Improved HBV-RNA quantification by HBV-RNA reverse transcription droplet digital PCR

EASL CONGRESS Milan, Italy 5–8 June 2024

Bernhard Kleter [1], Sanne van Gils [1], Joël Meyboom [1], Thierry Verbinnen [2], Oliver Lenz [3] and Leen-Jan Doorn [1]

[1] CerbaResearch NL, Rijswijk, The Netherlands, [2] Janssen Research & Development, Beerse, Belgium, [3] Janssen Pharmaceutica, Beerse, Belgium

Contact information
Leen-Jan.vandoorn@cerbaresearch.com
Visseringlaan 25, 2288ER Rijswijk, The Netherlands

Cerba Research
Your precision medicine partner

Introduction

HBV-RNA is a surrogate marker for cccDNA and used as an exploratory biomarker in chronic HBV infection. HBV-RNA can be specifically detected in plasma, often even when HBV DNA is completely suppressed by e.g. nucleos(t)ide analogue treatment.

Aim

An HBV-RNA RT droplet digital PCR (ddPCR) assay was developed to improve sensitivity and extend the lower quantifiable range as ddPCR is most effective at low target copy numbers.

Compare the validated HBV-RNA high sensitivity RT-qPCR assay and the HBV RNA RT-ddPCR assay in plasma samples of CHB patients.

Assess longitudinal HBV-RNA and HBV-DNA profiles in 6 patients. See Figure 4.

Method

133 plasma samples from patients with chronic hepatitis B participating in Janssen clinical trials, JADE (NCT03361956), OCTOPUS-1 (NCT05275023) and PENGUIN (NCT04667104) were tested for HBV RNA by both RT-qPCR and ddPCR. See Table 2 and 3.

From the OCTOPUS-1 and PENGUIN studies baseline samples from virologically suppressed patients were tested (HBV DNA <20 IU/mL detected or negative). In these 2 studies, 52 samples were tested with 200 µL due to sample volume shortage.

From JADE studies longitudinal samples were tested in which 28 samples were virologically suppressed.

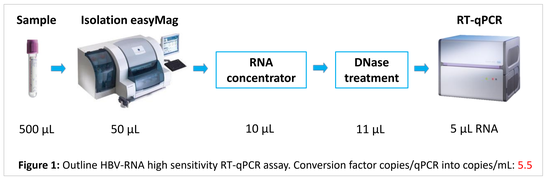

Figure 1: Outline HBV-RNA high sensitivity RT-qPCR assay. Conversion factor copies/qPCR into copies/mL: 5.5

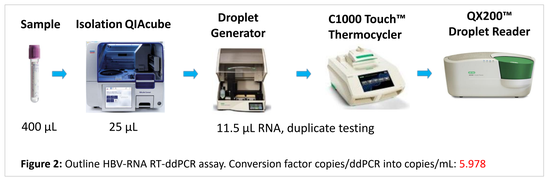

Figure 2: Outline HBV-RNA RT-ddPCR assay. Conversion factor copies/ddPCR into copies/mL: 5.978

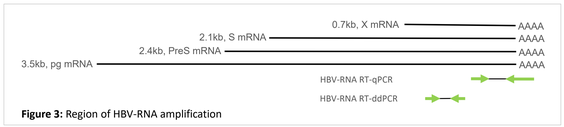

Figure 3: Region of HBV-RNA amplification

For assay characterization, a dilution series of HBV in vitro transcribed pregenomic RNA were tested by both HBV RNA assays. For determination of the LOB Armbruster's approach was used, 95% LOD by probit analysis, LLOQ and ULOQ according to FDA-guidelines. See Table 1.

Results

Table 1: Characteristics HBV RNA assays

Sensitivity	HBV RNA RT-qPCR copies/mL	HBV RNA RT-ddPCR copies/mL
LOB	-	4.4*
LOD	25	16**
LLOQ	869	141
ULOQ	>275.000.000	N.D.

**LOB*: ± 1 positive droplet/ddPCR; ***LOD*: ± 4 positive droplets/ddPCR

Table 2: HBV RNA testing

	HBV RNA RT-qPCR	HBV RNA RT-ddPCR
<LOD	65 (49%)	39 (29%)
LOD<x<LLOQ	53 (40%)	49 (37%)
>LLOQ	15 (11%)	45 (34%)

HBV RNA testing in 133 samples

Table 3: Comparison of HBV RNA testing in 133 samples

		HBV RNA RT-ddPCR		
		<LOD (<16cps/mL)	LOD<x<LLOQ	>LLOQ (>141cps/mL)
HBV RNA high sensitivity RT-qPCR	<LOD (25cps/mL)	35	25	5
	LOD<x<LLOQ	4	24	25
	>LLOQ (<869cps/mL)	0	0	15

Concordant 74 patients (56%)

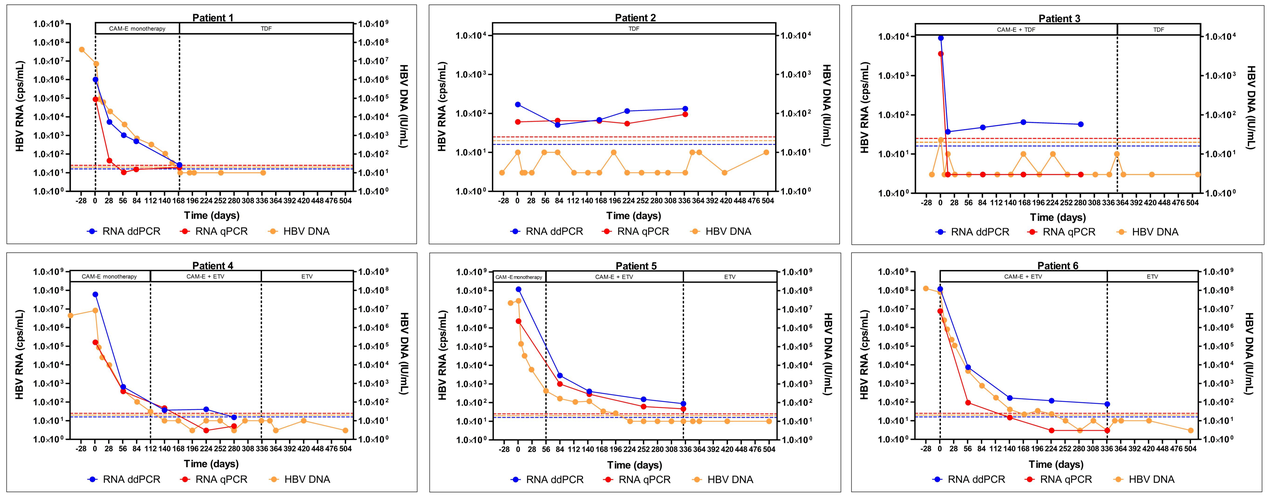

Figure 4: Longitudinal HBV-RNA and HBV-DNA profiles in 6 patients participating in the JADE study**.** LOD: RNA ddPCR 16 cps/mL, RNA qPCR 25 cps/mL, HBV DNA 20 IU/mL. Dotted lines represent LOD for each of the assays. For plotting purposes, imputation values were used when HBV-RNA RT-qPCR and HBV-DNA was <LOD.

Conclusions

Results show a clearly improved sensitivity and quantification of HBV RNA by the HBV-RNA RT-ddPCR assay. Due to the higher sensitivity in quantification of low HBV RNA concentrations, the response to treatment can be assessed for a longer period. This makes the HBV-RNA RT-ddPCR assay highly suitable for monitoring patients during antiviral treatment in clinical trials.

References

Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. *Clin Biochem Rev. 2008 Aug;29 Suppl 1(Suppl 1):S49-52. PMID: 18852857; PMCID: PMC2556583.*

Food and Drug Administration. Bioanalytical Method Validation: Guidance for Industry. *05/24/2018 www.fda.gov/media/70858/download*

Improved HBV-RNA quantification by HBV-RNA reverse transcription droplet digital PCR

EASL CONGRESS Milan, Italy 5–8 June 2024

Bernhard Kleter [1], Sanne van Gils [1], Joël Meyboom [1], Thierry Verbinnen [2], Oliver Lenz [3] and Leen-Jan Doorn [1]

[1] CerbaResearch NL, Rijswijk, The Netherlands, [2] Janssen Research & Development, Beerse, Belgium, [3] Janssen Pharmaceutica, Beerse, Belgium

Contact information
Leen-Jan.vandoorn@cerbaresearch.com
Visseringlaan 25, 2288ER Rijswijk, The Netherlands

Cerba Research
Your precision medicine partner

Introduction

HBV-RNA is a surrogate marker for cccDNA and used as an exploratory biomarker in chronic HBV infection. HBV-RNA can be specifically detected in plasma, often even when HBV DNA is completely suppressed by e.g. nucleos(t)ide analogue treatment.

Aim

An HBV-RNA RT droplet digital PCR (ddPCR) assay was developed to improve sensitivity and extend the lower quantifiable range as ddPCR is most effective at low target copy numbers.

Compare the validated HBV-RNA high sensitivity RT-qPCR assay and the HBV RNA RT-ddPCR assay in plasma samples of CHB patients.

Assess longitudinal HBV-RNA and HBV-DNA profiles in 6 patients. See Figure 4.

Method

133 plasma samples from patients with chronic hepatitis B participating in Janssen clinical trials, JADE (NCT03361956), OCTOPUS-1 (NCT05275023) and PENGUIN (NCT04667104) were tested for HBV RNA by both RT-qPCR and ddPCR. See Table 2 and 3.

From the OCTOPUS-1 and PENGUIN studies baseline samples from virologically suppressed patients were tested (HBV DNA <20 IU/mL detected or negative). In these 2 studies, 52 samples were tested with 200 µL due to sample volume shortage.

From JADE studies longitudinal samples were tested in which 28 samples were virologically suppressed.

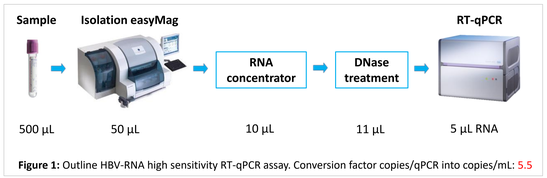

Figure 1: Outline HBV-RNA high sensitivity RT-qPCR assay. Conversion factor copies/qPCR into copies/mL: 5.5

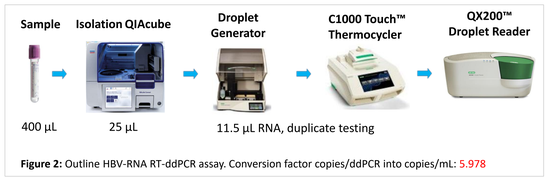

Figure 2: Outline HBV-RNA RT-ddPCR assay. Conversion factor copies/ddPCR into copies/mL: 5.978

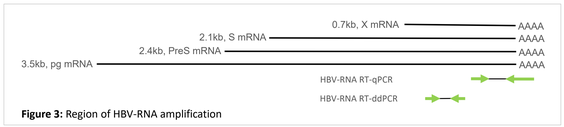

Figure 3: Region of HBV-RNA amplification

For assay characterization, a dilution series of HBV in vitro transcribed pregenomic RNA were tested by both HBV RNA assays. For determination of the LOB Armbruster's approach was used, 95% LOD by probit analysis, LLOQ and ULOQ according to FDA-guidelines. See Table 1.

Results

Table 1: Characteristics HBV RNA assays

Sensitivity	HBV RNA RT-qPCR copies/mL	HBV RNA RT-ddPCR copies/mL
LOB	-	4.4*
LOD	25	16**
LLOQ	869	141
ULOQ	>275.000.000	N.D.

**LOB*: ± 1 positive droplet/ddPCR; ***LOD*: ± 4 positive droplets/ddPCR

Table 2: HBV RNA testing

	HBV RNA RT-qPCR	HBV RNA RT-ddPCR
<LOD	65 (49%)	39 (29%)
LOD<x<LLOQ	53 (40%)	49 (37%)
>LLOQ	15 (11%)	45 (34%)

HBV RNA testing in 133 samples

Table 3: Comparison of HBV RNA testing in 133 samples

		HBV RNA RT-ddPCR		
		<LOD (<16cps/mL)	LOD<x<LLOQ	>LLOQ (>141cps/mL)
HBV RNA high sensitivity RT-qPCR	<LOD (25cps/mL)	35	25	5
	LOD<x<LLOQ	4	24	25
	>LLOQ (<869cps/mL)	0	0	15

Concordant 74 patients (56%)

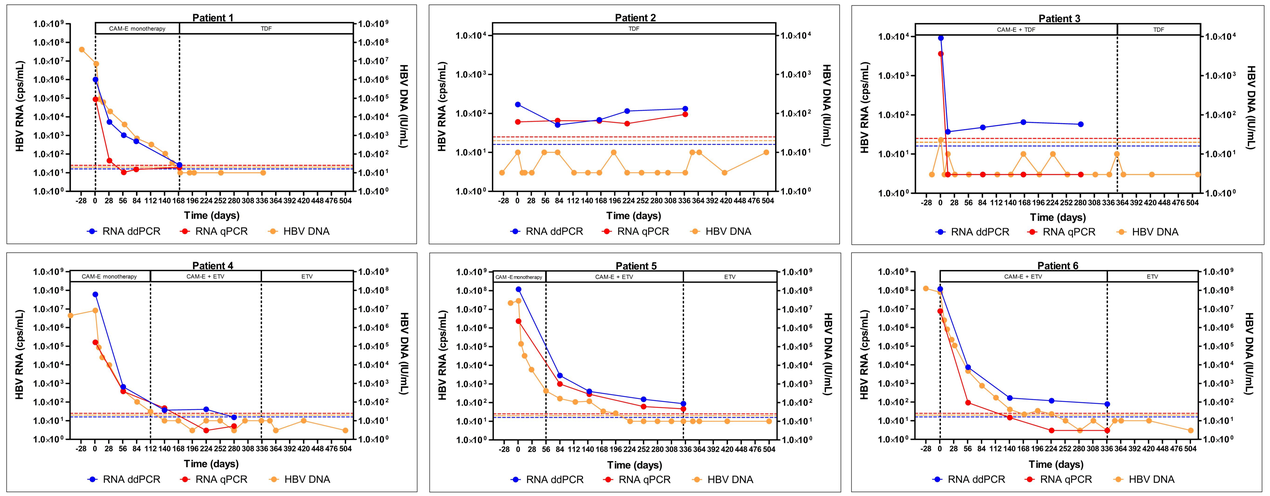

Figure 4: Longitudinal HBV-RNA and HBV-DNA profiles in 6 patients participating in the JADE study**.** LOD: RNA ddPCR 16 cps/mL, RNA qPCR 25 cps/mL, HBV DNA 20 IU/mL. Dotted lines represent LOD for each of the assays. For plotting purposes, imputation values were used when HBV-RNA RT-qPCR and HBV-DNA was <LOD.

Conclusions

Results show a clearly improved sensitivity and quantification of HBV RNA by the HBV-RNA RT-ddPCR assay. Due to the higher sensitivity in quantification of low HBV RNA concentrations, the response to treatment can be assessed for a longer period. This makes the HBV-RNA RT-ddPCR assay highly suitable for monitoring patients during antiviral treatment in clinical trials.

References

Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. *Clin Biochem Rev. 2008 Aug;29 Suppl 1(Suppl 1):S49-52. PMID: 18852857; PMCID: PMC2556583.*

Food and Drug Administration. Bioanalytical Method Validation: Guidance for Industry. *05/24/2018 www.fda.gov/media/70858/download*