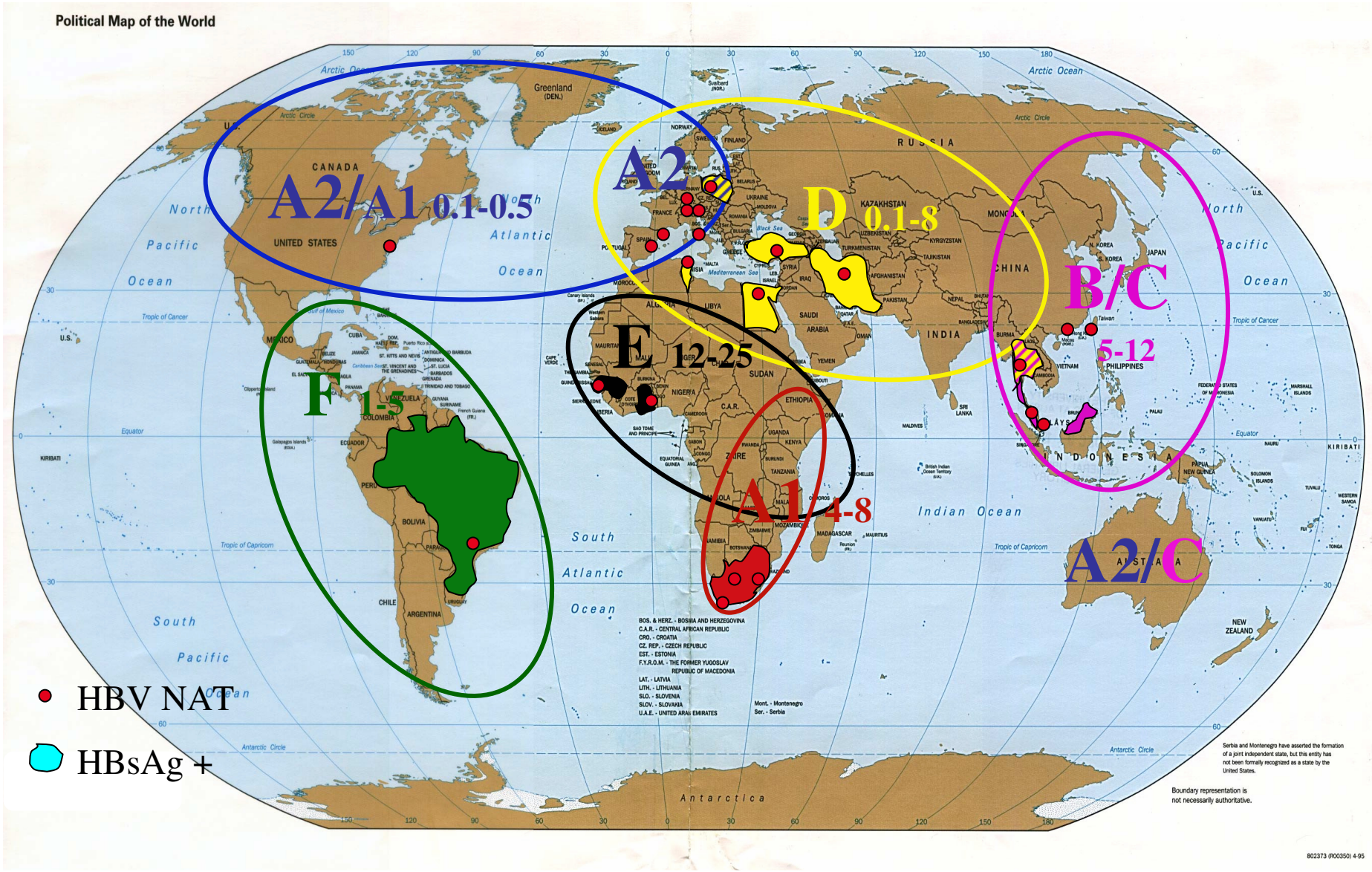
ISBT TTI WP
HBV safety subgroup

Review and update

Distribution of dominant HBV genotypes countries & centres participating in HBV studies



HBV DNA yield samples processed

Origin	N Samples	QPCR + (%)	BCP/PC	PreS/S	Full genome	Status
2006/2007	213	118	73	48	26	completed
Hong Kong	148*	97	35	37	9	completed
Taiwan	25	19	20	22	20	completed
Thailand	32	31	27	24	17	in progress
Malaysia	3	2	0	1	0	completed
Germany	1	1	1	1	1	completed
Poland	15	13	transfer	transfer	transfer	completed
Switzerland	15	3	3	3	2	in progress
South Africa	109	83	52	45	14	completed
ARC, USA	24	14	21	16	12	in progress
Total	**585**	**381(65)**	**232**	**197**	**101**	

Full genome sequences obtained in OBI according to genotype

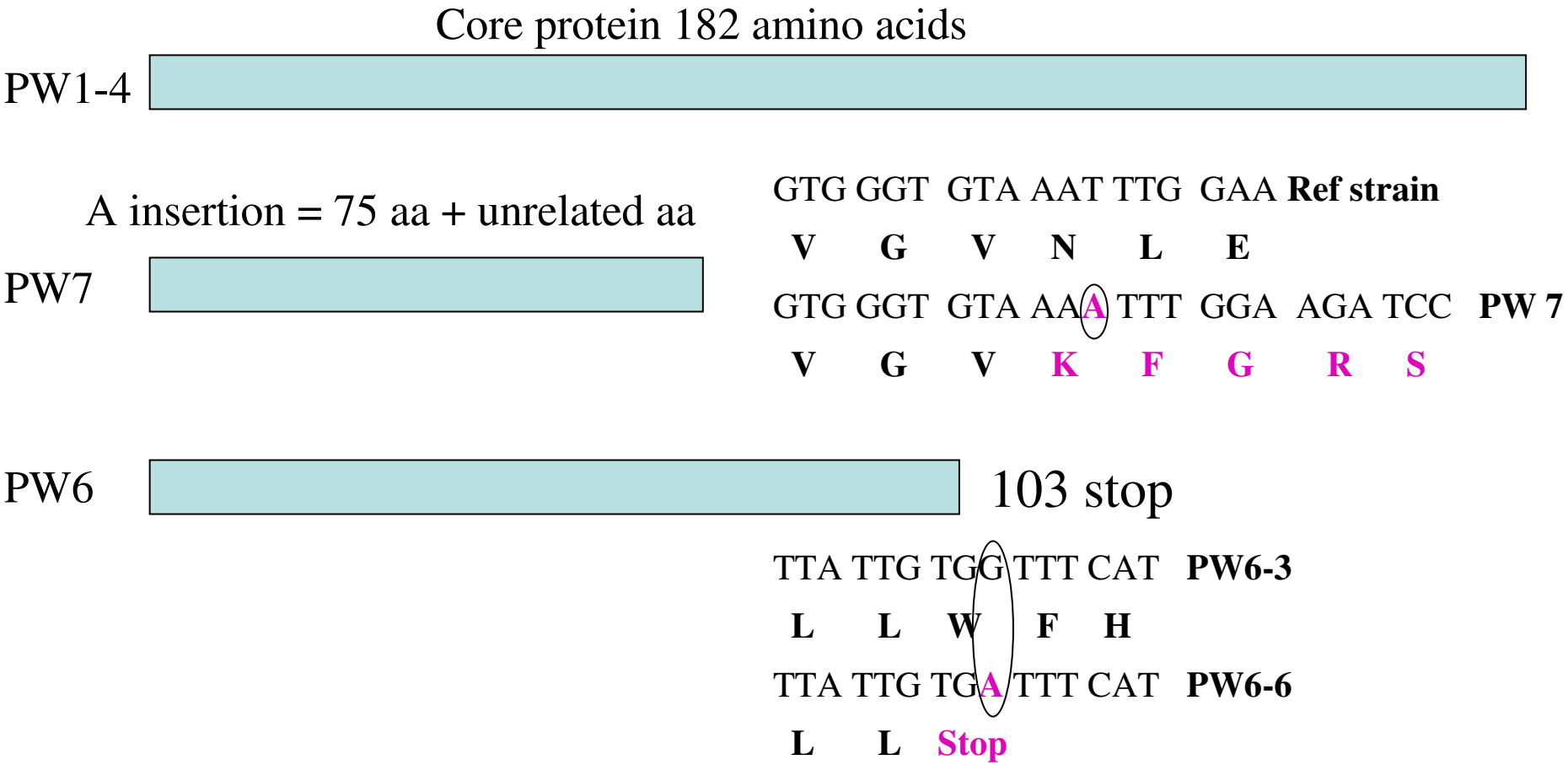
Genotype	N full genome
A1 (South Africa)	14
A2 (Europe/USA)	24
B (Far East)	34
C (Far East)	21
D (Europe/USA)	22
E (West Africa)	7

Immune pressure on Pre-S/S proteins (% aa substitutions)

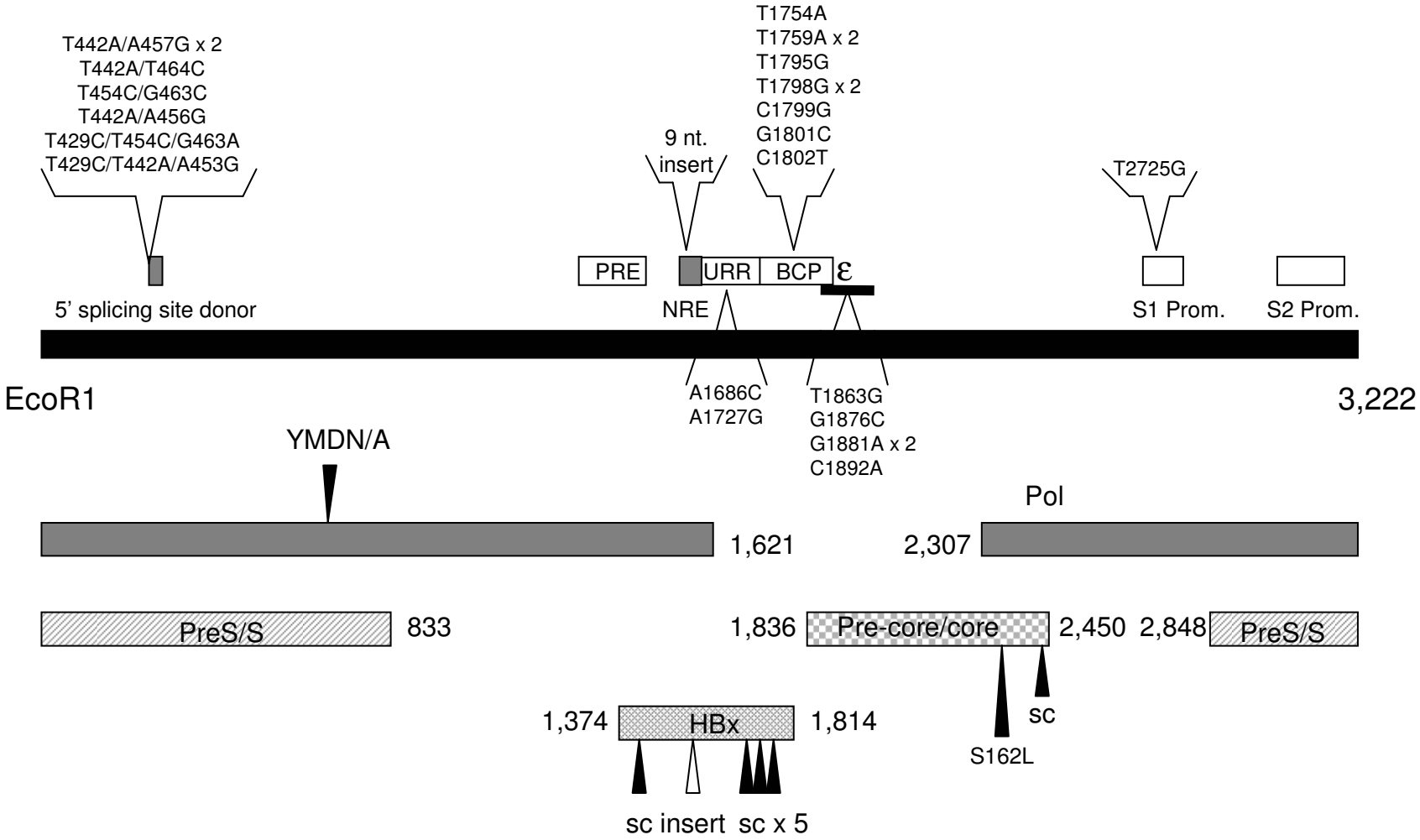
Genotype	A2	D	B	C	A1	E
N samples	13	39	16	8	13	9
Control	1.4*	0.8*	1.1*	1.2*	1.6	0.6
OBI	**3.4***	**3.9***	**3.2***	**3.3***	1.9	1.2#
Non-active	2.6	1.5	2.6	2.7	1.3	1.2
Active (C+H)	**5.7** *	**6.9** *	**4.2** *	**4.1**	1.9	1.1
Cellular	4.4	4.6	3.8	3.3	2.1	1.4
Humoral	**8.3**	**10.1** *	**5.3**	**6.1**	1.3	0

* $P < 0.001$ # $P = 0.008$

Truncated core protein: a potential mechanism of OBI genotype E



Mutations and amino acid substitutions found in 15 full genome genotype A1 OBI sequences



Development of a confirmatory algorithm for HBV DNA yield cases

Four steps

1. Confirm presence of HBV DNA
2. Generate serological marker data
3. Generate molecular data (sequencing)
4. Test follow-up sample

Diagnostic conclusion: donor information

1. Confirm presence of HBV DNA

A- Repeat discriminatory assay (Poisson distribution)?

B- Alternative NAT

- Commercial HBV NAT discriminatory
- In-house or commercial real time PCR (viral load)
- In-house amplification small amplicons (BCP/PC or S)

C- Viral concentration prior to HBV DNA testing

- Increase extraction volume 0.2 to 0.5 or 1ml
- Centrifugation ≥ 10,000g for ≥ 60 minutes
- Immuno-capture

Any of these procedures positive = HBV DNA confirmed

2. Correlate NAT results with serology

- Alternative HBsAg
  - Higher sensitivity
  - Different ability to detect variants
- Anti-HBc: when confirmed, indicates prior contact with HBV
- Anti-HBe when + can help confirm anti-HBc
- Anti-HBs
  - alone = vaccination
  - with anti-HBc = recovery from HBV infection
  - in vaccinee = breakthrough infection with variant

3. Follow-up sample

Timing: 4-12 weeks post-index sample

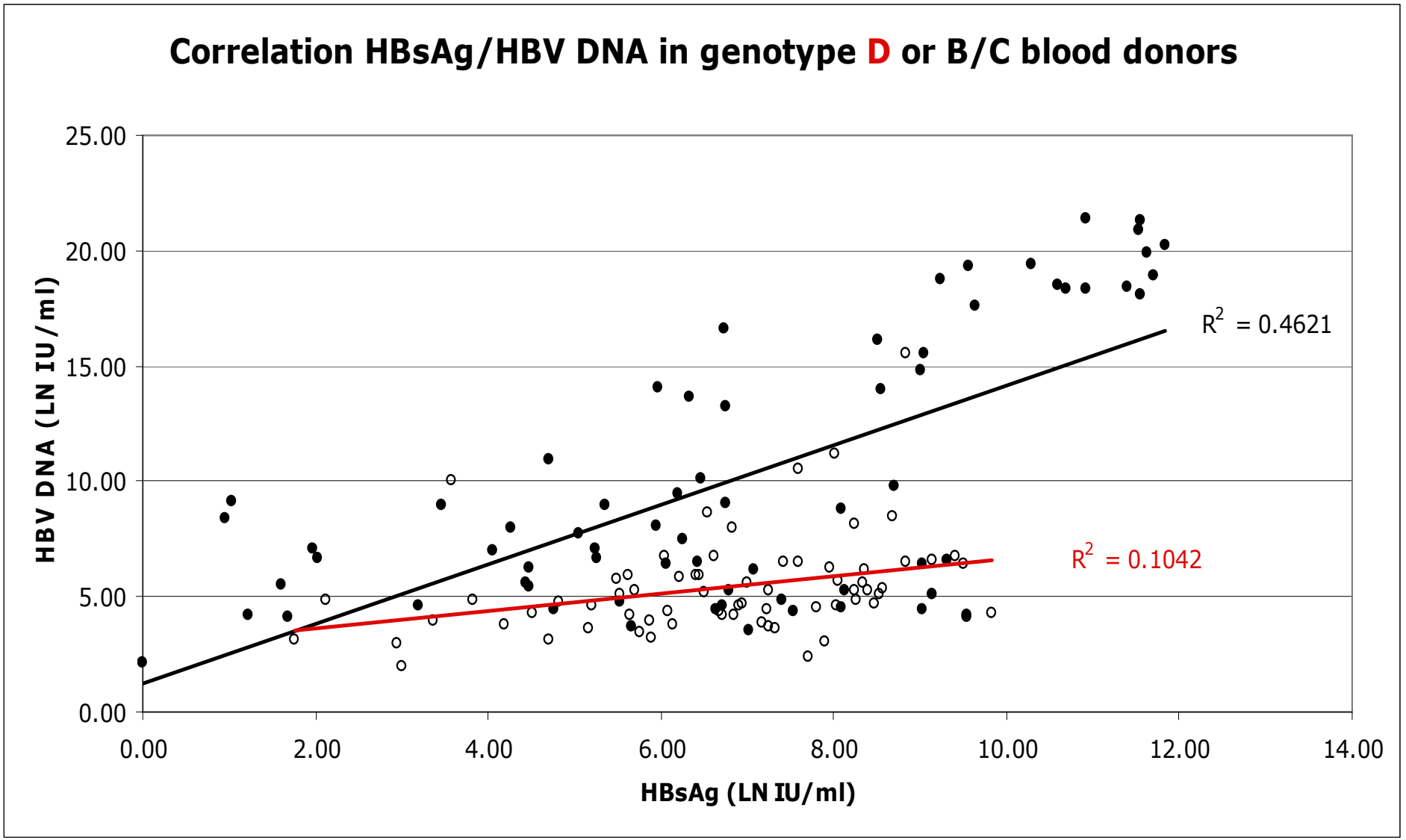
Assays

- NAT discriminatory
- NAT confirmatory
- HBsAg
- Anti-HBc
- Anti-HBs titre
- ALT level

HBsAg pos control samples

Origin	N samples	QPCR+	BCP/PC	Pre-S/S	Full genome	Status
Thailand	100					In progress
Malaysia	167	83/86*	82/86	71/86	54/86	completed
Italy	65					In progress
Poland	177	168	153/166	67/71	53/71	completed
South Africa	100	74	74	73	62	completed
Egypt	73	56	56	43	37	In progress
Turkey	199	176	165			In progress
Iran	200					In progress
Brazil 1	50	48		45		completed
Brazil 2	100					requested
Hong Kong	100					In progress
Taiwan	100					requested
Total	**1431**	**605**	**530**	**299**	**206**	

Correlation between HBsAg and HBV DNA



Training of scientists in NAT HBV yield confirmation

Country	Blood centre	Duration of training	Dates	Outcome
Brazil	Sao Paulo	1 week	Sept 2007	operational
Poland	Warsaw	1 week	Nov 2007	operational
Malaysia	Kuala Lumpur	3 weeks	July 2008	Setting up
Hong Kong	HK Red Cross	2 weeks	Sept 2008	Setting up
Italy	Lecco BTC	1 week	Dec 2008	Setting up

Publications 2008-2009

1. Candotti D, Grabarczyk P, Ghiazza P, Roig R, Casamitjana N, Iudicone P, Schmidt M, Brird A, Crookes R, Brojer E, Miceli M, Amiri A, Li C, Allain JP. Characterization of occult hepatitis B virus from blood donors carrying genotype A2 or genotype D strains. J Hepatol 2008; 49; 537-47.
2. Levicnik-Stezinar S, Rahne-Potokar U, Candotti D, Lelie N, Allain JP. Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients. J Hepatol. 2008; 48: 1022-5.
3. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxi A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trepo C, Villa E, Will H, Zanetti AR, Zoulim F. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol 2008; 49: 652-7.
4. Wendel S, Levi JE, Biagini S, Candotti D, Allain JP. A probable case of hepatitis B virus transfusion transmission revealed after a 13-month-long window period. Transfusion 2008; 48:1602-8.
5. Allain JP, Belkhiri D, Vermeulen M, Crookes R, Cable R, Amiri A, Reddy R, Bird A, Candotti D. Characterization of occult Hepatitis B virus strains in South African blood donors. Hepatology 2009; in press.
6. Allain JP, Candotti D. Diagnostic algorithm for HBV safe transfusion. Blood Transfusion 2009; in press.
7. Gonzalez R, Torres P, Castro M, Candotti D, Koppelman M, Zaaijer HL, Lelie N, Allain JP Echevarria JM. Efficacy of Hepatitis B virus DNA screening and characterization of acute and occult HBV infections among blood donors from Madrid, Spain. Transfusion, Submitted

Future work

- Complete genotype B/C studies in Far East (HK, MAL, SGP, TW, TH)
    - Control population (TH=100, HK=100, TW=50)
    - Sequential samples from Hong Kong
- Reference work for several countries (SW, HK, ARC)
- Continue training for reference labs (Italy, Switzerland)
- Publish - Far East data (2 articles)
    - HBsAg/DNA correlation