1. Procedure

Abbreviated Procedure

**Cepheid Xpert® Xpress SARS-CoV-2 Assay**

Sample preparation is performed by trained staff member. LIS orderable code is **NCOPCL**

Prepare samples inside the Biological Safety Cabinet (Class I or II)

**Nasopharyngeal aspirate specimens received at laboratory:**

1. Label tube of UTM with 2 specimen labels
2. Open UTM and use a clean sterile transfer pipette (supplied with Xpert kit) to transfer 300 µL of nasopharyngeal aspirate specimen into UTM. Use same pipette to repeat transfer of another 300 µL of aspirate into UTM (for a total 600 µL).
3. Cap tube of UTM.
4. Store specimen container with remaining volume of nasopharyngeal aspirate in current day’s specimen tray.

**Samples received at the laboratory already in UTM are ready for testing by Xpert Xpress SARS-CoV-2 Assay without further preparation.**

**At Biological Safety Cabinet:**

Print specimen labels and KEEP for results sheet print outs

1. Ensure appropriate PPE is worn including a new set of gloves.
2. Remove a new Xpert SARS-CoV-2 cartridge from the kit. Do not handle reaction tube on back of cartridge.
3. Remove one Auslab specimen label and apply to side of cartridge
4. Ensure UTM is secure and not cross-threaded and mix specimen by inverting UTM tube 5 times
5. Open cartridge lid
6. Use a clean transfer pipette (supplied in test kit) to transfer 300 µL (one draw) of specimen from the transport medium tube to the sample chamber with large opening in the cartridge.
7. Transfer the sample to the sample chamber of the cartridge by squeezing the pipette bulb until the pipette is completely empty.

8. Close the cartridge lid

**At GeneXpert System:**

1. Turn on GeneXpert instrument, then turn on system computer
2. Log-in to system software (password = cphd)
3. In the GeneXpert System Window click ‘Create test’ icon
4. Enter the Sample ID (lab number) by scanning lab number barcode from Auslab specimen label. Type surname in after the lab number is scanned.
5. Scan barcode from Xpert SARS-CoV-2 cartridge (system should automatically select Xpert® SARS-CoV-2 – this will always report results for all 3 target viruses)
6. Click ‘Start test’
7. Place cartridge in available module with green flashing indicator light
8. Open instrument module door of selected module (with blinking green light)
9. Load cartridge into module
10. Close module door and hold gently closed until door locks
11. The test starts and the green light stops blinking. The system software will display ‘Loading’, followed by ‘Sample Processing’ for the selected module.
12. When the test is finished, the green light turns off.
13. The module door can be opened, and the cartridge removed. Dispose of used cartridges Using EXTREME CAUTION in clinical waste. Under NO circumstances should the lid be opened.
14. A printed copy of results will generally be available automatically on the system printer.

Result Reporting:
1. Results should be entered manually into the Laboratory Information System where the GeneXpert is not interfaced and will be electronically transmitted where the instrument is interfaced.
2. Local procedures for the manual transcription of results should be followed where required.
3. Validate LIS report
4. **The laboratory performing GeneXpert testing should notify the requesting doctor of ALL results as per local policy regarding critical results**
5. **The referral lab performing supplementary testing will notify results to Public Health and Ministry of Health at the relevant hospital.**
6. Send all samples on to referral laboratory via normal courier method for further testing and or storage.
7. Scan the copy of the printed test report into LIS once the data entry has been checked and signed off by a second staff member.
Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

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. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Xpert® Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using the GeneXpert Dx.

### Test Frequency
As per local lab hours

### Expected Turnaround Time
Approximately 2 hours from time of sample receipt at the testing laboratory.

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<table>
<thead>
<tr>
<th>Xpert® SARS-CoV-2 Possible results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N gene positive</strong></td>
<td><strong>E gene positive</strong></td>
</tr>
<tr>
<td><strong>N gene positive</strong></td>
<td><strong>E gene negative</strong></td>
</tr>
<tr>
<td>SARS-CoV-2 RNA DETECTED</td>
<td>SARS-CoV-2 RNA DETECTED</td>
</tr>
<tr>
<td>SARS-CoV-2 RNA DETECTED</td>
<td>SARS-CoV-2 RNA DETECTED (Presumptive)</td>
</tr>
<tr>
<td>- Add comment SC2XD</td>
<td>- Add comment SC2XD</td>
</tr>
<tr>
<td>- Notify requesting clinician</td>
<td>- Notify requesting clinician</td>
</tr>
<tr>
<td>- Notify PHU via phone call and/or email as per routine process</td>
<td>- Notify PHU via phone call and/or email as per routine process - Refer specimen to ICPMR</td>
</tr>
<tr>
<td>- Add comment SC2XDP (see below)</td>
<td>- Add comment SC2XDP (see below)</td>
</tr>
<tr>
<td>- Notify requesting clinician</td>
<td>- Notify requesting clinician</td>
</tr>
<tr>
<td>- Notify PHU via phone call and/or email as per routine process</td>
<td>- Notify PHU via phone call and/or email as per routine process - Refer specimen to ICPMR</td>
</tr>
<tr>
<td>- Add comment SC2XN</td>
<td>- Add comment SC2XN</td>
</tr>
<tr>
<td>- Notify requesting clinician</td>
<td>- Notify requesting clinician</td>
</tr>
<tr>
<td>- No further referral unless other tests requested</td>
<td>- No further referral unless other tests requested</td>
</tr>
<tr>
<td>Action 1</td>
<td>Action 2</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Refer specimen to ICPMR for sequencing (may be batched referred)</td>
<td>for sequencing (may be batched referred)</td>
</tr>
<tr>
<td>Retain specimen frozen for 4 weeks</td>
<td></td>
</tr>
</tbody>
</table>

**Comments**

**DETECTED**


NATA/RCPA accreditation does not cover the performance of this service. For further information, please contact the laboratory on ……

**NOT DETECTED**


NATA/RCPA accreditation does not cover the performance of this service. For further information, please contact the laboratory on ……

**NOTE:** A DETECTED presumptive result is not reported in the test field. The following comment is added.

**DETECTED (presumptive)** -

SARS-CoV-2 (COVID) RNA - DETECTED (presumptive) Specimen referred for supplementary testing. Result to follow.

NATA/RCPA accreditation does not cover the performance of this service. For further information, please contact the laboratory on ……
## Xpert® SARS-CoV-2 Results & Interpretation

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
</table>
| **SARS-CoV-2 POSITIVE**    | The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected.  
• The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting  
• SPC: NA; SPC is ignored because coronavirus target amplification occurred  
• Probe Check: PASS; all probe check results pass |
| **SARS-CoV-2 PRESumptive POS** | The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.  
• The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting  
• SPC: NA; SPC is ignored because a target amplification has occurred.  
• Probe Check: PASS; all probe check results pass |
## Xpert Xpress SARS-CoV-2 Assay Method

### SARS-CoV-2 NEGATIVE

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
</table>
| The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. | - The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting  
- SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting  
- Probe Check: PASS; all probe check results pass |

### INVALID

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
</table>
| SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. | - SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting  
- Probe Check – PASS; all probe check results pass |

### ERROR

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
</table>
| Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. | - SARS-CoV-2: NO RESULT  
- SPC: NO RESULT  
- Probe Check: FAIL; all or one of the probe check results fail  
\(^1\) If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure. |

### NO RESULT

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
</table>
| Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. | A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.  
- SARS-CoV-2: NO RESULT  
- SPC: NO RESULT  
- Probe Check: NA (not applicable) |

### Responsibilities

- Perform and record daily and periodic maintenance tasks required for GeneXpert system and record in the Maintenance schedule spreadsheet.  
- Ensure that all reagents and samples are stored correctly  
- Ensure meticulous attention to potential for nucleic acid contamination when handling specimens and reagents for PCR tests  
- Perform notification of critical results and/or notification to Public Health as required
Competencies and Qualifications

- Staff require basic knowledge of preparing specimens for nucleic acid testing
- Cepheid GeneXpert System must only be operated by staff who have completed training and been authorised to perform the required assay

Definitions

PPE – Personal Protective Equipment (e.g. gloves, lab gown, goggles, BSC)
PCR – Polymerase Chain Reaction
RT-PCR – Reverse transcriptase PCR
RNA – Ribonucleic acid
BSC – Biological Safety Cabinet

References


Specimen Details

Specimen Collection

Nasopharyngeal aspirates and nasopharyngeal swab specimens can be collected using the healthcare facility’s standard procedures.

Collection using Copan FLOQSwab® is recommended, with samples placed into Copan UTM® (same as Xpert Viral Transport Medium). These materials are supplied from the laboratory on request, or may be ordered directly from Interpath.

An Xpert® Nasopharyngeal Sample Collection Kit for viruses (which includes FLOQSwab® and 3mL UTM) is available from Cepheid as product reference SWAB/B-100.
**Specimen Types**
This test is for use with specimens collected from human respiratory tract including:
- Nasopharyngeal aspirate or wash
- Nasopharyngeal or nose/throat swab in red topped UTM transport medium

**Specimen Rejection Criteria and Corrective Actions**
Proper specimen collection, storage, and transport are critical to the performance of this test.

**Specimen Transport**
Specimens should be transported at 2-8°C.

**Specimen Preparation**
Correct patient identity and labelling of specimen must be checked on arrival of sample at laboratory. Samples must be registered into the local LIS with test code NCOPCL prior to processing.

Sample preparation is performed by trained staff member.

Prepare samples inside the Biological Safety Cabinet.

Prepare one sample at a time. Remove gloves if contamination may have occurred, and when finished at BSC. Good laboratory practice includes changing gloves between handling patient specimens – use new gloves for handling each sample. Run UV lamp on BSC and disinfect surfaces when sample preparation steps have been completed at the end of the shift.

In event of contamination of work area, thoroughly clean the contaminated area (note: do not use bleach on stainless steel) and then 70% ethanol. Wipe work surfaces dry completely before proceeding.

Samples received at the laboratory already in UTM are ready for testing by Xpert Xpress SARS-CoV-2 assay without further preparation. Follow above procedure for sample preparation when Nasopharyngeal aspirates are received.

**Specimen Storage / Stability / Retention**
Swab specimens should be placed into transport medium (UTM) as soon as possible after collection.

Specimens placed in UTM following collection may be stored for up to 24 hours at 2-30°C, or up to 7 days at 2-8°C prior to testing with the Xpert Xpress SARS-CoV-2 Assay.

After testing, specimens are stored at 2-8°C for a maximum of 7 days before discard.
Quality Assurance

Stability
- A sample in UTM will remain suitable for testing for 7 days if stored at 2-8°C.
- For longer periods store sample frozen at -80°C.
- Avoid repeated freeze-thaw cycles.

QC of Shipments
Each new Lot number of GeneXpert cartridges will have negative and positive controls performed by the John Hunter Laboratory. This data will then be distributed to all laboratories confirming QC compliance for that lot number. The QC compliant lot number can then be ordered and used by other laboratories in NSWHP.

RCPA Enrolment for
As above for RCPA, rotating lab enrolment as interim.

Retests
Reasons to Repeat the Assay
If any of the test results mentioned below occur, repeat the test once according to instructions below:

- A **PRESUMPTIVE POS** result indicates the 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Only one of the SARS-CoV-2 nucleic acid target was detected (E gene) while the other SARS-CoV-2 nucleic acid target (N2 gene) was not detected.
- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

Retest Procedure
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.

4. Open the cartridge lid.

5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.

6. Close the cartridge lid.

To retest a non-determinate result (INVALID, NO RESULT, or ERROR) or a PRESUMPTIVE POS result, use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

Limitations

- Performance of the Xpert Xpress SARS-CoV-2 test has only been established in nasopharyngeal swab and nasal wash/aspirate specimens. Use of the Xpert Xpress SARS-CoV-2 test with other specimen types has not been assessed and performance characteristics are unknown.

- Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2 test but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.

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

- As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.

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

Xpert Xpress SARS-CoV-2 Kit Details

Principle of Assay

The Xpert® Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert® Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems.
The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the GeneXpert Dx System Operator Manual or the GeneXpert Infinity System Operator Manual.

The Xpert® Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The nasopharyngeal, nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL transport medium. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert® Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

**Reagents**

One Cepheid Xpert® Xpress SARS-CoV-2 single-use test cartridge is required for each sample. For samples in UTM®, no other reagents are required.

**Reagents Source**

Xpert® Xpress SARS-CoV-2 tests are supplied by Cepheid (GXFLU/RSV-CE-1) as a pack of 10 single-use tests.

**Reagent Preparation**

No reagent preparation is required.

**Reagent Storage**

Xpert® Xpress SARS-CoV-2 test cartridges are stored at 2-28°C.

**Reagent Stability**

Xpert® Xpress SARS-CoV-2 test cartridges may be used up to stated date of expiry.
# Standard Solutions
No standard solutions are required.

## Laboratory Apparatus
Cepheid Xpert® Xpress SARS-CoV-2 test cartridges are suitable for use on a Cepheid GeneXpert Dx System or GeneXpert Infinity System only.

## GeneXpert Instrumentation

### General
The Cepheid GeneXpert Dx System contains modules for loading and running a range of Xpert assays.

### Operating Parameters
Cepheid GeneXpert Dx System operates as a stand-alone benchtop instrument. It is not currently interfaced to LIS.

### Calibration Procedures
Cepheid recommends that the GeneXpert Dx system is checked for official calibration on an annual basis. A calibration check is performed by Cepheid Service Engineer for each test module yearly as part of service contract.

## 2. Risk

### Key Safety Information

<table>
<thead>
<tr>
<th>Key Safety Factors and Risks</th>
<th>PPE to use or controls in place</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Biological hazard from patient samples</td>
<td>• Specimen processing must be performed in Biological Safety Cabinet (Class I or II). Wear PPE (gloves, lab gown, safety glasses).</td>
</tr>
<tr>
<td>• Lysis reagent contains guanidinium thiocyanate</td>
<td>• Wear PPE; Careful disposal of clinical waste.</td>
</tr>
</tbody>
</table>
3. Further Information

For further information, please contact:

<table>
<thead>
<tr>
<th>Procedure Contact Officer</th>
<th>Position: Microbiology Lab Manager</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name: Jane Drury</td>
<td></td>
</tr>
<tr>
<td>Telephone: 02 4921 4398</td>
<td></td>
</tr>
<tr>
<td>Email: <a href="mailto:jane.kitcher@health.nsw.gov.au">jane.kitcher@health.nsw.gov.au</a></td>
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4. Version History

The approval and amendment history for this document must be listed in the following table.

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<th>Version No</th>
<th>Effective Date</th>
<th>Approved By</th>
<th>Approval Date</th>
<th>Procedure Author</th>
<th>Risk Rating</th>
<th>Sections Modified</th>
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<tbody>
<tr>
<td>1.1</td>
<td>01/05/2020</td>
<td>Robert Lindeman</td>
<td>01/05/2020</td>
<td>Jane Drury</td>
<td>Low</td>
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</tbody>
</table>