FOIA REQUEST: records re PURIFICATION OF "SARS-COV-2"

August 26th, 2021

Attn: FOIA Request
Department of General Services
c/o Division of Consolidated Laboratory Services
1100 Bank Street, Suite 420
Richmond, VA 23219
FOIA_DGS@dgs.virginia.gov

FOIA\Custodian of Records: This is a formal request for access to general records, reports, reference request forms. In accordance with the Department of General Services Responsibilities in Responding to Requests, The Department of General Services must respond to this request within five working days of receipt. "Day One" is the day after the request is received. The five-day period does not include weekends or holidays. made under Virginia's Freedom of Information Act**.**

If it is practically impossible to respond to the request within five days, please state in writing and explain the conditions which make the response impossible. An additional seven working days to respond to the request, gives the Department of General Services a total of 12 working days to respond, which follows procedure.

Description of Requested Records:

All studies and/or reports in the possession, custody or control of the Division of Consolidated Laboratory Services (DCLS) describing the **purification** of any **"COVID-19 virus**" (aka "SARS-COV-2", including any alleged "variants" i.e. "B.1.1.7", "B.1.351", "P.1") directly from a sample taken from a diseased human, where the patient sample was not first combined with any other source of **genetic** material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum).

Please note that I am not requesting studies/reports where scientists and researchers failed to **purify** the suspected "virus" (separate the alleged "virus" from everything thing else in the patient sample) and instead:

- cultured an unpurified sample or other unpurified substance, and/or
- performed an amplification test (i.e. a PCR test) on the total RNA from a patient sample or from a cell culture, or on genetic material from any unpurified substance, and/or
- fabricated a genome based on PCR-detected sequences in the total RNA from a patient sample or from a cell culture or from any unpurified substance, and/or
- produced electron microscopy images of unpurified things in a cell culture.

Clarification of Request

For further clarity, please note I am already aware that according to virus theory a "virus" requires host cells in order to replicate, and I am **not** requesting records describing the **replication** of a "virus" without host cells.

Further, I am **not** requesting records that describe a suspected "virus" floating in a vacuum; I am simply requesting records that describe its **purification** (**separation** from everything else in the human patient sample, as per standard operating procedure the Virology section at DCLS or laboratory practices for the purification of other very small things).

Please note that my request includes any study/report matching the above description, for example (but not limited to) any published peer-reviewed study authored by anyone, anywhere that DCLS is aware of.

Please also note that despite the fact that purification is an essential (but not sufficient) step in proving the existence of a disease-causing "virus", as of today 98 institutions globally (including the U.S. CDC, Public Health Agency of Canada, Australian Department of Health, New Zealand Ministry of Health, European Centre for Disease Prevention and Control, UK Department for Health and Social Care, Indian Council of Medical Research) have all failed to provide or cite any such records, therefore to my knowledge no such records exist and if they do exist I cannot access them until I am provided a citation or URL.

Therefore in the interest of citizens of the Commonwealth of Virginia and transparency and in accordance with the purposes of the legislation (Virginia's Freedom of Information Act), if any records match the above description of requested records and are currently available to the public elsewhere, please provide enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

Format:
Pdf documents sent to me via email; I do not wish for anything to be shipped to me.

Contact Information:
Last name: [redacted]
First name: [redacted]
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COMMONWEALTH *of*
VIRGINIA
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September 2, 2021

Via email: [redacted]

[redacted]

Dear [redacted]

I am responding to your request for information received by the Department of General Services (DGS) via email on August 26, 2021. In your request you asked for in brief, all studies and/or reports in possession, custody or control of the Division of Consolidated Laboratory Services describing the **purification** of any **COVID-19 virus**.

Please find attached documents responsive to your request.

I hope this information is helpful. Thank you for your inquiry.

Sincerely,

Dena Potter

Dena Potter
Director of Communications

/Attachments

Commonwealth of Virginia
Department of General Services
Division of Consolidated Laboratory Services
Richmond, Virginia

CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel

I. PURPOSE/APPLICATION:

A. This procedure is for the qualitative detection of nucleic acid from the 2019-novel Coronavirus (nCoV), termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), using real-time reverse transcription polymerase chain reaction (RT-PCR) amplification. Testing is performed for the purpose of patient diagnosis and surveillance of COVID-19 illness within Virginia at the direction of the Virginia Department of Health.

B. The RT-PCR test is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens collected from individuals who meet clinical and/or epidemiological criteria. Testing in the United States is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests by trained laboratory personnel who are proficient in performing real-time RT-PCR assays. The CDC 2019-nCoV RT-PCR Diagnostic Panel is only for use under a Food and Drug Administration (FDA) Emergency Use Authorization (EUA).

II. SUMMARY/SCOPE:

A. The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of COVID-19 illness, and is based on widely used nucleic acid amplification technology. The diagnostic panel contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) for the detection of SARS-CoV-2 RNA in respiratory specimens.

B. The SARS-CoV-2 oligonucleotide primers and probes target regions of the virus nucleocapsid (N) gene. Oligonucleotide primers and probe that target the human RNase P gene (RP) in human clinical specimens is included in the panel as an assay control to assess specimen integrity and assay performance. Purified RNA isolated from upper and lower respiratory specimens is reverse transcribed to cDNA and amplified in the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 software. If viral RNA is present in the clinical specimen, then the assay probes will anneal to specific target sequences located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument. Detection of viral RNA provides clinical, epidemiological and surveillance information for SARS-CoV-2.

C. Quality is assured through testing of positive and negative PCR controls along with a Human Specimen Control (HSC) as an extraction control and an RP within each clinical specimen.

D. DCLS validated three extraction methods for this procedure including:

1. Qiagen QIAamp DSP Viral RNA Mini Kit or QIAamp Viral RNA Mini Kit
2. MagMax Viral/Pathogen Nucleic Acid Isolation Kit using the ThermoFisher Kingfisher Flex Magnetic Particle Processors with 96 deep well head extraction platform
3. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™360 Magnetic Bead extraction platform

III. SAMPLE COLLECTION:

A. Specimen Type:

1. Sample types acceptable for testing:
    a. upper and lower respiratory specimens
        i. nasopharyngeal or oropharyngeal swabs
        ii. sputum
        iii. lower respiratory tract aspirates
        iv. bronchoalveolar lavage
        v. nasopharyngeal wash/aspirate or nasal aspirates
    b. respiratory specimens collected from individuals who meet 2019-nCoV clinical and/or epidemiological criteria. For example:
        i. clinical signs and symptoms associated with 2019-nCoV infection
        ii. contact with a probable or confirmed 2019-nCoV case
        iii. history of travel to geographic locations where 2019-nCoV cases were detected
        iv. other epidemiologic links for which 2019-nCoV testing may be indicated as part of a public health investigation
2. Swab specimens 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.
3. Place swabs immediately into sterile tubes containing 1-3 ml of appropriate transport media, such as viral transport media (VTM), Ames transport medium, phosphate buffered saline, or sterile saline.

B. Handling and Shipping Conditions:

1. Specimens can be stored at 2-8 ºC for up to 72 hours after collection.
2. Transport to DCLS refrigerated on ice packs.
3. The DCLS COVID-19 Submission Form (Qualtrax ID # 34293) is the preferred form to submit specimens for testing. However, the DCLS Test Request Form (Qualtrax ID #16857) can also be used.
4. The DCLS Clinical Microbiology/Virology Request Form (Qualtrax ID #

16857) has been discontinued, but will be accepted if submitted with specimens.

5. The submission form should be fully completed by the submitter and submitted with the specimen; OR the submitter may use Webvision or DCLS Connect to electronically enter information for specimen submission. Information necessary for proper specimen submission:
    a. Patient Information (name, address, age or date of birth)
    b. Submitter Information (name, address, telephone number)
    c. Patient Medical History (information relevant to diagnosis such as symptoms, date of onset, recent exposures, travel history)
    d. Outbreak Information, if applicable, (outbreak number, role of patient in outbreak)
    e. Test requested, specimen source, and date collected

   When specimens are received in Sample Support Services (SSS), a LIMS identification number will be assigned. The patient information and specimen metadata will be entered into LIMS, labels will be generated and placed on the specimen container and appropriate paperwork. The specimens will be stored refrigerated in a SSS refrigerated until retrieved by testing personnel, or the specimens will be delivered to the COVID extraction laboratory (room 268 A or B).

C. Storage Conditions:
    1. When specimen is received at DCLS, store at 2-8 ºC for up to 72 hours after collection.
    2. If a delay in extraction is expected, store specimens at -70ºC or lower.
    3. Store extracted nucleic acid at -70ºC or lower.
    4. Maintain RNA on a cold block or on ice during preparation to ensure stability.
    5. After testing is completed, specimens that are positive for SARS-CoV-2 will be aliquotted into cryovials and stored at -70°C or lower for long term storage, for at least three years. Samples must be disposed of as biohazardous waste in a red waste bin.
    6. *Chain of Custody (COC) samples are discarded according to Evidence Receipt/Storage and Disposition Procedure (Qualtrax ID # 1804)*

D. Rejection Criteria:
    1. After consultation with the Senior Scientist, Principal Scientist, Lead Scientist, or Group Manager, samples meeting the rejection criteria outlined below may still be tested and reported with additional disclaimers.
        a. Absence of or inconsistent labeling and identification:
            i. The absence of a name or unique identifier on specimen container.
            ii. More than one name on specimen container.
            iii. Name on paperwork is different from name on specimen

container.

b. Specimen submission form not properly filled out (e.g. patient name or address missing, etc.).
c. Specimen received in expired viral transport medium.
d. Specimen received without refrigeration.
e. Specimen received at the laboratory more than 72 hours after collection date.
f. Specimen with insufficient volume for testing.
g. When a sample is deemed unacceptable for testing, the submitter will receive a LIMS report explaining the reason for specimen rejection (Unsatisfactory for testing - reason).

IV. PERSONNEL QUALIFICATIONS:

A. *Procedures in* Molecular Detection and Characterization Group (MDC) *may only be performed by approved testing personnel. The list of testing personnel can be found in the DCLS Training Matrix. Testing personnel must comply with DCLS Competency (Qualtrax ID # 16472). Personnel will demonstrate competency twice during the first year. Competency assessment, with documentation, will be performed annually in subsequent years.*

V. INTERFERENCES/LIMITATIONS OF PROCEDURE:

A. This test has not been FDA cleared or approved; this test has been authorized by FDA under a EUA for use by laboratories certified under CLIA, 42 U.S.C. § 263a, to perform high complexity tests.
B. This test has been authorized only for the detection of nucleic acid from SARS CoV-2, not for any other viruses or pathogens.
C. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb3(b)(1), unless the authorization is terminated or revoked sooner.
D. Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time PCR reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). To mitigate this limitation, workflow in the laboratory proceeds in a unidirectional manner.
E. Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality.
F. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple

specimens (types and time points) from the same patient may be necessary to detect the virus.

G. A false-negative result may occur if a specimen is improperly collected, transported or handled. False-negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.

H. Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely when prevalence of disease is high. False-positive test results are more likely when prevalence is moderate to low.

I. If the virus mutates in the rRT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false-negative result. An interference study evaluating the effect of common cold medications was not performed.

J. Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and, during the course of infection, when these specimens are most likely to contain levels of viral RNA that can be readily detected.

K. Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.

L. The performance of this test has not been established for monitoring treatment of COVID-19 infection.

M. This test cannot rule out diseases caused by other bacterial or viral pathogens.

VI. SAFETY:

A. Attire and Personal Protective Equipment

1. *Totally enclosed shoes are required in this laboratory at all times.*

2. *The required minimum Personal Protective Equipment (PPE) in this laboratory is a lab coat and safety glasses.*

3. *Gloves are required when handling samples, infectious agents, chemicals, closing and moving regulated medical waste containers, and when working in a biological safety cabinet (BSC) or chemical fume hood. Nitrile gloves are preferred.*

NOTE: If latex gloves are in use, an alternative, non-latex glove must be available and laboratory door signage must reflect the usage of latex gloves.

4. Additional PPE that should be used when performing nucleic acid extractions, automated instrument loading, and specimen archiving in a Biosafety Level-2 (BSL-2) laboratory include:
    - fluid-impervious, back-closing gowns

- double gloves when working in the BSC
- face shields (if safety glasses fog due to face masks)
- disposable face masks

5. Additional PPE that should be used when performing nucleic acid extractions of lower respiratory specimens in a Biosafety Level-3 (BSL-3) laboratory include:
    - respirators: PAPR or CAPR, N-95 with safety glasses
    - fluid-impervious, back-closing gowns
    - double gloves when working in the BSC

B. *Safety precautions must be taken when handling reagents, samples, and equipment in this laboratory.*

C. Special Precautions

1. BSL-2+ work practices will be used in BSL-2 testing laboratories.
2. Lower respiratory specimens will be processed in a BSL-3 laboratory, using BSL-3 safety and work practices.
3. Vortex mixing will occur inside of the BSC.
4. Sealed rotors will be used for centrifugation steps, and will only be opened inside of a BSC.
5. Vacuum manifolds will only be used inside of the BSC.
6. All items will be decontaminated prior to removal from the BSC.
7. Specimen containers are only opened inside of a BSC prior to inactivation via lysis buffer treatment for at least 10 minutes. Inactivated specimens may be removed from the BSC for loading onto the instrument.
8. Closed specimen tubes can be handled on the benchtop for plate mapping preparations.
9. Sharp items are discarded in sharps containers. Broken glass is discarded in a broken glass box and the box should not be filled more than 3/4 full. If broken glass has come in contact with a sample then it is discarded in a sharps container. When ready to discard a sharps container, close the top securely and place it in a red regulated medical waste bin, or in the post lab, a designated cardboard box labeled with “regulated medical waste”.

D. Location of Eye Wash and Emergency Shower

1. *An eye wash/drench hose is present on each sink in this laboratory.*

2. *The emergency shower is located* in room 250/IV and MDC/134.

E. Hazards Associated With Procedure

1. Chemical Hazards

The following toxic, carcinogenic, or highly hazardous, ≤ 2, chemicals are associated with this procedure:

Chemical Name	Health Hazards	Flammability	Reactivity	Oxidizing Solid/ Liquid	Corrosive to Metals	Environ-mental Hazards	Fume Hood Required
Qiagen Buffer AVL*	1	N/A	N/A	N/A	N/A	3	No
Qiagen Buffer AW1*	2	N/A	N/A	N/A	N/A	N/A	No
Ethanol	2	2	N/A	N/A	N/A	N/A	No
Proteinase K	1	N/A	N/A	N/A	N/A	N/A	No
BTL, Viral/Pathogen Binding Solution	1	N/A	N/A	N/A	N/A	3	No
MagMax Viral/Pathogen Proteinase K	1	N/A	N/A	N/A	N/A	N/A	No
BTL, Viral/Pathogen Wash Buffer	2	N/A	N/A	N/A	N/A	N/A	No

*Contains chaotropic salt. Not compatible with disinfectants containing bleach.

2. Biological Hazards

a. Respiratory viruses, including influenza, SARS-CoV-2, and other viruses, are human pathogens.
b. All clinical specimens will be handled as potentially infectious materials using Universal Precautions as specified in the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030, www.osha.gov). Only personnel trained in handling infectious materials will be permitted to perform this procedure.
c. Aerosol barrier pipette tips will be used to prevent the generation of aerosols. Wash hands thoroughly after handling specimens, reagents, and equipment, after removing gloves, and before leaving the laboratory. Disinfect all bench tops and BSC after work is complete.
d. Specimen coolers and packages containing COVID-19 specimens are opened on the benchtop by SSS staff. Samples are then placed inside of the BSC for accessioning. All sample tubes are decontaminated prior to removal from the BSC and delivery to the testing laboratory.

3. Radiological Hazards
The following radiological hazards are associated with this procedure:
Not Applicable.

4. Safety Data Sheets/Pathogen Safety Data Sheets
The laboratory is responsible for maintaining a current, complete file of Safety Data Sheets (SDSs) related to this procedure. The SDSs are available to the analyst on computers throughout the laboratory at the following URL: https://msdsmanagement.msdsonline.com/21943a72-0bc7-4000-a405-4ba03280a52c/ebinder/?nas=True

F. Spill Response
1. *Small spills - handled by the laboratory staff (refer to SDS) or call Administration for Spill Response Team notification.*

2. *Large spills – call Administration for Spill Response Team notification.*

Refer to DCLS Safety Manual (Qualtrax ID # 1805) for additional safety information.

VII. EQUIPMENT & SUPPLIES, REAGENTS & STANDARDS:

For labeling requirements for purchased or prepared media/reagents/standards, refer to Measurement and Data Traceability (Qualtrax ID # 1789).

A. Equipment & Supplies: Store at room temperature unless otherwise specified
1. Specimen Extraction
a. Qiagen QIamp DSP Viral RNA or QIamp Viral RNA
i. QIAamp Mini Spin Columns with Wash Tubes. Store dry at 2–8°C
ii. Elution Tubes (1.5 ml)
iii. Lysis Tubes (2 ml)
iv. Wash Tubes (2 ml)
v. 1.5 ml microcentrifuge tubes
vi. Sterile, RNase-free pipets
vii. Sterile, RNase-free pipet tips with aerosol barriers
viii. Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
ix. For vacuum protocols:
a) QIAvac 24 Plus vacuum manifold (cat. no. 19413) or equivalent
b) VacConnectors (cat. no. 19407)
c) Vacuum Regulator (cat. no. 19530) for easy monitoring of vacuum pressures and easy releasing of vacuum
d) Vacuum Pump (cat. no. 84010 or equivalent pump capable

of producing a vacuum of –800 to –900 mbar)
e) Optional: VacValves (cat. no. 19408)
f) Optional: QIAvac Connecting System (cat. no. 19419)

b. Altria's Kingfisher Flex Magnetic Particle Processor with 96 deep well head extraction platform for use with MagMax Viral/Pathogen Nucleic Acid Isolation Kit.
   i. KingFisher™ deep-well 96 plate KingFisher Duo cap for elution strip
   ii. Adjustable micropipettors
   iii. Multi-channel micropipettors
   iv. MicroAmp™ Clear Adhesive Film
   v. Conical Tubes (15 mL)
   vi. Conical Tubes (50 mL)
   vii. Reagent reservoirs
   viii. Nonstick, RNase-Free Microfuge Tubes, 1.5 mL
   ix. Nonstick, RNase-Free Microfuge Tubes, 2.0 mL
   x. Vortex
   xi. 96 deep-well magnetic head
   xii. 96 deep-well heat block

c. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™360 Magnetic Bead extraction platform.
   i. Rack with Disposable Tips
   ii. low-well-plate (MICROTITER SYSTEM)
   iii. Magnetic Beads
   iv. deep-well-plate (riplate SW)

2. PCR Set up and Detection
   a. Vortex mixer
   b. Microcentrifuge
   c. Micropipettes (2 or 10 µL, 200 µL and 1000 µL)
   d. Multichannel micropipettes (5-50 µl)
   e. Racks for 1.5 mL microcentrifuge tubes
   f. 2 x 96-well -20°C cold blocks
   g. 7500 Fast Dx Real-Time PCR Systems with SDS 1.4 software
   h. Molecular grade water, nuclease-free
   i. 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
   j. DNAZapTM or equivalent
   k. RNase AWAY™ or equivalent
   l. Aerosol barrier pipette tips
   m. 1.5 mL microcentrifuge tubes (DNase/RNase free)
   n. 0.2 mL PCR reaction plates
   o. MicroAmp Optical 8-cap Strips

B. Reagents:

1. Specimen Extraction; All solutions should be stored at room temperature (15–25°C) unless otherwise stated. Follow manufacturer expiratory dates.

   a. Qiagen QIAmp DSP Viral RNA Mini Kit and QIAamp® Viral RNA Mini Kit:
   The follow reagents are stored at room temperature and expire per the kit expiration date:
      i. Buffer AVL
      ii. Buffer AW1 (concentrate)
      iii. Buffer AW2 (concentrate)
      iv. Buffer AVE
      v. Carrier RNA (poly A);
         a) Store at room temperature (15–25°C)
         b) Dissolve in 310 µl Buffer AVE**. Note:** This solution should be prepared fresh, and is stable at 2–8°C for up to 48 hours. Buffer AVE–carrier RNA develops a precipitate when stored at 2–8°C that must be re-dissolved by warming at 80°C ±3°C before use.
         c) Unused portions of carrier RNA dissolved in Buffer AVE should be frozen in aliquots at –25°C to –15°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times. DO NOT warm Buffer AVL–carrier RNA solution more than 6 times. DO NOT incubate at 80°C for more than 5 minutes. Frequent warming and extended incubation will cause degradation of the carrier RNA, leading to reduced recovery of viral RNA and eventually to false negative RT-PCR results, particularly when low-titer samples are used.
      vi. Ethanol (96–100%)
      Store the following at 2–8°C prior to use:
      vii. Qiagen QIAmp DSP Viral RNA Mini Kit spin columns

   b. ThermoFisher MagMax Viral/Pathogen Nucleic Acid Isolation Kit using Altria's Kingfisher Flex Magnetic Particle Processor with 96 deep well head extraction platform.
   The following reagents are stored at room temperature and expire per the manufacturer expiration date marked on the individual container.
      i. Binding Solution
      ii. Wash Buffer. Wash Solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

iii. Elution Solution
iv. Proteinase K
v. Total Nucleic Acid Binding Beads
vi. Ethanol, 100% (molecular biology grade)
vii. Nuclease-free Water

c. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™360 Magnetic Bead extraction platform.
   i. Poly(A) RNA; prepare according to manufacturer instructions; store in the dark; reconstituted Poly(A) is stable for 4 weeks at 4 °C; For long term storage store the reconstituted Poly(A) RNA in aliquots at -20 °C. Do not freeze the Poly(A) RNA aliquots after thawing.
   ii. Proteinase K; prepare according to manufacturer instructions; reconstituted Proteinase K is stable for 4 weeks at 4 °C; For long term storage store the reconstituted Proteinase K in aliquots at -20 °C. Do not freeze the Proteinase K aliquots after thawing.

   The follow reagents are stored at room temperature and expire per the kit expiration date:
   iii. Lysis Buffer 1; store in the dark; may form a precipitate upon storage. If necessary, warm to 55 °C to dissolve.
   iv. Binding Buffer 2
   v. Wash Buffers 3, 4 and 5
   vi. Elution Buffer 6
   vii. For long term storage it is recommended to store the reconstituted Poly(A) RNA and Proteinase K in aliquots at -20 °C. Do not freeze the Poly(A) RNA and Proteinase K aliquots after thawing.

2. PCR Set up and Detection; Prepare primers and probes per manufacturer instructions for use (CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, Revision 05) :
   a. 2019-nCoV_N1 Combined Primer/Probe Mix
   b. 2019-nCoV_N2 Combined Primer/Probe Mix
   c. Human RNase P Combined Primer/Probe Mix
   d. 2019-nCoV Positive Control (nCoVPC)
      i. Store all dried primers and probes and the positive control, nCoVPC, at 2-8°C until re-hydrated for use.
      ii. Note: Storage information is for CDC primer and probe materials obtained through the International Reagent Resource.
      iii. Protect fluorogenic probes from light.
      iv. Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during

preparation and use. **Consult most recent version of the package insert to confirm that dilution volumes have not changed.**

v. Do not refreeze probes.

vi. Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

e. Human Specimen Control (HSC); Store liquid HSC control materials at ≤ -20°C

f. ThermoFisher TaqPath™ 1-Step RT-qPCR Master Mix, CG

i. Store Master Mix at -20 ± 4°C, follow manufacturer expiration date.

g. Sterile, nuclease-free water (No Template Control)

i. Store at room temperature, follow manufacturer expiration date.

VIII. PROCEDURE:

A. Nucleic Acid Extraction: Perform one of the RNA extraction/purification procedures following the manufacturer's instructions for use with DCLS validated modification as specified:

1. Consult the FDA EUA website to confirm the most recent version of the IFU in use. https://www.fda.gov/media/134922/download
2. Qiagen QIAamp® DSP Viral RNA Mini Kit or QIAamp® Viral RNA Mini Kit.
   i. DCLS verified the performance of the both kits using 140-µL of sample and elution of viral RNA in 140-µL buffer.

3. MagMax Viral/Pathogen Nucleic Acid Isolation Kit using ThermoFisher's KingFisher™ Flex Magnetic Particle Processor with 96 deep well head extraction platform (standard volume: 200–400 µL).
   i. DCLS verified the use of the ThermoFisher KingFisher Flex using the automated program: "MVP_Flex 96DW" Program on the KingFisher Flex.
   ii. Procedure uses 400-µL patient sample
   iii. Processing plates include an additional Wash 3 Plate (500-µL 80% Ethanol) (in reference to #MAN0019181 rev. H)
   iv. Elution plate includes 100-µL Elution Solution

4. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™ 360 Magnetic Bead Extraction Platform; Purification Protocol for Viral DNA/RNA from 300 µl Plasma, Serum, Naso- or Oropharyngeal Swabs, BAL and Sputum Using the chemagic 360 with integrated chemagic Dispenser
   i. DCLS verified the use of the **Perkin Elmer Chemagic 360** for the preparation of RNA with the Chemagic Viral DNA/RNA 300 Kit special

H96 using automated program: Chemagic Viral 300 360 H96 drying prefilling VD141210.che Program **on the Perkin Elmer Chemagic 360**

ii. Sample input volume of 300-µL
iii. Master Mix combined with respiratory specimen includes 300-µL Lysis Buffer, 4-µL Poly(A) RNA and 10-µL Proteinase K, once combined, samples are incubated 10 min
iv. Processing plates include Low-Well Beads (150-µL), 3 Deep Well Washes
v. Elution Plate includes 100-µL Elution buffer

B. Perform PCR procedure using ThermoFisher TaqPath™ 1-Step RT-qPCR Master Mix per CDC; 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

IX. CALCULATIONS:

A. Refer to manufacturer IFU's for any relevant calculation instructions.

X. CALIBRATION, QUALITY CONTROL AND QUALITY ASSURANCE:

A. Refer to the manufacturer Instructions For Use, (CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, Revision 05) for Quality Control for the SARS-CoV-2 assay.

1. A minimum of one set of positive and negative controls is processed on each PCR plate. Controls must yield the appropriate result to release results for patient samples.
   a. No Template Control (NTC):
      i. The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA.
      ii. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit an amplification curve that crosses the cycle threshold, sample contamination may have occurred.
   b. 2019-nCoV Positive Control (nCoVPC):
      i. The nCoVPC consists of in vitro transcribed RNA.
      ii. The nCoVPC will yield a positive result with the following primer and probe sets: N1, N2, and RP.
      iii. A control preparation worksheet (Qualtrax ID #24640) is used to document the preparation of the positive PCR control.
   c. Human Specimen Control (HSC) (Extraction Control):
      i. HSC is used as a nucleic acid extraction procedural control to demonstrate successful recovery of nucleic acid as well as extraction reagent integrity. The HSC control consists of noninfectious cultured human cell material.
      ii. HSC is extracted with each round of nucleic acid extraction

and analyzed with concurrently extracted samples.

iii. Purified nucleic acid from the HSC should yield a positive result with the RP primer and probe set and negative results with all 2019-nCoV markers.

B. If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

C. RNase P (Extraction Control):

1. All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P gene.
2. Failure to detect RNase P in any clinical specimens may indicate:
    a. Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
    b. Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
    c. Improper assay set up and execution.
    d. Reagent or equipment malfunction.
    e. If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:
        i. If the 2019-nCoV N1 and N2are positive even in the absence of a positive RP, the result should be considered valid.
        ii. If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

XI. WASTE MANAGEMENT

DCLS manages all waste streams in compliance with local, state, and federal regulations.

A. Pollution Prevention

1. *As part of DCLS' Pollution Prevention efforts, procedures are aimed at the elimination or reduction of hazardous waste discharge at the point of generation.*

2. *Procedural decisions are based on the use of the least hazardous substance, limitations on the quantity ordered, the appropriate usage of the safety equipment, staff training, and competency assessment.*

3. *Training on waste management is provided to staff on an annual basis.*

B. Biological, Chemical, Radiological Waste Handling

1. *The safety office provides assistance in the development of waste handling and storage procedures and coordinates hazardous waste pick-ups.*

2. *A Waste Profile that is SOP-specific has been developed and approved. This information is listed on the DCLS Waste Profile Form (Qualtrax ID # 1646) which is attached to this SOP as Appendix 1.*

3. *This method does not generate any hazardous radiological waste.*

4. *This method generates the following hazardous chemical/biological (regulated medical waste)/radiological waste streams.*

   a. Chemical
      - Expired unused extraction reagents including Buffer AVL (50%-100% guanidinium thiocyanate), Buffer AW1 (50-100% guanidine hydrochloride), and AW2 (50% ethanol)
      - Unused, unopened or expired hazardous kit components (including BTL, Viral/Pathogen Binding Solution, MagMax Viral Pathogen Proteinase K, BTL, Viral/Pathogen Wash Buffer, Ethanol, PerkinElmer Binding Buffer 2, PerkinElmer Wash Buffer 3, PerkinElmer Wash Buffer 4, PerkinElmer Lysis Buffer, PerkinElmer Proteinase K, PerkinElmer Poly(A) RNA Buffer)
      - 70% Cleaning Grade Ethanol (used for PerkinElmer Intensive Clean), PerkinElmer bulk reagent mixed waste (from Prime or Check Manifolds procedure. Contains PerkinElmer Binding Buffer 2, PerkinElmer Wash Buffer 3, PerkinElmer Wash Buffer 4, and PerkinElmer Wash Buffer 5.)

   b. Biological ((regulated medical waste) if procedure generates biological waste or N/A):
      - *Gloves, disposable lab coats and other PPE should be disposed in the red regulated medical waste bins.*
      - Any waste that may have been in direct contact with samples
      - All testing materials used or generated in the BSC and items used during the processing of potentially infectious samples
      - All testing materials used or generated in Rooms 268A
      - Empty specimen containers and microcentrifuge tubes labeled with patient information

c. Radiological: Not applicable

Refer to the DCLS Safety Manual (Qualtrax ID # 1805) for Additional Safety information.

C. Solid Waste

Solid waste items that are associated with this procedure should be placed in trashcans for pick-up by BFM staff. The following items are considered solid waste: paper, paper towels, empty sample containers, food samples submitted for testing (that have tested negative) and the containers, expired media, non-infectious and non-chemical waste.

D. On-Site Autoclave Preparation

There are instances in which containers of potentially infectious materials may need to be autoclaved on site before being packaged for pick up by the regulated medical waste contractor or re-used in our laboratories.

- BSL-3 Laboratories

  The following items are routinely packaged in this laboratory and placed in the pass-through autoclave in BSL-3.

  (All waste generated in BSL-3)

Refer to the DCLS Safety Manual (Qualtrax ID # 1805) or the BSL-3 Biosafety Manual (Qualtrax ID # 8650) for detailed instructions on how to properly prepare materials for autoclaving.

XII. RECORDING AND REPORTING OF RESULTS:

A. Record procedural steps completed on the applicable worksheet as follows:

1. 2019-nCoV Manual Extraction Worksheet (Qualtrax ID# 34067)
2. COVID-19 KingFisher Flex Extraction (Qualtrax ID# 33746)
3. Perkin Elmer Chemagic Viral DNA_RNA 300 Extraction Worksheet (Qualtrax ID# 34019)
4. 2019-nCoV PCR Worksheet (Qualtrax ID# 33199)

B. Refer to the manufacturer Instructions For Use (IFU), (CDC; 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, Revision 05) for Results for the SARS-CoV-2 assay. The table below lists the expected results for the 2019-nCoV rRT-PCR Diagnostic Panel.

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation[a]	Report	Actions
+	+	±	2019-nCoV detected	Positive 2019-nCoV	Report results to CDC and sender.
If only one of the two targets is positive		±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance.
-	-	+	2019-nCoV not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.[b]
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

[a]Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

[b]Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that 2019-nCoV infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If 2019-nCoV infection is still suspected, re-testing should be considered in consultation with public health authorities.

C. Specimens that do not pass quality control requirements (unresolved) may include the following after comment on the patient report: Specimen did not pass quality control requirements, and collection of a new specimen for testing is recommended.

D. Ensure that QC materials are verified and results are second reviewed and approved before releasing patient reports.

E. Patient reports with CDC 2019-nCoV Assay results include the following disclaimer statements:
   1. The US Food and Drug Administration has made this test available under Emergency Use Authorization (EUA) for the duration of the COVID-19 declaration justifying emergency use of IVDs unless terminated or revoked. Results from this test should not be used as the sole basis for treatment or patient management decisions. A negative result does not exclude the possibility of COVID-19.

F. Fact sheets on the CDC 2019-nCoV Test for healthcare providers and patients can be accessed at:
   1. https://www.fda.gov/media/134920/download
   2. https://www.fda.gov/media/134921/download

XIII. REFERENCES:

1. CDC; 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel . Instructions for Use; CDC-006-00019; Revision 06; Effective 12/01/2020
2. CDC; 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel . Instructions for Use; CDC-006-00019; Revision 05; Effective 07/13/2020
3. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19); Updated Nov. 5, 2020; https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fguidelines-clinical-specimens.html
4. QIAamp DSP Viral RNA Mini Kit Handbook 03/2012
5. QIAamp Viral RNA Mini Handbook 07/2020
6. ThermoFisher/Applied Biosystems MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide; Catalog Number A42352 Pub. No. MAN0018073 Rev. C.0; 24 September 2020
7. Purification Protocol for Viral DNA/RNA from 300 µl Plasma, Serum, Naso- or Oropharyngeal Swabs, BAL and Sputum Using the chemagic 360 with integrated chemagic Dispenser; Version 200312; 2018

XIV. APPENDIX, TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA:

Appendix I. Waste Profile Form

Appendix I. DCLS Waste Profile

DCLS Waste Profile Form Richmond, VA		
Group: MDC	☒ New/Changed Waste Profile	
Contact: Sean Kelly ext 227		
SOP Name: CDC 2019-Novel Coronavirus Real Time RT-PCR Diagnostic Panel	SOP #:	

Waste Type (choose only one)		**Waste Composition**
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Residual respiratory clinical specimens (nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, NP aspirates, NP wash, bronchoalveolar lavage, tracheal aspirates, sputum, etc.)
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Mixed waste containing carrier RNA, Buffer AVL (50-100% guanidine hydrochloride/ guanidinium thiocyanate), Buffer AW1 (50-100%guanidine hydrochloride), ethanol, Buffer AW2 (50% ethanol), residual clinical sample. Biological specimen waste from MagMax extractions (plastics and liquid waste used to extract biological specimens) including BTL, Viral/Pathogen Binding Solution, MagMax Viral Pathogen Proteinase K, BTL, Viral/Pathogen Wash Buffer, Ethanol, and other kit components of MagMax Viral/Pathogen kit. All plates removed from the PerkinElmer instrument following a run (plates contain Wash Buffer 3, Wash Buffer 4, Wash Buffer 5, Magnetic Beads, Lysis buffer, and inactivated sample).
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Mixed waste containing specimen, Sputolysin, and 10x TE
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Mixed waste containing filtrate of washing solution, sample retentate, bleach rite, etc.
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Used consumables: transfer pipets, serological pipettes, gloves, aerosol barrier tips, conicals, microcentrifuge tubes, forceps, elution columns, etc. generated during processing
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Cleaning supplies used during sample processing and cleaning BSC: bench coat, BleachRite, Microchem, Dispatch wipes, WypAlls, etc.
☐Biological ☒Chemical ☐Radiological	☐Sink ☐Trash	**Unused expired reagents**: Buffer AVL (guanidinium thiocyanate), Buffer AW1 (50-100% guanidine hydrochloride), ethanol, Buffer AW2 (50% ethanol), 100% ethanol, PerkinElmer Binding Buffer 2, PerkinElmer Wash Buffer 3, PerkinElmer Wash Buffer 4, PerkinElmer Lysis Buffer, PerkinElmer Proteinase K, PerkinElmer Poly(A) RNA Buffer, BTL, Viral/Pathogen Binding Solution, MagMax Viral Pathogen Proteinase K, BTL, Viral/Pathogen Wash Buffer
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	**Unused expired reagents**: carrier RNA, Buffer AVE, HSC, 10x Tris-EDTA (TE), Sputolysin (Sodium Citrate, Dithiothreitol), solid or liquid media, polyvalent
☐Biological ☐Chemical ☐Radiological	☒Sink ☐Trash	**Unused expired reagents**: Unused Tris-EDTA (TE) Buffer, unused PerkinElmer Wash Buffer 5, unused PerkinElmer Elution Buffer, unused PerkinElmer Magnetic Beads liquid (decant liquid when beads have settled)
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	**BSL3 PPE**: Back closing gowns, gloves, N95 respirators, shoe covers.
☐Biological ☒Chemical ☐Radiological	☐Sink ☐Trash	70% Cleaning Grade Ethanol (used for PerkinElmer Intensive Clean), PerkinElmer bulk reagent mixed waste (from Prime or Check Manifolds procedure. Contains PerkinElmer Binding Buffer 2, PerkinElmer Wash Buffer 3, PerkinElmer Wash Buffer 4, and PerkinElmer Wash Buffer 5.)
☐Biological ☐Chemical ☐Radiological	☐Sink ☒Trash	Unused PerkinElmer Magnetic Beads (after decanting liquid into the sink), unused lyophilized Poly(A) RNA

Comments:

*Sink = non-hazardous aqueous solution or water soluble acid/base; Trash = solid waste

Commonwealth of Virginia
Department of General Services
Division of Consolidated Laboratory Services
Richmond, Virginia

ThermoFisher TaqPath™ COVID-19 Combo Kit Procedure for the Detection of 2019-nCoV RNA by RTPCR

I. PURPOSE/APPLICATION:

A. The purpose of this procedure is for the qualitative detection of nucleic acid from SARS-CoV-2 using real-time reverse transcription polymerase chain reaction (RT-PCR) amplification. Testing is performed for the purpose of patient diagnosis and surveillance of COVID-19 illness within Virginia at the direction of the Virginia Department of Health.

B. The RT-PCR test is intended for the qualitative detection of nucleic acid from SARS-CoV2 in upper respiratory and bronchoalveolar lavage (BAL) specimens collected from individuals who meet clinical and/or epidemiological criteria. Testing in the United States is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests by trained laboratory personnel who are proficient in performing real-time RT-PCR assays. The TaqPath™ COVID-19 Combo Kit is only for use under a Food and Drug Administration Emergency Use Authorization (EUA).

C. Deviations/modifications from the reference method validated and implemented by DCLS are listed in Section XIV, Table 1.

II. SUMMARY/SCOPE:

A. The ThermoFisher TaqPath™ COVID-19 Combo Real-Time RT-PCR test is a molecular *in vitro* diagnostic test that aids in the qualitative detection of SARS-CoV-2 RNA in respiratory specimens and the diagnosis of COVID-19 illness. The test is based on widely used nucleic acid amplification technology. The product contains primers and probes specific to three SARS-CoV-2 genomic regions and primers/probes for bacteriophage MS2.

B. The workflow begins with nucleic acid extraction from specimens in transport media. Nucleic acids are isolated and purified from specimens using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit using the Kingfisher Flex Magnetic Particle Processor with 96 deep well head extraction platform or the Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™360 Magnetic Bead extraction platform. The nucleic acid is reverse transcribed into cDNA, and amplified using the TaqPath™ COVID-19 RT-PCR kit with the Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument. In the process, the probes anneal to three (3) specific SARS-CoV-2 target sequences located between three (3) unique forward and reverse primers for the following genes: ORF1ab, N Gene and S Gene. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. During each amplification cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the Applied Biosystems 7500 Fast Dx Real-Time PCR

instrument. The data are analyzed, then interpreted by the Applied Biosystems™ COVID-19 Interpretive Software. Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

C. Quality is assured through testing of positive and negative PCR controls with each run. A negative and a MS2 phage positive extraction control is included in each extraction run, and the purified nucleic acid traction run is included on each PCR run as an internal process control for nucleic acid extraction.

D. DCLS validated two extraction methods for this procedure including:

1. MagMax Viral/Pathogen Nucleic Acid Isolation Kit using the ThermoFisher Kingfisher Flex Magnetic Particle Processors with 96 deep well head extraction platform.
2. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™360 Magnetic Bead extraction platform.

III. SAMPLE COLLECTION:

A. Specimen Type:

1. Sample types acceptable for testing per the IFU:
    a. upper respiratory specimens for example:
        i. nasopharyngeal or oropharyngeal swabs
        ii. nasal and mid-turbinate swabs
        iii. nasopharyngeal aspirate
    b. respiratory specimens collected from individuals who meet 2019-nCoV clinical and/or epidemiological criteria. For example:
        i. clinical signs and symptoms associated with 2019-nCoV infection
        ii. contact with a probable or confirmed 2019-nCoV case
        iii. history of travel to geographic locations where 2019-nCoV cases were detected
        iv. other epidemiologic links for which 2019-nCoV testing may be indicated as part of a public health investigation.
2. Swab specimens 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.
3. Place swabs immediately into sterile tubes containing 1-3 ml of appropriate transport media, such as viral transport media (VTM), Amies transport medium, phosphate buffered saline, or sterile saline.

B. Handling and Shipping Conditions:

1. Specimens can be stored at 2-8 ºC for up to 72 hours after collection.
2. Transport to DCLS refrigerated on ice packs.
3. The DCLS COVID-19 Submission Form (Qualtrax ID # 34293) is the preferred form to submit specimens for testing. However, the DCLS Test Request Form (Qualtrax ID #16857) can also be used. Specimens

submitted using the DCLS SARS-CoV-2 Sequencing Submission Form (Qualtrax ID 35889) may also require TaqPath COVID-19 PCR testing prior to initiating whole genome sequencing.

4. The DCLS Clinical Microbiology/Virology Request Form (Qualtrax ID # 16857) has been discontinued, but will be accepted if submitted with specimens.
5. The submission form should be fully completed by the submitter and submitted with the specimen; OR the submitter may use Webvision or DCLS Connect to electronically enter information for specimen submission. Information necessary for proper specimen submission:
   a. Patient Information (name, address, age or date of birth)
   b. Submitter Information (name, address, telephone number)
   c. Patient Medical History (information relevant to diagnosis such as symptoms, date of onset, recent exposures, travel history)
   d. Outbreak Information, if applicable, (outbreak number, role of patient in outbreak)
6. Test requested, specimen source, and date collected
7. When specimens are received in Sample Support Services (SSS), a LIMS identification number will be assigned. The patient information and specimen metadata will be entered into LIMS, labels will be generated and placed on the specimen container and appropriate paperwork. The specimens will be stored refrigerated in a SSS refrigerator until retrieved by testing personnel, or the specimens will be delivered to the COVID extraction laboratory (room 268A or B).

C. Storage Conditions:
1. When specimen is received at DCLS, store at 2-8 ºC for up to 72 hours after collection.
2. If a delay in extraction is expected, store specimens at -70ºC or lower.
3. Store extracted nucleic acid at -70ºC or lower.
4. Maintain RNA on a cold block or on ice during preparation to ensure stability.
5. After testing is completed, specimens that are positive for SARS-CoV-2 by PCR will be aliquotted into cryovials and stored at -70°C or below for long term storage. Specimens that are negative for SARS-CoV-2 by PCR are discarded after testing is complete unless other reflex testing is required. Samples must be disposed of as biohazardous waste in a regulated medical waste bin.
6. *Chain of Custody (COC) samples are discarded according to Evidence Receipt/Storage and Disposition Procedure (Qualtrax ID # 1804)*

D. Rejection Criteria:
1. After consultation with the Senior Scientist, Principal Scientist, Lead Scientist, or Group Manager, samples meeting the rejection criteria outlined below may still be tested and reported with additional disclaimers.

a. Absence of or inconsistent labeling and identification:
    i. The absence of a name or unique identifier on specimen container.
    ii. More than one name on specimen container.
    iii. Name on paperwork is different from name on specimen container.
b. Specimen submission form not properly filled out (e.g. patient name or address missing, etc.).
c. Specimen received in expired viral transport medium.
d. Specimen received without refrigeration.
e. Specimen received at the laboratory more than 72 hours after collection date.
f. Specimen with insufficient volume for testing.

2. When a sample is deemed unacceptable for testing, the submitter will receive a LIMS report explaining the reason for specimen rejection (Unsatisfactory for testing - reason).

3. PERSONNEL QUALIFICATIONS:

Procedures in Molecular Detection and Characterization Group (MDC) *may only be performed by approved testing personnel. The list of testing personnel can be found in the DCLS Training Matrix. Testing personnel must comply with the (insert group specific competency plan if there is one) and DCLS Competency (Qualtrax ID # 16472). Personnel will demonstrate competency twice during the first year. Competency assessment, with documentation, will be performed annually in subsequent years.*

4. INTERFERENCES/LIMITATIONS OF PROCEDURE:

A. This test has not been FDA cleared or approved; this test has been authorized by FDA under a EUA for use by laboratories certified under CLIA, 42 U.S.C. § 263a, to perform high complexity tests.

B. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

C. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb3(b)(1), unless the authorization is terminated or revoked sooner.

D. Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time PCR reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). To mitigate this limitation, workflow in the laboratory proceeds in a unidirectional manner.

E. Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly

recommended due to the importance of specimen quality.

F. Negative results do not preclude SARS-CoV2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.

G. The TaqPath™ COVID-19 RT-PCR Kit and the TaqPath™ COVID-19 RT-PCR Kit Advanced performance was established using nasopharyngeal and oropharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage samples only. Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the TaqPath™ COVID-19 RT-PCR Kit and the TaqPath™ COVID-19 RT-PCR Kit Advanced, but performance with these specimen types has not been established.

H. False-negative results may arise from improper sample collection, degradation of the SARS-CoV-2 RNA during shipping/storage, specimen collection after SARS-CoV-2 RNA can no longer be found in the specimen matrix, using unauthorized extraction or assay reagents, the presence of RT-PCR inhibitors, mutation in the SARS-CoV-2 virus, or failure to follow instructions for use.

I. False-positive results may arise from cross contamination during specimen handling or preparation, cross contamination between patient samples, specimen mix-up or RNA contamination during product handling.

J. Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely when prevalence of disease is high. False-positive test results are more likely when prevalence is moderate to low.

K. Test performance can be affected because the epidemiology and clinical spectrum of infection caused by 2019-nCoV is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and, during the course of infection, when these specimens are most likely to contain levels of viral RNA that can be readily detected.

L. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ COVID-19 RT-PCR Kit and the TaqPath™ COVID-19 RT-PCR Kit Advanced cannot rule out diseases caused by other bacterial or viral pathogens.

M. Limit of Detection of the the TaqPath™ COVID-19 Combo Kit is 10 GCE/reaction for BAL and Nasopharyngeal swab specimens.

VI. SAFETY:

A. Attire and Personal Protective Equipment

1. *Totally enclosed shoes are required in this laboratory at all times.*

2. *The required minimum Personal Protective Equipment (PPE) in this laboratory is a lab coat and safety glasses.*

3. *Gloves are required when handling samples, infectious agents, chemicals,*

closing and moving regulated medical waste containers, and when working in a biological safety cabinet (BSC) or chemical fume hood. Nitrile gloves are preferred.

NOTE: If latex gloves are in use, an alternative, non-latex glove must be available and laboratory door signage must reflect the usage of latex gloves.

4. Additional PPE that should be used when performing nucleic acid extractions, instrument loading, specimen archiving in a Biosafety Level-2 (BSL-2) laboratory this procedure include:
    - fluid-impervious, back-closing gowns
    - double gloves when working in the BSC
    - face shields (if safety glasses fog due to face masks)
5. Additional PPE that should be used when performing nucleic acid extractions of lower respiratory specimens in a Biosafety Level-3 (BSL-3) laboratory include:
    - respirators: PAPR or CAPR, N-95 with safety glasses
    - fluid-impervious, back-closing gowns
    - double gloves when working in the BSC
    - shoe covers

B. *Safety precautions must be taken when handling reagents, samples, and equipment in this laboratory.*

C. Special Precautions

1. BSL-2+ work practices will be used in BSL-2 testing laboratories.
2. Lower respiratory specimens will be processed in a BSL-3 laboratory, using BSL-3 safety and work practices.
3. Vortexing will occur inside of the BSC.
4. Sealed rotors will be used for centrifugation steps, and will only be opened inside of a BSC.
5. Vacuum manifolds will only be used inside of the BSC only.
6. All items will be decontaminated prior to removal from the BSC
7. Specimen containers are only opened inside of a BSC prior to inactivation via lysis buffer treatment for at least 10 minutes for the PerkinElmer Chemagic 360 extraction or 15 minutes for the KingFisher Flex extraction. Inactivated specimens may be removed from the BSC for loading onto the instrument.
8. Closed specimen tubes can be handled on the benchtop for plate mapping preparations.
9. Sharp items are discarded in sharps containers. Broken glass is discarded in a broken glass box and the box should not be filled more than 3/4 full. If broken glass has come in contact with a sample then it

is discarded in a sharps container. When ready to discard a sharps container, close the top securely and place it in a red regulated medical waste bin, or in the post lab, a designated cardboard box labeled with “regulated medical waste”.

D. Location of Eye Wash and Emergency Shower

1. *An eye wash/drench hose is present on each sink in this laboratory.*

2. *The emergency shower is located* in room 250/IV and MDC/134.

E. Hazards Associated With Procedure

1. Chemical Hazards

The following toxic, carcinogenic, or highly hazardous, ≤ 2, chemicals are associated with this procedure:

Chemical Name	Health Hazards	Flam-mability	Reactivity	Oxidizing Solid/ Liquid	Corrosive to Metals	Environ-mental Hazards	Fume Hood Required
Perkin Elmer Proteinase K*	1	N/A	N/A	N/A	N/A	N/A	No
BTL, Viral/Pathogen Binding Solution**	1	N/A	N/A	N/A	N/A	3	No
MagMAX Viral/Pathogen Proteinase K	1	N/A	N/A	N/A	N/A	N/A	No
BTL, Viral Pathogen Wash Buffer**	2	N/A	N/A	N/A	N/A	N/A	No
Ethanol***	2	2	N/A	N/A	N/A	N/A	No

*Incompatible with Bleach

**Incompatible with Acids and Bleach

***Incompatible with strong oxidizing agents, strong acids, acid anhydrides, acid chlorides

2. Biological Hazards

a. Respiratory viruses, including influenza, SARS-CoV-2, and other viruses, are human pathogens.
b. All clinical specimens will be handled as potentially infectious materials using Universal Precautions as specified in the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030, www.osha.gov). Only personnel trained in handling infectious materials will be permitted to perform this procedure.
c. Aerosol barrier pipette tips will be used to prevent the generation of aerosols. Wash hands thoroughly after handling specimens, reagents, and equipment, after removing gloves, and before leaving

the laboratory. Disinfect all bench tops and BSC after work is complete.

d. Specimen coolers and packages containing COVID-19 specimens are opened on the benchtop by SSS staff. Samples are then placed inside of the BSC for accessioning. All sample tubes are decontaminated prior to removal from the BSC and delivery to the testing laboratory.

3. Radiological Hazards

The following radiological hazards are associated with this procedure:
Not Applicable.

4. Safety Data Sheets/Pathogen Safety Data Sheets

The laboratory is responsible for maintaining a current, complete file of Safety Data Sheets (SDSs) related to this procedure. The SDSs are available to the analyst on computers throughout the laboratory at the following URL: https://msdsmanagement.msdsonline.com/21943a72-0bc7-4000-a405-4ba03280a52c/ebinder/?nas=True

F. Spill Response

1. *Small spills - handled by the laboratory staff (refer to SDS) or call Administration for Spill Response Team notification.*

2. *Large spills – call Administration for Spill Response Team notification.*

Refer to DCLS Safety Manual (Qualtrax ID # 1805) for additional safety information.

VII. EQUIPMENT & SUPPLIES, REAGENTS & STANDARDS:

For labeling requirements for purchased or prepared media/reagents/standards, refer to Measurement and Data Traceability (Qualtrax ID # 1789).

- Equipment & Supplies: Store at room temperature unless otherwise specified

1. Specimen Extraction

a. ThermoFisher Kingfisher Flex Magnetic Particle Processor with 96 deep well head extraction platform for use with MagMax Viral/Pathogen Nucleic Acid Isolation Kit.
   i. KingFisher™ deep-well 96 plate
   ii. KingFisher Duo cap for elution strip
   iii. Adjustable micropipettors
   iv. Multi-channel micropipettors
   v. MicroAmp™ Clear Adhesive Film
   vi. Conical Tubes (15 mL)

vii. Conical Tubes (50 mL)
viii. Reagent reservoirs
ix. Nonstick, RNase-Free Microfuge Tubes, 1.5 mL
x. Nonstick, RNase-Free Microfuge Tubes, 2.0 mL
xi. Vortex
xii. 96 deep-well magnetic head
xiii. 96 deep-well heat block

b. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™360 Magnetic Bead extraction platform.
i. Rack with Disposable Tips
ii. low-well-plate (MICROTITER SYSTEM)
iii. Magnetic Beads
iv. deep-well-plate (riplate SW)

2. PCR Set up and Detection
a. Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument (used with SDS Software v1.4.1)
b. ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)
c. JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)
d. Vortex mixer
e. Microcentrifuge
f. Centrifuge, with a rotor that accommodates standard and deepwell microplates
g. Single and multichannel adjustable pipettors (1 µL to 1,000.0 µL)
h. Racks for 1.5 mL microcentrifuge tubes
i. Cold block (96-well or 384-well) or ice
j. Molecular grade water, nuclease-free
k. 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite
l. bleach)
m. DNAZapTM or equivalent
n. RNase AWAY™ or equivalent
o. Aerosol barrier pipette tips
p. 1.5 mL microcentrifuge tubes (DNase/RNase free)
q. 0.2 mL PCR reaction plates
r. MicroAmp Optical 8-cap Strips
s. MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL. Note: plates without a barcode can be used.
t. MicroAmp™ Optical Adhesive Film
u. Laboratory freezers –30°C to –10°C and ≤ –70°C

- Reagents:
1. Specimen Extraction; All solutions should be stored at room temperature (15–25°C) unless otherwise stated. Follow manufacturer

expiration dates.

a. ThermoFisher MagMax Viral/Pathogen Nucleic Acid Isolation Kit using Altria's Kingfisher Flex Magnetic Particle Processor with 96 deep well head extraction platform.
   i. Binding Solution
   ii. Wash Buffer. Wash Solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.
   iii. Elution Solution
   iv. Proteinase K
   v. Total Nucleic Acid Binding Beads
   vi. Ethanol, 100% (molecular biology grade)
   vii. Nuclease-free Water

b. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™360 Magnetic Bead extraction platform.
   i. Poly(A) RNA; prepare according to manufacturer instructions; store in the dark; reconstituted Poly(A) is stable for 4 weeks at 4 °C; For long term storage store the reconstituted Poly(A) RNA in aliquots at -20 °C. Do not freeze the Poly(A) RNA aliquots after thawing.
   ii. Proteinase K; prepare according to manufacturer instructions; reconstituted Proteinase K is stable for 2 weeks at 4 °C; For long term storage store the reconstituted Proteinase K in aliquots at -20 °C. Do not freeze the Proteinase K aliquots after thawing.
   iii. Lysis Buffer 1; store in the dark; may form a precipitate upon storage. If necessary, warm to 55 °C to dissolve.
   iv. Binding Buffer 2
   v. Wash Buffers 3, 4 and 5
   vi. Elution Buffer 6
   vii. For long term storage we recommend to store the reconstituted Poly(A) RNA and Proteinase K in aliquots at -20 °C. Do not freeze the Poly(A) RNA and Proteinase K aliquots after thawing.

2. PCR Set up and Detection; Prepare RT-PCR reagents per manufacturer Instructions For Use (TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced* Instructions for Use) :
   TaqPath™ COVID-19 RT-PCR Kit; Store at –30°C to –10°C:
   a. COVID-19 Real Time PCR Assay Multiplex (ORF1ab, N gene, S gene, MS2). Thaw on ice and refer to the COVID-19 TaqPath Combo Kit 400_100 worksheet (Qualtrax ID # 34594)
   b. MS2 Phage Control. Thaw on ice and use as is per the COVID 19 automated extraction worksheets (Qualtrax ID # 34648 and

#34587).

c. TaqPath™ COVID-19 Control (1 x 10^4 copies/µL); Store at ≤ –70°C. Refer to the COVID-19 TaqPath Combo Kit 400-100 worksheet (Qualtrax ID # 34594) for control preparation.
d. TaqPath™ COVID-19 Control Dilution Buffer; Store at –30°C to –10°C
e. Nuclease-free Water

VIII. PROCEDURE:

A. Nucleic Acid Extraction: Perform one of the RNA extraction/purification procedures following the manufacturer's instructions for use with DCLS validated modification as specified:

1. Consult the FDA EUA website to confirm the most recent version of the IFU in use (https://www.fda.gov/media/136112/download).
2. MagMax Viral/Pathogen Nucleic Acid Isolation Kit using ThermoFisher's KingFisher™ Flex Magnetic Particle Processor with 96 deep well head extraction platform (standard volume: 200 – 400 µL).
    a. DCLS verified the use of the ThermoFisher KingFisher Flex using the automated program: "MVP_Flex 96DW" Program on the KingFisher Flex
    b. Sample input volume of 400µL.
    c. Extraction mixture combined with respiratory specimen includes 10µL Proteinase K and 550µL Binding Bead mixture. Once combined, samples are incubated 15 min prior to removal from the BSC.
    d. 10 µL Phage Control used
    e. Processing plates include an additional Wash 3 Plate (500µL 80% Ethanol) (in reference to #MAN0019181 rev. H)
    f. Elution plate includes 100µL Elution Solution

3. Perkin Elmer's Chemagic™ Viral DNA/RNA 300 Isolation Kit using Perkin Elmer Chemagic™ 360 Magnetic Bead Extraction Platform; Purification Protocol for Viral DNA/RNA from 300 µl Plasma, Serum, Naso- or Oropharyngeal Swabs, BAL and Sputum Using the chemagic 360 with integrated chemagic Dispenser
    a. DCLS verified the use of the **Perkin Elmer Chemagic 360** for the preparation of RNA with the Chemagic Viral DNA/RNA 300 Kit special H96 using automated program: Chemagic Viral 300 360 H96 drying prefilling VD141210.che Program **on the Perkin Elmer Chemagic 360**
    b. Sample input volume of 300µL
    c. Master Mix combined with respiratory specimen includes 300µL Lysis Buffer, 4µL Poly(A) RNA and 10µL Proteinase K, once combined, samples are incubated 10 min prior to removal from

the BSC.
d. 7.5 µL Phage Control used
e. Processing plates include Low-Well Beads (150µL), 3 Deep Well Washes
f. Elution Plate includes 100µL Elution buffer

B. Perform PCR procedure using TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced* Instructions for Use with DCLS validated modification as specified. Refer to the following sections:

1. Prepare the RT-PCR reactions (Refer to pages 34-36 of IFU, sections 4-5, for RNA preparation and reaction plate set-up)
   a. PCR reaction mixture (per sample) includes:
      - 6.25µL TaqPath 1-Step Multiplex Master Mix (No ROX, 4X)
      - 1.25µL COVID-19 Real-Time PCR Assay Multiplex
      - 7.5µL Nuclease-free water
   b. 10µL purified sample RNA used as template
   c. 10µL purified Negative Control (from RNA extraction) used for Negative Control reaction
   d. 2-µL Positive Control (diluted TaqPath COVID-19 control) + 8 uL nuclease-free water used as Positive Control reaction
   e. 10-µL nuclease-free water used as the Non-Template Control (NTC)

2. Set up and run the 7500 Fast Dx Real-Time PCR Instrument using the "TaqPath COVID-19 Kit" ABI template (refer to COVID-19 TaqPath Combo Kit 400_100 worksheet (Qualtrax ID #34594)

3. Analysis and results procedure:
   a. Interpretation of the results is performed by the Applied Biosystems™ COVID-19 Interpretive Software. For information about the Ct values that are used by the software to interpret results,refer to the Instructions for Use for "Ct cutoff values for assay targets".
   b. DCLS **does not** utilize the TaqMan SARS-CoV-2 RNase P assay; therefore, the COVID-19 Interpretive Software is used for data analysis and interpretation for patient reports.
   c. For detailed instructions about using the software, refer to AB COVID-19 Interpretive Software Job Aid (Qualtrax ID# 34604).
   d. For troubleshooting purposes only, refer to AB Design & Analysis Software Job Aid (Qualtrax ID# 34605).

IX. CALCULATIONS:

A. Refer to manufacturer IFU's for any relevant calculation instructions.

X. CALIBRATION, QUALITY CONTROL AND QUALITY ASSURANCE:

A. Refer to the manufacturer Instructions For Use, (TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced) for Quality Control for the SARS-CoV-2 assay.

1. For each RT-PCR reaction plate, include the following controls:
   a. One Positive Control
   b. One Negative Control from each extraction run. For example, if RNA samples from 4 extraction runs are combined on one 384-well RT-PCR reaction plate, then 4 Negative Control wells must be run on that 384-well reaction plate.

2. If any of the above controls do not exhibit the expected results as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

3. All control wells must pass for the real-time RT-PCR plate to be considered valid. Validation of results is performed automatically by the Applied Biosystems™ COVID-19 Interpretive Software based on performance of the Positive and Negative Controls.

4. Verify the performance of all testing reagents with control materials prior to releasing patient results.

XI. WASTE MANAGEMENT

DCLS manages all waste streams in compliance with local, state, and federal regulations.

A. Pollution Prevention

1. *As part of DCLS' Pollution Prevention efforts, procedures are aimed at the elimination or reduction of hazardous waste discharge at the point of generation.*

2. *Procedural decisions are based on the use of the least hazardous substance, limitations on the quantity ordered, the appropriate usage of the safety equipment, staff training, and competency assessment.*

3. *Training on waste management is provided to staff on an annual basis.*

B. Biological, Chemical, Radiological Waste Handling

1. *The safety office provides assistance in the development of waste handling*

and storage procedures and coordinates hazardous waste pick-ups.

2. *A Waste Profile that is SOP-specific has been developed and approved. This information is listed on the DCLS Waste Profile Form (Qualtrax ID # 1646) which is attached to this SOP as Appendix* I.

3. *This method does not generate any hazardous radiological waste.*

4. *This method generates the following hazardous chemical/biological (regulated medical waste)/radiological waste streams.*

   a. Chemical
      - Expired, unused extraction reagents, including Buffer AVL (50%-100% guanidinium thiocyanate),, Buffer AW1 (50-100% guanidine hydrochloride), and AW2 (50% ethanol)
      - Mixed waste containing Buffer AVL (50%-100% guanidinium thiocyanate), Buffer AW1 (50%-100% guanidine hydrochloride), 100% ethanol, Buffer AW2 (50% ethanol), viral transport media.

   b. Biological (regulated medical waste):
      - *Gloves, disposable lab coats and other PPE should be disposed in the red regulated medical waste bins.*
      - Any waste that may have been in direct contact with samples
      - All testing materials used or generated in the BSC and items used during the processing of potentially infectious samples
      - Empty specimen containers and microcentrifuge tubes labeled with patient information

   c. Radiological: Not applicable

Refer to the DCLS Safety Manual (Qualtrax ID # 1805) for Additional Safety information.

C. Solid Waste

Solid waste items that are associated with this procedure should be placed in trashcans for pick-up by BFM staff. The following items are considered solid waste: paper, paper towels, empty sample containers, food samples submitted for testing (that have tested negative) and the containers, expired media, non-infectious and non-chemical waste.

D. On-Site Autoclave Preparation

There are instances in which containers of potentially infectious materials may need to be autoclaved on site before being packaged for pick up by the regulated

medical waste contractor or re-used in our laboratories.

1. BSL-3 Laboratories
   The following items are routinely packaged in this laboratory and placed in the pass-through autoclave in BSL-3.

   All waste generated in BSL-3.

Refer to the DCLS Safety Manual (Qualtrax ID # 1805) or the BSL-3 Biosafety Manual (Qualtrax ID # 8650) for detailed instructions on how to properly prepare materials for autoclaving.

XII. RECORDING AND REPORTING OF RESULTS:

A. Record procedural steps completed on the applicable worksheet as follows:
   1. MS2 KF Flex Extraction (Qualtrax ID # 34587)
   2. MS2 PE chemagic Viral DNA_RNA 300 Extraction Worksheet (Qualtrax ID # 34648)
   3. COVID-19 TaqPath Combo Kit 400_100 (Qualtrax ID # 34594)

B. Refer to the manufacturer Instructions For Use (IFU), (TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced) for Results for the SARS-CoV-2 assay. The table below lists the expected patient results for the TaqPath™ COVID-19 Combo Kit.

ORF1ab	N gene	S gene	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	INVALID	NA	Repeat test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider collecting a new specimen.
Any result				INVALID	NA	The patient sample may be invalid because the same sample name (Sample ID) was assigned to multiple wells in the instrument software. In the **Samples** pane of the Home screen, review all samples with a status of **INVALID**. If there are duplicate sample names: In the instrument software, correct the sample names, for EDS files change the experiment name, save the file with a new file name, then import the corrected file into the interpretive software.
NEG	NEG	NEG	POS	VALID	SARS-CoV-2 Not Detected	Report results to the healthcare provider and appropriate public health authorities. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS			POS or NEG	VALID	SARS-CoV-2 Inconclusive[1]	1. Repeat test by re-extracting the original sample and repeating the RT-PCR. 2. After retesting one time, report results to the healthcare provider and appropriate public health authorities. **IMPORTANT!** Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time. If the repeat result remains inconclusive, the healthcare provider should conduct additional confirmation testing with a new specimen, if clinically indicated.
Two or more SARS-CoV-2 targets = POS			POS or NEG	VALID	Positive SARS-CoV-2	Report results to the healthcare provider and appropriate public health authorities.

[1] Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.

A. Ensure that QC materials are verified and results are second reviewed and approved before being released on patient reports.
   1. Import data to PCR COVID-19 Multi Results Cover Sheet (Qualtrax ID # 35047) and submit to reviewer.

B. Disclaimers and After Comments:

1. **Patient reports with TaqPath COVID-19 Combo Kit assay results** include the following disclaimer statements:

   i. *PCR COVID-19 Multi Disclaimer: The US Food and Drug Administration has made this test available under emergency use authorization (EUA) for the duration of the COVID-19 declaration justifying emergency use of IVDs unless terminated or revoked. Results from this test should not be used as the sole basis for treatment or patient management decisions. A negative result does not exclude the possibility of COVID-19.*

   Fact sheets on the TaqPath COVID-19 Combo kit for healthcare providers and patients can be accessed at https://www.fda.gov/media/136111/download, or https://www.fda.gov/media/136114/download.

2. If the initial result for a specimen is **inconclusive or invalid**, reflex testing may be performed using any COVID-19 test currently validated at DCLS.

   i. If initial testing is performed using the TaqPath COVID-19 Combo assay and repeat testing is performed using a different assay, apply the following after comment to the TaqPath COVID-19 Combo Assay step conclusion:

   REPEAT TESTING WILL BE PERFORMED. PLEASE REFER TO ADDITIONAL RESULTS FOR FINAL TEST REPORTING.

   ii. If initial testing and repeat testing are both performed using the TaqPath COVID-19 Combo assay and the test result remains inconclusive or invalid, apply the following after comment to the TaqPath COVID-19 Combo assay step conclusion:

   REPEAT TESTING WAS PERFORMED. THIS IS A FINAL TEST RESULT.

XIII. REFERENCES:

1. TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced* Instructions for Use; Publication Number MAN0019181
2. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19); Updated Nov. 5, 2020; https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-

specimens.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fguidelines-clinical-specimens.html
3. ThermoFisher/ appliedbiosystems MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide; Catalog Number A42352 Pub. No. MAN0018073 Rev. C.0; 24 September 2020
4. Purification Protocol for Viral DNA/RNA from 300 µl Plasma, Serum, Naso- or Oropharyngeal Swabs, BAL and Sputum Using the chemagic 360 with integrated chemagic Dispenser; Version 200312; 2018

XIV. APPENDIX, TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA:

Appendix I. Waste Profile Form

Appendix I. DCLS Waste Profile

DCLS Waste Profile Form Richmond, VA		
Group: MDC		☒ New/Changed Waste Profile
Contact: Sean Kelly ext 227		
SOP Name: ThermoFisher TaqPath COVID-19 Real Time PCR Assay	SOP #: 36677	
Waste Type (choose only one)		**Waste Composition**
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Residual respiratory clinical specimens (nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Biological specimen waste from MagMax extractions (plastics and liquid waste used to extract biological specimens) including BTL, Viral/Pathogen Binding Solution, MagMax Viral Pathogen Proteinase K, BTL, Viral/Pathogen Wash Buffer, Ethanol, and other kit components of MagMax Viral/Pathogen kit. All plates removed from the PerkinElmer instrument following a run (plates contain Wash Buffer 3, Wash Buffer 4, Wash Buffer 5, Magnetic Beads, Lysis buffer, and inactivated sample).
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Used consumables: transfer pipets, serological pipettes, gloves, aerosol barrier tips, conicals, microcentrifuge tubes, forceps, elution columns, etc. generated during processing
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	Cleaning supplies used during sample processing and cleaning BSC: bench coat, BleachRite, Microchem, Dispatch wipes, WypAlls, etc.
☐Biological ☒Chemical ☐Radiological	☐Sink ☐Trash	**Unused expired reagents**: PerkinElmer Binding Buffer 2, PerkinElmer Wash Buffer 3, PerkinElmer Wash Buffer 4, PerkinElmer Lysis Buffer, PerkinElmer Proteinase K, PerkinElmer Poly(A) RNA Buffer, BTL, Viral/Pathogen Binding Solution, MagMax Viral Pathogen Proteinase K, BTL, Viral/Pathogen Wash Buffer
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	**Unused expired reagents**: HSC, solid or liquid media, polyvalent
☐Biological ☐Chemical ☐Radiological	☒Sink ☐Trash	**Unused expired reagents**: Unused Tris-EDTA (TE) Buffer, unused PerkinElmer Wash Buffer 5, unused PerkinElmer Elution Buffer, unused PerkinElmer Magnetic Beads liquid (decant liquid when beads have settled)
☒Biological ☐Chemical ☐Radiological	☐Sink ☐Trash	**PPE**: Back closing gowns, gloves, N95 respirators, shoe covers.
☐Biological ☒Chemical ☐Radiological	☐Sink ☐Trash	70% Cleaning Grade Ethanol (used for PerkinElmer Intensive Clean), PerkinElmer bulk reagent mixed waste (from Prime or Check Manifolds procedure. Contains PerkinElmer Binding Buffer 2, PerkinElmer Wash Buffer 3, PerkinElmer Wash Buffer 4, and PerkinElmer Wash Buffer 5.)
☐Biological ☐Chemical ☐Radiological	☐Sink ☒Trash	Unused PerkinElmer Magnetic Beads (after decanting liquid into the sink), unused lyophilized Poly(A) RNA
Comments: *Sink = non-hazardous aqueous solution or water soluble acid/base; Trash = solid waste		

ThermoFisher TaqPath™ COVID-19 Combo Kit Procedure for the Detection of 2019-nCoV RNA by RTPCR

Table 1: DCLS modifications to the ThermoFisher TaqPath™ COVID-19 Combo Kit Procedure for the Detection of 2019-nCoV RNA*

Procedural Step:	Reference Method and Detail:	Does SOP reflect reference method?	List modification in DCLS Validated Method:	Does modification change chemistry of procedure and/or is modification specifically prohibited by reference method?	List explanation/justification for modifications that change the chemistry of the procedure and/or are specifically prohibited in the reference method:
TaqPath COVID-19 Combo Kit IFU #MAN0019181 rev. G, pg. 14: Instrument, assay and software compatibility	• Per IFU, the 7500 Fast-Dx Real-Time PCR Instrument with the SDS Analysis Software v1.4.1 data may be analyzed at a minimum with COVID-19 Interpretive Software version 1.3 if the test procedure **does not** include the TaqMan SARS-Cov-2 RNase P Assay, or with COVID-19 Interpretive Software version 1.4 if the test procedure **does**	Yes	• DCLS **does not** utilize the TaqMan SARS-CoV-2 RNase P assay; therefore, the COVID-19 Interpretive Software version 1.3 is used for data analysis and interpretation for patient reports	No	• COVID-19 Interpretive Software version 1.3 was verified by DCLS for data analysis, interpretation and reporting.

	include the TaqMan SARS-Cov-2 RNase P Assay				
TaqPath COVID-19 Combo Kit IFU #MAN0019181 rev. G, pg. 24: KingFisher Flex MagMax Viral/Pathogen Nucleic Acid Isolation Kit: Extract RNA – Automated Method (400-µL sample input volume)	• Automated Program: "MVP_2Wash_400" Flex Program from the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit product page **on the KingFisher Flex** • Processing plates include Wash 1 Plate (1000-µL Wash Solution) and Wash 2 Plate (1000-µL 80% Ethanol) • Elution Plate includes 50-µL Elution Solution	No	• Automated Program: "**MVP_Flex 96DW**" Program **on the KingFisher Flex** • Processing plates include an additional Wash 3 Plate (500-µL 80% Ethanol) • Elution plate includes 100-µL Elution Solution	Yes	• The processing protocol was used for the validation study. • The second 80% Ethanol wash (Wash 3 plate) is performed per the manufacturer product insert for the MagMax Viral/Pathogen Nucleic Acid Isolation Kit (MAN0018073, Rev. C.0), and was used for the validation study. • The elution volume was used for the validation study to provide additional extraction material for repeat testing and additional characterization, this elution volume is within the range specified in the manufacturer product insert for the MagMax Viral/Pathogen Nucleic Acid Isolation Kit (MAN0018073, Rev. C.0).
TaqPath COVID-19 Combo Kit IFU #MAN0019181 rev. G, pg. 24: KingFisher Flex MagMax	• Automated Program: MVP_2Wash_400 Flex Program from the MagMAX Viral/Pathogen II	No	• DCLS additionally verified the use of the **Perkin Elmer Chemagic 360** for the preparation of	Yes	• Manufacturer representatives programed the extraction protocol used for SARS-CoV-2 RNA on the Chemagic 360, **Chemagic Viral300 360 H96 drying prefilling VD141210.che,** upon instrument installation at DCLS in April 2020, this protocol was used in

Viral/Pathogen Nucleic Acid Isolation Kit: Extract RNA – Automated Method (400-µL sample input volume)	Nucleic Acid Isolation Kit product page • Sample input volume of 400-µL • 10-µL Proteinase K added to each sample well, followed by 550-µL Binding Bead Mix • 10-µL MS2 Phage Control Used • Processing plates include Wash 1 Plate (1000-µL Wash Solution) and Wash 2 Plate (1000-µL 80% Ethanol) • Elution Plate includes 50-µL Elution Solution		RNA with the Chemagic Viral DNA/RNA 300 Kit special H96 (Reference: PerkinElmer Purification Protocol for Viral DNA/RNA from 300-µL Plasma, Serum, Naso- or Oropharyngeal Swabs, BAL and Sputum using the Chemagic 360 with Integrated Chemagic Dispenser, Version 200312) • Automated extraction program: **Chemagic Viral300 360 H96 drying prefilling VD141210.che** Program **on the Perkin Elmer Chemagic 360** • Sample input		the validation. • The processing protocol with the **Perkin Elmer Chemagic 360** was adopted due to lack of access to automated instrumentation and reagents and supplies in the pandemic. The **Perkin Elmer Chemagic 360** processing protocol with the Chemagic Viral DNA/RNA 300 Kit special H96 was validated for use with the **TaqPath COVID-19 Combo Kit**. • The amount of phage control used was adjusted to maintain the ratio of phage to sample in the IFU #MAN0019181 rev. G assay. • The elution volume was standardized to obtain the same eluate across the KingFisher Flex and Perkin Elmer platforms for the TaqPath PCR assay, this elution volume is within the range specified in the manufacturer product insert for the purification of Viral DNA/RNA from specimens using the Chemaic 360 (Version 200312).

			volume of 300-µL • Master Mix combined with respiratory specimen includes 300-µL Lysis Buffer, 4-µL Poly(A) RNA and 10-µL Proteinase K, once combined, samples are incubated 10 min prior to removal from the BSC • 7.5-µL Phage Control Used • Processing plates include Low-Well Beads (150-µL), 3 Deep Well Wash • Elution Plate includes 100-µL Elution buffer		
TaqPath COVID-19 Combo Kit IFU #MAN0019181 rev. G, pg. 38: Prepare the	• PCR reaction mixture (per sample) includes: ○ 6.25-µL TaqPath 1-	No	• PCR reaction mixture (per sample) includes: ○ 6.25-µL TaqPath 1-	Yes	• The amount of Sample RNA, Positive Control and Negative Control added were used in the DCLS validation study. • The amount of template and control materials added maintains the same ratio of nucleic acid to reaction mixture

RT-PCR reactions (400 µL sample input, 960-well reaction plate, COVID-19 assay only)	Step Multiplex Master Mix (No ROX) (4X) ○ 1.25-µL COVID-19 Real-Time PCR Assay Multiplex ○ 12.5-µL Nuclease-free water • 5-µL purified sample RNA used as template • 5-µL purified Negative Control (from RNA extraction) used for Negative Control reaction • 2-µL Positive Control (diluted TaqPath COVID-19 control) + 3 uL nuclease-free water used as Positive Control reaction		Step Multiplex Master Mix (No ROX) (4X) ○ 1.25-µL COVID-19 Real-Time PCR Assay Multiplex ○ 7.5-µL Nuclease-free water • 10-µL purified sample RNA used as template • 10-µL purified Negative Control (from RNA extraction) used for Negative Control reaction • 2-µL Positive Control (diluted TaqPath COVID-19 control) + 8 uL nuclease-free water used as Positive Control reaction		used in the "200-µL sample input PCR reactions" described on page 34 of #MAN0019181. • The template amount used reflects the same input volume and elution volume ratios for the DCLS validated method (400-µL sample input, 100-µL elution) and the "200-µL sample input" Automated Method (200-µL sample input, 50-µL elution) on Pg. 21 of #MAN0019181 rev. G • DCLS added a PCR NTC to detect cross-contamination on the PCR plate

			• 10-µL nuclease-free water used as the Non-Template Control (NTC)		

*Qualifying External Components statement from CDC EUA procedure: “If a laboratory modifies this test by using unauthorized, alternative components (e.g., extraction methods or PCR instruments), the modified test is not authorized under this EUA. FDA’s Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency, updated May 11, 2020, does not change this. As part of this policy, FDA does not intend to object when a laboratory modifies an EUA-authorized test, which could include using unauthorized components, without 10 CDC-006-00019, Revision: 05 CDC/DDID/NCIRD/ Division of Viral Diseases Effective: 07/13/2020 obtaining an EUA or EUA amendment, where the modified test is validated using a bridging study to the EUA-authorized test.”