Truenat®

CMV

Chip-based Real Time PCR Test for Cytomegalo Virus

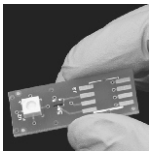
1. INTENDED USE

Truenat® CMV (REF 601590005 / 601590020 / 601590025 / 601590050 / 601590100 / 601590200) is a Chip-based Real Time Polymerase Chain Reaction (PCR) test for the quantitative detection of Cytomegalovirus in human serum / plasma / whole blood specimens and aids in diagnosis of infection with Cytomegalovirus. **Truenat® CMV** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. **Truenat® CMV** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

Cytomegalovirus (CMV) is a genus of viruses in the order Herpesvirales, in the family Herpesviridae, in the subfamily Betaherpesvirinae. Human cytomegalovirus (HCMV) is a human herpesvirus with a linear double stranded DNA which has the largest genomes, about 230 kbp in length, of any of the herpesviruses. In the United States, nearly one in three children are already infected with CMV by age five and over half of adults have been infected with CMV by age 40. Once CMV is in a person's body, it stays there for life and can reactivate. A person can also be re-infected with a different strain (variety) of the virus. Most of people with CMV infection have no symptoms and aren't aware that they have been infected. In some cases, infection in healthy people can cause mild illness that may include fever, sore throat, fatigue and swollen glands. Occasionally, CMV can cause mononucleosis or hepatitis (liver problem). People with weakened immune systems who get CMV can have more serious symptoms affecting the eyes, lungs, liver, esophagus, stomach, and intestines. Babies born with CMV can have brain, liver, spleen, lung, and growth problems. The most common long-term health problem in babies born with congenital CMV infection is hearing loss, which may be detected soon after birth or may develop later in childhood. People with CMV may pass the virus in body fluids, such as saliva, urine, blood, tears, semen, and breast milk. CMV is spread from an infected person in the following ways:

- From direct contact with saliva or urine, especially from babies and young children
- Through sexual contact
- From breast milk to nursing infants
- Through transplanted organs and blood transfusions



Viral culture, detection of CMV antibodies and, more recently, the detection of CMV DNA using molecular diagnostic techniques are the standard tools used for diagnosis of CMV infection. Molecular techniques such as polymerase chain reaction (PCR) or Real Time PCR are much more sensitive and specific. However PCR or Real Time PCR tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days.

The **Truelab®** Real Time micro PCR System enables decentralization and near patient diagnosis and treatment monitoring of Cytomegalovirus 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 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® CMV 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 Cytomegalovirus and runs on the **Truelab®** Real Time Quantitative 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® CMV** chip also stores information of used test to prevent any accidental re-use of the test.

3. PRINCIPLE OF THE TEST

Truenat® CMV works on the principle of quantitative 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® CMV** 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® CMV** chip and the test is started. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® CMV** chip to release the fluorophores in an exponential manner and the emitted light 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, a CMV "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and International Units (IU) per milliliter (IU/ml) is also displayed on the screen. Based on the Ct 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 network or 3G/GPRS network. Upto 20000 results in **Truelab® Uno Dx / Duo / Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this kit has been taken from the UL55 or gB gene coding for envelope glycoprotein B and RL11 gene coding for membrane glycoprotein RL11.

5. CONTENTS OF THE Truenat® CMV KIT

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

B. Package Insert

REF	601590005	601590020	601590025	601590050	601590100	601590200
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. STORAGE AND STABILITY

Truenat® CMV chip is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for 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 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.

8. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF623010001 / 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** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), Powder free disposable gloves, waste disposal container with lid.

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

Truenat® CMV requires purified nucleic acids from human serum / plasma / whole blood specimens collected in EDTA anticoagulant that are extracted using the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. Sample must be pre-treated using **Trueprep® AUTO** Universal Sample Pre-treatment Pack. Transfer 250 µL of whole blood or 500 µL of plasma/serum specimen using the transfer pipette provided into the Lysis buffer tube provided and mix well (Refer to the package insert of **Trueprep® AUTO** Universal Sample Pre-treatment Pack for further details).

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 30ºC and 40ºC.

Nucleic acid extraction: Use entire content from the Lysis Buffer tube containing specimen 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 lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 17).

10. 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.

11. 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® CMV** 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.

12. 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 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.

13. 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 bio-hazard waste container.

14. 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 "CMV" 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 "CMV" 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® CMV** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Place the **Truenat® CMV** 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.
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" (Section 17). 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® CMV** 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 17).
10. For **Truelab® Uno Dx,** slide the chip tray containing the **Truenat® CMV** 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® CMV** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 17).
14. Turn on **Truelab®** micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later. (Refer to **Truelab®** Analyzer manual).
15. Switch off the **Truelab®** Analyzer.

15. RESULTS & INTERPRETATIONS

Two amplification curves are displayed on the **Truelab®** Real Time micro PCR Analyzer screen when optical plot is selected to indicate the progress of the test. Both the target and the internal positive control (IPC) curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The Cycle threshold (Ct) will depend on the number of target nucleic acids in the sample. The 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 Ct value and the IU/ml 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.

16. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab®** Real Time micro PCR Analyzer is working accurately, run known PCR positive and negative samples from time to time.

17. DISPOSAL AND DESTRUCTION

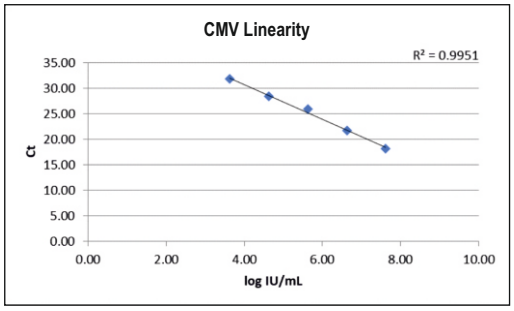
1. Submerge the used **Truenat® CMV** chip, microtube, microtube cap, transfer pipette, pipette tips, lysis buffer tube etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid 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.

18. SPECIFIC PERFORMANCE CHARACTERISTICS

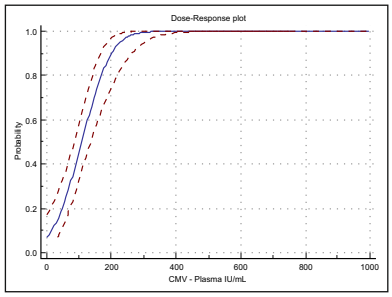
Analytical Exclusitivity (Primer specificity): The following viruses and microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine potential cross-reactivity in the **Truenat® CMV** assay. No interference in the performance of the **Truenat® CMV** assay was observed with the listed group of organisms.

Viruses	Microorganisms
Adenovirus	*Staphylococcus epidermidis*
Hepatitis C virus	*Mycobacterium gordonae*
Hepatitis B virus	*Neisseria gonorrhoeae*
Human Immunodeficiency virus-1	*Chlamydia trachomatis*
Human Immunodeficiency virus-2	*Candida albicans*
Epstein-Barr virus	*Staphylococcus aureus*
Herpes Simplex virus	*Mycobacterium tuberculosis*
Simian virus	
Human herpes virus 1	
Human herpes virus 2	
Human herpes virus 3	
Human herpes virus 4	
Vaccinia virus	
BK polyomavirus	
Human T-lymphotropic virus 1	

Linearity:
The linearity assay was performed according to CLSI Guidelines. Serial dilutions of CMV culture made from 4.23E+07 to 4.23E+03 IU/mL in negative human plasma and 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® CMV** test is found to be linear over 5 orders of magnitude (from 4.23E+07 IU/mL to 4.23E+03 IU/mL).



Limit of detection (Analytical Sensitivity):
The LoD was determined by testing dilutions of CMV NIBSC 1st International Standard diluted in Negative human plasma. The evaluation 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 95% probability of detection. The LoD for **Truenat® CMV** test was found to be 226.79 IU/mL.



CMV LoD: 226.79 IU/mL [95%CI: 186.90 - 303.36] IU/mL]

Robustness:
To determine whether the **Truenat® CMV** test showed any signs of carryover of PCR products between runs, alternating runs of positive samples and negatives samples were performed. The number of samples run was 20 positives and 20 negatives. The samples were tested by performing PCR on **Truelab®** Real Time micro PCR Analyzer using **Truenat® CMV** test. The **Truenat® CMV** test did not exhibit detectable carryover between positive and negative sample runs.

Reproducbility:
The purpose of this study is to determine the reproducibility of **Truenat® CMV** test between three different users (Inter user), between three different devices (Inter device) and between five consecutive days (Inter day) on 3 different titres of samples (High, Medium and Low). The Mean % CV values obtained for all titres has been calculated as Inter User (2.15), Inter day (1.63) and Inter Device (3.12) which were in the accepted range of ≤15% CV for **Truenat® CMV** test.

Interference:
The purpose of this study is to determine the effect of potentially interfering substances on the performance of **Truenat® CMV** assay. CMV Sample was spiked into known negative human plasma containing the respective interfering substances. Potentially interfering substances used are: Albumin - 9 g/dL, Billirubin - 20 mg/dL, Human DNA - 0.4 mg/dL and Haemoglobin - 500 mg/dL. The presence of any of the mentioned endogenous substances at the stated concentrations did not affect the performance of **Truenat® CMV** assay.

Precision:
Precision was tested by performing **Truenat® CMV** assay of High, Medium and Low titre CMV DNA for five consecutive days. Every day PCR for each titre DNA was run in triplicates. The %CV values obtained for High titre (0.99), Medium titre (1.25) and Low titre (2.17) were within the accepted range of ≤15% CV for **Truenat® CMV** assay.

Clinical validations:
A panel of 50 samples includes 20 positives and 30 negatives were run in parallel on 3 different lots of **Truenat® CMV** assay at Microbiological laboratory, Tamil Nadu and its performance was compared with CE marked CMV PCR kit as the reference test.

Truenat® CMV	**Reference CE marked kit**		
	Positive	Negative	Total
Positive	20	0	20
Negative	0	30	30
Total	20	30	50

Sensitivity: 100% (95% CI 83.16% to 100.00%)
Specificity: 100% (95% CI 88.43% to 100.00%)
Accuracy: 100% (95% CI 92.89% to 100.00%)

With the consideration of above data, **Truenat® CMV** test performed consistently in this study with observed sensitivity of 100% and specificity of 100% in comparison with CE marked CMV PCR kit and the inter lot variation data obtained was within the accepted range of ≤15% CV for **Truenat® CMV** test.

19. REFERENCES

1. Centers for Disease Control and Prevention. (n.d.). Cytomegalovirus (CMV) and Congenital CMV Infection. Retrieved from https://www.cdc.gov/cmv/index.html
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4. Herrmann, B., Larsson, V. C., Rubin, C. J., Sund, F., Eriksson, B. M., Arvidson, J., Yun, Z., Bondeson, K., & Blomberg, J. (2004). Comparison of a Duplex Quantitative Real-Time PCR Assay and the COBAS Amplicor CMV Monitor Test for Detection of Cytomegalovirus. Journal of Clinical Microbiology, 42(5), 1909-1914. doi:10.1128/JCM.42.5.1909-1914.2004.
5. Gault, E., Michel, Y., Dehe´e, A., Belabani, C., Nicolas, J., & Garbarg-Chenon, A. (2001). Quantification of Human Cytomegalovirus DNA by Real-Time PCR. Journal of Clinical Microbiology, 39(2), 772-775. doi:10.1128/JCM.39.2.772-775.2001.
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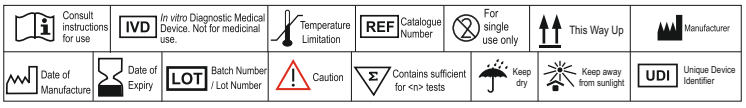
SYMBOL KEYS



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