

# Validation Of the Alternative NAT HBV Assay: A Highly Sensitive PCR Based Assay For HBV DNA

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## INTRODUCTION

Currently the blood supply in the US and several European and Asian countries is being screened by Nucleic Acid Techniques (NAT) test for HIV-1 and HCV. The NAT technologies have demonstrated the potential for substantial window period closure when compared to serology assays on the market. Nearly 80% of whole blood in the US is tested by the FDA approved Procleix<sup>®</sup> HIV-1/HCV qualitative assay. The next anticipated NAT assay-Procleix<sup>®</sup> Ultrio<sup>™</sup> Assay includes simultaneous testing for HBV DNA along with HCV and HIV-1 RNA is being co-developed by Gen-Probe, Inc. and Chiron Corp. HBV persistently infects 350 million people worldwide. The analytical sensitivity of Procleix<sup>®</sup> Ultrio<sup>™</sup> Assay for HBV DNA is in the range of 10-15 IU/ml, resulting in a window period reduction of 11-20 days. In Asian countries the yield cases through NAT testing is expected to be as high as 1 per 60,000 in pools of 50, and in the US 1 per 2,000,000 in pools of 16.

Nucleic acid tests by alternate technologies for all three viruses are required for clinical trials and are used as supplemental assays to confirm samples positive by the Procleix<sup>®</sup> Ultrio<sup>™</sup> Assay. We have developed and validated a Bead capture-Taqman<sup>®</sup> based Alternative NAT HBV assay for confirming Procleix<sup>®</sup> Ultrio<sup>™</sup> Assay positives.

Polymerase chain reaction (PCR), the basis for Taqman<sup>®</sup> assay while highly efficient at detecting small concentrations of genetic material, has proven to be very sensitive to potentially inhibiting substances. Endogenous substances, such as hemoglobin and lipids, therapeutic drugs and commonly used anticoagulants for specimen collection such as heparin and sodium citrate can potentially interfere with amplification. Specimen handling, storage conditions and freeze-thaws are also shown to decrease the level of viral DNA, and therefore the detection following PCR.

In consultation with Regulatory, Validation, Quality Assurance and Biostatistics groups, with a total of 2750 samples, an exhaustive validation protocol was developed and executed to evaluate the performance of the Alternative NAT HBV assay. Assay sensitivity and specificity were tested rigorously. Reproducibility studies were performed utilizing a collaboration with Bayer Reference Testing Laboratory in Berkeley, CA.

## OBJECTIVES

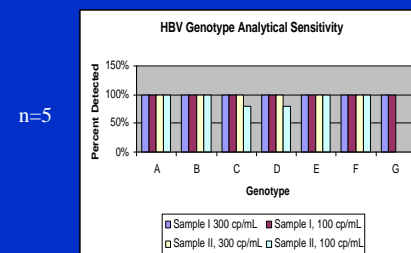
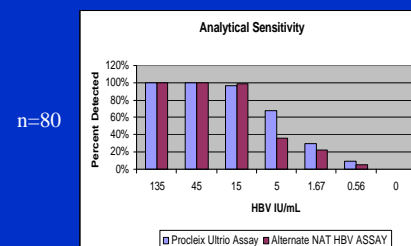
- Demonstrate the analytical and genotypic sensitivity of the Alternative NAT HBV Assay
- Demonstrate the Assay's tolerance for various anticoagulants and inhibitory factors present in specimens, as well as multiple freeze-thaws.
- Show Assay specificity against other blood borne pathogens
- Prove reproducibility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

## MATERIALS AND METHODS

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, 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 levels of hemoglobin, bilirubin, lipids, and protein	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

- ED<sub>50</sub> @ 4.16 [2.89-6.03]\* IU/mL
- ED<sub>95</sub> @ 22.87 [14.32-44.75] IU/mL
- \*95% confidence Interval

## RESULTS, continued

### Specificity

Condition	Spike Level of Condition	n=5			Pathogen	Results of test # Pos/Total tested
		% Hit @ 45 IU/mL	% Hit @ 15 IU/mL	% Hit @ 0 IU/mL		
Hemolyzed	Specified by Vendor	100%	100%	0%	HCV	0/25
Icteric	Specified by Vendor	100%	100%	0%	HTLV	0/25
Lipemic	Specified by Vendor	100%	100%	0%	Rubella	0/25
Propionibacterium spp	10 <sup>4</sup> 5 CFU/mL	100%	100%	0%	HAV	0/25
Corynebacterium spp	10 <sup>4</sup> 5 CFU/mL	100%	100%	0%	CMV	0/25
Micrococcus spp	10 <sup>4</sup> 5 CFU/mL	100%	100%	0%	EBV	0/25
S. aureus	10 <sup>4</sup> 5 CFU/mL	100%	100%	0%	HSV	0/25
S. epidermidis	10 <sup>4</sup> 5 CFU/mL	100%	100%	0%	HIV-1	0/25
Protein	75 g/L	100%	100%	0%	HIV-2	0/25
Lipids	2500 mg/L	100%	100%	0%		
Hemoglobin	900 mg/L	100%	100%	0%		
Bilirubin	50 mg/L	100%	100%	0%		

### Anticoagulants

Anticoagulant	n=5		
	% 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	n=5		
	% Hit @ 45 IU/mL	% Hit @ 15 IU/mL	% Hit @ 0 IU/mL
1	100%	100%	0%
3	100%	100%	0%
5	100%	100%	0%

### Reproducibility at Bayer Reference Testing Laboratory

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	120/120	0/24
1	2	120/120	0/24
2	1	120/120	0/24
2	2	120/120	0/24
Total		480/480	0/96

## RESULTS, continued

### Clinical Sensitivity

Sample	Alternate NAT HBV Results	HBsAg	Amplifier (cp/mL)	Genotype	Sample	Alternate NAT HBV Results	HBsAg	Amplifier (cp/mL)	Genotype
1	POS	Positive	1.18E+04	E	26	POS	Positive	1.48E+03	ND
2	POS	Positive	5.80E+03	D	27	POS	Positive	2.49E+03	C
3	POS	Positive	7.15E+03	D	28	POS	Positive	4.39E+03	A
4	POS	Positive	9.94E+03	D	29	POS	Positive	1.81E+03	C/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	4.09E+04	D/E	32	POS	Positive	3.23E+03	D
8	POS	Positive	1.57E+03	C/D	33	POS	Positive	2.18E+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	1.29E+03	C	37	POS	Positive	3.34E+03	D
13	POS	Positive	3.02E+03	D	38	POS	Positive	1.60E+03	A
14	POS	Positive	7.70E+06	C	39	POS	Positive	9.17E+03	D
15	POS	Positive	2.99E+03	F	40	POS	Positive	1.78E+04	F
16	POS	Positive	5.32E+03	F	41	POS	Positive	4.03E+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+09	B
20	POS	Positive	1.06E+04	D	45	POS	Positive	1.65E+03	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+03	D	48	POS	Positive	1.51E+05	A
24	POS	Positive	1.22E+03	E	49	POS	Positive	2.46E+04	A
25	POS	Negative	3.12E+04	D	50	POS	Positive	4.10E+03	A

## SUMMARY

- 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 assay specificity was confirmed using samples containing all major anticoagulants, as well as common interfering substances, blood borne pathogens, bacterial cultures, and multiple freeze thaws
- Data generated during the validation studies demonstrate that the Chiron Alternative NAT HBV Assay provides adequate sensitivity and specificity for use in confirmation of plasma specimens reactive by the Procleix<sup>®</sup> Ultrio<sup>™</sup> Assay or Discriminatory Assays under an investigational protocol.