



SELECTED OPPORTUNITIES IN INFECTIOUS DISEASES

Diagnosis of HEV infection (BIO17006 / BIO18236 / BIO18237)

Product factsheet

Stage:
Human POC

▶ Biomarker:

- ◆ ORF2i, ORF2g, ORF2c

▶ Technology:

- ◆ ELISA, Western Blot

▶ Information:

- ◆ Diagnostic
- ◆ Prognosis

▶ Sample:

- ◆ Blood

▶ Scientific and Clinical Rationale:

- ◆ HEV is responsible for 20 million infections and 70,000 deaths, with the highest prevalence in East and South Asia.
- ◆ Recently, a new diagnosis assay based on detection of the HEV antigen capsid protein has been developed (Wantai Biologicals), notably for laboratories with no molecular diagnosis facility.
- ◆ Growth of HEV in cell cultures has been difficult to obtain, limiting direct biochemical analysis of viral proteins and infectious material.

▶ POC:

- ◆ Robust cell culture system that allows production of large amounts of HEV particles and their biochemical analysis.
- ◆ Identification of three forms of HEV ORF2 capsid protein: two forms are associated with non-infectious viral material, and one form is associated with infectious particles.
- ◆ HEV-negative (n=5) vs HEV-positive patients (n=10) plasma and serum samples obtained via standard viral diagnostics following physician's order, in a non interventional study.
- ◆ Sera from HEV-positive patients display large amounts of ORF2 associated with non-infectious particles. This form of ORF2 is the main antigen recognized by the Wantai kit.

Product factsheet

Clinical State and Market Opportunity

▶ Clinical State:

- ◆ Global epidemic burden of 20,1 million people in 2010¹
- ◆ Mosts endemic outbreaks reported in Asia (China and India) and Africa (Somalia and Uganda)²
- ◆ Foodborne zoonotic HEV transmission in Eastern Asia and Europe³
- ◆ No indicated treatment of acute/chronic hepatitis E

▶ Clinical needs:

- ◆ Hepatitis E is an underdiagnosed disease
- ◆ Hepatitis E is not clinically distinguishable from other types of viral hepatitis
- ◆ Higher needs for field use tests, especially in endemic areas, settings with risks of water contamination, or in pregnant women

▶ Opportunity:

- ◆ Current tests: anti-HEV IgM, RT-PCR (HEV RNA)
- ◆ No tests against HEV viral particles

¹ Int J Infect Dis. 2013 Apr; Kumar S. et al., Hepatitis E virus: the current scenario, doi: 10.1016/j.ijid.2012.11.026.

² Int J Infect Dis. 2013 Apr;17(4):e228-33. doi: 10.1016/j.ijid.2012.11.026.

³ Virus Res., 2011 Oct, Meng XJ, From barnyard to food table: the omnipresence of hepatitis E virus and risk for zoonotic infection and food safety, doi: 10.1016/j.virusres.2011.01.016.

Unique Selling Points

▶ Priority or Patent:

- ◆ 3 patent families
 - ◆ EP17 305 097.2 on 2017/01/30
 - ◆ PCT/EP2018/052149 on 2018/01/29
 - ◆ EP18305918.7 on 2018/07/10
 - ◆ PCT/EP2019/068338 on 2019/07/09
 - ◆ EP18305917.9 on 2018/07/10
 - ◆ PCT/EP2019/068341 on 2019/07/09

▶ Product:

- ◆ Antibodies for diagnosis

▶ Scientific Publication(s):

- ◆ Hepatitis E Virus Lifecycle and Identification of 3 Forms of the ORF2 Capsid Protein. Montpellier et al. Gastroenterology. 2018 Jan;154(1):211-223.e8. doi: 10.1053/j.gastro.2017.09.020. Epub 2017 Sep 25.
- ◆ New insights into the ORF2 capsid protein, a key player of the hepatitis E virus lifecycle. Ankavay et al. Sci Rep. 2019 Apr 18;9(1):6243. doi: 10.1038/s41598-019-42737-2.

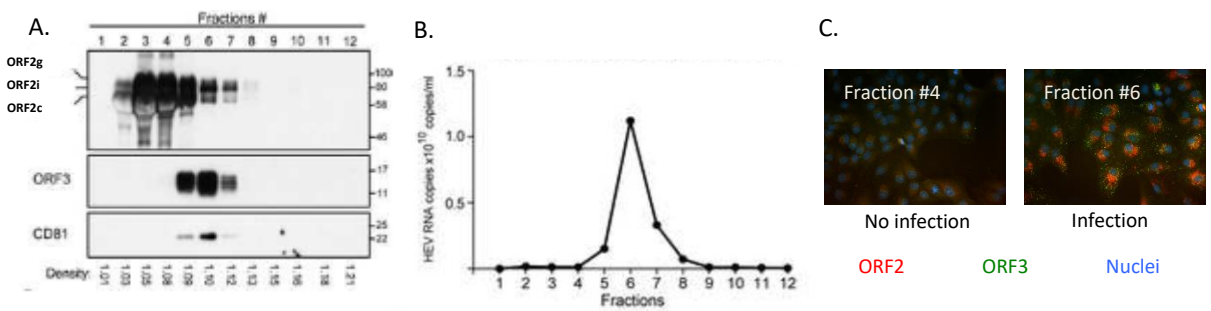
▶ Development opportunities

- ◆ Ongoing research open for partnering
- ◆ Access to scientific and clinical expertise on viral hepatitis

Proof of concept

▶ In vitro POC:

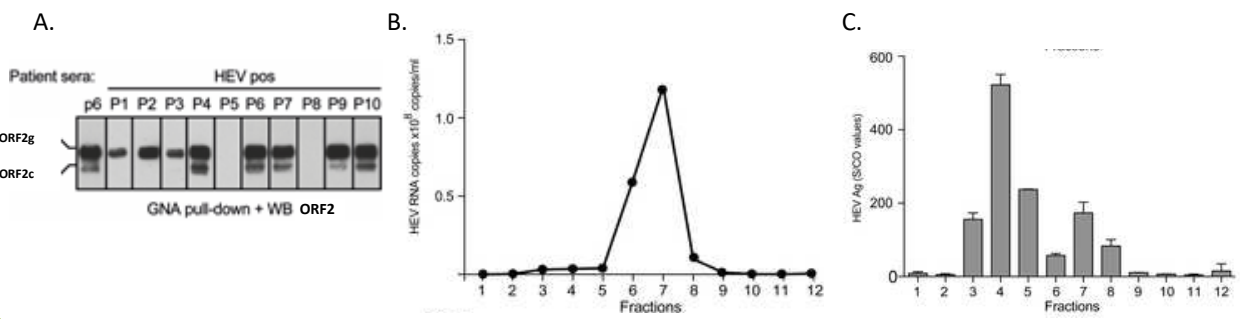
- ◆ **HEV produces large amounts of secreted capsid ORF2g & ORF2c that constitute non-infectious particles, and small amounts of capsid ORF2i that are assembled into infectious particles.**



Characterization of the different ORF2 products. (A) Density gradient of HEV-transfected PLC3 cell supernatant. Expression of ORF2, ORF3 and CD81 by WB. (B) Levels of HEV RNAs measured by RT-qPCR in A549 cells infected with an aliquot of each fraction. (C) A549 cells were infected with an aliquot of Fraction 4 and fraction 6. Five days post-infection, expression of viral proteins was analyzed by immunofluorescence.

▶ Human POC:

- ◆ **ORF2g and ORF2c proteins are the major HEV antigens in infected patients and are predominantly detected by the Wantai HEV-antigen ELISA^{Plus} assay**

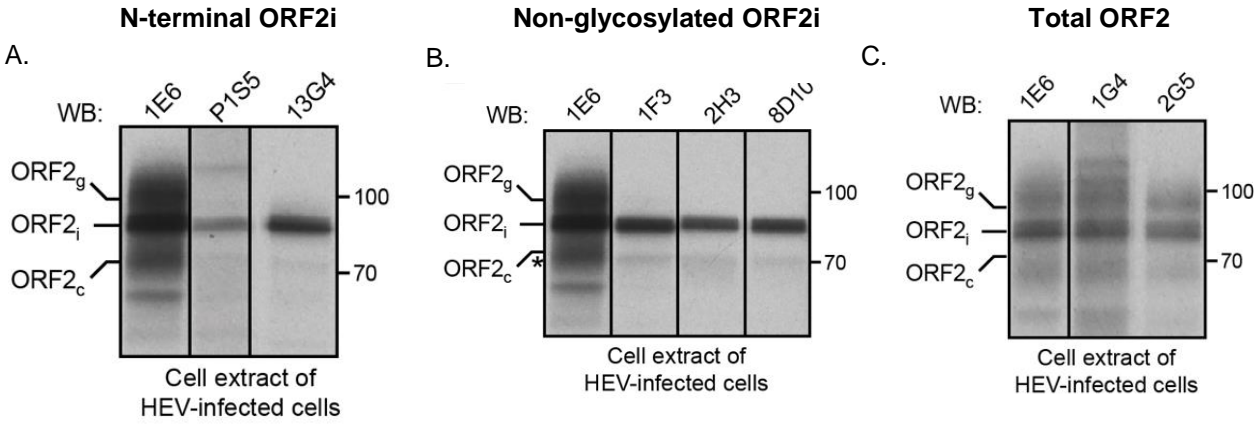


ORF2g and ORF2i proteins are secreted in large amounts in infected patients. (A) GNA pull-down on patient sera followed by INF-BM-** probing. Pull-down of PLC3/HEV supernatant was used as a positive control. (B) HEV RNA levels in each fraction of the P6 patient serum measured by RT-qPCR. (C) Detection of HEV Ag in each gradient fraction using the Wantai HEV-Ag ELISA^{Plus} kit. Results are expressed as signal to cut-off ratios (S/CO).

Proof of concept

► In vitro POC:

- ◆ Production of novel anti-ORF2 antibodies destined for use in a novel hepatitis E diagnostic test:



Characterization of the different ORF2 antibodies. Western blot assays were performed on HEV-infected cell lysates using hybridoma supernatants from mice immunized with different ORF2 peptides. (A) N-terminal ORF2i, (B) non-glycosylated ORF2i, (C) total ORF2. Monoclonal 1E6 antibody (Millipore), which recognizes all three forms of ORF2, was used as positive control.