COVID-19 specimen collection guidelines

Quest specimen requirements and acceptable supplies for SARS-CoV-2 RNA (COVID-19), Qualitative NAAT (test code 39448)

Quest does not manufacture the collection supplies used in testing. Due to extraordinary demand, we are temporarily unable to accept online orders for upper respiratory specimen collection and transport supplies. Please call your local order entry team for more information. You do not have to use supplies from Quest to send us samples for testing. Please refer to the information below and to the Quest Test Directory at TestDirectory.QuestDiagnostics.com for a list of acceptable specimen collection and transport supplies for COVID-19 testing.

The tests performed under this test code are being offered under an Emergency Use Authorization (EUA) by the FDA. The EUA stipulates the tests may be used only by Quest laboratories and only for the detection of nucleic acid from SARS CoV-2, not for any other viruses or pathogens. The authorization is valid only for the duration of the declaration that circumstances exist justifying the EUA for in vitro diagnostic tests for the detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act 21, U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

This guide is intended to describe the collection swabs and media to be used for upper respiratory specimens for SARS-CoV-2 (COVID-19) molecular (NAAT) testing.

For additional examples of acceptable swabs, visit the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2#whatif

Swab sample collections
- It is important that the swab be appropriate for the anatomic site on which it is used, and that the swab type is compatible with that platform.
- Flocked swabs are preferred.
- Use only sterile Dacron®, polyester, nylon, or rayon swabs with plastic shafts. Wired shaft swabs are acceptable but must be trimmed using sterile scissors.
- 3D-printed swabs are not acceptable.
- Note the stem/shaft must be flexible and long enough to collect the NP sample.
- If the applicator handle requires additional trimming, it is also important to perform the trimming with a sterile pair of scissors to prevent contamination of the sample.
- Calcium alginate swabs or swabs with wooden shafts are unacceptable as they may contain substances that inactivate some viruses and inhibit PCR testing (see https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).

The following specimen types are acceptable:

Collected by a healthcare professional (HCP):
- Nasopharyngeal (NP)
- Oropharyngeal (OP)

HCP-observed self-collection or HCP collected:
- Mid-turbinate (MT)
- Anterior nares (AN)

Note: The IDSA panel suggests collecting nasopharyngeal, or mid-turbinate, or nasal swabs rather than oropharyngeal swabs or saliva alone for SARS-CoV-2 RNA testing (https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/)

The current body of research shows mid-turbinate and anterior nares samples show greater sensitivity than oropharyngeal samples.1,2 Multiple specimens from the same patient may be taken with a single swab. If a separate swab is used for collecting specimens from two different locations in the same patient, both swabs may be placed in the same vial in order to conserve collection and assay supplies.

Other swab specimens, such as tongue or saliva, have decreased sensitivity and are unacceptable.3 More data are necessary to better understand the validity of buccal swabs, saliva, specimens, or other specimen types for COVID-19 testing.
Information on samples from anterior nares:

Anterior nares specimen collection instructions: Use a single swab for collecting specimens from both nares. Insert swab into 1 nostril straight back (not upwards). Once the swab is in place, rotate it in a circular motion 2 times and keep it in place for 15 seconds. Repeat this step for the second nostril using the same swab. Remove swab and insert the swab into an acceptable viral transport medium (including saline or PBS).

Acceptable swabs: Puritan® 6” Sterile Standard Foam Swab w/ Polystyrene Handle (SKU # 25-1506 1PF) and Copan® Foam swab single wrapped (1C055S01). Note: Other acceptable swabs include standard OP synthetic swabs w/ plastic/scored shaft.

Information on mid-turbinate specimen:

Mid-turbinate specimen: Collected by a healthcare professional or a patient self-collected sample is acceptable when the patient is in an appropriate clinical setting (such as a drive-thru testing site) and the collection is observed by the HCP.

Collection instructions: Use a collar/stopping point swab for collecting specimens from both nares of a symptomatic patient. Tilt patient head and insert swab into 1 nostril until the swab collar touches the outside of the nose. Once the swab is in place, rotate it in a circular motion 2 times and keep it in place for 15 seconds. Repeat this step for the second nostril using the same swab.

Remove the swab and insert the swab into an acceptable viral transport listed in this guide (including saline and PBS). Break the swab shaft against the side of the tube and close the lid.

Acceptable swabs: Contoured Adult Flocked Swab w/Stopper with 80 mm Breakpoint Copan® FLOQSwab (56380CS01) and MDL® NasoSwab™ A362CS02

Specimen stability is as follows:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature: 5 days</td>
<td>Frozen (-20 °C): 7 days</td>
</tr>
<tr>
<td>Refrigerated (2 °C–8 °C): 5 days</td>
<td>Frozen (-70 °C): Acceptable</td>
</tr>
</tbody>
</table>

Specimens should be transported to your local Quest Diagnostics laboratory according to standard operating procedures. Cold packs/pouches should be used if placing specimens in a lockbox for courier pick-up. STAT pick-up cannot be ordered for these tests.

NOTE: FOR TRANSPORT MEDIA, STRICTLY FOLLOW THIS GUIDE.

All swabs must be submitted in liquid transport media, as outlined below.

- Dry swab submissions are unacceptable and will be rejected
- Any swab submitted in media containing guanidinium isothiocyanate, guanidinium thiocyanate, guanidine isothiocyanate, guanidine thiocyanate, or like component is unacceptable and will be rejected
- Any tubes that lack labelling that includes media contents, lot, and expiration date may contain guanidine thiocyanate or a similar chemical; such vials are unacceptable and will be rejected

Viral transport media (VTM)

- 1 mL or 3 mL commercially available vials are acceptable (1 mL of volume may result in a Quantity Not Sufficient (QNS) if repeat testing of a specimen is needed. Therefore, 1 mL vials are not preferred and should only be used when other vials are not available).
- Only 3 mL vials of acceptable VTM may be split into 2 vials (1.5 mL each) using the vial filling process below.
- Clients interested in creating their own VTM should refer to the CDC website (https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf) and should follow the vial filling process outlined below:

Vial filling process:

- Note: Add the VTM, PBS, or saline in the sterile container before collecting the patient sample.
- Label an empty sterile container (eg, 10 mL falcon tube or equivalent, conical or round bottom) by placing the label close to the top end of the tube, ensuring enough space for the lab label. The label should include:
  - Lot #, expiration date, and manufacturer (for commercially available VTM, PBS, or saline)
  - The date of manufacture, and expiration date, if known (for client-created VTM)
Vial filling process (continued):

- Use a sterile environment (e.g., a laminar flow hood or biological safety hood) and add the appropriate volume of liquid to the sterile vial.
- Use 1.5 mL when splitting a 3 mL VTM tube (the mixing beads at the bottom of viral medium do not need to be transferred) or 2 mL to 3 mL of saline.
- Secure the lid of the tube until fully closed to prevent leakage. Liquid Amies - Specimens can be stored in liquid Amies media for up to 72 hours at 4 °C.

Saline or Phosphate Buffered Saline (PBS)
The FDA has indicated saline and PBS as acceptable transport media in situations where commercial viral transport media are unavailable for molecular RT-PCR SARS-CoV-2 assays (such as those in use for the Quest tests). Note, the FDA believes that for saline, a sterile plastic vial containing between 1 mL and 3 mL of phosphate buffered saline (PBS) 1X pH 7.4 (range of pH 7.2-7.4) or saline (0.85% to 0.90%) is appropriate (1 mL of volume may result in a Quantity Not Sufficient (QNS) if repeat testing of a specimen is needed. Therefore, 1 mL vials are not preferred and should only be used when other vials are not available). Collection should be conducted with a sterile swab.

- Quest Diagnostics will accept commercially available prefilled saline or PBS vials. In addition, for clients able to fill vials (using sterile laminar flow hood or a biological safety hood), Quest will accept 2 mL to 3 mL sterile PBS or sterile saline (0.85% or 0.9%) provided the vial filling process below is followed.

Acceptable SARS-CoV-2 (COVID-19) molecular (NAAT) specimen transport media/swabs/kits for test code 39448

Quest Phosphate Buffered Saline (PBS)
NP and AN Swab
Convenience kits for collecting and transporting upper respiratory specimens

NP kit ordering information:
PeopleSoft item #: 207128
PeopleSoft product ID: K162
Kit, PBS, Quest, 100/CA
Ordered by the EA (each)

AN kit ordering information:
PeopleSoft item #: 207621
PeopleSoft product ID: K165 KIT, ANTERIOR, NARES, PBS, 4PK
Ordered by the EA (each) in multiples of 4 only. For example, ordering 8 EA results in 2 kits of 4.
Acceptable SARS-CoV-2 (COVID-19) molecular (NAAT) specimen transport media/swabs/kits for test code 39448

VCM (Diagnostic Hybrids)
NP kit ordering information:
- PeopleSoft item #: 142059
- PeopleSoft product ID #: S05
- Quanum product ID #: S05
- Ordered by the EA (each)

Lesion swab kit ordering information (acceptable for OP):
- PeopleSoft item #: 142060
- PeopleSoft product ID #: S03
- Quanum product ID #: S03
- Ordered by the EA (each)

Note: both swabs in supply kit S03 are the same; only one is necessary for specimen collection.

Not supplied by Quest but acceptable media kits for SARS-CoV-2 (COVID-19) molecular (NAAT) testing

Amies liquid elution swab (ESwab)
ESwab with 1 mL of Amies fluid in the transport vial
(1 mL of volume may result in a Quantity Not Sufficient [QNS] if repeat testing of a specimen is needed. Therefore, 1 mL vials are not preferred and should only be used when other vials are not available.)

Routine swab (white cap)–OP/AN swab
Minitip swab (blue cap)–NP swab
Not supplied by Quest but acceptable media kits for SARS-CoV-2 (COVID-19) molecular (NAAT) testing

**UTM (Copan)**
- Combo collection kit NP, OP/AN 321C
- NP Collection Kit-305C
- OP/AN Collection Kit-306C
- UTM medium 3 mL-330C or 3U044n
- NP flocked swab-503CS501
- OP/AN flocked swab-519CS501

**UVT (BD)**
- Combo collection kit nasopharyngeal and oropharyngeal 3 mL-220527
- NP Collection Kit-220529
- OP/AN Collection Kit-220528
- UTM medium 3 mL-220220 (1 mL*-220244)
- NP flocked swab-220252
- OP/AN flocked swab-220250

**Cepheid® Xpert® Sample Collection Kit for Viruses**
- B-100 NP (new M-100 kit is equivalent)
- F-100 OP/AN

**Hardy Diagnostics Healthlink UTM**
- Hardy: 3C036NHL 3 mL: NP
- Hardy: 3C037NHL 3 mL: NP
- Hardy: 3C040NHL 1 mL*: NP
- Hardy: 3C038NHL 3 mL: OP/AN
- Hardy: 3C011NHL 1 mL*: OP/AN
- Hardy: 330CHL: 3 mL UTM
- Hardy: 3C039NHL 3 mL (NP and OP/AN)
- Hardy: 302CHL 3 mL (2 OP/AN with plastic applicator)

**M4 (Fisher/Remel)**
- Contains vancomycin, amphotericin B, and colistin and is suitable for transport of viruses, Chlamydiae, Mycoplasma and Ureaplasma)

**M4RT (Fisher/Remel)**
- Contains gentamicin and amphotericin B and is only suitable for transport of viruses and Chlamydiae

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*1 mL of volume may result in a Quantity Not Sufficient (QNS) if repeat testing of a specimen is needed. Therefore, 1 mL vials are not preferred and should only be used when other vials are not available.*
Not supplied by Quest but acceptable media kits for SARS-CoV-2 (COVID-19) molecular (NAAT) testing

M5 (Fisher/Remel)\(^{10}\)
Similar to the M4, but it does not contain gelatin

M6 (Fisher/Remel)\(^{10}\)
Contains gelatin, vancomycin, amphotericin B, and colistin for the transport of viruses, Chlamydiae, Ureaplasmas, and Mycoplasmas

Puritan\(^{®}\) UniTranz-RT Universal Transport Medium (UTM) with no swabs\(^{11}\)
1 mL* (UT 100); 3 mL (UT-300)

Puritan\(^{®}\) UniTranz-RT UTM with flocked swabs for NP (1 mL)* \(^{11}\)
Minitip (UT-116)
Ultrafine flocked swabs (UT-117)
Elongated swab (UT-367)

Puritan\(^{®}\) UniTranz-RT UTM with flocked swabs for NP (3 mL)\(^{11}\)
Ultrafine flocked swabs (UT-317)
Mini-tip Flock Swab (UT-316)

Puritan\(^{®}\) UniTranz-RT UTM with flocked swabs for OP/AN\(^{11}\)
Large flocked swab 1 mL* (UT-106)
Elongated flocked swab 3 mL (UT-306)

Puritan\(^{®}\) UniTranz-RT UTM with Polyester swabs for OP/AN (3 mL)\(^{11}\)
One swab (UT-361)
Two swabs (UT-362)
NP and OP/AN both 1 minitip and 1 standard (UT-366 and UT-302)

Starplex\(^{™}\) Scientific Multitrans\(^{™}\) with flocked swabs from Fisher Scientific\(^{12}\)
NP flocked: 23-038-096
NP and OP/AN flocked swab: 22-046-450

*1 mL of volume may result in a Quantity Not Sufficient (QNS) if repeat testing of a specimen is needed. Therefore, 1 mL vials are not preferred and should only be used when other vials are not available.
Not supplied by Quest but acceptable media kits for SARS-CoV-2 (COVID-19) molecular (NAAT) testing

<table>
<thead>
<tr>
<th>Media Kit</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Copan ESwab</strong></td>
<td>Flocked swab with 1 mL* of liquid Amies in a plastic, screw-cap tube</td>
</tr>
<tr>
<td>White cap (OP/AN flocked swab): Cat#480C or 4C012S.A</td>
<td></td>
</tr>
<tr>
<td>Green cap (minitip flocked swab for NP): Cat#481C</td>
<td></td>
</tr>
<tr>
<td>Blue cap (wire shaft/flexible minitip for NP): Cat#482C</td>
<td></td>
</tr>
<tr>
<td><strong>BD ESwab</strong></td>
<td>1 mL* of modified liquid Amies medium packaged with a nylon flocked swab</td>
</tr>
<tr>
<td>The system is available in 3 flocked swab formats:</td>
<td></td>
</tr>
<tr>
<td>- regular (white)</td>
<td>- minitip (green)</td>
</tr>
<tr>
<td>- flexible minitip (blue)</td>
<td></td>
</tr>
<tr>
<td>White (reg flocked swab) OP/AN: Cat#220245</td>
<td></td>
</tr>
<tr>
<td>Green (minitip flocked swab) NP: Cat#220246</td>
<td></td>
</tr>
<tr>
<td>Blue (flexible minitip) NP: Cat#220532</td>
<td></td>
</tr>
<tr>
<td><strong>Puritan® Opti-Swab</strong></td>
<td>1 mL* Liquid Amies Transport Medium w/ 6” elongated flock swab</td>
</tr>
<tr>
<td>Item # LA-106 for OP/AN swab</td>
<td></td>
</tr>
<tr>
<td><strong>Puritan® Opti-Swab</strong></td>
<td>1 mL* Liquid Amies Transport Medium w/ 6” minitip flock swab</td>
</tr>
<tr>
<td>Item # LA-116 for NP swab</td>
<td></td>
</tr>
<tr>
<td><strong>Puritan® Opti-Swab</strong></td>
<td>1 mL* Liquid Amies Transport Medium w/ 6” ultrafine flock swab</td>
</tr>
<tr>
<td>Item # LA-117 for NP swab</td>
<td></td>
</tr>
<tr>
<td><strong>NEST VTM</strong></td>
<td></td>
</tr>
<tr>
<td><strong>BD/BBL 0.85% Normal Saline</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Hardy Diagnostics R99 VTM</strong></td>
<td></td>
</tr>
</tbody>
</table>

*1 mL of volume may result in a Quantity Not Sufficient (QNS) if repeat testing of a specimen is needed. Therefore, 1 mL vials are not preferred and should only be used when other vials are not available.
Ref: Equivalency—Copan Universal Transport Medium (UTM)
The products described in the accompanying table are equivalent products. They are manufactured in identical fashion with all raw materials being utilized in all products being equivalent and at the same ratios.

Specifically, Copan Universal Transport Medium (UTM), BD UVT, Cepheid XPert Sample Collection Kit for Viruses, Hardy-HealthLink UTM and Quest VCM transports are equivalent products.

<table>
<thead>
<tr>
<th>Copan</th>
<th>Type</th>
<th>Description</th>
<th>Quest</th>
<th>Cepheid</th>
<th>Hardy/Healthlink</th>
<th>BD</th>
<th>DHI/Quidel</th>
<th>Fisher Healthcare</th>
</tr>
</thead>
<tbody>
<tr>
<td>305C</td>
<td>Sample collection kit (nasopharyngeal)</td>
<td>Nasopharyngeal collection kit: flexible minitip flocked swab + 3 mL UTM® tube</td>
<td>S05</td>
<td>SWAB/B-100</td>
<td>3C036NHL</td>
<td>220526/220531</td>
<td>403C</td>
<td>230001720</td>
</tr>
<tr>
<td>306C</td>
<td>Sample collection kit (OP/AN)</td>
<td>Oropharyngeal collection kit: regular flocked swab + 3 mL UTM® tube</td>
<td>S03</td>
<td>SWAB/F-100</td>
<td>3C038NHL</td>
<td>220528</td>
<td>402C</td>
<td>23001722</td>
</tr>
<tr>
<td>330C</td>
<td>Collection kit component</td>
<td>3 mL UTM® medium in 16x100 mm tube</td>
<td>NA</td>
<td>NA</td>
<td>330CHL</td>
<td>UVT</td>
<td>220244/220220</td>
<td>330C,DHI</td>
</tr>
<tr>
<td>503CS01</td>
<td>Collection kit component</td>
<td>Flexible minitip (nasopharyngeal) flocked swab</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>220252</td>
<td>503CS01,DHI</td>
<td>23600952</td>
</tr>
<tr>
<td>519CS01</td>
<td>Collection kit component</td>
<td>Regular (oropharyngeal) flocked swab</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>220250</td>
<td>NA</td>
<td>23600957</td>
</tr>
<tr>
<td>321C</td>
<td>Combo collection kit (NP and OP/AN)</td>
<td>Flexible minitip flocked swab + regular flocked swab + 3 mL UTM® tube</td>
<td>NA</td>
<td>NA</td>
<td>3C038NHL</td>
<td>220527</td>
<td>99-08021</td>
<td>NA</td>
</tr>
</tbody>
</table>

UNACCEPTABLE specimens for test code 39448

Calcium alginate swabs, wooden shafted swabs, charcoal medium, anaerobic swab/transport medium

Swab in Amies liquid transport
Amies in swabs is generally in a sponge so there is no fluid to test for COVID-19

Single or double swab (red cap)
Twisted wire shaft swab (green cap)

Swab in Amies gel transport
Gel is not acceptable for PCR
Single or double swab (blue cap)
Twisted wire shaft swab (yellow cap)

BD EZ Swab (single swab)21
Product code 220093
Available with liquid Stuart or liquid Amies media in more than 15 swab and shaft configurations, BD CultureSwab products are designed to meet a wide range of transport needs17

BD EZ Swab (double swab)22
Product code 220105
BD BBL™ CultureSwab™ EZ II collection and transport systems are simple-to-use, media-free systems that contain a patented polyurethane foam swab
### UNACCEPTABLE specimens for test code 39448, continued

Calcium alginate swabs, wooden shafted swabs, charcoal medium, anaerobic swab/transport medium

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>220235</td>
<td>Single swab, gel/no charcoal</td>
</tr>
<tr>
<td>220236</td>
<td>Double swab, Amies gel/no charcoal</td>
</tr>
<tr>
<td>220122</td>
<td>Double swab, Amies gel w/ charcoal</td>
</tr>
</tbody>
</table>

- The BD CultureSwab MaxV system is available with liquid Stuart or liquid Amies media, in single- or double-swab formats, for the transport of aerobes. Additionally, the BD CultureSwab MaxV (+) system is available in Amies gel medium without charcoal, in single- and double-swab formats, for the transport of aerobic and facultative anaerobic organisms.

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>220133</td>
<td>BD ProbeTec™ transport vials</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BBL BD CultureSwab™—Liquid Stuart (minitip swab-green cap)</td>
</tr>
<tr>
<td>220133</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quidel urethral swab</td>
</tr>
<tr>
<td></td>
<td>PeopleSoft #: 142058</td>
</tr>
<tr>
<td></td>
<td>PeopleSoft product ID #: S09</td>
</tr>
<tr>
<td></td>
<td>Quanum product ID #: S09</td>
</tr>
<tr>
<td></td>
<td>Mfg Part #: #99-08014-VCM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beaver</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PrimeStore-MTM™</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inveox transport media</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNA/RNA Shield™</td>
</tr>
</tbody>
</table>

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## UNACCEPTABLE specimens for test code 39448, continued

**Calcium alginate swabs, wooden shafted swabs, charcoal medium, anaerobic swab/transport medium**

<table>
<thead>
<tr>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruhof kits&lt;sup&gt;29&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clear and pink/red media</td>
</tr>
<tr>
<td>Abbott Multi-Collect&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>Babio VTM</td>
</tr>
<tr>
<td>MANTACC UTM&lt;sup&gt;31&lt;/sup&gt;</td>
</tr>
<tr>
<td>BIOER Technology RNAsafeguard Reagent/SARS-CoV-2 collection kit</td>
</tr>
<tr>
<td>NEST- ITM (inactivating transport medium)&lt;sup&gt;18&lt;/sup&gt;</td>
</tr>
<tr>
<td>ImproViral™ NAT VTM&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fillakit1X PBS&lt;sup&gt;33&lt;/sup&gt;</td>
</tr>
<tr>
<td>Huachenyang iClean VTM kit&lt;sup&gt;34&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
References

2. Zou L, Ruan F, and Huang M et al SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. DOI: 10.1056/NEJMmc2001737