Addis Ababa University

College of Health Science

School of Allied Health Sciences

Department of Medical Laboratory Sciences

Assessment of Transfusion Transmissible Infections Among Blood Donors (A six years study) and Strategy on Direct Laboratory Testing Cost of Blood Screening at National Blood Transfusion Service of Addis Ababa, Ethiopia.

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Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
BTS	Blood Transfusion Services
DNA	Deoxyribonucleic Acid
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ERCS	Ethiopian Red Cross Society
ERCS-NBBS	Ethiopian Red Cross Society-National Blood Bank Services
ETB	Ethiopian Birr
EU	European Union
FTA	Fluorescent Treponemal Antibody
HBV	Hepatitis B Virus
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HFBB	Health Facility-Based Blood Bank
HMIS	Health Management Information System
IRCRC	International Red Cross and Red Crescent
ISBT	International Society of Blood Transfusion
MOH	Ministry of Health
NAT	Nucleic Acid Amplification test
NBSS	National Blood Bank Services
NBTS	National Blood Transfusion Services
NRVD	Non-Remunerated Voluntary Donor
RBB	Regional Blood Bank
RHB	Regional Health Bureau
RIA	Radio Immunoassay
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagin
SOPs	Standard Operating Procedures
TPHA	TreponemaPallidumHaemagglutination Assay
TPI	TreponemaPallidium Immobilization
TTIs	Transfusion Transmissible Infections
USA	United State of America
VDRL	Venereal Disease Research Laboratory
VNRBD	Voluntary Non-remunerated Blood Donor
WHO	World Health Organization

Operational Definitions

Paid donors are donors who are paid or remunerated otherwise for their donation.

Quality assurance is a process through which the quality of blood transfusion services is checked continually

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 out of humanitarian concern.

Abstract

Background: Hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis are the most serious infections transmitted during blood transfusion. In such a resource limited setting, cheaper and feasible alternative strategies for blood donations testing are specifically required. However, updated data on the transfusion transmissible infections (TTIs), and cost effective strategies of blood screening are lacking.

Objective: To determine the prevalence of transfusion transmissible infections among blood donorsfrom July 2008 to July 2013and propose cost effective strategy of blood screening at National Blood Bank of Addis Ababa, Ethiopia.

Methodology:A retrospective analysis of blood donors' record covering the period from July 2008 to July 2013 was conducted. The data was collected from the National Blood Transfusion Services (NBTS) of Addis Ababa and includes category of all donors and result for TTI markers. In addition, direct laboratory costs of parallel versus sequential strategy of blood screening were compared. To compare the strategies we used the current price of the laboratory costs. Data was first exported to Excel spread sheet from the institution's data base and then finally exported to SPSS version 16 software (SPSS INC, Chicago, IL, USA) for analysis.Data analysis was performed using scores and odds ratio using same software to look for an association between dependent and independent variables. P values less than 0.05 were considered significant.

Result: A total of 173,207 consecutive blood donors were screened between 2008 and 2013.The overall seroprevalence rate ofHBV, HIV, HCV and syphilis of blood donors was 5.0%, 1.6%, 1.4% and 0.1%respectively. The HIV-HBV confection was higher among blood donors 135(41.79%) followed by HBV-HCV co-infection which accounts about 103(31.89%). Significantly increased seroprevalence of TTI's was observed in the age groups of 17-25 and 26-35 years. In this study, the difference in cost between the current in use strategy (Parallel) versus our proposed newly designed sequential testing algorithm was 746,773.90 ETB.

Conclusion: A significant percentage of the blood donors harbor TTIs. Higher prevalence of TTIs was observed among youths and replacement donors. The direct laboratory cost analysis using current in use strategy (parallel) was higher than the newly designed sequential testing algorithm. Thus, the new strategy can be implemented to make screening of TTIs cost effective in the face of the current effort of large mobilization of voluntary blood donors in the country.

1. Introduction

During World War II and the immediate post war period the demand for blood and blood components increased substantially. This resulted in the establishment and growth of blood banks transfusion services and other blood laboratory support services. For example by 1971, greater than 5400 organizations were involved in the field of transfusion medicine. Each year in the United States, 4 million patients receive transfusion of greater than 20 million units of whole blood and blood components [1]. Every year more than 80 million units of blood are collected worldwide [2]. Each transfusion carries a risk of transmitting blood-borne pathogens, including mainly human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis [3].

HIV is an RNA retrovirus belonging to the family of Lentiviruses that weakens the immune system and is the primary cause of acquired immunodeficiency syndrome (AIDS). Hepatitis B virus is a 42 nm double stranded DNA spherical particle with a double shell and Hepatitis C virus is classified among the flaviviridae which is an RNA-single stranded virus. Both HBV and HCV cause viral hepatitis in humans. The mode of transmission for HIV, HBV and HCV is the same and includes unsafe sexual contact, using sharp materials contaminated with body fluid, mother to child, and transfusion of blood and blood products. Laboratory diagnosis is based on serological tests to detect the specific antibody produced against the virus or directly detecting the antigen in body fluids and includes Enzyme Linked Immunosorbent Assay (ELISA), Radio Immunoassay (RIA), indirect immunofluorescence, immunodiffusion tests [4].

Syphilis is also a systemic disease caused by *Treponemapallidum* which can be spread by sexual contact, blood transfusion and via vertical transmission [5]. Laboratory diagnosis is based on serological test by using specific and non-specific test. Specific test include Treponema Pallidum Haemagglutination test (TPHA), Treponema Pallidium Immobilization test (TPI), Fluorescent Treponemal Antibody test (FTA), and Enzyme Immno Assay (EIA). Non-specific test used are Venereal Disease Research Laboratory Test (VDRL) and Rapid Plasma Reagin Test (RPR) [4].

Blood and its components are lifesaving; however, they are also associated with life threatening hazards such as transfusion transmitted infections. Quality and safe blood is paramount to all patients. Rigorous donor selection following established guidelines is crucial to exclude donors who have potential transfusion transmissible infections (TTI's) [6].

Unsafe blood remains a major threat for the global spread of transfusion transmissible infections (TTIs). Hepatitis B virus, hepatitis C virus, human immunodeficiency virus and syphilis are the most serious infections transmitted during blood transfusion. Complications associated with TTIs include long-term morbidity and mortality, delayed viremia, and hidden states; these make TTIs an important issue in transfusion medicine [7].

According to WHO, safe blood is a universal right, which means that blood that will not cause any harm to the recipient and that has been fully screened and is not contaminated by any blood borne disease such as HIV, hepatitis, malaria, or syphilis. WHO recommends that, at least, all donated blood should be screened forHBV, HCV and HIV, and Syphilis [8].

The demand for blood transfusion services in developing countries is high due to endemicity of infections causing anemia and high incidence of malnutrition. Surgical and obstetric emergencies associated with blood loss are also commonly encountered indications of blood transfusion [9].on the other hand, infections due to the HIV, HBV, and HCV are major public health problems worldwide. In sub-Saharan Africa, these infections are frequent among the general population and blood donors [10]. Syphilis also remains a serious public health problem in the region [11].

Blood safety remains an issue of major concern in transfusion medicine in Ethiopia where national blood transfusion services, policies, appropriate infrastructure, trained personnel and financial resources are inadequate [5].

Even if there are limited published studies in different places in the country, no studies have been conducted at particular place under study, and therefore this study was initiated. The purpose of this study was to determine the prevalence of transfusion transmissible infections and to propose cost-effective blood screening strategy at Federal Democratic Republic of Ethiopia Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia.

1.1 Statement of the problem

Blood transfusion is a highly effective means of transmitting HBV, HCV, HIV, and syphilis infection. HBV infection has a worldwide distribution. It is estimated that more than 2 billion people have been infected. Of these, approximately 240 million are chronically infected and at risk of serious illness and death from cirrhosis and hepatocellular carcinoma, diseases that are estimated to cause 500 000–700 000 deaths each year worldwide. About 150 million people are chronically infected with HCV. More than 350 000 people are estimated to die from HCV related liver diseases each year. An estimated 34 million persons currently are living with HIV infection [12, 13].

All countries in the African Region consider viral hepatitis an urgent public health issue. The burden of viral hepatitis, though not accurately known, is believed to be one of the highest in the world. Hepatitis A, B, C and E are the types mostly found in the Region. The prevalence of HBV is estimated at 8% in West Africa and 5-7% in Central, Eastern and Southern Africa. The prevalence of HCV is even higher in some areas, reaching levels of up to 10%. Similarly, 12.5% of patients who received blood transfusion are at risk of post- transfusion hepatitis. HBV is highly contagious and has a relatively higher prevalence in the tropics [5, 12].

Evidences showed about 10-15 % of HIV transmission in Africa had been related to blood transfusions. In sub-Saharan Africa and other resource-limited settings, transfusion-transmitted HIV infection persists, particularly among women and children. Syphilis also remains a serious public health problem. Prevalence of active syphilis infection among African countries was reported to be12.8% in Tanzania, and 3.8% in Kenya [5, 13, 14].

The high prevalence of HIV, HBV, HCV and syphilis has heightened the problems of blood safety in Ethiopia. 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 [5].

Most African countries are severely limited by the lack of financial and human resources, aggravated by inconsistent supplies of reagents. This limitation makes them dependent on external donations. The question is often raised as to how such blood services are sustained after external funding sources are no longer available. Thus, in Africa, there is an urgent need to create new simplified and pragmatic strategies that improve the cost-effectiveness of blood testing and ensure sustainability in the future [15].

Blood safety remains an issue of major concern in transfusion medicine in Ethiopia where national blood transfusion services, policies, appropriate infrastructure, trained personnel and financial resources are inadequate [5].Out of the total blood collected in the country, 71% was collected from Addis Ababa [16]. Even though there are limited and partial studies in the underlining problem in some parts of the country's blood banks service giving centers, there is no such study yet conducted in Addis Ababa despite its broader blood bank services.

Therefore, this study was conducted to determine the prevalence of TTIs and to propose cost-effective blood screening strategy at Federal Democratic Republic of Ethiopia Ministry of Health National Blood Bank Service, Addis Ababa, Ethiopia.

1.2 Significance of the Study

The study was significant in that it showed prevalence of transfusion transmissible infections at FederalMinistry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia and proposed cost-effective blood screening strategy. The proposed cost-effective strategy will ensure to economize the limited available resource in the country and the TTI's prevalence data will 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.

2. Literature Review

To improve blood transfusion safety, the World Health Organization recommends an integrated strategy including establishment of well-organized blood transfusion services, prioritization of blood donation from voluntary non-remunerated donors, screening of donated blood for at least the four major transfusion transmissible infections (HIV, HBV, HCV, and syphilis) with quality-assured assays, rational use of blood and implementation of effective quality control systems [3].

In the EU and the United States, due to continuous implementation and improvement of more sensitive serologic methods and nucleic acid amplification test (NAT), the residual risk of viral transmission decreased in 2000 to less than 1:250,000 for HCV and 1:1.3 million for HIV [17]. Africa faces the highest transfusion needs in the world, but also the highest prevalence of blood borne pathogens and the weakest transfusion programs. Most blood banks in Africa are small, hospital-based and relying on an important proportion of replacement donors, in contrast with western transfusion units organized with large pools of voluntary donors [3].

Prevalence of HIV, HBV, HCV virus and syphilis in the population of blood donors in Georgia has been investigated. Out of 4970 donors, 7.3% had anti-HCV (6.9% confirmed), HBsAg was positive in 4.1% (3.4% confirmed), seroprevalence of Syphilis was 2.3% and three individuals had HIV. Prevalence of HCV and HBV in Georgia is higher than national prevalence estimates of viral hepatitis in neighboring countries [18].

Seroprevalence and Trends in Transfusion Transmitted Infections among Blood Donors was studied in a University Hospital Blood Bank in India in duration of 5 years. The study revealed that of the total 39,060 donors, 25,303 (64.78%) were voluntary donors and the remaining 13,757 (35.22%) were replacement donors. The overall prevalence of HIV, HBsAg, HCV and syphilis were 0.44, 1.27, 0.23 and 0.28%, respectively. No blood donor tested showed positivity for malaria parasite [19].

Retrospective study carried out over a period of five year from January 2007 to December 2011 at blood bank, Sir Takhtsinhji general hospital attached with Government Medical College, in Bhavnagar (Gujarat) India showed that, out of 34051 donors, the prevalence of different TTIs was 825(2.42%). Of these, voluntary sero-reactive donors were 424(1.25%) and the remaining 401(1.18%) were replacement donors. The prevalence of TTIs in male donors was 809 (2.53%) and in female donors 27 (1.33%).

The trend of TTIs was decreasing over the period of five years. The most common TTI was HBV infection in both voluntary (1.06%) and replacement donors (2.69%) [20].

The finding of high proportions of replacement donors in transfusion programs has been a common challenge of developing countries. For example, during the period from January 2010 to December 2011, a total of 10,145 blood donations were processed at blood bank of FGPC hospital, Islamabad, Pakistan. Of these, 9573 (94%) blood donations were from replacement donors while a small proportion, 608 (6%), were contributed by voluntary non-remunerated blood donors (VNRBD). A total of 1,454 donations (14.34%) were found positive for at least one of the infections. The prevalence of HCV, HBV, HIV, Syphilis and Malaria was found to be 846 (8.34%), 397 (3.91%), 0 (0%), 90 (0.89%) and 121 (1.20%) respectively [21].

A relatively higher seroprevalence was documented for HBV by a study which was conducted to determine the Seroprevalence of transfusion transmissible infections among blood donors attending the Federal Medical Centre, Bida, Nigeria (between January 2011 and June 2011)on eight hundred blood donors, The overall prevalence of HBV, HIV and HCV infections were 14.4%, 1.0%, and 3.9% [22].

Another study among blood donors in Koudougou (Burkina Faso) showed that, from the total of 4,520 blood donors in 2009, 1,348 (29.82%) were infected with at least one pathogen and 149 (3.30%) had serological evidence of multiple infections. The overall seroprevalence rate of HIV, HBV, HCV and syphilis was 2.21%, 14.96%, 8.69% and 3.96%, respectively. Among blood donors with multiples infections, the most common dual or triple combinations were HBsAg-HCV (1.39%), HBsAg-syphilis (0.66%) and HBsAg-HCV-syphilis (0.11%). The highest prevalence of HBsAg and HIV were found among blood donors from rural areas and in the age groups of 20-29 years and >40 years old, respectively [23]. The HBV prevalence in this study is consistent with the Nigerian study in blood donors [22].

Similar study in Brunei Darussalam blood donors in 2011 revealed that out of 56,645 donation units, 874 donations units were positive (1.49%) for any of the screening tests: 520 (0.92%) for HBV, 175 (0.31%) for HCV, 173 (0.31%) for syphilis and 6 (0.01%) for HIV. A decreasing trend was noted for HBV from 1.15% to 0.53% over the five years. For HCV, there was only a slight decreasing trend while there were increasing trends for syphilis and HIV [6].

Seroprevalence of transfusion transmissible infections and evaluation of the pre-donation screening performance at the Provincial Hospital of Tete, Mozambique from February to May 2009 showed that of 750 consenting candidates (50.5% of voluntary donors), 71 (9.5%) were deferred by the questionnaire, including 38 specifically because of risk behavior for TTI. Of the 679 non-deferred candidates, 127 (18.7%) had serological confirmation of at least one TTI, with a lower prevalence in voluntary than in replacement donors (15.2% versus 22.4 %,). Seroprevalence of HIV, HBsAg and syphilis infections was 8.5%, 10.6 % and 1.2%. No confirmed HCV infection was found [3].

A retrospective analysis from the National Blood Transfusion Service in Eritrea showed out of a total of 29,501 units of blood 23,385(79%) were collected from voluntary blood donors and the rest 6,116(21%) units were collected from family replacement donors. The overall prevalence of TTI's were 3.8% with 3.5% in voluntary blood donors and 5.1% in family replacement donors. The sero-prevalence for TTI markers in the total population of blood donors, who are predominantly voluntary donors, was 0.18% HIV, 2.58% HBV, 0.57% HCV and 0.49% Syphilis [24].

Sero-prevalence of HIV, hepatitis B and C and syphilis among blood donors at ElObeid Teaching Hospital, West Sudan showed that out of 260 blood donors, all were males. The screening result for antibodies against HIV and *Treponemapallidum* was positive in 2 (0.8%) and 40 (15%) donors respectively. HBsAg was detected in 26 (10%) donors. Screening result for antibodies against hepatitis C virus was negative in all samples [9].

Several studies in Ethiopia have investigated the prevalence rates of TTIs. For example, a relatively older study conducted to assess the prevalence of infection with HIV, syphilis and HBV among Ethiopian blood donors at the blood bank of a regional hospital in Northwest Ethiopia in 1995 showed that the seroprevalence of HIV-1, syphilis and HBV out of a total of 2186 donors was 16.7%, 12.8% and 14.4%, respectively [25].

The study conducted in Jimma Zone, Southwest Ethiopia in 2001 revealed that (189/3394) 5.6% donors were positive for HIV upon screening by ELISA method [26].

Another study conducted from December 2002 to February 2003 in the northern part of Ethiopia has shown that the prevalence of HBsAg, was 4.7% (14/300) for Gondar College of Medical Sciences, 6% (6/100) 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 [11].

Taken together, the studies reviewed here showed that TTIs are of major concern to ensure blood safety by blood banks particularly in sub Saharan African countries. For performing serologic blood testing, most African countries are severely limited by the lack of financial and human resources, aggravated by inconsistent supplies of reagents. In some African countries, blood transfusion services have been put in place with massive financial and technical support of international partners from developed countries. The question is often raised as to how such blood services are sustained after external funding sources are no longer available. Thus, in Africa, there is an urgent need to create new simplified and pragmatic strategies that improve the cost-effectiveness of blood testing and ensure sustainability in the future [15].

Blood services in Ethiopia have for the past 30 years been mainly provided by the Ethiopian Red Cross Society (ERCS) through its 12 regional blood banks with replacement and directed donations in 35% of its 126 hospitals countrywide. However, there has been inadequacy and in-equitability in access to safe blood by the population, particularly in the regions. Only 24,000 units of blood were collected in 2004 (i.e. 0.3 units/1000 people) and of these 17,000 units (71% of the total) were collected from Addis Ababa. The shortage of blood supplies were more evident for the vast majority of the population (about 96%) residing outside Addis Ababa. On top of the shortage of blood donated nationwide, the prevalence of the major TTI's at the national blood bank (Hepatitis B Virus=5.23%, HIV=2.29% and Hepatitis C Virus=2.30%) indicates there is a high prevalence [16].

Other countries with limited resources like ours have tried to come up with a cost effective strategy. For example high Rates of HBV, HCV and HIV infections prevail among blood donors in Cameroon. The documented HBV, HIV, and HCV infection rates were 12.14% (n = 565), 4.44% (n = 206), and 1.44% (n = 67), respectively. Co infection with HIV and HBV was observed among 0.77% donors, followed by hepatitis B and C co-infection (0.21%) and HIV and HCV co-infection (0.06%). Triple infection with HIV-HBV-HCV was encountered in 2 donors. The HIV, HBV, and HCV infections lead to a destruction of one out of six sets of blood collected [11].

Consequently, they proposed new strategies for improving efficiency and management of blood donors in resource- limited settings and ensuring blood safety. Firstly, the screening tests should be performed in series. The next test will only be carried out if the previous one is negative.

The order in which the tests should be processed will be based on the prevalence of the infections found in the country starting from the most prevalent infection and proceed accordingly as per the magnitude of their prevalence. With regard to the Cameroon context, the HBV test was performed first, followed by the HIV test, and finally the HCV test. Other tests such as syphilis test can be performed depending on the local epidemiology. This strategy is more efficient in the sense that it avoids the wastage of materials, reagents, and time and is done on a small sample of blood [11].

Similarly, a pilot study to improve the cost-effectiveness of HIV, HBV, HCV, and syphilis testing of blood donations in Burkina Faso was carried outon500 blood donations from May to August 2002. By using the simultaneous strategy, the respective sero-prevalence of HBsAg, HIV, syphilis, and HCV among blood donors in Ouagadougou were estimated to be 19.2, 9.8, 1.6, and 5.2%. No significant difference between HIV, syphilis, and HCV prevalence rates was observed by using the sequential strategy (9.2, 1.9, and 4.7%, respectively). Whatever the strategy used, 157 blood donations (31.4%) were found to be reactive for at least one transfusion-transmissible agent and were thus discarded. The sequential strategy allowed a cost decrease of €908.6, compared to the simultaneous strategy. Given that approximately there are 50,000 blood donations annually in Burkina Faso, the money savings reached potentially €90,860[15].

Available studies documented the frequencies of TTIs in blood donors from different part of Ethiopia. Understanding the magnitude of TTIs in the past years from the blood bank which collects the majority of donations in the country (about 71%) and the making of cost effective blood screening strategy is a timely issue since there is an ongoing effort to mobilize large proportions of voluntary donations. Thus, designing a cost effective screening strategy is an important issue that should be addressed in such a resource limited setting to reduce the cost incurred during blood screening for TTI's. This study tried to address both.

3. Study Objectives

3.1 General Objective

- To determine the prevalence of transfusion transmissible infections among blood donors from July 2008 to July 2013and propose cost effective strategy of blood screeningat National Blood Bank of Addis Ababa, Ethiopia.

3.2 Specific Objectives

- To determine the prevalence of transfusion transmissible infections (HIV, HBV, HCV, and Syphilis) among blood donors at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.
- To estimate the direct laboratory cost of HIV, HBV, HCV, and Syphilis test (by estimating cost of reagent, distilled water, micro pipette tip, and labor) of blood donors at Federal Ministry of Health National Blood Transfusion Service Addis Ababa, Ethiopia from July 2008 to July 2013.
- To determine the associated factors with transfusion transmissible infections among blood donors at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.
- To propose cost-effective blood screening strategy for Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

3.3. Hypothesis

- The Prevalence of Transfusion Transmissible Infections are high in Ethiopia blood donors and the current strategy of direct laboratory testingis not cost effective.

4. Materials and Methods

4.1 Study design

A retrospective analysis of blood donors' records covering the period from July 2008 to July 2013 was conducted. The data was collected between March and April 2014, from the Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia and includes category of all donors, socio-demographic characteristics and result for TTI markers. Prevalence of TTI's(HIV, HBV,HCV, and Syphilis) were determined and direct laboratory cost of parallel blood screening strategy as well as sequential strategy of blood screening were compared.To compare the strategies we used the current price of the laboratory costs. The first strategy was a conventional simultaneous screening of these four blood borne infectious agents on each blood donation. The second strategy was a sequential screening starting by the most prevalent to the list prevalent accordingly and direct laboratory costs (cost of reagent, distilled water, micro pipette tip, and labor) of the two strategies were compared.

4.2 Study site

The study was conductedat the Federal Ministry of Health National Blood Transfusion Service in Addis Ababa, which is the capital city of Ethiopia. Blood transfusion services had been provided by the Ethiopian Red Cross Society (ERCS) through its 12 centers located in the capital and other cities in 8 of the 11 regions and covering the requirements of 52% of the hospitals in the country. The Ethiopian Red Cross society National Blood Bank Services (ERCS-NBBS) as one of the core activities of the ERCS was the sole organization providing blood bank services across the country since its establishment in 1969[16].

Currently, blood transfusion service is being provided by Federal Ministry of Health National Blood Transfusion Service. On average around 3500 peoples donate their blood per month.

4.3 Study period

The study was conducted betweenMarch and April 2014 on data of blood donors' records covering the period from July 2008 to July 2013.

4.4 Population

4.4.1 Source population

The source populations were records of all donated blood atthe Federal Democratic Republic of Ethiopia Ministry of Health National Blood Bank Service, Addis Ababa, Ethiopia.

4.4.2 Study population

The study populations were all donated blood with full socio-demographic data as well as their TTI markers during the study Period from July 2008 to July 2013.

4.5 Inclusion criteria

4.5.1. Inclusion criteria

All donated blood with completed data on TTI's and socio-demographic characteristics from a period from July 2008 to July 2013were selected for the study by convenient sampling techniques.

4.6Sample size and sampling techniques

All six years data was used to determine the prevalence of transfusion transmissible infections among blood donors. We used current price of the laboratory costs to propose cost effective blood screening strategy.

4.7 Sampling procedures

All donated blood atthe Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013were included.

4.8 Data collection method

The contents of structured checklist include socio demographic characteristics of blood donors, "ABO/Rh" blood types, sero status of the four TTI's (HIV, HBV, HCV, and Syphilis), and Current direct laboratory screening cost of each test. A number of inquiries that could address the objective of this study was gathered and adapted. The first draft checklist was an English version and this does not require translation into Amharic language for it was used and administered by professional data collectors alone. The checklist was pre-tested to ensure that the checklist is clear for data collectors.

4.9 Study Variables

4.9.1. Dependent variables

- Prevalence of transfusion transmissible infections.
- Strategy on direct laboratory costing of blood screening.

4.9.2. Independent variables

- Socio-demographic characteristics (age, sex, weight) and
- Blood group, RH, Type of Donation.

4.10 Data Management and analysis

Data from blood donors' records covering the period from July 2008 to July 2013was first exported to Excel spread sheet from the institution's data base and then finally exported to SPSS version 16 software (SPSS INC, Chicago, IL, USA) for analysis. Data cleaning was performed to check for accuracy and consistency. It includes category of all donors and result for TTI markers. In addition, a check list was used to extract current direct costs for the screening of the four TTI markers using parallel versus sequential testing strategy and determined the direct Laboratory cost. Data analysis was performed using scores and odds ratio using same software to look for an association between dependent and independent variables. P values less than 0.05 were considered as statistically significant.

4.11 Data quality assurance

Data was directly exported from the institution data base into excel then to SPSS carefully. Screening for TTIs was performed by experienced professionals at the blood bank laboratory following standard operating procedures (SOPs). Detailed testing procedure for the TTI markers is shown in Annex I.

4.12 Ethical considerations

Before the research work is conducted, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of College of Health Sciences, Department of Laboratory Sciences of Addis Abba University. A formal letter of cooperation was submitted and approval obtained from Federal Ministry of Health National Blood Transfusion Service.

4.13 Dissemination of results

This study on completion could serve as a reference material to researchers, experts and policy makers for intervention. To reach these bodies the finalized paper will be submitted to College of Health Sciences, Department of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material will be given to Federal Democratic Republic of Ethiopia Ministry of HealthNational Blood Transfusion Service. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant annual conferences, workshops and seminars.

5. RESULTS

5.1 Demographic characteristics of blood donors

As shown in Table 1, a total of 173,207 consecutive blood donors were screened at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia during the study period (June 2008-June 2013). Of these, 135,007 (77.9%) donors were males. The median age of the study subjects was 28 years (range 17 - 92 years).Of all donors,68146 (39.3%) were in the age group of 26-35 years, and 128,228 (74.0%) were replacement donors. Blood typing data showed, 71596 (41.3%) were blood group O, 160609 (92.7%) were Rh positive, and 61694 (35.6 %) were in the weight group of 56-65 kg.

Table 1. Socio-demographic characteristics of blood Donors at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia. From July 2008 to July 2013.

Variable	Frequency	Percent (%)
Age group		
17-25	66265	38.3
26-35	68146	39.3
36-45	28817	16.6
46-55	8490	4.9
56-65	1474	.9
>65	15	.0
Gender		
Female	38200	22.1
Male	135007	77.9
Blood Type		
A	51531	29.8
AB	10634	6.1
B	39446	22.8
O	71596	41.3
RH		
Negative	12598	7.3
Positive	160609	92.7
Type of Donation		
Mobile	23734	13.7
Replacement	128228	74.0
Voluntary	21245	12.3
Weight Group		
45-55	23419	13.5
56-65	61694	35.6
66-75	48781	28.2
76-85	27058	15.6
86-95	9467	5.5
>95	2788	1.6

5.2 Sero-prevalence of Transfusion transmissible infections (HBV, HIV, HCV, and Syphilis) and their Co-infections in Blood Donors

As shown in Table 2 below, the overall sero-prevalence rate ofHBV,HIV, HCV and syphilis of blood donors at Federal Ministry of Health National Blood Bank Service was 5.0%, 1.6%, 1.4% and 0.1% respectively.

Table 2. Sero-prevalence of Transfusion Transmissible Infections among Blood donors at Federal Ministry of Health National Blood Transfusion Service Addis Ababa, Ethiopia. From July 2008 to July 2013.

Transfusion Transmissible Infections (TTIs)	**Positive**		**Negative**		**Total**	
	Number	%	Number	%	Number	%
HBV	8743	5.0	164464	95.0	173207	100.0
HCV	2357	1.4	170850	98.6	173207	100.0
HIV	2848	1.6	170359	98.4	173207	100.0
Syphilis	93	0.1	173114	99.9	173207	100.0

Prevalence of co-infectionsof by HIV, HBV, HCV and syphilis among blood donors at Federal Ministry of Health National Blood Transfusion Service,in Addis Ababa during the study period is shown in Table 3. As shown in the table below HIV-HBV co-infection was higher among blood donors 135(41.79%) followed by HBV-HCV co-infection which accounts about 103(31.89%) and HIV-HCV accounting for 64 co-infections (19.81%).

Table 3.Prevalence of co-infections of HIV, HBV, HCV and syphilis among blood donors at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

Co-infections	Number	Percent
HIV– Syphilis	9	2.79
HIV–HBV	135	41.79
HIV–HCV	64	19.81
HBV–Syphilis	7	2.17
HBV– HCV	103	31.89
Syphilis–HCV	5	1.55
Total	323	100

5.3 Sero-positivity for HCV among blood donors

Bivariate and Multivariate analysis of socio–demographic characteristics of blood donors by HCV sero-positivity at Federal Ministry of Health, National Blood Transfusion Service is shown in Table 4 below. As depicted in the table, the seroprevalence of HCV was higher among donors in the age group of 26-35 years, followedby age group 36-45 years and among male blood donors. Seroprevalence of HCV was significantly higher among replacement donors which was 1924 (81.6%) followed by voluntary donors which accounted for 275 (11.7%) of the HCV positive donors. Analysis of data for HCV positivity and associated factors revealed that Age, sex, weight, Rh, and type of donation significantly associated with HCV seropositivity. However, only Age being in the age group 26-65, Rh positivity and Type of donation (replacement donation) remain significant factors in the multivariate model ($P<0.05$).

Table 4.Bivariate and Multivariate analysis of Socio–demographic characteristics of blood donors by HCV sero-positivity at Federal Ministry of Health National Blood Transfusion Service Addis Ababa, Ethiopia from July 2008 to July 2013.

Characteristics	HCV		Crude Odds Ratio (95% CI)	Adjusted Odds Ratio(95% CI)	P-value
	Positive Number (%)	Negative Number (%)			
Age group					
17-25	679 (28.8%)	65586 (38.4%)	Reference	Reference	
26-35	964 (40.9%)	67182 (39.3%)	1.386 (1.256- 1.530)	1.247 (1.122 -1.386)	.000**
36-45	530 (22.5%)	28287 (16.6%)	1.810 (1.614 -2.029)	1.635 (1.443 -1.853)	.000**
46-55	152 (6.4%)	8338 (4.9%)	1.761 (1.475 -2.103)	1.607 (1.336 -1.933)	.000**
56-65	32 (1.4%)	1442 (.8%)	2.144 (1.498 -3.067)	1.944 (1.354- 2.792)	.000**
>65	0 (.0%)	15 (.0%)	.000 (.000)	.000 (.000)	.999
Gender					
Female	478 (20.3%)	37722 (22.1%)	Reference	Reference	
Male	1879 (79.7%)	133128 (77.9%)	.898(.812 -.993)	1.022 (.9211-.135)	.679
Weight group					
45-55	242 (10.3%)	23177 (13.6%)	Reference	Reference	
56-65	840 (35.6%)	60854 (35.6%)	1.322 (1.145 -1.526)	1.089 (.939- 1.263)	.259
66-75	686 (29.1%)	48095 (28.2%)	1.366 (1.179 -1.583)	.995 (.850 -1.164)	.947
76-85	399 (16.9%)	26659 (15.6%)	1.433 (1.221 -1.683)	.978 (.822 -1.164)	.805
86-95	143 (6.1%)	9324 (5.5%)	1.469 (1.193 -1.809)	.988(.794 -1.231)	.916
>95	47 (2.0%)	2741 (1.6%)	1.642 (1.199- 2.250)	1.081(.782 -1.493)	.638
Blood Type					
A	700 (29.7%)	50831 (29.8%)	Reference	Reference	
B	528 (22.4%)	38918 (22.8%)	.878 (.726 -1.062)	.876 (.725 -1.060)	.174
AB	127 (5.4%)	10507 (6.1%)	.985 (.879 -1.104)	.987 (.880- 1.106)	.816
O	1002 (42.5%)	70594 (41.3%)	1.031 (.935 -1.136)	1.033 (.937- 1.138)	.514
Rh					
Positive	2211 (93.8%)	158398 (92.7%)	Reference	Reference	
Negative	146 (6.2%)	12452 (7.3%)	1.190 (1.006- 1.409)	1.194 (1.009 -1.413)	.039**
Type of Donation					
Voluntary	275(11.7%)	20970 (12.3%)	Reference	Reference	
Replacement	1924 (81.6%)	126304 (73.9%)	2.273 (1.932 -2.675)	1.958 (1.649- 2.324)	.000**
Mobile	158 (6.7%)	23576 (13.8%)	1.957 (1.608- 2.382)	1.808 (1.478- 2.212)	.000**

****P< 0.05 (statisticallysignificant association) for the Adjusted Odds Ratio**

5.4 Sero-positivity for HBV among blood donors

Socio–demographic characteristics of blood donors by HBV sero-positivity at Federal Ministry of Health, National Blood Transfusion Service is summarized in Table 5 as shown below. The seroprevalence of HBV was significantly higher among age group 26-35 years followed by age group 36-45 years, and among males.Replacement donors constituted a significantly higher proportion of the HBV seropositive blood donors followed by voluntary donors. Bivariate and multivariate analysis ofthe data revealed that age, sex, weight, Rh, and type of donation significantly associated with HBV seropositivity in the bivariate analysis. Age (26-45 & 56-65), sex (being male), weight (46-55 and >86), Rh positivity, and donor type (replacement) remain statistically significant after controlling for confounders in the multivariate model ($P<0.05$).

5.5 Sero-positivity for HIV among blood donors

The sero-prevalence of HIV was significantly higher among male blood donors than among female donors and also higher among donors in the age group 26-35 years followed by age group 36-45 years (Table 6). Sero-prevalence of HIV was significantly higher among replacement donors followed by voluntary donors. As shown in table 6, age (26-65), sex (male) and type of donation (replacement) independently associated with HIV seropositivity ($P<0.05$).

5.6 Sero-positivity for Syphilis among blood donors

The sero-prevalence of Syphilis was significantly higher among male blood donors than among female donors and also higher among donors in the age group 26-35than age group 36-45 years (Table 7).Seroprevalence of Syphilis was significantly higher amongreplacement donors followed by voluntary donorsandmobile donors. In the multivariate analysis, age and type of donation remain to be significantly associated with syphilis seropositivity ($P<0.05$) while that of sex disappeared.

Table 5.Bivariate and Multivariate analysis of Socio–demographic characteristics of blood donors by HBV sero-positivity at Federal Ministry of Health National Blood Transfusion Service Addis Ababa, Ethiopia from July 2008 to July 2013.

Characteristics	HBV		Crude Odds Ratio (95% CI)	Adjusted Odds Ratio(95% CI)	P-value
	Positive Number (%)	Negative Number (%)			
Age group					
17-25	2715 (31.1%)	63550 (38.6%)	Reference	Reference	
26-35	3763 (43.0%)	64383 (39.1%)	1.368 (1.301 -1.439)	1.164 (1.102- 1.229)	.000**
36-45	1780 (20.4%)	27037 (16.4%)	1.541 (1.449- 1.639)	1.289 (1.205 -1.378)	.000**
46-55	431 (4.9%)	8059 (4.9%)	1.252 (1.128 -1.389)	1.066 (.957- 1.188)	.243
56-65	54 (.6%)	1420 (.9%)	.890 (.676 - 1.171)	.734 (.557 -.967)	.028**
>65	0 (.0%)	15 (.0%)	.000 (.000)	.000 (.000)	.999
Gender					
Female	1092 (12.5%)	37108 (22.6%)	Reference	Reference	
Male	7651 (87.5%)	127356 (77.4%)	.490(.459-.522)	.542 (.507-.579)	.000**
Weight group					
45-55	851 (9.7%)	22568 (13.7%)	Reference	Reference	
56-65	3002 (34.3%)	58692 (35.7%)	1.356 (1.255 -1.466)	1.087 (1.004 -1.177)	.041**
66-75	2584 (29.6%)	46197 (28.1%)	1.483 (1.371 -1.605)	1.069 (.983 -1.163)	.119
76-85	1518 (17.4%)	25540 (15.5%)	1.576 (1.447 -1.717)	1.081 (.985- 1.186)	.100
86-95	597 (6.8%)	8870 (5.4%)	1.785 (1.603 -1.987)	1.209 (1.079- 1.354)	.001**
>95	191 (2.2%)	2597 (1.6%)	1.950 (1.659 -2.294)	1.311 (1.110- 1.549)	.001**
Blood Type					
A	2585 (29.6%)	48946 (29.8%)	Reference	Reference	
B	2083 (23.8%)	37363 (22.7%)	.905 (.819.- 999)	.911 (.825 -1.006)	.066
AB	485 (5.5%)	10149 (6.2%)	1.056 (.995-1.120)	1.060 (.999 -1.125)	.056
O	3590 (41.1%)	68006 (41.4%)	1.000 (.949-1.053)	1.000 (.949 -1.053)	.997
Rh					
Positive	8186 (93.6%)	152423 (92.7%)			
Negative	557 (6.4%)	12041 (7.3%)	1.161 (1.063-1.268)	1.154 (1.056 -1.260)	.001**
Type of Donation					
Voluntary	941 (10.8%)	20304 (12.3%)	Reference	Reference	
Replacement	7054 (80.7%)	121174 (73.7%)	1.789(1.657-1.931)	1.420 (1.309 -1.541)	.000**
Mobile	748 (8.6%)	22986 (14.0%)	1.424(1.291-1.571)	1.186 (1.072 -1.312)	.001**

****P< 0.05 (statisticallysignificant association) for the Adjusted Odds Ratio**

Table 6.Bivariate and Multivariate analysis ofSocio–demographic characteristics of blood donors by HIV sero-positivity at Federal Ministry of Health National Blood Transfusion Service Addis Ababa, Ethiopia from July 2008 to July 2013.

Characteristics	HIV		Crude Odds Ratio (95% CI)	Adjusted Odds Ratio(95% CI)	P-value
	Positive Number (%)	Negative Number (%)			
Age group					
17-25	668 (23.5%)	65597 (38.5%)	Reference	Reference	
26-35	1249 (43.9%)	66897 (39.3%)	1.833 (1.668 -2.015)	1.663 (1.503 -1.841)	.000**
36-45	725 (25.5%)	28092 (16.5%)	2.534 (2.279 -2.818)	2.285 (2.035 -2.567)	.000**
46-55	185 (6.5%)	8305 (4.9%)	2.187 (1.856- 2.578)	1.982 (1.670 -2.353)	.000**
56-65	21 (.7%)	1453 (.9%)	1.419 (.916- 2.198)	1.277 (.822- 1.984)	.276
>65	0 (.0%)	15 (.0%)	.000 (.000)	.000 (.000)	.999
Gender					
Female	637 (22.4%)	37563 (22.0%)	Reference	Reference	
Male	2211 (77.9%)	132796 (78.0%)	1.019(.932-1.113)	1.205(1.099-1.321)	.000**
Weight group					
45-55	295 (10.4%)	23124 (13.6%)	Reference	Reference	
56-65	987 (34.7%)	60707 (35.6%)	1.274 (1.118 -1.453)	1.009 (.882 -1.155)	.896
66-75	819 (28.8%)	47962 (28.2%)	1.339 (1.171 -1.530)	.903 (.783 -1.042)	.163
76-85	529 (18.6%)	26529 (15.6%)	1.563 (1.354 -1.804)	.979 (.839- 1.143)	.791
86-95	171 (6.0%)	9296 (5.5%)	1.442 (1.193 -1.743)	.893 (.731 -1.091)	.268
>95	47 (1.7%)	2741 (1.6%)	1.344 (.985- 1.833)	.817 (.5951.122)	.213
Blood Type					
A	875 (30.7%)	50656 (29.7%)	Reference	Reference	
B	650 (22.8%)	38796 (22.8%)	.918 (.777 -1.085)	.914 (.773 -1.081)	.293
AB	166 (5.8%)	10468 (6.1%)	.970 (.876 -1.074)	.970 (.875 -1.074)	.554
O	1157 (40.6%)	70439 (41.3%)	.951 (.870 -1.039)	.953 (.872 -1.041)	.285
Rh					
Positive	2644 (92.8%)	157965 (92.7%)	Reference	Reference	
Negative	204 (7.2%)	12394 (7.3%)	1.017 (.881- 1.174)	1.020 (.883- 1.178)	.789
Type of Donation					
Voluntary	215 (7.5%)	21030 (12.3%)	Reference	Reference	
Replacement	2450 (86.0%)	125778 (73.8%)	2.507 (2.156 -2.915)	1.987 (1.694 -2.331)	.000**
Mobile	183 (6.4%)	23551 (13.8%)	1.316 (1.079 -1.604)	1.151 (.940 -1.409)	.174

****P< 0.05 (statisticallysignificant association) for the Adjusted Odds Ratio**

Table 7.Bivariate and Multivariate analysis ofSocio–demographic characteristics of blood donors by Syphilis sero-positivity at Federal Ministry of Health National Blood Transfusion Service Addis Ababa, Ethiopia from July 2008 to July 2013.

Characteristics	Syphilis		COR (95% CI)	Adjusted ODR(95% CI)	P-value
	Positive Number (%)	Negative Number (%)			
Age group					
17-25	12 (12.9%)	66253 (38.3%)	Reference	Reference	
26-35	39 (41.9%)	68107 (39.3%)	3.162 (1.655- 6.038)	2.793 (1.429 -5.462)	.003**
36-45	27 (29.0%)	28790 (16.6%)	5.178 (2.623 -10.222)	4.820 (2.347- 9.898)	.000**
46-55	13 (14.0%)	8477 (4.9%)	8.467 (3.862-18.562)	8.126 (3.577- 18.462)	.000**
56-65	2 (2.2%)	1472 (.9%)	7.501 (1.677 -33.547)	6.928 (1.520- 31.579)	.012**
>65	0 (.0%)	15 (.0%)	.000 (.000)	.000 (.000)	.999
Gender					
Female	12 (12.9%)	38188 (22.1%)	Reference	Reference	.
Male	81 (87.1%)	134926 (77.9%)	.523 (.285-.960)	.624 (.337-1.157)	.134
Weight group					
45-55	9 (9.7%)	23410 (13.5%)	Reference	Reference	
56-65	34 (36.6%)	61660 (35.6%)	1.434 (.688- 2.991)	.822 (.390 -1.733)	.606
66-75	27 (29.0%)	48754 (28.2%)	1.440 (.677- 3.063)	.580 (.265 -1.267)	.172
76-85	14 (15.1%)	27044 (15.6%)	1.347 (.583 -3.111)	.452 (.189- 1.083)	.075
86-95	7 (7.5%)	9460 (5.5%)	1.925 (.717-5.170)	.621 (.224 -1.726)	.361
>95	2 (2.2%)	2786 (1.6%)	1.867 (.403- 8.647)	.572 (.121 -2.716)	.482
Blood Type					
A	28 (30.1%)	51503 (29.8%)	Reference	Reference	
B	15 (16.1%)	39431 (22.8%)	1.212 (.529 -2.774)	1.204 (.526 -2.758)	.660
AB	7 (7.5%)	10627 (6.1%)	.700 (.374 -1.310)	.696 (.372- 1.303)	.258
O	43 (46.2%)	71553 (41.3%)	1.105 (.687- 1.779)	1.110 (.690 -1.788)	.666
Rh					
Positive	86 (92.5%)	160523 (92.7%)	Reference	Reference	
Negative	7 (7.5%)	12591 (7.3%)	.964 (.446 -2.082)	.960 (.444-2.076)	.918
Type of Donation					
Voluntary	6 (6.5%)	21239 (12.3%)	Reference	Reference	
Replacement	85 (91.4%)	128143 (74.0%)	7.871 (1.937 -31.988)	4.546 (1.086 -19.022)	.038**
Mobile	2 (2.2%)	23732 (13.7%)	3.352 (.677 -16.610)	2.495 (.494- 12.591)	.268

****P< 0.05 (statisticallysignificant association) for the Adjusted Odds Ratio**

5.7 Current Estimation of Direct Laboratory Cost Analysis for Testing Transfusion Transmissible Infections

The current estimation of direct laboratory cost analysis for testing transfusion transmissible infections at the Federal Ministry of Health, National Blood Transfusion Service was performed by taking the estimated costs of reagents, distilled water, micro pipette tips, and its labor cost and summarized in tables 8 and 9. SOP's, manufacturer kit insert leaflets, purchase invoices of testing kits at National Blood Transfusion Service and manuals were referred for all cost of reagents, procedures, incubation time between testing in the laboratory and protocols for testing transfusion transmissible infections.

Twenty times testing hours were taken to establish the turnaround time by calculating the mean time taken for testing each transfusion transmissible infection starting at sample receipt in the laboratory to result submission to laboratory quality officer for Verification, approval, and dissemination for record and archiving. The established total testing time in minutes has been assumed and checked for a single investigator to perform a batch of test in this case, total minutes taken for four plates i.e. is a total of 384(96×4)test including control samples for HCV testing and has been estimated to come out about eight hours(480 minutes).The estimated time includes incubation time during the procedures,pipetting and adding samples and control samples into respective reaction well, adding (conjugate, substrate, and stop solution),washing between procedures, reading plate for result and recording has been taken into consideration.

The estimated cost analysis for reagent was calculated by the price of the kit for each test divided by the total number of test the kit is enough to do. Distilled water cost has been also estimated by calculating the volume utilized by a single well to wash in each test. Micro pipette tips used in each test has been calculated by taking total number of tips used to prepare a ninety six wells or one plate. The labor cost which is the other direct testing cost has been estimated by dividing the monthly income of the investigator in this case a salary of junior laboratory technologist which is (2250 Ethiopian Birr (ETB)) divided by thirty days and then by eight hours i.e. the time the investigator must cover in a day was taken into consideration. Similarly, the same consideration has been taken into account for HIV and HBV testing except the total time taken for HIV and HBV testing for four plates i.e. is a total of 384(96×4)test including control samples of testing has been estimated to come out about seven hours and thirty minutes i.e. (450 minutes) due to shorter incubation time during the testing procedures. Other steps and procedures listed above for HCV are similar for HIV and HBV testing expect differences in procedures and protocols for particular test in HIV and HBV.

For Syphilis test since the protocol is quite different from the above ELISA test for HCV, HIV, and HBV; the estimated time for testing for a batch of thirty samples including control sample has come out to be two minutes each on average. The estimated time is a time from sample receipt to the lab till result is made ready for submission to laboratory quality officer for Verification, approval, and dissemination for record and archive. The rest of cost analysis estimate is similar as for other tests listed above except for protocol difference.

The current estimated direct laboratory cost analysis using parallel screening strategy for all tests (HIV, HBV, HCV, and syphilis) has been depicted in the tables (Table 8, 9), for all samples at initial testing and those repeated positive samples for confirmation from an individual bag and test tube for checking if there is a mix up during sample preparation for serologic testing. Accordingly, the total estimated direct cost using the parallel strategy was17,579,297.78 for screening and 644789.34 birr for repeat testing adding up to a total of 18,224,087.12 birr. The parallel strategy is depicted in Figure 1.

The estimated direct laboratory cost analysis using newly designed screening strategy (algorithm)were depicted in Tables 10 and 11.The cost analysis was calculated the same way as in the parallel screening strategy, the only difference was that the estimated testing cost were done using the newly designed algorithm.Consequently, the total estimated direct cost using the newly proposed strategy was16839036.92 for screening and 638276.30 birr for repeat testing adding up to 17,477,313.22. Thus, the difference in cost between the current in use strategy (Parallel) versus our proposed newly designed sequential testing algorithm was 746,773.90 ETB. Figure 2 shows the newly proposed sequential TTIs testing algorism.

While we design our testing algorithm, we need to consider cost of testing in addition to the disease prevalence. As shown in the figure, the most prevalent TTI marker will be tested first and the bag discarded if positive. This continues sequentially from high to low prevalent markerbut we considered cost of testing as well. Even though the prevalence of HIV is higher than HCV in our study, the testing cost of HIV is three times bigger than testing cost of HCV and that is why we ordered HCV testing come second to HBV testing in the newly designed algorithm.

Table 8.Total TTI'S Test Done Including Control Samples using currently in use strategy(parallel) at Federal Ministry of Health, National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

Table 9.Total Repeated TTI'S Test Done Including Control Samples using currently in use strategy(parallel) at Federal Ministry of Health, National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

Table 10.Total TTI'S Test done Including Control Samples using Newly Designed Testing Algorithms at Federal Ministry of Health, National Blood Transfusion Service, and Addis Ababa, Ethiopia from July 2008 to July 2013.

Table 11.Total Repeated TTI'S Test doneIncluding Control Samples using Newly Designed Testing Algorithms at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

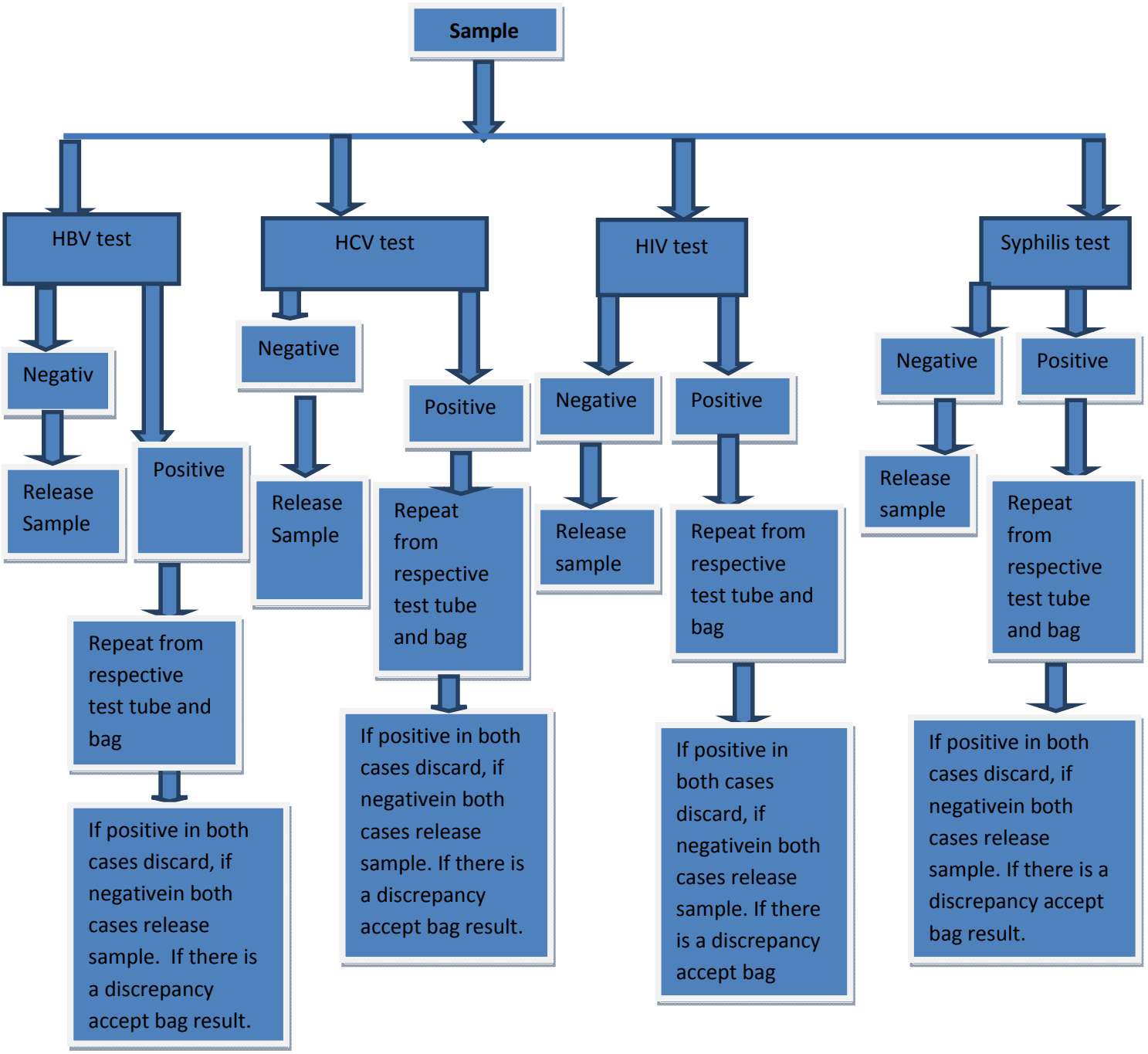

Figure1. Current parallel testing strategy at Federal Ministry of Health, National Blood Transfusion Service Addis Ababa, Ethiopia: All tests were performed at the same time (parallel).

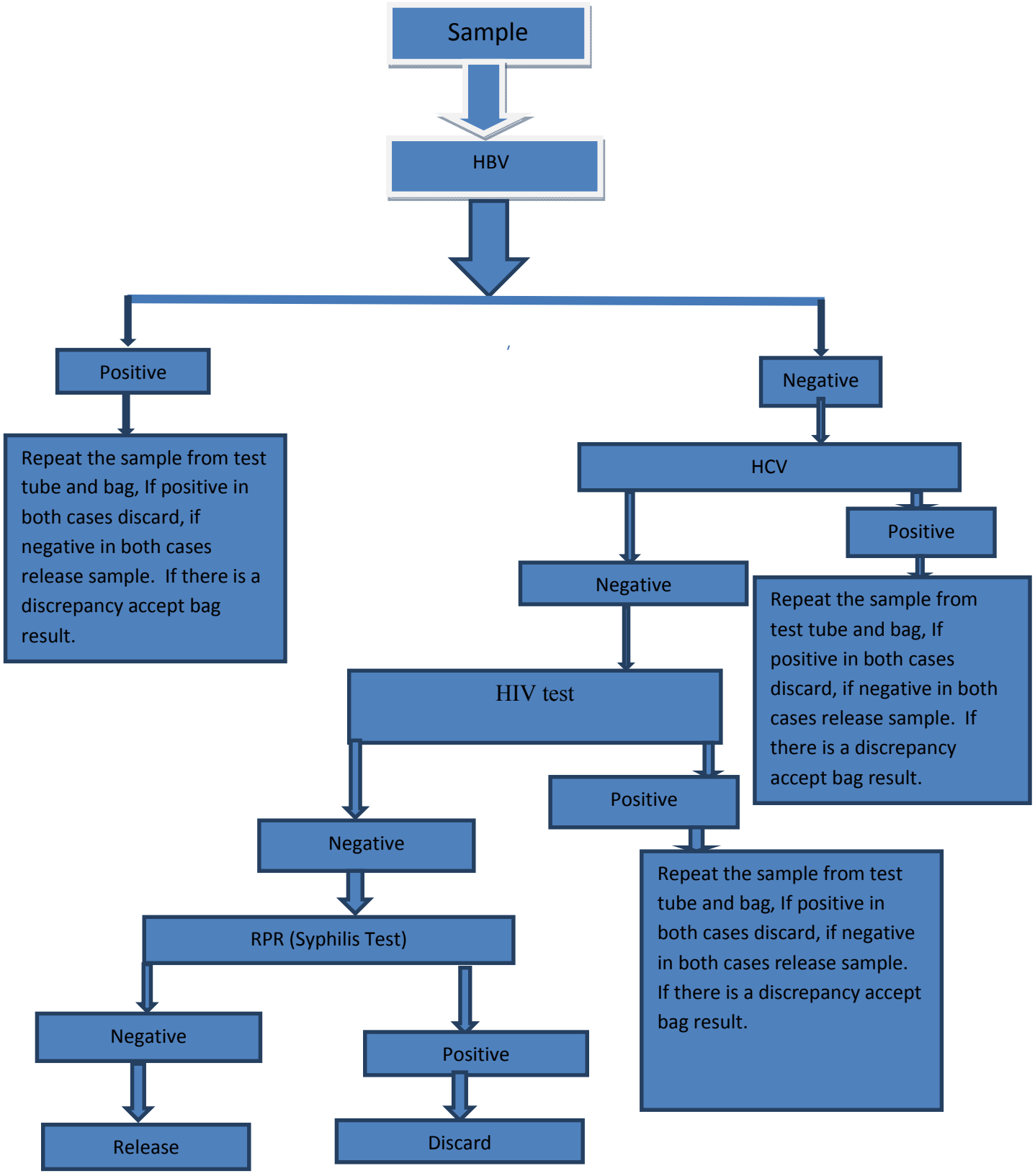

Figure 2. Newly designed testing algorithm: tests are done sequentially.

6. Discussion

In this study, the overall seroprevalence rate ofHBV, HIV, HCV and syphilis of blood donors at Federal Ministry of Health National Blood Bank Service was 5.0%, 1.6%, 1.4% and 0.1% respectively. Similar studies conducted in Gondar University Teaching Hospital, Northwest Ethiopiain 2010 using five years data has shownanoverall seroprevalence rate of HBV, HIV, HCV and syphilis to be4.7%, 3.8%, 0.7% and 1.3%, respectively[5]. Excepting for other transfusion transmissible infections listed above, HBV prevalence is in agreement with our study. Another study conducted in Koudougou (Burkina Faso) in comparison with our study has revealed a higher overall seroprevalence for HIV, HBsAg, HCV and syphilis, which was 2.21%, 14.96%, 8.69% and 3.96%, respectively [23].

A 5 Year Study in India showed, overall prevalence of HIV, HBsAg, HCV and syphilis were 0.44, 1.27, 0.23 and 0.28%, respectively, it is quite lower in prevalence compared to our study except for syphilis prevalence which is slightly higher than our study[20].

Another study conducted in the northern part of Ethiopia has shown the prevalence of HBsAg, was 4.7% (14/300) for Gondar College of Medical Sciences, 6% (6/100) for Bahirdar Hospital which is in agreement with our study, whereas prevalence of 3% (3/100) for Dessie and 14% (14/100) for the Mekele hospital blood banks are different from our study. The prevalence of HCV antibody was 2.3% (7/100) and 3% (3/100) for Gondar and Bahirdar, respectively, while 0% (0/200) for Dessie and Mekele Hospital blood banks. Compared to our finding these prevalences of HCV in Gondar and Bahirdar are higher [11]. Although, this multicenter study investigated smaller sample size, differences in prevalence of the TTIs might also indicate the epidemiology of these infections in the regions studied. As similar SOPs are being followed for testing TTIs in the blood banks, methodological variations may play little role. Same explanation can be given for the observed differences in HBV prevalence between our study and that of Tessema et al 2010 [5], who investigated five years data.

Prevalence of HIV, HBV, HCV virus and syphilis in the population of blood donors in Georgia has shown seroprevalence of 7.3% for anti-HCV (6.9% confirmed), HbsAg was positive in 4.1% (3.4% confirmed), seroprevalence of Syphilis was 2.3%, HIV (0.06%).Except for HBV and HIV which are slightly lower in prevalence compared to our study, the rest prevalence for HCV and syphilis are higher in prevalence than our study [19].

The findings in our study for all the TTI markers (except syphilis) are significantly higher compared to a retrospective analysis from the National Blood Transfusion Service in Eritrea. Fissehaye et al in their study showed out the sero-prevalence for TTI markers were 0.18% HIV, 2.58% HBV, 0.57% HCV and 0.49% Syphilis [24]. One of the possible explanation for the observed differences could be the Eritrean blood donors are predominantly voluntary donors (79%). In our case where the majorities (74%) are replacement donors, we have demonstrated that donor type significantly affects the prevalence of TTIs.

A study conducted at the national blood bank Addis Ababa, showed that prevalence of HBV (5.23%), HIV (2.29%) and HCV (2.30%) respectively. Compared to our study HBV prevalence was in agreement, whereas prevalence of HIV and HCV were slightly higher than our finding [16].

In our study, significantly increased seroprevalence of TTI's was observed in the age groups of 17-25 and 26-35 years. This finding is similar to a study conducted at Gondar University Teaching Hospital in Northwest Ethiopia, in India, and in China, in which higher prevalence was observed among youths [5, 27, and 17].

Co-infections observed in our study revealed that there is a higher prevalence than studies in Koudougou (Burkina Faso) which were HBsAg-HCV (1.39%), HBsAg-syphilis (0.66%) and HBsAg-HCV-syphilis (0.11%) [23]. Study conducted at Gondar University TeachingHospital in Northwest Ethiopia, Osogbo South-West Nigeria, were slightly in agreement with our study in the prevalence of co-infection of HIV– HBV which were(34%) and (33.3%) respectively[5,28].

Currently, TTI's screening at our study area (FMOH, National Blood transfusion service) showed that all tests on TTI's screening are done simultaneously or in parallel. Using this parallel screening strategy, the direct laboratory cost analysis of the last five years samples at initial screening was 17,579,297.78 in Ethiopian Birr (ETB).According to the current parallel testing strategy positive samples screened at initial phase are being repeated from their respective bag and test tube. The direct laboratory cost of repeated positive samples of the last five years samples were 644,789.34 Ethiopian Birr (ETB).The total direct laboratory cost of the last five years samples using this strategy was 18,224,087.12 ETB.

If the parallel testing Strategy were replaced by the newly proposed testing algorithm, the direct laboratory cost analysis of the last five years samples would been16, 839,036.92 ETB.

The direct laboratory cost of repeated positive samples of the last five years samples would be 638, 276.30(ETB).The total direct laboratory cost of the last five years samples using this strategy would be 17,477,313.22 ETB.In this study, the difference in cost between the current in use strategy (Parallel) versus our proposed newly designed testing algorithm (sequential) was 746,773.90 ETB. This showed that if the samples were tested by the newly designed algorithm, 746,773.90 ETB could have been saved. Currently the collection at NBTS of Addis Ababa alone is nearly equal with number of samples collected in all country side blood transfusion service centers. If we project this new testing algorithm nationally assuming same prevalence were observed at all other blood transfusion services in the country, more than 1,493,547.80 ETB could have been saved. If we in turn decide to stop repeating for samples already become positive at initial screening phase the money saving would be around 2,770,100.40 ETB nationally. The above cost analysis was only for direct laboratory cost incurred during testing in the laboratory and it did not include the indirect cost like electricity cost, water cost, infrastructure cost, machine depreciation cost, and administrative cost. Had those cost were included, the resource saving could have been by far more than what was estimated above nationally.

Similar studyin Burkina Faso showed that the sequential strategy allowed a cost decrease of €908.6, compared to the simultaneous strategy. Given that approximately there are 50,000 blood donations annually the money savings reached potentially €90,860[15]

Each country should decide on the TTIs to be screened for as part of the blood screening programme and develop a screening strategy appropriate to its specific situation. This will be influenced by the incidence and prevalence of infection, the capacity and infrastructure of the blood transfusion service, the costs of screening and the available resources [29].

7.Limitationsand Strength of the study

- The cost analysis is only focused on the direct laboratory cost alone.
- Since there were incomplete data, some variables such as Occupation, income, and address were omitted from the analysis as per the exclusion criteria.
- The data reported herein is very strong in that large data base which has never been reported thus far was included in the study.

8. Conclusions and recommendations

8.1 Conclusion

A significant percentage of the blood donors harbor transfusion-transmissible infections (with respective prevalences of 5.0%, 1.6%, 1.4% and 0.1% for HBV, HIV, HCV and syphilis). Transmission of transfusion-transmissible infections during the serologically negative window period still pose a threat to blood safety in environments where there is a high rate of transfusion-transmissible infection.

Higher prevalence of transfusion-transmissible infections was observed among youths and in replacement donors. The prevalence of HIV, HBV, HCV, and syphilis co-infection needs to be studied on a larger scale for the better understanding of the impact on clinical outcome and treatment response.

The direct laboratory cost analysis using current in use strategy (parallel) was higher than the newly designed testing algorithm. This effort is the first of its kind to quantify the direct laboratory testing costs and suggest a new cost effective testing strategy so as to complement the current donor mobilization effort of the Federal Ministry of Health by screening donors through wise utilization of limited resources.

8.2 Recommendations

- Since there is a substantial transfusion transmissible infections among replacement blood donors there is a need for strict selection of blood donors with the emphasis on mobilizing voluntary donors.
- Comprehensive screening of donors' blood for HIV, HBV, HCV and syphilis using standard methods are highly recommended to ensure the safety of blood for recipient.
- This observation was worrisome, since the most productive and economically viable age group of thepopulation was the worst hit. Therefore, there is the urgent need for renewed intensification of prevention programs aimed at changing high-risk behaviors.
- Mass immunization of HBV should be given to most productive and economically viable age group of the population to reduce the prevalence of the disease.
- Although, a well designed study including the indirect costs is needed to quantify all costs incurred for TTI testing, which can show us the cost that can be saved is much higher than reported here, we recommend the Federal Ministry of Health National Blood transfusion Service in particular and the rest of blood transfusion centers across the country to use the newly designed testing algorithm because it is economically efficient than the currently in use screening strategy (parallel). We would like to note that the sequence of tests depends on the prevalence of TTIs and cost of testing in the respective regions in Ethiopia where blood transfusion services are available. Thus, customizing this proposed algorithm is required based on the epidemiology of TTIs and cost of testing in the respective region.

9. References

1. Domen RE. Paid-versus-volunteer blood donation in the United States: A historical review. Transfus Med Rev. 1995; 9:53–59.
2. World Health Organization. Universal access to safe blood transfusion. Geneva:World Health Organization; 2008.
3. Stokx J, Gillet P, De Weggheleire A, Casas EC, Maendaenda R, Beulane AJ, et al. Seroprevalence of transfusion-transmissible infections and evaluation of the pre-donation screening performance at the Provincial Hospital of Tete Mozambique. BMC Infectious Diseases. 2011; 11:141-148.
4. Tapko JP. The road to a safe blood supply in the African region of the WHO: trends and current status: 1999-2006. Africa Sanguine. 2007; 10(1): 1-6.
5. Tessema B, Yismaw G, Kassu A, Amsalu A, Mulu A, Emmrich F, et al. Seroprevalence of HIV, HBV, HCV and syphilis infections among blood donors at Gondar University Teaching Hospital, Northwest Ethiopia: declining trends over a period of five years. BMC Infect Dis.2010; 10:111-117.
6. Teo KSK, Saparudin MS, Zaini Z, Morshidi MA, Metassan N, Jaberudin R,et al. Transfusion transmissible infections in Brunei Darussalam: A blood donor study, RIPAS Hospital, Bandar Seri Begawan, Brunei Darussalam Brunei. Int Med J. 2011; 7 (6): 321-327.
7. Saghir SAM, Al–Hassan FM,Alsalahi OSA,Alhariry AEA,Baqir HS. Frequencies of HBV, HCV, HIV, and Syphilis Markers among Blood Donors: A Hospital-Based Study in Hodeidah, Yemen. Trop J Pharm Res. 2012; 11 (1): 132 -136.
8. Manzoor I, Hashmi N, Ajmal SD, Fatima H, Rasheed Z,Syed S. Seroprevalence of Transfusion Transmissible Infections (TTIS) in Blood Donors.Biomedica. 2009; 25(10):154 – 158.
9. Elfaki AMH, Eldour AAA, and Elsheikh NMH. Sero-prevalence of immunodeficiency virus, hepatitis B and C and syphilis among blood donors at ElObeid Teaching Hospital, West Sudan. Sudan JMS.2008; 3(4):333-338.
10. Ymele FF, Keugoung B,Fouedjio JF,Kouam N, Mendibi S, and Mabou JD.High Rates of Hepatitis B and C and HIV Infections among Blood Donors in Cameroon: A Proposed Blood Screening Algorithm for Blood Donors in Resource-Limited Settings. Journal of Blood Transfusion. 2012: 1- 7.

11. Gelaw B, Mengistu Y. The prevalence of HBV, HCV and malaria parasites among blood donors in Amhara and Tigray regional states. Ethiop.J.Health Dev. 2007; 22(1):3-7.
12. World Health Organization. Prevention & Control of Viral Hepatitis Infection: Framework for Global Action. World Health Organization; 2012.
13. Centers for Disease Control and Prevention. Progress Toward Strengthening National Blood Transfusion Services -14 Countries, 2008–2010. MMWR.2011; 60: 1577-1582.
14. Dessie A, Abera B, Wale F. Seroprevalence of major blood-borne infections among blood donors at FelegeHiwot referral hospital, northwest Ethiopia. Ethiop.J.Health Dev. 2007;21(1):67-68.
15. Kania D, Sangaré L, SakandéJ, Koanda A, Nébié YK,Zerbo O, et al. A new strategy to improve the cost-effectiveness of human immunodeficiency virus,hepatitis B virus, hepatitis C virus, and syphilis testing of blood donations in sub-Saharan Africa: a pilot study in Burkina Faso. Transfussion.2009; 49(10):2237-40.
16. Gebreyigzaber H. Application of data mining technology in predicting the seroprevalence of hbv, hcv, hiv; the case of the national blood bank of Addis Ababa,Ethiopia. Available at :http://etd.aau.edu.et/dspace/bitstream/123456789/3547/1/HAFTOM%20GEBREGZIABHER.pdf accessed on January 3, 2014.
17. Li C, Xiao X, Yin H, He M, Li J,Dai Y, et al. Prevalence and prevalence trends of transfusion transmissible infections among blood donors at four Chinese regional blood centers between 2000 and 2010. Journal of translation Medicine. 2012; 10:176-185.
18. Butsashvili T. Tsertsvadze LA, McNutt G, Kamkamidze R,Gvetadze, andBadridzeN.Prevalence of Hepatitis B, Hepatitis C, Syphilis and HIV in Georgian Blood Donors. European Journal of Epidemiology. 2001; 17(7): 693-695.
19. Pallavi P, Ganesh CK, Jayashree K, Manjunath GV. Seroprevalence and Trends in Transfusion Transmitted Infections Among Blood Donors in a University Hospital Blood Bank: A 5 Year Study. Indian J Hematol Blood Transfus. 2011; 27(1):1–6.
20. ThakerMV,Shah PH, Gosalia VV, ValiyaLG, Suri SK. Seroprevalence of HIV, HbsAg, HCV, Syphilis in Blood Donors at Blood Bank in a Tertiary Care General Hospital – A Retrospective study . SEAJCRR. 2011; 2(3): 2319 – 2390.
21. Wayeed U,Khan H, Satti HS, Ansari MA, Malik MA , Zaheer HA.Prevalence of Transfusion Transmitted Infections among Blood Donors of a Teaching Hospital in Islamabad. Ann. Pak. Inst. Med. Sci. 2012; 8(4): 236-239.

22. Amiwero CE, Prescott RJ, George OA, Joy NI,Aisha M. Seroprevalence of transfusion transmissible infections among blood donors attending the Federal Medical Centre, Bida. Int. J. Mod. Biol. Res. 2013; 1:11-17.
23. Nagalo MB, Sanou M, Bisseye C, Kaboré MI, Nebie YK, Kienou K,et al.Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis among blood donors in Koudougou (Burkina Faso) in 2009. Blood Transfus. 2011; 9:419-424.
24. Fessehaye N,Naik D, Fessehaye T. Transfusion transmitted infections;A retrospective analysis from the National Blood Transfusion Service in Eritrea. Pan African Medical Journal. 2011; 9:40-46.
25. Rahlenbeck SI, Yohannes G, Molla K, Reifen R, and Assefa A.Infection with HIV, syphilis and hepatitis B in Ethiopia: a survey in blood donors.Int J STD AIDS. 1997; 8(4):261-264.
26. Belachew T, Gebre-Selassie S. Trends of HIV Seropositivity among Blood Donors over Six Years Period (1995-2000) in Jimma Zone, Southwest Ethiopia.Ethiopian J Health Sci. 2001; 11(2):97-109.
27. Ahmed Z,UmaruN,andShreesha N. Seroprevalence of transfusion transmitted infections among blood donors at Mangalore.MedicaInovatica. 2012;1(2):24-27.
28. Buseri FI, Muhibi MA,and Jeremiah ZA.Sero-epidemiology of transfusion-transmissible infectious diseasesamong blood donors in Osogbo, south-west Nigeria.Blood Transfus. 2009; 7: 293-299.
29. World Health Organization: Screening donated blood for transfusion-transmissible infections: recommendations. World Health Organization, Geneva; 2010.

Annex

Annex I: Tests protocol

Testing Protocol for Vironostika HIV Ag/Ab test

Principle of the Procedure

Vironostika^{R} HIV Ag/Ab is an ELISA based on a one-step ‘’Sandwich’’ principle.A mixture of HIV antigens and HIV antibodies coupled to horseradish peroxides (HRP) serves as the conjugate with tetramethylbenzidine (TMB) and peroxides as the substrate. Upon completion of the assay, the development of color indicates the presence of HIV antibody or HIV antigen, while no or low color development suggests the absence of HIV antibodies or antigens.

Assay Procedure

1. Fit the strip holder with the required number of microelisa strips.
2. Pipette 100ul specimen diluents into all wells, i.e. including control wells.
3. Pipette 50ul sample or control into assigned wells. Include three negative controls, one anti-HIV-1 positive control, one anti-HIV-2 positive control and one HIV-1 antigen positive control in each strip holder. Always pipette the controls after pipetting the samples. Fill any unused wells with specimen diluents in order to dissolve the conjugate sphere and prevent malfunction of the aspiration/wash system.
4. Mix well (e.g. using a microshaker, speed approximately 15 Hz (=900 revolutions per minute) for 15 seconds, or equivalent).
5. Wash and sock each well six times with phosphate buffer.
6. Pipette 100ul TMB substrate into each well. Do not mix or shake.
7. Incubate the strips at 15 to 30^{o}c for 30 +/- 2 minutes in the dark.
8. Stop the reaction by adding 100ul 1mol/l sulfuric acid to each well: plates should be read within 15 minutes.
9. Blank the reader on air, i.e. without strip holder and strips, and read the absorbance of the solution in each well at 450nm and 620 to 700nm as reference (dual wavelength).

Testing Protocol for HBsAg test

Test Principle

HBsAg ELISA kit uses polystyrene microwell strips pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample is added to the microwell together with a second antibody conjugated with horseradish peroxidase (HRP) and directed against a different epitope of HBsAg. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum protein and unbound HRP-conjugate,chromogen solutions containingtetramethybenzidine(TMB) and Urea peroxidase are added to the wells.in the presence of the antibody-antigen-antibody(HRP) sandwich immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid.The amount of color can be measured and is proportional to the amount of antigen in the sample. Wells containing samples negative for HBsAg remain colorless.

Procedure

1. Numbering wells: Set the strips needed in the strip-holder and number sufficient number of wells including three negative controls, two positive controls and one blank well.
2. Adding sample and HRP-conjugate: Add 50 ul of positive control,Negative control,and specimen into respective wells. Add 50ul HRP-conjugate to each well except the Blank and mix by tapping the plate gently.
3. Incubating: Cover the plate with the plate cover and incubate for 60 minutes at 37^{o}c. It is recommended to use water tank to assure the temperature stability and humidity during incubation. If dry incubator is used do not open the door frequently.
4. Washing: After the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted wash buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the strips plate down onto blotting paper or clean towel, and tap the plate to remove any remainders.
5. Coloring: Dispense 50ul of substrate solution B and after that 50ul of substrate solution A into each well including the Blank, and mix by tapping the plate gently. Incubate the plate at 37^{o}c for 15 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HRP-conjugate produces blue color in a positive control and HBsAg positive sample wells.

6. Stopping Reaction: Using a multichannel pipette or manually add 50ul stop solution into each well and mix gently. Intensive yellow color develops in positive control and HBsAg positive sample wells.
7. Measuring the absorbance: Calibrate the plate reader with the bank well and read the absorbance at 450nm. If the dual filter instrument is used, set the reference wavelength at 630nm.Calculate the cut-off value and evaluate the results.

Note: Read the results within 5 minutes after stopping the reaction.

Testing Protocol for HCV Ab

Test principle

This kit employs solid phase, indirect ELISA assay for detection of antibodies to HCV in two step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and non-structured regions of HCV (third generation HCV ELISA). During the first incubation step, anti-HCV specific antibodies, if present, will be bound to the solid phase pre coated HCV antigens. The wells are washed to remove unbound serum protiens, and rabbit anti-human IgG antibodies (anti-IgG) comjugated to horseradish peroxidase (HRP) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing tetramethylbenzidine(TMB) and urea per oxidase are added to the wells and in presence of the antigen –antibody –anti-IgG (HRP) immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antibody in the sample. Wells containing samples negative for anti-HCV remains colourless.

Procedure

1. Reagents preparation: Allow the reagents and samples to reach room temperature (18^{o}c-30^{0}c) for at least 15-30 minutes.
2. Numbering wells:Set the strips needed in strip-holder and number sufficient number of wells including three negative controls, two positive controle and one blank
3. Adding diluents: add 100ul specimen diluents in to each well except the blank
4. Adding sample: add 10ul of positive control, negative control, and specimen into their respective wells
5. Incubating (1): cover the plate with cover plate and incubate for 30minutes at 37^{o}c.
6. Washing (1): after the end of the incubation, remove and discard the plate cover. Wash each wall five times with diluted wash buffer.
7. Adding HRP-conjugate:add 100ul HRP-conjugate to each well except the blank
8. HRP-conjugate incubating (2): cover the plate with cover plate and incubate for 30minutes at 37^{o}c.
9. Washing (2): at the end of the incubation, remove and discard the plate cover. Wash each wall five times with diluted wash buffer
10. Coloring: dispense 50ul of substrate solution B after that 50ul of substrate solution A in to each well including the blank and mix by tapping the plate gently. Incubate the plate at 37 ^{o}c for 15 minutes avoiding light.
11. Stopping reaction: using a multichannel pipette or manually add 50ul stop solution in to ach well and mix by tapping the plate gently.
12. Measuring the absorbance: calibrate the plate reader with the blank well and read the absorbance at 450nm. Calculate the cut of value and evaluate the results. (note: read the absorbance within 5 minutes after stopping the reaction)

Testing protocol for non-treponemal slide agglutination test for detection of plasma regains in human serum

Principle of the method

The RPR-carbon is a non-treponemal slide agglutination test for the qualitative and semi-quantitative detection of plasma reagins in human serum. Carbon particles coated with a lipid complex are agglutinated when mixed with samples containing reagins of patient affected by syphilis.

Procedure

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 µL of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the RPR-carbon reagent vigorously or on a vortex mixer before using. Invert the dropper assembly and press gently to remove air bubbles from the micropipette.
4. Place the micropipette in a vertical position and perpendicular to the slide, and add one drop (20 µL) of this reagent next to the samples to be tested.
5. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
6. Place the slide on a mechanical rotator at 80-100 r.p.m. for 8 min (Note 1). False positive results could appear if the test is read later than 8 minutes.

"ABO/Rh" blood grouping testing protocol

"ABO" blood grouping

Principle

Qualitative test for determination of A and/or B antigens on human red blood cells. The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding ABO antigen. No agglutination generally indicates the absence of the corresponding ABO antigen.

Procedure

Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS.
2. Place on a labeled glass slide: 1 volume of Anti-ABO reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes over a diffuse light and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

"Rh" testing

Principle of the method

The reagent will cause direct agglutination (clumping) of test red cells that carry the D antigen and indirect agglutination of test red cells that are Category D in the antiglobulin phase of testing. No agglutination generally indicates the absence of the D antigen.

Slide Method

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS.
2. Place on a labeled glass slide: 1 volume of Anti-D reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2 -minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes over a diffuse light and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

Annex II: Data collection checklist

Annex III: Declaration

I, the undersigned, declare that this MSc proposal is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the proposal have been duly acknowledged.

Name of the candidate: JijigaEdosa(BSc)

Signature_____

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

This proposal has been submitted with my approval as university advisor.

Name of advisor: Dr. Aster Tsegaye(MSc, PhD)

Signature _____

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

Name of advisor: Mr.MelakuTamene(MSc, PhD candidate)

Signature _____

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

Table 8.Cost of Total TTI'S Test done Including Control Samplesusing currentlyin use strategy (parallel) at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

Type of Test	Total Test Done Including Control Samples	Reagent Cost Per Test	Total Reagent Cost	Distilled water Cost (Birr)			Micro pipette TIPs cost (Birr)			Total time taken in minutes to perform the test	Labor Cost (Birr)		Grand Total
				Required ml Per Test	Cost Per ml	Total Cost	Required No. Per test	Cost Per Test	Total Cost		Labor cost Per Minute	Total Labor cost	
HBV	184032	7.61	1400483.5	5.94	0.017	18583.6	1.33	0.53	97905.02	230040	0.16	36806.4	1553778.49
HIV	184032	57.35	10554235	12.5	0.017	39106.8	1.25	0.5	92016.0	215662	0.16	34505.92	10719863.92
HCV	184032	26.23	4827159.4	5.21	0.017	16299.7	1.33	0.53	97905.02	215662	0.16	34505.92	4975870.02
RPR	184032	1.072	197282.3	0	0	0	1	0.4	73612.8	368064	0.16	58890.24	329785.34
Grand Total													17,579,297.78

Table 9.Cost of Total Repeated TTI'S Test done Including Control Samples using currentlyin use strategy (parallel) at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

Type of Test	Total Test Done Including Control Samples	Reagent Cost Per Test in ETB	Total Reagent Costin ETB	Distilled water Cost		Micro Pipette Tips Cost					Labor Cost		Grand Total
				Required ml Per Test	Cost Per ml in ETB	Total Cost in ETB	Required No. Per test	Cost Per Test in ETB	Total Cost in ETB	Total time taken in minutes to perform the test	Labor cost Per Minute in ETB	Total Labor cost in ETB	
HBV	18578	7.61	141378.58	5.94	0.017	1876.01	1.33	0.53	9883.50	23222.5	0.16	3715.6	156853.68
HCV	5008	26.23	131359.84	5.21	0.017	443.56	1.33	0.53	2664.26	5868.75	0.16	939	135406.65
HIV	6052	57.35	347082.2	12.5	0.017	1286.05	1.25	0.5	3026	7092.18	0.16	1134.75	352529.00
Grand Total													644789.34

Table 10.Cost of Total TTI'S Test Done Including Control SamplesUsing Newly Designed Testing Algorithms at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

				Distilled water Cost			Micro pipette Tips cost				Labor Cost		
Type of Test	Total Test Done Including Control Samples	Reagent Cost in ETB	Total Reagent Cost in ETB	Required ml Per Test	Cost Per ml in ETB	Total Cost in ETB	Required No. Per test	Cost Per Test in ETB	Total Cost in ETB	Total time taken in minutes to perform the test	Labor cost/Min in ETB	Labor cost Per Minute in ETB	Grand Total
HBV	184032	7.61	1400483.52	5.94	0.017	18583.6	1.33	0.532	97905.02	230040	0.16	110419.2	1627391.29
HCV	175186	26.23	4595128.78	5.21	0.017	15516.2	1.33	0.532	93198.95	205296	0.16	98542.08	4802386.03
HIV	172765	57.35	9908072.75	12.5	0.017	36712.6	1.25	0.5	86382.5	205296	0.16	98542.08	10129709.89
RPR	169908	1.072	182141.38	0	0	0	1	0.4	67963.2	184032	0.16	29445.12	279549.69
Grand Total													16839036.92

Table 11.Cost of Total Repeated TTI'S Test Done Including Control Samples Using Newly Designed Testing Algorithms at Federal Ministry of Health National Blood Transfusion Service, Addis Ababa, Ethiopia from July 2008 to July 2013.

Type of Test	Total Test Done Including Control Samples	Reagent Cost in ETB	Total Reagent Cost in ETB	Distilled water Cost			Micro Pipette Tips Cost				Labor Cost		Grand Total
				Required ml Per Test	Cost Per ml in ETB	Total Cost in ETB	Required No. Per test	Cost Per Test in ETB	Total Cost in ETB	Total time taken in minutes to perform the test	Labor cost/Min in ETB	Labor cost Per Minute in ETB	
HBV	18578	7.61	141378.58	5.94	0.017	1876.01	1.33	0.532	9883.496	23222	0.16	3715.52	156853.60
HCV	4905	26.23	128658.15	5.21	0.017	434.44	1.33	0.532	2609.46	5748	0.16	919.68	132621.73
HIV	5988	57.35	343411.8	12.5	0.017	1272.45	1.25	0.5	2994	7017	0.16	1122.72	348800.97
Grand Total													638276.30

Annex II: Data collection checklist

S.No.	ID.No.	Socio demographic characteristics						TTIs				Cost of Laboratory tests (ETB)				
		age	Sex	Weight	Blood Type	RH	Type of Donation	HIV	HBV	HCV	Syphilis	Reagent cost	Micro pipette Tip cost	Labor cost	Distilled water cost	Remark