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Main topic : Animal Health

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, implying the need of efficient testing solutions. Ear notches are prime samples because their collection is easier thank to identification campaigns with ear tags. The objective is to remove permanently infected (PI) animals from the herd at an early stage.
To achieve that, eradication protocols based on both viral detection and serological testing are necessary. Real-time RT-PCR is widely used to detect the virus. Sample pooling allows to achieve the best eradication efficiency/cost ratio.
Optimized lab workflows are needed, as laboratories handle high number of samples. Test performance, robustness and accuracy are crucial to avoid missing PI animals.
Consequently, IDvet developed a new RT-qPCR kit, the ID Gene ™ BVD/BD Triplex 2.0 kit and a reliable direct lysis protocol which significantly simplifies the workflow in laboratories while increasing the results 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 ear notches rapid direct lysis.

MATERIALS & METHODS:
All reagents, the ID Gene™ BVD/BD Triplex 2.0 (product code: IDBVDV2) and the ID GENE™ Easy Preparation of ear notch samples 2.0 (product code: EZNOTCHV2), were used according to the manufacturer's instructions.
Briefly, the exogenous control and the Direct Lysis buffer were added to all ear notches samples, which were incubated for 15 min @100°C. After a cooling step, processed samples were either tested undiluted, either in pools of up to 25.
For comparison, samples were also processed with a classical nucleic acid purification using the ID GENETM Mag Fast Extraction Kit Fast (product code: MAGFAST).

RESULTS:
Regardless of the method of sample treatment, measured sensitivity and specificity on ear notches were 100%, for both individual and pool up to 25.
The new ID GENE™ Easy Preparation of ear notch samples 2.0 process offers a high robustness, and ensures a good stability of processed ear notches before qPCR analysis. The new inhibitor tolerant qPCR mix limits drastically the number of samples inducing inhibition.
Our new best-in-class RNA-based exogenous Non-Target Positive Control (NTPC) was shown to better detect possible RNA degradation occurring in some samples, perfectly mimicking BVDV signal inhibition pattern.
Compared to previous versions of the kits and to competitors' direct lysis processes, the combination of our 2.0 kits offers a better performance, giving earlier Cq values on BVDV target (mean ?Cq= - 2,9).

CONCLUSION:
This new qPCR is a highly performant solution for accurate and sure BVDV detection either in PI or transiently infected animals. This triplex qPCR includes both endogenous and a new state-of-the-art exogenous control, which provide reliable results and efficacy, especially when associated with the direct lysis kit ID GENE™ Easy Preparation of ear notch samples 2.0.
Furthermore, those two products offer extended pooling options, up to 25 ear notches and 100 for serum or plasma. They are validated by the French National Reference Laboratory (ANSES), accordingly to the local specifications for registration.