Supplementary guide

Quality assurance and quality control in L1 laboratories performing Xpert® Xpress SARS-CoV-2 testing

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Pacific Community
Communauté du Pacifique

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INTRODUCTION

The purpose of this document is to provide supplementary information on quality assurance and quality control for laboratory professionals to complement information in the standard operating procedures (SOPs) for Xpert® Xpress SARS-CoV-2 testing which has been shared to L1 laboratories in PICTs.

This document describes in detail the best practice for national laboratory verification in respect to Xpert® Xpress SARS-CoV-2, in which two scenarios are examined.

1. Emergency and low-resource settings where minimal quality control (QC) should be performed to preserve testing or patient cases, where reagents are limited by supply, or need for case testing outstrips reagent procurement.
2. Routine QC which is the ideal in a stable setting where procurement and demand are equal.

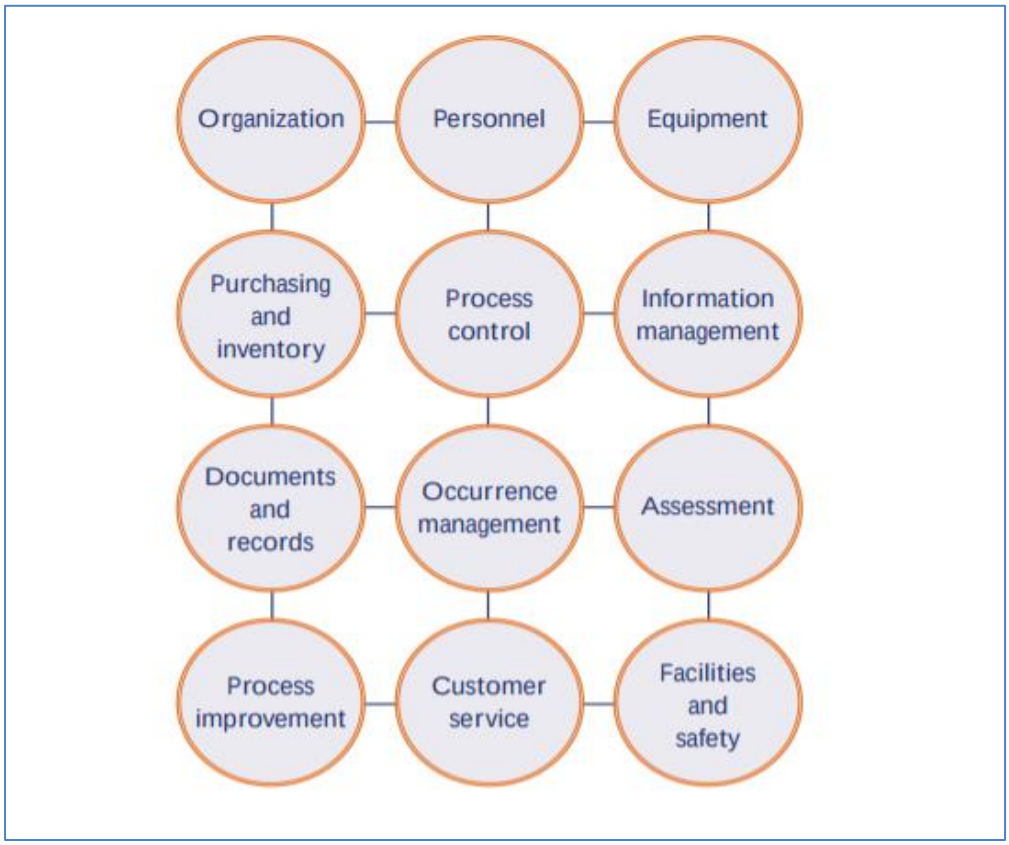
Considering that SARS-CoV-2, the virus that causes COVID-19, is a new emerging virus, the molecular assay Xpert® Xpress SARS-CoV-2 developed by Cepheid to detect the virus has been approved on 20 March 2020 by the US Food and Drug Administration (FDA) under the Emergency Use Authorization (EUA). Current information on the testing strategy and quality management may therefore change as new information on the virus becomes available.

For further reading *the Global Laboratory Initiative (GLI) guides*[2] *for Implementing a Quality Assurance System for Xpert® MTB/RIF Testing (Xpert® QA guide)* is highly transferable to Xpert® Xpress SARS-CoV-2 routine testing scenario.

Quality control (QC) and quality assurance (QA)

To achieve correct diagnosis, improvement of the Laboratory Quality Management System (QMS) using the 12 quality system essentials is crucial.[2,3] The 12 quality systems essentials are the building blocks for a quality management system in a clinical laboratory. Each quality system essential must be continuously monitored throughout the workflow path of laboratory processes in order to detect errors in the system and provide quality results.

Figure 1: The 12 quality system essentials



Source: Laboratory quality management system: handbook[4]

A good QMS program must:

- Evaluate the effectiveness of the policies and procedures of the laboratory.
- Identify and correct problems.
- Confidently provide accurate, reliable, and timely laboratory test results.
- Confidently show staff competency and performance results.

The pre-analytical, analytical, and post-analytical phases of the laboratory testing must be continuously monitored and strengthened to support provision of quality test results.

For this guide, assuring accuracy and reliability throughout the path of workflow depends on good management of the 12 quality system essentials. To ensure that the laboratory is operating smoothly, safely and effectively without any errors, QA needs to be re-enforced.

QA is an important element of the quality management system and includes documentation, SOPs, quality control samples, and external quality assessment schemes[4]

QC are procedures used in each assay to assure a test run is valid and results are reliable[5]. The goal of the QC is to detect, evaluate, and correct errors due to test system failure, operator performance, or environmental conditions before results are reported.

The laboratory is to have systems in place to evaluate the effectiveness of corrective actions about the quality control program, including:

- problems encountered during the evaluation of instruments using Xpert® Check kits;
- problems arising during external quality assessment (EQA); or
- problems encountered during the evaluation of patient test results.

1. Emergency and low-resource setting guidance for quality management systems

In order to implement an ongoing QA and QC for SARS-CoV-2 testing, and to ensure valid reliable test results are achieved, the following proposed steps have been generally agreed upon by the JIMT Laboratory Cell in collaboration with Cepheid and WHO.

Verification of GeneXpert® instrument module function

Essential **–** Xpert® check has been run within the previous year, with failing modules turned off until replaced and reverified.

Cartridge lot-to-lot verification

Optional depending on supply chain: This should only be followed where sufficient reagents have been delivered so that patient testing is not impacted. e.g. at least three months of test reagents that meet testing demands have been delivered.

To avoid excess loss of test reagents this is to be carried out by the central receiving laboratory in each country before distribution to sites. New lot verification ensures reagents have not been damaged in shipment. The protocol for a full-lot verification can be found at Cepheid website[6] and on page 34 of the SARS-CoV-2 SOP that has been shared.

To reduce the number of cartridges used in the lot-to lot-verification the protocol can be modified as below to only use 12 Xpert® Xpress cartridges. This does not change the acceptance criteria from the full unmodified protocol.

Modified lot- to-lot verification method

Prepare the control material.

1. Label three sterile, screw-capped tubes “A”, “B” and “C.”
2. In tube A create a 1:2 dilution of the positive AccuPlex reference material (DILUTED positive) in a test tube by taking 0.5 ml of the AccuPlex material and adding 0.5 ml of a negative patient specimen (can be pooled negative specimens).
3. Cap tube A tightly then mix thoroughly. The total volume in the tube A will be 1.0 ml.
4. In tube B prepare a pool of AccuPlex UNDILUTED positive reference material by pipetting out 0.5 ml from each of two AccuPlex positive reference material vials.
5. Cap tube B tightly then mix thoroughly. The total volume in the tube B will be 1.0 ml.
6. In tube C prepare a pool of AccuPlex NEGATIVE reference material by pipetting out 0.5 ml from each of two AccuPlex negative reference material.
7. Cap tube C tightly then mix thoroughly. The total volume in the tube C will be 1.0 ml.
8. For the final three samples select three, previously tested negative patient samples, each sample is to be added to a separate cartridge.

Prepare the testing cartridges

Note: only add reagents to cartridge when ready to run the tests. Do not set up cartridges more than 30 minutes before they can be run as this can lead to an increase in invalid test results[8].

1. Label 12 Xpert® Xpress SARS-CoV-2 cartridges #1 to #12.
2. Using pipette provided in Xpert® kit, add 0.3 ml into the cartridges as follows
   a. Tube A (diluted AccuPlex positive) = cartridge #1, #2 and #3.
   b. Tube B (full AccuPlex positive) = cartridge #4, #5 and #6.
   c. Tube C (AccuPlex negative) = cartridge #7, #8 and #9.
   d. Select three previously tested negative samples and take 0.3 ml from each and add it to cartridges #10, #11 and #12.
3. Run the Xpert® cartridges as per the product instructions for use.
4. Fill out the results in table below.

% Overall Agreement					
Cartridge #/Date	**SARS-CoV-2 positive testing result**	**% Agreement 100% for samples #1 - #3 and #4 - #6**	**Cartridge #/Date**	**SARS-CoV-2 negative testing result**	**% Agreement 100% for samples #7 - #9 and #10 - #12**
#1/			#7/		
#2/			#8/		
#3/			#9/		
#4/			#10/		
#5/			#11/		
#6/			#12/		
Comments			Comments		

Expected results

Acceptance criteria for the testing are:

- all tests should have a valid test result, no invalid, error or no results.
- all positive samples must be positive.
- all negative samples must be negative.

Verification results should be included in cartridge distribution details, either hard or soft copy, to all laboratories.

Incoming reagents failing verification should be reported immediately to local Xpert® focal person for technical support and/or Cepheid Technical Support depending on country quality program structure, and distribution and testing should be put on hold until resolution is found.

Operator verification

Essential **–** All staff must carry out Xpert® Xpress SARS-CoV-2 testing as per issued SOP. All staff should complete a competency examination as per country specific guidelines for laboratory competency examination. At a minimum, the operator should perform an Xpert®-Xpress SARS-CoV-2 test under supervision and demonstrate good performance as well as correctly answer theory questions about interpretation of test results. In-depth information about training and competency can be found in Chapter 4 of the Xpert®-QA-guide 2019.[1]

Initial training should be undertaken when a new test is introduced, followed by training at quarterly intervals for first year, and annually thereafter.

Expected results: Staff must pass the theoretical and practical part with 80% score in both sections. In case of failure, retraining must be completed, and staff retested prior to authorization to perform testing independently.

Laboratory validation - external quality assurance testing

Optional – depending on availability of blinded EQA panels. EQA testing should performed at least every four months using a panel supplied by a reference laboratory. If more than one person conducts tests within the laboratory, EQA panels should be rotated to different staff involved. Results must be reviewed by the laboratory and a failure to achieve passing score should be followed up with a root cause analysis and corrective actions that are monitored and signed off when completed. Two failures in a year should lead to test suspension.

Reagent verification - monthly QC

Essential – part of routine test monitoring. This does not require use of reagents outside of laboratory normal testing workload.

All Xpert® Xpress SARS-CoV-2 tests contain an internal sample processing control (SPC) that ensures all tests give accurate and correct results. This negates the need for other daily controls as all tests have the control inbuilt. A QC report of the SPC should be produced monthly and reviewed by a quality officer.

Instructions for generating a control trend report from "specimens" can be found from section 6.5 of the GeneXpert® manual (v4.8)[9] other software versions may vary please refer to the GeneXpert® manual for the specific software. Manual is usually found on the desktop of the computer supplied with the instrument.

Patient test verification – daily QC, internal controls

Essential - part of routine test monitoring. This does not require use of reagents outside of laboratory normal testing workload.

Sample Processing Control (SPC, non-infectious lyophilized spores of *Bacillus globigii*). The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time polymerase chain reaction (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 If the control is negative in a *negative* sample, the test is *invalid* The SPC passes if it meets the validated acceptance criteria.

Failure will result in an INVALID result and possibly an invalid code; refer to the Xpert®-Xpress SARS-CoV-2 SOP for corrective actions.

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.

Failure will result in an ERROR result and an error code; refer to the Xpert®-Xpress SARS-CoV2 SOP for corrective actions.

Routine test monitoring for quality assurance

Essential – part of routine test monitoring. This does not require use of reagents outside of laboratory normal testing workload.

Monthly key performance indicators (KPI) must be recorded and reported to the JIMT Laboratory Cell as well as country laboratory focal point.

GeneXpert® key performance indicators for Xpert® Xpress SARS-CoV-2:

1. Number of all tests run on system (this includes TB/HIV or other Xpert® tests).
2. Total number of failed tests on system (this includes TB/HIV or other Xpert® tests:
    - errors and the error code associated with the failed test
    - invalid and the invalid code associated with the failed test
    - no results.
3. Number of Xpert® Xpress SARS-CoV-2 runs.
4. Number of Xpert® Xpress SARS-CoV-2 negatives.
5. Number of Xpert® Xpress SARS-CoV-2 positives.
6. Number of presumptive positives.

The above can be simply generated by the laboratory directly from the GeneXpert® software using the Quick-Stats provided to automatically generate the KPI in correct format or by manually counting from the register.

If error rate is above 5% on machine or any module then further investigation using the error code is required for root cause analysis and corrective action.

2. Routine Xpert® Xpress SARS-CoV-2 guidance for quality management system

In order to implement an ongoing QA and QC for SARS-CoV-2 testing, and to ensure valid reliable test results are achieved, the following proposed steps have been generally agreed upon by the JIMT Laboratory Cell in collaboration with Cepheid and WHO.

Verification of GeneXpert® instrument module function

Essential – To be completed yearly as part of routine equipment maintenance as per manufacturer's instructions. Verification using Xpert® Check kit must be completed on all machine modules in use by the laboratory.

Cartridge lot-to-lot verification

Essential – New lot verification ensures reagents have not been damaged in shipment. The country can determine based on order size, internal distribution, storage and laboratory capacity whether the following should be carried out at the central receiving laboratory or by each laboratory individually, or in combination.

Lot-to-lot verification method

Details for lot-to-lot verification are the same as in section one of this document but complete with the recommended 20 samples instead of 12. The protocol for a full lot verification can be found at Cepheid website[7].

Please also refer to Annex A for full details of full lot verification, which the JIMT Laboratory Cell agreed, for Level 2 laboratories to conduct for the information of Level 1 laboratories in PICs.

Operator verification

Essential – Details for operator verification of competency is the same as section one of this document with the additional requirement of reproducibility of results. Please refer to Annex B for full details on reproducibility.

Laboratory validation -external quality assurance testing

Essential – depending on availability of blinded EQA panels. EQA testing should performed at least every four months using a panel supplied by a reference laboratory. If more than one person conducts tests within the laboratory, EQA panels should be rotated to different staff involved. Results must be reviewed by the laboratory and a failure to achieve passing score should be followed up with a root cause analysis and corrective actions that are monitored and signed off when completed. Two failures in a year should lead to test suspension.

Reagent verification - monthly QC

Essential – part of routine test monitoring. This does not require use of reagents outside of laboratory normal testing workload. Details for reagent verification are the same as in section one of this document.

Patient test verification – daily QC, internal controls

Essential – part of routine test monitoring. This does not require use of reagents outside of laboratory normal testing workload. Details for patient test verification are the same as in section one of this document.

Routine test monitoring for quality assurance
Essential – part of routine test monitoring. This does not require use of reagents outside of laboratory normal testing workload.

Supportive supervision and on-site evaluations
Essential – On-site supportive supervisory visits for assessments and training are especially critical during early stages of implementing a new test or procedure as they provide motivation and support to staff. On-site supervisory visits are a good opportunity to provide refresher training, mentoring, troubleshooting advice, and technical updates[1]. Strong relationships with GeneXpert® users encourage rapid reporting of any problems and enables rapid troubleshooting, re-training, and corrective actions. In addition to providing constructive feedback on performance, supportive supervision provides updates on technical guidelines and procedures, opportunities for training and assistance with reviews of quality indicators, results of proficiency testing and development of corrective actions.

In most settings, the central reference or supervisory laboratory will be responsible for planning and conducting the on-site evaluations. The supervisory visits should be planned at regular intervals with schedules communicated to sites in advance.

The on-site evaluations should use standardized checklists[1] and include discussions with GeneXpert® users, testing site management, review of the test site documentation, and observation of testing site operations. Any problems identified during the assessment should be discussed immediately with facility staff and a plan established for addressing problems. The testing site should receive interim feedback immediately after the supervisory assessment and subsequently a full supervisory visit report. The testing site should undertake and document any recommended corrective actions in a timely manner.

Implementing a Quality Assurance System for Xpert® testing:

1. Schedule supervisory visits according to national policy.
2. Prepare necessary documents for supervisory visits.
3. Undertake and document any recommended corrective actions.

Final Recommendations

- Ensure each PIC has an Xpert® quality manual that includes Xpert® Xpress SARS-CoV-2 testing, this may be part of a larger Laboratory Quality Manual which is updated to include Xpert® Xpress SARS-CoV-2 testing.

- Use existing Xpert® supervision schedules but expand to include Xpert® Xpress SARS-CoV-2 testing.
- Include Xpert® Xpress SARS-CoV-2 testing KPI in routine Xpert® data collection systems.
- Where possible do not duplicate tools and resources.

Annexes

Annex A: Lot-to-lot verification method

% Overall Agreement					
Cartridge #/Date	**SARS-CoV-2 positive sample testing result**	**% Agreement (acceptance criteria – 100% for specimens 1-5; 80% for specimens 6-10)**	**Cartridge #/Date**	**SARS-CoV-2 negative sample testing result**	**% Agreement (acceptance criteria – 100%)**
#1/			#11/		
#2/			#12/		
#3/			#13/		
#4/			#14/		
#5/			#15/		
#6/			#16/		
#7/			#17/		
#8/			#18/		
#9/			#19/		
#10/			#20/		
Comments			Comments		

Expected Results

Acceptance criteria for the testing are:

- No more than 1% invalid results.
- Less than 5% error results.
- Positive and negative results correlate to sample tested 100%.

Verification results done centrally should be included in cartridge distribution details either hard or soft copy to all laboratories.

Incoming reagents failing verification should be reported to local Xpert® focal person for technical support and/or Cepheid Technical Support immediately and distribution and testing should be put on hold until resolution is found.

Annex B: Operators verification and reproducibility

Reproducibility Procedure:

Essential – The protocol for lot verification and reproducibility can be found on the Cepheid website.[7]

1. Select two operators to repeat the Xpert® Xpress SARS-CoV-2 test on selected vials of AccuPlex positive reference material and AccuPlex negative reference material.
2. Do not allow either operator to know which reference material is positive or negative. Staff should work consecutively and not at the same time.
3. Using pipette provided, operator #1 add 0.3 ml of AccuPlex positive reference material to cartridge.
4. Using pipette provided, operator #1 add 0.3 ml of AccuPlex negative reference material to cartridge.
5. Repeat with operator #2.
6. Fill out results in the Table below.

Reproducibility					
Cartridge #/Date	**SARS-CoV-2 positive sample testing results**	**% Agreement (acceptance criteria – 100%)**	**Cartridge #/Date**	**SARS-CoV-2 negative sample testing results**	**% Agreement (acceptance criteria – 100%)**
Operator 1: Sample #1/			**Operator 1**: Sample #1/		
Operator 2: Sample #2/			**Operator 2**: Sample #2/		

Expected Results

Acceptance criteria for the testing are:

- All replicates of the positive samples should be 100% positive, and all replicates of the negative samples should be 100% negative.
- If test results fail to meet expected results, contact local Xpert focal person for technical support.

Note: This can only be carried out in laboratories where there is more than one certified operator. Where there is a single operator, the operator should process the four control tests, record in the above table, and return to the central laboratory quality officer for review.

References

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