NEW QPCR AND DIRECT LYSIS PROCESS FOR A FAST, RELIABLE & ACURATE DETECTION OF BVDV IN EAR NOTCHES SAMPLES

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INTRODUCTION

Bovine viral diarrhea (BVD) is one of the most important infectious cattle diseases worldwide.
In Germany, BVD is a notifiable disease, and a nationwide mandatory eradication program has been in place since 2011. Ear notches are prime samples because their collection is easier thanks to identification of animals with ear tags and simultaneous sampling just after birth. The objective is to remove permanently infected animals (PI animals) from the herd at an early stage.
Optimized lab workflows are needed, as laboratories handle high number of samples. Test performance, robustness and accuracy is crucial to avoid missing PI animals.
In this context, IDvet developed a new real-time RT-PCR kit, the ID Gene ™ BVD / BD Triplex 2.0 kit and a reliable direct lysis protocol which significantly simplifies the workflow in the laboratory while increasing the result accuracy (ID GENE™ Easy Preparation of ear notch samples 2.0).
The presentation shows validation data for the ID Gene ™ BVD / BD Triplex 2.0 and its companion kit for rapid direct lysis for ear notches.

CONCLUSION

The **ID Gene™ BVDV/BDV Triplex 2.0** kit offers:

- → **Excellent performance**: high analytical sensitivity ; LDPCR = 5 copies / PCR and LDMethod ≤ 25 TCID50/ ear notch when processed with the rapid direct lysis kit ID Gene™ Easy Preparation of Ear Notch Samples 2.0 (EZNOTCHV2).
- → Test **pools of up to 25 samples**, thanks to high kit sensitivity
- → **Optimal test reliability** thanks to endogenous and new best-in-class RNA-based exogenous internal controls which provide reliable results and efficacy, especially when associated with the direct lysis kit ID GENE™ Easy Preparation of ear notch samples 2.0
- → **Compatible** with the direct lysis kit available in liquid format with economical **room temperature shipping** worldwide
- → An included **positive amplification control (PAC).** Sold separately : a positive extraction control calibrated to validate the entire PCR process

ANALYSIS PROTOCOL

The **ID Gene™ BVD/BD Triplex 2.0 (IDBVDV2)** kit is a ready-to-use RT-qPCR kit assay detecting simultaneously BVD/BD virus, an endogenous and an exogenous internal positive controls.

EXTRACTION	
Sample preparation	Exogenous control and the Direct Lysis buffer were added to all individual ear notches samples
Incubation	15 min at 100°C / Cooling step
AMPLIFICATION	
Reaction condition	8µL of ready to use Mastermix was added to 5µL of each lysate from individual or up to 25 pooled samples
Amplification condition	Amplification were carried out using a rapid amplification program (65 min)

SAMPLE RESULT	BVDV/BDV SIGNAL	ENDOGENOUS CONTROL SIGNAL	EXOGENOUS CONTROL SIGNAL	INTERPRETATION
Positive	+	+/-	+/-	Valid; Animal detected as positive for BVDV/BDV No signal for endogenous and/or exogenous control because of competition
Negative	-	+	+	Valid; Animal detected as negative for BVDV/BDV
Degraded sample	-	-	+	Not Valid; Analytical process successful, no signal for endogenous control due to poor sample quality – other sample analysis is required
Partial PCR inhibition	-	+	-	Not Valid; no signal for exogenous control or Cq exogenous on sample > Cq exogenous on negative extraction control + 3 Dilute the extracted DNA 10 times in nuclease-free water

RESULTS

INCLUSIVITY

Analysis of 55 reference BVDV and BDV strains provided by the French Laboratory (LABOCEA Fougères) and by Friedrich Loeffer Institute (FLI, Gemany)

BVDV/BD ISOLATE	IDBVDV2 RESULTS
FLI (Germany)	DETECTED
LNR-BVD (France)	DETECTED
C strain FLI	DETECTED
Eystrup91 FLI	DETECTED
Alfort187 FLI	DETECTED
Koslov1128 FLI	DETECTED
Brescia FLI	DETECTED
Schweiz II FLI	DETECTED
Pader FLI	DETECTED
Bergen FLI	DETECTED
D4886/82/RO FLI	DETECTED
Uelzen FLI	DETECTED
Spante FLI	DETECTED
CongenitalTremor FLI	DETECTED
Kanagawa FLI	DETECTED
Moredun FLI	DETECTED
Rudolph FLI	DETECTED
Gifhorn FLI	DETECTED
Isard FLI	DETECTED
NADL FLI	DETECTED
Paplitz FLI	DETECTED
PI809 FLI	DETECTED
NC3807-1251/1 FLI	DETECTED
Egbert FLI	DETECTED
BO806-17 FLI	DETECTED
NC3807-8757 FLI	DETECTED
8644 FLI	DETECTED
Bure FLI	DETECTED
Walter FLI	DETECTED
PO1600 FLI	DETECTED
Hobi FLI	DETECTED
Giraffe FLI	DETECTED
NCP-2508-FCS FLI	DETECTED
Bohni FLI	DETECTED
018901 = BVD1 e/cythopathogene	DETECTED
08207-3 = BVD1 -b	DETECTED
163-5 = BVD1 -b	DETECTED
08167-1 = BVD1 -b	DETECTED
172-1 = BVD1 -e	DETECTED
176-2 = BVD1 -b	DETECTED

BVDV/BD ISOLATE	IDBVDV2 RESULTS
197-1 = BVD1 -b	DETECTED
207-1 = BVD1 -e	DETECTED
9001 = BVD1 -e	DETECTED
09005-4 = BVD1 -b	DETECTED
09002-1 = BVD1 -e	DETECTED
08185-1 = BVD1 -d	DETECTED
08158-5 = BVD1-a	DETECTED
08185-2 = BVD1 -l	DETECTED
13035-4 = BVD 2	DETECTED
4544-01 V12	DETECTED
14011 = BVD cythopathogene	DETECTED
07033-1 = BVD atypique PCR neg	DETECTED
VDVB 14 - 12	DETECTED
VDVB 10 - 5	DETECTED
VDVB 02/02/2007	DETECTED

The ID GeneTM BVD/BD Triplex 2.0 detects all the reference strains tested, which include both BVDV (Genotype 1 and 2) and BDV viruses, as well as circulating viral isolates

EXCLUSIVITY

The following 32 selected isolates were tested:

SAMPLE NUMBER	STRAINS	IDBVDV2 RESULTS
1-2	Influenza (H1N1, H5N2)	Not detected
3-4	Bovine Herpesvirus (1,4)	Not detected
5-8	Bovine Pneumovirus (3,A,B)	Not detected
9-10	PRRSV (EU and NA types)	Not detected
11-32	22 other viruses and bacteria from different species	Not detected

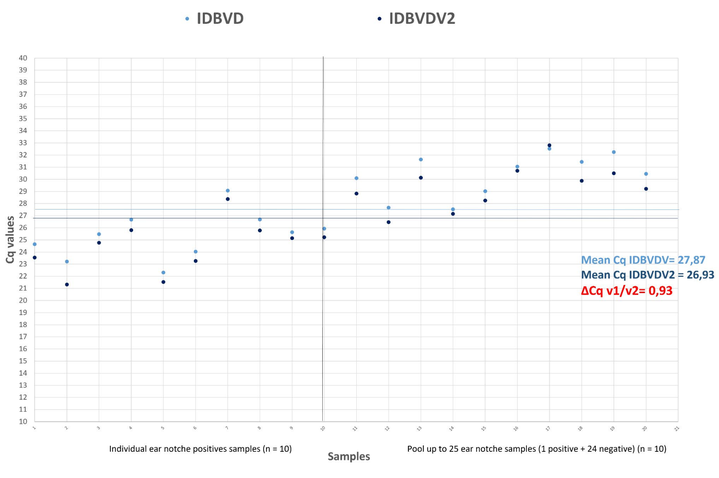
The ID Gene™ BVD/BD Triplex 2.0 kit did not show any cross-reactions with the 32 other pathogens tested

OVERALL PERFORMANCE

PCR CHARACTERISTICS	
Efficiency / R^2	100.6 % / 0,98
Limit of detection	5 copies/PCR with rapid amplification program
Robustness	Unaffected by all parameters tested (temperature and volume of sample)
METHOD CHARACTERISTICS (ID GENE™ EASY PREPARATION OF EAR NOTCH SAMPLES 2.0 : DIRECT LYSIS EXTRACTION)	
Experimental limit of detection on ear notch sample spiked with BVDV strain (C24V Oregon)	25 $TCID_{50}$/ear notch with rapid amplification program

COMPARISON WITH 2 PCR KITS

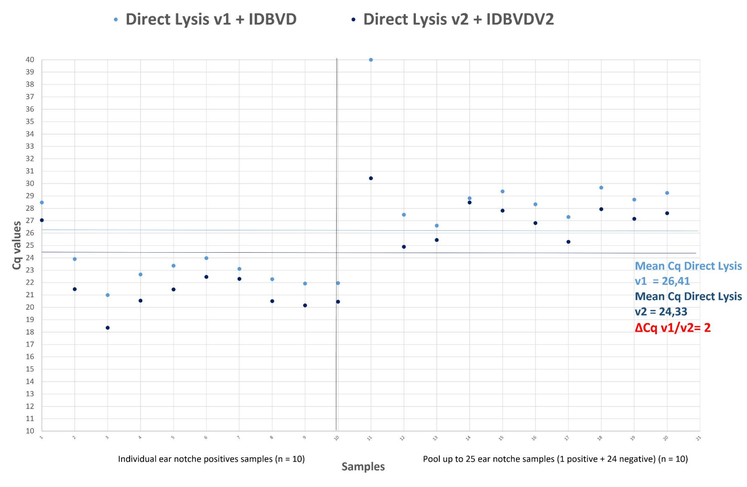
The performances of the new PCR kit were compared with the first version of ID GENE™ PCR kit (IDBVD) on 10 individual characterized field samples and 10 pooled to 25 samples. All individual or pooled samples were extracted with automated extraction method (with ID GENE™ MAG FAST Extraction Kit)



→ **New PCR kit (IDBVD2) gave lower Cq values than the former version of PCR kit (IDBVD; mean difference ΔCq = 1)**

COMPARISON OF BOTH DIRECT LYSIS PROTOCOLS

The performance of the two direct lysis protocols were compared on 10 individual characterized field samples and 10 pools of 25 samples. The lysis protocol v1 (Direct Lysis v1) has a preliminary incubation step (70° C, 30min) compared to the new version of the lysis protocol (Direct Lysis v2). Direct Lysis v1 was combined with first version of IDBVD kit whereas the new Lysis protocol v2 was evaluated with IDBVDV2.



- → **The Direct Lysis v1 and Direct Lysis v2 show excellent agreement**
- → **The Direct Lysis v2 gave lower Cq values than the Direct Lysis v1 (mean difference ΔCq = 2)**
- → **One inhibiting positive pooled sample (Cq > 40) was not detected with Direct Lysis v1 but found positive with Direct Lysis v2 which ascertain its better ability to manage the inhibitors present in the lysates.**