Sensitive detection of viral nucleic acids using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

Summary

- Detection of viruses in human biofluids is crucial to infectious disease research and management; however, it can be challenging to extract viral nucleic acid from samples that have a very low viral load.
- The new Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit is designed to extract viral nucleic acid from a variety of sample types with a range of viral loads.
- The MagMAX Viral/Pathogen Nucleic Acid Isolation Kit provides a sensitive and simple method for nucleic acid extraction from virus-containing samples, as demonstrated by successful detection of as few as 50 copies of input spiked into multiple sample types.
- Here we demonstrate the use of this kit and the Thermo Scientific™ KingFisher™ Flex instrument to extract nucleic acid from both cytomegalovirus (CMV) and Epstein-Barr virus (EBV)

Introduction

The detection of viruses in human biofluids is crucial to infectious disease research and management. Viral load testing is used to assess the efficacy of antiviral therapy, as well as to monitor disease progression and uncover the presence of an infection at an earlier stage. However, it can be challenging to extract sufficient viral nucleic acid from samples to meet a sensitivity threshold for an assay or test.

Viral load testing is useful for distinguishing between latent and active infections, as well as for mapping or defining the course of the infection. For example, CMV viral load tests are useful for deciding when to initiate preemptive therapy in organ transplant recipients and for distinguishing active disease from asymptomatic infection. The number of copies of CMV DNA can also help predict the development of active CMV disease; the higher the load value, the higher the risk of symptomatic disease [1]. Similarly, EBV viral load testing plays an important role in the diagnosis of EBV-associated posttransplant lymphoproliferative disorder in organ transplant recipients [2]. EBV has also been implicated as the underlying cause of multiple human cancers [3], so detection at an earlier stage (with a lower viral load) could help direct the need for early treatment.

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The new MagMAX Viral/Pathogen Nucleic Acid Isolation Kit is designed to extract viral nucleic acid from a range of sample types with a range of viral loads. Using a simplified, fast, and automation-friendly workflow, the kit can be used to extract nucleic acid from as few as 50 copies of virus contained in a 400 µL sample volume. Here we demonstrate the use of this kit and the KingFisher Flex instrument to extract nucleic acid from both CMV and EBV in a range of copies from multiple human sample types.

Materials and methods

Samples and controls

The extraction range was determined using a dilution series of known quantities of intact CMV and EBV controls (ZeptoMetrix, Cat. No. NATCMV-ERCM and NATEBV-ERCM) spiked into 400 µL of human blood, urine, and plasma.

Total nucleic acid extraction

Nucleic acid was extracted from each dilution of each sample type using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and KingFisher Flex instrument in 96-well plate format. Samples were eluted in 50 µL of elution solution to help maximize the concentration of the samples. The workflow is shown in Figure 1.

Viral detection by PCR

Real-time PCR was performed on 4 µL of each extract in duplicate, using CMV- and EBV-specific Applied Biosystems™ TaqMan® Assays and the TaqMan® Fast Virus One-Step Master Mix (with no preamplification step) on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System. The reactions were completed using fast cycling conditions: 1 cycle at 95°C for 20 sec, and 45 cycles at 95°C for 3 sec and 60°C for 30 sec. The number of cycles was increased from the standard 40 to 45 for enhanced sensitivity.

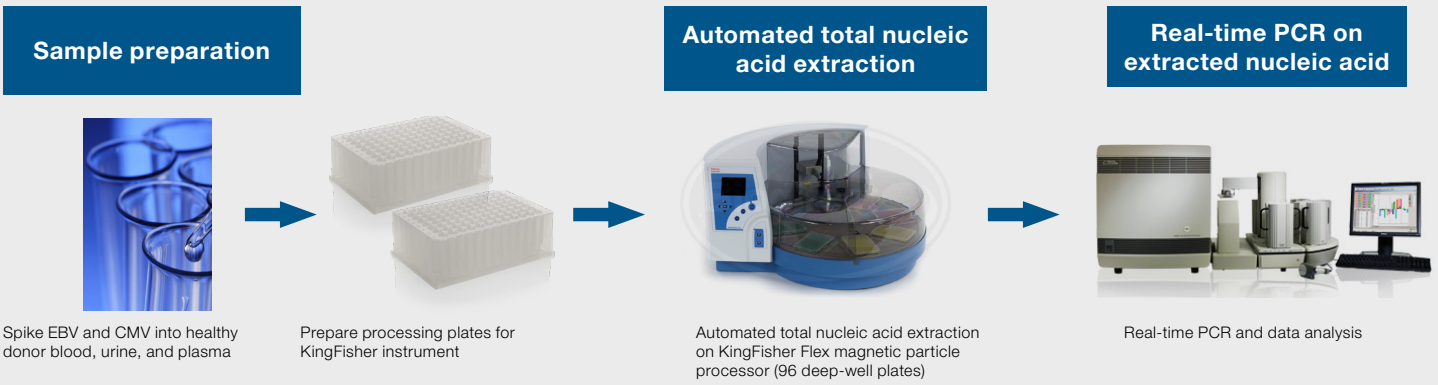

Figure 1. Workflow for extraction of viral total nucleic acid with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and analysis with real-time PCR.

Results

We successfully extracted viral nucleic acid from as few as 50 copies of virus using the MagMAX Viral/Pathogen kit, as demonstrated by real-time PCR results (Figure 2). Average C_t values increased at the expected rate as nucleic acid concentration decreased, indicating linear recovery. These results were consistent across sample types, demonstrating the robustness and flexibility of the protocol and reagents.

Conclusion

The MagMAX Viral/Pathogen Nucleic Acid Isolation Kit provides a sensitive and simple method for nucleic acid extraction from virus-containing samples, as demonstrated by successful detection of as few as 50 copies of input into multiple sample types. This is an efficient workflow that can address one of the critical issues in viral disease research and management.

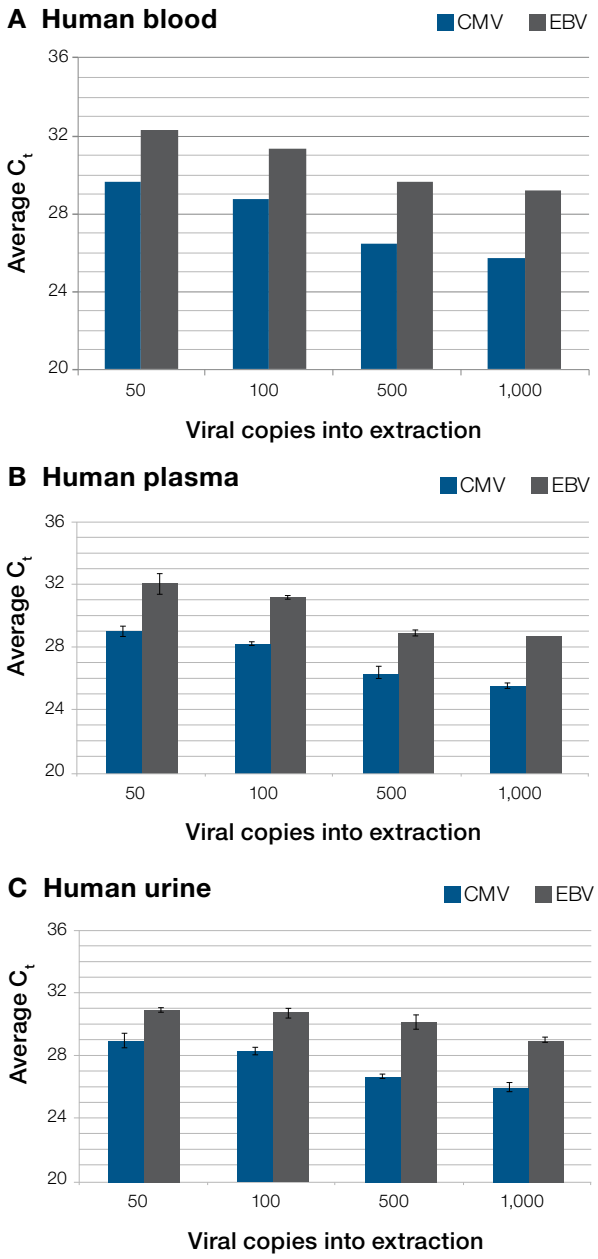

Figure 2. Average C_t values for a range of input copies of EBV and CMV in real-time PCR TaqMan Assays. Results from spike-in controls in **(A)** blood, **(B)** plasma, and **(C)** urine samples.

References

1. Humar A, Gregson D, Caliendo AM et al. (1999) Clinical utility of quantitative cytomegalovirus viral load determination for predicting cytomegalovirus disease in liver transplant recipients. *Transplantation* 68:1305–1311.
2. Gulley ML, Tang W (2010) Using Epstein-Barr viral load assays to diagnose, monitor, and prevent post-transplant lymphoproliferative disorder. *J Clin Microbiol* 23:350–366.
3. Young-Hyeh K (2015) EBV and human cancer. *Exp Mol Med* 47(1):e130.

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