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Prevalence of Transfusion Transmissible Infections and Its Cost Analysis among Voluntary Blood Donors at Shashemane and Wolaita Sodo Blood Banks, Southern Ethiopia.

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This is to certify the thesis prepared by BILEN ASRAT, entitled: **"Prevalence of Transfusion Transmissible Infections and Its Cost Analysis Among Voluntary Blood Donors at Shashemane and Wolaita Sodo Blood Banks"** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Laboratory Management and Quality Assurance) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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Acronyms / Abbreviations

BTS	Blood Transfusion Services
HBV	Hepatitis B Virus
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
MOH	Ministry of Health
NBS	National Blood Bank Services
RBB	Regional Blood Bank
RHB	Regional Health Bureau
SOPs	Standard Operating Procedures
TTIs	Transfusion Transmissible Infections
USA	United States of America
VNRBD	Voluntary Non-remunerated Blood Donor
WHO	World Health Organization

Abstract

Background: Blood transfusion is a vital healthcare intervention, but the risk of infections like Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Immunodeficiency Virus (HIV), and syphilis remains a major concern due to prolonged viremia and latent phases. Cost analysis, particularly the Activity-based costing model (ABC), aids in financial decision-making by linking costs to specific activities. Despite having these challenges, there is not well-documented evidence, particularly in the southern part of our country. Hence, this study aims to determine the prevalence of transfusion-transmissible infections among blood donors and its cost of analysis in Shashemane and Wolaita Blood Banks.

Methods: A cross-sectional study was conducted from March to May 2024 at Shashemane and Wolaita Sodo Blood Banks, involving 568 consecutive blood donors. Data were collected using a semi-structured questionnaire by trained personnel. Transfusion-transmissible infections (TTIs) were tested using Chemiluminescent Microparticle Immunoassay. The cost of TTIs was analyzed using Activity-Based Costing (ABC) to trace individual infections, and costs were compared between sequential and simultaneous testing approaches.

Result: From the total number of 568 blood units collected, 34 donors were positive for any of the TTI tested, giving an overall positivity rate of 6.0%. The sero-prevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV), Syphilis, and Human Immunodeficiency Virus (HIV) was 18(3.2%), 7(1.2%), 6(1.1%), 3(0.5%), respectively. In this study, by using the activity-based cost analysis method, the single cost of Hepatitis B virus, Hepatitis C virus, Syphilis, and Human Immunodeficiency Virus was 1.8926$, 2.9628$, 1.8231$, and 1.9631$. There was a difference in cost between the simultaneous versus newly sequential testing algorithms was $1,183.66.

Conclusion: The prevalence of transfusion-transmitted infections (TTIs) was higher in this study area than in other regions of Ethiopia. The simultaneous testing strategy incurred higher costs than the sequential testing strategy. This suggests that the newly proposed testing method is a more cost-effective solution compared to the parallel or current testing method.

Keywords: Sero prevalence, Cost analysis, transfusion-transmissible infections, voluntary blood donors, Shashemane and Wolaita Sodo blood banks

1 Introduction

1.1 Background

Blood transfusions are transfusions of whole blood or its components, which have become a lifesaving medical procedure in the modern-day health care system (1,2). Blood transfusion is a therapeutic process; however, if the blood is contaminated, it can be fatal instead of saving a life. The emergence of transfusion-transmissible infections (TTIs) has led to a change in blood transfusion practices all around the world, and has placed safety and the protection of human life at the forefront. (3,4).

Blood transfusion poses a potential threat of transmitting serious infections, including hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), syphilis, cytomegalovirus (CMV), herpes simplex virus (HSV), and Epstein–Barr virus (EBV), as well as toxoplasmosis and malaria. Consequently 5), the World Health Organization (WHO) suggested that all donated blood must undergo screening for transfusion-transmissible infections (TTIs) caused by pathogens such as HBV, HCV, HIV, and syphilis (6). It is essential to conduct screenings for malaria and other diseases in areas where these diseases are common (7).

Human Immunodeficiency Virus, Hepatitis B Virus, and Hepatitis C Virus are the causative agents of acquired immune deficiency syndrome (AIDS), and hepatitis B and C infections, respectively. These infections are capable of causing long-term carrier states, prolonged viremia and infectivity, chronic disorders, along with high rates of morbidity and mortality due to chronicity, liver cirrhosis, hepatocellular carcinoma (HCC), and opportunistic infections. These viruses can be transmitted through direct exposure to infected blood and blood derivatives, organ transplantation, hemodialysis, intravenous drug use, blood transfusion, tattooing, and sexual contact. However, the latter is not the common mode of HCV transmission (8).

Also, Syphilis, which is caused by Treponema pallidum (a type of Spirochete bacteria), can be transmitted through various means such as sexual activity, vertical transmission from mother to fetus, blood transfusion, and direct contact with infectious lesions. The infection has the potential to result in acute illness, long-term complications, infertility, medical and psychological consequences, and even death (9).

There are several methods to screen transfusion transmissible infections but according to this study assay for Hepatitis B virus, Hepatitis C virus, HIV and Syphilis are screened by using HBsAg qualitative assay, Anti-HCV assay, HIV antigen-antibody combination assay, and syphilis TP assay by an automated chemiluminescent microparticle immunoassay (CMIA) analyzer using (HBsAg qualitative II kit, anti-HCV kit, and HIV Ag/Ab Combo kit, syphilis TP, respectively, Abbott Diagnostics) (10).

Cost analysis is a systematic approach that facilitates the provision of an analysis or forecast of information, which is crucial for making financial decisions. It serves as a valuable tool in evaluating management efficiency and calculating the breakeven point. By utilizing cost analysis, the laboratory manager can establish charges that adequately cover the costs and develop policies for future laboratory services (11).

There are different types of costing methods, but according to my study, there are two types of costing methods to trace a single test: these are the traditional costing model and the activity costing model.

The traditional costing method can trace direct costs associated with single cost drivers such as the number of tests performed or labor hours. It is easy to apply but often lacks accuracy, especially in healthcare settings where resources are used unevenly across different tests or procedures. So there is a need for an accurate system, such as ABC, that uses more cost drivers to allocate overhead costs more accurately(12).

Activity-based costing model is a method that focuses on the relationship between costs and activities, offering financial information to managers. In this method, costs are calculated more precisely by assigning overhead costs to the activities that generate those costs. Therefore, ABC has a tendency to identify activities that drive overall costs in a test. This model effectively captures the manpower, equipment, and activities within a specific section. By providing precise cost data, ABC costing enhances efficiency and effectiveness, ultimately aiding in the accomplishment of the laboratory's strategic goals (12,13). According to my research, the activity-based costing method is more preferable than the traditional testing method for obtaining a more accurate cost of a single test.

1.2 Statement of the problem

Blood donation saves the lives of millions of people worldwide; however, the patients are at a potential risk of contracting transfusion-transmitted infections (TTIs), which in turn impose serious challenges to the medical providers for the availability of safe and affordable blood products (14).

WHO recommends that all blood donations be screened for infections before use. Before the use of blood products, these common transfusion-transmissible infections (HIV, hepatitis B, hepatitis C, and syphilis) should be screened. Blood screening should be performed according to quality system requirements. 99.8% of the donations in high-income countries and 99.9% in upper-middle-income countries are screened for basic quality procedures, as compared to 83% in lower-middle-income countries and 76% in low-income countries. The prevalence of transfusion-transmissible infections in blood donations in high-income countries is considerably lower than in low and middle-income countries (15).

Currently, about 80% of the world's population has access to only 20% of the world's blood supply. Low and middle-income countries (LMIC) often are challenged to meet transfusion needs. The approaches to securing a safe and adequate blood supply in high-income countries (HIC) are not appropriate, practical, or validated for the broad range of Low and middle-income countries (LMIC) settings. Inadequate or inconsistent supplies and acute shortages of blood for transfusion in low-resource settings, often coupled with questionable quality, pose real risks to the health of patients who need transfusion (16).

Africa has the most extreme need for blood transfusions in the world, while also having the highest prevalence of blood-borne diseases and some of the weakest transfusion programs(17).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 (18).

Human Immunodeficiency Virus was said to be tested in all of the units, and problems in the supply of HIV testing kits were observed. There is also a shortage of manpower in most of the centers. This presents a major challenge to the transfusion services in Ethiopia as well as other

underdeveloped countries (19), with the potential for unscreened blood to put patients at risk of acquiring TTIs and consequently acting as a source of infection to the community, leading to an increased disease burden that may incur further costs for diagnosis and treatment throughout the country (20, 21).

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 disease transmission (22).

Currently, Ethiopia has been implementing several programs to reduce the burden of blood-borne pathogens among the population. These include, but not limited to, public awareness creation on transmission and prevention of blood-borne infection, free HIV testing and counseling, ANC screening of pregnant women for syphilis, for HIV and HBV, immunization of children, and risk groups such as health workers against HBV, and universal access to HIV antiviral therapy (23). However, Most African countries are severely limited by the lack of financial and human resources, aggravated by inconsistent supplies of reagents for performing serologic blood testing. In some African countries, blood transfusion services have been put in place with massive financial and technical support from 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 (24).

Even though there are a few studies conducted in Ethiopia on TTIs among blood donors, most of them used retrospective blood donors' data and also proposed cost cost-effective method for screening of TTI (25). So I prefer these areas because there is limited study about the prevalence of transfusion-transmissible infection in this study area over others.

Therefore, this study determines the magnitude of transfusion transmissible infections among voluntary blood donors and analyzes costs by using the Activity-based costing method to determine the single cost of TTIs in Shashemane and Wolaita Sodo Town.

1.3 Significance of the study

Estimating the prevalence of TTIs, namely HBV, HCV, HIV, and syphilis among blood donors can identify the problem of unnoticeable infections in healthy-looking members of the general population of Shashemane town and Wolaita town, and also provide data that is important in formulating the strategies for improving the management of a safe blood supply. In addition, it can give us a guide to the current magnitude of some sexually transmitted infections in the community.

The study is significant as it proposes a cost-effective blood screening strategy using the Activity-Based Costing method and performing a sequential testing method for TTIs. This proposed strategy will help economize the limited resources available in the country.

Also, it provides information on associated risk factors of TTIs, which can be helpful to responsible bodies for awareness creation or health education.

Finally, all information can also be used as reference data for further similar studies and for planning health development activities.

2. Literature review

2.1 Prevalence of transfusion-transmissible infection in the blood bank

According to the World Health Organization's global hepatitis report in 2017, every year around 118.5 million units of blood are collected worldwide. Along with a large number of people donating blood, TTIs continue to be a big threat globally. Globally, an estimated 257 million, 71 million, and 36.7 million people were living with chronic HBV, HCV, and HIV infections, respectively. The epidemic caused by HBV affects mostly the African Region, with a prevalence of 6.1%. On the other hand, HCV infection affects all regions, with the highest prevalence in the Eastern Mediterranean region at 2.3% and the European region at 1.5% of prevalence. Around 1.34 million deaths were attributed to hepatitis, from which cirrhosis (720,000 deaths), and hepatocellular carcinoma (470,000 deaths). Globally, due to unsafe injections (5%), around 1.75 million new HCV infections occurred worldwide (26).

Retrospective study was carried out by Glynn SA. on a total of 1.9 million US volunteer blood donors with 1 or more nonautologous donations over a period of five year from January 1991 to December 1996, which asses Changes in rates of HIV, HTLV, HCV, and HBV infections. The result showed that the prevalence of HIV decreased in first-time donors from 0.030% to 0.015%, HCV prevalence decreased from 0.63% to 0.40% and Trends were not statistically significant for the proportion of first-time donors with hepatitis B surface antigen (HBsAg) or HTLV (27).

Another retrospective study was done by Farshadpour F et al. on a total of 293454 blood donors in south Iran over a period of 10 years from 2004 to 2014, which shows that the overall seroprevalence rates of HBV, HCV, and HIV were 0.15%, 0.1% and 0.004%, respectively. The highest seroprevalence was found for HBV, followed by HCV and HIV. These infections were more prevalent in male, low-educated, and first-time donors. The highest HCV seroprevalence was observed among donors aged 20 to 40 years, while HBV seroprevalence increased with age. The seroprevalence rates of HBV and HCV from 2004 to 2014 showed significant decreasing trends from 0.460% to 0.060% and 0.329% to 0.045%, respectively. Whereas, HIV infection had a slight but not significant decline from 0.0173% in 2004 to 0.0028% in 2014 (28).

A cross-sectional study titled 'Seven-Year Trends in the Prevalence of Transfusion-Transmissible Viral Infections in Yazd Blood Transfusion Organization (2004–2010)' by

Shahshahani H.J. et al. in Iran, reported that the prevalence rate of HBV, HCV, and HIV infection decreased during these years (From 0.37%, 0.14% and 0 percent in 2004 to 0.14%, 0.05% and 0 in 2010, respectively). Both hepatitis B and C infections were significantly more in first-time blood donors with a BSc or BA educational level. The prevalence rate of hepatitis B was significantly higher in donors with less than 20 years old and female donors. The prevalence rate of hepatitis C was higher in the 30-39 age group (29).

A study in Brunei Darussalam by Teo KSK et al. from 2005 to 2009 revealed that out of 56,645 donations 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 (30). Another study was done by Afolabi AY et al. in 2010 that discussed transfusion transmissible viral infections among potential blood donors in Ibadan, Nigeria, which shows that 507 potential blood donors were recruited and tested for HIV, HBsAg, and anti-HCV using a commercial ELISA test kit in strict compliance with the manufacturer's procedures. Overall results show rates of 2.0%, 5.9% and 1.4% for HIV, HBsAg, and HCV, respectively. Also, the highest prevalence rates were recorded among the age group 26 to 35 years at 2.6%, 7.2%, and 2.1% for HIV, HBV, and HCV, respectively. Furthermore, higher prevalence rates were noted among unmarried individuals at 2.6%, 6.8% and 2.1% for HIV, HBV, and HCV, respectively (31).

A retrospective study by Fessehaye N et al.from 2006 to 2009 in Eritrea discussed that a total of 29,501 units of blood were collected those, 23,385(79%) were voluntary blood donors, and the rest 6,116(21%) units were collected from family replacement donors. The overall prevalence of TTIs was 3.8% with 3.5% in voluntary blood donors and 5.1% in family replacement donors. The seroprevalence for TTI markers was 0.18% HIV, 2.58% HBV, 0.57% HCV, and 0.49% Syphilis (32).

2.2 Prevalence of transfusion-transmissible infection in Ethiopia

A three-year retrospective study conducted by Biadgo B. et al. at the North Gondar Blood Bank in Ethiopia from 2010 to 2012 included a total of 6,471 blood donors. Overall, 424 (6.55%) of the blood donors were sero-reactive for at least one transfusion-transmissible viral infection. Of

all study participants, 233 (3.6%) were sero-reactive for HBV, 145 (2.24%) were sero-reactive for HIV, and 51 (0.8%) were sero-reactive for HCV. Four (0.062%) of the study's participants were co-infected: 3 (75%) with HBV-HCV and 1 (25%) with HIV-HBV-HCV. Being a farmer, unemployed or employed donor was significantly associated with transfusion-transmissible viral infections compared to being a student donor (33).

Another four-year retrospective study was conducted by Mohammed Y. et al. from 2010 to 2013, which discussed the seroprevalence of transfusion-transmitted infections among blood donors at the Jijiga Blood Bank in Ethiopia. The study reported a total of 4224 people donated blood, and the overall prevalence of transfusion-transmitted infection was 487/4224 (11.5 %). The prevalence for HBsAg, HCV, HIV, and Syphilis antibodies was 460 (10. 9 %), 17 (0.4 %), 6 (0.1 %), and 4 (0.1 %), respectively. The majority, 460/487 (94.5 %) of infections were HBsAg. Statistically significant difference was observed in the number of donations as well as sero-positivity from 2010 to 2013, in Trends of HBsAg from year to year, HIV was seen as the age of donors increased, and there was also a statistically significant difference in the prevalence of HBsAg distribution by sex (34).

Another retrospective cross-sectional study was conducted by Abdella et al. in Ethiopia, which measured the prevalence of transfusion-transmissible viral infections from 2014 to 2019. A total of 554,954 blood donors from fourteen blood bank facilities were included in the study. The overall sero-prevalence of HBV, Treponema pallidum, HIV, and HCV was 2.4%, 0.9%, 0.4% and 0.4% respectively. The prevalence of TTIs was comparatively higher in 2014, 5.70% and lowest in 2019, 3.40 %(35).

A retrospective study conducted by Shiferaw E. et al. from 2014 to 2018 at the Bahir Dar District Blood Bank showed that, from a total of 35,435 blood donors 2130 (6.0%) of them had serological evidence for at least one infection and 50 (0.14%) of them were confirmed as having multiple infections. The sero-prevalence of HBV, HCV, HIV, and syphilis was 3.9%, 0.6%, 0.5% and 1.2% respectively. From those who had co-infection majority of them, 22 (44.0%), were attributed to HBV-Syphilis co-infection, and 1 (2.0%) study participant was co-infected with HBV-HIV- Syphilis infection. There was an increment in the overall prevalence of transfusion-transmissible infection; 183 in 2014/2015 to 624 in 2017/2018. The sero-prevalence of HBV shows a significant increment with respect to the year of donation. On the other hand,

HCV and HIV sero-prevalence show an increasing trend from 2014 and a decrease in 2018. The sero-prevalence of syphilis was 67 (1.3%) in 2015 and doubled in 2016, 138 (1.5), but subsequently decreased to 110 (1.1%) in 2017, and in 2018 it was 114 (1.0%) (36).

2.3 Cost -Analysis of transfusion transmissible infection in Blood banks

An institution-based cross-sectional study was conducted by Tsehay S. et al. in 2018 among 414 voluntary blood donors at the Hawassa Regional Blood Bank in Ethiopia. The study showed that the overall seropositivity of transfusion-transmissible infections was 7.0% and the positivity rates of hepatitis B virus, syphilis, and Plasmodium falciparum were 5.6%, 1.0%, and 0.5%, respectively. The cost per test of each transfusion-transmissible infection was US$5.04 for human immunodeficiency virus, US$4.61 for hepatitis B virus, US$5.11 for hepatitis C virus, US$4.75 for syphilis, and the cost per test of the malaria rapid diagnostic test was US$4.74.In addition, the total cost of laboratory incurred for transfusion-transmissible infections screening is estimated to be US$213,634.5 per year (37).

Another study was done by Kania et al. in 2002 that discussed the cost-effectiveness of HIV, HBV, HCV, and syphilis testing of blood donations in Burkina Faso was carried out on 500 blood donations. By using the simultaneous strategy, the respective sero-prevalence of HBsAg, HIV, syphilis, and HCV among blood donors in Ouagadougou was 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 are made annually in Burkina Faso, the money savings reached potentially €90,860 (24). The order in which the tests should be processed will be based on the prevalence of the infections found in the country of Burkina Faso, starting from the most prevalent infection and proceeding accordingly, as per the magnitude of their prevalence. With regard to the Burkina Faso context, the HBV test was performed first, followed by the HIV test, and finally the HCV test, and then syphilis. This strategy is more efficient in the sense that it avoids the wastage of materials, reagents, and other resources (24).

3. Objectives

3.1 General objectives

- To assess the magnitude of transfusion-transmissible infections among voluntary blood donors at Shashemane and Wolaita Sodo Blood Banks and their cost analysis from March to May 2024.

3.2 Specific objectives

- To determine the magnitude of transfusion-transmissible infections (HBV, HCV, HIV, and Syphilis) among blood donors at Shashemane and Wolaita Soda Blood Banks.
- To determine the cost analysis of transfusion-transmissible infections (HBV, HCV, HIV, and Syphilis) and compare sequential versus simultaneous testing strategies among blood donors at Shashemane and Wolaita Soda Blood Banks.

3.3 Hypothesis

The Prevalence of transfusion-transmissible infections is high in Shashemane and Wolaita Sodo blood donors, and the current simultaneous strategy of testing is not cost-effective.

4. Materials and Methods

4.1 Study design and period

A prospective cross-sectional study was conducted among apparently healthy volunteer blood donors from March to May, 2024G.C at Shashemane Blood Bank and Wolaita Sodo Blood Bank.

4.2 Study area

The study was done in two blood banks, the first one is Shashemane Blood Bank which is located in Shashemane Town, in West Arsi Zone of Oromia Regional State, Ethiopia. The town is located 250 km from Addis Ababa, the capital city of Ethiopia. Based on the 2007 census, the population was 100,454 (male 50,654 and female 49,800). However, the Ethiopian Statistical Service (ESS), in 2023, the total population was 218,997 (male 109,293 and female 109,704). Shashemane Blood Bank collects around 10,000 blood units annually at mobile and center sites of blood collection to satisfy the blood transfusion need (38).

The other study area was Wolaita Sodo Blood Bank, which is located in Wolaita Sodo town, in Wolaita zone of South Ethiopia Regional State, Ethiopia. The town is located is 328 km south of the capital city of Ethiopia. Based on the 2007 Census conducted by the CSA, this town has a total population of 76,050, of whom 40,140 are men and 35,910 are women. According to the Ethiopian Statistical Service (ESS) however, in 2023, the total population was 213,467 (male 108,161 and female 105,306). On average, Wolaita Sodo Blood Bank collects around 3500 blood units annually at mobile and center sites of blood collection to satisfy the blood transfusion need (39).

The decision to focus on the Wolaita and Shashemane blood banks is driven by the scarcity of research in these specific regions regarding TTI's prevalence.

4.3 Population

4.3.1 Source population

- The source population was all potential blood donors who came to donate blood at Shashemane and Wolaita Sodo Blood Banks.

4.3.2 Study population

- The study population was all volunteer blood donors who donated blood at Shashemane and Wolaita Sodo Blood Banks during the study period.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion Criteria

- All participants who fulfilled the national and regional blood bank criteria and who have informed consent to participate in the study were included.

4.4.2 Exclusion Criteria

- Participants who did not meet the national and regional blood bank criteria and were unwilling to give informed consent were excluded.

4.5 Study Variables

4.5.1 Dependent Variables

- Prevalence of transfusion transmissible infections (HBV, HCV, HIV, and Syphilis)
- Strategy on cost analysis of blood screening and compare the sequential strategy and the simultaneous strategy

4.5.2 Independent Variables

- Socio-demographic: Age, Sex, marital status, Residence, Educational Status, Occupational status.

- Behavioral Factors: Multiple sexual partners, history of tattooing, Ear piercing, Nose piercing, History of sharing sharp materials.
- Other associated Factors: History of surgery, History of direct contact with the blood or open sores of an infected person, family history of HIV or HBV

4.6 Measurement and Data Collection

4.6.1 Sample Size Determination

Sample size was calculated based on a single population formula by considering 95% Confidence interval, assuming the prevalence of transfusion transmissible infection among blood donors at Jigjiga blood bank was 14.1%, and the margin of error (d), was 3% (40).

$$\mathbf{n}=\frac{Z^2\ \boldsymbol{\alpha/2}\ p\ (1-p)}{d^2}$$

Where:-

Z = Z statistic for a level of confidence (95% level of confidence; z=1.96)

P (expected prevalence or proportion) = 14.1%=0.141

d (marginal error) = 3% which is 0.03

$$\mathbf{n}=\frac{Z^2\ \boldsymbol{\alpha/2}\ p\ (1-p)}{d^2}$$

$$n=\frac{((1.96)^2 \times 0.141)\ (1-0.141))}{(0.03)^2}$$

$$n=\frac{((1.96)^2 \times (0.0141*0.859))}{(0.03)^2}$$

$$n=\frac{(3.8416 \times 0.121)}{0.0009}$$

$$n= 516.48=51$$

By assuming a 10% non-response rate, the final sample size was **568**

The final sample size was proportionately allocated to the two selected blood banks based on their blood donor flow in the service and the annual achievements.

4.6.2 Sampling method

During the study period, all voluntary blood donors who arrived consecutively at blood banks and mobile collection sites such as schools, governmental offices, churches, and charity groups were included. A convenient sampling technique was employed to select 568 eligible blood donors for donation. The final sample size was proportionately allocated to two selected blood banks based on their donor flow and their annual achievements. Specifically, all blood donors who donated at Wolaita Sodo Blood Bank (168 donors) and Shashemane Blood Bank (400 donors) were selected consecutively until the required sample size was reached.

4.7 Data Collection Technique and Laboratory Diagnosis

4.7.1 Data collection technique

A semi-structured questionnaire to collect information about associated factors and to assess the socio-demographic characteristics of the study participants was used. The questionnaire was adapted from different literature and prepared in the English language by the principal investigator in simple, understandable language, then translated to Amharic and the local language, Oromiffa. To confirm its consistency, the questionnaire was translated back to English. This makes the questionnaire simple to understand for data collectors and respondents. After written consent was obtained, data collection was started when the participants started giving blood donation, and the questionnaire was completed. After that, the collected blood is transported to the testing site of the laboratory.

Data was collected by four data collectors (one BSc nurse, two health officers, and one laboratory technician) who work in the blood bank. For data collection, a face-to-face interview using a semi-structured questionnaire was conducted to gather sociodemographic, associated factors, and lab investigation of blood for the donors.

4.7.2 Laboratory diagnosis.

Chemiluminescent microparticle immunoassay (CMIA) is a detection technology used to measure analyte concentration. The CMIA technology detects the presence of antigens, antibodies, and analytes in patient samples.

The sample and the paramagnetic microparticles coated with capture molecules are dispensed into the reaction vessel (RV). The vortexer mixes the reaction mixture. Then the reaction mixture is incubated. The analyte in the sample binds to the capture molecules on the paramagnetic microparticles and forms an immune complex. A magnet attracts the paramagnetic microparticles (which are bound to the specific analyte) to the wall of the RV. The wash zone assembly washes the reaction mixture to remove unbound materials. Additional assay processing can now occur. Then the pipettor dispenses a chemiluminescent, acridinium-labeled conjugate into the RV. The conjugate binds to the immune complex to complete the reaction mixture. The vortexer mixes the reaction mixture. The reaction mixture is incubated. After that, the wash zone assembly washes the reaction mixture to remove unbound materials. Then, the Pre-Trigger Solution nozzle dispenses the Pre-Trigger Solution (hydrogen peroxide) into the reaction mixture. The vortexer mixes the reaction mixture. The Trigger Solution nozzle dispenses the Trigger Solution (sodium hydroxide) into the reaction mixture. The Trigger Solution creates an alkaline environment that, with the exposure to peroxide in the Pre-Trigger Solution, causes the acridinium dye to undergo an oxidative reaction. The oxidative reaction causes a chemiluminescent reaction to occur. N-methylacridone forms and releases energy (light emission) as N-methylacridone returns to its ground state. The CMIA optical system measures the chemiluminescent emission (activated read) over a predefi ned time period to determine a result.

4.7.3 Cost analysis procedure

Every fixed and every variable cost was calculated. Building, equipment and employee salaries are examples of fixed costs, whereas the cost of supplies and materials is an example of a variable cost. The depreciation cost was subtracted from the asset's original cost to determine the cost of the plant asset.

The building's useful life is predicted by the engineers to be 50 years, and the construction cost was $48,885.27, which is determined from previous literature because both study areas are collection sites only, and the tests are performed in the Hawassa blood bank. The useful life of all equipment and the original cost of supplies and materials were determined by referring to the relevant voucher, current market value, and previous literature. The automated analyzer known

as Alinity i, which is a product of Abbott which performs Chemiluminescent microparticle immunoassay (CMIA).

The accumulated depreciation cost is calculated by using Straight-Line Depreciation. This method evenly spreads the cost of the asset over its useful life.

The construction of the building commenced in 2013, coinciding with the purchase of the refrigerator in the same year. Consequently, the depreciation cost for both assets began in 2014. As for the centrifuge and automated analyzer, they were acquired in 2021. Hence, the depreciation cost for these items initiates from the year 2022. The depreciation of the building and refrigerator costs is calculated from 2014 and accumulated until 2021 (Table 1).

Table 1: Accumulated Depreciation-Assets

Asset	**Life Span year**	**Cost in $**	**Accumulated depreciation year**					**Book Value in $**
			2014	**2021**	**2022**	**2023**	**2024**	
Building	50	48,885.27	977.71	7,821.68	8,799.39	9,777.1	10,754.81	38,130.46
Automated analyzer	20	68,325.13			3,416.26	6,832.32	10,248.78	58,076.35
Centrifuge	7	535.63			76.52	153.04	229.56	306.07
Refrigerator	12	215.95	17.99	143.92	161.91	179.9	197.99	17.96
	12	345.51	28.79	230.32	259.11	287.9	316.69	28.82

When conducting tests for Transfusion-Transmissible Infections (TTIs), we consider both direct labor costs (like lab technicians' and technologists' salaries) and indirect labor costs (such as those for the CEO, cleaner, and security). These indirect labor costs are not directly involved in the testing process but are factored into the overall expenses. We allocate these labor costs to each test conducted daily to figure out the portion of salary attributed to each individual test. All salaries are obtained from the testing site, Hawassa blood bank (Table 2).

Table 2: Direct labor cost and indirect labor cost

	A in $	B in No	C=(A*b)/30 in$	D in No	E=C/D in $
Professional salary	**Salary $**	**No of staff**	**Salary per day in $**	**No of tests per day**	**Salary per test in $**
Salary of CEO	97.65	1	3.26	150	0.0217
Salary of a laboratory technologist	87.67	2	5.84	150	0.0389
Salary of a laboratory technician	69.1	2	4.60	150	0.0306
Salary of a cleaner	20.6	3	2.06	150	0.0137
Salary of security	28.79	4	3.84	150	0.0256

Utility expenses, considered as overhead costs in the ABC costing method, are part of the operational expenses in the blood bank, which contribute. To determine the monthly utility expense, we divide the annual expense by the number of months. Then, we allocate the monthly average cost, and then it is divided by the quantity of samples tested each day, helping us find the utility expense per test (Table 3).

Table 3: Utility expense

	A	B=A/12	C	D=B/C
Utility	**Annual expense In $**	**Average expense In $**	**Test done per day**	**Utility cost per test in $**
Electricity	567.07	47.26	150	0.32
Water	114.04	9.5	150	0.06
Telephone	365.45	30.45	150	0.20

In this study, we analyze the variable costs associated with operating the Alinity i analyzer, focusing on materials and supplies required for testing four transfusion-transmissible infections (TTIs) which are HV kit, HBV kit, HCV kit, syphilis kit, prob conditioner, trigger, pre trigger, con wash buffer, RX vessel, sample cup. It also included controls, calibrators, and maintenance.

Each Alinity i kit is designed to perform 100 tests. The materials and supplies consumed per test constitute the variable cost for each TTI, and this cost varies depending on the specific test. To determine the variable unit cost, we calculate the total cost of the kit and divide it by the number of tests (i.e., 100), yielding the per-test cost for each TTI (Table 4).

Table 4: Variable cost

Material and supplies

	A	**B**	**C**	**D=B*C/A**
Materials and supplies	**Quantity**	**Total cost in $**	**Quantity per test**	**Unit cost in $**
HIV kit	100	120.39	1	1.20
HBV kit	100	113.09	1	1.13
HCV kit	100	219.73	1	2.20
Syphilis kit	100	105.67	1	1.06
Prob conditioner	975	20.94	1	0.0214
Trigger	975	21.25	1	0.0217
Pre trigger	975	18.61	1	0.0190
Con-wash buffer	1000	20.79	1	0.0207
RX vessel	500	37.96	1	0.0759
Sample cup	500	17.61	1	0.0352
Pipet	100	18.31	1	0.1831
Cotton	1000	2.80	1	0.0028
Alcohol	500	14	1	0.028

The other variable costs include controls and calibrators. Controls are run daily, so their cost is calculated on a daily basis. The machine is calibrated once a month, so the cost for calibrators is calculated on a monthly basis. To begin the calculation, 1 drop is approximately 50 µl. For one run, about 6 drops are needed. The calculation is as follows:

1 drop= 5µl 1ml=1000µl

6 drop= x x= 300µl

X= 300µl x=0.3 ml

According to the above calculation, 0.3 mL is needed for one run, whether for the control or the calibrator.

The entire vial of control contains about 10 mL. To calculate how many days the whole vial will last, we need to determine how many runs can be performed using the vial, since controls are run once a day. The calculation is as follows.

1day= 0.3ml

X=10ml

X=33.33= 33 days

Table 5: Controls

Supplies	**Number of days**	**Total cost**	**Cost per day**	**Number of test per day**	**Cost per test**
HIV control	33 days	21.47	0.65	150	0.0043
HBV control	33 days	19.61	0.59	150	0.0039
HCV control	33 days	20	0.61	150	0.0041
Syphilis control	33 days	21.32	0.65	150	0.0043

The entire vial of calibrator contains approximately 3 ml. We will now calculate how many months the vial will last, given that the calibrator is used once per month

. This calculation will be done in terms of months as follows:

1 month= 0.3ml

X=3ml X=10 months

Table 6: Calibrators

Supplies	Number of months	Total cost	Cost per month	Number of test per month	Cost per test
HIV calibrator	10	21.42	2.142	4500	0.00048
HBV calibrator	10	19.46	1.946	4500	0.00043
HCV calibrator	10	19.95	1.995	4500	0.00044
Syphilis calibrator	10	21.27	2.127	4500	0.00047

The procedures outlined above represent a systematic approach aimed to determining of the cost associated with conducting a single TTI test. This involves accounting for various factors such as building costs, equipment costs, direct and indirect labor costs, overhead costs, which is utility expenses, material and supply costs. Once the cost per test for TTIs is established, the next step involves a comparative analysis. Specifically, we examine the cost implications of testing 568 units of blood using two different approaches: sequential testing order and simultaneous testing order.

The other variable cost is the maintenance cost.

One of the variable costs associated with the Alinity I machine is the daily maintenance cost, which includes the use of Alinity I wash solution.

- The wash solution is supplied in 2-liter (2000 ml) bottles, each costing $105.
- On average, 30 ml of this solution is consumed per day for maintenance procedures.

To determine how many days a single bottle lasts:

Total volume per bottle = 2000 ml

Daily usage = 30 ml

Number of days=66.7≈67days

- Therefore, the daily cost of the wash solution is calculated as:

105$/67 days= $1.57per day

- An average of 150 tests are performed per day, the maintenance cost per test is:

1.57$/150 test= **0.01$per test**

Another variable maintenance cost is associated with the weekly probe conditioning procedure required for the Alinity I machine. This process involves the use of a probe conditioning solution, which is essential for maintaining the performance of the two probes in the machine.

- The probe conditioning solution is supplied in 1-liter (1000 ml) bottles, each costing $100.
- Each maintenance cycle (performed weekly) requires 100 ml of solution: 50 ml per probe, for 2 probes.
- To determine how many weeks one bottle lasts:

  Total volume = 1000 ml

  Weekly usage = 100 ml

  Number of weeks=10cycle= 10 weeks

- So, the weekly cost of the probe conditioning solution is:

  100$/10week=10$ per week

- An average of 1,050 tests per week, the maintenance cost per test is:
  10$/1050 tests =0.0095 $

Table 7: Maintenance cost

Supply	Number of days/ weeks	Total cost	Cost per day or cost per week	Number of tests per day or per week	Cost per test
Washing solution daily	67 days	105$	1.57$	150	0.01$
Probe conditioning for weekly maintenance	10 weeks	100$	10$	1050	0.0095

4.8 Data Quality Assurance

During the data collection period, data was checked by the supervisor daily for completeness, inconsistencies, and cleanness and finally, the principal investigator monitored the overall quality of data collection and handling.

4.8.1 Pre-analytical phases

Before the data collection, the data was checked by the supervisor daily for completeness, inconsistencies, and cleanness.

4.8.1 Analytical

During the data collection, the data was collected by choosing a comfortable place for the interview to allow maximum concentration and interest in the study topic. Any error found during the process will be corrected immediately.

4.8.3 Post-analytical

After completion of the data collection, the data was checked to ensure that the full questionnaire was recorded completely

4.9 Data analysis

Each completed questionnaire was checked for completeness, consistency, and it was coded and entered by using EpiData Version 3.1 software, and then it was exported to SPSS version 23.0 for analysis. Data cross-checking was also carried out before analysis. Both descriptive and analytical statistical procedures were carried out. For descriptive analysis, frequencies, mean, standard deviations, and percentages were used to describe the study population with socio-demographic and other relevant variables. For analytical analysis, a binary logistic regression model was used. The degree of association between independent and dependent variables was assessed through binary logistic regression. Variables with a P-value less than 0.25 during the bivariate analysis were considered as candidates for multivariate logistic regression analysis. Variables in the multivariate analysis model with a p-value of <0.05 with a 95% confidence interval and minimum detectable adjusted odds ratio will be considered as statistically significant determinant factors for the dependent variable. Finally, the result will be presented narratively, by tables and charts, and the Cost analysis will be computed using the ABC costing method to find the single cost of TTIs by computing all variable and fixed costs.

4.10 Ethical considerations

Before the research work was conducted, ethical clearance was obtained from the Departmental Research and Ethical Review Committee of the College of Health Science, Department of Medical Laboratory Science of Addis Ababa University ethical clearance reference number was Ref.no.MLS/177/24. A formal letter was submitted to Shashemane Blood Bank and Wolaita Sodo Blood Bank. Consent was signed before sample Collection, and the participants' information was used only for study purposes. Respondents were not identified by name, and the participant had the right to discontinue the participation.

4.11 Dissemination of Results

After the completion of the study, the findings were presented and submitted to the Addis Ababa University Department of Medical Laboratory Science as a partial fulfillment of the Master of Science in Laboratory Management and Quality Assurance. It is also planned to communicate with Shashemane Blood Bank, Wolaita Sodo Blood Bank, and any concerned bodies with documentation. It could serve as a reference material to researchers, experts, and policymakers for intervention

4.12 Operational Definitions

Original cost: The original costs are the costs at which the equipment is purchased

Sequential strategy: The tests start with the most prevalent infection. If the result is reactive, the sample is discarded. If not, it goes to the next prevalent TTI. Tests performed series way

Simultaneous strategy: all TTIs screening in a parallel way.

Self jobs: are job that travels from one city to another, which include merchants and car drivers

5. Result

5.1 Socio-demographic characteristics

In this study, 568 voluntary blood donors were approached to participate and fill out the questionnaire, and the positive results were obtained from the Hawassa blood bank. Of the 568 voluntary blood donors, 57.6% (n=327/568) were males. The mean age of the study participants with standard deviation was 23.18±6.97 years (range 18-54 years). Most study subjects, 66.2 % (n=376/568), were found within 18-24 years of age. Regarding marital status, 77.3 %(n=439/568) of the participants were never married, 85.9% (n=488/568) were lived in urban, regarding to educational status 55.8% (n=317/568) were secondary education level and majority 60.2 %(n= 342/568) of the study participants were students (Table 8).

Table 8: Socio-demographic characteristics of voluntary blood donors in Shashemane blood bank and Wolaita Sodo blood bank, Southern Ethiopia, March-May 2024

Characteristics	Category	Frequency	Percent
Sex	Male	327	57.6
	Female	241	42.4
Age	18 – 24 years	376	66.2
	25 – 34 years	146	25.7
	35 – 44 years	36	6.3
	45 – 54 years	10	1.8
Marital status	Single	439	77.3
	Married	105	18.5
	Divorced	16	2.8
	Widowed	8	1.4
Residence	Urban	488	85.9
	Rural	80	14.1
Educational Status	No formal education	21	3.7
	Primary education	43	7.6
	Secondary Education	317	55.8
	College and above	187	32.9
Occupation	Government worker	88	15.5
	Private company employee	64	11.3
	Self-job	48	8.4
	Student	342	60.2
	Unemployed	8	1.4
	Others	18	3.2

5.2 Behavioral and other associated factors of the study participant

Out of the total participants, 147 (25.9%) had either ear, nose or any other piercing on their body, 51 (9.0%) had a tattoo, 119 (21.0%), and 35 (6.2%) had shared sharp materials like razor for shaving and shares tooth brushes, respectively. Of total participant 176 (31.0%) had multiple sexual partners, 15(2.6%) had previous surgery, 337 (59.3%) had first time blood donors, 51 (9.0%) had family history of HIV/HBV, 57 (10.0%) had direct contact with the blood or open sores of an infected person (Table 9).

Table 9: Behavioral and other associated factors of blood donors in Shashemane blood bank and Wolaita Sodo blood bank, Southern Ethiopia, March-May 2024

Characteristics	Frequency	Percentage
Ear piercing, nose piercing, or any Body piercing	147	25.9
Tattooing	51	9.0
Sharing sharp materials like razors for shaving	119	21.0
Sharing a toothbrush with another person	35	6.2
Having multiple sexual partners in life	176	31.0
History of previous surgery	15	2.6
First-time blood donation	337	59.3
Multiple-time blood donation	231	40.7
Family history of HIV/HBV	51	9.0
History of direct contact with the blood or open Sores of an infected person	57	10.0
History of sharing needles, syringes, or other drug injection equipment's	41	7.2
Vaccination history of hepatitis B	69	12.1
Losing more than 10 kg of weight within 6 months	39	6.9

5.3 Sero-Prevalence of Transfusion Transmissible Infections

The total number of 568 blood units collected from 34 donors was positive for any of the TTI tested, giving an overall prevalence rate of 6.00% (34). Hepatitis B virus was the most prevalent of the TTIs, which is 3.2% (18) among blood donors. The Seroprevalence of HBV, HCV, Syphilis, and HIV was 18(3.2%), 7(1.2%), 6(1.1%), and 3(0.5%), respectively (Figure 1).

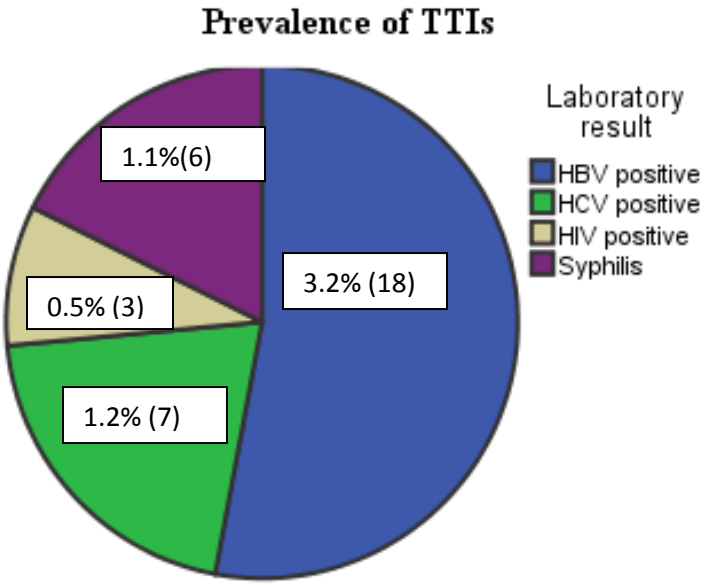

Figure 1: Sero-prevalence of HBV, HCV, HIV, and Syphilis among voluntary blood donors at Shashemane blood bank and Wolaita Sodo blood bank, 2024

Among those who have the TTIs, 76.5% (26) were males, 70.6% (24) were the age group between 18-24 years interval,79.4%(27) were single or never married, 85.3% (29) were lived in urban,52.9% (18) were students, 44.1% (15) were secondary education students and according to occupation 52.9% (18) were students (Table 10).

Table 10: Socio-demographic characteristics of Seroprevalence of transfusion transmissible infections among those who have TTIs in Shashemane blood bank and Wolaita Sodo blood bank, Southern Ethiopia, March-May 2024

Characteristics	Category	Positive for TTIs, % (n)
Sex	Male	76.5% (26)
	Female	23.5% (8)
Age	18 – 24 years	70.6% (24)
	25 – 34 years	23.5% (8)
	35 – 44 years	5.9% (2)
Marital status	Single	79.4% (27)
	Married	17.7% (6)
	Divorced	2.9% (1)
Occupation	Government worker	20.6% (7)
	Private company employee	8.9% (3)
	Self-job	11.8% (4)
	Student	52.9% (18)
	Unemployed	2.9% (1)
	Others	2.9% (1)

Among those who have the TTIs,17.6% had history of ear piercing, 7.8% had history of tattooing, 35.3% had history of sharing sharp materials like razor, 5.9% had history of sharing tooth brush, 55.9% had history of multiple sexual partners, 2.9% had history of previous surgery, 64.7% are first time donors, 11.8 had history of sharing needles, syringes, or other drug injection equipments, 20.6% had family history of HBV/HIV, 17.6% had history of direct contact with the blood or open sores of an infected person, 2.9% had Vaccination history of hepatitis B, 5.9% had Loose more than 10 KG weight with in 6 months (Table 11).

Table 11: Behavioral and other associated factors of Seroprevalence of transfusion transmissible infections among those who have TTIs in Shashemane blood bank and Wolaita Sodo blood bank, Southern Ethiopia, March-May 2024

Characteristics	Category	Positive for TTIs, % (n)
History of ear piercing, nose piercing, or any Body piercing	Yes	17.6% (6)
	No	82.4% (28)
History of tattooing	Yes	7.8% (4)
	No	88.2% (30)
History of sharing sharp materials like razors for shaving	Yes	35.3% (12)
	No	64.7%(22)
History of sharing a toothbrush with another person	Yes	5.9% (2)
	No	94.1% (32)
History of having multiple sexual partners in life	Yes	55.9% (19)
	No	44.1% (15)
History of previous surgery	Yes	2.9% (1)
	No	97.1% (33)
History of blood donation	Yes	35.3% (12)
	No	64.7% (22)
History of sharing needles, syringes, or other drug injection equipment	Yes	11.8% (4)
	No	88.2% (30)
Family history of HIV/HBV	Yes	20.6% (7)
	No	79.4 % (27)
History of direct contact with the blood or open Sores of an infected person	Yes	17.6% (6)
	No	82.4% (28)
Vaccination history of hepatitis B	Yes	2.9% (1)
	No	97.1% (33)
Losing more than 10 kg of weight within 6 months	Yes	5.9% (2)
	No	94.1 (320)

5.4 Factors associated with Transfusion Transmissible Infections

In the bivariate analysis, variables such as Sex of blood donors, History of sharing sharp materials, History of having multiple sexual partners, Family history of HIV or HBV, History of direct contact with blood or open sore of infected person, Vaccination of HBV, were significantly associated with TTIs (Table 11).

In multivariate analysis, variables such as the Sex of blood donors, History of having multiple sexual partners, History of direct contact with blood or open sore of an infected person, Vaccination of HBV, were significantly associated with TTIs.

In this study, several factors were found to be positively associated with the seroprevalence of transfusion-transmissible infections (TTIs). Males were significantly more likely to be affected by TTIs compared to females. Female participants were 65.1% less likely to be seropositive (AOR = 0.349; 95% CI: 0.150–0.814; p = 0.015), indicating that being male was positively associated with a higher likelihood of TTI infection.

People with a history of sharing sharp materials were 1.821 times more likely to be affected by TTIs than those with no history of sharing sharp materials (AOR = 1.821; 95% CI: 0.846–3.923; p = 0.126), suggesting a positive association, although it was not statistically significant.

Participants who had a history of having multiple sexual partners were 2.692 times more likely to be affected by TTIs compared to those with no such history (AOR = 2.692; 95% CI: 1.302–5.566; p = 0.008), indicating a strong and statistically significant positive association.

Individuals with a family history of HIV or HBV were 2.061 times more likely to be affected by TTIs than those without such a family history (AOR = 2.061; 95% CI: 0.754–5.634; p = 0.159), showing a positive association, though not statistically significant.

Participants who had a history of direct contact with blood or open sores of an infected person were 3.420 times more likely to be affected by TTIs compared to those without such exposure (AOR = 3.420; 95% CI: 1.066–10.971; p = 0.039), indicating a strong and statistically significant positive association.

Individuals who were not vaccinated for hepatitis B were 11.745 times more likely to be affected by TTIs than those who were vaccinated (AOR = 11.745; 95% CI: 1.332–103.583; p = 0.027), indicating a very strong and statistically significant positive association between lack of vaccination and increased TTI infection (Table 12).

Table 12: Simple (bivariate) and multiple (multivariable) analysis of factors associated with TTIs among voluntary blood donors in Shashemane blood bank and Wolaita Sodo blood bank, Southern Ethiopia, March-May 2024.

Variable	Category	Seroprevalence of TTIs		COR (CI, 95%)	p-value	AOR (CI, 95%)	p-value
		Positive (%)	Negative (%)				
Sex	Male	26(8.0%)	301(92.0%)	1		1	
	Female	8(3.3%)	233(96.7%)	0.397(0.177,0.89)	0.026	0.349(0.150,0.814)	0.015**
Sharing sharp materials	Yes	12(10.1%)	107(89.9%)	2.177(1.044,4.54)	0.038	1.821(0.846,3.923)	0.126
	No	22(4.9%)	427(95.1%)	1		1	
Having multiple sexual partners	Yes	19(10.8%)	157(89.2%)	3.042(1.51,6.14)	0.002	2.69(1.302,5.566	0.008**
	No	15(3.8%)	377(96.2)	1		1	
Family history of HIV or HBV	Yes	7(13.7%)	44(86.3%)	2.887(1.19,7.01)	0.019	2.06(0.754,5.634)	0.159
	No	27(5.2%)	490(94.8%)	1		1	
direct contact with blood or an open sore of infected person	Yes	6(10.5%)	51(89.5%)	2.029(0.80,5.13)	0.135	3.42(1.066,10.971)	0.039**
	No	28(5.5%)	483(93.5%)	1		1	
Vaccination of HBV	Yes	1(1.4%)	68(98.6%)	1		1	
	No	33(6.6%)	466(93.3%)	4.815(0.65,35.78)	0.125	11.745(1.33,103.6)	0.027**

** There is a statistically significant association between the variables & the Prevalence of TTIs

5.5 Cost of laboratory for tests

The estimated book value, or current cost, of the facility was $38,130.46. By dividing the book value by the total number of samples taken in the number of days in the year to get the cost per sample, it came out to be 0.71 for each sample. The cost of the building for each of the four TTI tests was 0.17 because the building costs are split among all tests, which are HBV, HCV, HIV,

and Syphilis. Other fixed costs of equipment were calculated based on the test utilization, and all of the TTI tests use the equipment equally, so the cost was distributed among the four TTIs. Since all of the equipment was used in the TTI analysis in the same amount, the cost of all of the equipment was determined to be $0.27142 per sample (Table 13).

Table 13: Cost per test of fixed assets

Name of the asset	Book value In $	No of days per year	No of tests per day	Cost per sample in $	No of tests used it	Cost per test in $
Building	38,130.46	360	150	0.71	4	0.18
Automated analyzer	58,076.22	360	150	1.075	4	0.27
Centrifuge	306.07	360	150	0.00567	4	0.00141
Refrigerator	46.78	360	150	0.00087	4	0.00022

The indirect labor cost like salary paid for the CEO, salary of the cleaner, salary of the security was distributed to all TTI and found to be $0.01525 other direct labor cost such as salary of laboratory of technologist, salary of laboratory technician for the TTI was calculated to be $ 0.017375 and Utility expense for TTI was $0.145. The cost of materials used for all TTI was $0.1293. Variable cost of each test was found to be $1.2048 HIV, $1.1343 HBV, $2.2045 HCV, and $1.0648 Syphilis. So, the cost per test for each TTI is calculated by summing up all the fixed and variable costs for each TTI and was $1.9631 for HIV, $1.8926HBV, $2.9628 for HCV, and $1.8231Syphilis. During the study period, $1 was converted to about 115.77 Ethiopian Birr; hence, all study expenses were reported using $ (Table 14).

Table 14: Total cost per test of all TTI

Test	Building cost in $	Equipment cost in $	Indirect labor costs $	Direct labor costs $	Utility expense in $	Material cost in $	Variable cost in $	Total cost in $
HIV	0.18	0.27142	0.01525	0.017375	0.145	0.1293	1.2048	1.9631
HBV	0.18	0.27142	0.01525	0.017375	0.145	0.1293	1.1343	1.8926
HCV	0.18	0.27142	0.01525	0.017375	0.145	0.1293	2.2045	2.9628
Syphilis	0.18	0.27141	0.01525	0.017375	0.145	0.1293	1.0648	1.8231

5.6 Cost analysis using simultaneous screening strategy and newly sequential testing strategy

The current simultaneous testing strategy tests all TTIs (HIV, HBV, HCV, Syphilis) at once or in parallel. Using a simultaneous screening strategy for all tests (HIV, HBV, HCV, and syphilis) has been depicted in Figure 2. A new sequential testing strategy tests one blood-borne infectious agent after the other. The four TTIs are tested in the following order, which starts from the most prevalent TTIs: HBV, HCV, syphilis, and finally HIV. Only blood units found negative for HBV were tested for HCV, then only those that were negative for HCV were screened for syphilis, and finally, only those that were negative for syphilis were assessed for HIV (Figure 2).

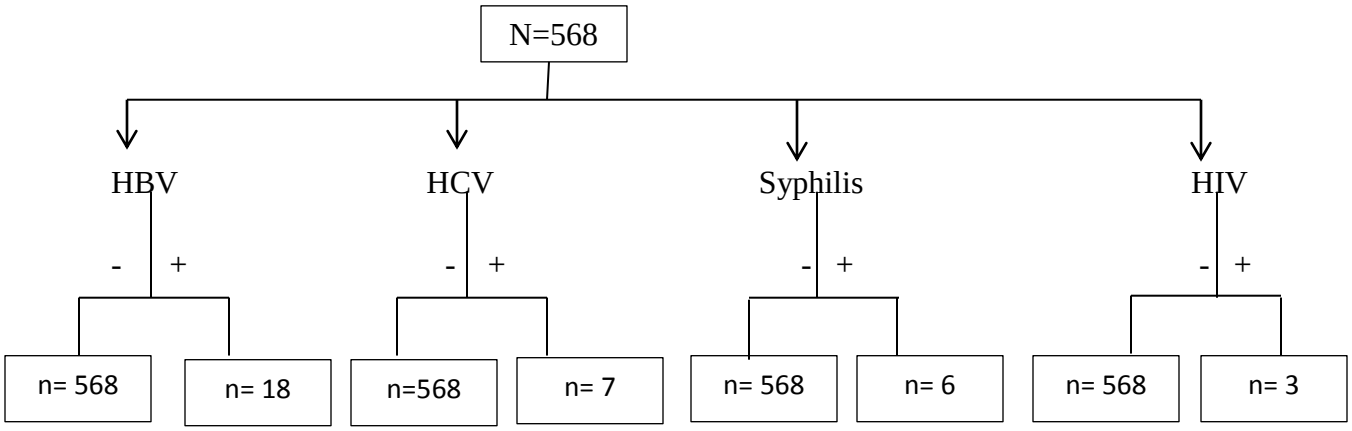

Figure 2: Results of the parallel or simultaneous strategies for the HBV, HCV, syphilis, and HIV screening performed from 568 blood donations

In a parallel testing strategy, all 568 blood samples were simultaneously tested for each of the four transfusion-transmissible infections (TTIs): hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and syphilis (Figure 3).

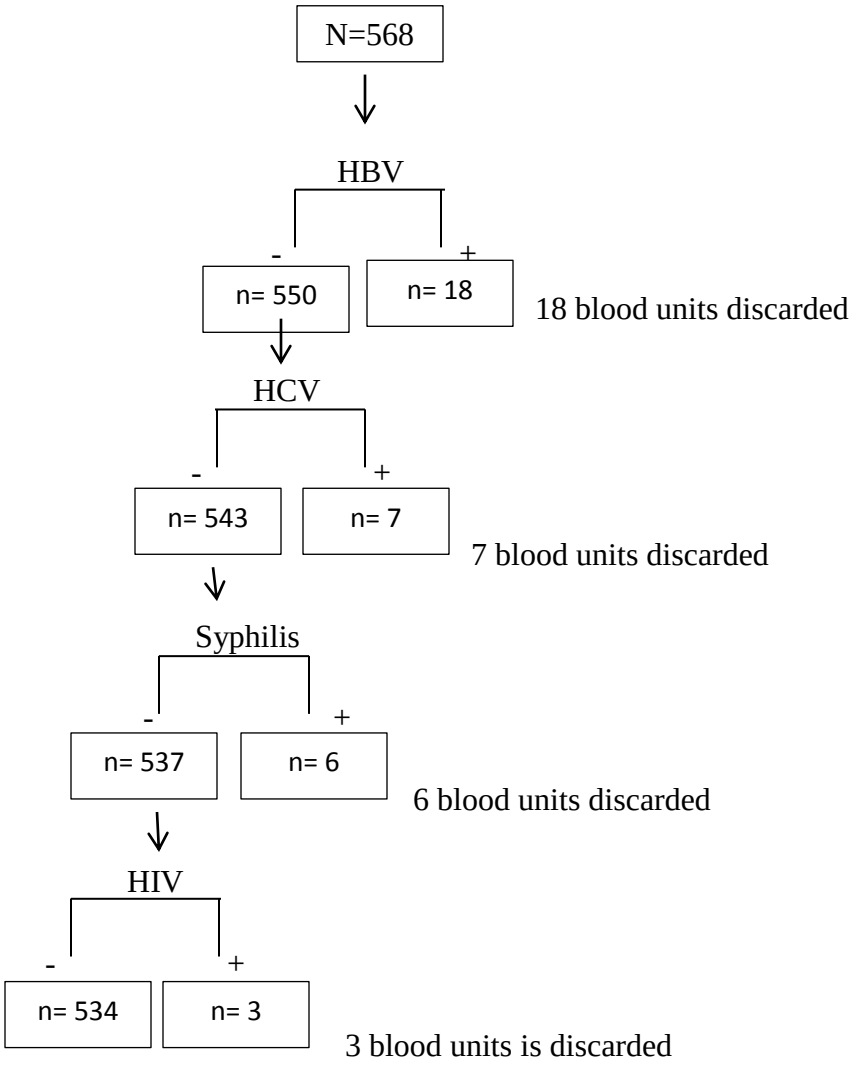

Figure 3: Results of the serious or sequential strategies for the HBV, HCV, syphilis, and HIV screening performed from 568blood donations

We tested all 568 samples for HBV, as it was the most prevalent infection according to our study (Table 15). Out of these, 18 samples tested positive for HBV and were therefore discarded,

leaving 550 samples. We then tested these 550 samples for HCV, the second most prevalent infection. Seven samples tested positive for HCV and were discarded, leaving 543 samples. Next, we tested the remaining 543 samples for syphilis and identified 6 positive cases, which were also discarded. This left us with 534 samples, which we then tested for the least prevalent infection was HIV (Table 16).

Table 15: Simultaneous blood testing cost

TTIs	**Unit cost $**	**Number of samples tested**	**Total cost**
HBV	1.8926	568	1,075
HCV	2.9628	568	2,682.87
Syphilis	1.8231	568	1,035.52
HIV	1.9631	568	1,115.04
Total			**5,908.43**

Table 16: Sequential Blood Testing Cost

TTIs	**Unit cost $**	**Number of samples tested**	**Total cost**
HBV	1.8926	568	1,075
HCV	2.9628	550	1,629.54
Syphilis	1.8231	543	989.94
HIV	1.9631	534	1,048.29
Total			**4,724.77**

As indicated in Tables 15 and 16, the cost of the simultaneous strategy was higher than that obtained with the sequential strategy. From a total of 568 blood units. The simultaneous testing strategy for detecting Transfusion Transmissible Infections (TTIs) comes with a total cost of $5,908.43, whereas the sequential strategy is costed as $4,724.77. This reveals a cost differential of approximately $1,183.66 between the two approaches.

6. Discussion

Blood transfusion is undergoing a significant transformation in response to the growing variety of transfusion-transmissible infections (TTIs). The increasing diversity of these infections presents a substantial challenge, especially when considering the inevitable impact on both the complexity of screening processes and the associated costs. This issue is particularly pronounced in resource-limited areas, such as those in sub-Saharan Africa, where the financial and logistical burdens of ensuring safe blood transfusions are already considerable (20).

In this study, Moreover, there was a significant involvement of students in voluntary blood donation programs, indicating a positive engagement from a younger age. TTIs are more prevalent among individuals aged 18-24, as well as males and those who are single. These patterns could stem from various factors such as lifestyle choices, lack of awareness about safe practices, or limited access to healthcare services. Addressing these vulnerabilities may require targeted interventions including education campaigns, increased access to testing and treatment, and support for safer behaviors.

The seroprevalence of TTIs in male blood donors was high in our study compared to the study conducted in North East Ethiopia, and lower in male donors in the study conducted in North West Ethiopia. Additionally, there is variation in occupational status in our study; students are more prone to acquiring TTIs compared to other occupations, while a study in North East Ethiopia showed that students were less susceptible compared to other occupations. Another study in North West Ethiopia similarly found that students are more susceptible than others, similar to our findings (22,25)

In the current study, from the total of 568 blood donors, the overall seroprevalence of transfusion transmissible infections was 6.00% which is almost similar to a study done in Pakistan (5.8%), a study done in North East Ethiopia (6.25%) (14,25). But the result of overall prevalence transfusion transmissible infections is lower than study done Nigeria (19.3%), the study done in North West Ethiopia (9.5%), the study done in Wolaita Sodo (29.5%), the study in Dire Dawa Ethiopia (7.06%) (41, 22, 45, 18). The observed differences in the overall prevalence of transfusion-transmitted infections (TTIs) across multiple research studies may be attributed to several factors. These include variations in the size of the sample population, the demographic

composition of the study participants, the effectiveness of initial donor screening procedures, the methodology utilized in the research, and the duration over which the study was conducted. Each of these elements could potentially influence the accuracy and representativeness of the findings regarding TTI prevalence (41).

However, the present study showed the overall prevalence of TTI was higher than compared with a study done in East Africa in Eritrea (3.8%), and (20). The finding of the current study was higher around the world, which is the study done in Pakistan (3.53%) and the study done in Iran (0.254%) (1, 28). The variation in seroprevalence rates observed among different studies could stem from a range of factors. These may include differences in socio-demographic profiles and economic conditions of the populations studied, as well as variations in societal and cultural behaviors and risk factors related to transfusion-transmitted infections (TTIs). Furthermore, levels of awareness within the population regarding TTIs and associated preventive measures could also contribute to these differences. Additionally, the efficacy of initial screening protocols for blood donors and the diagnostic techniques employed for screening purposes may also play a role in influencing the observed prevalence rates.

In our study, we observed prevalence rates of 3.2% for HBV, 1.2% for HCV, 1.1% for syphilis, and 0.5% for HIV among blood donors. These figures indicate a higher prevalence compared to the seroprevalence rates reported for the general population of blood donors in Eritrea, which stand at 0.18% for HIV, 2.58% for HBV, 0.57% for HCV, and 0.49% for syphilis. This discrepancy suggests that our study population exhibits elevated rates of all TTI markers in comparison to the broader blood donor population in Eritrea (20).

The comprehensive seroprevalence rates for HIV, HBV, and syphilis among blood donors at Gondar University Teaching Hospital were 3.8%, 4.7%, and 1.3%, respectively. A comparison between this study and ours reveals lower prevalence rates for HBV, HIV, and syphilis from Gondar (22).

In this study, voluntary blood donors who had multiple sexual partners were significantly associated with TTIs. This finding is consistent with studies conducted in the Eastern Ethiopia blood bank (44), the North East Ethiopia blood bank (25), and Kenya (43). Transfusion-transmitted infections can also be transmitted through sexual contact, so it's not surprising that

higher levels of sexual activity are linked to a greater prevalence of these infections. In Africa, the primary way of acquiring sexually transmitted infections is through sexual activity, making having multiple sexual partners a major risk factor for acquiring sexually transmitted infections as well.

Currently, TTI's screening at our study area indicates that all TTI tests are conducted concurrently or simultaneously. This screening strategy involves the simultaneous assessment of multiple TTI markers. During our investigation, a total of 568 blood units undergo simultaneous testing for transfusion-transmitted infections (TTIs), ensuring efficient screening. The expenditure incurred for this parallel screening process amounted to $4,720.08.

If we were to substitute the existing simultaneous testing strategy with the newly suggested sequential algorithm for testing transfusion-transmitted infections (TTIs), the projected cost for conducting TTI screening would amount to $4,724.77. This represents a variance of $1,183.66 compared to the cost incurred for screening 568 blood units under the current methodology. The sequential algorithm entails a step-by-step approach to TTI testing, potentially optimizing resource utilization and cost-effectiveness in the screening process.

The annual accomplishments across both study regions signify a total of 13,500 blood units. If implementing the innovative sequential testing algorithm for transfusion-transmitted infections (TTIs), it is projected that we could realize an annual cost reduction amounting to approximately $28,132.76, which is 3,256,929.62Birr according to current exchange. This assumption is founded on the expectation that the sequential testing methodology would enhance efficiency and streamline resource allocation within the TTI screening process, ultimately leading to substantial financial savings on an annual basis.

A study conducted in Burkina Faso was observed that the cost associated with the simultaneous strategy exceeded that of the sequential strategy. In particular, the sequential method led to an overall cost reduction of €908.7 for 500 blood units tested. Extrapolating this result to the total number of donations in Burkina Faso (around 50,000 donations/year), potential annual savings of €90,870 per year could be achieved by using the sequential testing strategy, compared to the simultaneous strategy (24).

An additional study carried out in Ethiopia analyzed a total of 173,207 blood units. The total cost of implementing the parallel strategy was 18,224,087.12 birr, while the total estimated cost using the newly developed algorithm was 17,477,313.22 birr. This resulted in a cost difference of 746,773.90 ETB between the two strategies, demonstrating that the sequential testing algorithm is more economically advantageous compared to the parallel strategy (42).

7. Limitations and Strengths of the study

7.1 Strength of the study.

- The study used the Activity-Based Costing (ABC) method for cost analysis in testing TTIs such as HBV, HCV, HIV, and Syphilis. ABC costing is effective because it traces all activities involved in the testing process, offering a more accurate understanding o f where costs are incurred.
- The study was conducted by prospectively collecting blood donors' data, but a different type of study conducted in Ethiopia was utilized secondary, and it avoids incompleteness and increases data accuracy.

7.2 Limitation of the study

- These results may, however, not exactly reflect the prevalence of the blood donor population due to the donor selection processes involved. There was no follow-up on each donor. This could have led to a slight overestimate

8. Conclusion and recommendation

8.1 Conclusion

Overall, the magnitude of transfusion-transmitted infections (TTIs) such as HBV, HCV, Syphilis, and HIV was higher in the study area compared to other regions of Ethiopia. It's worth noting that the blood donors involved in this study were individuals who willingly donated blood and were seemingly healthy. However, the findings revealed a concerning prevalence of these diseases among voluntary donors.

The cost analysis utilizing Activity-Based Costing (ABC) for the current parallel testing strategy was found to be greater than that of the newly devised sequential testing algorithm. This suggests that the newly proposed testing method may offer a more cost-effective solution compared to the existing parallel approach.

8.2 Recommendation

Priority attention should be directed towards addressing HBV and the other TTI infections within the Shashemane and Wolaita Sodo. This entails advocating for comprehensive safe sex education within educational institutions, raising awareness among the population, especially among single males aged 18-24, regarding transmission methods, and encouraging early adoption of HBV screening tests followed by vaccination. Such measures are imperative for minimizing the burden of transfusion-transmitted infections (TTIs) among blood donors.

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Annexes

Annex-I Information sheet

Introduction:

Hello; my name is Bilen Asrat I am MSC student at Addis Ababa university health since college medical laboratory department. I am doing a research entitled the prevalence of transfusion transmissible viral infection and its cost analysis Among Voluntary Blood Donors at shashemane Blood Bank and Wolaita Sodo Blood Bank. I'm going ask you to participate in this research project. You can decide whether or not to take part in the study.

Aim of the study:

The main objective of the study is to determine the prevalence of transfusion transmissible infection and its cost analysis among voluntary blood donors at shashemane and Wolaita Sodo Blood Banks.

Risk associated with the study:

You will not be at any physical or psychological risk and should experience no discomfort resulting from the research procedures

Confidentiality of your information:

I would like to indicate that your name will not be written in the form and I assure you all the information you give will be kept strictly confidential. The researchers conducting this study will review your records and follow the progress of the research, but nothing that can be used to identify you will be used in reports of this study. You have the Right to Refuse or withdraw at any time.

Person to contact: If you have any question you can contact the principal investigator

Principal investigator Bilen Asrat

Email:
Tel: +251918698365

Annex-II Consent Form

Here the undersigned agree on terms and the conditions and give my consent to participate in the study to assess the prevalence of transfusion transmissible viral infection and its cost analysis Among Voluntary Blood Donors at shashemane Blood bank and Wolaita Sodo Blood Bank. I understand that my participation is voluntary and there is no serious procedure that harms.

Do you agree to answer the following questions to the best of your ability?

Yes_____ No_____

Thank you for participation in the study!

Participant's signature: _____ Date: _____

Participant's Code _____

Annex-III English questioner

Data Collection Tools (Questionnaire)

Interviewer Name _____place of interview_____

Date of Interview_____ study Code number_____

A. Donor's Socio- Demographic Characteristics

No	Question	Respondent answer
1	Age	_____ In a year
2	Sex	1. Male 2. Female
3	Marital status	1. Single 2. Married 3. Other (Divorced, widowed)
4	Religion	1. Orthodox 2. Muslim 3. Protestant 4. Catholic 5. Other
5	Residence	1. Urban 2. Rural
6	Occupation	1. Government worker 2. Private company employee 3. Self-job 4. Student 5. Unemployed 6. Other_____
7	Education status	1. No form education 4. College and above 2. Primary 3. Secondary

B. Donor's behavioral and other factors associated with TTIs

No	Question	Respondent Answer
1	History of ear piercing, nose piercing or any Body piercing	1. Yes 2. No
2	History of a tattooing	1. Yes 2. No
3	History of sharing sharp materials	1. Yes 2. No
4	History of sharing toothbrush	1. Yes 2. No
5	History of having multiple sexual partner life	1. Yes 2. No
6	If yes in number 4 , how many sexual partner in life	1. two 3 more than three 2 three
7	History of previous surgery	1. Yes 2. No
8	History of Tissue (Cornea) or Organ transplant	1. Yes 2. No
9	History of blood donation	1. Yes 2. No
10	History of sharing needles, syringes, or other drug injection equipments	1. Yes 2. No
11	Family history of HIV/HBV	1. Yes 2. No
12	History of direct contact with the blood or open sores of an infected person	1. Yes 2. No
13	Vaccination history of hepatitis B	1. Yes 2. No
14	Have you lost more than 10kg weight in the last 6 months?	1. Yes 2. No

Annex-IV Amharic questioner

ጥያቄ መጠይቅ ዝርዝር

የጠያቂው ስም_____ የቃለ መጠይቁ ቦታ_____

የቃለ መጠይቁ ቀን_____ የተቹሙ መለያ ቁጥር_____

ሀ. የለጋሽ የስነ ህዝብ መረጃ መጠይቅ

ቁጥር	**ጥያቄ**	**የምላሽ ሰጪ መልስ ኮድ** _____
1	ዕድሜ	_____
2	ጸታ	1.ወንድ 2.ሴት
3	የጋብቻ ሁኔታ	1.ያላገባ /ች 2.ያገባ /ች 3.ሌላ (የተፋታ/ች፣ ባሏ የሞተባት፣ሚስቱ የሞተችበት)
4	ሃይማኖት	1.አርቶዶክስ 2.ሙስሊም 3.ፕሮቴስታንት 4.ካቶሊክ 5.ሌሎች
5	መኖሪያ	1.ከተማ 2.ገጠር
6	ሥራ	1.የመንግስት ሰራተኛ 2. የግል ድርጅት ሰራተኛ 3. የግል ስራ 4.ተማሪ 5.ስራ አልባ 6.ሌሎች
7	የትምህርት ሁኔታ	1. መደበ ት/ትያልተማረ/ች 2. የመጀመሪያ ደረጃ ት/ት 3.ሁለተኛ ደረጃ ት/ት 4.ኮሊጅ እና ከዛ በላዩ

ለ. የለጋሾች ባህሪ እና ሌሎች ከቲቲአይ ጋር የተያያዙ መጠይቆች

ቁጥር	ጥያቄ	የምላሽ ሰጪ መልስ
1	ጆሮ ፣ አፍንጫ ወይም ማንኛውም አካል ተበስተው ያዉቃሉ	1.አዎ 2.አይደለም
2	ንቅሳት አለብዎት	1.አዎ 2.አይደለም
3	እንደ ምላጭ ያሉ ስለታም ቁሳቁሶችን ከሌሎች ሰዎች ጋር በጋራ ተጠቅመዉ ያዉቃሉ	1.አዎ 2.አይደለም
4	የጥርስ ብሩሽን ከሌላ ሰው ጋር በጋራ ተጠቅመዉ ያዉቃሉ	1.አዎ 2.አይደለም
5	በህይወትዎ ውስጥ ብዙ የፍቅር ጓደኛ ኖሮት ያዉቃል	1.አዎ 2.አይደለም
6	ጥያቄ ቁጥር 5 አዎ ከሆነ ከስንተ ሰዉ ጋር	1.ሁለት 2.ሶስት 3.ከሶስት በላይ
7	ቀዶ ጥገና አርገዉ ያቃሉ	1.አዎ 2.አይደለም
8	የቲሹ (ኮርኒያ) ወይም የአካል ክፍል ንቅለ ተከላ አርገዉ ያዉቃሉ	1.አዎ 2.አይደለም
9	ደም ለግሰዉ ያዉቃሉ	1.አዎ 2.አይደለም
10	መርፌ፣ ሲሪንጅ ወይም ሌላ የመድኃኒት መዉጊያ መሣሪያዎችን ተጋርተዉ ያዉቃሉ	1.አዎ 2.አይደለም
11	እድሜ ማራዘሚያ የሚጠቀም ወይም ጉበት በሽታ ያለበት የቤተሰብ አባል አለ	1.አዎ 2.አይደለም
12	ከታመመ ሰው ቁስሎች ወይም ደም ጋር ቀጥተኛ ግንኙነት ኖሮዎት ያዉቃል	1.አዎ 2.አይደለም
14	ባለፉት 6 ወራት ከ10 ኪሎ ግራም በላይ ክብደት ቀንሰዋል	1.አዎ 2.አይደለም

Annex-V Afan oromo questionnaire
Maqaa Gaafataa _____ bakka gaaffii_____

Guyyaa Gaaffiin godhame_____ Lakkoofsa koodii qorannoo_____

Lakkoofsa koodii baankii dhiigaa_____

A. **<u>Gaaffii Odeeffannoo Dimogiraafii Arjoomtootaa</u>**

Lakk.	**Gaaffilee**	**Deebii**
	Umurii	_____Waggaadhaan
	Saala	1. Dhiira 2. Dhalaa
	Haala Gaa'elaa	1. Qeenxee 2. Kan fuudhe/heerumte 3. Kan biroo (adda bahe/kan irra du'e)
	Amantaa	1. Ortodoksii 2. Musliima 3. Pirotestaantii 4. Kaatoolikii 5. Kan biro
	Mana jireenyaa	1. Magaalaa 2. Baadiyyaa
	Hojii	1. Hojjetaa mootummaa 2. Hojjetaa dhaabbata dhuunfaa 3. Daldalaa 4. Barataa 5. Hojii dhabeeyyii 6. Kan biro
	Haala barnootaa	1. Dubbisuu fi barreessuu 2. Dubbisuu fi barreessuu kan hin dandeenye

B. Amala arjoomtootaa fi gochoota biroo TTI waliin walqabatan

Lakk.	Gaaffilee	Deebii
1	Gurra, funyaan ykn kutaan qaamaa kamiyyuu ni hurrattee beektaa?	1. Eeyee 2. Lakkii
2	Tattoo qaama irra qabdaa?	1. Eeyee 2. Lakkii
3	Wantoota qara qaban kan akka billaa/murtuu areedahaadachuuf itti fayyadaman ni beektaa?	1. Eeyee 2. Lakkii
4	Buraashiin ilkaanii nama biraa waliin itti fayyadaman ni beektaa?	1. Eeyee 2. Lakkii
5	Jireenya kee keessatti hiriyoota ykn jaalalle lamaa oli ni qabdaa?	1. Eeyee 2. Lakkii
6	Gaaffii lakkoofsa 5 Yoo eeyyee ta'e, namoota meeqa?	1. Lama 2. Sadii 3. Sadii ol
7	Baqaqsanii yaaluun siif raawwatame beektaa?	1. Eeyee 2. Lakkii
8	Tishuu (korniyaa) ykn qaama namaa bakka buusuun siif hojjettamee jiraa?	1. Eeyee 2. Lakkii
9	Kana dura dhiiga arjoomtee beektaa?	1. Eeyee 2. Lakkii
10	Meeshaalee akka billaa(murtuu), marfee qoricha ittiin fudhatan nama biro waliin fayyadamte beektaa?	1. Eeyee 2. Lakkii
11	Maatii keessa namni HIV/AIDS qabu jiraa?	1. Eeyee 2. Lakkii
12	Madaa ykn dhiiga nama dhukkubsatee waliin kallattiin tuttuqii goote beektaa?	1. Eeyee 2. Lakkii
13	Nama dhukkubsataa sifilisii ykn HIV ta'e waliin walqunnamtii saalaa raawwattee ni beektaa?	1. Eeyee 2. Lakkii
14	Talaallii hepatitis B fudhattee beektaa?	1. Eeyee 2. Lakkii
15	Ji'oota jahan (6) darban keessatti 10 kg ol hir'istee jirtaa?	1. Eeyee 2. Lakkii

Annex-VI National blood bank criteria

- Donor should be in age group of 18 to 65, not less than 45 Kg
- Donation should be done once in three month
- Temperature and pulse should be normal
- Hemoglobin of donor should not be less than 12 g/dl for female and 13 g/dl for male
- The systolic and diastolic blood pressures are within normal limits without medication
- Donor should be free from acute respiratory disease
- Donor should be free from hepatitis
- History of abortion should be more than 6 month
- History of transfusion should be more than 6 month
- History of surgery should be more than 12 month
- Donor should be free from history of typhoid with in 12 month
- Donor should be free from history of malaria with in 3 month
- Tattooing with in 6 months
- Free from Rabies vaccination with in 1 year after vaccination

Annex-VII : Laboratory analysis

The Alinity i system (Abbott Laboratories, IL, USA), a recently launched automated immunoassay analyzer utilizes chemiluminescent microparticle immunoassay (CMIA) principle, by using anti-analyte coated paramagnetic microparticles and anti-analyte acridinium-labeled conjugates.

Immunochemistry offers simple, rapid, robust yet sensitive, and easily automated methods for routine analyses in clinical laboratories. Immunoassays are based on highly specific binding between an antigen and an antibody. An epitope (immunodeterminant region) on the antigen surface is recognized by the antibody's binding site. The type of antibody and its affinity and avidity for the antigen determines assay sensitivity and specificity. Depending on the assay format, immunoassays can be qualitative or quantitative. They can be used for the detection of antibodies or antigens specific for bacterial, viral, and parasitic diseases as well as for the diagnosis of autoimmune diseases. Immunoassays can measure low levels of disease biomakers and therapeutic or illicit drugs in patient's blood, serum, plasma, urine, or saliva. Immunostaining is an example of an immunochemical technique, which combined with fluorescent labels allows direct visualization of target cells and cell structures.

1. **HIV**

The ARCHITECT HIV Ag/Ab Combo assay is a chemiluminescent microparticle immunoassay (CMIA) for the simultaneous qualitative detection of HIV p24 antigen and antibodies to human immunodeficiency virus type 1 and/or type 2 (HIV-1/HIV-2) in human serum or plasma. The ARCHITECT HIV Ag/Ab Combo assay is intended to be used as an aid in the diagnosis of HIV-1/HIV-2 infection and as a screening test for donated blood and plasma. An ARCHITECT HIV Ag/Ab Combo result does not distinguish between the detection of HIV p24 antigen, HIV-1 antibody, or HIV-2 antibody.

Test principle

The ARCHITECT HIV Ag/Ab Combo assay is a two-step immunoassay to determine the presence of HIV p24 antigen and antibodies to HIV-1 (Group M and 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, assay diluent, and paramagnetic microparticles are combined. HIV p24 antigen and HIV-1/HIV-2 antibodies present in the sample bind to the 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 HIV p24 antibody [mouse, monoclonal]). Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists 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 reaction 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.

Specimens that are initially reactive in the ARCHITECT HIV Ag/Ab Combo assay should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HIV p24 antigen and HIV-1/HIV-2 antibodies. However, as with all immunoassays, the ARCHITECT HIV Ag/Ab Combo assay may yield nonspecific reactions due to other causes, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be investigated further with sensitive, supplemental HIV-specific tests, such as immunoblots, antigen tests, and HIV nucleic acid tests. Supplemental testing of repeat-reactive specimens obtained from individuals at risk for HIV infection usually confirms the presence of HIV antibodies or HIV antigen, and HIV nucleic acid. A full differential diagnostic work-up for the diagnosis of AIDS and AIDS-related conditions includes an examination of the patient's immune status and a clinical history.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT HIV Ag/Ab Combo assay is that a single sample of each control be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. The ARCHITECT HIV Ag/Ab Combo Control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated. It is recommended that each laboratory establish a control range for the ARCHITECT HIV Ag/Ab Combo Positive Control 1 when a new lot of ARCHITECT HIV Ag/Ab Combo reagents is used.

Interpretation of Results

- Specimens with S/CO values < 1.00 are considered nonreactive (NR).
- Specimens with S/CO values ≥ 1.00 are considered reactive (R).

2. HBV

The ARCHITECT HBsAg Qualitative assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma.

Test principle

The ARCHITECT HBsAg Qualitative assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. (Note: Ancillary wash buffer is added in a second incubation step, so the assay file performs a two-step assay protocol).

Sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled 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 units (RLUs). A direct 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 curve. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT HBsAg Qualitative assay is that a single sample of each control be tested once every 24 hours each day of use. If your laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures.

Interpretation of Results

Initial Result (S/CO)	Instrument Interpretation	Retest Procedure
< 1.00	NONREACTIVE	No retest required
≥ 1.00	REACTIVE	Retest in duplicate

Instrument Interpretation	Specimen Classification
Both results nonreactive	Specimen considered nonreactive for HBsAg
One or both results reactive	Specimen considered repeatedly reactive for HBsAg - Confirm by a neutralizing assay

3. HCV

The ARCHITECT Anti-HCV assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibody to hepatitis C virus (anti-HCV) in human serum and plasma.

Test principle

The ARCHITECT Anti-HCV assay is a two-step immunoassay, using chemiluminescent microparticle immunoassay (CMIA) technology, for the qualitative detection of anti-HCV in human serum and plasma. In the first step, sample, recombinant HCV antigen coated paramagnetic microparticles and Assay Diluent are combined.

Anti-HCV present in the sample binds to the HCV coated microparticles. After washing, anti-human acridinium-labeled conjugate is added in the second step. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-HCV in the sample and the RLUs detected by the ARCHITECT i* System optics.

The presence or absence of anti-HCV in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from a previous

ARCHITECT Anti-HCV calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the specimen is considered reactive for anti-HCV.

QUALITY CONTROL PROCEDURES

NOTE: It is recommended that the ARCHITECT Anti-HCV Positive Control and the Negative Control be run to verify the calibration. The recommended control requirement for the ARCHITECT Anti-HCV assay is a single sample of both ARCHITECT Anti-HCV Controls tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory specific procedures. Ensure that assay Control values are within the ranges specified in the Controls package insert.

Interpretation of Results

- Specimens with S/CO values < 1.00 are considered nonreactive by the ARCHITECT Anti-HCV assay and need not be tested further.
- Specimens with S/CO values ≥ 1.00 are considered reactive by the ARCHITECT Anti-HCV assay. • All initially reactive specimens should be retested in duplicate. If both retest values are nonreactive, the specimen must be considered nonreactive for anti-HCV. If either of the retest values is reactive, the specimen must be considered repeatedly reactive for anti-HCV by the criteria of ARCHITECT Anti-HCV.
- Repeatedly reactive anti-HCV specimens should be investigated further in supplemental tests such as other HCV specific immunoassays and immunoblot assays or a combination thereof and/or NAT tests.

4. Syphilis

The ARCHITECT Syphilis TP assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibody to Treponema pallidum (TP) in human serum and plasma on the ARCHITECT i System as an aid to diagnosis of syphilis.

The ARCHITECT Syphilis TP assay is a two-step immunoassay for the qualitative detection of antibody to TP in human serum or 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 and TpN47) and Assay Diluent are combined. Anti-TP antibodies

present in the sample bind to the TP coated microparticles. After washing, the acridinium-labeled anti-human IgG and IgM conjugate is added in the second step. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists 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 anti-TP antibodies in the specimen is determined by comparing the chemiluminescent signal in the reaction 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 specimen is considered reactive for anti-TP.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Syphilis TP assay is that a single sample of each control be tested once every 24 hours each day of use for each reagent lot. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. Ensure that assay control values are within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Interpretation of Results

- Specimens with S/CO values < 1.0 are considered nonreactive by the ARCHITECT Syphilis TP assay.
- Specimens with S/CO values ≥ 1.0 are considered reactive by the ARCHITECT Syphilis TP assay

Declaration

I, the undersigned declare that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. I also agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: **Bilen Asrat (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Abay Sisay (MSc, PhD, Assitant Professor)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor **Zemenu Tamir (MSc, PhD, Assitant Professor)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.