PERFORMANCE EVALUATION OF NEW AUTOMATED IMMUNOASSAY TESTS, VIDAS® ANTI-HEV IGM AND IGG ASSAYS, IN EUROPEAN AND NON-EUROPEAN POPULATIONS

Florence Abravanel [1,2]*, Nadège Goutagny[3]*, Sébastien Lhomme[1,2], Corinne Perret[3], Mathilde Chenet[3], Françoise Vischi[1], Alexandre Aversenq[1], Sandrine Bourg[3], Aude Chapel[3], Nathalie Dehainault[3], Laurence Mercier[3], Francoise Luciani[3], Catherine Pothion[3], Emile Eichenlaub[3], XinXin Zhang[4], Pierre Roques[5], Jean-Marc Dugua[3], Jacques Izopet[1,2]

[1] CHU Toulouse, Hôpital Purpan, Laboratoire de virologie, National Reference Center for Hepatitis E, F-31300 France, [2] INSERM, U1043, Centre de Physiopathologie de Toulouse Purpan, Toulouse, F-31300 France, [3] R&D Immunoassay and Clinical Affairs, Biomérieux SA, Chemin de l'Orme, Marcy l'étoile, France , [4] Department of Infectious Diseases, Rui Jin Jospital, Shanghai, China, [5] UMR1184: Inserm, Université Paris Sud, CEA, Fontenay-aux-Roses, France, * These authors contributed equally to this work.

BACKGROUND

Hepatitis E is now recognized as the most common cause of acute viral hepatitis in the world. Hepatitis E is a disease present both in developing and developed countries. It mainly induces acute self-limiting disease (0.2- 4 % mortality), but it can present fulminant forms in pregnant women and infants (10-25% mortality in developing countries) or patients with pre-existing liver disease (11% mortality). In industrialized countries, evolution towards chronic infection is seen (60% in solid-organ transplant).

Hepatitis E virus (HEV) is a non-enveloped virus with a 7.2-kb positive-sense single-stranded RNA genome containing three open reading frames (ORFs) . ORF1 encodes the nonstructural polyprotein, ORF2 encodes the virus capsid protein while ORF3 encodes a small protein involved in virion morphogenesis and release. Human HEV viruses belong to one of four main genotypes, each with a distinct geographic distribution (Figure1). In highly endemic areas, genotypes 1 and 2 are transmitted between humans by the faecal-oral route. By contrast, HEV infections that occur in industrialized countries are due to zoonotic transfer of genotypes 3 and 4. The spread of HEV infection is still underestimated, because of differences in the specificity and sensitivity of diagnostic assays, and because most infections are subclinical and asymptomatic.

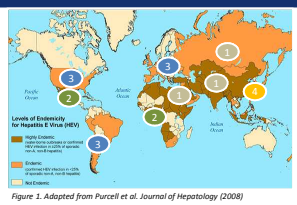


Figure 1. Adapted from Purcell et al. Journal of Hepatology (2008)

MEDICAL NEEDS

- **Differential diagnosis of viral** (non-A, non-B, non-C) **and non- viral infection** in patients presenting with signs of hepatitis
- HEV Diagnosis to **address relevant treatment or follow-up**, especially in the case of pregnant women or immunodepressed patients
- **Characterization of the hepatitis E infection** (viremic and post-viremic phase).

CLINICIAN NEEDS

- **Current diagnosis** relies on:
  - PCR (during viremic phase)
  - IgM serology to detect the initial short-lived IgM response
  - IgG serology to detect long-lasting IgG antibodies
- In order to provide accurate results, **main needs for serology** are:
  - A **robust and automated technology**
  - A **single use reagent** with high sensitivity and specificity
  - A quantitative assay for IgG serology to further define of immune correlate

- Ready-to-use
- Single-use test
- Reliable
- Time to result ≤ 40min

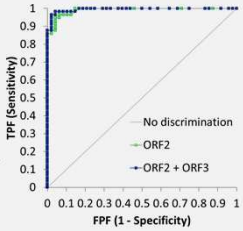
VIDAS Anti HEV-IgM

Assay characteristics and performances

- **QUALITATIVE assay**
- **Clinical cut-off value:** 1.00
- **Precision:** Repeatability is 4.1-9.9% and between-lot within-instrument is 7.4-13%
- **Interferences:** No interference with Hepatitis A, B, C, CMV, Dengue, Malaria, EBV and Rheumatoid factor. Interference for 1/10 sample for HIV.

Research Issue: Impact of the antigen on assay performances

- **CONTEXT:** Different concurrent test have distinct antigenic format. **What is the input of antigen(s) in assay performances ?**
- **MATERIAL:** 2 prototypes VIDAS ORF2 vs ORF2+ORF3
- **STUDY:** ROC curve analysis of sensitivity/specificity using European population with clinical signs and characterized by PCR (National Reference Center for Hepatitis E)
- **CONCLUSION:** **ORF2+ORF3 format presents slightly better sensitivity and specificity compared with the ORF2 format.**
  → **Choice of the ORF2+3 format**



Research issue: Sensitivity towards all genotypes

- **CONTEXT:** For HEV, 4 genotypes were described but only 1 serotype.
  Are the performances of the VIDAS Anti-HEV IgM assay equivalent for samples from different origin?
- **MATERIAL**: Samples from Burkina Faso, China and Europe, tested with VIDAS® Anti-HEV IgM assay.
  - **Europe: 459 samples,** characterized for hepatitis E by PCR and Wantai HEV IgM assay, from immunocompetent patients with clinical symptoms (National Reference Center for Hepatitis)
  - **China: 156 samples** characterized for hepatitis E using Wantai HEV IgM assay.
  - **Burkina Faso: 989 samples** characterized for hepatitis E using Wantai HEV IgM assay.
- **STUDY:** Analyses of Positive and Negative agreement with Wantai HEV IgM assay.

For European samples, data were analysed in term of infectious profile, i.e considering samples from Viremic phase (PCR+), Post-viremic phase (PCR-, Wantai IgM+) and no recent infection (PCR-/Wantai IgM-).

Europe		Infectious profile			
		Viremic phase *PCR+*	Post-viremic phase *PCR-, Wantai IgM+*	No recent infection *PCR-/Wantai IgM-*	Total
VIDAS Anti-HEV IgM	Positive	83	42	2	127
	Negative	2	29 (*)	301	332
Total		85	71	303	459
Performances		%	[IC95%]		
Positive agreement for viremic phase		97.65 %	[91.76 ; 99.71] %		
Positive agreement for post-viremic phase		59.15 %	[47.54 ; 69.83] %		
Negative agreement		99.34 %	[97.64 ; 99.92] %		

(*) These samples from patients in a post-viremic phase showed residual IgM that were not detected by the VIDAS® Anti-HEV IgM assay. 25/29 samples were positive with the VIDAS® Anti-HEV IgG assay.

China		Wantai HEV IgM		
		Positive	Negative	Total
VIDAS Anti-HEV IgM	Positive	53	2	55
	Negative	1	100	101
Total		54	102	156
Performances		%	[IC95%]	
Positive Agreement		98%	[90.2 ; 99.7] %	
Negative Agreement		98%	[93.1 ; 99.5] %	

Burkina Faso		Wantai HEV IgM	
		Negative	Total
VIDAS Anti-HEV IgM	Positive	16	16
	Negative	973	973
Total		989	989
Performances		%	[IC95%]
Negative agreement		98%	[97.4 ; 99] %

CONCLUSION:
High Positive and Negative Agreement (>97% cases) for VIDAS Anti-HEV IgM assay with commercially available HEV assays in European and non-European populations

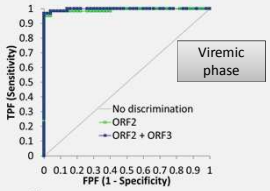
VIDAS Anti HEV-IgG

Assay characteristics and performances

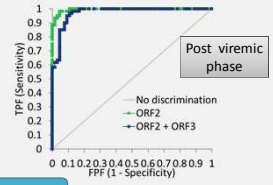
- **QUANTITATIF assay**
- **Linearity** of the assay in the measuring range: 0.05 to 10.00 U/mL
- **Clinical cut-off value :** 0.56U/ml
- **Precision** Repeatability is 4.6-7.8% and between-lot within-instrument is 9.1-11.9%
- **Interferences:** No interference with Hepatitis A and C, Dengue, CMV, VIH, EBV and Rheumatoid factor. Interference for 1/10 sample for Hepatitis B and 3/26 samples for malaria.

Research Issue: Impact of the antigen on assay performances

- **CONTEXT:** Literature suggests an impact of the antigenic format on diagnostic test sensibility
  → **What is the input of ORF3 vs ORF2 antigens?**
- **MATERIAL:** 2 prototypes VIDAS ORF2 vs ORF2+ORF3
- **STUDY:** ROC curve analysis of sensitivity/specificity using 2 types of samples
  - 113 samples with clinical symptoms (viremic phase) from the National Reference Center for Hepatitis E
  - 125 samples from the French Blood Bank, without clinical symptoms (post viremic phase).



- **CONCLUSION:**
  - **Similar sensibility/specificity for samples with clinical symptoms (viremic phase).**
  - **ORF2 format presents a better sensibility compared with the ORF2+ORF3 format for post viremic samples**
    → **Choice of the ORF2 format**



Research issue: Sensitivity towards all genotypes

- **CONTEXT:** For HEV, 4 genotypes were described but only 1 serotype.
  Are the performances of the VIDAS Anti-HEV IgG assay equivalent for samples from different origin?
- **MATERIAL:** Samples from Burkina Faso, China and Europe, tested with VIDAS® Anti-HEV IgG assay.
  - **Europe: 457 samples,** characterized for hepatitis E by PCR and Wantai HEV IgG assay, from immunocompetent patients with clinical symptoms (National Reference Center for Hepatitis)
  - **China: 156 samples** characterized for hepatitis E by Wantai HEV IgG assay
  - **Burkina Faso: 963 samples** characterized for hepatitis E by Diapro HEV IgG assay
- **STUDY:** Analyses of Positive and Negative agreement with Wantai or Diapro HEV IgG assay.

Europe		Wantai HEV IgG		
		Positive	Negative	Total
VIDAS Anti-HEV IgG	Positive	145	11	156
	Negative	5	296	301
Total		150	307	457
Performances		%	[IC95%]	
Positive Agreement		96.67 %	[92.39 ; 98.91] %	
Negative Agreement		96.42 %	[93.68 ; 98.20] %	

China		Wantai HEV IgG		
		Positive	Negative	Total
VIDAS Anti-HEV IgG	Positive	93	1	94
	Negative	7	55	62
Total		100	56	156
Performances		%	[IC95%]	
Positive Agreement		93.0%	[86.3 ; 96.6] %	
Negative Agreement		98.2%	[90.6 ; 99.7] %	

Burkina Faso		Diapro HEV IgG		
		Positive	Negative	Total
VIDAS Anti-HEV IgG	Positive	338	9 *	347
	Negative	62 *	554	616
Total		400	563	963
Performances		%	[IC95%]	
Positive Agreement		84.50%	[80.6 ; 87.7] %	
Negative Agreement		98.40%	[97.0 ; 99.2] %	

** 7/9 and 60/62 are consistent between VIDAS and WANTAI results*

CONCLUSION:
High Positive and Negative Agreement (>93% cases) for VIDAS Anti-HEV IgG assay with commercially available HEV assays in European and non-European populations

CONCLUSION

In conclusion, the present data indicated that the VIDAS HEV IgG and IgM showed excellent clinical performance, similar to the commercially available assays, in European and non European populations. These new assays will strengthen the diagnostic arsenal for HEV infection with a RAPID (<40min) and AUTOMATED detection of anti-HEV antibodies