

Are hepatitis E virus (HEV) ELISAs suitable for screening and monitoring infection?

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Introduction

Hepatitis E virus (HEV), a non-enveloped, single-stranded positive-sense RNA virus classified in the family *Hepeviridae*, is a major enterically transmitted pathogen causing self-limiting, acute hepatitis.

Generally, HEV-specific immunoglobulin M (IgM) and G (IgG) -antibodies are detectable at the onset of disease. The titres of IgM decline rapidly during early convalescence while IgG responses have been shown to persist for different periods of time (1, 2). However, commercial ELISA test systems for the detection of anti-HEV antibodies seem to reflect this course of immune response differently and also vary considerably in their sensitivity and specificity.

In the present study, we evaluated two currently available commercial enzyme-linked immunosorbent assays (ELISAs) from MP Diagnostics (formerly Genelabs Diagnostics), Singapore (MPD) and from Mikrogen, Germany (MK) with regard to their suitability for screening and monitoring HEV infection.

Materials & Methods

Blood samples: a) Blood samples from 14 patients with acute hepatitis E confirmed by the presence of HEV RNA in sera or stools by RT-PCR according to Inoue et al. (3); b) Clinical follow-up samples from 4 of the patients over different time-periods; c) 13 blood samples IgM positive in MPD HEV IgM ELISA-screening; d) 86 blood samples from patients with antibodies against hepatitis A, B, C virus or Cytomegalovirus (CMV) (and/or) respectively; e) 200 samples from blood donors (Bavarian Red Cross). **ELISAs:** MP Diagnostics (MPD) HEV ELISA (IgG) and MPD HEV IgM ELISA from MP Biomedicals, Singapore. *recomWell* HEV IgG and *recomWell* HEV IgM from Mikrogen (MK), Germany.

Results

Sensitivity of ELISAs in blood samples from patients with acute hepatitis E

Blood samples from 14 patients with acute hepatitis E were tested with MPD- and MK-ELISA. Sensitivities of the ELISAs relative to RT-PCR were 100% / 100% for IgG and 78.6% / 100% for IgM with MPD / MK, respectively (Tab. 1). 13 RT-PCR products were sequenced, showing genotype 1 in six and genotype 3 in seven cases.

Tab. 1 - Sensitivity of HEV-ELISAs compared to RT-PCR

	Mikrogen <i>recomWell</i> HEV IgG (n=14)	Mikrogen <i>recomWell</i> HEV IgM (n=14)	MP Diagnostics HEV ELISA IgG (n=13)	MP Diagnostics HEV ELISA IgM (n=14)
negative	0	0	0	3 ^{a)}
borderline	1	0	/	/
positive	13	14	13	11
sensitivity (%)	100.0	100.0	100.0	78.6

a) genotype 3 according to sequence
/ no borderline results in this test

Sera from patients with antibodies against hepatitis A, B, C virus or CMV

Additional 13 samples that were positive for IgM-antibodies in MPD-ELISA-screening but negative by RT-PCR were also tested by MK-ELISA. Only 4 of 13 (30.8%) were confirmed (data not shown). In order to check, if this discrepancy is due to differences in specificity, we tested 86 blood samples with antibodies against hepatitis A, B, C virus or CMV and found 7.0% / 10.5% reactive in IgG and 3.5% / 1.2% in IgM with MPD / MK, respectively (Tab. 2A).

Tab. 2A - Reactivity rate of HEV ELISAs in blood samples with antibodies against hepatitis A, B, C virus or CMV

(n=86)	Mikrogen <i>recomWell</i> HEV IgG	Mikrogen <i>recomWell</i> HEV IgM	MP Diagnostics HEV ELISA IgG	MP Diagnostics HEV ELISA IgM
negative	77	85	80	83
borderline	1	0	/	/
positive	8	1	6	3
reactivity rate (%)	10.5	1.2	7.0	3.5

Tab. 2B - Reactivity rate of HEV ELISAs in samples from blood donors

(n=200)	Mikrogen <i>recomWell</i> HEV IgG	Mikrogen <i>recomWell</i> HEV IgM	MP Diagnostics HEV ELISA IgG	MP Diagnostics HEV ELISA IgM
negative	176	198	192	193
borderline	5	1	/	/
positive	19	1	8	7
reactivity rate (%)	12.0	1.0	4.0	3.5

/ no borderline results in this test

The values correspond to test results of 200 samples from blood donors, where the rate of seroprevalence tested with MPD / MK- ELISA was 4.0% / 12.0% for IgG- and 3.5% / 1.0% for IgM-antibodies (Tab 2B).

Course of HEV antibody response

Clinical follow-up samples were collected from 4 of the patients from Tab. 1 over different time-periods of 21 to 520 days. The course of IgM- and IgG antibody-response was reflected differently by the two ELISAs, with IgG antibody response decreasing with time in MPD-ELISA and increasing in MK-ELISA (Fig. 1).

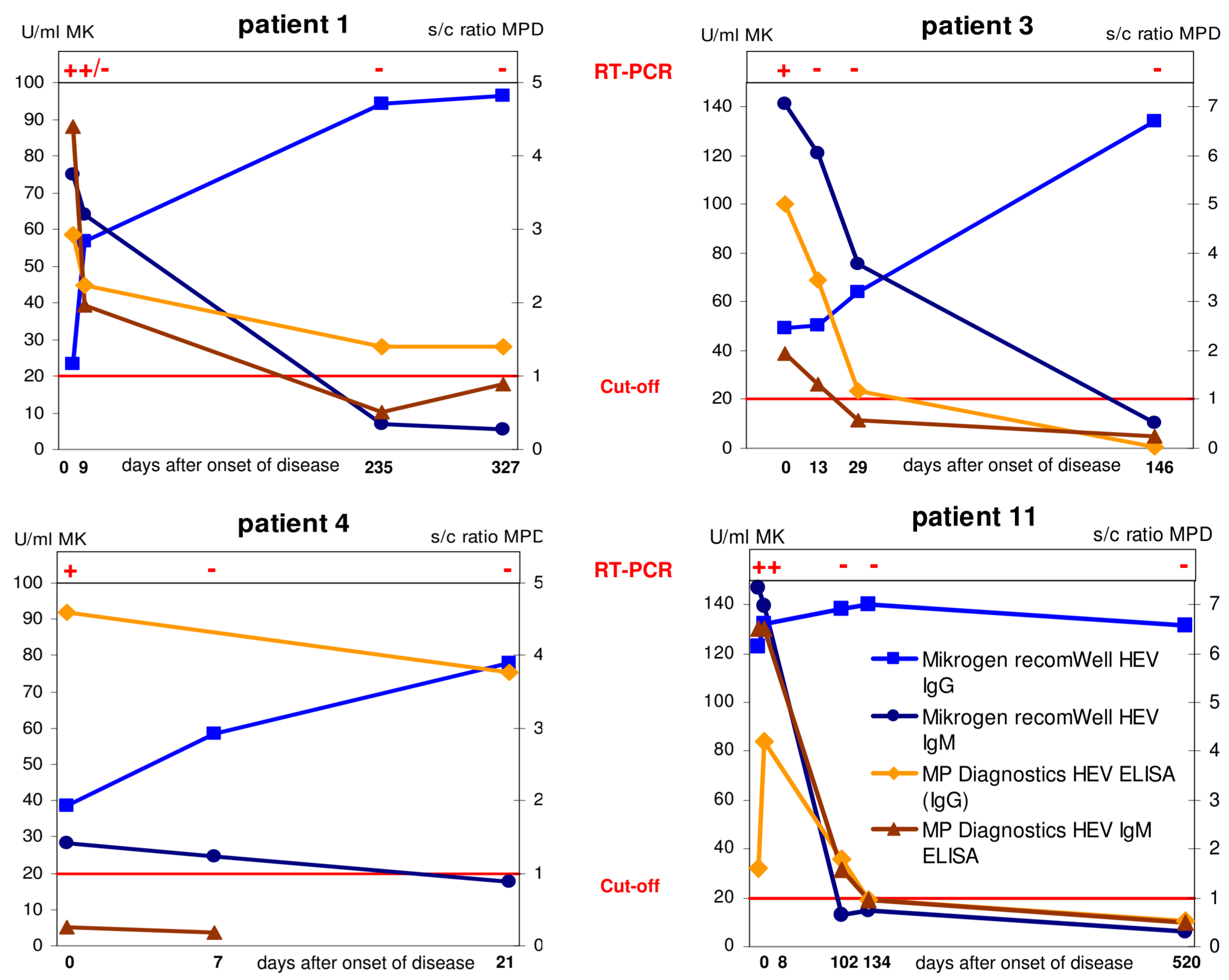


Fig. 1 - Course of anti-HEV immune response (IgG/IgM) of 4 patients with acute HEV infection according to ELISAs from Mikrogen (MK), Germany and MP Diagnostics (MPD), Singapore. Cut-off: red line; RT-PCR results indicated in red.

Discussion

Testing samples from patients with acute HEV, the MK-ELISA shows a higher sensitivity for IgM-antibodies (Tab. 1). This finding is confirmed by the examination of follow-up sera, where IgM-antibody values of MPD-ELISA decrease under cut-off earlier than MK IgM ELISA values (Fig. 1).

Additionally, because of the early degeneration of IgG-antibody values in MPD-ELISA, a passed HEV-infection will not be detectable shortly after onset of disease, while MK ELISA still detects IgG antibodies after up to 520 days (Fig. 1).

The reactivity rates of both ELISAs in samples from blood donors correspond to those from patients with antibodies against hepatitis A, B, C virus or CMV (Tab. 2), indicating that there is no significant cross-reactivity with antibodies against these viruses. But the high reactivity-rate of IgM-antibodies by MPD-ELISA (Tab. 2) points to unspecific results in MPD HEV IgM ELISA. MPD-ELISA antibody reactivity rates of 3.5% (IgM) and 4.0% (IgG) in blood donors seem to be inconsistent. This discrepancy could also be due to the short-term traceability of IgG-antibodies by MPD-ELISA (Fig. 1).

Conclusions

- **MK *recomWell* HEV IgM seems to be more sensitive and more specific than MPD HEV IgM ELISA**
- **Anti-HEV IgG-antibodies are detectable over a much longer time period by MK *recomWell* HEV IgG than by MPD HEV ELISA**
- **Seroprevalence in blood donors (Bavarian Red Cross) of 12% (MK *recomWell* HEV IgG) seems to be more plausible than 4% (MPD HEV ELISA)**

References

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