BVDV control — 10 good reasons for using the *virotype* BVDV RT-PCR Kit

QIAGEN offers complete solutions for molecular veterinary testing and animal pathogen research. We are a dependable partner when it comes to analysis, diagnostics, and development for veterinary medicine. Our products help to reliably identify poultry and livestock diseases.

1. Highly reliable assay for BVDV control

virotype® BVDV RT-PCR Kit is a real-time RT-PCR test kit for rapid and safe detection of the bovine viral diarrhoea virus (BVDV). The kit is approved in Germany by the Friedrich-Loeffler-Institut (FLI) and in Switzerland by the Federal Veterinary Office (BVET).

2. Certified quality — made in Germany

QIAGEN operates to the highest standards of quality meeting GMP and GLP guidelines. Our R&D, production, and sales departments are ISO 9001:2008 certified.

3. Dependable test procedure

QIAGEN's *virotype* BVDV RT-PCR Kit enables the reliable detection of RNA from bovine viral diarrhoea virus in whole blood, plasma, serum, milk, and ear tissue samples from cattle (individual and pooled samples).

The high sensitivity of the *virotype* BVDV RT-PCR Kit permits blood samples (whole blood, plasma, serum) to be tested in pools consisting of up to 50 individual samples, ear tissue samples in pools of up to 25 individual samples, and milk in pools of up to 100 individual samples. Our *virotype* Tissue Lysis Reagent Buffer also allows ear tissue samples to be analyzed easily and inexpensively without the need for time-consuming RNA extraction.

4. High specificity and sensitivity

virotype BVDV RT-PCR Kit has been designed as 'panpesti' real-time RT-PCR. It specifically detects viral RNA of the pestivirus genus. The probe is positioned in a highly conserved region of the 5' untranslated region (5' UTR) of the genome and shows excellent correlation with all known pestivirus species (1). *virotype* BVDV RT-PCR Kit detects genotypes 1 and 2 of the bovine viral diarrhoea virus (BVDV 1 and BVDV 2), as well as the atypical pestiviruses HoBi and Giraffe. There are no known cross-reactions with other non-pestiviruses.

The high analytical sensitivity of *virotype* BVDV RT-PCR Kit has been demonstrated with a titration series of BVDV in vitro RNA (2×10^8 copies/µl) using ten-fold dilutions. The titration series ▶

demonstrates the *virotype* BVDV RT-PCR Kit can detect as low as 1–10 copies per assay. The RNA copy number correlates excellently with the amplification results and the coefficient of correlation is 0.997.

5. Highly reliable detection of BVDV in calves in the diagnostic gap

Only real-time RT-PCR can ensure the reliable identification of BVDV in calves in the diagnostic gap. When using BVDV antigen-ELISA, persistently infected (PI) animals may be incorrectly diagnosed as negative, jeopardizing the success of controls (2). In addition, studies in Germany (3) and New Zealand (4) have revealed that when using the E^{rns} ELISA on calves, incorrect negative findings may be produced following the intake of colostrums (5). Therefore, the antigen ELISA is not permitted in Germany for testing blood samples of calves less than 30 days of age (6).

In June 2012, the BVDV steering committee of New Zealand issued an update on a precautionary calf age limit of 35 days for using BVD antigen ELISA on ear punch samples (7).

When analyzing ear tissue samples, rapid lysis mainly detects PI animals. *virotype* BVDV RT-PCR Kit enables PI animals to be separated from transiently BVDV-infected livestock by using the C_T level for the BVDV fluorescence signal. Expensive follow-ups on positive animals with a C_T <30 are no longer necessary (8).

6. Simple testing with ear tissue samples

- No sample preparation necessary
- Short protocol — add 200 µl ready-to-use *virotype* Tissue Lysis Reagent Buffer and incubate for 45 min
- Ear tissue samples can be left in containers or taken out of the container and placed in a sample test tube
- Sample lysate can be directly added to the PCR master mix

Unlike ELISA, PCR results are available on the very first day. No overnight incubation is required.

7. Certified batch quality and long shelf life

We deliver batches with a minimum shelf life of nine months. Shelf life from production is 24 months.

Every test kit is accompanied by a Certificate of Analysis, which therefore does not need to be separately downloaded from the internet. In addition, a detailed validation report is available that can be used for lab accreditation purposes.

Studies carried out with tissue sampling ear tags, by various ear-tag manufacturers (including Caisley and Allflex), confirmed the reliable detection of BVDV after the ear tissue samples had been stored for three weeks at room temperature and after a week at 37°C in sampling vials.

8. Highly suitable for BVD control

Livestock epidemic control programs in Europe have, for over a decade, predominately employed real-time PCR as the method of choice to test individual and pooled samples for viruses such as bluetongue virus (BTV), BVDV, classical swine fever virus (CSFV), and influenza A. *virotype* BVDV RT-PCR Kit is highly suitable for BVD control and following its launch in 2007 has replaced the

BVD^{Erns} ELISA in many laboratories. In Switzerland alone, more than 75% of all ear tissue samples are tested for BVD using real-time PCR.

Established

Real-time RT-PCR is an established technique and the equipment required is already available in many laboratories worldwide. In addition, QIAGEN is a leading global provider of sample and assay technologies with many years of experience.

Flexible

BVDV RNA can be detected in serum, plasma, milk, full blood, and ear tissue samples. The samples can be analyzed manually, semi-automatically, or fully automatically without laborious time-consuming sample preparation. QIAGEN offers high-quality manual and automated sample preparation and assay technologies for low- and high-throughput workflows.

Time saving

virotype BVDV RT-PCR Kit can be performed with other *virotype* real-time RT-PCR test kits [Schmallenberg virus (SBV), CSFV, porcine reproductive and respiratory virus (PRRSV), influenza A, BTV] in one cycler run.

Ultra-efficient

virotype BVDV RT-PCR Kit is very economical (see Table 1).

Table 1. Sample throughputs in BVDV detection with *virotype* BVDV RT-PCR Kit and one laboratory assistant

Manual	Semi-automated	Fully automated
Approximately 1000 samples/day	Approximately 2000 samples/day	More than 2000 samples/day

Minimized risk of contamination

The contamination risk is minimized by the use of the *virotype* BVDV RT-PCR Kit one-step single tube RT-PCR.

Inexpensive

Pools of up to 25 ear tissue samples, 50 blood samples, and 100 milk samples can be tested by using *virotype* BVDV RT-PCR Kit. These pooling options allow unbeatable economic testing compared to individual tests performed using ELISA.

In Germany, the prevalence of PI cattle after the first year of the eradication program was 0.38%. A similar prevalence can be expected for other countries.

Available

The BVDV^{Erns} antigen ELISA is patented. This means there is only one supplier. Laboratories opting for this testing system make themselves dependent in terms of cost and availability on a single provider. Many veterinary laboratories experienced in connection with the IBRgE ELISA and PRRS ELISA have experienced how disadvantageous this situation can be. In contrast, a number of approved test systems exist for BVDV assays using RT-PCR.

Method of the future

Real-time RT-PCR used to study pool and individual samples is the method of the future and already common for testing CSFV, influenza A, SBV, and BTV.

Environmental protection

BVDV antigen ELISA entails the expensive storage and disposal of large quantities of sample diluent, washing solution, and conjugate, as well as substrate and stop solution. In contrast, *virotype* BVDV RT-PCR Kit is far simpler to use and causes much less waste (see Table 2).

Table 2. A comparative analysis when testing 4500 ear tissue samples

BVDV antigen ELISA	*virotype* BVDV RT-PCR Kit
9 test kits at 1.5 kg	1 test kit (480 reactions) at 85 g
4500 ml water to dilute the washing solution	900 ml *virotype* Tissue Lysis Reagent

Availability and logistics

QIAGEN guarantees the reliable provision and fast delivery through its worldwide sales channels and distribution network.

9. More than just a test kit

We provide complete solutions for reliable BVDV diagnosis.

Our BVDV Pool-Manager software features include the simple automatic control of laboratory equipment, the easy production and dissolution of pools, and the tracking of sample IDs. The software can also be used for other pooled PCR protocols. Furthermore, an English-speaking expert technical service team is available to answer any queries you have about the software and kits.

10. We are here to help you

QIAGEN is your highly reliable partner when it comes to analysis, diagnostics, and development for veterinary medicine. We provide a full range of services for each *virotype* BVDV RT-PCR Kit and we are easy to reach for individual expert advice.

Just call us or send us an email.

Labs with experience of the *virotype* BVDV RT-PCR Kit

virotype BVDV real-time RT-PCR Kit is increasingly replacing other methods of testing for BVDV. Laboratories in Germany and abroad rely on the high quality of the *virotype* BVDV RT-PCR Kit, including:

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References

1. Hoffman, B., Depner, K., Schirrmeier, H., and Beer, M. (2006) A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. Journal of Virological Methods, Volume **136**, Issues 1–2, pp. 200–209.

2. Fux, R., Pfahler, C., Hellwig, M., Birlbauer, R., and Wolf, G. (2008) Ohrgewebediagnostik von BVDV-PI-Kälbern: Ist eine Sensitivität von 100% möglich? Arbeitskreis für veterinärmedizinische Infektionsdiagnostik (AVID), **27**. Arbeits- und Fortbildungstagung Virologie, Bad Staffelstein/Kloster Banz, 17.–19. 09.

3. Christian, J., Kupča, A., Drdlicek, J., and Bogner, K. H. (2010) Fallbericht eines mittels ERNS-ELISA in der Ohrstanze negativ untersuchten BVD-Virämikers, Arbeitskreis für veterinärmedizinische Infektionsdiagnostik (AVID), **29**. Arbeits- und Fortbildungstagung Virologie, Bad Staffelstein/Kloster Banz, 15.–17. 09.

4. Witzel Ini-Isabee., and Hinrich, V. (2012) BVD Antigen Detection - Exploring the Diagnostic Gap. American Association of Veterinary Laboratory diagnosticians, 55th Annual Meeting, Greensboro, NC, 144.

5. Fux, R., and Wolf, G. (2012) Transient elimination of circulating bovine viral diarrhoea virus by colostral antibodies in persistently infected calves: a pitfall for BVDV-eradication programs? Veterinary Microbiology **161** 13–19.

6. Proceedings Bovine Virusdiarrhoe in „Amtliche Methodensammlung" Friedrich-Loeffler-Institut 2011, p.121-122, www.fli.bund.de/fileadmin/dam_uploads/Publikationen/Amtliche_Methodensammlung/Methodensammlung_201112.pdf.

7. BVD Steering Committee (2012) Precautionary calf age limit for BVD Antigen ELISA on ear punch samples, www.controlbvd.org.nz/sites/default/files/domain-23/Calf%20testing%20update_June2012.pdf.

8. Schroeder, C., Gaunitz, C., Dierchen, B., Labitzke, M., and Gabert, J. (2009) Testing Mini-Ear Samples for BVDV – Experiences from Germany, American Association of Veterinary Laboratory Diagnosticians, San Diego.

Ordering Information

Product	Contents	Cat. no.
virotype BVDV RT-PCR Kit (24)	For 24 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280373
virotype BVDV RT-PCR Kit (96)	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280375
virotype Tissue Lysis Reagent (25 ml)	Reagent for RNA isolation for downstream detection of BVDV RNA (25 ml)	289991
virotype Tissue Lysis Reagent (100 ml)	Reagent for RNA isolation for downstream detection of BVDV RNA (100 ml)	289992
virotype Tissue Lysis Reagent (250 ml)	Reagent for RNA isolation for downstream detection of BVDV RNA (250 ml)	289993

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

virotype BVDV RT-PCR Kit is for veterinary use only.

In Germany: Registered in accordance with §17c of the German Law on Animal Diseases. Registration No.: FLI-B 451

In Switzerland: Approved by the Federal Veterinary Office (BVET)

Learn more about BVDV testing at www.qiagen.com/virotype-BVDV-RT-PCR-Kit.

Sample & Assay Technologies