Performance of Xeno Internal Positive Controls with variable thermal cycling conditions and enzymes

Introduction

The Applied Biosystems™ VetMAX™ Xeno™ Internal Positive Control (IPC) assay is used to monitor the post-lysis sample preparation process and provide a control for the recovery of nucleic acid throughout the sample preparation protocol. This is achieved by spiking in a known copy number of the IPC template (RNA or DNA) into the sample lysis reaction and comparing the recovery of nucleic acids from post-lysis, downstream PCR products to an extraction control.

The Xeno assay has been developed to detect the Xeno IPC template (RNA or DNA) in a background of host- and pathogen-specific nucleic acids. The assay consists of primers and probes at limiting concentrations, which can be multiplexed with a primary target assay. Since the IPC is only meant to serve as a control, it is important to make sure that the Xeno assay is specific for only the Xeno IPC template (RNA or DNA) and does not interfere with the detection of the primary target nucleic acid. The IPC template is a synthetically designed sequence, which is regularly checked against the most commonly used nucleotide sequencing databases to help ensure specificity.

Materials and methods

The quantitative PCR (qPCR) experiments were performed to provide data showing whether Xeno IPC is capable of amplifying effectively when multiplexed with complex assays using a variety of master mixes, as well as a range of annealing temperatures and times. These conditions were meant to replicate a variety of common scenarios at animal health molecular testing labs. The following variables were analyzed:

- Both qPCR and quantitative reverse transcription PCR (qRT-PCR) master mixes using the default thermal cycling parameters for each master mix; six different master mixes tested in total
- A range of annealing temperatures in 2-degree increments from 54 to 62°C
- Three commonly used annealing times—30, 60, and 90 sec

- Applied Biosystems™ VetMax™ Xeno™ IPC–VIC™ Assay with complex DNA and RNA assays:
  - Bluetongue virus RNA assay, consisting of 4 primers and 3 probes, was tested for pathogen detection
  - *Mycoplasma hyopneumoniae* DNA assay, consisting of 8 primers and 2 probes, was tested for pathogen detection
- Assays were evaluated at 3 different concentrations of Xeno IPC DNA or RNA (10,000, 1,000, and 100 copies)
- All testing was performed in triplicate
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System with VeriFlex™ heat block was used for all qPCR experiments

Results

In the figure below (Figure 1), amplification of Xeno IPC is compared across a range of template concentrations, annealing times, and annealing temperatures. Figure 1A shows Xeno IPC RNA amplification using Applied Biosystems™ Path-ID™ Multiplex One Step RT-PCR Master Mix and Figure 1B shows Xeno IPC DNA amplification with Applied Biosystems™ Path-ID™ qPCR Master Mix. The results show that the C_t values obtained for a given copy number of the Xeno IPC RNA and DNA templates were very similar at different annealing times. In addition, it was shown that varying the annealing temperature had only a minor effect on Xeno IPC template amplification. These results highlight that for the given master mix, robust amplification across all tested conditions was obtained.

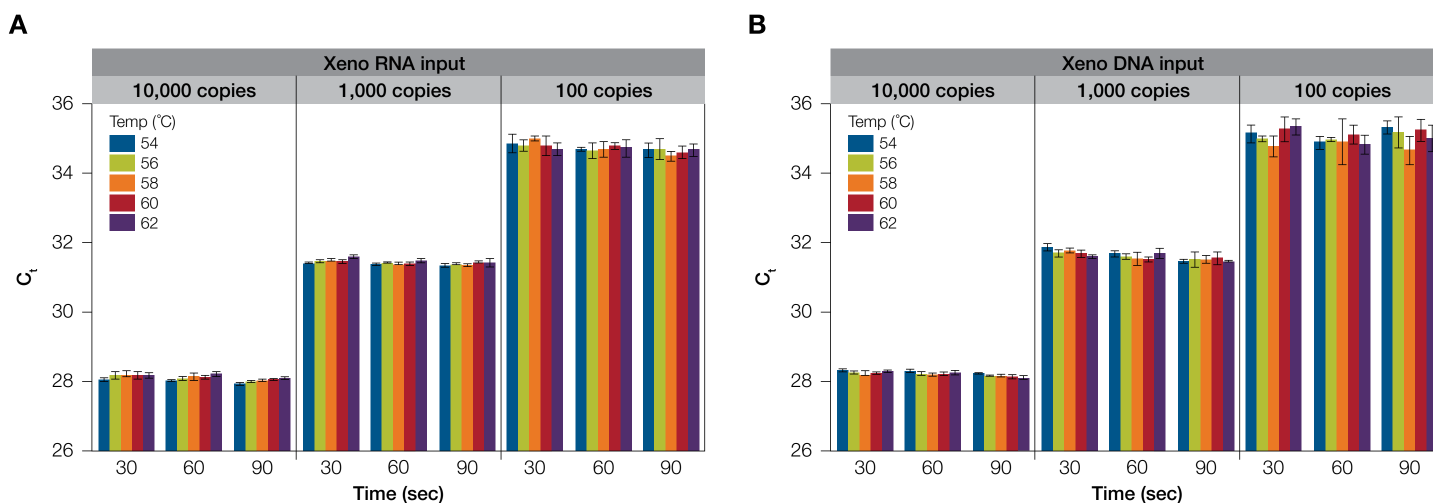

Figure 1. Amplification of (A) Xeno IPC RNA and (B) Xeno IPC DNA at 3 different concentrations using Path-ID Multiplex One Step RT-PCR master mix and Path-ID qPCR Master Mix, respectively. The y-axis represents the C_t values, and the x-axis represents the copy numbers (10,000, 1,000, or 100 copies) and annealing times (30, 60, 90 sec) tested. The colored bars represent the different annealing temperatures (54–62°C) used in these experiments.

Figure 2 compares the amplification of Xeno IPC RNA and DNA templates at 3 different concentrations using 3 different master mixes. As expected, the recorded C_t values increased with decreasing Xeno IPC template concentration. For the majority of master mixes tested, this experiment shows that the Xeno IPC RNA and DNA templates can be reproducibly amplified irrespective of the template concentration and annealing temperature tested in this study. For one tested master mix (E, Figure 2), Xeno IPC DNA amplification was negatively impacted when conditions were more stringent than recommended, i.e., 62˚C. This resulted in higher mean C_t values with large standard deviations, compared to all other master mixes tested.

Conclusions

The Xeno IPC assay demonstrates effective functionality in a variety of thermal cycling conditions, including multiple annealing temperatures and times, as well as high and low IPC template concentrations. In addition, multiple qRT-PCR and qPCR master mixes can be successfully used with reproducible amplification of the Xeno IPC template. The performance of the Xeno IPC assay, using a wide range of conditions and enzymes, exemplifies its flexibility and compatibility, suggesting that these products can be seamlessly incorporated into different qPCR workflows. As is the case with all new reagents, the Xeno IPC assay should be evaluated with your existing qPCR workflow in order to help ensure optimal performance. For more information regarding testing of this product in your specific workflow, please see the Applied Biosystems™ white paper entitled 'Incorporating VetMAX Xeno IPC and assay into existing qPCR workflows'.

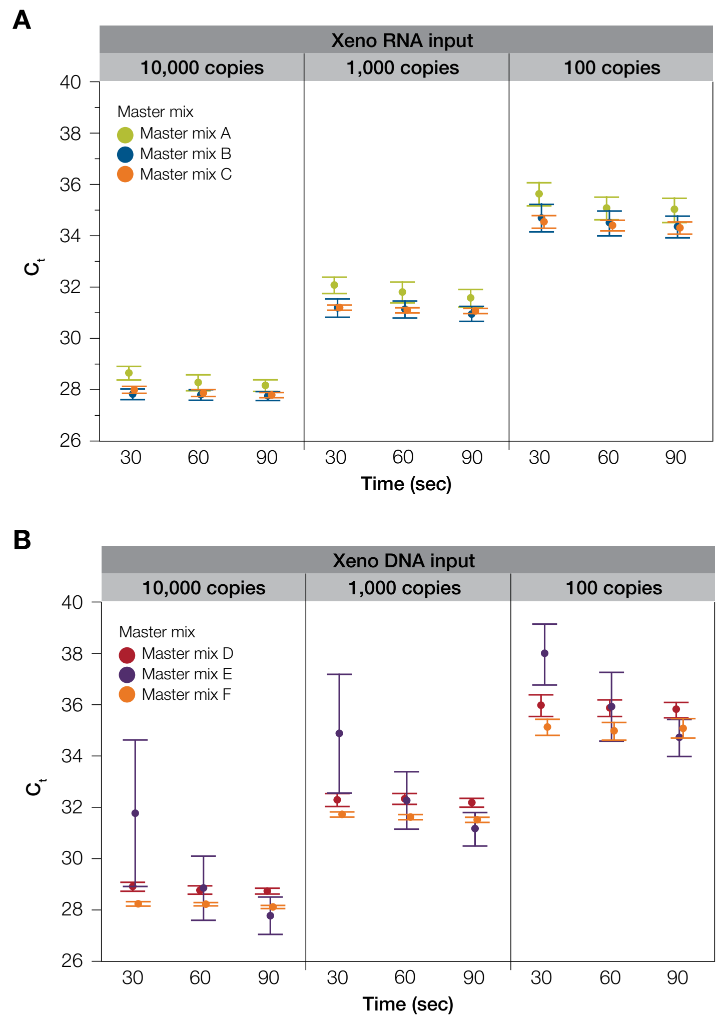

Figure 2. Comparison of (A) Xeno IPC RNA and (B) Xeno IPC DNA amplification across different master mixes for each tested concentration of Xeno template. The y-axis represents the C_t values. The x-axis is grouped into three segments. Each segment represents a tested Xeno (RNA or DNA) template concentration (10,000, 1,000, or 100 copies). Multiple annealing times (30, 60, and 90 seconds) were employed for each attribute evaluated. Each data point represents a mean C_t value of a range of temperatures tested from 54˚C to 62˚C. The standard deviations in C_t values from using different master mixes (A–F) are depicted by diffrent colors in the graph above.

applied biosystems

Ordering information

Product	Quantity	Cat. No.
VetMAX Xeno Internal Positive Control RNA	100 reactions	A29763
VetMAX Xeno Internal Positive Control DNA	100 reactions	A29764
VetMAX Xeno Internal Positive Control—VIC Assay	100 reactions	A29765
VetMAX Xeno Internal Positive Control—LIZ Assay	100 reactions	A29766
VetMAX Xeno Internal Positive Control RNA	500 reactions	A29761
VetMAX Xeno Internal Positive Control DNA	500 reactions	A29762
VetMAX Xeno Internal Positive Control—VIC Assay	500 reactions	A29767
VetMAX Xeno Internal Positive Control—LIZ Assay	500 reactions	A29768

Find out more at **thermofisher.com/xeno-ipc**

ThermoFisher SCIENTIFIC

For Veterinary Use Only. For *In Vitro* Use Only. Products may vary by country; products may not be available in your geographic area.
© 2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. **COL31282 0816**