OMNIgene®•ORAL

Stability, recovery efficiency and detection limit of viral DNA in OMNIgene®•ORAL (OM-505)

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OMNIgene®•ORAL (OM-505) is designed to collect and stabilize microbial DNA and RNA from oral fluids. In this document, we demonstrate that OMNIgene•ORAL stabilizes viral DNA for up to 21 days at room temperature. We also show that viral DNA collected and stabilized in OMNIgene•ORAL does not adversely affect detection of viral DNA by downstream applications such as PCR.

Introduction

Accurate detection of viruses and bacteria by molecular techniques greatly depends on the quality and quantity of the sample after collection. Poorly preserved samples may provide misleading results including false negatives or false positives. Such bias may result in misdiagnosis, improper selection and/or dosage of therapies (e.g., antibiotics or antivirals), and may skew the results of epidemiology studies.

Typical sources of error in samples that are not properly stabilized and preserved are:

- Selective overgrowth of a subset of microorganisms
- Formation of spores
- Chemical and enzymatic degradation of nucleic acids
- Impaired ability to detect targets of lower abundance

Current strategies to mitigate these stability challenges include storing and transporting samples at low temperatures, accelerating turn-around times during processing and drying the samples over solid matrix (i.e., paper). These strategies are expensive, impractical and in some cases inefficient.

Preserved samples must yield reliable results from a variety of downstream applications, especially when the microbe of interest is present in low quantities. Therefore, preservation methods must not introduce inhibition.

OMNIgene•ORAL is an all-in-one system for the collection and stabilization of microbial DNA and RNA from oral fluids. The kit stabilizes nucleic acids at ambient temperature, enabling cost-effective transportation over prolonged time and distance. This method allows for the painless and non-invasive collection of high quality samples that facilitate nucleic acid detection from DNA and RNA viruses and bacteria, without inhibition, even in low copy numbers, across a variety of molecular methods (e.g., RT-qPCR or the Abbott *m*2000*rt* RealTi*me* System).

This technical bulletin describes the stability and recovery efficiency of viral DNA spiked into oral fluid samples collected with the OMNIgene•ORAL kit. Inactivated Epstein Barr-Virus (EBV) was used as a target for detection using the Corbett Life Sciences Rotor-Gene™ instrument. In-house PCR protocol was used to analyze EBV DNA from spiked oral fluid samples collected in OMNIgene•ORAL (OM-505).

Materials and methods

Inactivated EBV virus (NATtrol EBV, Zeptometrix) was used in all experiments. NATtrol EBV is prepared from highly purified virus, isolated from cell culture, which is chemically and enzymatically treated to alter its surface proteins rendering it unable to infect a host cell. While modified on its surface, the virus still retains an intact and complete viral genome, making it an ideal control[1].

Purified viral DNA from NATtrol EBV was used to optimize and validate the conditions for a quantitative PCR (qPCR). Serial dilutions of EBV DNA were tested in triplicate with custom designed primers for the LMP-2A EBV gene (Forward: 5'-CATGTTCTGGGTCAAAAGG and Reverse: 5'-TGCTGATTCTGCTAACACTG). PCR reaction: in a 25 µL reaction volume; 0.2 U Taq Polymerase, 200nM primers, 1.5 mM $MgCl_2$,

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1 µM Syto®9 dye (Invitrogen), PCR Buffer without $MgCl_2$ (Roche), 0.1 mg/mL BSA. PCR conditions were used: initial denaturation for 5 minutes at 95°C (1 cycle); denaturation for 30 seconds at 95°C, annealing 40 seconds at 58°C and extension for 30 seconds at 72°C (40 cycles), final extension for 7 minutes at 72°C. All qPCR runs were performed on the Corbett Life Sciences Rotor-Gene™ and the data was analyzed using the default quantitation analysis with the built-in software, Rotor-Gene™ 6000 ver. 1.7. The qPCR assay resulted in a 4 log linear dynamic range (80 - 20,000 copies cp of EBV virus per reaction), a reaction efficiency of 1.01 ±0.07, R^2 of 0.98, no detectable amplification in the no-template control and a single amplified product with the expected theoretical melting temperature.

"Sample" in this document refers to oral fluids collected in OMNIgene•ORAL per kit instructions. To determine the limit of detection (LOD) of the assay, samples were spiked with known amounts of EBV NATtrol: very low copy: 320 cp/mL; low copy: 1,600 cp/mL; medium copy: 3,200 cp/mL; high copy: 16,000 cp/mL. To determine the ability of OMNIgene•ORAL to stabilize viral nucleic acids, samples were spiked with 3,200 cp/mL of EBV NATtrol. Samples were then incubated at room temperature (22°C to 24°C) for 0, 7 and 21 days prior to purification. In all cases, viral DNA was extracted from a sample aliquot of 250 µL using the Qiagen QIAamp Virus MinElute Spin Kit, then immediately frozen until ready to use. EBV DNA was detected using the EBV qPCR protocol described above.

The ΔCq method[2] was used to evaluate the extraction efficiency and possible changes over time in EBV DNA content. The reference Cq value for the experimental control was obtained using an equivalent amount of purified EBV DNA (standard curve). Due to inherent variability in qPCR amplification, a ΔCq threshold of ±1 cycle was used, whereby any samples demonstrating more than 1 cycle difference between sample and standard was considered significant.

Results

After OMNIgene•ORAL/oral fluid samples were spiked with either very low, low, medium or high quantities of EBV NATtrol, they were extracted and quantified as described above. All data were analyzed using the ΔCq method (Figure 1). In all cases, less than 1 cycle difference was observed between the control and EBV spiked samples. Greater variability among the very low EBV spiked sample group was observed as compared to other groups; however the detection was still within the established threshold. These results indicate that viral DNA from oral fluids collected with OMNIgene•ORAL can be reliably amplified, even in samples with a very low viral load.

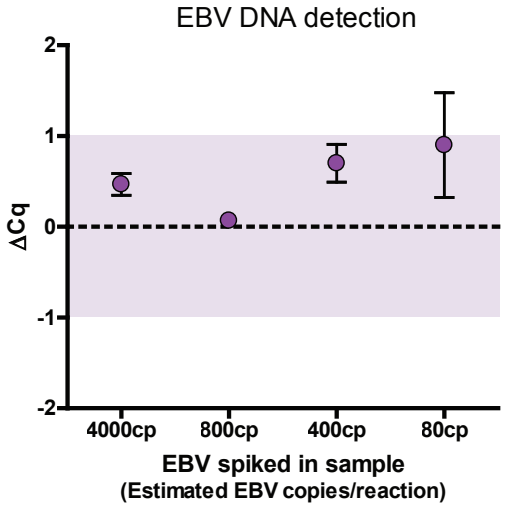

***Figure 1**: qPCR detection of EBV DNA in spiked OMNIgene•ORAL/oral fluid samples. ΔCq = Cqsample - Cqcontrol. ΔCq values are presented as a mean of 3 experimental replicates with standard error of mean.*

Spiked samples were stored at room temperature and viral DNA was extracted at T= 0, T= 7 days or T= 21 days. No differences in the recovered amount of EBV viral DNA were observed between the samples processed 0, 7 or 21 days after collection (Figure 2). These results indicate that viral DNA in oral fluid samples collected with OMNIgene•ORAL is stable for up to 3 weeks at room temperature.

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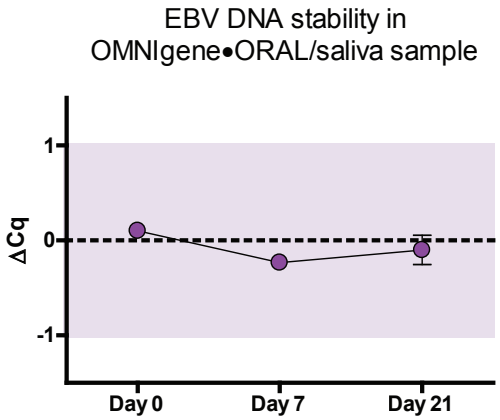

***Figure 2**: Room temperature stability of EBV DNA in OMNIgene•ORAL/ oral fluid samples. ΔCq values are presented as a mean of 3 experimental replicates with standard error of mean; 800 cp/reaction.*

Conclusions

1. OMNIgene•ORAL (OM-505) enables successful PCR amplification of even very low viral loads of a human DNA virus.
2. OMNIgene•ORAL (OM-505) stabilizes viral DNA from human viruses in oral fluid when stored for up to 21 days at room temperature.

References:

1 http://www.zeptometrix.com/nattrol.htm

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