OCTOBER 11-12, 2024 MILAN, ITALY
DeltaCure 3rd International Meeting
P15
Poster for you

Hepatitis Delta Virus RNA quantification: a story about fruitful collaboration between private company and academic laboratory

Athenaïs Gerber [1], Marie Breuer [2], Valérian Delagarde [1], Karin Rottengatter [2], Samira Dziri [1], Emmanuelle Vincent [2], Emmanuel Gordien [1], Frédéric Le Gal [1], Ségolène Brichler [1]

(1) Avicenne Hospital, Virology Unit, French National Reference Centre for HDV (F-NRC), Bobigny, France (2) altona Diagnostics GmbH, Hamburg, Germany

Introduction

- HBV-HDV co-infection is responsible for the most severe form of viral hepatitis. For patients with positive anti-HDV antibodies, HDV RNA detection and quantification in plasma is mandatory to identify an active infection, and to monitor the response to antiviral treatment.
- Several commercial or in-house RT-qPCR tests are used worldwide, but some tests encounter difficulties to quantify certain HDV strains, due to the high genetic diversity (8 genotypes and multiple subgenotypes) and strong secondary structure of HDV RNA.

Initial altona kits evaluation by the F-NRC

Sept-2022

RealStar® HDV RT-PCR 1.0
Extraction: m2000sp (Abbott)

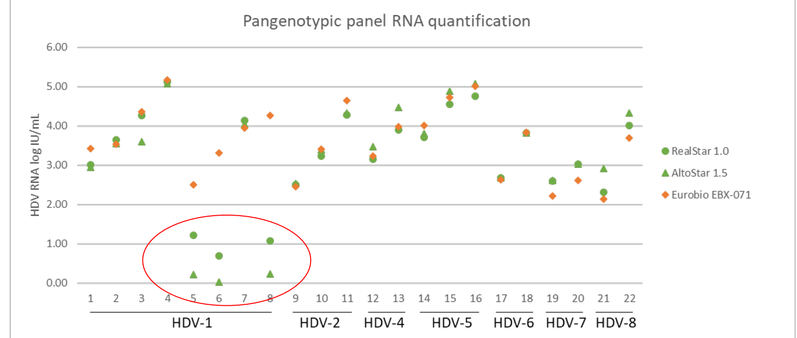
July-2023

AltoStar® HDV RT-PCR 1.5

- **F-NRC pangenotypic HDV panel:** 24 plasma samples
  - 2 negatives, 8 HDV-1, 3 HDV-2, 2 HDV-4, 3 HDV-5, 2 HDV-6, 2 HDV-7, 2 HDV-8
  - Viral loads ranging from 2 to 5 log IU/mL
  - Complete sequences available

→ Severe underquantification of 3 HDV-1 samples (figure 1)

Figure 1 **F-NRC HDV panel quantification**

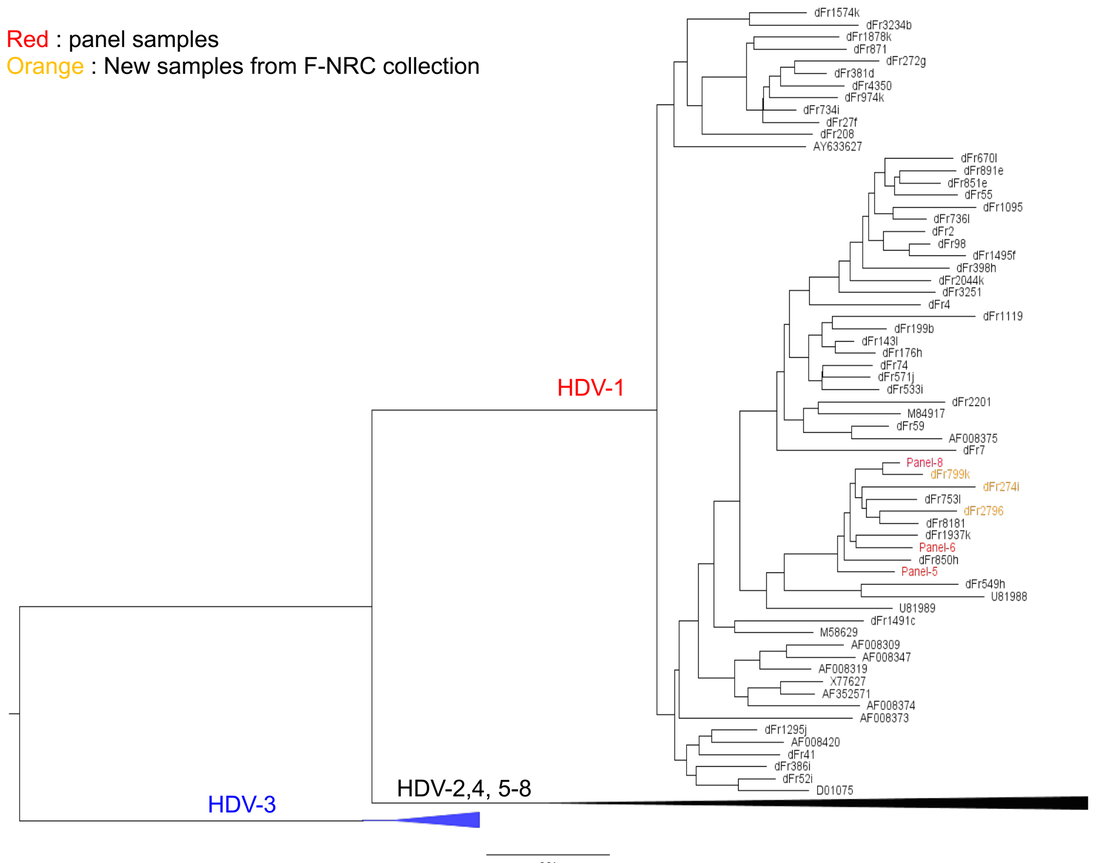


F-NRC and altona's R&D joint investigation

Avicenne F-NRC

- Repeat testing on new aliquots of the panel → reproducible results
- Test 50 HDV-1 samples from the F-NRC collection, choosing specifically patients born in sub-Saharan Africa → 3 new problematic samples
- Phylogenetic analysis → the 3 problematic samples along with the 3 identified earlier all belong to the same HDV-1 small cluster (figure 2)

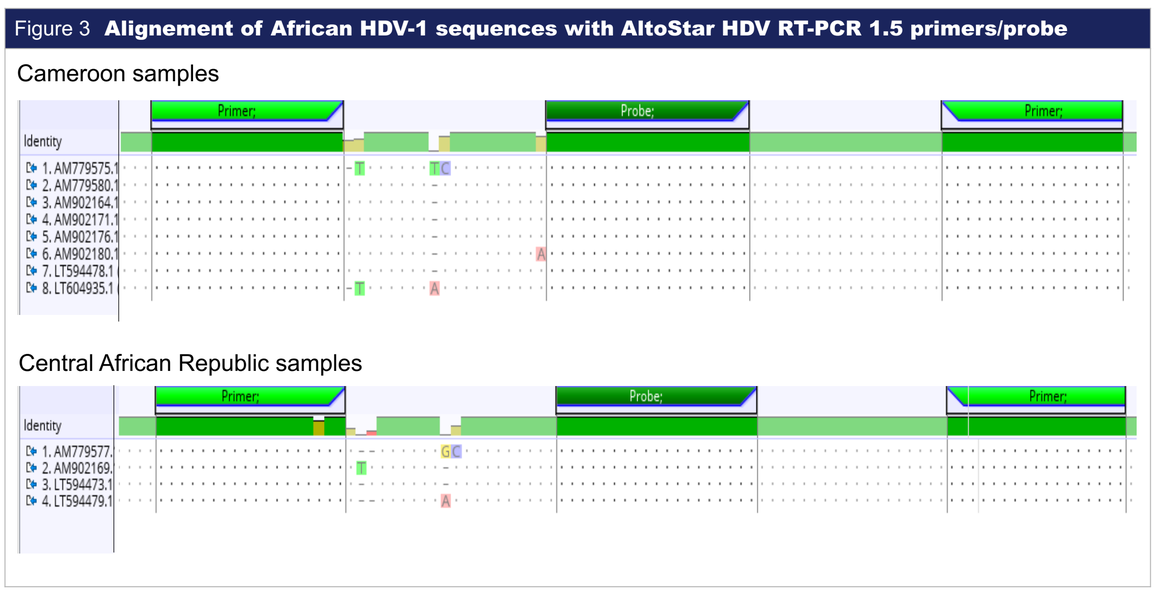
Figure 2 **HDV phylogenetic tree**



altona R&D Department

- *In silico* analyses
  - Alignment of AltoStar® primers/probe with the 3 F-NRC panel sequences (provided by F-NRC) → 100% match (figure 3)
  - Alignment of AltoStar® primers/probe with 487 sequences retrieved from NCBI databases and from the HDV_Web_Server (323 HDV-1, 24 HDV-2, 14 HDV-3, 38 HDV-4, 18 HDV-5, 16 HDV-6, 46 HDV-7, 7 HDV-8) → 100% match
  - Analysis of HDV-1 RNA secondary structure: ongoing
- Test different protocol conditions (extraction and PCR temperatures, reagents concentrations, primers/probe sequences) → addition of a new primer

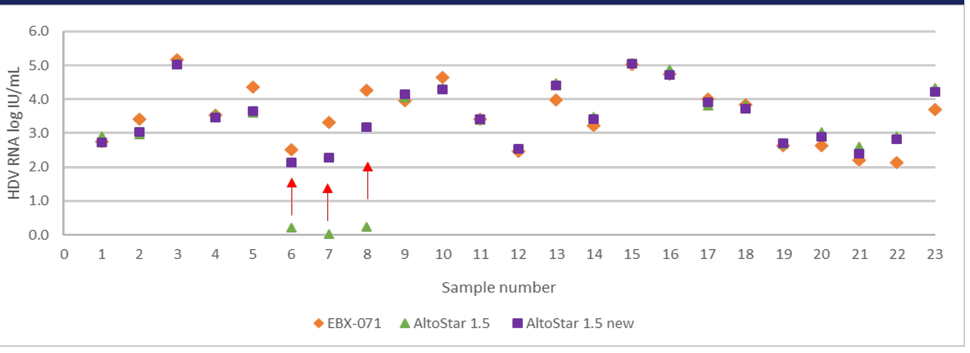
Figure 3 **Alignement of African HDV-1 sequences with AltoStar HDV RT-PCR 1.5 primers/probe**



New AltoStar® version evaluation

- Panel + 23 samples from the F-NRC collection (Cameroon origin) → correct quantification (with a mean difference of 0.3 log IU/mL *vs* EurobioPlex EBX-071)

Figure 4 **F-NRC HDV panel quantification**



Conclusion

- Secondary structure analyses are ongoing to identify the precise mechanism for the underquantification of these HDV-1 strains.
- This story underlines how academic and private labs can collaborate in an exemplary manner to provide clinicians and patients the best biological tools.

Contact:
segolene.brichler@aphp.fr

HDV reference center
Avicenne Hospital

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

altona DIAGNOSTICS