VetLine

Hepatitis E Virus ELISA

(HEVVT0780)

Performance Characteristics

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1 Introduction

Hepatitis E virus (HEV) is one of the main causes of acute hepatitis worldwide. Reports of HEV infections are increasing exponentially. The World Health Organization (WHO) estimates that 20 million HEV infections worldwide occur annually, of which approximately 3 million are symptomatic and over 55,000 are fatal.

Hepatitis E virus is a small (32-34 nm) non-enveloped, single-stranded RNA virus that belongs to the family Hepeviridae. The genome comprises 7.2 kb, and codes for three partially overlapping open reading frames (ORF1, ORF2 and ORF3).

So far 4 human pathogenic genotypes have been described (HEV-1, HEV-2, HEV-3 and HEV-4). As studies have shown pronounced cross-reactions between them, currently only one HEV serotype is assumed.

HEV-1 occurs predominantly in Asia and Africa, HEV-2 in Africa and Mexico, while HEV-3 is observed worldwide and HEV-4 predominantly in Asia and Europe. HEV-1 and HEV-2 are restricted to humans, while HEV-3 and HEV-4 additionally infect various animal species. HEV-3 and HEV-4 have been repeatedly detected in animals, particularly in domestic pigs, wild boar and pork. Domestic pigs and wild pigs infected with the hepatitis E virus do not show any clinical symptoms, they do not contract hepatitis themselves.

Infection routes known to date comprise fecal-oral transmission by contaminated water (HEV-1 and HEV-2), food-borne infection acquired from food of animal origin (HEV-3 and HEV-4), via contaminated blood transfusions or organ transplants and via vertical transmission from mother to child. The incubation period of HEV is 2-8 weeks.

The course of HEV infection in humans can vary considerably depending on the infecting genotype.

In general, a broad spectrum of clinical symptoms can be identified. HEV-1 and HEV-2 infections are usually acute and self-limiting. So far, no cases of chronic hepatitis caused by genotypes 1 and 2 have been described. However, infection with HEV-1 may be life-threatening for pregnant women. In areas with endemic distribution of HEV-1 a high proportion of fulminant hepatitis in expectant mothers in the last trimester of pregnancy has been reported. The death rate was up to 30 %.

In immunocompetent patients, HEV-3 and HEV-4 infections mostly progress asymptomatically. Symptomatic infections are usually acute and self-limiting and can hardly be distinguished from symptoms of hepatitis A infections (icterus, upper abdominal pain, fever, fatigue, etc.). Persons with existing liver damage and immunosuppressed patients have an increased risk of developing chronic hepatitis. Chronic infections, mainly caused by HEV-3, may evoke life-threatening liver cirrhosis, even without observed symptoms.

In regions endemic for HEV-1 and HEV-2 unboiled tap water as well as ice cubes made from it should be avoided to prevent from HEV infection. In Germany and other industrial countries with HEV-3 and HEV-4 distribution, especially pig and game products should only be consumed well cooked. HEV can be inactivated by heating the food above 71 °C for at least 20 minutes.

Table 1: Hepatitis E virus - Symptoms and Transmission routes

Species	Disease	Symptoms (e.g.)	Transmission route
Hepatitis E Virus (HEV)	Hepatitis E	icterus, upper abdominal pain, fever, fatigue ➢mostly asymptomatic in immunocompetent patients (HEV-3, HEV-4) ➢severe disease seen in pregnancy (HEV-1), immunosuppressed patients or patients with pre-existing liver damage (HEV-3, HEV-4)	waterborne, fecal-oral (HEV-1, HEV-2) food-borne (HEV-3, HEV-4)

The presence of pathogen or infection may be identified by

- RT-PCR
- Antigen detection
- Serology: detection of antibodies by ELISA, immunoblot

2 Intended Use

The NovaTec VetLine Hepatitis E Virus ELISA is intended for the qualitative determination of antibodies against Hepatitis E Virus in veterinary serum.

3 Principle of the Assay

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Test Description

The reproducibility of the NovaTec VetLine Hepatitis E virus ELISA kit was determined by comparing 12-24 replicates of at least 2 different samples in one assay (within-run) and by comparing at least 2 different samples assayed in 12 different runs (between-run).

In accordance with the procedures prescribed in the instruction, the mean ($\bar{x}$) and the standard deviation (s) of the absorbance values (E) were determined.

The CV value [%] was then calculated using the following formula:

$$CV = s/\bar{x} \times 100\ \%$$

Acceptance Criterion: CV < 15 %

Results

Table 2: Within-Run Precision

Sample	n	Mean (E)	CV [%]
1	24	0,470	4,62
2	24	0,171	7,73
3	24	1,106	3,80

Table 3: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	4,13	8,21
2	12	14,35	5,06
3	12	25,31	3,69

Conclusion

The acceptance criterion was met for all samples.

4.2 Analytical Specificity

4.2.1 Interference from Hemoglobin, Bilirubin and Triglycerides

Introduction

Increased concentrations of possible interference materials such as bilirubin, triglycerides and hemoglobin may interfere with immunoassays creating false-negative or false-positive results.
In order to investigate this topic a literature research in combination with investigation of nearly 100 parameters were performed.

Material and Test Condition

Different members of the NovaLisa® and Novatec VetLine ELISA line were used including assays for the detection of different antibody isotypes (IgA, IgM, IgM, IgM + IgM, total antibody with protein A/G) to bacteria, viruses, fungi, parasites and worms as well as an antigen ELISA for the detection of TSH, Dirofilaria and FeLV.

Defined positive resp. negative or equivocal samples were used.

A certain amount of the potentially interfering substance was added to each sample. The final concentration of each substance was in a pathological range as also described by competitors (10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides). The results obtained with the sample with added “interfering substance” should be 60-140 % of the result of the untreated sample in order to fulfil the specifications.

Conclusion

The internal specifications of 60-140 % were always fulfilled.
Interferences with hemolytic, lipemic or icteric samples were not observed up to a concentration of 10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides.
These results are also in agreement with literature data.

This conclusion is valid for the NovaLisa® as well as for the VetLine version of the assays.

Dimeski, G. (2008) Interference Testing, Clin Biochem Rev 29, S43 – S48
Tate, J. and Ward, G. (2004) Interferences in Immunoassay, Clin Biochem Rev 25, 105 – 120

4.2.2 Cross-Reactivity

Cross reactions, especially against spirochaetes, cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

The purpose of this study was to determine the efficiency of the assay to discriminate between positive and negative clinical samples.

The **VetLine Hepatitis E Virus (HEV) ELISA** and the human **NovaLisa® Hepatitis E Virus (HEV) IgG ELISA** share similar components since they are based on the same platform.
The only difference is the conjugate:
Protein A/G Conjugate in case of the VetLine assay and **anti-human IgG Conjugate** in case of the NovaLisa® assay.

Due to this fact, the Diagnostic Sensitivity and Specificity of the CE marked NovaLisa® assay gives a rough indication of the expected veterinary data situation.

To evaluate the diagnostic performance of the Hepatitis E Virus (HEV) IgG ELISA, internal studies were conducted by NovaTec, either in comparison to an immunoassay already established on the market, or by using well defined samples.

Material

Hepatitis E Virus (HEV) IgG		Lot:	HEVG-012
Production date:	2018-04	Expiry date:	2019-04-30
Hepatitis E Virus (HEV) IgG		Lot:	HEVG-014
Production date:	2018-07	Expiry date:	2019-07-31
Hepatitis E Virus (HEV) IgG		Lot:	HEVG-016
Production date:	2019-03	Expiry date:	2020-03-31

Mikrogen assays (CE marked):

*recom*Well HEV IgG	Lot:	EHE011701
	Expiry Date:	2018-06
*recom*Well HEV IgG	Lot:	EHE031701
	Expiry Date:	2018-08
*recom*Well HEV IgG	Lot:	EHE101701
	Expiry Date:	2019-03
*recom*Well HEV IgG	Lot:	EHE081802
	Expiry Date:	2020-01
*recom*Well HEV IgG	Lot:	EHE101801
	Expiry Date:	2020-03
*recom*Line HEV IgG/IgM	Lot:	LHE031803
	Expiry Date:	2019-08

228 samples

- 57 defined HEV IgG/IgM samples
- 128 potentially cross-reactive samples
- 29 pregnancy samples
- 14 defined EQAS samples ("Virusimmunologie – Hepatitis E Virus (Ak)" (348), www.instandev.de)

Table 3: Diagnostic Sensitivity and Specificity
(Equivocal results were not included in the calculations)

		Demand		
		positive	negative	**Σ**
NovaTec VetLine Hepatitis E Virus ELISA	positive	71	156	**72**
	negative	0	157	**156**
	Σ	**71**	**157**	**228**

Conclusion

Diagnostic Sensitivity: 100.0 % (95 % confidence interval: 94.94 % - 100.0 %)
Diagnostic Specificity: 99.36 % (95 % confidence interval: 96.5 % - 99.98 %)