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Roche

Elecsys® Anti-HEV IgM

Immunoassay for the qualitative detection of IgM antibodies to the hepatitis E virus (HEV) in human serum and plasma

Summary

HEV is the etiological agent of hepatitis E and is regarded as an emerging pathogen of global public health concern.[1,2] HEV, which is classified as species *Paslahepevirus balayani* in the family Hepeviridae, genus *Paslahepevirus*, includes 8 genotypes, of which HEV 1-4 are the most frequently detected globally. HEV-1 and HEV-2 infect only humans, whereas HEV-3 and HEV-4 infect humans but also several animal species such as pig, boar, rabbit and deer.[3-9] It is estimated that HEV-1 and HEV-2 account for approximately 20.1 million HEV infections, 3.4 million symptomatic cases, 70,000 deaths, and 3000 stillbirths annually.[10,11]

HEV is an icosahedral, non-enveloped, positive-sense, single-stranded RNA virus with a diameter of 27-34 nm. The RNA genome of 7.2 kb has 3 open reading frames (ORFs): ORF1, encoding non-structural proteins involved in viral replication; ORF2, encoding the viral capsid protein important for virion assembly and immunogenicity; and ORF3, encoding a protein essential for virus release.[1,3,12-16]

HEV-1 and HEV-2 are commonly found in developing countries with poor sanitation,[1,4,17] and they are typically transmitted through the fecal-oral route. HEV-3 and HEV-4 are prevalent in both developing and developed nations and they are transmitted zoonotically.[1,13,18] In several countries, occasional HEV-3 transmission through blood transfusion has been reported.[1,12,19]

HEV infection usually causes a mild or subclinical infection with a self-limiting illness that lasts from 2 to 6 weeks.[20,21] Symptomatic hepatitis E is similar to other acute hepatitis infections (fatigue, nausea, vomiting as well as jaundice and elevated liver enzymes).[20] High-risk populations are immunocompromised patients,[16,20,22,23] patients with underlying liver conditions, and elderly people and pregnant women.[3,12,14,24-26] In pregnant women, HEV-1 infection may lead to severe clinical outcomes with a mortality rate of up to 30%.[5,14,20,27,24,25] Vertical transmission from mother to fetus can cause premature birth and perinatal mortality.[28-31] Acute and chronic HEV-3 and HEV-4 infections have also been associated with extra-hepatic manifestations, especially neurological and renal disorders.[16,20,26,31]

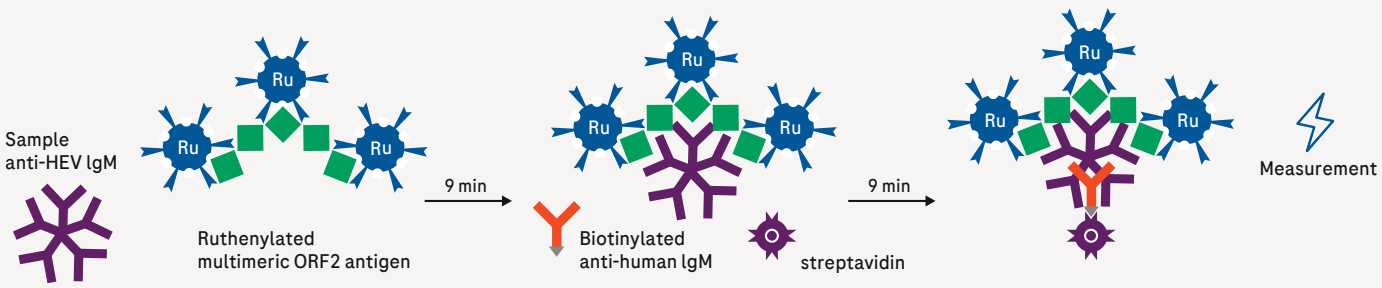
Around 3 weeks post-infection, HEV RNA becomes detectable in blood and stool, with viremia lasting approximately 3-6 weeks, and shedding of virus in stool for approximately 4-6 weeks.[31] IgM antibodies against the HEV capsid protein are detectable in serum after 1-4 weeks for up to 6-9 months post infection, and are a key marker of recent or current infection.[3,12,16,25,26,31,32] Anti-HEV IgG antibodies appear around the same time or soon after anti-HEV IgM antibodies. They are an indicator of recent and past infection and usually persist for several years.[12,25,26,31,33]

Testing for hepatitis E is recommended in all patients presenting symptoms consistent with acute hepatitis, patients with unexplained flares of chronic hepatitis, in all immunosuppressed patients with unexplained abnormal liver function tests, and in case of suspected drug-induced liver injury (DILI).[16,31] In pregnant women, antenatal screening for HEV antibodies should be considered.[34] Acute HEV infection can be diagnosed by the detection of anti-HEV antibodies (IgG, IgM, or both) in serum or plasma, in combination with testing for HEV RNA. Serological testing alone relies upon the combined detection of anti-HEV IgM and rising anti-HEV IgG titers.[31] Past infection is determined by the presence of anti-HEV IgG.[20,31,35,36]

The Elecsys® Anti-HEV IgM assay uses recombinant proteins based on structural domains of HEV ORF2 (genotype 1 and 3) as antigens in a μ-capture assay format for the qualitative detection of IgM antibodies to HEV. Qualitative measurement of IgM antibodies to HEV is intended as an aid, in conjunction with other laboratory results and clinical information, in the diagnosis of acute HEV infection (e.g., by detecting anti-HEV IgM antibodies during acute infection, in combination with rising titers of IgG antibodies to HEV or HEV RNA), as part of the differential diagnosis of acute hepatitis to enable timely initiation of medical interventions. Testing for HEV infection, including anti-HEV IgM antibodies, is also indicated in pregnant women.

Electrochemiluminiscence immunoassay (ECLIA)[37]

Test principle: µ-capture assay (testing time: 18 mins)



1st incubation (9 minutes)
10 µL* / 6 µL** of sample are automatically prediluted 1:20 with Diluent Universal. HEV specific recombinant antigen labeled with a ruthenium complex is added. Anti-HEV IgM antibodies present in the sample bind to the ruthenium-labeled HEV specific recombinant antigen.

2nd incubation (9 minutes)
After addition of monoclonal biotinylated human-IgM-specific antibodies and streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Measurement
The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

* on **cobas**® e 411 analyzer and **cobas** e 601 / 602 modules ** on **cobas** e 402 and **cobas** e 801 analytical modules

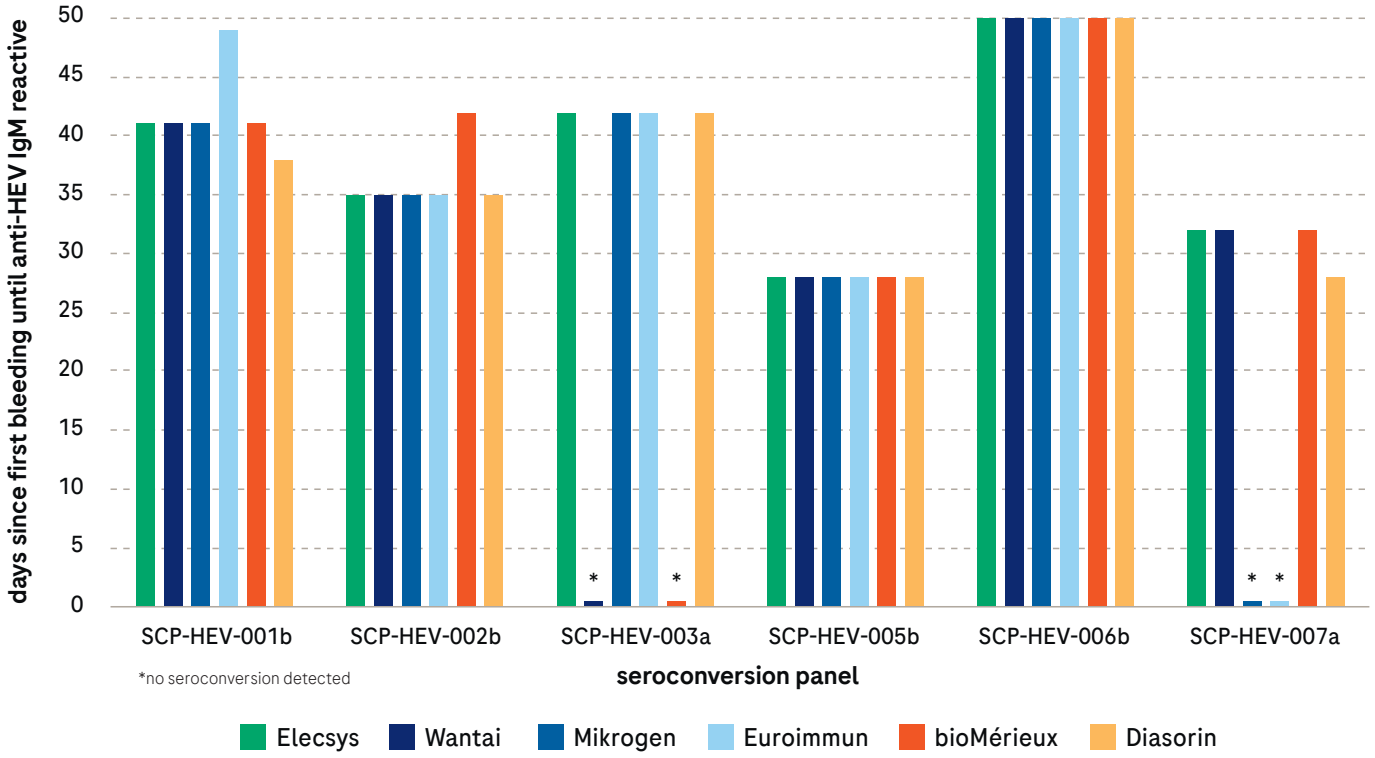
Elecsys Anti-HEV IgM assay characteristics[37]

Systems	**cobas**® e 411 analyzer **cobas** e 601 / **cobas** e 602 modules	**cobas** e 402 analytic module **cobas** e 801 analytic module
Testing time	18 minutes	
Test principle	µ-capture assay, qualitative	
Calibration	Individual 2-point calibration	
Traceability	Roche reference standard	
Interpretation	COI* < 1.0 = non-reactive for anti-HEV IgM COI ≥ 1.0 = reactive for anti-HEV IgM	
Specimen types	Serum collected using standard sampling tubes or tubes containing separating gel. Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA and Na-citrate plasma. Plasma tubes containing separating gel can be used.	
Sample volume	10 µL	6 µL
Onboard stability	4 weeks	16 weeks
Intermediate precision in positive samples	**cobas** e 411: CV** 2.4 – 3.0 % **cobas** e 601/602: CV 5.9 – 6.8 %	CV 5.1 – 6.1 %

* cutoff index; ** coefficient of variation

Seroconversion sensitivity[37]

Seroconversion sensitivity of the Elecsys Anti-HEV IgM assay was shown by testing 6 commercial seroconversion panels in comparison to 5 other registered anti-HEV IgG assays. The Elecsys Anti-HEV IgM assay detected anti-HEV IgM in 36 out of a total of 88 panel members, while the comparison assays detected 27 (one panel not detected, bioMérieux VIDAS Anti-HEV IgM), 29 (one panel not detected, Wantai HEV IgM ELISA), 30 (+ 3 borderline, one panel not detected, Euroimmun Anti-HEV ELISA IgM), 35 (one panel not detected, Mikrogen recomWell HEV IgM), and 41 (DiaSorin Liaison Murex Anti-HEV IgM), respectively.



Relative sensitivity[37]

A total of 657 samples (440 samples from patients with presumed acute HEV infection and 217 HEV RNA positive samples) were tested with the Elecsys Anti-HEV IgM assay and 3 commercially available anti-HEV IgM assays at 3 different study sites. Samples from patients with presumed acute hepatitis E infection included 252 samples from Europe (endemic for HEV genotype 3) and 188 samples from Vietnam and Bangladesh (endemic for HEV genotype 1). Additionally, 50 samples (confirmed genotype 4) were measured at one study site in China with the Elecsys Anti-HEV IgM assay and 3 anti-HEV IgM assays commercially available in China. Samples were considered positive if the result was reactive in all of the comparator assays.

Cohort	N	Confirmed positive samples*	Elecsys Anti-HEV IgM congruent positive	Sensitivity (95 % CI)
Presumed acute*	440	359	354	98.6 % (96.8 – 99.5 %)
Presumed acute**	50	49	49	100 % (92.7 – 100 %)
HEV RNA positive	217	69	68	98.6 % (92.2 – 100 %)
Overall	**707**	**477**	**471**	**98.7 % (97.3 – 99.5 %)**

* included 252 samples from Europe (endemic for HEV-3) and 188 samples from Vietnam and Bangladesh (endemic for HEV-1);
** from China (confirmed genotype 4); CI: confidence interval, 2-sided

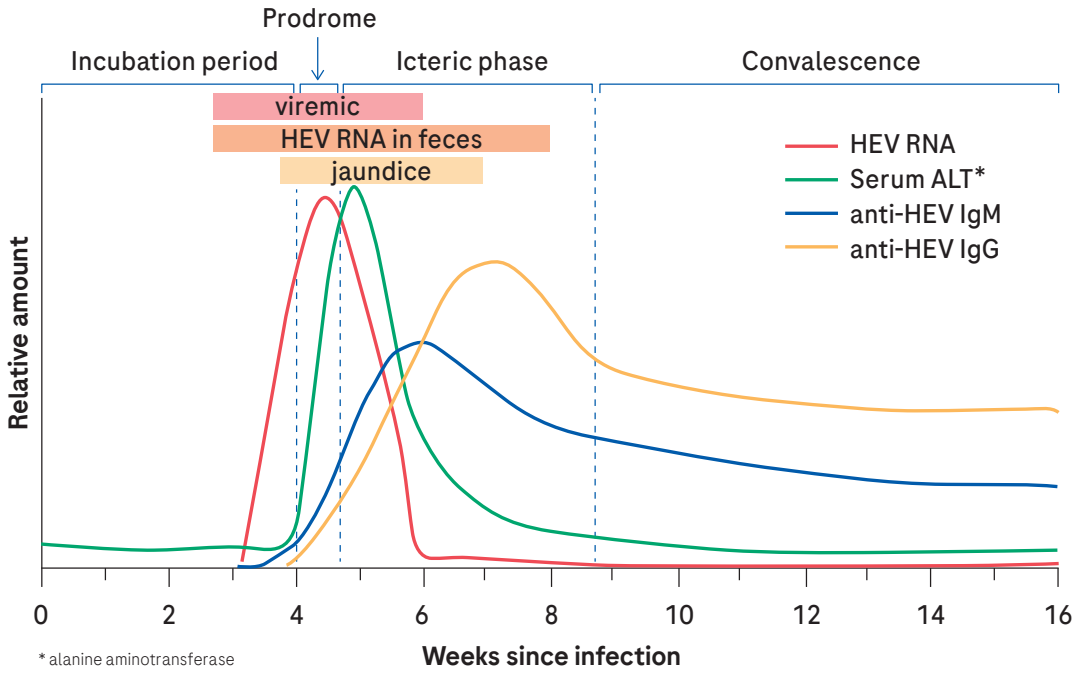
Relative specificity[37]

A total of 8011 samples from blood donors, diagnostic routine (suspected for viral hepatitis), and pregnant women were tested at 4 centers in Europe with the Elecsys Anti-HEV IgM assay and 3 commercially available anti-HEV IgM assays. Samples were considered negative for anti-HEV IgM if they were non-reactive in 2 out of 3 comparator assays.

Cohort	N	Confirmed negative samples	Elecsys Anti-HEV IgM non-reactive	Specificity (95 % CI)
Blood donors	5040	4995	4977	99.64 % (99.43 – 99.79 %)
Daily routine	2427	2375	2348	98.86 % (98.35 – 99.25 %)
Pregnant women	544	531	531	100 % (99.31 – 100 %)
Overall	**8011**	**7901**	**7856**	**99.43 % (99.24 – 99.58 %)**

CI: confidence interval, 2-sided

Course of HEV infection and diagnostic markers [2,7,16]



* alanine aminotransferase

Order information

Product	Material configuration	Material Number
Elecsys Anti-HEV IgM [a)]	100 tests	09 056 246 190
Elecsys Anti-HEV IgM [b)]	300 tests	09 056 254 190
PreciControl Anti-HEV IgM	16 × 1.0 mL	09 056 289 190

a) for use on the **cobas**® e 411 analyzer and the **cobas** e 601 / 602 modules; b) for use on the **cobas** e 402 and **cobas** e 801 analytical units

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37 Elecsys Anti-HEV IgM (Mat. Nos. 09044639190 & 09044647190) method sheets. V1 2023-09.

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