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Enable the Right Result the First Time with Xeno Internal Positive Control

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ABSTRACT

Misdiagnosis of one sick animal can endanger the health of an entire herd, affecting the producer's livelihood, and potentially damage a lab's reputation. When using real-time PCR (qPCR) for molecular diagnostic testing, false negatives rarely result from a lack of sensitivity. Rather, the biological material used to test these animals can contain a variety of compounds that inhibit the actual PCR chemistry. A simple way to test for PCR inhibition is to include an internal positive control. Our newly released VetMAX™ Xeno™ Internal Positive Control (IPC) products, for both DNA and RNA testing, can be easily integrated into any qPCR workflow, greatly reducing the likelihood of costly false negatives and thus providing confidence in the accuracy of our customer's qPCR test results.

Although Xeno has been incorporated into many of our existing Animal Health diagnostic products, these new products, which include Xeno IPC templates and Xeno IPC assays, are now conveniently available as standalone products that are directly orderable from our catalog. The Xeno IPC assay is a highly specific primer/probe mix that detects the Xeno IPC and is available in VIC and LIZ dye channel formats for flexibility of multiplexing with a variety of diagnostic assays. The Xeno IPC template, available as either DNA or RNA, was designed as a unique synthetic sequence and was evaluated against a comprehensive nucleotide database to show no off-target alignments to sequences relevant to animal health. The Xeno IPC template serves as both an internal positive control for the recovery of nucleic acid during the isolation process, and as a positive control for qPCR. It is a very effective control for qPCR inhibition, which enables the customer to be easily alerted to problematic test results, reducing the likelihood of false-negatives. Xeno IPC performance was tested and shown to be compatible with a wide variety of veterinary sample matrices and qPCR master mixes. Nucleic acid isolation was performed using MagMAX™ Total Nucleic Acid Isolation Kit and MagMAX™ Pathogen RNA/DNA Kit with an automated Applied Biosystems™ MagMAX™ Express-96 instrument. Real-time PCR was performed on a Applied Biosystems™ 7500 Fast real-time PCR system and on an Applied Biosystems™ QuantStudio™ 5 real-time PCR system.

Recommended by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and used in our USDA-licensed kits, Xeno IPC is a trusted product intended for use in a growing Animal Health diagnostic market seeking improved quality control.

INTRODUCTION

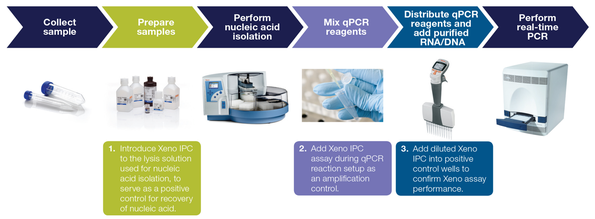
Animal sample matrices used for diagnostic qPCR testing may contain a variety of PCR inhibitors that can potentially lead to false-negative results. In an effort to provide a solution to help diagnose farm animal health issues more accurately, we've developed an internal positive control that can be easily integrated into existing TaqMan™-based workflows to test for the presence of PCR inhibitors, thereby lowering the risk of false-negative results.

MATERIALS AND METHODS

Xeno IPC assay and control sequences were designed to have no homology with known microbial sequences and were evaluated against a comprehensive microbial database of 24,262,358 sequences for off-target alignments. All experiments were performed using VetMAX Xeno Internal Positive Control – VIC™ Assay and VetMAX Xeno IPC RNA or DNA. Nucleic acid isolation was performed using MagMAX Total Nucleic Acid Isolation Kit for fecal samples and MagMAX Pathogen RNA/DNA Kit for oral fluid samples with an automated MagMAX Express 96 instrument. During isolation, 20,000 copies of Xeno IPC DNA were spiked directly into the lysis solution. VetMAX™ *M. hyopneumoniae* Reagents, VetMAX™ MAP (Johne's) Reagents and VetMAX™ Bluetongue Virus (BTV) Reagents were used to amplify *Mycoplasma hyopneumoniae* (M. hyo) DNA, *Myobacterium avium* subspecies *paratuberculosis* (MAP) DNA and BTV RNA, respectively. BTV and *M. hyo* reagents were used as complex RNA and DNA pathogen assays that were multiplexed with the Xeno IPC assay for experiments represented in figures 4 and 5. Real-time PCR was performed on a 7500 Fast real-time PCR system or on a QuantStudio 5 real-time PCR system with a VeriFlex™ heat block for experiments that required a temperature gradient. Real-time PCR reactions were run according to the manufacturer's recommended thermal cycling protocol for each master mix tested.

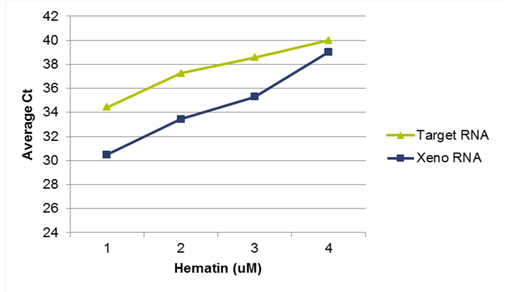
RESULTS

Figure 1. Xeno IPC Real-Time PCR Workflow



- Xeno IPC DNA or RNA is spiked into the lysis solution during nucleic acid isolation to control for recovery of nucleic acid and to monitor for PCR inhibition.
- Xeno IPC assay is a primer/probe mix that is multiplexed with the primary target assay at a limiting concentration, to prevent negatively impacting amplification of the primary target.
- Amplification of Xeno IPC should occur with equal efficiency in all samples and controls. If the Ct value for Xeno IPC in the test sample is delayed by more than 1.5 Ct relative to that of a negative extraction control (NEC), this indicates the presence of a PCR inhibitor in the test sample.
- Strong signal for the target may affect the Xeno IPC negatively, but this does not affect the result.

Figure 2. Monitors for PCR Inhibition



Graph shows the effect of increasing inhibition on amplification of a Bovine Respiratory Syncytial Virus (BRSV) target and subsequent effect on Xeno IPC. 100 copies per reaction of BRSV RNA and 1,000 copies per reaction of Xeno IPC were exposed to increasing levels of hematin (0–4 µM), a known PCR inhibitor found in blood. The data show that Xeno IPC follows the target's trend of increasing Ct values due to inhibition and therefore can be used as an indicator of inhibition in the reaction.

Figure 3. Compatibility with Complex Sample Types

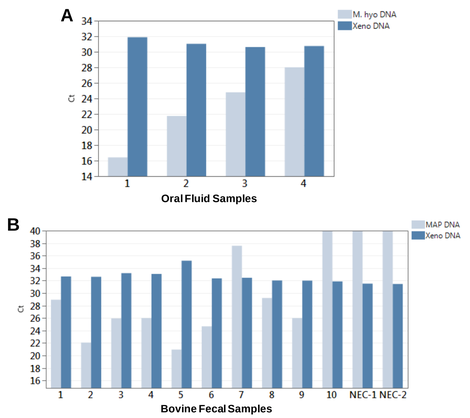
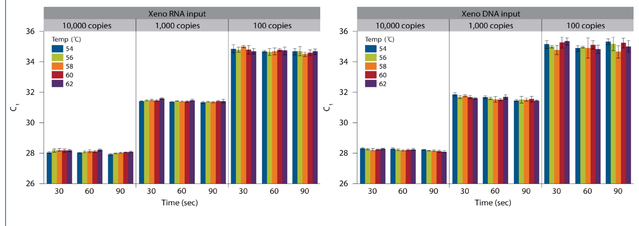


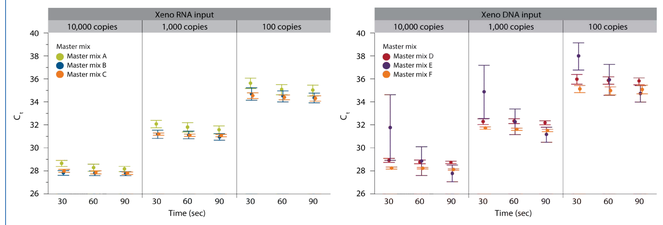
Figure 3 shows that Xeno IPC is compatible with complex veterinary sample types such as oral fluids (A) and feces (B), that may contain high levels of PCR inhibitors. By design, the Xeno IPC assay components are at limiting concentrations, therefore high levels of target DNA in the sample can reduce amplification of Xeno IPC as shown in graph B, sample 5. Sample 10 is MAP-negative with no indication of PCR inhibition based on Ct value for Xeno IPC in sample compared to NEC.

Figure 4. Amplification of Xeno IPC RNA and DNA using Variable Thermal Profiles



Varying the thermal cycling conditions such as annealing temperature (54-62ºC) and time (30-90 sec), had very little impact on Xeno IPC template amplification in the presence of a complex pathogen target assay. Path-ID™ Multiplex One-Step RT-PCR and Path-ID qPCR master mixes were used to amplify Xeno IPC RNA and DNA, respectively.

Figure 5. Performance of Xeno IPC with Various Master Mixes



To demonstrate the flexibility and compatibility of the Xeno IPC assay, a series of Thermo Fisher Scientific and competitor-branded RT-PCR and qPCR master mixes were tested with Xeno IPC across a range of commonly used thermal cycling conditions. These graphs demonstrate reproducible amplification of Xeno IPC for the majority of master mixes shown, irrespective of template concentration, annealing temperature and times tested. For master mix E, Xeno IPC DNA amplification was negatively impacted when conditions were more stringent than recommended. Each data point represents a mean Ct value of a range of temperatures tested from 54-62ºC.

CONCLUSIONS

- Xeno IPC assay demonstrates effective functionality, flexibility and compatibility with a variety of real-time PCR reagents under various thermal cycling conditions, including variable annealing temperature (54-62ºC) and time (30-90 sec).
- Xeno IPC is easily integrated into any qPCR workflow to provide confidence that qPCR results are accurate and actionable, by helping to reduce the likelihood of false-negatives.
- Xeno IPC is recommended for use by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and used in the USDA-licensed kits available through Thermo Fisher Scientific.

ACKNOWLEDGEMENTS

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