EQA PROGRAM FOR COVID-19 PCR TESTING LABS
(Retesting method)

1. Introduction:

EQA (external quality assessment) is one of the pre-requisite for quality assurance of any clinical laboratory including COVID-19 PCR testing labs. Various methods of such programs can be adopted by participating lab as per their availability.

Proficiency testing (PT) is one of the preferred method for EQA (External quality assessment). Participation in a PT program enables a laboratory to compare its performance against other laboratories using similar methods/reagents/instruments.

PT/EQA is, however, not available for many tests, one of them being newly introduced tests such as COVID 19 PCR testing. For such tests, laboratories should, when appropriate and practical, implement an alternative assessment procedure (AAP).

AAPs may often use patient specimens, which have certain advantages over the manufactured materials frequently used in PT.

- Matrix effects are reduced when patient specimens are used.
- The steps in the preanalytic phase of clinical patient testing—specimen acquisition, transportation, and processing—are not evaluated by PT programs using manufactured testing materials, because the preanalytic phase of PT differs from patient testing.In contrast, variables related to preanalytic processing may be evaluated by an AAP that uses patient specimens. Patient specimens used for AAPs require attention to stability during storage and transportation between laboratories, to minimize introduction of additional variability not related to clinical testing performance.

If an AAP can be traced to a reference method, accuracy can be assessed. Even if neither interlaboratory comparison nor evaluation ofaccuracy is practical for a particular test, it is still worthwhile to use an AAP to complement QC, becauseQC is not perfectly sensitive or specific. In-house AAPs provide more timely data than does a PT program.

Among many AAP method, **split sample method (split sample with another laboratory**) is much common in practice. This procedure is used for externally verifying test results by sending aliquots of samples to another laboratory(ies) for testing.

When adopting split-sample procedures, laboratories should consider their institutions' requirements for informed patient consent and maintenance of patient confidentiality.

2. Procedure:

2.1 Reference lab:

NPHL acts as reference lab for retesting of COVID-19 PCR tests by split sample analysis method.

2.2 Frequency:

The testing laboratories will have to send samples once a month.

2.3 Number of samples:

At least five negative samples and up to five positive samples need to be sent to reference lab per batch (each month).

The number of samples may be more if clinically indicated.

2.4 Time of sample shipment:

If the participant lab has proper sample storage facility (-80 degree freezer required, if samples have to be stored for months), samples can be sent to reference lab at a certain date (25th of the particular month).

If such storage facility is not available, samples can be shipped any time of the month as per participating lab's convenience. More often, the time when positive samples are reported is presumed to be right time as negative samples are usually present all month round.

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2.5. Packaging:

The samples must be triple layer packaged as per biosafety protocol. Temperature should be maintained at 2 to 8 degree Celsius.

2.6 Participating lab must duly fill the EQA form (provided in annex II) and send it along with samples. The lab ID of samples sent must be matched completely in forms and sample containers.

2.6 Communication to reference lab:

Communication must be done with reference lab before samples are dispatched. A mail to official mail address (nphl@nphl.gov.np) is mandatory. Verbal communication with designated personnel (information officer- contact details present at nphl website) in addition to mail is recommended.

** Please metion QC COVID as subject while communicating via mail for any issues regarding to this EQA program*

3.0 Evaluation of results:

3.1 Evaluation procedure

- Samples will be retested at reference lab by same method (qPCR) technique
- To evaluate the contribution of chance to agreement between two sets of data, the kappa statistic will be used.
- The kappa statistic compares observed agreement with agreement that might be expected by chance.
- Kappa values range from +1 (complete agreement) through 0 (no agreement beyond that expected by chance) to –1 (complete disagreement—which in the laboratory setting suggests systematic reversal of results, perhaps by clerical or programming error).
- Kappa values above 0.8 can be considered excellent analytic agreement, and those between 0.6 and 0.8 can be considered to be reasonable agreement.

3.2 Reporting of results:

- The report of retesting (split sample analysis) will be provided within 15 days of sample receipt at reference lab.
- A standardized format will be used to provide reports to participating labs (provided in annex III)
- Reports will be made available via mail.

3.3. Documentation:

- Results of retesting EQA should be documented and retained by the laboratory (both reference lab and participating lab) so that trends can be identified.
- Corrective action in response to unacceptable results should be documented by participating lab.

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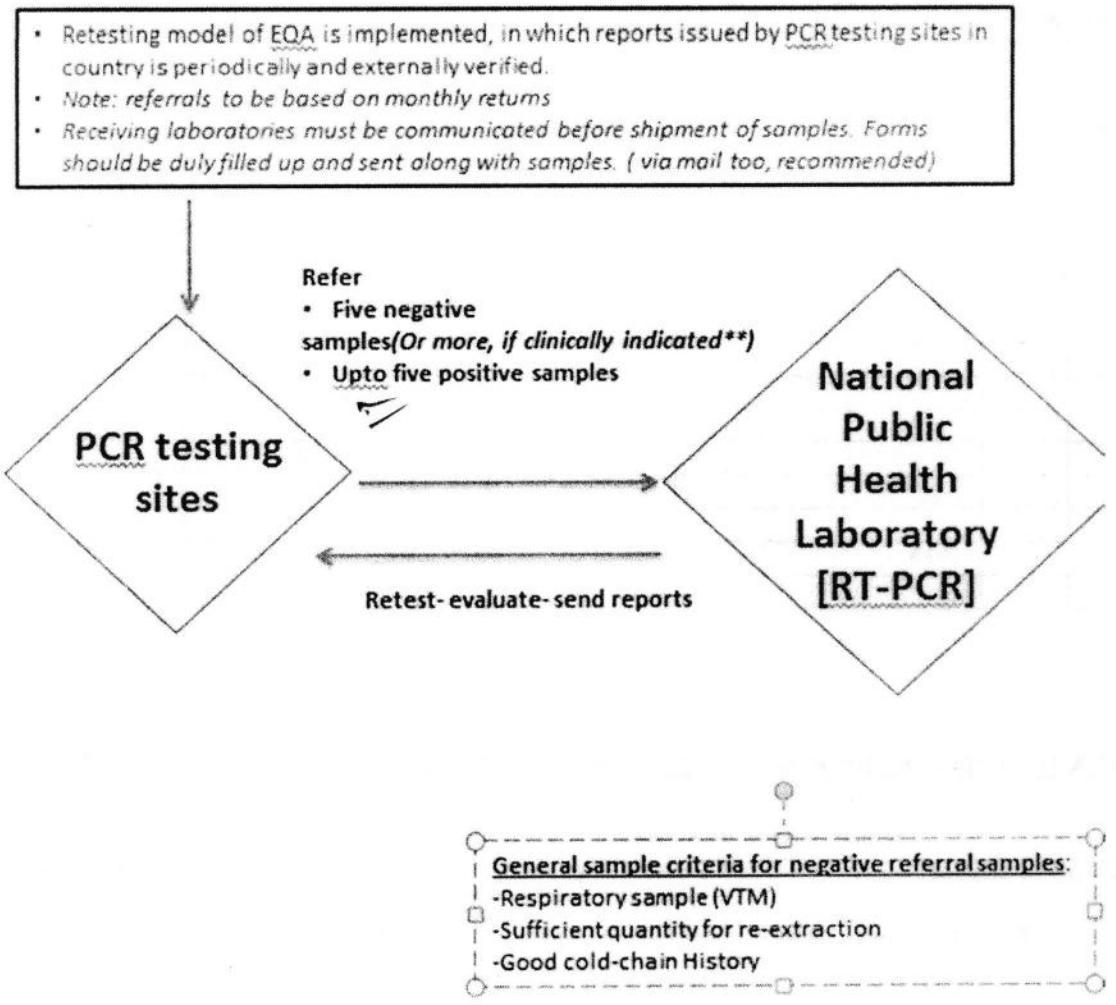
ANNEX I: Flowchart for Retesting (split sample analysis) samples of COVID19 PCR

COVID-19: Routine referral of NEGATIVE samples for confirmatory analysis



Note: Samples must be transported by the referring laboratory using proper containment for a Category B Infectious Agent (as per UN & IATA regulations) and be accompanied by a chain-of-custody form.

** *Potential clinical indications could include: deteriorating clinical picture, repeated indeterminate PCR test results, etc.*

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ANNEX II: Form to be filled by participating lab

PCR lab(Name/ address): Month/ Year: Mailing date: Form completed by: Verified by:	Test information:						For NPHL staff only
		kit name	lot no	expiry date	Platform used	Sample ID (if different kits being used)	Received date: examined by: date examined:
	Extraction						
	PCR						

SN	Sample ID	Patient information			Sample type	Test result	Remarks (mention CT values in case of positive samples)
		Name	Address	Age/Sex			

ANNEX III: Reporting form to be used by reference lab (NPHL)

Summary of results of External Quality Assessment of COVID-19 PCR testing

Sample type:	Number of Samples			Condition of samples when received (Circle the alternatives)	Agreement of results (NPHL vs Testing site)	
	Received	Tested	Rejected	Poor/ Satisfactory/ Good	Total number of results agreed	Total number of results disagreed

Table 1: Comparison of site results and NPHL results

		NPHL results		Total
		Negative	Positive	
Site results	Negative			
	Positive			
	Total			
Percentage agreement =				
Kappa* =				
Strength of agreement =				

Discussion/Recommendations:

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