Sacace BIOTECHNOLOGIES

IVD For *in Vitro* Diagnostic Use

CE

HGV Real-TM

Handbook

Real Time PCR kit for the Qualitative detection of Hepatitis G Virus (HGV) in human plasma

REF **V2-50FRT**

Σ **50**

NAME

HGV Real-TM

INTENDED USE

HGV Real-TM is a Real-Time test for the Qualitative detection of Hepatitis G Virus in human plasma and simultaneous detection of a HGV-specific Internal Control (IC), by dual color detection.

PRINCIPLE OF ASSAY

HGV Real-TM Test is based on three major processes: isolation of *virus* RNA from specimens, one-step reverse transcription of the RNA and Real Time amplification of the cDNA. *HGV* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers and detection via fluorescent dyes. These dyes are linked with probes of oligonucleotides which bind specifically to the amplified product. The real-time PCR monitoring of fluorescence intensities allows the accumulating product detection without reopening of reaction tubes after the PCR run. **HGV Real-TM** PCR kit is a qualitative test which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the process of each individual sample extraction and serves also to identify possible reaction inhibition.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (V2-50FRT)

"Controls ":

- **Negative Control (C-),** 1,2 ml*
- **Pos *HGV* RNA-rec,** 0,1 ml **
- **HGV IC RNA,** 2 x 0,28 ml ***
- **RNA-buffer**, 1,2 ml
- **cDNA HGV/IC (C+),** 0,1 ml

"HGV Real-TM": Real Time amplification kit

- **RT-G-mix-2**, 0,015 ml
- **RT-PCR-mix-1 HGV,** 0,6 ml
- **RT-PCR-mix-2,** 0,3 ml
- **TaqF Polymerase,** 0,03 ml
- **M-MLV Revertase,** 0,015 ml

Contains reagents for 50 reactions

* *must be used in the isolation procedure as Negative Control of Extraction.*
** *must be used in the isolation procedure as Positive Control of Extraction.*
*** *add 10 µl of Internal Control RNA during the RNA purification procedure directly to the sample/lysis mixture*

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- RNA extraction kit
- Biosafety cabinet
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g); Eppendorf 5415D or equivalent
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Disposable gloves, powderless
- Tube racks
- 70% Ethanol (freshly prepared mixture of reagent grade 96% ethanol and distilled water)
- Acetone
- Refrigerator
- Freezer

Zone 2: RT and amplification:

- Real Time Thermalcycler
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

STORAGE INSTRUCTIONS

HGV Real-TM kit must be stored at -20°C. The kit can be shipped at 2-8°C for 3-4 days but should be stored at -20°C immediately on receipt.

STABILITY

HGV Real-TM is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

QUALITY CONTROL

In accordance with Sacace’s ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

IVD

***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

The user should always pay attention to the following:

- Clinical specimens from suspect influenza A (H1N1) cases should be performed in a BSL2 laboratory with BSL3 practices (enhanced BSL2 conditions). Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

HGV Real-TM can analyze RNA extracted from plasma:

Note: Handle all specimens as if they are potentially infectious agents.

- EDTA tubes may be used with the **HGV Real-TM**. Follow sample tube manufacturer's instructions.
- Whole blood collected in EDTA should be separated into plasma and cellular components by centrifugation at 800-1600 x g for 20 min within six hours. The isolated plasma has to be transferred into a sterile polypropylene tube. Plasma may be stored at 2-8°C for an additional 3 days. Alternatively, plasma may be stored at -18°C for up to one month or 1 year when stored at -70°C.
- Do not freeze whole blood.
- Specimens anti-coagulated with heparin are unsuitable for this test.
- Thaw frozen specimens at room temperature before using.
- Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma may be transported at 2-8°C or frozen.
- Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

RNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the "SAMPLE COLLECTION, STORAGE AND TRANSPORT" paragraph, could be used.

The following isolation kits are recommended:

⇒ **Ribo-Sorb-100** (Sacace, REF K-2-1/100): sample volume 100 µl

⇒ **Ribo Virus 50/100** – spin column extraction kit (Sacace, REF K-2-C): sample volume 150 µl

⇒ **Magno-Virus** – Magnetic RNA/DNA extraction kit (Sacace REF K-2-16) sample volume 500 µl or 1000 µl

⇒ **SaMag Viral Nucleic Acids Extraction kit** (Sacace, REF SM003)

Please carry out DNA extraction according to the manufacture's instruction.

RNA is extracted from each clinical sample in the presence of **Internal Control** (**10 µl** of **IC** is added to each sample).
Transfer **100 µl** of **Negative Control** to the tube labeled C–.
Transfer **90 µl** of **Negative Control** and **10 µl** of **Positive Pos *HGV* RNA-rec** to the tube labeled PCE.

RT AND AMPLIFICATION

Total reaction volume is **25 µl**, the volume of RNA sample is **10 µl**.

1 Prepare the reaction mix for required number of samples.

2 For N reactions mix in a new tube:

10*(N+1) µl of RT-PCR-mix-1,

5.0*(N+1) µl of RT-PCR-mix-2

0.5*(N+1) µl of TaqF Polymerase

0.25*(N+1) µl of RT-G-mix-2

0.25*(N+1) µl of MMIv

3 Vortex the tube, then centrifuge shortly. Add **15 µl** of prepared reaction mix into each tube.

4 Using tips with aerosol filter add **10 µl** of RNA samples obtained at the stage of RNA isolation and mix carefully by pipetting**.**

N.B. If the Ribo-Sorb isolation kit is used as a RNA extraction kit, re-centrifuge all the tubes with extracted RNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. N.B. don't disturb the pellet, sorbent inhibit reaction

5 Prepare for each panel 2 controls:

- add **10 µl** of **RNA-buffer** to the tube labeled PCR Negative Control;
- add **10 µl** of **cDNA HGV/IC (C+)** to the tube labeled $C_{pos/IC}$;

Create a temperature profile on your Real-time instrument as follows:

	***Rotor type instruments*[1]**				***Plate type or modular instruments*[2]**			
Stage	***Temp, ℃***	***Time***	***Fluorescence detection***	***Cycle repeats***	***Temp,℃***	***Time***	***Fluorescence detection***	***Cycle repeats***
Hold	50	15 min	–	1	50	15 min	–	1
Hold	95	15 min	–	1	95	15 min	–	1
Cyclin g	95	5 s	–	5	95	5 s	–	5
	60	20 s	–		60	25 s	–	
	72	15 s	–		72	15 s	–	
Cycling 2	95	5 s	–	40	95	5 s	–	40
	60	20 s	FAM(Green), JOE(Yellow)		60	30 s	FAM, JOE/HEX/Cy3	
	72	15 s	–		72	15 s	–	

[1] For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)
[2]For example, SaCycler-96™ (Sacace), iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied), SmartCycler® (Cepheid), LineGeneK® (Bioer)

INSTRUMENT SETTINGS

Rotor-type instruments (RotorGene 3000/6000, RotorGene Q)

Channel	Threshold	More Settings/ Outlier Removal	Calibrate / Gain Optimisation…	Slope Correct
FAM/Green	*0.03*	*10 %*	from 5Fl to 10Fl	*On*
JOE/Yellow	*0.03*	*10 %*	from 5Fl to 10Fl	*On*

Plate- or modular type instruments (iQ5, Mx300P, ABI 7500, SmartCycler)

For result analysis, set the threshold line at a level corresponding to 10–20% of the maximum fluorescence signal obtained for Pos C+ sample during the last amplification cycle.

RESULTS ANALYSIS

1. The results are interpreted by the device software through the presence of crossing of fluorescence curve with the threshold line. Put the threshold line at such level where curves of fluorescence are linear.

   cDNA of HGV is detected on the JOE (Yellow)/HEX/Cy3 channel and *IC* on the FAM (Green) channel.

Results are accepted as relevant if both positive and negative controls of amplification and extraction are passed (see table 1).

Table 1. Results for controls

Control	Stage for control	Ct channel Fam (Green)	Ct channel Joe (Yellow)/ HEX/Cy3	Interpretation
NCE	RNA isolation	Pos (< 33)	Neg	Valid result
Pos *HGV*-RNA-rec	RNA isolation	Pos (< 31)	Pos (< 31)	Valid result
NCA	Amplification	Neg	Neg	Valid result
cDNA HGV/IC (C+)	Amplification	Pos (< 31)	Pos (< 31)	Valid result

PERFORMANCE CHARACTERISTICS

Analytical sensitivity

The kit **HGV Real-TM** allows to detect *HGV* in 100% of the tests with a sensitivity of not less than 50 copies/ml. The detection was carried out on the control standard and its dilutions by negative plasma using the “Magno-Virus” extraction kit (Sacace REF K-2-16/1000) starting from a sample volume of 1 ml.

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *HGV* primers and probes. The specificity of the kit **HGV Real-TM** was 100%. The potential cross-reactivity of the kit **HGV Real-TM** was tested against the group control (HAV, HCV, HBV, HDV, HIV, HSV ½, EBV, CMV and other ones). It was not observed any cross-reactivity with other pathogens.

TROUBLESHOOTING

1. Weak or absent signal of the IC (Fam (Green) channel): retesting of the sample is required.
    - The PCR was inhibited.
        - ⇒ Make sure that you use a recommended RNA extraction method and follow the manufacturer's instructions.
        - ⇒ If the Ribo-Sorb isolation kit is used as a RNA extraction kit, re-centrifuge all the tubes before pipetting the extracted RNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent inhibit reaction.
    - The reagents storage conditions didn't comply with the instructions.
        - ⇒ Check the storage conditions
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the PCR conditions and for the IC detection select the fluorescence channel reported in the protocol.
    - The IC was not added to the sample during the pipetting of reagents.
        - ⇒ Make attention during the RNA extraction procedure.
2. Weak (Ct > 37) signal on the Joe (Yellow)/Cy3/HEX channel: retesting of the sample is required.
3. Joe (Yellow)/Cy3/HEX signal with Negative Control of extraction.
    - Contamination during RNA extraction procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        - ⇒ Use only filter tips during the extraction procedure. Change tips among tubes.
        - ⇒ Repeat the RNA extraction with the new set of reagents.
4. Any signal with Negative PCR Control.
    - Contamination during PCR preparation procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        - ⇒ Pipette the Positive controls at the end.
        - ⇒ Repeat the PCR preparation with the new set of reagents.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
	Expiration Date	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
IC	Internal Control	IVD	For *in Vitro* Diagnostic Use

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