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EN

REF 1N1232

STAT-NAT® SN200 HHV-6

Lyophilized mix for quantitative detection of HHV-6 (Human betaherpesvirus 6) in Real Time PCR

REAGENT: 6 Master Mix miniplate

BUFFER : 3 x 1.5 mL Reconstitution Buffer

Σ 96

IVD

CE

NOTE: This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

INTENDED USE

The STAT-NAT® SN200 HHV-6 is an automated, in vitro nucleic acid amplification assay for the detection and the quantification of Human betaherpesvirus 6 (HHV-6) DNA, extracted from human samples[1,2]. The assay is based on Real-time PCR and it is performed on an automated extraction and nucleic acid amplification system. The assay is intended for use as an aid in the diagnosis of HHV-6 infections.
For professional use only.

PRINCIPLE

STAT-NAT® SN200 HHV-6 kit is based on Real-Time PCR testing and SENTINEL CH. S.p.A. proprietary freeze-dried amplification reagents that guarantee the sensitivity and the specificity of the reaction without intermediate manual steps for setting up reaction mixtures. The kit consists of 6 miniplates, each containing 16 optimized freeze-dried master mixes (96 reactions total) targeting simultaneously the specific regions of U67 and U31 genes (labelled with FAM fluorophore) allowing fast and simple results evaluation, and the STAT-NAT® SN200 HHV-6 Reconstitution Buffer.
Primers and probes specific for an exogenous Internal Control (IC) (labelled with HEX fluorophore), are present in each reaction mixture. This provides indications on the functionality of the system and on the absence of inhibitors of polymerase activity, which could cause false negatives.
To perform the analysis, the STAT-NAT® SN200 HHV-6 kit is intended to be used on the SENTiNAT® 200 automated system and self-contained assay-specific STAT-NAT® SN200 HHV-6 Plugin in conjunction with the STAT-NAT® SN200 HHV-6 Controls kit REF.1N1233, the STAT-NAT® SN200 HHV-6 Calibrator kit REF.1N1234 and a dedicated DNA extraction kit.
The EDX HHV-6 standard (Exact Diagnostics), calibrated against the *1st WHO International Standard for HHV-6B, 15/266*), was used as reference materials.

REAGENTS

STAT-NAT® SN200 HHV-6 kit consists of:

- **REAGENT: 6 miniplates x 16 master mix wells**

The kit includes 6 aluminum pouches labelled *Master Mix miniplate*, containing a single miniplate with 16 lyophilized Master Mixes and a small orange desiccant sachet.
Each PCR well contains:
• dNTPs (dATP, dCTP, dGTP and dTTP);
• Hot Start (Taq) Polymerase;
• Specific primers and probe for U67 gene;
• Specific primers and probes for U31 gene;
• Specific primers and probe for IC;
• Reaction buffer.
Lyophilized Master Mix must be stored at +15/+30 °C. Use only undamaged packages.
On board miniplate stability: stable up to 7 days if placed on the specific miniplate carriers and loaded on SENTiNAT® 200 automated system.

- **BUFFER: 3 x 1.5 mL**

The kit includes 3 STAT-NAT® SN200 HHV-6 Reconstitution Buffer tubes (1.5 mL) in a liquid form, labelled as HHV-6 RB.
Buffers must be stored at +15/+30 °C. Use only undamaged packages.
After opening, recap STAT-NAT® SN200 HHV-6 Reconstitution Buffer and store it at +15/+30 °C.
In use vial stability: stable up to expiry date indicated on the package if stored at +15/+30 °C.

All the kit components are barcoded to be automatically read by SENTiNAT® 200 automated system.

CALIBRATION

To generate valid results, a test calibration must be completed using only the calibrator vials included in the STAT-NAT® SN200 HHV-6 Calibrator kit (REF. 1N1234), provided separately.
A new calibration curve has to be done every 90 days or with every new lot of STAT-NAT® SN200 HHV-6.

QUALITY CONTROL

Use only the Positive Controls (PCs, HHV-6 High Positive Control and HHV-6 Low Positive Control) included in STAT-NAT® SN200 HHV-6 Controls kit (REF. 1N1233), provided separately.
The PCs provide indications on the functionality of the system.

It is necessary to validate each run using:
- No Template Control (NTC) (STAT-NAT® SN200 Control Reconstitution Buffer included in REF. 1N1233);
- HHV-6 High Positive Control and HHV-6 Low Positive Control (included in STAT-NAT® SN200 HHV-6 Controls kit REF. 1N1233).
If required by laboratory's guidelines, include a negative control (NC) in each run.
A verified negative sample can be used as NC.

SAMPLE

Whole blood should be collected using EDTA tubes as laboratory procedure.
After collection, the human whole blood and plasma must be stored as described below:
- Whole blood is stable for up to 24 hours at room temperature or for up to 72 hours at 2- 8 °C prior to DNA extraction[3,4];
- Plasma is stable for up to 5 days at 2-8 °C and longer if frozen at –20 °C or below[3,4].

INSTRUMENTATION AND MATERIALS REQUIRED BUT NOT PROVIDED

Positive controls kit: STAT-NAT® SN200 HHV-6 Controls kit – REF. 1N1233.
Calibrator kit: STAT-NAT® SN200 HHV-6 Calibrator kit - REF. 1N1234.
Consumables/Accessories: Hamilton MIC Tubes & V-Caps – REF DS0009, SENTiNAT® 200 extraction kit consumable set – REF.1N1004, Elution plates – REF. 1N1008 and Deep Well Plate – REF. DS0005.
General molecular laboratory equipment: biosafety cabinet (laminar flow hood) for samples handling, centrifuge/micro-centrifuge, vortex mixer, variable volume pipettes, sterile disposable plastics.
Extraction Kit: SENTiNAT® X48 Pathomag Extraction kit – REF. 1N1009.
Personal protective equipment (PPE): as gloves, laboratory coats, safety glasses, facemasks.

Validated automated system: SENTiNAT® 200, and the dedicated instrument consumables.
Software platform and Analysis Software: FastFinder (UgenTec), the assay-specific STAT-NAT® SN200 HHV-6 Plugin REF PLSN20008. The analysis software can be supplied on request. Fill out the online request form on https://www.ugentec.com/create-account to set up your account.
CAUTION: Refer to FastFinder (UgenTec) website to ensure you are using the most current version of the analysis software.

WARNINGS AND PRECAUTIONS
- The STAT-NAT® SN200 HHV-6 kit – REF. 1N1232 is exclusively for IVD use with SENTiNAT® 200 system only.
-Read all the instructions contained in the kit insert before performing the test;
-Comply with the kit expiration date;
-Do not mix up reagents for amplification (i.e. buffer) or consumables from other commercial kits;
-Do not mix up reagents or consumables from kits with different Lot Number;
-Do not use any reagents if the safety seal is broken or if the packaging is damaged upon arrival;
-Do not use consumables or reagents if the protective pouch is open or broken upon arrival;
-Minimum specimen volume is 700 µL. It is possible to use different tube size and different specimen carriers as indicated in SENTiNAT® 200 Operator's Manual;
-The MSDS are available at www.sentineldiagnostics.com or at your local supplier;
-Keep all miniplates, with lyophilized Master Mix, protected from light and humidity in their aluminum envelopes;
-Avoid microbial, ribonuclease (RNase) and deoxyribonuclease (DNase) contamination of all reagents and consumables;
-In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the consumables and reagents required for testing, personal protective equipment such as gloves and lab coats, and the SENTiNAT® 200 System are not contaminated;
-Wash hands thoroughly after performing the test;
-Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or reagents are being handled.
-Always use personal protective equipment for the individual protection;
-The product must be handled by staff trained in molecular biology techniques, such as nucleic acids extraction, amplification, detection and in automation;
-Results obtained with the STAT-NAT® SN200 HHV-6 assay should be interpreted in conjunction with other clinical and laboratory findings;
-As with other tests, negative results do not rule out the HHV-6 infections;
-Mutations within the target regions of the HHV-6 DNA covered by the STAT-NAT® SN200 HHV-6 kit may affect primers and/or probes pairing resulting in the under-estimation of viral nucleic acids detection;
-False negative or invalid results may occur due to interference. The Internal Control is included in STAT-NAT® SN200 HHV-6 to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
-**CAUTION** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens[5], Biosafety Level 2[6] or other appropriate biosafety practices[7,8] should be used for materials that contain or are suspected of containing infectious agents.

INSTRUCTIONS FOR USE
Miniplates highlights:
1. Ensure that before using pouches, they are always well sealed and that the desiccant sachets are still inside. Use only undamaged packages;
2. Cut the aluminum pouches at the point indicated by the lateral notches;
3. Remove the miniplates from the pouches immediately before use;
4. Examine the lyophilized Master Mix before use to verify that the content has a solid and white appearance. Please discard the product that appears with signs of moisture contamination (i.e. change of colour, collapsing product, etc…);
5. Make sure Lyophilized Master Mix is at the bottom of the test well before to load the miniplates into the specific carriers;
6. Waste the aluminum pouches and their content if the desiccant sachets turn from orange to green.
7. All reagents, correctly stored at +15/+30 °C, are stable up to the expiration date indicated on the package;

Warning: Do not remove the aluminum foil from the miniplates top. Do not shake or invert the miniplates.

For a detailed description of how to load the STAT-NAT® SN200 HHV-6 kit miniplate and buffers on the SENTiNAT® 200 system, refer to the SENTiNAT® 200 Operator's Manual.

PROCEDURE
SENTiNAT® 200 application and sample-to-result run
1. The STAT-NAT® SN200 HHV-6 kit can process a maximum of 48 samples per run;
2. The required minimum sample volume is 700 µL using primary sterile tubes. Refer to the SENTiNAT® 200 Operator's Manual for acceptable tube size and appropriate sample rack type;
3. For a detailed description of how to operate the SENTiNAT® 200 system, refer to the SENTiNAT® 200 Operator's Manual.

Reaction set up
1. For the DNA extraction from human samples, refer to the SENTiNAT® 200 Operator's Manual;
2. Follow the instruction for use (IFU) of STAT-NAT® SN200 HHV-6 Controls kit and SENTiNAT® 200 Operator's Manual to prepare the PCs and the NTC (STAT-NAT® SN200 Control Reconstitution Buffer)
3. Follow the IFU of STAT-NAT® SN200 HHV-6 Calibrator kit and SENTiNAT® 200 Operator's Manual to prepare the Calibration curve, if required;
4. Include a NC if required;
5. Follow the SENTiNAT® 200 Operator's Manual and software instructions for the instrument deck preparation.

INTERPRETATION OF RESULTS
Data interpretation requires analysis software. Follow standard laboratory practices for transfer, reporting and storage of results.
The below paragraph describes the different steps to analyze the results with the analysis software. For detailed instructions on any of the steps, including screenshots, refer to the technical note and FastFinder IFU, accessible from the support menu in FastFinder platform.
Data Analysis
- Start up the FastFinder software and log in;
- Select Dashboard menu;
- Select the data file to be analysed into Open Analysis section;
- Check/Set up the plate layout into PCR setup section. Verify the correct selection of assay and samples position;

- Check controls and resolve any uncertain results. Refer to FastFinder IFU for detailed instructions
- Authorize, reject or restart the analysis;
- Select Analysis results into Archive menu and download the results using the Export file(s) column or send your results directly to your LIS.

PERFORMANCES [9,10]

Linearity[11]

Linearity of the STAT-NAT® SN200 HHV-6 kit was investigated using a panel of 9 levels of the EDX HHV-6 standard (Exact Diagnostics, calibrated against the *1st WHO International Standard for HHV-6B, 15/266*), ranging from $4{,}29x10^8$ to $5x10^1$ IU/mL. The assay present linear trend from $1x10^8$ to $5x10^1$ IU/mL.

Analytical Sensitivity[12]

The Limit of Detection (LoD) and the Limit of Quantitation (LoQ) were evaluated using a dilution panel of the EDX HHV-6 standard, from 100 IU/mL to 1000 IU/mL;
The LoD and LoQ were calculated on several replicates of samples, the results that show a 95% probability to have a positive result are summarized in **Table A**.

Table A. LoD and LoQ

	Result	
	LoD	LoQ
Whole Blood	490 IU/mL	560 IU/mL
Plasma	380 IU/mL	380 IU/mL

Precision[13]

In this study, the closeness of agreement between measured quantities obtained by replicate measurements on the same analyte under specified conditions was evaluated. **Table B** summarizes precision measurements intended as repeatability and reproducibility studies.

Table B. Precision measurement studies intended as repeatability and reproducibility studies

Measurement	Criteria for acceptance	Pass (Yes/No)
Repeatability	CV% < 10%	Yes
Reproducibility	CV% < 10%	Yes

Cross-reactivity[9,10]

Analytical specificity was demonstrated using a panel of 22 different pathogens. No cross-reactivity was observed with any of the organisms tested, as indicated in **Table C**, confirming 100% analytical specificity of the STAT-NAT® SN200 HHV-6 assay.

Table C. *In vitro* cross-reactivity evaluation

Pathogen	Cross-Reactivity (Yes/No)
Enterovirus	NO
Adenovirus	NO
Streptococcus pneumoniae	NO
Herpes Simplex Virus 1	NO
Herpes Simplex Virus 2	NO
Varicella-Zoster virus	NO
Epstein-Barr virus	NO
Human herpes virus 8	NO
Human immunodeficiency virus 1	NO
Human immunodeficiency virus 2	NO
Cytomegalovirus	NO
Staphylococcus aureus	NO
Streptococcus pyogenes	NO
Staphylococcus epidermidis	NO
BK polyomavirus	NO
Hepatitis B virus	NO
Enterococcus faecalis	NO
Klebsiella pneumoniae	NO
Human betaherpesvirus 7	NO
JC polyomavirus	NO
Parvovirus B19	NO
Toxoplasma gondii	NO

Interferences[9,10]

The STAT-NAT® SN200 HHV-6 assay was evaluated in the presence of typical exogenous and endogenous interfering substances in selected human samples. A list of the interferents is reported in **Table E**.

Table E. List of the tested interferents

Interfering Substances	Concentration Tested
Valganciclovir	10 µg/mL
Prednisone	22,2 µg/mL
Cidofovir	20 µg/mL
Cefotaxime	214 µg/mL
Mycophenolate mofetil	40 µg/mL
Vancomycin	50 µg/mL
Tacrolimus	100 ng/mL
Famotidine	200 µg/mL
Valacyclovir	100 µg/mL
Leflunomide	100 µg/mL
Triglycerides	500 mg/dL
Conjugated bilirubin	0,25 g/L
Unconjugated bilirubin	0,25 g/L
Albumin	58,7 g/L
Hemoglobin	0,25 g/L
Human genome	2 mg/L

Results obtained from this study shows an irrelevant interfering effect of the endogenous or exogenous molecules on kit analytical sensitivity.

CLINICAL EVALUATION

A panel of 100 positive and negative samples (whole blood and plasma samples), was tested using a commercially available predicate device. The positive and negative predicted agreement are reported in **Table F**.

Table F: Clinical performances

Parameter	Calculated agreement	
	Positive Predicted Agreement	Negative Predicted Agreement
Whole Blood	96%	100%
Plasma	96%	100%

TROUBLESHOOTING

Problem 1: Weak or no signal in Positive Control:

1. Real-Time PCR conditions do not comply with the instructions in the kit insert:
- The Positive Control was not added to the reaction. Repeat test;
- Check the performance of the SENTiNAT® 200 and carry out the instrument calibration.

2. Primers/probes degradation: the reagent storage conditions do not comply with the instructions in the kit insert:
  - Check the kit storage conditions;
  - Check the kit expiration date.

Problem 2: Weak or no signal in Internal Control.

1. Inhibitory effect of the sample: DNA with a low quality extraction. The result is INVALID:
- Ensure to follow carefully the instructions reported in the kit insert;
- Repeat the test using the same extracted DNA sample. If the result is still negative, repeat the extraction step using the same primary sample. Otherwise, collect a new primary sample and repeat the test.

2. Primers/probes degradation: the reagent storage conditions do not comply with the instructions in the kit insert:
- Check the kit storage conditions;
- Check the kit expiration date.

Problem 3: FAM, HEX signal in NTC or in Negative Control.

1. Contamination during the Real-Time PCR preparation procedure: all results are INVALID:

- Clean the workbench and all the instruments;
- Handle Positive Control carefully avoiding contamination;
- Repeat Real-Time PCR using a new set of reagents.

Problem 4: Fluorescence intensity variability or absence of FAM, HEX signal.

1. Humidity damage for Lyophilized mix: the reagent storage conditions do not comply with the instructions in the kit insert:
- Check the kit expiration date;
- Check the kit storage conditions; ensure that the pouch is always well sealed and that the desiccant sachet is still inside;
- Check if the desiccant sachet turns from orange to green.

2. Inhibitory effect of the sample: DNA with a low quality extraction. The result could be a false negative. The result is INVALID:
- Ensure to follow the instructions reported in the kit insert carefully.

Problem 5: No signal at all.

1. Check the performance of the SENTiNAT® 200 instrument:
- Carry out the instrument calibration.

2. Primers/probes degradation: the reagent storage conditions do not comply with the instructions in the kit insert:
- Check the kit storage conditions;
- Check the kit expiration date.

Problem 6: Error message given by the SENTiNAT® 200 instrument.

Consult the Instrument Operator's Manual or contact the local technical support.

Problem 7: Ct > 30 in the Internal Control.

Sample with low quality extracted DNA or errors in sample dispensing in the reaction set up.
- Repeat the test using the same extracted DNA sample. If the IC Ct result is still > 30, repeat the extraction step using the same primary sample. Otherwise, collect a new primary sample and repeat the test.

CAUTION: For the SENTiNAT® 200 instrument please refer to manual troubleshooting

WASTE MANAGEMENT

– The reagents of the kit are not classified as dangerous according to Regulation EC 1272/2008 (CLP). Adopt good working practices, so that the product is not released into the environment. Recover if possible. In so doing, comply with the local and national regulations currently in force.

– Manage and waste all the biological samples as potentially infectious. All the material that come in contact with the biological sample must be treated with 0.5% sodium hypochlorite for at least 30 minutes or sterilized in autoclave at 121 °C for 30 minutes and then wasted.

BIBLIOGRAPHY

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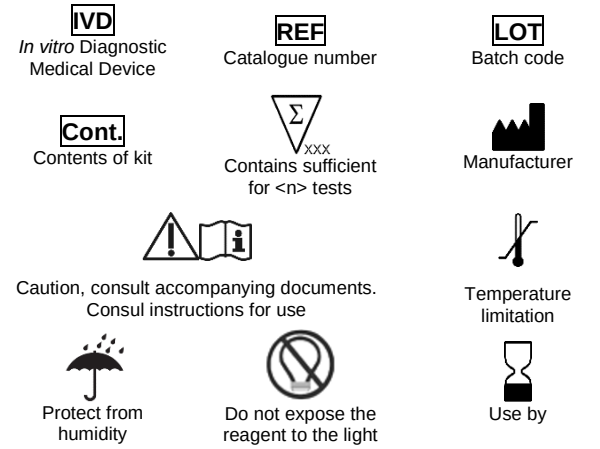
Explanation of symbols EN

REAGENT / CONTROL
The terms refer to the: single reagent/control



In case of incident, please contact Sentinel Diagnostics (contact details at www.sentineldiagnostics.com) or your local representative. For customers in the European Union: if, in the course of using this device, you have reason to believe that a serious incident has occurred, report it to the manufacturer and to your national authority.

Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin.