

## Effect of Grey Zone Sample Testing of Transfusion Transmissible Infectious Diseases by Enzyme Linked Immunosorbent Assay in Blood Safety - Experience at a Tertiary Care Hospital Blood Bank from Western Odisha

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### Original Research Article

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**Abstract:** Enzyme-linked immunosorbent assay is a standard protocol adopted in all blood bank centres in Odisha for screening blood units for transfusion transmissible infections (TTIs). But donors who are at the time of donation in window period, give ubiquitous readings and show results in grey zone. Repeat testing of these samples can help in reducing the risk of TTIs. This is especially cost effective in developing countries where nucleic acid amplification testing for TTIs has not been adopted universally. Till date most of the studies has been done regarding the utility of grey zone samples and its application in TTI screening procedures. So this study has been taken up to find out the utility of repeat testing for these grey zone samples and its role in enhancing the sensitivity of current Elisa technology used for blood donor screening.

**Keywords:** Enzyme linked immunosorbent assay, Transfusion transmissible viral infections, Grey zone, Blood safety.

### INTRODUCTION

Blood transfusion is a life-saving procedure and plays a very vital role in the medical / surgical management of most of the patients. Among the various adverse effects of transfusion, transfusion transmitted infections (TTI) are the most important, which include, Hepatitis B virus (HBV), Hepatitis C virus (HCV), human immunodeficiency virus (HIV), hemoparasites and Syphilis. In spite of advanced screening test technologies, recipients still have an increased risk of becoming infected by the various transmissible infections.

The scenario becomes denser in developing countries, as improvised testing technologies for screening of TTIs leads to increased cost of blood components for the patients. So, the transmission of various viral infections continues to be a serious threat to safe blood transfusion in these countries. The task of screening blood donors is more challenging as these developing countries account for more than 90% of all new HIV cases worldwide [1].

The risk of TTIs is estimated to be 1 in 6,77,000 units for HIV, 1 in 1,03,000 for HCV, and 1 in 63,000 for HBV and the risk of transmission of these infectious agents through infected blood products exceeds that of any other exposures [2]. In multiple transfused hemophiliac patients, the prevalence of HCV was found to be as high as 23.9% [3]. Several tools have been implemented for preventing TTIs ranging from donor selection to donor testing. Screening of blood donors for infectious markers is done by immunoassay in the form of antigen/antibody detection

methods such as latex agglutination, Immunochromatography, enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA) or by genetic tests like nucleic acid amplification technology (NAT) assay. Drug and Cosmetic Act as well as supreme court guidelines in India has made testing of HIV, HBV, HCV, syphilis, and malaria mandatory on all blood donations done in blood banks [4].

Screening of all blood donors is done complying with the strategy I as laid down by world health organization (WHO), in India. Strategy I of World Health Organization mentions subjecting all blood donors sample to one-time ELISA for screening purposes and marking samples with optical density (OD) above or equal to cut-off OD as reactive and samples below cut-off OD as nonreactive [5]. In literature there is paucity regarding detection of grey zone sample and its application in TTI screening procedures in blood bank set up.

Therefore, it becomes very necessary to assess the utility of grey zone calculation and its role in improvising the current screening methodologies. Therefore, we share our study which was undertaken to analyze the importance of grey zone testing of TTI of apparently healthy blood donors and its role in enhancing the sensitivity of current ELISA technology at our blood bank.

**MATERIALS AND METHODS**

This is a prospective study which was performed on blood donors coming for blood donation in the department of Transfusion Medicine of a tertiary care center in western Odisha, in VSSIMSAR, Burla and was conducted for a period of one year seven months from the period April 2017 to November 2018.

Written informed consent was obtained from all the donors. Blood donors, fulfilling the criteria for donor selection as per the selection criteria laid down by Drugs and Cosmetics Act, 1940 and Rules, 1945 were considered for the present study. A total of 21,181 blood donors were screened during the study period. The donors were either voluntary or replacement donors. Voluntary donors are those who voluntarily donated their blood either at the blood bank or at voluntary blood donation camps, whereas the replacement donors were either relatives or friends of patients. Immunoassay in the form of ELISA was done for screening all blood samples by an automatic instrument (MAGO4, Transasia and semi-automated lab system (Thermofischer)) strictly following the manufacturer's guideline. HIV screening was performed by GEN 3 ELISA kits (ERBALISA) for HBV (fourth generation kit (ERBALISA) and HCV (GEN3 Version 2 (ERBALISA)). Validation of each test was performed according to manufacturer's instruction. Quality control of ELISA testing was done by preparing Levy-Jennings chart by simultaneously running the in-house borderline

positive controls for 30 consecutive runs as mentioned in National Aids Control Organization guidelines [5]. External quality control assay was done with samples received from CMC, Vellore. All the samples with optical density (OD) more than the cut-off were considered as reactive and those blood units were discarded and donors were notified as per departmental standard operating procedure. Grey zone was calculated as 10% below the cut-off OD. All the samples with OD between cut-off value and  $0.9 \times$  cut-off value were marked as grey zone samples and were quarantined. All the grey zone samples were retested in duplicate for their respective viral marker using the same ELISA kits the next day. On repeat testing, the grey zone samples showing both OD values below  $0.9 \times$  cut-off value were marked as nonreactive and the blood units were included in the inventory. If on repeat testing the grey zone sample showed one or both OD value above the cut-off value it was marked as reactive and blood units were discarded and donors were notified. The grey zone sample showing one or both OD value again in grey zone on repeat testing was marked as indeterminate and blood unit was discarded, but the donor was documented as nonreactive.

**RESULTS**

Of the 21,181 healthy donors screened for mandatory infectious markers during the study period, HIV reactivity was found in 8 (0.03%) donors with HBV and HCV in 73 (0.34%) and 2 (0.009%) donors, respectively. Excluding all reactive samples, about 10 (0.04%) more samples were found to lie in a grey zone with 7 belonging to HBV, 3 belonging to HIV, and none belonging to HCV. On repeat testing of these grey zone samples, 3 were found to be reactive with 2 for HBV, 1 for HIV, and none for HCV. None of the grey zone samples showed indeterminate results on repeat testing.

**Table-1: TTD marker seroreactivity in grey zone samples**

TTD Marker	Grey zone sample	Repeat nonreactive	Repeat seroactive	Repeat Grey Zone(Indeterminate)
HIV	3	2	1	0
HBV	7	5	2	0
HCV	0	0	0	0
Total	10	7	3	0

HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus  
 HBV: Hepatitis B Virus; TTD: Transfusion Transmitted Disease

**Table-2: Total yield of seroreactivity in grey zone samples**

TTD Marker	First Time Seroactive	Repeat reactive of grey zone donors	Total yeild on grey zone testing
HIV	8(0.03%)	1	9
HBV	73(0.34%)	2	75
HCV	2(0.008%)	0	2
Total	83(0.39%)	3(0.01%)	86(0.4%)

HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus  
 HBV: Hepatitis B Virus; TTD: Transfusion Transmitted Disease

The inclusion of criteria of grey zone calculation in routine ELISA screening test at our set up increased the reactivity of infectious markers from 0.39% to 0.42%.

## DISCUSSION

Though NAT technology is an advanced screening method for screening of all seropositive donors in the context of reducing the window period still in developing countries like India, installation of this technique in all blood bank centres is not feasible due to cost factor. So, Elisa test still remains a prominent screening tool in most blood bank centres in India.

The patients with sickle cell anemia and thalassemia who are recipients of frequent blood component transfusion are the most vulnerable group for transfusion of TTIs. Some of the potential factors like genetic variation, immunologically silent carriers, procedural errors, variation in window period of the infectious agents etc still pose a threat to the screening procedures. In our study we could not confirm the results due to inability to follow up the seropositive donors. So, in our centre it is not possible to comment on the test results in grey zone.

NAT test is not routinely practiced for screening due to non-affordability. Immunological assays like ELISA serves as a main screening tool in blood bank setup in developing countries. To improve the sensitivity of ELISA many methods such as the inclusion of borderline reactive control samples in each run to minimize batch to batch, as well as day to day variation in testing are adopted. These borderline reactive samples are also able to detect minor variation in the assay procedure [5]. The other method to enhance the sensitivity of ELISA as screening assay is by subjecting the sample lying in grey zone for a repeat testing.

Pereira *et al.* illustrated that ELISA-based screening test for TTI in blood banks involve some amount of uncertainty especially around the cut-off zone used for calculating the reactive samples [6]. Anitha M *et al.* and Archana S. *et al.* has also reported that, the estimation of grey zone samples with repeat testing can further enhance the safety of blood transfusion in resource poor developing nations where more sophisticated and sensitive methods such as nucleic acid amplification test (NAT) is not available in most of the blood banks[7,8].

In a study in Turkey they have found 70% false positivity on testing grey zone samples, out of these only 2% were confirmed by advanced tests. In our study we have found total 10 samples in grey zone for all these three viral markers [9]. In other studies, samples in grey zone were much higher; the reason for

this may be due to use of different types of company kits.

## CONCLUSION

In spite of all best efforts, to achieve a zero risk through transfusion still remains a distant cry. So, proper donor screening, sensitive screening assays and effective pathogenic inactivation procedures can minimize the risk of TTI to a great extent. Repeat testing of grey zone samples will help in improving the safety of blood transfusion.

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