1. INTENDED USE
Truenat™ CT (REF 601140005 / 601140020) is a chip-based Real Time Polymerase Chain Reaction (PCR) test for the semiquantitative detection of Chlamydia trachomatis 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.

Truenat™ CT runs on the Truelab™ Real Time Quantitative micro PCR Analyzers. *Urine specimen can be processed only on Trueprep™ AUTO protocol (see section 12).

2. INTRODUCTION
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 uherthritis, pelvic inflammatory disease, ectopic pregnancy, neonatal pneumonia and neonatal conjunctivitis,
- Serovars L1, L2 and L3 — lymphogranuloma venerenum (LGV).

Each year, there are an estimated 357 million new infections with 1 of 4 sexually transmitted infection (STIs): chlamydia, gonorrhoea, syphilis and trichomoniasis. Chlamidia is the most prevalent STI in various countries. 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 inclusions 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. A few molecular / NAAT based tests are available commercially for detection of Chlamydia trachomatis. 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 and detection of Chlamydia infection 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/AUTO Sample Prep Device and room temperature stable Truenat™ micro PCR chips and Trueprep™ MAG/AUTO 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™ CT is a disposable, room temperature stable, micro PCR chip with dried MgCl2 in reaction well and freeze dried PCR reagents for performing Real Time PCR test for Chlamydia trachomatis 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. The Truenat™ CT chip also stores information of used chips to prevent any accidental re-use of the chip.

NOTE: Trueprep Uno / Truelab Uno Dx / Truelab Duo / Truelab Quattro / Trueprep AUTO / Trueprep MAG / Trupet™ / 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/CH/E2007, 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 2313/CH/E2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST
Truenat™ CT 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™ MAG Sample Prep Device and Trueprep™ MAG Blood Sample Prep Kit or using Trueprep™ AUTO Universal Cartridge based Sample Prep Device and Trueprep™ AUTO Universal Cartridge Based Sample Prep kit. The Truenat™ CT 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 chip and the test is started. A positive amplification causes the dual labeled fluorescent probe in the Truenat™ CT 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. exceeded 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 ‘DETECTED’ or ‘NOT DETECTED’ result is displayed and in positive cases, semiquantitative 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 Trueprep™ micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 3G/GPRS network. Upto 5000 test results in Trueprep Uno to 20,000 results in Trueprep Uno Dx/Duo/Quattro can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION
The target sequence for this assay is a region within the cryptic plasmid DNA of C. trachomatis.

5. CONTENTS OF THE Truenat™ CT KIT
A. Individually sealed pouches, each containing
   1. Truenat™ CT micro PCR chip.
   2. Microtube with freeze dried PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch.
B. Package Insert

   REF 601140005  601140020
   5T  20T

6. CONTENTS OF THE Trueprep™ MAG Swab Specimen Pre-treatment Pack (only for Trueprep™ MAG users)
A. Sample Pre-treatment tubes (contains lysis cum transport medium).

   REF 602025WV20
   20T

7. CONTENTS OF THE Trueprep™ AUTO Universal Sample Pre-treatment Pack (only for Trueprep™ AUTO users)
A. Lysis Buffer.
B. Disposable transfer pipette (graduated).

   REF 60205AB05  60205AB20
   5T  20T

8. CONTENTS OF THE Trueprep™ AUTO Transport Medium for Swab Specimen Pack (only for Trueprep™ AUTO users)
A. Transport Medium for Swab specimen tubes (contains transport medium).

   REF 60206TS05  60206TS20
   5T  20T

9. STORAGE AND STABILITY
Truenat™ CT is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for upto six (6) months at temperatures up to 40°C and one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

Trueprep™ MAG Swab specimen Pre-treatment Pack, Trueprep™ AUTO Transport Medium for Swab specimen Pack and Trueprep™ AUTO Universal Sample Pre-treatment Pack is stable for two (2) years from the date of manufacture if stored between 2-40°C. It is also stable for one (1) month at temperatures up to 45°C.
10. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

- **Truelab™ Real Time micro PCR Workstation** (Ref. 603010001/62301001/633010001/64301001)
- **Truprep™ MAG / AUTO Sample Prep Device** (Ref. 603040001/603041001)
- **Truelab™ Uno/Truepet™ Uno Dx / Truelab™ Duo/Truepet™ Quattro Real Time micro PCR Analyzer** (Ref. 603020001/603021001/603022001/603023001)
- **Truelab™ Microtip Stand** (Ref. 603070001)

Also required additionally are: **Truprep™ MAG Blood Sample Prep Kit** (Ref. 602010005/REF. 602010050) / **Truprep™ AUTO Universal Cartridge Based Sample Prep Kit** (Ref. 60203AR05/REF. 60203AR25), **Truenat™ Universal Control Kit** (Ref. 601100008), DNase and RNase-free pipette tips with filter barrier which may also be procured from Molbio, Powder free disposable gloves, Nylon flocced swabs, urine collection cup and waste disposal container with lid.

11. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep™ MAG

Swab specimen must be collected as per standard procedures using a standard nylon flocced swab. Insert the swab with specimen into the Sample pre-treatment tube for swab specimen 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 of the swab as per the section on “Disposal and Destruction” (Section 20). Tightly close the cap of the Sample pre-treatment tube.

- **Sample Storage and Transportation:**
  - Sample Pre-treatment 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 entire content from the Sample pre-treatment tube containing specimen for further procedure with the Trueprep™ MAG Sample Prep Device and Trueprep™ MAG Blood Sample Prep Kit. Transfer the entire content into the extraction tube. ▲ Dispose of the Sample pre-treatment tube as per the section on “Disposal and Destruction” (Section 20). Start the extraction from step 4 of “the sample extraction and purification procedure” in section 13 of the package insert of 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.

12. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep™ AUTO

Swab specimen must be collected as per standard procedures using a standard nylon flocced 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 of the swab as per the section on “Disposal and Destruction” (Section 20). Tightly close the cap of the Transport Medium for Swab Specimen Tube.

- **For Urine specimen,** collect about 10 ml of first flow of urine (ensuring at least 2 hours gap from last urination) in a urine collection cup. Transfer 1 ml from the cup to the lysis buffer tube and mix well after tightly closing the cap. Dispose off urine collection cup 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 the entire content from the Transport Medium for Swab Specimen Tube into the Lysis Buffer Tube. Use the entire content of lysis buffer tube containing urine sample/Transport Medium for Swab Specimen for further procedure with the Trueprep™ AUTO Universal Cartridge Based Sample Prep Device and Trueprep™ AUTO Universal Cartridge Based Sample Prep Kit (Refer to the User Manual of Trueprep™ AUTO Universal Cartridge Based Sample Prep device and the package insert of Trueprep™ AUTO Universal Cartridge Based Sample Prep kit for details) ▲ Dispose of the Transport Medium for Swab Specimen Tube, lysis buffer tube and transfer pipette after use, as per the section on “Disposal and Destruction” (Section 20).

13. SAFETY PRECAUTIONS

- **In vitro diagnostic use only.**
- **Do not perform the test in the presence of reactive vapours (e.g. from Sodium hypochlorite, acids, alkalis or aldehydes) or dust.**
- **Dispose of the swab as per the section on “Disposal and Destruction” (Section 20).**

14. PROCEDURAL PRECAUTIONS

- 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.
- Do not perform the test in the presence of reactive vapours (e.g. from Sodium hypochlorite, acids, alkalis or aldehydes) or dust.
- While retrieving the Truenat™ CT 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.

15. PROCEDURAL LIMITATIONS

- **Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.**
- **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-quantification of or a failure to detect the presence of the concerned pathogen.**
- 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.
- 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

- **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].**
- **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 bio-hazard waste container.**

17. TEST PROCEDURE

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

- **Switch on the Truelab™ Analyzer.**
- **For Truelab™ Uno/Uno Dx, also switch on the touch screen.**
- **Select User and enter password.**
- **For Truelab™ Uno/Uno Dx, select the test profile for “Chlamydia” to be run from the Profiles Screen on the Analyzer screen.**

For Truelab™ Duo/Quattro, select the Bay (Ide1/2) for Duo and (Ide1/2/3/4) for Quattro from the Status Screen to view the Profiles Select. Test the profile for “Chlamydia” to be run from the Profiles Screen on the Analyzer screen.

- **Place the patient details as prompted in the Truelab™ Analyzer screen.**
- **Press Start Reaction.**
- **For Truelab™ Uno/Uno Dx, open the chip tray.**

For Truelab™ Duo/Quattro, the chip tray opens automatically on tapping the “Start Reaction” button.

- **Open a pouch of Truecn™ CT and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.**
- **Label the chip with the patient ID using a marker pen at the space provided on the back side of the chip.**
- **Place the Truecn™ CT 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.**
- **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” (Section 20). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified DNA from the Elute Collected 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 Truecn™ CT 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).

For Truelab™ Uno/Uno Dx, slide the chip tray containing the Truecn™ CT chip-based Real Time PCR test loaded with the sample into the Truelab™ Analyzer.
Analytical Exclusivity (Primer specificity):

3. Samples and reagents of human and animal origin, as well as contaminated
2. Disinfect the solutions and/or solid waste containing biological samples before
1. Submerge the used

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 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.
• By each new user prior to performing testing on clinical specimen.

20. DISPOSAL AND DESTRUCTION

1. Submerge the used Truenat™ CT chip, microtube, microtube cap, transfer pipette,
pipette tips, nylon flocked swab, Sample pre-treatment tube for swab specimen,
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
disposing them according to local regulations.
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
disposed according to local regulations after decontamination by immersion in a
freshly prepared 0.5% 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
dispensed according to the local regulations.

21. SPECIFIC PERFORMANCE CHARACTERISTICS

Standard used: The assay was standardized from the DNA isolated from standard
culture of Chlamydia trachomatis Serovar E ATCC® VR-348BD™ strain DNA.

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 assay. No interference in the performance of the Truenat™ CT assay was observed with the listed organisms.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomonas vaginatis</td>
<td>Simian virus</td>
</tr>
<tr>
<td>Enterobacter anitratus</td>
<td>Human Papilloma virus</td>
</tr>
<tr>
<td>Enterooccus faecalis</td>
<td>Human Papilloma virus</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>Gardenella vaginalis</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>Vaginalis B virus</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Herpes Simplex virus</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Epstein-Barr virus</td>
</tr>
</tbody>
</table>

Linearity & Assay range:
The linearity assay was performed according to CLSI Guidelines. Serial dilutions of the

Limit of detection (Analytical Sensitivity):
The LOD was determined by testing dilutions of Chlamydia trachomatis Serovar E
ATCC® VR-348BD™ strain DNA. Probit analysis of the data was used to determine the
concentration of the respective DNA with 95% probability. LOD was determined to be
569.91 copies/ml for Chlamydia trachomatis Serovar E ATCC® VR-348BD™ strain.

Robustness:
To determine whether the Truenat™ CT 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™ CT test did not exhibit detectable carryover from
positive to negative samples.

Reproducibility:
The reproducibility of Truenat™ CT assay was determined between three different
users and between three different devices. Three different titres of samples (High,
Medium and Low) were used for this study. The variation in the standard deviation
between the users and devices were calculated. The standard deviation values
obtained for both three user study and three device variation study was within the
accepted range of =<1.5 Ct.

Interference:
The purpose of this study is to determine the effect of potentially interfering
substances on the Truenat™ CT assay. For this study medium 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 extraction on Trueprep AUTO Sample Prep
Device. DNA was eluted and PCR was performed on Truelab Uno Dx real time micro
PCR analyzer using Truenat™ CT chips. The presence of blood till 30% did not interfere with the performance of Truenat™ CT assay. The standard deviation values
obtained were within the accepted range of =<1.5 Ct for Truenat™ CT assay.

Accuracy of Truenat™ CT assay:
Accuracy was determined by performing DNA extractions and Truenat™ CT PCR for
varying titres of samples over 5 consecutive days. The standard deviation values
obtained were within the accepted range of =<1.5 Ct.

Precision of Truenat™ CT assay:
Precision was tested by performing Truenat™ CT assay of High, Medium and Low
titre DNA for five consecutive days. Every day PCR for each titre DNA was run in
duplicates. The standard deviation values obtained were within the accepted range of
=<1.5 Ct.

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