DSI SRL

Extraction of Performance Evaluations

Products:
DS-EIA-anti-HEV-G, cat# E-151

2. Performance characteristics

Laboratory trials of the kit "DS-EIA-ANTI-HEV-G" were carried out under routine manufacture conditions using 1469 samples (Table 2).

Table 2

Samples used for the kit "DS-EIA-ANTI-HEV-G" evaluation

The category of samples	The number of samples
1. Negative for antibodies to hepatitis E virus	
a) unselected donors (including 1st time donors)	1288
b) clinical patients	44
c) cross reactive samples:	
pregnant women;	20
sera samples with rheumatoid factor;	28
patients infected with HBV;	12
infected with HCV;	8
infected with HAV	9
2. Positive for antibodies to HEV samples:	36
3. Hepatitis E Mixed Titer Panel (ZeptoMetrix – cat. K-ZMC003, lot 0802-272-00032)	24
6. Standard	
WHO REFERENCE REAGENT FOR HEPATITIS E VIRUSANTIBODY, human serum, NIBSC, cat. 95/584	
Total number of samples	1469

NIBSC – National Institute of Biological standards and controls, Great Britain

2.1. Analytical sensitivity.

Evaluation of analytical sensitivity was carried out using "WHO REFERENCE REAGENT FOR HEPATITIS E VIRUS ANTIBODY, human serum" (cat. № 95/584, NIBSC, UK). Eight dilutions in sample dilution buffer were prepared with concentration of antibody to HEV: 100.0 units/ml, 50.0 units/ml, 25.0 units/ml, 12.5 units/ml, 6.25 units/ml, 3.125 units/ml, 1.563 units/ml, 0.782 units/ml and tested in 10 repeats. Samples with OD/Cut-off ≥1.0 are considered as positive. The last positive dilution was considered to be the criteria of the kit analytical sensitivity. The analytical sensitivity of "DS-EIA-ANTI-HEV-G" was 1.563 units/ml according to "WHO REFERENCE REAGENT FOR HEPATITIS E VIRUS ANTIBODY, human serum" (NIBSC, UK). Results presented in Table 3.

Table 3

Evaluation of the kit "DS-EIA-ANTI-HEV-G" analytical sensitivity

Samples №	No. of replicates	Concentration antibody (units/ml)	(OD/Cut off)
1	10	100.0	**12.13±0.20**
2	10	50.0	**11.60±0.21**
3	10	25.0	**10.21±0.24**
4	10	12.5	**9.18±0.23**

5	10	6.25	**6.17±0.15**
6	10	3.125	**3.23±0.09**
7	10	1.563	**1.25±0.09**
8 (Negative)	10	0.782	0.38±0.07

2.2. Diagnostic sensitivity

2.2.1. Testing of panels

Sensitivity of "DS-EIA-ANTI-HEV-G" was evaluated using "Hepatitis E Mixed Titer Panel» (ZeptoMetrix, USA) in comparison with test «recomWell HEV IgG» (lot EHE10091, exp. 2010-09, Mikrogen, Germany). Samples with OD/Cut-off ≥1.0 are considered as positive. Results presented in tables 4.

Table 4

Results of ZeptoMetrix "Hepatitis E Mixed Titer Panel K-ZMC003» testing (OD/Cut-off)

Panel member	"recomWell HEV IgG"	"DS-EIA-ANTI-HEV-G"
1	**3.3**	**8.8**
2	0.5	0.1
3	**2.5**	**6.2**
4	**6.4**	**11.1**
5	**4.5**	**10.9**
6	**1.1**	**5.4**
7	**2.2**	**6.9**
8	0.3	0.0
9	**2.5**	**11.3**
10	**2.3**	**7.4**
11	**1.5**	**3.8**
12	**2.7**	**9.8**
13	**3.1**	**10.3**
14	**5.1**	**10.5**
15	**2.2**	**1.1**
16	**1.6**	**3.1**
17	**1.3**	**1.2**
18	0.2	0.0
19	**1.4**	**1.7**
20	**1.1**	0.3
21	**5.2**	**11.3**
22	0.3	0.0
23	**2.9**	**3.1**
24	**6.9**	**11.7**

"DS-EIA-ANTI-HEV-G" revealed 95% relative sensitivity with "recomWell HEV IgG.

2.2.2. Testing of positive for antibodies to hepatitis E virus samples

For the diagnostic sensitivity assessment 36 specimens from patients with hepatitis E. Most of samples were provided by Institute of Poliomyelitis and Viral Encephalitis nam. M.P. Chumakov (Moscow, Russia). Presence of specific antibodies for hepatitis E virus was confirmed by "recomWell HEV IgG". Samples with OD/Cut-off > 1,0 are considered as positive.

Results of testing of 36 known positive samples with "DS-EIA-ANTI-HEV-G" and test of comparison "recomWell HEV IgG" are presented in Table 5. Diagnostic sensitivity of "DS-EIA-ANTI-HEV-G" was 100 % with 100 % concordance with test of comparison.

Table 5

Results of testing positive for antibodies to HEV samples

№ samples	"DS-EIA-ANTI-HEV-G" OD/Cut-off	"recomWell HEV IgG" OD/Cut-off
1	11.1	7.5
2	10.0	7.3
3	10.9	4.2
5	9.9	6.6
7	11.3	5.1
8	11.8	9.5
9	10.8	5.5
10	9.7	7.5
12	11.1	4.8
13	8.3	2.1
14	5.2	1.1
15	6.5	1.4
16	10.7	5.0
17	2.1	1.2
18	4.8	5.3
19	10.8	7.1
20	6.7	1.2
21	7.9	1.5
22	10.7	2.5
23	9.3	1.7
24	8.4	1.9
25	10.4	2.9
26	8.7	2.3
27	8.1	1.6

28	11.8	8.4
29	5.7	1.4
30	7.8	1.8
31	10.8	3.5
32	4.5	1.6
33	11.7	4.2
34	5.5	2.1
35	10.3	3.8
36	6.5	1.5

2.3. Diagnostic specificity

Specificity was evaluated regarding the frequency of occurrence of false-positive results when testing negative samples. Evaluation of diagnostic specificity was carried out using different target categories of population: unselected donors (n = 1288), clinical patients (n = 44), cross reactive samples: pregnant women (n = 20), sera samples with rheumatoid factor (n = 28), patients infected with HBV (n = 12), patients infected with HCV (n=8), patients infected with HAV (n=9). Donor sera were obtained from the Regional Blood Bank (N. Novgorod) and from the subdivision of the Regional Blood Bank (Dzershinsk). Sera from clinical patients were received from Institute Regional Clinical Hospital nam. Semashko N.A. (N.Novgorod), sera of pregnant women – from antenatal clinics of N.Novgorod, sera of HBV, HCV, HAV infected patients – from the Research Institute of Virology n. D.I. Ivanovskyi of the Russian Academy of Medical Sciences. Results are represented in Table 6.

Table 6

Evaluation of diagnostic specificity of the kit "DS-EIA-ANTI-HEV-G"

Tested categories	The number of samples	The number of first-positive samples	The number of first -positive samples which were tested and found negative whith references "recomWell HEV IgG"	Specificity, %
Unselected donors (including 1st time donors)	1288	42	22	98.3
Clinical patients	44	3	3	93.2
Pregnant women	20	2	2	90.0
Sera samples with rheumatoid factor	28	1	1	96.4
Patients infected with HBV	12	0	0	100
Patients infected with HCV	8	0	0	100
Patients infected with	9	0	0	100

Cut-Off = average OD value of the Negative Control + A

Where A is a coefficient defined by manufacturer during quantitative ROC analysis. The coefficient value is indicated in the instruction for use.

6.2. Interpretation of results

Samples are considered positive if their OD value exceed or equal the Cut-off value.

7. Conclusion

The following data was received during the trials of the enzyme immunoassay "DS-EIA-ANTI-HEV-G":

	Parameters	The object of study	Obtained characteristics
1	Diagnostic sensitivity	Positive for antibodies to HEV samples from patients with hepatitis E (n=36)	100%
		ZeptoMetrix "Hepatitis E Mixed Titer Panel K-ZMC003" (n=24)	95%
2	Analytical sensitivity	WHO REFERENCE REAGENT FOR HEPATITIS E VIRUS ANTIBODY, NIBSC, cat. № 95/584.	1.563 units/ml
3	Specificity	Blood donors (n=1288)	97.5%
		Serum samples with potential eross reactivity: Clinical patients (n=44)	93.2%
		Pregnant women (n=20)	90%
		Samples containing rheumatoid factor (n=28)	96.4%
		Patients with infectious diseases (HBV, HCV, HAV) (n=29)	100%
		Total (n=1409)	97.3%
4	Equivalency of serum/plasma testing	Serum/plasma	100%
5	Equivalency of testing serum/plasma with anticoagulants	Serum/plasma with anticoagulants	100%

High sensitivity at detection of antibodies to hepatitis E in combination with high specificity and reproducibility of results allows using the kit "DS-EIA-ANTI-HEV-G" as an aid in the diagnosis of patients suspected of having hepatitis E.

Revision: 002

The head of the production control division № 3 **N.G. Belova**
"13" January 2012

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DSI SRL

Extraction of Performance Evaluations

Products:
DS-EIA-anti-HEV-M, cat# E-152

Performance characteristics

Sensitivity and specificity

«DS-EIA-ANTI-HEV-M" system's trials were conducted with help Center for Disease Control, Atlanta, USA. The following groups of sera were used in the trials:

1. Healthy donors serum samples (n=600)
2. Serum samples, containing different infection markers:

- Anti-HAV-IgM positive (n=27)
- HbsAg positive (n=109)
- Anti-HCV IgG positive (n=323)
- CMV PCR positive (n=28)

3. HEab Panel (Centers for Disease Control and Prevention, USA): chimpanzee serum samples containing and not containing HEV antibodies (n=8). HEab panel (CDC, USA) consists of chimpanzee serum samples, not containing (sera #1 and #2) anti-HEV IgG, containing anti-HEV IgM and IgG (serum #3), and containing anti-HEV in various concentration (sera #4-8). All positive sera were collected during 1 year period after infection.
4. Manufacturer's standard sample (SOP K+ No 2.02.04 RPC DS). This sample is human serum, containing human IgM, tested with "Anti HEV IgM" (Genelabs Diagnostics, Singapore). The sample has been designed for technological control and evaluation of "DS-EIA-ANTI-HEV-M". SOP K+ is characterized in its product passport and should be tittered according to the instructions manual.

Sensitivity and specificity of the «DS-EIA-ANTI-HEV-M" was tested on serum samples containing and not containing HEV antibodies from HEab and SOP K+

Results of the trials are shown in Table #2

Table 2

Sensitivity and specificity evaluation of 3 laboratory batches of the «DS-EIA-ANTI-HEV-M" kit.

Serum Number	SOP K+ 2.02.04		HEab (CDC, USA)	
	SOP K+ titer	OD/Cut-off	OD (mean±δ of 3 laboratory batches)	OD/Cut-off
1	whole	2.15	0.019±0.003	0.091
2	1:2	5.01	0.023±0.004	0.0109
3	1:4	3.60	0.395±0.045	1.881
4	1:8	2.03	0.025±0.003	0.120
5	1:16	1.09	0.045±0.006	0.200
6			0.028±0.003	0.130
7			0.035±0.005	0.170
8			0.022±0.003	0.110

As it is shown in the table, sensitivity of the «DS-EIA-ANTI-HEV-M" is 100%. Positive results are obtained with all positive sera from panels HEab and SOP K+. The OD in cases of negative sera was lower than cut-off that shows on 100% specificity of the assay.

Comparative evaluation of diagnostic performance of "DS-EIA-ANTI-HEV-M" and "Anti HEV EIA IgM" Genelabs, Singapore has been carried out using the same SOP K+ and HEab panels. All serum samples were tested with both kits. Results are shown in table #3.

Table 3

Comparative evaluation of diagnostic performance of «DS-EIA-ANTI-HEV-M" and "Anti HEV EIA IgM"

Serum Number	"DS-EIA-Anti-HEV-M"		"Anti HEV EIA IgM"	
	SOP K+ titer	HEab	SOP K+	HEab
	OD/Cut-off		OD/Cut-off	
1	Whole/5.15	0.091	1:2/6.15	0.083
2	1:2/5.0	0.109	1:4/4.62	0.129
3	1:4/3.6	1.880	1:8/2.94	1.214
4	1:8/2.03	0.120	1:16/1.50	0.220
5	1:16/1.09	0.200		0.180
6		0.130		0.165
7		0.170		0.270
8		0.110		0.090

Shown data suggest that diagnostic performance of both compared kits is equal.

Different groups of the population with various infectious diseases or blood donors were tested to test system's specificity. Obtained data are shown in table #4.

Table 4

Anti-HEV reactivity of the sera collected from different groups of the population.

Patients groups	"DS-EIA-Anti-HEV-M"		
	Total studied sera	HEV Ab IgM positive sera number	
		Amount	percentage
Blood donors	600	2	0.3
Hep. A patients	27	0	0
Hep. B patients	109	1	0.9
Hep. C patients	323	2	0.6
CMV patients	28	0	0
total	987	5	0.5

As it can be seen from the Table #3, «DS-EIA-ANTI-HEV-M" has revealed 0.3% of positive sera among blood donors, 0-0.9% among other acute hepatitis patients. All positive samples were tested with Anti HEV EIA IgM kit. All sera showed negative results in the reference kit, thus we can suggest that relative specificity is 99.5%

Table #5 shows results of the comparative study of "Anti HEV EIA IgM" and "DS-EIA-ANTI-HEV-M". 10 HEV PCR positive serum samples used in the study(gift from Prof. Mihailov) were collected at early stages of acute hepatitis E.

Data show that both kits revealed positive results in all samples. OD values were very close in both kits. Shown results indicate that both systems have similar sensitivity parameter.

Table 5

Comparative evaluation of anti-HEV IgM detection with «DS-EIA-ANTI-HEV-M" and "Anti HEV EIA IgM" in serum samples collected at early stages of acute hepatitis E.

Serum Number	"DS-EIA-Anti-HEV-M"		"Anti HEV EIA IgM"	
	OD	Result	OD	Result
1	0.540	+	0.854	+
2	0.810	+	1.032	+
3	2.300	+	1.124	+
4	0.450	+	0.496	+
5	2.800	+	2.000	+
6	1.940	+	1.856	+
7	0.860	+	0.920	+
8	0.357	+	0.550	+
9	0.281	+	0.350	+
10	2.318	+	2.000	+
Cut-off	0.245		0.219	

Thus shown data suggest that «DS-EIA-ANTI-HEV-M" system has high diagnostic performance. «DS-EIA-ANTI-HEV-M" is suitable for screening studies, diagnostic purposes and studies necessary for research of HEV infection.

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"15" 06 2005

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