GUIDELINES FOR NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME

FOR

STI/TTI SEROLOGY

First Edition 2013

Public Health Laboratory Services
Ministry of Health
Thimphu, BHUTAN

World Health Organization

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ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
EQA	External Quality Assurance
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
NEQAS	National External Quality Assessment Scheme
NSRL	National Serology Reference Laboratory
PHL	Public Health Laboratory
QA	Quality Assurance
QC	Quality Control
QI	Quality Improvement
RPR	Rapid Plasma Reagin
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
TTI	Transfusion Transmitted Infection
TPHA	Treponema Pallidum Hemagglutination Assay

1. Introduction

Sexually transmitted infections (STIs) and Transfusion Transmissible Infections (TTIs), particularly HIV/AIDS if left unmanaged have far reaching health, psycho-social and economic consequences and an additional burden of disease to the national health system. Thus testing for STIs and TTIs for clinical diagnosis and transfusion safety are very essential. Therefore to ensure reliable test results, establishment of quality assessment scheme becomes a crucial component of laboratory quality assurance programme.

The availability of excellent tests in the market does not automatically guarantee reliable laboratory results. This is because many steps are involved from receipt of a specimen, analysis and reporting of the test result and each of these steps sometimes can go wrong. Therefore it is imperative that a continuous mechanism of monitoring and improvement is in place in the country, conducted by Public Health Laboratory. Such a periodic monitoring mechanism is an important step towards achieving high-quality laboratory performance nationwide. To avoid confusion following are distinct definitions of the three commonly used terms:

1. *Quality Control (QC):* - comprises of all those measures that must be taken during each test run to verify that the test is working properly. It includes ensuring correct temperature conditions, kit controls, etc. it indicates that the test run was valid and has produced acceptable results. It does not guarantee the accuracy of results and reports provided to the physician.

2. *Quality Assurance (QA):* - it is the total process that guarantees the accuracy of the results and reports provided. It involves inspecting specimens, reviewing transcriptional details, reliability of assays used and verifying the final reports and results

3. External Quality Assessment (EQA): - It is an external evaluation of a laboratory's performance using known but undisclosed panel samples. Quality assessment is undertaken at periodic intervals to evaluate the effectiveness of the QA program of a participating laboratory. EQA allows participating laboratories to assess their performance levels in comparison to others in the network so that corrective action and preventive action (CAPA) can be implemented for improvement.

2. Objectives

1. Assess the performance of testing centers
2. Compare the inter-laboratory performance.
3. Identify common errors and provide corrective measures
4. Institute and upgrade uniform quality system in each Laboratory in the country.

3. Structure of NEQAS

3.1 Organizing laboratory

Public Health Laboratory as mandated by the ministry of Health, RGoB shall be the organizing laboratory for the NEQAS in STIs and TTIs namely HIV, Hepatitis B, Hepatitis C and syphilis serology.

3.2 Participating centres

All the testing centers shall be mandated to participate in the NEQAS program. These centers include National, regional, district laboratories, blood centres, Voluntary Counseling and Testing Centers (VCTs) and in future the private laboratories.

3.3 Frequency of Assessment

NEQAS shall be conducted bi-annually.

3.4 Roles and responsibilities

3.4.1 The Organizing laboratory –PHL

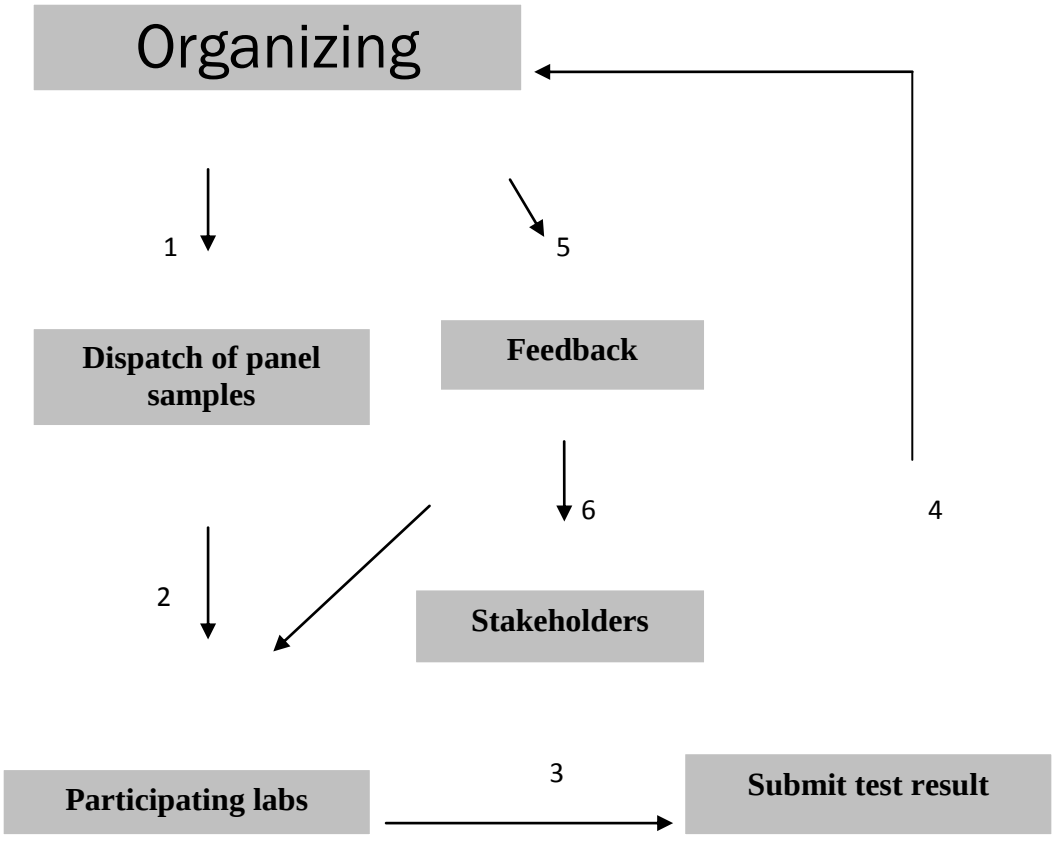
- Shall have the necessary expertise in the field.
- Develop, review and update the guidelines.
- Provide training to participating laboratories (Pre-NEQAS and /or Post NEQAS)
- Shall be acquainted with available screening and diagnostic assays used in the country
- Shall be responsible in preparing, packaging and dispensing the panel specimens.
- Shall be responsible for receiving the results, analyzing data and preparing and dispatching reports.
- Provide necessary feedback to all testing centers and conduct follow-up visits

3.4.2 Participating Centers

Participating centers should understand the objectives of NEQAS and enroll in the program. They will be responsible for the following;

- The staff of the testing centers should read and be aware of all the documents, including the NEQAS guidelines, instructions in the NEQAS, forms accompanying the panel specimens and feedback reports
- Test the panel specimens in the same manner as any routine specimen
- Return the completed test result form to organizing laboratory within the given deadline
- Study the feedback report and recommendations provided by organizing laboratory and bring in corrective actions and preventive measures.

Figure 1: - NEQAS Flow Process



4. Design of NEQAS

4.1 Panel specimens

The panel specimens comprises of positive and negative serum (liquid), containing the following;

- Antibodies to HIV 1 & 2
- Hepatitis B surface antigen
- Antibody to Hepatitis C
- Antibody to Treponema Pallidum

4.2 Panel specimen preparation and Stability test

The detailed procedure for panel sample preparation, homogeneity test and stability test is described in **Annexure 1 & 2**

4.3 Panel packaging and transport

The vial shall be packed in plastic zip-lock pouch and put in double-bubbled plastic bag with adsorbent paper at ambient temperature in compliance with local postal regulations for transportation of infectious substances.

The packing label shall include name of the scheme, contact details of the consignee and that of the organizing laboratory.

Also included in the package (package insert) will be the instruction for handling panel samples **(Annexure 3).** The transportation of the panel shall be contracted to registered national courier service.

4.4 Result submission

Each participating center shall send the filled result form to the PHL either through Fax, Email or by Post.

4.5 Data Analysis

The data analysis and use of the suitable scoring index is an important step in the process of EQAS. It is used for judgment of individual laboratory performance. In this program the performance shall be assessed based on scoring criteria and percentage accuracy as follows:

4.5.1 Accuracy in Percentage

Accuracy is calculated using the following formula: -

$$\text{Accuracy in (\%)} = \frac{\text{Total number of correct results}}{\text{Total no of panel samples}} \times 100$$

4.5.2 Scoring criteria

A scoring scheme shall also be used to assess the performance of the participant center.

Graded scores shall be given based on

a. The results and

b. The mention by the center 'to refer reactive or positive or indeterminate results for confirmatory testing'.

This is used so that participants shall be encouraged to refer all difficult, positive, equivocal or indeterminate samples to differentiate true positivity from cross reactivity to as part of good laboratory practices.

Sl.No	***PHL***	***Participating Lab***	***Score***
1	Strong Positive	Positive and referral to PHL for confirmatory test mentioned	2
		Positive but no referral to PHL for confirmatory test mentioned	1
		Indeterminate and referral to PHL for confirmatory test mentioned	1
		Indeterminate but no referral to PHL for confirmatory test mentioned	0
		Negative	0
2	Weakly Positive	Positive and referral to PHL for confirmatory test mentioned	2
		Positive but no referral to PHL for confirmatory test mentioned	1
		Indeterminate and referral to PHL for confirmatory test mentioned	2
		Indeterminate and no referral to PHL for confirmatory test mentioned	1
		Negative	1
3	Negative	Positive and referral to PHL for confirmatory test mentioned	0
		Negative	2

4.6 Feedback Report

The feedback report will be sent to the participating Centers in two formats; preliminary report and summary report.

In the incidence of non-conformity results the preliminary report (**Annexure 6)** shall be sent immediately after receiving the individual panel report**.** However, **s**ummary report (**Annexure 7)** shall be sent once analysis is completed.

The summary reports contains the performance reports of the testing Laboratory based on the misclassification score and accuracy percentage as shown above.

The preliminary and summary report will be sent to all participating Centers by fax or email. The summary report will be shared with concerned program.

5. Interventions

If non conforming results are found during preliminary checking, the organizing lab will follow up with the participant lab immediately with preliminary report to verify the results. After verification, the testing center shall take immediate action to rectify the problem. The organizing lab with support from concerned program shall analyze the cause of poor performance and take remedial actions to improve the NEQAS program.

6. Participants' response time

All participating centers shall fill the test results and other required information in the report form (**Annexure 6).** The participants should also retain and document a copy of the report form.

The turn-around time should be calculated from the time the sample is dispatched from the organizing lab till the time the report is received. This will enable organizing lab to make each participating center aware of the time to report their results.

A maximum of one month from sample dispatch shall be allowed for the testing center to report their results to the reference lab. Any delay in reporting shall not be entertained and the particular testing center shall be scored zero for which the responsible staff of the testing center shall be liable for explanation to the concerned authority.

7. Test kits performance

Brand name of test kits with the methods/principle, etc. (**Annexure 6**), used by participating laboratories should be recorded. This will give an overview of different type of test kits used and its performance, which may be helpful in future for evaluation of the test kits.

8. On-site evaluation

Onsite supervisory visit shall be carried out in those labs that are consistently performing poorly based on proficiency score index using standard checklist **(Annexure 7).**

9. Confidentiality

Confidentiality will be maintained for all participating centers using individual testing center's identification code.

10. References

1) UNAIDS 2008 report on global AIDS epidemic, WHO.

2) Health worker's manual on syndromic management of sexually transmitted infection-2002, National STD/AIDS program, Public Health Division, Ministry of Health and Education, Thimphu, Bhutan.

3) India: World Health Organization (WHO), Regional office for Southeast Asia, New Delhi, 2003.

4) Guidelines for organization national External Quality assessment schemes for HIV serology testing, UNAIDS/96.5.

5) Regional External Quality Assessment Scheme, National Institute of Health, Bangkok Thailand.

6) Quality Control Sample; Quality Assurance of HIV testing, NIH; Bangkok; Thailand.

7) Guidelines for the management of sexually transmitted infections; world health organization; 2001.

11. Annexure

Annexure: 1

Panel sample preparation procedures

1. Preparation of negative stock solution

Collect large volume of blood sample from blood bank for serum and plasma extraction by re-calcification. The negative stock must be tested for all STI serology before proceeding for panel preparation.

Re-calcification procedures

- Make a 2 mol/l solution of $CaCl_2.2H_2O$ by adding 3g of $CaCl_2.2H_2O$ to 10 ml of distilled water in the plastic bottle.
- Add 0.5 ml of the freshly prepared $CaCl_2$ solution to 100 ml of volume of plasma. The final concentration should be 0.01M $CaCl_2$.
- Mix the solution and plasma properly and incubate in a water bath at 37ºC for 1 hour. Large volume may require several hours to clot plasma.
- If plasma has not clotted, add more $CaCl_2$ (not exceeding a total of 1% of the 2 mol/l solution) and incubate the mixture further.
- When the plasma has clotted, remove mixture from the water bath and transfer plasma to the bottle and place in a freezer at – 20ºC over night.
- Remove the bottle from the freezer; allow thawing at room temperature.

2. Preparation of positive stock samples

Use strong known positive samples (HIV, HBV, HCV and syphilis) for the preparation of positive stock. Use the negative stock to dilute positive stock.

3. Dilution of positive stock samples

- Carry out serial 2-fold (for rapid test kit) and 10-fold dilution (for ELISA) as shown in Figure 2.
- Add 1ml of known positive serum in the first tube and 1 ml of negative serum OR 100ul of known positive serum in the first tube and 900ul of negative serum in all the rest of the tubes.
- Pipette out 1 ml/100ul of serum from tube 1 and transfer to tube 2.
- Mix well, transfer 1 ml/100ul serum to tube 3.
- Repeat the step and continue till the last tube.
- Discard 1ml/100ul of mixed serum from the last tube.
- Test mix serum from each test tube by the ELISA and rapid test kit to determine the appropriate concentration (OD value).
- Test mix serum from each tube by the rapid test kit to determine detection titer level.
- Record results in worksheet (**Annexure 8**)

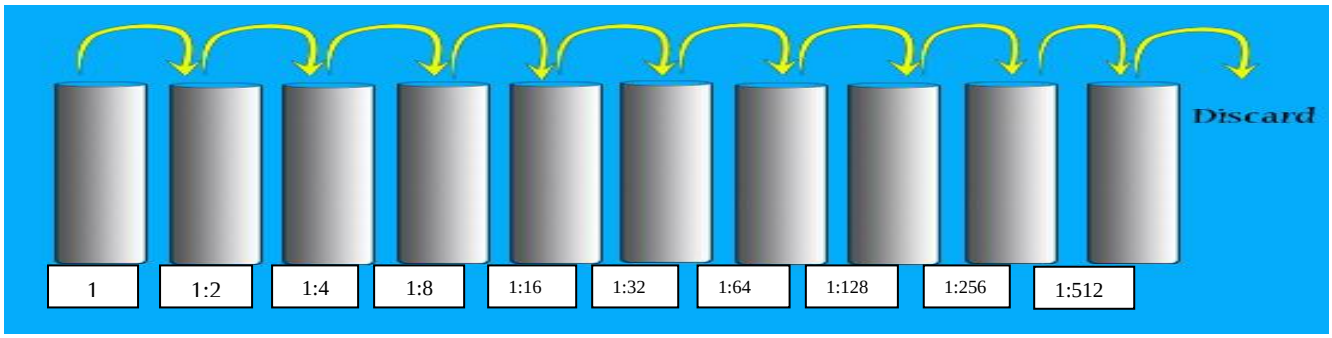


Figure 2: Dilution of positive stock

4. Selection of known positive stock samples for panel

The composition of positive panels should consist of strong reactive, weak reactive and negative samples for every test concerned. However, weak positive should be the priority by large to assess the proficiency.

5. Heat inactivation

Set the water bath at a temperature of 56ºC and monitor the temperature with a thermometer. Once the temperature has reached 56ºC, place the selected positive samples in the water bath and heat for 60 minutes. *Note: Do not heat samples beyond 56ºC and do not heat-inactivate negative panel because heating may cause false positive reactions.*

6. Filtration

Centrifuge the heat inactivated positive and negative samples at 10,000 RPM at 4˚C for 10 minutes and filter the samples through a membrane filter of 0.45µ pore size. Filter small volume at a time to avoid clogging.

7. Preservation

To inhibit the growth of bacteria contaminants in the sample, use chemical disinfectant as preservative/biocides. The commonly used preservatives are 0.05% of Proclin 300, and 0.05% Bronidox L (5 bromo-5- nitro-1,3-dioxane in propylene glycol. Add 0.05 ml (50 µl) of preservatives to every 100ml of serum and mix well.

8. Characterization of panel samples

Characterize the panel to be tested for range of diagnostic test kits available with participating prior to distribution. The ranges of test used should be adequate to establish the sera as positive, negative, or indeterminate. A selection of specimens should be made to compose a particular panel for the distribution. Record the results (**Annexure 9)** for traceability and to use as an expected result for preliminary report to participants.

9. Aliquot and Labeling

Dispense 500ul the panel samples into leak-proof screw-capped plastic micro vials with O-ring. Properly label the samples with a consecutive number for each specimen. The labeling should not be washed off or removed from vial.

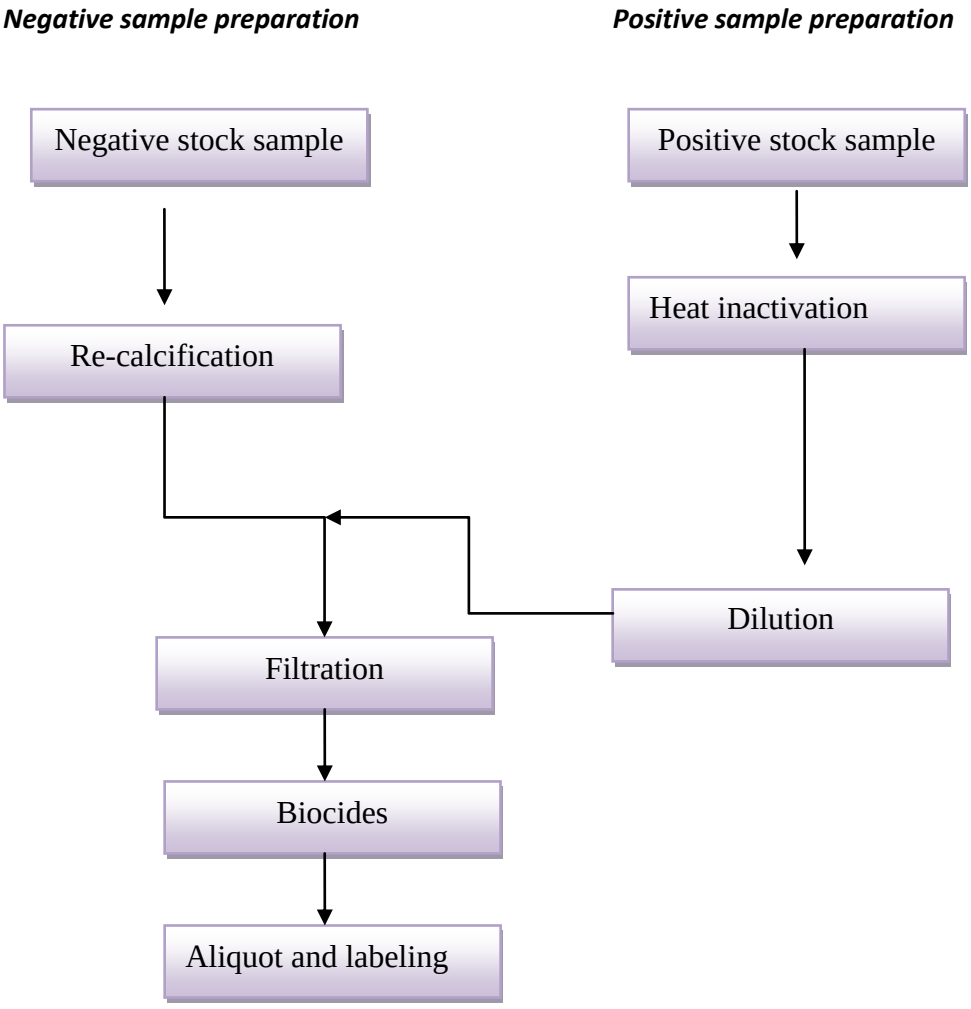


Figure 3: Flow chart for panel specimen preparation

Annexure: 2

TRANSPORTATION STABILITY CHECK FORM (For Organizing Lab)

Sl.No	S/Co ratio					
	Returned sample			Retained sample(4C)		
	Duplicate 1	Duplicate 2	Mean (u1)	Duplicate1	Duplicate 2	Mean (u2)
1						
2						
3						
4						
5						
6						
7						
8						

Package Insert

(Please read the instructions before performing tests)

- Keep NEQAS panel in the refrigerator at 2-8˚C until ready to perform test
- The NEQAS panel comprises 6-8 coded samples, each containing 0.5 ml
- All samples contain 0.05% Proclin300 as preservative which does not interfere with the test methodologies
- The positive samples have been inactivated by heating at 56˚C for 1 hour; however, it should be handled as potently infectious specimen
- Vortex samples for few second before testing
- The samples have to be processed in the same way as routine specimens to reflect day-to-day functions of your laboratory
- Record results in the form provided.
- Complete the form for each type of test performed
- Fax result form at **02 332464** or attached as an e-mail message kelfinx_srmc@ymail.com to organizing lab before deadline at _____

PRELIMINARY REPORT FORM

LAB NO: -……

Sl. No	PHL Result	Test Parameters				
		HIV	HBsAg	HCV	RPR	TPHA
		Participating Lab Result				
SR.00.00.00						
SR.00.00.00						
SR.00.00.00						
SR.00.00.00						
SR.00.00.00						
SR.00.00.00						
SR.00.00.00						
SR.00.00.00						

Recommendations: -

……

……

……

..

******In compliance to the requirements of the EQAS programme the identity of participating laboratories is kept confidential******

(In-charge)

Infectious Disease Serology Reference Laboratory

PHL, DoPH

MoH

Annexure: 5

SUMMARY REPORT FORM

Trial no: -……

La No: -……

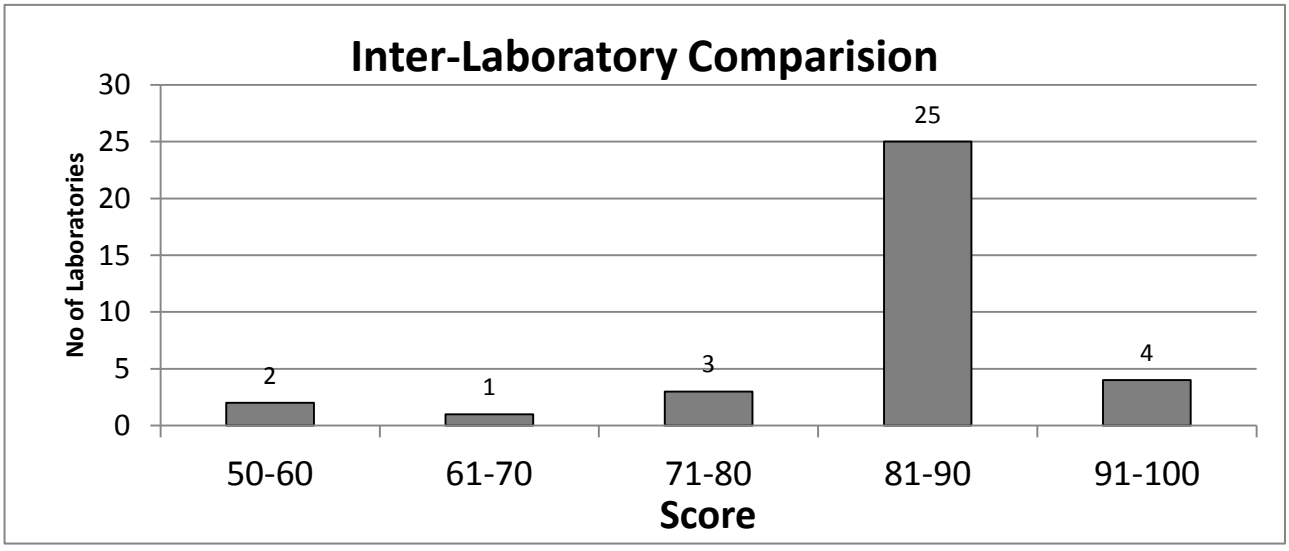
Sl. No	PHL	Lab Result	Score
SR. 2013/10/01			
SR. 2013/10/02			
SR. 2013/10/03			
SR. 2013/10/04			
SR. 2013/10/05			
SR. 2013/10/06			
SR. 2013/10/07			
SR. 2013/10/08			

Total Score: -

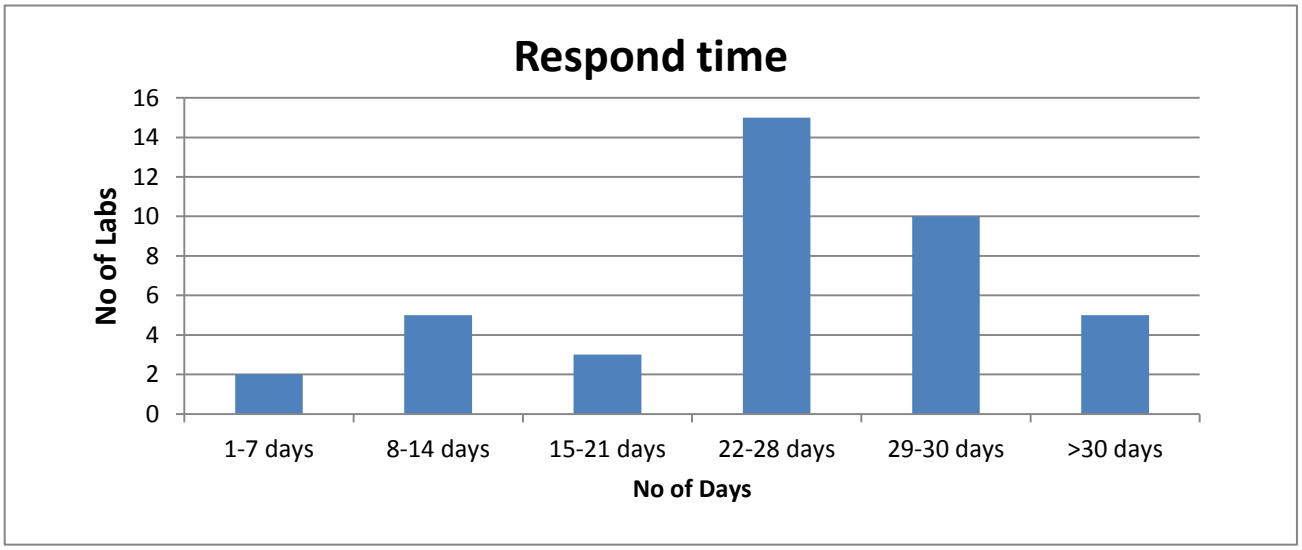
Efficiency/ Accuracy

Lab	Test Method	No of specimen tested	TP	TN	FP	FN	Accuracy (%)
1	Rapid						

Inter-Laboratory Comparison chart



Participants Respond time



Recommendations: -

……

……

……

******In compliance to the requirements of the EQAS programme the identity of participating laboratories is kept confidential******

(In-charge)

Infectious Disease Serology Reference Laboratory

PHL, DoPH

MoH

Annexure: 6

REPORTING FORM

Name of Hospital: -……..District: -…… Lab CODE: -……..

Address

Name of Contact Person: -…….. Mobile: -……..

Phone: -…….. Fax: -…….. E-mail: -……..

Test Result

Sample ID	**Anti-HIV (P/N)**	**Anti-HCV (P/N)**	**HBsAg (P/N)**	**RPR (R/NR)**	**TPHA (P/N)**
SR.00.00.00					
SR.00.00.00					
SR.00.00.00					
SR.00.00.00					
SR.00.00.00					
SR.00.00.00					
SR.00.00.00					
SR.00.00.00					
Kit Used: -					
Manufacturer: -					
Expiry date: -					
Performed By: -					

(Note: - P-Positive, N-Negative, IND-indeterminate/Intermediate-Reactive, NR-Non-reactive)

******Comments on your results and give details of any further testing you may do or send away to confirm any of the results******

Comments: -

……

……

……

Received(panel) date: -

Test date: -

Dated signature of Technician

NEQAS Assessment checklist for Supervisory Visit

1. **Name of the Hospital: -**
2. **DMO/CMO/Superintendent: -**
3. **Name of Laboratory Technician: -**
4. **Head of Laboratory/Contact person: -**
5. **Name and designation of supervisor: -**

Tick the most appropriate one and provide comments where necessary. *Y: Yes*

N: No P: Partial NA: Not Applicable

Section	ITEM	Y	N	P	Comment
A. Personnel and Organization					
A.1	Does the laboratory have sufficient staff?				
A.2	Are the laboratory technicians aware of NEQAS activities that PHL conducts half-yearly?				
A.3	Have deputies/alternates for all key functions been identified, if the authorized personnel are not available?				
B. Training					
B.1	Has there been any change in the staff since last supervisory visit?				
B.2	Has new staffs received proper training? Has each laboratory personnel participated in refresher training/workshops within past two years?				
B.3	Does the laboratory have training records of the laboratory personnel?				
C. Waste Management					
C.1	Is a system in place for handling of the waste in accordance with the local regulatory requirements?				
C.2	Is the waste segregated at source in appropriate containers including needles & sharps?				
C.3	Is the waste decontaminated & autoclaved before disposal?				
C.4	Are the equipments used for the disposal of waste (e.g. Autoclave) validated for their performance?				
D. Specimen Shipment					
D.1	Does the laboratory ship specimens to a reference laboratory for confirmation?				
D.2	Does specimen transport follow proper organization, packaging, shipping to ensure integrity, timely and safe transfer?				

Section	ITEM	Y	N	P	Comment
D.3	Are the specimens accompanied with complete details(name, specimen ID, test, other details)				
E. Standard Operating procedures/ Manuals/Guidelines					
E.1	Does the lab have SOP for specimen collection and handling?				
E.2	Does the lab have SOP for equipment maintenance, calibration, operation and cleaning?				
E.3	Does the lab have SOP for test procedures?				
E.4	Does the laboratory have NEQAS guideline?				
E.5	Is the SOP reviewed & updated regularly?				
F. Documentation					
F.1	Does the lab have organisation charts, personnel qualifications, training, experience and job descriptions stored?				
F.2	Are the records and reports of calibration and maintenance of equipments and validation of computerised systems archived?				
F.3	Are the Internal and external quality control, and inspection records archived?				
G. Quality assurance					
G.1	Does the laboratory check the quality of new batch of rapid test kits?				
G.2	Are the records of Internal quality control retained?				
G.3	Does the laboratory visually cross-check test results by another staff in the same laboratory?				
G.4	Has the laboratory participated in External Quality Assessment by Proficiency Panel Testing by PHL this year / last year?				
H. Safety measures					
H.1	Do the Laboratory personnel use PPE every time serology work is performed?				
H.2	Is there Autoclave in the Laboratory?				
H.3	Does the laboratory monitor the function of autoclave? [If yes, how- mention in comment.]				
H.4	Does laboratory worker use disinfectant? Mention kind of disinfectant used?				
H.5	Is there a basin or a sink where worker can wash hand at every interruption of work?				
H.6	Are workers wearing lab coats & removed prior to leaving the laboratory?				
H.7	Is biohazard waste bin with lid available?				
H.8	How often do the laboratory workers take medical examination including for HIV & HBsAg?				
I. Stock Register					
I.1	Is there stock register in place to monitor stock balance?				
I.2	Is the test kit used in First-In-First-Out (FIFO) basis?				
I.3	Has the laboratory technician maintained the register properly?				
I.4	Does the Laboratory have logbook for equipment maintenance?				

Section	ITEM	Y	N	P	Comment
J. Logbook					
J.1	Do the Laboratory have logbook for equipments like refrigerator?				
J.2	Do the Laboratory have logbook for equipment maintenance?				
J.3	Is the Logbook properly entered and up-to date?				

Annexure: 8

Positive stock preparation log (For ref lab)

Panel no: -……

Test	Result	*Stock Dilution*											
		1:2	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**	**1:512**	**1:1024**	**1:2048**	**1:4096**
HIV													
HBsAg													
HCV													
TPHA													
RPR													

(Note: - P-Positive, N-Negative)

PANEL SPECIMEN CHARACTERIZATION FORM (for organizing lab)

Panel no: - …….

Sample ID	***Anti-HIV***		***Anti-HCV***		***HBsAg***		***RPR***	***TPHA***	
	Test Type								
	Rapid	*EIA*	*Rapid*	*EIA*	*Rapid*	*EIA*	*Rapid*	*Rapid*	*GPA*
SR.00.00.00									
SR.00.00.00									
SR.00.00.00									
SR.00.00.00									
SR.00.00.00									
SR.00.00.00									
SR.00.00.00									
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Public Health Laboratory
Department Of Public Health
Ministry of health
Thimphu Bhutan