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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

EN

REF 1N1244

STAT-NAT® SN200 HHV-7

Dried Oligonucleotides mix for quantitative detection of HHV-7 (Human herpes virus 7) in Real Time PCR

REAGENT: 3 x HHV-7 Oligo Mix

BUFFER : 1 x 1.5 mL Reconstitution Buffer

Σ 48

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-7 is an *in vitro* primers and probes assay to be used with STAT-NAT® SN200 DNA Mix or STAT-NAT® DNA Mix Flex for the detection and the quantification of Human herpes virus 7 (HHV-7) DNA, extracted from human samples[1,2].

The assay is based on Real-time PCR and it is compatible with manual, semi-automatic, and automatic systems. The assay is intended for use as an aid in the diagnosis of HHV-7 infections.

For professional use only.

PRINCIPLE

STAT-NAT® SN200 HHV-7 kit is a primers and probes mix included in the Oligonucleotides (Oligo) assays line. The assay is based on Real-Time PCR testing and it works in conjunction with the DNA Mix that uses a SENTINEL CH. S.p.A. proprietary freeze-dried amplification reagents, which guarantees the sensitivity and the specificity of the reaction without intermediate manual steps for setting up reaction mixtures. The kit consists of 3 tubes, containing primers and probes mix for a minimum of 16 reactions each, targeting simultaneously the specific regions of U56 gene and U57 gene (labelled with FAM fluorophore) allowing fast and simple results evaluation, and the STAT-NAT® SN200 Reconstitution Buffer.

Primers and probes specific for an exogenous Internal Control (IC) (labelled with HEX fluorophore), are present in each Oligo tube. 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-7 kit is intended to be used:

- in manual or semi-automatic system using the STAT-NAT® DNA Mix Flex kit REF.1N201, in conjunction with the STAT-NAT® SN200 HHV-7 Controls kit REF.1N1245, the STAT-NAT® SN200 HHV-7 Calibrator kit REF.1N1246 and a dedicated DNA extraction kit;
- on the SENTiNAT® 200 automated system and self-contained assay-specific STAT-NAT® SN200 HHV-7 Plugin using the STAT-NAT® SN200 DNA Mix kit REF.1N1900 or STAT-NAT® DNA Mix Flex kit REF.1N201, in conjunction with the STAT-NAT® SN200 HHV-7 Controls kit REF.1N1245, the STAT-NAT® SN200 HHV-7 Calibrator kit REF.1N1246 and a dedicated DNA extraction kit.

The EDX HHV-7 standard (Exact Diagnostics) was used as reference materials.

REAGENTS

STAT-NAT® SN200 HHV-7 kit consists of:

- **REAGENT: 3 x tubes HHV-7 Oligo Mix**

The kit includes 3 aluminum pouches labelled “HHV-7 Oligo Mix”, containing a single tube with the dried HHV-7 Oligo Mix and a small orange desiccant sachet.

Each tube contains:

- Specific primers and probe for U56 gene;
- Specific primers and probe for U57 gene;
- Specific primers and probe for IC;

Dried Oligo Mix must be stored at +15/+30 °C. Use only undamaged packages.

- **BUFFER: 1 x 1.5 mL**

The kit includes: 1 STAT-NAT® SN200 Reconstitution Buffer tubes (1.5 mL) in a liquid form.

Buffers must be stored at +15/+30 °C. Use only undamaged packages.

After opening, recap STAT-NAT® SN200 Reconstitution Buffer and store it at +15/+30 °C.

In use tube stability: stable up to expiry date indicated on the package if stored at +15/+30 °C

The Oligo Mix tubes 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 tubes included in the STAT-NAT® SN200 HHV-7 Calibrator kit (REF. 1N1246), provided separately.

A new calibration curve has to be done as follow:

- For manual and semi-automatic assay, calibration is required for each run;
- For automatic system, calibration is required every 90 days or with every new lot of reagents.

QUALITY CONTROL

Use only the Positive Controls (PCs – HHV-7 High Positive Control and HHV-7 Low Positive Control) included in STAT-NAT® SN200 HHV-7 Controls kit (REF. 1N1245), 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. 1N1245);
- HHV-7 High Positive Control and HHV-7 Low Positive Control (included in STAT-NAT® SN200 HHV-7 Controls kit REF. 1N1245).

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

DNA Mix Kit: STAT-NAT® SN200 DNA Mix – REF.1N1900 or STAT-NAT® DNA Mix Flex – REF. 1N201

Positive controls kit: STAT-NAT® SN200 HHV-7 Controls kit – REF. 1N1245.

Calibrator kit: STAT-NAT® SN200 HHV-7 Calibrator kit - REF. 1N1246.

General molecular laboratory equipment: biosafety cabinet (laminar flow hood) for samples handling, centrifuge/micro-centrifuge, vortex mixer, variable volume pipettes, sterile disposable plastics.
Personal protective equipment (PPE): as gloves, laboratory coats, safety glasses, facemasks.
Extraction Kit: Nucleic acid extraction should be performed by CE-IVD validated extraction kits available at the market according to protocols for the particular clinical material extraction. The kit was validated with the SENTiNAT® X48 Pathomag Extraction kit – REF. 1N1009.
Validated Real-Time PCR Thermal Cycler: SENTiNAT® MICRO.
Consumables/Accessories for SENTiNAT® MICRO: MIC Tubes and Caps - REF DS0100.
Validated automated system, Software platform and Analysis Software: SENTiNAT® 200, and the dedicated instrument consumables. FastFinder (UgenTec), the assay-specific STAT-NAT® SN200 HHV-7 Plugin PLSN20012. 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. Refer to FastFinder (UgenTec) website to ensure you are using the most current version of the analysis software
Consumables/Accessories for SENTiNAT® 200 system: 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.

WARNINGS AND PRECAUTIONS
-The STAT-NAT® SN200 HHV-7 kit – REF. 1N1244 is exclusively for IVD use.
-Read all the instructions contained in the kit insert before performing the test;
-Comply with the kit expiration date;
-Do not mix up reagents (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 consumables or reagents if the protective pouch is open or broken upon arrival;
- Refer to the specific extraction kit and/or semi-automatic system for the minimum specimen volume and tube size to be used.
- Refer to the specific extraction kit and automatic system for the minimum specimen volume and tube size to be used. Minimum specimen volume for SENTiNAT® 200 system 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 tubes, with dried Oligo 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-7 Oligo assay, in conjunction with STAT-NAT® SN200 DNA Mix or STAT-NAT® DNA Mix Flex, should be interpreted in conjunction with other clinical and laboratory findings;
-As with other tests, negative results do not rule out the HHV-7 infections;
-Mutations within the target regions of the HHV-7 DNA covered by the STAT-NAT® SN200 HHV-7 Oligo assay, in conjunction with STAT-NAT® SN200 DNA Mix or STAT-NAT® DNA Mix Flex, 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-7 Oligo assay 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.

PROCEDURE
HHV-7 Oligo Mix reconstitution
1. Centrifuge the HHV-7 Oligo Mix tubes prior to opening to ensure that primers and probes mix is at the bottom of the tube;
2. Reconstitute the HHV-7 Oligo Mix tubes with 450 µL of STAT-NAT® Reconstitution Buffer.
3. Cap the tube and vortex for 15 seconds until the dried DNA is resuspended;
4. Centrifuge for few seconds at medium speed to remove any residue from the cap and eliminate bubbles/foam;
5. Wait for at least 15 minutes before use;

Reaction set up
The STAT-NAT® SN200 HHV-7 oligo assay can be used in manual/semi-automatic or automatic system. Follow the instructions below for a detailed description of how to operate:
- Real-Time PCR cycling parameter setting for manual/semi-automatic system:
1. Turn Real-Time PCR thermal cycler and computer on and open the dedicated software program;
2. Set the detector for the HHV-7 target probe with reporter "FAM";
3. Set the detector for the IC of reaction with reporter "HEX";
4. Set the Passive Reference "none", if requested;
5. For basic information regarding the setup and programming of the different Real-Time PCR instruments, please refer to the user manual of the specific instrument;
6. Perform the HHV-7 Real-Time PCR assay using the thermal profile shown in **Table A** and **Table B**.

Table A. Real-Time PCR thermal profile

Real-Time PCR protocol and settings				
Step	Cycle number	Temperature	Time	Data collection
1	1	95°C	2 min	Off
2	10	95°C	15 sec	
		60°C	1 min	
3	35	95°C	15 sec	Off
		60°C	1 min	On

Table B. Real-Time PCR settings

Sample Volume	Ramp Rate
25 µL	Instrument default

- Manual reaction set up to obtain a HHV-7 Master Mix:

1. Take one amber glass vial of lyophilized STAT-NAT® DNA Mix Flex REF. 1N201;
2. Examine the lyophilized DNA 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…);
3. Open the cap carefully. Exercise caution when peeling off the metal seal as it has sharp edges;
4. Add 450 µL of reconstituted HHV-7 Oligo Mix to the amber glass vial (**Figure 1**);
5. Gently swirl the vial until the lyophilized mix is completely dissolved and any particulate is disappeared (**Figure 1**). Do not shake or invert.
6. Make sure that there are no air bubbles; if so, remove them by aspiration with the tip of a pipette.

It is possible to freeze/thaw the solution for maximum 2 times. Do not mix freshly prepared and thawed HHV-7 Master Mixes in the same run.

Figure 1: DNA Mix reconstitution

7. Once the lyophilized DNA Mix is completely dissolved, using the reconstituted HHV-7 Oligo mix, proceed immediately with the reaction set up;
8. It is strongly advised to use all the resuspended HHV-7 Master Mix; if not, recap the vial and place it at -20 °C right after the use.
9. Extract DNA from the human samples using a dedicated extraction kit;
10. Prepare the PCs as indicated in the Instruction for Use (IFU) of STAT-NAT® SN200 HHV-7 Controls kit –REF. 1N1245;
11. Use STAT-NAT® Control Reconstitution Buffer (included in the STAT-NAT® SN200 HHV-7 Controls kit - REF. 1N1245) as NTC;
12. Prepare the STandard points (STD point) from the reconstituted HHV-7 Standard tube as indicated in the IFU of STAT-NAT® SN200 HHV-7 Calibrator kit –REF. 1N1246;
13. Prepare the reaction mix in PCR tubes or plates as indicated in **Table C** to generate a Calibration curve for quantitative analysis;

Table C. Reaction volumes for Calibration curve. It is recommended to carry out at least two replicates for each STD point.

Components per test tube	Volume per test tube/reaction
STAT-NAT® Reconstituted Master Mix	15 µL
STD point	10 µL
Final Reaction volume	**25 µL**

A total 5 STD points, at least in duplicate, should be prepared

14. Prepare the reaction mix in PCR tubes or plates as indicated in **Table D** to analyze unknown samples (extracted DNA), PCs or NTC or NC;

Table D. Reaction volumes for unknown samples, PCs, NTC, NC

Components per test tube	Volume per test tube/reaction
STAT-NAT® Reconstituted Master Mix	15 µL
Extracted DNA or PC or NTC or NC	10 µL
Final Reaction volume	**25 µL**

15. Include a NC if required
16. Make sure that there are no air bubbles; if so, remove them by aspiration with the tip of a pipette.

- Semi-automatic reaction set up:

1. For a detailed description of how to operate the semi-automatic system, refer to the Operator's Manual of the instrument
2. Follow all the steps of the *Manual reaction set up to obtain a HHV-7 Master Mix* section of this IFU.

- Automatic reaction set up using STAT-NAT® DNA Mix Flex kit REF. 1N201:

For a detailed description of how to operate the automatic system, refer to the Operator's Manual of the instrument.

Automated application and sample-to-result run:

1. Pipette the reconstituted HHV-7 Master Mix into the dedicated tube and load the tube on the instrument following the software instructions indicated for the Master Mix Resuspension Buffer;
2. The HHV-7 Master Mix tube can process a maximum of 16 samples per run, on SENTiNAT® 200;
3. Refer to the instrument Operator's Manual for acceptable sample volume, tube size and appropriate sample rack type;
4. For the DNA extraction from the human samples, refer to the dedicated extraction kit;
5. Follow the IFU of STAT-NAT® SN200 HHV-7 Controls kit – REF. 1N1245 and instrument Operator's Manual to prepare the PCs and the NTC (STAT-NAT® SN200 Control Reconstitution Buffer);
6. Follow the IFU of STAT-NAT® SN200 HHV-7 Calibrator kit – REF. 1N1246 and instrument Operator's Manual to prepare the Calibration curve, if required;
7. Include a NC if required;
8. Follow the instrument Operator's Manual and software instructions for the instrument deck preparation.

SENTiNAT® 200 Reaction set up using STAT-NAT® SN200 DNA Mix kit REF. 1N1900:

1. Each STAT-NAT® SN200 HHV-7 Oligo Mix in conjunction with STAT-NAT® SN200 DNA Mix, can process a maximum of 16 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. Load the reconstituted HHV-7 Oligo Mix on the instrument following the software instructions indicated for the *Master Mix Resuspension Buffer*;
4. Follow the IFU of STAT-NAT® SN200 HHV-7 Controls kit and SENTiNAT® 200 Operator's Manual to prepare the PCs and the NTC (STAT-NAT® SN 200 Control Reconstitution Buffer);
5. Follow the IFU of STAT-NAT® SN200 HHV-7 Calibrator kit and SENTiNAT® 200 Operator's Manual to prepare the Calibration curve, if required;

6. Include a negative control (NC) if required;
7. Follow the SENTiNAT® 200 Operator's Manual and software instructions for the instrument deck preparation.

INTERPRETATION OF RESULTS

- Interpretation of results in Manual/semi-automatic system:

The analysis of the results is carried out directly on the specific management software;
Set threshold values automatically;
FAM signal indicates the successful amplification of the specific sequence for HHV-7 identification;
HEX signal indicates the successful amplification of the specific sequence for IC.
It is necessary to validate each diagnostic run as indicated in Quality Control section;
Check the amplification curves for PCs, NTC and NC as indicated in **Table E**.

Table E: Interpretation of results

	Interpretations	
	SENTiNAT® MICRO FAM (Ct)	Result
STD point 1	7.8 ± 1.5	VALID
STD point 2	11.5 ± 1.5	VALID
STD point 3	15.2 ± 1.5	VALID
STD point 5	22.5 ± 1.5	VALID
STD point 6	26.3 ± 1.5	VALID
Pcs (HHV-7 High Positive Control)	16.4 ± 2	VALID
Pcs (HHV-7 Low Positive Control)	23.3 ± 2	VALID
NTC / NC	No Signal	VALID
NTC / NC	Signal	INVALID

STD point 4 is not used for calibration

The entire session is to be considered INVALID and to be repeated in the event that:
- the NTC/NC provided a signal
- At least one of the PCs is out of the expected Cts
- At least one of the STD point is out of the expected Cts

Check the values reported in **Table F** for the calibration curve.

Table F

Expected values for the calibration curves	
$R^2 > 0.98$	Slope < -3.0

The calibration curve is not valid and should be repeated if R^2 and Slope are not within the expected values.

Check the IC of each sample. The expected threshold cycle (Ct) of the IC is 22.5 ± 2. In case the Ct value is outside of the range or no HEX signal is present, check the troubleshooting section.
The **Table G** shows the validity of diagnostic test run.

Table G

FAM (HHV-7 target)	HEX (IC)	Result	Interpretation
YES	YES	VALID	Positive
YES	NO	VALID	Positive*
NO	YES	VALID	Not detectable#
NO	NO	INVALID	Invalid

* In case of high concentrated samples, an inhibition of the Internal Control amplification can occur.
# In samples resulting negative for HHV-7 DNA target, it is not excluded that there is HHV-7 DNA concentration lower than the system's sensitivity limit.

Laboratories must determine the appropriate cut-off of HHV-7 DNA level for clinical decision making in treatment of patients. Follow institutional/society guidelines as appropriate.

- Interpretation of results on automatic system:

Data interpretation requires analysis software. Follow standard laboratory practices for transfer, reporting and storage of results.
For a detailed description of how to interpret the results for an automatic system, refer to the Operator's Manual of the instrument.
The below paragraph describes the different steps to analyse the results with the SENTiNAT® 200 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-7 assay was investigated using a panel of 8 levels of the EDX HHV-7 standard, ranging from $1x10^2$ to $1x10^7$ copies/mL (cps/mL). The assay present linear trend from $1x10^2$ to $1x10^7$ cps/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-7 standard, from $1x10^2$ cps/mL to $1x10^3$ cps/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 H**.

Table H. LoD and LoQ

	Sample	Result (cps/mL)	
		LoD	LoQ
SENTiNAT® Micro	**Whole Blood**	250	350
	Plasma	202	300
SENTiNAT® 200	**Whole Blood**	250	350
	Plasma	202	300

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 I** summarizes precision measurements intended as repeatability and reproducibility studies.

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

Measurement	Criteria for acceptance	Pass (Y/N)
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 J**, confirming 100% analytical specificity of the STAT-NAT® SN200 HHV-7 assay.

Table J. *In vitro* cross-reactivity evaluation

Pathogen	Cross-Reactivity (Y/N)
Enterovirus	N
Adenovirus	N
Streptococcus pneumoniae	N
Herpes Simplex Virus 1	N
Herpes Simplex Virus 2	N
Toxoplasma gondii	N
Human herpesvirus 6	N
Human herpesvirus 8	N
Human immunodeficiency virus 1	N
Human immunodeficiency virus 2	N
Cytomegalovirus	N
Staphylococcus aureus	N
Streptococcus pyogenes	N
Staphylococcus epidermidis	N
BK polyomavirus	N
Hepatitis B virus	N
Enterococcus faecalis	N
Klebsiella pneumoniae	N
Toxoplasma gondii	N
JC polyomavirus	N
Epstein Barr Virus	N
Parvovirus B19	N

Interferences[9,10]

The STAT-NAT® SN200 HHV-7 assay was evaluated in the presence of typical exogenous and endogenous interfering substances in the selected human samples. A list of the interferents is reported in **Table K**.

Table K. 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 L**.

Table L: Clinical performances

Parameter	Calculated agreement	
	Positive Predicted Agreement	Negative Predicted Agreement
Whole Blood	96%	100%
Plasma	98%	100%

TROUBLESHOOTING

Problem 1: Weak or no signal in Standard/Positive Control:

1. Real-Time PCR conditions do not comply with the instructions in the kit insert:
- The Standard/Positive Control was not added to the reaction. Repeat test;
- For manual/semi-automatic system:
  - Check the Real-Time PCR cycling parameters protocol and select fluorescence channels reported in the kit insert;
  - Check the performance of the thermalcycler and carry out the instrument calibration.
- For automatic system:
  - Check the performance of the automatic system, such as SENTiNAT® 200 instrument, 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 use a validated DNA extraction method and 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.
3. Real-Time PCR conditions do not comply with the instructions in the kit insert of manual/semi-automatic system:
- The Internal Control was not added to the reaction. Repeat test;
- Check the Real-Time PCR cycling parameters protocol and select fluorescence channels reported in the kit insert;
- Check the performance of the thermalcycler and carry out the instrument calibration.
4. Wrong selection of channel/filter in manual/semi-automatic system. Real-Time PCR conditions do not comply with the instructions in the kit insert:
- Check the Real-Time PCR cycling parameters protocol and select fluorescence channels reported in the kit insert

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 use a validated DNA extraction method and follow the instructions reported in the kit insert carefully.

3. Air bubbles trapped in the PCR tubes in manual/semi-automatic system:

- Remove air bubbles before starting the Real-Time PCR run.

4. The Lyophilized Mix is not well reconstituted:

- Repeat the Real-Time PCR procedure carefully.

Problem 5: No signal at all.

1. Check the performance of the automatic system, such as SENTiNAT® 200 instrument, or thermalcycler 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 automatic system, such as SENTiNAT® 200 instrument, or the Real-Time PCR instrument.

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

Problem 7: Ct > 30 in the Internal Control.

1. 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.

Problem 8: Duplicate samples do not reproduce identical results in manual/semi-automatic system.

The Ct values of identical samples may differ in individual reactions. Ct variations > ± 2 values suggest pipetting errors or other differences between duplicate samples.

CAUTION: For the semi/automatic and/or automatic system, such as SENTiNAT® 200 instrument, please refer to the troubleshooting section in the manual.

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.

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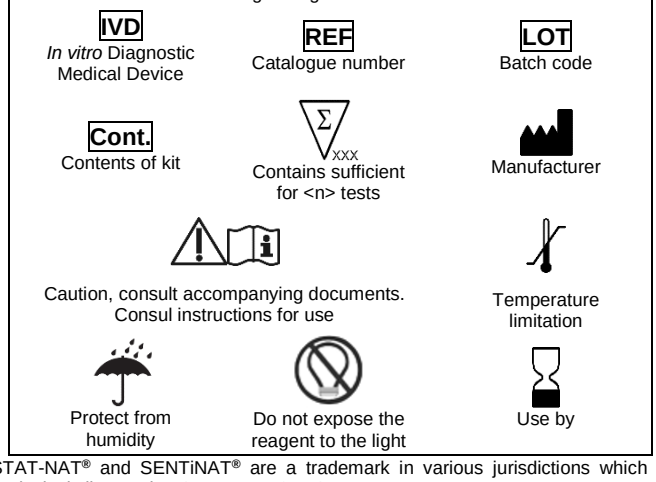
Explanation of symbols EN

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



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

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.

SENTINEL DIAGNOSTICS
SENTINEL CH. S.p.A.
Via Robert Koch, 2
Milano (MI) 20152 Italy
Ph. +390234551495
www.sentineldiagnostics.com