Sample Processing

The type of specimen(s) required:

1) nasopharyngeal, oropharyngeal swab, nasal swab

i. Use only synthetic fiber (Dacron or flocked) swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 3 ml of viral transport media. For initial testing, collecting nasopharyngeal swab specimens are preferred (refer to FDA).
ii. A nasopharyngeal, oropharyngeal swab collected by a healthcare professional
iii. A nasal mid-turbinate swab collected by a healthcare professional or by a supervised onsite self-collection (using a flocked tapered swab); or
iv. An anterior nares (nasal swab) specimen collected by a healthcare professional or by onsite or home self-collection (using a flocked or spun polyester swab);
v. Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) specimen collected by a healthcare professional.
vi. Transfer in 4 ºC condition and deep freeze at -70 ºC if it takes longer than 48 hours

Table 1. Specimen requirements

Specimen Type	Nasopharyngeal Swab, Nasal swab, or Oropharyngeal swab collected according to standard technique and immediately placed in 1-3 mL of transport media.
Minimum Sample Volume	0.3 mL (300 µL)
Transport and Storage	Samples should be processed and tested with the GG COVID-19 Quadplex Real-Time RT-PCR as soon as possible.
	If storage is required, samples can be held:
	•At room temperature for up to 4 hours (15-25ºC)
	•Refrigerated for up to 2 days (2-8ºC)
	•Frozen (≤-15ºC or ≤-70ºC) for up to 30 days

Note)

Refer to: Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical- specimens.html

Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

2) Lower respiratory tract specimens

(1)Bronchoalveolar lavage, tracheal aspirate
-2 to 3 mL should be collected in a sterile, leak-proof, screw-cap sputum collection cup or sterile, dry container.
-If clinically indicated (eg, if the patient is undergoing invasive mechanical ventilation), collection and testing of a lower respiratory tract aspirate or bronchoalveolar lavage sample should be performed.

(2)Sputum
-The patient should rinse his or her mouth with water and then expectorate deep cough sputum directly into a 50-mL sterile, leak-proof, screw-cap sputum collection cup or sterile, dry container containing 3 ml of sampling solution. If the sputum is not collected in the sampling solution, 2-3 ml of the sampling solution can be added into the tube before testing, or add sputum digestive reagents of equal volume of sputum.
-Sputum can also be treated with a phosphate buffer containing 1 g/L of protease K in an equal volume of sputum.
-Only patients with a productive cough should undergo sputum collection.

Sputum recommended processing protocol*:

- proteinase K and DNase I treatment (PK-DNase): NucliSENS easyMAG protocol (bioMérieux SA, Marcy-l'Etoile, France).

- 100 uL of proteinase K (1 mg proteinase K/1 mL proteinase K buffer; Promega, Madison, WI, USA) added to 100 µL of sample and incubated for 15 min at 55℃.

- mix samples by vortexing every 5 min.

- 20-µL of DNase I solution (1 U/µL) is added for every 100 µL of sample, followed by incubation at 37℃ for 30 min

2) treatment with a phosphate buffer containing 1 g/L of protease K in an equal volume of sputum and vortexed for 1 min. The cell lysates are centrifuged and clarified supernatants transferred to sterile microtubes.