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Original Article

ELISA screening results for HAV, HBV, HCV, HIV: A retrospective study

Olgun Goktas[1], Ismail Emre Deniz[2], Ilker Ercan[3]

ABSTRACT

Objective: To retrospectively evaluate the hepatitis A, B, C, and human immunodeficiency virus screening results using the ELISA method in primary care.

Methodology: This study was conducted retrospectively using the available recorded data of individuals registered with the Bursa Uludag University Family Health Center who underwent ELISA measurement between January 2020 to December 2024. The study included data from a total of 2,918 individuals over the age of 18 who met the inclusion criteria. In statistical comparisons, α was taken as 0.05. Data analysis was performed using the IBM SPSS Statistics 28 statistical package program.

Results: Of the 2918 individuals who participated in the study, 1528 (52.4%) were female, and 1390 (47.6%) were male. The median age of the participants was 24 (18-83). Of the participants, 1355 (46.4%) were healthcare workers, while 1563 (53.6%) were not. Hospital referrals were made in 37 (1.3%) individuals, while 2881 (98.7%) were not referred. Anti-HAVIgM and HBsAg positivity was significantly higher in the non-healthcare worker group compared to the healthcare worker group (p=0.013, p=0.038). Anti-HBcIgG, anti-HBs positivity, and Hepatitis-B vaccination were higher in the healthcare worker group (p<0.001). There was a statistically significant difference between HBsAg and anti-HBs (p<0.001). Hospital referral rates were higher in those positive for Hepatitis A, B, C, and HIV antibodies, while they were significantly lower in those with anti-HBs positivity and those vaccinated against Hepatitis-B (p<0.001).

Conclusion: ELISA screenings, Hepatitis-B antibody levels and vaccination status were higher in healthcare workers, while Hepatitis-B antigen levels were higher in non-healthcare workers. Hospital referral rates were also higher in those with high Hepatitis A, B, C, and HIV antibody levels. Our ELISA screening results for infections in family medicine are important for diagnosis, treatment, follow-up, and referral to other clinics.

KEYWORDS: Enzyme-linked immunosorbent assay (ELISA), Hepatitis A virus (HAV), Hepatitis-B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV).

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1. Olgun Goktas
Associate Professor,
Uludag University Family Health Center,
Nilufer, Bursa, Turkey.
2. Ismail Emre Deniz
Department of Biostatistics,
Institute of Health Sciences,
3. Ilker Ercan
Professor, Department of Biostatistics,
Faculty of Medicine,
2-3: Bursa Uludag University, Bursa, Turkey.

Correspondence:

Dr. Olgun Goktas
Associate Professor,
Uludag University Family Health Center,
Nilufer, Bursa, Turkey.
Email: olgun_goktas@hotmail.com

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INTRODUCTION

Enzyme-linked immunosorbent assays (ELISA) detect antigen-antibody interactions using enzyme-labeled conjugates and enzyme substrates that produce color changes. Today, ELISA techniques are widely used for the diagnosis and monitoring of a variety of diseases, including those related to endocrine, metabolic, cardiovascular, and microbiological disorders. ELISA testing is one of the most sensitive methods for measuring substances such as peptides, proteins, and hormones, and can detect even the smallest concentrations ranging from 5 pg/ml to 10 pg/ml. The sensitivity of ELISA tests is determined by the characteristics of the antibody-antigen interaction. To ensure accurate and high-quality results for the diagnosis and monitoring of disorders, standardized ELISA procedures and well-trained personnel are vital. This includes appropriate sample collection, transportation, analysis, and capacity.

Regular in-service training helps ensure consistent processes, criteria, and results. Improving laboratory conditions and addressing potential sources of error can increase patient comfort, reduce the burden on national health systems, and produce more reliable results.[1]

ELISA is a laboratory technique that detects antibodies, antigens, proteins, and hormones in body fluids such as blood, plasma, urine, saliva, and cerebrospinal fluid.[2] Hepatitis B (HBV), C (HCV), and human immunodeficiency virus (HIV) can coexist in drug users, requiring community-based treatment and monitoring.[3] Accurate diagnosis of viral hepatitis, which can be classified as hepatitis A, B, C, D, and E, is crucial for appropriate treatment.[4]

ELISA has higher sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) ratios in detecting HBV, HCV, and HIV, and has higher diagnostic accuracy and lower error rates compared to rapid diagnostic techniques.[5] ELISA tests are reported to be effective in identifying varicella zoster virus,[6] in determining the clinical characteristics of parameters in HCV,[7] in demonstrating tumor markers in liver cancer with co-infection of Hepatitis-B and C viruses,[8,9] and in evaluating DNA in severe viral infections.[10] The Dried Blood Blot (DBS) based ELISA method has shown good performance in simultaneously screening anti-HIV, anti-HCV, and anti-TP (anti-treponema pallidum) antibodies.[11] In populations at risk of HIV transmission, the sensitivity, specificity, and robustness of the ELISA test in evaluating reciprocal antibody endpoint titers in human serum are highlighted.[12] Hepatitis infections are globally prevalent in occupations such as cleaning workers and require preventive measures.[13]

The World Health Organization's "Global Status Report on Blood Security and Supply 2021" provides comprehensive data and analysis on blood security and supply worldwide. It covers key areas such as the organization and management of national blood transfusion services, global blood collection trends, donor profiles, and laboratory screening of blood donations for infectious diseases. The report aims to guide policymakers and health professionals in strengthening blood security measures and ensuring adequate blood supplies.[14]

ELISA is used in family medicine practice to screen individuals and patients for HAV, HBV, HCV, and HIV infections. These infections, usually sexually transmitted, pose a risk to the general population and especially to healthcare personnel, medical, dental, and nursing students. ELISA screenings and follow-up of results in family medicine are important for the detection, differential diagnosis, and other health-affecting conditions of these infections. Early treatment should be provided in the primary care process by identifying the contributing factors. While there are reports in the literature regarding the factors causing and affecting these infections and their consequences, studies on infection status in specific regions and populations are insufficient. Furthermore, the numerous diseases and causes of these infections, and their combinations, create different situations and require careful analysis during the decision-making process. In this respect, the evaluation of registered individual data from family medicine, which has a large population, is important in contributing to this gap in the literature.

In this study, we aimed to evaluate the ELISA screening results for HAV, HBV, HCV, and HIV infections performed on individuals registered in a family health center.

METHODOLOGY

This study was conducted retrospectively using existing recorded data from individuals registered at Uludağ University Family Health Center. The study retrospectively evaluated the results of ELISA blood tests for HAV, HBV, HCV, and HIV infections administered to individuals registered at the Family Health Center between January 1, 2020 to December 31, 2024, over five-year. Data from 2918 registered individuals over the age of 18 were examined. Data were obtained anonymously from the Family Medicine Information System (AHBS). The analysis considered factors such as whether registered individuals were healthcare professionals, antigen and antibody marker results of infections from ELISA tests, vaccination status, treatment, follow-up, and hospital referrals. Analyses were performed using SPSS 28.0 (Statistical Packages of Social Sciences).

Ethical approval: The study was performed after approval from the Health Research Ethics Committee of Bursa Uludag University, Faculty of Medicine (Ref. Dated February 4, 2026/ decision no:2026/57-3/1) following the Declaration of Helsinki.

Statistical analysis: The Shapiro-Wilk test was applied to examine whether the continuous variable conformed to a normal distribution. Since it did not conform to a normal distribution, descriptive values were presented as median (minimum:maximum). Categorical data are given as n (%); Pearson Chi-Square test and Fisher's Exact test were used to analyze categorical data. α=0.05 was used for statistical comparisons. Data analysis was performed using the IBM SPSS Statistics 28 statistical package program.

Inclusion and exclusion criteria: Individuals registered at the family health center between 2020 and 2024, and who had their HIV, HAV, HBV, and HCV measurements using the ELISA method taken were included in the study. Individuals who migrated or left the region for various reasons, or individuals located outside the region, or whose ELISA measurements were not available, were excluded from the study.

RESULTS

A total of 2918 individuals participated in the study. The median age of the participants was 24. The minimum age was 18, while the maximum age was 83. Of the participants, 1390 (47.6%) were male and 1528 (52.4%) were female. 1355 (46.4%) of the participants were healthcare professionals, while 1563 (53.6%) were not. 37

Table-I: Percentage distribution of infections.

Variable	*n (%)*
Anti-HAVIgM	
No	2907 (99.6%)
Yes	11 (0.4%)
Anti-HAVIgG	
No	1697 (58.2%)
Yes	1221 (41.8%)
Anti-HBcIgM	
No	2912 (99.8%)
Yes	6 (0.2%)
Anti-HBcIgG	
No	1404 (48.1%)
Yes	1514 (51.9%)
HbsAg	
No	2906 (99.6%)
Yes	12 (0.4%)
AntiHBs	
No	1165 (39.9%)
Yes	1753 (60.1%)
Hepatitis-B vaccination status	
No	1210 (41.5%)
Yes	1708 (58.5%)
Anti-HCV	
No	2914 (99.9%)
Yes	4 (0.1%)
Anti-HIV	
No	2913 (99.8%)
Yes	5 (0.2%)
Total	2918 (100%)

(1.3%) individuals were referred to a hospital, while 2881 (98.7%) individuals were not referred.

When infection status was examined; Anti-HAVIgM; positive in 11 (0.4%) individuals, negative in 2907 (99.6%) individuals. Anti-HAVIgG; positive in 1221 (41.8%) individuals, negative in 1697 (58.2%) individuals. Anti-HBcIgM; positive in 6 (0.2%) individuals, negative in 2912 (99.8%) individuals. Anti-HBcIgG; positive in 1514 (51.9%) individuals, negative in 1404 (48.1%) individuals. HBsAg; positive in 12 (0.4%) individuals, negative in 2906 (99.6%) individuals. Anti-HBs; positive in 1753 (60.1%) individuals, negative in 1165 (39.9%) individuals. Hepatitis-B vaccine; 1708 (58.5%) individuals tested positive, while 1210 (41.5%) did not. Anti-HCV was positive in 4 (0.1%) individuals and negative in 2914 (99.1%) individuals. Anti-HIV was positive in 5 (0.2%) individuals and negative in 2913 (99.8%) individuals (Table-I).

There is a statistically significant difference between HBsAg and anti-HBs ($p<0.001$). The proportion of anti-HBs positive individuals was 8.3% in HBsAg positive individuals, while it was 60.3% in HBsAg negative individuals. Similarly, the proportion of anti-HBs positive individuals was 91.7% in HBsAg negative individuals, while it was 39.7% in HBsAg negative individuals. These findings indicate that HBsAg positivity is significantly lower in anti-HBs positive individuals and significantly higher in anti-HBs negative individuals (Table-II).

In cases where HBsAg and anti-HBs were negative, Hepatitis-B vaccination was present in 12 (1.0%) individuals, while it was not present in 1142 (99.0%) individuals (Table-III).

There is a statistically significant difference between anti-HAVIgM status and hospital referral ($p<0.001$). The hospital referral rate was 100.0% in anti-HAVIgM positive individuals, while it was 0.9% in anti-HAVIgM negative individuals. The rate of individuals without hospital referral was 0.0% in anti-HAVIgM positive individuals, while it was 99.1% in anti-HAVIgM negative individuals. These findings indicate that anti-HAVIgM positivity is significantly higher in the group that received hospital referrals, while the rate of anti-HAVIgM positivity is significantly lower among those who did not receive hospital referrals.

There is a statistically significant difference between anti-HBs status and hospital referral ($p<0.001$). The hospital referral rate was 0.7% in anti-HBs-positive

Table-III: Hepatitis-B vaccination status while HBsAg and Anti-HBs are negative.

Hepatitis-B vaccination status	*n (%)*
No	1142 (99.0%)
Yes	12 (1.0%)

Table-II: Investigation of the relationship between HBsAg and Anti-HBs.

		Anti-HBs		
		No	*Yes*	*p*
HBsAg	No	1154 (39.7%)	1752 (60.3%)	<0,001
	Yes	11 (91.7%)	1 (8.3%)	

individuals, while it was 2.1% in anti-HBs-negative individuals. The rate of non-hospital referral was found to be 99.3% among individuals who were anti-HBs positive, while it was 97.9% among individuals who were anti-HBs negative. These findings indicate that anti-HBs positivity was significantly higher in the group that did not receive hospital referrals, while the rate of anti-HBs positivity was significantly lower among those who were referred to hospitals.

There is a statistically significant difference between anti-HCV status and hospital referral (p<0.001). The hospital referral rate was 75.0% in anti-HCV positive individuals, while it was 1.2% in anti-HCV negative individuals. The rate of individuals without hospital referral was 25.0% in anti-HCV positive individuals, while it was 98.8% in anti-HCV negative individuals. These findings indicate that anti-HCV positivity is significantly higher in the group that was referred to the hospital, while the rate of anti-HCV positivity is significantly lower among those who were not referred.
There is a statistically significant difference between anti-HIV status and hospital referral (p<0.001). The hospital referral rate was 100.0% in anti-HIV positive individuals, while it was 1.1% in anti-HIV negative individuals. The rate of individuals without hospital referral was 0.0% in anti-HIV positive individuals, while it was 98.9% in anti-HIV negative individuals. These findings indicate that anti-HIV positivity is significantly higher in the group that received hospital referrals, while the rate of anti-HIV positivity is significantly lower among those who did not receive hospital referrals.

There was no statistically significant difference between anti-HAV IgG status and hospital referral (p=0.066) (Table-IV).

There is a statistically significant difference between HBsAg status and being a healthcare worker (p=0.038). The proportion of healthcare workers among HBsAg-positive individuals was 16.7%, while this figure was 46.6% among HBsAg-negative individuals. The proportion of non-healthcare workers among HBsAg-positive individuals was 83.3%, compared to 53.4% among HBsAg-negative individuals. These findings indicate that HBsAg positivity is significantly higher in the non-healthcare worker group, while the rate of HBsAg positivity is significantly lower among healthcare workers.

There is a statistically significant difference between anti-HBs status and being a healthcare worker (p<0.001).

Table-IV: Examining the relationship between hospital referrals and infections.

		Referral to Hospital		
Infections		*No*	*Yes*	*p*
Anti-HAVIgM	No	2881 (99.1%)	26 (0.9%)	<0.001
	Yes	0 (0.0%)	11 (100.0%)	
Anti-HAVIgG	No	1670 (98.4%)	27 (1.6%)	0.066
	Yes	1211 (99.2%)	10 (0.8%)	
Anti-HBclgM	No	2881 (98.9%)	31 (1.1%)	<0.001
	Yes	0 (0.0%)	6 (100.0%)	
Anti-HBclgG	No	1379 (98.2%)	25 (1.8%)	0.017
	Yes	1502 (99.2%)	12 (0.8%)	
HBsAg	No	2881 (99.1%)	25 (0.9%)	<0.001
	Yes	0 (0.0.%)	12 (100.0%)	
Anti-HBs	No	1140 (97.9%)	25 (2.1%)	<0.001
	Yes	1741 (99.3%)	12 (0.7%)	
Hepatitis-B vaccination status	No	1182 (97.7%)	28 (2.3%)	<0.001
	Yes	1699 (99.5%)	9 (0.5%)	
Anti-HCV	No	2880 (98.8%)	34 (1.2%)	<0.001
	Yes	1 (25.0%)	3 (75.0%)	
Anti-HIV	No	2881 (98.9%)	32 (1.1%)	<0.001
	Yes	0 (0.0.%)	5 (100.0%)	

Table-V: Examining the relationship between infections based on the status of being a healthcare worker.

		Healthcare Worker		
Infections		*No*	*Yes*	*p*
Anti-HAVIgM	No	1553 (53.4%)	1354 (46.6%)	0,013
	Yes	10 (90.9%)	1 (9.1%)	
Anti-HAVIgG	No	922 (54.3%)	775 (45.7%)	0,327
	Yes	641 (52.5%)	580 (47.5%)	
Anti-HBclgM	No	1562 (53.6%)	1350 (46.4%)	0,103
	Yes	1 (16.7%)	5 (83.3%)	
Anti-HBclgG	No	940 (67.0%)	464 (33.0%)	<0,001
	Yes	623 (41.1%)	891 (58.9%)	
HBsAg	No	1553 (53.4%)	1353 (46.6%)	0,038
	Yes	10 (83.3%)	2 (16.7%)	
Anti-HBs	No	1111 (95.4%)	54 (4.6%)	<0,001
	Yes	452 (25.8%)	1301 (74.2%)	
Hepatitis-B vaccination status	No	1151 (95.1%)	59 (4.9%)	<0,001
	Yes	412 (24.1%)	1296 (75.9%)	
Anti-HCV	No	1561 (53.6 %)	1353 (46.4%)	1,000
	Yes	2 (50.0%)	2 (50.0%)	
Anti-HIV	No	1559 (53.5%)	1354 (46.5%)	0,381
	Yes	4 (80.0%)	1 (20.0%)	

The proportion of healthcare workers among anti-HBs positive individuals was 74.2%, while this proportion was 4.6% among anti-HBs negative individuals. The proportion of non-healthcare workers among anti-HBs positive individuals was 25.8%, while this proportion was 95.4% among anti-HBs negative individuals. These findings indicate that anti-HBs positivity is significantly higher in the healthcare worker group, while it is significantly lower among non-healthcare workers.

There is a statistically significant difference between Hepatitis-B vaccination status and being a healthcare worker ($p<0.001$). The proportion of healthcare workers among vaccinated individuals was 75.9%, while among unvaccinated individuals, the proportion of healthcare workers was 4.9%. The proportion of non-healthcare workers among vaccinated individuals was 24.1%, while among unvaccinated individuals, the proportion of non-healthcare workers was 95.1%. These findings indicate that Hepatitis-B vaccination is significantly higher in the healthcare worker group, while it is significantly lower among non-healthcare workers.

We also found that there was no statistically significant difference between anti-HAVIgG, anti-HBcIgM, anti-HCV, and anti-HIV status and being a healthcare worker ($p=0.327$, $p=0.103$, $p=1.000$, $p=0.381$, respectively) (Table-IV).

DISCUSSION

In this study, Hepatitis-B antibody levels and vaccination status were higher in healthcare workers as determined by ELISA testing. Conversely, Hepatitis-B antigen levels were higher in non-healthcare workers. Hospital referral rates were lower in those with high Hepatitis-B antibody levels and those who were vaccinated against Hepatitis-B. Hospital referral rates were also higher in those with high Hepatitis-B antigen levels and high levels of HAV, HBV, HCV, and HIV antibodies.

The contagiousness of viral infections and their co-occurrence with bacterial infections necessitate the optimization of clinical treatment strategies. Studies using ELISA in HIV patients report a high incidence of hepatitis and syphilis co-infection, highlighting the importance of serological tests for early diagnosis of these infections.[15] Although Hepatitis-B infection is controlled by regular vaccination programs, the

variability of antibody levels over time necessitates the measurement of HBV infection using a reliable method.[16]

In congenital hypothyroidism, serum TSH levels can be determined,[17] IL-17 and eotaxin levels can be determined in allergic asthma attacks and remission,[18] and insulin and insulin-like factors can be detected with high sensitivity using ELISA during pregnancy.[19] While PCR results gave false negative results in the laboratory diagnosis of Crimean-Congo hemorrhagic fever (CCHV) virus, ELISA yielded accurate results.[20] In our study, the diagnostic accuracy rates of hepatitis and HIV infections with the ELISA test were high and similar to the results in the hospital for referred patients. In rickettsial infections, the ELISA test was also found to have high sensitivity, specificity and diagnostic accuracy, and this feature was comparable to reference standards.[21]

It is emphasized that the immunosuppressive effect of ruxolitinib, used for myeoproliferative neoplasms, reactivates the Hepatitis-B virus, and that screening for Hepatitis-B infection is necessary to control this effect. Clinicians are strongly advised to screen for HBV to reduce complications and ensure maximum treatment.[22] The relationship of Vitamin-D with female hormones such as Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and Anti-Müllerian Hormone (AMH) has been demonstrated using the ELISA method.[23]

A study conducted on pregnant women and students indicated that standard tests may be insufficient to detect the antigen due to the presence of the transmembrane domain in the S gene of HBV. The study reported that measurements were made using the ELISA method and that the antigen and vaccination status were determined more reliably.[24] In a study conducted on dental patients, HBaAg and anti-HCV levels were first measured with ICT and then confirmed with ELISA.[25]

ELISA testing is used to report co-infections of herpes simplex virus (HSV), herpes zoster (HZ), HBV, HCV, HIV infections, and psoriasis.[26] In a study in Italy, anti-HBs levels were measured in serum samples using ELISA, and the presence of anti-HBc and HBsAg was evaluated. The study reported that the prevalence of anti-HBs was 30%, and a significant decrease in seropositivity was observed with increasing age. It is stated that as a result of the regular HBV vaccination strategy, anti-HBc and HBsAg markers show a decrease, especially in younger age groups.[27] In our study, while anti-HAVIgM and anti-HBcIgM levels were quite low, anti-HAVIgG and anti-HBcIgG levels were positive in approximately half of the cases.

In India, medical students who donated blood were tested for anti-HBsIgG, HBsAg, and HIV levels using ELISA. Only one student was found to have HIV infection, while more than half lacked protective anti-HBs titers against HBV and did not have sufficient immunity. The study's results highlight the importance of ELISA screening for evaluating anti-HBs titers.[28] In our study, anti-HIV was positive in only 5 individuals, while the vast majority had negative results.

In a similar study, ELISA hepatitis markers detected infection in less than half of healthcare workers, while more than half showed results indicating acquired immunity.[29] In our study, 58.5% of cases had received the Hepatitis-B vaccine, while the remaining cases were unvaccinated. There was a statistically significant difference between HBsAg and anti-HBs in our study. Only 12 cases (0.4%) were found to be HBsAg positive, while 1753 individuals (60.1%) were anti-HBs positive. In cases where both HBsAg and anti-HBs were negative, only 12 individuals had received the Hepatitis-B vaccine, while the other 99% did not. Therefore, it is important to routinely screen for Hepatitis-B infections using reliable ELISA methods and to administer necessary preventive Hepatitis-B vaccinations.

In our study, HBsAg positivity was higher in non-healthcare workers. Conversely, Hepatitis-B vaccination status and anti-HBs positivity were higher in healthcare workers. In our study, hospital referral rates were higher in cases of anti-HAVIgM, anti-HBcIgM, anti-HCV, anti-HIV, and HBsAg positivity. Conversely, hospital referral rates were lower in those who were anti-HBs positive and those who had been vaccinated against Hepatitis-B.

In a local population study, a fairly common genome of HCV was tested by ELISA. HCV prevalence was determined to be 13.67%, while anti-HCV levels were higher in men than in women, and coinfection was detected in half of the patients.[30] In one study, approximately half of the hospitalized patients with chronic kidney disease were positive for HCV infection by ELISA.[31] In our study, anti-HCV was positive in only 4 individuals, and the vast majority had negative results.

A study of HIV and HBV prevalence among adolescents in Nigeria using ELISA techniques found that HIV prevalence was higher in males than in females. There was no difference between genders in HBV infection. However, factors such as age, sexual activity, and blood transfusions were associated with an increased risk for both infections. The study highlights the urgent need for education on safe practices, routine screening, and vaccination programs to reduce the risks of HIV and HBV.[32]

Our study found differences in ELISA test results for HAV, HBV, HCV, and HIV infection markers, Hepatitis-B vaccination status, hospital referral status, and between healthcare professionals and non-healthcare professionals in our region. It is crucial to make informed decisions about the varying ELISA test levels in data obtained from ELISA screening results in primary healthcare settings within family medicine practice. Evaluations, follow-up, and treatment of patients when referring to secondary and tertiary healthcare institutions are vital. Sharing information on infection marker levels and influencing factors in ELISA screening test results for different conditions in the literature is important. Considering that ELISA test infection screening results in blood can lead to different outcomes and abnormal processes for various reasons, we emphasize the importance of a holistic approach by family physicians and their collaboration with other clinics.

Strengths and Limitations: The strengths of the study are the frequent and practical use of the HAV, HBV, HCV, and HIV screening results using the ELISA method in family medicine and the creation of a large database for research. Since our study is retrospective, the possibility that some of the results of consultations at other clinics were not included in our records may have created a limitation. Additionally, individuals who left our region or migrated abroad may have also created limitations.

CONCLUSION

ELISA screenings showed that Hepatitis-B antibody levels and vaccination status were higher in healthcare workers, while Hepatitis-B antigen levels were higher in non-healthcare workers. Hospital referral rates were also higher in those with high Hepatitis A, B, C, and HIV antibody levels. Our ELISA screening results for infections in family medicine are important for diagnosis, treatment, follow-up, and referral to other clinics.

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