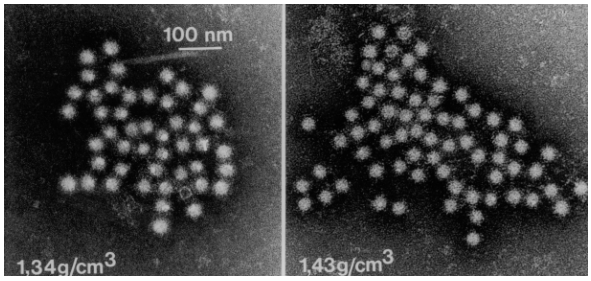
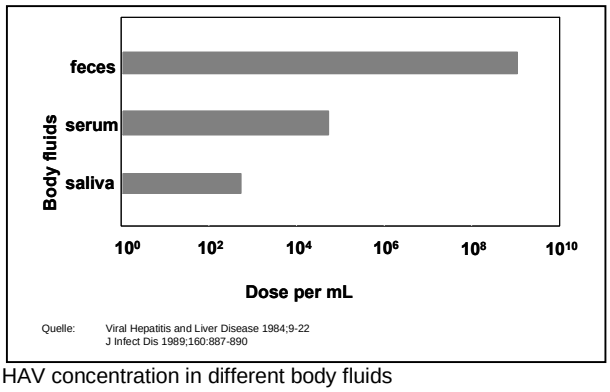
Hepatitis A Virus Antigen ELISA E12

Hepatitis A is caused by HAV, a 27-nm ribonucleic acid (RNA) virus that is classified as a picornavirus. Only one serotype has been observed among HAV isolates collected from various parts of the world. HAV causes both acute disease and asymptomatic infection. HAV does not cause chronic infection. Total antibody to HAV develops in response to infection and confers lifelong immunity from future HAV infection.



Electron microscopic picture of HAV particles with bound antibodies.

HAV is transmitted by different ways, close personal contact e.g. sexual contacts or exposure to blood are only two possible ways. Main source of infection is food or water contaminated by feces. Feces can contain up to 10^8 infectious virions/ml and are the primary source of HAV. Viremia occurs during the preclinical and clinical phases of illness.

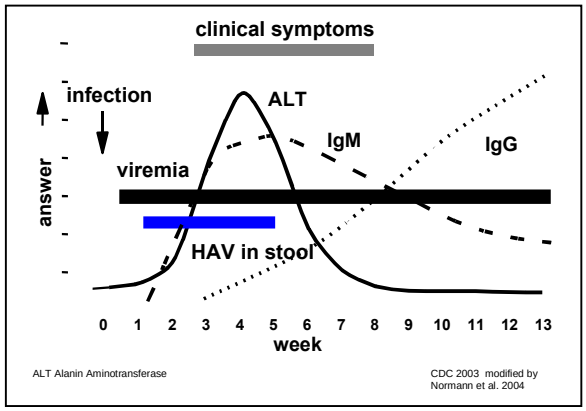


HAV concentration in different body fluids

Virus has also been found in saliva and urine during the incubation period in experimentally infected animals, but transmission by saliva or urine has not been reported to occur. Main excretion of HAV occurs before the onset of clinical symptoms. Therefore persons with slight pathology in or just coming from high risk areas should be tested immediately for HAV to prevent endemic outbreaks.

Mediagnost HAV ELISA E12

Highly specific monoclonal antibodies allow sensitive detection of HAV in stool and other matrices. Prepare a 20% (w/v) suspension of stool in Dilution Buffer. Centrifuge the suspension with at least 2400 x g for 10 minutes at room temperature. The clear supernatant can be used in the test. If required repeat the centrifugation. Supernatants of cell culture and cell lysates can be used directly. If required, they can be concentrated i.e. by ultracentrifugation.



Assay Features E12

- ✓ Detection limit of 10^4-10^5 virus particles
- ✓ Intra-assay variance of control serum ≤ 4%
- ✓ 91% of the ELISA-positive samples were also HAV-PCR positive
- ✓ Control serum included, inactivated

Mediagnost ELISA E12 was approved by Paul-Ehrlich Institute, Germany (212a/95).

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Mediagnost HAV Antigen ELISA E12

Reagent preparation:	Dilution:	
Conjugate Concentrate A	1:100 with Dilution Buffer D: Dilute only the volume used in the test	
Neutralising serum C	1:10 with Dilution Buffer D: "	
Washing Buffer G	1:20 with Aqua dest.	
Stool samples	Prepare a 20% (w/v) suspension of stool in Dilution Buffer D. Centrifuge the suspension with at least 2400 x g for 10 minutes at room temperature. The clear supernatant can be used in the test.	
Pipette	Reagents	Position
50 µl	Pipette Dilution Buffer D or Neutralising Dilution Buffer C (1:10 Dilution of the Neutralising Serum C)	In all wells required
50 µl	Add Dilution Buffer D (Negative-Control) in positions:	A1/A2
50 µl	Add HAV-Antigene B (Positive-Control) in positions:	B1/B2
50 µl	Add Samples in	following wells
Cover the wells with the sealing tape		
Incubation: 2 h at 37°C		
3x 300 µl	Aspirate the contents of the wells and wash 3 x with 300 µl Wash Buffer G	each well
100 µl	Diluted Conjugate Solution A	each well
Cover the wells with the sealing tape.		
Incubation: 2 h at 37°C		
3x 300 µl	Aspirate the contents of the wells and wash 3 x with 300 µl Wash Buffer G	each well
100 µl	Substrate Solution E	each well
Incubation: 30 min in the Dark at RT		
100 µl	Stopping solution F	each well
Measure the absorbance within 30 min at 450 nm (Referece wavelengt between 570 nm and 620 nm).		