Comparative Analysis of Diagnostic Performance: HAV IgG/IgM Combo Rapid Test Cassette and ELISA HAV Test

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Abstract

The Hepatitis A Virus (HAV) leads to acute liver disease, Hepatitis A, predominantly is transmitted *via* the fecal-oral route through contaminated water, food, or direct person-to-person contact. While most cases of Hepatitis A are acute with rare fatal outcomes, the disease can manifest as a mild to severe symptoms or even result in acute liver failure, which can be fatal. The World Health Organization (WHO) attributed approximately 1,300 deaths to Hepatitis A in 2019. Notably, countries with low endemicity, especially in Europe and the Americas, have recently reported a surge in Hepatitis A cases, particularly among men who have sex with men. These surges underline the importance of laboratory-based diagnostics to support public health responses during outbreaks.

Given the current epidemiological situation in many countries, there is a heightened demand for rapid, scalable diagnostic methods to optimize vaccination efforts targeting high-risk populations. Conventionally, acute Hepatitis A diagnosis relies on detecting serum anti-HAV IgM, an antibody generally present at the onset of symptoms and persisting 2-9 months post-infection. This study evaluates the performance of the HAV IgG/IgM combo rapid test cassette manufactured by Hangzhou AllTest Biotech Co., Ltd, comparing it against the ELISA method. Preliminary results affirm the product's adherence to professional *in vitro* diagnostic use, underscoring its potential in expediting Hepatitis A diagnosis during outbreaks.

Keywords: Hepatitis A virus; Rapid test; Enzyme-linked immunosorbent assay

Editorial Note

About HAV

Hepatitis A Virus (HAV), a nonenveloped, single-stranded RNA member of the Picornaviridae family, causes acute infectious Hepatitis [1]. The primary mode of transmission involves an uninfected (and unvaccinated) individual consuming contaminated food or water. Risk factors also include poor sanitation, subpar personal hygiene, and certain sexual practices such as oral-anal sex.

Unlike Hepatitis B and C, Hepatitis A does not cause chronic liver disease. However, it can still cause debilitating symptoms, occasionally culminating in fulminant hepatitis (acute liver failure), which is often fatal. WHO's 2016 data suggest that Hepatitis A accounted for 0.5% viral hepatitis-induced mortality, with a worldwide death toll of 7,134. The disease can manifest sporadically or as an epidemic, often with cyclic recurrences. Large-scale outbreaks, like Shanghai's 1988 epidemic, can affect hundreds of thousands, perpetuated by person-to-person transmission or contaminated food and water. Prompt HAV detection and treatment are imperative.

Clinical manifestations of HAV infection

In children under six, nearly 70% of acute HAV infections are asymptomatic. Conversely, roughly the same percentage of adults exhibit symptoms like jaundice and elevated aminotransferases [2]. Typical adult manifestations encompass sudden fever, malaise, nausea, vomiting, abdominal discomfort, dark urine, and jaundice [3]. Other documented manifestations encompass cholestasis, recurring infections, and autoimmune phenomena like chronic autoimmune hepatitis [4].

HAV diagnostic methods

Clinical Hepatitis A presentations are often indistinguishable from other acute viral hepatitis forms. Hence, a specific diagnosis necessitates HAV-specific Immunoglobulin G (IgG) antibody detection in the bloodstream. Supplementary diagnostics like Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) are also utilized to detect HAV RNA, but they may mandate specialized laboratory facilities.

Serologic testing

Hepatitis A's clinical symptoms often overlap with several other febrile and gastrointestinal illnesses, making differentiation challenging; As a result, serological testing becomes pivotal [5]. These tests are designed to detect host-produced antibodies in response to HAV infections. The IgM anti-HAV antibody test, especially, is vital as it confirms an acute HAV infection. Nearly all patients with acute Hepatitis A register detectable IgM anti-HAV levels, making this test an effective tool for confirming diagnosis.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

RT-PCR is a laboratory technique used to detect and amplify RNA sequences, including those of viruses, in biological samples. It is pivotal for pinpointing HAVRNA in blood or fecal samples. This intricate process involves several steps. First, the viral RNA is extracted from the sample using a specialized kit or protocol. The extracted RNA is then converted into complementary DNA (cDNA) using an enzyme called reverse transcriptase. The cDNA is then amplified using PCR, which involves repeatedly heating and cooling the sample to denature and anneal the DNA strands, respectively. During each cycle of amplification, the amount of DNA in the sample is doubled, resulting in a large number of copies of the target DNA sequence.

Treatment

Rest and hydration are vital in supporting the body's immune response and alleviating symptoms associated with the infection, including fatigue and nausea. It is advisable to avoid alcohol consumption until symptoms subside. Non-prescription pain relievers like acetaminophen can be used to relieve fever and body aches, but it is crucial to consult a healthcare professional beforehand. Some over-the-counter drugs, such as ibuprofen or acetaminophen, can potentially harm the liver in high doses. To ensure liver safety while managing infection symptoms, healthcare providers may advise lower medication doses. In cases of severe symptoms, hospitalization may be necessary to provide intravenous fluids, nutrition support, and manage complications like liver failure.

Prevention

Prevention of HAV infection involves a comprehensive approach encompassing vaccination, hygiene practices, and sanitation measures. Vaccination is the primary method of prevention, as the HAV vaccine effectively stimulates the production of protective antibodies. It is recommended for high-risk individuals, including travelers to endemic areas, healthcare workers, men who have sex with men, and those with chronic liver disease. Hygiene practices, such as frequent and proper handwashing with soap and water, are crucial in reducing fecal-oral transmission. Public health campaigns should emphasize the importance of hand hygiene and provide education on effective handwashing techniques. Furthermore, improving sanitation infrastructure, including access to safe drinking water and proper waste disposal systems, is vital in minimizing fecal contamination and reducing HAV transmission. Governments and public health authorities should prioritize investments in sanitation facilities, particularly in areas with limited resources, to effectively prevent HAV infection.

Evaluation of AllTest HAV IgG/IgM combo rapid test cassette

Objective: The evaluation aims to explore the reliability and performance of Hangzhou AllTest Biotech Co., Ltd.'s HAV IgG/IgM Combo Rapid Test Cassette for the qualitative detection of IgG and IgM antibodies against the Hepatitis A virus in human blood samples [6-8].

Method: We performed the rapid *in vitro* diagnostic test device, designed to detect Hepatitis A Virus (HAV) antibodies (IgG and IgM) in whole blood, serum, or plasma samples, against a benchmark commercial Dengue ELISA test to validate its performance.

Materials and directions for using: The test kits include a test cassette, buffer, droppers, and a package insert. It uses a proprietary technology that combines the principles of immune-chromatography and fluid dynamics, leveraging a recombinant HAV antigen bound to a membrane in the test zone. A specimen is added to the specimen well of the cassette, where it reacts with mouse anti-human IgG coated particles in the test. Similarly, the HAV IgM test involves adding a specimen to the specimen well of the cassette, where it reacts with mouse anti-human IgM coated particles in the test.

A positive result is indicated by the formation of a colored line in the test zone, while a negative result is indicated by the absence of a colored line in the test zone. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred [9,10].

Performance characteristics: The HAV IgG/IgM combo rapid test cassette (Whole Blood/Serum/Plasma) was compared against a leading commercial ELISA HAV test. The rapid test cassettes delivered an impressive sensitivity rate of 96.2% and a specificity of 98.6%, implying its accuracy in HAV diagnostics (Tables 1 and 2).

Method		ELISA		Total results
HAV IgG/IgM combo rapid test cassette (Whole Blood /Serum/ Plasma)	Results	Positive	Negative	
	Positive	371	3	374
	Negative	8	316	324
Total results		379	319	698

Note: Relative sensitivity: 97.9% (95%CI: 95.5%-99.0%); Confidence intervals: Relative specificity: 99.1% (95%CI: 97.3%-99.8%)

Table 1: HAV IgG combo rapid test cassette.

Method		ELISA		Total results
HAV IgG/IgM combo rapid test cassette (Whole Blood /Serum/ Plasma)	Results	Positive	Negative	
	Positive	111	5	116
	Negative	5	576	581
Total results		116	581	697

Note: Relative sensitivity: 95.7% (95%CI: 90.2%-98.6%); Confidence intervals: Relative specificity: 99.1% (95%CI: 98.0%-99.7%) Overall accuracy: 98.6% (95%CI: 97.4%-99.3%)

Table 2: HAV IgM combo rapid test cassette.

Expected values

The HAV IgG/IgM combo rapid test cassette (Whole Blood/Serum/Plasma) was benchmarked against a leading commercial ELISA HAV test, resulting in a correlation exceeding 98% between these two systems.

Conclusion

The HAV IgG/IgM combo rapid test cassette (Whole Blood/Serum/Plasma) has proven its efficacy under standard experimental conditions of this comparison study. Not only did the tests demonstrate excellent accuracy and specificity, but its ability to simultaneously detect both IgG and IgM antibodies for Hepatitis A offers convenient, speed, and cost-effectiveness.

Based on the results from the tested samples, we can conclusively state that the HAV IgG/IgM combo rapid test cassette, developed by AllTest, meets the professional standards for *in vitro* diagnostic use. It stands as a reliable tool for detecting specific antibodies related to the Hepatitis A virus.

References

1. Cuthbert JA (2001) Hepatitis A: old and new. Clin Microbiol Rev 14: 38-58.
2. Jeong SH, Lee HS (2010) Hepatitis A: clinical manifestations and management. Intervirology 53: 15-19.
3. Shin EC, Jeong SH (2018) Natural history, clinical manifestations, and pathogenesis of Hepatitis A. Cold Spring Harb Perspect Med 8: a031708.
4. Schiff ER (1992) Atypical clinical manifestations of Hepatitis A. Vaccine 10: S18-S20.
5. Matheny SC, Kingery JE (2012) Hepatitis A. Am Fam Physician 86: 1027-1034.
6. Burucoa C, Axon A (2017) Epidemiology of *Helicobacter pylori* infection. Helicobacter 22:e12403.
7. Pucułek M, Machlowska J, Wierzbicki R, Baj J, Maciejewski R, et al. (2018) Helicobacter pylori associated factors in the development of gastric cancer with special reference to the early-onset subtype. Oncotarget 9: 31146-31162.
8. Ansari S, Yamaoka Y (2017) Survival of *Helicobacter pylori* in gastric acidic territory. Helicobacter 22: e12386.
9. Ciaula AD, Baj J, Garruti G, Celano G, Angelis MD, et al. (2020) Liver Steatosis, gut-liver axis, microbiome and environmental factors: A never-ending bidirectional cross-talk. J Clin Med 9: 2648.
10. Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R (2020) Gastric cancer: Epidemiology, risk factors, classification, genomic characteristics and treatment strategies. Int J Mol Sci 21: 4012.