

# Validation of the investigational assay in clinical cohorts

**RHU CirB-RNA**

**International workshop on viral biomarkers**

September, 7<sup>TH</sup> 2023

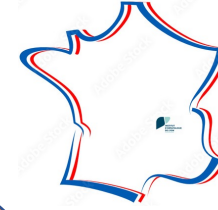
**Dr. Caroline SCHOLTES (PharmD PhD)**

## France-Italy

Roche Investigational Assay



Testing on the Cobas 6800  
in the Clinical Virology Lab (Lyon)



## Hospices Civils de Lyon



- Hepatology Department
- Infectious Diseases Department
- Paediatric Gastroenterology, Hepatology and Nutrition Department

*Samples,  
Clinical data*



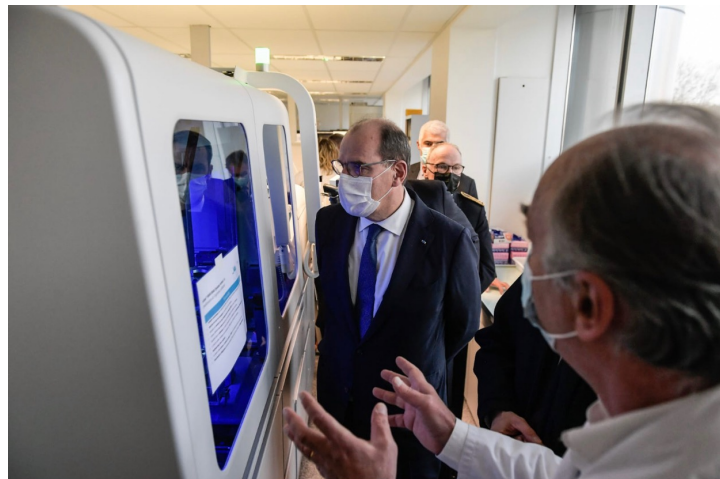
## Italy

- Milano
- Palermo

# Performance of the cobas® HBV RNA automated investigational assay for the detection and quantification of circulating HBV RNA in chronic HBV patients

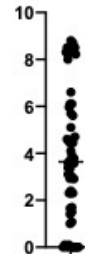
Scholtès C. *et al.* *Journal of Clinical Virology* 150-151 (2022) 105150

cobas 6800®



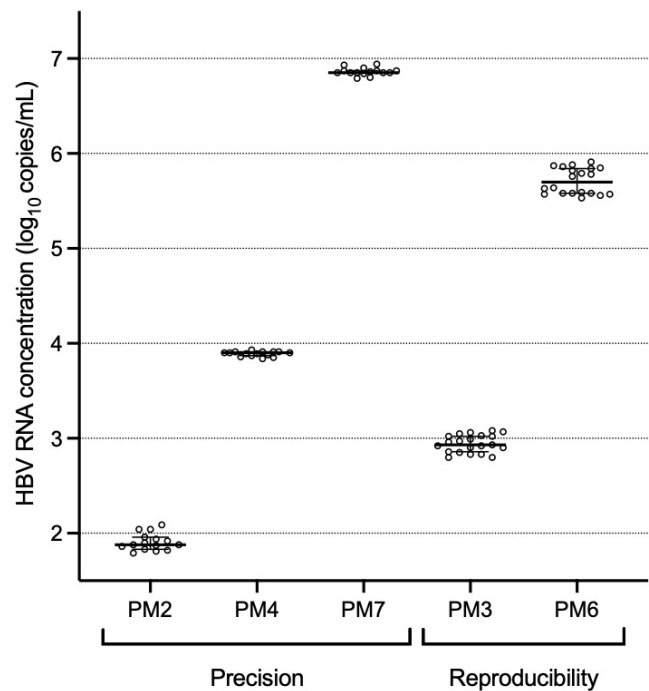
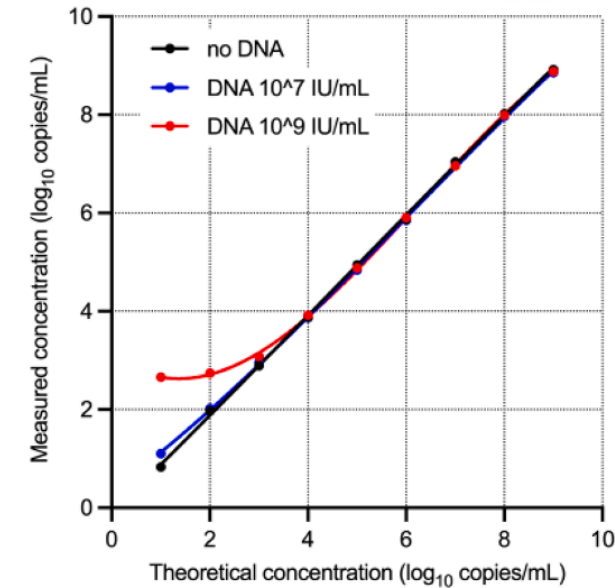
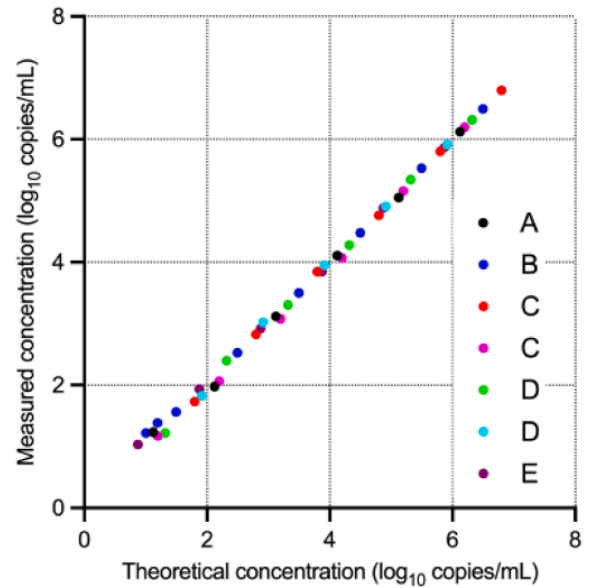
**Table S1. Patient sample characteristics**

		Untreated <sup>a</sup>	NUC-treated	Total
	N	36	20	56
	Age range (years)	19-77	19-72	19-77
Genotype	A	3	2	5
	B	4	2	6
	C	4	3	7
	D	11	4	15
	E	12	4	16
	F	1	2	3
	G	0	1	1
	unknown	1	2	3
HBeAg status	positive	13	8	21
	negative	23	12	35
HBV DNA	>LLOQ	36	12	48
	<LLOQ, >LOD	0	5	5
	<LOD	0	3	3
	not done	0	1	1
	mean HBV DNA (log <sub>10</sub> IU/mL) <sup>b</sup>	5.49	2.97	4.86



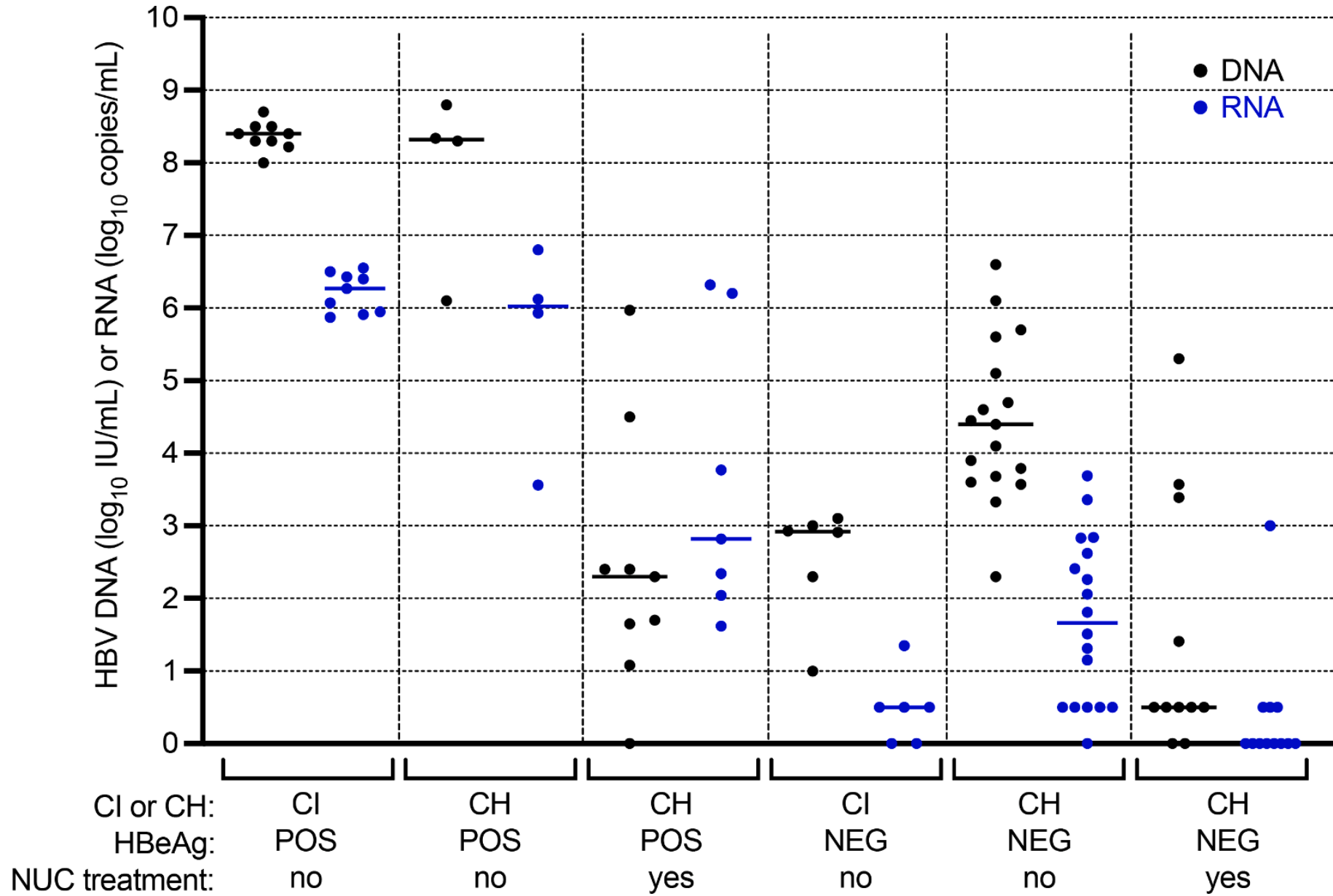
**Table 1**  
Analytical sensitivity.

HBV RNA concentration (copies/mL)	N positive/N valid replicates (plasma)	% positive (plasma)	N positive/N valid replicates (serum)	% positive (serum)
20	84/84	100	84/84	100
15	84/84	100	84/84	100
10	83/83	100	84/84	100
5	82/84	97.6	83/84	98.8
2.5	75/83	90.4	75/84	89.3
1.25	65/84	77.4	49/84	58.3
0	0/84	0	0/84	0
LOD by PROBIT analysis (95% Reactive Rate)	3.3 copies/mL (95% CI: 2.6 – 4.8 copies/mL)		3.3 copies/mL (95% CI: 2.7 – 4.5 copies/mL)	
LOD by Hit Rate	5 copies/mL (97.6%)		5 copies/mL (98.8%)	



The Roche HBV RNA investigational assay meets all the analytical criteria required:

- high throughput,
- precise
- sensitive
- broad linear range
- genotype inclusive
- specific



# The CirB-RNA Prospective clinical cohort, => real-life



## • HBV infected Patients:

- Acute hepatitis B
- Chronic infection: all phases of infection and disease as defined by the EASL guidelines

	HBsAg positive Chronic <i>infection</i>	HBsAg positive Chronic <u>hepatitis</u>	HBsAg negative Chronic <i>infection</i>	HBsAg negative Chronic <u>hepatitis</u>
HBsAg	High	High/Intermediate	Low	Intermediate
HBeAg	Positive	Positive	Negative	Negative
HBV DNA	>10E7 IU/mL	10E4-10E7 IU/mL	<2,000 IU/mL <sup>oo</sup>	>2,000 IU/mL
ALT	Normal	Elevated	Normal	Elevated*
Liver disease	None/minimal	Moderate/severe	None	Moderate/severe
<b>Old terminology</b>	Immune tolerant	Immune reactive HBeAg positive	Inactive carrier	HBeAg negative Chronic hepatitis

EASL 2017 CPG HBV, J Hepatol 2017;  
Volume 67, Issue 2, Pages 370-398 (August 2017)  
DOI: 10.1016/j.jhep.2017.03.021

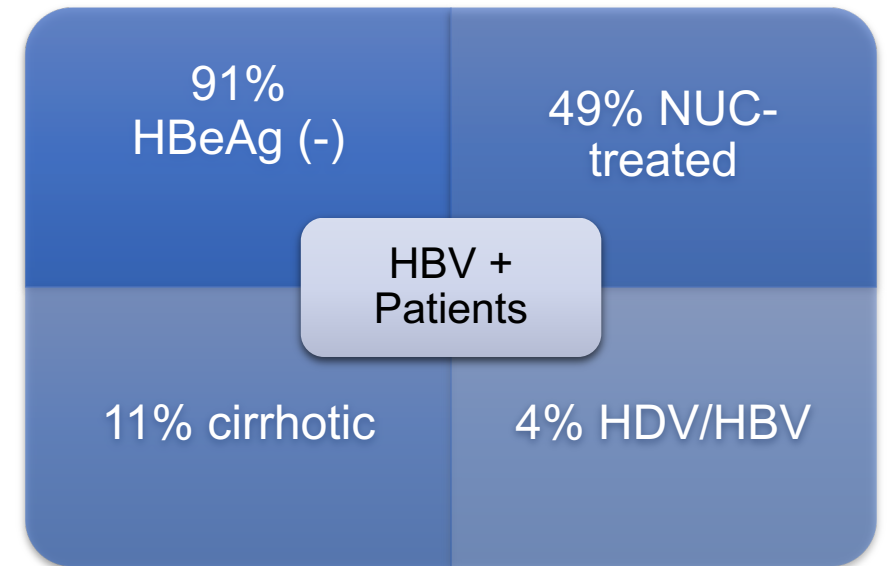
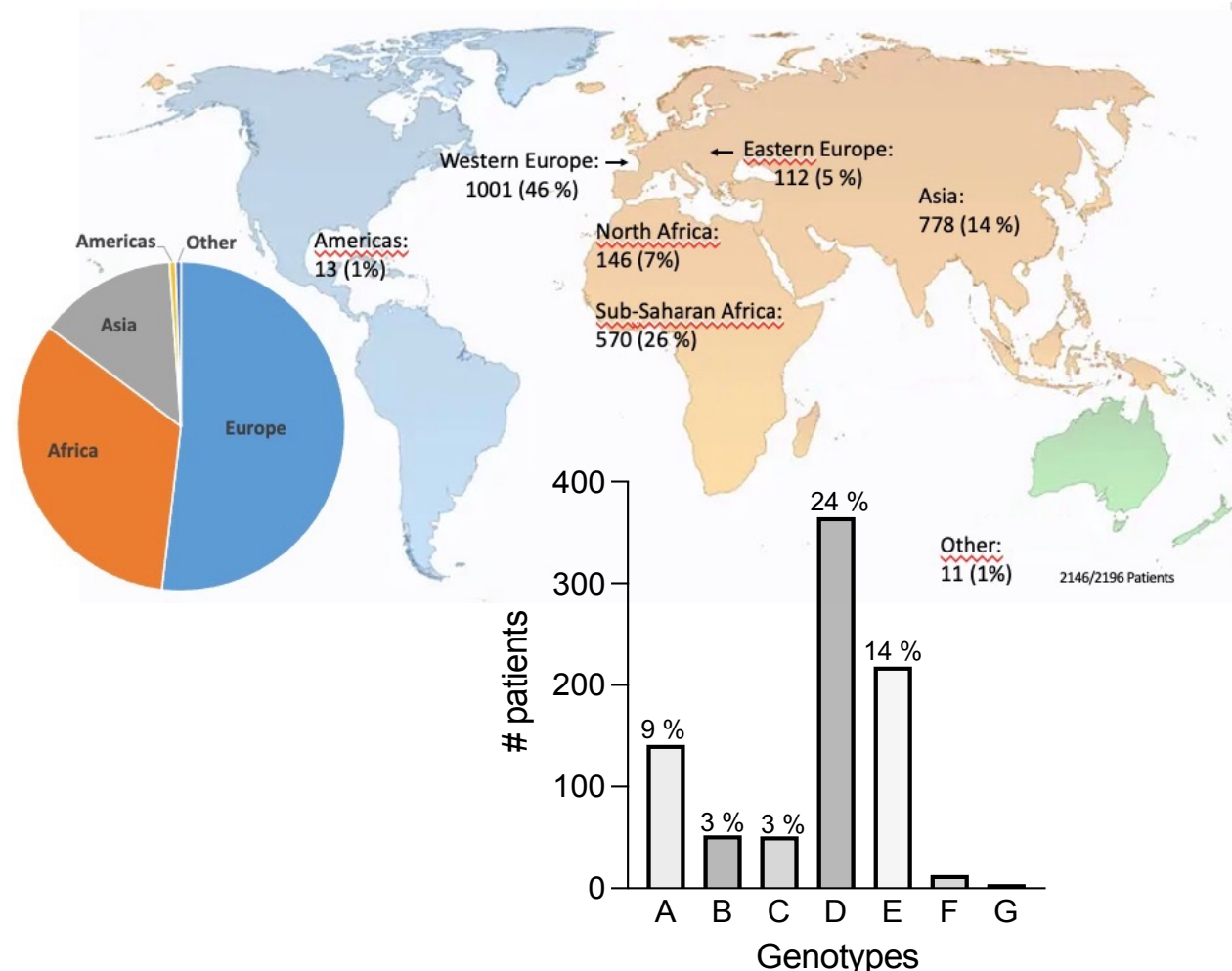
- Patients with HBsAg loss (functional cure): either spontaneously or after treatment with currently available antivirals (NUCs or IFN)
- Co-infections with HDV, HCV, HIV

# Cross-sectional study of serum HBV RNA and HBcrAg in the first 1500 patients enrolled in the RHU real-life prospective cohort

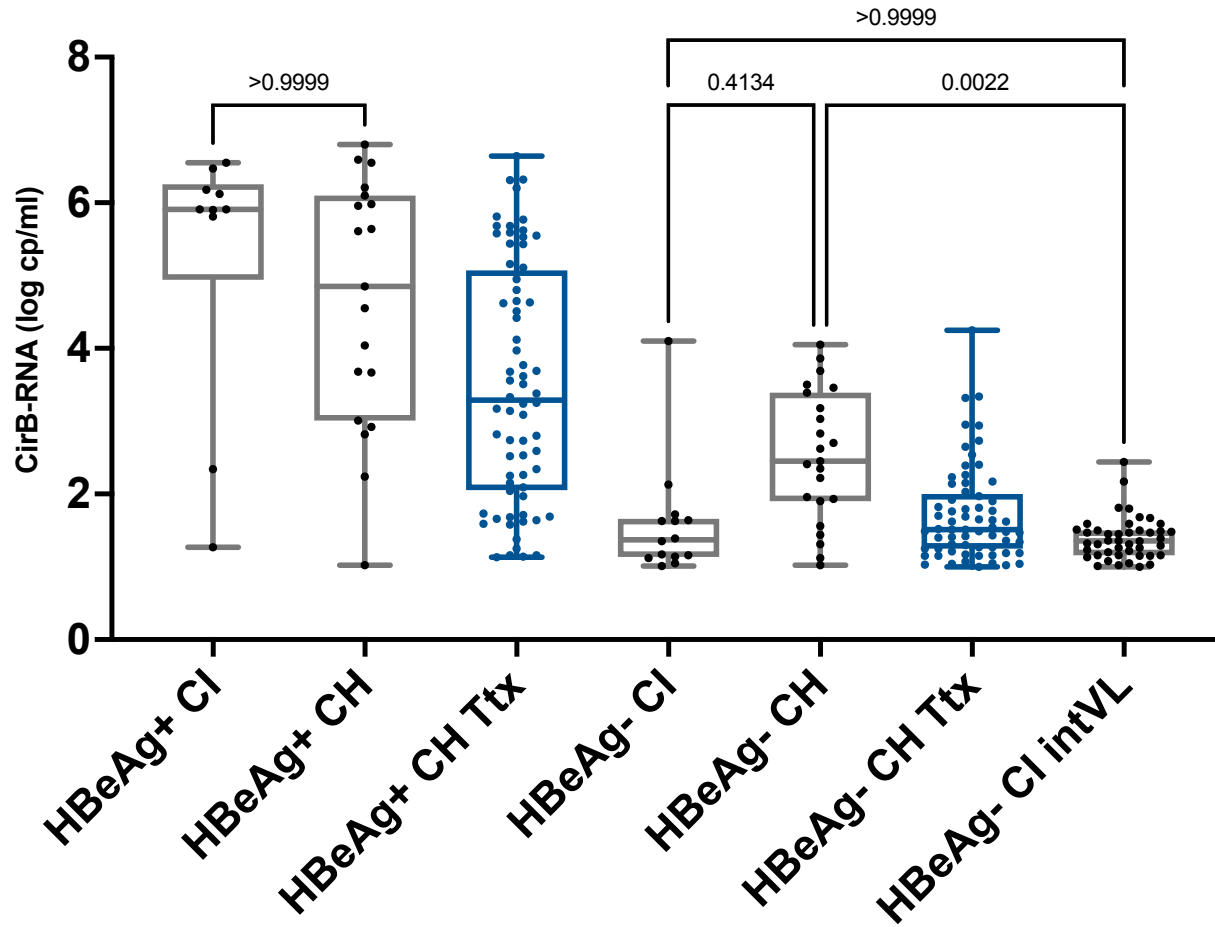


The baseline results presented here were obtained in the first 1503 patients enrolled in Lyon and Milan until April 2021

## Geographical origin & HBV genotypes distribution



# CirB-RNA according to CHB phases



CirB-RNA positive in:

- ✓ 92% of HBeAg(+)\*
- ✓ 37% of HBeAg(-)
- ✓ 38% of NUC-treated patients

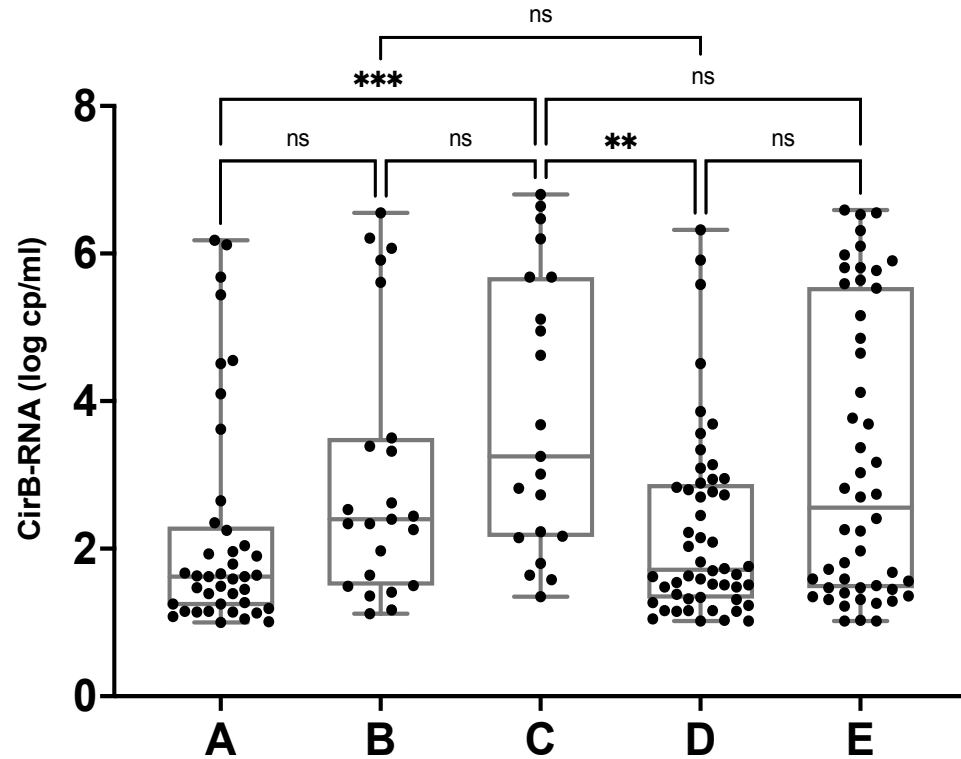
\* all the negative ones were NUC-suppressed

CI: chronic infection; CH: chronic hepatitis;  
Ttx: NUC-treatment;

intVL: patients with serum HBV DNA values >2000 IU/ml and <20000 IU/ml



# CirB-RNA according to HBV genotype



Genotype C infected patients showed the highest median values for CirB-RNA (w/o difference in serum HBV DNA)

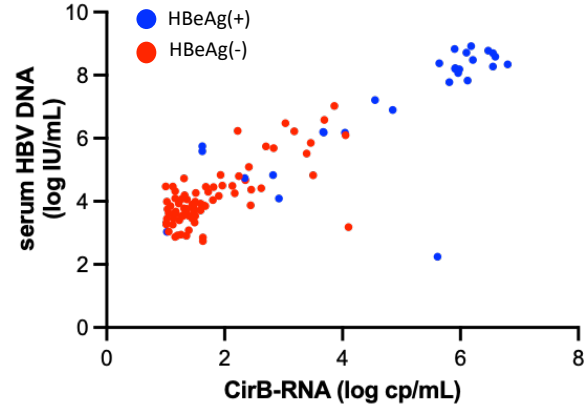
Kruskal Wallis test,  $\alpha$  threshold=0.5. \* $p$ <0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001

# Correlations between CirB-RNA & serum HBV markers



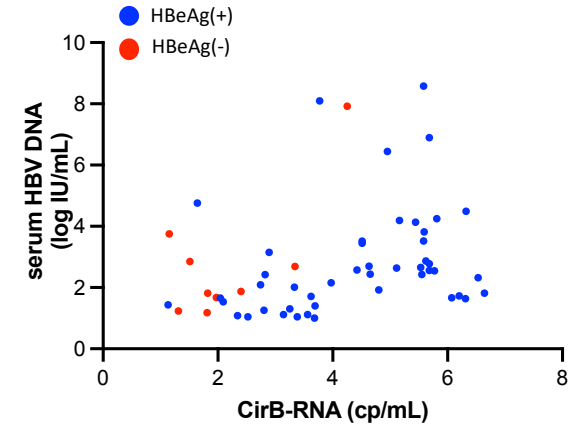
## Untreated patients

	HBeAg(+)	HBeAg(-)
Serum HBV DNA	R=82; p<0.0001	R=0.59; p<0.0001



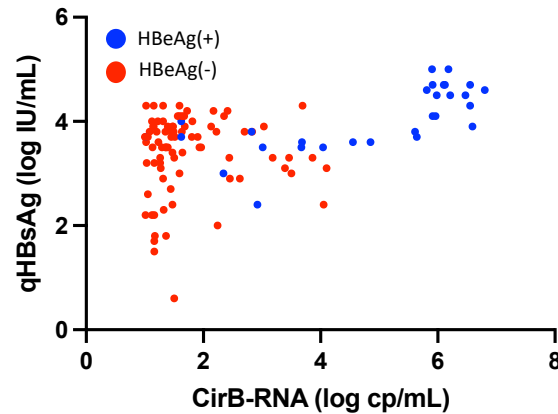
## NUC-treated patients

	HBeAg(+)	HBeAg(-)
Serum HBV DNA	R=42; p<0.003	-

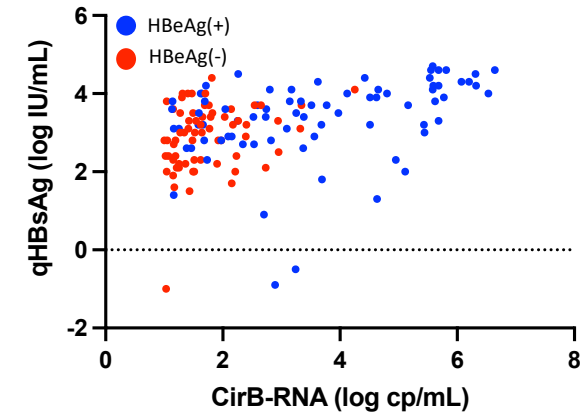


Serum HBV DNA

	HBeAg(+)	HBeAg(-)
qHBsAg	R=0.70; p<0.0001	R=0.08; p=ns



	HBeAg(+)	HBeAg(-)
qHBsAg	R=0.48; p<0.0001	R=0.30; p=0.01



qHBsAg

# Correlation between CirB-RNA and HBcrAg



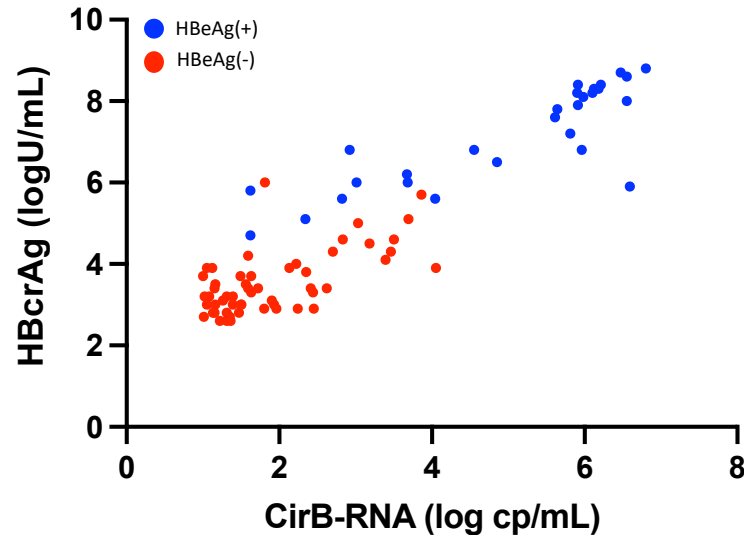
Hepatitis B core-related antigen (HBcrAg): serological biomarker that reflects intrahepatic HBV cccDNA activity

*Testoni, J Hepatol 2020*

CirB-RNA positive – HBcrAg positive: 370 patients (25%)

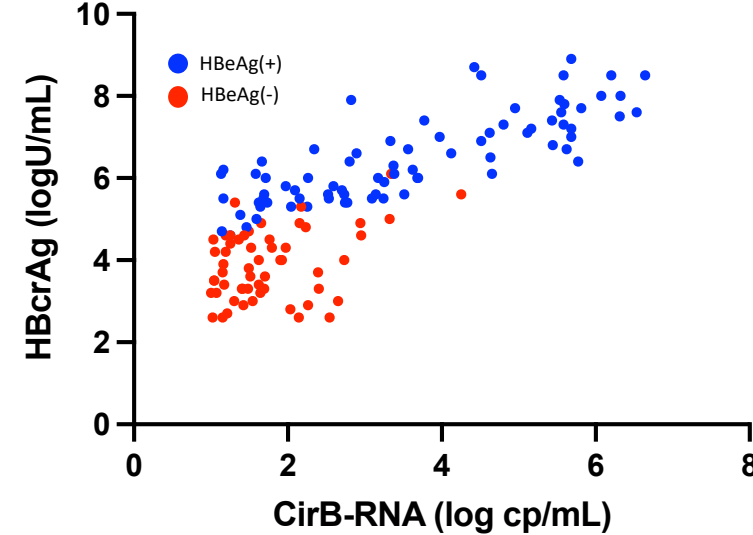
Untreated patients, n=133

	HBeAg(+)	HBeAg(-)
HBcrAg	R=0.79; p<0.0001	R=0.54; p<0.0001



NUC-treated patients, n=237

	HBeAg(+)	HBeAg(-)
HBcrAg	R=0.81; p<0.0001	R=0.26; p=0.05



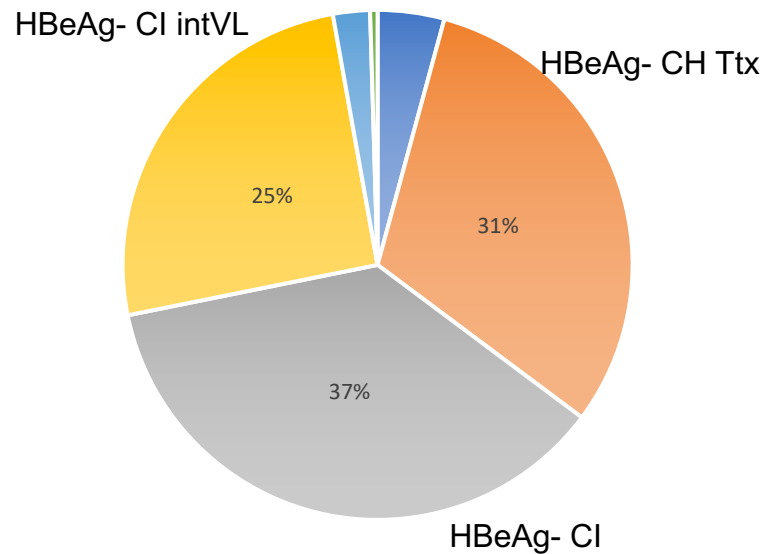
CirB-RNA positively correlated with serum HBcrAg in untreated HBeAg(-) and (+) subgroups, and in NUC-treated HBeAg(+) patients

# Discrepancies between CirB-RNA and HBcrAg quantification

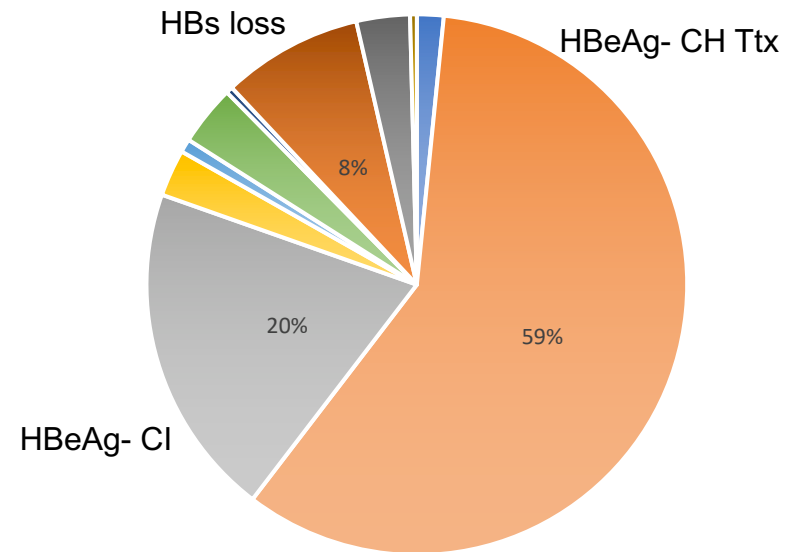


**Subgroups of HBeAg(-) patients show discordant results in CirB-RNA and HBcrAg detection**

**CirB-RNA positive – HBcrAg negative:  
213 patients (14%)**

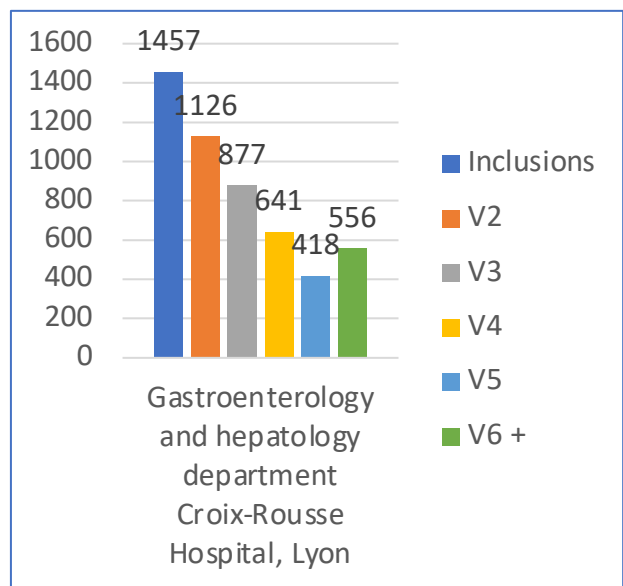
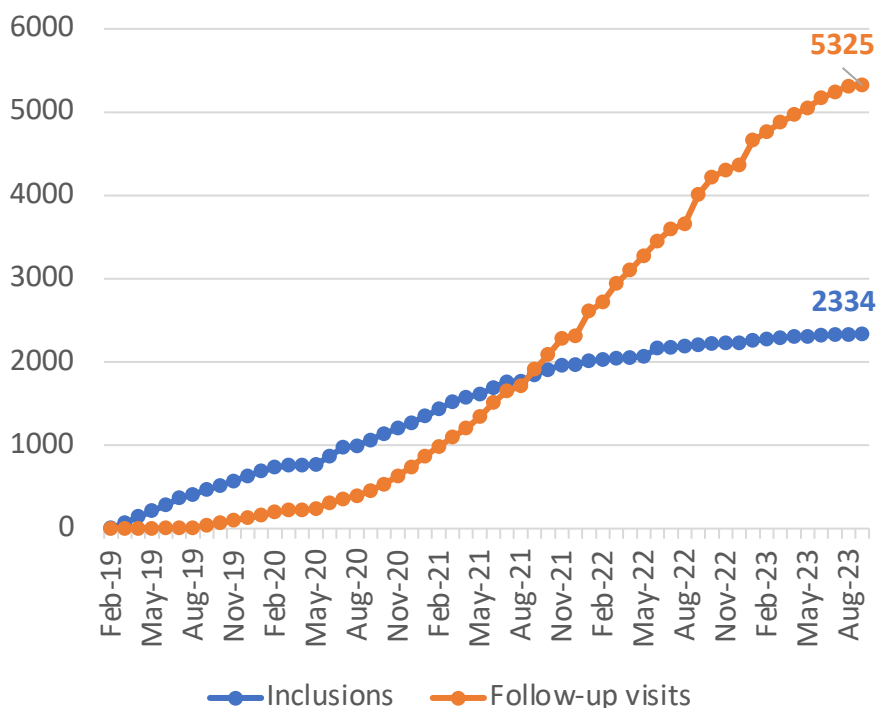


**CirB-RNA negative – HBcrAg positive:  
250 patients (17%)**



# Perspectives

Identify which clinical context will benefit from HBV RNA quantification?



- **Follow-up visits** will ascertain the predictive value of these novel biomarkers in **refining subcategories** of patients and **predicting functional cure** and also help clarify if it can be used to guide **treatment discontinuation**
- Deep analyses of **selected patients** with discrepancy detection in CirB-RNA and HBcrAg will inform on the differential biological/clinical information provided by CirB RNA and HBcrAg assays

Do CirB RNA reflect cccDNA transcriptional activity?

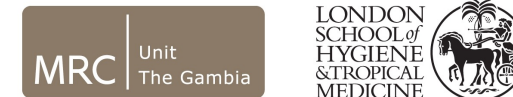
# Analysis of CirB-RNAs in specific patients' populations



Cohort #1: untreated chronically infected patients from Sub-Saharan Africa (Gambia)  
190 patients, 95 liver biopsies



**B. Testoni**



Cohort #2: liver transplanted patients, prospective study of HBV kinetics (Ecogrefe study, France)  
41 patients with FU, 2 time-points

**B. Testoni**

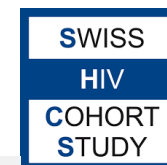


Cohort #3: Long-term kinetics of circulating HBV RNA level among persons living with HIV and HBV treated with tenofovir in the Swiss HIV cohort Study (Switzerland, Paris)  
455 patients with FU, up to 5 time-points

**L. Begré**

*u<sup>b</sup>*

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Cohort #4: HBV/HDV co-infected patients (Lyon-Milan):  
380 patients


**P. Lampertico**





# Analysis of CirB-RNAs in specific patients' populations





 Cohort #5: NUC-treated cirrhotic patients with HCC (Milan, Italy)  
24 patients with FU, 2 time-points

 Cohort #5: untreated HBeAg negative CHB patients with or without ALT flares (Pisa, Italy)  
117 patients with FU, up to 5 time-points

 Cohort #6: HBeAg negative infection patients, never treated with or without HBsAg loss and patients with persistent viremia (<20000 IU/ml + normal ALT) (Pisa, Italy)  
346 patients with FU, up to 112 time-points

 Cohort #7: treated HBeAg negative CHB patients with or without cirrhosis (Pisa, Italy)  
249 patients with FU, at least 6 time-points

 Cohort #8: CHB patients vaccinated with VVX001 (Viravaxx, Austria)  
32 patients with FU, up to 10 time-points

 Cohort #9: Proof of concept trial IP-cure B



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# Aknowledgments



*all the patients*



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