR testing process

 Substitution Options
Learn about substitution options for the Open Mix and Match style CDC test

Return to the Main Menu  
Additional Resources  
Return to FDA FAQ
Specimen Collection

Specimen collection is the process of obtaining a sample from a patient, usually by swabbing the nose or mouth, then placing the swab in a tube that is commonly filled with liquid (media) which maintains the sample for transport to the lab.

Originally, specimen collection for SARS-CoV-2 testing required a specialized Nasopharyngeal (NP) swab and Viral Transport Media (VTM)/Universal Transport Media (UTM).

Options for swabs and media are below.

For more information please see the FDA FAQ on this topic under, "What if I Do Not Have…?" It is important that the swab type be appropriate for the anatomic site on which it is used, i.e. only a Nasopharyngeal swab should be used to obtain a Nasopharyngeal specimen.”
## Specimen Collection: Swabs

### Choices for Swabs*

<table>
<thead>
<tr>
<th>NasoPharyngeal (NP) Swab</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OroPharyngeal (OP) Swab</strong></td>
</tr>
<tr>
<td><strong>Mid-Turbinate (MT) Swab</strong></td>
</tr>
<tr>
<td>Anterior Nares (“Nasal”) Swab</td>
</tr>
</tbody>
</table>

- Flocked, tapered swab
- Round Foam
- Spun Polyester

---

* For more information please see the [FDA FAQ on this topic](https://www.fda.gov) under, “What if I Do Not Have...?”

It is important that the swab be appropriate for the anatomic site on which it is used, and that the swab type (e.g. polyester vs rayon) is compatible with that platform. Rayon swabs may not be compatible with all molecular testing platforms. Analytical testing should be performed to confirm compatibility with individual platforms.

---

*Additional Resources*

- [Return to Specimen Collection](#)
- [Return to PCR Testing Process “Collect Specimen from Patient”](#)
- [Return to the Main Menu](#)
- [Return to FDA FAQ](#)

Released 6/3/2020
Specimen Collection: Media

Some transport media may contain guanidine thiocyanate, which produces a dangerous chemical reaction releasing cyanide gas when exposed to bleach (sodium hypochlorite). These media may not be compatible with in vitro diagnostic products that do not utilize guanidine thiocyanate during sample processing.

*WARNING: Do not use PrimeStore MTM with the Hologic Panther or Panther Fusion Systems due to a disinfecting step involving bleach that is specific to the platform. When the bleach interacts with the guanidine thiocyanate in the transport media, it produces dangerous cyanide gas.

<table>
<thead>
<tr>
<th>Choices for Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTM/UTM</td>
</tr>
<tr>
<td>Liquid Amies-based Media</td>
</tr>
<tr>
<td>• e.g., Eswab</td>
</tr>
<tr>
<td>Nucleic Acid Transport Media</td>
</tr>
<tr>
<td>• e.g., Primestore MTM*</td>
</tr>
<tr>
<td>Saline Solution</td>
</tr>
<tr>
<td>• Normal saline</td>
</tr>
<tr>
<td>• Phosphate-buffered saline (PBS)</td>
</tr>
</tbody>
</table>

Additional Resources

Return to Specimen Collection
Return to PCR Testing Process “Collect Specimen from Patient”
Return to the Main Menu

Released 6/3/2020
What Is RT PCR?

RT-PCR stands for “Reverse-transcriptase polymerase chain reaction” (commonly referred to as “PCR”)

RT-PCR is a method used to detect RNA nucleic acids (a type of genetic material)

• PCR is used to detect the presence of SARS-CoV-2, the virus that causes COVID-19 disease
• Finding SARS-CoV-2 genetic material in a specimen indicates that a person has been infected with the virus
Overview of System Types

*Select a system type for more information*

- **Closed System**
  - Extraction and Amplification

- **Open Mix and Match**
  - Extraction
  - Amplification

Return to the Main Menu

Additional Resources

Return to FDA FAQ

Released 6/3/2020
Closed System

Instruments

A single dedicated instrument performs extraction and amplification

Extraction and PCR System

Supplies and Reagents

Some materials may be specific to the instrument platform

Proprietary Reagent Kit

Proprietary Reagent Cartridge

Collection and Media

General Reagents

Return to the Main Menu

Return to Overview of System Types

Additional Resources

Return to FDA FAQ

Released 6/3/2020
Open Mix and Match (CDC Design)

**Instruments**
*Extraction and amplification performed separately*

- **Extraction System**
  ![Extraction System](image1.png)

- **PCR System**
  ![PCR System](image2.png)

**Supplies and Reagents**
*Some materials may be usable on more than one system*

- **Primers and Probes**
  ![Primers and Probes](image3.png)

- **Master Mix**
  ![Master Mix](image4.png)

- **Collection and Media**
  ![Collection and Media](image5.png)

- **General Reagents**
  ![General Reagents](image6.png)

Return to the Main Menu
Return to Overview of System Types
Additional Resources
Return to FDA FAQ
Released 6/3/2020
Select the STEPs above for more information about the PCR testing process.

STEP 1
Collect Specimen from Patient

STEP 2
RNA Nucleic Acid Extraction

STEP 3
RT-PCR Amplification

STEP 4
Results Reporting
INPUT FOR THIS STEP? A sample from the patient to test for the presence of SARS-CoV-2

WHAT SUPPLIES ARE NEEDED?
A swab to collect the sample.

Transport Media in a collection tube to transport the sample without degradation. The swab carrying the sample is placed in a collection tube filled with transport media.

WHAT IS THE OUTCOME? A sample for PCR analysis
INPUT FOR THIS STEP? A sample for PCR analysis

WHAT SUPPLIES ARE NEEDED?
Extraction System
Extraction Reagents
• Lysis Buffer
• RNA Extraction Control
• Human Specimen Control
• Positive Control specific to SARS-CoV-2

WHAT IS THE OUTCOME? Extracted nucleic acids
INPUT FOR THIS STEP? Extracted nucleic acid

WHAT SUPPLIES ARE NEEDED?

PCR REAGENTS
- PCR buffer
- dNTPs (building blocks of nucleic acids)
- Reverse Transcriptase (RT)
- No Template Control
- Polymerase (Enzyme)
- Positive Control (specific to SARS-CoV-2)
- Primers and Probe (SARS-CoV-2 specific)

WHAT IS THE OUTCOME? Fluorescence output signal indicating presence of SARS-CoV-2 RNA
INPUT FOR THIS STEP?
Fluorescence output signal

WHAT IS THE OUTCOME?
Report of output indicating presence of SARS-CoV-2 virus
Substitution Options*

*Substitution Options are illustrated with the CDC test for this tool. Labs can identify and validate alternative options to other authorized assays.

Select a Test Component on the left to explore possible substitutions for the Open Mix and Match style CDC molecular test.
Primers and Probes are the key reagents for direct detection and identification of a given target sequence.

Targets listed in the CDC EUA

- CDC N1
- CDC N2
- RNaseP

CDC Vetted Acceptable Manufacturers (per CDC guidance)

- BioSearch Technologies
- Integrated DNA Technologies

Additional Resources

Return to the Main Menu
Return to Substitution Options
Additional Resources
Return to FDA FAQ

Released 6/3/2020
The master mix contains the reagents for the PCR process. Alternatives will need to be validated with the PCR system.

Master mixes listed in the CDC EUA

- Quantabio qScript XLT
- Quantabio UltraPlex
- Promega GoTaq
- Thermofisher TaqPath

Validation Needed

Equivalent Master Mix

CAUTION
The PCR system is the instrument performing amplification.

**PCR system listed in the CDC EUA**

- Applied Biosystems 7500 Fast Dx Real-Time PCR

**Acceptable Alternatives (per FDA FAQ*)**

- Applied Biosystems 7500 Fast Real-Time PCR
- Applied Biosystems QuantStudio 6 Flex
- Applied Biosystems QuantStudio Dx
- QIAGEN Rotor-Gene Q MDx

* Check [FDA FAQ](https://www.fda.gov) for updates after 6/3/2020
Controls are the reagents that monitor the extraction and amplification process.

Controls listed in the CDC EUA

- CDC Positive Control
- CDC Extraction Control

Acceptable Alternatives (per FDA FAQ*):

- BEI Resources (N1, N2 Positive Control)
- Integrated DNA Technologies (N1, N2, Rnase P Positive Control)
- Human RNA (Rnase P Positive Control, Extraction Control)
- Twist Bioscience (Synthetic RNA Controls)

* Check FDA FAQ for updates after 6/3/2020
The Extraction system is the instrument performing extraction. The Extraction kit contains the reagents for the Extraction process.

**Extraction Systems/kits listed in the CDC EUA**
- QIAGEN/QIAmp DSP Viral RNA Mini Kit
- QIAGEN/QIAamp Viral RNA Mini Kit
- QIAGEN EZ1 Advanced XL/EZ1 DSP Virus Kit, Buffer AVL
- QIAGEN EZ1 Advanced XL/EZ1 Virus Mini Kit v2.0, Buffer AVL

**Acceptable Alternatives (per FDA FAQ*)**
- QIAGEN QIAcube/QIAmp DSP Viral RNA Mini Kit
- QIAGEN QIAcube/ QIAmp Viral RNA Mini Kit
- Roche MagNA Pure LC/Total Nucleic Acid Kit
- Roche MagNA Pure Compact/Nucleic Acid Isolation Kit I
- Roche MagNA Pure 96/DNA and Viral RNA Small Volume Kit
- bioMérieux NucliSENS easyMAG/EasyMAG Extraction Reagents, Biohit Pipette Tips
- Beckman RNAdvance Viral XP extraction kit
- bioMérieux EMAG/EasyMAG Extraction Reagents, EMAG 1000μl Tips
- KingFisher Flex/Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 Kit
- Applied Biosciences MagMAX Express/Viral/Pathogen Ultra Nucleic Acid Isolation Kit
- Promega Maxwell RSC 48/Maxwell RSC Viral Total Nucleic Acid Purification Kit

* Check [FDA FAQ](https://www.fda.gov) for updates after 6/3/2020
General consumable laboratory supplies used in PCR

- Molecular grade water, nuclease-free
- Aerosol barrier pipette tips
- Microcentrifuge tubes
- DNA Decontamination Reagent (DNAZap)
- Surface Decontaminant (RNAse Away)
- PCR Reaction Plates
- Vortex Mixer
- Microcentrifuge
- Micropipettes
- 10% Bleach
- MicroAmp Optical 8-cap Strips
Additional Resources

• [FDA Frequently Asked Questions on Diagnostic Testing for SARS-CoV-2](#)

• [CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel](#)