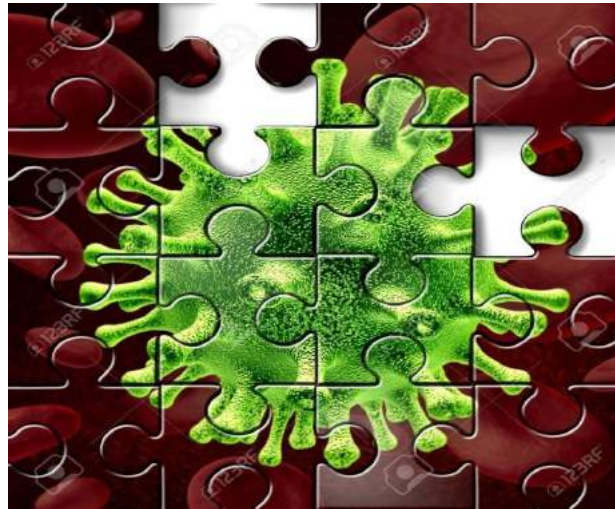


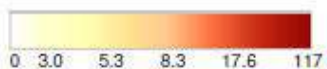
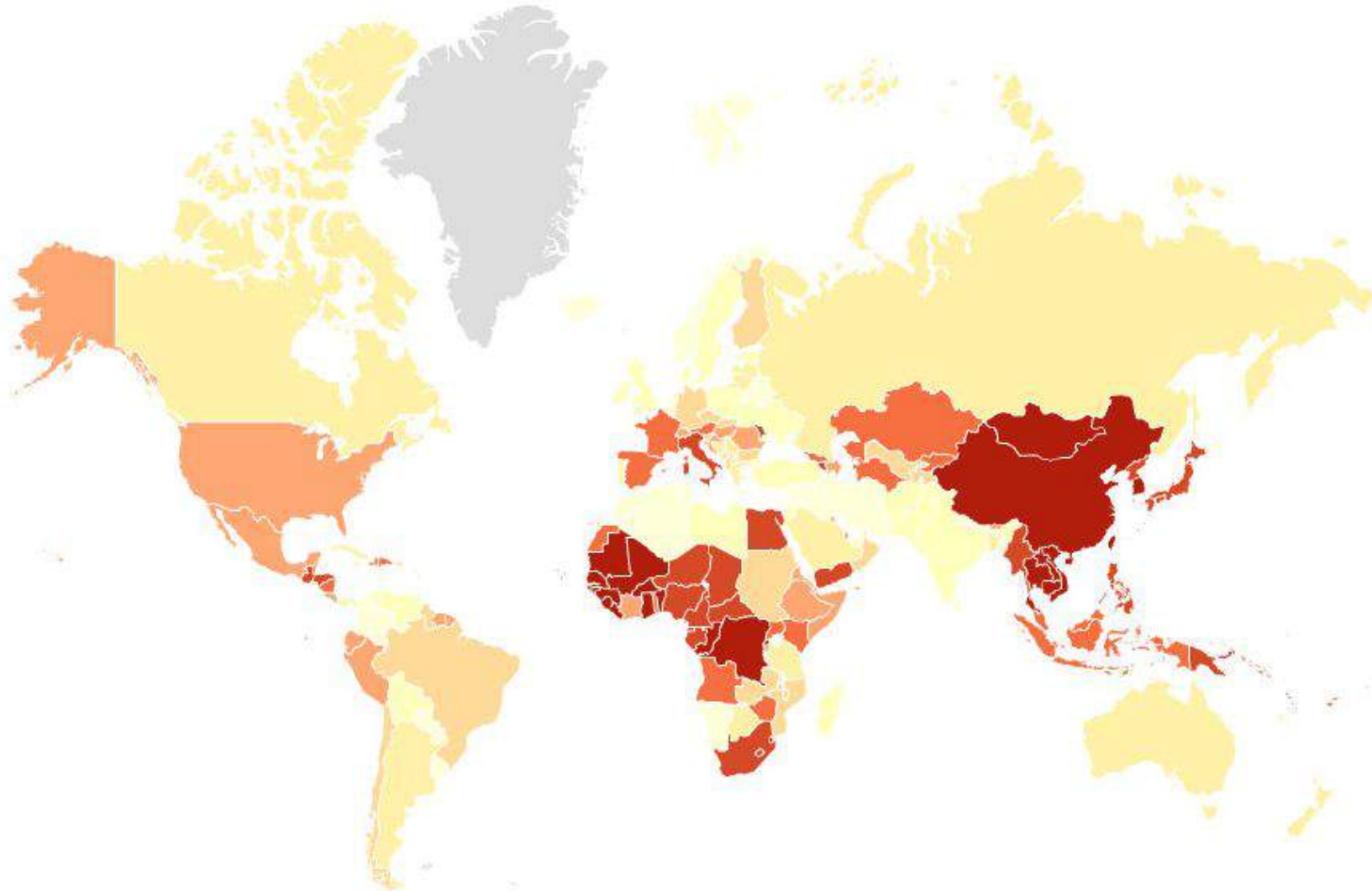
Genomic characterization of HBV and HDV strains, novel tools for a long lasting question

LIA and ANRS France-Brazil



Isabelle Chemin, DR2 Inserm U1052-CRCL

Hepatocellular carcinoma Worldwide incidence

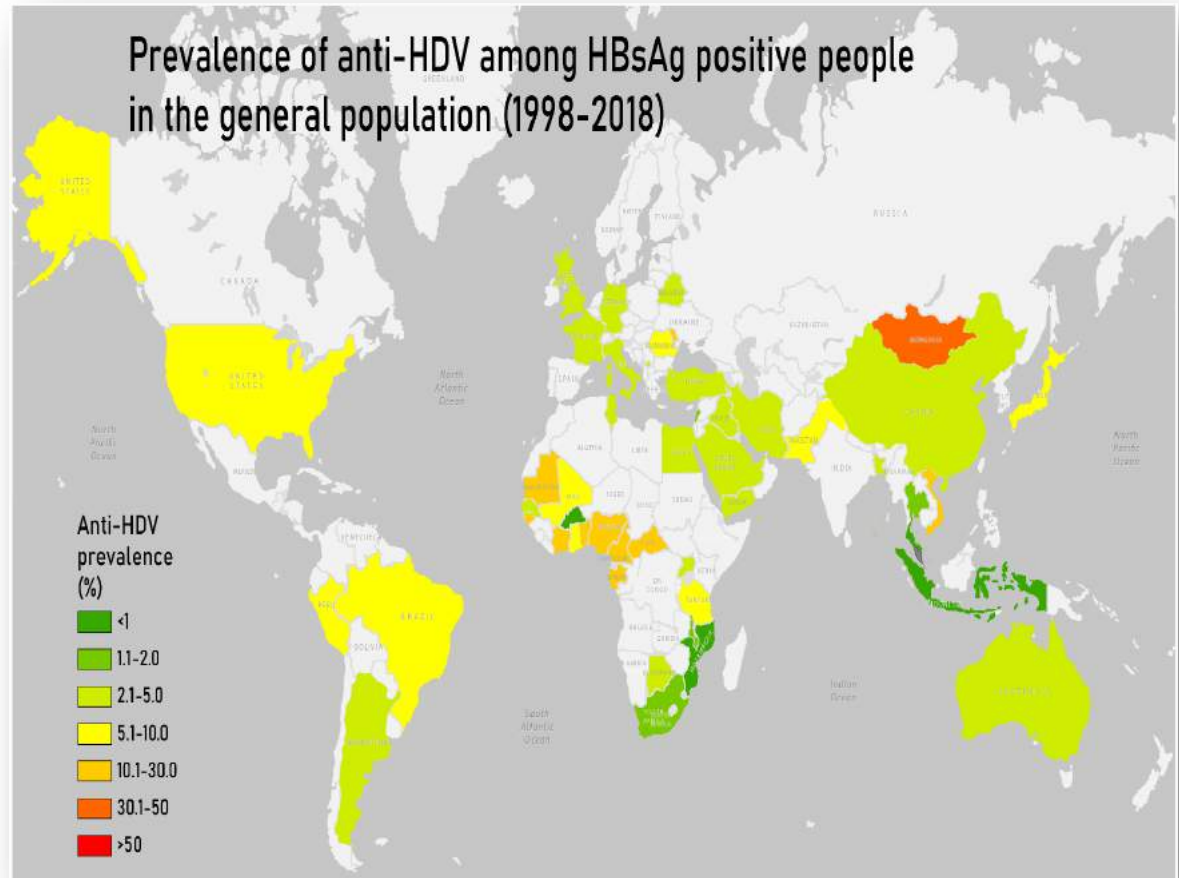


**Mondial HCC incidence in Men (number of cases for 100
000, 2008 (GLOBOCAN 2008, CIRC)**

Global prevalence of hepatitis D virus (HDV) infection: systematic review and meta-analysis

Stockdale et al. 2020, J Hepatol

- An estimated 12 million people worldwide have HDV infection antibodies
- Significant gaps in available data
- Anti-HDV increases the risk of cirrhosis and HCC in patients with chronic HBV infection



	N studies	Pooled OR	95% CI
Liver cirrhosis	29	6.7	4.4 - 10.2
Hepatocellular carcinoma	20	4.8	3.2 - 7.3

Development of a quantitative PCR for hepatitis Delta diagnosis

C. Scholtes - P. Dény

AHU, Laboratoire de Virologie Nord, Hôpital de la Croix Rouse

- **Automated Nucleic acid extraction**

Tested on

- **EasyMag**, BioMérieux
- M2000sp, Abbott
- Versant Siemens

- **Internal controls**

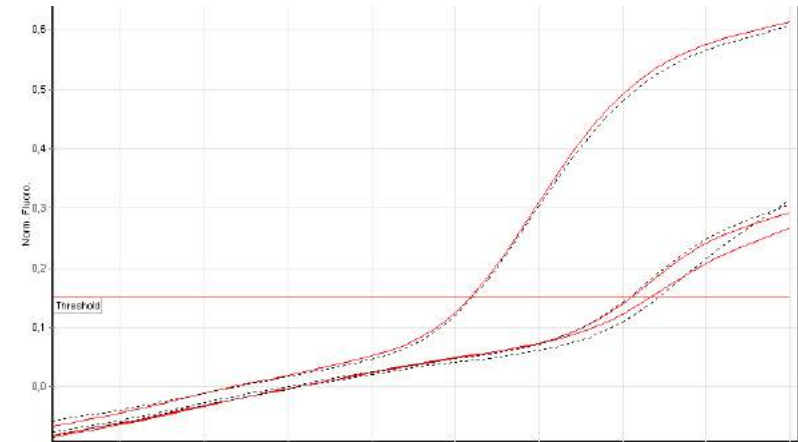
- Encapsulated RNA commercial (Eurobio)
- For extraction, RT et and amplification

- **RT-PCR 1 step**

Tested on

- **RotorGene**, Qiagen
- M2000rt, Abbott

- Optimized probe with **LNA nucleosides**

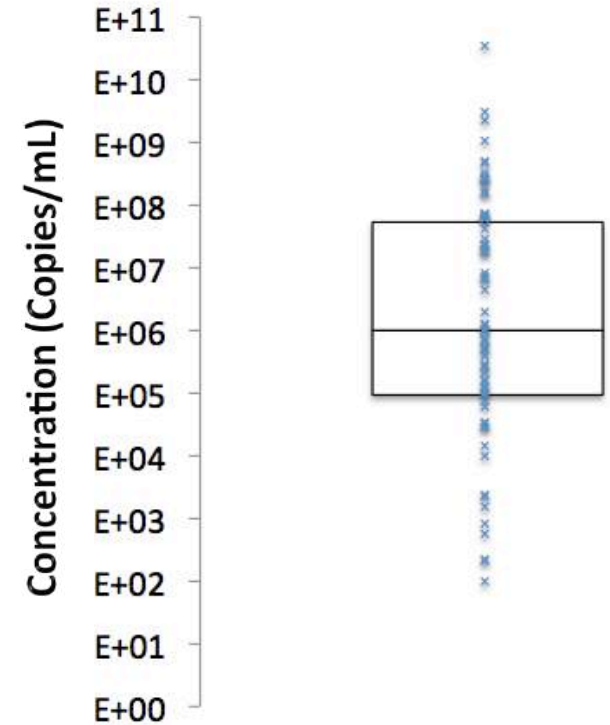
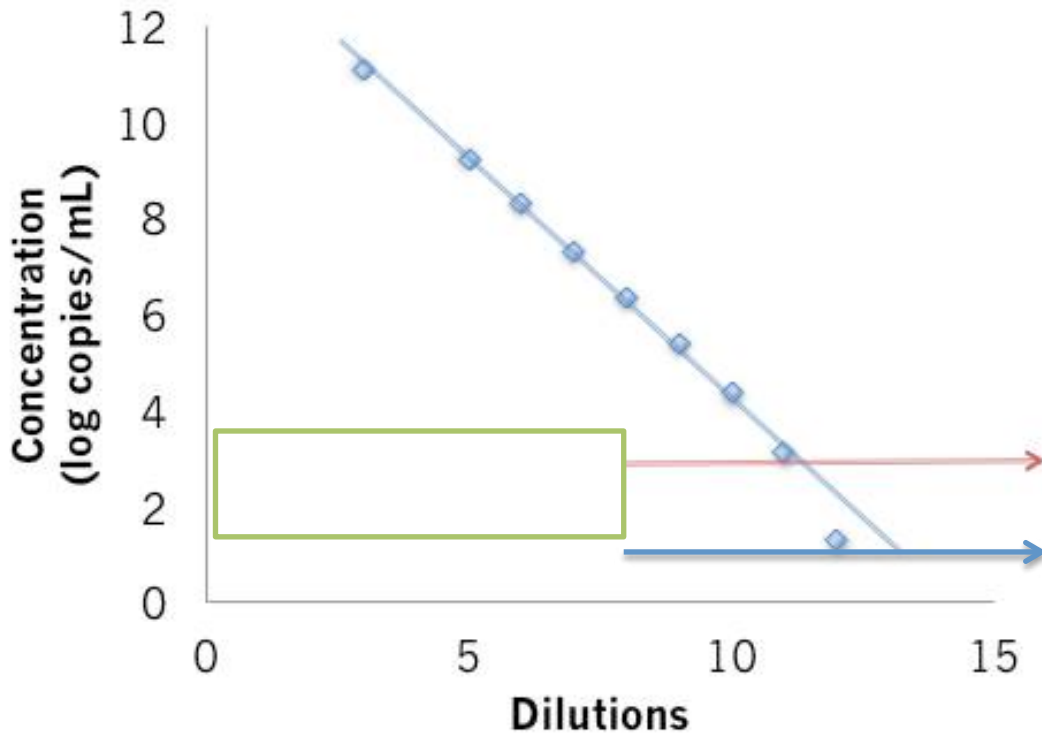


Quantification standard : HDV-1 RNA transcript *in vitro*

Large linearity range

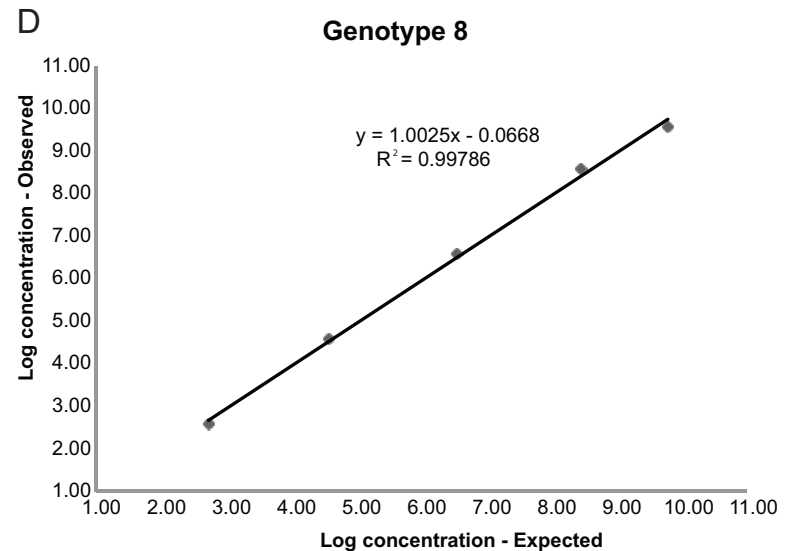
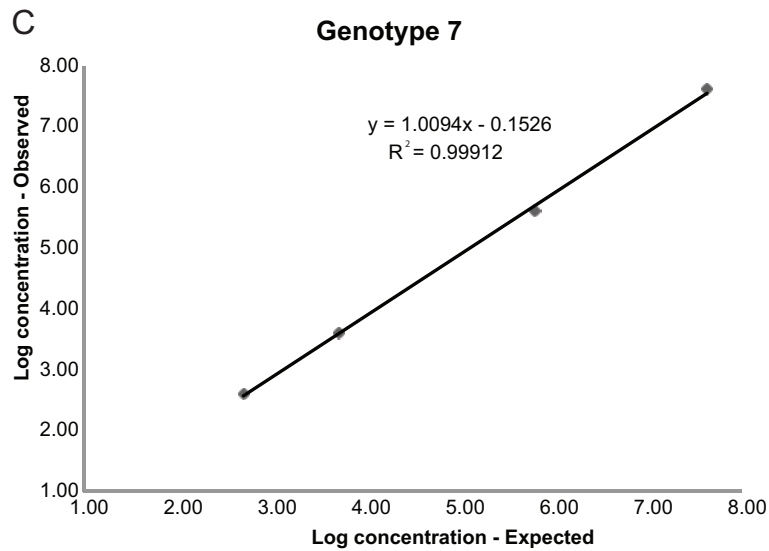
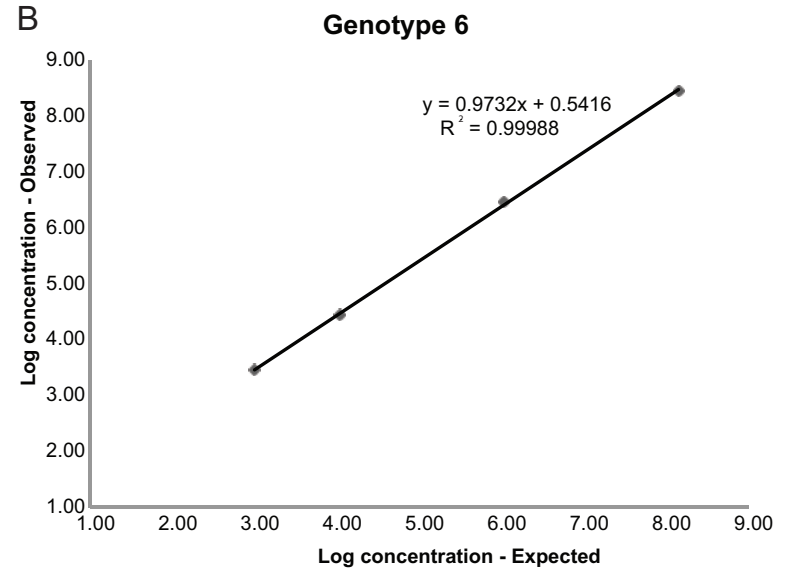
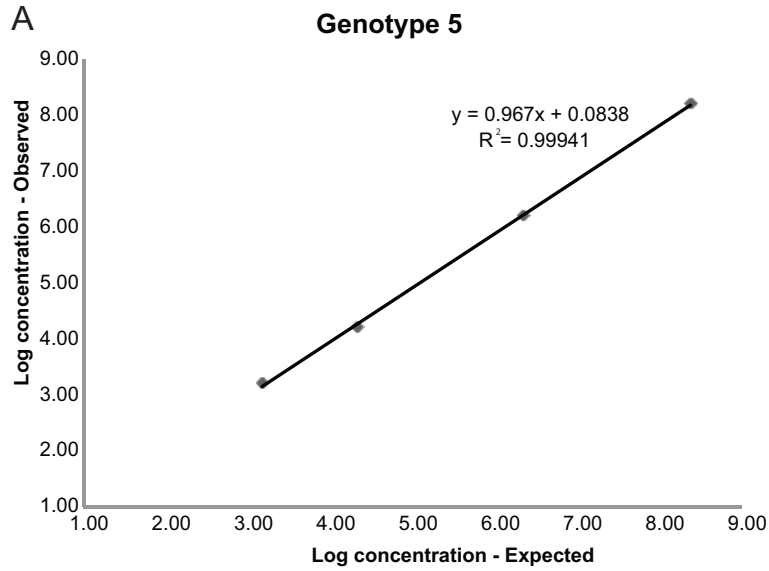
500 copies/mL to $1,7 \times 10^{11}$ copies/mL

Gamme HDV1



Limit of quantification LOQ :
500 copies/mL (2,7 log)

Limit of detection LOD :
150 copies/mL

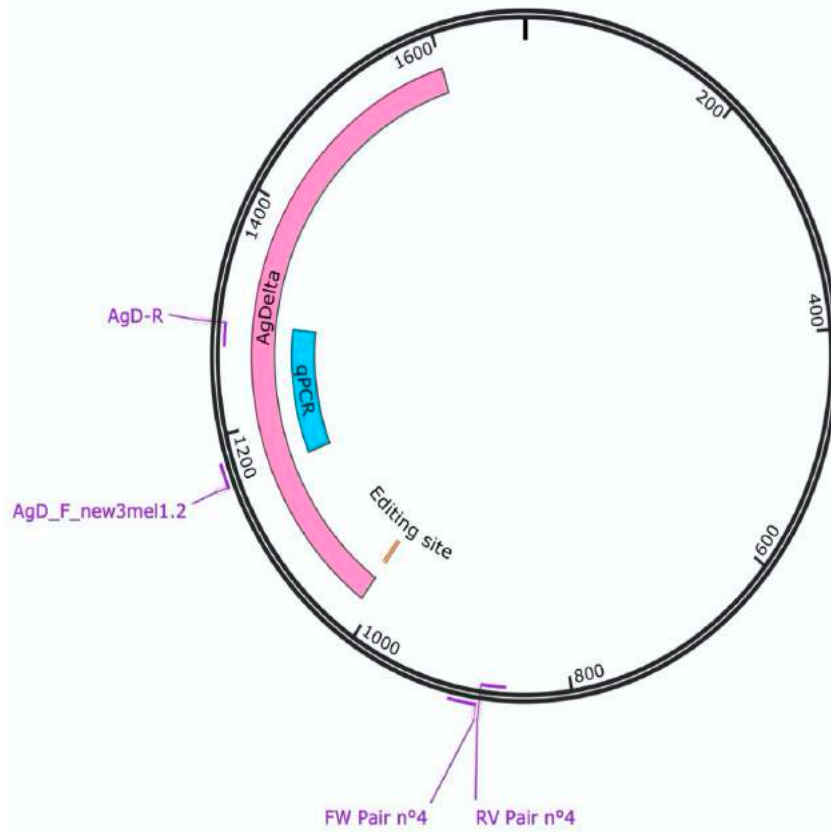


Different HDV genotypes in different regions!

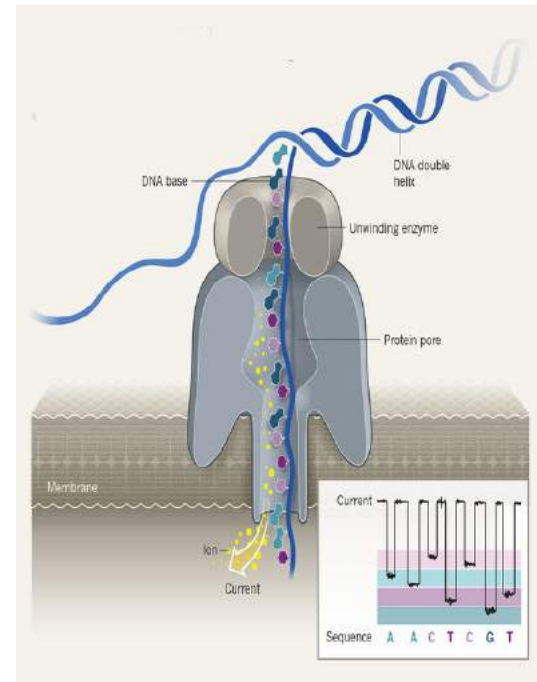


.Paraná R, Pujol FH. Clinical and Virological Heterogeneity of Hepatitis Delta in the Amazonia: More Questions Than Answers. Clin Liver Dis. 2019;13(2):62-65.

Complete HDV genome amplification from Amazonian clinical samples

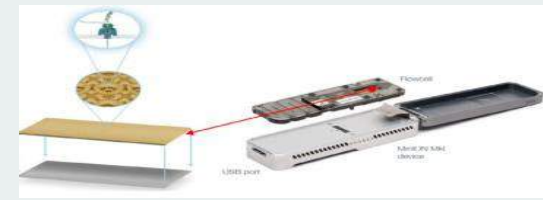


ONT long read SEQUENCING



=> Adaptation of HDV-probe

Sequencing of the new HBV/HDVs



"primer walking"
sequencing
(Sanger)

One shot
whole HBV/HDV
genome sequencing
(MinION)

- Full characterization of the new HBV/HDVs
- Identification of quasi species
- Identification of splicing/deletion/recombinations events

- MinION technology was validated on SARS-COV2
- The use of this new technology could represent a valid support for the full characterization of the putative HBV/HDV genotypes interactions
 - Faster = sequencing of the entire viral genome in one run
 - Cheaper = the price of one run is less expensive compared to the dozens of Sanger sequencing runs required to cover the whole genome
 - This technology can be easily transferred to any lab in low/medium resource countries

ANRS Project: Re-evaluation of HBV and HDV co-infections in the Brazilian Amazon (M Schinoni-I Chemin)

Most severe cases including fast progressive and fulminant hepatitis associated with **co-infection HBV/F and HDV/3**

1. Standardization of quantitative HDV RNA testing Including for HDV/3
2. New HBV markers for hepatitis B and Delta in Brazil; quantitative HBsAg, HBcrAg, HBV RNA
3. Molecular epidemiology and role of intergenotypic interactions HBV/HDV in severity and progression of HDV

- ✓ Systematic review ongoing in coll with IARC (C De Martel)
- ✓ Nanopore Minion pipeline developed (C Scholtès, C Charre, C Goldsmith)

Recent papers :

Long read sequencing and de Novo assembly of HBV identifies 5mCpG in CpG islands

C Goldsmith, D Cohen, A Dubois, MG Martinez, K Petitjean, A Corlu, H Hernandez Vargas, Chemin. bioRxiv 2020

Hepatitis B virus American genotypes : Pathogenic variants?(review)

Pujol F, Jaspe RC, Loureiro CL, Chemin I. Clin Res Hepatol Gastroenterol. 2020 Jun 15:S2210-7401(20)30145-5. Review.

Evaluation of NGS-based approaches for SARS-CoV-2 whole genome characterisation

C Charre, C Ginevra, M Sabatier, H Regue, G Destras, S Brun, G Burfin, C Scholtes, F Morfin, M Valette, B Lina, A Bal, L Josset
Virus Evolution, 2020



*Laboratory of Molecular Biology
in Rio Branco /ACRE*

THANK YOU FOR YOUR ATTENTION

**Center of
Research on
Cancerology of
Lyon (CRCL)**

Damien Cohen
Anaëlle Dubois
Dr Janet Hall
Dr Chloe Goldsmith
Pr Fabien Zoulim

All of U1052 ;)

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