AmpliTest *BVDV*
(Real Time PCR)

Diagnostic kit for detection of RNA sequences
specific for the ***BVDV (Bovine Viral Diarrhoea Virus)***
using the **Real Time PCR** technique

Cat. No.	*Quantity*
RV01-100	**100 assays**

The diagnostic kit is intended for professional use in specialised clinical, diagnostic and research laboratories.
We strongly recommend that you read the user manual attached to the diagnostic kit before first use.

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TRANSPORT

The **AmpliTest *BVDV* (Real Time PCR)** kit is transported in dry ice. Immediately unpack the kit after delivery. Immediately inform the manufacturer if the tape securing the transport box or the seal of the commercial packaging are damaged or there is no dry ice left in the styrofoam transport box.

KIT CONTENTS

Component	***Description***	**RV01-100** ***100 assays***	***Tube lid colour***
RM	Reaction Mixture	2 × 550 µL	Green
PC	Positive Control	1 × 300 µL	Red
NC	Negative Control	1× 300 µL	Blue
IC	Internal Control	2 × 750 µL	Transparent
RT	Reverse Transcriptase	1 × 20 µL	Black
Rin	RNA Inhibitor	1 × 40 µL	Yellow
WATER	Water	1 × 1500 µL	White

STORAGE AND TRANSPORTATION CONDITIONS

- The kit components should be stored at -20°C.
- **AVOID THE EXPOSURE OF THE RM COMPONENT TO LIGHT**.
- Avoid repeated freezing/thawing cycles of the kit components (especially the RM component). The kit is stable after 10 repeated freezing/thawing cycles.
- **IC** and **PC** components contain RNA: they must be protected from ribonucleases.
- The kit is stable under recommended storage conditions until the expiry date stated on the label.
- Do not use kit components after the expiry date.

PRODUCT DESCRIPTION

Application

The **AmpliTest *BVDV* (Real Time PCR)** kit is designed to detect RNA sequences specific for the BVD virus (Bovine Viral Diarrhoea Virus) which causes Bovine Viral Diarrhoea and Mucosal Disease. The presence of the pathogen-specific sequences is assayed in RNA preparations extracted from bovine tissues. The diagnostic kit is intended for *in vitro* diagnostics. The kit is a qualitative type: it is used to confirm or exclude the presence of the viral RNA in the RNA samples.

Method principle

The BVD virus is an RNA-type virus of the *Flaviviridae* family. Its detection by the Real Time PCR technique requires a reverse transcription process consisting in the synthesis of cDNA molecules based on the RNA sequences. The **AmpliTest *BVDV* (Real Time PCR)** kit is a one-step type kit: reverse transcription process and amplification of BVDV-specific sequences occur in one tube.

The **AmpliTest *BVDV* (Real Time PCR)** kit contains three pairs of primers that allow the amplification of sequences specific for three known virus genotypes (BVDV1, BVDV2 and BVDV3). Accumulating amplicons (the amplified fragments of the viral genome) are detected owing to specific TaqMan® probes which are hydrolysed during the Real Time PCR reaction. After hydrolysis, there are FAM, Cy5® and Texas

Red® fluorescent dyes (for BVDV1, BVDV2 and BVDV3, respectively) released from the probes after their hydrolysis detected by the optical system of the Real Time PCR device. Carefully designed, the probes and primers ensure high specificity of the reaction.
To increase the reliability of the results, the **AmpliTest *BVDV* (Real Time PCR)** kit contains an internal control system that can be used to monitor the proper course of nucleic acid extraction and the Real Time PCR reaction. In addition to the probes and primers for detection of the pathogen-specific sequences, the **RM** component contains the probe and primers for amplifying and detecting the internal control. Internal control detection occurs due to the release of the HEX fluorescent dye from the probe. A positive result for internal control amplification confirms the proper course of nucleic acid extraction and the Real Time PCR reaction.

ADDITIONAL INSTRUMENTS AND MATERIALS

The **AmpliTest *BVDV* (Real Time PCR)** kit allows a complete diagnostic procedure (detecting the presence of the BVD virus in the samples) **only** in conjunction with the kit for RNA extraction from the sample and the Real Time PCR device.

NOTE 1: The **AmpliTest *BVDV* (Real Time PCR)** kit is compatible with Real Time PCR devices having detection of HEX, FAM, Cy5® and Texas Red® fluorescent dyes. A full list of compatible Real Time PCR devices can be found on the manufacturer's website.

NOTE 2: The **AmpliTest *BVDV* (Real Time PCR)** kit does not contain the ROX reference dye.
The addition of ROX dye to the reaction mixture is not possible. The corresponding detection channel is reserved for the Texas Red® dye released from the probe that detects sequences specific for the BVDV3 genotype.

TECHNICAL SPECIFICATION

Quality control	Complies with ISO 13485: Medical devices - Quality management systems
Optimal RNA amount added to the reaction	to 1 µg

RNA EXTRACTION

Due to the high susceptibility of RNA to degradation, the time between collecting the biological material and RNA extraction should be as short as possible. Until RNA extraction, the biological material should be properly protected (stored at -20°C, possibly at 2–8°C, preferably in an environment inhibiting nuclease activity, e.g. in the presence of EDTA). The improper storage of the biological material may result in RNA degradation, which will lead to false negative results. The amount of the material required depends on the RNA extraction method.
For RNA extraction and purification silica-based RNA extraction kits are recommended. These kits ensure good-quality of RNA samples. RNA samples should be stored at -20°C (short storage period) or at -80°C (long storage period). Avoid repeated freezing and thawing of RNA samples and protect from contact
with nucleases. For this, use materials (water, tubes, pipette tips, etc.) free of nucleases (confirmed by appropriate certificates).
The internal control system included in the kit can be used to control the RNA extraction process. For this, the **IC** component should be added to the sample at the initial stage of RNA extraction. The volume

of the **IC** component depends on the volume of the elution buffer used for the RNA extraction. Add 5 µL of the **IC** component for every 50 µL of the elution buffer.

NOTE 3: Other extraction methods can cause contamination of RNA samples with compounds that significantly reduce the efficiency of the PCR reaction. This leads to lower sensitivity of the assay and, in extreme situations, complete inhibition of cDNA amplification.

NOTE 4: During RNA extraction, the homogenisation of solid biological material (e.g. internal organs, biopsies) is particularly important. Care must be taken to ensure that RNA does not degrade during homogenisation.

REAL TIME PCR REACTION

1. Determine the number of RNA samples to be analysed (**n**).
2. Thaw the kit components, except for the **RT** and **Rin** components. After thawing, mix the tubes thoroughly and briefly spin them.
   Store thawed components at 2–8°C or in ice.
   AVOID THE EXPOSURE OF THE RM COMPONENT TO LIGHT.

NOTE 5: Keep **RT** (reverse transcriptase) and **Rin** (RNA inhibitor) below 0°C. These components are temperature sensitive and may become inactivated after heating.

3. Determine the amount of the RNA sample added to the reaction (**x** µL).

NOTE 6: Optimal amount of RNA added to the reaction should not exceed 1 µg. Large amounts of RNA added to the PCR reaction may reduce reverse transcription efficiency and increase the detection limit of the test.

OPTION I: The internal control was added to the sample during the RNA extraction process.

NOTE 7: Proceed according to Option I, if the internal control system is used to control the proper course of nucleic acid extraction and of the Real Time PCR reaction.

4.1 In a nuclease-free tube, mix the following components:

RM	(***n*** + 3) × 11 µL
RT	(***n*** + 3) × 0.2 µL
Rin	(***n*** + 3) × 0.4 µL
Water	(**n** + 3) × (8.4 - ***x***) µL

4.2 Add (20 - **x**) µL of the mixture to **n** + 2 reaction tubes.
4.3 Add **x** µL of the RNA samples to **n** reaction tubes.
4.4 Add **x +** µL of the **NC** component (negative control) to one of the two remaining reaction tubes and **x** µL of the **PC** component (positive control) to the other one. Then go to Step 5.

OPTION II: The internal control was not added to the sample during the RNA extraction process.

NOTE 8: Proceed according to Option II, if the internal control system is used only to monitor the proper course of the Real Time PCR reaction.

4.1 In a nuclease-free tube, mix the following components:

RM	(***n*** + 3) × 11 µL
RT	(***n*** + 3) × 0.2 µL

Rin	(***n*** + 3) × 0.4 µL
Water	(**n** + 3) × (7.9 - ***x***) µL

4.2 Add (19.5 - **x**) µL of the mixture to **n** + 2 reaction tubes.

4.3 Add **x** µL of the RNA sample and **0.5** µL of the **IC** component (internal control) to **n** reaction tubes.

4.4 Add (**x + 0.5)** µL of the **NC** component (negative control) to one of the two remaining reaction tubes (**x + 0.5)** µL of the **PC** component (positive control) to the other one. Then go to Step 5.

5. Close the reaction tubes and centrifuge them shortly.

6. Place the reaction tubes into the reaction device and run the amplification program according to the following amplification profile.

Step	Temperature	Time	Data Collection	Cycles
Reverse transcription	45°C	15 min		1
Initial denaturation	95°C	5 min		1
Denaturation	95°C	10 s		45
Amplification	58°C	25 s	FAM, Cy5®, Texas Red® and HEX	

NOTE 9: The FAM channel is used to detect the sequences specific for BVDV1, the Cy5® channel for detecting sequences specific for BVDV2, sequences specific for BVDV3 are detected in the Texas Red® channel. The HEX channel is used to detect the internal control.

NOTE 10: It is recommended that you prepare the Real Time PCR reaction in the laminar chamber to reduce the risk of reaction mixtures contamination.

ANALYSIS EVALUATION

The table below shows the correct Real Time PCR result:

	Type of Sample	FAM	Cy5®	Texas Red®	HEX	Result
Sample Results	Positive Control	+	+	+	-	Valid
	Negative Control	-	-	-	+	Valid
	Assay 1	-	-	-	+/-	Negative
	Assay 2	+	-	-	+/-	Positive (the presence of BVDV1)
	Assay 3	-	+	-	+/-	Positive (the presence of BVDV2)
	Assay 4	-	-	+	+/-	Positive (the presence of BVDV3)
	Assay 5	+	+	-	+/-	Positive (the presence of BVDV1 or BVDV2)*
	Assay 6	-	+	+	+/-	Positive (the presence of BVDV2 or BVDV3)*
	Assay 7	+	-	+	+/-	Positive (the presence of BVDV1 or BVDV3)*
	Assay 8	+	+	+	+/-	Positive (the presence of BVDV1, BVDV2 or BVDV3)*

+ means the PCR product amplification recorded as a fluorescence increase with Cq value ≤ 40

- means no PCR product amplification and no fluorescence increase or the PCR product amplification recorded as a fluorescence increase with Cq value > 40

* Due to the similarity between some variants of the BVD virus of different genotypes, it may happen that a signal confirming the presence of a specific type of virus will be detected simultaneously in two or three channels. In this case, the appropriate genotype is the one for which the Cq value is the lowest. For example, if a signal was detected simultaneously in the FAM and Cy5® channels and the Cq value for these channels was 32 and 25, respectively, the viral genotype present in the sample would be BVDV2.

ADDITIONAL REMARKS

- The TaqMan® probes have dark quenchers that do not generate additional fluorescent signals. For devices which require it (e.g. RotorGene 6000), select the **None** option as the quencher type.
- The Real Time PCR device should be calibrated (*color compensation* should be carried out) for fluorescent dyes.
- The total volume of the reaction mixture is 20 µL. Change of this volume may have a significant impact on some test parameters, including the detection limit.

WARNINGS AND PRECAUTIONS

- Take special care during RNA extraction. The biological material obtained from animals is considered as **potentially infectious material**.
- RNA extraction and detection of the RNA sequences specific for the BVD virus should be done by properly trained personnel in a professional laboratory which is allowed to work with potentially infectious material. Special attention should be paid to prevent cross-contamination of the RNA preparations during RNA extraction.
- Protect the RNA preparation from nucleases (use properly certified nuclease-free materials). RNA should be stored in frozen form. Avoid repeated freezing and thawing cycles.
- To increase RNA stability, preparations that improve RNA stability and inhibit nuclease activity can be used.
- To minimise the false results, adhere to the following rules:
  - If possible, designate the separate stands for RNA extraction as well as preparation and performance of the Real Time PCR reaction;
  - Change protective clothing and gloves between the stages of RNA extraction and preparation of the Real Time PCR reaction;
  - Bench surfaces should be washed with RNA removing/destructive agents;
  - Use only sterile filter tips for automatic pipettes;
  - The **PC** component should be added to the reaction mixture as the **last one**. Before adding it, if possible, close the tubes with the RNA preparations and the **NC** component;
- Do not eat, drink or smoke while using the kit;
- Wash your hands immediately after work with the kit;
- After contact of the skin or eyes with the kit components, gently wash them with water;

It is recommended that you dispose of the kit after its expiry date or of any unused kit components as a laboratory waste.

TROUBLESHOOTING

Real Time PCR reaction

Problem			Possible cause	Suggestions
	FAM	HEX		
Incorrect fluorescence reading for positive control	-	+	The **NC** component instead of the **PC** component was added to the reaction tube.	Repeat the Real Time PCR reaction for the positive control.
	-	-	No **PC** component added or the **RM** component is of poor quality.	Repeat the Real Time PCR reaction for the positive control. If you receive an incorrect result again, assume the poor quality of the **RM** component (improper storage, improper transport conditions or exceeding the expiry date).
Incorrect fluorescence reading for negative control	+	+	Contamination of the **RM** or **NC** component.	Repeat the Real Time PCR reaction for the negative control. If you receive an incorrect result again, assume the contamination of the **RM** or **NC** component.
	+	-	The **PC** component instead of the **NC** component was added to the reaction tube.	Repeat the Real Time PCR reaction for the negative control.
	-	-	No **NC** component added or the **RM** component is of poor quality.	Repeat the Real Time PCR reaction for the negative control. If you receive an incorrect result again, assume the poor quality of the **RM** component.
Incorrect fluorescence reading for the tested sample	-	-	No internal control **IC** was added at one of the assay steps.	Determine if the **IC** internal control was added at the intended stage of the assay. Repeat the Real Time PCR reaction with the internal control added to the reaction mixture.
			Low RNA extraction yield and/or Real Time PCR inhibitors present. Too high RNA concentration. Poor quality of the **RM** component.	Measure the concentration of the RNA preparation and, if necessary, repeat the reaction with less RNA added to the reaction. In the absence of amplification in the HEX channel for RNA preparations assayed and for the negative control, assume the poor quality of the **RM** component. In the case of amplification in the HEX channel for the negative control and no amplification in the HEX channel for RNA preparations tested, assume contamination of the RNA preparation with PCR inhibitors or low efficiency of nucleic acid extraction.

Nucleic acid extraction

Problem	Possible cause	Suggestions
Significantly higher Cq values obtained in the HEX channel for the samples assayed in comparison with the Cq value obtained for the negative control (ΔCq> 2)	Low efficiency of RNA extraction from the sample assayed.	To determine the reasons for the low efficiency of RNA extraction, please contact the manufacturer of the nucleic acid extraction kit.

CUSTOMER SERVICE

- Any problems or irregularities that arise during the use of the diagnostic kit can be reported by phone or email.
- Orders for the **AmpliTest** kits can be placed by email.

CONTACT

Support and customer care

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