CE

Truenat®

HPV-*HR*

Chip-based Real Time Duplex PCR Test for Human Papillomavirus High Risk Types 16, 31 and 18, 45

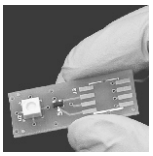
1. INTENDED USE

REF **Truenat® HPV-*HR* (REF 601220005 / 601220020 / 601220025 / 601220050 / 601220100 / 601220200)** is an automated point of care or near patient Chip-based Real Time Polymerase Chain Reaction (PCR) test for the semi-quantitative detection of **high risk Human Papillomavirus (HPV) types 16, 18, 31 and 45** in female cervical specimens collected by a clinician. It aids in the differential diagnosis of symptomatic or asymptomatic infection with high risk HPV types 16, 31 and 18, 45. **Truenat® HPV-*HR*** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers.

IVD **Truenat® HPV-*HR*** 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.

2. INTRODUCTION

Human papillomavirus (HPV) is a non-enveloped, double stranded DNA virus from the papillomavirus family. It infects squamous epithelia including the skin and mucosae of the upper respiratory and anogenital tracts. There are approximately 170 types of HPV, of which about 40 are sexually transmitted and infect the anus and genitals. Although most infections are asymptomatic and self-limiting, genital infection by HPV is associated with genital warts and anogenital cancers in both men and women. HPV viruses are classified as either 'high-risk' or 'low-risk' types depending on their association with the development of cancer. Low-risk HPV types, such as HPV 6 and 11, can cause common genital warts or benign hyperproliferative lesions with very limited tendency to malignant progression. Persistent infection by high-risk HPV types such as HPVs 16, 18, 31 and 45 is associated with the occurrence of pre-malignant and malignant lesions that are detectable in more than 90% of cervical cancers. Cervical cancer is one of the most common types of cancer in women worldwide. High-risk HPV types are also associated with many penile, vaginal, vulvar, anal, head and neck carcinomas and contribute to over 40% of oral cancers. Although persistent infection with oncogenic HPV types is the most common risk factor in its etiology of cervical cancer and its precursor lesions, only a very small proportion of infections advance to these disease states. Upwards of 70% of healthy young adults, will clear HPV infections within 12 to 24 months. In the subset of adults with persistent infection, progression to clinical infection may take years, providing opportunities for detection and treatment of pre-cancerous lesions. Cervical cancer has in the past been shown to be highly preventable when cytological and HPV screening programs are deployed to assist the detection and treatment of pre-cancerous lesions. Screening can also detect cancer at an early stage and treatment has a high potential for cure.



Cervical screening using a Papanicolaou (Pap) test or liquid-based cytology to detect abnormal cells that may develop into cancer has greatly reduced the incidence and fatalities of cervical cancer. However, these tests require interpretation by highly trained cytopathologists and have a high rate of false negatives. HPV is exceptionally difficult to culture *in vitro* and a demonstrable antibody response is not found in all patients infected with HPV. Nucleic acid (DNA) testing is a highly sensitive and specific method for determining the presence of infection with high-risk HPV types in cervical specimen. However, nucleic acid based molecular tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days to weeks leading to high losses to follow-up.

The **Truelab®** Real Time Quantitative micro PCR System enables decentralization and near patient diagnosis of high-risk HPV types 16, 18, 31 and 45 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, mains / 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 Kits 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 results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.

Truenat® HPV-*HR* is a disposable, room temperature stable, Chip-based Real Time PCR test with dried $MgCl_2$ in reaction well and freeze dried PCR reagents in microtube for performing Real Time PCR test for detection and diagnosis of HPV types 16, 18, 31 and 45 and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. It requires only six (6) µL of purified DNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information including standard values for quantitation. The **Truenat® HPV-*HR*** chip also stores information of used test to prevent any accidental re-use of the test.

3. PRINCIPLE OF THE TEST

Truenat® HPV-*HR* works on the principle of Real Time Polymerase Chain Reaction. The DNA from the patient sample is first extracted using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. The **Truenat® HPV-*HR*** chip is placed on the chip tray of the **Truelab®** Real Time micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed using the provided micropipette and tip into the microtube containing freeze dried 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® HPV-*HR*** chip and the test is started. A positive amplification causes the labeled fluorescent probes in the **Truenat® HPV-*HR*** Chip-based Real Time PCR test 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). 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, a HPV-*HR* "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, semi-quantitative result 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 via Bluetooth using the **Truelab® micro PCR** printer or transferred to the lab computer/or any remote computer via Wifi or 3G/GPRS network. Upto 20,000 results in **Truelab® Uno Dx/Truelab® Duo/Truelab® Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequences for this assay are regions within the E6 and E7 genes of high risk HPV types 16,18,31 and 45.

5. CONTENTS OF THE Truenat® HPV-*HR* KIT

A. Individually sealed pouches
B. Package Insert
   Each individually sealed pouch contains:
   1. **Truenat® HPV-*HR*** micro PCR chip (1 Nos.)
   2. Microtube with freeze dried PCR reagents (1 Nos.)
   3. DNase & RNase free pipette tip (1 Nos.)
   4. Desiccant pouch (1 Nos.)

REF	601220005	601220020	601220025	601220050	601220100	601220200
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.

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

7. CONTENTS OF THE Trueprep® AUTO Transport Medium for Swab Specimen Pack

A. Transport Medium for Swab specimen tubes (contains transport medium).
B. Package Insert.

REF	60206TS05	60206TS20	60206TS25	60206TS50	60206TS100	60206TS200
Σ	5T	20T	25T	50T	100T	200T

8. STORAGE AND STABILITY

Truenat® HPV-*HR* chip is stable for two (2) years from the date of manufacture if

stored between 2-30°C. It is also stable for upto one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

Trueprep® AUTO Transport Medium for Swab Specimen Pack and **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.

9. 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 / Truelab® Duo / Truelab® Quattro** Real Time 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** Transport Medium for Swab Specimen Pack (REF 60206TS05 / 60206TS20 / 60206TS25 / 60206TS50 / 60206TS100 / 60206TS200), **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), **Truenat®** Positive Control Kit - Panel III (REF 801030008), Powder free disposable gloves and waste disposal container with lid.

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

Swab specimen must be collected as per standard procedures using a standard nylon flocked swab. Insert the swab with specimen into the Transport Medium for Swab Specimen Tube provided and mix well by repeatedly twirling the swab in the buffer solution. Gently break the handle of the nylon swab at the break point, leaving the swab containing the specimen in the Transport Medium for Swab Specimen Tube. Tightly close the cap of the Transport Medium for Swab Specimen Tube (Refer to the package insert of **Trueprep® AUTO** Transport Medium for Swab Specimen Pack for further details).

⚠ Dispose off the remaining part of the swab after use, as per the section on "Disposal and Destruction" (Section 18).

Sample Storage and Transportation:

Transport Medium for Swab Specimen decontaminates the specimen and makes it ready for storage/transportation/extraction. The specimen in this form is stable for:

Stability temperature	Working period
2°C to 8°C	07 days
Room temperature (22°C±2°C)	3 weeks

Nucleic acid extraction: Transfer 500 µL from the Transport Medium for Swab Specimen Tube into the Lysis Buffer Tube 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 the Transport Medium for Swab Specimen Tube and Lysis Buffer Tube as per the section on "Disposal and Destruction" (Section 18).

11. 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 **Truelab® Real Time micro PCR System** before use.
5. All materials of human origin should be handled as though potentially infectious.
6. Do not pipette any material by mouth.
7. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
8. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

12. 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® HPV-*HR*** 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.

13. 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.

14. 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 biohazard waste container.

15. 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 "HPV" 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 "HPV" 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 Test.
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 Test" button.
7. Open a pouch of **Truenat® HPV-*HR*** 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® HPV-*HR*** 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 PCR reagents in the microtube stand provided along with the **Truelab®** Real Time micro PCR workstation after ensuring that white pellet of freeze dried 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 18). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified DNA 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® HPV-*HR*** 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 18).
10. For **Truelab® Uno Dx**, slide the chip tray containing the **Truenat® HPV-*HR*** 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® HPV-*HR*** Chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 18).

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 the **Truelab**® Analyzer manual).
15. Switch off the **Truelab**® Analyzer.

16. RESULTS & INTERPRETATIONS

Three amplification curves are displayed on the **Truelab**® Real Time Quantitative micro PCR Analyzer screen to indicate the progress of the test. Either or 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 viral load as "HIGH (Ct<20)","MEDIUM (20≤Ct<25)", "LOW (25≤Ct<30)" or " VERY LOW (Ct ≥ 30)" 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.

17. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab**® Real Time Quantitative micro PCR Analyzer is working accurately, run positive and negative controls from time to time. **Truenat**® Positive Control Kit - Panel III (REF 801030008) containing Positive Control and Negative Control must be ordered separately. It is advisable to run controls under the following circumstances. ● Whenever a new shipment of test kits is received. ● When opening a new test kit lot. ● If the temperature of the storage area falls outside of 2-30°C. ● By each new user prior to performing testing on clinical specimen.

18. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as **Truenat**® **HPV-*HR*** chip, microtube, microtube cap, pipette tips, remaining part of nylon flocked swab, Transport Medium for Swab Specimen Tube, 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.

19. SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Exclusitivity (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 potential cross-reactivity in the **Truenat**® **HPV-*HR*** assay. Results obtained showed no cross reactivity of the assay with the listed organisms.

Bacteria	Bacteria	Virus
Acinetobacter anitratus	*Escherichia coli*	Adenovirus
Chlamydia trachomatis	*Gardnerella vaginalis*	Hepatitis B virus
Enterobacter cloacae	*Neisseria gonorrheae*	Hepatitis C virus
Salmonella enterica	*Trichomonas vaginalis*	Human Immunodeficiency virus
Staphylococcus aureus	*Enterococcus faecalis*	Epstein-Barr virus
Candida albicans	*Streptococcus mutans*	Herpes Simplex virus
		Simian virus
		Cytomagalovirus

Linearity:

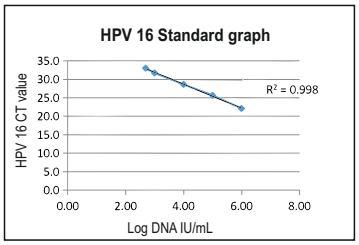
Serial dilutions of the NIBSC 1st WHO International Standard for HPV 16 DNA, NIBSC 1st WHO International Standard for HPV 18 DNA, HPV Type 31, pHPV31 (ATCC® 65446™) and NIBSC 1st WHO International Standard for HPV 45 DNA were made and nucleic acids were extracted on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truelab**® **Uno Dx** Real Time micro PCR Analyzer.

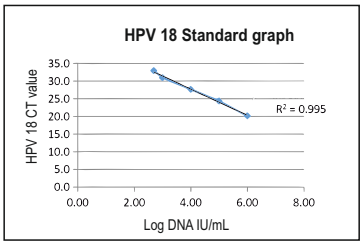
The assay is found to be linear over 5 orders of magnitude (from 1.00E + 06 IU/ml to 5.00E + 02 IU/ml) for the NIBSC 1st WHO International Standard for HPV 16 DNA.

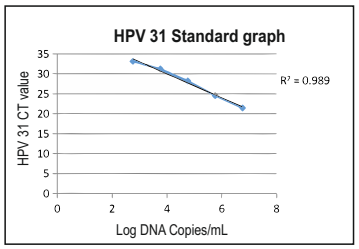
The assay is found to be linear over 5 orders of magnitude (from 1.00E + 06 IU/ml to 5.00E + 02 IU/ml) for the NIBSC 1st WHO International Standard for HPV 18 DNA.

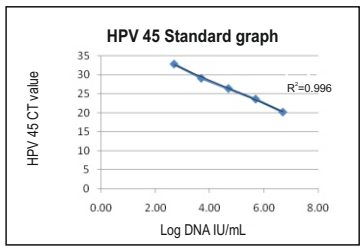
The assay is found to be linear over 5 orders of magnitude (from 5.79E + 06 copies/ml to 5.79E + 02 copies/ml) for pHPV31 (ATCC® 65446™) for HPV 31 DNA.

The assay is found to be linear over 5 orders of magnitude (from 5.00E + 06 IU/ml to 5.00E + 02 IU/ml) for the NIBSC 1st WHO International Standard for HPV 45 DNA.









Limit of detection (Analytical Sensitivity):

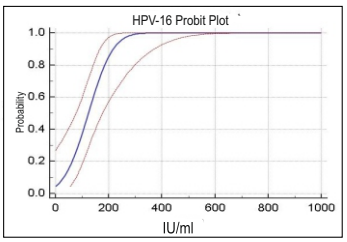
The LoD was determined by testing the dilutions of NIBSC 1st WHO International Standard for HPV 16 DNA, NIBSC 1st WHO International Standard for HPV 18 DNA, HPV Type 31 pHPV31 (ATCC® 65446™) DNA and NIBSC 1st WHO International Standard for HPV 45 DNA. Probit analysis of the data was used to determine the concentration of the respective DNA with 95% probability of detection.

The LoD was found to be 242.86 IU/ml of NIBSC 1st WHO International Standard for HPV 16 DNA.

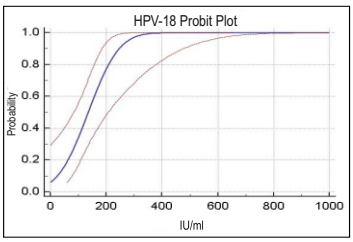
The LoD was found to be 277.44 IU/ml of NIBSC 1st WHO International Standard for HPV 18 DNA.

The LoD was found to be 400.94 Copies/ml of pHPV31 (ATCC® 65446™) DNA for HPV 31 DNA.

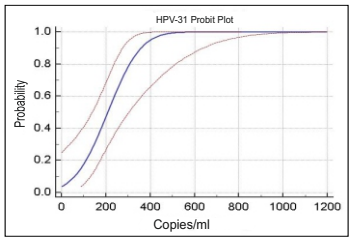
The LoD was found to be 294.49 IU/ml for NIBSC 1st WHO International Standard HPV 45 DNA by **Truenat**® **HPV-*HR*** assay.



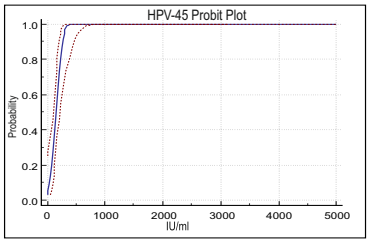
LoD = 242.86 CI [185.72 - 431.24]



LoD = 277.44 CI [205.95 - 560.33]



LoD = 400.94 CI [304.97 - 741.65]



LoD = 294.49 CI [226.61 - 542.52]

Robustness:

To determine whether the **Truenat**® **HPV-*HR*** Chip-based Real Time PCR test showed any signs of carryover between the runs, alternating runs of positive and negatives samples were performed. 10 positive samples and 10 negative samples were used for the study. The **Truenat**® **HPV-*HR*** test did not exhibit detectable carryover between positive and negative PCR runs.

Reproducbility:

The purpose of this study is to determine the Repeatability and Reproducibility of **Truenat**® **HPV-*HR*** test. The study was performed on a sample panel comprising of 2 negative specimens, 2 low positive specimens (with a concentration of analyte just above the assay cutoff) and 2 moderate positive specimens (with the concentration approximately two to three times the assay cutoff). The study was spread over a period of 5 days. Every day each sample panel member was tested which includes nucleic acid extraction on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truelab**® Real Time micro PCR analyzer using three different lots of reagents, at three different sites, by three users. The %CV values obtained for

Low Positive Specimen (HPV 16/31) : Inter Lot (3.18), Inter device / Inter User / Inter Site (2.95) and Inter Day(2.86)

Low Positive Specimen (HPV 18/45) : Inter Lot (2.78), Inter device / Inter User / Inter

Site (2.99) and Inter Day (3.13)
Moderate Positive Specimen (HPV 16/31) : Inter Lot (2.36), Inter device / Inter User / Inter Site (2.37) and Inter Day(1.96)
Moderate Positive Specimen (HPV 18/45) : Inter Lot (2.46), Inter device / Inter User / Inter Site (2.38) and Inter Day (2.42) were within the accepted range of ≤15% CV for **Truenat® HPV-*HR*** assay.

Interference:
The purpose of this study is to determine the effect of potentially interfering substances on the **Truenat® HPV-*HR*** assay. The low load samples were used for this study. To the samples different concentrations of blood ranging from 5%, 10% and 30% were spiked and then the samples were subjected to nucleic acid extraction on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device. The PCR was performed on **Truelab® Uno Dx** Real Time micro PCR Analyzer using **Truenat® HPV-*HR*** chips. The presence of blood till 10% did not interfere with the performance of **Truenat® HPV-*HR*** assay.

Precision:
Precision was tested by performing **Truenat® HPV-*HR*** assay of High, Medium and Low titre DNA for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The %CV values obtained for
HPV-16: High titre (1.49), Medium titre (0.64) and low titre (2.83)
HPV-18: High titre (0.68), Medium titre (0.69) and low titre (3.94)
HPV-31: High titre (1.50), Medium titre (1.15) and low titre (3.29)
HPV-45: High titre (0.83), Medium titre (0.71) and low titre (1.38)
were within the accepted range of ≤15% CV for **Truenat® HPV-*HR*** assay.

Effect of Endogenous substances:
The interference of endogenous substances on the performance of **Truenat® HPV-*HR*** was done by spiking the endogenous substances (Leukocytes - 1.00E+06 Cells/mL, Whole Blood - 2.5% v/v) at abnormally high levels to a sample panel comprising of 3 different HPV negative and 3 different positive specimens spiked with HPV 16 and 18 NIBSC standards at a concentration at 3 x LOD. Further, the specimens in the sample panel were extracted on **Trueprep® AUTO** Sample Prep Device followed by PCR on **Truelab®** Real Time micro PCR analyzers using **Truenat® HPV-*HR*** test. The performance of the **Truenat® HPV-*HR*** assay was not affected by the endogenous substances mentioned under the stated experimental conditions.

Effect of Exogenous substances:
The interference of exogenous substances on the performance of **Truenat® HPV-*HR*** was determined by spiking the exogenous substances (Acetic acid – 2%, Clingen – 0.50%, Candid B – 0.25%, Micogel – 0.25%, Lubic gel – 0.50%, Carex – 2.50% and Quadriderm – 0.25%) at abnormally high levels to a minimum of 3 different negative as well as 3 different positive specimens spiked with HPV 16 and 18 NIBSC standards at a concentration at 3 x LOD. Further, the specimens in the sample panel were extracted on **Trueprep® AUTO** Sample Prep Device followed by PCR on **Truelab®** Real Time micro PCR analyzers using **Truenat® HPV-*HR*** test. The performance of the **Truenat® HPV-*HR*** assay was not affected by the exogenous substances mentioned under the stated experimental conditions.

Equivalence between Contrived and Clinical specimens:
The equivalence between contrived and clinical specimens was demonstrated on a sample panel comprising of 7 contrived (plasmid samples) and 7 clinical specimens. Serial dilutions of clinical and contrived specimens were made in the viral transport media. Further, the specimens in the sample panel were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truelab®** Real Time micro PCR analyzers using **Truenat® HPV-*HR*** test. Both HPV contrived and clinical specimens showed equivalence in performance by **Truenat® HPV-*HR*** assay.

Genotype Panels:
The performance of **Truenat® HPV-*HR*** on a standard performance panel comprising of the respective genotypes in order to show diversity of variations within the genotypes was tested using HPV 16,18, 45 genotypes from NIBSC and HPV 31 from ATCC. The testing have been performed using 2 different lots of **Truenat® HPV-*HR*** tests. The CV values obtained were within the accepted range of ≤15%.

Clinical validation :
The study was conducted at the Health Promotion clinic of National Institute of Cancer Prevention and Research (NICPR), Noida. A total of 615 cervical samples were collected in duplicate by alternate sampling and analyzed for both HC2 (HC2 was performed according to manufactures instructions (Qiagen, USA) on the Digene Hybrid Capture system Model No. DML-2000, Germany) and **Truenat® HPV-*HR***. All the samples positive by either HC2 or **Truenat®** were further analyzed using Sacace 14 Real-TM Quant kit (Sacace Biotechnologies, Italy) for quantitative detection and genotyping of 14 HPV strains (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).
The HPV-HR DNA test was positive in 78 (12.7%) by HC2 and 49 (8%) by **Truenat®** out of 615 cervical samples. The sensitivity, specificity, positive predictive value, negative predictive value and concordance between HC2 and **Truenat®** is shown in Table 1. After validating all HPV positive samples and including only **Truenat® HPV-*HR*** assay claimed genotypes, the revised 2x2 matrix table with observed performance parameters is given in Table 2. The sensitivity, specificity, PPV and NPV of **Truenat® HPV-*HR*** were found to be 97.7% (95% CI: 88-99.9%), 98.9% (95% CI: 97.7-99.6%), 87.8% (95% CI: 75.2-95.4%) and 99.8% (95% CI: 99-100%) respectively, thereby maintaining the consistency of the **Truenat® HPV-*HR*** results (sensitivity and PPV) when they were compared to HC2 in Table 1.

Table 1: Comparison of HC2 and Truenat® test results

HC2	Truenat®	
	All 13 genotypes of HC2 are included	Only 4 high risk types; 16,31,18,45 of HC2 are included
Sensitivity (95% CI)	51.3% (39.7 - 62.8%)	97.5% (86.8 - 99.9%)
Specificity (95% CI)	98.3% (96.8 -99.2%)	NA *
PPV (95% CI)	81.6% (68 -91.2%)	90.7% (77.9 -97.4%)
NPV (95% CI)	93.3% (90.9 -95.2%)	NA *
Agreement (kappa)	92.36% (0.59)	-

*NA: Not applicable as Specificity and NPV cannot be calculated as PCR was done only in positive cases

Table 2: Validated results of positive samples of HC2 and Truenat® were performed using 14 Real-TM Quant kit

Sacace 14 Real-TM Quant kit (Real Time PCR)	Truenat®	
	4 HR types are included	All 14 HR types are included
Sensitivity (95% CI)	97.7%(88-99.9%)	69.2%(57.8 - 79.2%)
Specificity (95% CI)	98.9%(97.7-99.6%)	98.9%(97.6 - 99.6%)
PPV(95% CI)	87.8%(75.2-95.4%)	90% (79.5 - 96.2%)
NPV(95% CI)	99.8%(99-100%)	95.7%(93.6 - 97.2%)
Agreement (Kappa)	98.86 % (0.92)	-

PPV: positive predictive value, NPV: negative predictive value

20. REFERENCES

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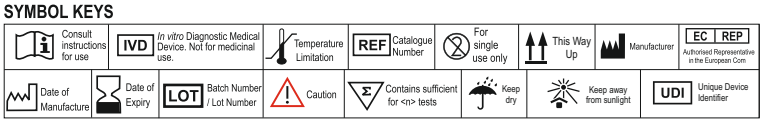
SYMBOL KEYS



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