ADIAVET™ BTV TYPE 8 REAL TIME

TEST FOR THE DETECTION OF THE BLUE TONGUE VIRUS OF TYPE 8 BY REAL-TIME ENZYMATIC AMPLIFICATION (RT-PCR TEST)

References:
ADI381-50 (50 reactions)

NOTE
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English version
NE381-08
2016/07

ADIAVET™ BTV TYPE 8 REAL TIME

I. Revision history

N/A	Not Applicable (first publication)
Correction	Correction of document anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: minor typographical, grammar and formatting changes are not included in the revision history.

Release Date	Part Number	Change type	Change summary
2012/05	NE381-06	Technical change	Addition of "Extraction with DNA/RNA magnetic beads kit" paragraph, in page 12, § V-5.
2014/12	NE381-07	Technical change	Addition of "Index of symbols" section, in page 17.
2014/12	NE381-07	Technical change	Removal of reference ADI381-100 (100 reactions)
2016/07	NF381-08	Administrative	Changing logos
2016/07	NF381-08	Administrative	Biosearch legal mention
2016/07	NF381-08	Administrative	Addition of table "Analysis options according to the specimen" §I.3.
2016/07	NF381-08	Technical change	Revision of interpretation result §VII.2.B.

II. General information

1. Purpose of the test

ADIAVET™ BTV TYPE 8 REAL TIME kit is intended to detect the Bluetongue Virus (BTV) serotype 8 using real-time Polymerase Chain Reaction (PCR) technology from whole blood specimen of bovine and ovine.

2. Pathogen

The bluetongue virus is a non-contagious viral arthropod-borne infectious disease due to an Orbivirus (family Reoviridae, virus ARN), mainly transmitted by hematophageous midges from Culicoides genus. The disease is found in countries where these midges are prevalent and clinical cases have been reported in Africa, the Middle East, the USA, Asia and southern Europe. It induces serious syndromes by ovine (fever, oedema, slimming, mortality 1 to 10%), but it is mainly asymptomatic by caprine, domestic or wild ruminants, which are the virus reserve.

The clinical expression is widely dependent on the environmental parameters (nutritional state, parasitism and bacterial infections concomitant) and on the individual sensitivity. 26 distinct serotypes exist inducing partial or no cross protections between them.

Under the natural conditions, the dissemination is exclusively the fact of infected biting midge or the seed of infected males. The diffusion of the disease thus is largely influenced by the activity of the midge.
Transmission by pregnant ewes has also been described. Transmission by contaminated blood injection is possible when needles and syringes are re-used.

Samples for virus detection are bloods of animals with anticoagulants (EDTA). Virus is detected by isolation on embryonated eggs, *in vitro* cell culture, immunofluorescence on cell culture or by PCR.

3. Description and purpose of the test

This test is based first on the reverse transcription (RT) of RNA into complementary DNA. Then, cDNA is amplified (PCR) by a DNA polymerase using specific primers. Both enzymatic reactions occur in the same tube (One-step RT-PCR).
Amplified products are detected in real-time thanks to a specific labelled hydrolysis probe (5'-exonulease technology).

The ADIAVET™ BTV TYPE 8 REAL TIME kit enables the simultaneous detection of:

- The Bluetongue Virus Type 8 (probe labelled in FAM),
- The GAPDH, an internal control of extraction and amplification steps specific from an endogenous RNA (probe labelled with a fluorochrome with the same spectra as VIC and HEX).

ADIAGENE validated the test using RNA purification kits (Qiagen, Macherey-Nagel). Other purification kits can be used if they have been validated by the user.

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of sample is possible*, up to
Whole blood	☑	5

* It depends on the epidemiological case, on the quality of the specimen and specific directives that exist in some countries (follow them).

This test is specific to the serotype 8.
No cross-reaction has been observed with other types of BTV.
No cross-reaction has been observed with EHDV strains

III. Material and reagents

1. Reagents provided with the kit

Designation	Reagents	ADI381-50
A5	Amplification solution	1 x 1000 µl green tube
BTV T8 CTL+	Positive control Bluetongue Virus Type 8	1 purple tube

2. Validity and storage

On receipt, the kit should be stored at **<-15°C**.
It is recommended to make fractions of A5 solution if it should be defrosted more than 3 times. **Do not defrost reagents more than 3 times.**
Realtime reagents are susceptible to light: store them in the darkness.
The A5 reagent is ready to use for PCR reaction.
Do not mix reagents of two different batches.

3. Use of BTV T8 CTL+

Add 200 µl of Nuclease-free water to the BTV T8 CTL+ tube included in the kit. Homogenize tube contents using a mixer such as vortex, at least 20 seconds. Aliquot this solution by 6 or 12 µl and store them to <-15°C.
For each analysis, we recommend to use 5 µl of BTV T8 CTL+ in a well.

4. Equipment required but not supplied

Material should be Nuclease-free (e.g. autoclaved 25 minutes twice at +120°C or once 60 minutes at +121°C)
- Thermal cycler with consumables for real-time PCR: 0.2 ml PCR tubes or closed 96-wells PCR plates with optical quality
- Class II Microbiological Safety Cabinet
- A centrifuge for microtubes or 96-wells plates
- A heating block or water bath (+56°C or +70°C)
- Instrument for homogenous mixing of tubes
- 96 wells plates agitator (for 96-wells plates RNA extraction)
- 96 wells plates, Elisa-like (for 96-wells plates RNA extraction)
- 1 - 10 µl pipette, 20 - 200 µl pipette and 200 - 1000 µl pipette
- Multichannel pipette 1000 µl (for 96-wells plates RNA extraction)
- Nuclease-free filter tips
- Nuclease-free microtubes: 1.5 ml and 2 ml
- Powder-free latex gloves
- 96-100% ethanol solution
- Nuclease-free water
- PBS 1X buffer pH 7.4
- DMSO (dimethylsulfoxide)
- **Material needed for individual column extraction**
  - QIAamp® Viral RNA kit (Qiagen, 50 extractions: ref. 52904; 250 extractions: ref. 52906)

or
  - Nucleospin® RNA Virus (Macherey-Nagel, 10 extractions: ref. 740956.10; 50 extractions: ref. 740956.50; 250 extractions: ref. 740956.250)
- **Material needed for 96-wells plates extraction**
  - Nucleospin® 96 Virus kit (Macherey-Nagel, 2x96 extractions: ref. 740691.2; 4x96 extractions: 740691.4)
  - MN Square-well Block (Macherey-Nagel, 4 plates: ref. 740476), optionnal

or
  - QIAamp® 96 DNA Blood (4x96 extractions, ref. 51161; 12x96 extractions, ref. 51162); Buffer AVL + carrier (155 ml) (ref. 19073); Qiafilter (24x96 extractions) (ref. 120010); S-Block (ref. 19585)
  - S-Block (Qiagen, 24 plates: ref. 19585), optionnal
- **Automated DNA/RNA extraction kit (magnetic beads)**
  See the NEKF user manual available on the web site mentioned on the certificate of analysis included in the used ADIAVET™ kit.

IV. Recommendation before the analysis of samples

1. Precautions

Adiagène has elaborated this PCR test with the use of Qiagen and Macherey-Nagel extraction kits. Other extraction kits can be used with a previous validation.

Follow the supplier's instructions for the storage, the preparation and the use of the extraction reagents.

Some kits include and/or need the use of toxic reagents. These reagents should be use with gloves and into chemical cabinet.

We strongly recommend that only appropriately trained personnel perform this test. Ensure the accuracy and precision of the micropipettes used. The quality of the obtained results depends upon rigorous respect of good laboratory practices.

The PCR generates large amount of amplified DNA. A few molecules of amplified products are sufficient to generate a positive result. It is important to reserve 2 rooms, one for manipulation of samples to be tested, and another one for amplified products analysis. Do not open the PCR tubes after amplification.
Samples for analysis should be handled and disposed of as biological waste. **Take all measures of security and confinement required for the manipulation of the concerned biological agents.**

We recommend using fractions of demineralised and saline water and to autoclave them twice 25 minutes at +120°C or once 60 minutes at +121°C. Take a new fraction for each new manipulation to avoid contamination.

Before starting the test, read the entire protocol and scrupulously respect it.

2. Storage of samples and DNA extracts

Samples can be stored a couple of days at +2/8°C. After 2 days, we recommend to store them at <-15°C
Samples of blood with anticoagulant reagent must not be frozen.
Extracted RNAs are quite sensitive molecules. Extraction is made at room temperature and should be performed as fast as possible to avoid degradations. We then recommend to read the entire protocol before starting the test and to respect it rigorously. Crude extracts should be stored at the end of extraction on melting ice or at +2/8°C for few hours, then at <-15°C.

3. Samples preparation

Bloods should have been sampled in an anticoagulant tube (EDTA) under a microbiological safety cabinet.
See § IV for the extraction and purification of RNA.

4. Controls to include

The use of controls allows verifying the reliability of the results.
The controls are included per trial of analysis. A trial is defined as all the samples treated in the same conditions.

All the steps of the analysis procedure (extraction+amplification), for all the serotypes of samples, are validated with the association of the controls included in the kit.

- The internal endogenous control (GAPDH) naturally found in the samples allows verifying the extraction and amplification steps of each sample.
- The BTV T8 CTL+ allows validating the amplification of the target.

Other controls must or could be added:

- **Negative control of extraction (required)**

To verify the absence of cross-contamination, at least one negative control must be included per trial (e.g. the normative requirement and recommendation for the development and the validation of veterinary PCR NF U47-600 suggests the use of 1 negative control for 24 samples

or 4 negative samples for a 96 wells-plate). This control could be a negative matrix, or a buffer used for dilutions.

- **Positive control of extraction (recommended)**

A positive control could be added in each trial. The control is a sample including the BTV Type 8. It could come from a positive sample available in the laboratory or from a negative sample spiked with a solution of BTV Type 8. This positive control will be closed to the limit of detection of the method. It will inform about the fidelity of the obtained results between different trials.

V. Extraction and purification

1. Using QIAamp® Viral RNA kit

All the centrifugations are performed at room temperature.

	Bloods on anticoagulant tube (EDTA)
Lysis	Place **100 µl** of blood (individual or pools of 5) in a microtube. For negative extraction controls, place **100 µl** of **buffer PBS 1X** in a microtube.
	Add **560 µl** of **buffer AVL + RNA carrier**. Homogenize ~15 seconds. Check if the mix is homogeneous. Incubate at room temperature during 10 minutes. Briefly centrifuge.
Binding preparation	Add **560 µl** of **ethanol 100%**. Homogenize by pipetting (~10 times) or by using a mixer such as vortex (~15 seconds). Briefly centrifuge.
Transfer to columns and binding to the membrane	Identify columns, apply **630 µl** of the **obtained solution** to the corresponding column. Centrifuge 1 minute at 6 000 g. Increase centrifugation times if the mix is too viscous, hard to pipette and/or likely to clog the column. Change the collection tube, put the rest of the mix on the column and centrifuge 1 minute at 6 000 g.
1st wash	Change the collection tube and add **500 µl** of **buffer AW1**. Centrifuge 1 minute at 6 000 g.
2nd wash	Change the collection tube and add **500 µl** of **buffer AW2**. Centrifuge 3 minutes at 20 000 g.
Column dry step	Change the collection tube. Centrifuge 1 minute at 14 000 g.
Elution	Transfer the column to a microtube. Add **40 µl** of **buffer AVE**. Incubate ~1 minute at room temperature and centrifuge 2 minutes at 6 000 g.
Storage	Close the tubes, identify and store on ice if using immediately or at <-15°C.

2. Using Nucleospin® RNA Virus kit

All the centrifugations are performed at room temperature.
Before the beginning of extraction, pre-warm the RAV1 buffer + RNA carrier at +56°C.

	Bloods on anticoagulant tube (EDTA)
Lysis	Place **100 µl** of **blood** (individual or pools of 5) in a microtube. For negative extraction controls, place **100 µl** of **buffer PBS 1X** in a microtube.
	Add **560 µl** of **buffer RAV1 + RNA carrier** pre-warmed at +56°C. Homogenize ~15 seconds. Check if the mix is homogeneous. Incubate at room temperature during 10 minutes. Briefly centrifuge.
Binding preparation	Add **560 µl** of **ethanol 100%**. Homogenize by pipetting (~10 times) or by using a mixer such as vortex (~15 seconds). Briefly centrifuge.
Transfer to columns and binding to the membrane	Identify columns, apply **630 µl** of the **obtained solution** to the corresponding column Centrifuge 1 minute at 8 000 g. Increase centrifugation times if the mix is too viscous, hard to pipette and/or likely to clog the column. Change the collection tube and put the rest of the mix on the column and centrifuge 1 minute at 8 000 g.
1st wash	Change the collection tube and add **500 µl** of **buffer RAW**. Centrifuge 1 minute at 8 000 g.
2nd wash	Change the collection tube and add **630 µl** of **buffer RAV3**. Centrifuge 1 minute at 8 000 g.
Column dry step	Change the collection tube. Centrifuge 5 minutes at 11 000 g.
Elution	Transfer the column to a microtube. Add **50 µl** of **Nuclease-free water**. Incubate ~1 minutes at room temperature and centrifuge 1 minute at 11 000 g.
Storage	Close the tubes, identify and store on ice if using immediately or at <-15°C.

3. Using QIAamp® 96 DNA Blood kit

Caution: S-Block plates included in the "QIAamp® 96 DNA Blood" kits have several functions. They are used as mix plates or recovery plates. Once they have been used, they can be emptied, decontaminated with HCl 0.4 M during 1 minute, washed with distilled water and autoclaved.

All centrifugations are achieved at 5900 tr/min (5600 to 5800 g) and at room temperature.
Before the beginning of extraction, pre-warm the AVE buffer or Nuclease-free water at +70°C.

	Bloods on anticoagulant tube (EDTA)
Lysis	Place **100 µl** of **blood** in each well of a Round-well Block plate. For extraction negative controls, use **100 µl** of **buffer PBS 1X**.
	Add **400 µl** of **buffer AVL + RNA carrier**. Close the plate with an adhesive seal AirPore tape. Mix ~15 seconds with a plate agitator. Incubate 10 minutes at room temperature.
Binding preparation	Place **400 µl** of **ethanol 100 %** in an S-Block plate. Cover with Qiafilter plate. Carefully remove the adhesive seal of the Round-well Block plate containing the samples and transfer the **whole content** of each well in the Qiafilter plate. Centrifuge 2 minutes.
Transfer to columns and binding to the membrane	Remove the Qiafilter plate. Homogenize the mix 5-times (very important) with a multichannel pipette P1000. Transfer the **whole mix** on the QIAamp® 96 plate after having put it on a new S-Block plate. Place a new adhesive seal AirPore tape on the plate. Centrifuge 2 minutes. If the whole mix has not filtered, centrifuge one more time 3 minutes.
1st wash	Place the QIAamp® 96 plate on a new S-Block plate. Remove the adhesive seal of the QIAamp® 96 plate. Add **500 µl** of **buffer AW1** in each well. Place a new adhesive seal AirPore tape on the plate. Centrifuge 2 minutes.
2nd wash	Remove the adhesive seal of the QIAamp® 96 plate. Add **900 µl** of **buffer AW2**. Place a new adhesive seal AirPore tape on the plate. Centrifuge 5 minutes.
Column dry step	Put the QIAamp® 96 plate on an empty and dry 96-wells plate (ELISA-like). Centrifuge 10 minutes.
Elution	Remove the adhesive seal of the "QIAamp® 96 plate". Put the QIAamp® 96 plate on the Elution microtubes CL plate. Put **100 µl** of **buffer AVE** or **Nuclease-free water** pre-warmed at +70°C in each well of the QIAamp® 96 plate. Centrifuge 2 minutes
Storage	Remove the QIAamp® 96 plate. Close the Elution microtubes CL plate with the Caps for Strips. Store it on melting ice if analysis is immediately achieved, then at <-15°C.

4. Using Nucleospin® 96 Virus

Three MN Square well block plates are included in each kit. They are used as mix plates or recovery plates. Once they have been used, they can be emptied, decontaminated with HCl 0.4 M during 1 minute, washed with distilled water and autoclaved.

All centrifugations are achieved at 5900 tr/min (5600 to 5800g) and at room temperature.
Before the beginning of extraction, pre-warm:

- the RAV1 buffer + RNA carrier at +56°C.
- the Nuclease-free water at +70°C.

	Bloods on anticoagulant tube (EDTA)
Lysis	Place **100 µl** of **blood** in each well of a Round-well Block plate. For extraction negative controls, use **100 µl** of **buffer PBS 1X**.
	Add **400 µl** of **buffer RAV1 + RNA carrier** pre-warmed at +56°C + **20 µl of proteinase K**. Close the plate with an adhesive seal Self-adhering PE Foil. Mix ~15 seconds with a plate agitator. Incubate 10 minutes at +70°C.
Binding preparation	Place **400 µl** of **ethanol 100 %** in an MN Square well Block plate. Carefully remove the adhesive seal of the Round-well Block plate containing the samples and transfer the **whole content** of each well in the MN Square well Block plate containing ethanol. Homogenize the mix 5-times (very important) with a multichannel pipette P1000.
Transfer to columns and binding to the membrane	Place a Nucleospin® Virus Binding plate (blue) on a new MN Square well Block plate. Transfert the **whole mix** with a multi pipette P1000 on the Nucleospin® Virus Binding plate. Place a new adhesive seal Self adhering PE Foil on the plate. Centrifuge 2 minutes. If the whole mix has not filtered, centrifuge one more time 3 minutes.
1st wash	Place the Nucleospin® Virus Binding plate on a new MN Square well Block plate. Remove the adhesive seal from the Nucleospin® Virus Binding plate. Add **500 µl** of **buffer RAW** in each well. Place a new adhesive seal Self adhering PE foil on the plate. Centrifuge 2 minutes.
2nd wash	Remove the adhesive seal of the Nucleospin® Virus Binding Plate. Add **900 µl** of **buffer RAV3** in each well. Place a new adhesive seal Self adhering PE Foil on the plate. Centrifuge 5 minutes.
Column dry step	Place the Nucleospin® Virus Binding Plate on an empty and dry 96 well plate (ELISA-like). Centrifuge 10 minutes.
Elution	Place the Nucleospin® Virus Binding Plate on the Rack plate with MN tube strips. Remove the adhesive seal from the plate. Add **100 µl** of **Nuclease-free water** pre-warmed at +70°C in each well of the Nucleospin® Virus Binding plate. Do not use the **buffer RE.** Centrifuge 2 minutes.
Storage	Remove the Nucleospin® Virus Binding plate. Close the Rack plate with MN tube strips with Caps for strips. Store it on melting ice if analysis is immediately achieved, then at <-15°C.

5. Using DNA/RNA magnetic beads kit

See the NEKF user manual available on the web site mentioned on the certificate of analysis included in the used ADIAVET™ kit.

VI. Amplification

a - Determine the number samples analysed including the controls (e.g. positive and negative extraction controls, positive control of amplification (BTV T8 CTL+) and PCR reagent control (NTC)).

b - **Denaturation of viral RNAs**
For each sample, including extraction negative controls, place 1.6 µl of DMSO in a 0.2 ml-microtube and add 16 µl of ARNs. For the BTV T8 CTL+, add 10% of the aliquot volume (§ II.3.).
Centrifuge the microtubes. Heat the microtubes 3 minutes at +95°C, then immediately place them on melting ice until use.

c- Defrost the A5 solution reagent at room temperature. Homogenize. Dispense **20 µl** of A5 solution in each PCR tubes or PCR plate wells with a micropipette with a Nuclease-free tip.

d- **Immediately replace the A5 solution tube at <-15°C and in darkness.**

e- For each sample, the BTV T8 CTL+, the extraction negative control (required) and the extraction positive control (recommended) add **5 µl** of denatured purified extract to the 20 µl of A5 solution.
For the PCR reagent control (NTC), nothing is added to the A5 solution.
Immediately replace purified RNA extracts at -20°C +/-5°C or at -70°C +/- 10°C. Take care to have no bubbles in the bottom of the wells.

f- Store the plate or the tubes on melting ice or at +2/8°C until the cycler is programmed and start quickly the run after you have placed the plate or the tubes in the cycler.

The BTV Type 8 target is read in FAM. The Internal Control is read in VIC or HEX. The Quencher is non fluorescent. The solution contains a passive reference ROX for the ABI machines. Fluorescence is read during the elongation step (1 minute at 60°C).

The following program is defined for **ABI Prism** thermalcyclers (like 7500, StepOne...) from **Applied Biosystems** (check the "emulation 9600" option if available), for the **Rotorgene** from **Qiagen** and for the **Chromo 4** from **Biorad**:
10 minutes 45°C
10 minutes 95°C
15 seconds at 95°C and 1 minute at 60°C during 40 cycles

This program is concerning the **MX3000P** and **MX3005P** of **Stratagene**:
10 minutes 45°C
10 minutes 95°C
30 seconds at 95°C and 1 minute at 60°C during 40 cycles

Roche diagnostic: LightCycler 2*, LightCycler 480*
**** NOTE:*** *The use of LightCycler thermalcyclers requires a calibration manipulation. Adiagene will furnish process chart and reagents required for this calibration.*

Contact us if you wish to use other thermalcyclers.

VII. Interpretation of results

1. Definitions

The **« base line »** corresponds to the background of fluorescence and qualifies the non-characteristic part of the curve observed during the first cycles.

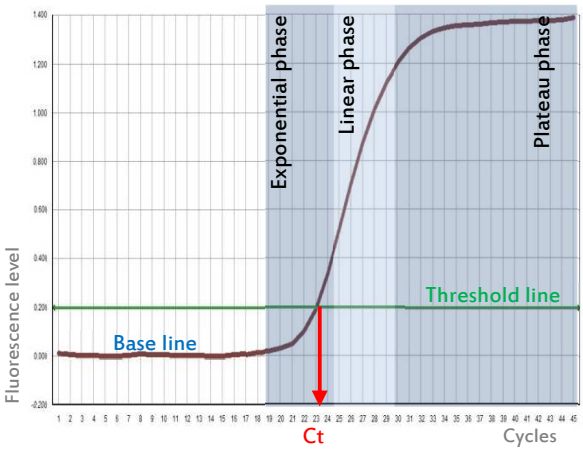
The **« Characteristic amplification curve »** qualifies a fluorescence curve with an exponential phase, a linear phase and a plateau phase.

The **« threshold line »** has to be placed over the background in the exponential phase of a characteristic amplification curve or a group of characteristic amplification curves.

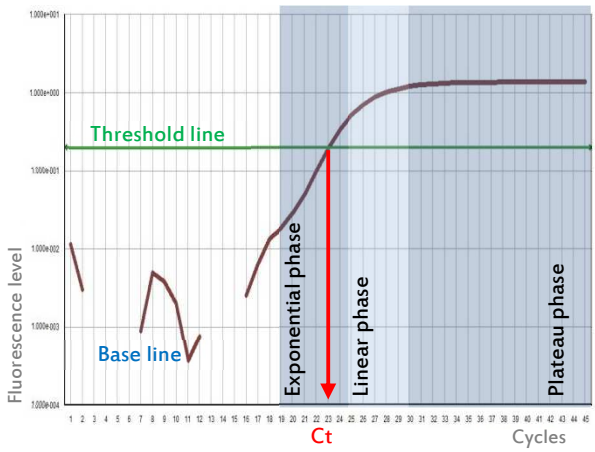
The **« threshold cycle » (Ct)** of a well corresponds, for each detected fluorophore, at the crossing point of the threshold line with the fluorescent curve. The Ct value expressed by the machine for each well depends on the threshold position and on the quantity of target sequences initially present in the PCR well.

Example of characteristic amplification curve

Arithmetic scale of Y axis



Logarithmic scale of Y axis



2. Validation and interpretation of results

Display the FAM curves from the plate and set the threshold value as indicated above.
Proceed in the same mean for the VIC or HEX curves.

A. Test validation (40 cycles)

The test is valid if:

- the NTC and the extraction negative controls have all undetermined values (UNDET) for the BTV T8 target (in FAM) and internal control (in VIC),
- The BTV T8 CTL+ shows Ct values around the values (+/-2 Ct) of the certificate of analysis of the kit.

B. Result interpretation

Sample considered		BTV T8 target (FAM)	Internal control (VIC)
Case 1	BTV type 8 positive	Ct < 34	Ct < 40
Case 2	BTV type 8 negative	Undet.	Ct < 40
Case 3	BTV type 8 weak positive	34 < Ct < 40	Ct < 40
Case 4	Undetermined	Undet.	Undet.

Case 1: The result can be given as "presence of the BTV type 8 genome". Be careful, other genotypes of the BTV virus can be present in the analysed sample.

Case 2: The result can be given as "absence of the BTV type 8 genome".

Case 3: The sample is considered as "a weak positive for BTV type 8". The infection is recent or old.

Case 4: The results can't be interpreted. In the case, we recommend first to repeat the test with RNA pure and diluted 1/5 in sterile Nuclease-free water. Then, if the sample is still not valid, a new extraction of the total RNAs by diluting the blood with a 1/2 rate in (calcium-free and magnesium-free) PBS (50 µl of EDTA blood + 50 µl PBS 1X) is recommended.
If the result is again undetermined, the sample will be considered as non-usable (inhibitors of PCR, lysed sample...). In this case, please contact the reference laboratory or authority of your country and ask for a new sample.

Note:

This RT-PCR test does not exclude the presence of other BTV serotypes than serotype 8 in the analysed sample.

In case of negative result, a RT-PCR for the BTV group can be performed on the RNA extracts to determine the presence of the genome of other BTV serotypes.

In this case, the interpretation of the results is:

Interpretation		Results obtained with **ADIAVET™ BTV REAL TIME kit (ref. ADI352)**	Results obtained with **ADIAVET™ BTV TYPE 8 REAL TIME kit (ref. ADI381)**
		BTV target (FAM)	**BTV T8 target (FAM)**
Case 1	BTV type 8 positive	Ct < 34	Ct < 40
Case 2	BTV Non type 8 positive		Ct > 40
Case 3	BTV type 8 weak positive	34 < Ct < 40	Ct < 40
Case 4	BTV positive, undetermined type		Ct > 40
Case 5	Negative	Ct > 40	Purposeless

VIII. Index of symbols

Symbol	Meaning
REF	Catalogue number
	Manufacturer
	Upper temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
Σ	Contains sufficient for <n> tests
	Keep away from sunlight
VET	For veterinary in vitro use only – For animal use only

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