Truenat®

H3N2/H1N1

Chip-based Real Time Duplex PCR Test for H3N2 and H1N1

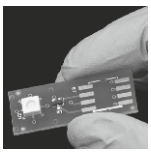
1. INTENDED USE

Truenat® H3N2/H1N1 (REF 601310005 / 601310020 / 601310025 / 601310050 / 601310100 / 601310200) is a Chip-based Real Time Duplex Reverse Transcription Polymerase Chain Reaction (RT-PCR) test for the semi-quantitative detection of H3N2 and H1N1 virus in human throat and nasal swab specimens and aids in differential diagnosis of infections with H3N2 and/or H1N1 virus. **Truenat® H3N2/H1N1** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® H3N2/H1N1** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

Influenza virus is a single stranded RNA virus of the family Orthomyxoviridae. Among the four types of Influenza viruses A,B,C and D, only Influenza A and B viruses cause seasonal epidemics of disease called flu in people. Of the two, only Influenza A viruses cause flu pandemics. Influenza A viruses are divided into subtypes based on two proteins on their surface, hemagglutinin (H) and Neuraminidase (N). H3N2 and H1N1 are currently the commonly circulating Influenza A viruses in people causing flu. The common symptoms of flu are fever, sore throat, muscle pains, severe headache, coughing, and weakness and fatigue. Severe symptoms of influenza includes pneumonia, which can be fatal, particularly in young children and the elderly. The transmission of influenza virus happens through the air by cough or sneezes creating aerosols containing the virus, and from infected birds through their droppings and also be transmitted by saliva, nasal secretions, faeces and blood. Infections either occur through direct contact with these bodily fluids, or by contact with contaminated surfaces. Hundreds of thousands of people die year on year because of epidemics of seasonal flu and millions are killed during pandemic years.

Early and correct identification of the Influenza virus is important for effective treatment and case management. Rapid point-of-care or immunofluorescence tests designed for direct detection of H3N2/H1N1 viruses have low sensitivity and specificity and yield "false negative" results and therefore not recommended for diagnostic purposes. In line with WHO recommendations, molecular diagnostics are currently the method of choice for influenza virus detection and differentiation. However, molecular tests for H3N2/H1N1 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 **Truelab®** Real Time Quantitative micro PCR System enables decentralization and near patient detection and differential diagnosis of infections with H3N2 and/or H1N1 virus 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® H3N2/H1N1 is a disposable, room temperature stable, micro PCR chip with dried $MgCl_2$ in reaction well and freeze dried PCR reagents for performing Real Time RT-PCR test for H3N2/H1N1 virus and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. 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® H3N2/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® H3N2/H1N1 works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) based on Taqman chemistry. 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 **Truenat® H3N2/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 micropipette and tip into the microtube containing freeze dried RT-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® H3N2/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 RT enzyme and further thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® H3N2/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, H3N2/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 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 sequence for this assay is the *HA* gene of H3N2 genome and *NP* and *HA* gene of H1N1 genome.

5. CONTENTS OF THE Truenat® H3N2/H1N1 KIT

A. Individually sealed pouches, each containing
   1. **Truenat® H3N2/H1N1** micro PCR chip.
   2. Microtube with freeze dried RT-PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch

B. Package Insert

REF	601310005	601310020	601310025	601310050	601310100	601310200
Σ	5T	20T	25T	50T	100T	200T

7. 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

8. 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

9. STORAGE AND STABILITY

Truenat® H3N2/H1N1 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 Universal Sample Pre-treatment Pack and **Trueprep® AUTO** Transport Medium for Swab Specimen 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.

10. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

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

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device

(REF603041001/603042001).
2. **Truelab® Uno Dx/Truelab® Duo/Truelab® Quattro** Real Time micro PCR Analyzer (REF603021001/603022001/603023001).
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 (REF60205AB05 / REF60205AB20 / REF60205AB25 / REF60205AB50 / REF60205AB100 / REF60205AB200), **Trueprep® AUTO** Transport Medium for Swab Specimen Pack (REF60206TS05 / REF60206TS20 / REF60206TS25 / REF60206TS50 / REF60206TS100 / REF60206TS200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), **Truenat®** Positive Control Kit - Panel I (801010008) and **Truenat®** Positive Control Kit - Panel V (801050008), 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 20).

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 up to 3 days at 40ºC and 1 week at 30ºC.

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 20).

13. 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.
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.

14. 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® H3N2/H1N1** 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.

15. 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.

16. 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.

17. TEST PROCEDURE

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

1. Switch on the **Truelab®** Analyzer .
2. Select user and enter password.
3. For **Truelab® Uno Dx**, select the test profile for "H3N2-H1N1" to be run from the Profiles Screen on the Analyzer screen. For **Truelab® Duo/Quattro**, select the Bay (Idle1/2) for **Duo** and (Idle1/2/3/4) for **Quattro** from the Status Screen to view the Profiles Screen. Select the test profile for "H3N2-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® H3N2/H1N1** and retrieve the Chip-based Real Time PCR test and the microtube.
8. Place the **Truenat® H3N2/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 press the chip to ensure that it has seated in the chip tray properly.
10. 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 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 20). 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 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® H3N2/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 20).
11. For **Truelab® Uno Dx,** slide the chip tray containing the **Truenat® H3N2/H1N1** Chip-based Real Time PCR test loaded with the sample into the **Truelab®** Analyzer. Press Done on the "Please Load Sample" Alert message. For **Truelab® Duo/Quattro**, select "YES" at the Please load Sample prompt. Chip tray will close automatically and the reaction will start.
13. Read the result from the screen.
14. 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.
15. Take out the **Truenat® H3N2/H1N1** Chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 20).
16. 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).
17. Switch off the **Truelab®** Analyzer.

18. RESULTS & INTERPRETATIONS

Three 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 Ct will depend on the number of target RNA 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 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.

19. 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 (801010008) and **Truenat**® Positive Control Kit - Panel V (801050008) 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.

20. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as **Truenat**® **H3N2/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.

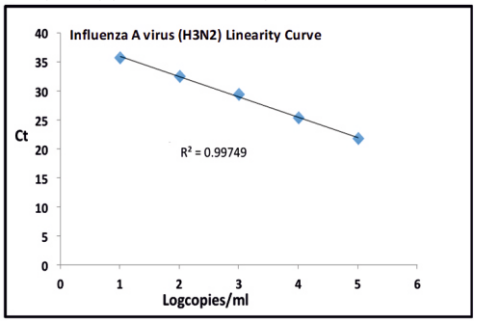
21. SPECIFIC PERFORMANCE CHARACTERISTICS

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**® **H3N2/H1N1** assay. Result obtained showed no cross reactivity of the **Truenat**® **H3N2/H1N1** assay with the listed organisms.

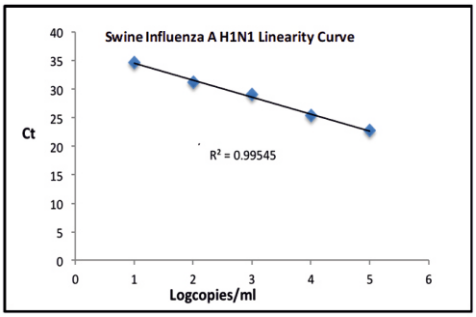
Organism	Organism
Klebsiella pneumoniae	*Mycobacterium leprae*
Bordetella pertussis	*Neisseria meningitidis*
Neisseria cinerea	*Brucella species*
Corynebacterium xerosis	Human metapneumovirus
Mycoplasma pneumoniae	Vaccinia virus
Staphylococcus aureus	SARS COV 2
Streptococcus pneumoniae	Epstein-Barr virus (EBV)
Pseudomonas aeruginosa	Cytomegalovirus
Haemophilus parainfluenzae	Human respiratory syncytial virus
Mycobacterium gordonae	Human herpesvirus 6
Haemophilus influenzae type B	MERS-CoV
Mycobacterium tuberculosis	Influenza B virus

Linearity:

The linearity analysis was performed according to CLSI Guidelines. Serial dilutions of *Influenza A virus* (H3N2) (ATCC® VR-822™) from 1.31x10^{7} to 1.31x10^{3} copies/mL was 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**® analyzer. The assay is found to be linear over 5 orders of magnitude (from 1.351E+07 copies/mL to 1.31E +03 copies/mL) for Dilution panel from *Influenza A virus* (H3N2) (ATCC® VR-822™)

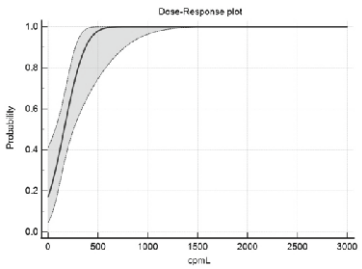


The linearity analysis was performed according to CLSI Guidelines. Serial dilutions of *Swine Influenza A H1N1-Zeptometrix* from 1.35 x107 to 1.35x103 copies/mL was made and nucleic acids were extracted on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab**® analyzer. The assay is found to be linear over 5 orders of magnitude (from 1.35E+07 copies/mL to 1.35E +03 copies/mL) for Dilution panel from *Swine Influenza A H1N1-Zepto*

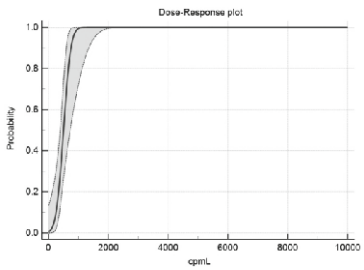


Limit of detection (Analytical Sensitivity):

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 10 times followed by PCR on **Truelab**®. 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 434 copies/mL for the H3N2 and 821 copies/mL for the H1N1 for IVT RNA by **Truenat**® **H3N2/H1N1** test.



H3N2 LoD = 434 cp/mL (95% CI – 312 to 931 cp/mL)



H1N1 LoD = 821 cp/mL (95% CI – 640 to 1527 cp/mL)

Robustness:

To determine whether the **Truenat**® **H3N2/H1N1** Chip-based Real Time PCR test showed any signs of carryover of PCR products between runs. Potential sample carryover within the **Truenat**® **H3N2/H1N1** test was evaluated by testing alternate positive followed by negative samples. The number of samples run was 10 positives and 10 negatives. The **Truenat**® **H3N2/H1N1** test did not exhibit detectable carryover from positive to negative samples.

Reproducibility:

The purpose of this study is to compare the functional performance of the **Truenat**® **H3N2/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 H3N2 as Inter User (2.74), Inter day (3.60) and Inter Device (2.13) and for H1N1 as Inter User (2.16), Inter day (2.62) and Inter Device (3.91) which were in the accepted range of ≤15% CV for **Truenat**® **H3N2/H1N1** assay.

Interference:

The purpose of this study is to determine the effect of potentially interfering substances on the **Truenat**® **H3N2/H1N1** assay. For this study medium load samples were used. To the samples different concentrations of blood ranging from 5%, 10% and 30% was spiked and then the samples were subjected to sample prep on **Trueprep**® **AUTO** Sample Prep Device. RNA was eluted and PCR was performed on **Truelab**® **Uno Dx** real time micro PCR analyzer using **Truenat**® **H3N2/H1N1** chips. The presence of blood till 30% did not interfere with the performance of **Truenat**® **H3N2/H1N1** assay. The CV values obtained were within the accepted range of ≤15% for **Truenat**® **H3N2/H1N1** assay.

Precision:

Precision was tested by performing **Truenat**® **H3N2/H1N1 assay** of assay of High, Medium and Low titre RNA for five consecutive days. Every day PCR for each titre RNA was run in duplicates for H3N2 and H1N1. The %CV values obtained for High titre (1.93), Medium titre (1.72) and low titre (0.92) for H3N2 while High titre (2.58), Medium titre (4.10) and low titre (1.35) for H1N1 were within the accepted range of ≤15% CV for **Truenat**® **H3N2/H1N1** assay.

Clinical validation:

A panel of 40 samples were run in parallel using **Truenat**® **H3N2/H1N1** on 3 different lots of tests and its performance was compared with NIV Combo PCR In-house for negatives as the reference tests. **Truenat**® **H3N2/H1N1** test performed consistently

in this study with observed sensitivity of 100% and specificity of 100% in comparison with respective reference NIV Combo PCR In-house Kits. The inter lot variation data obtained was within the accepted range of ≤15% CV for **Truenat® H3N2/H1N1** test.

22. REFERENCES

1. WHO Influenza Fact Sheet http://www.who.int/influenza/en/
2. CDC Influenza Fact. Sheet https://www.cdc.gov/flu/index.htm
3. http://www.who.int/csr/disease/swineflu/en/
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SYMBOL KEYS

Consult instructions for use	IVD *In vitro* Diagnostic Medical Device. Not for medicinal use.	Temperature Limitation	REF Catalogue Number	For single use only	This Way Up	Manufacturer
Date of Manufacture	Date of Expiry	LOT Batch Number / Lot Number	Caution	Contains sufficient for <n> tests	EC REP Authorised Representative in the European Community	

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