Testing Supply Substitution Strategies

U.S. FOOD & DRUG ADMINISTRATION

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.

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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

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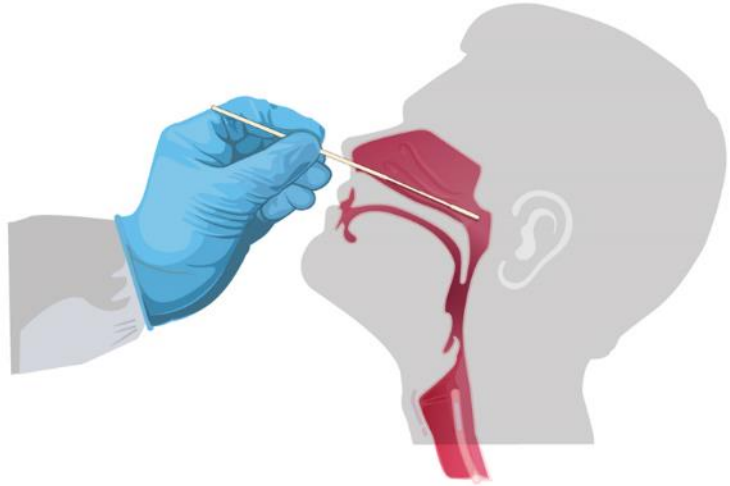
Specimen Collection

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).

Options for swabs and media are below.

Explore acceptable choices for swabs

Explore acceptable choices for media

For more information please see the FDA FAQ on this topic under "Testing Supply FAQ." 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."

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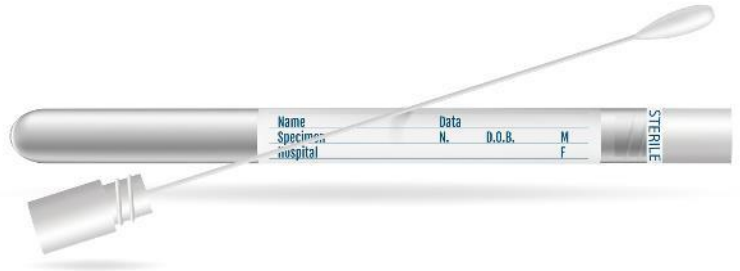
Specimen Collection: Swabs

Choices for Swabs*			
Naso**P**haryngeal (NP) Swab	**O**ro**P**haryngeal (OP) Swab	**M**id-**T**urbinate (MT) Swab • Flocked, tapered swab	Anterior Nares ("Nasal") Swab • Round Foam • Spun Polyester



** For more information please see the FDA FAQ on this topic under "Testing Supply FAQ."*
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.

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Specimen Collection: Media

Choices for Media			
VTM • e.g., Universal Transport Media (UTM)	Liquid Amies-based Media • e.g., Eswab	Inactivating Transport Media • e.g., Primestore MTM*	Saline Solution • Normal saline • Phosphate-buffered saline (PBS)

As explained in FDA's COVID-19 Transport Media Policy, all transport media, including saline, should be sterile to avoid contamination of the specimen.

WARNING: Media containing guanidine thiocyanate or similar chemicals produce **a potentially hazardous chemical reaction that releases cyanide gas when exposed to bleach.**

Please see the full Warning statement related to this issue for more information, including a list of some media and devices where extra care should be taken because of this issue.

See WARNING Statement

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WARNING - Specimen Collection: Media

Many inactivating transport media (ITM) contain guanidine thiocyanate or similar chemicals making them incompatible with certain in vitro diagnostic platforms, including those that use bleach (sodium hypochlorite), and with laboratory processes that use bleach. See warning below.

WARNING: Media containing guanidine thiocyanate or similar chemicals, including Longhorn PrimeStore MTM, Zymo DNA/RNA Shield, as well as that found in the Spectrum Solutions Saliva Collection Devices, should not be used with in vitro diagnostic platforms that use bleach (sodium hypochlorite) in the testing platform or during laboratory processes.

Media containing guanidine thiocyanate or similar chemicals produce **a potentially hazardous chemical reaction that releases cyanide gas when exposed to bleach.** Molecular platforms that use bleach in a disinfecting step include the **Hologic Panther** and **Panther Fusion** systems.

Additionally, media containing guanidine thiocyanate or similar chemicals should not be used with tests that are not designed for use with such chemicals. If you have questions about compatibility with guanidine thiocyanate media and your EUA test or test platform, please contact the test or platform manufacturer for further recommendations.

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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 is infected with the virus

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Overview of System Types

***Select** a system type for more information*

Open Mix and Match

Closed System

Extraction and Amplification

Extraction

Amplification

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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

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Open Mix and Match (CDC Design)

Instruments

Extraction and amplification performed separately

Extraction System

PCR System

Supplies and Reagents

Some materials may be usable on more than one system

Primers and Probes

Master Mix

Collection and Media

General Reagents

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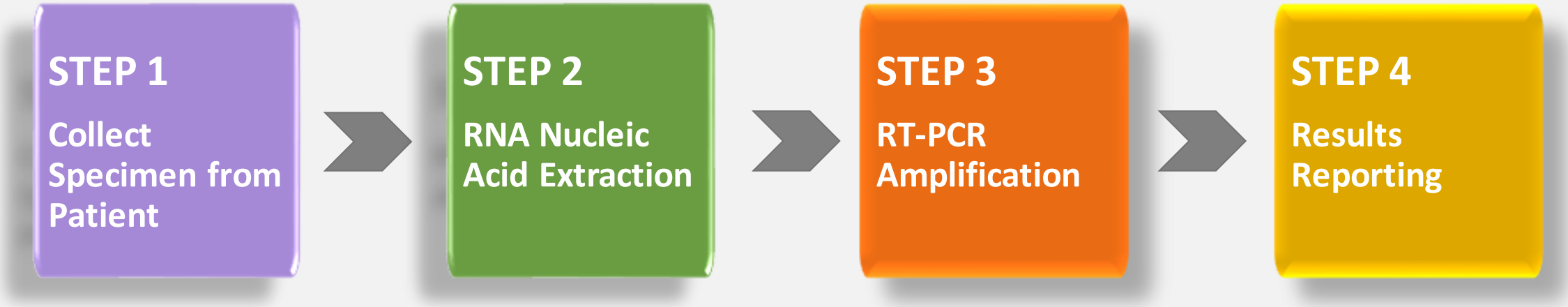
PCR Testing Process

Schematic of SARS-CoV-2 Molecular Diagnostic Assay



Select the STEPs above for more information about the PCR testing process

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1. Collect Specimen from Patient → 2. RNA Nucleic Acid Extraction → 3. RT-PCR Amplification → 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

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1. Collect Specimen from Patient → **2. RNA Nucleic Acid Extraction** → 3. RT-PCR Amplification → 4. Results Reporting

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

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1. Collect Specimen from Patient → 2. RNA Nucleic Acid Extraction → **3. RT-PCR Amplification** → 4. Results Reporting

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

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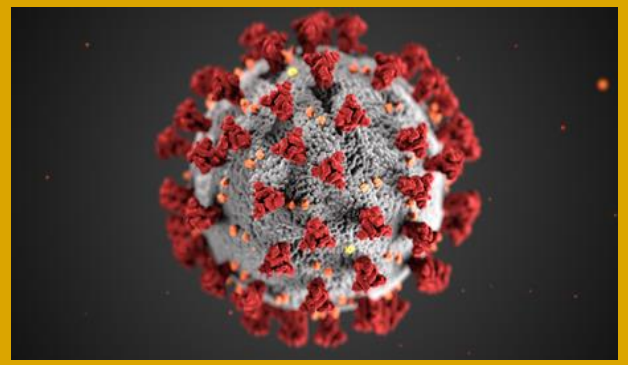
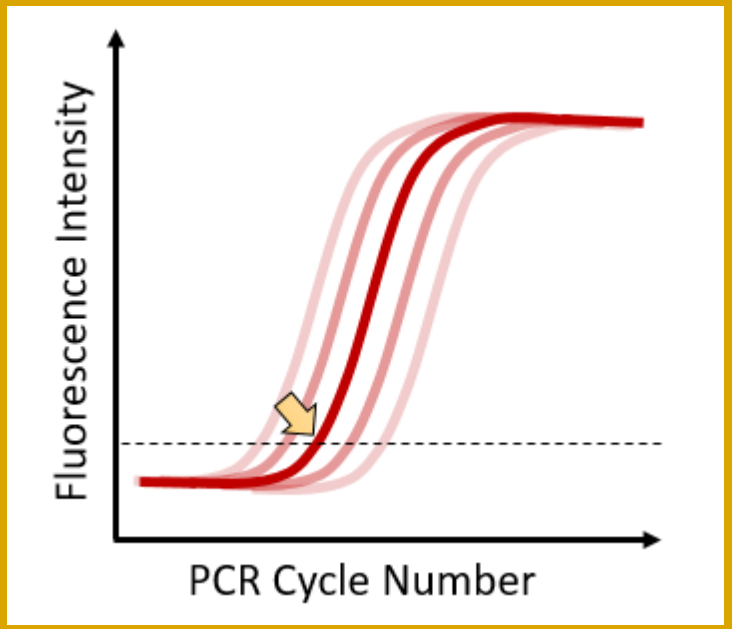
1. Collect Specimen from Patient → 2. RNA Nucleic Acid Extraction → 3. RT-PCR Amplification → **4. Results Reporting**

INPUT FOR THIS STEP?
Fluorescence output signal

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





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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 PCR TEST COMPONENT

- Primers and Probes
- Master Mix
- PCR System
- Controls
- Extraction System/Kits
- General Consumables

Select a Test Component on the left to explore possible substitutions for the Open Mix and Match style CDC molecular test

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SELECT A PCR TEST COMPONENT

- Primers and Probes
- Master Mix
- PCR System
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- General Consumables

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

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SELECT A PCR TEST COMPONENT

- Primers and Probes
- Master Mix
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- General Consumables

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

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SELECT A PCR TEST COMPONENT

- Primers and Probes
- Master Mix
- PCR System
- Controls
- Extraction System/Kits
- General Consumables

The PCR system is the instrument performing amplification.

PCR system listed in the CDC EUA

- Applied Biosystems 7500 Fast Dx Real-Time PCR

Alternatives (see 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 for updates after 6/3/2020***

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SELECT A PCR TEST COMPONENT

- Primers and Probes
- Master Mix
- PCR System
- Controls
- Extraction System/Kits
- General Consumables

Controls are the reagents that monitor the extraction and amplification process.

Controls listed in the CDC EUA

- CDC Positive Control
- CDC Extraction Control

Alternatives (see 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***

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SELECT A PCR TEST COMPONENT

- Primers and Probes
- Master Mix
- PCR System
- Controls
- Extraction System/Kits
- General Consumables

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

Alternatives (see FDA FAQ*)

- ✓ *QIAGEN QIAcube/QIAmp DSP Viral RNA Mini Kit*
- ✓ *QIAGEN QIAcube/ QIAamp 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*

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SELECT A PCR TEST COMPONENT

- Primers and Probes
- Master Mix
- PCR System
- Controls
- Extraction System/Kits
- General Consumables

General consumable laboratory supplies used in PCR

General consumables listed in the CDC EUA

- 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

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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
- Enforcement Policy for Viral Transport Media During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency - Guidance for Commercial Manufacturers, Clinical Laboratories, and Food and Drug Administration Staff (July 2020)

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