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Comparative Evaluation of Immunochromatographic Test and ELISA for Detection of HBsAg and Anti-HCV Among Patients at a Tertiary Care Hospital in South Kashmir

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Abstract

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections remain significant public health concerns in Kashmir. Rapid immunochromatographic tests (ICTs) allow decentralized screening, but their accuracy compared with enzyme-linked immunosorbent assay (ELISA) requires local validation. ***Aim:*** To evaluate the diagnostic performance of commonly used rapid ICTs against ELISA for detecting hepatitis B surface antigen (HBsAg) and anti-HCV antibodies in patients attending a tertiary care hospital in South Kashmir. ***Materials and Methods***: This retrospective cross-sectional diagnostic accuracy study included 3,951 paired serum samples tested by ICT and ELISA between January 2024 and June 2025. ELISA was the reference standard. Sensitivity, specificity, predictive values, likelihood ratios, Cohen's kappa, and McNemar's exact test were calculated with Wilson 95% confidence intervals. Subgroup analyses were conducted by sex and age (<20 vs. ≥20 years). ***Results:*** ELISA identified HBsAg prevalence of 5.7% (95% CI: 5.0–6.5) and anti-HCV prevalence of 11.0% (95% CI: 10.1–12.1). For HBsAg, ICT showed 89.3% sensitivity and 99.95% specificity (LR+ ≈ 1,664; LR− = 0.11), with strong agreement (κ ≈ 0.936). McNemar's test indicated significantly more false negatives than false positives ($p \approx 1\times10^{-5}$). For anti-HCV, ICT sensitivity was 94.7% and specificity 99.4% (LR+ ≈ 158.6; LR− ≈ 0.05), with strong agreement (κ ≈ 0.943) and no significant discordance (p ≈ 0.88). No significant differences were observed by sex or age. ***Conclusion:*** Rapid ICTs demonstrated excellent specificity and high sensitivity for hepatitis screening. However, excess false-negative HBsAg results support reflex ELISA testing of ICT-negative samples, particularly in high-risk groups, to reduce missed diagnoses.

Keywords: Hepatitis B virus (HBV), Hepatitis C virus (HCV),Diagnostic accuracy, Sensitivity and specificity.

INTRODUCTION

Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are leading causes of liver-related morbidity and mortality worldwide, contributing substantially to cirrhosis and hepatocellular carcinoma [1,2]. Globally, an estimated 250 million people live with chronic HBV infection and approximately 71 million with chronic HCV infection, with South Asia bearing a disproportionate share of this burden [3]. In India, and particularly in the Kashmir region, transmission is driven by injection drug use, unsafe medical injections, and inadequate infection control practices. Reported regional seroprevalence estimates range from 1–5% for HBsAg and 2–10% for anti-HCV [4,5].

Serological detection remains the cornerstone of hepatitis screening. While ELISA-based assays offer high sensitivity and reliability, they require laboratory infrastructure, trained personnel, and batch processing, which may delay diagnosis in resource-limited or high-throughput settings [6]. In contrast, immunochromatographic tests (ICTs) provide rapid, point-of-care results and are widely deployed under India's National Viral Hepatitis Control Program [7]. However, the diagnostic performance of ICTs varies across manufacturers and settings, with reported sensitivities ranging from 50–95% for HBsAg and 70–98% for anti-HCV [8–10]. False-negative results, in particular, pose a risk of delayed diagnosis and ongoing transmission.

Given the rising burden of viral hepatitis among young adults in Kashmir and the extensive reliance on ICTs for screening, local validation of test performance is essential [4,5]. This study aimed to evaluate the diagnostic accuracy of commonly used ICTs against ELISA for detection of HBsAg and anti-HCV in a large cohort from a tertiary care hospital in South Kashmir, with additional assessment of agreement metrics and subgroup variations by sex and age.

MATERIALS AND METHODS

Study Design and Setting

A retrospective, cross-sectional diagnostic accuracy study was conducted at the Department of Microbiology, Government Medical College Hospital, Anantnag, South Kashmir. The study adhered to the STARD 2015 reporting guidelines for diagnostic accuracy studies [11].

Study Population

Residual serum samples from patients tested for HBsAg and/or anti-HCV between 1 January 2024 and 30 June 2025 were eligible. Inclusion criteria comprised availability of paired ICT and ELISA results for the same sample. Samples with incomplete test pairs, hemolysis, or non-human origin were excluded. Consecutive non-probability sampling of all eligible samples yielded a final sample size of 3,951.

Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee of Government Medical College, Anantnag (IEC/GMC/2024/012). As the analysis was retrospective and based on de-identified laboratory records, informed consent was waived in accordance with Indian Council of Medical Research guidelines.

Description of Diagnostic Tests

The index tests were commercially available immunochromatographic card tests for HBsAg and anti-HCV (e.g., SD Bioline, Alere), performed according to manufacturer instructions by trained laboratory personnel. Results were read visually at 15–20 minutes. The reference standard comprised third- or fourth-generation ELISA kits (e.g., Erba Lisa, Transasia) processed in batches with appropriate positive and negative controls. ICT and ELISA results were generated as part of routine workflow, with no post-hoc reinterpretation.

Data Collection and Statistical Analysis

De-identified demographic and laboratory data (age, sex, ICT result, ELISA result) were extracted from laboratory registers into a spreadsheet and analyzed using SPSS version 20 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as medians with interquartile ranges (IQR), and categorical variables as frequencies and percentages. Diagnostic indices were derived from 2×2 contingency tables. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard formulas, with Wilson 95% confidence intervals [12]. Positive and negative likelihood ratios (LR+ and LR−) were calculated using exact cell counts; likelihood ratio confidence intervals were estimated using the method of Simel et al. Agreement between ICT and ELISA was assessed using Cohen's kappa with 95% confidence intervals [13]. Discordance was evaluated using McNemar's exact binomial test, given the small number of discordant pairs [14]. Subgroup analyses by sex and age (<20 vs. ≥20 years) were performed using chi-square tests. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Cohort Characteristics

A total of 3,951 paired serum samples were analyzed. Of these, 1,996 (50.5%) were from male patients and 1,955 (49.5%) from female patients. The median age was 23 years (IQR: 19–28). By ELISA, 225 samples (5.7%) were positive for HBsAg and 436 (11.0%) were positive for anti-HCV (Table 1).

Table 1: Cohort Characteristics (N = 3951)

Total Patients	Male	Female	ELISA Positive HBsAg	ELISA Positive Anti HCV
3951	1996 (50.5%)	1955 (49.5%)	225 (5.7%)	436 (11.0%)

Diagnostic Performance for HBsAg

Among 225 ELISA-confirmed HBsAg-positive samples, ICT correctly identified 201 true positives and missed 24 cases, yielding a sensitivity of 89.3% (95% CI: 84.7–92.8). Among 3,726 ELISA-negative samples, ICT correctly classified 3,724 as negative, with two false-positive results, corresponding to a specificity of 99.95% (95% CI: 99.8–100.0). The PPV and NPV were 99.0% and 99.4%, respectively. The LR+ was approximately 1,664.3, reflecting near-perfect specificity, while

the LR− was 0.11. Agreement between ICT and ELISA was strong (Cohen's κ ≈ 0.936). McNemar's exact test demonstrated a statistically significant excess of false-negative over false-positive ICT results (FP = 2 vs. FN = 24; two-sided $p \approx 1\times10^{-5}$), indicating systematic under-detection of HBsAg by ICT (Table 2).

Table 2. Diagnostic Performance of ICT for HBsAg (Reference: ELISA)

ICT Result	ELISA Positive (225)	ELISA Negative (3726)
Positive	201	2
Negative	24	3724

Sensitivity:	89.3%	(95%CI:	84.7–92.8)
Specificity:	99.95%	(95%CI:	99.8–100.0)
PPV:			99.0%
NPV:			99.4%
LR+:	≈1,664.3;	LR−:	≈0.11
Cohen's	κ:	≈	0.936

McNemar's exact p-value: $\approx 1\times10^{-5}$

Diagnostic Performance for Anti-HCV

For anti-HCV, ICT detected 413 true positives and 23 false negatives among 436 ELISA-positive samples, giving a sensitivity of 94.7% (95% CI: 92.1–96.5). Of 3,515 ELISA-negative samples, 3,494 were correctly identified as negative, with 21 false positives, resulting in a specificity of 99.4% (95% CI: 99.0–99.6). The PPV was 95.2% and the NPV was 99.3%. The LR+ was approximately 158.6 and the LR− was 0.05. Agreement was strong (κ ≈ 0.943). McNemar's exact test showed no significant asymmetry between false-positive and false-negative results (FP = 21 vs. FN = 23; two-sided p ≈ 0.88) (Table 3).

Table 3. Diagnostic Performance of ICT for Anti-HCV (Reference: ELISA)

ICT Result	ELISA Positive (436)	ELISA Negative (3515)
Positive	413	21
Negative	23	3494

Sensitivity:	94.7%	(95%CI:	92.1–96.5)
Specificity:	99.4%	(95%CI:	99.0–99.6)
PPV:			95.2%
NPV:			99.3%
LR+:	≈158.6;	LR−:	≈0.05
Cohen's	κ:		≈0.943

McNemar's exact p-value: ≈0.88

Subgroup Analyses

HBsAg sensitivity was slightly higher in females (91.8%) than males (87.4%) and in individuals aged ≥20 years (90.6%) compared with those <20 years (86.8%), but these differences were not statistically significant (p = 0.21). Anti-HCV sensitivity showed minimal variation by sex or age, with no significant subgroup differences (Table 4).

Table 4. Shows the Sensitivity of ELISA in terms of gender

Marker	Sex	Sensitivity % (95% CI)	ELISA positive
HbsAg	Male	87.4 (80.5–92.5)	127
	Female	91.8 (84.5–96.2)	98
Anti-HCV	Male	95.2 (91.7–97.4)	249
	Female	94.1 (89.7–96.9)	187

Table 5.Shows the Sensitivity of ELISA in terms of Age Group.

Marker	Age Group	Sensitivity % (95% CI)	ELISA positive
HbsAg	<20 years	86.8 (77.1–93.2)	76
	≥20 years	90.6 (84.8–94.6)	149
Anti-HCV	<20 years	95.7 (90.3–98.5)	117
	≥20 years	94.4 (91.2–96.6)	319

DISCUSSION

In this large hospital-based evaluation from South Kashmir, rapid ICTs demonstrated excellent specificity (>99%) and high sensitivity for detection of both HBsAg and anti-HCV when compared with ELISA. The sensitivity for HBsAg (89.3%) was within the range reported in prior Indian and international studies, while anti-HCV sensitivity was higher (94.7%), consistent with recent meta-analytic estimates [8,10,17].

A key finding was the statistically significant excess of false-negative ICT results for HBsAg, as demonstrated by McNemar's exact test. This asymmetry indicates that while false positives are rare, a meaningful proportion of true HBsAg-positive cases may be missed if ICT is used as the sole screening tool. In contrast, no such systematic discordance was observed for anti-HCV. These results reinforce concerns that ICT-based screening, particularly for HBV, may under-detect low-titer or early infections, potentially delaying diagnosis and facilitating ongoing transmission in high-risk populations [4,5,18].

Subgroup analyses suggested modest variations in sensitivity by sex and age, with slightly lower HBsAg sensitivity among males and individuals under 20 years of age, although these differences were not statistically significant. Such patterns may reflect biological or behavioral factors and warrant further investigation in larger, prospective studies.

Compared with published benchmarks from blood donor and hospital-based studies in India, the specificities observed here exceed 95%, supporting the continued use of ICTs for initial triage under national screening programs [7,23–25]. However, the demonstrated false-negative burden for HBsAg underscores the need for reflex ELISA testing of ICT-negative samples in clinically suspected or high-risk cases. Strengths of this study include its large sample size and comprehensive evaluation of agreement and discordance metrics. Limitations include its retrospective design, single-center setting, and lack of nucleic acid testing for confirmatory diagnosis.

CONCLUSION

Rapid immunochromatographic tests show excellent specificity and good sensitivity for hepatitis screening in South Kashmir. However, the significant excess of false-negative ICT results for HBsAg highlights the need for reflex ELISA testing, particularly in high-risk or clinically suspected cases, to minimize missed diagnoses and support hepatitis elimination goals.

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