MultNAT HIV-1 Viral Load Assay

[PRODUCT NAME]
MultNAT HIV-1 Viral Load Assay

[SPECIFICATION]
Σ 20

[INTENDED USE]
MultNAT HIV-1 Viral Load Assay is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma or serum of HIV-1-infected individuals using the automated MultNAT Molecular Diagnostic Testing System for specimen processing, amplification and detection. The test can quantitate HIV-1 RNA over the range of 50 to 1×10^7 copies/mL(cps/mL), and is validated for specimens across Group M, N and O.
This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiretroviral therapy by measuring changes in plasma HIV-1 RNA levels during the course of antiretroviral treatment.
The MultNAT HIV-1 Viral Load Assay is not intended to be used as a donor screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

[MATERIALS PROVIDED]
The MultNAT HIV-1 Viral Load Assay kit contains sufficient reagents to process 20 specimens or quality control samples. The kit contains the following:

No.	Name	Specification	Quantity	Main components
1	HIV-1-Cartridge	1 test/cartridge	20 cartridges	Specific primers and probes, deoxyribonucleoside triphosphate (dNTP), DNA polymerase, reverse transcriptase, Uracil-DNA Glycosylase (UDG)
2	HIV-1-RNA Extraction Solution	2 mL/tube	20 tubes	Guanidine salt, Magnet beads, Isopropanol
3	HIV-1-QS	1 test/tube	20 tubes	HIV-1 Quantitation Standard (QS) Armored RNA
4	HIV-1-Positive Control	1.2 mL/tube	1 tube	Pseudovirion contains HIV-1 target-specific fragment
5	HIV-1-Negative Control	1.2 mL/tube	1 tube	Pseudovirion does not contain target-specific fragment

Notes: The components in different batches of kits cannot be used interchangeably.

[TEST PRINCIPLE]
Ustar MultNAT Molecular Diagnostic Testing System automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex samples using real-time reverse transcriptase PCR (RT-PCR). The systems consist of an instrument preloaded software for running tests and viewing the results. The systems require the use of single-use disposable Ustar cartridges that hold the RT-PCR reagents and host the RT-PCR processes. Because the cartridges are self-contained, cross-contamination between samples is minimized.
The MultNAT HIV-1 Viral Load Assay includes reagents for the detection of HIV-1 RNA in specimens and HIV-1 Quantitation Standard (HIV-1-QS) used for quantitation of HIV-1 RNA. The HIV-1-QS are also used to monitor the presence of inhibitor(s) in the RT and PCR reactions. Specimen preparation is automated using Ustar MultNAT Molecular Diagnostic Testing System with amplification and detection. The MultNAT HIV-1 Viral Load Assay is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide probe specific to the target. The MultNAT HIV-1 Viral Load Assay permits automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation Standard (QS) Armored RNA. The Master Mix reagent contains primers and probes specific for both HIV-1 RNA and HIV-1 QS RNA. The Master Mix has been developed to ensure equivalent quantitation of group M, N and O subtypes of HIV-1. The detection of amplified DNA is performed using a target-specific and a QS-specific dual-labeled oligonucleotide probe that permit independent identification of HIV-1 amplicon and HIV-1 QS amplicon. The quantitation of HIV-1 viral RNA is performed using the HIV-1 QS. It compensates for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantitation of HIV-1 RNA in each specimen. The HIV-1 QS is a non-infectious Armored RNA construct that is added to each specimen at a known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification and detection steps of cleaved dual-labeled oligonucleotide detection probes. Ustar MultNAT Molecular Diagnostic Testing System calculates the HIV-1 RNA concentration in the test specimens by the HIV-1 signal.

[STORAGE AND STABILITY]
1. Storage condition: store the assay at 2~8℃.
2. Validity period: 6 months (provisional). See the label for production and expiry date.
3. Transport at 2~37℃ within 15 days does not affect the assay performance.

[SAMPLE COLLECTION AND HANDLING]
1. Sample type
Human plasma or serum
a) Plasma
Collect 2 mL venous blood in an EDTA anticoagulant blood collection tube (*Do not use heparin anticoagulant tube), and immediately turn the tube upside down 5~6 times to mix the anticoagulant and blood well. Wait 5~10 minutes, and then transfer the isolated plasma into a sterile centrifuge tube (1.5 mL).
b) Serum
Draw 2 mL venous blood by a disposable syringe to a sterile dry tube. Then, place the tube at room temperature (20~35℃) for 30~60 minutes OR centrifuge the tube (horizontal centrifuge, 1500 rpm) for 5 minutes immediately after collection. Transfer the isolated serum into a sterile centrifuge tube (1.5 mL).
3. Sample storage
Collected samples should be sent for testing as soon as possible. If instant testing cannot be carried out: Plasma sample collected in an EDTA anticoagulant tube needs to be refrigerated between 2-8 ℃ for not more than three (3) days; Serum sample needs to be refrigerated between 2-8 ℃for not more than three (3) days, or below -20℃ within three (3) months.

4. Sample transportation
Transport samples in sealed pot or foam box filled with ice.

[APPLICABLE INSTRUMENT]
MultNAT HIV-1 Viral Load Assay is to be used with MultNAT Molecular Diagnostic Testing System.

[TEST PROCEDURE]
1. Preparing the Specimen
1.1 If using frozen specimens, place the specimens at room temperature (20~35 ℃) until completely thawed before use.
1.2 Vortex plasma or serum for 15 seconds before use. If the specimen is cloudy, clarify by a quick spin.
2. Preparing the Cartridge
2.1 Wear protective disposable gloves.
2.2 Inspect the test cartridge for damage. If damaged, do not use it.
2.3 Open the lid of the test cartridge.
Fully mix the HIV-1-RNA Extraction Solution until there is no visible brown sediment nor agglomerates in the tube. Using the pipette to transfer 2 mL HIV-1-RNA Extraction Solution into the HIV-1-QS tube to vortex and dissolve HIV-1-QS until there is no purple solid particles. The above mixture is transferred into HIV-1-cartridge. Then using the pipette to transfer 0.4 mL plasma or serum from the collection tube into the test cartridge.
2.4 Close the cartridge lid and mix it gently.
2.5 Load the cartridge into the Ustar MultNAT Molecular Diagnostic Testing System.
2.6 Scan QR code (Quick Response code) . Please align the QR code with the red dot in the center of the scan area.
2.7 Enter the sample information.
2.8 Click start detection.
3. Test Result
After testing, the results will be displayed and saved automatically. For details, please refer to Section [*Interpretation of the Test Results*].
4. Quality control testing
4.1 Using the pipette to transfer 2 mL HIV-1-RNA Extraction Solution into the HIV-1-QS tube to vortex and dissolve HIV-1-QS until there is no purple solid particles. The above mixture is transferred into HIV-1-cartridge.
4.2 Using the pipette to transfer 0.4 mL HIV-1-Positive Control or HIV-1-Negative Control into the test cartridge.
4.3 The rest of the operations are the same as the steps 2.4-2.8 in Section [Assay Protocol], including 2. Preparing the Cartridge

[INTERPRETATION OF RESULTS]
The results are interpreted automatically by the Ustar MultNAT Molecular Diagnostic Testing System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window. Possible results are shown in Table 1.

Table 1. MultNAT HIV-1 Viral Load Results and Interpretation

TEST Result	Interpretation
POSITIVE: XX cps/mL	1. XX cps/mL>1×10^7 cps/mL, QS is positive. HIV-1 RNA is detected above the analytical measurement range. 2. XX cps/mL < 50 cps/mL, QS is positive. HIV-1 RNA is detected below the analytical measurement range. 3. 50 cps/mL<XX cps/mL<1×10^7 cps/mL, QS is positive. HIV-1 RNA is detected within the analytical measurement range.
NEGATIVE	HIV-1 RNA is not detected, QS is positive.
INVALID	Presence or absence of HIV-1 RNA cannot be determined, QS is negative.
NO RESULT	Insufficient data were collected. For example, the operator stopped a test that was in progress.

1. Conditions That Require a Retest
1.1Reasons to Repeat the Assay
If any of the following conditions occur, please retest with a new HIV-1-Cartridge.
•The XX cps/mL>1×10^7 cps/mL indicates that HIV-1 RNA is detected above the analytical measurement range. We recommend the operator to dilute the sample and retest it with a new HIV-1-Cartridge.
• An INVALID result indicates that the QS Cts are not within valid range, or the sample was not properly processed , or PCR was inhibited.
• A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or a power failure occurred.
1.2 Retest Procedure
For retest of an INVALID or NO RESULT result, use a new cartridge (do not re-use the cartridge).
• Remove a new cartridge from the kit.
• See Section [*Assay Protocol*].

[LIMITATIONS]
1. Inappropriate collection, transport and treatment of samples or low viral load may yield false negative result.
2. Other unverified interfering substances or amplification inhibitors may cause false negative results.
3. Mutation of target nucleic acid to be tested or the sequence alteration caused by other reasons may yield false negative result.

[PERFORMANCE CHARACTERISTICS]
1. Limit of Detection (LOD): 50 cps/mL.
2. Linear range: 50 cps/mL to 1×10^7 cps/mL.
3. Cross-reactivity
None of the organisms tested showed cross reactivity including *Human Immunodeficiency virus 2*, *Human T-cell lymphotropic virus 1*, *Human T-cell lymphotropic virus 2*, *Candida albicans*, *Cytomegalovirus*, *Epstein-Barr virus*, *Hepatitis A virus*, *Hepatitis B virus*, *Hepatitis C virus*, *Herpes simplex virus 1*, *Herpes simplex virus 2*, *Human herpes virus 6*, *Influenza A*, *Staphylococcus aureus*.
4.Endogenous substances including albumin (9 g/dL), bilirubin (20 mg/dL), hemoglobin (500 mg/dL), human DNA (0.4 mg/dL), triglycerides (3000 mg/dL) and drugs prescribed to HIV-1 infected patients including zidovudine, saquinavir, ritonavir, clarithromycin, abacavir sulfate, peginterferon 2b, ribavirin, tenofovir disoproxil fumarate, lamivudine, (3TC), indinavir sulfate, ganciclovir, valganciclovir HCl, acyclovir, raltegravir, stavudine (d4T), efavirenz, lopinavir/Ritonavir, enfuvirtide (T-20), ciprofloxacin, nevirapine, nelfinavir mesylate, azithromycin, valacyclovir HCl, fosamprenavir calcium, interferon alfa-2b were shown to not interfere with the quantification of the MultNAT HIV-1 Viral Load Assay or impact the assay specificity.

[PRECAUTIONS]
1. For research use only. Please read this IFU carefully prior to use. Ustar shall not assume any responsibility for the false results and corresponding consequences due to improper handling of the assay or any problems not derived from the performance defects of the assay.

2.Testing

1) This assay is a single-use product. Disinfect the worktable and necessary articles with 1% sodium hypochlorite solution, 75% alcohol solution or ultraviolet lamp regularly.
2)Take corresponding laboratory quality control measures according to relevant national regulations to avoid false-positive results caused by laboratory pollution.
3)Manage protective facilities used by operators, such as gloves, laboratory clothes, etc. in different area to avoid an introduction of pollution which will lead to the error of test results.
4)Do not squeeze the middle and lower parts of HIV-1-Cartridge when using it.
5)HIV-1-RNA Extraction Solution contains insoluble particles. Mix well before pipetting to guarantee all magnetic beads are transferred to cartridge.
6)Keep intact and clean the QR code on the cap of HIV-1-Cartridge. Do not scrawl on it or cover, remove it.
7)Sent HIV-1-Cartridge for amplification and detection immediately after sample loading. If it needs to be stored, it should be stored at 2~8 ℃ and the storage time should not exceed two (2) hours (before inserting the cartridge into the instrument module, shake and mix it well).
8)Follow this IFU during operation. Do not put the cartridge into the instrument module until sample information has been input.
9)Do not add HIV-1-Positive Control, until other cartridges have been used.
10)Centrifuge the HIV-1-Positive Control before opening the cap, and shorten the time to open the cap as much as possible.
11)Do not open module cover of applicable instrument during test.
12)Do not open the cap of HIV-1-Cartridge after testing.

3.Result viewing

Test result is automatically stored in the applicable instrument at the end of test. Previous test results can be viewed in the [*View*] interface of the applicable instrument.

4.Operation

1)When reagent enters eyes or mouth by mistake, or contaminates the skin, rinse with plenty of water immediately, and seek for help from medical professionals if necessary.
2)Before the HIV-1-Cartridge is tested on the instrument, make sure that the outer wall of the cartridge is free of liquid and other adherents.

5.Storage and use

1)Store the assay as required in this IFU.
2)To prevent the reagent from going bad, take out only the required amount of reagent for each test. The rest should be stored according to conditions specified in this IFU.
3)Do not use the HIV-1-Positive Control for purposes not described in this IFU (such as dilution or add it to samples) in order to avoid polluting the test environment.
4)Do not use reagents beyond their expiry date.
5)Do not mix components from different kit lots. Do not replenish reagents in this assay.
6) Please check before use that the HIV-1-Cartridge has no scratches or cracks.

6.Disposal

1)Do not open the cap of used cartridge, and directly dispose of the cartridge as medical waste.
2) All samples and other materials should be disposed of in accordance with the [*Medical Waste Management Regulations*][1] after use.

[REFERENCE]

1.Medical waste management regulations: The State Council of the People's Republic of China promulgated on June 16, 2003.

[EXPLANATION OF SYMBOLS]

	Use-by date		Do not re-use
	Temperature limit		Consult instructions for use
	Keep away from sunlight		Manufacturer
	Date of manufacture	LOT	Batch code
	Contains sufficient for <*n*> tests		Keep dry
REF	Catalogue number		Do not use if package is damaged
	Biological risks		

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[INSTRUCTION VERSION AND MODIFICATION DATE]

Approved on May 17, 2023. Version: A0