Erciyes Med J 2021; 43(5): 465–9 • DOI: 10.14744/etd.2021.34682
ORIGINAL ARTICLE – OPEN ACCESS

An Evaluation of the Effect of the Clinical Features of Patients and the Drugs Used on the False-Positive EIA Test for HIV

Filiz Orak[1], Adem Doğaner[2], Kezban Tülay Yalçınkaya[1], Murat Aral[1]

ABSTRACT

Objective: Human immunodeficiency virus (HIV) false positivity is one of the common misdiagnoses in laboratories. Thus, only positive results obtained by confirmatory tests should be considered positive while other types of screening tests should instead be referred to as reactive. This study investigated false HIV positivity via the sociodemographic characteristics of the patient, clinical diagnosis, and the relationship with the drugs used.

Materials and Methods: From January 2017 to October 2018, 25,180 patients were tested for HIV with the fourth-generation antigen/antibody EIA. Reactive test results were sent to the Public Health Institution in Turkey to confirm the test results with immunoblot or polymerase chain reaction.

Results: Of the samples, 23 (0.091%) and 113 (0.44%) were found to be HIV-positive and HIV-negative. Confirmation tests were performed on 136 samples. Consequently, 113 samples were confirmed to be negative and, therefore, considered as false positives. A distributional relationship was found between HIV false positivity and patients hospitalized in clinics of infectious diseases, hematology, and orthopedics (χ^2=49.048; p=0.001), and patients diagnosed with neoplasm or soft tissue infection (χ^2=51.699; p=0.001). Moreover, the rate of false positivity significantly increased with the use of antibiotics, steroidal/nonsteroidal drugs, immunoglobulin preparations, and antithrombotic drugs.

Conclusion: Since HIV tests used in a low prevalence population are commonly testing low-risk individuals, the reactive tests can lead to false-positive results rather than true-positive. Thus, screening test results alone should not be relied upon for this diagnosis.

Keywords: Anti-HIV positivity, cross-reactions, enzyme immunoassay, human immunodeficiency virus, tests

Cite this article as:
Orak F, Doğaner A, Yalçınkaya KT, Aral M. An Evaluation of the Effect of the Clinical Features of Patients and the Drugs Used on the False-Positive EIA Test for HIV. Erciyes Med J 2021; 43(5): 465-9.

[1]Department of Microbiology, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey
[2]Department of Biostatistics and Medical Informatics, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey

Submitted
19.11.2020

Accepted
18.01.2021

Available Online Date
12.07.2021

Correspondence
Filiz Orak,
Kahramanmaraş Sütçü İmam University, Department of Microbiology, Kahramanmaraş, Turkey
Phone: +90 506 337 10 46
e-mail: drfilizorak@hotmail.com

INTRODUCTION

Human immunodeficiency virus (HIV) is an enveloped retrovirus from the lentiviral subfamily. The virus causes a chronic disease characterized by acquired-immune deficiency syndrome (AIDS), which is accompanied by opportunistic infections as a result of immune system suppression. Two types of this virus, HIV-1 and HIV-2, exist.

HIV-1 is the most common type of virus worldwide. HIV-2 is most common in West Africa. However, it has also been isolated in Europe, Brazil, and India. HIV has a core region that is surrounded by an envelope employing glycoproteins known as gp120 and gp41. The genome contains gag, pol, and env genes; six regulatory genes; and many accessory genes. Initial testing methods used viral lysates as antigens, the second-generation testing combined recombinant HIV proteins and synthetic peptides, and the third-generation testing coidentified IgG and IgM by the sandwich method. Today, the fourth-generation enzyme immunoassay (EIA) methods are commonly used to simultaneously detect the p24 antigen and the HIV enveloped antibodies (1). The interference event, also called cross-reaction, continues despite the increase in sensitivity and specificity of the EIA techniques over the years. Therefore, the obtained result is considered a cross-reaction when a reactive test result is obtained even though the person is not infected with HIV.

Substances that alter the measurable concentration of the analyte tested or alter antibody binding can potentially lead to cross-reactivity, which has been termed a reactive result (2). In the case of a cross-reaction, regardless of analyte concentration, the presence of hemolysis, lipemia, anticoagulant, and storage in the sample is important. The analytical dependent factors are endogenous substances such as polyreactive antibodies, autoantibodies (heterophile), rheumatoid factor, or anti-animal (mouse) antibodies used for therapeutic purposes. In addition, the binding of Ca^{+2}/Mg^{+2} ions to drugs or proteins in the serum can alter the antigen structure and the measurable analyte density (3). False-positive HIV EIA tests are often correlated with autoimmune disease, renal failure, blood transfusion, multiple pregnancies, lymphoma, multiple sclerosis, liver disease, viral diseases, malignancy, tuberculosis, hemodialysis, cystic fibrosis, and recent rabies or influenza vaccination (1, 4). Furthermore, the use of biotin has been reported recently to may cause cross-reactions in tests (5). In 2018, the Center for Disease Control

Table 1. Sociodemographic characteristics of patients

	Patient group	Control group	Test statistics	p
Age, Mean±SD	44.88±19.85	43.77±21.61	0.401[a]	0.689
Gender, n (%)				
Male	36.00 (48.60)	38.00 (51.40)	0.880[b]	0.480
Female	77.00 (50.00)	77.00 (50.00)		

SD: Standard deviation; a: Independent-sample t-test; b: Chi-square test; α=0.05

updated their HIV diagnosis algorithm, which led to the identification of acute HIV-1 infection and resulted in a faster and more accurate differential diagnosis of HIV-2 infection. According to this algorithm, samples with a reactive screening test (fourth-generation antigen/antibody EIA) should be confirmed with the HIV-1/2 antibody discriminant rapid confirmation test. No additional testing is required if positivity is detected. The presence of acute infection should be excluded by testing the HIV RNA if both HIV-1 and HIV-2 tests are negative or indeterminant in the HIV-1/2 differential rapid confirmation tests (6). The most important disadvantages of antibody detection methods such as line immunoassay (LIA) and western blot (WB), which have similar sensitivity to first-generation EIA, are that they cannot detect positivity in the early period of the infection. No diagnostic method currently exists to detect infection in the first 8–10 days, known as the window period following infection. The detection of viral RNA can be achieved by nucleic acid tests (NAT) after an average of 10 days of infection at the end of the window period. The detection of the p24 antigen can be achieved by EIA 4–10 days thereafter. In the early period, the p24 antigen detection or NAT should be performed for diagnosis when antibody development cannot be monitored. However, due to the short-term and low sensitivity of the p24 antigen detection, HIV RNA detection is the most commonly used method in the diagnosis of acute HIV infection (1).

Thus, the study aims to evaluate the effect of the clinical features of patients and their used drugs on the false-positive EIA test for HIV.

MATERIALS and METHODS

Study Place and Design

A retrospective study was carried out at the Microbiology Laboratory of Kahramanmaraş Sütçü İmam University. The study included cases of false positives for HIV testing with the routinely used fourth generation antigen/antibody EIA.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of Kahramanmaraş Sütçü İmam University and strictly followed the institution's ethical guidelines. (Ethical identification number: meeting no, 2019/12; meeting date, 3 July 2019; decision no: 08). Written informed consent was obtained from all participants.

Patients and Data Collection

Between January 2017 and October 2018, 25,180 patients who applied to the Kahramanmaraş Sütçü İmam University Hospital clinics with different complaints were subjected to fourth-generation HIV enzyme-linked immunosorbent assay (ELISA) tests. All patients' data were collected from electronic health records. The data involved patients' demographic information (age and gender), laboratory tests, the type of drugs used, and the clinics applied.

In line with the national guidelines, the ELISA test was repeated using another test kit or a second blood sample was taken when reactivity was detected with the Liaison XL ELISA (DiaSorin, Italy). The sample was sent to the confirmation center, the Turkish Public Health Agency, if reactive results were obtained in two of the three tests. Tests that were found to be negative as a result of the confirmation test were considered as HIV false-positive. The results of patients who were HIV-negative with EIA tests and HIV-positive with confirmation tests were excluded from the study.

The patients were divided into two groups (HIV false-positive group and HIV-negative group). The sociodemographic characteristics, clinical diagnosis, and the drugs used by the patients were compared according to groups.

Statistical Analysis

The suitability of the variables to the normal distribution of the data was examined using the Kolmogorov–Smirnov test. Group comparisons of variables with abnormal distribution were performed using the independent-samples t-test. The distribution among categorical variables was examined using the chi-square test and exact test. The statistically significant value was $p<0.05$. Data were analyzed with IBM SPSS statistical software (IBM SPSS for Windows v. 22, IBM Corporation, Armonk, NY, USA).

RESULTS

Of the 136 samples found to be reactive with the EIA test, 23 (0.091%) and 113 (0.44%) samples were confirmed positive and negative, respectively, according to the results from the HIV Confirmation Center. The HIV false-positive test population consisted of 36 (48.60%) females and 77 (50%) males. The mean age of the patients and the control group was 44.7±4.3 and 43.77±21.61 years old, respectively (Table 1). According to the statistical analysis, no relationship exists between the sociodemographic characteristics of the patients and HIV false positivity. However, a distributive relationship was noted between HIV false positivity and diseases. Accordingly, HIV false positivity was significantly increased in patients with a neoplasm or soft tissue infection (χ^2=51,699; p=0.001) compared with other patients. Similarly, a distributional relationship was found between the clinics of the patients and HIV false positivity. The misleading test results were found to be higher in hematology, orthopedics, and infectious diseases clinics compared to other clinics (χ^2=49,048; p=0.001). While no distributive relationship was found between the drugs used and the false positivity, the rate of false positives was found to be higher in patients receiving antibiotics, steroidal/nonsteroidal drugs, immunoglobulin preparations, and antithrombotic drugs (Table 2; Fig. 1, 2).

DISCUSSION

Serological tests are the most commonly used tests in the diagnosis of HIV infection. The type of test, the ease of use of the test, the infrastructure of the laboratory, and the technical competence of

Table 2. Comparison of HIV false-positive and HIV-negative groups according to the sociodemographic characteristics and clinical diagnosis of the patients and the drugs used

	HIV false-positive		HIV-negative		χ^2	p
	n	%	n	%		
Disease[a]					**51.699**	**0.001***
Diabetes mellitus	7	70.0	3	30.0		
Bone fractures	5	100.0	0	0.0		
Soft tissue infection	13	76.5	4	23.5		
Hypothyroidism	7	77.8	2	22.2		
Hyperlipidemia + cardiovascular disease	9	36.0	16	64.0		
Liver disease	15	57.7	11	42.3		
Rheumatoid arthritis	2	20.0	8	80.0		
Infertility treatment	12	54.3	11	45.6		
Neoplasm	20	80.0	5	20.0		
Pregnancy	7	25.0	21	75.0		
Other	32	36.0	57	64.0		
Drugs[b]					16.255	0.180
Antibiotic	14	87.5	2	12.5		
Antidiabetic	7	77.8	2	22.2		
Antiepileptic	4	100.0	0	0.0		
Antihypertensive	6	54.5	5	45.5		
Antineoplastic	2	100.0	0	0.0		
Anticoagulant–antithrombotic	6	85.7	1	14.3		
Immunoglobulin preparations	3	100.0	0	0.0		
Steroidal–nonsteroidal anti-inflammatory	20	76.9	6	23.1		
Vitamin B	3	100.0	0	0.0		
Hypolipidemic	3	100.0	0	0.0		
Other	13	61.9	8	38.1		
Clinic					**49.048**	**0.001***
Emergency	5	83.3	1	16.7		
Neurosurgery	2	50.0	2	50.0		
Internal medicine	3	75.0	1	25.0		
Dermatology	2	40.0	3	60.0		
Endocrinology and metabolic disease	5	71.4	2	28.6		
Infectious disease and clinical microbiology	13	81.3	3	18.8		
Gastroenterology	8	34.8	15	65.2		
Surgery	2	22.2	7	77.8		
Breast surgery	5	31.3	11	68.8		
Chest disease	0	0.0	0	0.0		
Eye disease	1	100.0	0	0.0		
Hematology–oncology	9	90.0	1	10.0		
Gynecology	22	37.3	37	62.7		
Cardiovascular surgeon	2	40.0	3	60.0		
Cardiology	7	70.0	3	30.0		
Ear–nose–throat disease	3	50.0	3	50.0		
Nephrology	0	0.0	1	100.0		
Neurology	4	57.1	3	42.9		
Orthopedics	8	88.9	1	11.1		
Pediatrics	1	12.5	7	87.5		
Plastic surgery	3	60.0	2	40.0		
Rheumatology	0	0.0	4	100.0		
Medical oncology	6	75.0	2	25.0		
Urology	2	40.0	3	60.0		

a: Patients may have multiple diseases. Therefore, the total number of n can be exceeded. b: Patients may use more than one drug, and the total number of n can be exceeded. *: Chi-square test; exact test; α=0.05

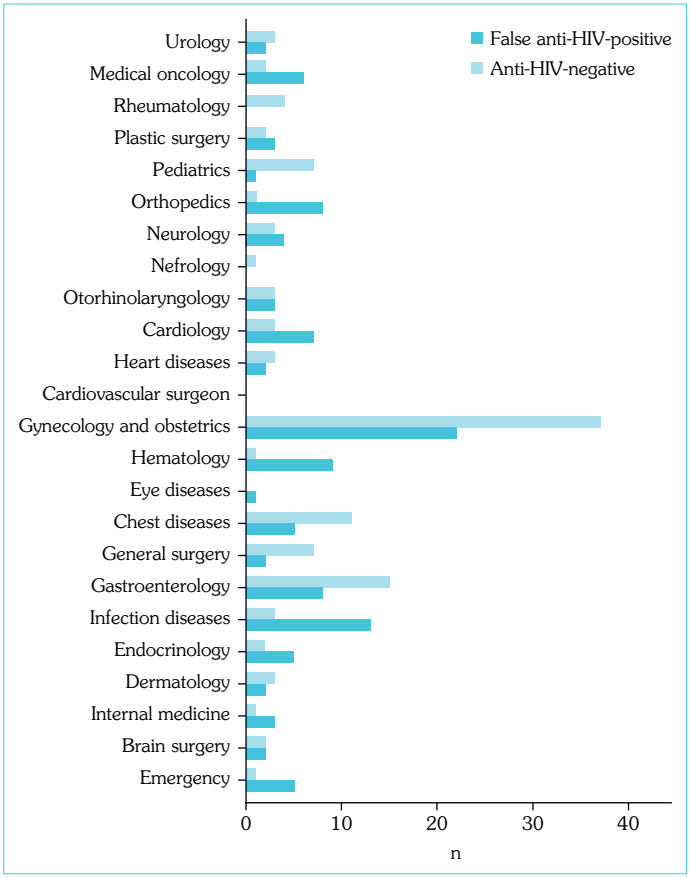

Figure 1. Clinical diagnoses of patients with false HIV positivity

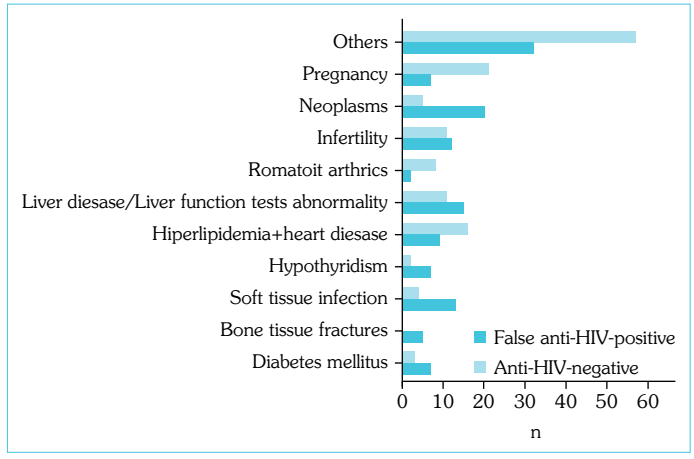

Figure 2. Clinical diagnoses of false-positive and HIV-positive patients that may cause cross-reactivity

the staff who will perform the test are all variables that are used for the selection of the appropriate test. Interpretation of all tests should be performed within the time specified in the kit and should not be evaluated before or after the incubation periods. The sensitivity of the screening tests and the specificity of the confirmatory tests are expected to be high. EIA tests are fast, safe, and economical for the diagnosis of HIV infection. However, antibody tests could be negative during the window period, even with active viral replication. Furthermore, the possibility of false negativity was considerably reduced by the fourth-generation EIA tests as they had the shortest window period and could detect p24 antigens as well as HIV-1 and HIV-2 antibodies (1).

False positives could exist due to biological causes, test kits, reagents, or devices. The EIA tests are considered screening tests while the antibody discriminant confirmation tests (i.e., WB, LIA, and indirect immune-fluorescence antibody test) are used for confirmation purposes. Among the screening tests used, the fourth-generation EIA tests were found to be higher in sensitivity than other tests and could detect infection at an early stage on day 14. Tests, such as WB or LIA, that only detect antibodies will yield negative or indeterminate results if the reactivity detected by the fourth-generation EIA is due to the antigen. The second sample can be confirmed to be positive for antibodies approximately 2 weeks later. Alternatively, p24 antigen or HIV RNA can be examined in a sample with antigen reactivity (1). The positive predictive value of the screening tests is decreased in regions with low prevalence. Therefore, rapid tests based on immunochromatography were developed to be used as an alternative to classical EIA tests for the screening of difficult-to-reach populations or occupational contact with blood and body fluids in health personnel and in cases of emergency where the mother is infected with HIV during childbirth. However, this evaluation is subjective in these tests. False positivity has occurred using rapid diagnostic tests due to misinterpretation, cross-reactions of blood products, and positive reactivities in the commercial products. Thereby, interpreting weak positive test lines as reactive rather than indeterminate for rapid diagnostic tests increased the risk of misinterpreting test results. Moreover, several inflammatory reactions appeared to induce the formation of heterophile antibodies, which could result in cross-reactions (6). Heterophilic antibodies bind noncompetitively to the conjugate, enzyme, or other moieties. In addition, false negativity can be observed by EIA as B cell dysfunction and rheumatoid factor positivity. In the early immune response, activation of CD + 5 B-lymphocytes leads to the production of broad-spectrum antibodies, which caused nonspecific cross-reactions (7, 8). Pregnancy is one of the most frequently listed causes of HIV false-positive reactions. First-generation EIA tests may lead to a cross-reaction due to alloimmunization during pregnancy from contamination with viral antigens obtained from cell culture with cellular proteins. This study found that HIV false positivity was in 25% of pregnant women (9). In Africa, schistosomiasis and other helminth infestations led to polyclonal B cell reactivation, which was associated with false reactivity in the HIV EIA tests (10). Moreover, antischistosomal antibody and high rheumatoid factor titer (>80) could cause false positivity, and cross-reactivity between *Schistosoma mansoni* and HIV-1 antigen has been reported (11). B cell activation during malaria infection may produce false-positive results with first- or second-generation rapid diagnostic tests. Moreover, the antiplasmodium antibodies cross-reacted with the gag antigens in the WB test (7, 12). Doing the separation process via prior extraction of the analyte from the sample and gel chromatography or removal of the nonspecific antigens via the addition of an immobilized protein A suspension onto the Sepharose beads was recommended in the prevention of common cross-reactions. The competition of antibodies against animal proteins with the analyte is prevented by PEG 6000 precipitation (3). Contamination with bacterial proteins during the synthesis of recombinant HIV antigens could cause false positivity in rapid diagnostic tests (7). Furthermore, HIV tests conducted following influenza or rabies vaccination (especially in the first 6 weeks) resulted in indeterminate results if obtained with the WB test (13). In addition, the rabies virus has a glycoprotein similar

to the gp120 antigen (14). Craske et al. (15) suggested that pseudoepitopes would form during recombinant antigen production due to protein differences between the eukaryotic and prokaryotic cells, which could leave to cross-reaction in HIV tests.

In a positive HIV WB test, the presence of the p31 band would indicate the potential for HIV infection in low-risk populations. Kleinman et al. (16) found a 4.8% false positivity in the WB tests, which suggested that repeated HIV false positivity of blood donors by third-generation EIA methods could be prevented by thiocyanate. Thiocyanate inhibits the weak binding of early polyspecific antibodies (16, 17). Hemolysis, lipemia and hyperbilirubinemia, hypergammaglobulinemia, or hyperproteinemia may indicate possible interactions in serum samples above a certain threshold. Thus, increasing the signal-to-cut off index >1.0 in HIV screening tests was suggested to reduce the number of false positives by 76% (13).

In societies where the seroprevalence was low, the possibility of reactive results with a single test was low if the person had not engaged in high-risk behavior (1). In the current study, the HIV prevalence was found to be 0.091%. This result suggested a high rate of false-positive results in a low-risk population. The probability of false positivity increased in patients with deterioration of liver function due to neoplasm, liver tumors, or other illnesses. In addition, false HIV positivity was detected in all five patients with bone fractures. Furthermore, the use of antibiotics, steroidal/nonsteroidal drugs, immunoglobulin preparations, and antithrombotic drug use were found in most of the patients with false-positive HIV results. Supporting the current findings, a false test result was obtained with the use of anabolic steroids in a case report (2). Finally, this article demonstrated that false positives are very common, and no patient should be told that they are HIV-positive until this test has been confirmed to be positive by either immunoblot or polymerase chain reaction because this is a life-threatening diagnosis.

CONCLUSION

Reactive results can affect the patients' psychological condition and can also affect treatment planning and even cause treatment delay. Thus, HIV prevalence in the community, national diagnostic algorithms, and producer validation reports should be taken into account for the selection of HIV tests to be used in laboratory settings.

Ethics Committee Approval: The Kahramanmaraş Sütçü İmam University Clinical Research Ethics Committee granted approval for this study (date: 03.07.2019, number: 08).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – FO; Design – FO; Supervision – KTY; Materials – KTY, FO; Data Collection and/or Processing – FO; Analysis and/or Interpretation – AD; Literature Search – FO, KTY; Writing – FO; Critical Reviews – KTY, MA.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. T.R. Ministry of Health General Directorate of Public Health. HIV / AIDS Diagnostic Manual, Ankara 2019. Available from: URL: https://hsgm.saglik.gov.tr/depo/birimler/Bulasici-hastaliklar-db/duyurular/hiv-aids-tani-klavuzu/HIV___AIDS_Tani_Klavuzu_Ek_47016636.pdf. Accessed Jan 11, 2020.
2. Tsybina P, Hennink M, Diener T, Minion J, Lang A, Lavoie S, et al. Repeated false reactive ADVIA centaur® and bio-rad Geenius™ HIV tests in a patient self-administering anabolic steroids. BMC Infect Dis 2020; 20(1): 9. [CrossRef]
3. Tate J, Ward G. Interferences in immunoassay. Clin Biochem Rev 2004; 25(2): 105–20.
4. Whithead P. Immunoassay – Sources and effects of immunoassay interference. ElgaViolia, 2020. Available from: URL: https://www.elgalabwater.com/blog/immunoassay. Accessed Jan 11, 2020.
5. Bowen R, Benavides R, Colón-Franco JM, Katzman BM, Muthukumar A, Sadrzadeh H, et al. Best practices in mitigating the risk of biotin interference with laboratory testing. Clin Biochem 2019; 74: 1–11. [CrossRef]
6. Centers for Disease Control and Prevention (CDC). Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations, 2014. Available from: URL: https://www.cdc.gov/hiv/pdf/testing/cdc-hiv-fact-sheet-false-positive-test-results.pdf. Accessed Jan 11, 2020.
7. Klarkowski D, O'Brien DP, Shanks L, Singh KP. Causes of false-positive HIV rapid diagnostic test results. Expert Rev Anti Infect Ther 2014; 12(1): 49–62. [CrossRef]
8. Vojtek I, Buchy P, Doherty TM, Hoet B. Would immunization be the same without cross-reactivity? Vaccine 2019; 37(4): 539–49. [CrossRef]
9. Doran TI, Parra E. False-positive and indeterminate human immunodeficiency virus test results in pregnant women. Arch Fam Med 2000; 9(9): 924–9. [CrossRef]
10. Langenberg MCC, Hoogerwerf MA, Koopman JPR, Janse JJ, Kos-van Oosterhoud J, Feijt C, et al. A controlled human *Schistosoma mansoni* infection model to advance novel drugs, vaccines and diagnostics. Nat Med 2020; 26(3): 326–32. [CrossRef]
11. Everett DB, Baisely KJ, McNerney R, Hambleton I, Chirwa T, Ross DA, et al Association of schistosomiasis with false-positive HIV test results in an African adolescent population. J Clin Microbiol 2010; 48(5): 1570–7.
12. Stempel JM, Mora Carpio AL, Puga D, Perloff S. False positive fourth generation HIV test in a patient with severe malaria. Int J Infect Dis 2019; 83: 86–7. [CrossRef]
13. Robinson MA, Nagurla SR, Noblitt TR, Almaghlouth NK, Al-Rahamneh MM, Cashin LM. Falsely positive fourth generation ADVIA Centaur® HIV Antigen/Antibody Combo assay in the presence of autoimmune hepatitis type I (AIH). IDCases 2020; 21: e00886. [CrossRef]
14. McKenna PM, Pomerantz RJ, Dietzschold B, McGettigan JP, Schnell MJ. Covalently linked human immunodeficiency virus type 1 gp120/gp41 is stably anchored in rhabdovirus particles and exposes critical neutralizing epitopes. J Virol 2003; 77(23): 12782–94. [CrossRef]
15. Craske J, Turner A, Abbott R, Collier M, Gunson HH, Lee D, et al. Comparison of false-positive reactions in direct-binding anti-HIV ELISA using cell lysate or recombinant antigens. Vox Sang 1990; 59(3): 160–6. [CrossRef]
16. Kleinman S, Busch MP, Hall L, Thomson R, Glynn S, Gallahan D, et al. False-positive HIV-1 test results in a low-risk screening setting of voluntary blood donation. Retrovirus Epidemiology Donor Study. JAMA 1998; 280(12): 1080–5. [CrossRef]
17. Bouillon M, Aubin É, Roberge C, Bazin R, Lemieux R. Reduced frequency of blood donors with false positive HIV-1 and -2 antibody EIA reactivity after elution of low-affinity nonspecific natural antibodies. Transfusion 2002; 42(8): 1046–52. [CrossRef]