Sacace BIOTECHNOLOGIES

HCV Genotype Plus Real-TM

HANDBOOK

Real Time PCR Kit for qualitative detection and differentiation of hepatitis C virus (HCV) genotypes 1a, 1b, 2, 3, 4, 5a, 6

REF R1-Gen-6

Σ 50

NAME

HCV Genotype Plus Real-TM

INTRODUCTION

The hepatitis C virus is an enveloped RNA virus with a diameter of about 50 nm, classified as a separate genus (Hepacivirus) within the Flaviviridae family. The genomic organization and sequence of HCV resembles that of the pestiviruses and flaviviruses.

HCV is classified into six major genotypes (designated 1-6), many subtypes (designated a, b, c, etc.), and about 100 different strains (numbered 1,2,3, etc.) based on the genomic sequence heterogeneity.

Genotypes 1-3 have a worldwide distribution. Types 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. Type 2 is less frequently represented than type 1. Type 3 is endemic in south-east Asia and is variably distributed in different countries. Genotype 4 is principally found in the Middle East, Egypt, and central Africa.

The determination of the infecting genotype is important for the prediction of response to antiviral treatment: genotype 1 and 4 are generally associated with a poor response to interferon alone, whereas genotypes 2 and 3 are associated with more favourable responses. At patients with subtype 1b the disease progresses to a chronic condition 90 % of cases, in that time as with genotypes 2 and 3b in 33-50 %. In a number of works it is mentioned, that infection with 1b genotype have heavier current of disease with development of a cirrhosis and hepatocarcinoma. When using combination therapy with interferon and ribavirin, patients with genotypes 2 or 3 generally are treated for only 24 weeks, whereas it is recommended that patients infected with genotype 1 receive treatment for 48 weeks.

The International Consensus European Association for the Study of the Liver (EASL) recommends before beginning of antiviral therapies to carry out a liver biopsies and to determine HCV genotype.

INTENDED USE

kit **HCV Genotype Plus Real-TM** is a Real-Time test for the determination of HCV-RNA genotypes 1a, 1b, 2, 3, 4, 5a and 6 in the human plasma and simultaneous detection of Internal Control (IC).

The **HCV Genotype Plus Real-TM** is not meant to be used for screening of blood, plasma, serum or tissue donors for HCV, but only for determining the genotype(s) of hepatitis C virus in plasma from HCV-infected individuals.

PRINCIPLE OF ASSAY

HCV Genotype Plus Real-TM is based on three major processes:

1. isolation of *HCV* RNA from specimens;
2. reverse transcription of the RNA;
3. real time PCR:
    - **PCR-mix-1-FRT HCV 1b/3** with primers and probes for subtypes 1b, 3;
    - **PCR-mix-1-FRT HCV 1a/2** with primers and probes for subtypes 1a, 2;
    - **PCR-mix-1-FRT HCV 4/IC** with primers and probes for subtypes 4 and Internal Control;
    - **PCR-mix-1-FRT HCV 5a/6** with primers and probes for subtypes 5a, 6;

Internal Control (IC) serves as an amplification control for each individually processed specimen and to identify possible inhibition. IC is detected in a channel other than the HCV RNA.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (R1-Gen-6)

Part N° 1 – **"Controls"**

- **Negative Control***, 0,5 ml;
- **HCV Rec IC**** (Internal Control), 0,5 ml.

** must be used during the sample preparation procedure: add 100 µl of C– (Negative Control) to the tube labeled Cneg;*

*** must be used during the sample preparation procedure (see RNA isolation).*

Part N° 2 – **"Reverta-L":**

- **RT-G-mix-1**, 5 x 0,01 ml;
- **RT-mix**, 5 x 0,125 ml;
- **Reverse transcriptase** (M-MLV), 0,03 ml;
- **TE-buffer**, 1,2 ml.

Contains reagents for 60 tests.

Part N° 3 – **"HCV Genotype Plus Real-TM":**

- **PCR-mix-1-FRT HCV 1b/3**, 0,6 mL
- **PCR-mix-1-FRT HCV 1a/2**, 0,6 mL
- **PCR-mix-1-FRT HCV 4/IC**, 0,6 mL
- **PCR-mix-1-FRT HCV 5a/6**, 0,6 mL
- **RT-PCR-mix-2-TM**, 4 x 0,3 mL.
- **TaqF Polymerase**, 4 x 0,03 mL
- Controls:
  - **HCV cDNA C+ 1b/3**, 0,2 mL;
  - **HCV cDNA C+ 1a/2**, 0,2 mL;
  - **HCV cDNA C+ 4**, 0,2 mL;
  - **HCV cDNA C+ 5a/6**, 0,2 mL;
  - **TE-buffer**, 0,5 mL.

Contains reagents sufficient for 50 samples.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder-free gloves and laboratory coat.
- Automatic adjustable pipettes (from 5 to 20 µl and from 20 to 200 µl).
- Disposable tips with aerosol barriers (100 or 200 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Real Time PCR instrument.
- Disposable polypropylene microtubes for PCR or PCR-plate
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.
- RNA isolation kit

STORAGE INSTRUCTIONS

Part N° 1 – **"Controls"** must be stored at 2-8°C.

Part N° 2 – **"Reverta-L"** must be stored at -20°C

Part N° 3 – **"HCV Genotype Plus Real-TM"** must be stored at -20°C.

The **HCV Genotype Plus Real-TM** kit can be shipped at 2-8°C but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY

HCV Genotype Plus Real-TM Test is stable up to the expiration date indicated on the kit label. All components of the **HCV Genotype Plus Real-TM** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT tubes are to be kept away from light.

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

The user should always pay attention to the following:

- 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

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

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

1. EDTA tubes may be used with the **HCV Real-TM Genotype Plus**. Follow sample tube manufacturer's instructions.
2. 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.
3. Do not freeze whole blood.
4. Specimens anti-coagulated with heparin are unsuitable for this test.
5. Thaw frozen specimens at room temperature before using.
6. 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.
7. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

Serum can also be used as starting material on some occasions. In this cases the analytical sensitivity of the kit is the same, but the clinical sensitivity may be significantly decreased because of the precipitation of viral particles during the clot retraction phase of serum preparation.

RNA ISOLATION

The following isolation kits are recommended*:

- ⇒ **Ribo Virus 50** – spin column extraction kit (Sacace, REF K-2-C/50)
- ⇒ **DNA/RNA Prep** – (Sacace, REF K-2-9)
- ⇒ **Ribo-Sorb** (Sacace, REF K-2-1)
- ⇒ **SaMag Viral Nucleic Acids Extraction kit** (Sacace, REF SM003)

Please carry out the RNA extraction according to the manufacturer's instructions. Add 10 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture.

** if other manufacturers' RNA isolation kits are used, please contact our customer care service at specialists@sacace.com*

REVERSE TRANSCRIPTION PROTOCOL

Reverse Transcription:

1) Thaw **RT-G-mix-1** and **RT-mix**, vortex and centrifuge briefly.
2) Prepare Reaction Mix: for 12 reactions, **add 5,0 *µl* RT-G-mix-1** into the tube containing **RT-mix** and vortex for at least 5-10 seconds, centrifuge briefly. Add **6 *µl* M-MLV** into the tube with Reagent Mix, mix by pipetting, vortex for 3 sec, centrifuge for 5-7 sec (must be used immediately after the preparation).

*If it is necessary to test less than 12 samples add for each sample (N) in the new sterile tube **10*N µl** of **RT-mix, 0,4*N µl** of **RT-G-mix-1** and **0,5*N µl** of **M-MLV**. Mix by pipetting, vortex for 3-5 sec, centrifuge briefly.*

Table 1. Reverse transcription reaction mix preparation for less than 12 samples

Reagent volume for one reaction (µl)	***10,00***	***0,4***	***0,5***
Clinical samples	***RT-mix***	***RT-G-mix-1***	***MMlv***
4	*60**	*2,4*	*3,0*
5	*70*	*2,8*	*3,5*
6	*80*	*3,2*	*4,0*
7	*90*	*3,6*	*4,5*
8	*100*	*4,0*	*5,0*

** The volumes are calculated considering one negative control of extraction and reagents for one extra reaction*

3) Add **10 *µl*** of **Reaction Mix** into each sample tube.
4) Pipette **10 *µl*** of **RNA** samples to the appropriate tube. (*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)*.Carefully mix by pipetting.
5) Place tubes into thermalcycler and incubate at 37^{o}C for 30 minutes.
6) Dilute 1: 2 each obtained cDNA sample with TE-buffer (add **20 *µl* TE-buffer** to each tube). Vortex and centrifuge briefly the tubes.
7) cDNA specimens could be stored at -20^{o}C for a week or at -70^{o}C up to one year.

REAL TIME PCR

1. Thaw PCR reagents, vortex and centrifuge briefly the tubes.
2. Prepare 4 PCR tubes for each sample and mark the tubes properly (for ex.“No. sample 1b/3”, “No. sample 1a/2”, “No. sample 4/IC”, “No. sample 5a/6”).
3. Prepare 4 Master mixes: “1b/3”, “1a/2”, “4/IC” and “5a/6”. To do this, add for each sample in the new sterile tube:
    - **10 µl** of **PCR-mix-1-FRT HCV** genotype **1b/3** (or **1a/2** or **4/IC** or **5a/6**)
    - **5 µl** of **RT-PCR-mix-2-TM**,
    - **0,5 µl** of **polymerase (TaqF)**
4. Thoroughly vortex. Make sure there are no drops on the walls of the tubes; otherwise, centrifuge briefly.

Table 2. Master Mixes preparation

Reagent volume for one reaction (µl)		***10,00***	***5,00***	***0,50***
Clinical samples	***PCR reactions****	***PCR-mix-1-FRT HCV genotype***	***RT-PCR-mix-2-TM***	***Polymerase (TaqF)***
4	*6*	*60*	*30*	*3,0*
5	*7*	*70*	*35*	*3,5*
6	*8*	*80*	*40*	*4,0*
7	*9*	*90*	*45*	*4,5*
8	*10*	*100*	*50*	*5,0*
9	*11*	*110*	*55*	*5,5*
10	*12*	*120*	*60*	*6,0*
11	*13*	*130*	*65*	*6,5*
12	*14*	*140*	*70*	*7,0*

** minimum one Pos PCR control and one Negative extraction control must be performed for each Master Mix*

5. Pipette **15 µl** of Master **Mix** and **10 µl** of **cDNA** sample to the appropriate tube as indicated in the Diagram 1.
6. Perform for each PCR-mix-1 at least one negative control of extraction (PCR control - TE-buffer is also recommended to be used) by adding to the appropriate tube of **10 µl** of **Control** (all tubes in the position 13 of the Diagram 1).
7. Perform the Positive Control for each PCR-mix-1:
    - Add **10 µl** of Positive Control **cDNA HCV** genotypes **1b/3** to the tube with “1b/3” reaction mix labeled C+1b/3 (1st tube in the position 14 of the Diagram 1).
    - Add **10 µl** of Positive Control **cDNA HCV** genotypes **1a/2** to the tube with “1a/2” reaction mix labeled C+1a/2 (2nd tube in the position 14 of the Diagram 1).
    - Add **10 µl** of Positive Control **cDNA HCV** genotype **4/IC** to the tube with “4/IC” reaction mix labeled C+4/IC (3rd tube in the position 14 of the Diagram 1).
    - Add **10 µl** of Positive Control **cDNA HCV** genotype **5a/6** to the tube with “5a/6” reaction mix labeled C+5a/6 (4th tube in the position 14 of the Diagram 1)

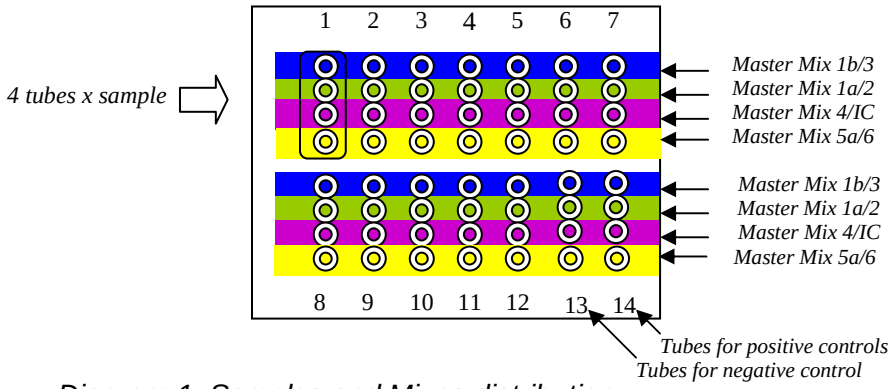

Diagram 1. Samples and Mixes distribution

8. Close tubes and transfer them into the Real Time PCR instrument.

Table 3. Real Time PCR Temperature profile.

	Rotor type instruments[1]				Plate type or modular instruments[2]			
Stage	**Temp, °C**	**Time**	**Fluorescence detection**	**Cycle repeats**	**Temp, °C**	**Time**	**Fluorescence detection**	**Cycle repeats**
Hold	95	15 min	–	1	95	15 min (900 s)	–	1
Cycling	95	5 s	–	5	95	5 s	–	5
	60	20 s	–		60	20 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), CFX/iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied Biosystems), SmartCycler® (Cepheid), LineGeneK® (Bioer)

DATA ANALYSES

The fluorescence curves are analyzed with the software of Real Time PCR instruments on the 2 channels (FAM/Green and Joe/Yellow/HEX/Cy3).

Table 4. HCV genotype detection

Tube	1b/3	1a/2	4/IC	5a/6
FAM (Green)	1b	1a	IC	5a
JOE (Yellow)/HEX/Cy3	3	2	4	6

The result of the experiment can be accepted only if the Positive and Negative Controls runs are valid (see table 5).

Table. 5 Results of controls

Reaction Mix	1b/3		1a/2		4/IC		5a/6	
Channel	FAM (Green) Ct	Joe(Yellow)/ HEX/Cy3 Ct	FAM (Green) Ct	Joe(Yellow)/ HEX/Cy3 Ct	FAM (Green) Ct	Joe(Yellow)/ HEX/Cy3 Ct	FAM (Green) Ct	Joe(Yellow)/ HEX/Cy3 Ct
Neg. Control (Extraction)	-	-	-	-	<40	-	-	-
Neg. Control (PCR)	-	-	-	-	-	-	-	-
$C+_{1b/3}$	<37	<37	*	*	*	*	*	*
$C+_{1a/2}$	*	*	<37	<38	*	*	*	*
$C+_{4/IC}$	*	*	*	*	<37	<37	*	*
$C+_{5a/6}$							<37	<37

The results of the samples are interpreted through the presence of crossing of fluorescence curve with the threshold line (see table 6).

Table 6. Examples of samples results.

Tube	Channel	Ct	Result
1b/3	FAM	-	Genotype 3
	JOE/HEX/Cy3	27,7	
1a/2	FAM	-	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,2	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	22,1	Genotype 1b, 2
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	32,1	
4/IC	FAM	35,4	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	30,4	Genotype 1b, 1a
	JOE/HEX/Cy3	-	
1a/2	FAM	26,6	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,0	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	28,0	Genotype 1b, 4
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,7	
	JOE/HEX/Cy3	22,9	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	39,4	Genotype 4*
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,3	
	JOE/HEX/Cy3	22,3	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	-	Genotype 1a
	JOE/HEX/Cy3	-	
1a/2	FAM	32,0	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,5	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	-	Genotype 5a
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,2	
	JOE/HEX/Cy3	-	
5a/6	FAM	28,0	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	22,1	Genotype 1b, 6
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,4	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	27,6	

Tube	Channel	Ct	Result
1b/3	FAM	-	Genotype 2
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	32,1	
4/IC	FAM	35,0	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	-	not detected (negative)
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	-	
4/IC	FAM	35,4	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	-	Genotype 2, 4*
	JOE/HEX/Cy3	-	
1a/2	FAM	38,9	
	JOE/HEX/Cy3	33,0	
4/IC	FAM	35,8	
	JOE/HEX/Cy3	21,1	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

Tube	Channel	Ct	Result
1b/3	FAM	-	repeat test
	JOE/HEX/Cy3	-	
1a/2	FAM	-	
	JOE/HEX/Cy3	-	
4/IC	FAM	-	
	JOE/HEX/Cy3	-	
5a/6	FAM	-	
	JOE/HEX/Cy3	-	

* *Ct of the 4th genotype is lower than Ct of 1st for more than 15 cycles: the results for the genotypes 1a and 1b must not be considered. The result must be given as "positive for genotype 4".*

SPECIFICATIONS

The sensitivity of the kit **HCV Genotype Plus Real-TM** was evaluated using serial dilutions of recombinant positive controls. The analytical specificity of the kit was determined using the recombinant positive controls with high HCV concentrations (not less than 10^8 copies/ml). The diagnostic specificity and sensitivity of the kit **HCV Genotype Plus Real-TM** were analyzed also on clinical samples (blood plasma). 245 patients having diagnosis of viral hepatitis C, represented by different genotypes (1a (43 samples), 1b (66 samples), 3a (68 samples), 2 (57 samples), 4 (5 samples), 1a+1b (6 samples) were included in the trial group. As a kit of comparison the HCV Genotype Kit (Sacace) with electrophoresis detection as well as the sequencing (for samples not identified using HCV Genotype Kit) were used. The control group was composed by blood plasma taken from patients with hepatitis of other etiology and samples taken from healthy persons – donors, total 30 samples. There were used 2 control panels QCMD (Quality Control for Molecular Diagnostics) containing different genotypes of the virus – "QCMD 2006 Hepatitis C Virus Genotype Proficiency Panel" and "QCMD 2007 Hepatitis C Virus Genotype Proficiency Panel".

Results:

The analytical sensitivity of the kit **HCV Genotype Plus Real-TM** was 1000 IU/ml. Using the recombinant positive controls with high HCV concentrations and blood samples taken from patients with high viral load, it was shown the complete absence of cross-reactions between the above mentioned genotypes. In the control group none of the specimens showed any reactivity with **HCV Genotype Plus Real-TM** kit**.** The diagnostic specificity of the tested **HCV Genotype Plus Real-TM** kit was 100% and the diagnostic sensitivity was 100%.

TROUBLESHOOTING

1. Weak (Ct > 40) or no signal of the IC (Fam channel tube 4/IC) for the Negative Control of extraction.
    - The PCR was inhibited.
        - ⇒ Make sure that you use a recommended RNA extraction method and follow to the manufacturer's instructions.
        - ⇒ Re-centrifuge all the tubes before pipetting of 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 select for the IC detection 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 > 38) or no signal of the Positive Controls.
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
    - Incorrect pipetting of reagent.
        - ⇒ Check your pipetting scheme (see Diagram 1)
3. Any signal with Negative Control of extraction (ecc. Fam channel tube 4/IC).
    - 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 between tubes.
        - ⇒ Repeat the RNA extraction with the new set of reagents.
4. Any signal with Negative Control of PCR (TE-buffer).
    - 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 last.
        - ⇒ 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	**RUO**	For Research Use Only

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX™ and iQ5™ are registered trademarks of Bio-Rad Laboratories
* Rotor-Gene™ is a registered trademark of Qiagen
* MX3005P® is a registered trademark of Agilent Technologies
* ABI® is a registered trademark of Applied Biosystems
* LineGeneK® is a registered trademark of Bioer
* SmartCycler® is a registered trademark of Cepheid

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314892926
mail: ***info@sacace.com*** *web:* ***www.sacace.com***