Technical Note

Efficient Viral Nucleic Acid Purification with the Thermo Scientific KingFisher Pure Viral NA Kit

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Goal

The Thermo Scientific™ KingFisher™ Pure Viral NA Kit enables nucleic acid (NA) purification from a wide variety of cell-free samples, such as plasma, serum, saliva and urine, as well as from nasal, buccal and urogenital swabs. This technical note describes viral DNA and RNA purification from plasma samples with the KingFisher Pure Viral NA Kit in combination with Thermo Scientific™ KingFisher™ magnetic particle processors. The results indicate that purification is sensitive and the purified viral NA is free of contaminants and inhibitors.

Introduction

The KingFisher Pure Viral NA Kit is designed to purify viral nucleic acids from various sample materials, including typical cell-free body fluids, such as plasma, as well as from different swab samples. Robust automatic viral RNA or DNA purification is a prerequisite for fast and sensitive detection and identification of viruses from various sources. Using the KingFisher Pure Viral NA Kit in combination with the Thermo Scientific™ KingFisher™ Flex or Thermo Scientific™ KingFisher™ Duo magnetic particle processors, purification is both rapid and requires very little hands-on time. The KingFisher Flex enables nucleic acid purification of up to 96 samples per run, while a lower throughput instrument, the KingFisher Duo, is available for up to 12 samples per run.

Materials and Methods

Human plasma samples were spiked with a known titer of Hepatitis B virus (HBV) or Hepatitis C virus (HCV). Dilution series from 10^2 to 10^7 copies/mL were produced by diluting plasma samples infected with HBV. Competitor comparison was performed from the plasma samples spiked with 10^7 copies/mL of HCV and the purifications were carried out according to manufacturers' instructions. Viral nucleic acids were purified from 200 µL aliquots of infected plasma samples using the KingFisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) and the KingFisher Flex instrument.

One run on the KingFisher Flex or KingFisher Duo lasts approximately 40 minutes. After purification, the viral nucleic acids were eluted into 100 µL of nuclease-free water. The volume can, however, be adjusted. Reverse transcription of RNA from HCV samples was performed with Thermo Scientific™ RevertAid™ Premium

Reverse Transcriptase. qPCR was carried out on the Thermo Scientific™ PikoReal™ Real-Time PCR System or on the Applied Biosystems® 7500 Real-Time PCR System with Thermo Scientific™ Maxima™ Probe qPCR Master Mix.

Results

Viral DNA and RNA were efficiently extracted from HBV- and HCV-spiked plasma samples, respectively, using the KingFisher Pure Viral NA Kit.

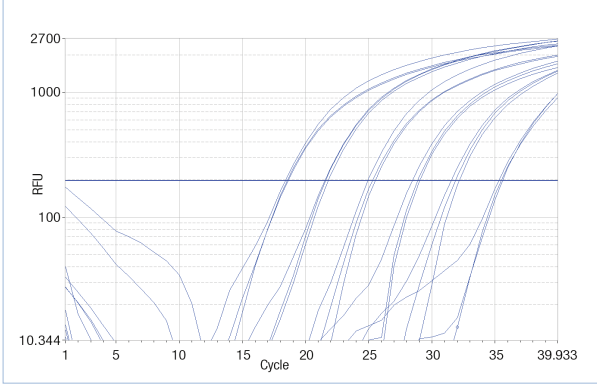

Figure 1. Amplification curves from virus NA purified from plasma (HBV; 10^2 to 10^7 copies/mL) on the PikoReal Real-Time PCR System. qPCR efficiency was 96.277%; R2 = 0.9959.

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The copy number of HBV viruses specifically detected by qPCR was in a linear range from samples including 10^2 to 10^7 copies/mL (Figure 1). Extracted yields were highly repeatable and reproducible as shown in Figure 2. Similar sensitivity was achieved testing a wide variety of DNA and RNA viruses (HIV, HBV, HCV, HPV, CMV, and EBV) and different samples (plasma, serum, buccal and nasal swabs, and urine), for which data is not shown.

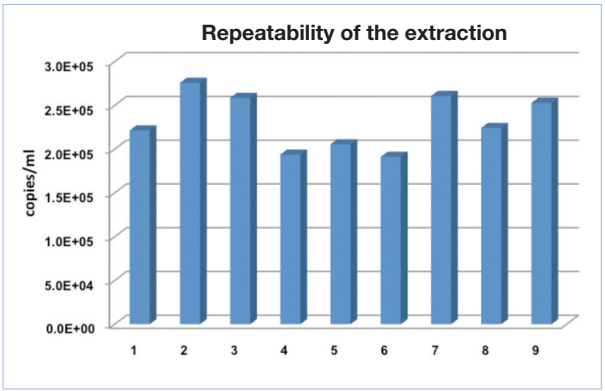

Figure 2A. Repeatability of the purified NA yield within an experiment. DNA was extracted from nine parallel HBV-spiked plasma samples and quantified by absolute quantification in a qPCR experiment on the Applied Biosystems® 7500 Real-Time PCR System (two technical repeats, SD of Cq < 0.2). The variation of the calculated quantities gave a CV of 13.5%.

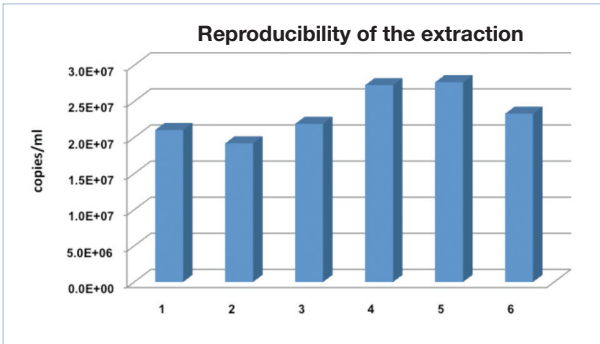

Figure 2B. Reproducibility of the purified NA yield in six independent purifications. Human plasma was spiked with 10^7 copies/mL HBV, and DNA was extracted from the samples on six different days. Copy numbers were determined by absolute quantification in a qPCR experiment on the Applied Biosystems® 7500 Real-Time PCR System (three parallel extraction samples in each experiment and two technical repeats in qPCR, SD of Cq < 0.2). The variation of the calculated quantities gave a CV of 5%.

Viral RNA was efficiently extracted from plasma samples infected with 10^7 copies/mL of HCV using the KingFisher Pure Viral NA Kit. Competitor comparison indicates that the KingFisher Pure Viral NA Kit is more sensitive compared to the three competitive magnetic bead kits (Figure 3).

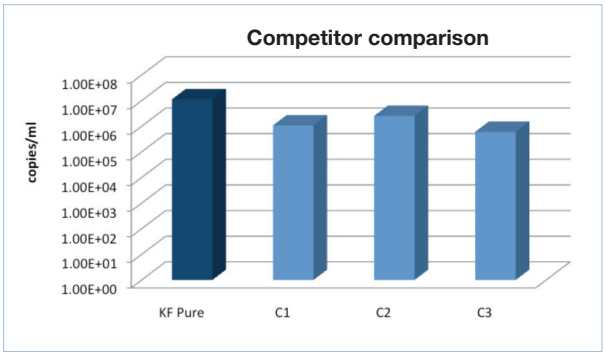

Figure 3. The purified NA yield from experiments comparing four different magnetic bead kits: Human plasma was spiked with 10^7 copies/mL HCV, and RNA was extracted with the KingFisher Pure Viral NA Kit (KF Pure), and competitor kits (C1 to C3). Results are based on qPCR absolute quantification experiments. Note the logarithmic scale on the vertical axis (copies/mL).

Conclusions

The KingFisher Pure Viral NA Kit provides efficient and sensitive nucleic acid purification from various samples. The purified NA is of high integrity, and contaminants and inhibitors are washed away during the process. The eluate is suitable for direct use in different downstream applications.

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