Truenat®

HIV-1/HIV-2

Chip-based Real Time Duplex PCR Test for HIV-1/HIV-2

1. INTENDED USE

REF **Truenat® HIV-1/HIV-2 (REF 601540005 / 601540020 / 601540025 / 601540050 / 601540100 / 601540200)** an automated point-of-care or near patient Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test for the quantitative detection and differential diagnosis of infection with human immunodeficiency virus type 1 and 2 (HIV-1 and 2) in whole plasma and aids in the monitoring of the HIV-1 and 2 viral loads in patients with HIV-1 and/or HIV-2 infection. **Truenat® HIV-1/HIV-2** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® HIV-1/HIV-2** is a single use *in vitro* diagnostics test meant for professional use in near-patient, laboratory or any healthcare settings, by healthcare professionals or any user appropriately trained by a representative of Molbio Diagnostics.

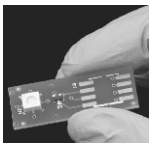
IVD

2. INTRODUCTION

The human immunodeficiency virus (HIV) is a lentivirus (part of the family Retroviridae) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). HIV continues to be a significant public health burden, having claimed more than 34 million lives so far. As per WHO, at the end of 2014 there were approximately 36.9 million people living with HIV and about 2.0 million people becoming newly infected with HIV globally. By mid-2015, 15.8 million people living with HIV were receiving antiretroviral therapy (ART) globally. Expanding ART to all people living with HIV and expanding prevention choices could help avert 21 million AIDS-related deaths and 28 million new infections by 2030.

HIV can be divided into two major types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 is more common and the more pathogenic strain, causing the majority of HIV infections accounts for around 95% globally. HIV-1 is further subdivided into four distinct groups or clades known as M, N, O and P. HIV-2 is less pathogenic and less prevalent. HIV-2 strains are classified into five subtypes; A through E; only subtypes A and B viruses are predominant. HIV-2 is estimated to be more than 55% genetically distinct from HIV-1. It progresses more slowly than HIV-1, resulting in fewer deaths. However, without treatment, most people living with HIV-2 will eventually progress to AIDS and die from the disease. While many commonly used antiretroviral drugs are active against HIV-2, non-nucleoside reverse transcriptase inhibitors (NNRTIs) like nevirapine and efavirenz do not work against it. The best way to treat HIV-2 has been less clearly defined than HIV-1.

In high-income countries, plasma viral load assays are used in combination with CD4 cell counts to determine when to initiate the therapy and when a regimen is failing. Viral replication in the presence of ART favors selection of resistance mutations and treatment failure. Viral load is a very useful tool for monitoring; unfortunately the full benefits of viral load monitoring tests using molecular tests have not yet reached the majority of HIV infected patients who live in countries with limited resources because of the costs and technical constraints. Molecular tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure, also the turnaround time for HIV result could take a few days.



The **Truenat®** point-of-care real time PCR system enables decentralization and near patient diagnosis and viral load monitoring of HIV-1 and/or HIV-2 by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable and battery operated **Truelab®** Real Time Quantitative micro PCR Analyzer and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and room temperature stable **Truenat®** micro PCR chips and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR result in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.

Truenat® HIV-1/HIV-2 is a disposable, room temperature stable, chip-based Real Time RT-PCR test with dried MgCl2 in reaction well and freeze dried RT-PCR reagents in microtube for performing a Real Time RT-PCR test for detection of HIV-1 and/or HIV-2 virus and runs on the **Truelab®** Real Time micro PCR Analyzer. All components of **Truenat®** pouch are nuclease-free. It requires only six (6) µL of purified RNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information. The **Truenat® HIV-1/HIV-2** chip also stores information of used chip to prevent any accidental re-use of the chip

NOTE :Truelab®/ Truenat® / Trueprep® / Truepet® are all trademarks of Molbio Diagnostics Private Limited.

The Truelab® Real Time micro PCR Analyzer is protected by the following patents and patents granted: IN 2313/CHE/2007 (Patent No. 281573), WO2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat® micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat® HIV-1/HIV-2 works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) based on Taqman chemistry. The patient sample (whole plasma) is first pre-treated using the **Trueprep® AUTO** Universal Sample Pre-treatment Pack. The RNA from the patient sample is first extracted using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. The cartridge from the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit contains pre-loaded Internal Positive Control (IPC), composed of known concentration of DNA, trehalose, PBS buffer and amaranth dye, which is co-extracted along with sample nucleic acids, thereby validating the process from extraction to PCR run. The RNA extract is analyzed using the **Truenat® HIV-1/HIV-2** Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test and the **Truelab®** Real Time Quantitative micro PCR Analyzer. The **Truenat® HIV-1/HIV-2** chip is placed on the chip tray of the **Truelab®** Real Time Quantitative micro PCR Analyzer. Six (6) µL of the purified RNA is then dispensed using the provided calibrated micropipette and tip into the microtube containing freeze dried RT-PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. ⚠ No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the **Truenat® HIV-1/HIV-2** chip and the test is inserted in the **Truelab®** Real Time micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® HIV-1/HIV-2** chip to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample). Ct value is linearly correlated with amount of target RNA copies present in the sample, enabling quantitative estimation of the analyte. Standard values for every batch are preset in the **Truenat® HIV-1/HIV-2** chip and the analyzer automatically compares these with the Ct value of the test sample to provide a quantitative result. In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, HIV-1/HIV-2 "DETECTED" or "NOT DETECTED" result is displayed, and in positive cases, Ct values and International Units (IU) per milliliter (IU/mL) is also displayed on the screen. Based on the detection of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes – from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed using the **Truelab®** micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 4G/3G/GPRS network. Upto 20,000 results in **Truelab® Uno Dx/Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this assay is the *pol* gene for HIV-1 and 5' UTR for HIV-2 genome.

5. CONTENTS OF THE Truenat® HIV-1/HIV-2 KIT

A. Individually sealed pouches
B. Package insert

Each individually sealed pouch contains:

1. **Truenat® HIV-1/HIV-2** micro PCR chip (1 No.)
2. Microtube with freeze dried RT-PCR reagents (1 No.)
3. DNase and RNase free pipette tip (1 No.)
4. Desiccant pouch (1 No.)

Pack sizes of **Truenat® HIV-1/HIV-2 KIT**

REF	601540005	601540020	601540025	601540050	601540100	601540200
Σ	5T	20T	25T	50T	100T	200T

6. CONTENTS OF THE Trueprep® AUTO Universal Sample Pre-treatment Pack

A. Lysis buffer.
B. Disposable transfer pipette (graduated).
C. Package insert.

Pack sizes of **Trueprep® AUTO Universal Sample Pre-treatment Pack**

REF	60205AB05	60205AB20	60205AB25	60205AB50	60205AB100	60205AB200
Σ	5T	20T	25T	50T	100T	200T

7. CONTENTS OF THE Trueprep® AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit

A. The reagent pack contains the following reagents

No.	Contents	Purpose
1.	Wash Buffer A	To wash inhibitors from the sample
2.	Wash Buffer B	To wash inhibitors from the sample
3.	Elution Buffer	To elute nucleic acids
4.	Priming Waste	To purge residual liquid from tubing

B. The cartridge pack contains the following:

No.	Contents	Purpose
1.	Cartridge	Cartridges containing immobilized internal control (IPC) for extraction
2.	Elute collection tube (ECT)	Capped tubes for collection and storage of extracted nucleic acids
3.	Elute collection tube (ECT) label	To label Elute Collection Tube (ECT)
4.	Disposable transfer pipette	To pierce the seal of elute chamber and to transfer extracted nucleic acids from elute chamber of cartridge into the Elute Collection Tube (ECT)

C. Disposable transfer pipettes (graduated) - 3 mL
D. Reagent reset card-1 No.
E. Package insert

Pack sizes of **Trueprep® AUTO Universal Cartridge Based Sample Prep Kit**

REF	60203AR05	60203AR25	60203AR50	60203AR100	60203AR200
Σ	5T	25T	50T	100T	200T

Pack sizes of **Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit**

REF	60207AR05	60207AR25	60207AR50	60207AR100	60207AR200
Σ	5T	25T	50T	100T	200T

7. STORAGE AND STABILITY

1. **Truenat® HIV-1/HIV-2** test is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.
2. **Trueprep® AUTO** Universal Sample Pre-treatment Pack is stable for two (2) years from the date of manufacture if stored between 2-40°C. It is also stable for one (1) month at temperatures upto 45°C. Do not freeze.
3. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit is stable for two (2) years from the date of manufacture if stored between 2°C to 40°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to light.
4. Do not open the pouch until ready to test. ⚠ Make sure to start the test promptly after 30-60 seconds of adding the elute to the microtube.
5. Do not use the pouch if torn.
6. Do not use pouches that have passed the expiration date.

8. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

REF **Truelab®** Real Time micro PCR Workstation (REF 623010001 / 633010001 / 643010001 / 653010001) consisting of

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF 603041001 / 603042001).
2. **Truelab® Uno Dx / Duo / Quattro** Real Time Quantitative micro PCR Analyzer (REF 603021001 / 603022001 / 603023001).
3. **Truelab®** micro PCR Printer (REF 603050001).
4. **Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab®** Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep® AUTO** Universal Sample Pre-treatment Pack (REF 60205AB05 / 60205AB20 / 60205AB25 / 60205AB50 / 60205AB100 / 60205AB200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF 60203AR05 / 60203AR25 / 60203AR50 / 60203AR100 / 60203AR200) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF 60207AR05 / 60207AR25 / 60207AR50 / 60207AR100 / 60207AR200), powder free disposable gloves, waste disposal container with lid and sodium hypochlorite.

9. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep® AUTO/AUTO v2

Truenat® HIV-1/HIV-2 requires purified nucleic acids from whole plasma collected in EDTA anticoagulant that are extracted using the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. Sample must be pre-treated using **Trueprep® AUTO** Universal Sample Pre-treatment Pack. Transfer 500 µl of plasma using the transfer pipette provided into the lysis buffer tube provided and mix well (Refer to the package insert of **Trueprep® AUTO** Universal Sample Pre-treatment Pack for further details).

Sample Storage and Transportation:

Sample pre-treatment decontaminates the specimen and makes it ready for storage/ transportation/extraction. The specimen in this form is stable for up to 3 days at 40°C and 1 week at 30°C.

Nucleic acid extraction: Use entire content from the Lysis Buffer tube containing specimen for further procedure with the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit (Refer to the User Manual of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and the package insert of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit for details). ⚠ Dispose off lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 17).

10. SAFETY PRECAUTIONS

IVD 1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20-30°C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the user manuals, package inserts and Material Safety Data Sheets (MSDS) of all the components of the **Truenat®** point-of-care real time PCR system before use.
5. Good laboratory practices are recommended to avoid contamination of specimens or reagents.
6. All materials of human origin should be handled as though potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
9. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.
10. Do not substitute assay components / reagents with any other components / reagents.
11. Each single-use **Truenat®** chip is used to process one test. Do not reuse chip.

11. PROCEDURAL PRECAUTIONS

1. Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
2. Do not perform the test in the presence of reactive vapours (e.g. from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the **Truenat® HIV-1/HIV-2** chip, microtube and the DNase and RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.
4. Ensure that the colour of the desiccant pouch is orange after opening a sealed **Truenat®** chip pouch. If the colour of the desiccant pouch changes from orange to white due to the absorption of moisture, do not use the contents of the **Truenat®** chip pouch.

12. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **Truenat®** assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or

specimens.

4. A specimen for which the **Truenat®** assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **Truenat®** assay should be interpreted in the context of other clinical and laboratory findings.

13. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite [10 times dilution of 5% sodium hypochlorite (household bleach)] before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves should be disposed off as potentially bio-hazardous waste e.g. in a bio-hazard waste container.

14. TEST PROCEDURE

(Please also refer the **Truelab®** Real Time Quantitative micro PCR Analyzer user manual)**.**

1. Switch on the**Truelab®** analyzer.
2. Select Username and enter password.
3. For **Truelab® Uno Dx**, select the test profile for "HIV 12" to be run from the profiles screen on the analyzer screen. For **Truelab® Duo/Quattro**, select the bay (I/II) for **Duo** and (I/II/III/IV) for **Quattro** from the status screen to view the profiles screen. Select the test profile for "HIV 12" to be run from the profiles screen, on the analyzer screen.
4. Enter the patient details as prompted in the **Truelab®** analyzer screen.
5. Press start reaction.
6. For **Truelab® Uno Dx,** press the eject button to open the chip tray. For **Truelab® Duo/Quattro**, the chip tray opens automatically on tapping the "Start Reaction" button.
7. Open a pouch of **Truenat® HIV-1/HIV-2** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip. Do not open the pouch until ready to test.
8. Place the **Truenat® HIV-1/HIV-2** chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the analyzer. Gently place the chip on the chip tray by aligning it in the slot provided.
9. Place the microtube containing freeze dried RT-PCR reagents in the microtube stand provided along with the **Truelab®** Real Time micro PCR workstation after ensuring that white pellet of freeze dried RT-PCR reagents remains at the bottom of the microtube. Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 17). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified RNA from the elute collection tube into the microtube. Allow it to stand for 30-60 seconds (in-use time) to get a clear solution. ⚠ Do not mix it by tapping, shaking or by reverse pipetting. Using the same filter barrier tip, pipette out six (6) µL of this clear solution and dispense into the centre of the white reaction well of the **Truenat® HIV-1/HIV-2** chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well. Dispose off the microtip as per the section on "Disposal and Destruction"(Section 17).
10. For **Truelab® Uno Dx**, slide the chip tray containing the **Truenat® HIV-1/HIV-2** Chip-based Real Time PCR test loaded with the sample into the **Truelab®** analyzer. Press "YES" on the "Please Load Sample" prompt. For **Truelab® Duo/Quattro**, select "YES" at the "Please Load Sample" prompt. Chip tray will close automatically and the reaction will start. ⚠ Make sure to start the test promptly after 30-60 seconds of adding the elute to the microtube.
11. Read the result from the screen.
12. After the reaction is completed, for **Truelab® Uno Dx,** push the eject button to eject the chip tray. For **Truelab® Duo/Quattro**, tap the "Open/Close Tray" button to eject the chip tray.
13. Take out the **Truenat® HIV-1/HIV-2** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 17).
14. Turn on **Truelab®** micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later. (Refer to **Truelab®** analyzer manual).
15. Switch off the **Truelab®** analyzer.

15. RESULTS AND INTERPRETATIONS

Three amplification curves are displayed on the **Truelab®** analyzer screen to indicate the progress of the test. Both the target and the internal positive control (IPC) curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The Cycle threshold (Ct) will depend on the number of target nucleic acids in the sample. The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen would also display the Ct values and viral loads in International Units per milliliter (IU/mL) for positive specimen. The result screen also displays the validity of the test run as "VALID" or "INVALID". Invalid samples have to be repeated with fresh specimen from the sample preparation stage.*While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

16. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab®** Real Time micro PCR Analyzer is working accurately, run known PCR positive and negative samples from time to time.

17. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat® HIV-1/HIV-2** chip, microtube, microtube cap, transfer pipette, pipette tips, lysis buffer tube etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of water).
4. Do not autoclave materials or solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

18. SPECIFIC PERFORMANCE CHARACTERISTICS

Traceability to the WHO Standard: The **Truenat® HIV-1/HIV-2** assay is standardized to the HIV-1 RNA 4th International Standard with NIBSC (NIBSC Code:16/194) and for Human Immunodeficiency Virus type 2 RNA (HIV-2) 2nd International Standard with NIBSC (NIBSC Code: 16/296).

Analytical Exclusivity (Primer specificity):

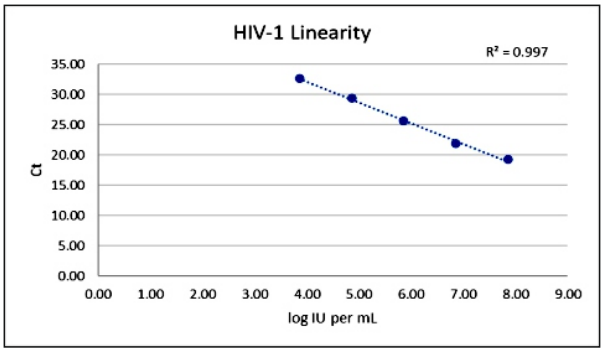
The following viruses and microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine for potential cross-reactivity in the **Truenat® HIV-1/HIV-2** assay.

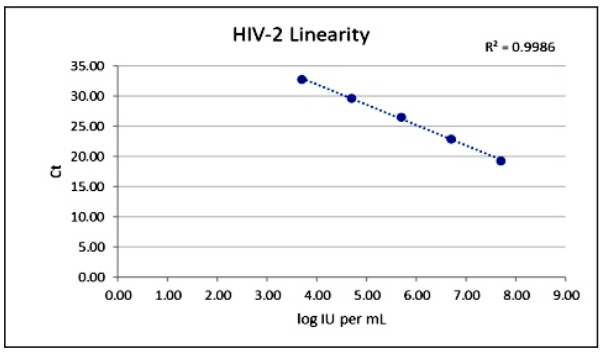
Microorganisms	Microorganisms
Adenovirus	Human herpes virus 4
Hepatitis B Virus	Vaccinia virus
Hepatitis C Virus	BK polyomavirus
Human T-lymphotropic virus 1	*Staphylococcus epidermis*
Cytomegalovirus	*Chlamydia trachomatis*
Epstein-Barr Virus	*Candida albicans*
Herpes Simplex Virus	*Staphylococcus aureus*
Simian Virus	*Mycobacterium tuberculosis*
Human herpes virus 1	*Mycobacteriumgordonae*
Human herpes virus 2	*Neisseria gonorrhoeae*
Human herpes virus 3	

No cross reactivity of the **Truenat® HIV-1/HIV-2** assay was observed with the listed organisms.

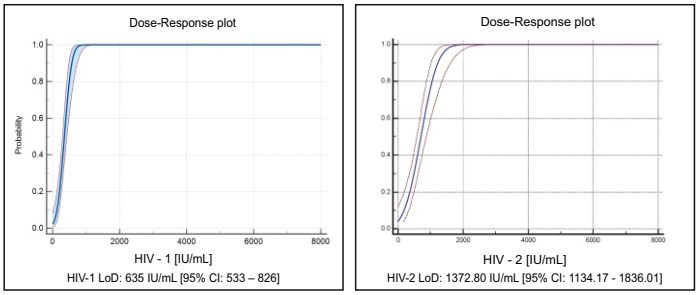
Linearity:

To determine the linear range for **Truenat® HIV-1/HIV-2** test**,** Serial dilutions of HIV-1 and HIV-2 In-House Armored RNA were made from 7.18E+07 to 7.18E+03 IU/mL for HIV-1 and 5.00E+07 to 5.00E+03 IU/mL for HIV-2 and the nucleic acids were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truelab®** Real Time micro PCR analyzer. The **Truenat® HIV-1/HIV-2** test is found to be linear over 5 orders of magnitude (from 7.18E+07 to 7.18E+03 IU/mL) for HIV-1 armored RNA and (from 5.00E+07 to 5.00E+03 IU/mL) for HIV-2 armored RNA.





Limit of Detection (LoD):
The LoD was determined by testing dilutions of HIV-1 and HIV-2 NIBSC international Standards diluted in Negative human plasma. The evaluation was performed according to CLSI guidelines. Probit analysis of the data was used to determine the concentration of the RNA that could be detected with 95% probability of detection. The LoD for HIV-1 with NIBSC 4th International Standard was found to be 635 IU/mL and the LoD for HIV-2 with 2nd WHO International Standard was found to be 1372.80 IU/mL by the **Truenat® HIV-1/HIV-2** test.



Robustness:
To determine whether the **Truenat® HIV-1/HIV-2** Chip-based Real Time Duplex PCR test showed any signs of carryover between the runs, alternate positive and negative samples were extracted and further tested the same by PCR. 20 positive samples and 20 negative samples were used for the study. The **Truenat® HIV-1/HIV-2** test did not exhibit detectable carryover contamination from positive to negative samples.

Reproducibility:
The purpose of this study is to compare the functional performance of the **Truenat® HIV-1/HIV-2** assay using three different titres of samples on **Truelab®** Real Time micro PCR analyzer. High, Medium and Low titre samples were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device and tested among three different users (Inter user), on three different devices (Inter device) and on 5 consecutive days (Inter day) to check the variability. Mean %CV values for all titres has been calculated for HIV-1 as Inter User (1.99), Inter day (2.13) and Inter Device (2.68) and for HIV-2 as Inter User (2.63), Inter day (1.89) and Inter Device (1.61) which were in the accepted range of ≤15% CV for **Truenat® HIV-1/HIV-2** assay.

Interfering Substances:
The purpose of the study is to determine the effect of potentially interfering substances on the performance of **Truenat® HIV-1/HIV-2** assay. Quantified HIV-1 and HIV-2 Samples were spiked into known negative human plasma containing the respective interfering substances. Potentially interfering substances used are: Albumin: 9 g/dL, Billirubin: 20mg/dL, Human DNA: 0.4 mg/dL, Hemoglobin: 500 mg/dL . The samples were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device and PCR was performed on **Truelab®** Real Time Quantitative micro PCR analyzer using **Truenat® HIV-1/HIV-2** assay. The presence of any of the mentioned endogenous substances at the stated concentrations did not affect the performance of **Truenat® HIV-1/HIV-2** assay.

Precision:
Precision was tested by performing **Truenat® HIV-1/HIV-2** assay with extracted RNA of High (2.0E+07 IU/mL), Medium (2.0E+05 IU/mL) and Low (2.0E+04 IU/mL) titres for HIV-1 while High (2.0E+06 IU/mL), Medium (2.0E+04 IU/mL) and Low (2.0E+03 IU/mL) titres for HIV-2 for five consecutive days. Every day PCR for each titre RNA was run in triplicates. The mean %CV values obtained for HIV-1 High titre (1.65), Medium titre (1.79) and low titre (4.20) while for HIV-2 High titre (1.01), Medium titre (1.24) and low titre (2.34) were within the accepted range of ≤15% CV for **Truenat® HIV-1/HIV-2** assay.

Clinical Validations:
Totally 110 comparative runs were performed to assess the sensitivity and specificity on three different manufacturing lots of **Truenat® HIV-1/HIV-2** assay at AIIMS (All India Institute of Medical Sciences, New Delhi) against the AIIMS, comparative HIV-1/HIV-2 RT-PCR assay.
Sensitivity: Positive samples containing viral loads ranging from ~4,00,000IU/mL to ~4,000IU/mL were tested. 25 out of 25 positive runs for HIV-1 and HIV-2 respectively are correlated between the methods giving a sensitivity of 100% for the **Truenat® HIV-1/HIV-2** assay.
Specificity: 60 negative runs correlated between the methods, depicting 100% specificity for the **Truenat® HIV-1/HIV-2** assay.
Conclusion:
Satisfactory results were seen in the specificity and sensitivity of **Truenat® HIV-1/HIV-2** assay and overall concordance of viral load estimation was in agreement with the AIIMS, comparative HIV RT-PCR assay.

19. REFERENCES

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SYMBOL KEYS

Consult instruction for use.	IVD In vitro Diagnostic Medical Device. Not for medicinal use.	LOT Batch number/ Lot number.	REF Catalogue number.	UDI Unique Device Identifier.	This way up.	Manufacturer.	Caution.	NON STERILE Non sterile.
Contains sufficient for <n> tests	Temperature limitations.	Date of manufacture.	Date of expiry.	For single use only.	Keep dry.	Keep away from sunlight	Device for near-patient testing	

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Registered Office & Manufacturing Unit:
Plot No. L-46, Phase II D,
Verna Industrial Estate, Verna,
Goa - 403 722, INDIA
www.molbiodiagnostics.com
Email: sales@molbiodiagnostics.com (Sales Enquiries)
customersupport@molbiodiagnostics.com (Feedback and Customer Support)

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