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Hepatitis A serological assays: A review of evaluation literature

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Hepatitis A serological assays: A review of evaluation literature

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Contents

Background	1
Scope of review	3
Findings	4
Other HAV diagnostics outside of review	6
Conclusions	7
References	8

Background

Hepatitis A virus (HAV) is a single stranded RNA virus from the *Picornaviridae* family, which can cause acute hepatitis. The virus is transmitted primarily by the faecal-oral route. Children are often asymptomatic or have mild disease, whilst clinical disease is more common in adults. Infection with HAV is usually self-limiting and the symptoms of HAV infection include fever, nausea, abdominal pain, loss of appetite, and jaundice (1). However, complications can arise causing considerable morbidity, which in turn can lead to an economic burden (2).

There are approximately 1.5 million cases of clinical hepatitis A worldwide (3). The prevalence of the disease is highest in developing countries, where most children are exposed to HAV infection. Adults are increasingly affected by the disease in the developed world, due to better hygiene which reduces the risk of infection in childhood, along with increased international travel to countries with high disease prevalence and reduced sanitation facilities. Groups at most risk of HAV infection in the UK include sewage workers, people living in residential centres, men who have sex with men, overseas travellers, and injecting drug users (IDUs) (1). In the UK from October to December 2004, there were 109 laboratory reports of HAV, although an extra 133 were formally notified (4). There has been a decrease in HAV infection in the last seven quarters when compared to the equivalent quarters in the previous year (4). There were no documented IDU cases in the third or fourth quarter of 2004, suggesting that IDU outbreaks have been controlled (4;5).

The incubation period for HAV is two to six weeks (2). Anti-HAV IgM and IgG are detectable at the onset of symptoms (reviewed in (6)), although virus can be detected in the blood and faeces sooner (10 to 12 days post-infection) (3). Anti-HAV IgM continues to be detected in infected individuals for between two and nine months post-infection (7), after which it declines. Therefore anti-HAV IgM is useful for diagnosing acute or recent infection. Anti-HAV IgG offers life long immunity from infection.

Vaccination elicits an anti-HAV IgG response and confers immunity. Current vaccines include HAVRIX[®] (SmithKline Beecham Biologicals) and VAQTA[®] (Merck & Co., Inc) and have been shown to be safe, efficacious and to offer long-lasting protection (8).

Immune electron microscopy (9;10), antigen assays (radioimmunoassay (RIA) and (11;12), enzyme immunoassay (EIA)) (13), and RT-PCR (reverse transcriptase polymerase chain reaction) (13;14) may be used to detect HAV in faecal samples. However, these methods are usually confined to research laboratories where they are particularly applied to viral shedding and HAV outbreak studies. RT-PCR has also been used to detect HAV RNA in serum in a study that investigated the duration of viraemia (15). More recently a TaqMan assay has been developed to rapidly detect HAV in environmental, food and clinical samples (16).

Liver function tests may be performed to assist in making a diagnosis of hepatitis, but for specific confirmation of hepatitis A, the diagnosis is usually by serology. Anti-HAV can be detected by a number of serological techniques including RIA, complement fixation and immune adherence haemagglutination (IAHA). At present commercial microplate EIAs and assays carried out on random access automated systems are the serological methods used by most clinical laboratories for detecting anti-HAV.

Acute HAV infection is usually confirmed by anti-HAV IgM detection. Past infection or vaccination can be determined by total anti-HAV assays. Commercial assays for total anti-HAV can be used for pre-vaccination screening. There are currently no assays designed to distinguish between antibodies produced following vaccination and antibodies produced as a result of natural infection. At four weeks following vaccination, IgM is either not detected or is present at borderline levels (17); therefore anti-HAV IgM assays may help to discriminate between natural infection and response to vaccination.

Scope of review

This review concentrates on evaluation literature related to anti-HAV total and IgM microplate EIAs and automated systems, since these are preferentially used in diagnostic laboratories. Evaluations of RIAs compared with IAHA have been undertaken (18;19), but these tests are now rarely employed in diagnostic settings. This review also focuses on assays that use human serum or plasma, although it is possible to detect anti-HAV in whole blood (20), saliva (21) and urine (22;23) specimens and therefore eliminate the need for venepuncture.

This review covers seven peer-reviewed papers published between 1983 and 2003, and from these the available sensitivities and specificities of four anti-HAV total assays and three anti-HAV IgM assays have been recorded (Table 1 and 2). There were no papers identified in 2004 and 2005 which described evaluations of commercial anti-HAV assays.

There were nine peer-reviewed papers on evaluations of in-house EIAs and other assays in development, which are described later.

The papers were retrieved from PubMed using the keywords; HAV, Hepatitis A, evaluation, kit, assay and serology. The main difficulty in assessing the papers was due to the nature of the specimen panels used. In most studies the HAV status of the specimens was unknown, and the results were compared with the results from other assays. Therefore the sensitivity and specificity is shown as concordance with the assay(s) used for comparison (reference standard), instead of calculating absolute sensitivity and specificity by using a panel with confirmed anti-HAV status.

Findings

The evaluation results of the anti-HAV total assays are presented in Table 1. The four assays evaluated were automated anti-HAV total assays (Abbott IMx HAVAB, Abbott AxSYM HAVAB, Roche Enzymun anti-HAV and Roche Elecsys anti-HAV). The specificities ranged from 95.4 to 99.5% and the concordance with the reference standard using a population with unknown anti-HAV status ranged from 96.8 to 99.9%. There were no sensitivity values given as such, but rather serial bleeds from vaccinees or dilutions compared to a WHO standard were used to assess sensitivity. The IMx HAVAB and the Enzymun anti-HAV showed equivalent sensitivity and specificity to other methods. The Elecsys and Enzymun assays were found to be comparable to each other and both superior to the AxSYM.

The evaluation results of anti-HAV IgM assays are presented in Table 2. Of the three anti-HAV IgM assays evaluated, one was a microplate ELISA (Organon Tecknika), and two were automated IgM assays (Abbott IMx HAVAB-M and Roche Enzymun IgM anti-HAV). Of the anti-HAV IgM assays, there was only one sensitivity score of 95 to 96% for the Organon IgM ELISA. Specificities for the IgM assays ranged from 97.9 to 100% and the concordance with the reference standard using a population with unknown anti-HAV IgM status ranged from 97.5 to 100%. The Organon IgM ELISA was found to be less sensitive than the reference test, but the automated IgM assays gave comparable performance to the reference tests.

Presently, only the Elecsys and AxSYM automated systems have assays commercially available for anti-HAV total and anti-HAV IgM. Automated systems can be quicker and require less operator intervention.

Report 05107: Hepatitis A serological assays: A review of the evaluation literature

Table 1. Commercially available anti-HAV total antibody assays

First author	Year	Test	Manufacturer	Sensitivity	Specificity	Specificity of samples with interfering substances	Clinical evaluation / unknown population	Assay used for comparison	Authors conclusions
Robbins	1991	IMx HAVAB - MEIA	Abbott	18-25 WHO U/l (no sensitivity panel used)	99.5% A RIA only (657/660) (This was called specificity group in the paper, but 55.9% of specimens were reactive)	100% (185/185)	99.9% A (1376/1377)	Abbott HAVAB RIA & HAVAB EIA	More sensitive than HAVAB RIA and EIA
Hess	1995	Enzymun anti-HAV	Boehringer Mannheim (now Roche)		95.4% (329/345) A		96.8% A (1773/1835)		High sensitivity*, reproducibility and specificity
Wiedmann	2003	Enzymun Anti-HAV Elecsys Anti-HAV AxSYM HAVAB 2.0	Roche Roche Abbott	Panel: Serial bleeds from 20 HAV vaccinees at pre-vaccination, 2 weeks, 1 month, and 6 months post vaccination, and also 1 month after administration of a booster at 7 months. Results: all 3 assays detected seroconversion at month 1, whilst the AxSYM detected seroconversion in 55% of recipients at 2 weeks postvaccination, and the other detected seroconversion in all vaccines at week 2. Mean total anti-HAV titres were lower in the AxSYM when compared to the other 2 assays					Elecsys and Enzymun assays are comparable to each other and both superior to the AxSYM.

Notes: A - in agreement with the assay used. Discrepant samples not always confirmed pos or neg

MEIA - microparticle enzyme immunoassay

* from endpoint dilution studies

Column headings as per the papers

Table 2. Commercially available anti-HAV IgM assays

First author	Year	Test	Manufacturer	Sensitivity	Specificity	Specificity of samples with interfering substances	Clinical evaluation / unknown population	Assay used for comparison	Authors conclusions
Supran	1983	IgM ELISA	Organon	95-96% A (94)	100% A (80)			In-house RIA	There were undetected specimens in the weakly positive category, therefore this EIA is less sensitive than the RIA.
Fayol	1991	IMx HAVAB-M - MEIA	Abbott				97.5% A(156/160)	Abbott HAVAB-M RIA	Comparable performance with the HAVAB-M RIA
Eble	1991	IMx HAVAB-M - MEIA	Abbott	Equivalent or 2-fold greater sensitivity than HAVAB-M EIA (no sensitivity panel used)	100% (301/301) A (5 of these reactive in all assays) 100% (200/200) A M-EIA only	288/288 (100%) A (2 reactive in all a	99.8% (981/983) A EIA 99.8% (489/490) A RIA	Abbott HAVAB-M RIA & HAVAB-M EIA	Excellent correlation with the HAVAB EIA and RIA and better reproducibility
Chernesky	1991	IMx HAVAB-M - MEIA	Abbott		100% (However sensitivity and specificity results from paper taken from the comparisons with the unknown popn)	100%	100% (211/211)	Abbott HAVAB-M RIA	Equal accuracy to exiting tests
Hess	1995	Enzymun-Test IgM anti-HAV	Boehringer Mannheim (now Roche)		97.9% (854/872) A		97.6% A (1617/1656)	Abbott, Sorin and Amerlite	Comparable to other anti-HAV tests*

Notes: A - in agreement with the assay used. Discrepant samples not always confirmed pos or neg

MEIA - microparticle enzyme immunoassay

* from endpoint dilution studies

Column headings as per the papers

Other HAV diagnostics outside of review

During the literature search, other types of serological assays for the detection of anti-HAV antibodies include a rapid test dot immunogold filtration assay (DIGFA) for the detection of IgM antibody (24), which eliminates the need for expensive laboratory equipment. The sensitivity and specificity of this test was 86.27% and 90.12% respectively. Anti-HAV antibodies can also be detected by immunofluorescence (25). Synthetic peptides have been used for the detection of HAV antibodies using biosensor technology based on surface plasmon resonance (SPR) (26). A specificity of 100% and a sensitivity range of 48% to 96% was achieved, depending upon what peptide was used. Even though this assay is in the developmental stage, biosensors can be more rapid than EIAs when they are fully automated and have potential clinical value, however their expense may prove unpopular. None of these assays are as yet commercially available.

Due to non-specific polyclonal activation, where anti-HAV IgM may be produced by an infectious agent other than HAV, an IgG HAV avidity assay has been developed (27) to assist in diagnosing acute infection. Low-avidity antibodies can be detected in acute infection and the avidity becomes higher in individuals with past infection. The authors showed a difference ($P < 0.001$) in anti-HAV IgG avidity between acute infection and previous infection, and similar results were demonstrated in a different study (28). However, this assay format is not yet commercially available and this phenomenon of non-specific anti-HAV IgM production needs to be studied further.

Other EIAs have been developed 'in-house', with comparable results to commercially available EIAs. These include an EIA using monoclonal antibodies (29), a total competitive ELISA (30), a solid-phase antibody capture haemadsorption assay (31) and an anti-HAV IgM capture assay (32).

Conclusions

HAV testing is important for diagnosis of acute disease, pre and post vaccination testing, outbreak investigations and other epidemiological studies. The peer-reviewed literature indicates that only a small number of evaluations have been undertaken to assess the performance of commercially available HAV assays. It is clear that a more up-to-date independent and comprehensive evaluation should take place, especially since a number of the assays previously evaluated are no longer available.

Such an evaluation needs to include a specimen panel with known anti-HAV status established according to an agreed methodology so that absolute sensitivities and specificities can be determined and thus allow kits to be directly compared. This avoids the difficulties associated with evaluating a test and deriving potentially incorrect sensitivities and specificities on the basis of direct comparison with other tests with higher or lower performance. Discrepant results are then difficult to interpret if no further testing is performed to confirm the specimen status.

In addition there are evaluation examples where kits from the same company are directly compared against each other, which may lead to bias. For example evaluations of the Abbott HAV assays on automated analysers (AxSYM and IMx), used the Abbott HAVAB EIA or RIA for comparison (33-36).

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