SUPPLEMENTAL MATERIAL

Table S1. ROC curve analyses based on testing 816 sera for anti-HEV IgM and IgG.

	Cut-off	Area under ROC curve +/- standard error	95% confidence interval	N reactive (N Single reactive)	N non-reactive (N False negative)
Anti-HEV IgM					
Mikrogen	1.0	0.981 +/- 0.006	0.968- 0.994	35[1] (13[2])	781[5] (10)
DSI	1.0	0.9934 +/- 0.003	0.988- 0.999	46[1] (13[2])	769[5] (1[2])
Euro-immun	1.0	0.983 +/- 0.011	0.962 – 1.00	16[3] (0)	800[7] (18)
Axiom	1.0	0.9149 +/- 0.034	0.848-0.982	21[3] (2)	795[7] (16)
DiaPro	1.0	0.9641 +/- 0.018	0.930-0.998	49[1] (18[2])	767[5] (3)
Anti-HEV IgG					
Mikrogen	1.0	0.9639+/- 0.008	0.949-0.979	138[4] (4)	678[8] (76[9])
DSI	1.0	0.9711+/- 0.008	0.955-0.987	160[4] (4)	656[8] (55[9])

Euro-immune	0.8	0.9554+/- 0.009	0.938-0.973	95[5] (4)	721[1] (117[10])
Axiom	1.0	0.9887+/- 0.005	0.978-0.999	215[6] (11)	601[11] (7)
DiaPro	1.3	0.9969+/- 0.001	0.995-0.999	233[6] (23)	583[11] (1)

[1]11 HEV RNA positive sera; [2] one HEV RNA positive serum; [3]10 HEV RNA positive sera; [4]14 HEV RNA positive sera; [5]15 HEV RNA positive sera; [6]22 HEV RNA positive sera; [7]16 HEV RNA positive sera; [8]12 HEV RNA positive sera; [9]8 HEV RNA positive sera; [10]7 HEV RNA positive sera; [11]4 HEV RNA positive sera

Table S2. Results obtained from analyzing 500 serum samples from blood donors and 361 serum samples from patients for anti-HEV IgM with five assays.

	Blood donor samples (No total positive; % total positive) (N= 500)	Patients with suspected hepatitis E (No total positive; % total positive) (N= 137)	Patients with liver disease (No total positive; % total positive) (N= 156)	Liver transplant recipients (No total positive; % total positive) (N= 23)	TOTAL (No total positive; % total positive) (N= 816)

Anti-HEV IgM

Reactive in five assays	1 (0.2%)	13^1 (9.5%)	0	0	14^1 (2%)
Reactive in four assays only	0 (1; 0.2%)	1^2 (14; 10%)	0	0	1^2 (15; 1.8%)
Reactive in three assays only	1 (2; 0.4%)	6 (20; 15%)	1 (0.6%)	0	8 (23; 2.8%)
Reactive in two assays only	4 (6; 1.2%)	5 (25; 18%)	2 (3; 1.9%)	0	11 (34; 4.2%)
Reactive in one assay only	11^2 (17; 3.4%)	26^3 (51; 37%)	7 (10; 6.4%)	2 (8.7%)	46^4 (80; 9.8%)
Non-reactive in five assays	483^5 (97%)	86^5 (63%)	146^4 (94%)	21^3 (91%)	736^6 (90%)
Concordance in five assays	484 (97%)	98 (71%)	146 (94%)	21 (91%)	749 (87%)
Concordance in four assays	484 (97%)	100 (73%)	146 (94%)	21 (91%)	751 (87%)
Concordance in three assays	485 (97%)	106 (77%)	147 (94%)	21 (91%)	759 (88%)
Concordance in two assays	489 (98%)	111 (81%)	149 (96%)	21 (91%)	770 (89%)

[1] 9 samples positive for HEV RNA; [2] one sample positive for HEV RNA; [3] two samples positive for HEV RNA; [4] 3 samples positive for HEV RNA; [5] 4 samples positive for HEV RNA; [6] 13 samples positive for HEV RNA

Table S3. Results obtained from analyzing 500 serum samples from blood donors and 361 serum samples from patients for anti-HEV IgG with five assays.

	Blood donor samples (No total positive; % total positive) (N= 500)	Patients with suspected hepatitis E (No total positive; % total positive) (N= 137)	Patients with liver disease (No total positive; % total positive) (N= 156)	Liver transplant recipients (No total positive; % total positive) (N= 23)	TOTAL (No total positive; % total positive) (N= 816)
Anti-HEV IgG					
Reactive in five assays	20 (4%)	52[1] (38%)	9[2] (5.8%)	4[2] (17%)	85[3] (10%)
Reactive in four assays only	13[2] (33; 6.6%)	19[2] (71; 52%)	12 (21; 13%)	0 (4; 17%)	44[4] (129; 16%)
Reactive in three assays only	15 (48; 10%)	8 (79; 58%)	2 (23; 15%)	0 (4; 17%)	25 (154; 19%)
Reactive in two assays only	31[5] (79; 16%)	12[4] (91; 66%)	11[2] (34; 22%)	2 (6; 26%)	56[6] (210; 26%)
Reactive in one assay only	27 (106; 21%)	2 (93; 68%)	15 (49; 31%)	3 (9; 39%)	47 (257; 31%)
Non-reactive in five assays	394 (79%)	44[2] (32%)	107[2] (68%)	14[2] (61%)	559[7] (69%)
Concordance in five assays	414 (83%)	96 (70%)	116 (74%)	18 (78%)	644 (80%)
Concordance in four assays	427 (85%)	115 (84%)	128 (82%)	18 (78%)	688 (84%)
Concordance in three assays	442 (88%)	123 (90%)	130 (83%)	18 (78%)	713 (87%)
Concordance in two assays	473 (95%)	135 (99%)	141 (90%)	20 (87%)	769 (94%)

16   [1]12 samples positive for HEV RNA; [2]one sample positive for HEV RNA; [3]14 samples positive for HEV RNA; [4]2samples positive for HEV RNA; [5]4 samples positive

17   for HEV RNA; [6]7 samples positive for HEV RNA; [7]3 samples positive for HEV RNA

Table S4. Results with indicated assay given as sample OD/cut-off OD from testing for anti-HEV IgM and IgG of consecutive sera according to time of sampling for 27 patients

Patient no	Period between samples	Mikrogen IgM/IgG	DSI IgM/IgG	Euro-immun IgM/IgG	Axiom IgM/IgG	DiaPro IgM/IgG	HEV RNA/ genotype (gt)
1	-	**8.6/12.5**	**10.2/8.4**	**7.7/4.6**	**13.0/14.5**	**15.0/11.0**	+ /gt3
	4 months	**5.1/7.7**	**8.6/10.9**	**6.2/4.0**	**10.1/27.5**	**12.8/9.1**	+
	5 months	**3.7/8.2**	**8.4/11.1**	**5.6/4.2**	**8.6/26.7**	**12.3/9.0**	-
	7 months	**2.9/7.7**	**8.0/10.8**	**4.1/3.5**	**5.7/27.1**	**11.9/8.9**	-
2	-	**9.8/14.8**	**11.4/11.6**	**9.7/4.3**	**12.6/20.5**	**13.5/9.1**	+/ gt1
	3 weeks	**4.2/8.6**	**10.9/11.6**	**10.0/5.0**	**12.9/22.2**	**12.8/9.0**	+
6	-	**4.1/2.2**	**10.8**/0.7	**9.5/1.2**	**4.3/6.3**	**12.9/4.4**	+ /gt3
	1 week	**6.6/2.3**	**10.5**/0.8	**6.1/1.2**	**4.3/5.5**	**12.9/4.2**	+
9	-	**9.7/10.7**	**10.3/9.6**	**7.6/3.8**	**8.5/15.8**	**12.6/8.9**	+ /gt3
	2 weeks	**10.0/2.3**	**8.9/1.9**	**5.4/1.1**	**6.3/10.8**	**12.2/4.3**	+
10	-	0.4/0.2	0.0/0.0	0.0/0.1	0.0/0.0	0.2/0.4	-
	4 years	**2.7/12.2**	**2.8/11.3**	**1.2/4.6**	**1.9/28**	**9.4/8.8**	+/gt3
13	-	**2.4/12.0**	**5.2/10.3**	**1.3/4.1**	**8.4/27.1**	**6.1/8.9**	+/gt3
	2 months	0.8/**12.0**	0.6/**10.9**	0.1/**3.1**	0.7/**28.0**	1.7/8.7	+

21	-	**1.4**/0.8	**5.3**/0.5	0.4/0.3	0.2/**14.6**	**2.3**/**5.5**	-
	3 weeks	**1.6**/**1.1**	**5.9**/0.4	0.4/0.3	0.2/**13.0**	**2.5**/**4.9**	-
23	-	**1.1**/**1.5**	**2.5**/**5.0**	0.2/0.2	**3.7**/**28.0**	**2.1**/**6.8**	-
	5 months	0.4/**1.1**	0.9/**4.0**	0.3/0.4	**2.0**/**28.0**	0.5/**6.0**	-
25	-	**1.1**/**1.1**	**2.3**/**1.6**	0.3/0.4	0.0/**28.0**	**1.6**/**6.5**	-
	1 month	0.8/**1.4**	**2.8**/**1.8**	0.2/0.5	0.0/**27.8**	**1.8**/**7.2**	-
29	0	0.8/5.6	0.1/8.9	0.1/1.6	0.1/28.2	1.5/8.7	-
	2 years	0.8/6.6	0.2/9.3	0.1/1.9	0.0/28.0	1.4/9.1	-
30	-	0.6/**2.7**	0.8/**9.5**	0.2/**1.0**	0.2/**28.0**	1.3/**8.3**	-
	2 weeks	**1.1**/**3.8**	**1.1**/**10.3**	0.2/**1.2**	0.1/**28.0**	**1.5**/**8.5**	-
36	-	0.3/**2.4**	0.3/**11.0**	0.1/**1.0**	0.0/**28.0**	**1.1**/**8.6**	+
	1 week	0.2/**3.2**	0.3/**10.7**	0.1/**1.0**	0.0/**28.0**	0.9/**9.1**	+
41	-	**2.4**/**1.4**	**1.2**/**2.4**	0.2/0.6	0.1/**28.0**	**1.0**/**7.7**	-
	18 months	0.6/**1.4**	**1.3**/**2.2**	0.3/0.5	0.1/**28.0**	0.9/**8.1**	-
47	-	0.1/**2.0**	0.0/**5.8**	0.0/**1.5**	0.0/**28.0**	0.8/**8.8**	+
	2 months	0.2/**2.7**	0.2/**4.4**	0.0/**1.7**	0.0/**27.1**	0.7/**8.5**	-

	6 months	0.2/**1.7**	0.1/**3.9**	0.0/**1.2**	0.0/**27.2**	0.6/**7.8**	-
	12 months	0.1/**1.5**	0.0/**3.6**	0.0/**1.1**	0.0/**27.2**	0.4/**7.5**	-
51	-	0.3/**5.2**	0.4/**8.5**	0.1/**1.3**	0.0/**28.0**	0.7/**8.3**	-
	5 years	0.7/**3.1**	0.2/**6.0**	0.0/**1.4**	0.0/**28.0**	0.3/**8.8**	-
57	-	1.3/**7.4**	0.5/**10.8**	0.1/**3.5**	0.0/**28.0**	0.4/**9.0**	-
	1 months	0.7/**5.8**	**1.7**/**8.3**	0.1/**2.4**	0.0/**28.0**	0.5/**8.9**	-
	3 months	**1.0**/**6.2**	0.2/**8.9**	0.2/**2.2**	0.1/**27.3**	0.6/**9.0**	-
75	-	0.1/**2.0**	0.0/**6.3**	0.0/**1.0**	0.0/**28.0**	0.2/**8.5**	-
	8 months	0.2/**2.4**	0.0/**8.6**	0.1/**1.0**	0.0/**27.7**	0.4/**8.3**	-
79	-	0.2/**1.6**	0.0/**6.6**	0.0/0.7	0.1/**28.0**	0.4/**8.7**	-
	13 months	0.2/**2.1**	0.0/**4.5**	0.0/**0.9**	0.1/**28.0**	0.3/**8.4**	-
84	-	0.2/0.9	0.0/**1.9**	0.1/0.4	0.0/**27.8**	0.2/**7.8**	-
	14 months	0.2/**2.9**	0.0/**3.8**	0.0/0.6	0.0/**27.7**	0.3/**8.7**	-
100	-	0.2/**2.4**	0.7/**7.2**	0.1/**1.1**	0.0/**28.0**	0.3/**8.8**	-
	19 months	0.1/**1.8**	0.0/**6.1**	0.1/**0.9**	0.0/**28.0**	0.3/**8.7**	-
	23 months	0.2/**3.0**	0.2/**8.6**	0.0/**1.4**	0.0/**28.0**	0.2/**8.7**	-
	28 months	0.1/**4.0**	0.1/**10.1**	0.0/**1.4**	0.0/**26.9**	0.3/**8.8**	-
104	-	0.2/0.5	0.0/0.9	0.0/0.3	0.0/**11.2**	0.3/**2.6**	+

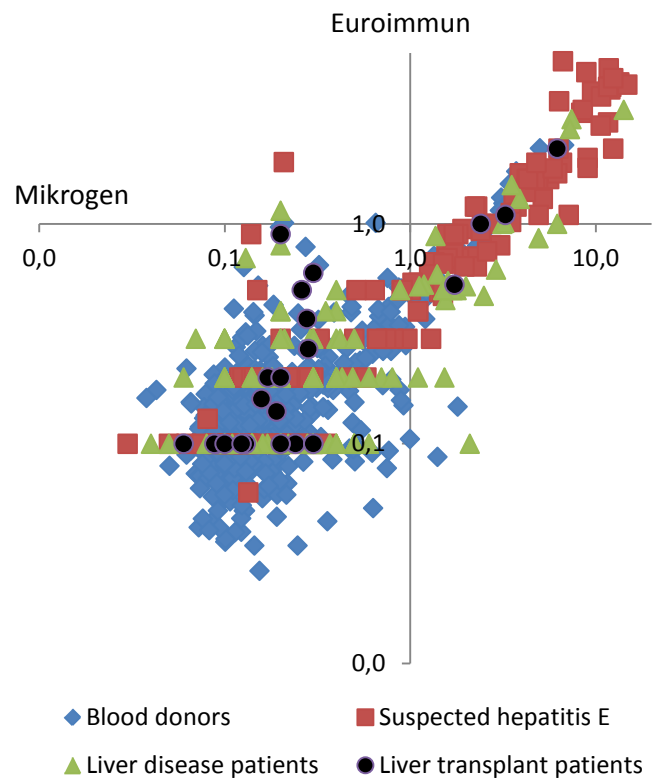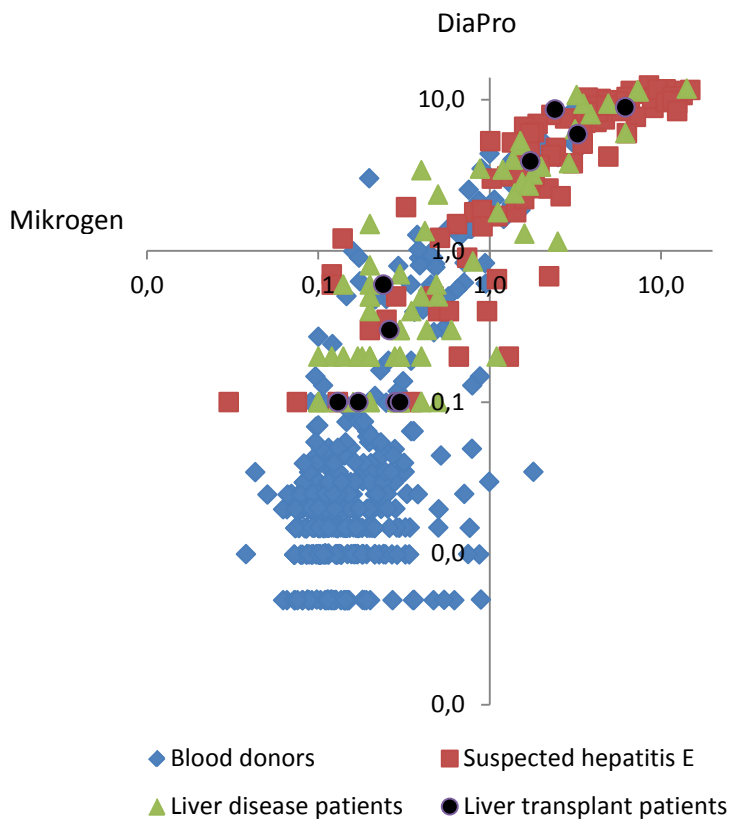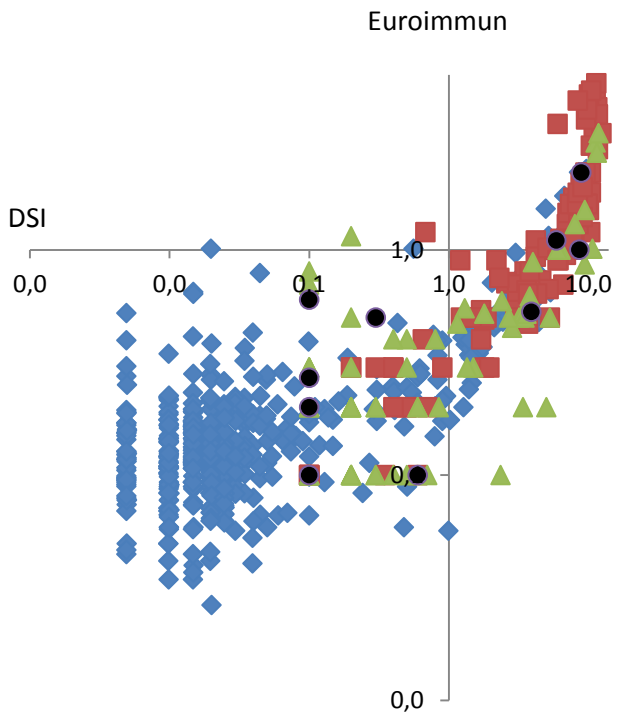|							
	3 months	0.2/0.3	0.0/0.3	0.0/0.2	0.0/**7.8**	0.2/**1.7**	+
	6 months	0.3/0.6	0.0/0.3	0.0/0.2	0.0/**6.3**	0.3/**1.7**	-
111	-	0.4/0.2	**1.3**/0.0	0.0/0.1	0.0/0.1	0.3/0.9	-
	3 months	0.9/0.2	0.1/0.0	0.1/0.1	0.1/0.1	0.2/0.9	-
115	-	**2.1**/0.1	0.0/0.0	0.1/0.1	0.1/0.8	0.2/0.3	-
	2 weeks	**2.2**/0.1	0.0/0.0	0.0/0.1	0.2/0.4	0.3/0.3	-
117	-	0.1/**1.8**	0.0/**2.6**	0.0/0.6	0.0/**27.5**	0.2/**8.4**	-
	2 weeks	0.2/**2.6**	0.0/**2.3**	0.0/**0.8**	0.0/**27.4**	0.2/**9.0**	-
132	-	0.3/**1.3**	0.2/0.2	0.1/0.3	0.0/**27.8**	0.2/**8.8**	-
	3 weeks	0.3/0.8	0.2/0.9	0.1/0.4	0.0/**28.0**	0.1/**8.8**	-
134	-	0.5/**4.4**	0.0/**9.2**	0.0/**1.2**	0.0/**28.0**	0.2/**8.6**	+
	1 month	**1.1**/**4.8**	0.0/**9.1**	0.0/**1.9**	0.0/**28.0**	0.2/**8.5**	-
180	-	0.1/**1.0**	0.0/**3.0**	0.0/0.5	0.0/**27.8**	0.2/**6.9**	-
	1 year	0.1/**1.2**	0.0/**2.2**	0.0/0.4	0.0/**27.5**	0.1/**6.7**	-

Supplemental figure legends

Figure S1 a-j. Log[OD values/cutoff values] for each of the 861 sera with two assays being compared in each graph. Sera represented in the lower left quadrant are negative in both assays, those in the upper right quadrant are positive with both assays. Sera represented in the lower right quadrant are positive with the assay indicated on the X-axis, and negative in the assay on the Y-axis. Those in the upper left quadrant are negative with the assay indicated on the X-axis, and positive with the assay indicated on the Y-axis.

Figure S2. Regression analysis of log[OD values of dilutions/cut-off value of the assay] in relation to log[dilution] for determination of end-point titers for anti-HEV IgM in WHO reference reagent NIBSC code: 94/584 with the indicated assay.

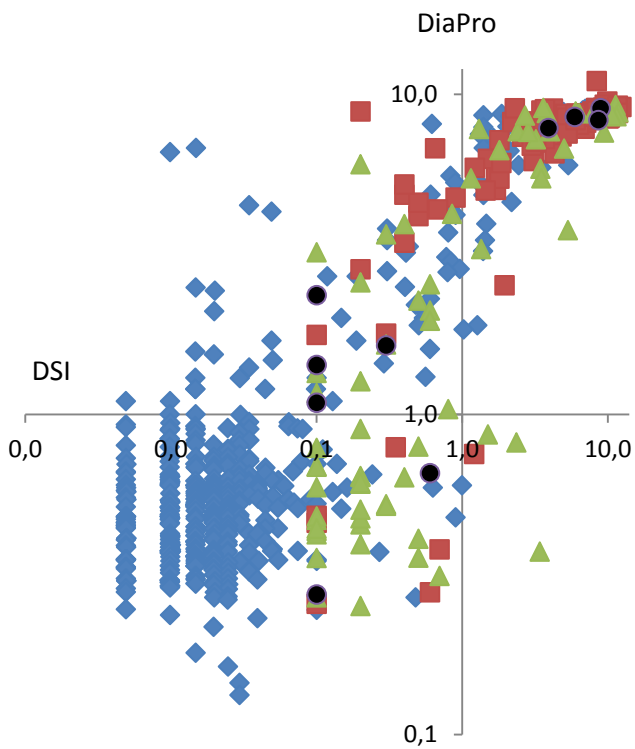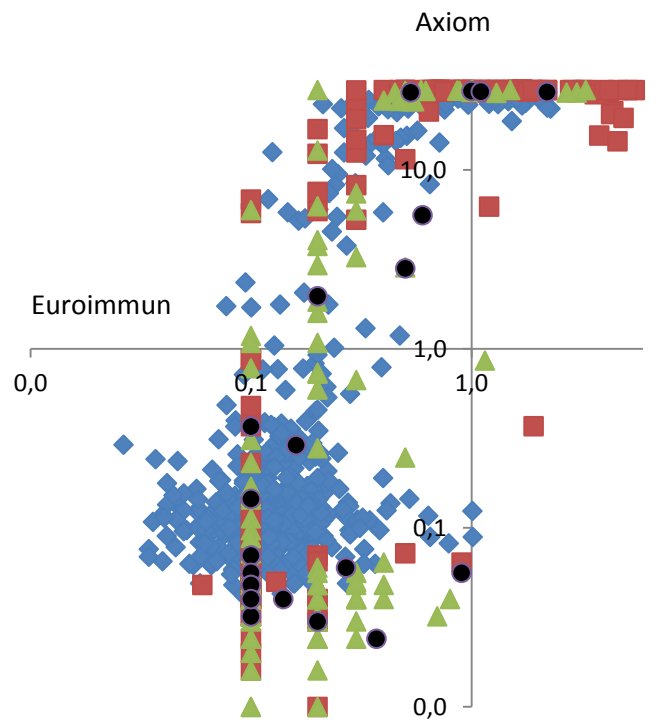Figure S3. Age distribution of blood donors and patients analysed for anti-HEV IgM, IgG and HEV RNA.

DiaPro

Mikrogen

- ◆ Blood donors
- ■ Suspected hepatitis E
- ▲ Liver disease patients
- ● Liver transplant patients

Euroimmun

Mikrogen

- ◆ Blood donors
- ■ Suspected hepatitis E
- ▲ Liver disease patients
- ● Liver transplant patients

Axiom

Mikrogen

- ◆ Blood donors
- ■ Suspected heptatitis E
- ▲ Liver disease patients
- ● Liver transplant patients

DiaPro

Mikrogen

- ◆ Blood donors
- ■ Suspected hepatitis E
- ▲ Liver Disease patients
- ● Liver transplant patients

Euroimmun

DSI

Axiom

DSI

DiaPro

DSI

Axiom

Euroimmun
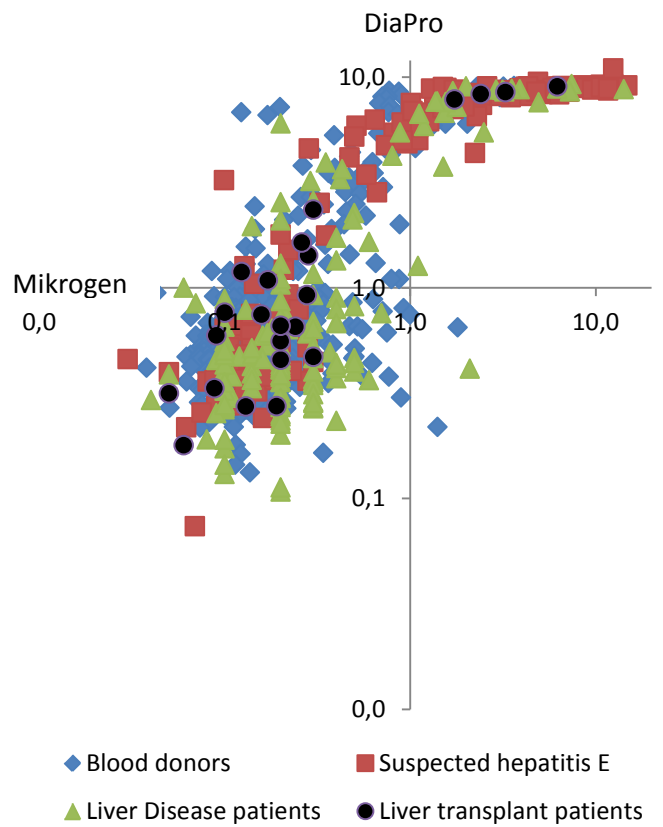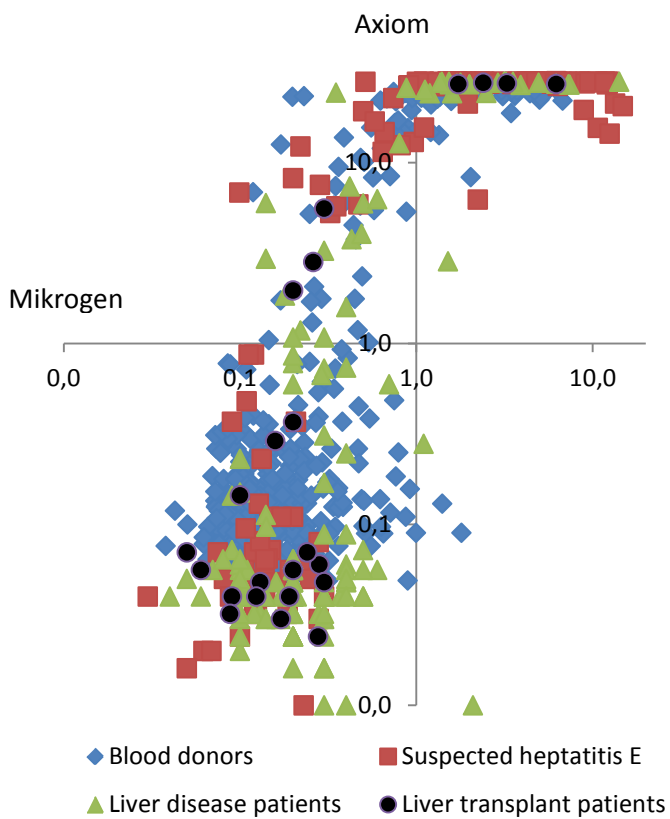
Blood donors
Suspected hepatitis E
Liver disease patients
Liver transplant patients
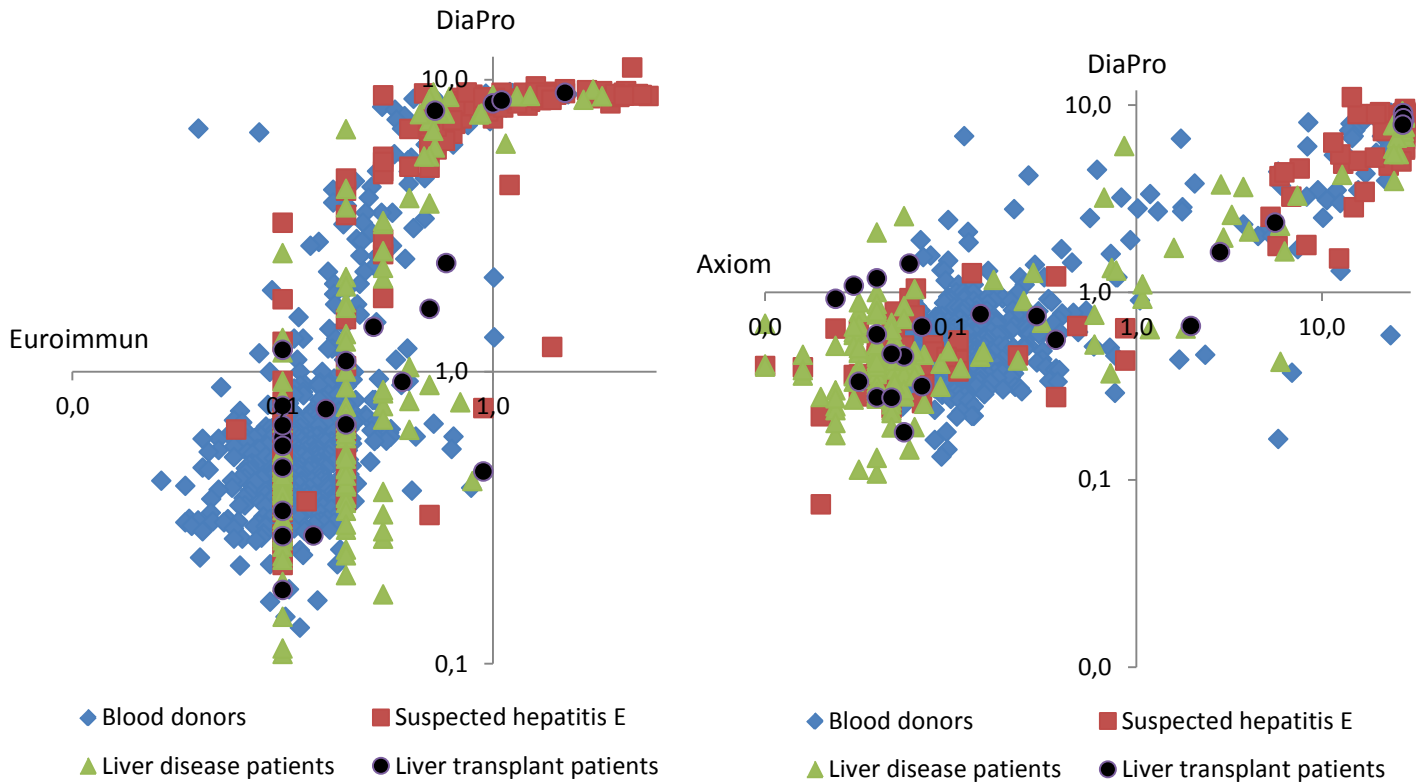
Figure S1 a-j. Log[OD values/cutoff values] for each of the 861 sera with two assays being compared in each graph. Sera represented in the lower left quadrant are negative in both assays, those in the upper right quadrant are positive with both assays. Sera represented in the lower right quadrant are positive with the assay indicated on the X-axis, and negative in the assay on the Y-axis. Those in the upper left quadrant are negative with the assay indicated on the X-axis, and positive with the assay indicated on the Y-axis.
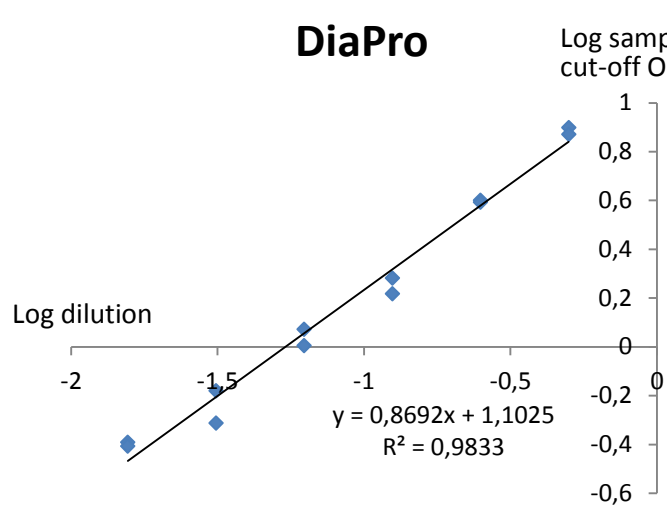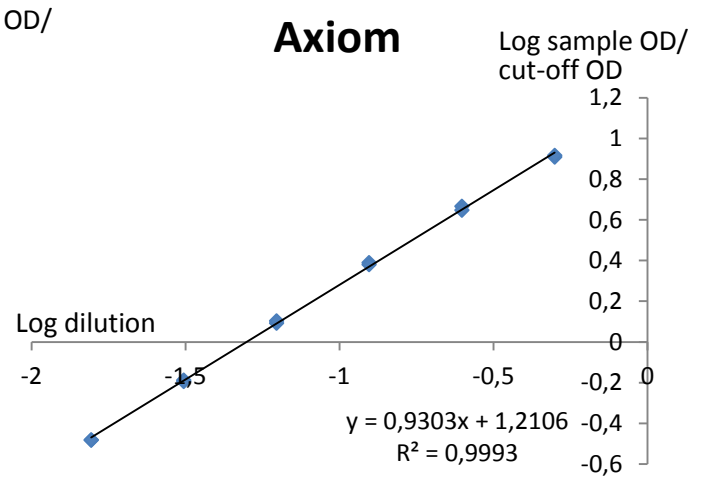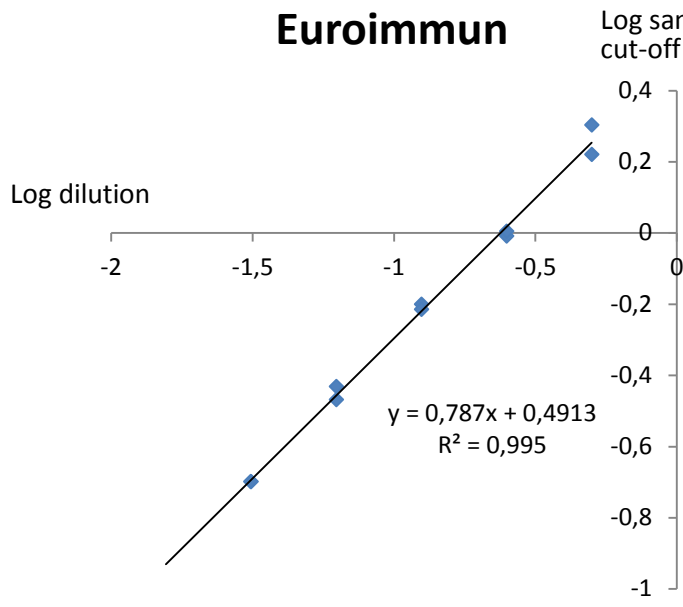
Mikrogen

Log sample OD/ cut-off OD

Log dilution

y = 0,6454x + 0,5114
R² = 0,9817

DSI

Log sample OD/ cut-off OD

Log dilution

y = 1,2511x + 1,3763
R² = 0,941

Euroimmun

Log sample OD/ cut-off OD

Log dilution

y = 0,787x + 0,4913
R² = 0,995

Axiom

Log sample OD/ cut-off OD

Log dilution

y = 0,9303x + 1,2106
R² = 0,9993

DiaPro

Log sample OD/ cut-off OD
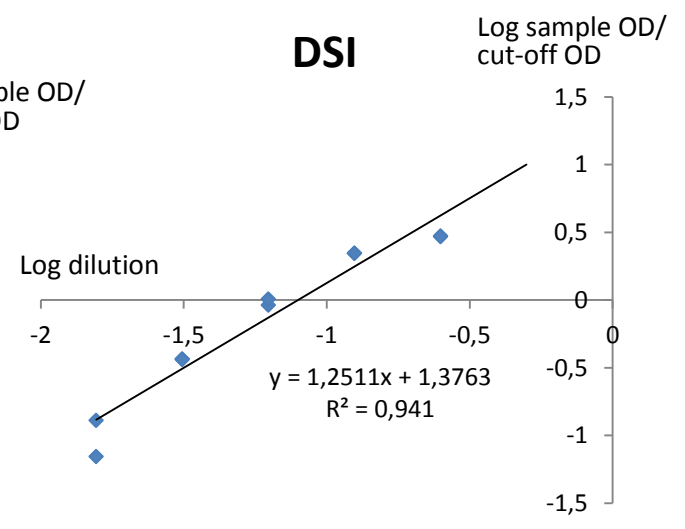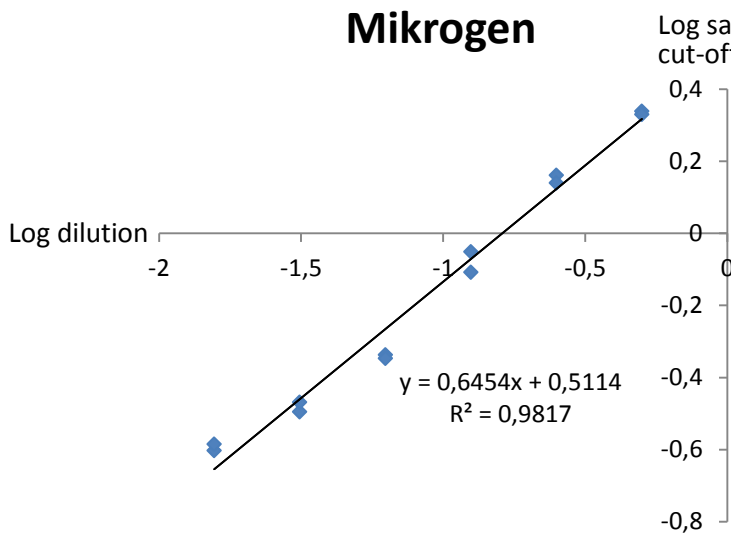
Log dilution

y = 0,8692x + 1,1025
R² = 0,9833

Figure S2. Regression analysis of log[OD values of dilutions/cut-off value of the assay] in relation to log[dilution] for determination of end-point titers for anti-HEV IgM in WHO reference reagent NIBSC code: 94/584 with the indicated assay.
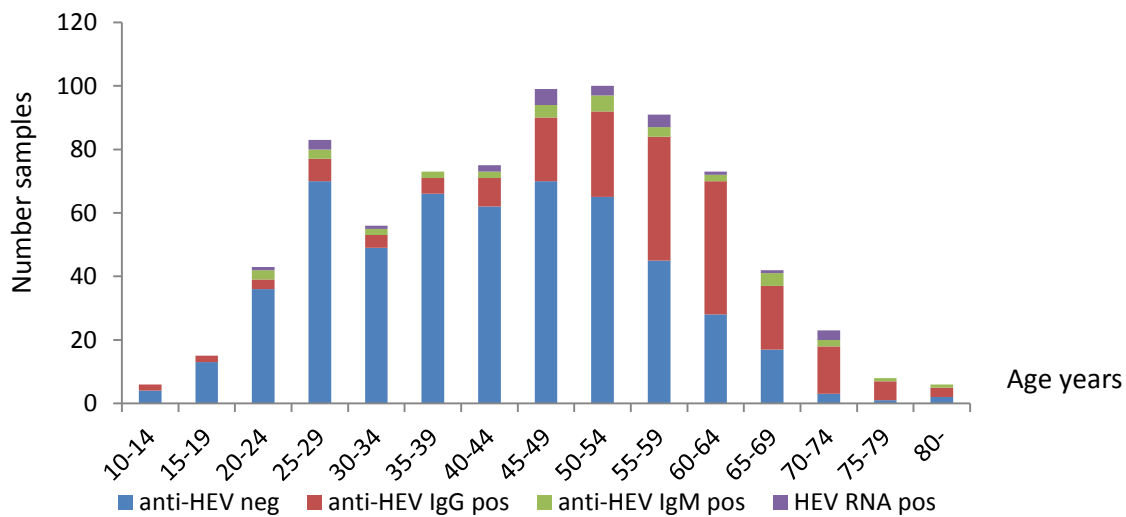

Figure S3. Age distribution of blood donors and patients analysed for anti-HEV IgM, IgG and HEV RNA.