Hepatitis B Virus Nucleic Acid Amplification Technology: Potential Uses at a Blood Center

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Nucleic Acid Amplification Testing (NAT) Potential for Blood Screening:

- Power of NAT to identify infectious donations during the infectious part of the seronegative “window” period
- Power of NAT to identify donations from low level carriers
- Potential of NAT to reenter donors with false positive serologic tests

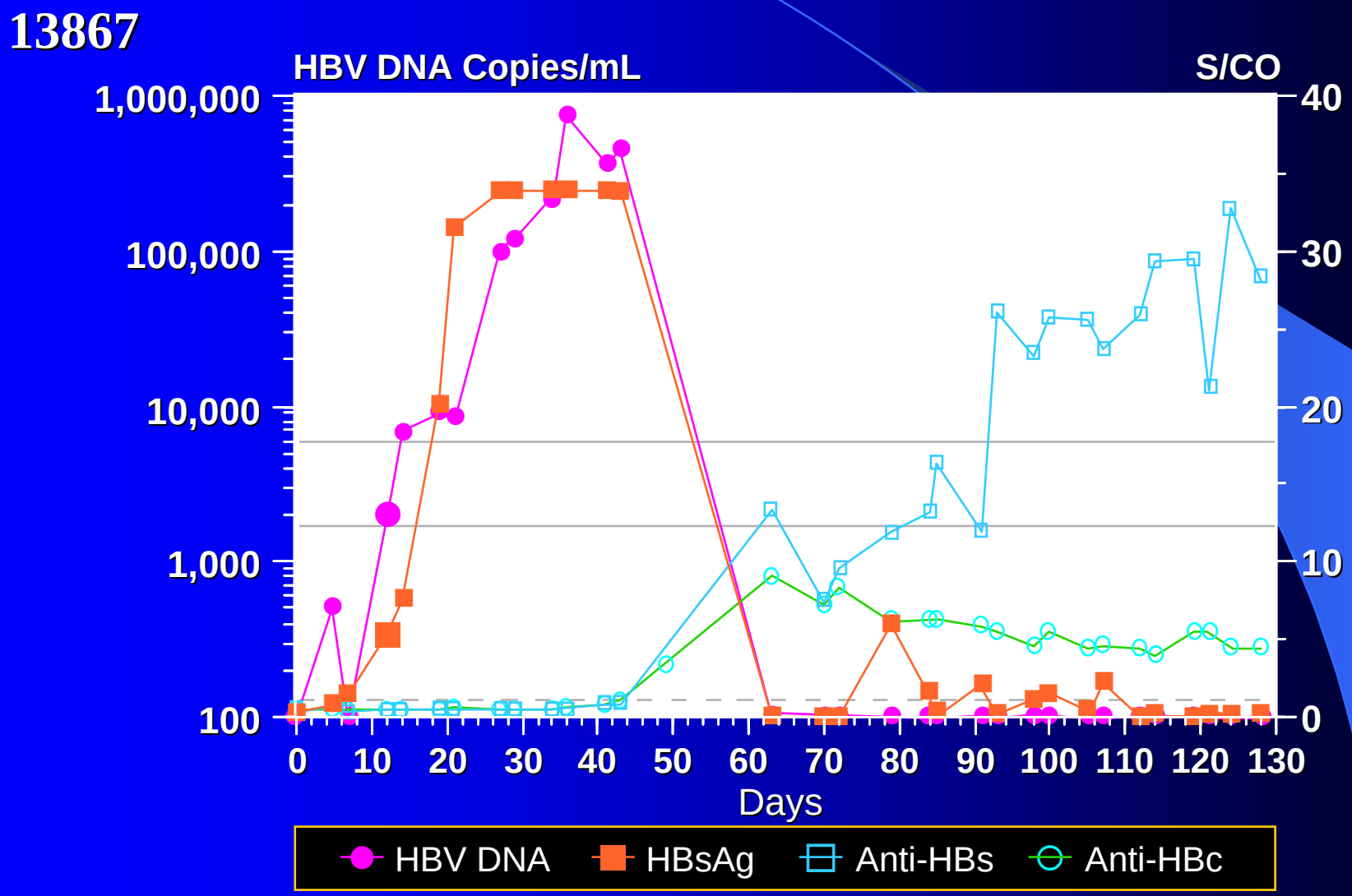
Risk vs. Safety of a Blood (or Plasma) Transfusion Using a Volunteer, Unpaid, Repeat USA Donor

	Risk	Safety
HIV ½	**1:676,000**	**99.9999%**
HBV	**1:66,000**	**99.9985%**
HCV	**1:125,000**	**99.9992%**
HTLV I/II	**1:641,000**	**99.9999%**
Cumulative	**1:38,500**	**99.997%**

Schreiber et al. New Engl J Med 11/21/96

(after implementing HIV p24 Ag and anti-HCV 3.0 testing)

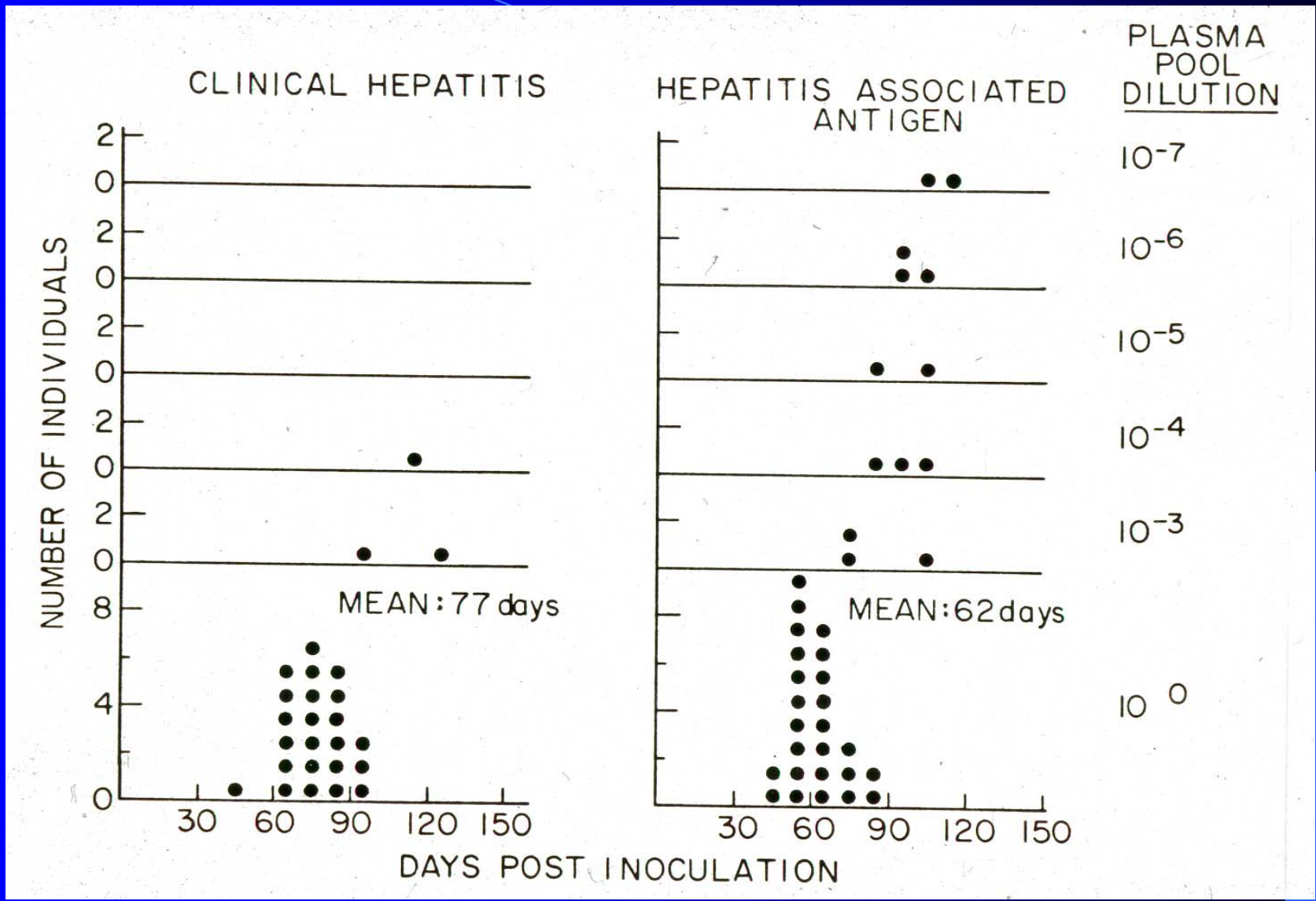
Representative Results from SC Panels Based on NGI's PCR




I'M O.K!
I'M CHRONIC!
90%
5-10%
1-3%
R.I.P.

Risk of Becoming a Chronic HBsAg Carrier by Age at Infection

McMahon BJ, et al. *J Infect Dis*. 1985;151:599-603.


CLINICAL HEPATITIS
HEPATITIS ASSOCIATED ANTIGEN
PLASMA POOL DILUTION
NUMBER OF INDIVIDUALS
0
4
8
2
0
2
0
2
0
2
0
2
0
MEAN: 77 days
MEAN: 62 days
30 60 90 120 150
30 60 90 120 150
DAYS POST INOCULATION
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{0}

Clinical Trial Summary

- Improved safety of blood donations by HBV MP-NAT
- Initiated August 2002 at 5 U.S. sites, completed April 2003.
- 704,902 specimens tested with HBV MP-NAT, of which 581,790 were included in the sensitivity and specificity analysis*.

**Specimens were excluded if no electronic results for HBsAg and anti-HBc were available from the site.*

Summary of Clinical Study Results

Results			Total
Anti-HBc	HBsAg	DNA	
—	—	—	578,671
+	+	+	84
+	+	—	16
+	—	—	2,988
+	—	+	1
—	+	+	3
—	+	—	4
—	—	+	23
		Total	581,790

Eligible for follow-up

Additional Index Testing & Follow-up Protocol

- Action
  - Test index donation by Alternate NAT*
  - If Alternate NAT positive, quantitate
  - Enroll in 6 month follow-up study
    - Weekly draws x 4, then monthly draw x 5
- Testing included the following:
  - IgM anti-HBc
  - anti-HBc (total)
  - anti-HBs
  - HBsAg
  - HBV DNA

*by National Genetics Institute

Potential Window Cases

- 23 Donors were HBV DNA + / HBsAg - / Anti-HBc –
  - 14 were enrolled into the follow-up study

    9 donors declined follow-up (presumed false positive by additional index testing)
- Of the 14 enrolled donors,
  - 2 confirmed window period cases
  - 12 shown false positive due to persistently negative Anti-HBc, HBsAg and HBV DNA (negative by alternative NAT on index specimens)

Follow-up Subject AA110001

Day	Anti-HBc	HBsAg	HBV DNA	Anti-HBs	IgM Anti-HBc	Quant. copies/mL
Index	NR	NR*	Positive	Not Done	Not Done	2,000
17	NR	Positive	Positive	NR	NR	120,000
40	NR	Positive	Positive	NR	NR	3.1E9
48	RR	Positive	Positive	NR	NR	3.5E9
55	RR	Positive	Positive	NR	RR	4.7E8
202	RR	Positive	Positive	NR	RR	>5.0E9

26 year old male repeat donor with no known risk factors

*Ortho HBsAg Test System 2

Follow-up Subject DA120001

Day	Anti-HBc	HBsAg *	HBV DNA	Anti-HBs titer	IgM Anti-HBc	Quant. copies/mL
Index	NR	NR	Positive	2340	Not Done	200
8	NR	NR	Positive	1980	NR	700
22	RR	NR	Positive	2730	NR	200
29	RR	NR	Negative	3350	RR	100
57	RR	NR	Negative	112	RR	Not Done
168	NR	NR	Negative	55	RR	Not Done

49 year old female, repeat donor, health care worker, states history of vaccination. She had a negative anti-HBs result 8 weeks retrospective to index.

* Abbott Auszyme

Additional Window Cases detected by sites continuing to use HBV NAT

- 3 sites elected to continue HBV MP-NAT under IND
  - April 2003 – present
- 3 additional window period cases detected in approximately 1 million donations screened

Window case summary

1st Positive post DNA detection

Donor ID	HBsAg	Anti-HBc	DNA index (copies/mL)
AA110001	Day 17	Day 48	2000
DA120001	NR through day 168	Day 22	200
LA150001	Day 7	Day 26	61,000
LA150002	Day 7	Day 28	2,300
LA151303	Pending	Pending	37,000

HBV MP-NAT yield for window period cases

- Clinical study yield

  2 / 0.7 million (1/350K)

- Continuing site data

  3 / 1.0 million (1/330K)

- Yield of HBV MP-NAT:
  - Approximately equal to HCV MP-NAT
  - Greater than HIV MP-NAT

Results of NAT Screening in the US

From: M Busch EPFA (Paris May 2004)

Stramer, Glynn, Kleinman, Caglioti, Strong, Busch . NEJM, in press

Gandhi, Strong, Kleinman et al. Blood102 (11):192A, 2003

*Morb Mortal Wkly Rep **52:**1160*

Virus	Dates	Units Tested	NAT+/Ab-
HCV	4-10/99 to 12/03	53.3 million	230 **(1/230,000)**
HIV	4/99-12/00 to 12/03	50.3 million	18 **(1/3.1 million)**
HBV	8/02 to 12/03	1.7 million	5 **(1/340,000)**
WNV	7/03 to 11/03	4.8 million	968 **(1/5,000)**

Conclusions re HBV NAT

1. **Some donors with HBV are identified despite negative HBsAg (and anti-HBc) testing.**
2. **A few donors with anti-HBc also have HBV DNA.**
3. **HBV NAT may permit reentry of some anti-HBc reactive donors.**
4. **If HBV follows a transfusion, consider other routes of transmission besides the transfusion.**