

## **Supplementary Section:**

### **Section 1: Alternate Swabs**

#### **Nasopharyngeal swab**

The recommended specimen collection technique is a nasopharyngeal swab taken by a healthcare provider. This technique requires a flocked swab to be inserted into one nostril parallel to the palate until it reaches the nasopharynx. After waiting three to five seconds for the swab to absorb secretions, the swab is gently rotated, removed and immediately placed into a sterile tube containing viral transport media. This collection technique is commonly performed on adults.

#### **Oropharyngeal (OP) or throat swab**

If a nasopharyngeal swab cannot be conducted, the recommended next step is for an oropharyngeal swab (OP), also known as a throat swab, to be collected by a healthcare provider. This technique requires a flocked swab to contact and rubbed against the posterior pharynx and tonsillar pillars, while avoiding the tongue and teeth. After the swab has absorbed secretions, it is removed and immediately placed into a sterile tube containing viral transport media

#### **Nasal mid-turbinate (NMT) swab**

Nasal mid-turbinate swabs are another option for upper respiratory specimen collection for COVID-19. The CDC has recently updated their guidelines on specimen collection to include that if NP and OP samples cannot be collected, NMT specimens may be collected onsite by either the patient, via a self-swab, or a healthcare worker. NMT specimens are collected by inserting a flocked swab into the nostril parallel to the palate until the mid-turbinate is reached (swabs meant for NMT samples will have a collar or stopping point). After waiting three to five seconds for the swab to absorb secretions, the swab gently rotated, removed and immediately placed into a sterile tube containing viral transport media. The NMT swab technique traverses less distance than the NP swab technique, resulting in less discomfort and the possibility of a self-swab collection method.

#### **Anterior Nares or Nasal Swabs**

Anterior-Nares (AN)swabs, or nasal swabs, have also been approved by the CDC as an acceptable specimen type to be taken onsite via a patient self-swab or by a healthcare worker if NP and OP swabs are unavailable. AN specimens are collected by inserting a round foam swab into one of the nares, or nostrils, and making contact with the wall of the nostril. Once against the wall of the nostril, the swab is run along the wall of the nostril until the swab has absorbed nostril mucosa. The swab is removed and inserted into the second nostril, repeating the same procedure. After both nostril specimens have been collected on the same swab, the swab is

immediately placed into a sterile tube containing viral transport media. This method of collection is the least discomforting out of the previous listed upper respiratory specimen collection techniques, allowing for patient self-swab.

### **Nasopharyngeal wash/aspirate**

A nasopharyngeal wash is conducted with a saline filled syringe attached to tubing. The tubing is inserted into one nostril and guided into the nasopharynx. The saline is instilled into the nasopharynx, and then it is aspirated gently back into the syringe while the tube is rotated and gently removed. The solution in the syringe is now the specimen and is immediately injected into a sterile sputum cup or leak-proof container. If possible, the procedure should be repeated on the second nostril.

A nasopharyngeal aspirate specimen requires a catheter and suction device. The catheter is inserted into a nostril and guided to the nasopharynx. Suction is applied, and the specimen is collected directly into a sterile container. Nasopharyngeal aspirates are usually used for younger children and infants.

### **Sputum**

Sputum is only collected onsite if the patient has a productive cough. To collect a sputum sample, the patient first rinses their mouth and is then asked to cough deeply and expectorate the produced sputum directly into a sterile container.

### **Tracheal aspirate**

A tracheal aspirate specimen is only used as a specimen when it is clinically indicated (e.g., those receiving invasive mechanical ventilation). A tracheal aspirate specimen is collected by inserting a catheter into the trachea and saline solution is instilled. The solution is immediately aspirated, and the catheter is removed. The solution aspirated is now the specimen and is injected into a sterile container.

### **Bronchoalveolar lavage (BAL) fluid**

Bronchoalveolar lavage uses a bronchoscope to inject saline solution into a small section of a patient's lung. The solution aspirated is now the specimen and is aspirated out of the lung and placed into a sterile container.

## **Section 2: Details on Extraction Methods**

### **Magnetic bead purification**

The magnetic bead isolation method uses superparamagnetic beads that are pre-functionalized to bind to viral RNA. The specimen is lysed and then mixed with the magnetic beads. The magnetic beads are then held in place by an external magnet, and a series of wash steps occur. Following the wash steps a solution is introduced that releases the viral RNA from the bead, and the now isolated viral RNA solution is collected for analysis. Magnetic beads offer highly efficient target capture and concentration, but there is a risk of magnetic bead contamination in the isolated viral RNA solution.

### **Spin Basket or Spin Column**

The spin basket or spin column method uses membranes, made of materials like silica or charged polymers, in columns or baskets and centrifugal force or vacuum to isolate viral RNA. The specimen is lysed and introduced to the top of the column or basket. Centrifugal force or a vacuum is applied to pull the lysis through the membrane. As the lysis is pulled to the bottom of the column, the viral RNA remains attached to the membrane. Several wash steps occur, until only viral RNA is attached to the membrane. A solution is introduced that releases the RNA from the membrane, and the now isolated viral RNA solution is eluted off the column or basket and collected for analysis. The spin column or spin basket method of viral RNA isolation is easy to implement; however, it does require a centrifuge or vacuum. In addition, the membranes can be clogged depending on the specimen used.

### **Organic Extraction**

The organic extraction method uses a phenol or chloroform solution and centrifugal force to isolate viral RNA. The specimen is lysed, and then the lysis is introduced into the phenol or chloroform solution and centrifuged. The solution separates into three distinct layers, with the top layer containing the viral RNA. This layer is collected, and then the viral RNA is isolated through alcohol precipitation and rehydration. The organic extraction method is the most robust method for isolating viral RNA; however, it is more manually intensive than the other methods.

<b>Company</b>	<b>Extraction Platform</b>	<b>Kit/card</b>	<b>Isolation Method</b>	<b>Automated Only</b>
Roche	MagNA Pure LC	MagNA Pure Total Nucleic Acid Kit	Magnetic beads	Yes
Roche	MagNA Pure Compact	MagNA Pure Nucleic Acid Isolation Kit	Magnetic beads	Yes
Roche	MagNA Pure 96	MagNA Pure 96 DNA and Viral NA Small Volume Kit	Magnetic beads	Yes
QIAGEN	QIAcube	QIAamp® DSP Viral RNA Mini Kit or QIAamp® Viral RNA Mini Kit	Spin Column	No
QIAGEN	EZ1 Advanced XL	EZ1 DSP Virus Kit and Buffer AVL (supplied separately) for offboard analysis. Card: EZ1 Advanced XL DSP Virus Card	Magnetic beads	Yes
QIAGEN	EZ1 Advanced XL	EZ1 DSP Virus Kit and Buffer AVL (supplied separately) for offboard analysis. Card: EZ1 Advanced XL DSP Virus Card	Magnetic beads	Yes
Roche	MagNA Pure LC	MagNA Pure Total Nucleic Acid Kit	Magnetic beads	Yes
Roche	MagNA Pure Compact	MagNA Pure Nucleic Acid Isolation Kit	Magnetic beads	Yes

**Table S1: Summary of Extraction Platforms**