

Title: Diagnostic performance of a fully automated hepatitis E virus antibody immunoassay

Running title: Performance of automated HEV antibody tests

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Table 1 – Raw data for the comparison of the Mikrogen and DiaSorin HEV IgG immunoassays. The cut-off for positive results is 20 U/ml and 0.3 IU/ml for Mikrogen recomWell IgG and DiaSorin LIAISON® Murex anti-HEV IgG, respectively. * These samples should be quoted as borderline in the recomWell HEV IgG test (≥ 20 U/ml and ≤ 24 U/ml). Abbreviations: qual., qualitative; +, positive.

Number	Mikrogen recomWell HEV IgG		Mikrogen recomLine HEV IgG	DiaSorin LIAISON® Murex anti-HEV IgG	
	[U/ml]	qual.	qual.	[IU/ml]	qual.
1	29.0	+	+	0.5	+
2	80.4	+	+	1.3	+
3	79.7	+	+	2.4	+
4	>125	+	+	>10	+
5	>125	+	+	>10	+
6	113.8	+	+	4.5	+
7	>125	+	+	3.7	+
8	>125	+	+	>10	+
9	89.3	+	+	2.4	+
10	103.2	+	+	>10	+
11	103.2	+	+	5.4	+
12	103.2	+	+	>10	+
13	71.4	+	+	1.3	+
14	98.8	+	+	2.9	+
15	91.2	+	+	3.4	+
16	80.0	+	+	1.9	+
17	98.8	+	+	>10	+
18	89.4	+	+	3.1	+
19	35.1	+	+	0.8	+
20	92.3	+	+	>10	+
21	25.4	+	+	0.5	+
22	92.3	+	+	>10	+
23	183.2	+	+	>10	+
24	40.7	+	+	0.6	+
25	59.5	+	+	1.1	+
26	54.3	+	+	0.8	+
27	69.4	+	+	1.1	+
28	107.0	+	+	>10	+
29	107.0	+	+	>10	+
30	96.0	+	+	3.6	+
31	26.1	+	+	0.7	+
32	107.0	+	+	4.7	+

Number	Mikrogen recomWell HEV IgG		Mikrogen recomLine HEV IgG	DiaSorin LIAISON® Murex anti-HEV IgG	
	[U/ml]	qual.	qual.	[IU/ml]	qual.
33	107.0	+	+	>10	+
34	117.5	+	+	3.8	+
35	117.5	+	+	>10	+
36	71.7	+	+	1.3	+
37	82.3	+	+	1.5	+
38	32.1	+	+	0.5	+
39	98.4	+	+	6.3	+
40	98.4	+	+	>10	+
41	34.4	+	+	0.49	+
42	40.9	+	+	1.5	+
43	79.4	+	+	1.2	+
44	98.4	+	+	3.5	+
45	46.2	+	+	0.8	+
46	96.4	+	+	4.4	+
47	96.4	+	+	>10	+
48	41.2	+	+	0.7	+
49	41.9	+	+	0.8	+
50	31.0	+	+	0.5	+
51	74.4	+	+	1.6	+
52	92.4	+	+	5.9	+
53	27.2	+	+	0.6	+
54	28.1	+	+	0.5	+
55	51.3	+	+	1.1	+
56	97.6	+	+	>10	+
57	97.6	+	+	>10	+
58	97.6	+	+	>10	+
59	23.9*	+	+	0.4	+
60	>125	+	+	>10	+
61	>125	+	+	6.5	+
62	23.9*	+	+	0.3	+
63	44.7	+	+	0.8	+
64	73.0	+	+	1.6	+
65	>125	+	+	>10	+
66	>125	+	+	>10	+
67	101.7	+	+	7.2	+
68	101.7	+	+	3.6	+
69	21.2*	+	+	0.3	+
70	>125	+	+	>10	+
71	>125	+	+	>10	+
72	>125	+	+	>10	+
73	36.1	+	+	0.9	+
74	60.2	+	+	1.5	+
75	49.1	+	+	0.8	+
76	85.5	+	+	1.9	+
77	106.7	+	+	>10	+
78	34.2	+	+	0.6	+
79	86.4	+	+	2.6	+
80	122.7	+	+	8.5	+
81	34.6	+	+	0.6	+
82	35.7	+	+	0.5	+
83	73.3	+	+	1.6	+
84	25.4	+	+	0.4	+
85	>125	+	+	>10	+
86	>125	+	+	5.0	+
87	>125	+	+	>10	+
88	103.2	+	+	9.8	+

Number	Mikrogen recomWell HEV IgG		Mikrogen recomLine HEV IgG	DiaSorin LIAISON® Murex anti-HEV IgG	
	[U/ml]	qual.	qual.	[IU/ml]	qual.
89	98.8	+	+	>10	+
90	92.3	+	+	>10	+
91	43.0	+	+	0.7	+
92	38.5	+	+	0.4	+
93	37.8	+	+	0.4	+
94	>125	+	+	>10	+
95	58.5	+	+	1.3	+
96	>125	+	+	5.2	+
97	63.9	+	+	1.4	+
98	84.7	+	+	2.0	+
99	106.7	+	+	>10	+
100	66.3	+	+	1.4	+

Table 2 - Raw data for the comparison of the Mikrogen, DiaSorin and Wantai HEV IgM immunoassays. The cut-off for positive results is 20 U/ml and 1 [Index] for Mikrogen recomWell HEV IgG and DiaSorin LIAISON® Murex anti-HEV IgM, respectively. Only sera that had a discrepant result between the recomWell HEV IgM ELISA and the DiaSorin assay were re-screened in the Wantai HEV IgM assay (positive = absorbance value of the sample/absorbance value of the cut-off ≥ 1). * These samples should be quoted as borderline in the recomWell HEV IgM test (≥ 20 U/ml and ≤ 24 U/ml). Abbreviations: qual., qualitative; +, positive; -, negative; n.t., not tested).

Number	Mikrogen recomWell HEV IgM		Mikrogen recomLine HEV IgM	DiaSorin LIAISON® Murex anti-HEV IgM		Wantai HEV-IgM ELISA		RealStar® HEV RT- PCR Kit 2.0	Mikrogen recomWell HEV IgG	
	[U/ml]	qual.	qual.	[Index]	qual.	sample/ cut-off	qual.	RNA	[U/ml]	qual.
1	114.5	+	+	3.1	+	n.t.	n.t.	n.t.	85.2	+
2	83.7	+	-	2.8	+	n.t.	n.t.	n.t.	85.2	+
3	>125	+	-	2.9	+	n.t.	n.t.	n.t.	>125	+
4	100.7	+	+	1.7	+	n.t.	n.t.	n.t.	98.7	+
5	35.6	+	-	0.5	-	0.29	-	-	98.8	+
6	34.5	+	-	0.8	-	0.44	-	-	98.8	+
7	68.7	+	+	3.3	+	n.t.	n.t.	n.t.	91.2	+
8	43.4	+	-	0.6	-	3.98	+	-	98.8	+
9	24.4	+	-	0.8	-	1.2	+	-	103.2	+
10	33.7	+	-	1.3	+	n.t.	n.t.	n.t.	89.3	+
11	45.4	+	-	1.1	+	n.t.	n.t.	n.t.	>125	+
12	>125	+	+	9.1	+	n.t.	n.t.	n.t.	>125	+
13	21.1*	+	+	0.97	-	2.6	+	-	>125	+
14	>125	+	+	9.1	+	n.t.	n.t.	n.t.	>125	+
15	39.7	+	+	1.6	+	n.t.	n.t.	n.t.	79.7	+
16	93.4	+	+	5.4	+	n.t.	n.t.	n.t.	92.3	+
17	187.5	+	+	6.9	+	n.t.	n.t.	n.t.	183.2	+
18	98.4	+	+	1.7	+	n.t.	n.t.	n.t.	117.5	+
19	57.5	+	-	1.9	+	n.t.	n.t.	n.t.	117.5	+
20	35.7	+	+	1.5	+	n.t.	n.t.	n.t.	14.7	-
21	28.7	+	+	0.99	-	1.08	(+)	-	96.0	+
22	>125	+	+	4.0	+	n.t.	n.t.	n.t.	107.0	+
23	72.8	+	-	2.2	+	n.t.	n.t.	n.t.	107.0	+
24	34.2	+	-	1.4	+	n.t.	n.t.	n.t.	107.0	+
25	22.1*	+	-	0.8	-	1.68	+	-	107.0	+
26	28.7	+	-	<0.1	-	0.03	-	-	4.0	-
27	83.3	+	+	3.2	+	n.t.	n.t.	n.t.	96.4	+
28	24.2	+	-	<0.1	-	0.02	-	-	2.3	-
29	120.1	+	+	4.6	+	n.t.	n.t.	n.t.	96.4	+
30	23.3*	+	+	0.6	-	0.22	-	-	32.1	+

Number	Mikrogen <i>recomWell</i> HEV IgM		Mikrogen <i>recomLine</i> HEV IgM	DiaSorin LIAISON® Murex anti-HEV IgM		Wantai HEV-IgM ELISA		RealStar® HEV RT- PCR Kit 2.0	Mikrogen <i>recomWell</i> HEV IgG	
	[U/ml]	qual.	qual.	[Index]	qual.	sample/ cut-off	qual.	RNA	[U/ml]	qual.
31	116.7	+	+	5.9	+	n.t.	n.t.	n.t.	98.4	+
32	21.1*	+	-	0.6	-	0.06	-	-	92.4	+
33	27.3	+	-	1.8	+	n.t.	n.t.	n.t.	51.3	+
34	104.4	+	+	1.6	+	n.t.	n.t.	n.t.	92.4	+
35	33.3	+	+	1.4	+	n.t.	n.t.	n.t.	51.3	+
36	46.3	+	-	1.1	+	n.t.	n.t.	n.t.	97.6	+
37	43.2	+	-	1.0	+	n.t.	n.t.	n.t.	>125	+
38	>125	+	+	8.7	+	n.t.	n.t.	n.t.	27.5	+
39	37.5	+	-	0.8	-	1.66	+	-	>125	+
40	22.3*	+	+	1.8	+	n.t.	n.t.	n.t.	3	-
41	>125	+	+	6.6	+	n.t.	n.t.	n.t.	>125	+
42	>125	+	+	3.0	+	n.t.	n.t.	n.t.	>125	+
43	24.5	+	-	0.8	-	0.02	-	-	63.9	+
44	30.5	+	-	0.6	-	1.18	+	-	101.7	+
45	52.0	+	-	1.1	+	n.t.	n.t.	n.t.	101.7	+
46	>125	+	+	7.0	+	n.t.	n.t.	n.t.	>125	+
47	43.0	+	-	1.4	+	n.t.	n.t.	n.t.	>125	+
48	25.1	+	-	0.7	-	0.68	-	-	85.5	+
49	27.0	+	-	0.7	-	0.02	-	-	49.1	+
50	66.1	+	+	2.2	+	n.t.	n.t.	n.t.	106.7	+
51	31.4	+	-	0.9	-	1.12	+	-	106.7	+
52	>125	+	+	8.2	+	n.t.	n.t.	n.t.	122.7	+
53	>125	+	+	2.2	+	n.t.	n.t.	n.t.	>125	+

Table 3 – Raw data of the HEV antibody negative samples included in the study.

Number	Mikrogen <i>recomWell</i> HEV IgG	DiaSorin LIAISON® Murex anti-HEV IgG	Mikrogen <i>recomWell</i> HEV IgM	DiaSorin LIAISON® Murex anti-HEV IgM
	[U/ml]	[IU/ml]	[U/ml]	[Index]
1	1.9	<0.1	3.1	<0.1
2	1.5	0.2	2.8	<0.1
3	5.2	<0.1	3.5	<0.1
4	9.2	0.3	2.8	0.2
5	1.5	<0.1	3.8	0.1
6	2.6	<0.1	7.8	<0.1
7	3.4	<0.1	4.3	0.1
8	2.5	<0.1	2.2	<0.1
9	2.1	<0.1	3.0	0.4
10	2.1	<0.1	10.6	<0.1
11	2.7	<0.1	2.2	<0.1
12	2.5	<0.1	5.4	<0.1
13	2.2	<0.1	1.5	<0.1
14	10.3	0.1	5.4	0.2
15	2.8	<0.1	2.0	<0.1
16	3.4	<0.1	1.5	<0.1
17	2.9	<0.1	2.5	<0.1
18	1.7	<0.1	7.9	0.3
19	2.6	<0.1	3.2	<0.1
20	1.9	<0.1	2.5	<0.1
21	8.8	0.1	2.7	<0.1
22	1.8	<0.1	1.9	<0.1
23	1.2	<0.1	1.7	<0.1
24	2.0	<0.1	2.5	<0.1
25	2.2	<0.1	1.8	<0.1
26	1.2	<0.1	2.1	<0.1
27	1.3	<0.1	2.0	<0.1
28	2.2	<0.1	2.4	<0.1
29	1.7	<0.1	2.8	<0.1
30	14.6	0.2	2.1	<0.1
31	11.2	0.1	2.7	<0.1
32	12.0	0.2	2.3	<0.1
33	19.2	0.2	1.8	<0.1
34	2.0	<0.1	2.4	<0.1
35	4.5	<0.1	4.6	<0.1
36	9.9	<0.1	8.2	<0.1
37	5.4	<0.1	4.3	<0.1
38	5.1	0.2	2.6	<0.1
39	6.1	<0.1	4.4	<0.1
40	3.6	<0.1	6.7	0.8
41	2.3	<0.1	7.9	0.4
42	3.6	<0.1	5.8	<0.1
43	5.5	<0.1	4.4	0.1
44	3.4	<0.1	2.3	<0.1
45	4.3	<0.1	5.7	<0.1
46	3.2	<0.1	4.3	0.3
47	3.9	<0.1	2.4	<0.1
48	5.0	0.1	5.2	0.1
49	3.1	<0.1	3.4	<0.1

Table 4 – Raw data of the HEV IgG linearity studies. For all three investigated samples individual as well as mean values are given. The cut-off for positive results is 20 U/ml and 0.3 IU/ml for Mikrogen recomWell HEV IgG and DiaSorin LIAISON® Murex anti-HEV IgG, respectively. * These measurements should be quoted as borderline in the recomWell HEV IgG test (≥ 20 U/ml and ≤ 24 U/ml). Some sera dilutions around the cut-off of the recomWell HEV IgG test were also re-analyzed by immunoblot. Abbreviations: +, positive; -, negative; n.t., not tested.

Sample	Dilution	Mikrogen recomWell HEV IgG		Mikrogen recomLine HEV IgG	DiaSorin Murex anti-HEV IgG	
		[U/ml]	Mean [U/ml]		[IU/ml]	Mean [IU/ml]
# 1	1:1	81.9	81.9	n.t.	>10	>10
		81.9			>10	
	1:2	81.9	81.9	n.t.	9.5	>10
		81.9			>10	
	1:4	81.9	81.9	n.t.	4.6	4.6
		81.9			4.6	
	1:8	81.9	81.9	n.t.	2.0	2.0
		81.9			2.0	
	1:16	57.7	56.4	+	1.0	1.0
		55.0			1.0	
	1:32	34.2	34.1	-	0.5	0.5
		34.1			0.5	
	1:64	19.6	18.9	-	0.2	0.2
		18.2			0.2	
# 2	1:1	79.0	79.0	n.t.	>10	>10
		79.0			>10	
	1:2	79.0	79.0	n.t.	4.7	5.1
		79.0			5.5	
	1:4	79.0	79.0	n.t.	2.3	2.3
		79.0			2.3	
	1:8	65.9	66.9	n.t.	1.1	1.2
		67.8			1.2	
	1:16	44.2	42.0	+	0.6	0.6
		39.7			0.6	
	1:32	24.6	23.6*	+	0.3	0.4
		22.5*			0.4	
	1:64	14.0	14.3	n.t.	0.2	0.2
		14.6			0.2	
# 3	1:1	79.0	79.0	n.t.	9.4	9.2
		79.0			9.1	
	1:2	79.0	79.0	n.t.	3.8	3.6
		79.0			3.4	
	1:4	79.0	79.0	n.t.	1.7	1.7
		79.0			1.6	
	1:8	61.4	60.0	n.t.	0.9	0.9
		58.7			0.8	
	1:16	39.0	38.7	+	0.4	0.4
		38.3			0.4	
	1:32	24.6	24.1	-	0.3	0.3
		23.5*			0.3	
	1:64	13.9	13.7	n.t.	0.1	0.2
		13.5			0.2	