GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysgol Hywel Dda University Health Board

LABORATORY PROCEDURE

Detection of Flu A, Flu B, RSV and SARS-CoV-2 using Cepheid Xpert Xpress

Edition no.	007
Date of Issue	12/05/25
Review Interval	Biennially
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Copy	1 of 2
Location of Copies	1. Q-Pulse
	2. Laboratory Procedure File, WGH

Stage	Risk Assessment	Manual Handling
Preparation	<5	<5
Instrumentation	<5	<5
Chemical	<5	<5
Sample	<5	<5
Disposal	<5	<5

Key	Risk Assessment/Manual Handling Score
<5	<5 No Action
6-10	6-10 Action within 12 months
10+	10+ Urgent Action Required

CONTENTS

0 Introduction

0.1 Scope and purpose

This SOP describes the procedure for using the Cepheid Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 test kit for the qualitative detection of nucleic acid from Flu A, Flu B, RSV and SARS-CoV-2 in throat swabs collected from individuals displaying symptoms of respiratory infection.

This procedure applies to all staff involved in the running and operation of the GeneXpert Xpress system.

0.2 Responsibility

The laboratory manager is responsible for the implementation and maintenance of this procedure in conjunction with senior staff in the department.
BMS, laboratory technicians and MLA staff may carry out the procedures in which they have been trained and assessed as competent.

0.3 References

- EXPAT685 ISO 15189:2012
- UMMIC018 GeneXpert Reference Guide
- UMMIC019 Quick Reference Instructions for Xpert® Xpress
- UMMIC021 Maintenance of GeneXpert
- EXMIC1038 Cepheid Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 kit insert

0.4 Definitions

- IQC – Internal quality control
- LIMS – Laboratory information management system
- WGH – Withybush General Hospital

0.5 Related document

- MPPAT628 External quality assurance in the pathology department
- MPPAT606 Procedure for assuring the quality of examinations
- LPMIC007 Specimen Collection and Handling
- LPPAT601 Waste management
- MPPAT625 Procedure for the Control of Clinical Material
- LPMIC071 Procedure for the IQC of all examinations
- LIPAT630 Pathology Services Handbook
- LPMIC082 Cepheid GeneXpert
- LFMIC074 Molecular IQC Record Sheet
- COMIC001 COSHH assessment review – Microbiology
- LFMIC055 Consumables Log
- LFMIC056 Reagent Log
- RAMIC016 Risk Assessment - Acceptance Testing of Reagents
- LIMIC027 Acceptance Testing in Microbiology
- EXMIC1119 Cepheid Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 kit insert
- LIMIC042 Instructions for the preparation of stock reagent from patient samples for the IQC and acceptance testing of respiratory PCR assays

1 Clinical relevance/Purpose of the examination

Respiratory viruses cause significant morbidity and mortality in both healthy and vulnerable individuals. Clinically it can be difficult to diagnose the specific cause of any given respiratory infection due to similarities in presenting features, especially in the immunocompromised. Due to the availability in antiviral treatment for influenza it is important to rapidly diagnose the cause of an infection to instigate proper patient management, infection control measures and where possible antiviral treatment. Molecular techniques have been shown in numerous studies to be both sensitive and specific for detecting respiratory viruses when compared to traditional laboratory techniques, and they are now becoming more routinely used in diagnostic laboratories

SARS-CoV-2 is a newly identified strain of betacornavirus which emerged in China in 2019. Rapid spread of the infection quickly resulted in a pandemic and consequent global health emergency. Infection with the SARS-CoV2 virus has been shown to cause the COVID-19 syndrome. COVID-19 disease may consist of a relatively mild flu-like illness, which can progress to severe pneumonia, respiratory distress, renal failure and death in vulnerable populations.

2 Principle and method of the procedure used for examinations

The Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the Flu A, Flu B, RSV and SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/ aspirate specimens collected from individuals suspected of COVID-19 by their healthcare provider. The Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 test is performed on GeneXpert Xpress System.

The GeneXpert Xpress System 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.
The Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 test includes reagents for the detection of RNA from Flu A, Flu B, RSV and SARS-CoV-2 in nasopharyngeal swab specimens. Results from this test are reported on a qualitative basis (presence/ absence of the gene/s).

Negative results do not preclude Flu A, Flu B, RSV and 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

3 Performance characteristics

See operating manual for performance specifications.

4 Specimen requirements and means of identification

4.1 Type of specimen

Dry throat swabs. After collection the dry swab sample can be held for up to 14 days at 15-28ºC. The swab can be stored in lysis buffer at 2-8ºC for up to a year or frozen at -20ºC or -80ºC.

4.2 Patient preparation

No specific patient preparation is required.

4.3 Type of container and additives

Specimens should be taken using a dry swab. Swabs received in any medium are not suitable for testing.

4.4 Means of identification

All samples and request forms must be labelled with unequivocal identification criteria. Please refer to MPPAT613 Patient sample and request form identification criteria for further details.

5 Equipment and reagents

5.1 Equipment

GeneXpert Instrument System

Screw capped containers for lysis buffer sample preparation
Filtered tips (200µl - 1000µl)
Calibrated pipette (200µl - 1000µl)

5.2 Reagents, standards or calibrants and internal control materials

5.2.1 Reagents

The Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

- Cartridges with integrated reaction tubes
- Disposable transfer pipettes

Also required, but not supplied with the kit:

- Lysis buffer.

The Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 cartridges and reagents should be stored at 2-28ºC.

- Do not open a cartridge lid until you are ready to perform testing
- Do not use a cartridge that is wet or has leaked
- Do not use a cartridge that has been dropped after removing it from the packaging
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results
- Do not use a cartridge that has a damaged reaction tube

- Use the cartridge within 30 minutes of opening the cartridge lid.

5.2.2 Calibrators

N/A

5.2.3 Acceptance testing

Acceptance testing is performed using confirmed previously positive patient samples. These may also be used to test the analyser post intervention from an engineer, mechanical failure, continuous invalid results, or any other intervention which may give rise to suspect results. The results must be recorded to ensure that there are no errors.

Acceptance testing is performed using confirmed previously positive patient samples on opening of each new batch and recorded on LFMIC056 and LFMIC055 as appropriate. Please refer to RAMIC016 Risk assessment – Acceptance testing of reagents.

Each batch of Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 kit with the same lot number will have user acceptance tests conducted upon arrival to the laboratory.

Each kit that has a different lot number must have user acceptance tests conducted per batch. As there are no positive and negative controls supplied with the kit this will be conducted using Third Party Reference Material. Each time the controls are used they will be placed in a different module within the analyser. This will be recorded to ensure all modules within the GeneXpert, Cepheid are being used and ensuring that each module is functioning as required.

6 Environmental and safety controls

Samples, reagents, and quality control materials must be stored so as to ensure their integrity. In particular, samples must be stored in such a way that cross contamination is not possible. All areas where consumables are stored are monitored for temperature. All equipment undergoes portable appliance testing (electrical safety) which is undertaken by the Health Boards estates department at pre-defined intervals depending on the equipment concerned.

Material safety data sheets (MSDS) are obtained for all reagents, quality controls, chemicals etc as applicable and from these COSHH assessments are completed. Manual handling and risk assessments are also completed. All MSDS, manual handling assessments, risk assessments and COSHH assessments can be found in the documents module of Q-Pulse. All documents are controlled.

6.1 Electrical

Potential electrical hazards exist behind covers and panels. Keep doors, covers, and panels closed during normal operation.

Do not operate the system if any of the assemblies have been removed. Removing assemblies from their normal positions may create electrical hazards.

6.2 Biological

 **Positive results are indicative of presence of Flu A, Flu B, RSV and SARS-CoV-2-RNA.**

This method should be carried out using principles of good laboratory practice at containment levels appropriate to the specimen type.

- The opening of packages and pre preparation of swabs from patients with suspected Flu A, Flu B, RSV and SARS-CoV-2 will be carried out in a MSC safety cabinet within CL2.
- If specimen boxes/bags are received these are opened in MSC and the boxes are placed in red sack, disinfected and sent for recycling.
    - All specimens received from patients with known or confirmed positive for Flu A, Flu B, RSV and SARS-CoV-2 are processed in a Class 1 MSC cabinet within CL2+.
- Only fully trained and competent staff must undertake the work; in addition to this the level of training provided should be appropriate to the level of risk and the complexity of the procedures being undertaken.
- As lysis buffer contains Guanidinium thiocyanate it should be disposed of in sealed containers at a licensed waste disposal site. Dispose of contents/container in accordance with local/regional/national/international regulations.
- Caution when dealing with accidental release/spillage of lysis buffer. Wear protective equipment (gloves and safety glasses). Isolate spill area. Collect with absorbent material and place in a container for disposal. Wash spill area with 70% ethanol then disinfect the surface with a 1% (w/v) sodium hypochlorite (bleach) solution or an equivalent disinfectant. If staff come into contact with lysis buffer please seek first aid immediately.

DO NOT add bleach to spilled lysis buffer – a dangerous reaction may occur.

7 Calibration Procedure

7.1 Calibration

N/A

7.2 Metrological traceability

Please refer to MPPAT606 Procedure for assuring the quality of examinations for further information.

Results are assessed periodically and recorded on LFMIC081 Measurement uncertainty – qualitative. Completed records are stored in the Q-Pulse audit module.

NB. To access completed records from the Q-Pulse audit module: In the search facility change the scheduled date to ‘This year’ from the drop down list (or last year if you want to view last year’s audit) and then in the keywords box type in LFMIC081.

8 Instructions for Performance of the Examination

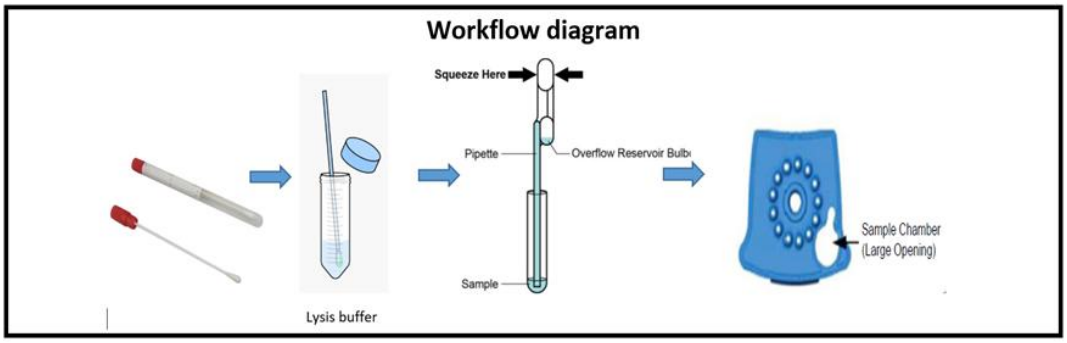
8.1 Sample processing

8.1.1 Sample handling

All specimens received should be opened in a MSC safety cabinet in the enhanced CL2+ laboratory.

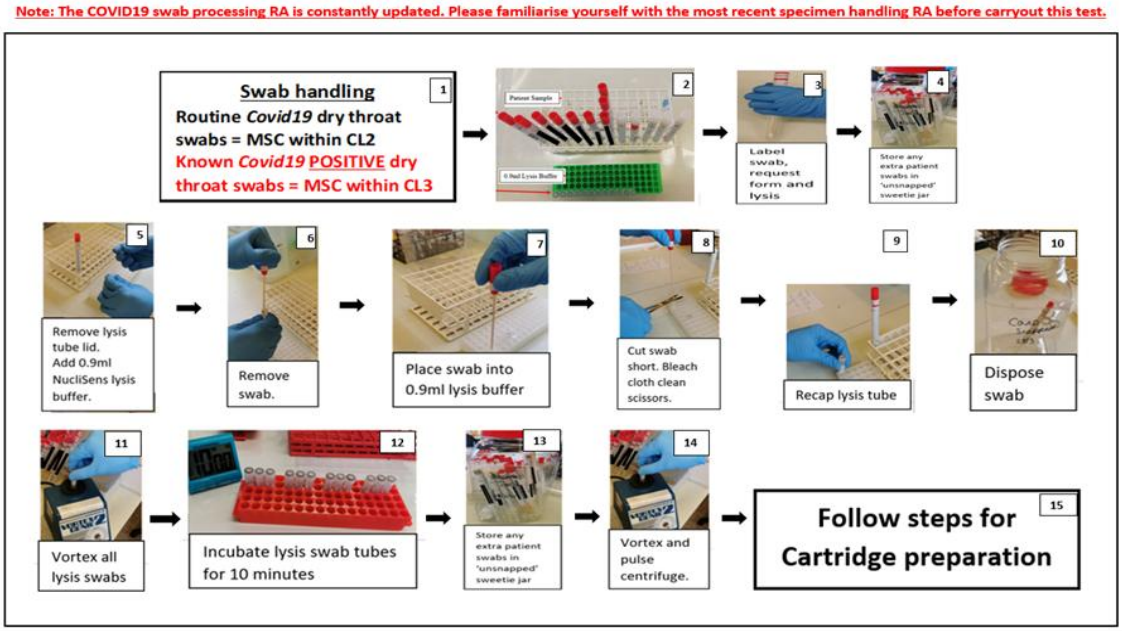
8.1.2 Sample processing

The processing of throat swabs will enable extraction of the viral nucleic acid for the subsequent detection of Flu A, Flu B, RSV and SARS-CoV-2 virus.



8.1.3 Swab preparation

NB. This document is also available as LIMIC039



1. If specimen boxes/bags are received these are opened in MSC and the boxes are disposed of appropriately.

2. The swabs and their corresponding request forms are allocated episode/accession numbers. The forms will be used to book the specimens onto LIMS.
3. For each sample, label 1x 0.9ml lysis tube with the patient's name and episode number (barcode).
4. Place any 'doubled up' patient swabs into the 'Unsnapped' samples sweetie jar.
5. Fill each lysis tube with 0.9ml lysis buffer using pipette and filtered tips.
6. Ensuring all patient demographics and episode number on the lysis buffer tube and dry swab match.
    - Remove the lid from the lysis tube.
    - Remove swab from tube. Refrain from touching the end of the flocked swab with your gloves. If this occurs change gloves immediately.
    - Place swab into lysis buffer and snap/cut swab into 0.9ml lysis buffer, recap & vortex well (ensure swab is snapped/cut off as short as possible to allow space for pipette tip to reach liquid).
    - Place the snapped swab into the COVID19 sweetie jar with in the cabinet. Repeat steps 5 until all swabs are snapped.
7. Start timer and all 0.9ml lysis buffers containing samples should be left at room temperature (20ºC - 25ºC) for 10 minutes.
8. After the 10-minute incubation period, vortex all 0.9ml lysis tubes for a second time and pulse centrifuge to remove liquid from the cap.

8.1.4 Cartridge preparation

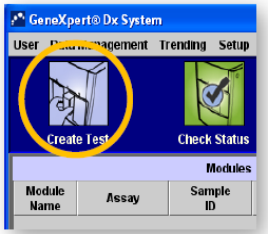
1. From the Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 test kit remove required number of a new cartridges and new transfer pipettes.
2. Label all **sides** of cartridges with their corresponding sample lab number.
   Note: Ensure that you check the lab numbers on sample lysis tube and cartridge correspond to the swab being tested.
3. Open the cartridge lid and processed swab/lysis buffer tube.
4. Using aseptic technique, Fill pipette with sample:
    - Remove pipette from wrapper and squeeze the top bulb of the pipette completely and then place the pipette tip in the processed swab/lysis buffer vial.
    - Release the top bulb of the pipette to fill the pipette with sample from the patient swab/lysis buffer tube.
5. Transfer the sample to the cartridge by squeezing the top bulb completely again to empty the contents of the pipette into the large cartridge opening (sample chamber).
6. Close the cartridge lid and place the cartridge into the GeneXpert instrument within 30 minutes.

8.2 Running the test on the GeneXpert system

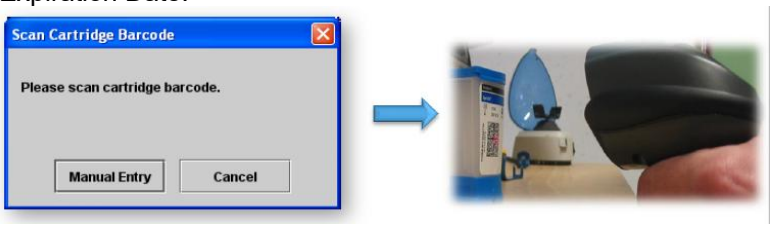
Put on clean pair of gloves and lab coat (if necessary).

1. First turn on the instrument and then turn on the computer.
   The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows desktop.

2. Log on to the GeneXpert Instrument System software using your allocated Cepheid user name and password.
3. In the GeneXpert System window, click Create Test (GeneXpert Dx).



4. **Note: Ensure that the correct sample test has been opened as some machines have different assays loaded.** Scan or type in the Patient laboratory number. The Patient laboratory number is associated with the test results and is shown in the View Results window.
5. Scan the barcode on the Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.



Note: If the barcode on the Xpert Flu A, Flu B, RSV and SARS-CoV-2 cartridge does not scan, click on MANUAL ENTRY and type the 2 line numbers of the cartridges, if this is not accepted then set up a new test.

6. Click on **START TEST** and open the instrument module door with the blinking green light and load the cartridge.
7. Close the door with a **firm click**. The test starts and the green light stops blinking. The test will take approximately 50 minutes and when it is finished, the light turns off.

   Note: While a test is running, you can start another test.
8. When the test is complete the screen test will change to COMPLETE and the door unlocks. Wait until the system releases the door lock before opening the module door. Then remove the cartridge.

9. The used cartridges should be disposed in the appropriate specimen waste containers as per laboratory guidelines

The empty test module can now be used for the next test cartridge.

8.3 Viewing Test Results

The GeneXpert System provides automatic interpretation but pending further assessment of low level positives, some manual re-interpretation of low level positives is required prior to reporting (see Result Interpretation). Results should be reviewed in the **View Results** window. The Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

1. Click the View Results icon to view results. To visualise one particular test result, click on **VIEW TEST**
2. Double click on the test you want to see.
3. The screen will show you:
    - Information about the test
    - Real-Time PCR curves
    - Interpretation of the result 'Test Result'

8.4 Maintenance and Troubleshooting

Follow link for maintenance procedure or refer to UMMIC021
https://www.ghdonline.org/uploads/10-Maintenance_of_GeneXpert.pdf

9 Quality assurance procedures

9.1 Internal quality control

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC) within each cartridge.

Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

Run confirmed previously positive patient samples at the start of each new lot. Record results on LFMIC074 Molecular IQC Record Sheet (failures recorded on page 2).

9.2 External quality assurance

This is monitored by regular returns to the NEQAS Molecular detection of SARS-CoV-2 and Molecular detection of respiratory viruses EQA schemes. Refer to LPMIC071 for further details

For further information please refer to MPPAT628 External Quality Assurance in the Pathology department.

10 Interferences and cross reactions

- Manufacturer advises the Xpert Xpress Flu A, Flu B, RSV and SARS-CoV-2 test is carried out on samples such as nasopharyngeal swab and nasal wash/aspirates. However, a thorough verification has been completed by PHW using dry throat swabs with this kit and performance was satisfactory in comparison to reference molecular assays for Flu A, Flu B, RSV and SARS-CoV-2.
- 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 SARSCoV-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.

11 Principle of procedure for recording and calculating results

11.1 Result interpretation

The results are interpreted automatically by the GeneXpert Xpress System and are clearly shown in the **View Results** window. The system provides test results based on the detection of respective gene targets according to the algorithms Click on the Analyte result tab and view the table. This will give the sample CT values and result. See Appendix One for examples of results to expect.

11.1.1 Negative results

Negative results are reported on LIMS and authorized on the bench. Negative results do not preclude Flu A, Flu B, RSV and SARS-CoV-2 infection and should not be used as the sole basis for treatment ot other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

11.1.2 Positive results

NB. All SARS-CoV-2 positive results are referred PENGU. Positive Flu A are referred to virology UHW

11.1.3 Tests interpreted as Invalid/Error/No Result entry

These results are retested.

11.2 Positive and Negative result entry

Results are sent to LIMS via interface.

11.3 Reasons to repeat the test

- Any **low level positive** (see table 1 for definition) regardless of on platform interpretation (May be **POSITIVE** or **PRESUMPTIVE POSITIVE**) will be sent to Cardiff Virology for Seegene testing. But reporting should not be delayed pending a result.
- 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, or the maximum pressure limits were exceeded.
- **NO RESULT – REPEAT TEST** 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.

11.3.1 Retest Procedure for Specimens with INVALID, ERROR, or NO RESULT- REPEAT TEST

1. Obtain the leftover specimen and in a MSC safety cabinet within CL2+ invert specimen 5 times to mix.
2. Follow section 8.1.4 Cartridge preparation.
3. Repeat test

11.2 Measurement Uncertainty of measured quantity values

Please refer to MPPAT606 Procedure for assuring the quality of examinations for further information.

Results are assessed periodically and recorded on LFMIC081 Measurement uncertainty – qualitative. Completed records are stored in the Q-Pulse audit module.

NB. To access completed records from the Q-Pulse audit module: In the search facility change the scheduled date to ‘This year’ from the drop down list (or last year if you want to view last year’s audit) and then in the keywords box type in LFMIC081.

12 Reporting results

12.1 Biological reference intervals or clinical decision values

N/A

12.2 Reportable interval of examination results

N/A.

12.3 Determination of quantitative results outside the measurement interval

N/A

12.4 Alert/critical values

Inform Infection Control of any Positive results from Ward patients

12.5 Responsibilities of personnel in authorising, reporting and monitoring of reports

All results where GeneXpert Systems are interfaced to LIMS are automatically sent to LIMS from the Cepheid instrument.
All negative results are automatically authorised and sent out.

12.5.1 Authorisation

All results are authorised automatically at the bench

12.5.2 Report Issue (Interim/Final/Additional)

All results are reported on LIMS using the RESPR test set..

12.5.3 Reporting to other departments

Positive results are reported to health protection via automated methods via TrakCare lab data via Labexpert and Tarian.

13 Laboratory clinical interpretation

Clinical interpretation of results is available via Consultant Microbiologists 24/7 who can advise clinicians on the evaluation and interpretation of the results of laboratory examinations including precision and accuracy of methods, significance of results in relation to the laboratory's reference values, clinical significance of the requested procedure and its fitness for purpose and any further procedures that may be helpful.

14 Potential sources of variation

Sources of variation may include biological, rhythmical/cyclical or random e.g., pre-analytical sources of variation, analytical variation etc. Please refer to MPPAT606 Procedure for assuring the quality of examinations" for further details.