Truenat®

CT/NG

Chip-based Real Time Duplex PCR Test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

1. INTENDED USE

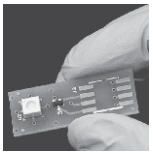
Truenat® CT/NG (**REF 601160005 / 601160020 / 601160025 / 601160050 / 601160100 / 601160200**) is a Chip-based Real Time Polymerase Chain Reaction (PCR) test for the semi-quantitative detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female endocervical and vaginal swab specimens, male urethral swab specimen and male and female Urine specimen. It aids in the diagnosis of symptomatic or asymptomatic infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae.* **Truenat® CT/NG** runs on **Truelab®** Real Time micro PCR Analyzers. **Truenat® CT/NG** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

Each year, there are an estimated 357 million new infections with 1 of 4 sexually transmitted infection (STIs): chlamydia, gonorrhoea, syphilis and trichomoniasis. Chlamydia and Gonorrhoea are the most prevalent STI in various countries. *Chlamydia trachomatis* is a Gram-negative bacterium, one of four bacterial species in the genus *Chlamydia*. The bacteria exist as obligate intracellular parasites of eukaryotic cells due to their inability to synthesize ATP. It includes three human biovars:

• Serovars Ab, B, Ba, or C - cause trachoma: infection of the eyes, which can lead to blindness • Serovars D-K- cause urethritis, pelvic inflammatory disease, ectopic pregnancy, neonatal pneumonia and neonatal conjunctivitis.

• Serovars L1, L2 and L3-lymphogranuloma venereum (LGV). Uncomplicated *Chlamydia trachomatis* infections can be treated and cured with antibiotics. Untreated infection can result in serious complications such as pelvic inflammatory disease, infertility, and ectopic pregnancy in women, and urethritis, epididymitis and orchitis in men. Screening for *Chlamydia trachomatis* is thus especially recommended in pregnant women. Several methods are available for the detection of *C. trachomatis* in clinical specimens. These methods include direct Giemsa's staining of infected tissue, detection of chlamydia inclusion bodies in infected culture cells using fluorescent antibody stain, direct antigen detection using fluorescent antibody stain and nucleic acid amplification tests (NAATs). Culture is highly specific but is less sensitive when applied in routine clinical practice. The performance of NAATs with respect to overall sensitivity, specificity, and ease of specimen transport is better than that of any of the other tests available for the diagnosis of chlamydial infections.



Neisseria gonorrhoeae is a species of Gram-negative bacteria responsible for the sexually transmitted infection (STI) gonorrhea. These non-motile cocci are facultatively intracellular and typically appear in pairs (diplococci). An estimated 78 to 88 million cases of gonorrhea occur each year. Uncomplicated gonorrhea infections can be treated and cured with antibiotics. If left untreated, *N. gonorrhoeae* infections can disseminate to other areas of the body, causing inflammation of the epididymis or pelvic inflammatory disease or throughout the body, affecting joints and heart valves in both men and women. The current methods for detection of *N. gonorrhoeae* include microscopy, culture, immunoassays and nucleic acid amplification tests (NAATs). Culture methods can have good clinical sensitivity, but are highly dependent on proper specimen handling. Improper specimen storage and transport can lead to false negatives because of loss of organism viability. The performance of NAATs with respect to overall sensitivity, specificity, and ease of specimen transport is better than that of any of the other tests available for the diagnosis of gonococcal infections. Early, accurate and differential diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* is necessary to initiate appropriate treatment, check transmission of the disease and prevent complications. A few molecular/NAAT based tests are available commercially for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae.* However, 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.

The **Truelab®** Real Time micro PCR System enables decentralization and near patient diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* 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® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and room temperature stable **Truenat®** micro PCR chips and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.

Truenat® CT/NG 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 *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and runs on the **Truelab®** Real Time 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® CT/NG** chip also stores information of used test to prevent any accidental re-use of the test.

3. PRINCIPLE OF THE TEST

Truenat® CT/ NG works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The DNA 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® CT/NG** 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® CT/NG** chip and the test is started. A positive amplification causes the labeled fluorescent probe in the **Truenat® CT/NG** chip to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample). 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, Chlamydia or Gonorrhea "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/ Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for ***Chlamydia trachomatis*** assay is a region within the cryptic plasmid DNA and ompA gene. While the target sequence for ***Neisseria gonorrhoeae*** is porB gene and opaE gene.

5. CONTENTS OF THE Truenat® CT/NG KIT

A. Individually sealed pouches, each containing a
   1. **Truenat® CT/NG** micro PCR chip.
   2. Microtube with freeze dried PCR reagents.
   3. DNase and RNase free pipette tip.
   4. Desiccant pouch.

B. Package Insert.

REF	601160005	601160020	601160025	601160050	601160100	601160200
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® CT/NG 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.

9. 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 III (REF 801030008), Powder free disposable gloves, 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. After mixing, squeeze out the excess liquid from the swab by pressing it a few times against the inside wall of the tube. ⚠Dispose off the swab as per the section on "Disposal and Destruction" (Section 18). Transfer 500 µL from the Transport Medium for Swab Specimen Tube into the Lysis Buffer 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).

For Urine specimen: Collect about 10 ml of first flow of urine (ensuring atleast 2 hours gap from last urination) in a urine collection cup. Transfer 0.5 ml from the cup to the lysis buffer tube and mix well after tightly closing the cap (Refer to the package insert of **Trueprep® AUTO** Universal Sample Pre-treatment Pack for further details). Dispose off urine collection cup 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 up to 3 days at 40ºC and 1 week at 30ºC.

Nucleic acid extraction: Use the entire content of lysis buffer tube containing Transport Medium for Swab Specimen/urine sample 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, lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section18).

11. 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.

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® CT/NG** micro PCR chip, microtube 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.

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 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 "Ngonorrhoeae-Chlamydia" 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 "Ngonorrhoeae-Chlamydia" 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® CT/NG** and retrieve the micro PCR chip, microtube and the DNase & RNase free pipette tip.
8. Place the **Truenat® CT/NG** Chip-based Real Time PCR test 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 is seated in the chip tray properly.
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 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" (Section18). 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 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® CT/NG** 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" (Section18).
10. For **Truelab® Uno Dx,** slide the chip tray containing the **Truenat® CT/NG** 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.
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® CT/NG** Chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section18).
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 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 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

17. 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 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 **Truenat**® **CT/NG** chip, microtube, microtube cap, transfer pipette, pipette tips, cervical swab, Transport Medium for Swab Specimen Tube, lysis buffer tube, urine collection cup 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.

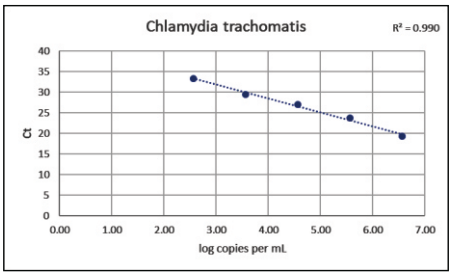
19. SPECIFIC PERFORMANCE CHARACTERISTICS

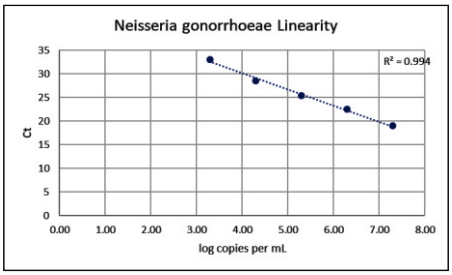
Analytical Exclusivity (Primer specificity): The following viruses and microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine potential cross-reactivity in the **Truenat**® **CT/NG** assay. Results obtained showed no cross reactivity of the **Truenat**® **CT/NG** assay was observed with the listed organisms.

Bacteria	Viruses
Acinetobacter anitratus	Adenovirus
Candida albicans	Cytomegalovirus
Enterobacter cloacae	Hepatitis B virus
Salmonella enterica	Hepatitis C virus
Staphylococcus aureus	Human Immunodeficiency virus
Streptococcus mutans	Epstein-Barr virus
Escherichia coli	Herpes Simplex virus
Gardnerella vaginalis	Simian virus
Trichomonas vaginalis	Human Papilloma virus
Enterococcus faecalis	

Linearity:

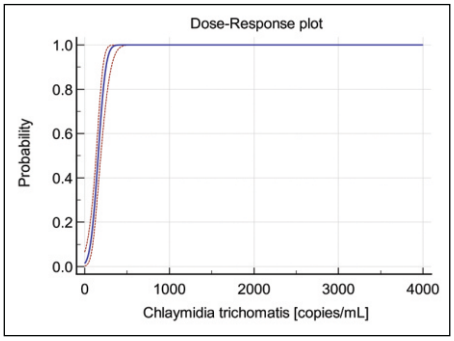
Serial dilutions of *Chlamydia trachomatis* Serovars E made from 3.65E+06 to 3.65E+02 copies/mL and *Neisseria gonorrhoeae* made from 1.95E+07 to 1.95E+03 copies/mL, nucleic acids were extracted on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truelab**® Real Time micro PCR Analyzer. The **Truenat**® **CT/NG** assay is found to be linear over 5 orders of magnitude (from 3.65E+06 copies/mL to 3.65E +02copies/mL) for *Chlamydia trachomatis* Serovar E ATCC® VR-348BD™ strain DNA. Similarly, the assay is found to be linear over 5 orders of magnitude (from 1.95E+07 copies/mL to 1.95E+03 copies/mL) for *Neisseria gonorrhoeae* (Zopf) Trevisan ATCC® 700825D-5™ strain DNA.



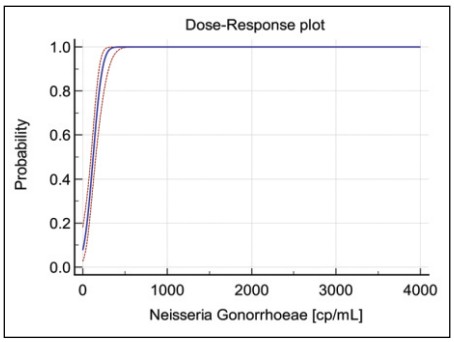


Limit of Detection:

The LoD was determined by testing dilutions of *Chlamydia trachomatis* Serovars E ATCC® VR-348BD™ strain DNA and *Neisseria gonorrhoeae* (Zopf) Trevisan ATCC® 700825D-5™ strain DNA. The testing was performed according to CLSI guidelines. Probit analysis of the data was used to determine the concentration of the DNA that could be detected with a positivity rate of 95%. The LoD was found to be 274.67 copies/mL for *Chlamydia trachomatis* Serovar E ATCC® VR-348BD™ strain DNA and 264.65 copies/mL for *Neisseria gonorrhoeae* (Zopf) Trevisan ATCC® 700825D-5™ strain DNA by **Truenat**® **CT/NG** assay.



LoD: 274.67 copies/mL [95% CI: 233.36 - 349.31]



LoD: 264.65 copies/mL [95% CI: 218.06 - 352.96]

Robustness:

To determine whether the **Truenat**® **CT/NG** Chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternate positive and negatives samples were extracted and further tested the same by PCR. 20 positive samples and 20 negative samples were used for the study. The **Truenat**® **CT/NG** test did not exhibit detectable carryover between positive and negative PCR runs.

Reproducibility:

The purpose of this study is to compare the functional performance of the **Truenat**® **CT/NG** assay using three different titres of samples on **Truelab**® **Uno Dx** 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 *Chlamydia* as Inter User (1.25), Inter day (2.34) and Inter Device (1.12) and for *Neisseria* as Inter User (1.06), Inter day (2.09) and Inter Device (2.1) which were in the accepted range of ≤15% CV for **Truenat**® **CT/NG** assay.

Interference:

The purpose of this study is to determine the effect of potentially interfering substances on the **Truenat**® **CT/NG** assay. For this study low load samples were used. To the samples different concentrations of blood ranging from 5%, 10% and 30% were spiked and then the samples were subjected to sample prep on **Trueprep**®

AUTO Universal Cartridge Based Sample Prep Device. DNA was eluted and PCR was performed on **Truelab® Uno Dx** Real Time micro PCR Analyzer using **Truenat® CT/NG** chips. The presence of blood till 10% did not interfere with the performance of **Truenat® CT/NG** assay. The %CV values obtained were within the accepted range of ≤15%.

Precision:
Precision was tested by performing **Truenat® CT/NG** assay with extracted DNA of High (3.65E+05 copies/mL), Medium (3.65E+04 copies/mL) and Low (3.65E+03 copies/mL) titres for *Chlamydia* while High (1.95E+05 copies/mL), Medium (1.95E+04 copies/mL) and Low (1.95E+03 copies/mL) titres for *Neisseria* for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The %CV values obtained for High titre (3.84), Medium titre (2.23) and Low titre (3.58) for *Chlamydia* while High titre (3.05), Medium titre (2.71) and Low titre (2.68) for *Neisseria* were within the accepted range of ≤15% CV for **Truenat® CT/NG** assay.

Clinical Validation 1:
A total of 50 samples were used for testing on **Truenat® CT/NG** assay on 3 different lots of **Truenat® CT/NG** kits at Suyog Life Care, Pune, Maharashtra and compared against CE marked *Chlamydia trachomatis* PCR Kit & CE marked *Neisseria gonorrhoeae* PCR Kit as the reference tests.

Truenat® CT/NG	**CE marked *Chlamydia trachomatis* PCR Kit**		
	Positive	Negative	Total
Positive	10	0	10
Negative	0	35	35
Total	10	35	45

Sensitivity: 100% (95% CI 69.15% to 100%)
Specificity: 100% (95% CI 90.00% to 100%)
Accuracy: 100% (95% CI 92.13% to 100%)

Truenat® CT/NG	**CE marked *Neisseria gonorrhoeae* PCR Kit**		
	Positive	Negative	Total
Positive	10	0	10
Negative	0	35	35
Total	10	35	45

Sensitivity: 100% (95% CI 69.15% to 100%)
Specificity: 100% (95% CI 90.00% to 100%)
Accuracy: 100% (95% CI 92.13% to 100%)

With the consideration of above data, **Truenat® CT/NG** test performed consistently in this study with observed sensitivity of 100% and specificity of 100%. The inter lot variation data obtained was within the accepted range of ≤15% CV for **Truenat® CT/NG** test.

Clinical Validation 2:
A panel comprising 117 Urine and 65 swab specimens were run in parallel using **Truenat® CT/NG** at Proxilis SA and compared against *Chlamydia trachomatis* PCR Kit & *Neisseria gonorrhoeae* PCR Kit as the reference tests.

For Urine Specimen:
a) Detection of *Chlamydia trachomatis* target by Truenat® CT/NG test and the Reference test in Urine Specimens

Truenat® CT/NG	***Chlamydia trachomatis* PCR Kit**		
	Positive	Negative	Total
Positive	20	0	20
Negative	0	97	97
Total	20	97	117

Sensitivity: 100% (95% CI 83.16% to 100.00%)
Specificity: 100% (95% CI 96.27% to 100.00%)
Accuracy: 100% (95% CI 96.90% to 100.00%)

b) Detection of *Neisseria gonorrhoeae* target by Truenat® CT/NG test and the Reference test in Urine Specimens

Truenat® CT/NG	***Neisseria gonorrhoeae* PCR Kit**		
	Positive	Negative	Total
Positive	11	0	11
Negative	0	106	106
Total	11	106	117

Sensitivity: 100% (95% CI 71.51% to 100.00%)
Specificity: 100% (95% CI 96.58% to 100.00%)
Accuracy: 100% (95% CI 96.90% to 100.00%)

For Swab Specimens:
a) Detection of *Chlamydia trachomatis* target by Truenat® CT/NG test and the Reference test in Swab Specimens

Truenat® CT/NG	***Chlamydia trachomatis* PCR Kit**		
	Positive	Negative	Total
Positive	10	0	10
Negative	0	55	55
Total	10	55	65

Sensitivity: 100% (95% CI 69.15% to 100.00%)
Specificity: 100% (95% CI 93.51% to 100.00%)
Accuracy: 100% (95% CI 94.48% to 100.00%)

b) Detection of *Neisseria gonorrhoeae* target by Truenat® CT/NG test and the Reference test in Swab Specimens

Truenat® CT/NG	***Neisseria gonorrhoeae* PCR Kit**		
	Positive	Negative	Total
Positive	15	0	15
Negative	0	50	50
Total	15	50	65

Sensitivity: 100% (95% CI 78.20% to 100.00%)
Specificity: 100% (95% CI 92.89% to 100.00%)
Accuracy: 100% (95% CI 94.48% to 100.00%)

Truenat® CT/NG test gave satisfactory results in comparison to *Chlamydia trachomatis* PCR Kit and *Neisseria gonorrhoeae* PCR Kit as reference tests in both urine and swab specimens.

20. REFERENCES

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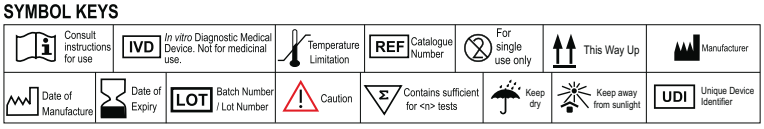
SYMBOL KEYS



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