Applications

5/04 epMotion® 5070 No. 81

High-throughput, fully automated Real Time PCR diagnostics of HBV and Salmonella

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Introduction

Polymerase Chain Reaction (PCR) is a well established and popular technique to amplify and detect specific RNA and DNA sequences. The invention of Real-Time quantitative PCR (RT-qPCR) has overcome several limitations of the conventional PCR and lead the way to implement nucleic acid amplification techniques (NATs) into the field of pathogen diagnostics [1]. In the Real-Time qPCR the amplified product is detected using fluorescent dyes. These fluorescent dyes are linked to oligonucleotide probes, which bind specifically to the amplified PCR product. Changes in the fluorescence intensities are monitored during the PCR reaction (in „real time“), reflecting the accumulation of the specific PCR product. This allows not only highly sensitive and specific detection of the target sequences, but also makes possible a very accurate quantitation of the target sequence. In addition, the whole process is vastly accelerated and the possibility for cross contamination, leading to false positive results, is minimised [2, 3, 4].

Here we demonstrate that it is now possible for a routine diagnostic laboratory to use Real-Time qPCR technology for a fast, reliable, and highly sensitive and specific detection and quantitation of viral and bacterial pathogens.

We used two commercially available Real-Time PCR assays (RealArtTM PCR Kits, artus GmbH) together with the state of the art robotic pipetting system (ep*Motion* 5070, Eppendorf) [5], which allows the automated and reliable set up of the reactions in a high throughput format.
The hepatitis B virus (HBV) titers in the human plasma samples have been determined using the RealArtTM HBV RG PCR assay (artus GmbH). Stool samples have been tested for the presence of Salmonella DNA using the RealArtTM Salmonella RG PCR assay (artus GmbH).
The PCR runs have been performed on the quantitative Real-Time PCR instrument Rotor-Gene 3000 (Corbett Research).

Materials and Methods

The ep*Motion*® 5070 procedures
The worktable of the Liquid Handling Workstation ep*Motion* 5070 (Eppendorf) was equipped with the following labware: 50 µL filter, PCR-grade tips (epT.I.P.S.® Motion), TwinTec PCR Plate 96 and ThermoRack with the Safe-Lock tubes 1.5 ml. For the PCR reaction the 100 µl Rotor-Gene tubes (Corbett Research) were used in a special thermorack with the cooling function (Eppendorf). The general worktable setup is shown in the Figure 1.

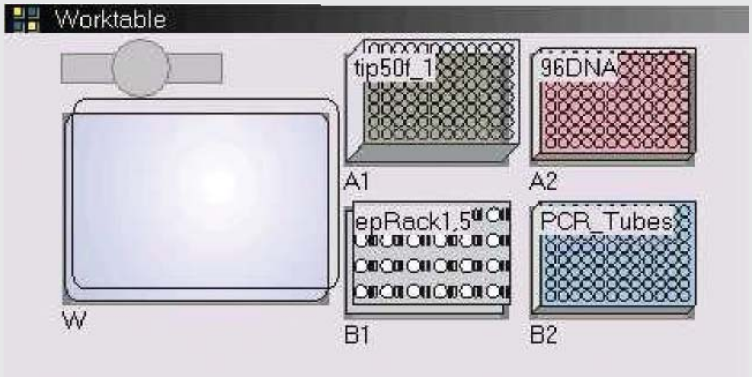

Fig. 1: The ep*Motion*® 5070 worktable setup.
Screenshot from the ep*Motion* Method Editor. Ts_50 - single channel dispensing tool (Eppendorf). A2: 96 well plate with the DNA from the serum samples, B1- RT-PCR reagents: RealArtTM Master Mix (incl. Internal Control).

eppendorf

Real Time PCR

For the Real Time PCR setup the RealArtTM HBV RG PCR Kit was used. The kit contains a Master Mix with reagents and enzymes for the specific amplification of a 134 bp region from the HBV genome and the direct detection of this specific amplicon in the fluorescence channel A.FAM. In addition the system contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an Internal Control (IC) in the fluorescence channel A.JOE. External positive controls (HBV RG QS 1-4) are supplied allowing the determination of the absolute viral DNA copy number.

To show the high precision and contamination free liquid handling the 50 µl PCR reaction was automatically pipetted using the Liquid Handling Workstation ep*Motion* 5070 as follows: 20 µl of water were added to 30 µl Master Mix for the No Template Control (NTC) whereas for the positive controls 20 µl of the Quantitation Standards (QS 1-4) were added. The No Template Control and the Quantitation Standards were pipetted in alternation by the Workstation. The complete PCR samples were run according to the Real Time PCR software on the Rotor Gene 3000 system (Corbett, Sydney, Australia) with the maximum capacity of 72 samples. The HBV and control specific signals were obtained in the A. FAM and A. JOE channels respectively and the data was evaluated using the Rotor-Gene analysis software.

This high precision and contamination free liquid handling was also tested for much smaller volumes using a second detection kit: 15 µl of the Master Mix (RealArtTM Salmonella RG PCR reagents) and 5 µl of the Quantitation Standards (QS 1-4). Also here the high reproducibility and signal quality was achieved (Fig. 4 and 5).

Results and Discussion

In Quantitation Standard replicates the presence of the HBV genomic DNA was detected and quantified (Fig. 2; e.g. Salmonella: Fig. 4). The dynamic detection range in the HBV experiment was between 10^2 and 10^5 IU/µl (as defined by the quantitation standards) setting this technology far below the detection limits for any immuno-serological method [4]. In addition a very high reproducibility and excellent signal conformity of the sample replicates indicates a very precise sample preparation during the reagent pipetting. No cross contamination was observed between quantitation standards and all negative controls. Positive signals for the Internal Control in channel A. JOE ruled out the possibility of PCR inhibition (Fig. 3 (HBV) and Fig. 5 (salmonella)). This was ensured by the unique free-jet dispensing technology and the optical sensor-based, no-contact liquid level detection system employed in the ep*Motion* 5070.

Conclusion

In summary, we report in this study the use of a fully automated Real Time qPCR protocol for the high-throughput quantitative viral RNA respectively DNA as well as bacterial DNA testing. The state-of-the-art automated system, ep*Motion* 5070 (Eppendorf), is shown to provide very precise and reproducible performance in the pipetting process of the sensitive qPCR samples with conjunction with the commercially available PCR systems on the Rotor Gene (artus GmbH and Corbett Research). The reliability and error free execution of the complex steps provided by this system are the significant advantages for the most molecular biology and clinical research applications employing Real Time qPCR.

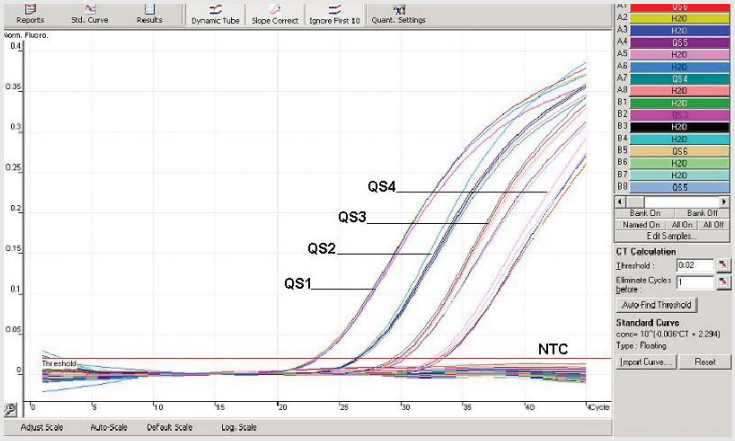

Fig. 2: A FAM channel for direct detection of the HBV-specific PCR product.
Note the reproducibility of the sample replicates. No contamination were detected.
QS = quantitation standard, NTC = no template control

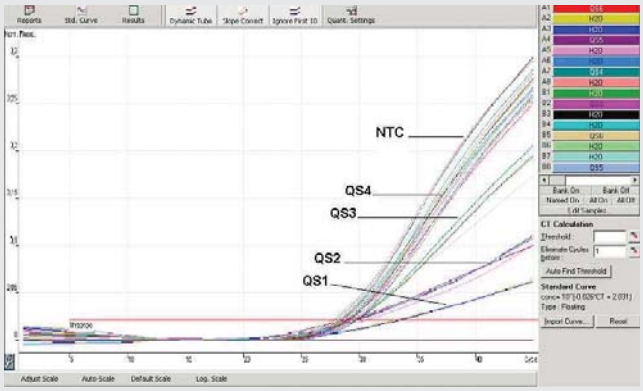

Fig. 3: A.JOE channel for detection of the internal control samples in presence of the specific standard PCR products.
A positive signal from the internal control rules out the possibility of PCR inhibition when no signal is present from the HBV PCR.
QS = quantitation standard, NTC = no template control

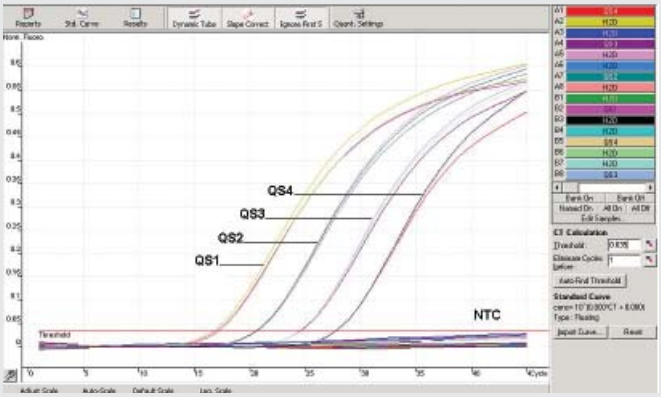

Fig. 4: A.FAM channel for direct detection of the Salmonella PCR product.
During the sample preparation the components were scaled down by 60% as compared to the HBV samples (Fig. 2). Note comparable high reproducibility of sample replicates.
QS = quantitation standard, NTC = no template control

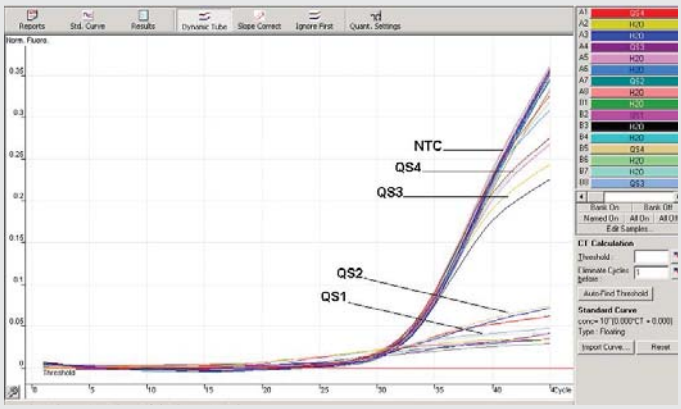

Fig. 5. A.JOE channel for detection of the internal control samples in presence of the specific standard PCR products.
A positive signal from the internal control rules out the possibility of PCR inhibition when no signal is present from the Salmonella PCR.
QS = quantitation standard, NTC = no template control.

References

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[5] Apostel F. (2003) Facilitating PCR setup via an automated liquid handling system. International Biotechnology Laboratory; 12: 1-2

Ordering information

	Order no.	
epMotion® 5070	**International**	**Brinkmann USA**
Liquid Handling Workstation 5070 basic device incl. control panel, software, Optical Sensor, waste box, MMC(tm) and reader, operating instructions 50/60 Hz, 100 - 130 V	5070 000.018	960000005
twin.tec PCR plate 96, skirted (Wells colourless)	0030 128.648	951020303
Safe-Lock micro test tubes (colourless)	0030 120.086	022363204
Thermoblock for PCR, 96 wells	5075 766.000	960002083

For the ordering information of the dispensing tools, accessories and consumables please refer to *www.eppendorf.com or www.epmotion.com.*

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