

# 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**

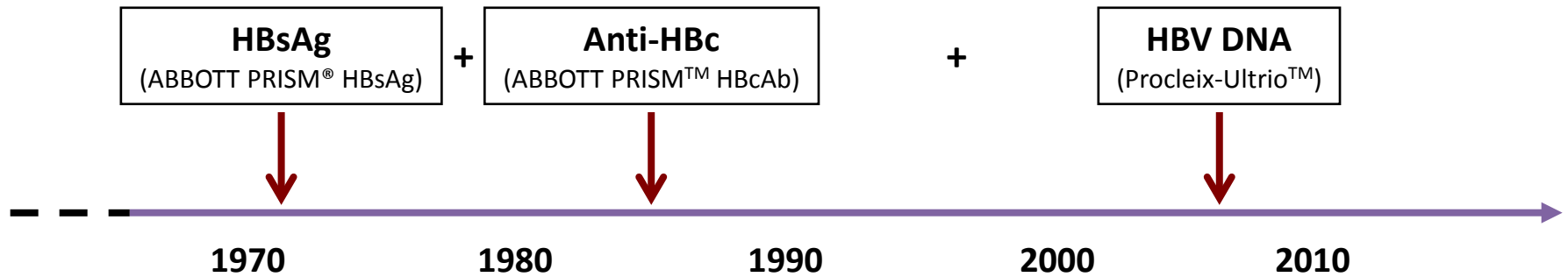
Institut National de la Transfusion Sanguine  
Dept. Agents Transmissibles par le Sang  
Paris, France



# 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:**

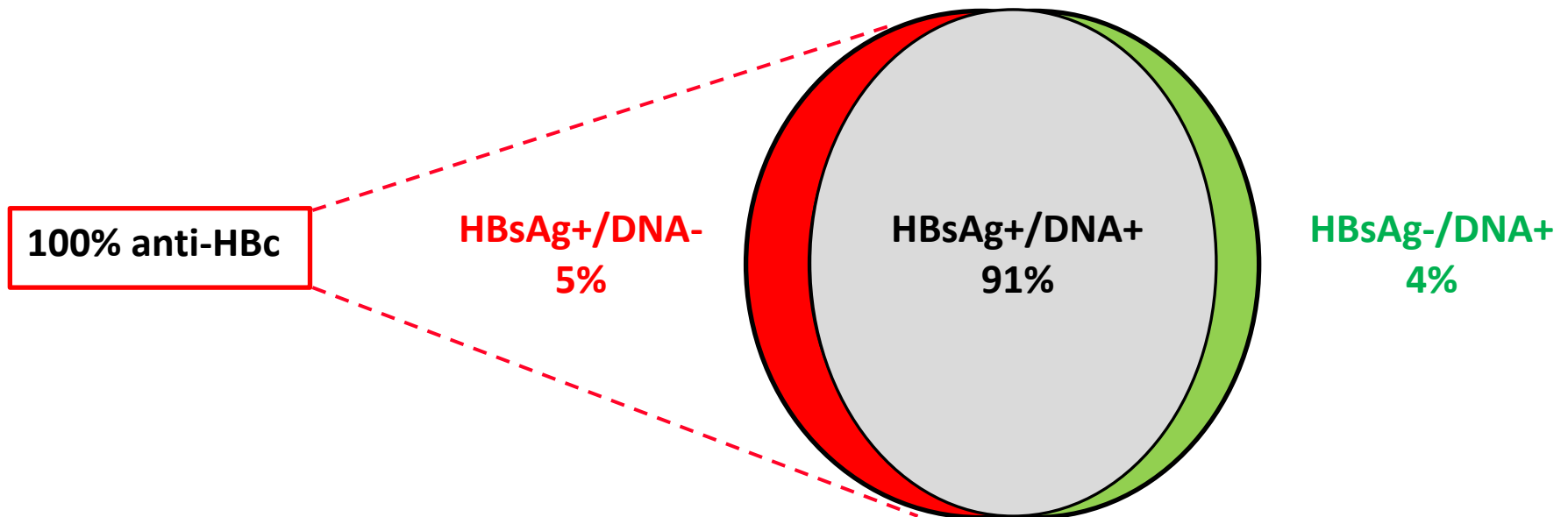
- High cost
- Redundancy of HBsAg and HBV DNA direct markers

# 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** ( $\approx 1/10,000$ )



# 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
<b>NAT* neg.</b>	41 (5%)	13 (32%)	20 (49%)	8 (19%)
<b>NAT pos.</b>	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

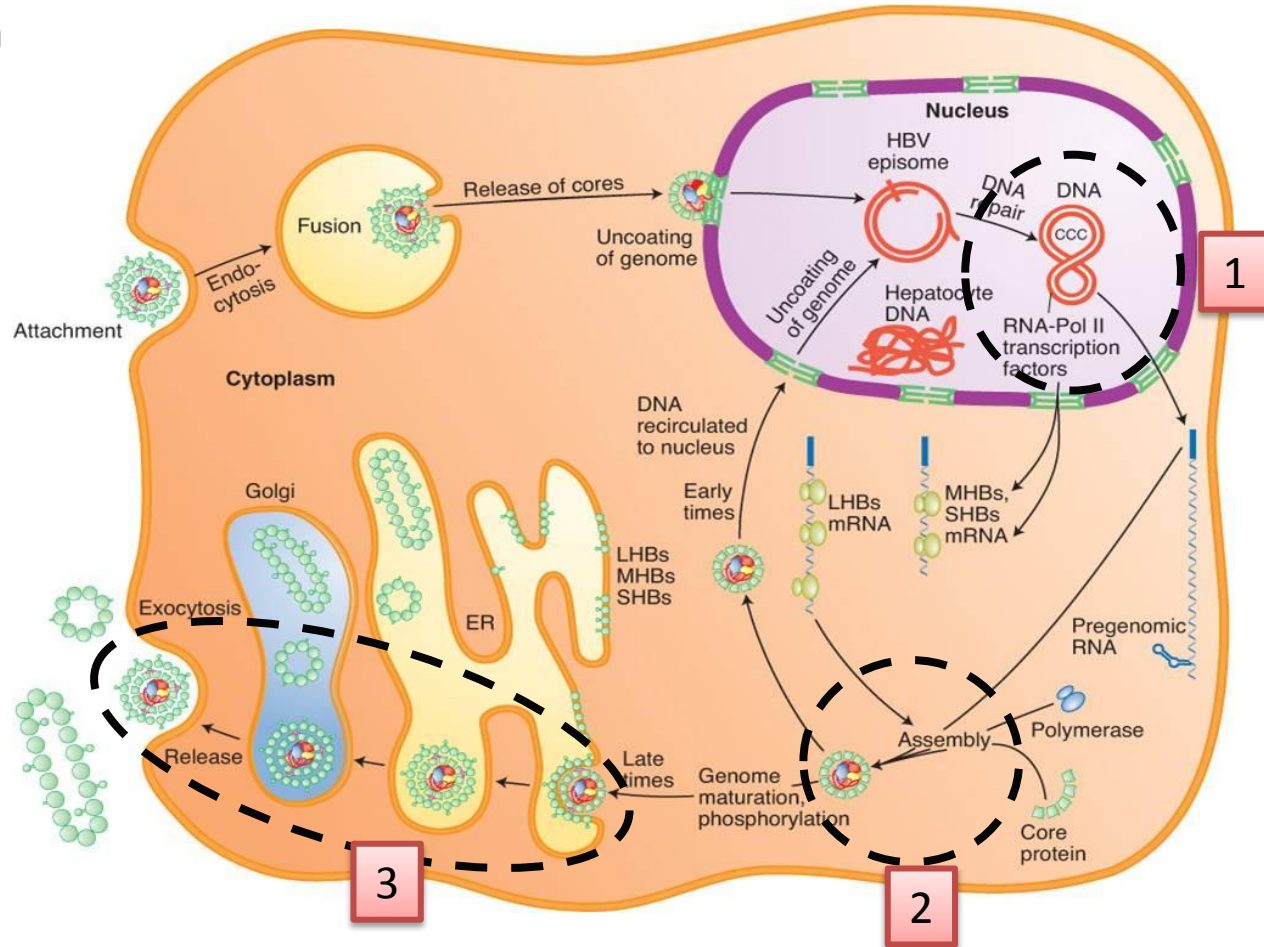
- HBV genotypes

- **Hypotheses:**

- NAT failure

- Impaired viral replication

- **Infectivity?**

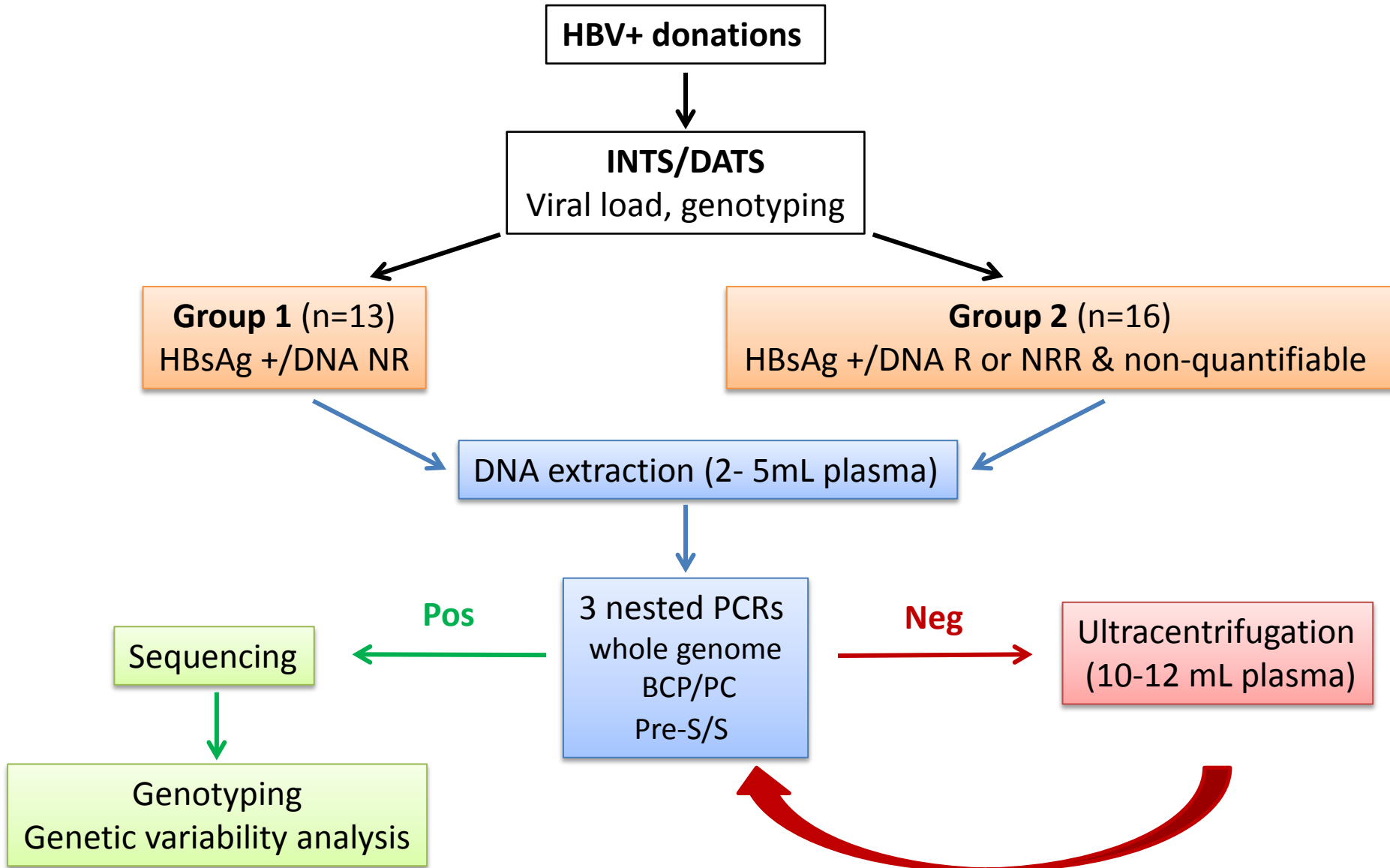


# Objectives

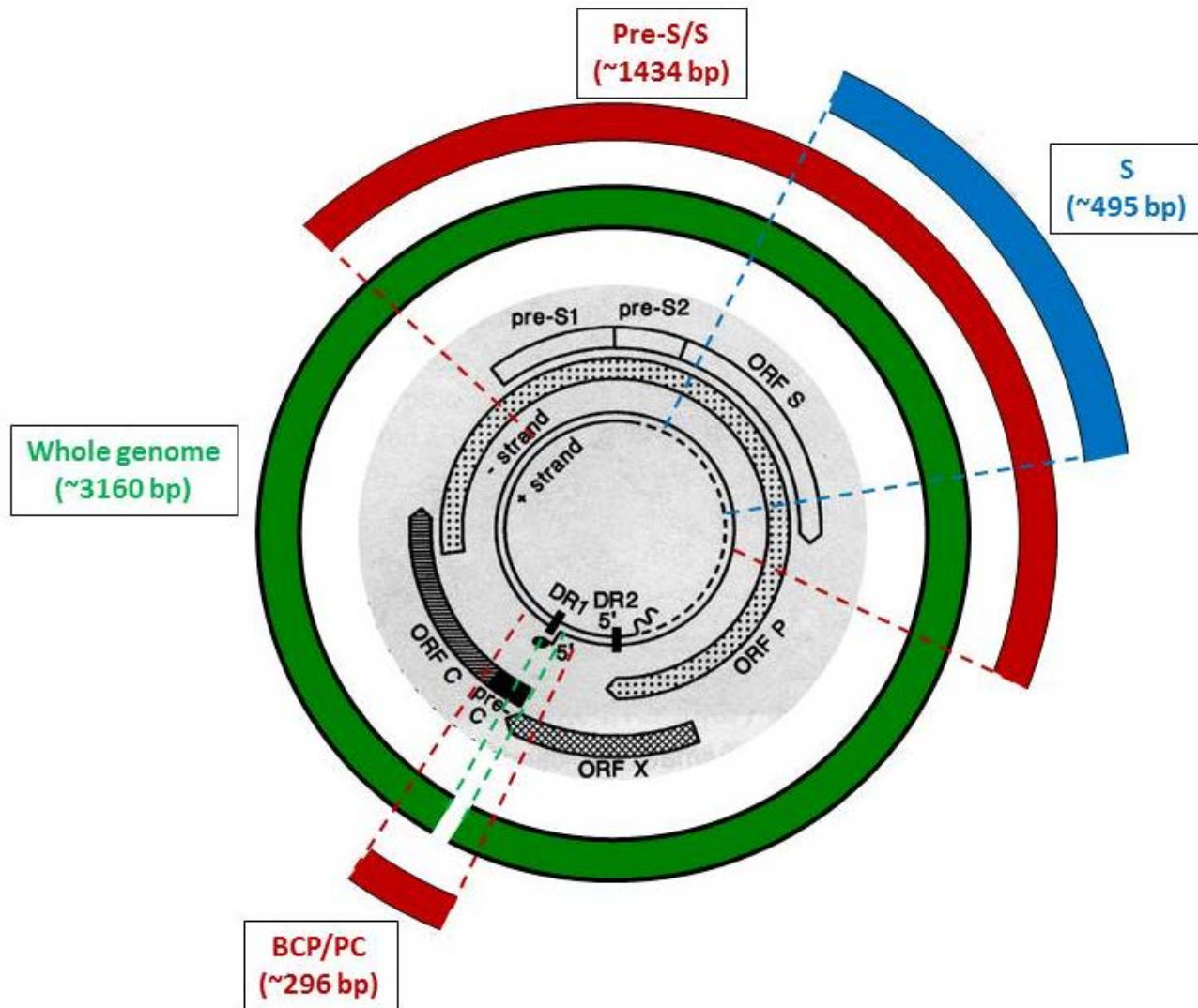
- Prevalence of HBsAg+/ NAT non-reactive or non-repeatable reactive donations
- Detect and/or confirm HBV DNA presence
- Evaluate and compare performance of NAT assays to detect these samples
- Perform genetic characterization of the viral strains associated with this phenotype
- Evaluate viral replicative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



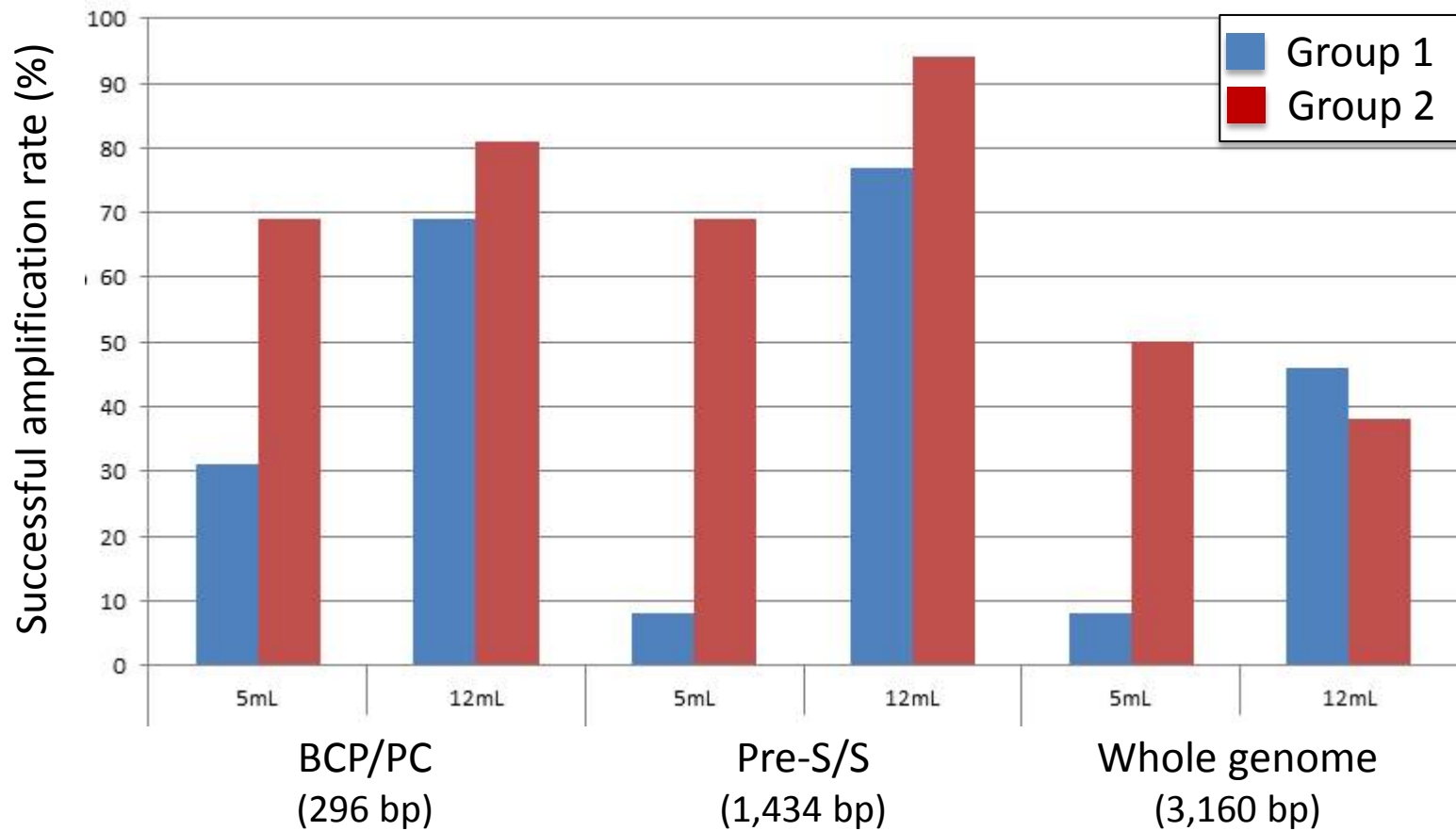
# Study design



# HBV DNA amplification



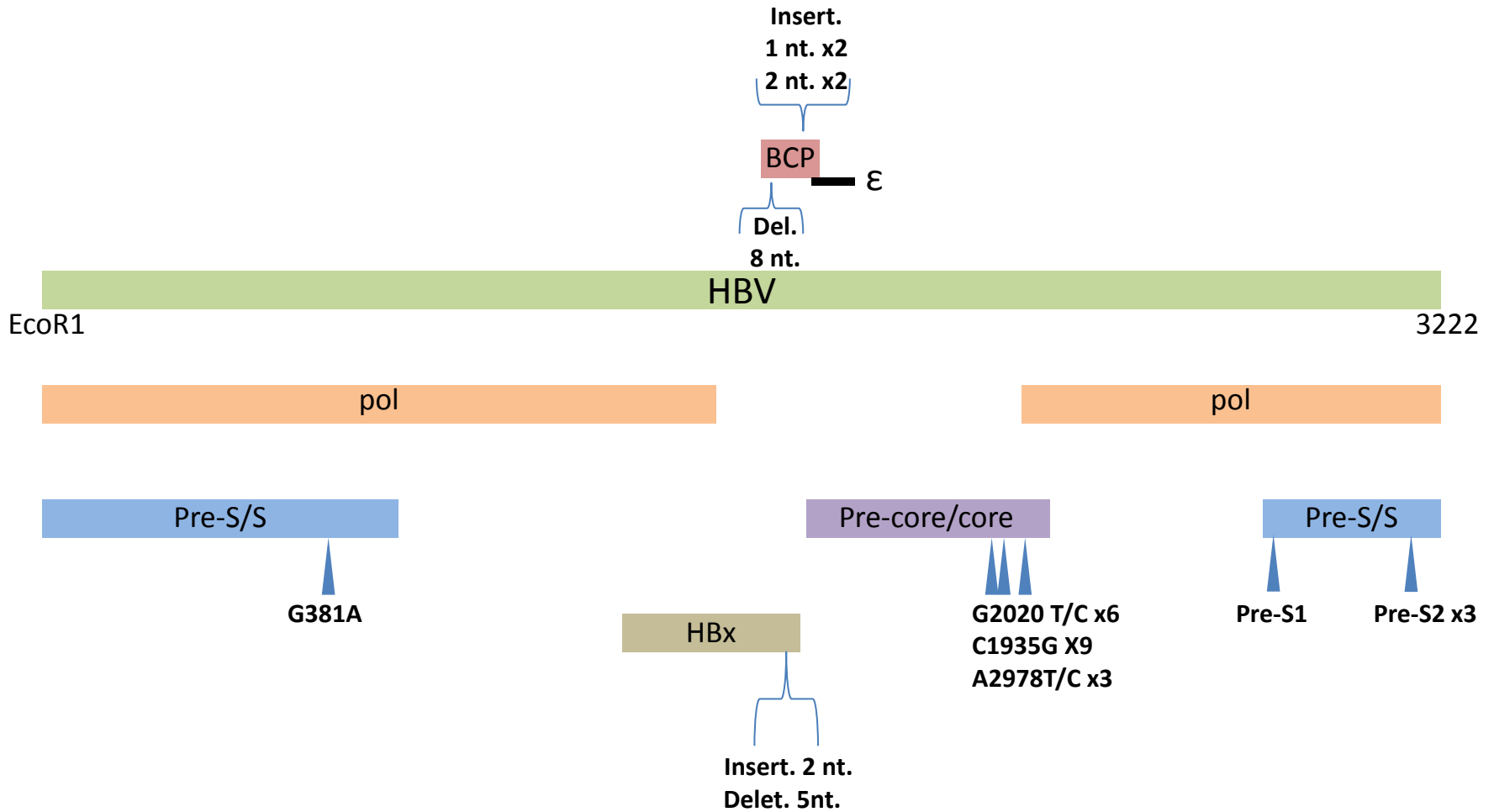
# HBV DNA amplification performance



# Preliminary results

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

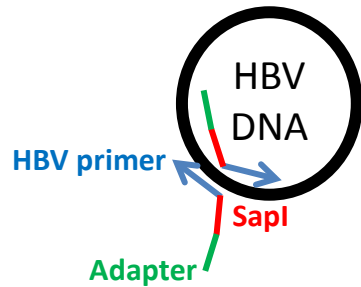
# Sequences analysis



# Construction of HBV replicons

## Method 1

1st PCR amplification with HBV-specific primers



2nd PCR amplification using adapters



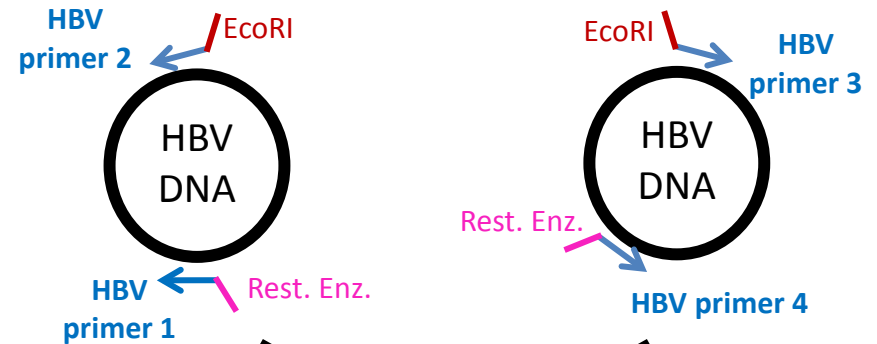
Huh7 transfection & re-circularization with SapI



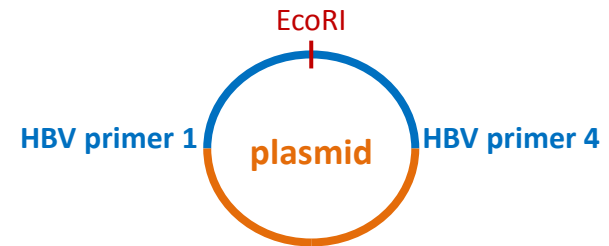
HBV genome expression & replication

## 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**