CE

Truenat™

H1N1

Chip based Real-Time PCR test for H1N1

1. INTENDED USE

Truenat™ H1N1 (REF 601070005 / 601070020) is a chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RTPCR) test for the diagnosis 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 micro PCR Analyzer.

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 17,000 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 and 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 Truelab™ Real Time micro PCR System enables decentralization and near patient diagnosis of H1N1 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 micro PCR Analyzer and Trueprep™ MAG Sample Prep Device and room temperature stable Truenat™ micro PCR chips and Trueprep™ MAG 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, micro PCR chip with dried down PCR reagents for performing Real Time RTPCR test for H1N1 virus and runs on the Truelab™ Real Time 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™ H1N1 chip also stores information of used chips to prevent any accidental re-use of the chip.

NOTE : Truelab™ / Truelab™ Uno / Trueprep™ MAG / Truepet™ / Truenat™ are all registered trademarks of Molbio Diagnostics (P) Limited.

The Truelab™ Real Time micro PCR Analyzer is protected by the following patents and patents pending: IN 2313/CHE/2007, WO 2009/047804 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 (real time RTPCR). The RNA from the patient sample is first extracted using Trueprep™ MAG Sample Prep Device and Trueprep™ MAG Blood sample prep kit and six (6) µL of the purified RNA is then dispensed into a microtube containing freeze dried PCR reagents, including reverse transcriptase (RT). After allowing approximately 20 seconds for the dried PCR reagents to get hydrated with the RNA sample, the entire contents is pipetted out and dispensed into the reaction well of the Truenat™ H1N1 chip. The Truenat™ H1N1 chip is then inserted in the Truelab™ Real Time micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place.

A positive amplification causes the dual labeled fluorescent probe in the Truenat™ H1N1 chip to release the fluorophore 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. (ie 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. Based on the detection of the internal positive control (IPC), human RNase P, the validity of the test run is also displayed. 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 GPRS network. Upto 5000 test results can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequences for this triplex 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, each containing a
   1. Truenat™ H1N1 micro PCR chip.
   2. Microtube with freeze dried RT PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch.

B. Package Insert.

C. Sample Pretreatment Pack for swabs specimen.
   a. Nylon flocked swabs.
   b. Sample pretreatment tubes (contains lysis cum transport medium).

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6. STORAGE AND STABILITY

Truenat™ H1N1 is stable for one year from the date of manufacture if stored between 2-30°C. It is also stable for upto three (3) months at temperatures up to 40°C. Avoid exposure to light.

7. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab™ Real Time micro PCR Workstation (REF 603010001) consisting of

1. Trueprep™ MAG Sample Prep Device (REF 603040001).
2. Truelab™ Real Time micro PCR Analyzer(REF 603020001).
3. Truelab™ micro PCR Printer (REF 603050001).
4. Truepet™ Precision Micropipettes - 6µL, 50µL, 100µL, 500µL, 1000µL. (REF 604010006 / 604020050 / 604030100 / 604040500 / 604051000).

Also required additionally are: Trueprep™ MAG Blood Sample Prep Kit (REF 602010050), Truenat™ Universal Control Kit (REF 601100008), DNase and RNase-free pipette tips (2 - 200µL / 200 - 1000µL microtips) with filter barrier, which may also be procured from Molbio (REF 604072200 / REF 604062010 respectively), Powder free disposable gloves, waste disposal container with lid.

8. SPECIMEN COLLECTION AND PREPARATION

Nasal/ Throat swabs specimen must be collected as per standard procedures using the nylon flocked swabs provided. Insert the swab with specimen into the Sample pretreatment tube provided and mix well. Discard the swab and tightly close the cap of the pretreatment tube.

Specimen Storage and Transportation:

Sample Pretreatment decontaminates the specimen and makes it ready for extraction. The specimen in this form is stable for up to 3 days at 40° C, 1 week at 30°C, and 1 year at -20°C

For nucleic acid extraction: Use 100µL of the fluid from the Sample pretreatment tube for further procedure with the Trueprep™ MAG Sample Prep Device and Trueprep™ MAG Blood Sample Prep Kit (Refer to the User Manual of Trueprep™ MAG Sample Prep device and the package insert of Trueprep™ MAG Blood Sample Prep Kit for details).

9. 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 and package inserts 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.

10. 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 and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.

11. 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.

12. TEST PROCEDURE

(Please also refer Section 4 in the Truelab™ Real Time micro PCR Analyzer user manual)

1. Switch on the Truelab™ Real Time micro PCR Analyzer touch screen.
2. Select user and enter password.
3. Select the test profile for "H1N1" on the Analyzer screen.
4. Enter the patient details as prompted in the Truelab™ Real Time micro PCR Analyzer screen.
5. Press Start Reaction.
6. Press the eject button to open the chip tray.
7. Open a pouch of Truenat™ H1N1 and retrieve the micro PCR chip and the microtube.
8. Label the chip and the tube with the patient ID using a marker pen at the space provided on the back side of the chip and the space on the microtube label.
9. Place the Truenat™ H1N1 micro PCR 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. Using the filter barrier tip provided in the pouch, pipette six (6) µL of the purified RNA from the Elute Collection Tube into the microtube containing the freeze dried RTPCR reagents. Allow twenty (20) seconds for the elute to hydrate the contents. With the same tip mix the contents twice gently by pipetting in and out. pipette six (6) µL of the mixture and dispense into the centre of the white reaction well of the Truenat™ H1N1 micro PCR chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well.
11. Slide the chip tray containing the Truenat™ H1N1 micro PCR chip loaded with the sample, into the Truelab™ Real Time micro PCR Analyzer.

12. Press the power button on the Analyzer. The green LED should glow.
13. Press "Done" on the "Please Load Sample" Alert message.
14. Observe the optical plot for any irregularities (Refer to the Truelab™ Real Time micro PCR Analyzer manual).
15. Read the result from the screen at end of the test.
16. Turn on Truelab™ micro PCR printer and select print on the screen for printing out hard copy of the results.
17. 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 15).

13. RESULTS & INTERPRETATIONS

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 time taken (Ct) of the specimen 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 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.

14. 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 Universal Control kit (REF 601100008) 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.

15. DISPOSAL AND DESTRUCTION

1. Submerge the used Truenat™ H1N1 micro PCR chip in freshly prepared 1% sodium hypochlorite solution for 20 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 contaminated fluid or 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.

16. SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of detection

To evaluate the limit of detection of Truenat™ H1N1 assay , RNA was extracted from cultured H1N1 virus, serially diluted and subjected to real-time PCR on standard commercially available real time PCR and also on the Truenat™ H1N1 using the Truelab™ Real Time micro PCR Analyzer.

Table 1: Ct values obtained from serial dilutions of cultured virus

Dilution	Truenat™ H1N1 micro PCR assay	Commercial PCR Test	
	SW infA/SW H1	SW infA	SWH1
Dil 1	22.5	21	25
Dil 2	27	25	30
Dil 3	32	28	32
Dil 4	37	32	35
Dil 5	ND	37	ND
Dil 6	ND	ND	ND

Serial dilutions of RNA upto 35 Ct could be detected on Truenat™ H1N1 micro PCR. The results were found to be in agreement with established Centers for Disease Control and Prevention (CDC) guidelines for H1N1 detection.

Clinical Sensitivity & Specificity for H1N1

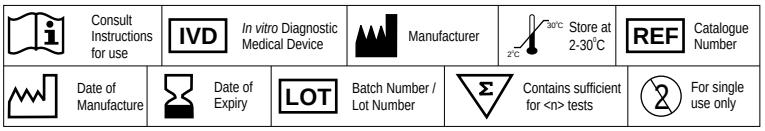
Throat and nasal swabs (99) were processed by the Department of Virology at the National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore on a commercial PCR machine as per WHO/CDC protocol for H1N1 detection and using the Truenat™ H1N1 test on the Truelab™ Real Time micro PCR Analyzer.

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).

17. REFERENCES

1. http://www.who.int/csr/disease/swineflu/en/
2. Drexler, Jan Felix, et al. "Poor clinical sensitivity of rapid antigen test for influenza A pandemic (H1N1) 2009 virus." Emerging infectious diseases 15.10 (2009): 1662.
3. Kok, Jen, et al. "Comparison 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 (2010): 290-291.
4. http://www.cdc.gov/flu/professionals/diagnosis/molecular-assays.htm

SYMBOL KEYS:



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