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PREVALENCE OF TRANSFUSION TRANSMISSIBLE INFECTIONS, ABO BLOOD GROUPING AND ANTIBODY SCRENNING AMONG BLOOD DONORS AT DEFENSE HEALTH MAIN DEPARTMENT BELLA BLOOD BANK CENTER OF ADDIS ABABA, ETHIOPIA.

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This is to certify that the thesis prepared by Benyam Belay entitled: prevalence of transfusion transmissible infections among blood donors at Defense Health Main Department Bella Blood Bank Center of Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for master of science degree in clinical laboratory science (Hematology and Immunohematology)complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviation

AFRTH	Armed forces referral and teaching hospital
CMIA	Chemiluminecent Microparticle Immunoassay
ELISAs	Enzyme linked immunosorbet assay
ENDF	Ethiopian National Defense Force
ERCS	Ethiopia red cross society
FMoH	Federal Ministry of Health
GARPR	Global aid Response progress report
HBsAg	Hepatitus B surface antigen
HBV	Hepatitus B virus
HCV	Hepatitus C virus
HIV	Human Immune deficiency virus
LIAs	Line immunoassays
NAT	Nucleic acid testing
NBBS	National blood bank service
RLUs	Relative Light units.
SOP	Standard operating procedure.
TP	Treponemal Pallidum
TTI	Transfusion Transmissible Infection

ABSTRACT

Background: The presence of blood borne infections in blood cells or plasma of asymptomatic donors is the major risk factors for transmitting infectious agents through blood transfusion.Common infectious agents include Hepatitis B virus,Hepatitis C virus , Human immunodeficiency virus and Syphilis .

Objective: To determine prevalence of transfusion transmissible infections ,ABO blood grouping and Antibody screening among blood donors at Defense Health Main Department Bella Blood Bank center of Addis Ababa, Ethiopia.

Methodology: A retrospective study on the magnitude of TTIs among blood donors was conducted from January2010 to April2019 at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia. The socio-demography, TTI, ABO typing and antibody screen data was first entered into Excel spread sheet from the institutions file by removing any identifier, cleaned and then finally exported to SPSS Version24 software for analysis.Proportion and P-value was used to calculate statistical significances. P-value<0.05 was considered as statistically significant.

Result: The overall prevalence of TTI was 99(10.8%) out of 927 voluntary donors. The prevalence is 93(11.8%)in male and 6(4.2%) in Female. Majority affected agegroup were 46-58(16.4%) followed by 36-45(10.4) .The prevalence of HIV,HBV, HCV and Syphilis was 8(0.9%),46(5%),23(2.5%)and 22(2.4%) respectively.Five out of 927(0.53%) had multiple infections, the most common combinations were HBV-HCV 2(0.21%), HBV-Syphilis 2(0.21%) and Syphilis-HCV 1(0.1%). Antibody screening profile revealed 1(0.1%)positive.

Conclusion: Th e overall prevalence of TTI was 10.8. promoting of health education among the donor community and enhancement of voluntary donation is recommended.

Keywords: TTI, ABO,Antibodyscreen,voluntary blood donors.

1.INTRODUCTION

1.1 Background

Blood transfusion is a life saving intervention and millions of lives are saved each year globally through this procedure. However blood transfusion are associated with certain risks which can lead to adverse consequences. It may cause acute or delayed complications and carries the risk of the transmission of infections. Globally, more than 81 million units of blood are donated each year (1).

The presence of blood borne infections in blood cells or plasma of asymptomatic donors is the major risk factors for transmitting infectious agents through blood transfusion. Although blood transfusion service is mandatory to save the life of many patients who suffer from the loss of blood, it is an ideal vehicle for transmission of any infectious organism that may present in the blood (2).

Transfusion transmissible infectious agents such as Human Immunodeficiency virus(HIV), Hepatitis B virus(HBV), Hepatitis C virus(HCV) and Syphilis are among the greatest threats to blood safety for recipient and pose a serious public health problem(3).

Evaluation of data on the prevalence of transfusion transmissible infections namely HIV, HBV, HCV and Syphilis antibodies among blood and plasma donors permit an assessment of the occurrence of infections in the blood donor population and consequently the safety of the collected donations. It also gives an idea of the epidemiology of these diseases in the community(4).

Transfusion associated infections continue to be a big threat Globally, transfusion of contaminated blood causes up to 16 million new infections with hepatitis B, and 5 million new infections with hepatitis C every year. With each blood unit transfused, there is always a 1% likelihood of transfusion-linked risks including transfusion-transmitted infections. Prevention of TTIs in developed countries has been achieved by reducing unnecessary transfusions, using only regular voluntary donors, excluding donors with specific risk factors and systematic screening of all donated blood for infection. By contrast, in many developing countries none of these interventions is applied uniformly and the risk of transfusion-transmitted infections remains high (5-7).

An effective donor screening protocol for donor selection, proper counseling of donor, sensitive screening tests and effective discarding techniques for reactive units can ensure a reduction in the risk of acquiring TTIs.(8).

Screening for blood-transmissible pathogens is a critical aspect of blood safety. Where effective screening programs exist,the risk of transmission of transfusion transmissible infections(TTIs) has been reported to progressively and significantly decrease(9,10).This has been clearly demonstrated in westerncountries, where the most reliable screening methods are used, including nucleic acid amplification testing technologies,which allow for very early detection of various infectious agents (11).Recommended reference screening tests like enzyme immunoassays (EIA) or nucleic acid testing (NAT) are technically, logistically and financially still far beyond reach of many resource constrained blood banks (12). In such settings with limited capacity and low throughput, WHO accepts the use of rapid and simple serological assays for TTI screening, provided that they are quality assured, locally validated and quality controlled. Rapid test based screening protocols tend to be used increasingly in African blood banks(13).

Thus, continuous monitoring of the magnitude of transfusion-transmissible infections in blood donors is important for estimating the risk of transfusion and optimizing donor recruitment strategies to minimize infectious diseases transmission. Therefore, this study was conducted to determine the prevalence and trends of HIV, HBV, HCV and syphilis infections among blood donors at Defense Health Main Department Bella Blood Bank center of Addis Ababa, Ethiopia.

1.2 Statement of the problem

Blood Banks are an essential part of each and every hospital with basic purpose of the provision of blood transfusion services. Blood transfusion services are required in a number of clinical conditions like Anemia, Thalassemia, Hemophilia or may be required in gynecological problems e.g., in case of labor as well as Intrauterine death. It may be also indicated when surgery of patients is unavoidable because during surgery there may be some blood loss during surgery and to cope up with the loss transfusion of blood or blood products is unavoidable (14).

Safe blood transfusion is of at most importance as an unsafe blood transfusion bears lot of burden on human and economy. Morbidity and mortality associated with transfusion-transmitted infections reveal long-term effects on the recipients, their families, along with the community. Apparently healthy donor can transmit an infection during asymptomatic phase, further increasing the prevalence of various infections in the general population.In terms of economy, the additional transmission of infections through blood transfusions puts burden on medical care services, decreases productivity of society, and increases number of dependents to be taken care of. It aggravates crisis of providing good health and social services to large population with financial burden on the nation's economy (15).

Developing Nations largely fail to meet the key requirements for modern blood transfusion system that is, the collection of safe and locally sufficient blood supply from non-remunerated blood donors with in a formal organization and legislative frame work. The underlying problems are structural and attributable to various causes. Blood safety is threatened by a combination of factors due to poverty,including lack of infrastructures, frequent electricity break down,the unavailability of properly trained professionals, an in adequate supply of instruments and laboratory reagents and also difficulties in mobilizing voluntary donors (16).

In sub-Saharan African countries, factors contributing to transfusion-related transmissions include a high prevalence of HIV and other transfusion transmitted infections in the general and blood donor populations, inadequate screening facilities and lack of infrastructure and capacity to ensure sustainable operations (17).

Unsafe blood transfusions have contributed to the enormous burden of HIV infections in sub- Saharan Africa and still continue to add to this burden. The risk of HIV infection through unsafe blood and blood products is exceptionally high 95–100% compared to other common routes of HIV exposure: for example, 11–32% for mother-to-child transmission and 0.1%–10% for sexual contact. Sub-Saharan Africa has a particularly high level of a transfusion associated HIV compared with other regions due to a higher risk of infected blood being transfused (18).

Worldwide about 350 million people have chronic Hepatitis B virus infection; about 125 million have been infected with Hepatitis C virus, putting viral HBV and HCV infections among the worlds greatest infectious disease problems. These diseases are therefore important candidate for public health measures aimed at prevention,early diagnosis and treatment(19).

Hepatitis-B virus infection results in broad spectrum of disease from sub-clinical infection to fulminate hepatitis. It can progress to chronic active hepatitis, cirrhosis of liver and hepatocellular carcinoma. Globally more than one million deaths occur from complication of HBV infection every year (20).

The WHO (1997) estimates that approximately 3% of the world's population is to be infected with HCV and viremia persists in over 80%. In developing countries, the prevalence of antibodies to HCV is much higher, with reported rates reaching 4% to 6% in some parts of Africa and the Middle East (21).

Syphilis is also a systemic disease caused by Treponema pallidum which can be spread by sexual contact, blood transfusion and via vertical transmission (22). In sub-Saharan Africa, syphilis remains a serious public health problem. The prevalence of active syphilis infection among African countries was 12.8% in Tanzania (23),and 3.8% in Kenya (24). A study conducted to assess the prevalence of infection with HIV, syphilis and HBV among Ethiopian blood donors in 1995 showed that the seroprevalence of HIV-1, syphilis and HBV was 16.7%,12.8% and 14.4%, respectively (25). Regular monitoring of these viral markers and syphilis among blood donors in our country contributes the national effort of ensuring the safety of this life saving service. This study is one such effort in the less investigated blood donor population at one of the military hospital affiliated blood bank.

1.3 Significance of the study

Understanding the prevalence of Transfusion Transmitted infection and its associated factors, and identifying the most common TTIs are critical in the development of screening protocols and proper preventive measures in blood bank programs. Therefore, assessing the prevalence of TTIs and identifying common causes of TTIs in a blood bank service program may promote effective use of blood as a therapy.The TTI's prevalence data will also help in continuous monitoring of the magnitude of transfusion transmissible infections in blood donors. It will further help in estimating the risk of transfusion and optimizing donor recruitment strategies to minimize infectious diseases transmission.

Information is very scarce on the prevalence of HIV, HBV, HCV and syphilis among blood donors at Defense Health Main Department Bella Blood Bank center of Addis Ababa, Ethiopia. It should be noted that testing for HIV, HBV, HCV and syphilis infections is useful for epidemiological monitoring and public health planning. As a result of this dearth of information, guidelines and other adequate information on the preventive and control measures are limited. Thus this study provides information on the prevalence of HIV, HBV, HCV and syphilis in blood donors for epidemiological purpose and to be used as reference for future studies. The study also avails data on the frequency of blood groups and antibody screen among the donor population.

2. LITRATURE REVIEW

A study conducted at coastal Karnataka, India in the months of April and May 2015, showed that out of 500 blood donors the highest number of donors, 249 (49.8%) belonged to the age group of 21-30 years and the lowest number of donors, 7 (1.4%) belonged to the age group of 51-55 years**.**Of them 485 (97.0%) were male donors and 15 (3.0%) were female donors. Most of the blood donors, 219 (43.8%) belonged to O blood group, followed by blood group A ,135(27.0%) donors, group B 120 (24.0%) and only 26 (5.2%) donors belonged to AB blood group. Of the total donors, 2 (0.4%) of them were positive for HBsAg. Both of them belonged to the age group 21-30 years (0.8%) and both of them were males (0.41%).One of them was of O blood group (0.45%) and the other was of B blood group (0.83%) (26).

Another study conducted at Pakistan in 2016, involving a total of 16,602 donors; of whom 16,557 were males and 45 females, with a mean age of 28.6 ± 2 years (range 18 to 55 years), In this study in which 95% were replacement blood donors,973 (5.8%) were reactive in the screening assays, among them 58 (0.35%) were reactive in more than one assay. The prevalence of HCV, HBV, HIV, syphilis and malaria in the study population was 1.8, 1.7, 0.04, 2.1 and 0.07% respectively (27).

A total of 2616 donors over a period of 3 years were included in aretrospective study conducted at hospital blood bank of Pt. Jawaharlal Nehru Government Medical College, Chamba (H.P), India from January 2015 to December 2017. Of them, only 847 (32.37%) were voluntary donors and 1769 (67.62%) were replacement donors Male donors comprised 97.2% of total donors with 2543 donors whereas female donors were only 73 forming 2.79%of total donors. The overall TTI prevalence was higher (0.26%) among replacement donors in comparison with voluntary donors (0.07%).Out of 2616 blood samples, 8 were HBsAg positive, showing a seroprevalence of 0.3%. All the eight HBsAg positive samples were male donors. There was 1 replacement donor showing seropositivity for HCV in 2017, comprising 0.03% (28).

A Nigerian study done at Nigeria Tertiary Hospital in July 03, 2017 ; comprises 108 blood donors screened at the Madonna University Teaching Hospital (MUTH) Elele, The prevalence rates of HIV, HBsAg and HCV were 5.6%, 4.6% and 2.8%, respectively. All of the subjects tested negative to syphilis. The percentage positive based on the donor

source was 4.6%, 4.6% and 0.9% for HIV, HBsAg and HCV respectively among the paid donors, 1% for HIV among the voluntary donor and 1.9% for HCV among the family replacement donors (FRD) (29).

Another Nigerian study conducted from January 2012 to December 2012 showed that prevalence of TTIs recorded for HBsAg and HCV was 2.0% (29/1419) and 2.0% (28/1419) respectively. Among all donors, it was nil for syphilis (0%).Commercial donors recorded the highest percent prevalence of all TTIs while voluntary donors had the least. Traders and students had a higher prevalence of TTIs among the occupational groups while laborers and the unemployed recorded the least. Blood Group A Rhesus D positive donors recorded the highest prevalence of TTIs while blood Groups A and B Rhesus D negative and AB Rhesus D positive donors recorded the least. There were no significant differences in the prevalence rates of TTIs compared by donor type, gender and occupational groups ($P > 0.05$)(30).

In a study conducted at Hawassa blood bank center, Southern Ethiopia in 2015 a total of 384 blood donors were screened during the study period. These donors were 67.2% (258) males and 32.8% (126) were females with 2:1 ratio. The median age of the participants was 22 years (range 18–54 years). Most donors were in the 18–24 age group (N = 331, 86.2%). From total donors, 91.9% (N = 353) were voluntary and 8.1% (N = 31) were family replacement donors .The ABO and Rh profile of the participants were 41.4% (N= 159) O, 31.5% (N = 121) A, 21.9% (N= 84) B, 5.2% (N = 20) AB, and 7.0% (N = 27) were Rh negative. In addition, many of the donors were above grade twelve (N = 291, 75.8%) and were students (N = 330, 85.9%) The overall prevalence of TTI was 7.29% (N = 28). The seroprevalence of HIV, HBV, HCV, syphilis and malaria was 1.6% (N = 6), 4.2% (N = 16), 0.5% (N = 2), 0.8% (N= 3), and 0.3% (N = 1), respectively two donors had co-infections; one each 0.5% with HIV- HBV (0.26%) and HBV- HCV (0.26%) (31).

According to a retrospective study conducted in Jijiga blood bank starting from January 2010 to December 2013, out of 4224 blood units collected, 487 units tested positive for any of the TTI giving an overall positivity rate 11.5 %. No co-infection reported during this study period. Of all the TTI, hepatitis B form majority of infection 460/4224

(10.9 %), followed by hepatitis C 17 (0.4 %), while the least percentage was detected for HIV and syphilis 6 (0.1 %) and 4 (0.1 %) respectively (32).

Serological evidence of infection with at least one pathogen was recorded in 963(14.1%) donors by another retrospective study conducted in Jijiga from January 2010 to December 2014. The study which included a total of 6827 consecutive blood donors, donors detected 73(1.07%) donors with multiple infections. The overall seroprevalence of HIV, HBV, HCV and syphilis was 3.16%, 9.48%, 0.73% and 0.73% respectively. Among those with multiple infections, the most common combinations were HIV-HBV 41/73(56.2%). Blood group "O positive" was the most common with 51.62% followed by "A positive". Moreover, significantly declining trends of HIV, HCV and syphilis seropositivity were observed over the study period (33).

A multicenter study conducted from 2002 to February 2003 in the northern part of Ethiopia has shown that the prevalence of HBsAg was 4.7%(14/300)for Gonder college of Medical Science,6% For Bahirdar hospital,3%(3/100) for Dessie and 14%(14/100)for the Mekele hospital blood banks.The prevalence of HCV antibody was 7%(7/100) and 3%(3/100) for Gondar and Bahirdar, respectively, while 0% (0/200) for Dessie and Mekele hospital blood banks (34).

In a retrospective study conducted in Dire Dawa blood bank starting from Julay 2010 to June 2013,total of 6376 blood donors were tested, large proportionout 5647(88.57%) were replacement donors and 729(11.43%) were voluntary donors. The majority of them were male, 5430(85.16%), and aged between 18–32 years, 4492(70.45%). A total of 450(7.06%) donors had serological evidence of infection with at least one pathogen. The overall positivity rates of HBV, HIV, HCV and syphilis were 4.67%, 1.24%, 0.96%, and 0.44% respectively.Trends for transfusion-transmissible infections showed a significant decrease from 9.51% in 2010 to 6.95% in 2013 with the least prevalence in 2012 (5.90%) (P=0.004)(35).

In a study conducted at Addis Ababa,Ethiopia blood bank in 2017 out of 156729 blood donors, 14757 (9.41%) had serological evidence of infection with at least one pathogen and 29 (0.19%) had multiple infections. The overall seroprevalence of HIV, HBV and HCV was 2.29%, 5.23%, and 2.30% respectively. The seropositivity of TTIs was

significant in business owners, students, civil servants, unemployed individuals,drivers and age groups 25 to 34 and 35 to 44 years (36).

According to the studies reviewed above the overall magnitude of TTIs as well as individual viral markers and syphilis vary from place to place. As far as my literature search goes there is no published study on the magnitude of TTIs from Bella blood bank center of Addis Ababa, a gap this study is trying to address.

3. OBJECTIVES

3.1General objective

To determine Prevalence of transfusion transmissible infections,ABO blood grouping and Antibody screening among blood donors at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia.

3.2 Specific Objective

To Determine the prevalence of HIV, HBV, HCV and syphilis among blood donors at Defense Health Main Department Bella Blood Bank center of Addis Ababa, Ethiopia.

To Determines the prevalence of co-infections and trends of HIV, HBV, HCV and Syphilis infections over a study period among blood donors.

4. MATERIAL AND METHODS

4.1 Study Design

A retrospective study was conducted from January 2010 to February 2019 G.C at Defense Health Main Department Bella Blood Bank center Addis Ababa ,Ethiopia .

4.2 Study Area

The Ethiopian National Defense Blood Bank center was established in July 2007 G.C by the U.S Department of Defense HIV/AIDS Prevention Program in collaboration with FDRE Defense force health main department in order to provide safe blood and blood products to all medical facilities and field medical units throughout the country.The ENDF is serving as a training center for medical technical personnel supporting the ENDF blood program. Through excellence and innovation, the ENDF blood bank center tries to provide the best possible service to all defense medical facilities and to be model Blood bank and transfusion centers throughout Ethiopia. ENDF blood bank services operate under the direction of the defense health commandant. The commandant of the ENDF is a member of the Defense Forces Counsel.

The ENDF has two blood banks, five blood transfusions centers and many Hospitals such as Armed forces referral and teaching hospital (AFRTH), four Command Referral Hospitals and one Air Force Hospital located across Ethiopia. The roles and responsibilities of these health facilities are to provide sustainable supply of blood and blood products, quality assured, preventive and curative health services to the active army and their dependents in order to ensure ENDF operational readiness.Blood and blood products are crucial for ENDF health services. To ensure adequate and quality blood supply, the establishment of satellite transfusion sites near ENDF troop concentrations is imperative. The ENDF currently collaborates with the national blood bank service (NBBS) to supplement its blood supply. The Ethiopian red Cross Society (ERCS) used to provide blood transfusion services throughout Ethiopia since 1969. In 2010, the ERCS transferred the national Blood Program to NBBS within the Federal Ministry of Health

(FMOH). Soon after the NBBS assumed responsibility for the national blood supply, it began close collaborative efforts with the ENDF. In particular, the ENDF allows active recruitment and collection of blood donors aboard military facilities for the NBBS, especially at times of increased demand. In return, the NBBS provides blood products to ENDF for the transfusion of military personnel. Recognizing this mutually beneficial relationship and the opportunity for greater areas of collaboration, a formal Statement of Intent for Collaboration between the NBBS and the ENDF was signed by the Director of the NBBS and the Commandant of the ENDF Health Main Department on August 29, 2014.Given the limits of the NBBS to meet all ENDF blood requirements, a self-sustaining ENDF military blood program is required and deemed necessary by the ENDF. Under the PEPFAR program, collaboration between the ENDF and U.S. began to establish a safe, standardized, self-sufficient military blood program capable of collecting, testing, and distributing blood to military hospitals and supporting civilian hospitals'blood requirements during an emergency(37).

4.3 Study period

The data was collected from the blood bank log books from April 2019 to May 2019 GC.

4.4 Population

4.4.1 Source population

The source populations were data from all ⑦⑥❶⑤❶⓪③ ⑥⑤⑥⑨⑩ ❸⑥ ❷⓪⑩⓪❶⑥ Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia from January 2010 to February 2019 G.C.

4.4.2 Study population

The study populations were data from all individuals who donated blood with complete data on socio-demographic as well as TTI markers during the study Period.

4.5 Inclusion and exclusion criteria

4.5.1 Inclusion criteria

All Donated blood data on TTI 's and socio-demographic characteristics from a period of January 2010 to February 2019 were selected for the study.

4.5.2 Exclusion criteria

Donated blood records with incomplete data were excluded.

4.6 Study variable

4.6.1 Dependent variable

The prevalence of HIV, HBV, HCV, Syphilis .

4.6.2 Independent variables

- Age
- Sex
- ABO and RH Blood group
- Occupation
- Ab Screening

4.7 Sample size determination.

The Data is collected from all individuals who donated blood and have fully recorded data at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia from January 2010 to February 2019.

4.8 Sampling method

Convenient sampling technique is employed. All donated blood at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia from January 2010 to February 2019 and who fulfilled the inclusion criteria were included.

4.9 Measurement and data collection

4.9.1 Data Collection procedure

Data on socio-demographic variables and laboratory test results were collected from blood donors registration folder using data extraction format.Collected data were then cross checked for completeness,entered in to Excel and then transferred to SPSS version 24 for analysis.

4.9.2 Laboratory Analysis.

Blood samples were tested using Abbott automated chemiluminescence microparticle immunoassay for Qualitative detection of HIV, Hepatitis B, Hepatitis C and Syphilis

infection, ABO/ Rh Blood grouping and Antibody screening is also performed following standard operating procedures (SOP).

General Principal of Chemiluminecent

Chemiluminescent is light produced by a chemical reaction; the energy levels are identical with those involved in Fuorescence phenomena, the only difference being the mode of excitation. Chemiluminescent reactions generally yield a product in an electronically excited state producing visible light. As a general rule, only quite exothermic reactions can generate the required energies. Therefore, most Chemiluminescent reactions use oxygen, hydrogen peroxide, or similar potential oxidants. As a principle in bioluminescence and Chemiluminescent reactions, at least two reagents, A and B react to form a product C, some fraction of which is present in an electronically excited state, C*, which may subsequently relax to the ground state emitting a photon: $A+B \rightarrow C \rightarrow C+hn$

Hence, the luminescence process takes place at the rate of a chemical reaction and the factors that affect emission intensity are in fact a combination of chemical reaction rate and luminescence considerations (38).

Principle and Interpretation of chemiluminescent microparticle immunoassay test for HIV detection .

Is a two-step immunoassay to determine the precence of HIV p24 antigen and antibodies to HIV-1(GroupMand group O)and HIV-2 in Human serum and plasma using CMIA technology with flexible assay protocols,referred to as Chemiflex. in the first step, sample, ARCHITECT i Wash buffer, assay diluents and paramagnetic microparticles are combined.HIVp24 antigen and HIV-1/HIV-2 antibodies present in the sample bind to HIV-1/HIV-2 antigen and HIV P24 monoclonal(mouse)antibody coated microparticles.After washing ,the HIV p24 antigen and HIV-1/HIV-2 antibodies bind to the acridinium-labeled conjugates(HIV-1/HIV-2 antigens;recombinant,synthetic peptides and HIVp24antibody(mouse,monoclonal).Following another wash cycle,pre-trgger and trigger solutions are added to the reaction mixture.The resulting chemiluminescent reaction is measured as relative light unit (RLUS).Adirect relationship exist between the amount of HIV antigen and Antibodies in the sample and

the RLUS detected by the ARCHITECT I system optics.The presence or absence of HIV p24 antigen or HIV-1/HIV-2 antibodies in the specimen is determined by comparing the chemiluminescent signal in the reacton to the cutoff signal determined from an ARCHITECT HIV Ag/Ab Combo Calibration. Specimens with signal to cutoff(S/CO)values greater than or equal to 1.00 are considered reactive for HIV p24 antigen or HIV-1/HIV-2 antibodies.Specimens with S/CO values less than 1.00 are considered nonreactive for HIV p24 antigen or HIV-1/HIV-2 antibodies.

Principle and Interpretation of chemiluminescent microparticle immunoassay test for HBsAg detection .

The ARCHITECT HBsAg QualitativeII assay is one –step immunoassay for qualitative detection of HBsAg in Human serum and plasma using CMIA technology,with flexible assay protocols,referred to as Chemiflex.

In ARCHITECT HBsAg QualitativeII assay,sample,anti-HBscoated paramagnetic microparticles and ant-HBs acridinium-labeled conjugate sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labled conjugate.After washing,ancillary wash buffer is added to the reaction mixture.following another wash cycle,pre-trigger and trigger solutions are added to the reaction mixture.The resulting chemiluminescent reaction is measured as relative light unit(RLUs).Adirect relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT I System optics.

The presence or absence of HBsAg in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal,the sample is considered reactive for HBsAg.

Principle and Interpretation of chemiluminescent microparticle immunoassay test for HCV detection .

The ARCHITECT HCV assay is Two-step immunoassay ,using chemiluminescent micoparticle immunoassay(CMIA)technology,for the qualitative detection of anti-HCV in Human serum and plasma.Sample,recombinant HCV antigen coated para magnetic micoparicles and assay diluents are combined.The ant-HCVpresent in the sample binds to

the HCV Coated microparticles.After washing,Anti-Human acridinium-labled conjugate is added to creat areaction mixture.Following another wash cycle.pre-triggerd and Triggerd solutions are added to the reaction mixture.

The resulting chemiluminescent reaction is measured as relative light unit(RLUs).There is a direct relationship between the amount of anti-HCV in the sample and the RLUs detected by the ARCHITECT I system optics.

The presence or absence of HCV in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal,the sample is considered reactive for HCV.

Principle and Interpretation of chemiluminescent microparticle immunoassay test for Syphilis detection .

The ARCHITECT I Syphilis TP assay is a two-step immunoassay for the qualitative detection of antibody to TP in Human serum and plasma using CMIA technology with flexible assay protocols,referred to as Chemiflex.in the first step;sample, microparticles Coated with recombinant TP antigens(TpN15,TpN17,TpN47) and assay diluents are combined.Anti-TP antibodies present in the sample bind to the TP coated microparticles. After washing ,the acridinium-labeled anti-humanIgM and IgG conjugates is added in the second step.Following another wash cycle,pre-trgger and trigger solutions are added to the reaction mixture.The resulting chemiluminescent reaction is measured as relative light unit (RLUS).Adirect relationship exist between the amount of anti-TP antibodies in the sample and the RLUS detected by the ARCHITECT I optical system . The presence or absence of ant-TP antibodies in the specimen is determined by comparing the chemiluminescent signal in the reacton to the cutoff signal determined from a previous ARCHITECT Syphilis TP Calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for anti-TP.

Principle and Interpretation of ABO blood grouping

Blood group of the donor was determined from sample obtained by finger pricking as well as from segment . Forward ABO Blood grouping is determined by testing unknown

red cells against known anti-A, Anti-B antibodies. The presence or absence of agglutination of the red cells indicates the Presence or absence of corresponding antigen.

Principle and Interpretation of Rh typing

The presence of the anti D antigen is determined by testing the test blood cells against the antibody with a known anti D specificity. The presence or absence of agglutination of the red cells indicates the Presence or absence of corresponding antigen.

Principle and Interpretation of Antibody screening

Serum or plasma is systematically tested against panoscreen reagent red blood cells. Agglutinationof one or more of the panoscreen cells at any phase,or hemolysis at the saline or potentiated phases of testing,constitutes a positive test and is the result of a reaction between an antigen and its respective antibody.No agglutination or no hemolysis indicates either the absence of antibody,providing the test red cells possess the corresponding antigen,or that an antibody,if present,is in concentrations too low to be detected by the serologic techniques employed.Once an antibody is detected,it can be identified by testing the sample with panocell reagent red blood cells. At Bella blood bank only detection step was carried out, for the first time in our country.

4.10 Data Quality Assurance

Data were extracted carefully with double checking, cleaned and analyzed using SPSS version 24 statistical package.To ensure the quality of data entered into the computer, by two people independently cross-checked each entry.

4.11 Data analysis and Interpretation

Data from blood donor's records covering the period from January 2010 to February 2019 were entered in to excel and exported to SPSS for analysis. Data cleaning was performed to check for accuracy and consistency. Descriptive statistics was employed to determine the prevalence of TTIs. Binary and multiple regression analysis were employed to assess association of dependent and independent variables. Odds ratio and P-values were used to assess the strength of the association. p-value less than 0.05 was considered as statistically significance at 95% confident interval .

4.12 Operational Definition

Replacement donation: donation of blood for relatives or friends to replace blood used from blood bank stocks.

Safe blood: A blood that is free from transfusion transmissible diseases, drugs, alcohol, chemical substances, or other extraneous factors that might cause harm or danger to the recipient.

Transfusion Transmissible Infections: are infections like HBV, HIV, HCV and Syphilis.

Voluntary(non-remunerated donation): is free donation of blood by volunteers humanitarian concern.

4.13 Ethical consideration

Ethical clearance was obtained from Departmental Research and Ethical review committee (DRERC) of the Department of Medical Laboratory Sciences,College of Health Sciences, Addis Ababa University .A formal letter of cooperation was submitted and approval obtained from Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia. Only codes were used to extract data from records by removing any identifier information. Hard copies were locked and electronic files password protected to ensure confidentiality of data.

4.14 Dissemination of results

The research findings will be presented to Addis Ababa University, College of Health Science, Department of Medical Laboratory Sciences,at public defense. The findings will be shared to Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia. The result will also be communicated through presenting at scientific conferences and effort will be made to publish findings in peer reviewed journals.

5. RESULT

5.1 Socio-demographic and Blood typing data of donors

A Total of 927 blood donors were screened during the study period among these 85.1%(789) were male and 14.9%(138) were female with almost 6:1.The age of donors ranged from 19 to 58. The majority of donors 334(37.0%) were in the age group 26 to 35. All the donors were voluntary blood donors. The ABO and RH profile of the participants were 401(43.5%)O, 267(28.8%)A, 201(21.6%)B, 58(6.3%)AB. 852(91.9%) were RH positive and 75(8.1%) were RH negative. Antibody screening profile of participants were 926(99.9%)negative, 1(0.1%)positive. Majority of the donors were government workers 865(93.3%) and the remaining 62(6.7%) were students. Table 1 summarizes Socio-demographic and bloodgroup data of donors.

Table 1 Socio-demographic and blood group characteristics of blood donors at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia,January 2010-February 2019 (n=927)

Variables		**Frequency**	**Percent**
Age	19-25	80	8.6
	26-35	343	37.0
	36-45	334	36.0
	46-58	170	18.3
	Total	927	100.0
Gender	Male	789	85.1
	Female	138	14.9
	Total	927	100.0
Occupation	Governmet	865	93.3
	Student	62	6.7
	Total	927	100.0
ABO Blood grouping	A	267	28.8
	B	201	21.6
	AB	58	6.3
	O	401	43.3
	Total	927	100.0
RH typing	Positive	852	91.9
	Negative	75	8.1
	Total	927	100.0
Antibody Screening	Positive	1	0.1
	Negative	926	99.9
	Total	927	100
	TOTAL	927	100.0

5.1. Magnitude of transfusion transmissible infections

The Overall prevalence of TTI was 99(10.8%) among 927 apparently healthy donors. The prevalence of HIV,HBV,HCV and Syphilis was 8.0(0.9%), 46(5%), 23(2.5%), and 22(2.4%) respectively(Table 2).Five out of 927(0.53%)had multiple infections, of them the most common combinations were HBV-HCV 2(0.21%),HBV-Syphilis 2(0.21%) and Syphilis-HCV 1(0.1%).

Table 2 Prevalence of HIV,HBsAg,HCV,Syphilis among blood donors at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia January 2010-February 2019 (n=927)

Type of TTI		**Frequency**	**Percent**
HIV	Positive	8.0	0.9
	Negative	919	99.1
	Total	927	100.0
HBV	Positive	46	5.0
	Negative	881	95.5
	Total	927	100.0
HCV	Positive	23	2.5
	Negative	904	97.5
Syphilis	**Total**	927	100.0
	Positive	22	2.4
	Negative	905	97.6

The prevalence of HIV was 1/138(0.7%) and 7/789(0.9%) in female and male donors respectively,P-value=0.953. The prevalence of HCV was 2/138(1.4%) in female 21/789(2.7%) in male respectively,p-value=0.24. Males were found to have a significantly higher HBV prevalence than females 1/138(0.7%) in female and 45/789(5.7%)in male,p-value=0.042.The prevalence of Syphilis was 2/138(1.4%) in female and 20/789(2.5%) male,p-value =0.45.

Moreover the prevalence of HIV was highest in age group 46-58 (2.9%) and lowest in 19-25(0.0%).The prevalence of HBV was highest in age group 46-58 (7.6%) and lowest in 19-25(0.0%).The prevalence of HCV was highest in age group 46-58 (4.1%) and lowest in 19-25(0.0%).The prevalence of Syphilis was highest in age group 19-25(3.8%) and lowest in age group 46-58 (1.8%).

Table 3 Association of HIV with Socio-demographic factors among voluntary blood donors at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia, January 2010-February 2019 (n=927)

		HIV (N),%	**P-value**
Age group	19-25	0(0%)	
	26-35	1(0.3%)	0.99
	36-45	2(0.5%)	0.041
	46-58	5(2.9%)	0.065
Gender	Male	7(0.9%)	0.953
	Female	1(0.7%)	
Occupation	Government	8(0.9%)	0.99
	Student	0(0.0)	

Table 4 Association of HBV and HCV with sociodemographic factor among voluntary blood donors at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia, January 2010-February 2019 (n=927)

		HBV Frequency	**p-value**	**HCV Frequency**	**p-value**
Age group	19-25	0(0.0%)		0(0.0)	
	26-35	14(4.1%)	0.99	10(2.9%)	0.99
	36-45	19(5.7%)	0.27	6(1.8%)	0.62
	46-58	13(7.6%)	0.5	7(4.1%)	0.15
Gender	Male	45(5.7%)	0.042	21(2.7%)	0.24
	Female	1(0.7%)		2(1.4%)	
Occupation	Government	46(5.3%)	0.99	23(2.6%)	0.99
	Student	0(0.0%)		0(0.0%)	

Table 5 Association of Syphilis with socio-demographic factors among voluntary blood donors at Defense Health Main Department Bella Blood Bank center Addis Ababa, Ethiopia,January 2010-February 2019 (n=927)

		Syphilis	**p-value**
Age group	19-25	3(3.8%)	0.29
	26-35	8(2.6%)	0.51
	36-45	8(2.4%)	0.6
	46-58	3(1.8%)	-
Gender	Male	20(2.5%)	0.45
	Female	2(1.4%)	-
Occupation	Government	22(2.5%)	0.99
	Student	0(0.0%)	-

Declining trends of HIV, HBV, HCV prevalence were observed over the nine years study period. The prevalence of HIV was 2.2% in 2010 and showed zero prevalence for consecutive years afterwards. The prevalence of HBV slightly increase from 6.2% in 2010 to 6.7% in 2011 and decreased after ward and increases at 2016 showing prevalence of 6.9%. HCV prevalence decreased from 3.1% in 2010 to 2.7% in 2011, 0.0% in 2012, 2014,and 2015,3.4% in 2016 and increases to 4.1%% in 2017 and decreases afterward. Similarly, the prevalence of syphilis decreased from 1.4% in 2010 to 0.0% in 2011 up to 2015 and increases to 5.4%,5.5%,6.4%,11.1% consecutively.

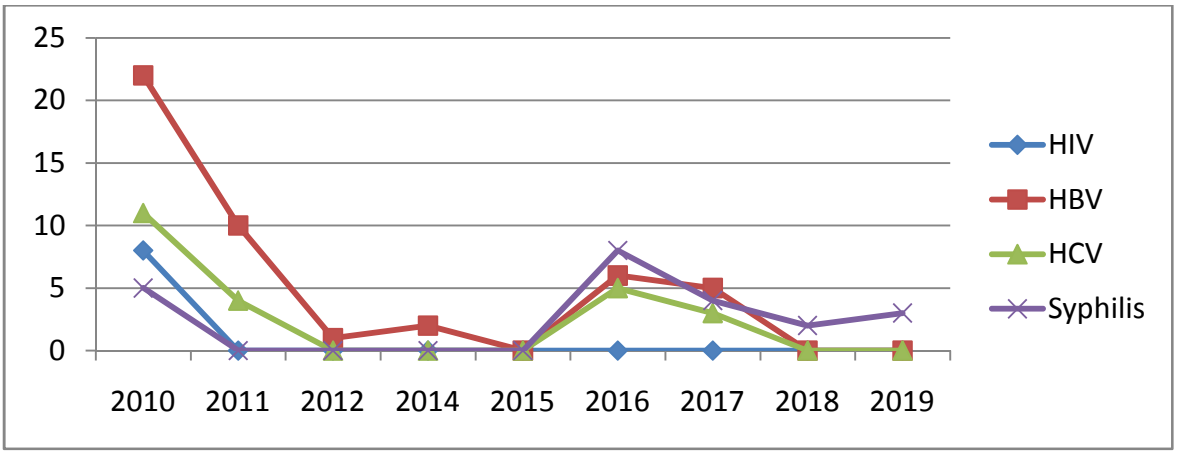

Figure 1 Line graph showing trends of TTIs over a study period among voluntary blood donors.

6.DISCUSSION

This study aimed at determining the magnitude of four TTIs that are routinely screened in the blood banks of Ethiopia. The study was carried out in Bella Blood bank which is one of the less investigated blood banksthat gives service for the military population and their families in Addis Ababa,In this study the overall prevalence of Transfusion transmitted infections was 99(10.8%). This prevalence is low compared with the overall prevalence of the study done in Nigeria19.3%(39), Islamabad,Pakistan14.34%(40)A possible reason for the discrepancy may be due to characteristics of the donor types, Study participants in the previous study were commercial donors where as only voluntary donors were included in this study.

However, the present study showed the overall prevalence of Transfusion transmitted infections was higher compared with the study done in ✋⑦③ ❸⓪ ⑩⑥❸ Indian study was showed the overall TTI was 2.22%(42) , in Gujrat, India 0.93% (43).

The present study showed prevalence of Human immune deficiency virus 8(0.9%), Hepatitis B virus 46(5%),Hepatitis C virus 23(2.5%) ,Syphilis 22(2.4%).The overall prevalence in male 93/789(11.8%) was higher than in Females 6/138(4.2%).This result is consistence with previous study done in India the male 98.38% and female 1.62% .the possible reason for discrepancy may be due to low number of female participants. Another study conducted in Easter Ethiopia was higher among the male donors 53/408(13.1%) compared to female donors.

The current study finding indicated high prevalence of HIV 8(0.9%) when compared with the study done in India(0.27%)(44), Pakistan(0.06%)(45),China0.86%(46), Namibia0.3% (47)Yemen 0.14%(48), China 0.08% (49) . Even if the finding was higher than other countries finding, it was low compared with the prevalence of HIV that was reported from Mozambique 8.5%(50),Ghana 4.9%(51),Nigeria13.2%(52) and a study from various populations in Ethiopia13.3%(53)

The prevalence of HBV infection was 46(5%)among blood donors in this study.The finding showed high prevalence compared with the study done in BahirDar 4.11%(54), Canada0.09%(55), India1.93%(56). The finding agrees with the study done in Sudan 5%

(57) .On the contrary the prevalence of HBV infection in the current study showed low prevalence when compared with the study done in Nigeria26.0%(58),Jos,Nigeria 14.3%(59), BurkinaFaso 14.96%(60), the possible reason of discrepancy may be study design, duration of the study,donor type and type of study participants characteristics.

The prevalence of HCV infection was 23(2.5%) among blood donors. This study finding showed low prevalence compared with study done in Islamabad,Pakistan 8.34%(61), Ghana 5.6%(62) and BurkinaFaso 8.6%(63) while it agrees with the study done in Gabonese 2.78%(64)But low prevalence was observed in study done in Gonder, Ethiopia0.8%(65),Jijiga,Ethiopia0.7%(66),Cameroon1.3%(67),Enugu1.1%(68)

The prevalence of Syphilis infection was 22(2.4%)among blood donors of the current study.This study finding showed low prevalence compared with study done in EquatorialGuinea21.5%(69), Ghana7.5%(70)and Cameroon5.7(71) while it agrees with the study done in Nigeria2.6%(72), Sudan2.7%(73).But the present study findings was higher when compared with findings from Ereteria0.5%(74) and Kenya1.2%(75).

The present study prevalence was low compared with study done in Sudi arabia, which reported a prevalence of 21.3% transfusion transmissible infections among blood donors (76).Another study reported from Addis Ababa, National Blood Bank of Ethiopia shows similar prevalence of 9.5%(77) When compared to other studies conducted in other African countries, the prevalence of TTI in this study is high compared to the study conducted in Uganda 5.7%(78).

7.Strength and Limitation of the study

7.1 Strength of the study

The strength of the study is mostly reported transfusion transmitted infections like HIV, HBV, HCV, Syphilis are included in a less investigated setting. Also Screening of TTI is done by the most sensitive and specific method. Antibody screen data is reported for the first time.

7.2 Limitation of the study

The Limitations of the study is related to retrospective nature of the study in that it did not include information about some Socio demographic characteristics like marital status, educational background and behavioral patterns of blood donors.

Service inconsistency in the blood bank due to reagent unavailability is one of the major problem for the sample size to be small.

However, the data generated in this study provides firsthand information to create awareness in the donor population in this military hospital.

8.Conclusion and Recommendation

8.1 Conclusion

In general, the overall prevalence of TTI was 99(10.8%) out of 927 apparently healthy donors. The prevalence of HIV, HBV, HCV and Syphilis was 8.0(0.9%), 46(5%), 23(2.5%), and 22(2.4%), respectively in the study area, HBV being commonest TTI among voluntary donors, followed by HCV and Syphilis. The study showed low participants of female donors. The blood donors in this study were all voluntary donors,who are apparently healthy. Antibody screening profile of participants were 926(99.9%) negative and 1(0.1%)positive .In this study low percentage of multiple infections 5/927 (0.53%) was observed compared with other studies.

8.2 Recommendation

Effort on promoting of the blood bank activity,motivating,educating and creating awareness in the community should be made to maintain voluntary donors. Regular monitoring of TTIs also in other blood transfusion centers of Ethiopia is needed to ensure safety of blood transfusion from these infections need to be conducted to monitor the overall status of the concerned infections.

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10. ANNEXES

10.1 Standard operating Procedures For Chemiluminescent micro particle immunoassay tests.

Chemiluminescent micro particle immunoassay test for qualitative detection of syphilis antibody.

1. Before loading the ARCHITECT syphilis TP reagent kit on the system for the first time, the micro particle bottle requires mixing to resuspend microparticles that have settled during shipment. after the first time the micropartcles have been loaded, no further mixing is required.

-once the micro particles have been resuspend,place a septum on the the bottle.

2. Load the ARCHITECT syphilis TP reagent kit on the ARCHITECT i system.

-Verify that all necessary assay reagents are present.

-Ensure that septums are present on all reagent bottles.

3. Order calibration if necessary then Order tests.

4.The minimum sample cup volume is calculated by the system. no more than ten replicates may be sampled from the same sample cup. To minimize the effect of evaporation, verify adequate sample cup volume is present before running the test.

-Priority;150 micro litter for the first ARCITECT Syphilis TP test plus 30 micro litters for each additional ARCHITECT syphilis TP test from the same sample cup.

-Less than or equal to 3 hours on boared;150 micro litter for the first ARCHITECT syphilis TP test plus 30 micro litters for each additional ARCHITECT syphilis TP test from the same sample cup.

-Greater than 3 hours on boared; additional sample volume is required.

-If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

5. Prepare calibrator and controls.

-Mix ARCHITECT syphilis TP Calibrator and controls by gentle inversion before use.

-To obtain the recommended volume requirements for the ARCHITECT syphilis TP Calibrator and controls,hold the bottles vertically and dispense 5 drops of calibrator or 6. drops of each control in to each respective sample cup.

7. Load samples and press RUN.

Chemiluminescent microparticle immunoassay test for simultaneous detection of HIV p24 antigen and antibodies to HIV-1/HIV-2 .

1.Before loading the ARCHITECT HIV Ag/Ab combo reagent kit on the system for the first time, the micro particle bottle requires mixing to resuspend microparticles that have settled during shipment.

-Invert the microparticle bottle 30 times.

-Visually inspect the bottle to ensure microparticles are resuspended.if the microparticles remain adhered to the bottle,continue inverting the bottle until the microparticles have been completely resuspended.

-If the microparticles do not resuspend,do not use.

-once the microparticles have been resuspended,remove and discared the cup.wearing clean gloves,remove a septum from the bag.carefully snap the septum on to the top of the bottle.

2. Order calibration if necessary then Order tests.

3. Load the ARCHITECT HIV Ag/Ab combo reagent kit on the ARCHITECT i system.

-verify that all necessary reagents are present.ensure the septums are present on all reagent bottle.

4.The minimum sample cup volume is calculated by the system. no more than ten replicates may be sampled from the same sample cup. To minimize the effect of evaporation, verify adequate sample cup volume is present before running the test.

-Priority;150 micro litter for the first ARCITECT HIV Ag/Ab combo test plus 30 micro litters for each additional ARCHITECT syphilis TP test from the same sample cup.

-Less than or equal to 3 hours on boared;150 micro litter for the first ARCHITECT HIV Ag/Ab combo test plus 100 micro litters for each additional ARCHITECT HIV Ag/Ab combo test from the same sample cup.

-Greater than 3 hours on boared; additional sample volume is required.

-If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

5. Prepare calibrator and controls.

- ARCHITECT HIV Ag/Ab combo Calibrator 1and controls shold be mixed by gentle inversion prior to use.

-To obtain the recommended volume requirements for the ARCHITECT HIV Ag/Ab combo Calibrator 1 and controls, hold the bottles vertically and dispense 20 drops of calibrator or 10 drops of each control in to each respective sample cup.

6. Load samples and press RUN.

Chemiluminescent micro particle immunoassay test for Qualitative detection Hepatitis B surface antigen.

1. Before loading the ARCHITECT HBsAg qualitative II reagent kit on the system for the first time, the micro particle bottle requires mixing to resuspend microparticles that have settled during shipment. after the first time the micropartcles have been loaded, no further mixing is required.

-Invert the microparticle bottle 30 times.

-Visually inspect the bottle to ensure microparticles are resuspended.if the microparticles remain adhered to the bottle,continue inverting the bottle until the microparticles have been completely resuspended.

-If the microparticles do not resuspend,do not use.

-once the microparticles have been resuspended,remove and discared the cup.wearing clean gloves,remove a septum from the bag.carefully snap the septum on to the top of the bottle.

2. Load the ARCHITECT HBsAg qualitative II reagent kit on the ARCHITECT i system.

-Verify that all necessary assay reagents are present.

-Ensure that septums are present on all reagent bottles.

3. Order calibration if necessary then Order tests.

4.The minimum sample cup volume is calculated by the system. no more than ten replicates may be sampled from the same sample cup. To minimize the effect of evaporation, verify adequate sample cup volume is present before running the test.

-Priority;125 micro litter for the first ARCITECT HBsAg qualitative II test plus 75 micro litters for each additional HBsAg qualitative II test from the same sample cup.

-Less than or equal to 3 hours on boared;150 micro litter for the first HBsAg qualitative II test plus 75 micro litters for each additional HBsAg qualitative II test from the same sample cup.

-Greater than 3 hours on boared; replace with a fresh sample (patient specimens,controls and calibrators).

-If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

5. Prepare calibrator and controls.

-Mix ARCHITECT HBsAg qualitative II Calibrator and controls by gentle inversion before use.

-To obtain the recommended volume requirements for the ARCHITECT HBsAg qualitative II Calibrator and controls,hold the bottles vertically and dispense 11 drops of each calibrator and 6 drops of each control in to each respective sample cup.

6. Load samples and press RUN.

Chemiluminescent micro particle immunoassay test for Qualitative detection of antibody to Hepatitis C virus.

1. Before loading the reagent kit on the system for the first time, the micro particle bottle requires mixing to resuspend microparticles that have settled during shipment.

-Invert the microparticle bottle 30 times.

-Visually inspect the bottle to ensure microparticles are resuspended.if the microparticles remain adhered to the bottle,continue inverting the bottle until the microparticles have been completely resuspended.

-If the microparticles do not resuspend,do not use.

-once the microparticles have been resuspended,remove and discared the cup.wearing clean gloves,remove a septum from the bag.carefully snap the septum on to the top of the bottle.

2. Load the reagent kit on the ARCHITECT i system.

-verify that all necessary reagents are present.

-ensure the septums are present on all reagent bottle.

3. Order calibration if necessary then Order tests

4..The minimum sample cup volume required to perform asingle anti-HCVtest on the ARCITECT i system is 150 micro litter for the first anti-HCVtest plus 20 micro litters for additional anti-HCVtest from the same sample cup. No more than ten replicates may be sampled from the same sample cup. verify adequate sample cup volume is present before running the test.the minimum sample cup volume is calculated by the system and displayed on the patient,calibrator and control order screen and on the order list report.

-For the sample that is priority loaded,with 3 or fewer replicates ordered,asmaller sample cup volume that is displayed on the ored screen may be used.in this case,the minimum sample cup volume is 70 micro litters for the first anti-hcv test plus 20 microlitters for additional replicate.

-To minimize the effect of evaporation,all samples(patient specimens,calibrator and controls)must be tested within 3 hours of being placed on boared the ARCHITECT I system. if the sample is on boared the system for longer than 3 houres,replace with fresh sample.

-If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

5. Prepare ARCHITECT Anti-HCV calibrator and controls.

-Mix calibrators and controls by gently inversion before use.

-Hold bottles vertically and dispense recommended volumes in to each respective sample cup.

-Recommended volumes for each calibrator 5 drops and for each control 6 drops.

6. Load samples and press RUN.

10.2 Standard operating Procedures For direct ABO blood grouping.

10.2.1 First ABO blood grouping(finger pricking)

1.Receive first ABO sample with donor card from the donor screening room.

2.Check the samples for any abnormality, if there is any abnormality obtain sample from the respective bag segment.

3.Arrange the samples according to the sequence of donors' card number

4.Label the slides according to the donors' card sequence number

5.Add one drop of anti-A in the first raw, anti-B in the second raw (repeat this step depending on the number of samples to be run)

6. Mix well the donors sample with saline

7.Add one drop of the donors sample on anti-A and anti-B respectively

8.Put the slide on the shaker for 3-5 minute at 100 RPM

9.Observe for the presence of agglutination or hemolisis.

10.Record the ABO result on First ABO result form .

11.Record the result on donor card with pencil.

10.2.2 Second ABO blood grouping(segment)

1.Take samples from ABO storage refrigerator

2.Arrange samples according to their sequence order

3.Check samples for any abnormality, if any abnormality seen take sample from segment andreport the occurrence

4.Label three test tubes with proper donor pack number for Serum/plasma, red cell suspensionand Rh typing

5.Centrifuge at 3500 rpm for 5-10 minutes

6.Separate Serum/plasma from red cell

7.Prepare 3-5% washed red cells suspension. (Refer SOP LAB-06 for preparation of 3-5% Redcell suspension)

8.Properly label ABO slides with donor's pack number

9.Add one drop of anti A with the first row, anti B with the second row (repeat this stepdepending on the Number of sample to be run)

10. Add one drop of red cell suspension to be tested on its corresponding sequence

11.Put the ABO slide on Mechanical rotator at 80-100 rpm for 8 minute

12.Examine each well for agglutination

13.Read, grade degree of reaction and record results on result form for ABO and Rh typing

10.3 Standard operating Procedures For RH typing.

1.Add one drop of anti D in labeled test tube for Rh test

2.Add one drop of 3-5% red cell suspension

3.Mix well and centrifuge at 1000 rpm for 60 seconds

4.Macroscopically observe for agglutination and record results on the result form for ABO Reading and Rh typing form

5.If there is no agglutination keep test tube for D_u testing

6.Add one drop of anti D in a test tube which was previously negative for anti D testing

7.Centrifuge at 1000 rpm for 1 minute

8.Examine for agglutination macroscopically

9.Examine for agglutination microscopically if agglutination is not visible macroscopically

10.Mix thoroughly and incubate at 37^0c for 30 minutes if negative

11.Wash test red cells 4 times and decant the supernatant

12.Add one drop of anti-human globulin reagent

13.Mix gently and centrifuge at 1000 rpm for 15 second

14.Suspend the cell button gently, examine for agglutination macroscopically

15. Examine for agglutination microscopically if agglutination is not visible macroscopically

16. Interpret and record the result on ABO grouping and Rh typing result form

10.4 Standard operating Procedures for Antibody screening.

1.Lable one test tube for each Panoscreen vial,and if performed,one additional tube for an autologous control.

2.Place 2-3drops of the serum or plasma to be tested into each of tubes.Adding 3 drops may enhance reactivity.

3.Gently invert each Panoscreen vial several times to achieve a complete suspention of red blood cells.

4.Add 1drop of Panoscreen red cells to the appropriately labeled tubes.if an autologous control is to be run in parallel.add 1drop of a2-4% saline suspention of autologous red blood cells to the appropriate tube.mix the contents of each tube thoroughly.

5.Centrifuge each tube.examine the supernatant fluid for hemolysis.Gently suspend each red blood cell button and examine for agglutination.record results.

6.Add Potentiator,if used to each tube in amount specified by the manufacturers product insert.NOTE:if desired,all tubes may be incubated at room temperature(18-30°c)for 5-30 minutes,centrifuged and examined for agglutination prior to the addition of a potentiator or incubation at 36-38°c.This may enhance reactivity.

7.Mix the contents of each tube thoroughly.incubate at 36-38°c for 30-60 minutes.NOTE:Depending on the potentiator employed,the tubes may be incubated for shorter period of time.consult the manufacturer product insert for optimal incubation time for potentiator employed.

8.Centrifuge eachtube.examine the supernatant for hemolysis.Gently suspend each red blood cell button and examine for agglutination.record results.

9.Wash the red blood cells aminimum of three times with saline,being careful to decant completely after each wash.

10.Add Anti-Human Globulin to each tube in the amount specified by the manufacturers product insert.

11.Centrifuge each tube.Gently suspend each red blood cell button and examine for agglutination.record results.Negative reaction may be examined with an optical aid.

12.Confirm the validity of all negative or weakly positive reaction with IgG sensitized antiglobulin control red blood cells.

DECLARATION

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate; _____(B.Sc.)

Signature :_____

Date of submission:_____

This thesis have be submitted with our approval as advisors.

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