

III. HBV NUCLEIC ACID TESTING

**Edward Tabor, M.D., Associate Director for Medical Affairs
Office of Blood Research and Review, CBER, FDA**

65th Meeting
March 16, 2000
Holiday Inn, Silver Spring
8777 Georgia Avenue
Silver Spring, MD

Revised January 5, 2000

Summary of a Workshop on Implementation of Nucleic Acid Testing

December 14, 1999
Bethesda, MD

Edward Tabor, M.D.

A Workshop on Implementation of Nucleic Acid Testing (NAT) was sponsored by CBER, FDA on December 14, 1999 at Masur Auditorium at the National Institutes of Health campus. Participants included IND sponsors who are testing blood and plasma donations by NAT, representatives of blood and plasma trade organizations, foreign regulatory officials, and FDA staff. The goals of the workshop were to discuss the progress and problems of putting NAT systems in place to detect viruses in blood and plasma.

It should be noted that, in the United States, it is expected that >99% of blood and plasma collected will be tested by NAT for both HCV and HIV-1 by the end of 1999. The only material not tested is thought to consist of collections by some small facilities and collections by the military.

Some of the important items discussed at the workshop were:

- **“Phase II” testing; returning HCV and HIV-1 NAT results before any components are transfused:** At present, fewer IND holders than expected have reached Phase II testing (i.e. completion of testing and delivery of results before Platelets are out-of-date), although all are expected to reach this point in the coming months. AIBC is already at Phase II for all testing. ARC hopes to have all testing at Phase II by January. ABC now has 60% of its testing in Phase II and they hope to have all in Phase II within six months.
- **Extent of testing by ARC:** It was indicated in the discussion that the ARC is currently only doing HCV and HIV-1 NAT on “consenting donors;” that is, 1-2% of ARC donors are NOT tested by NAT screening because they did not consent. ARC is planning to change this situation in the future.
- **Movement toward license applications for NAT:** Among the major IND holders, one stated that they were planning to submit a license application for pool testing soon and another said they would not submit a license application for pool testing until they had had a chance to prepare data for a similar and simultaneous application for individual unit testing (“single donor testing” or SDT). However, FDA is working to encourage the early submission of license applications by all holders of INDs under which large-scale screening by NAT is occurring.

- **Single donor testing:** The ultimate goal to improve sensitivity of NAT screening would be to progress from minipool testing to "single donor testing" or SDT. Concerns about this include increased cost, increased space needs, and increased personnel needs to do the testing. Although research on SDT is being actively conducted by at least one of the major IND holders, and it is definitely NOT being done by another of the major IND holders because of patent restrictions, it appears that some time will elapse before SDT is generally available.

- **HBV NAT:** Concern had been felt prior to the workshop that pressure to develop and implement NAT screening might occur sooner than practicable if the Japanese regulatory authorities required it for plasma or plasma derivatives imported to Japan, as had been suggested by rumors. However, during the workshop, an official of the Japanese regulatory agency stated that Japan would not require HBV NAT for plasma until U.S. manufacturers were able to do such testing. (However, Japan has been requiring HBV NAT testing for Whole Blood donations since October 1999.) Germany is not planning to require HBV NAT testing at present because they feel that the test is not sensitive enough yet. The Swiss Red Cross have withdrawn a request that ABC Recovered Plasma be tested for HBV by NAT.

Participants felt that the yield with HBV NAT would be low in the U.S. because of the combination of low viral load during the HBV window period and the lesser sensitivity of HBV NAT compared to HCV NAT. However, the rate of detection of HBV by NAT screening was higher than expected in preliminary results. In minipool NAT testing conducted under INDs so far, NGI detected HBV DNA in 11 of 43,000 donations; Centeon detected HBV DNA in 56 out of 3 million units between April 1998 to November 1999 (resulting in the interdiction of 236 units as a result of lookback actions). The 56 units detected by NAT were all HBsAg-negative and would not have been interdicted without NAT screening. Thus, further studies and further analysis of available data may be needed before the value of HBV NAT testing can be accurately assessed.

Currently licensed HBsAg screening tests made by some manufacturers are already so sensitive that they can detect samples in which the viral load is 1,000 copies/ml. Thus, the only undetected window period cases would contain <1,000 copies/ml. For this reason, NAT minipool testing for HBV would have to be very sensitive to be useful, and even a 20-sample pool dilution would be inadequate. (This would be true despite the fact that one manufacturer reported detecting as few as 10 copies/ml of HBV DNA.)

One participant suggested that, as an alternative to HBV NAT screening, an equivalent number of window period donations could be interdicted if the required cut-off for HBsAg immunoassay sensitivity were moved by FDA to the level of the most sensitive tests now on the market, since some manufacturers have succeeded in improving the sensitivity of their tests to a level beyond that available at the time the tests were first licensed. The relative benefits of the more sensitive HBsAg immunoassays and minipool NAT testing cannot be stated precisely without additional studies.

• **Increasing sensitivity of NAT for HCV and HIV-1:** During the past several years, most IND sponsors have dramatically increased the sensitivity of NAT. At this workshop, one manufacturer reported that their 95% detection limits for both HCV and HIV-1 were 5-6 copies/ml. The detection prevalence for HCV reported at this meeting from various studies (all on plasma that was negative when “pre-screened” by other screening tests) ranged from 1 unit per 3,000 units to 1 unit per 300,000. The detection prevalence for HIV-1 on pre-screened plasma ranged from 1 unit in 20,000 to 1 unit in 700,000.

• **NAT testing for parvovirus B19:** NAT screening of minipools of plasma for further manufacturing to detect parvovirus B19 DNA is considered by FDA to be an in-process control rather than a donor screening test. Although NAT screening of minipools will not eliminate parvovirus B19 because the virus is so widespread, such screening would lower the viral load in the manufacturing pools to the point where the manufacturing process could eliminate the infectivity. The possibility of limiting the levels of parvovirus B19 to $<10^4$ genome equivalents per ml in all manufacturing pools was discussed. Three manufacturers presented data on such testing that indicated that this limit is technically feasible. Suitable test validation studies were also discussed.

**The epidemiology of virus transmission by plasma derivatives:
clinical studies verifying the lack of
transmission of hepatitis B and C viruses and HIV type 1**

E. Tabor

From the Office of Blood Research and Review, Food and Drug Administration, Bethesda, Maryland.

Address reprint requests to: Edward Tabor, MD, FDA/CBER, HFM-300, 1401 Rockville Pike, Suite 400N, Rockville, MD 20852-1448.

Presented at Food and Drug Administration Blood Products Advisory Committee meetings, June 20, 1997, and December 11, 1998.

Received for publication November 2, 1998; revision received April 2, 1999, and accepted April 6, 1999.

TRANSFUSION 1999;39:1160-1168.

Infectious HBV Window Period and its Projected Reduction By Nucleic Acid Amplification Testing

BD Rawal, SH Kleinman, MC Kuhns, EW
Fiebig, MP Busch

for the Retrovirus Epidemiology Donor Study
and Abbott Laboratories

Introduction

- Hepatitis B Virus Transmission During pre-HBsAg Window Period (WP) Occurs in 1:66,000 Donations
 - Based on (4/100,000 PY incidence x 58d Pre-HBsAg WP)
- Potential Application of Nucleic Acid Amplification Testing (NAT) for HBV Donor Screening
 - Extent of WP Closure vs Assay Sensitivity
 - Single vs Pooled Donation Screening
 - Impact on Retention of HBsAg and Anti-HBc tests

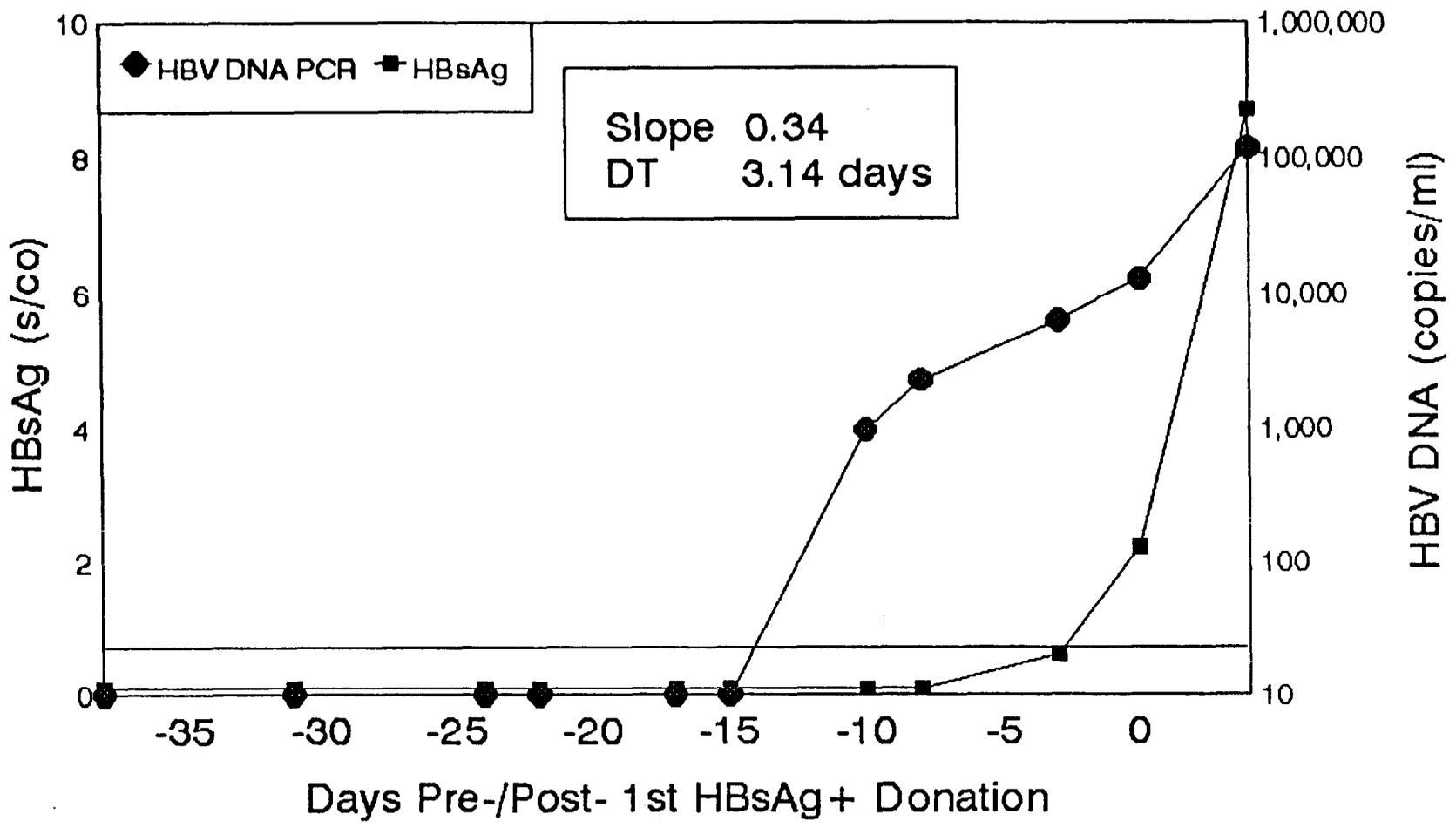
Objectives

- Characterize Kinetics of HBV DNA in SC Plasma Donors Relative to HBsAg Seroconversion and Infectivity
 - Quantitate HBV DNA at HBsAg Detection Point
 - Measure Virus Doubling Time in pre-HBsAg WP
- Develop Model to Project
 - Potential Impact of NAT on HBV Donor Screening
 - Optimal NAT Sensitivity for Testing Pooled or Single Donation Samples
- Define Chimpanzee Infectious Dose Equivalent for HBV DNA Copy Numbers

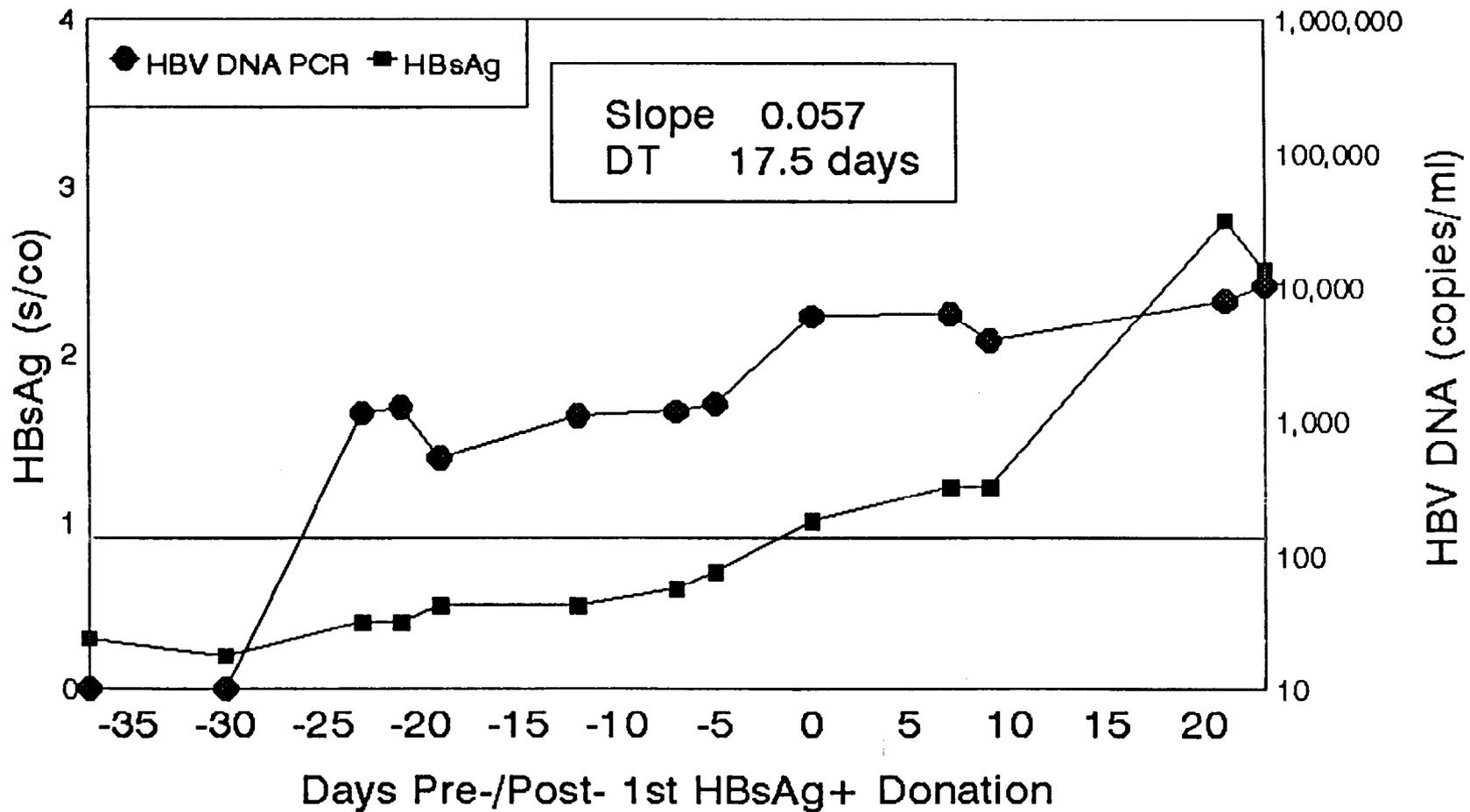
Methods -1

- 17 HBV SC (BBI) Panels (N=173)
 - HBsAg (Auszyme)
 - HBV DNA (Roche Quantitative PCR -400 Copy Sensitivity)
 - Regression Analysis of HBV DNA vs HBsAg
 - Calculate HBV Doubling Time (t) Days
 - $t=1/\text{slope}(\text{Log ln of HBV DNA/ml during pre/post HbsAG SC phase})$

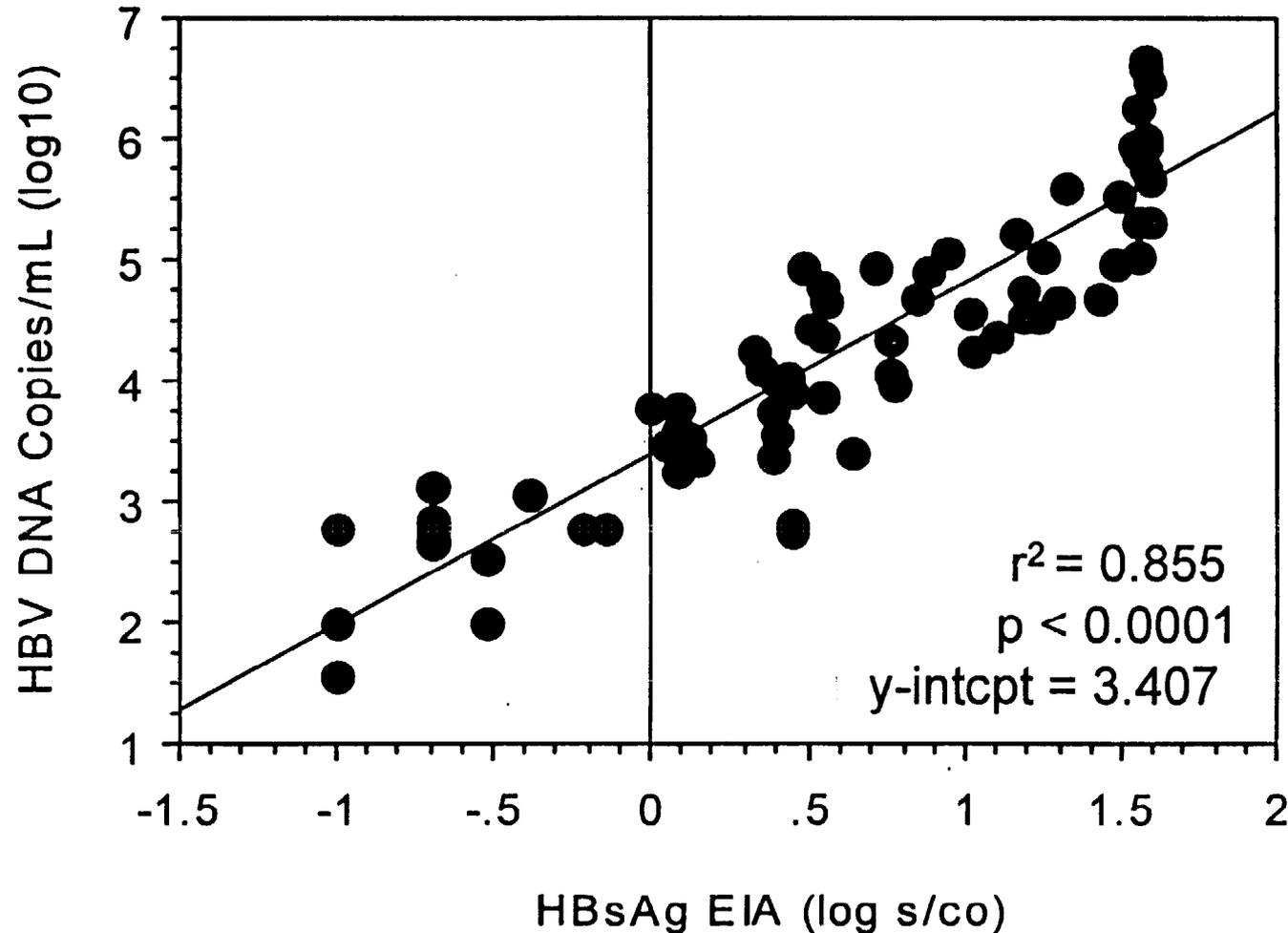
Hepatitis B Virus Seroconversion Panel (Rapid Doubling Time)



Hepatitis B Virus Seroconversion Panel (Slow Doubling Time)

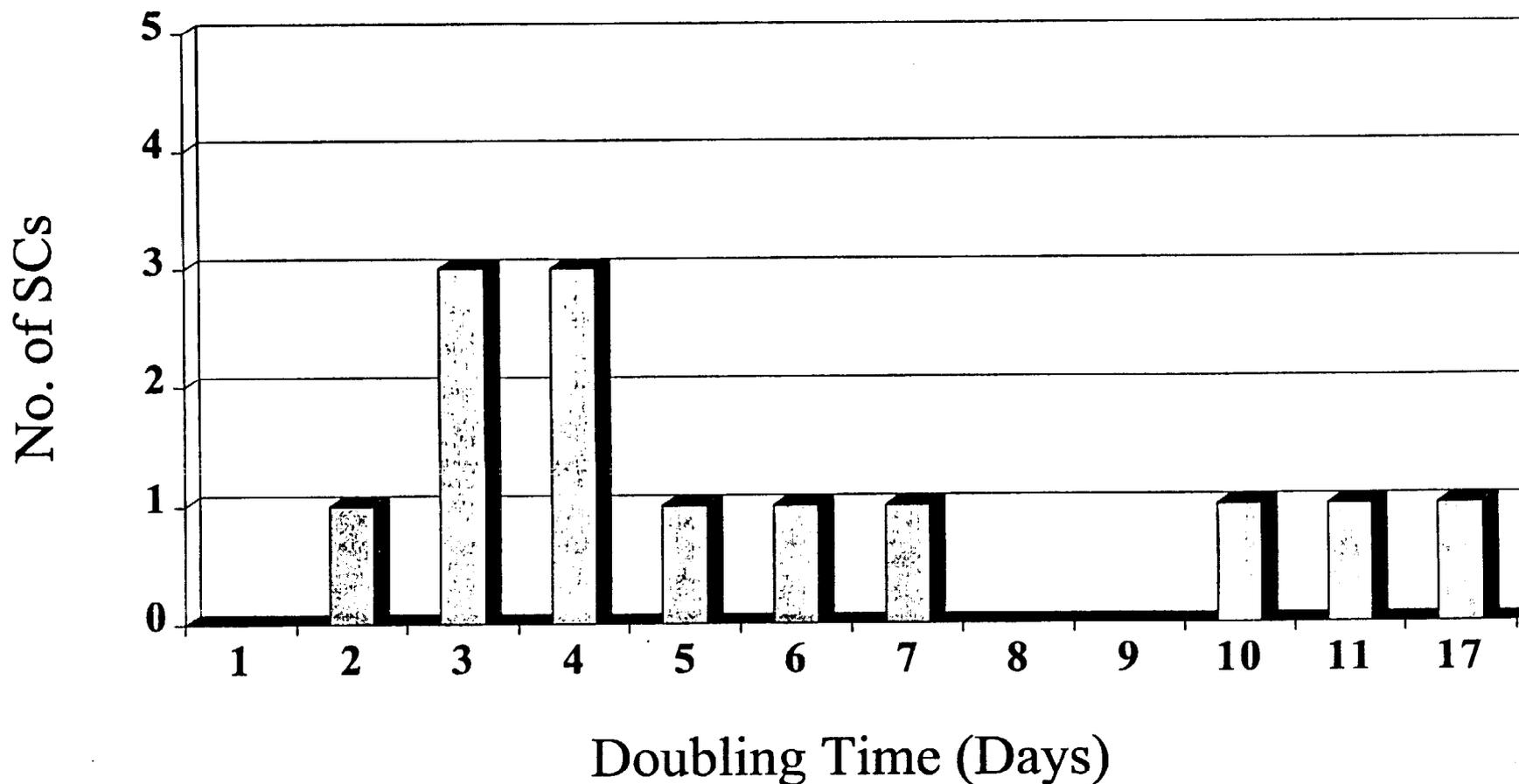


Relationship between HBV DNA and HBsAg levels (76 Samples, 17 HBV SCs)



Distribution of HBV Doubling Time During Primary Infection

13 HBV SC Panels with ≥ 2 PCR(+) pre-HBsAg Specimens

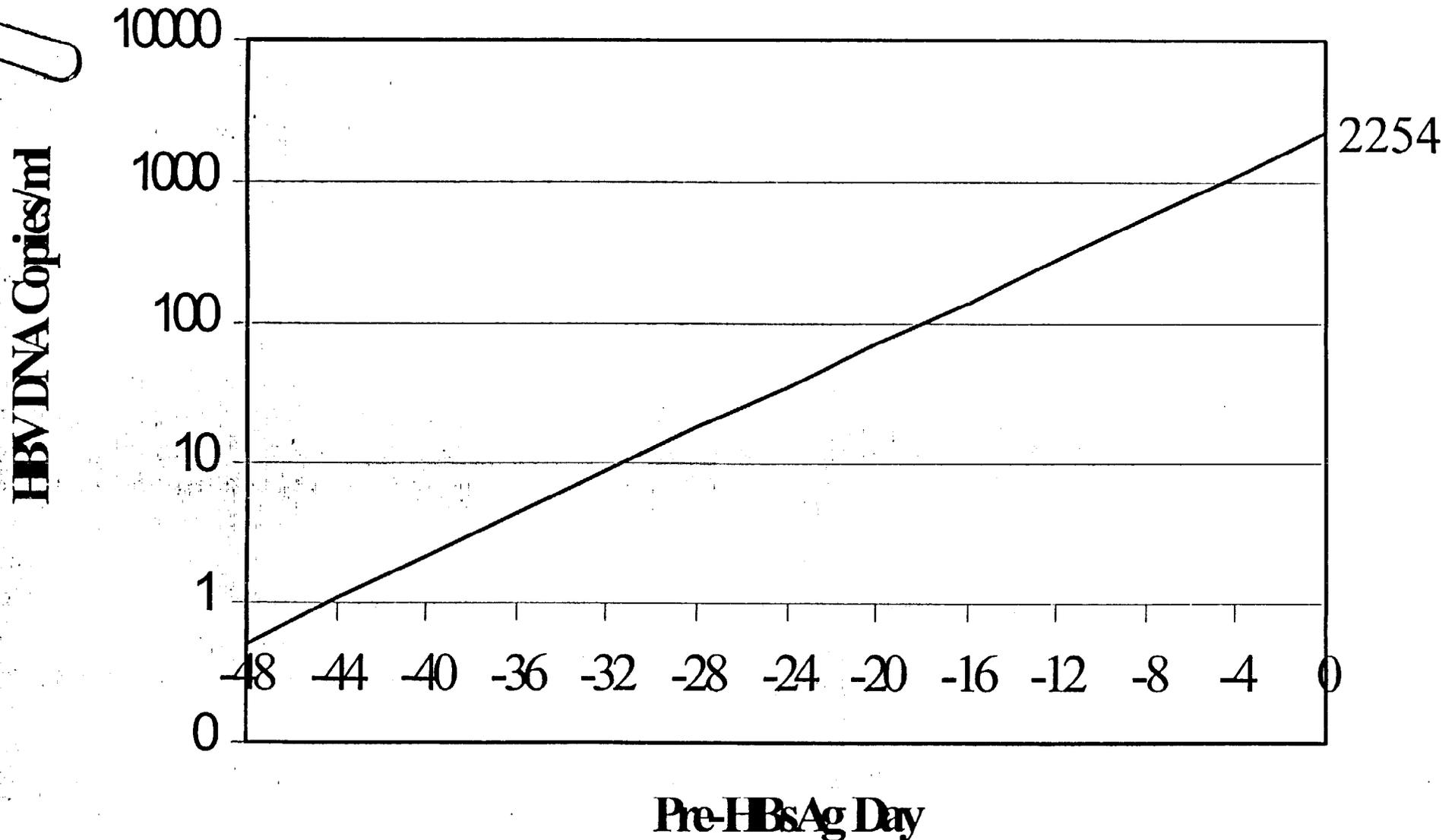


Model to Estimate Virus Load & Pre-HBsAg Window

Doubling Time ~4 Days

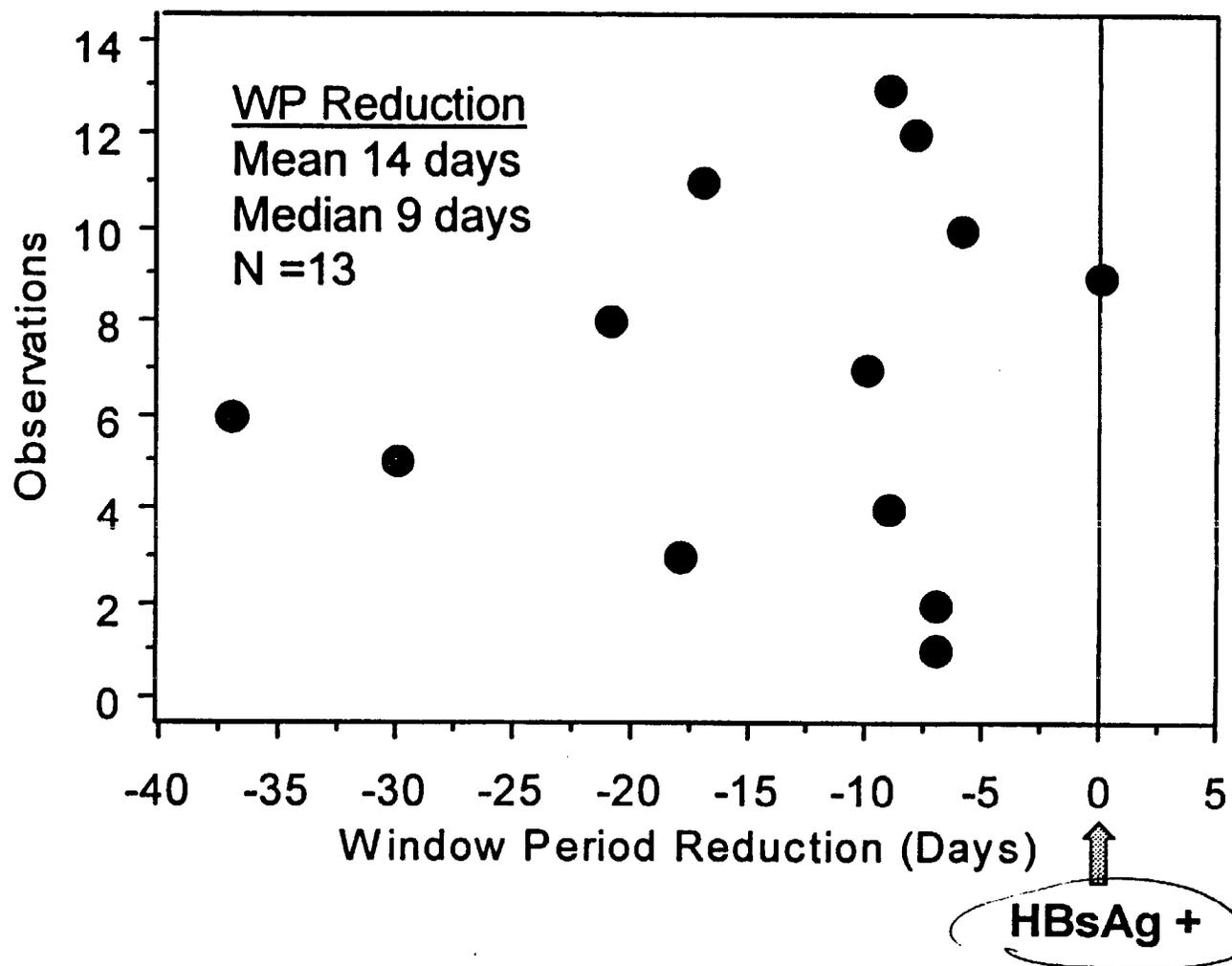
(N=17 SC Persons)

HBV DNA at HBsAg SC= 2254 (1257-4045) copies/ml



Window Period Reduction from HBsAg Positivity

Abbott Research PCR (HBV DNA 1 CID/mL ~20 copies/mL)



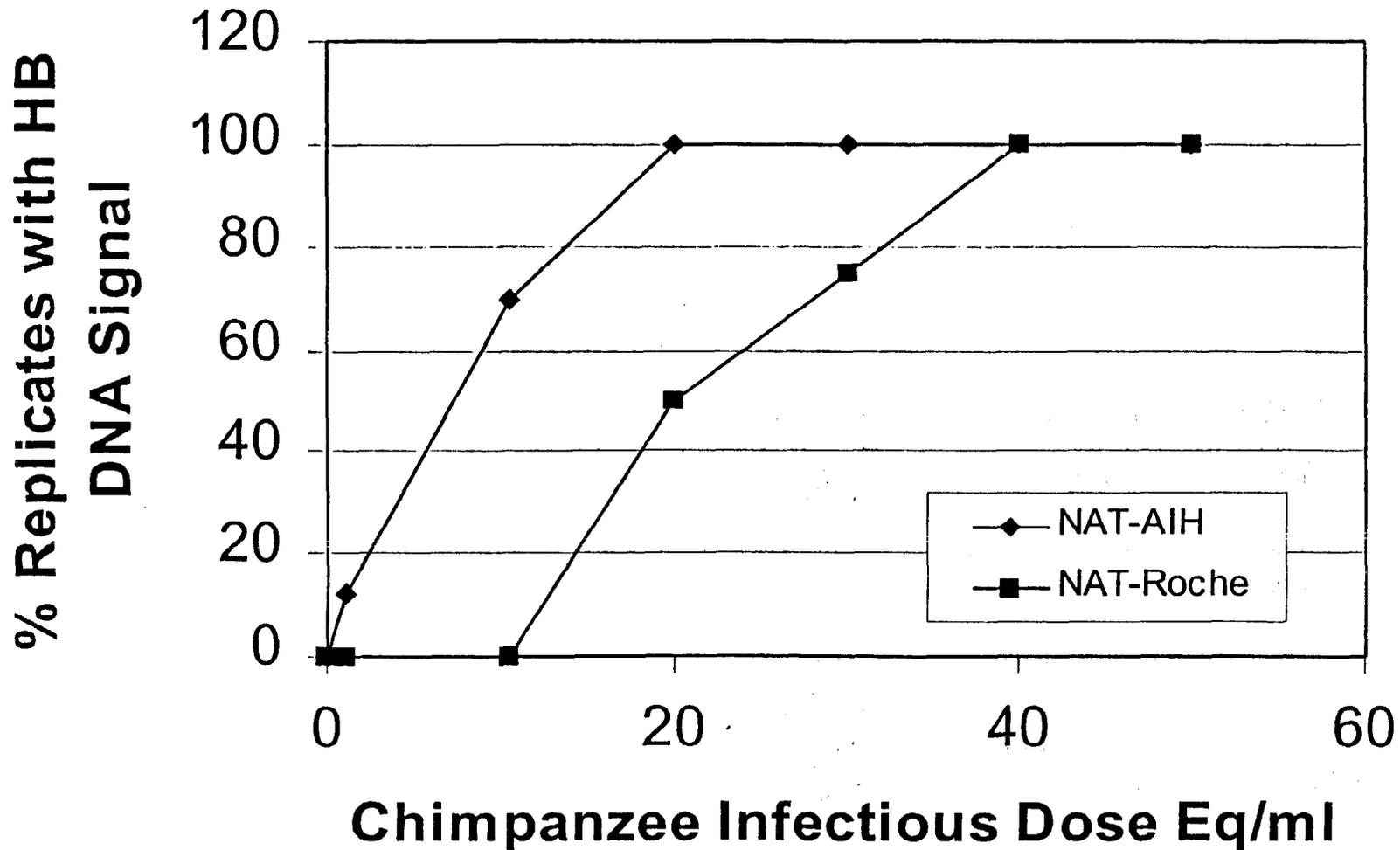
HBV WP Closure (pre-HBsAg) by 3 HBV DNA NAT Assays

Assay	No. Panels	Analytical Sensitivity	Mean W.P. Closure
Roche Amplicor PCR	15	~400 gEq/mL	7 days
Abbott Research PCR	13	~ 20 gEq/mL	14 days
GenProbe TMA	18	~ 20 gEq/mL	13 days

Methods - 2

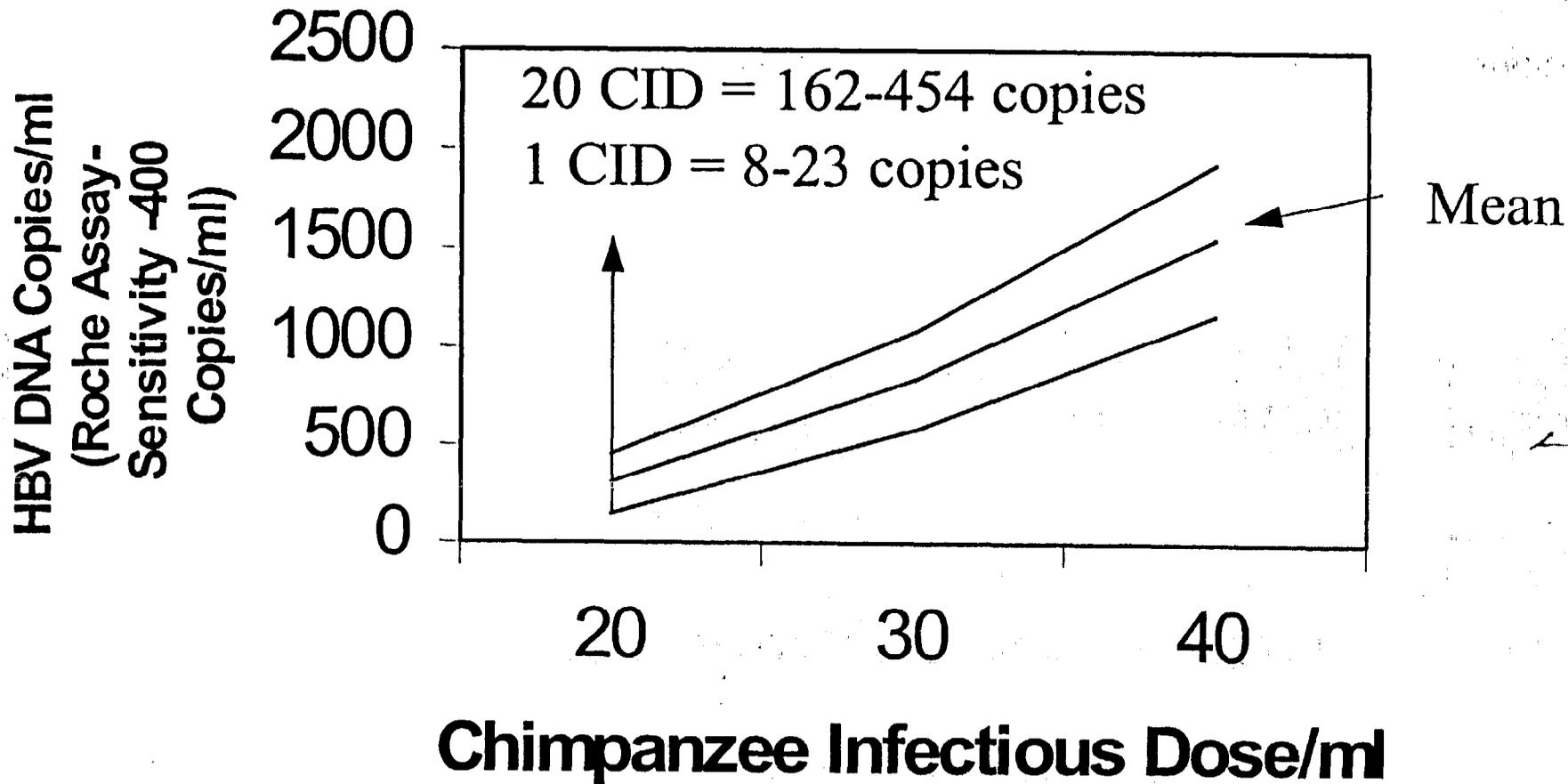
- Define Chimpanzee Infectious Dose Equivalents for HBV DNA Assay (NAT) Sensitivity Levels
 - 20 replicates at 50,40,30,20,10.5 & 0 CID/ml in a Pedigreed Panel were tested by 2 NAT Protocols
 - Roche Monitor (Sensitivity >400 Copies/ml)
 - Abbott in-House (Sensitivity of >20 Copies/ml)

Frequency of Detection of HBV DNA in Infected Chimpanzee Specimens by Two NAT Protocols (20 Replicates)



Equivalence of HBV DNA Copies and Chimpanzee Infectious Doses (CID)

(20 Replicates Tested)

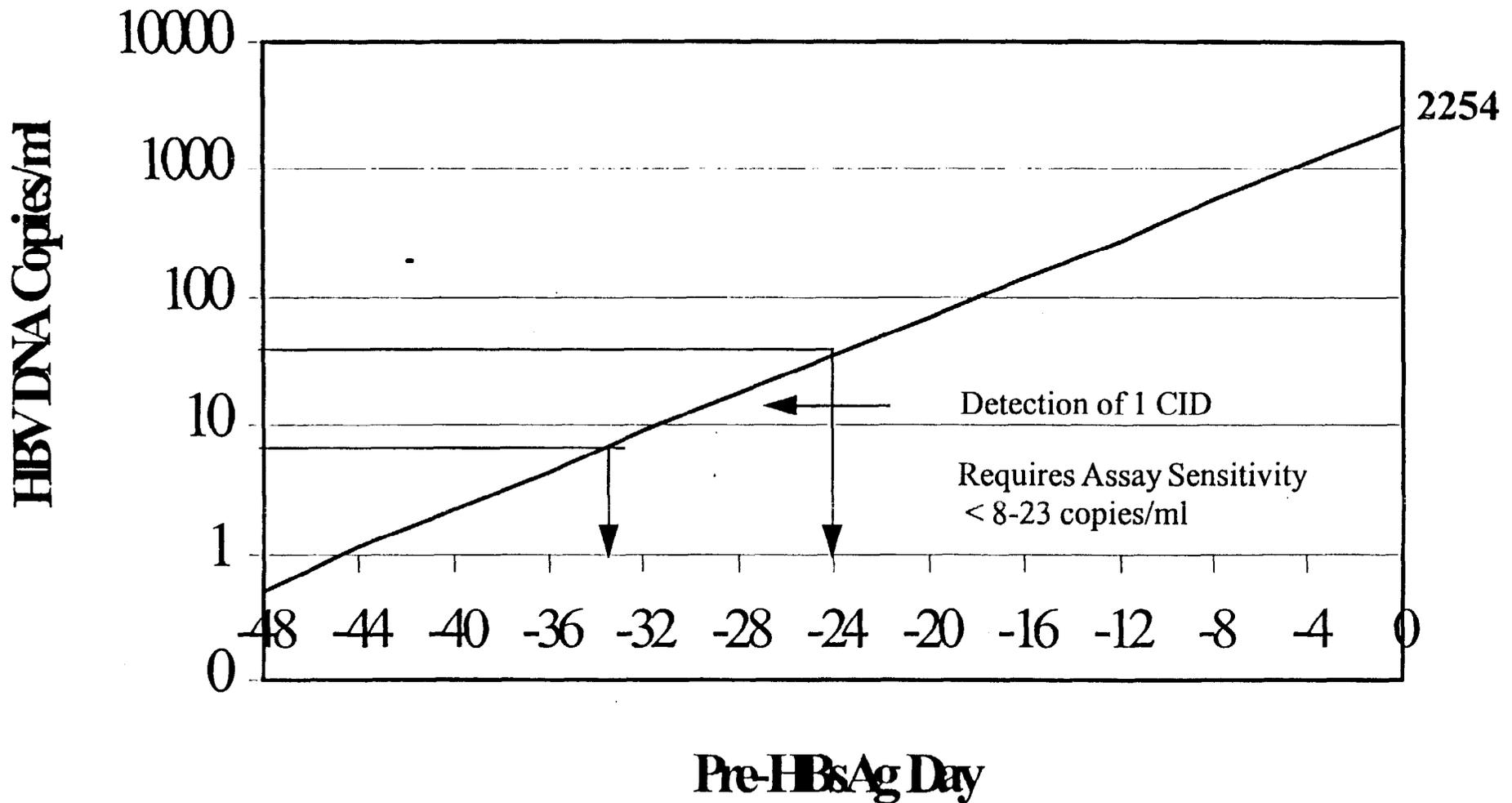


Model to Estimate Virus Load & Pre-HBsAg Window

Doubling Time ~ 4 Days

(N=17 SC Donors)

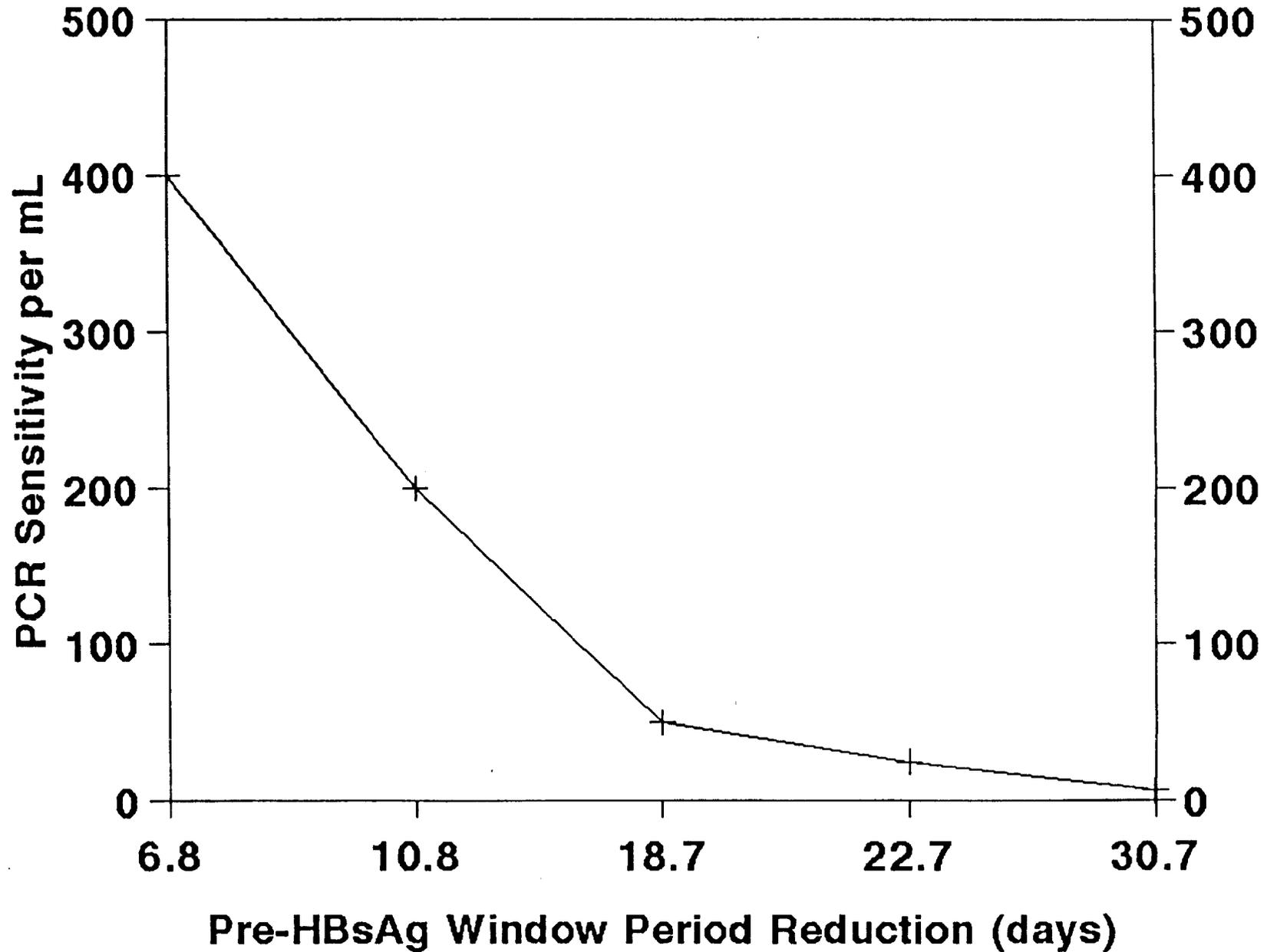
HBV DNA at HBsAg SC= 2254 (1257-4045) copies/ml



Methods - 3

- Access Potential Role of NAT in HBV Donor Screening
 - Estimate Duration of HBV DNA & Infectivity Detection Pre-HBsAG
 - Compare Observed vs Estimated WP Reduction
 - Calculate Sensitivity Limitations of NAT for Testing Pooled or Single Donations

PCR Sensitivity and pre-HBsAg Window Period Reduction (days)



HBV Window Period Closure Relative to NAT-Pool Size

Assuming Assay Sensitivity of 20 gEq / mL

Pool Size	Sensitivity	W.P. Closure	Yield per Year *
1	20 gEq/mL	24 days	75
20	400	7 days	22
100	2,000	0.3 days	1

* Assumes HBV IR of 9.5 / 100,000 py and 12 million allogeneic donations per year

Conclusions

- Model of HBsAg / HBV DNA Kinetics in Primary Infection
 - Correlates NAT End-Point Sensitivity and HBV Infectivity (CID)
 - Defines Level of Pre-HBsAg Infectious WP Reduction --NAT Testing of Single Donations
 - Evaluates Potential of NAT For Testing Pooled Donations

Relationship Between HBV DNA and HBsAg Among HBsAg(+) Donors with Primary vs Chronic Infection

HBV Stage	HBsAg (+) Samples	HBV DNA N (%)	HBV DNA Concentration (1,000 per mL)	
			Mean	Range
Primary	57	57 (100)	441	2 - 4,668
Chronic	200	139 (70)	257	0.5 - 144,000

* Based on HBV DNA (+) samples only

REDS Investigation of HBV DNA in anti-HBc Reactive Whole Blood Donors

