



Hepatitis B virus (HBV) detection & quantitation using real-time qPCR (SYBR Green format).

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This protocol describes the real-time quantitative assay for hepatitis B virus (HBV) detection and quantitation suitable for all HBV genotypes. Hepatitis B virus is a double stranded DNA virus and a species of the genus Orthohepadnavirus, which is likewise a part of the Hepadnaviridae family of viruses. HBV is classified as the type species of the Orthohepadnavirus, which contains three additional species: The Ground squirrel hepatitis virus, Woodchuck hepatitis virus and the Woolly monkey hepatitis B virus. The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins. These serotypes are based on a common determinant (a) and two mutually exclusive determinant pairs (d/y and w/r). The viral strains have also been divided into ten genotypes (A–J) and forty subgenotypes according to overall nucleotide sequence variation of the genome. The genotypes have distinct geographical distributions and are associated to differing disease severity, disease progression, treatment response, vaccination success and likelihood of complications. The serotypes and genotypes do not correspond. Genotype D has 10 sub-genotypes.

Oligonucleotide primers

Name	Target	Sequence*	bp	%GC	Tm	Position†	Amplicon	Ref
HBVrt-F	HBV	5'-CCT-ggY-TAT-CgY-Tgg-ATg-TgT-3'	21	54	59	362-382	116	1
HBVrt-R	HBV	5'-ggA-CAK-ACg-ggC-AAC-ATA-CCT-3'	21	55	57	457-477		

* Reverse oligonucleotide primer sequences given in this table are the reverse-complement of the sequence present in the alignments and as they should be ordered for synthesis. Hairpin (Hair), homodimer (Hm) and heterodimer (Ht) ΔQ 's are given in kcal/mol.

† Primer binding sites given on table are based on the WHO International Standard for HBV reference sequence (GenBank: KY003230.1).





qPCR components and conditions

Uses Biorad iTaq (Cat. No.: 1725150) qPCR Master Mix.

dH ₂ O	cf	3.7 µL
Master mix	1 x	5 µL
10 µM Forward oligo	150 nM	0.15 µL
10 µM Reverse oligo	150 nM	0.15 µL
Template	10 ng	1 µL
		vf: 10 µl



Run generic program in Applied Biosystems 7500



Total time: 2:10 hrs		
95 °C	3 min	40 cycles
94 °C	15 sec	
60 °C §	60 sec	
95 °C	15 sec	
60 °C	20 sec	
Ramp §	60 min	
95 °C	15 sec	

§ Data acquisition

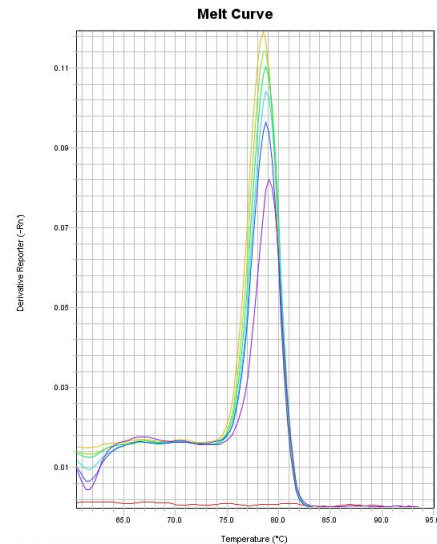
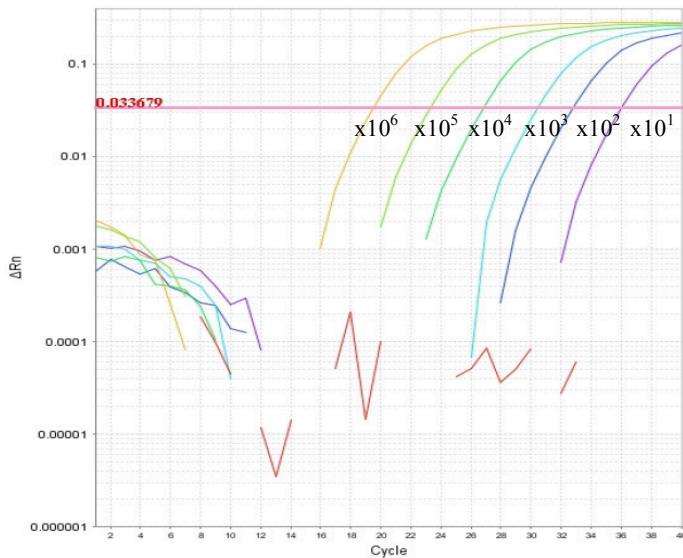
Titration curve preparation for quantitative analysis of viral titres

	Plasmid stock	dH ₂ O vol	Ct
Working	2x10 ⁷ cp/µL	-	-
1 st	2x10 ⁶ cp/µL	10 µL	19.48
2 nd	2x10 ⁵ cp/µL	10 µL	23.19
3 rd	2x10 ⁴ cp/µL	10 µL	26.79
4 th	2x10 ³ cp/µL	10 µL	30.43
5 th	2x10 ² cp/µL	10 µL	32.76
6 th	2x10 ¹ cp/µL	10 µL	35.96

Note: Add 90 µL to each of the 6 PCR 0.2 mL tubes. Take 10 µL of initial working stock (at 2x10⁷ cp/µL) and dispense into 1st PCR tube, wash tip 30 times, cap, vortex for 10 seconds and spin down for 10 seconds. Retrieve 10 µL from volumetric centre of PCR tube and dispense into 2nd PCR tube repeating the same procedure for further dilutions.



Performance summary



Standard curve: $m = -3.2$, $Y = 40.54$, $R^2 = 0.995$
 Target amplicon T_m : $78.5^\circ\text{C} \pm 0.3 \text{ SD } (78.2-78.8^\circ\text{C})$
 Limit of detection (LODet): $2 \times 10^1 \text{ cp}/\mu\text{L}$
 Limit of discrimination (LODis): $2 \times 10^1 \text{ cp}/\mu\text{L}$

Notes

1. Clean workbench with 0.1% NaOCl 0.1% followed by 70% Ethanol before and after work.
2. Preparation of all mastermixes should only be performed in the pre-PCR workbench.
3. Addition of sample DNA should only be performed in the pre-PCR workbench.
4. Addition of positive template DNA should be performed on instrument (post-PCR) area.
5. All mastermixes should be prepared on ice to prevent excess evaporation.
6. Vortex and spin all mastermixes before and after aliquoting to PCR tubes.



References

1. Sitnik R, Paes A, Manguera CP, Pinho JR. A real-time quantitative assay for hepatitis B DNA virus (HBV) developed to detect all HBV genotypes. Rev Inst Med Trop Sao Paulo. 2010 May-Jun;52(3):119-24. PubMed PMID: 20602019

Revision history

- 1.0 Original document.
- 2.0 Formatting changes to oligonucleotide sequences.
- 3.0 Optimized protocol.
- 4.0 Changes to document format only.

