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Evaluation of Four Enzyme-Immunoassay Diagnostic Kits Available in the Market of Uzbekistan for Detection of Anti-Hcv Antibodies as a Part of the Quality Assurance Program for the Hepatitis C Elimination Campaign in Uzbekistan

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Abstract

When diagnosing viral hepatitis, the leading directions of laboratory service work are the determination of the most informative methods of detecting infection markers, determination of criteria for objective evaluation of the results obtained, development of optimal algorithms of laboratory tests, and implementation of quality improvement systems for detecting infection markers. Therefore, affordable and high-quality ELISA test kits play an important role in the primary diagnosis of HBV and HCV infection. Each country participates in or develops its own laboratory quality assurance programs to ensure that tests are performed following manufacturer-provided specifications.

The purpose of the study was to conduct incoming quality control of enzyme immunoassay test kits to diagnose HCV infection using a qualifying panel of sera. For this purpose, four diagnostic test kits available in the market of the Republic of Uzbekistan were evaluated for the detection of antibodies to the hepatitis C virus by enzyme immunoassay. The need for research in this direction is confirmed by the results obtained and trends over time.

Introduction

Infection with the hepatitis C virus (HCV) is a significant public health threat, with an estimated 71 million people having chronic HCV infection globally [1]. Highly effective HCV treatments have made the global elimination of viral hepatitis possible [2]. The proper screening with a sensitive and specific diagnostic HCV test is vital for HCV elimination programs[3]. Despite the availability of HCV diagnostic tests, the percentage of individuals in low-and-middle-income countries aware of their status falls below 5%[4]. Underdiagnosis is also indicated as a gap in the care-and-treatment cascade in the United States [5]. In this regard, using quality HCV diagnostic assays followed by referral to treatment is vital for reaching the 2030 target indicators for eliminating HCV infection.

Affordable and quality enzyme immunoassays (EIA) play a vital role in the primary diagnosis of HCV infection. Each country participates in or develops its laboratory testing quality assurance programs to ensure tests are performed within the manufacturer's specifications. This study was conducted to evaluate the characteristics of four available EIAs in the market in Uzbekistan for detecting HCV antibodies. The study is a part of a quality assurance program aiming to develop a national HCV elimination program.

Methods

Evaluation panel

The evaluation panel was kindly provided by the Division of Viral Hepatitis, US Centers for Disease Control and Prevention (CDC, Atlanta, USA). The panel consisted of 60 well-characterized samples, which included 31 anti-HCV positive samples and 29 anti-HCV negative samples.

Anti-HCV EIA testing kits selected for evaluation

Four anti-HCV EIA kits available in the market of Uzbekistan were evaluated: (1) ELISA-ANTI-HCV (Diagnostic systems, Nizhny Novgorod, Russian Federation); (2) BEST-ANTI-HCV (VECTOR BEST, Novosibirsk, Russian Federation); (3) EIA-anti HCV (Institute of Chemistry of plant substances, Tashkent, Uzbekistan); (4) ELISA-Recombinant anti-HCV-strip (RADIOPREPARAT, Tashkent, Uzbekistan).The characteristic of the diagnostic test kits presented in **Table 1**.

Table 1: Manufacturers set characteristics of anti-HCV diagnostic test kits.

Test kit	Manufacturer characteristic		CE Marked	WHO prequalified
	Sensitivity, %	Specificity, %		
ELISA-ANTI-HCV, DS	100.0%	99.8%	Yes	No
BEST-ANTI-HCV, VB	100.0%	100.0%	No	No
EIA-anti HCV, ICPS	100.0%	99.0%	No	No
ELISA-Recombinant anti-HCV-strip, RP	100.0%	97.0%	No	No

Evaluation procedure

The testing procedure was performed according to the manufacturers' instructions. The optical density (OD) was measured in a plate reader (ELx808 Absorbance Reader, BioTek USA). Signal-to-Cutoff Ratio (SCR) above 1.0 is considered a positive result per the manufacturer's specifications.

Statistical analysis

Data analysis was performed using R software with the use of R-studio GUI, "rel" and "epiR" libraries [6,9]. The sensitivity and specificity with 95% CI were calculated. Based on the study results, the kappa statistics for inter-reader agreement between reference classification and classification [10]. The positive predictive value (PPV) and Negative Predictive Value (NPV) were calculated for the population previously reported in Uzbekistan using 7.0% prevalence of HCV infection and a population of one million [11].

Results

Performance of anti-HCV EIA diagnostic test kits

The sensitivity and specificity for four tested anti-HCV EIA test kits are presented in **Table 2**. The sensitivity varied from the highest 100.0% by ELISA-ANTI-HCV from "Diagnostic Systems" to the lowest 83.9% by BEST-ANTI-HCV by "Vector Best" with 93.5% by EIA-anti HCV from ICPS and 96.8% from ELISA-Recombinant anti-HCV-strip from "Radiopreparat." The specificity for all assays was 96.55%. The PPVs varied from 68.6% to lowest 64.7% while NPV varied from 100.0% to 98.75%. As presented in **Table 2**, the kappa statistics varied from 0.801 to 0.967, with the highest score by ELISA-ANTI-HCV from "Diagnostic Systems."

Table 2: Performance of anti-HCV EIA diagnostic test kits.

Test kit	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV*, % (95% CI)	NPV, % (95% CI)	Kappa (95% CI)
ELISA-ANTI-HCV, DS	100.0 (88.78, 100.0)	96.55 (82.24, 99.91)	68.57 (68.29, 68.86)	100.0 (N/A, N/A)	0.967 (0.9003, 1.0)
BEST-ANTI-HCV, VB	83.87(66.27, 94.55)	96.55 (82.24, 99.91)	64.66 (64.35, 64.97)	98.75(98.73, 998.78)	0.801 (0.648, 0.954)
EIA-anti HCV, ICPS	93.55 (78.58, 99.21)	96.55 (82.24, 99.91)	67.12 (66.82, 67.41)	99.5(99.49, 99.51)	0.900 (0.787, 1.0)
ELISA-Recombinant anti-HCV-strip, RP	96.77(83.3, 99.92)	96.55 (82.24, 99.91)	67.86 (67.57, 68.15)	99.75(99.74, 99.76)	0.933 (0.84, 1.0)

Notes: * The PPV/NPV were calculated for the population with a 7.0% prevalence of HCV infection and one million population.

Discussion

WHO recommends that diagnostic assays for HCV "meet minimum acceptance criteria of either WHO prequalification of in vitro diagnostics (IVDs) or a stringent regulatory review for IVDs. All IVDs should be used following manufacturers' instructions and, where possible, at testing sites enrolled in a national or international external quality assessment scheme [12]. According to the WHO data on the meta-analysis of reported EIA/RDTs IVDs studies worldwide, the overall sensitivity and specificity of HCV antibody tests varied from 0.22 to 1.00. In contrast, specificity varied from 0.77 to 1.0, underscoring the need for an independent evaluation before wide-scale use in a country. The Uzbekistan population has an estimated 7.0% HCV infection prevalence; therefore, screening for HCV infection by the EIA with NPV estimated from 98.7 to 100.0% makes the avail-

able market assays acceptable for routine screening of anti-HCV **(Table 2)**.

The kappa statistics values are generally interpreted as >0.6 as a poor predictor of positivity based on the comparator assay, 0.61-0.80 as a substantial predictor based on the comparator assay, and 0.81-1 as a test being strongly concordant. In our study, only the ELISA-ANTI-HCV by "Diagnostic Systems" performed with acceptable results with a kappa value of 0.967. While all assays had a kappa score above 0.80, we recommend a higher concordance to an FDA-approved test as the cutoff to ensure a lower level of false negatives.

The requirements for the EIA diagnosing test kits may vary depending on the purpose of the kits and their role in the diagnostic algorithm. It is strongly recommended to validate test kits before utilization in a laboratory. This ensures that the performance characteristics described by the manufacturer are valid in the testing laboratory and that the performance claims are accurate. Our laboratory used non-CE marked, except ELISA-ANTI-HCV, DS test-system, and non-WHO prequalified kits. This necessitated the need to verify the manufacturer's claims with a well-characterized set of specimens to ensure the specificity and sensitivity of the assay compared to FDA-approved tests [13].

The limitations of the study are: (1) limited number of samples in the reference panel, which precludes a strong conclusion on the performance of the test kits; (2) information regarding the characterization of samples used by manufacturers is missing, potentially biasing the sensitivity/specificity claims by the manufacturers; (3) potential issues in temperature maintenance of the kits during storage and delivery to the end-user in some more distant regions of the country. More information is required to make a stronger conclusion on the applicability of the test kits for HCV testing, as well as a requirement for developing an in-country broad HCV panel for registration of the kits, lot verification, and other purposes.

Conclusion

The WHO calls for eliminating HCV infection, a feasible goal that requires consolidation of the public health efforts in laboratory testing and referral to treatment and care. In this regard, the first initial screening with affordable, reliable assays serves as a baseline activity. The current report is an initial activity in this effort to establish the targeted laboratory activities for assuring the proper EIA assay used for primary screening of HCV infection in Uzbekistan as a part of the HCV elimination program. Considering limitations of this study, a broader assessment with an extended panel is required for establishing an in-country quality assurance program. The Research Institute of virology is currently procuring anti-HCV positive and HCV RNA positive units of blood to develop a more extensive set of reference panels that can be used to assess further, the suitability of available HCV screening EIAs for use in the elimination program in Uzbekistan.

Disclaimers

The findings and conclusions in this report do not necessarily reflect the official position of the Centers for Disease Control and Prevention, or the authors' affiliated institutions. Use of trade names and commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, or the US Department of Health and Human Services.

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