IVD For *in Vitro* Diagnostic Use

CE

TBEV, B.burgdorferi, A.phagocytophilum, E.chaffeensis / E.muris Real-TM

Handbook

Real-Time PCR test for qualitative detection of RNA of tick-borne encephalitis virus (TBEV), Borrelia burgdorferi, Ehrlichia chaffeensis and Ehrlichia muris and DNA of Anaplasma phagocytophilum in biological materials

REF V59-100FRT

Σ 100

NAME

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM

INTENDED USE

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris **Real-TM** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of RNA of *tick-borne encephalitis virus* (*TBEV), Borrelia burgdorferi sl* (Ixodes tick-borne borreliosis (ITB) pathogen), *Ehrlichia chaffeensis* and *Ehrlichia muris (*human monocytic ehrlichiosis (HME) pathogens) and DNA of *Anaplasma phagocytophilum (*human granulocytic anaplasmosis (HGA) pathogen) in biological materials (ticks, blood, cerebrospinal fluid, and autopsy material) by using real-time hybridization-fluorescence detection.

PRINCIPLE OF ASSAY

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris nucleic acid detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM*** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM*** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris nucleic acid detection includes:

a) RNA/DNA extraction from biological material simultaneously with the Internal Control;

b) reverse transcription of cDNA on RNA template;

c) PCR with real-time detection of cDNA/DNA amplification products.

MATERIALS PROVIDED

Part N° 1 – **“Reverta-L”**

Reagent	***Volume (ml)***	***Amount***
RT-G-mix-1	0,01	10 tubes
RT-mix	0,125	10 tubes
Reverse transcriptase (M-MLV)	0,06	1 tube
TE-buffer	1,2	2 tubes

Part N° 2 – **“Real-TM PCR kit”**

Reagent	***Volume (ml)***	***Amount***
PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E. m.*	0.6	2 tubes
PCR-mix-1-FRT *B.b. sl* / IC	0.6	2 tubes
RT-PCR-mix-2	0.3	4 tubes
TaqF Polymerase	0.03	4 tubes
Positive Control cDNA *TBEV, B.b. sl, A.ph., E.ch. / E.m.* / IC (C+*TBEV, B.b. sl, A.ph., E.ch. / E.m.* /IC)	0.2	2 tubes
DNA-buffer	0.5	2 tubes
Internal Control (IC)*	0.12	10 tubes

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM PCR kit is intended for 120 reactions (including controls).

** add 10 µl of Internal Control-STI-87-rec during the RNA/DNA extraction directly to the sample/lysis mixture.*

MATERIALS REQUIRED BUT NOT PROVIDED

- RNA/DNA extraction kit.
- Reverse transcription kit
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 6000 (Corbett Research, Australia), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2 ml) or microplate.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

STORAGE INSTRUCTIONS

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM must be stored at -20°C. The shelf life of reagents before and after the first use is the same, unless otherwise stated. The kit should be transported at 2–8 °C for no longer than 5 days but should be stored at -20°C immediately on receipt.

STABILITY

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM Test 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. The shelf life of reagents before and after the first use is the same, unless otherwise stated. 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. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

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:

- 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

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM PCR kit is intended to analyze RNA/DNA extracted from:

- *Tick suspension*

Tick pools of no more than 10 specimens or a single tick (preferably for the *Dermacentor* genus) can be used for analysis.

Place ticks into Eppendorf tubes, add 500 µl of 96 % ethanol, and vortex. Spin the tube with ticks for 3-5 s then remove liquid with a vacuum aspirator. Add 500 µl of 0.15 M NaCl or phosphate buffer, vortex, and spin for 3-5 s. Remove liquid with a vacuum aspirator.

Use a sterile porcelain mortar and a pestle to prepare tick suspension. Homogenize ticks in 300 µl (a single *Ixodes* tick), 500 µl (a single *Dermacentor* tick), or 1 ml (tick pool) of 0.15 M NaCl or phosphate buffer then centrifuge at 5,000 rpm for 2 min. Take 100 µl of the supernatant for RNA/DNA extraction from *Ixodes* ticks or 50 µl of the supernatant for RNA/DNA extraction from *Dermacentor* ticks.

Add glycerol (10% v/v) to the tube with the remained suspension, stir, and freeze at or below minus 16 °C for further use.

- *Cerebrospinal fluid (CSF) and leukocyte fraction of blood*

Take a blood specimen in the morning after overnight fasting to a tube with 6 % EDTA in the ratio 1:20. Invert the closed tube several times. To obtain the leukocyte fraction of blood, transfer 1.5 ml of the blood with EDTA to an Eppendorf tube and centrifuge at 800 rpm for 10 min. Then transfer 500-600 µl of the upper plasma layer with leukocytes to an Eppendorf tube and centrifuge at 13,000 rpm for 10 min. Remove and discard the supernatant. Use cell pellet and 200 µl of supernatant above it for RNA/DNA extraction.

Centrifuge 1-1.5 ml of CSF at 13,000 rpm for 10 min. Remove and discard the supernatant. Use the cell pellet and 200 µl of supernatant above it for RNA/DNA extraction.

- *Internal organs of animals and autopsy material*

Homogenize internal organs of animals and autopsy material with a porcelain mortar and a pestle and prepare 10 % suspension using sterile saline (0.15 M NaCl) or phosphate buffer. Take 50 µl of the suspension for RNA/DNA extraction.

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.

Sacace Biotechnologies recommends to use the following kit:

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

Please carry out the RNA extraction according to the manufacturer's instructions.

Internal Control (IC): *add 10 µl of Internal Control RNA during the RNA isolation procedure directly to the sample/lysis mixture.*

PROTOCOL

Reverse Transcription:

1) 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. This mix is stable for 1 month at -20°C. **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-G-mix-1 with RT-mix** and **0,5*N µl** of **M-MLV**).*

2) Add **10 µl** of **Reaction Mix** into each sample tube.
3) Pipette **10 µl 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.
4) Place tubes into thermalcycler and incubate at 37°C for 30 minutes.
5) Incubate the tubes at 95°C for 5 min.
6) Dilute 1: 2 each obtained cDNA sample with TE-buffer (add **20 µl TE-buffer** to each tube).

cDNA specimens could be stored at -20°C for a week or at -70°C during a year.

Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

All obtained cDNA samples should be examined in two tubes – one with **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and the other one with **PCR-mix-1-FRT *B.b. sl* / IC.**

1. Prepare the reaction mixture for the required number of reactions. To do this, mix **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.,* polymerase (TaqF),** and **RT-PCR-mix-2** in one tube and **PCR-mix-1-FRT *B.b. sl* / IC, polymerase (TaqF), and RT-PCR-mix-2 FEP/FRT** in the other tube.

   Reagent volumes per one reaction as follows:

   - **10 µl of PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.* or PCR-mix-1-FRT *B.b. sl* / IC,**
   - **5 µl of RT-PCR-mix-2,**
   - **0.5 µl of polymerase (TaqF).**

Do not store the prepared reaction mixture.

PCR run should include amplification reactions for six control points: negative control of extraction (C-), positive control of RT-PCR (C+$_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ \mathrm{STI}}$), and negative control of RT-PCR (NCA) for two reaction mixtures (**PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and **PCR-mix-1-FRT *B.b. sl* / IC**).

2. Transfer **15 µl** of the prepared mixture to each tube.
3. Add **10 µl** of **cDNA** obtained from clinical or control samples at the reverse transcription stage to the prepared tubes using tips with aerosol barrier.
4. Carry out the control amplification reactions:

NCA	-Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
C+*TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI	-Add **10 µl** of **Positive Control cDNA *TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI** to the tube labeled C+$_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ \mathrm{STI}}$ (Positive Control of Amplification).

Perform the amplification reaction immediately after cDNA samples and controls are added to the reaction mixture.

Amplification

1. Create a temperature profile on your instrument as follows:

	Rotor-type Instruments[1]			Plate-or modular type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Cycles**	***Temperature, °C***	***Time***	**Cycles**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	10 s	5	**95**	10 s	5
	60	30 s		**60**	35 s	
	72	15 s		**72**	15 s	
3	**95**	10 s	40	**95**	10 s	40
	56	30 s *fluorescent signal detection*		**56**	35 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

[1] For example Rotor-Gene™ 6000/Q (Corbett Research, Qiagen)
[2] For example SaCycle-96 (Sacace), CFX96/iQ5™ (BioRad); Mx3000P™Mx3005P™ (Stratagene), Applied Biosystems® 7500 Real Time PCR (Applera), SmartCycler® (Cepheid)

Fluorescent signal detection is enabled in the channels designed for the FAM/Green, JOE/Yellow/HEX, and ROX/Orange fluorophores for the tubes with the **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and for the FAM/Green and JOE/Yellow/HEX fluorophores for the tubes with the **PCR-mix-1-FRT *B.b. sl* / IC**.

2. Insert the tubes into the reaction module of the instrument. If amplification is carried out simultaneously for both PCR-mixes-1, the tubes with PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.* should be inserted first.
3. Run the amplification program with fluorescence detection.
4. Analyze results after the amplification program is completed.

DATA ANALYSIS

The fluorescent signal intensity is detected in two or three channels depending on the PCR-mix-1 used.

PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*

- The signal from the *TBEV* cDNA amplification product is detected in the FAM/Green channel;
- The signal from the *A.phagocytophilum* DNA amplification product is detected in the JOE/Yellow/HEX channel;
- The signal from the *E.chaffeensis / E.muris* cDNA amplification product is detected in the ROX/Orange channel.

PCR-mix-1-FRT *B.b. sl* / IC

- The signal from the Internal Control cDNA amplification product is detected in the FAM/Green channel;
- The signal from the *Borrelia burgdorfer sl.* cDNA amplification product is detected in the JOE/Yellow/HEX channel.

RESULT INTERPRETATION

The results are interpreted by the Instrument software by the crossing (or not-crossing) of the fluorescence curve with a threshold line and are shown as the presence (or absence) of Ct (threshold cycle) in the result grid.

Principle of interpretation:

- *TBEV* cDNA is **detected** in a sample if its Ct is defined in the result grid in the FAM/Green channel (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *A.phagocytophilum* DNA is **detected** in a sample if its Ct is defined in the result grid in the JOE/Yellow/HEX channel (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *E.chaffeensis / E.muris* cDNA is **detected** in a sample if its Ct is defined in the result grid in the ROX/Orange channel (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *Borrelia burgdorferi sl.* cDNA is **detected** in a sample if its Ct is defined in the result grid in the JOE/Yellow/HEX channel (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**).

Moreover, the fluorescence curve of every studied sample should cross the threshold line at the exponential growth stage.

- *Borrelia burgdorferi sl.* cDNA is **not detected** in a sample if its Ct is not defined in the result grid in the JOE/Yellow/HEX channel while Ct in the FAM/Green channel is less than 38 (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**).
- *TBEV A.phagocytophilllum, and E.chaffeensis / E.muris* cDNA/DNA are **not detected** in a sample if Ct values are not defined in the result grid in the appropriate channels (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- The result is **invalid** if Ct of a sample is absent in the channels for specific pathogen detection whereas in the FAM/Green channel (with the use of **PCR-mix-1-FRT *B.b. sl* / IC)** Ct is also absent or greater than 38. It is necessary to repeat the PCR test for such samples.

The result of the analysis is considered reliable only if the results of both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct.

Table.1 Results for controls

Control	Stage for control	Ct value			Interpretation
		FAM/Green	JOE/Yellow/HEX	ROX/Orange/TexasRed	
PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*					
		Detection of *TBEV*	**Detection of *A.phagocyto-phillum***	**Detection of *E.chaffeensis / E.muris***	
C–	RNA/DNA extraction	Absent	Absent	Absent	OK
NCA	Amplification	Absent	Absent	Absent	OK
C+ ***TBEV, B.b. sl, A.ph., E.ch. / E.m.*** **/ STI**	Amplification	<31	<31	<31	OK
PCR-mix-1-FRT *B.b. sl* / IC					
		Detection of IC	**Detection of *B.burgdorferi sl***	–	
C–	RNA/DNA extraction	<33	Absent	–	OK
NCA	Amplification	Absent	Absent	–	OK
C+ ***TBEV, B.b. A.ph., E.ch. / E.m.*** **/ IC**	Amplification	<30	<30	–	OK

Results for test samples

PCR-mix-1-FRT	Signal in channel (Ct)		
	FAM/Green	JOE/Yellow/HEX	ROX/Orange/TexasRed
TBEV, A.ph., E.ch. / E.m.	Detection of *TBEV*	Detection of *A.phagocytophillum*	Detection of *E.chaffeensis / E.muris*
	<39	<39	<39
***B.b. sl* / IC**	Detection of IC	Detection of *B.burgdorferi*	–
	<38	<39	–

SPECIFICATIONS

Sensitivity

The analytical sensitivity of ***TBEV, B. burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*** **Real-TM** PCR kit is specified in the table below.

Clinical material	DNA extraction kit	Reverse transcription kit	Analytical sensitivity, GE/ml*	Pretreatment of biological material
Ticks of *Ixodes* and *Dermacentor* genera	DNA/RNA-prep	REVERTA-L	10^3	Indicated sensitivity can be reached only if the specified pretreatment instructions are followed and the specified specimen volume is used

* Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample.

Specificity

The analytical specificity of ® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E. chaffeensis / E.muris*** **Real-TM** PCR kit is ensured by selection of specific primers and probes as well as by selection of strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Analitival specificity was studied on the following microorganisms:

- *flaviviruses* (*West Nile*, *Langat, Powassan, Japanese encephalitis, and Omsk hemorrhagic fever viruses*);
- *spirochaetes* (*Borrelia miyamotoi; Treponema pallidum; Leptospira interrogans, L.kirshneri; and L. borgpetersenii*);
- *rickettsiae* of spotted fever group (*Rickettsia conorii* spp*. caspia* and *R.heilongiangensis*; *Coxiella burnetii*; and *Bartonella henselae* and *B.quintana).*

No false-positive results were observed during examination of DNA of the above-mentioned organisms, ticks (*Ixodes persulcatus*, *I. ricinus*, *Dermacentor reticulatus*, and *D. marginatus*), rodents (*Clethrionomys glareolus* and *Apodemus agrarius)*, as well as human DNA.

The clinical specificity of ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris Real-TM*** PCR kit was confirmed in laboratory clinical trials.

TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

- If the Ct value of the Positive Control of amplification (C+$_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ STI}$) is absent or greater than the specified in Table 1 (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***) or in the FAM and JOE channels (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**), PCR should be repeated for all samples in which specific cDNA/DNA detected in the appropriate channel was not found.
- If the Ct value of the Negative Control of extraction (C-) (in the FAM, JOE, ROX channels with **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and in the JOE channel with **PCR-mix-1-FRT *B.b. sl* / IC**) and/or Negative Control of amplification (NCA) (in all channels) is defined in the result grid, PCR should be repeated for all samples in which specific cDNA DNA detected in the appropriate channel was found.
- If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
	Consult instructions for use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative control of Extraction
IC	Internal Control	**C+** *TBEV, B.b. sl, A.ph., E.ch / E.m.* / **STI**	Positive Control of Amplification
	Expiration Date	IVD	For *in Vitro* Diagnostic Use

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