Molecular characterization of hepatitis B virus strains infecting blood donors with high HBsAg and undetectable HBV DNA levels: implications for blood safety and screening policy

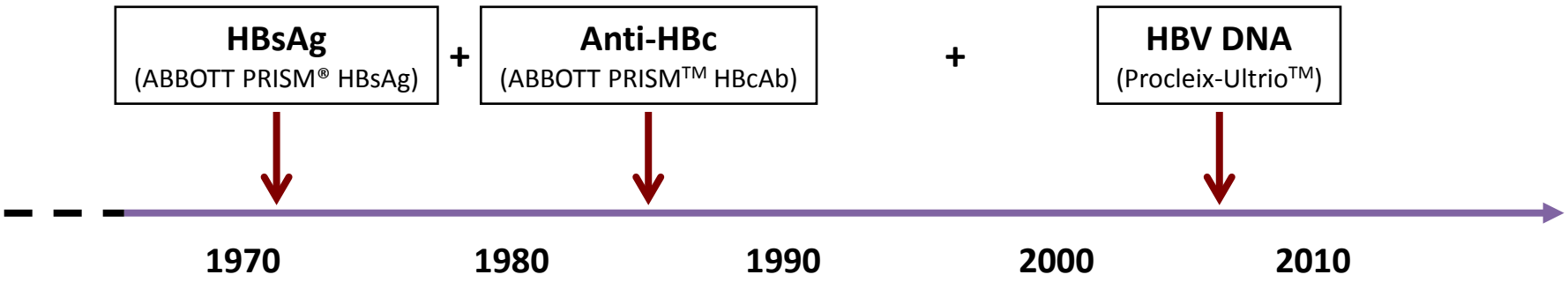
D Candotti

Institut National de la Transfusion Sanguine
Dept. Agents Transmissibles par le Sang
Paris, France

Relative efficacy of HBV screening assays

HBV infection features	Detected by		
	HBsAg	Anti-HBc	HBV NAT
Window period	*No*	*No*	**Yes**
Primary OBI	*No*	*No*	**Yes**
2nd window period	*No*	**Yes**	**Yes**
Chronic infection	**Yes**	**Yes**	**Yes**
Anti-HBc+ OBI	*No*	**Yes**	**Yes**
Anti-HBs only OBI	*No*	*No*	**Yes**
Anti-HBc only	*No*	**Yes**	*No*
HBsAg only	**Yes**	**?**	*No*

HBV screening in French blood donations



- High sensitivity and adequate specificity
- Pre-seroconversion window period & occult infections

→ **Estimated HBV residual risk: 1 in 4 millions donations**

But:

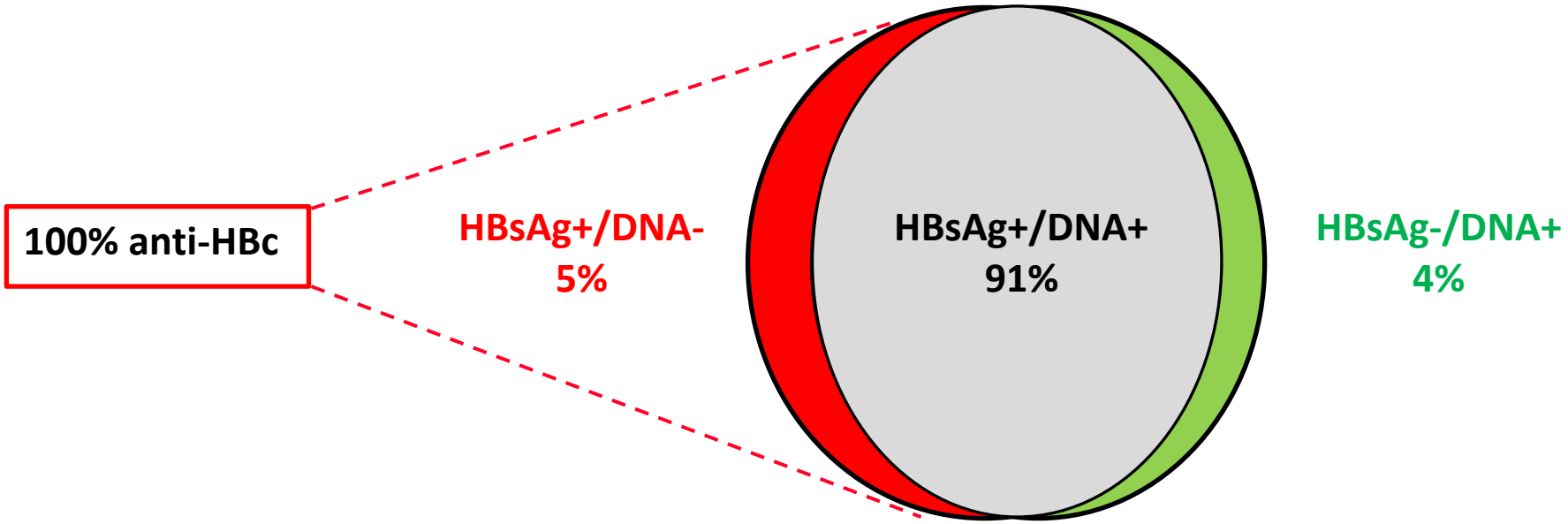
- High cost
- Redundancy of HBsAg and HBV DNA direct markers

Maintaining HBsAg testing?

- Cost reduction of blood testing
- Complementarity of anti-HBc and HBV DNA testing
  (Enjalbert et al. Transfusion 2014;54:2485-95)
- Anti-HBc testing issues on blood availability in high endemic settings
- **Potential impact on blood safety?**

Distribution of HBV markers in French blood donors

- Period: 2010-2013
- Excluding overseas territories
- 10 186 279 donations tested → **806 HBV reactive (≈ 1/10,000)**



HBsAg & HBV DNA discrepant levels in 740 samples confirmed HBsAg+

Sample screening	Number (%)	HBV DNA load (IU/mL) (COBAS TaqMan HBV; LOQ 6 IU/mL)		
		Undetected	< 6	≥ 6
NAT* neg.	41 (5%)	13 (32%)	20 (49%)	8 (19%)
NAT pos.	699 (95%)			
• HBsAg < 100 IU/mL	58 (8%)	1 (2%)	12 (21%)	45 (77%)
• HBsAg > 100 IU/mL	641 (87%)	13 (2%)	27 (4%)	601 (94%)

*NAT: Procleix-Ultrio (LOD 12 IU/mL)

Hypotheses

- **Ratio: 1 viral particle / 1,000-10,000 HBsAg**

  - Natural course of infection

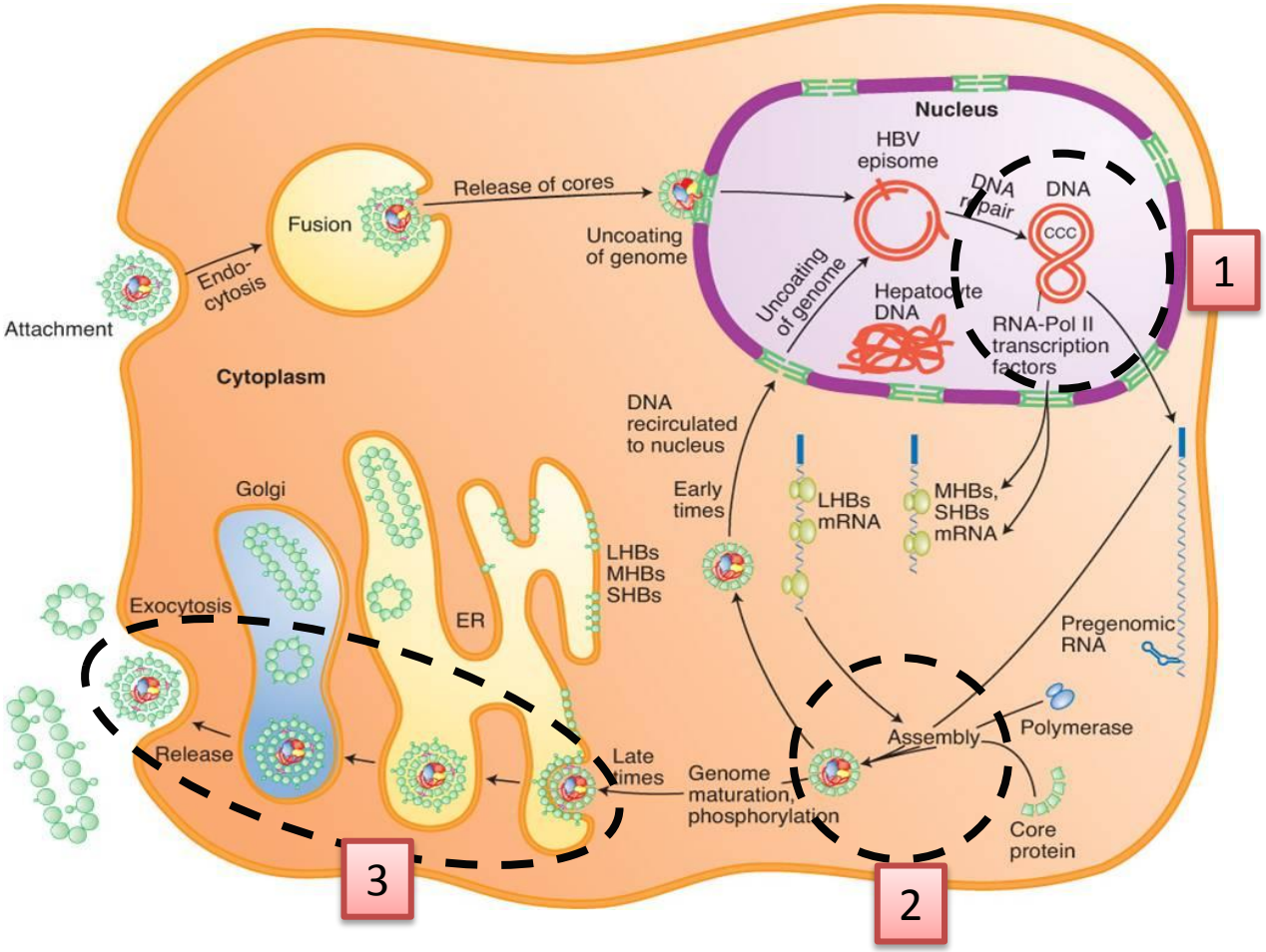
  - HBV genotypes

- **Hypotheses:**

  - NAT failure

  - Impaired viral replication

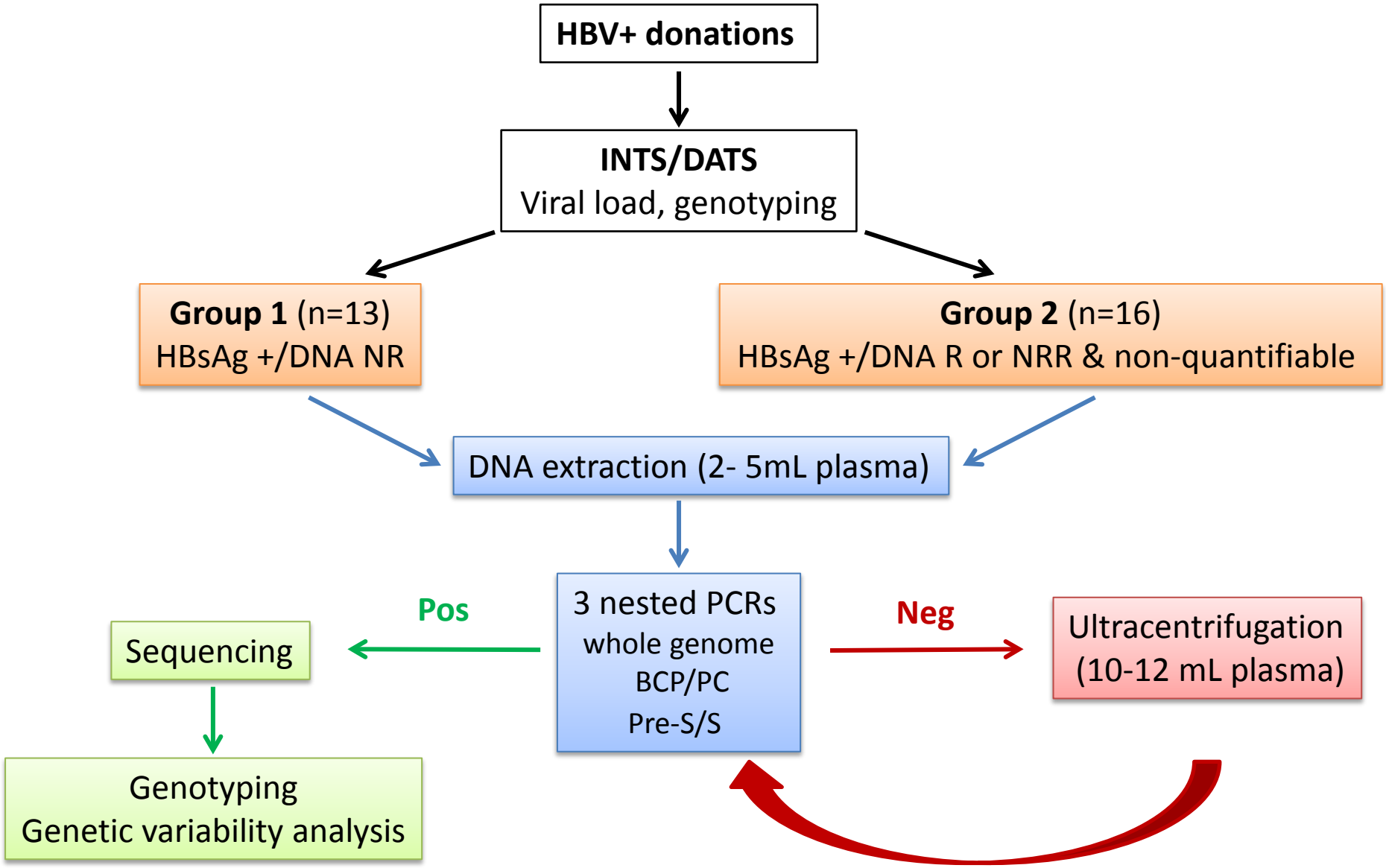
- **Infectivity?**



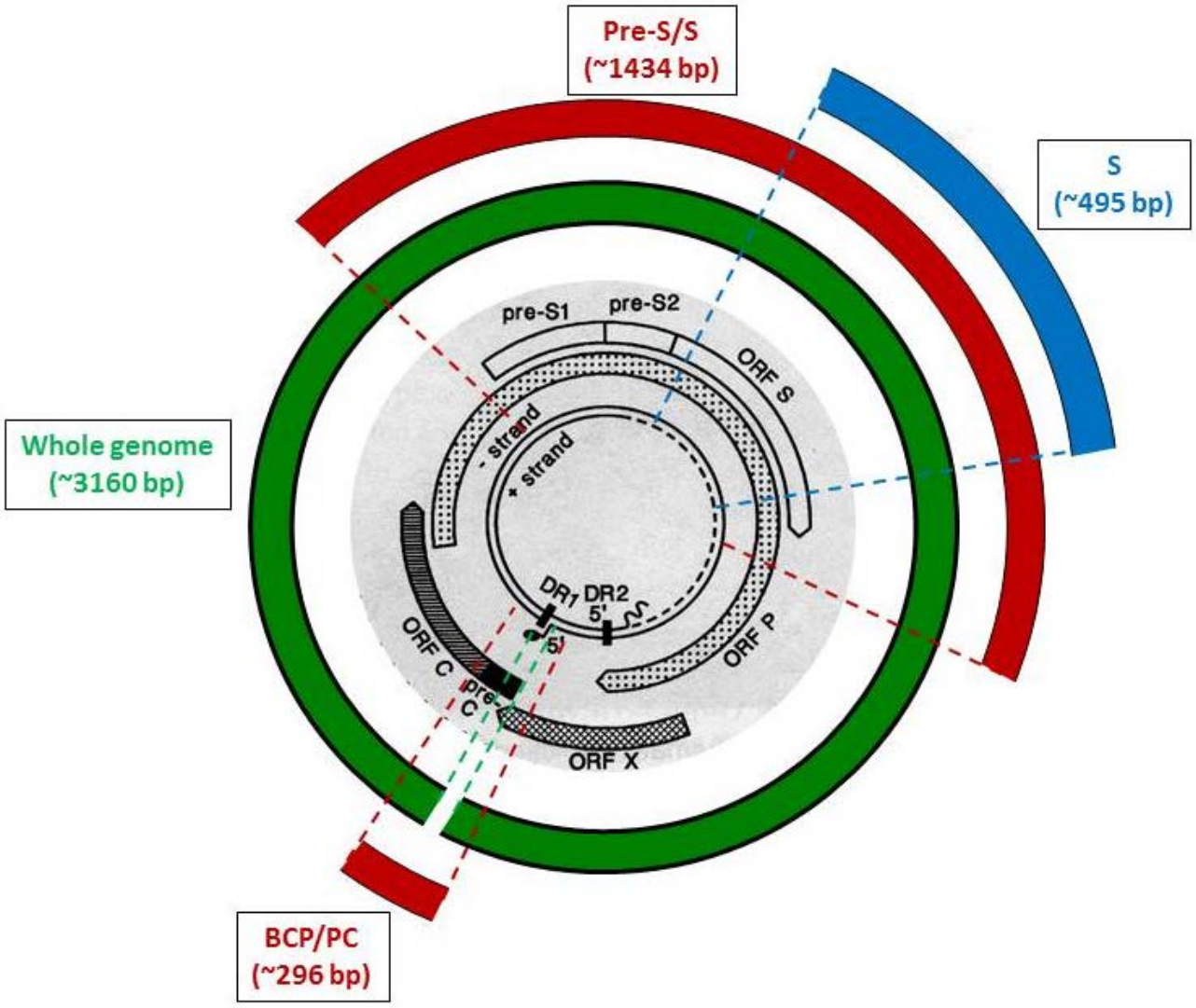
Objectives

- Prevalence of HBsAg+/ NAT non-reactive or non-repeatable reactive donations
- Detect and/or confirm HBV DNA presence
- Evaluate and compare performance of NAT assays to detect these samples
- Perform genetic characterization of the viral strains associated with this phenotype
- Evaluate viral replicative properties *in vitro* as a surrogate marker of infectivity

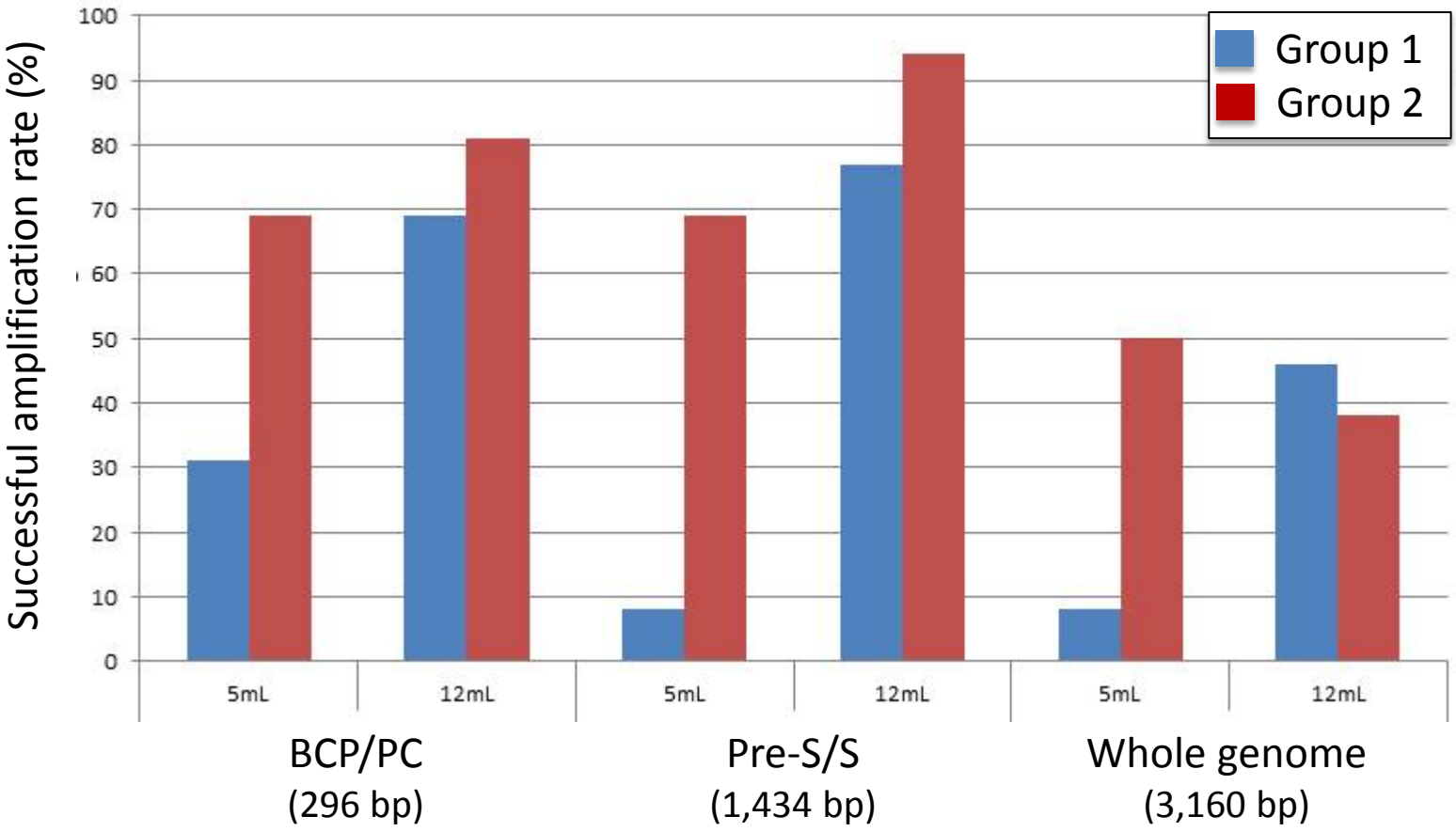
Study design



HBV DNA amplification



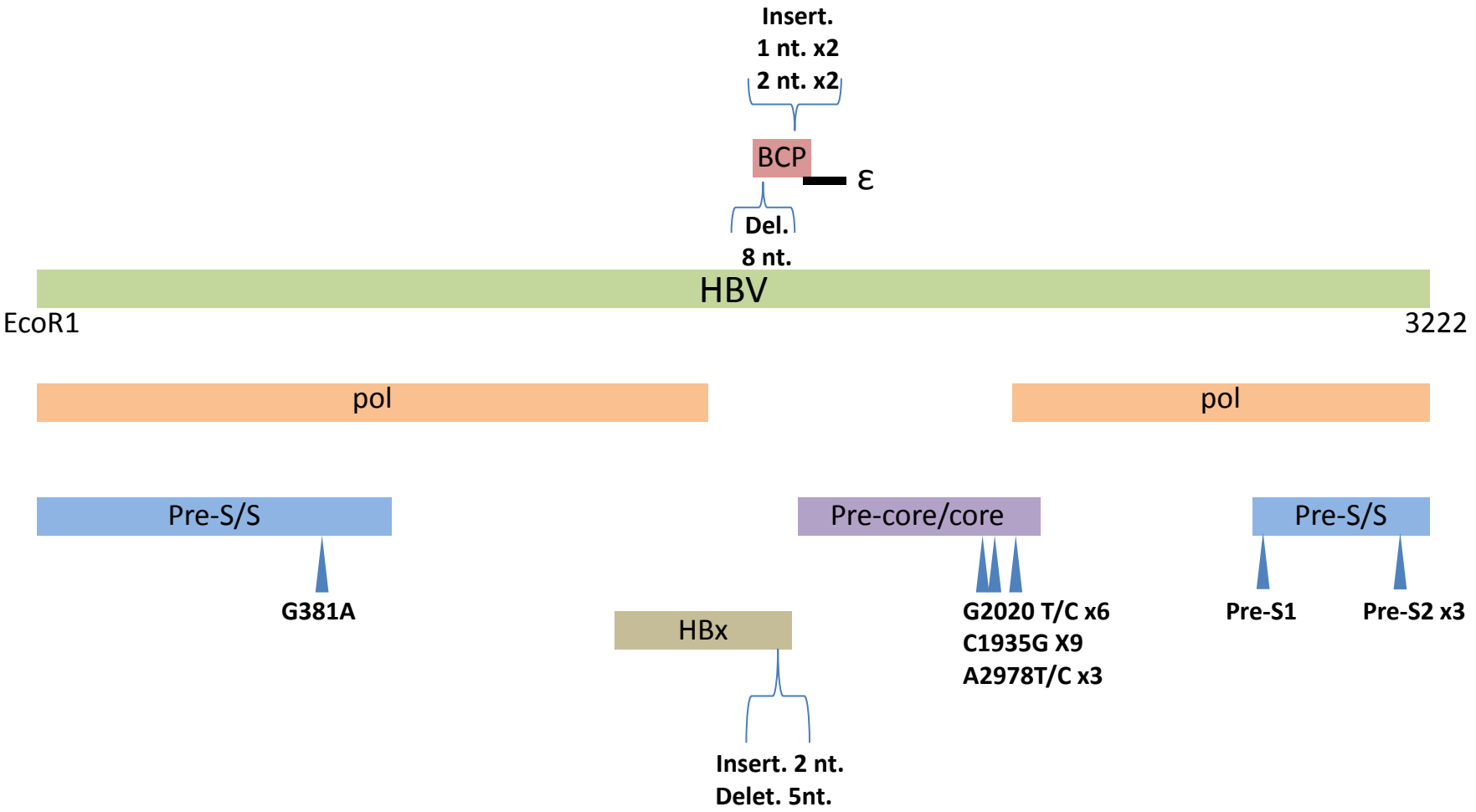
HBV DNA amplification performance



Preliminary results

	Group 1 (n = 13)	Group 2 (n = 16)	Total (n = 29)
Age (y) (mean; range)	34 (19 – 59)	35.5 (18 – 61)	34.8 (18 – 61)
HBsAg (ng/mL) (median; range)	1,355 (110 – 39,500)	2,113 (150 – 19,030)	1,881 (110 – 39,500)
HBV DNA confirmed	**12 (92%)**	**15 (94%)**	**27 (93%)**
HBV genotypes			
• A	-	9	9 (35%)
• B	1	-	1 (4%)
• C	2	1	3 (11%)
• D	7	2	9 (35%)
• E	1	3	4 (15%)

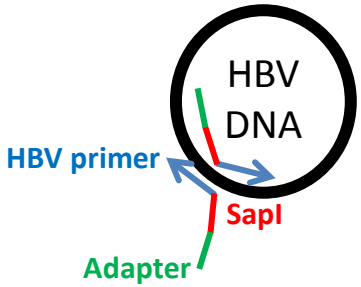
Sequences analysis



Construction of HBV replicons

Method 1

1st PCR amplification with HBV-specific primers



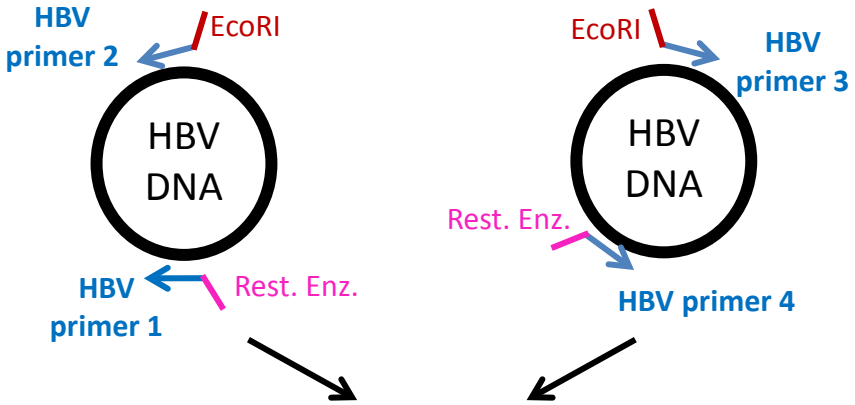
2nd PCR amplification using adapters

Huh7 transfection & re-circularization with SapI

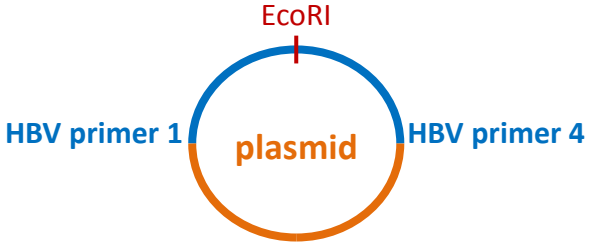
HBV genome expression & replication

Method 2

2 distinct PCR amplifications



Cloning of 1.2 HBV construct



Huh7 transfection

HBV genome expression & replication

Preliminary conclusions & perspectives

- **Conclusions:**
  - Extremely low level of HBV DNA confirm in >90% of ID-NAT non-reactive blood donations with concomitant high HBsAg levels
  - Phenotype not associated with donor age or HBV genotype
  - Impaired viral replication rather than NAT failure is suggested
  - Mutations potentially affecting viral replication identified

- **Perspectives:**
  - Increase the number of samples and controls of various genotypes
  - Collaborative study (Croatia, Poland, Switzerland, South Africa, Malaysia,…)
  - Develop an *in vitro* HBV replication system
    - functional characterization of HBV variants
    - evaluation of infectious risk
    - increase knowledge about distinct molecular control of viral replication & HBsAg production →potential clinical implications
  - Funding

Acknowledgements

INTS
DATS/CNR Hépatites en Transfusion
Paris, France
S. Laperche
A. Servant-Delmas
L. Boizeau
C. Leclerc

Inter-regional Blood Transfusion SRC
Berne, Switzerland
C. Niederhauser
P. Gowland

Croatian Institut of Transfusion Medecine
Zagreb, Croatia
M. Miletic Lovric
I. Mihaljevic

Dept. Virology
Institute of Haematology & Transfusion Medicine
Warsaw, Poland
P. Grabarczyk
A. Kopacz

South African NBS
Johannesburg, South Africa
M. Vermeulen
A. Saville

National Blood Centre
Kuala Lumpur , Malaysia
A. Bon
A. Fread

Grifols
Diagnostic Solutions Inc