Introduction
Healthcare Personnel (HCPs) who collect, handle, transport or test clinical specimens from a patient under investigation (PUI) for Coronavirus Infectious Disease-19 (COVID-19) with the SARS-CoV-2 virus should adhere rigorously to the following precaution measures and biosafety practices listed in https://www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html. HCPs caring for patients with COVID-19 are at elevated risk of exposure.
HCPs should be prepared to:

✔ **Identify** patients in accordance to the CDC Case definition: https://www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html

✔ **Isolate** patients by placing a facemask on the patient and isolate in an examination room with the door closed. It is recommended patients be isolated in an Airborne Infection Isolation Room (AIIR) or if not available, placed in room where the exhaust is not recirculated within the building without HEPA filtration.

✔ **Inform** both in-house infection prevention (IP) personnel and the local health department (LHD). (http://www.dhhs.ne.gov/Pages/LHD)

✔ **Initiate** collection of specimens use appropriate Personal Protective Equipment (PPE) for respiratory pathogens.

Testing at Nebraska Public Health Laboratory
Testing at the NPHL will be prioritized due to the limited testing available to public health laboratories across the nation. Nebraska Public Health Laboratory will test only when approved by LHD and assigned a PUI number. Specimens will be tested within 24hr of receipt at NPHL (Monday through Friday) or within 48hr of receipt (Saturday through Sunday). STAT courier services and testing will depend on the gravity of the case and must be cleared by the LHD or NE DHHS. Call ordering physician or LHD for results. Please do not call NPHL due to workload.

Priorities for testing may include:
- **Inpatients:** for suspected COVID-19, rule out flu and RPP, then order COVID-19
- **Outpatients:** vulnerable or high-risk populations with a clinical diagnosis of COVID-19, after ruling out alternative diagnoses (negative flu/RPP), will be considered for testing
  - Healthcare workers
  - Public safety (EMS, law enforcement, firefighters)
  - Nursing home, group home, daycare attendees or employees
  - Older adults (65 years of age and older)

Testing at Commercial Laboratories
Commercial laboratories have come on board nationally to test for the COVID-19. Therefore, patients should call their clinician or LHD to discuss symptoms before a test can be ordered. Physicians should consider:

- If lab test is warranted, assess whether clinical diagnosis of COVID-19 infection suffices, or if laboratory diagnosis is even necessary.
- Patients with a clear source of exposure (e.g., household member of a known lab-confirmed case) and a clinical presentation consistent with COVID-19 are now a lower priority for testing.
- Prioritize limited laboratory testing capacity for patients with the highest pre-test probability (the most severely ill who fit COVID-19 profile) and lacking a clearly identifiable source/exposure and flu/RPP tests negative.

Generally, most facilities have an established relationship with a commercial laboratory. If not already established, the facility should set up a commercial account to arrange computer access and courier pickup. Standard patient collection centers and other phlebotomy sites provided by commercial labs traditionally cannot collect specimens.
Commercial laboratories currently experience the same bottlenecks with delayed results. Further details can be found at the commercial laboratory’s website or contact them directly.

**Respiratory Specimen Collection**

Maintain proper infection control ([https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4](https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4)) when collecting specimens or performing other aerosolizing procedures. Use appropriate PPE-following standard, contact, airborne precautions plus eye protection including eye protection such as goggles and/or disposable face shield, respirator-preferably N-95, N-99 or Powered Air Purifying Respirator (PAPR), a long-sleeved gown, and gloves. The surgical mask can be used when triaging the patient, however CDC recommends the respirator when possible to collect actual specimen due to the risk of aerosolization.

A video on PPE donning and doffing is available at: [https://www.youtube.com/watch?v=bG6zISnenPg](https://www.youtube.com/watch?v=bG6zISnenPg)
A video for NP swab collection is available at: [https://www.youtube.com/watch?v=hXohAo1d6tK](https://www.youtube.com/watch?v=hXohAo1d6tK)

Collect one (1) Nasopharyngeal (NP) swab. NOTE: The oropharyngeal swab (OP) has been discontinued. Facilities must first perform a risk assessment to assess if collection or testing can be safely performed. If a facility is unable to safely collect or perform multiplex PCR, see notes below. Specimens should be collected as soon as possible once a PUI is identified to fit the CDC criteria regardless of the time of symptom onset.

**Nasopharyngeal**

- Only one NP swab is required as long as the viral transport medial (VTM) vial contains 2-3mL of media to perform both the multiplex Respiratory Pathogen Panel (RPP) and the COVID-19 NPHL test if requested. Collect additional NP swabs if vial contains <1mL transport media, if additional testing is sent to commercial laboratories outside of Nebraska or if rapid detection tests require extracting reagent be added to the original specimen.
- Use only swabs designed for NP collection, usually a mini-tipped synthetic fiber swab with thin plastic shaft. Do not use calcium alginate swabs or swabs with wooden shafts used for bacterial cultures, as they may contain substances that inactivate some viruses and inhibit PCR testing. Kits containing both swabs and viral transport media (VTM, UTM, VCM or M4) may be available from the LHD but should be reserved for only severe cases.
- HCP should stand to the side, not directly in front of the patient when collecting to avoid aerosols. Insert a swab into the nostril parallel to the palate. Gently hold, then rotate swab to absorb secretions. Slowly withdraw the swab. **Do not sample the nostrils or tonsils.**
- Place swab immediately into a sterile tube containing 2-3 ml of VTM. Aseptically cut swab stick off to permit tightening of the cap. If the swab has a break line, cover vial opening with gauze and hold away from HCPs and patients, to break off swab handle.
- **Close cap tight and straight on vial (tilted caps will leak), make sure shaft of swab is short enough not to be in contact with lid, thus preventing a secure seal and leakage in transport.**
- Place patient’s label on the specimen container (i.e., primary container) after collection only after name has been confirmed with patient ID. Label must contain patients first and last name, date of birth, time and date of collection, source and collector initials.
- **Ideally specimens should be refrigerated at 2-8°C immediately after collection. Couriers should be instructed to transport on ice or frozen gel packs. Courier arrangements are addressed below. If courier or FedEx arrangements cannot be made within 72h, the specimen should be frozen at -20°C, and shipped on Dry Ice. Write “Frozen Specimen” on NPHL Test Order form.**
- Specimens can be kept frozen at -20°C for up to 30 days.

**Lower Respiratory**

- The induction of sputum is not recommended. However, patients for whom it is clinically indicated (e.g., those receiving invasive mechanical ventilation), a lower respiratory tract aspirate or bronchoalveolar lavage sample can be collected and tested in-house for bacterial or fungal pathogens.
- Consult infectious diseases/infection control if other specimen types such as sputum can be safely collected for in-house testing. Sputum can be obtained if PUI has a productive cough and can produce sputum.
- If the patient can produce sputum, have the patient rinse their mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof screw-cap collection cup or a sterile dry container.
If the specimen is collected using an aerosol-generating procedure (such as tracheal intubation, non-invasive ventilation, tracheotomy, cardiopulmonary resuscitation, manual ventilation before intubation or bronchoscopy), the PUI should ideally be in an AIIR. If this is not possible than they should be placed in a private room with the door closed. The exhaust from this room should not recirculate throughout the facility without HEPA filtration.

During specimen collection only the PUI and essential HCP should be in the room wearing appropriate PPE.

If required, lower respiratory tract specimens ordered for bacterial or fungal culture should be forwarded to the in-house laboratory. If specimens are routinely forwarded to an off-site reference laboratory, contact the reference laboratory first for acceptability. Specimen containers and reference laboratory order forms should be clearly labeled with “PUI for COVID-19.”

Rapid Respiratory Panel (RPP) To Rule-Out Other Pathogens

Nebraska state epidemiologist may recommend the RPP to rule-out more common pathogens for patients who do not have history of direct contact or travel to an “at risk” country. Whereas, a RPP may not be necessary for patients who do have known close contacts. Ultimately, the decision will be made after the local health department and NE DHHS have discussed the patient’s symptoms and demographics with the physician or infection control. RPP orders sent to commercial labs outside of Nebraska tends to provide a longer turn-around-time (TAT) of 2-4 days. Consider using reference laboratories within Nebraska with a shorter TAT and can reflex the same specimen to NPHL.

Packing, Shipping and Transport to NPHL

Packaging, shipping, and transport of specimens from a PUI to NPHL must follow shipping regulations for UN 3373 Biological Substance, Category B. All personnel who package and transport specimens (including couriers) need to be trained in safe handling practices and spill decontamination procedures. Laboratories should be proactive and calculate the most efficient means of transporting specimens to NPHL, prior to the arrival of the first suspected PUI. Call NPHL Client Services at (866) 290-1406 to ask what the ground options are for your location. Client Services hours are 24/7 Monday-Friday, open Saturday/Sunday until 3pm. To make arrangements after 3pm on Saturday or Sunday, call the client services pager at 402-888-2086. Consider shipping by FedEx in an insulated Category B shipper using FedEx Priority shipping (NOT Overnight) for delivery by 10:30am to NPHL client services, if distance is problematic and ground shipping not sufficient.

Place each specimen in leak-proof specimen bag (i.e., secondary container) with adsorbent material. Seal.

DO NOT use a pneumatic-tube system to transport these specimens in-house.

Ideally, specimens should bed immediately refrigerate at 2-8°C upon collection. Keep refrigerated at all times, including transport.

If frozen before shipment, transport on dry ice and document “Frozen Specimens” in NUlirt batch list or on NPHL Test Request form.

Rigorous measures are being taken to bring up a COVID-19 test code in NPHL’s NUlirt Order Entry system, with complete documentation of patient demographics and symptomology. Facilities are encouraged to sign-up on http://nphl.org/philp.cfm Orders placed through NUlirt will be printed out as a batch list.

If using the NPHL Test Request Form http://nphl.org/forms.cfm, legibly complete all demographics including correct patient address. Check line with “COVID-19” test order found under the Molecular Virology section of the NPHL Test Request form found on the website listed above.

Write “PUI for COVID-19” and PUI number on the top of all forms and document name of NE DHHS staff who approve testing.

Place NPHL form in separate pocket on outside of specimen bag.

Specimens sealed in secondary biohazard bags with appropriate paperwork, can be given directly to a routinely scheduled NPHL ground courier. No special ambient Category B boxes are required unless shipping by FedEx. For FedEx shipping, a box marked UN3373 with Styrofoam and gel pack is required.

When NPHL couriers are not scheduled routinely, specimens should be arranged by calling Client Services support. Main Line: (402) 559-2440, Toll-Free: (866) 290-1406 or Client Service Pager: (402) 888-2086.

If NPHL ground courier is unable to transport to NPHL within a reasonable time, please call the NPHL emergency pager at (402) 888-5588 to arrange FedEx shipments.

Requests for results must go through the local health departments.
In-House Clinical Laboratory Testing

When indicated, clinical laboratories should continue to perform routine hematology, urinalysis, and clinical chemistry studies. Microbiology laboratories can perform diagnostic tests on blood, sputum, urine or stool specimens. Facilities must first perform a Risk Assessment to identify the tasks that create aerosols (below) and mitigate prior to testing in a clinical settings, also known as biosafety level-2 (BSL-2). One method of mitigation is to enhance biosafety precautions by implementing enhanced BSL-3 practices. Ideal BSL-3 practices include wearing respiratory protection (such as a fit-tested N-95 respirator or surgical mask if N-95 not available although this is not as protective), a face shield or goggles, and work in a Biological Safety Cabinet (BSC). To use the BSC, work slowly and methodically, from dirty to clean, and remove gloves immediately after every exit. See BSC just-in-time training at: https://www.youtube.com/watch?v=96-aZLom340

Not all enhancements may be possible, but all conceivable measures must be taken to protect the HCP. The following activities that involve manipulation of potentially infected respiratory specimens should be performed in a certified Class II BSC:

✔ Performing rapid diagnostic test kits such as those used for RSV, Strep A, and influenza kits (all respiratory specimens testing should be manipulated inside the BSC).
✔ Adding specimen aliquots to test analyzers e.g. multiplex PCR cartridges.
✔ Aliquoting, vortexing and/or diluting specimens.
✔ Inoculating bacterial or mycological culture media.
✔ Nucleic acid extraction procedures.
✔ Preparation and chemical- or heat-fixing of smears for microscopic analysis.
✔ Opening of sealed rotor centrifuge cups or centrifuged specimen containers in unsealed rotor cups.

BSC NOTE: Remove gloves upon every exit of the cabinet, use good glove-glove technic, move slowly not to aerosolize what has contaminated the gloves.

Facilities performing the following activities causing aerosolization but are unable to use a BSC must consider enhancing precaution when working on the bench. Upon performing a risk assessment consider using face shield or goggles and N95 (or surgical mask if N95 are not available or in short supply although this is not as protective), and performed behind a Plexiglass tabletop splash guard if possible:

✔ Performing any rapid diagnostic test kit such as those used for RSV, Strep A, or influenza kits in a laboratory, clinic settings or doctor’s office where a BSC is not available.
✔ Vortexing stools or other specimens without caps on an open bench top
✔ Loading and unloading of automated tests e.g. multiplex PCR panel
✔ Working with multi-plex instruments when kits or panels lodge, are stuck or broken and require additional manipulation
✔ Laboratorian is immunosuppressed or has a co-morbidity

Notes:
If a facility is unable to safely collect specimens, notify the LHD for directions to alternate collection locations.

Questions to NPHL should go through npnl@unmc.edu.

If a laboratory test confirms the presence of another respiratory pathogen such as the influenza virus, RSV, or Streptococcus pneumonia, but clinical suspicion remains high for either a co-infection or a secondary infection, then consideration for testing for the virus causing COVID-19 should be discussed with public health officials.

Laboratory waste can be handled as all other medical waste. Use two red liner bags, tie with an overhand balloon knot, place waste and sharps waste inside double bags. Contact medical waste courier for specific requirements.

BioFire Diagnostics has reported that the coronavirus targets on their panels do not cross-react with the COVID-19 disease virus. If the PCR assay is negative for all targets, call your local health department for advice on whether to forward the respiratory specimen to NPHL.

If your facility is unable to safely perform multiplex PCR analysis utilizing BSL-3 practices, the specimen can be submitted to Regional Pathology Services for PCR analysis. Please write possible COVID-19 PUI” on top and check “Respiratory Pathogen Panel” on the Regional Pathology Services (RPS) Clinical Test Request Form found at http://www.reglab.org/reglab/assets/File/Green%20Clinical%20Req%20Fillable.pdf
Other resources:
https://emergency.cdc.gov/han/2020/HAN00430.asp Updated March 17, 2020
http://dhhs.ne.gov/Pages/News-Releases.aspx
https://www.biofiredx.com