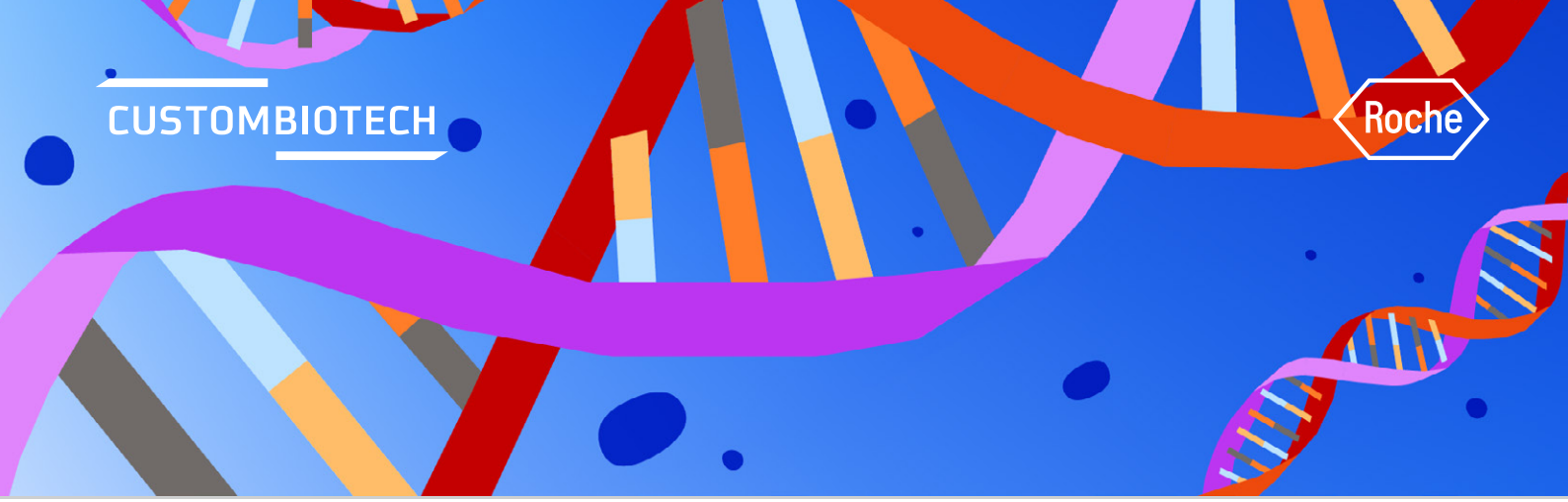

KAPA3G HotStart DNA Polymerase performance with common sample types used in infectious disease testing

Application Note

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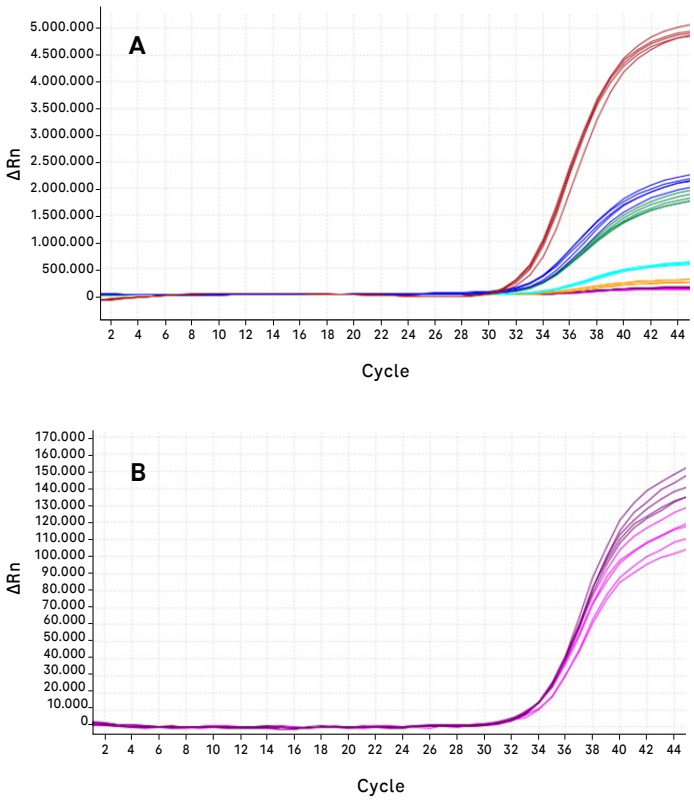
Shining Light on a hidden pandemic

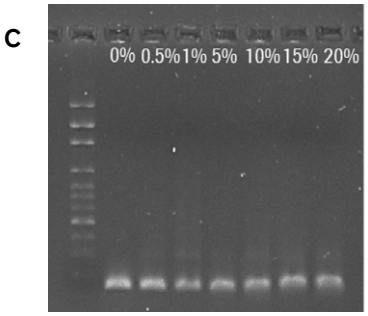
In 2023 the WHO estimated that over 1 million sexually transmitted infections (STIs) are acquired every day worldwide[1]. Often asymptomatic, STIs have been considered a "hidden epidemic" for over 20 years[2] and prevalence continues to grow[3]. STIs affect the health and wellness of people from all socioeconomic categories. Timely diagnosis is crucial to determining an ideal treatment plan and, in some cases, preventing the further development of antimicrobial resistance (AMR) or drug-resistant pathogens[4].

Molecular diagnostic assays to detect viral or bacterial infections, such as STIs, commonly use blood or urine. For example, samples derived from the components of whole blood are used in the detection and/or monitoring testing of infections by Epstein-Barr virus (EBV), hepatitis B virus (HBV), herpes simplex virus (HSV), human immunodeficiency virus (HIV) and many more. Urine samples are often used in the diagnosis of chlamydia and gonorrhea, as well as urinary tract infections (UTIs). Both blood and urine samples can present challenges for assay developers aiming to deliver a meaningful and timely diagnosis. These sample types require cumbersome purification or nucleic acid isolation steps before they can be used in polymerase chain reactions (PCR) or reverse transcription-polymerase chain reactions (RT-PCR). Rising demand for shorter turnaround times and simpler protocols has shifted focus to developing assays that reduce or eliminate sample purification steps. However, using cruder sample preparation methods inevitably introduces inhibitors that can cause inconsistent results in assays using conventional reagents. Next-generation enzymes with increased tolerance to common PCR inhibitors overcome this challenge. In the next sections, we discuss the robust performance of KAPA3G HotStart DNA Polymerase in assays containing unpurified blood and urine samples.

KAPA3G HotStart DNA Polymerase tolerates up to 20% blood

Blood contains known PCR inhibitors such as hemoglobin, lactoferrin, and IgG. Therefore, PCR-based diagnostics to detect DNA and RNA in blood samples typically require a purification or isolation step to rid the sample of these compounds[5]. The process is cumbersome and costs the user significant amounts of time and money. To investigate the feasibility of eliminating these cost drivers, we experimented with spiking various amounts of whole blood into the final reactions of a detection assay using KAPA3G HotStart DNA Polymerase. While the introduction of whole blood into the reactions did significantly reduce the overall fluorescence (Fig. 1A), reactions containing up to 15% or 20% whole blood still showed consistent and repeatable real-time results, just at a lower overall fluorescence (Fig. 1B). The reduced fluorescence may be more the result of fluorescent probe quenching than inhibition of the DNA polymerase itself. The consistent PCR product formation across all tested reaction conditions (Figure 1C) corroborates this possibility and ultimately demonstrates the strong inhibitor tolerance of KAPA3G HotStart DNA Polymerase, even with 20% whole blood in the reaction.





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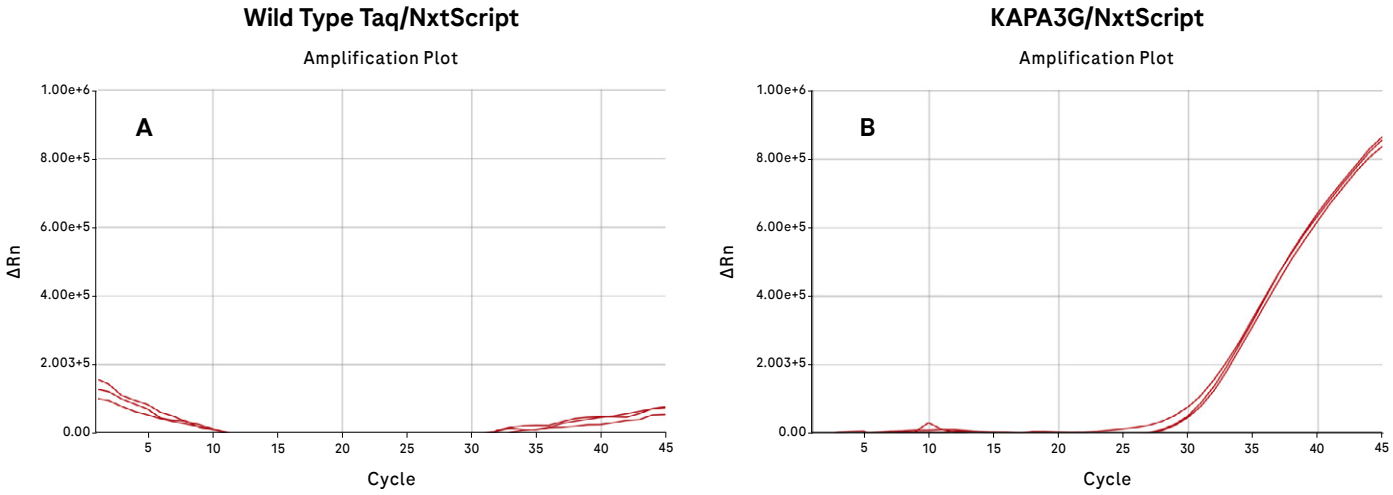
A) Amplification curves for reactions containing whole blood. KAPA3G HotStart DNA Polymerase was used to amplify viral DNA in the presence of increasing amounts of whole blood. Whole blood was spiked into reactions at concentrations of 0% (red), 0.5% (Blue), 1.0% (green), 5.0% (cyan), 10% (orange), 15% (purple), and 20% (pink) of the total reaction volume. B) Rescale of the plot from Figure 1A with the reactions containing 15% (purple) and 20% (pink) whole blood.
C) Agarose gel showing consistent fragment generation.

PCR cycling conditions: Initial denaturation hold: 3 min at 95 °C,
45 cycles: denaturation: 1 second at 95 °C, annealing/extension: 10 seconds at 60 °C

Using unpurified urine speeds up turnaround time

Urine is a common sample type for UTI and certain STI testing. Many assay developers seek to use unpurified urine to simplify and accelerate diagnostic tests. As with blood, using unpurified urine introduces inhibitors such as urea and various highly concentrated salts into the assay[5,6]. To explore the performance limits of NxtScript Reverse Transcriptase and KAPA3G HotStart DNA Polymerase in detecting RNA in urine, we maximized sample volume in an RT-PCR assay. Reaction mixtures in these experiments consisted of 75% unpurified urine.

Conventional enzymes did not tolerate the inhibitors found in the unpurified urine samples (Fig. 2A). In contrast, NxtScript Reverse Transcriptase coupled with KAPA3G HotStart DNA Polymerase, showed excellent detection of viral RNA in the unpurified urine samples, even when samples accounted for 75% of the total reaction mixture (Fig. 2B). Using such a large sample volume in the reactions was possible because both enzymes and master mixes were highly concentrated. That concentration and the inhibitor tolerance of these reagents create space in a reaction for greater sample volumes, which improves detectability.



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A) Amplification curves from reactions using wild-type Taq and NxtScript Reverse Transcriptase to detect viral RNA in an unpurified urine sample. No amplification was observed. B) Amplification curves from reactions containing KAPA3G HotStart DNA Polymerase and NxtScript Reverse Transcriptase run on the same unpurified urine sample. All samples show consistent and robust amplification.

RT-PCR cycling conditions: RT hold: 5 min at 55 °C, initial denaturation hold: 1 min at 95 °C, **45 cycles:** denaturation: 10 s at 95 °C, annealing/extension: 30 s at 60 °C

Conclusion

Detecting STIs is just one application where molecular diagnostics makes an impact in healthcare. Some of the challenges discussed here impact many other applications, including tests for respiratory and, hospital acquired infections. Next-generation raw materials that are tolerant to common PCR inhibitors can save time and money by reducing turnaround times and simplifying workflows. Furthermore, developers can achieve more design goals during optimization by coupling multiple features of these reagents, including high concentration, inhibitor tolerance, and speed. Results from the described experiments demonstrate that KAPA3G HotStart DNA Polymerase is well-suited to meet the requirements of modern centralized and point-of-care molecular diagnostic assays.

Ordering information

Product	Pack size	Catalog number
KAPA3G HotStart Master Mix	custom fill	09084711103
KAPA3G HotStart DNA Polymerase	custom fill	08918651103
Related products		
KAPA3G PCR Buffer	custom fill	09160914103
NxtScript Reverse Transcriptase	custom fill	07051166103
NxtScript 2G Reverse Transcriptase	custom fill	09085220103

Interested in learning about other ways KAPA3G HotStart DNA Polymerase or other Roche CustomBiotech enzymes can reduce the turnaround time of your assay? Follow the QR code to find more information on our website. Additional data on the inhibitor tolerance, speed, robustness, and other performance parameters of KAPA3G HotStart DNA Polymerase are available upon request.

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