International Journal of Scientific Research in Science and Technology
Available online at : www.ijsrst.com
OPEN ACCESS
Print ISSN: 2395-6011 | Online ISSN: 2395-602X
doi : https://doi.org/10.32628/IJSRST52310571

Evaluation of Sensitivity and Specificity of Third and Fourth Generation ELISA in detecting Hepatitis B Virus among Blood Donors

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ARTICLEINFO

Article History:

Accepted: 01 Oct 2023
Published: 09 Oct 2023

Publication Issue
Volume 10, Issue 5
September-October-2023

Page Number
512-516

ABSTRACT

Aim

To compare the 4th generation ELISA with 3rd generation ELISA for early detection of HBV infection among voluntary blood donors and for specificity.

Material and methods

779 samples of voluntary blood donors were tested for HBsAg by 3rd generation ELISA and 4th generation ELISA.

The results were compared and discrepant samples were further tested by NAT (RT-PCR) and HBe antigen and HBc antibody by CMIA.

Results

279 were found HBsAg reactive and 500 samples were found non–reactive by third generation ELISA.

Out of these 279 we found 3 samples to be non-reactive by fourth generation ELISA.

Discrepant samples were then tested for NAT which also gave comparative results with fourth generation ELISA. Two samples were further tested for marker study. Marker study also showed that these two samples were negative for HBV infection.

Conclusions

The results of 4th generation ELISA, NAT and HBV markers were found comparable in our prospective study

We had 3 false positives with III generation ELISA.

Thus 4th generation kit for HBsAg is more specific than 3rd generation ELISA and comparable with better method like NAT.

In India where NAT testing is costly and not mandatory, 4th generation ELISA can be preferred as it is will decrease false positive and rejection of donors

Keywords : Hepatitis B, HBsAg, 3rd generation ELISA, 4th Generation ELISA.

I. INTRODUCTION

According to World Health Organization (WHO), blood donation should be absolutely voluntary, non-remunerative and the motive should be purely altruistic to help the unknown recipient.[1]
WHO, in addition to promoting unpaid voluntary blood donation, recommends all donations should be mandatorily screened for highly prevalent infections which are likely to be transmitted by blood transfusion. Transfusion-transmissible infections such as HIV, HCV and HBV are among the greatest threats to blood safety and potential serious chronic sequelae associated with readily transmitted agents.

In India, it is mandatory to test every unit of blood collected for Hepatitis B, Hepatitis C, HIV, Syphilis and Malaria. If a donor is found to be positive to any of these five infections, their blood is considered infectious and discarded.[2]

The mandatory blood screening for HBV, HIV and HCV is done by serological tests for HBsAg and antibodies to HIV 1&2 and HCV.

The hepatitis B virus (HBV) causes the disease hepatitis B. The hepatitis B virus is unique among human viral pathogens as it is a DNA virus that replicates via an RNA intermediate, and thus belongs to the reverse transcribing DNA and RNA viruses. HBV is part of the *Hepadnaviridae* family of viruses, which consists of genotype A-H[3,4]

HBV is transmitted via blood or other body fluids and can survive outside the body for at least seven days. The most common transmission types are perinatal mother-to-infant transmission or horizontal transmission between children up to the age of 5 years.
Sources of infection are also medical surgery instruments, tattooing needles or razors that are contaminated with blood[5].

In India, we currently use 3rd generation ELISA KITS for HBV which will detect HBsAg.

The implementation of new highly sensitive 4th generation ELISA tests has significantly enhanced early detection of HBV infection compare with 3rd generation ELISA.

The 4th generation ELISA assays for HBV ultrasensitive kit detection of various subtypes of HBsAg and the most part of variants HBV strains thus detect in the window period to about 24 days, as compared 37 days with 3rd generation ELISA.[6]
Hence, this study is aimed to compare the 4th generation ELISA with third generation ELISA for early detection of HBV infection among voluntary blood donors and for specificity.

Objectives

Comparative evaluation of sensitivity and specificity of third and fourth generation ELISA in detecting Hepatitis B virus among blood donors.

Materials and Methods

This study was conducted over a period from May 2023 to July 2023 in Surat Raktadan Kendra and Research centre, a voluntary blood bank in Surat, Gujarat, India, which is the first, oldest and one of the largest voluntary blood banks in Surat.

Sample collection

Serum was separated from donated samples of voluntary blood donors in a sterile plain test tube and stored at -20°C for ELISA and PCR tests.

1) All blood units were tested for HBsAg using 3rd generation ELISA (Merlisa HBsAg kits manufactured by Meril diagnostics).[7]

Principle

Merilisa HBsAg is based on microwells coated with monoclonal anti-HBsAg antibody. The Conjugate is polyclonal anti-HBsAg labelled with horseradish peroxidase. HBsAg if present binds to anti-HBsAg. Conjugate is added which in turn binds to any specific antigen already bound to the antibody on the well. Unbound Conjugate is washed away and a solution containing 3, 3', 5, 5'-tetramethylbenzidine. (TMB) and hydrogen peroxide is added to the wells. Wells with bound Conjugate develop a blue to bluish green colour which is converted to yellow to orange when the reaction is stopped with Sulphuric acid. After incubation the reaction is stopped with sulphuric acid and the colour is read spectrophotometrically. The intensity of colour is directly proportional to the concentration of HBsAg in the sample. Wells containing negative samples remain colourless.

2) In addition, donor units were screening with 4th generation HBsAg ELISA (Monolisa HBsAg ULTRA ELISA kits manufactured by Bio Rad).[8]

Principle

Monolisa™ HBsAg ULTRA assay is a one-step enzyme immunoassay based on the principle of the "sandwich" type using monoclonal antibodies and polyclonal antibodies selected for their ability to bind themselves to the various subtypes of HBs Ag now recognized by the WHO and the most part of variant HBV strains.

The Monolisa HBsAg ULTRA solid phase is coated with monoclonal antibodies.

The Monolisa HBsAg ULTRA conjugates are based upon the use of monoclonal antibodies from mouse and polyclonal antibody from goat against the HBsAg. These antibodies are bound to the peroxidase.

3) All discrepant samples were tested by Nucleic acid testing (NAT). NAT by RT-PCR is a molecular technique for screening blood donations to reduce the risk of Transfusion Transmitted Infections (TTIs) in the recipients, thus providing an additional layer of blood safety. It was introduced in the developed countries in the late 1990s and early 2000s and presently around 33 countries in the world have implemented NAT for human immunodeficiency virus (HIV) and around 27 countries for hepatitis B virus (HBV).[9]

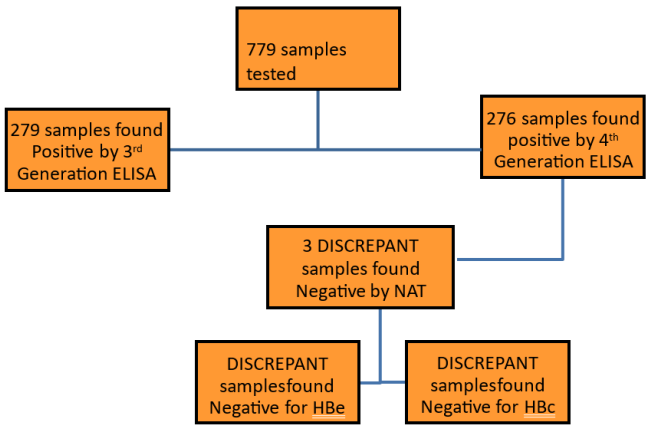
The AltoStar HBV PCR kit 1.5 is an in-vitro diagnostic test for the detection and quantification of HBV specific DNA in human EDTA plasma within the AltoStar® _Workflow (for details see chapter 6.4 AltoStar® _Workflow). It is based on real-time PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of HBV specific target sequences and fluorescently labelled target specific probes for the detection of the amplified DNA.[10]

Though not mandatory in India, Surat Raktadan Kendra does perform NAT testing on all samples.

Study Protocol

1)

Sample size: 779 samples
Step – 1: Testing all 779 samples using third generation ELISA
Step – 2: Testing all 779 samples using fourth generation ELISA
Step – 3: Testing of **DISCREPANT** samples using NAT (PCR)
Step – 4: Marker (HBe Antigen and HBc Antibody) study of **DISCREPANT** samples by CMIA (Carbonylmetallo-immunoassay) – Outsourced to Desai Metropolis Lab, Surat.



II. RESULTS AND INTERPRETATION

Table – 1: Sero-prevalence found in third and fourth generation ELISA

Samples	Third generation ELISA	Fourth generation ELISA
HBV Positive	279	276
HBV Negative	500	500

Table – 2: Tests performed on DISCREPANT samples

No. of Discrepant samples by III gen vs. IV gen ELISA	Tests Performed		
	NAT	HBe Antigen Assay	HBc Antibody Assay
3	Non - Reactive	Non - Reactive	Non - Reactive

III.DISCUSSION

Total 779 samples were included in the study.

279 were found HBs Ag reactive and 500 samples were found non – reactive by third generation ELISA.

Out of these 279 we found 3 samples to be non-reactive by fourth generation ELISA. Discrepant samples were then tested for NAT which also gave comparative results with fourth generation ELISA. Out of these three samples one sample was having insufficient volume, thus only two samples were further tested for marker study. Marker study also showed that these two samples were negative for HBV infection.

!) Conclusion

- The results of IV generation ELISA, NAT and HBV markers were found comparable in ourprospective study
- We had 3 false positives with III generation ELISA.
- Thus 4th generation kit for HBsAg is more specific than 3rd generation ELISA and comparable with better method like NAT.
- In India where NAT testing is costly and not mandatory, 4th generation ELISA can be preferred as it is will decrease false positive and rejection of donors

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Abbreviations

HIV - Human Immunodeficiency Virus
HCV - Hepatitis C Virus
HBV - Hepatitis B Virus
HBsAg - Hepatitis B surface Antigen
ELISA - Enzyme linked Immunosorbent Assay
NAT - Nucleic Acid Testing
PCR - Polymerase Chain Reaction
CMIA - Carbonyl Mettalo Immunoassay
HBe antigen- Hepatitis B envelope antigen
HBc antibody - Hepatitis B core antibody

Cite this article as :

Shreya P Desai, "Evaluation of Sensitivity and Specificity of Third and Fourth Generation ELISA in detecting Hepatitis B Virus among Blood Donors ", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 10 Issue 5, pp. 512-516, September-October 2023. Available at doi : https://doi.org/10.32628/IJSRST52310571
Journal URL : https://ijsrst.com/IJSRST52310571