CE

Truenat®

H1N1

Chip-based Real Time PCR Test for H1N1

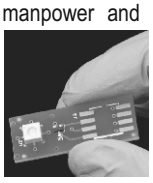
1. INTENDED USE

REF **Truenat® H1N1 (REF 601070005 / 601070020 / 601070025 / 601070050 / 601070100 / 601070200)** is an automated point-of-care or near patient Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test for the semi-quantitative detection of H1N1 virus in human throat and nasal swab specimen and aids in the diagnosis of infection with H1N1. **Truenat® H1N1** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® H1N1** 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 second flu pandemic or swine flu pandemic in 2009 was an influenza pandemic involving a novel influenza A (H1N1) virus (the first was the 1918 flu pandemic) with about 17,0000 recorded cases of death. First described in April 2009, the virus appeared to be a new strain of H1N1 of Swine origin. Unlike most strains of influenza, H1N1 can infect people of all ages equally. Even in the case of previously healthy persons, a small percentage develop pneumonia or acute respiratory distress syndrome (ARDS). This manifests itself as increased breathing difficulty and typically occurs 3-6 days after initial onset of flu symptoms. The pneumonia caused by this virus can be either direct viral pneumonia or a secondary bacterial pneumonia. Similar to other influenza viruses, H1N1 is typically contracted by person-to-person transmission through respiratory droplets from coughing and sneezing. Symptoms usually last for 4-6 days. Rapid influenza A antigen diagnostic tests (RIDTs) and direct and indirect immunofluorescence tests for influenza A are widely available but have variable sensitivity (10-70%) and are non-specific for detecting H1N1 influenza in clinical specimen. Viral isolation and nucleic acid amplification tests, such as real-time PCR, are the most reliable diagnostic tests for H1N1. Since a negative viral culture does not exclude infection with H1N1, Real Time Reverse Transcription PCR is the recommended method for confirmation of infection with H1N1. However viral culture or Real-Time PCR 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.



The **Truenat®** point-of-care real time PCR system enables decentralization and near patient diagnosis of and detection of H1N1 by making real time PCR technology rapid, simple, robust and user friendly 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® H1N1 is a disposable, room temperature stable, Chip-based Real Time PCR test with dried $MgCl_2$ in reaction well and freeze dried RT-PCR reagents in microtube for performing Real Time PCR test for H1N1 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® H1N1** chip also stores information of used chips 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® H1N1 works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) based on Taqman chemistry. The patient sample (human oropharyngeal and nasopharyngeal swab specimen) 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 RNA extract is analyzed using the **Truenat® H1N1** Chip-based Real Time Duplex Reverse Transcription Polymerase Chain Reaction (RT-PCR) test and the **Truelab®** Real Time Quantitative micro PCR Analyzer. The **Truenat® H1N1** chip is placed on the chip tray of the **Truelab®** Real Time 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 PCR reagents, including reverse transcriptase (RT) 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® H1N1** chip and the test is inserted in the **Truelab®** Real Time Quantitative micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the Reverse Transcriptase (RT) enzyme and further thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® H1N1** 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, H1N1 "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 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 are conserved sequences of swine influenza A virus (swInfA) nucleocapsid gene, the H1N1 swine influenza A virus (swH1) hemagglutinin gene and human RNase P. Detection of the human RNase P gene serves as a full process internal positive control (IPC) for proper swab collection, nucleic acid extraction and PCR.

5. CONTENTS OF THE Truenat® H1N1 KIT

A. Individually sealed pouches
B. Package Insert

Each individually sealed pouch contains:

1. **Truenat® H1N1** 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® H1N1**

REF	601070005	601070020	601070025	601070050	601070100	601070200
Σ	5T	20T	25T	50T	100T	200T

6. CONTENTS OF THE Trueprep® AUTO Universal Sample Pre-treatment Pack (for Extraction with Trueprep® AUTO/AUTO v2)

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 Transport Medium for Swab Specimen Pack (for Extraction with Trueprep® AUTO/AUTO v2)

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

Pack sizes of **Trueprep® AUTO Transport Medium for Swab Specimen Pack**

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

8. 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

9. STORAGE, HANDLING AND STABILITY

1. **Truenat® H1N1** test 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.
2. **Trueprep® AUTO** Universal Sample Pre-treatment Pack, **Trueprep® AUTO** Transport Medium for Swab Specimen Pack and **STABILYSE®** Prep Free Pack are 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.

10. 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 I (REF 801010008), Powder free disposable gloves, waste disposal container with lid.

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

Oropharyngeal or nasopharyngeal 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 19).

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 with cap, lysis buffer tube with cap and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 19).

12 SAFETY PRECAUTIONS

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.

IVD 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.

13. 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® H1N1** micro PCR 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.

14. 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.

15. 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.

16. 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 "H1N1" 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 "H1N1" 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**® **H1N1** and retrieve the micro PCR chip, microtube and DNase and RNase free pipette tip. Do not open the pouch until ready to test.
8. Place the **Truenat**® **H1N1** 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 19). 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**® **H1N1** 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 19).
10. For **Truelab**® **Uno Dx**, slide the chip tray containing the **Truenat**® **H1N1** 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**® **H1N1** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 19).
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.

17. RESULTS AND INTERPRETATIONS

Two amplification curves are displayed on the **Truelab**® **Real Time micro PCR 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 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 microbial 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.

18. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab**® Real Time micro PCR Analyzer is working accurately, run positive and negative controls from time to time. The **Truenat**® Positive Control Kit - Panel I (REF 801010008) 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.

19. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as **Truenat**® **H1N1** chip, microtube, microtube cap, pipette tips, nylon flocked swab, Sample pre-treatment tube, Transport Medium for Swab Specimen 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.

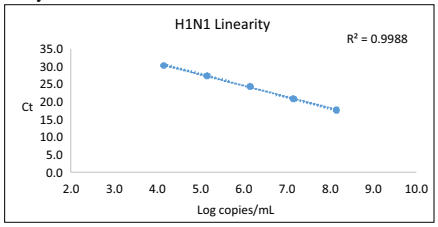
20. SPECIFIC PERFORMANCE CHARACTERISTICS

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**® **H1N1** assay. No interference in the performance of the **Truenat**® **H1N1** assay was observed with the listed organisms.

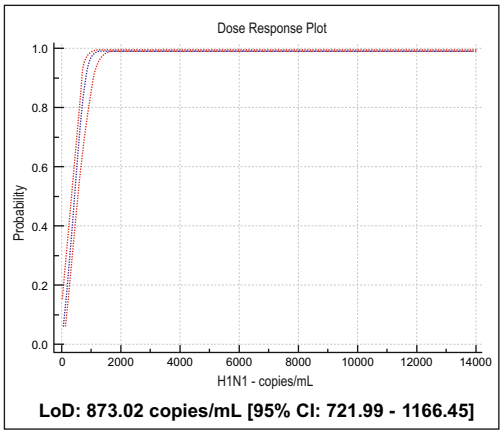
Organisms	Organisms
E.coli	Herpes Simplex Virus-1
Staphylococcus epidermidis	Herpes Simplex Virus-2
Mycobacterium tuberculosis	Epstein Barr virus
Mycobacterium gordonae	Human immunodeficiency virus
Neisseria gonorrhoeae	Hepatitis B virus
Chlamydia trachomatis	Hepatitis C virus
Candida albicans	Parvovirus
Staphylococcus aureus	Adenovirus
Enterobacter aerogenes	Cytomegalovirus
Klebsiella pneumoniae	Influenza B Virus
Human herpes virus 8	Human herpes virus 3
Vaccinia virus	Human herpes virus 4
Alphapapillomavirus 9	Human herpes virus 6
Human DNA (Various Samples)	Alphapapillomavirus 7

Analytical Specificity (Interference study) :
For this study, two different loads of samples were used. To the samples different concentrations of interfering substances such as mucin, blood, azithromycin and oseltaminir were spiked and then the samples were subjected to sample prep on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device. RNA was eluted and PCR was performed on **Truelab**® devices using **Truenat**® **H1N1** chips. The presence of above mentioned potential interference substances did not interfere with the performance of **Truenat**® **H1N1** assay.

Linearity:
The Linearity analysis was performed according to CLSI Guidelines. Serial dilutions of RNA from 1.0E+08 to 1.0E+04 copies/mL were made and nucleic acids were extracted on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab**® **Uno Dx** Real Time micro PCR Analyzer using **Truenat**® **H1N1** test. The assay is found to be linear over 5 orders of magnitude (from 1.0E+08 to 1.0E+04 copies/mL) for the H1N1 IVT RNA by **Truenat**® **H1N1** test.



Limit of detection (Analytical Sensitivity):
The LoD was determined by making dilutions of IVT RNA sample and performing nucleic acid extractions on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device for each of the dilution 24 times followed by PCR on **Truelab® Uno Dx** Real Time micro PCR Analyzer. Probit analysis of the data was used to determine the concentration of the RNA with 95% probability of detection. The LoD was found to be 873.02 copies/mL for the H1N1 IVT RNA by **Truenat® H1N1** test.



LoD: 873.02 copies/mL [95% CI: 721.99 - 1166.45]

Robustness:
To determine whether the **Truenat® H1N1** Chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternating runs of positive samples and negatives samples were performed. 20 positive samples and 20 negative samples were used for the study. The **Truenat® H1N1** test did not exhibit detectable carryover contamination between positive to negative sample runs.

Reproducibility:
The purpose of this study is to compare the functional performance of the **Truenat® H1N1** 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 Inter User (1.75), Inter day (1.45) and Inter Device (1.57) which were in the accepted range of ≤15% CV for **Truenat® H1N1** assay.

Precision of Truenat® H1N1 assay:
Precision was tested by performing **Truenat® H1N1** assay with extracted RNA for varying titres of samples five consecutive days. The %CV values obtained for High titre (0.55), Medium titre (1.13) and low titre (2.05) were within the accepted range of ≤15% CV for **Truenat® H1N1** assay.

Clinical validation 1:
Throat and nasal swabs (99) were processed by the **Department of Virology at the National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore on a commercial PCR machine** as per WHO/CDC protocol for H1N1 detection and using the **Truenat® H1N1** test on the **Truelab®** Real Time micro PCR Analyzer.
The sample panel had 24 confirmed H1N1 positive samples and 71 confirmed H1N1 negative samples based on the WHO/CDC protocol. The **Truenat® H1N1** test was found to have a sensitivity of 100% (24/24) and a specificity of 95.77% (68/71).

Clinical validation 2:
A panel of 151 samples comprising of 86 negative and 65 positive specimens were tested on three different manufacturing lots of **Truenat® H1N1 assay** at ICMR National Institute of Virology, Pune against the Gold standard (WHO/CDC protocol).

	Gold standard (WHO/CDC protocol)		
Truenat® H1N1 test	Positive	Negative	Total
Positive	64	1	65
Negative	0	86	86
Total	64	87	151
Sensitivity: 100% (95% CI 94.40 % to 100%)			
Specificity: 98.85% (95% CI 93.76% to 99.97%)			

With the consideration of above data, **Truenat® H1N1** performed well in this study with observed sensitivity of 100% and specificity of 98.85%.

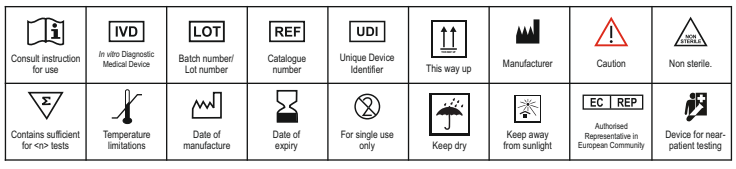
21. REFERENCES

1. http:// www.who.int/csr/disease/swineflu/en/.
2. Drexler, Jan Felix, et al. (2009). Poor clinical sensitivity of rapid antigen test for influenza A pandemic (H1N1) 2009 virus. Emerging Infectious Diseases, 15.10 : 1662.
3. Kok, Jen et al. (2010). Comparision of a rapid antigen test with nucleic acid testing during cocirculation of pandemic influenza A/H1N1 2009 and seasonal influenza A/H3N2. Journal of Clinical Microbiology, 48.1:290-291.

22. Revision History

Section	Description of the changes
Throughout	Symbols are added as per Regulatory requirements
3,7	Section is updated
8,14	Section are added
22	Added Revision History table
Symbol keys	Symbol keys are updated

SYMBOL KEYS



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