Human Cytomegalovirus DNA Quantitative Fluorescence Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number

S3014E

Package Specification

48 tests/kit

Intended Use

The diagnostic kit is an in vitro nucleic acid amplification test for the detection of Human Cytomegalovirus (HCMV) DNA in human urine, serum and peripheral blood samples. It is intended for use as an aid in the diagnosis of an HCMV infection and for observing drug efficacy.

For in vitro diagnostic use only. For professional use only.

Summary

Human cytomegalovirus (HCMV), also called cell inclusion body virus, is a double helix DNA virus and belongs to β genus of herpes virus family, which causes infected cells to enlarge and has a huge intranuclear inclusion. The ways of infection of HCMV is mainly through contact, blood transfusion, intrauterine and birth canal, and the infections are commonly found in fetus, newborns, pregnant women, etc. If the pregnant women are infected, it may cause their newborns congenital monstrosity. When the organism immune deficiency or immune system is under inhibitory state, people can be easily infected by HCMV, such as the patients receiving immunosuppressive therapy after transplantation of organ, the patients receiving malignant tumor chemotherapy and AIDS patients, etc. If these patients are infected by HCMV, it usually causes high mortality and serious diseases.

Clinical tests suggest that HCMV infection hasn't specific manifestations and it causes harm to multiple organs, especially the liver and lung. A statistical analysis of positive and negative rate of HCMV in various kinds of clinical indications shows HCMV infection is probably related with various diseases, such as Cytomegalovirus hepatitis, Infant hepatitis syndrome, liver dysfunction, pneumonia, Bronchitis, Upper respiratory tract infections, enteritis, enterocolitis, diarrhea, hematosepsis, heart failure, etc.

Test Principle

The diagnostic kit uses a nucleic acid lysis buffer to allow rapid lysis and release of HCMV DNA in test samples. By applying real-time fluorescence quantitative PCR technology, this test utilizes a pair of specific primers which are designed to target at a conserved sequence of HCMV-DNA, and a specific fluorescence probe, accompanied with other ingredients in HCMV-PCR mix, to achieve fast detection of HCMV-DNA through fluorescent signal changes.

The PCR detection system uses UNG enzyme + dUTP contamination-proof system, which can fully degrade possible PCR amplifited products, to avoid a false positive result.

The PCR detection system uses internal control to judge whether the internal control is normal by monitoring the presence of PCR inhibitors, to avoid a false negative result.

Components of the Diagnostic Kit

No.	Reagent Name	Specification & Qty.	Main Ingredients
1	HCMV-Lysis Buffer	2.5 mL/tube x 1 tube	KCl, SDS, Surfactin
2	HCMV-Enzyme Mix	96 μL/tube x 1 tube	DNA polymerase, uracil DNA glycosylases
3	HCMV-Internal Control	50 μL/tube x 1 tube	Cloning plasmid containing target gene fragment
4	HCMV-PCR Mix	912 μL/tube x 2 tubes	Primer, probe, dNTPs, Mg^{2+}, PCR buffer
5	HCMV-Quantitative Reference A (4.0E+07copies/mL)	50 μL/tube x 1 tube	Cloning plasmid containing target gene fragment
6	HCMV-Quantitative Reference B (4.0E+06copies/mL)	50 μL/tube x 1 tube	Cloning plasmid containing target gene fragment
7	HCMV-Quantitative Reference C (4.0E+05copies/mL)	50 μL/tube x 1 tube	Cloning plasmid containing target gene fragment
8	HCMV-Quantitative Reference D (4.0E+04copies/mL)	50 μL/tube x 1 tube	Cloning plasmid containing target gene fragment
9	HCMV-Negative Control	50 μL/tube x 1 tube	HCMV negative specimen (inactivated)
10	HCMV-Positive Control	50 μL/tube x 1 tube	HCMV positive specimen (inactivated)
11	Concentrate	5 mL/tube x 1 tube	PEG-600, NaCl, Purified water

Note:

1. Do not mix or exchange components from different kits.
2. All biological materials in the diagnostic kit have been inactivated. Please refer to the specified value in the kit for the concentration of HCMV- Quantitative Reference A~D.
3. Reagent required but not provided: Sterile saline, Red Blood Cell Lysis Buffer (Reference Number: S1004E-48) manufactured by Sansure Biotech.

Precautions

1. For *in vitro* diagnostic use only. Please read the product manual carefully before operation.
2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory; laboratory personnel must receive professional training; test processes must be performed in separated regions; all consumables should be for single use only after sterilization; required instruments and devices as specified in Test Method should be used for each stage of the experimental operation; all lab devices required in different processes and regions should not be cross-used.
4. All specimens for detection should be handled as potentially infectious and they should be handled in a proper way. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.
5. Before use, all reagents must be fully thawed at room temperature and mixed thoroughly.
6. After the addition of the sample in the tube the resulting solution is to be considered potentially biohazardous, handle the reagent with appropriate precautions and good laboratory practice.
7. The safe disposal of the reagents supplied must be carried out according to the instruction contained in the specific Safety Data Sheets and in compliance with the national regulations on disposal of potentially hazardous waste.
8. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established; If you have any questions about the test or the results, please contact Sansure`s customer service hotline +86-731-88883176-6116 or send an email to info@sansure.com.cn/ support@sansure.com.cn.

Storage

The diagnostic kit should be stored in a sealed pouch at -25°C to -15°C and protected from light. The shelf life of the kit is 12 months. The freeze/thaw cycles should not exceed 3.

Compatible Instrument

The diagnostic kit is compatible to Applied Biosystems 7500, Roche LightCycler® 480, Stratagene Mx3000P and CFX96 Deep Well Dx ORM.

Specimen Requirements

1. Applicable specimen types: urine, serum and peripheral blood specimens.
2. Collection of specimen:
   2.1 Collection of urine specimen: Use urine collection cup to collect the middle phase of morning urine, absorb 5 mL of the sample and inject into 5 mL sterile centrifuge tube, seal and deliver to test.
   2.2 Collection of serum specimen: use sterile syringe to withdraw 2 mL of vein blood from the subject, then inject into sterile collection tube. Place it at room temperature for up to 4 hours to allow the serum to be separated or directly centrifuge the sample at 1600rpm for 5 minutes to allow the separation of serum. Transfer to 1.5 mL sterile centrifugal tube, seal for testing.
   2.3 Collection of peripheral blood specimen: use sterile syringe to withdraw 2 mL of vein blood from subject, insert into sterile collection tube containing EDTA or Sodium citrate anticoagulant, and immediately mix by gentle inversion.
3. Storage and delivery of specimen: specimen collected through above mentioned method can be used for immediate diagnosis, or saved at temperature of 2~8°C (no longer than 24 hours). If specimen needs to be saved for 2 years, storage temperature should be below -20°C. Avoid repeated freezing and thawing. Delivery of sample should be transported in the way of sealed in iced pitcher with ice or sealed in foam box with ice.

Test Method

1. Preparation of reagent (perform at "reagent preparation region")

1.1 Take out all the components out off the diagnostic kit, and equilibrate them at room temperature. Allow the reagents to equilibrate at room temperature, and then vortex each of them respectively for future use.

1.2 Preparation of PCR-Mastermix Solution: According to the quantity of test samples, HCMV-Negative controls, HCMV-Positive controls and HCMV-Quantitative Reference A~D, take out the corresponding quantity of HCMV-PCR Mix (38 μL/test), HCMV-Enzyme Mix (2 μL/test) and HCMV-internal control (1 μL/test) in portion, fully mix them into PCR-Mastermix Solution, and centrifuge them instantaneously for future use.

2. Processing and loading of specimen (perform at "specimen processing region")

2.1 Pre-treatment of specimens

2.1.1 Urine specimen: Take 1 mL of urine specimen, centrifuge at 12,000rpm for 5 minutes, discard the above layer of clear fluid, add 50 μL of HCMV-Lysis Buffer into the precipitate. Fully vortex or pipette the liquid up and down to mix and use it as under-test specimen.

2.1.2 Serum specimen: Take 100 μL of serum specimen, add the same portion of concentrate, centrifuge at 12,000 rpm for 5 minutes, discard the above layer of clear fluid, add 50 μL of HCMV-Lysis Buffer into the precipitate. Pipette it up and down for a couple of times to shatter the precipitate, and use it as under-test specimen.

2.1.3 Peripheral blood specimen: it is recommended to use Sansure's Red blood cell lysis buffer to handle the specimen and use the abstracted DNA as sample.

2.1.3.1 Take the peripheral specimens and mix them thoroughly (If there are residual whole blood samples on the tube wall after vortex, centrifuge it at a low speed). Use the pipette to inhale and inject the specimens for 2-3 times (When the specimens stay stable completely, the white blood cell in the whole blood will settle naturally, which causes nonuniform specimens, then the specimens should be mixed thoroughly). Take 1 mL of red blood cell lysis buffer into a 2 mL centrifuge tube and load 800 μL of the whole blood sample. Cover the tube lid and mix it thoroughly (It is recommended to vortex it for 20-30 times). Centrifuge it at 12,000 rpm for one minute and then discard the supernatant.

2.1.3.2 Add 1 mL of red blood cell lysis buffer into the precipitation and mix them thoroughly (It is recommended to spin down for 20-30 times to make sure there are no obvious red bulk precipitations). Centrifuge it at 12,000 rpm for 3 minutes and discard the supernatant.

2.1.3.3 Add 1 mL of saline, but there is no need to vortex. Centrifuge it at 12,000 rpm for 10 minutes and discard the supernatant (During the process of the whole blood samples, when discard the supernatant, please be careful to remove the supernatant and don't absorb the precipitation, otherwise there will be a Ct value delay or a false negative result). The white precipitations at the bottom of the tube are the extracted white blood cells and can be used for nucleic acid extraction.

2.1.3.4 Add 400 μL of nucleic acid lysis buffer into the precipitation and vortex them thoroughly. Process them at a constant temperature of 75°C for 10 minutes and then centrifuge them immediately for future use.

2.1.4 HCMV-Negative Control, HCMV-Positive Control and HCMV-Quantitative References: Take respectively 10 μL of HCMV-Negative Control, HCMV-Positive Control and HCMV-Quantitative References A-D. Mix them respectively with 10 μL of HCMV-Lysis Buffer for future use.

2.2 Loading of specimen

2.2.1 Add 10 μL of the test specimen, HCMV-Negative Control, HCMV-Positive Control and HCMV-Quantitative References A~D processed as per the above method into each PCR reaction tube.

2.2.2 Hold them for 10 minutes, and add 40 μL of PCR-Mastermix Solution to each tube. Absorb and inject them for 2~3 times, then cover the tubes (after removing bubbles), and centrifuge at 2000 rpm for 30 seconds.

3. PCR Amplification (perform at "Amplification and analysis region") (refer to user manual of each instrument to adjust the settings)

3.1 Place PCR reaction tube into the sample well of amplification device, set in corresponding sequence of HCMV-Negative Control, HCMV-Positive Control, HCMV-Quantitative References A~D and unknown samples, and set sample name and concentration of quantitative references.

3.2 Select PCR test channel

For Applied Biosystems 7500, Stratagene serials equipment and CFX96 Deep Well Dx ORM:

a. Select FAM (Reporter: FAM, Quencher: None) channel to test HCMV-DNA.

b. Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None), test internal control.

c. Reference fluorescence (Passive reference) set as none. Set sample volume as 50.

For Roche Light Cycler 480:

Select New Experiment, on the setting panel of setup "Detection format", in the drop-down menu, select Dual Color Hydrolysis Probe/UPL Probe. In the drop-down menu of Customize: a. Select FAM channel detect HCMV-DNA. b. select VIC/HEX/Yellow 555 channel detect internal control. c. Set reaction Volume as 50.

3.3 Setting of cycle parameters: (different equipment, different time parameter, please see the table below)

3.3.1 Parameter settings for Applied Biosystems 7500, Stratagene equipment:

	Step	Temperature	Time	Recycle Qty.
1	UNG Enzyme reaction	50°C	2 min.	1
2	Taq Enzyme activation	94°C	5 min.	1
3	Denaturation	94°C	15 sec.	45
	Annealing, extension and collection of fluorescence	57°C	30sec.*	
4	Device cooling	25°C	10 sec.	1

*Note: Due to the device Applied Biosystems 7500 own reason, the setting cannot be 30 sec., but can be set to 31 sec.)

3.3.2 Parameter setting for Roche LightCycler 480 equipment (if parameter not listed, select the equipment default value)

Program	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Cycles	Analysis Mode
1	50°C	None	00:02:00	1	None
2	94°C	None	00:02:00	1	None
3	94°C	None	00:00:05	45	Quantification
	57°C	Single	00:00:30		
4 (optional)	25°C	None	00:00:10	1	None

When the setting are completed, save settings and operate the reaction procedure.

4. Result Analysis (refer to user manual of each instrument to adjust the settings)

After the reaction completes, results will be saved automatically. Based on the analyzed diagram to adjust Start value, End value and Threshold value of Baseline (user can adjust them according to actual situation, Start value can be set between 3~15, and End value can be set between 5~20, adjust amplification curve of negative control to make it flat or lower than threshold line), click “Analyze” to implement the analysis, make each parameters satisfy the requirement of the below mentioned “2.5 Quality Control”, then go to “Plate” window to record the detected Ct value and quantitative result.

5. Quality Control

5.1 HCMV-Negative Control: no display of Ct value, but internal standard is detected as positive, Ct value ⩽ 40.

5.2 HCMV-Positive Control: detect concentration value is between 1.11E+05~1.11E+06 copies/mL.

5.3 Four references: should detect as positive, and correlation coefficient of standard curve is R2 ⩾ 0.98.

5.4 The above requirements must be satisfied at the same time in the same test, otherwise, the test is treated as invalid, and needs to be re-tested.

Reference Scope

Through the research of reference values to determine Ct reference value of the detection target gene of the diagnostic kit is 39, Ct reference value of detection internal control is 40.

Explanation of Detection Result

1. Qualitative result

1.1 For samples of detected Ct value ≤ 39, reported as HCMV DNA positive.

1.2 For samples of detected Ct value > 39, and the detected internal control is positive (Ct value ≤40), reported as HCMV DNA is lower than diagnostic lower limit; and if Ct value > 40 or no display, then the sample’s detection result is invalid. Reason should be searched and excluded and perform repeated test on the sample (if the test result from repeated test still shows invalid, please contact Sansure Biotech.)

2. Quantitative result

2.1 For sample of detected value which is between 4.00E+02 ~4.00E+09 copies/mL , report the corresponding test result.

2.2 For sample of detected value which is > 4.00E+09 copies/mL, mark clearly on the report as > 4.00E+09 copies/mL. If accurate quantitative is required, dilute the sample to 4.00E+09 copies/mL or below and then repeat the test.

2.3 For sample of detected value < 4.00E+02 copies/mL, and the detected internal control is positive (Ct value ≤40), reported that HCMV DNA is below diagnostic lower limit.

Limitation of Detection Method

Detection result of sample is related with sample collection, processing, delivery and storage quality, any mistakes in the circle will lead to incorrect detection result. If there is cross-contamination occurring during sample processing, false-positive result might happen.

Product Performance Index

When the kit is used to detect the enterprise’s work references, the consistency rate for both negative and positive reaches 100%; linear quantitative references L1-L5 are all tested positive; the coefficient of correlation between the logarithm value of detection concentration and the logarithm value of theoretical concentration is ≥0.97. Precision test shows excellent reproducibility in both intra-batch and inter-batches with its coefficient of variation of Ct value <10%, and its coefficient of variation of concentration <50%. The linear range of this kit is determined to be between 4.00E+02 copies/mL and 4.00E+09 copies/mL. The sensitivity for the kit is 4.00E+02 copies/mL. It shows no cross-reaction with pathogens such as HBV, HCV, HIV, HSV-1, HSV-2, EBV and HPV. If the presence in specimen does not exceed 300μmol/L for total bilirubin , 6mmol/L for triglyceride and 20g/L for hemoglobin, no obvious interference on test results is observed.

Bibliography

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Symbols

Symbols	Meanings	Symbols	Meanings
IVD	*In Vitro* Diagnostic Medical Device		Date of Manufacture
	Use-by date		Consult Instructions for Use
	Temperature Limit		Manufacturer
LOT	Batch Code	REF	Reference Number
Σ	Contains sufficient for <n> tests	EC REP	Authorized representative in the European Community /European Union
	Caution	CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.
CONTROL -	Negative Control	CONTROL +	Positive Control
LYSIS BUFFER	Lysis Buffer	INTER CONTROL	Internal Control
ENZYME MIX	Enzyme Mix	PCR MIX	PCR Mix
Quant. Ref. A Copies/mL	Quantitative Reference A (4.0E+07copies/mL)	Quant. Ref. B Copies/mL	Quantitative Reference B (4.0E+06copies/mL)
Quant. Ref. C Copies/mL	Quantitative Reference C (4.0E+05copies/mL)	Quant. Ref. D Copies/mL	Quantitative Reference D (4.0E+04copies/mL)
Concentrate	Concentrate	VERSION	Version
	Keep away from light	21 PAP	PAP21: Not corrugated cardboard

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