

# Performance Characteristics of the Qualitative and Quantitative Target-Capture PCR Based HBV NAT Assay

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## ABSTRACT

**Background:** The application of nucleic acid amplification technology (NAT) has dramatically improved the detection of HIV-1 and HCV at earlier stages of infection. The next anticipated NAT assay includes testing for HBV DNA. Here we describe the development of a Target-Capture PCR technology based NAT assay for HBV.

**Materials and Methods:** A magnetic bead based protocol was used for isolation of HBV DNA target along with a related internal control in a single tube from 0.5 mL plasma. Region X of the isolated target on the beads was amplified by PCR technology. The qualitative assay was validated for Analytical and Clinical sensitivity, Specificity, and Reproducibility. The quantitative assay utilizes a 12 member panel of standards tested in triplicates in the range of 50-10<sup>5</sup> IU/mL. A blinded panel of HBV samples by QCMD were tested for detection and quantitation.

**Results:** The analytical sensitivity of the Qualitative Target-Capture PCR HBV assay determined with WHO HBV standard indicated >95% positivity at 15 IU/mL. The assay detects all known genotypes, has very high specificity and tolerates a variety of anti-coagulants, interfering substances, and plasma from pathological conditions. The Quantitative assay estimates HBV in the unknowns in the range of 10<sup>2</sup>-10<sup>9</sup> IU/mL. The QCMD BBV results indicate the detection of the lowest copy member and also very close estimation of IU/mL for the A genotype.

**Conclusion:** Using a combination of magnetic bead based target capture and real-time PCR amplification-detection a rapid, sensitive, user-friendly, accurate assay for HBV DNA detection and quantitation has been developed.

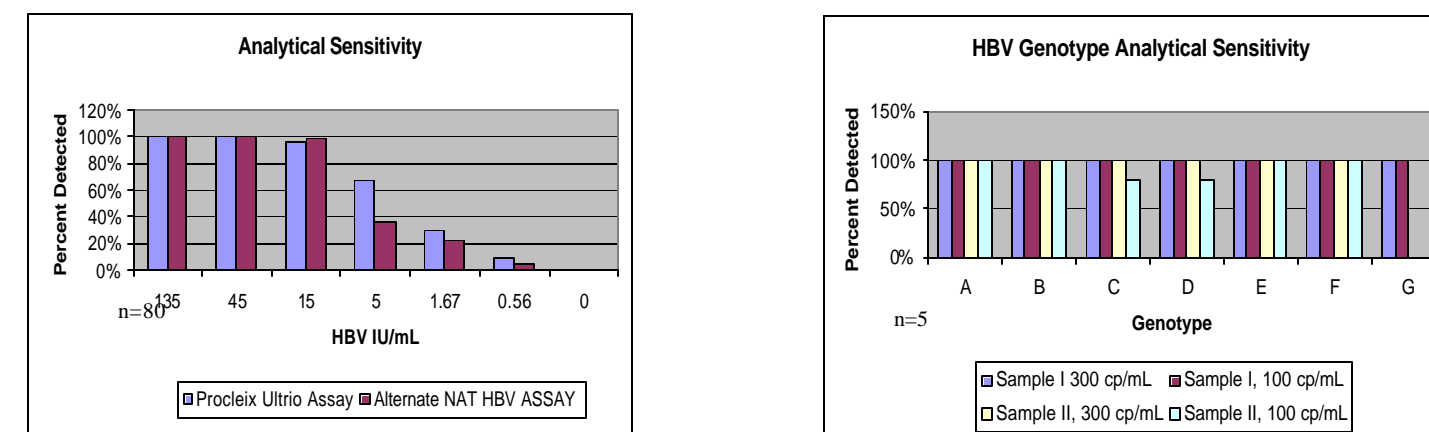
## METHODS

A magnetic bead based protocol was used for isolation of HBV DNA target along with a related internal control in a single tube from 0.5 mL plasma. Region X of the isolated target on the beads was amplified by PCR technology. The qualitative assay was validated for Analytical and Clinical sensitivity, Specificity, and Reproducibility. The quantitative assay utilizes a 12 member panel of standards tested in triplicates in the range of 50-10<sup>5</sup> IU/mL. A blinded panel of HBV samples by QCMD were tested for detection and quantitation.

Test Condition	Copy Levels Tested	Number of Replicates
Analytical Sensitivity Using dilutions of WHO HBV Standard	135,45,15,5,1.67, .56 IU/mL	n=80
Analytical Sensitivity Study for HBV Genotypes	300, 100 copies/mL	n=5
Clinical Sensitivity Using Naturally Infected Specimens	Dynamic Range of copy levels and genotypes	50 individual specimens
Specificity of random donor specimens using 2 lots of reagents	negative	500 individual specimens per lot
Specificity for other blood borne pathogens	negative for HBV, Positive for HCV, HTLV, Rubella, HAV, CMV, EBV, HSV, HIV-1, HIV-2	Number of specimens per pathogen: HCV(5), HTLV(5), Rubella(5), HAV(5), CMV(5), EBV(2), HSV(5), HIV-1(2), HIV-2(2) n=5 for each specimen
Anticoagulant Study including K2EDTA, K3EDTA, CPD, CPDA-1, CP2D, ACD, PPT, Serum and Na Heparin	45, 15, and 0 IU/mL	n=5
Potentially interfering substances including hemolyzed, icteric, and lipemic samples as well as specimens spiked with Propionibacterium spp., Corynebacterium spp., Micrococcus spp., Staphylococcus aureus, and Staphylococcus epidermidis. Also elevated lev	45, 15, and 0 IU/mL	n=5
Freeze-thaw study required samples undergo 1,3, and 5 freeze-cycles prior to testing	45, 15, and 0 IU/mL	n=5
Reproducibility Study included testing 2 lots of reagents using 2 operators on 2 days	45 and 0 IU/mL	n=480 @ 45 IU/mL and n=96 @ 0 IU/mL

## RESULTS

### Analytical Sensitivity



Analytical Sensitivity Estimates of the Chiron Alternate NAT HBV Assay

	Alternate NAT (IU/mL)	Procleix® Ultrio™ (IU/mL)
ED <sub>50</sub>	4.16 [2.89-6.03]*	2.71 [1.87-3.91]
ED <sub>95</sub>	22.87 [14.32-44.75]	14.91 [9.45-28.52]

\*95% confidence Interval

### Reproducibility

Each Operator Evaluated 8 Reagent Combinations, n=15 at 45 IU/mL and n=3 at 0 IU/mL

Operator	Day	# Hits/# tested at 45 IU/mL	# Hits/# tested at 0 IU/mL
1	1	120	24
1	2	120	24
2	1	120	24
2	2	120	24
Total		480	96

Testing Performed at Bayer Reference Testing Laboratory in Berkeley, CA

### Specificity

Condition	Spike Level of Condition	% Hit @ 45 IU/mL	% Hit @ 15 IU/mL	% Hit @ 0 IU/mL
Hemolyzed	Specified by Vendor	100%	100%	0%
Icteric	Specified by Vendor	100%	100%	0%
Lipemic	Specified by Vendor	100%	100%	0%
Propionibacterium spp	10 <sup>6</sup> CFU/mL	100%	100%	0%
Corynebacterium spp	10 <sup>6</sup> CFU/mL	100%	100%	0%
Micrococcus spp	10 <sup>6</sup> CFU/mL	100%	100%	0%
S. aureus	10 <sup>6</sup> CFU/mL	100%	100%	0%
S. epidermidis	10 <sup>6</sup> CFU/mL	100%	100%	0%
Protein	75 g/L	100%	100%	0%
Uridic	3000 mg/dL	100%	100%	0%
Hemoglobin	500 mg/dL	100%	100%	0%
Bilirubin	20 mg/dL	100%	100%	0%

### Anticoagulants

Anticoagulant	% Hit @ 45 IU/mL	% Hit @ 15 IU/mL	% Hit @ 0 IU/mL
K2EDTA	100%	100%	0%
K3EDTA	100%	100%	0%
CPD	100%	100%	0%
CPDA-1	100%	100%	0%
CP2D	100%	80%	0%
ACD	100%	100%	0%
PPT Tubes	100%	100%	0%
Serum	100%	100%	0%
Na Heparin	100%	100%	0%

### 1000 Random Donors Tested Using 2 Reagent Lots

Lot	Results of test # Pos/Total tested
1	0/500
2	0/500

### Freeze-thaw Study

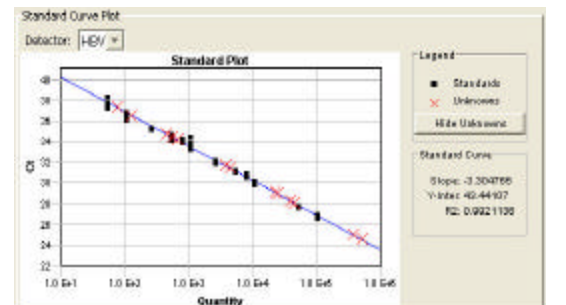
# of Freeze-thaws	% Hit @ 45 IU/mL	% Hit @ 15 IU/mL	% Hit @ 0 IU/mL
1	100%	100%	0%
3	100%	100%	0%
5	100%	100%	0%

### Clinical Sensitivity

Sample	Alternate NAT HBV Results	HBsAg	Amplicor (cp/mL)	Genotype	Sample	Alternate NAT HBV Results	HBsAg	Amplicor (cp/mL)	Genotype
1	POS	Positive	1.18E+04	E	26	POS	Positive	1.48E+03	HD
2	POS	Positive	5.80E+03	D	27	POS	Positive	2.45E+03	C
3	POS	Positive	7.15E+03	D	28	POS	Positive	4.36E+03	A
4	POS	Positive	5.34E+06	D	29	POS	Positive	1.81E+03	G/A
5	POS	Positive	9.72E+03	D/A	30	POS	Positive	7.91E+03	D/A
6	POS	Positive	3.02E+03	C/D	31	POS	Positive	1.48E+05	A
7	POS	Positive	4.08E+04	D/E	32	POS	Positive	3.23E+03	A
8	POS	Positive	1.57E+03	C/D	33	POS	Positive	2.10E+03	E/A
9	POS	Positive	1.84E+03	C/D	34	POS	Positive	1.04E+04	D
10	POS	Positive	3.32E+04	A	35	POS	Positive	4.29E+03	A
11	POS	Positive	5.71E+05	A	36	POS	Positive	4.21E+03	C/B
12	POS	Positive	1.23E+05	C	37	POS	Positive	3.34E+03	D
13	POS	Positive	3.02E+05	D	38	POS	Positive	1.80E+03	A
14	POS	Positive	7.70E+05	C	39	POS	Positive	9.17E+03	D
15	POS	Positive	2.88E+03	F	40	POS	Positive	1.76E+04	F
16	POS	Positive	5.32E+06	F	41	POS	Positive	4.00E+07	F
17	POS	Positive	1.51E+03	F	42	POS	Positive	6.47E+03	A
18	POS	Positive	1.71E+04	A	43	POS	Positive	1.80E+03	C
19	POS	Positive	1.37E+04	D	44	POS	Positive	1.40E+03	B
20	POS	Positive	1.06E+04	D	45	POS	Positive	1.65E+08	C
21	POS	Positive	3.87E+03	C	46	POS	Positive	1.97E+04	A
22	POS	Positive	2.30E+04	A	47	POS	Positive	6.30E+09	C
23	POS	Positive	8.70E+08	D	48	POS	Positive	1.51E+05	A
24	POS	Positive	1.22E+03	E	49	POS	Positive	2.40E+04	A
25	POS	Negative	3.12E+04	D	50	POS	Positive	4.19E+03	A

### Quantitative HBV NAT Assay

A 12-member panel ranging from 50 IU/mL to 100,000 IU/mL is run in triplicates. A standard curve is then created plotting Ct vs. Quantity, which allows for Ct-based quantitation of the panel members.



### Standardization Against the WHO Standard

WHO Standard Concentration IU/mL	Predicted	Observed	Accuracy %
1000	1000	1063	106
135	135	191	142
45	45	63	140

### Quantitation of 2003 QCMD HBV Panel

QCMD Code	SubType	Concentration Target-Capture PCR (IU/mL)	HBV Concentration (cp/mL) Calculated from Target-Capture PCR Results*	Published QCMD Results	Accuracy %
HBV-01	A	547,239	1.4E+06	1.0E+06	140
HBV-02	A	288	7.4E+02	1.0E+03	74
HBV-03	-	0	0	0	100
HBV-04	D	14,265	3.70E+04	1.0E+05	37
HBV-05	A	4,077	1.00E+04	1.0E+04	100
HBV-06	A	1,155	3.00E+03	3.0E+03	100
HBV-07	A	87	226	200.00	113
HBV-08	A	39.445	1.00E+05	1.0E+05	100

\* Each result is the average of triplicate determinations in IU/mL, converted to cp/mL using the formula: 1 IU = 2.6 Cps

### Istituto Superiore di Sanità (Italy) Blinded HBV EQA/1 Study

Sample	Expected	IC Ct	HBV Ct	Qualitative		Quantitative	
				Result	IU/mL	StDev	%CV
ISS 1	100	42.11	37.85	+	104	41.61	40%
ISS 2	0	38.89	50.00	-	0	0.00	NA
ISS 3	1000	40.31	33.37	+	1606	121.19	8%
ISS 4	100	41.63	37.08	+	104	29.94	29%
ISS 5	0	40.66	50.00	-	0	0.00	NA
ISS 6	1000	37.12	33.25	+	1405	422.95	0.30

## OBJECTIVES

- Demonstrate the analytical and genotypic sensitivity of the Alternative NAT HBV Assay
- Demonstrate the Assay's tolerance for various anticoagulants and inhibitory factors present in specimens, as well as multiple freeze-thaws.
- Show Assay specificity against other blood borne pathogens
- Prove reproducibility utilizing multiple lots of reagents, on multiple days and multiple technicians
- Show efficacy of the quantitative Target-Capture PCR HBV Assay

## SUMMARY AND CONCLUSIONS

- An Alternative NAT HBV assay has been validated using an exhaustive 2750 test protocol, and meets the criterion for sensitivity, specificity and reproducibility
- Using the WHO HBV Standard as a sensitivity panel, the Alternative NAT HBV Assay detected 100% at 45 IU/mL and 99% at 15 IU/mL
- The Alternate Assay is being used as a supplemental test to confirm samples positive by the Procleix® dHBV assay. The testing of TMA positive HBV samples will be performed at the Bayer Reference Testing Laboratory
- The Quantitative Target-Capture PCR HBV Assay has been successfully validated against the QCMD panel and is currently in use to support Procleix® Ultrio™ Assay and Procleix® TIGRIS® System clinical trials