

Effect of Grey Zone Samples Testing by Enzyme Immunoassay and Its Impact on Enhancing Blood Safety: Experience from Tertiary Care Hospital based Blood Bank in Central Gujarat

Ashu Dogra¹, Devanshi Gosai², Jasmin Jasani³

¹Associate Professor in Department of Transfusion Medicine, ²Assistant Professor in Department of Pathology,

³Professor in Department of Pathology, SBKSMIRC, Sumandeep University

Abstract

Background : Enzyme linked immunosorbent assay is a standard protocol adopted by majority of Blood Banks for screening of blood units for transfusion transmissible infections. The grey zone testing of samples increases sensitivity of ELISA tests which in turn enhances the safe blood transfusion practices. The present study was thus designed to know the effect of repeat testing of Grey zone Blood Donor samples for improving the sensitivity of ELISA Screening methods in blood banks.

Methods:- All Blood Donor Samples which were 10% below the cut- off value were marked as Grey zone samples and were repeated for ELISA screening . Interpretation of Repeat tests was done as per NACO and WHO guidelines and the samples which recorded their one or both O.D value above cut- off were labelled as reactive and samples which recorded one or both O.D value in grey zone were marked as indeterminate.

Results and Conclusion: In present study, a total of 10,425 Blood Donors were screened for TTI' s and out of 10,425 (15) Blood donor samples were found in grey zone. The results of repeat testing showed that 9 (60%) Blood Donor samples were found to be confirm reactive for TTI. Grey zone sample testing should be done routinely as a measure to enhance sensitivity of ELISA testing of Blood Donors in developing countries.

Key words:- ELISA, Grey Zone, Transfusion transmitted infection,

Introduction

Blood transfusion is a life saving procedure for the management of clinical and surgical patients . The use of Blood and components should always be done judiciously , weighing their benefits against the risks involved since Blood transfusion is associated with the risk of disease transmission. The collected units should be tested with highly sensitive and specific testing kits before transfusion. The important diseases transmitted through blood are HIV (Human Immunodeficiency virus), HBV (Hepatitis B virus), HCV (Hepatitis C virus), Malarial parasites and Syphilis. ⁽¹⁾

Following Blood transfusion , there is a risk of transfusion- transmitted infectious diseases, despite the use of current advanced screening technologies. The risk

of Transfusion Transmitted Infection is estimated to be 1 in 6,77,000 units of HIV , 1 in 1,03,000 for HCV, and 1 in 63000 for HBV.⁽²⁾ Since transfusion of blood and components involves entry of maximum viral load particles to be transmitted so sensitive and specific donor screening strategies are needed for safe blood transfusion to the patients. ⁽³⁾

The various screening methods currently used for diagnosis of Transfusion Transmitted Infections are either Immunoassays e.g Enzyme linked Immunosorbent assay (ELISA) or Chemiluminiscence Assays (CLIA) and Molecular techniques like Nucleic Acid Testing (NAT).(4) ELISA is the most commonly performed screening test at blood banks and tertiary care centers testing a large number of samples a day. It is sensitive, specific and cost -effective.

As per Drugs and cosmetics act, 1940 and rule, 1945, the testing of Blood and components for viral markers of HIV, HBV, HCV, are mandatory in blood bank. Screening of all blood donors in India is done by complying with NACO and WHO guidelines. According to these guidelines samples with optical density (O.D) Values above or equal to cut-off are considered as reactive samples and those below the cut-off value as non reactive. Samples with optical density 10% below the cut off are marked as grey zone samples. Testing of grey zone samples enhances the sensitivity of screening of blood by ELISA and CLIA techniques as they identify seroconverters early in the course of disease and thus contributes to provide safe blood for use. ⁽⁶⁾

Since there is very less data to know usefulness of grey zone testing in screening of transfusion transmitted infections. The present study thus aims to know the utility of grey zone calculation of Blood Donor Saamples and its role in increasing the sensitivity of ELISA technology used for blood donor screening at our blood bank.

Material and Methods

The present study was a prospective analytical study carried out in Department of Transfusion Medicine, SBKSMIRC, from January 2017- December 2029. All Blood Donors were explained about Blood Donation Process and an informed consent was obtained from blood donors that their blood samples will be screened for Transfusion transmitted infections like; HIV, HBV, HCV, Syphilis and Malarial Parasite.

In our blood bank, Immunobased assays, (ELISA) Enzyme Linked Immunosorbent assay (Lisquant 4000 and Lisawash 4000) was used for screening of all blood samples strictly following manufacturers guidelines. HIV screening was performed by fourth generation Elisa kits (Qualisa), for HBV 3rd generation Elisa kits (Qualisa) were used, for HCV 3rd generation Elisa kits (Qualisa) were used. Validation and Quality control of ELISA was done by preparing Levy – Jenning charts and using west gard rules by running 30 borderline positive controls as per guidelines and procedures in National AIDS control organization (NACO) Quality Manual⁽⁶⁾

The test run samples with OD value more than cut-off were considered reactive units. All reactive units were discarded and reactive donors were notified as per departmental standard operating procedures. Grey zone is defined as values 10 % below the cut off margin. Grey zone values are calculated with each day of run and samples falling in Grey zone area are removed from inventory to be discarded as per departmental standard operating procedures. All initial grey zone samples are tested in duplicate and those samples showing one or both values above cut off were labelled as reactive units and those donors are notified and recalled for counselling and further management. The Grey zone samples which were showing one or both OD value again as grey zone on duplicate testing was marked as indeterminate. Indeterminate donors were documented as nonreactive and informed to visit Blood Bank for repeat testing after 6 months.

Results and Discussion

Among the 10,425 blood donors donating the blood during 3 years of study i.e. from January 2017 to December 2019, 50 (0.47%) donors were reactive for HBsAg, HCV, and HIV in the TTI Screening tests and 15 (0.0015%) donors samples were Grey Zone positive. Of total 50 TTI Positive Blood Donors, 25(50%) were positive for HBsAg, 8 (16%) were positive for HIV, 6 (12%) were positive for HCV and 10 (20%) were positive for VDRL and one positive (2%) for Malaria.

Donors having their TTI result in the Grey zone were 15, of which HBsAg was 07 (46.6%), HCV 05 (33.33%), HIV 2 (13.33%), VDRL 1 (6.66%). **Table 1.**

On repeat testing of 15 Grey Zone samples, confirm reactive HBsAg samples were 4 and indeterminate was 1. For HIV Confirm reactive samples were 2, For HCV Confirm reactive sample were 2 and indeterminate were 3, and for VDRL Confirm reactive samples were 1.

Table 2

Table no.1: Shows number of TTI positive and grey zone samples in first run on Blood Donors , (n= 10,425)

Test	Sero reactive samples in first run (%)	Grey zone samples in first run. (%)
HIV	08 (16%)	02(13.33%)
HBV	25(50%)	07(46.6%)
HCV	06 (12%)	05(33.33%)
VDRL	10 (20%)	01(6.66%)
Malarial Parasite	01(2%)	00

Table no. 2: Shows interpretation of Grey zone samples on repeat testing.

TTI Marker	Sample in Grey zone .	Repeat non reactive	Repeat Seroreactive	Repeat Grey zone (Indererminate)
HIV	02	--	02	--
HBV	07	02	04	01
HCV	05	--	02	03
VDRL	01	--	01	--
Malarial parasite	00	--	--	--
Total	15	02	09	04

There are very few research studies conducted on reporting significance of Grey zone positive samples in increasing sensitivity of immunobased assays like ELISA and CLIA.^{(3) (4)} The present study reported 0.14% (15/10,425) samples in Grey zone .

NAT testing though improves blood safety by decreasing the window period of infection but it is not routinely used in many blood banks because of economic reasons. The tests based on immunological assays like ELISA /CLIA testing remains one of the economical methods for screening of donor blood samples. To enhance the sensitivity of ELISA / CLIA as screening assay repeat testing of samples with O.D value 10 % below cut off should be practiced.

In one study by Pereire etal it was reported that there can be possibility of reporting precarious result values around the cut off zone in TTI screening , used for calculating the reactive samples. ⁽⁷⁾ The study thus concluded that samples in Grey zone were not always

confirmatory source of test methods for notifying the donor about his TTI positive status, as chances , for these tests as confirmed positive is low. This study when compared with other Indian studies in analyzing the impact of Grey zone on TTI ,it was observed in one study conducted by Anitha et al. that 144 Blood Donors (n=21,908) were in Grey Zone. Repeat testing when done on all these Grey zone samples , 35(24.30%) samples were found to be reactive for TTI and 18 (12.5%) were in grey zone.

In our study out of 15 samples which were initially in grey zone 9 samples were reported confirm reactive and four samples were in repeat grey zone/Indeterminate.

One study conducted in Turkey reported 70% false positivity on repeat testing of grey zone samples⁽⁸⁾

In present study, 0.14% samples were reported in Grey Zone and 60% of Grey zone samples reported, were repeat reactive for either of viral markers.

In similar studies Grey Zone repeat testing was 4.6-75%. (9,10,11).

Repeat reactivity in grey zone testing is astounding condition and thus the more stringent screening technologies and donor selection methods is the need of hour in developing countries.

The only limitation in present study was the nonavailability of performing the confirmatory assays for Reactive and Indeterminate units and lack of follow up to look for seroconversion of infection for those donors whose samples were in Grey zone of ELISA.

Conclusion

Repeat testing of Grey zone samples will help in improving the safety of Blood transfusion in developing countries where more sensitive supplementary assay such as NAT technology is very expensive.

Ethical approval: Taken from ethics committee of sumandeep Vidyapeeth university.

Conflict of Interest : Nil

Funding: Self Funded.

References :-

1. Dhar G, Sarkar AP, Sen S. et.al; Patterns of infective sero positivity among blood donors in a rural medical college regional blood transfusion centre: a prospective study, *Nepal Journal of Medical Science*.2013; **02**:03-08.
2. Schreiber GB, Busch MP, Kleinman SH, et.al The risk of transfusion transmissible viral infections. The retrovirus epidemiology donor study. *New*

3. Solanki A, Singh A, Chaudhary R, . Impact of grey zone sample testing by enzyme linked immunosorbent assay in enhancing blood safety: Experience at a tertiary care Hospital in North India.. *Asian journal of transfusion Science*. 2016; **10**:71-74.
4. Anitha M, Sreedhar Babu K, Praveen M.D, et.al .Effect of grey zone sample testing in transfusion transmissible infectious diseases on safety of blood- experience of a tertiary care referral teaching hospital blood bank from south India, *International journal of research in Medical sciences*. 2017;**05(08)**:3488-3442.
5. Malik V. Drugs and cosmetics act , I 1940, 13th edition, Eastern book company : lucknow ,2001.
6. Rao KS. Guidelines on HIV testing . National AIDS Control Organization Ministry of Health and family welfare India 2007.
7. Pereira P, Westgard JO, Encarnacao P, et.al. The role of uncertainty regarding the results of screening immunoassays in blood establishments, *Transfus Apher Science*.**2015**; **52**:252-5
8. Chen D, Kaplan LA. Performance of a new generation chemiluminescent assay for Hepatitis B surface antigen, *Clinical Hematology*. 2006;**52**: 1592-8.
9. Acar A, Kemahli S, Altunay H, et al. HBV, HCV, And HIV seroprevalence among blood donors in Istanbul, Turkey: How effective are the changes in the national blood transfusion policies? *Brazilian Journal of Infectious Disease*. 2010;14:41-6.
10. Verma A, Girish S, Chaudhary R, Grey zone samples Assessment in ELISA Screening of Blood Donors Enhances Blood safety . 4th IAS Conference on HIV pathogenesis, Treatment and Prevention 2007; CBD065.
11. Detection and False negativity due to ELISA Kit Based Criteria for HIV, HBsAg & HCV . Available from <http://www.bmstindia.org/scandocs>.