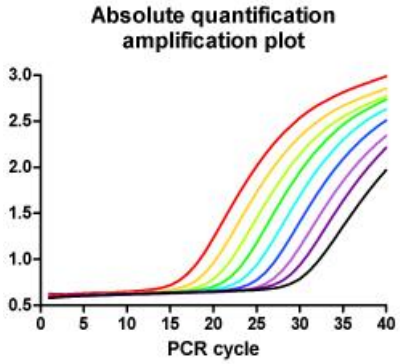

GUIDANCE MANUAL

ON

QUALITY CONTROL EVALUATION OF HBV, HCV & HIV-1 MOLECULAR DIAGNOSTIC KITS

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AMENDMENT SHEET

S.NO	Date	Page no.	Revision No.	Nature of Amendment Section/ details	Authorization

FOREWORD

In developing countries the major hurdle in treatment of a disease is improper diagnosis. Many times it has been observed that the technology used for the diagnosis of a disease has not been selected properly and is not an advanced one. It has been witnessed in many infections including critical infections like HIV, HCV and HBV that serology tests fail to detect window period cases. Therefore, blood safety is a major challenge in India because of the high sero-prevalence of HIV (0.3%), HCV (0.7%) and HBV (1.4%) in blood donor population and relatively low percentage (55%) of voluntary blood donors. In order to narrow the infectious period (window) between the time of viral exposure and the time a virus can be serologically detected, blood centres of the developed countries started nucleic acid testing (NAT) technology for screening HIV, HCV and HBV infections in late 90s but, developing countries including India are still in the process of implementing this technology. There is the potential that NAT testing could completely eradicate the transfusion risk of HIV, HCV and HBV. The test is thus likely to add substantially to the safety of blood transfusions and has been implemented by all blood suppliers and transfusion services in the United States. The potential benefits of this testing for blood safety are great and the risk is considered minimal.

ABBREVIATIONS USED

DCG(I)	Drugs Controller General of India
FP	False Positive
FN	False Negative
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
NAT	Nucleic Acid Testing
NABL	National Accreditation Board for Testing and Calibration Laboratories
NIB	National Institute of Biologicals
NIBSC	National Institute of Biological Standards and Control
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
SRRDU	Sample Receipt and Report Dispatch Unit
TP	True Positive
TN	True Negative

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The hard work and sincere efforts of all lab staff members are highly acknowledged.

I hope the guidance manual will serve as a Reference Document to all stakeholders for required information on QC evaluation of molecular diagnostic kits.

Dr. Reba Chhabra
S-II & Lab. Head
NAT Laboratory

CONTENTS

PURPOSE

The purpose of this guidance manual is to provide information to manufacturers/ suppliers of molecular Diagnostic Kits and blood banks about Quality Control evaluation of molecular diagnostic Kits at NIB.

SCOPE

1. To provide information about requirements of samples and documents for performance evaluation of molecular diagnostic kits.
2. To inform about the technical procedures followed at NIB for evaluation of molecular diagnostic kits.
3. This information will save time of everyone involved.

INTRODUCTION

National Institute of Biologicals is an autonomous institute under the Ministry of Health & Family Welfare set up for the quality assessment of biological products which are to be marketed in India. The institute works in coordination with the Regulatory Authorities such as Central Drugs Standard Control Organization (CDSCO) and Indian Pharmacopoeia Commission.

Nucleic acid based testing (NAT) laboratory has been established for quality control evaluation of molecular diagnostic kits intended to be used for (i) viral load monitoring for Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human immunodeficiency virus-1 (HIV-1) (ii) Blood donor screening for HBV, HCV and HIV-1 infections.

FLOW CHART: Movement of Sample / Kit / Information for QC Evaluation

The molecular diagnostic kits for Quality Control Evaluation are forwarded by offices of DCG (I) / ADC (I) / CDSCO to National Institute of Biologicals, Noida. These are received by Sample Receipt and Report Dispatch Unit (SRRDU) at NIB. The SRRD Unit after completing its own formalities forwards these kits to NAT Laboratory.

Sample(s) forwarded by DCG (I) / ADC (I) / CDSCO

Sample(s) received at SRRDU (NIB)

Sample(s) received in NAT Laboratory

QC Testing/ Performance evaluation

Reporting / Issuing Certificate of Analysis

CoA to office of the Director

CoA sent for updating at NIB website

CoA sent to SRRDU (NIB)

CoA  **sent to DCG (I)**

NUMBER OF TESTS REQUIRED FOR PERFORMANCE EVALUATION

S.no	Name of the Product	Intended use	Quantity Required	
			Testing	Retained
1	Molecular Diagnostic kit	Viral load monitoring / Quantitation	48 Tests	48 Tests
2	Multiplex molecular Diagnostic kit	Blood donor screening	96 Tests	96 Tests
Note: At the time of kit/sample submission to NIB, the remaining shelf life of the kit should be at least 60% of the total shelf life.				

DOCUMENTS REQUIRED FOR PERFORMANCE EVALUATION (TO BE SUBMITTED BY THE SUPPLIER / SAMPLE SUBMITTER TO NIB)

1. Evaluation fee (Testing fee)
2. Test license
3. Certificate of Analysis from the manufacturer
4. Batch release certificate from the manufacturer
5. Kit brochure/ package insert/ leaflet showing indented use of kit.
6. Dossier (For new sample, to be submitted once for that particular sample)
7. Dossier review fee (for item at s.no.5 above)
8. Calibration certificate of the instruments to be used for evaluation (If installed by the supplier at NIB)
9. OPTIONAL: Previous regulatory approval if any (from any country)
10. OPTIONAL: List of users in India and abroad.
11. OPTIONAL: Whether listed in WHO list of prequalified diagnostic products if yes, proof document with validity period.

PROCEDURE FOR KIT / SAMPLE RECEIVING

Sample / Kit brought to LAB by SRRDU

Cold chain maintained

If yes, tally details of kit label with SRRDU register record

Record the details of kit in sample receipt register

Store the kit as per manufacturer's instructions

Report deficiencies if any, to SRRDU (NIB)

QUALITY ASSURANCE OF MOLECULAR DIAGNOSTIC KITS

Quality assurance ensures the reliability of the test results of molecular diagnostic kits. It minimizes the variability arising due to various factors. The main feature of quality assurance is result interpretation based on reference data.

The evaluation of molecular diagnostic kits is carried out with plasma panel prepared from the plasma collected from various blood banks located in NCT of Delhi and other states. These panel members have been characterized for HBV, HCV and HIV-1 and stored at -20°C.

Parameters which affect quality assurance

1. **Performance Evaluation:** The parameters such as sensitivity and specificity must be determined as per SOP/WHO guidelines.
2. **Validation of Tests:** Tests must be validated before implementation and records of such validation should be maintained in the laboratory.
3. **Verification of results:** The evaluation test results must be verified by the Analyst/Senior Analyst.
4. **Certificate of Analysis (CoA):** The performance evaluation results should be presented on an approved CoA format. The CoA must be signed by the Analyst and Senior Analyst/Head of Laboratory. The CoA must be approved by the Director, NIB before its release.
5. **Turn-around time:** The performance evaluation should be accomplished with in a stipulated time.
6. **Equipment:** The equipment used for quality control evaluation must be calibrated and regular maintenance practices should be followed as per quality policy.

S.no	Name of the Product	Intended use	Turnaround time (Working days)
1	Molecular Diagnostic kit	Viral load monitoring / Quantitation	30

2	Multiplex molecular Diagnostic kit	Blood donor screening	30

Procedure for preparation of Plasma panels for evaluation of molecular diagnostic kit

Seroreactive (HBV / HCV / HIV-1) plasma samples

↓

Nucleic acid extraction as per kits instructions

↓

Amplification with respective reagents and Target detection
As per manufacturers' instructions

↓

If, Target detected (If found NAT positive)

↓

Included in respective panel

↓

Panel used for respective molecular diagnostic kit evaluation

Performance evaluation of molecular diagnostic kits

Plasma panel composition and size for evaluation of molecular diagnostic kits

S.No	Name of the Product	Intended use	Panel size & composition		
			Positives	Negatives	Total
1	Molecular Diagnostic kit	Viral load monitoring / Quantitation	20	15	35
2	Multiplex molecular Diagnostic kit	Blood donor screening	60	15	75

Molecular diagnostic Kit (Received for evaluation)

Assignment of appropriate plasma panel

Extraction of nucleic acid as per manufacturers' instructions

1. **By automated system for the kits meant for totally closed automated system**
2. **Manually for the kits meant for amplification and detection in closed system**

Amplification & Detection as per manufacturers' instructions

Printing raw data after completion of run

Data

compilation in data compilation format

Preparation of Certificate of Analysis (CoA)

Format: Data Compilation

National Institute of Biologicals
NAT Laboratory
FINAL RESULT SHEET
HBV/HCV/HIV-1

S.No	Sample ID	Lab status	Lot result	Repeat.1	Repeat.2
n = 35 for single marker kit					
n = 75 for Multiple x kit					

Note: TND = Target not detected

Compiled by: Name & Signature

Calculation of Sensitivity & Specificity

Sensitivity = {TP / (TP + FN)} X 100

Where TP = True Positive and FN = False Negative.

Sp

Where TN = True Negative and FP = False Positive.

True Positive (TP): When a plasma panel member originally positive for a particular marker is reported positive for that marker by the kit under evaluation.

False Negative (FN): When a plasma panel member originally positive for a particular marker is reported negative for that marker by the kit under evaluation.

True Negative (TN): When a plasma panel member originally negative for a particular marker is reported negative for that marker by the kit under evaluation.

False Positive (FP): When a plasma panel member originally negative for a particular marker is reported positive for that marker by the kit under evaluation.

Manual CoA
NATIONAL INSTITUTE OF BIOLOGICALS
(Ministry of Health & Family Welfare)

F. No.:

Dated:

CERTIFICATE OF ANALYSIS

1. **Date of sample receipt** :
2. **Start Date of analysis** :
3. **CDR No.** :
4. **Analytical report no.** :
5. **Product and Dosage form** :
6. **Name of the manufacturer** :
7. **Batch / Lot No.** :
8. **Manufacturing Date** :
9. **Expiry Date** :
10. **Whether Legal/ Regulatory or Non Regulatory** :

S.No.	Test(s) Conducted	Specifications (As per Lab SOP No. SOP/NAT/28)	Results
1.	**Sensitivity**	**100 %**	• True Positive (TP)= • False Negative (FN) = • **Sensitivity** = {TP/(TP + FN)} X 100 =
2.	**Specificity**	**100 %**	• True Negative (TN) = • False Positive (FP) = • **Specificity** = {TN/(TN + FP)} X 100 =

Conclusion: The Batch/Lot No…….. …..of the above named product…….. as per the requirement(s) of Lab SOP is of standard quality/Not of standard quality

Signature of the Analyst
Name:
Designation:

Signature of the Lab. Head
Name:
Designation:

Date: Date:

Online CoA

NATIONAL INSTITUTE OF BIOLOGICALS
(Ministry of Health & Family Welfare)

Dated:

CERTIFICATE OF ANALYSIS

1. **Date of sample receipt :**
2. **Start Date of analysis :**
3. **CDR No. :**
4. **Analytical report no. :**
5. **Product and Dosage form :**
6. **Name of the manufacturer :**
7. **Batch / Lot No. :**
8. **Manufacturing Date :**
9. **Expiry Date :**
10. **Whether Legal/ Regulatory or Non Regulatory :**

S.No.	Test(s) Conducted	Specifications (As per Lab SOP No. SOP/NAT/28)	Results
1.	Sensitivity	100 %	
2.	Specificity	100 %	

Conclusion: The Batch/Lot No…….. …..of the above named product…….. as per the requirement(s) of Lab SOP is of standard quality/Not of standard quality

Signature of the Analyst
Name:
Designation:
Date:

Signature of the Lab. Head
Name:
Designation:
Date:

List of equipment available in NAT Lab

1. Biosafety cabinet
2. Micropipettes
3. Dry bath incubator
4. Water bath
5. Centrifuge
6. Thermal cycler
7. Realtime PCR system (i-cycler)
8. DNA sequencer
9. MilliQ water purification system
10. Ice flacking machine
11. Gel documentation system
12. Microwave oven
13. Electrophoresis system
14. Weighing balance
15. Spectrophotometer
16. pH meter
17. Magnetic stirrer

List of Standard Operating Procedures

S.No	SOP ID	Title
1	SOP/NAT/01	Extraction of Hepatitis-B virus DNA
2	SOP/NAT/02	Master mix preparation for Hepatitis-B Virus detection by PCR
3	SOP/NAT/03	Nucleic acid based detection of Hepatitis B virus in human serum and plasma by PCR
4	SOP/NAT/04	Extraction of Hepatitis-C Virus and Human Immunodeficiency Virus-1 RNA
5	SOP/NAT/05	Master-mix preparation for Hepatitis-C Virus detection by PCR
6	SOP/NAT/06	Nucleic Acid-based detection of Hepatitis-C Virus in human serum and plasma by PCR
7	SOP/NAT/07	Master-mix preparation for Human Immunodeficiency Virus-1 detection by PCR
8	SOP/NAT/08	Nucleic Acid-based detection of HIV-1 in human serum and plasma by PCR
9	SOP/NAT/09	Agarose Gel Electrophoresis of amplified (Polymerase Chain Reaction) product
10	SOP/NAT/10	Safety measures
11	SOP/NAT/11	Waste Management
12	SOP/NAT/12	Calibration and maintenance of pipettes
13	SOP/NAT/13	Segregation of areas in NAT Laboratory
14	SOP/NAT/14	Operation and maintenance of Thermal cycler
15	SOP/NAT/15	Operation and maintenance of Dry bath Incubator
16	SOP/NAT/16	Operation and maintenance of pH meter
17	SOP/NAT/17	Operation and maintenance of weighing balance
18	SOP/NAT/18	Operation and maintenance of microcentrifuge
19	SOP/NAT/19	Operation, maintenance and calibration of Water Bath
20	SOP/NAT/20	Operation and maintenance of gel documentation system
21	SOP/NAT/21	Operation and maintenance of vortex shaker
22	SOP/NAT/22	Operation, maintenance of Biosafety cabinet
23	SOP/NAT/23	Performance evaluation report preparation
24	SOP/NAT/24	Kit receiving for evaluation
25	SOP/NAT/25	Collection and transportation of blood/plasma bags
26	SOP/NAT/26	Washing of laboratory glassware
27	SOP/NAT/27	Autoclaving (Sterilization)
28	SOP/NAT/28	Evaluation of Molecular diagnostic Kits
29	SOP/NAT/29	Characterization

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