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Performance Evaluation of the Newlink One Step HBsAg and HCV Combo Test (Whole Blood/Serum/Plasma, REF: L11-HBC02C4) via Clinical Validation

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Abstract: This study assessed the clinical performance of the Newlink One Step HBsAg and HCV Combo Test (Whole Blood/Serum/Plasma), a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen (HBsAg) and antibodies to Hepatitis C Virus (HCV). Using commercial EIA/CMIA as the reference standard for HBsAg and EIA/ECLIA for HCV, accuracy was evaluated with human whole blood, serum, and plasma specimens. The validation included a large cohort: 2,926 specimens (669 HBsAg positive, 2,257 negative) for Hepatitis B and 2,062 specimens (540 HCV antibody positive, 1,522 negative) for Hepatitis C. The combo test demonstrated high diagnostic accuracy. For HBsAg detection, relative sensitivity was 99.7% (95% CI: 98.9%-100%), relative specificity was 99.8% (95% CI: 99.6%-100%), and overall accuracy was 99.8% (95% CI: 99.6%-99.9%). For HCV antibody detection, relative sensitivity exceeded 99.9% (95% CI: 99.3%-100%), relative specificity was 99.9% (95% CI: 99.6%-100%), and overall accuracy was >99.9% (95% CI: 99.7%-100%). No cross-reactivity was observed with a panel of potential interfering substances or other common infectious agents. The test also showed excellent within-run and between-run precision. In summary, the One Step HBsAg and HCV Combo Test is a reliable, accurate and practical point-of-care tool for simultaneous screening of Hepatitis B and C, supporting timely clinical management.

1. Introduction

Viral hepatitis, mainly due to Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV), poses a significant worldwide health burden and may lead to chronic liver disease, cirrhosis, and hepatocellular carcinoma[1]. The presence of Hepatitis B surface antigen (HBsAg) in blood serves as a key indicator of acute or chronic HBV infection[2]. HCV infection, often asymptomatic initially, is generally identified through detection of anti-HCV antibodies[3]. Early diagnosis is critical for timely antiviral treatment, preventing disease progression and reducing transmission[4].

Conventional laboratory techniques for HBsAg and anti-HCV detection, such as enzyme immunoassays (EIA) and chemiluminescent immunoassays (CMIA/ECLIA), which provide high sensitivity and specificity but require specialized equipment, trained staff and prolonged turnaround times[5,6]. These limitations restrict their use in resource-limited environments, primary care settings and point-of-care screening. Rapid diagnostic tests (RDTs), based on immunochromatographic principles, deliver qualitative results within 10–20 minutes and are highly valuable for screening across varied healthcare contexts[7,8]. However, rigorous validation against established reference methods is essential to ensure RDT reliability.

The Newlink One Step HBsAg and HCV Combo Test (Whole Blood/Serum/Plasma) allows simultaneous qualitative detection of HBsAg and anti-HCV antibodies from fingerstick or venous whole blood, serum, or plasma. This study aims to thoroughly evaluate the test's clinical performance, including sensitivity, specificity, accuracy, precision, and cross-reactivity by using EIA/CMIA/ECLIA as reference standards, thereby confirming its suitability as a rapid and dependable screening tool.

2. Experimental Procedures

2.1 Source of Clinical Specimens

The specimens used in this evaluation were derived from both symptomatic and asymptomatic individuals, as outlined in the clinical study methodology. The One Step HBsAg and HCV Combo Test (from Newlink Biotech Co., Ltd) is designed for use with human whole blood (collected via venipuncture or fingerstick), serum, or plasma specimens, which aligns with its intended use description. All specimens included in the analysis had been previously characterized; a specimen was classified as positive or negative based on the results from the reference methods (commercial EIA/CMIA for HBsAg and EIA/ECLIA for HCV antibody), as stated in the clinical report. The evaluation was conducted across multiple international clinical sites, including Huzhou Jiaotong Hospital (China), Hopital St Louis (France), General Hospital Jesenice (Slovenia), Public Health Institute Kragujevac and University Clinical Center Kragujevac (Serbia), and Metagenomix and University of KwaZulu-Natal (South Africa).

2.2 Test Kits and Procedures

The investigational device used in this study was the Newlink One Step HBsAg and HCV Combo Test (Whole Blood/Serum/Plasma), Ref: L11-HBC02C4. As reference standards, commercially available EIA/CMIA kits were used for HBsAg detection and EIA/ECLIA kits for anti-HCV detection, with all reference testing performed strictly according to the manufacturers' protocols.

The rapid combo test was carried out following the manufacturer's instructions. Briefly, all components and specimens were first equilibrated to room temperature. Approximately 50 µL of whole blood, serum, or plasma was then applied to the specimen well (S) of the test cassette using the provided capillary tube or dropper. Immediately after, one drop (about 40 µL) of the supplied assay buffer was added to the same well. Timing began upon buffer addition, and results were read at 10 minutes; readings taken after 20 minutes were considered invalid and were not recorded.

Interpretation was based on the following criteria: a positive result for HBsAg and/or HCV was indicated by the presence of both a colored control line (C) and a colored test line (T). A negative result was defined by the appearance of only the control line (C). If the control line (C) failed to appear, the result was considered invalid, and the test was repeated with a new cassette. All testing was performed by trained personnel under appropriate biosafety conditions.

3. Performance Analysis

3.1 Analysis of Performance Characteristics

3.1.1 Key Diagnostic Metrics

The performance of the One Step HBsAg and HCV Combo Test compared to the reference methods is summarized in the following contingency Tables 1-2 and metrics.

Table 1: Clinical Performance for HBsAg Detection vs. EIA/CMIA

Method		EIA/CMIA		Total Results
One Step HBsAg and HCV Combo Test (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	667	4	671
	Negative	2	2253	2255
Total Results		669	2257	2926
Relative Sensitivity		99.7% (95%CI*: 98.9%-100%)		
Relative Specificity		99.8% (95%CI*: 99.6%-100%)		
Overall accuracy		99.8% (95%CI*: 99.6%-99.9%)		

Relative Sensitivity: 99.7% (95% Confidence Interval [CI]: 98.9% - 100%)
Relative Specificity: 99.8% (95% CI: 99.6% - 100%)
Overall Accuracy: 99.8% (95% CI: 99.6% - 99.9%)

Table 2: Clinical Performance for HCV Antibody Detection vs. EIA/ECLIA

Method		EIA/ECLIA		Total Results
One Step HBsAg and HCV Combo Test (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	540	1	541
	Negative	0	1521	1521
Total Results		540	1522	2062
Relative Sensitivity		>99.9% (95%CI*: 99.3%-100%)		
Relative Specificity		99.9% (95%CI*: 99.6%-100%)		
Overall accuracy		>99.9% (95%CI*: 99.7%-100%)		

Relative Sensitivity: >99.9% (95% CI: 99.3% - 100%)
Relative Specificity: 99.9% (95% CI: 99.6% - 100%)
Overall Accuracy: >99.9% (95% CI: 99.7% - 100%)

The results demonstrate exceptionally high sensitivity and specificity for both analytes, meeting and exceeding common performance thresholds for rapid diagnostic tests.

3.1.2 Precision

Precision was evaluated through within-run and between-run studies.

Within-run precision was evaluated by testing 20 replicates each of four distinct specimens containing varying concentrations of HBsAg and HCV antibody. All replicates were correctly identified as negative or positive, resulting in 100% agreement across repeated measurements.

Between-run precision was assessed by performing 20 independent assays on the same four specimens with different HBsAg and HCV antibody levels, using three separate lots of the One Step HBsAg and HCV Combo Test over a 10-day period. All specimens were consistently identified correctly in every run, achieving 100% accuracy across different lots and testing days.

3.1.3 Cross-reactivity and Interfering Substances

The specificity of the test was further investigated.

Cross-reactivity: The HBsAg component showed no cross-reactivity when tested with specimens positive for HAMA, Rheumatoid Factor, HAV, Syphilis, HIV, H. Pylori, MONO, CMV, Rubella, HCV, HEV, and TOXO. Similarly, the HCV component showed no cross-reactivity with specimens positive for HAMA, RF, HBsAg, HBsAb, HBcAg, HBcAb, HBeAb, Syphilis, HIV, H. Pylori, MONO, CMV, Rubella, and TOXO.

Interfering Substances: Potential endogenous and exogenous interferents were spiked into negative and positive specimens at high physiological or supraphysiological concentrations. No interference was observed from Acetaminophen (20 mg/dL), Acetylsalicylic Acid (20 mg/dL), Ascorbic Acid (2 g/dL), Creatinine (200 mg/dL), Bilirubin (1 g/dL), Caffeine (20 mg/dL), Gentisic Acid (20 mg/dL), Albumin (2 g/dL), Hemoglobin (1000 mg/dL), or Oxalic Acid (60 mg/dL).

3.2 Discussion

3.2.1 Performance Characteristics

The One Step HBsAg and HCV Combo Test exhibited outstanding diagnostic performance, with sensitivity and specificity values above 99.5% for both pathogens. This performance is comparable to, and in some metrics superior to, other rapid tests described in the literature. The ability to use multiple specimen types (fingerstick blood, venous blood, serum, plasma) significantly enhances its flexibility and applicability in various clinical and field settings, from hospital emergency departments to remote community screening programs. The high accuracy and rapid turnaround time (10 minutes) make it an excellent tool for simultaneous screening of two major blood-borne viruses, enabling prompt triage, patient counseling, and initiation of confirmatory testing and care pathways.

3.2.2 Limitations

As a qualitative screening assay, this test is not designed to determine quantitative viral load or antibody titer. A negative result does not completely rule out infection, particularly in the early "window period" before seroconversion for HCV or during the late stages of HBV infection when HBsAg levels may be very low. Any reactive result must be confirmed with supplemental tests as per national guidelines. Adherence to the specified procedure, particularly regarding sample volume (50 µL) and reading time (10-20 minutes), is critical to avoid invalid or erroneous results.

3.2.3 Comparison with Other Diagnostic Methods

While laboratory-based EIA/CMIA/ECLIA remain the standard for high-throughput, quantitative, or confirmatory testing, this rapid combo test fills a vital niche for point-of-care screening. It offers a clear advantage in settings where laboratory infrastructure is unavailable or where immediate results can directly impact clinical management decisions, such as in prenatal screening, prior to blood donation, or in outreach programs for high-risk populations.

4. Conclusion

The Newlink One Step HBsAg and HCV Combo Test (Whole Blood/Serum/Plasma) has been clinically validated as a highly sensitive, specific, accurate, and reliable rapid immunochromatographic assay for the simultaneous detection of HBsAg and anti-HCV antibodies.

Its excellent diagnostic performance, combined with its operational simplicity, rapid result availability, and flexibility in accepting multiple specimen types, establishes it as a powerful tool for the rapid screening of Hepatitis B and C infections. Widespread adoption of this test can facilitate earlier diagnosis, improve linkage to care, and contribute significantly to global efforts to control and eliminate viral hepatitis.

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