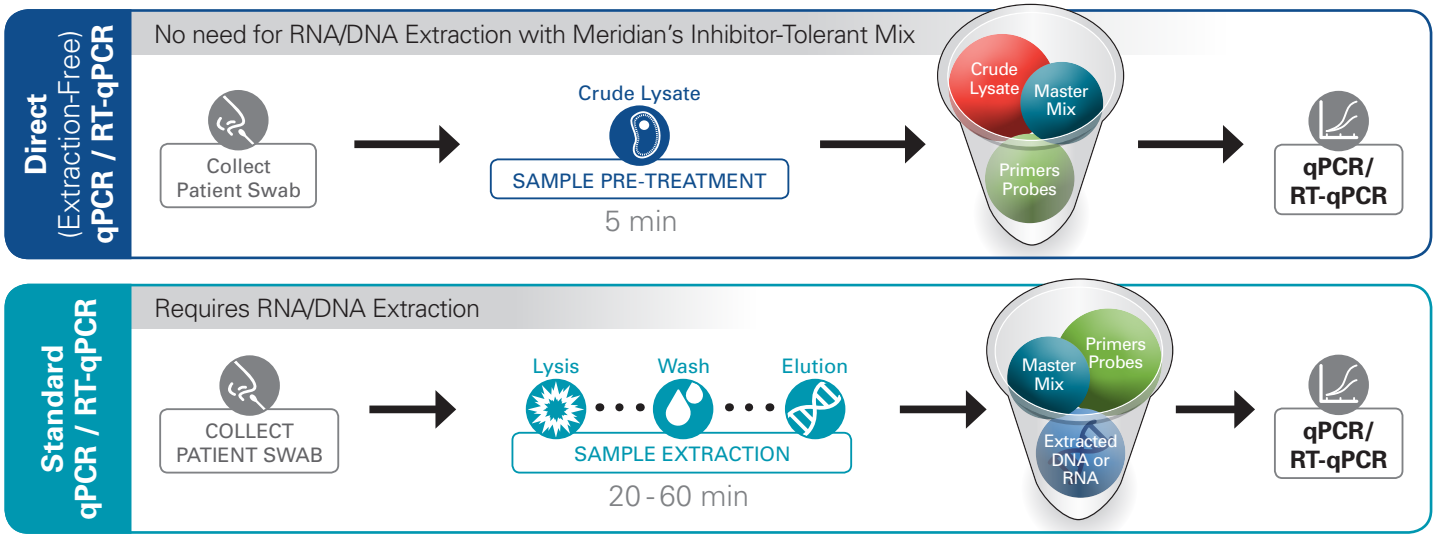
Inhibitor-Tolerant qPCR & RT-qPCR Mixes

Optimized master mixes formulated for direct amplification of crude samples (e.g. sputum, stool, saliva, urine and blood)

A variety of different inhibitors inherent to clinical and environmental samples can negatively impact the sensitivity and accuracy of a molecular assay. Conventionally, to overcome this, specimens undergo processing prior to testing. However, extraction technologies are not 100% efficient which can impact the amount of target nucleic acid available for testing, and some inhibitors can co-elute following purification and induce false-negative assay results.

Meridian's Inhibitor-Tolerant qPCR/RT-qPCR formulations are designed for developing qualitative multiplex assays that require minimal sample processing and fast turn-around times (TAT). The mixes can be used for direct amplification from crude lysates or inhibitor-rich samples such as urine, cerebral spinal fluid (CSF), blood, sputum, saliva and stool.

Direct Amplification Workflow



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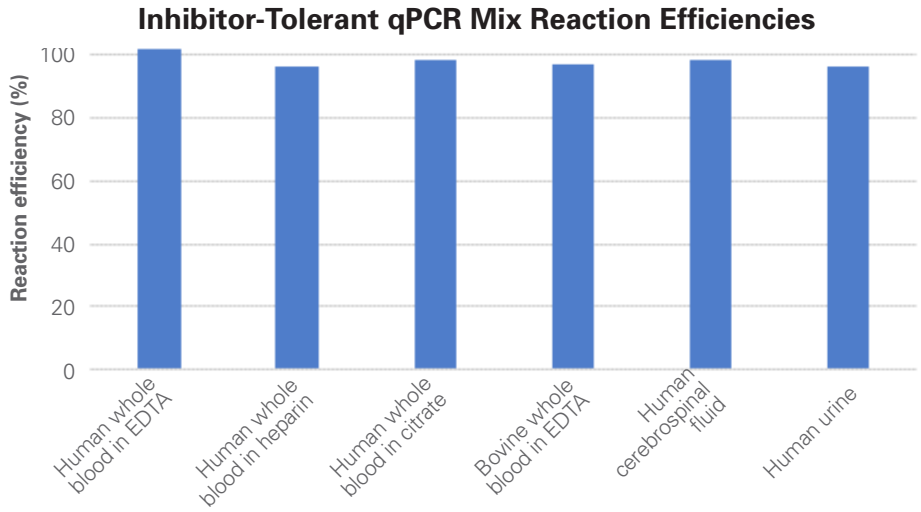
LIFE DISCOVERED. LIFE DIAGNOSED.

Inhibitor-Tolerant qPCR Mix

- Ready-to-use qPCR mix engineered with a proprietary buffer system designed for overcoming common PCR inhibitors
- Allows for DNA amplification direct from crude lysate or unprocessed samples such as urine, cerebral spinal fluid (CSF), milk and blood
- Ideal for developing qualitative assays where sample is limited
- Eliminates need for sample extraction and speeds up assay TAT

PRODUCT	CAT NO.	VOLUME	REACTIONS
Inhibitor-Tolerant qPCR Mix	MDX013	5 mL	500 Rxn
		100 mL	10,000 Rxn

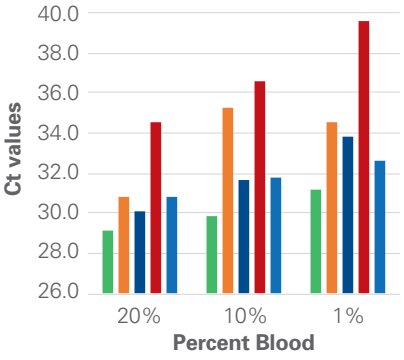
High qPCR efficiency across a range of inhibitors



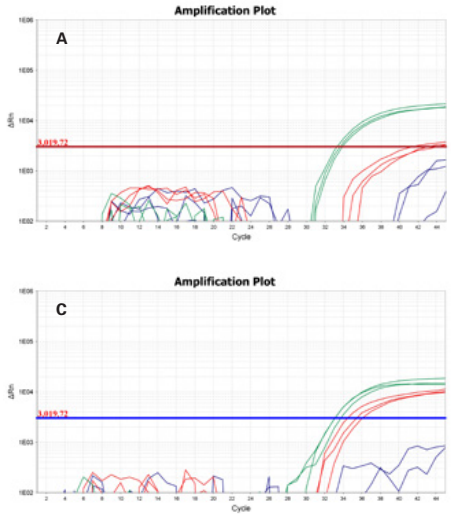
Reaction efficiencies were determined from reactions containing a variety of known PCR inhibitors ranging from whole blood to biofluids (20% in reaction). The results demonstrate that the reaction efficiency of the Inhibitor-Tolerant qPCR Mix remained within 90-110% in the presence of a wide range of common PCR inhibitors.

Efficient amplification from various liquid sample types (spinal fluid, urine, milk and blood).

WHOLE BLOOD SPECIMENS

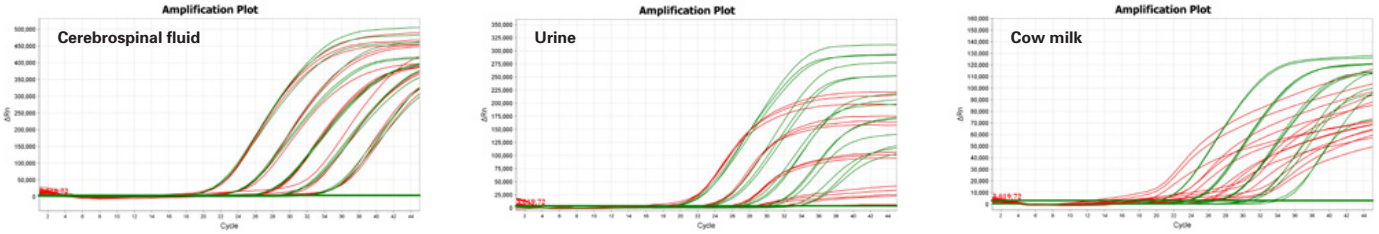


Direct detection of GC-rich (64%) DNA from 1% to 20% whole blood using Inhibitor-Tolerant qPCR Mix (green) and mixes from other suppliers.



Direct detection of (A) 25% (B) 46% and (C) 64% GC-rich DNA from 20% whole blood using Inhibitor-Tolerant qPCR Mix (green) and mixes from suppliers R (red) and T (blue).

SPINAL FLUID, URINE AND MILK SPECIMENS



A 10-fold serial dilution of genomic DNA was spiked into cerebrospinal fluid, human urine or cow whole milk and the DNA was amplified, using Inhibitor-Tolerant qPCR Mix (green) and KAPA Probe Force Mix (red). The results illustrate that Inhibitor-Tolerant qPCR Mix is more sensitive than KAPA Probe Force Mix, as lower dilutions could be detected with better efficiencies, across all three sample types.

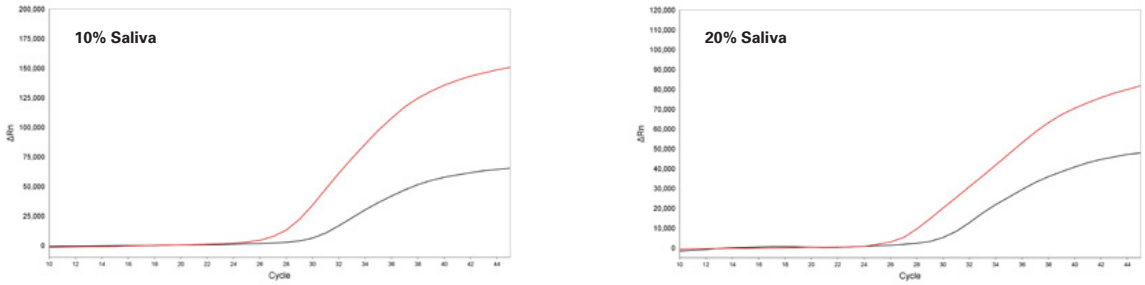
Inhibitor-Tolerant RT-qPCR Mix

- Designed for amplification from crude samples without the need for complex or time consuming extraction
- 4x concentration master mix allows for greater sample volume
- Single use one-step mix. Only add primers, probes and clinical samples
- Suitable for single and multiplex detection of RNA and DNA pathogens

PRODUCT	CAT NO.	VOLUME	REACTIONS
Inhibitor-Tolerant RT-qPCR Mix	MDX016	5 mL	1,000 Rxn
		50 mL	10,000 Rxn

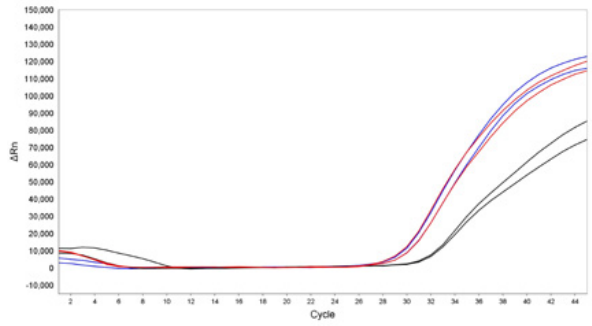
Inhibitor-Tolerant RT-qPCR Mix exhibits a high tolerance to PCR inhibitors from clinical samples

SALIVA SPECIMENS

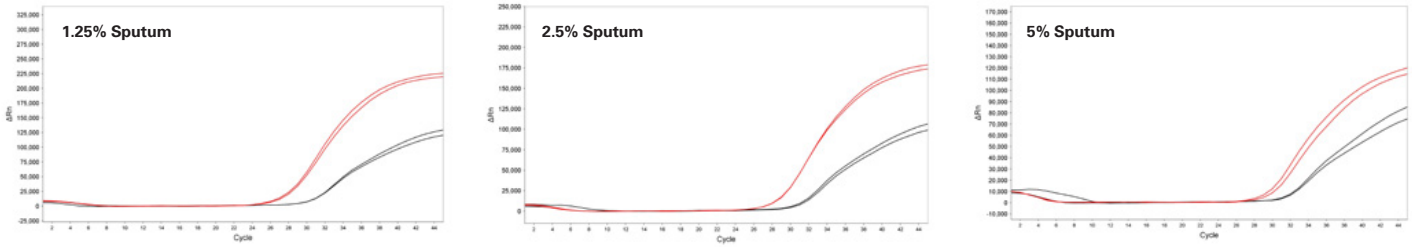


MDX016 (red) and MDX032 (**black**) amplification traces of Influenza A spike in presence of saliva swabs (COPAN ESwab 359C) at 10% (left) and 20% (right) final concentration. With earlier Ct (approx. 4 Ct) and higher fluorescence (approx. +50%), the results demonstrate the superiority of Inhibitor-Tolerant RT-qPCR Mix (MDX016) against a standard RT-qPCR Mix (MDX032) for the detection of viral RNA in presence of saliva swab resuspension in UTM.

SPUTUM SPECIMENS

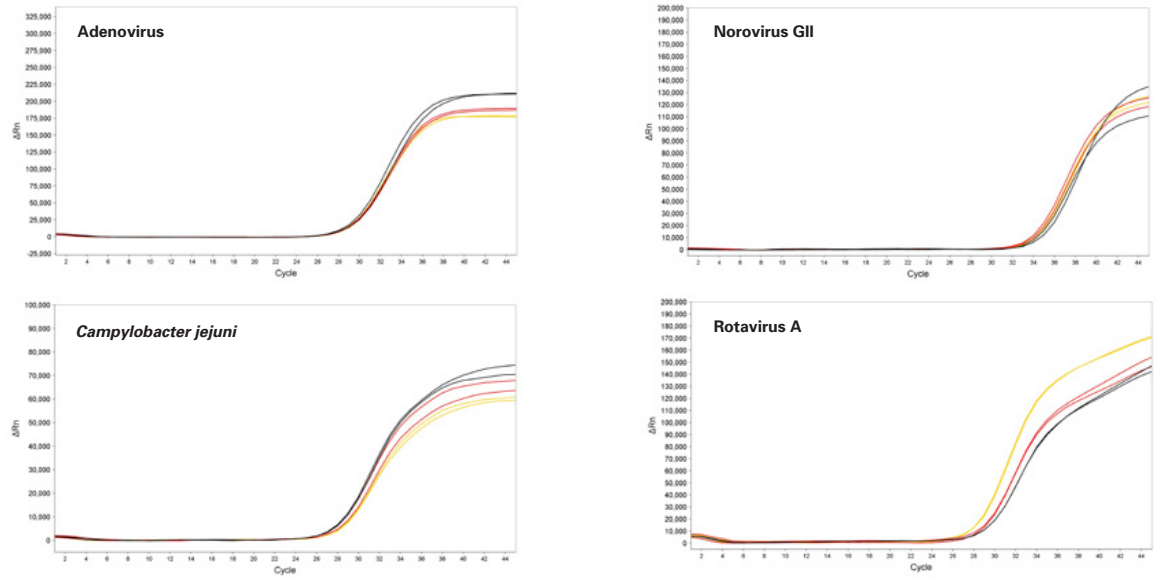


Amplification profile of inactivated influenza virus spiked into samples containing 5% sputum or no sputum. The data illustrates that the performance of Inhibitor-Tolerant RT-qPCR Mix (MDX016) in the presence of 5% artificial sputum (red) is the same Fast One-Step RT-qPCR Mix (MDX032) with no sputum (blue). In contrast, the sensitivity and performance of Fast One-Step RT-qPCR Mix (MDX032) significantly decreases in the presence of 5% artificial sputum (**black**) compared to no sputum or compared to Inhibitor-Tolerant RT-qPCR Mix (MDX016) with sputum.



Amplification profiles of inactivated influenza virus spiked into samples containing various concentrations of sputum (1.25% - 5%). Results demonstrate that MDX016 (red) exhibits a higher tolerance to inhibitors present in artificial sputum (Kirchner, S. *et al. J Vis Exp* (64) 2012) compared to the standard Fast One-Step RT-qPCR Mix MDX032 (**black**).

STOOL SPECIMENS



Amplification profiles of 4 pathogens (RNA: Norovirus and Rotavirus, DNA: Adenovirus, *C. jejuni*) in a multiplex reaction containing 5% (yellow), 10% (red) and 20% (**black**) stool extract. The results demonstrate the multiplexing capability of MDX016 in the presence of inhibitors found in stool (up to 20% final volume).

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