Evaluation of the yield of HBV DNA-positive, seronegative donors using an automated HIV-1/HCV/HBV triplex NAT assay

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Ultrio Study Objective

- Evaluate the "yield" of HBV DNA positive donations that are HBsAg and anti-HBc nonreactive using a combination of individual donation and mini-pool (MP) NAT of 16 donations with the Ultrio reagents and TIGRIS platform to supplement the existing Gen-Probe Ultrio license for an HBV DNA screening claim
- Determine rate/characteristics of HBV yield donors
- HBV screening claim approved by FDA on 8/12/08 for MPs up to 16 donations

Scope

- 3 of the 5 ARC NTLs involved (Charlotte, Detroit and St. Louis)
  - All donations tested for HIV-1/HCV/HBV (triplex Ultrio assay) on the TIGRIS instruments using both
    - ID NAT for a target of 600,000 donations
    - MP NAT for a minimum of 2 million donations
  - HIV-1/HCV (duplex) NAT was discontinued during the evaluation
    - Other NTLs continued using HIV-1/HCV (duplex) using the manual platform (eSAS)
  - Testing of WNV assay continued without change
    - TIGRIS or eSAS

Expected Yield

Confirmation of MP NAT Reactivity for ID NAT Yield

- ID NAT: up to 5 window period (WP) donations
  - IND studies: yield of 1:100,000-1:200,000
- MP NAT: 1 additional WP donation
- Dilutional studies to determine reactivity of ID NAT yield samples using pool sizes of: 4, 8, 16

Results (1/28/08-1/5/09)

- Donations tested by MP NAT = 3,118,368
  - 1640 Rx MPs resolved to a Rx donation = 0.05%
  - Unresolved pool rate = 0.21%, or 413/194,898
- Donations tested by ID NAT = 576,490
  - 945 Rx IDs = 0.16%
- Total tested = 3,694,858
  - 2585 Ultrio Rx dtns = 0.07%
  - 2119 (82%) discriminated = 0.06%
  - 455 nondiscriminated, or 0.01% (1:8120) of total tested
    - MP NAT = 65 (**1:47,974**)
    - ID NAT = 390 (86%, **1:1478**)
    - 431 eligible for follow up of which 120 have been submitted for reentry
  - 11 QNS for discriminatory testing

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Discriminated Results (1/28/08-1/5/09)

- 2119 (82%) discriminated, or **0.06%** of total tested
  - 2083 (98%) concordant serologic results from 2060 donors
    - 426 HBV, 231 HIV, 1426 HCV
  - 3 HIV of which **2 confirmed** (**1:1,847,429**); 1 false pos
  - 42 HCV of which **15 confirmed** (**1:246,324**); 27 false pos
  - 30 HBV of which **9 confirmed** (**1:410,540**); 21 false pos
    - 8 MP pos (**1:389,796**), 1 ID pos (**1:576,940**)
    - 6 anti-HBs pos donors with likely vaccine breakthrough (**1:270,956** assuming 44% donors vaccinated; **1:228,680** MP only)
      - 2/5 developed HBsAg; 4/5 developed anti-HBc
    - 3 anti-HBs neg window period donors (**1:689,707** assuming 56% donors unvaccinated; **1:873,143** MP only)
      - 1/2 developed HBsAg; 2/2 developed anti-HBc

Results of Dilutions for 019 Yield Donor S/CO values

	Undilute	*1:4*	*1:8*	*1:16*
ARC dHBV TIGRIS	**26.55**	**0.06**	**0.14**	**0.19**
GP dHBV eSAS	**23.28**	**19.49**	**0.03**	**0.03**
GP Ultrio eSAS	**13.10**	**0.08**	**9.81**	**0.09**

HBV Yield Demographic/Risk Info

Donor	Donor Status	Sex	Age	City/State	Risk Factors/Comments
013 MP+, anti-HBs+	Repeat 11/18/05	M	27	Ann Arbor/MI	Donor received HBV vaccine 2000-2001; sexual partner HBV chronic carrier; donor ≥ 34 days HBV DNA (100-200 copies/mL); no HBsAg/anti-HBc in >9 months f/u
042 MP+, anti-HBs+	Repeat 2/10/07	M	28	Olmsted Falls/OH	Paramedic in urban setting; donor received HBV vaccination; ≥ 75 days HBV DNA (200-45,000 copies/mL); HBsAg SC at day 75 (54 days); anti-HBc SC at day 107
003 MP+, anti-HBs-; strong seroconv	Repeat 5/21/05	F	37	Marietta/GA	HBV vaccine in 1980s; sexual partner HBV chronic carrier; donor 44 days HBV DNA (200-4800 copies/mL); anti-HBc SC at day 70; no HBsAg for 320 days f/u
019 ID+ (MP-), anti-HBs -	Repeat 1/15/08	F	44	White House/TN	Donor denies risk factors and no history of HBV vaccination; refused to enroll in f/u; 100 copies/mL at index
011 ID+ (MP+), anti-HBs+	Repeat 3/29/07	F	17	Centralia/IL	Donor received HBV vaccine 10-11 yrs prior to index donation; sexual partner HBV chronic carrier; donor ≥ 137 days HBV DNA (100-50,000 copies/mL); HBsAg SC at day 108 (60 days rx); anti-HBc SC at day 168

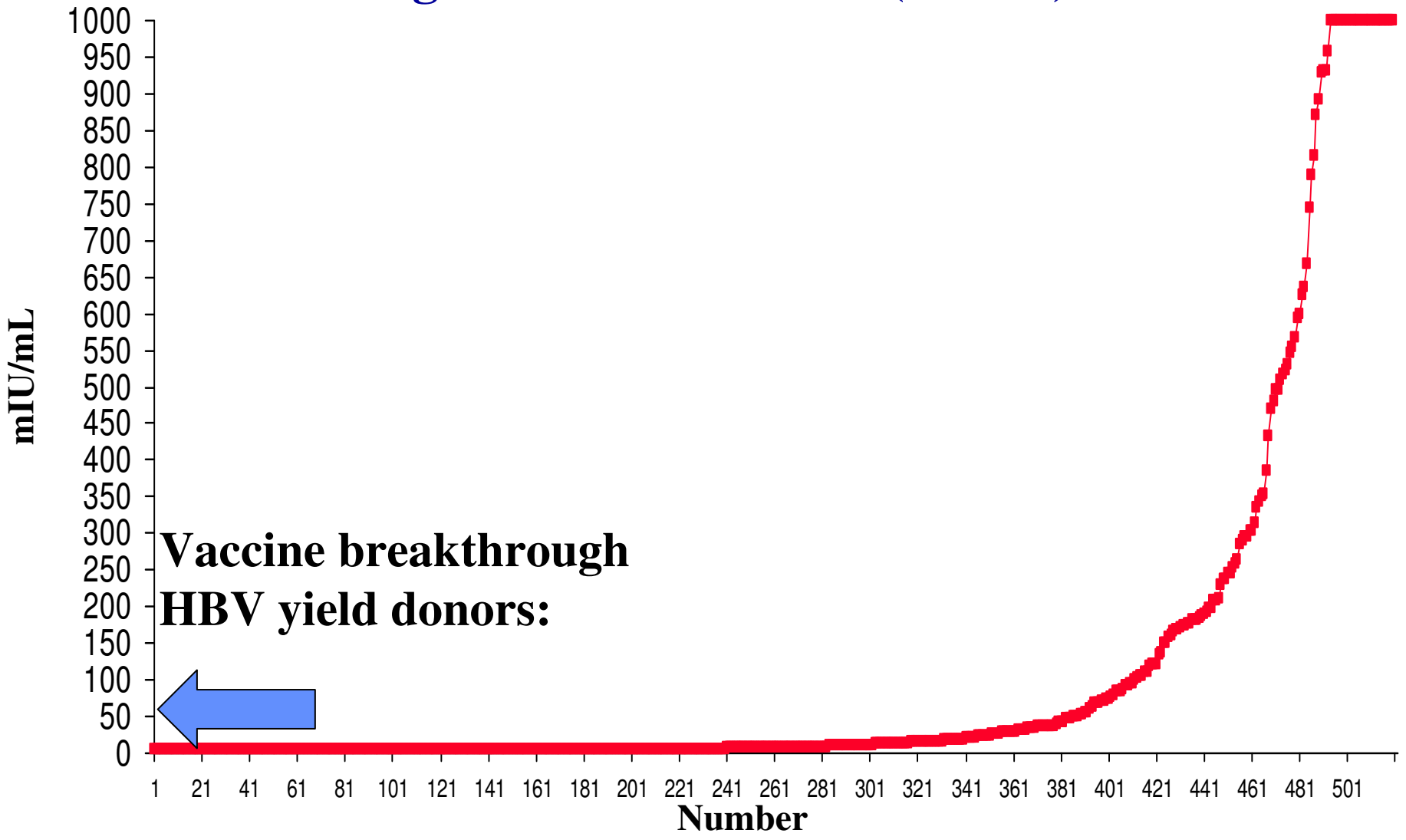
HBV Yield Demographic/Risk Info

Donor	Donor Status	Sex	Age	City/State	Risk Factors/Comments
055 MP+, anti-HBs-	First-Time	M	20	Batesville/MS	Donor in Job Corps; no history of HBV vaccine (vaccinated after finding out of DNA+ result); ≥ 75 days HBV DNA with high-titer viremia (200-10^8 copies/mL); HBsAg SC at day 41 (123 days rx); anti-HBc SC at day 70
074 MP+, anti-HBs-	Repeat 05/17/08	F	24	Coral Springs/FL	Iranian donor; cannot recall HBV vaccination history; sexual partner HBV chronic carrier; HBV DNA ≥ 73 days (200 copies/mL); no HBsAg for >116 days f/u; anti-HBc SC at day 73
001 MP+, anti-HBs+	Repeat 03/31/08	M	22	Boston/MA (NY)	College student who received HBV vaccine 1991-1992; HBV DNA ≥ 49 days (viral load pending); sexual partner HBV chronic carrier no HBsAg for >91 days f/u; anti-HBc SC at day 69
029 MP+, anti-HBs+	Repeat 04/03/07	M	19	Colerain/NC	HIV infected donor (RNA + Ab); unable to be contacted for participation in f/u and confirmation of vaccination

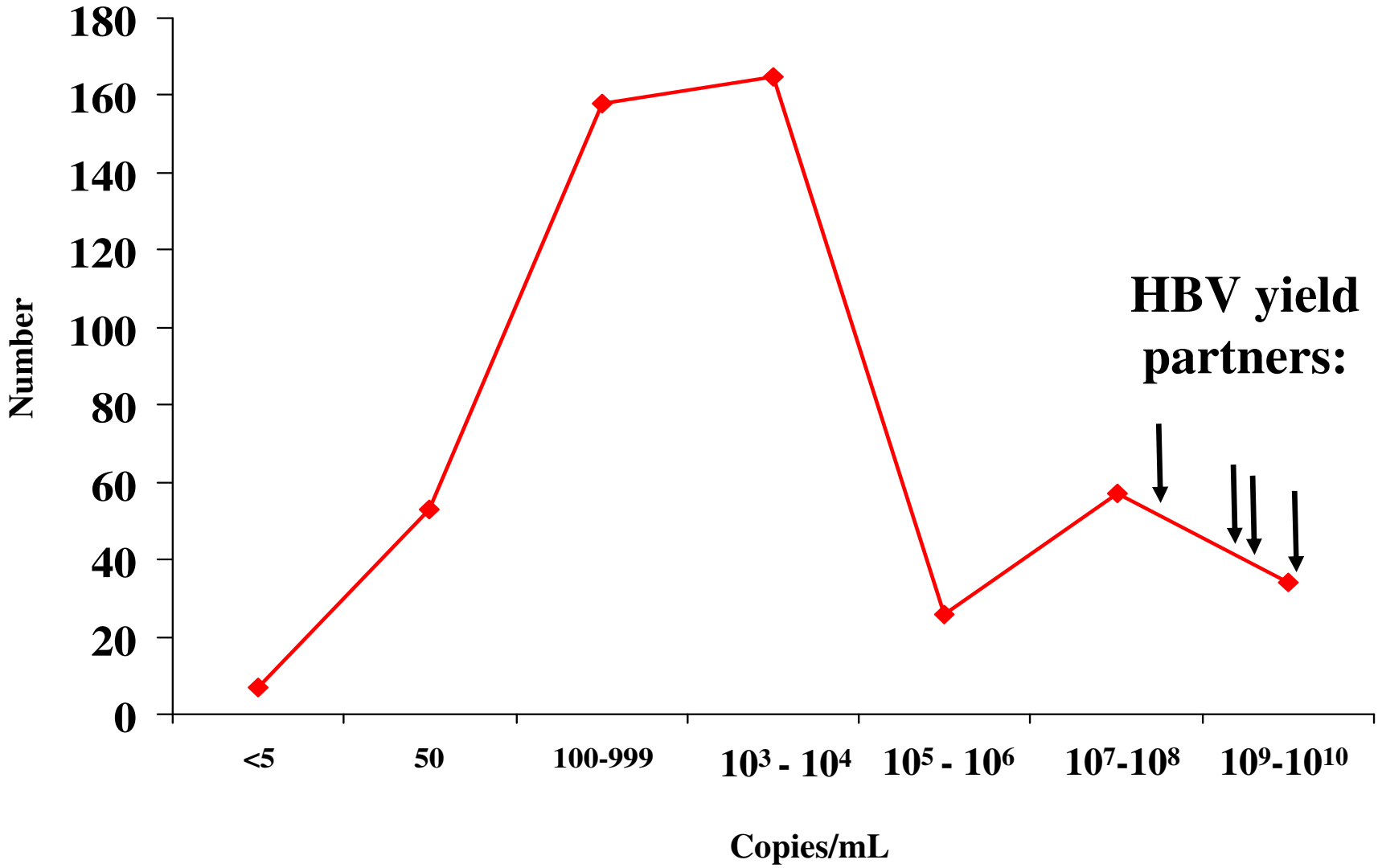
Virologic/Serologic Markers in HBV DNA Yield Donors

Donor	Anti-HBs mIU/mL @ index	Time (days) followed	Viral Load Range (c/mL)	Duration (days) DNA pos	HBsAg first pos day (duration)	Anti-HBc first pos day	Anti-HBc IgM
013	+; 43	243	100-200	≥ 34	-	-	-
042	+; 33	133	800-45,000	≥ 75	75 (54)	107	+
003	+; 3	320	200-4800	= 44	-	70	+
011	+; 11	189	100-50,000	≥ 137	108 (60)	168	+
055	-	>175	>10^8	≥ 75	41 (123)	70	+
074	-	>116	200	≥ 73	-	73	+
001	+; 100 (day 45)	>115	100	≥ 49	-	69	+

Anti-HBs Concentrations in HBsAg and Anti-HBc Negative Blood Donors (N=520)



HBV DNA Viral Loads among HBsAg and anti-HBc Positive Donors (N=500)



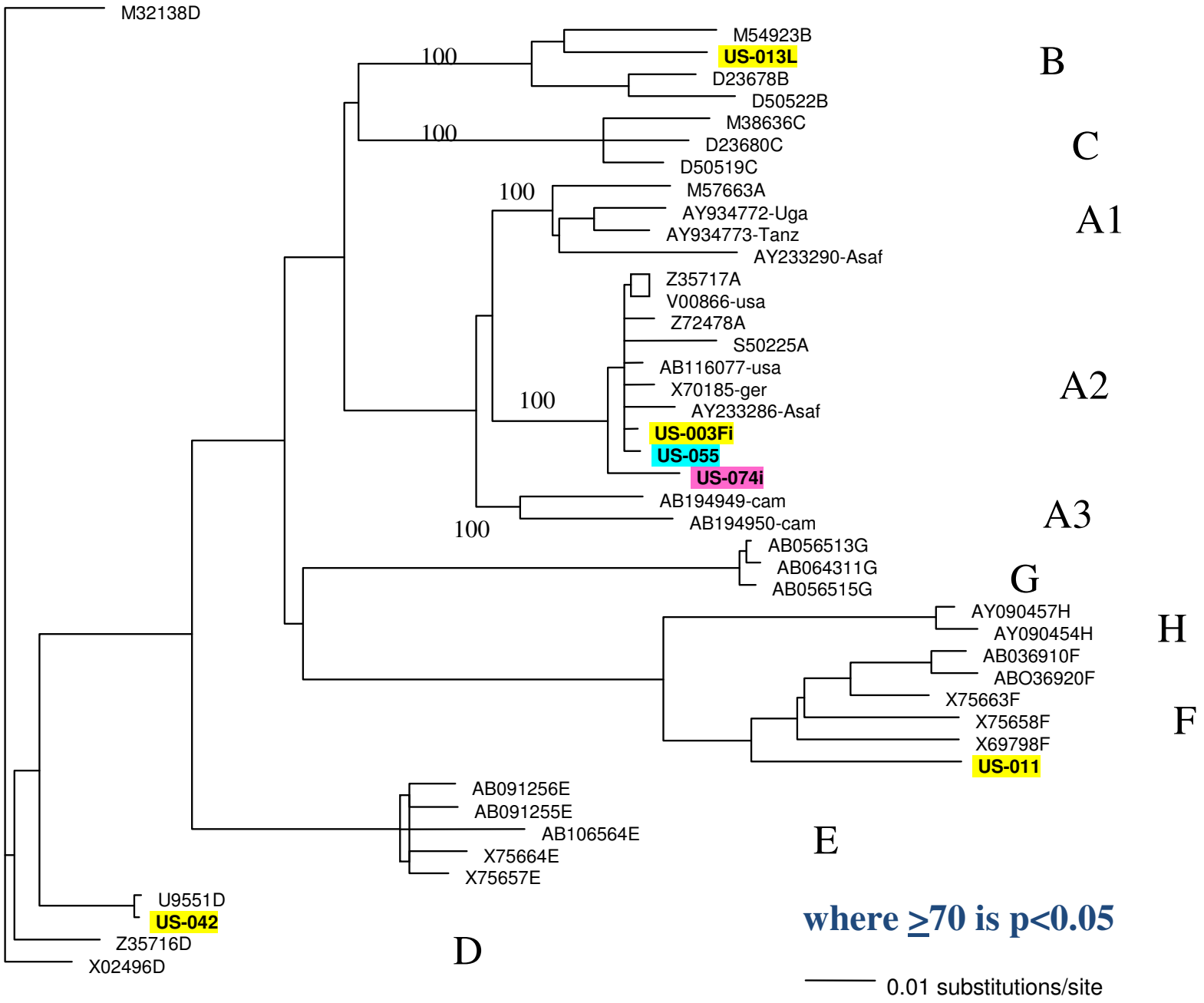
HBV Additional Studies

- Performed by 3 groups
  - Prof Wolfram Gerlich
    - Institute for Medical Virology, Univ of Giessen, Germany
    - Viral loads, genotype, subtype, sequence analysis
  - Dr. Paul Coleman
    - Abbott Laboratories
    - Anti-HBc IgM (ARCHITECT), sequence analysis
  - Prof JP Allain
    - Laboratory of Molecular Virology, Depart Haematology, Univ of Cambridge, UK
    - Viral loads, genotype, sequence analysis
    - Sequencing
      - Full length, preS/S, BPC/PC

Sequencing of HBsAg with Genotype and Subtype

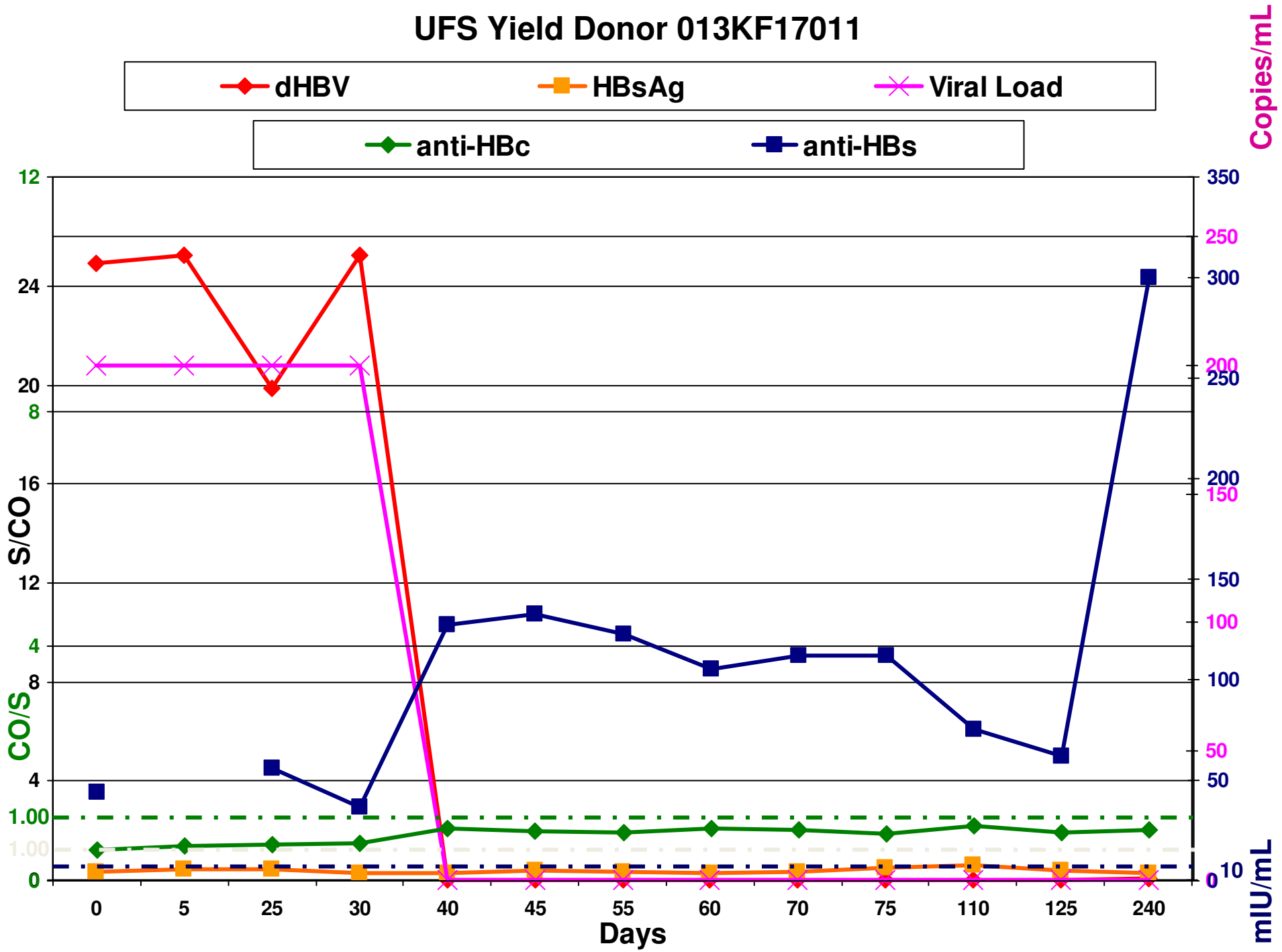
Donor or Partner	DNA Viral Load (copies/mL)	HBsAg Conc. (ng/mL)	Genotype	Subtype	Mutation (No. and Location)
Donor - 003	1,200, 86		WT A2 (Central Europe, N Amer)	*Pending*	5 - sN59D; sT143A; sS210N; sL22S; sK122E
Partner - 003	2.7 x10^9	67,300	WT A2	*Pending*	2 – preS V172M and rtC6Y
Donor - 011	2,700, 13		WT F1 (American Indians of Central America)	*Pending*	2 - S and preS domain stop codons @ sY71 Stop and preS F24L
Partner - 011	2.4 x10^{10}, 2.6 x 10^8	100,600	WT F1	*Pending*	1 - T173M Not present in other F1 strains
Donor - 013	35		WT B2 (East Asia)	adw2	1 - sF220L (rtL229V) Not present in other B2 strains
Partner - 013	8.0 x10^9, 1.8 x 10^6		WT B2	adw2	1 - sF220L (rtL229V) Not present in other B2 strains
Donor - 042	65, 230, 43		WT D3 (Mediterranean) (preS domain)	ayw3	2 - sT125M and sP127T
			WT A2 (S domain)	ayw3	2 - sG44R and sT143A
Donor - 001	11		C2 (N China, Korea)	adw2	1 – G145R; vaccine escape mutation
Partner - 001	1.9 x 10^8		C2)	adw2	1 – G145R; vaccine escape mutation

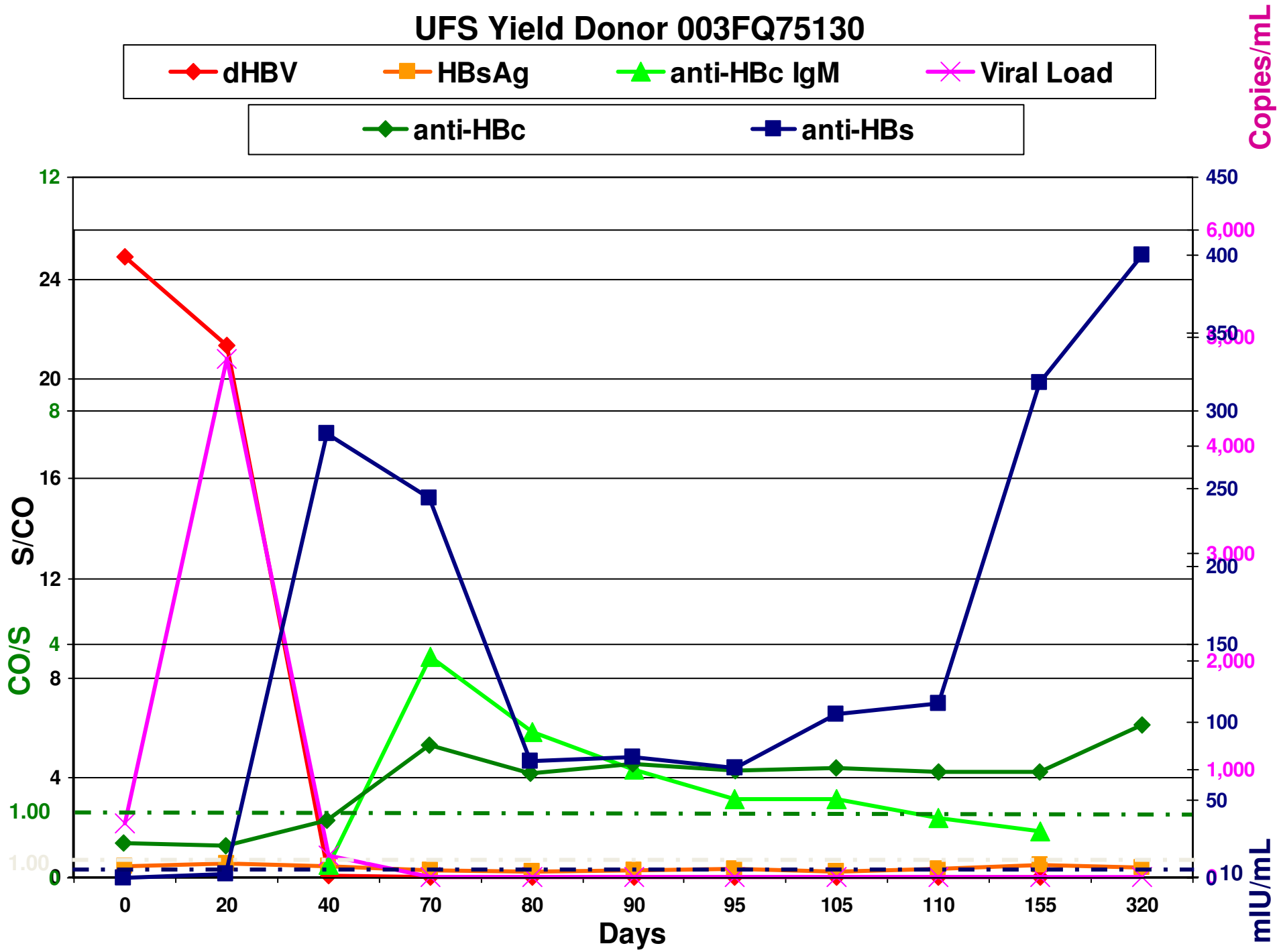
HBV Bootstrap Analysis for 6 cases with full genome sequences

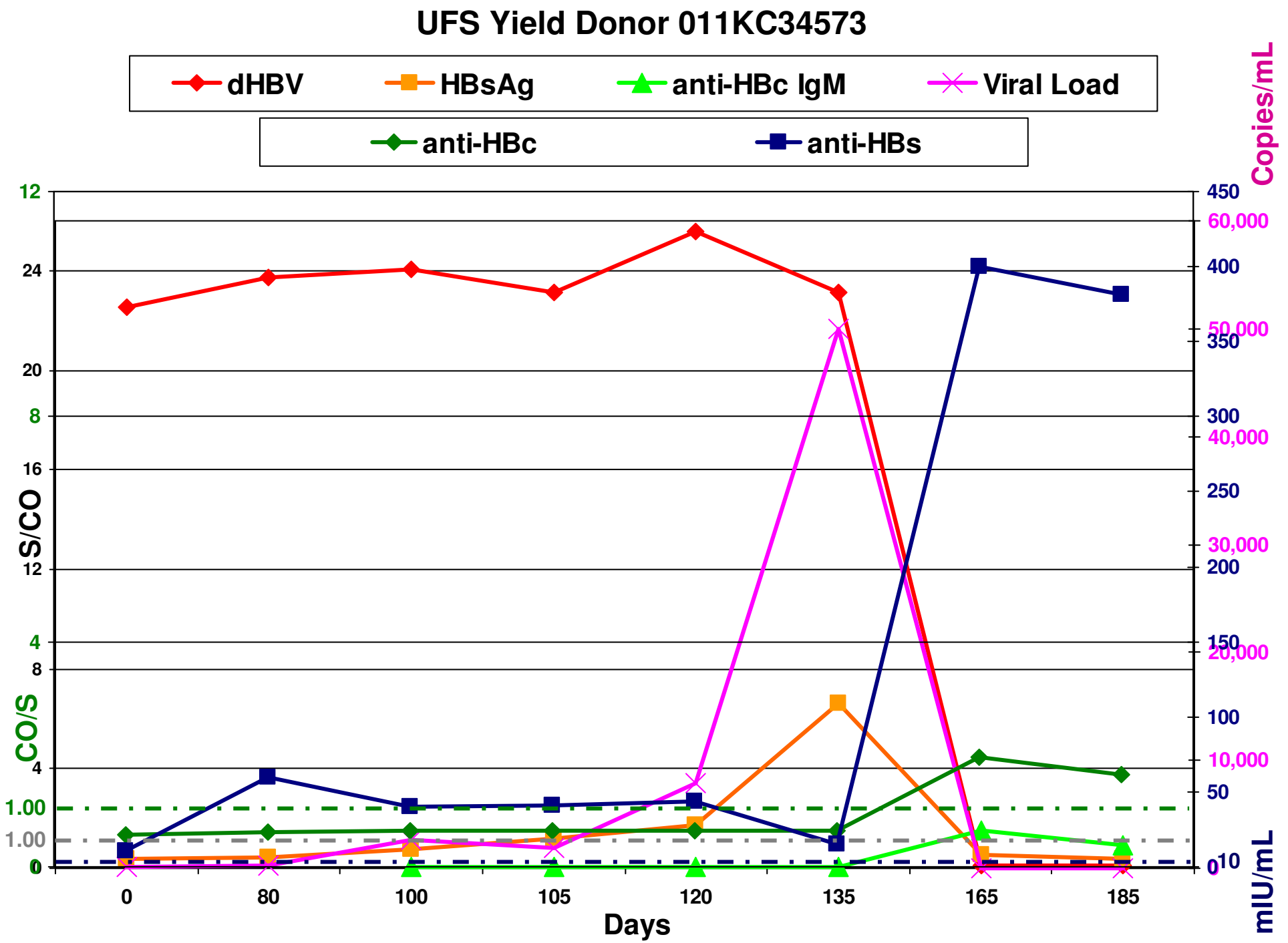


Virologic/Serologic Profiles

- Breakthrough cases with likely infection via a sexual partner
  - All partners tested and confirmed to have high levels of HBV DNA (>10^7 copies/mL), HBsAg, anti-HBc but **no** anti-HBs, and when sequencing complete, same virus as the donor
  - Donors with and without HBsAg development
- Breakthrough case occupational exposure
- Acute infection in the absence of vaccination


UFS Yield Donor 013KF17011
dHBV
HBsAg
Viral Load
anti-HBc
anti-HBs
Copies/mL
S/CO
CO/S
mIU/mL
Days
12
24
20
8
16
12
4
8
4
1.00
1.00
0
350
250
300
200
250
200
150
150
100
100
50
50
10
0
0
5
25
30
40
45
55
60
70
75
110
125
240


UFS Yield Donor 003FQ75130
dHBV
HBsAg
anti-HBc IgM
Viral Load
anti-HBc
anti-HBs
Copies/mL
S/CO
CO/S
mIU/mL
Days
12
24
20
8
16
12
4
8
4
1.00
1.00
0
450
6,000
400
350
5,000
300
4,000
250
3,000
200
150
2,000
100
1,000
50
0
10
0
20
40
70
80
90
95
105
110
155
320


UFS Yield Donor 011KC34573
dHBV
HBsAg
anti-HBc IgM
Viral Load
anti-HBc
anti-HBs
S/CO
CO/S
Copies/mL
mIU/mL
Days
0
80
100
105
120
135
165
185
12
24
20
8
16
12
4
8
4
1.00
1.00
0
450
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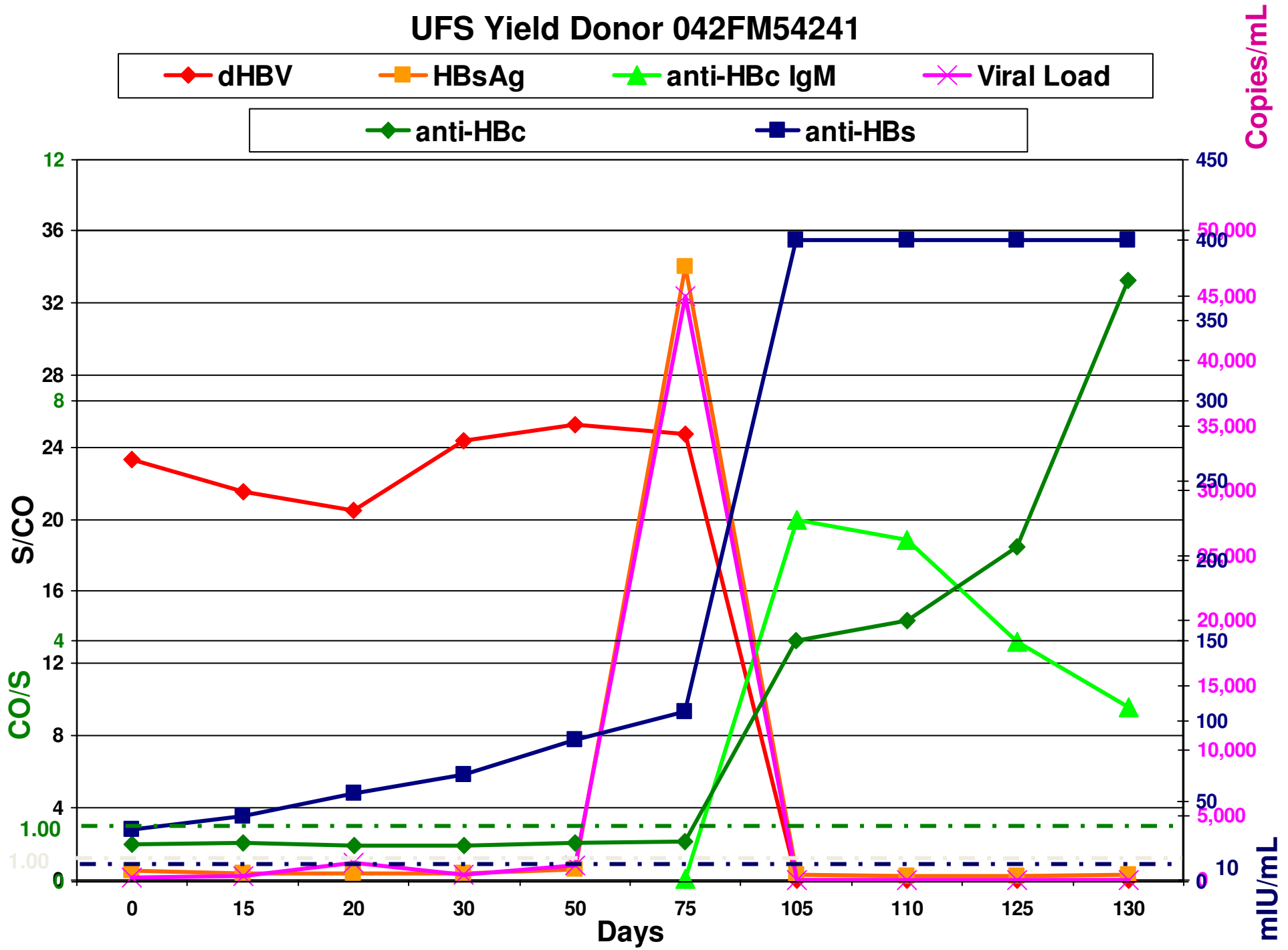
200
150
20,000
100
10,000
50
10
0


UFS Yield Donor 042FM54241
dHBV
HBsAg
anti-HBc IgM
Viral Load
anti-HBc
anti-HBs
S/CO
CO/S
Copies/mL
mIU/mL
Days
12
36
32
28
8
24
20
16
4
12
8
4
1.00
1.00
0
450
400
350
300
250
200
150
100
50
10
0
50,000
45,000
40,000
35,000
30,000
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10,000
5,000
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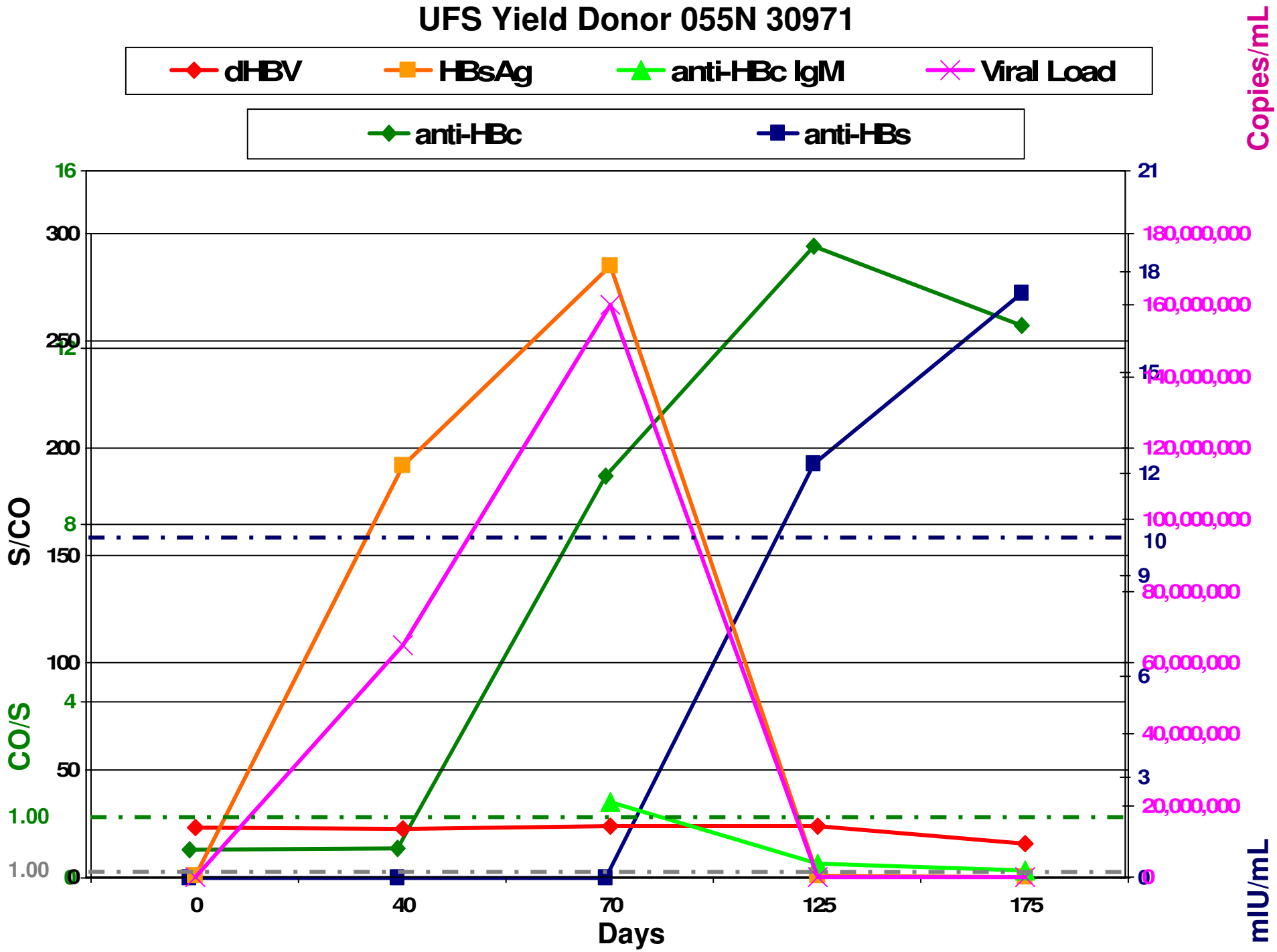
15
20
30
50
75
105
110
125
130


UFS Yield Donor 055N 30971
dHBV
HBsAg
anti-HBc IgM
Viral Load
anti-HBc
anti-HBs
S/CO
CO/S
Copies/mL
mIU/mL
Days
0
40
70
125
175
16
300
250
12
200
8
150
100
4
50
1.00
1.00
0
21
180,000,000
18
160,000,000
15
140,000,000
120,000,000
12
100,000,000
10
9
80,000,000
60,000,000
6
40,000,000
3
20,000,000
0

Comparison of HBV DNA Yields in US Studies

	ABC Roche Trials (1, 6, 24)
1:352,451	(2/704,902) COBAS AmpliScreen trial
1:425,730	(7/2,980,103) + extended COBAS AmpliScreen testing
1:72,336	(1/72,336) MPX trial
1:381,555	**(8/3,052,439) Combined**
1:610,488	***(5/3,052,439) Combined (– PRISM HBsAg RRs)***
	ABC GP Trial (1, 8) 9 sites; 5/07 to 7/16/08
1:282,984	**(4/1,131,937) vs GSC 3.0 and PRISM HBsAg**
	ARC GP Trial (1, 16) 1/29/08 to 1/5/09
1:410,540	**(9/3,694,858) vs PRISM HBsAg**

Summary

- Performance of duplex (eSAS) and triplex (ULTRIO®-TIGRIS) is comparable regarding MP NAT specificity and HIV-1/HCV NAT yield
- 9 HBV DNA pos/HBsAg and anti-HBc neg donations produced comparable yields to other HBV yield studies performed in the US
  - **1:410,540** yield rate
  - 8 of 9 yield donors were detected by MP NAT; **1:389,796** yield rate
    - 7 with long-term follow up
      - All with detectable HBV DNA for 34-137 days
      - 3 with HBsAg at 41-75 days after DNA (duration 54-123 days)
      - 6 with anti-HBc SC at days 69-168
  - 6 of 9 were immunized individuals having anti-HBs at index or shortly thereafter; although these represent acute infections, dynamics of infection differ, and infectivity of such donations is unknown; **1:270,956** yield rate (or **1:228,680** for MP only)
  - 3 of 9 were window period donors; **1:689,707** yield rate (or **1:873,143** for MP only)

Conclusions re HBV NAT

- ID NAT modeling studies (nonvaccinated donors)
  - Lowest residual risk; highest yield = **1:466,000 – 1:713,000**
  - Logistically not feasible for either platform
  - Modeling cannot predict total yield due to vaccinated donors with differing kinetics early in infection
- MP NAT modeling studies (nonvaccinated donors)
  - MPX: MP 6 highest yield = **1:830,000 (= 1:713,000)**
  - Ultrio: MP 8 = MP 16 = **1:1,345,000 – 1:2,000,000**
- MP NAT observed yield (both vaccinated and nonvaccinated)
  - MP NAT at various pool sizes = **1:300,000-1:600,000**
  - Data do not suggest benefit of MP 8 vs MP 16
  - ARC study; MP 16 detected 8 of 9 units
    - 9th yield unit **only** detected by ID NAT (not detected MPs 4, 8 or 16)
- High yield of MP 16 indicates value in comparison to ultra-sensitive HBsAg assays
  - Total yield = **1:410,540**
  - MP 16 yield = **1:389,796**
  - Vaccinated MP 16 yield = **1:228,680**; infectivity ?
  - Nonvaccinated MP 16 yield = **1:873,143**

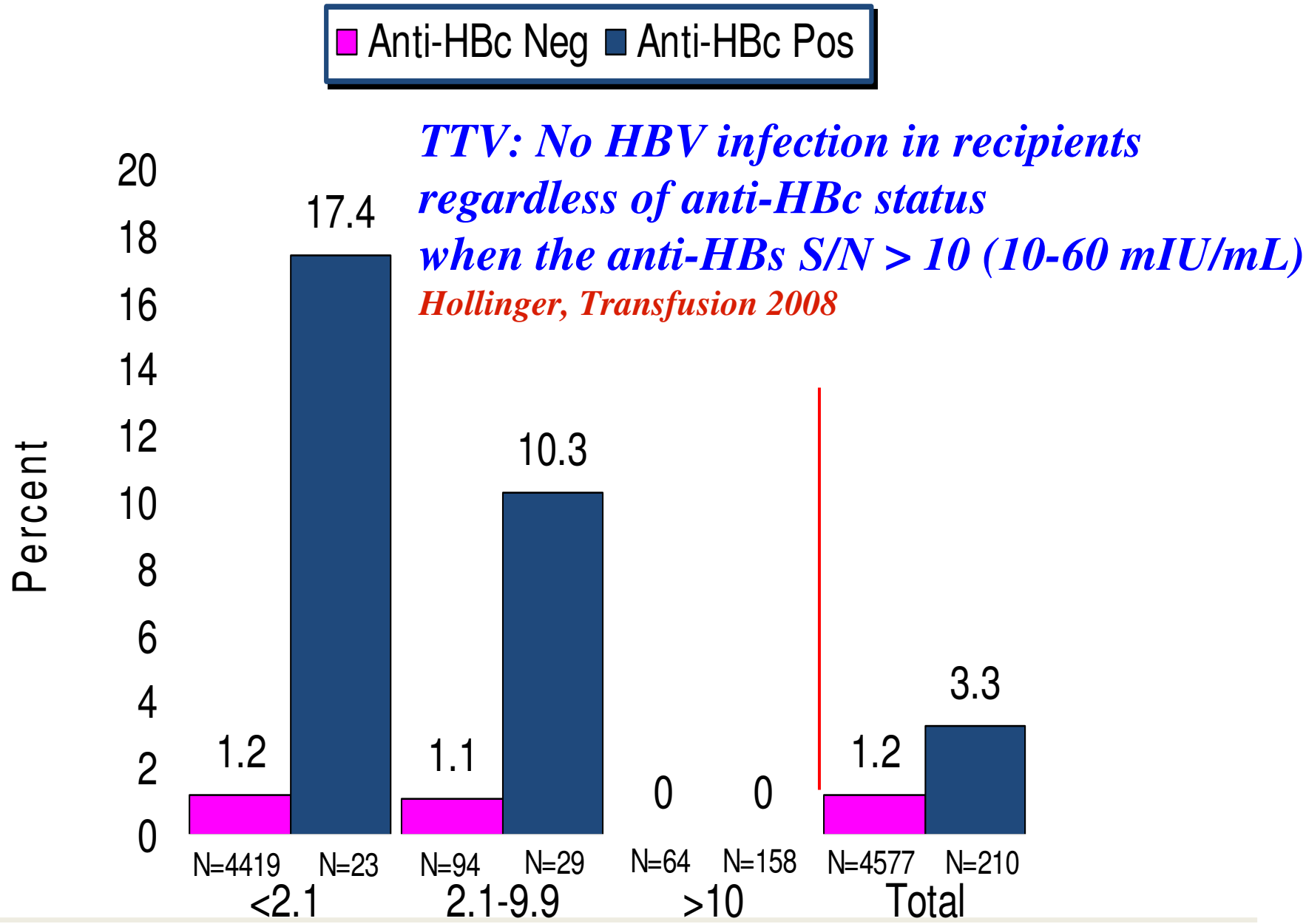
Are anti-HBs positive units infectious?

HBV Transmission from Low Level HBV DNA Pos "Occult" Donor

Levicnik-Stexinar et al., J Hepat 2008:48

	HBsAg	Anti-HBc	Anti-HBc IgM	Anti-HBs (mIU/mL)	HBV DNA (IU/mL)	Genotype
Donor (index)	Neg	Pos	NT	12-29	180	D
Recip* #1 4 days	Pos	Pos	Pos	Neg	NT	
8 days	Neg	Pos	Pos	Neg	185	D
Recip #2 7 days	Pos	Neg	Neg	Neg	$1.1x10^8$	D
14 days	Neg	Pos	Pos	Neg	Neg	

* Neg pretransfusion



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Results of JRC LB Study

Component Infectivity from ID NAT + Repository					
Anti-HBc in donors	No. donors	No. products tested	Infection		Infection Rate
			yes	no	
Low titer	29	33	1	32	3%
Negative	20	22	11	11	50%
Unknown	6	8	0	8	
Total	55	63	12 (100-380 c/mL)	51	19%

Of 12 infectious components, 11 (92%) were window period and 1 (8%) was anti-HBc positive (jumbo FFP)

Results of JRC LB Study

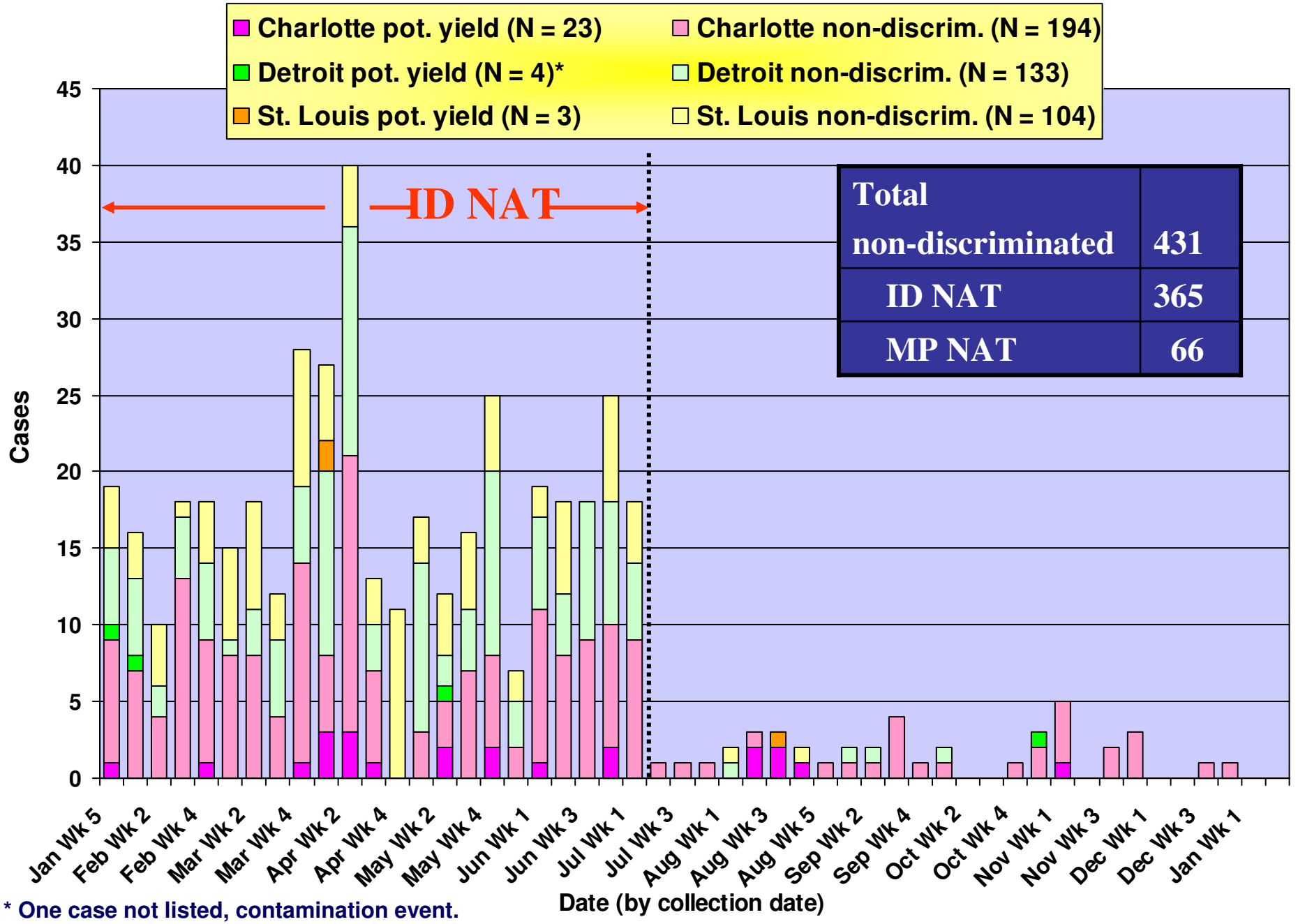
Anti-HBc in donors	Infectious	Anti-HBs in donors	
		Pos	Neg
Low titer	Yes	0	1
	No	11	12
Negative	Yes	0	8
	No	0	8
Unknown	Yes	0	0
	No	5	2
Total	Yes (12)	0	9 (29%)
	No (51)	16 (100%)	22

Additional Slides for questions

Additional Results (JPA) and Conclusions to Date

- Full sequences obtained for 6 (013, 003, 055, 074, 011, 042)
  - 1 each F, B, D and 3 each A2; half representing genomes not typically found in the US (majority in US is A2 in Caucasians, A1 in Blacks and B/C in Asians)
- Pre-S/S or BCP/PC sequences for 8 (above + 019 + 001)
  - 7 WT (compared to ref strains in GenBank); all major hydrophilic regions (MHRs) of the S protein conserved. Also, core, pol and X genes conserved; no stop codons
  - 1 S protein vaccine escape mutation (001)
- 4 (013, 003, 011, 042) are likely breakthrough infections, since no MHR mutations and all WT; one (001) vaccine escape
  - Inability of background levels of vaccine-derived anti-HBs to neutralize infectious virus
  - Low level anti-HBs might be sufficient to complex HBsAg making it undetectable but not sufficient enough, or too low, to prevent infection and the detection of HBV DNA
  - Public health implications; vaccine does prevent chronic HBV but not infection (all of these donors did clear virus and had truncated HBsAg responses, if present); more frequent boosters may be warranted
- 3 likely window period cases (019, 055 and 074)

Impact of ID NAT



* One case not listed, contamination event.

Specificity Comparisons

Duplex eSAS – MP NAT (16); 3 NTLs to 12/07

- 33,868,522 screened = 99.9974% (99.9972%-99.9975%)*

Ultrio TIGRIS – MP NAT (16); 3 NTLs

- 3,118,368 screened = 99.9973% (99.9966%-99.9978%)*

Ultrio TIGRIS – ID NAT; 3 NTLs

- 576,490 screened = 99.9297% (99.9225%-99.9364%)*

*95% CIs by the binomial distribution

Concordant Ultrio/Serology
2083 Results/2060 Donors

No. Reactive	HBV	HIV	HCV (+anti-HBc)
St Louis	63*	23	333*
Detroit	78	22*	222*
Charlotte	285* (67%)	186* (81%)	871* (61%)
Total	426	231	1,426**
Rate	1:8673	1:15,995	1:2591

*12 donations reactive for both HIV and HCV Ab + NAT
5 donations reactive for both HBV and HCV Ab + NAT
6 donations reactive for both HBV and HIV Ab + NAT

** 327 (23%) HCV reactives also anti-HBc reactive

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Ultrio Nonreactive – Serology Reactive (Confirmed Positive); N=5666 from 5662 Donors

	HBsAg (+)	Anti-HCV (+)	Anti-HIV (+)
ID NAT (-) (N=576,490)	134 (25)	455 (71)	339 (2)
MP NAT (-) (N=3,118,368)	655 (153)	2615 (446)	1468 (27)
TOTAL	789 (178)	3070 (517)	1807 (29)
Rate False Pos Serology (ID or MP NAT -)	1:5289 1:6212	1:1501 1:1438	1:1710 1:2164
Rate of Conf'd Pos Serology (ID or MP NAT -)	1:23,060- 1:20,381	1:8120 1:6992	1:288,245- 1:115,495

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Comparison of HBV DNA Reactivity in Anti-HBc Reactive Donors

(with removal of all HBsAg, HCV and HIV reactives)

	22 Reactive Ultrio MP NAT of 6573 anti-HBc RRs	12 Reactive Ultrio ID NAT of 1447 anti-HBc RRs	34 Total Reactive of 8020 anti-HBc RRs
No. (%) dHBV Pos (No. PCR* Pos/No. Tested)	18 (0.33%) (5/9 = 56%)	9 (0.62%) (6/7 = 86%)	27 (0.34%) (11/16 = 69%)
Adjusted	10 (0.15%)	8 (0.55%)	19 (0.23%)

*Multiprep Ampliscreen HBV ID NAT

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HBV Sensitivity of NAT Systems according to the PIs

- ID NAT Ultrio/TIGRIS = 10.40 IU/mL
- MP NAT 8 Ultrio/TIGRIS = 83.20 IU/mL
- MP NAT 16 Ultrio/TIGRIS = 166.40 IU/mL

- ID NAT MPX/s 201 = 3.80 IU/mL
- MP NAT 6 MPX/s 201 = 22.80 IU/mL

Table 1 HBV DNA Yield Samples
Index and Follow up Results
Linauts et al. Transfusion July 2008

		INDEX SAMPLE – HBsAg & anti-HBc NR		FOLLOW UP SAMPLE: DAYS REACTIVE (DAYS OF FOLLOW UP)					DONOR HISTORY
	DRAW DATE	anti-HBs mIU/mL	HBV DNA c/mL	HBsAg	anti-HBc IgM	anti-HBc Total	anti-HBs	DNA	
1	6/11/2003	51	61,000	7 (54)	26-54 (54)	26-54 (54)	0-54 (54)	0-7 (54)	27 YEAR OLD MALE REPEAT DONOR-MULTIPLE MALE SEXUAL PARTNERS
2	4/24/2004	NT	37,000	14 (177)	NR to 177	28-177 (177)	<14-177 (177)	0-21 (177)	50 YEAR OLD MALE REPEAT DONOR, NO RISK FACTORS IDENTIFIED
3	7/24/2003	NT	2300	7-21 (134)	28-63 (63)	28-134 (134)	63-91 (91)	0-28 (134)	29 YEAR OLD MALE REPEAT DONOR ACUPUNCTURE 8 WEEKS PRIOR TO DONATION
4	9/6/2002	NT	2000	17-200 (200)	55-200 (200)	48-200 (200)	NR to 200	0-200 (200)	26 YEAR OLD MALE REPEAT DONOR, NO KNOWN RISK

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Table 1 HBV DNA Yield Samples
Index and Follow up Results
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		INDEX SAMPLE – HBsAg & anti-HBc NR		FOLLOW UP SAMPLE: DAYS REACTIVE (DAYS OF FOLLOW UP)					DONOR HISTORY
	DRAW DATE	anti-HBs mIU/mL	HBV DNA c/mL	HBs Ag	anti-HBc IgM	anti-HBc Total	anti-HBs	DNA	
5	10/24/2006	NR	270						UNABLE TO ENROLL IN FOLLOW UP
6	6/11/2005	NR	200						39 YEAR OLD MALE REPEAT DONOR REPORTED VACCINATION HISTORY 3 DAYS PRIOR TO DONATION. COULD NOT CONTACT FOR F/U;
7	9/25/2002	2340	200	NR to 167	29-167 (167)	22-167 (167)	0-167 (167)	0-22 (167)	49 YEAR OLD FEMALE REPEAT DONOR, HISTORY OF VACCINE BUT WITH NEGATIVE TITER 8 WEEKS PRIOR TO INDEX
8 MPX*	7/10/2005	RR	<LOD	NR to 59	NT	NR to 59	0 (0)	0-59 (59)	PHILLIPPINE IMMIGRANT; NO HISTORY OF VACCINATION

* Nonreactive by MP COBAS AMPLISCREEN; Reactive IDT using multiprep procedure

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Table 2 Window Period Samples
Repeat Viral Load, Genotype and PRISM Results
Linauts et al. Transfusion July 2008

Donor #	Draw Date	HBV DNA Quantitation original copies/mL	HBV DNA Quantitation 2007 repeat copies/mL	HBV genotype	PRISM S/CO
1	6/11/2003	61,000	31,000	H	1.22, 1.16, 1.16
2	4/24/2004	37,000	3850	C	0.63
3	7/24/2003	2300	2480	B	1.90, 1.99, 1.97
4	9/6/2002	2000	ND	ND	1.11, 1.23, 1.09
5	10/24/2006	270	<200	NA	0.22
6	6/11/2005	200	<200	NA	0.81
7	9/25/2002	200	ND	NA	0.22
8	7/10/2005	<LOD	<200	NA	0.19