MOLECULAR DIAGNOSIS OF HEPATITIS B INFECTION IN THE TRANSFUSIONAL SETTING

Dra. Emma Castro and

Dra. Rocío González

HBV Blood Donor Testing in Spain

- ***Mandatory:***

  HBsAg

- ***Not mandatory:***

  – ***HBc-Ab; ALT.***

  – ***NAT-HBV***

Spanish Blood Centers Statistics, 2004.

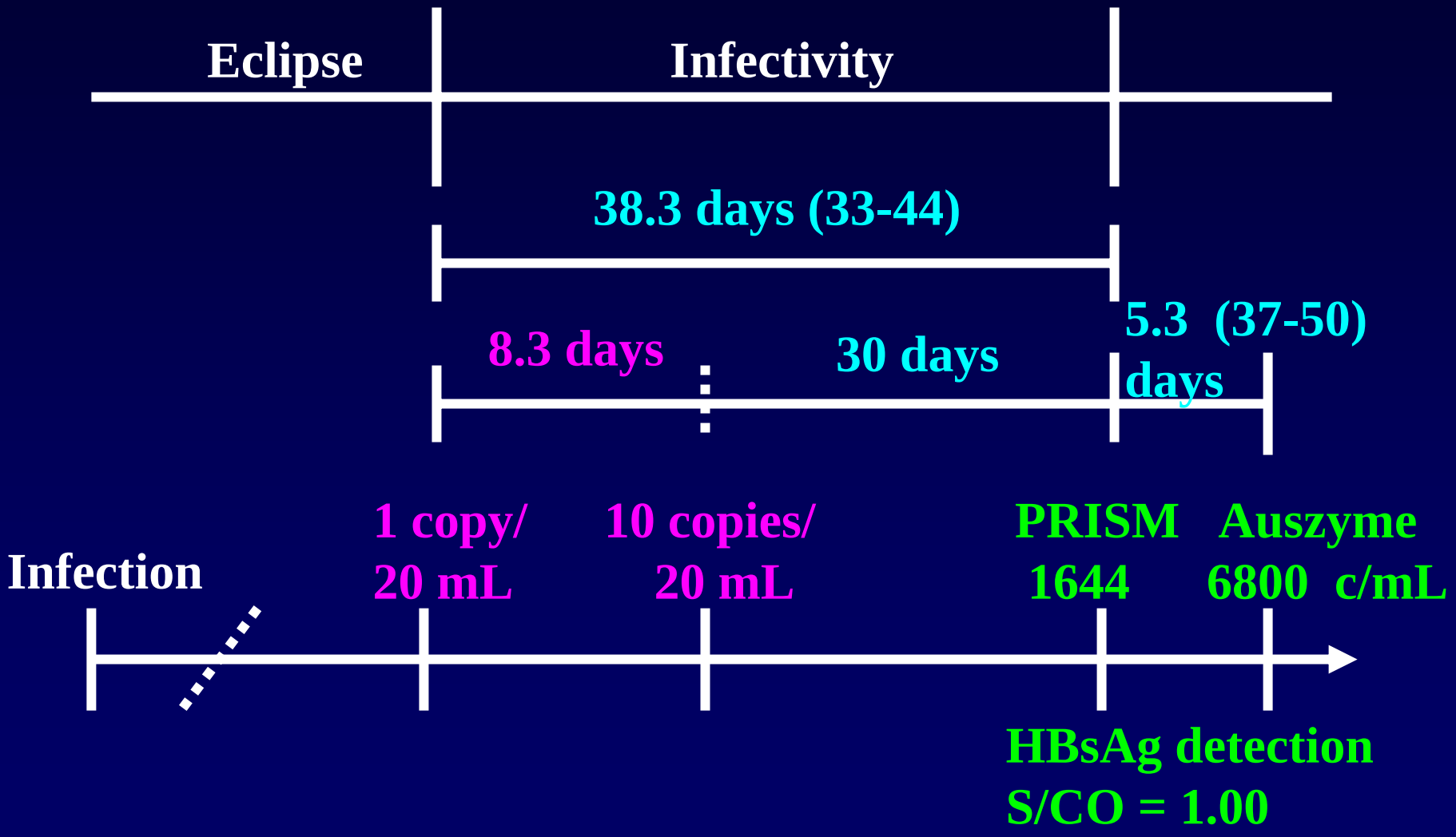
	U.x 100.000 SPAIN		U.x 100.000 MADRID	Nº Donations tested	Confirmed donations
HIV-Ab	5,77	1/20,000	5,89	1610824	93
HCV-Ab	30,27	1/3,333	37,15	1608733	487
HBsAg	36,75(*)	(0.04%) 1/2,700	55,27	1610824	592
SIFILIS	16,82	1/5,900	16,76	1610826	271

(*) Range: 8,03 Vasque Country up to 56,20 Canary Islands

HBc-Ab. Consequences of implementation in Spain.

- HBc-Ab prevalence among blood donors :
  - 6,1% (130/2161) Inst. Carlos III-1996
  - 4,2% (651/15545) BST – 2005
- Re-entry of donors with HBs-Ab:
  - Loss of 2,8 blood donors /1000 (BST: 45/15545)
- Do not avoid window period transmission.

Window Periods in HBV
Kleinman and Busch, JCV 35 Suppl 2006



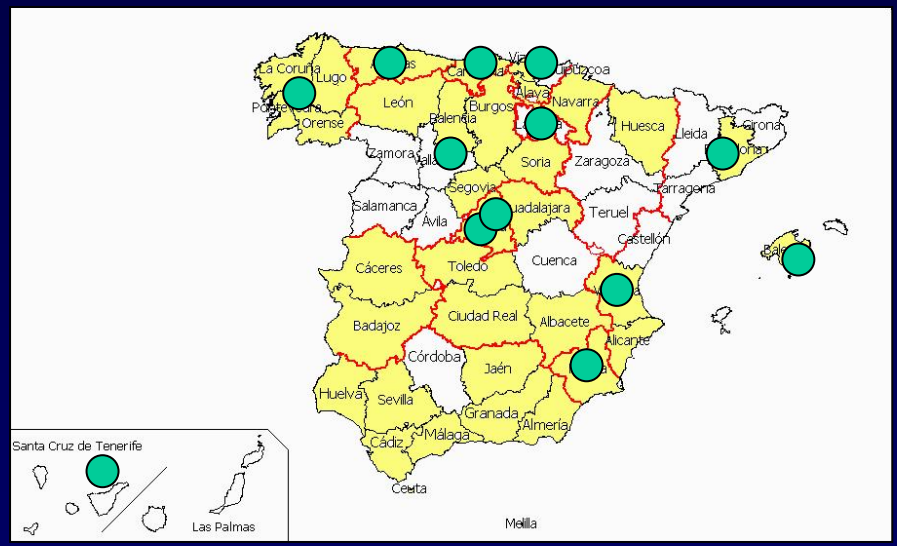
Analytical sensitivity analysis HBV-DNA assays by Sanquin on PeliCheck (n=24)

geq/mL	AmpliScreen *5 IU/mL*	Ultrio *11 IU/mL*
1,000	**100 %**	**100 %**
300	**100 %**	**92 %**
100	**100 %**	**83 %**
30	**83 %**	**33 %**
10	**27 %**	**4 %**
3	**21 %**	**4 %**
1	**21 %**	**0 %**
0.3	**0 %**	**0 %**

Impact = Decreased value of MP NAT

HBV-NAT in Spain.

- **80% of blood donations screened by HBV-NAT.**
  - **TMA (CHIRON) 40%, PCR (ROCHE) 25%, "IN HOUSE"15%**
- **2004:**
  - **CT Comun.Madrid**
  - **CT Cantabria**
  - **CT Galicia**
- **2005:**
  - **CT Baleares**
  - **CT Cruz Roja Madrid**
  - **CT Murcia**
  - **CT Canarias**
- **2006:**
  - **CT Asturias, BST Cataluña, CT Valencia, CT Pais Vasco, CT Castilla-León, CT La Rioja**



Observed yield vs.calculated

	VHB	VIH	VHC
Donations 1997-1999 (*)	1/180.000	1/1.000.000	1/240.000
Donations 1999-2001 (¡)	-	-	1/338.000
Donations 2000-2002(Ŧ)	1/100.000	1/1.000.000	1/400.000
Observed (Dic-2005)	1/142.800	1/450.338	1/381.264

(*) Alvarez M y cols Transfusion 2002;42:994-8
(¡) Eiras A y cols Transfusion 2003;43:713-20
(Ŧ) Alvarez M y cols Eurosurveillance 2005;10(2):11-16

Ultrio-HBV-NAT testing data 2004 - 2005

- NAT HBV Screening of 394,564 blood donations by Ultrio assay.
- Confirmation and characterization of all the cases.

Methods

- Screening: EIA/CHLIA + HIV-HCV- HBV-NAT
- IR: re-tested + dHXV. Index and/or follow-up samples tested by alternate NAT and extended HBV markers.
- Viral load: quantitative real time PCR (Chiron) or enhanced sensitivity limiting dilution TMA (Chiron/Gen-Probe).
- A 252 base pair fragment of HBsAg “a” determinant amplified for sequence analysis by enhanced PCR.

Yield of HBV-DNA screening (Ultrio) (until December 2005)

Pool size	pre (post) HBsAg window phase infections	anti-HBc reactive occult HBV infections	Total yield HBV-NAT HBsAg negative	donations tested
MP-NAT (1:8)	1	5	6	237,357
yield rate	1:237,400	1: 47,500	1:39,600[a]	
ID-NAT	3 (1)	11	15	157,207
yield rate	1:52,402	1:14,300	1:10,500[a]	
total	4 (1)	16	21	394,564
yield rate	1:98,600	1:24,700	1:18,800	

a) $p<0.0001$

MP-NAT Yield Case#1 genotype D wildtype Madrid Community Blood Center

A high viral load (~100,000 geq/ml) window phase donation

day	HBsAg PRISM S/CO	Ultrio 1:8 S/CO	Ultrio ID S/CO	ALT IU/ml	Anti-HBc	Anti-HBe	Anti-HBs mIU/ml
0	0.63	15.6[a]	15.8	23	neg	neg[b]	neg
32	0.30		15.7	122	pos	neg	>1000
57	0.30		0.06	10	pos	neg	>1000
175	0.32		0.12	39	pos	pos	>1000

a) HBV-DNA ~15.000 IU/ml, ~100,000 geq/ml
b) Also HBeAg negative
Torres,P personal communication.

ID-NAT Red Cross–Madrid Yield Case #1
Acute infection with HBV genotype F/adw4q-

González et al, Transfusion 2006, 46, 1138-1141

Days after index donation	ALT (UI/ml)	HBsAg		HBV DNA				Anti-HBc	Anti-HBc IgM	Anti-HBs (UI/l)	HBeAg
		PRISM (S/CO)	Auszyme (A) (% neutr.)	TMA (S/CO)	n-PCR	b-DNA (IU/ml)	Gtp				
0	22	Neg (0.7)		Pos (13.5)	Neg			Neg	Neg	Neg	Neg
4	21	Pos (1.4)	Pos (0.037) (99)	Pos (16.1)	Pos	$7.8x10^2$		Neg	Neg	Neg	Neg
11	20	Pos (12.1)	Pos (0.327) (99)	Pos (15.0)	Pos	$5.4x10^2$	F adw4	Neg	Neg	Neg	Neg
51	264	Pos (379)	Pos (>2.000) (95)	Pos (16.4)	Pos	$6.6x10^6$	F adw4	Pos	Pos	Neg	Pos
91	74	Neg (0.6)		Pos (16.0)	Neg			Pos	Pos	Pos (2.4)	Neg

- Index donation in 1:8 pool, 1 in 10 reactive with ULTRIO assay
- Confirmed with Alternate NAT
- Anti-HBc positive in 3rd sample
- Genotype F/adw4q- ;Fragment 333nt, ORF S; HBsAg amino acids 112 to 212
- HBV DNA load 1260 geq/ml in enhanced TMA and 136 IU/ml in Q-PCR

ID-NAT Red Cross–Madrid Yield Case #2
Acute infection with HBV type D/ayw3

González et al, Transfusion 2006, 46, 1138-1141

Days after index donation	ALT (UI/m)	HBsAg		HBV DNA				Anti-HBc	Anti-HBc IgM	Anti-HBs (UI/l)	HBeAg
		PRISM (S/CO)	Auszyme (A) (% neutr.)	TMA S/CO	n-PCR	IU/ml	Gtp				
0	9	Neg (0.3)		Pos (14.9)	Neg			Neg	Neg	Neg	Neg
7	5	Neg (0.4)		Pos (15.6)	Pos	$1.2x10^3$	D/ ayw3	Neg	Neg	Neg	Neg
33	8	Pos (59.3)	Pos (1.670) (100)	Pos (15.2)	Pos	$7.1x10^3$		Neg	Neg	Neg	Neg
77	12	Neg (0.3)		Neg (0.3)	Neg			Pos	Pos	Pos (8.7)	Neg

- Index donation in 1:8 pool: 1of 10 reactive in ULTRIO Assay
- Confirmed with Alternate NAT
- Anti-HBc positive on 3rd sample
- HBV DNA load 44 geq/ml in enhanced TMA and 22 IU/ml in Q-PCR

ID-NAT Red Cross–Madrid Yield Case #3
Acute infection with HBcAb IgM positive

Día	ALT (UI/L)	TMA-VHB POS	d-VHB	PCR-VHB nested	anti-HBc	anti-HBc IgM	HBeAg	anti-HBe	anti-HBs (mUI/ml)
0	32	11.9 Pos	10/10	Neg	Pos	Pos	Neg	Pos	263
12	20	14.2 Pos	1/1	Neg	Pos	Pos	Neg	Pos	405
38	25	0.07 Neg	1/2	Neg	Pos	Pos	Neg	Pos	684
56	28	9.8 / 0.12 Pos / Neg	0/1	Neg	Pos	Pos	Neg	Pos	706

- Gonzalez R, personal communication.

ID-NAT Mallorca Yield Case #1
Acute infection with HBV type F

Case #1	Date	HBsAg ORTHO S-3 (S/CO)	HBsAg PRISM (S/CO)	HBV-DNA ULTRIO (S/CO)	dHBV
Index Donation*	Day 1	0.3 (Neg)	Pos (1.1)	14.04 (Pos)	Pos
1st Sample	Day 5	Neg		Pos	
2nd Sample	Day 8	Pos (1.2)		Pos	
3rd Sample	Day 20	Pos			

*HBV-DNA concentration 230 IU/ml in Q-PCR (Chiron)
Sedeño M, personal communication.

OCCULT HBV YIELD CASES UNDER STUDY. SPAIN 2005 (INCOMPLETE)

Donor	HBsAg S/CO	Ultrio S/Co	Q-PCR cps/ml	Ampliscr Sanquin	S_PCR estimate Sanquin	IgM anti-HBc	Anti-HBc	Anti HBs IU/L	Anti-Hbe	ALT U/L	Pre-core mutant	genotypeS "a" mutant
Mad -1	0.30	16.6	30	pos	100-300	neg	pos	17	neg	12		D, yes
Mad -2	0.38	16.2	650	pos	300-1000	neg	pos	5.7	pos	65	yes	D, yes
Mad -3	0.27	13.8	100	pos	30-100	neg	pos	neg	neg	13		D, yes
Mad -4	0.23	15	30	pos/neg	10-30	neg	pos	22.3		26	no	D, yes
San -1	Neg/ 0.22	6.1	<300	pos	10-30			neg			yes	D, yes
San -2	neg	1.01/ 15		pos	<10							
San -3	neg						pos					
CR-1	0.25	13.2				neg	pos	neg	neg	15		A, ND
CR-2	0.38	10.9	4.8 *	pos		neg	pos	17		13		D, ND
CR-3	0.31	9.16	36 *	pos		neg	pos	149		16		A, ND
CR-4	0.3	15	58*	pos		neg	pos	22	pos	29		D, No
CR-5	0.26	14	40*	pos		neg	pos	neg		23		D, Yes
CR-6	0.26	14.7	895*	pos		neg	pos	7.5		14		D, Yes
CR-7	0.25	14.6		pos		neg	pos	2.1		23		D, Yes
CR-8	0.29	14		neg+		neg	pos	9		19		

* Limiting dilution TMA

Amino acid substitutions in HBsAg ‘a’ determinant in nine Spanish blood donors with occult HBV infection

amino acid nr:	116	120	130	140	pre-C	mIU/ml
genotype D	TSTG	PCRTCTTPAQ	GTSMYPSCCC	TKPSDGNCTC	mutant	aHBs
8323344 donor 1A	n...	.*YPL*...tv.	....S.Y...	I...A....		
9977414 donor 1B	n...	.*YP*..*I*.tv.	....S.Y...	I...A....		17
3404193 donor 2	n...	....*F*M.t..	...Ih.....	.....R....	Yes	5.7
0943514 donor 3	...*V* S....M....	....	...t......			neg
3596585 donor 4	....	...*P*.*I*.t..	E...f*A*....	...L...*Y*..	No	22
4120755 donor 5A	..*R*.	T..N.M*LN*.P	....S.F...	....	yes	neg
4220755 donor 5B	..*R*.	T..s.Mi*H*.P	....S.F...	....		
5570587 donor 6	....	....	....	....		22
5580467 donor 7	..*A*.	....	....	....		neg
5585979 donor 8	....	....	......Y...	....		7.5
5581462 donor 9	....	.....M....	....	....ER....		2.1

X known mutation associated with occult hepatitis B;
or described in patients simultaneously testing positive for HBsAg and anti- HBs

X variant not previously reported

x naturally occurring variant, not associated with impaired detection;
either present in the reference HBV strains, or described without association with occult hepatitis

C Cysteine substitution affecting HBsAg loops

Conclusions

- Good correlation between WP Found and Predicted (1:98,600 vs. 1/100,000 donations).
- ID-NAT significantly more effective than MP-NAT in detecting low viral load WP and OBI donations.
- ID-NAT detected low viral load OBI carriers. A few were dHBV negative with inconsistent S/CO values.
- Numerous amino acid changes identified in OBI cases, many of which have not previously been described.
- But still, the most important question is:

  Are late stage occult HBV individuals really infectious?