

Delhi - National Capital Region: Screening Of Plasma For HIV- Antibodies

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Abstract: Immunoassays are valuable tools for the detection of blood borne diseases which are, per se, widely used for measuring specific antigen(s) and / or antibodies of viral pathogens present in the plasma sample of asymptomatic donors all over the world. These are quick and accurate tests used in the laboratory to detect specific viral infection(s). In the past three decades a large number of different principle based immunoassays for screening of blood borne diseases like Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) have come in the Indian market viz Rapid, Enzyme Linked Immunosorbent Assay (ELISA), Enzyme Linked Fluorescent Assay (ELFA) and Chemi-luminescence Immuno Assay (CLIA). Hence, quality of these kits are tested before their entry into the market for safe guarding of patients. Quality of these kits require a set of disease specific performance panel comprising of true negative and true positive samples. In present study, HIV reactive plasma samples, as per blood banks reports, were collected from fourteen different hospitals and blood banks of Delhi and National Capital Region (NCR) during the year 2009 to 2017. The reactivity status of these collected plasma samples at source were again re-checked at National Institute of Biologicals (NIB) and found that percentage of concordance results of screening assays for HIV were increased from 47% in the year 2009 to 2015 to 82 % in the year 2016 to 2017. It shows that the hospitals and blood banks of Delhi & NCR have been improving their screening testing procedure(s) by using appropriate kit(s) and through skill development of technical personnel and documentation practices but still more efforts are also required to achieve a target for about 100% concordance results.

Keywords: Quality Assurance; Quality Control; Immunoassay; Accreditation; Human Immunodeficiency Virus (HIV)

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I. Introduction

Screening for Transfusion-Transmissible Infections (TTIs) to exclude blood donations at risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that blood transfusion is as safe as possible. Effective screening of the most common and dangerous TTIs can reduce the risk of transmission to very low and undetectable levels [1]. Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are common and dangerous TTIs. Hence, transfer of safe blood, blood components or blood products from one donor to recipient requires proper screening of donor blood for HIV, HBV & HCV. Historically, blood donor screening was based on using health histories, a self-deferral process and pre-selected donor groups to enhance blood safety. But these ancient practices has not reduced the number of TTIs rather increased the same since the beginning of routine blood transfusion in the late 1930s [2]. Till then there have been significant developments in blood transfusion services. According to the World Health Organization (WHO) all donated blood need to be tested for absence of HIV, Hepatitis B, Hepatitis C, Treponema pallidum (syphilis) [3] and even as per Drugs & Cosmetic Acts 1940 & Rules 1945, Government of India, the donated blood should be free from Hepatitis B surface antigen and Hepatitis C Virus antibody, HIV I and HIV II antibodies, syphilis and malarial parasite [4].

These blood-borne viruses per se are transmitted through contaminated blood transfusion, parenteral route, contaminated syringes, unprotected sexual intercourse, contaminated surgical equipment or other sharp instruments etc. Not only do they establish asymptomatic persistent infections with occasional sequelae, but they also cause significant morbidity and mortality when transmitted through transfusion of blood and blood products [5]. The screening for these viral infections are performed by immunoassay, an important strategy for the safe transfusion, prevention and cure for public health. An immunoassay basically a bioanalytical test measures the presence or concentration of protein (analytes) in the sample(s) of interest by using specific antibody or antigen [6]. In India different kinds of immunoassays / platforms viz Rapid, Enzyme Linked

Immunosorbent Assay (ELISA), Enzyme Linked Fluorescent Assay (ELFA) and Chemo-luminescence Immuno Assay (CLIA)[7] have been used in the hospitals / blood banks for screening of donated blood / plasma. Immunoassays have progressed dramatically in scope and utility since their development in the year 1959 [8]. Rapid and Conventional ELISA have been the prominent technology and used by the maximum blood banks & hospitals. ELISA is commonly used as a diagnostic tool to detect and / or quantify disease in early stage and it helps a clinician for treatment and save lives. It is also used as a quality control measure in various industries and in biomedical research as an analytical tool [9]. Similarly, rapid assays also work on the same principle of antigen-antibody reactions. This assay has a quick turnaround time, can be applied outside the sophisticated laboratory, is a very convenient tool to transport and used at rural, urban, remote, and even tropical areas as an assay does not need electricity. Some rapid kits use dried blood spots, oral fluid, urine etc. as a sample. A Rapid assay is in low cost, gives fast result does not require highly skilled specialist even few tests can be performed by clinician at their clinic like malaria rapid diagnostic test, pregnancy test and glucose strip test etc. Either of two techniques are used in Rapid immunoassay i.e. lateral flow rapid tests or flow through rapid tests [10]. Chemiluminescence Immunoassay (CLIA) is another promising format and applied widely to the clinical diagnosis [11]. It is a quick, simple, human error free as it performs complete sample processing, ultra-sensitive, clear interpretation of results, wide range of applications, broad linear range, high throughput and quality to detect low abundance of the biological molecules. Now a days CLIA is well established as one of the alternative methods of conventional or traditional ELISA for detection and quantitation of samples having antigen and / or antibodies [10] but for operation of this type of high-ended equipment(s) it requires well trained manpower for highly sophisticated infrastructure, specific reagents and consumables etc.

All the above discussed assays have their own disadvantages also. The cost per test for rapid tests may exceed traditional testing methods such as ELISA. Most rapid tests have limited shelf life that place increased demands on procurement and distribution systems. They are mainly qualitative tests, producing only "YES/NO" answers that may yield less information than the existing laboratory-based quantitative tests. It requires subjective interpretation, which may result in reader variation in results. In many cases, rapid tests are less sensitive or less accurate compared to existing reference-level laboratory tests. These are not amenable for high throughput testing. They require extensive and robust quality control and quality assurance mechanisms [12].

ELISA has certain limitations such as tedious/laborious assay procedure [13] require sophisticated laboratory infrastructure, highly skilled manpower etc. CLIA also has limitations like high cost, closed analytical system [14], require highly sophisticated laboratory infrastructure, highly trained technical staff for each high-ended-platform etc. Often, not as sensitive as gold standard.

Apart from above, error(s) can occur from the collection of samples to hand over the right report to right person. Most errors are due to pre-analytical factors (46-68.2% of total errors), while a high error rate (18.5-47% of total errors) has also been found in the post-analytical phase [15]. In year 2015, Miligy DA published the finding by studying 1600 testing procedures which showed that the 35.7% encountered errors was in pre- and 50% was in post-analytic phases of testing cycle. While the number of test errors encountered in the analytic phase represented only 14.3 percent of total errors. About 85.7 percent of total errors were of non-significant implication on patient's health being detected before test reports have been submitted to the patients. On the other hand, the number of test errors that have been already submitted to patients and reach the physician represented 14.3 percent of total errors. Only 7.1 percent of the errors could have an impact on patient diagnosis.

Proper storage of the sample, Human Anti-Mouse Antibodies which comes under Heterophile antibodies, autoantibodies / naturally occurring antibodies, passive immunization, sample with microbial contamination, Hook effect, breakdown / denaturation of antigen and some other interfering substances like dietary hCG, sodium azide can also cause false results which can affect the accuracy of the results particularly antibody detection based assays.

National Institute of Biologicals (NIB), NOIDA being the sole quality control laboratory in the country has been designated as Central Medical Device Testing Laboratory for immunodiagnostic kits, has initiated to compile the data for HIV reactive plasma samples collected from different hospitals and blood banks of Delhi - NCR versus their results obtained at NIB during the year 2009 to 2017.

II. Methods

During present study left over plasma samples were collected from 14 Hospitals / Blood Banks (A to N) of Delhi and National Capital Region and strictly followed the International guidelines (WHO) as well as NACO guidelines for transportation of infectious material and also followed the CDC manual for biosafety. The protocol used in this work was approved by Institutional Ethics Committee of the Institute and experiment performed as per laboratory written procedures. Details of the plasma bag viz name of the blood bank, blood bag number, test results at source and all other information were documented and also the record for any delay in transport or leakage during transportation etc. are maintained.

Collected plasma were tested on three different WHO/FDA approved/recommended ELISA kits for each marker and then further tested for cross reactivity with other Sexually Transmitted Diseases since 2009 to 2017. The testing was performed for each marker as per laboratory Standard Operating Procedure and Manufacturer's Instructions (Kit Insert / Instruction For Use) provided with respective kits.

III. Results & Discussion

During the year 2009 to 2017 laboratory has collected total 349 HIV-Ab reactive plasma bags claimed by different hospitals / blood banks of Delhi & NCR, however, they obtained over all 54.15% (189/349) concordance with NIB results. Out of this 46.73% (129/276) concordance results was obtained during the year 2009 to 2015 and 82.19% (60/73) concordance results was observed during the year 2016 and 2017 (Table-1). It is evident that the hospitals and blood banks of Delhi & NCR are improving their screening testing procedure(s), kit(s) used, skill development of technical personnel and documentation. On other hand, these data also shows that how much number of prestigious plasma bags have been discarded by the hospitals / blood banks due to use of poor quality test kits / wrong testing / wrong interpretation and reporting of results as positive which are actually false positive etc.

Laboratory testing of viral infections is a highly complex process and, although laboratory services are relatively safe, yet they are not as safe as they could or should be. Clinical laboratories have long focused their attention on quality control methods and quality assessment programs dealing with analytical aspects of testing [15].

As laboratory data are extensively used in medical practice; consequently, laboratory errors have a tremendous impact on patient safety. Therefore, programs designed to identify and reduce laboratory errors, as well as, setting specific strategies are required to minimize these errors and improve patient safety [16]. Errors due to analytical problems have been significantly reduced over time, but there is evidence that, particularly for immunoassays, interference may have a serious impact on patients [15]. Up to 70 percent of errors associated with lab results happened way before the tests are even analysed, according to a report from ECRI Institute's (formerly the Emergency Care Research Institute) Patient Safety Organization. Further getting a delayed diagnosis or misdiagnosis, particularly for what could be life-threatening conditions, can create significant emotional distress for patients [17].

For achieving a goal of about 100% concordance results mean near by 100% accurate results of hospitals or blood banks they need to establish the system which ensures that all donated blood is screened for TTI is a core component of every national blood programme. Globally, however, there are significant variations in the extent to which donated blood is screened, due to the type of screening strategies adopted and the overall quality and effectiveness of the blood screening process [3].

The accuracy of any laboratory investigation depends upon several factors like collection of sample at appropriate time, collection of specimen in specific container, storage & transportation of sample, technique used for testing etc. The clinician should keep the possible laboratory errors in mind while interpreting the laboratory investigations [18]. In case of ELISA contamination of substrate, conjugate, other reagents; preparation of accurate reagents as suggested by manufacturers, insufficient washing, dirty plate from the bottom, air bubbles on the well(s), incorrect wavelength, incorrect reference wavelength, highly haemolysed sample, lipemic sample, improper setting of volume in pipette; calibration of pipette(s), incubator, ELISA washer, ELISA reader are the common source of errors in the laboratories. In summary, regular training of lab personnel and their participation in national or international proficiency testing, implementation of barcodes system, accreditation of the lab etc. will further improve the quality of the lab HIV results and save the wasting of precious blood by falsely reporting as positive.

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Table-1: Hospital or Blood Bank wise / Year wise reactivity status of HIV-Ab samples collected from Blood Banks of Delhi-NCR during the year 2009 to 2017

Year (Jan-Dec)	Center wise True HIV-Ab Reactive Samples % (Percentage)														Total Percentage (Year wise)
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
2009	(06/23) 26	(05/09) 56	(00/02) 00	(03/05) 60	(07/07) 100	--	--	--	--	--	--	--	--	--	(21/46) 46
2010	(09/28) 32	--	--	--	--	--	--	--	--	--	--	--	--	--	(09/28) 32
2011	(01/03) 33	--	(06/16) 37	--	--	--	--	--	--	--	--	--	--	--	(07/19) 37
2012	--	--	(09/10) 90.00	--	--	--	--	--	--	--	--	--	--	--	(09/10) 90
2013	(02/03) 67	(02/02) 100	(15/26) 58	--	--	(06/06) 100	(01/04) 25	(01/02) 50	(03/11) 27	(00/01) 00	(01/01) 100	--	--	--	(31/56) 55
2014	--	(01/01) 100	(11/13) 85	--	--	(04/04) 100	(01/03) 33	--	(07/30) 23	(04/11) 36	(05/09) 56	(03/05) 60.00	--	--	(36/76) 47
2015	--	--	(05/11) 45	--	--	(02/03) 67	(01/05) 20	(00/01) 00	(08/21) 38	--	--	--	--	--	(16/41) 38
2016	--	--	(05/07) 71	--	--	(01/01) 100	(02/02) 100	(00/01) 00	(04/06) 67	--	(04/06) 67	--	--	--	(16/23) 70
2017	--	--	(19/24) 79	--	--	--	(10/10) 100	--	(02/03) 67	--	(03/03) 100	--	(05/05) 100	(05/05) 100	(44/50) 88
Total Percentage (Hospital or Blood Bank wise)	(18/57) 32	(08/12) 67	(70/109) 64	(03/05) 60.00	(07/07) 100	(13/14) 93	(15/24) 62	(01/04) 25	(24/71) 34	(04/12) 33	(13/19) 68	(03/05) 60	(05/05) 100	(05/05) 100	Grand Total = 349 plasma bags

- Reactivity status of Plasma bag in concordant with source
- Number of Reactive Plasma bag collected from source
- % (Percentage Rounded off)

From 2009 to 2015: 129/276 = 47%

From 2016 to 2017: 60/73 = 82%

From 2009 to 2017: 189/349 = 54%

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