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Acknowledgment

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## Abbreviation

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ARPCFT</td>
<td>Automated Reiter Protein Complement Fixation Test</td>
</tr>
<tr>
<td>BFP</td>
<td>Biological False Positive</td>
</tr>
<tr>
<td>DGM</td>
<td>Dark Ground Microscopy</td>
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<tr>
<td>EIA</td>
<td>Enzyme Immuno Assay</td>
</tr>
<tr>
<td>FTA</td>
<td>Fluorescent Treponemal Antibody</td>
</tr>
<tr>
<td>FTA-ABS</td>
<td>Fluorescent Treponemal Antibody-Absorption</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C Virus</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immuno Deficiency Virus</td>
</tr>
<tr>
<td>MHA-TP</td>
<td>Microhemagglutination assay for antibody to <em>T. pallidum</em></td>
</tr>
<tr>
<td>NAT</td>
<td>Nucleic Acid Test</td>
</tr>
<tr>
<td>NBBS</td>
<td>National Blood Bank Service</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PS</td>
<td>Primary Syphilis</td>
</tr>
<tr>
<td>RPCFT</td>
<td>Reiter Protein Complement Fixation Test</td>
</tr>
<tr>
<td>RPR</td>
<td>Rapid Plasma Reagin</td>
</tr>
<tr>
<td>RSSS</td>
<td>Reverse Sequence Screening for Syphilis</td>
</tr>
<tr>
<td>RST</td>
<td>Rapid Syphilis Test</td>
</tr>
<tr>
<td>SIFA</td>
<td>Surface Immuno Fluorescence Assay</td>
</tr>
<tr>
<td>SS</td>
<td>Secondary Syphilis.</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
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<tr>
<td>STI</td>
<td>Sexually Transmitted Infection</td>
</tr>
<tr>
<td>STS</td>
<td>Serologic Test for Syphilis</td>
</tr>
<tr>
<td>TPHA</td>
<td><em>Treponemapallidum</em> hemagglutination Assay</td>
</tr>
<tr>
<td>TPI</td>
<td><em>Treponemapallidum</em> Immobilization</td>
</tr>
<tr>
<td>TPLA</td>
<td><em>Treponemapallidum</em> Latex Agglutination</td>
</tr>
<tr>
<td>TPPA</td>
<td><em>Treponemapallidum</em> Particle Agglutination</td>
</tr>
<tr>
<td>TTI</td>
<td>Transfusion Transmitted Infection</td>
</tr>
<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
</tr>
<tr>
<td>WB</td>
<td>Western Blot</td>
</tr>
</tbody>
</table>
Operational Definition

- **Sensitivity**: How accurate is the test in correctly diagnosing the presence of a condition.
- **Specificity**: How accurate is the test in correctly diagnosing the absence of a condition.
- **Positive predictive value**: How likely is someone with a positive test result to actually have the characteristic.
- **Negative predictive value**: How likely is someone with a negative test result to actually not have the characteristic.
- **Kappa value**: Measure of inter-rater agreement for categorical scales when there are two raters (where \( \kappa \) is the lower-case Greek letter 'kappa').

- **Screening**: Detect early disease or risk factors for disease in large numbers of apparently healthy individual
- **Diagnosis**: Establish the presence (or absence) of disease as a basis for treatment decisions in symptomatic or screen positive individuals (confirmatory test).
- The agreement of the results according to their \( \kappa \) values was categorized as:
  
  Perfect (0.81–1.0), Substantial (0.61–0.8), Moderate (0.41–0.6), Fair (0.21–0.4), Slight (0–0.2) and Poor (<0).

- **Traditional algorithm**  
  Non treponemal test --- Treponemal test

- **Reverse algorithm**  
  Treponemal test --- Nontreponemal test --- Treponemal test
Abstract

**Background:** Serological diagnosis of syphilis is based on demonstration of specific treponemal antibody and non treponemal antibodies (reagin) in body fluids. Accurate and reliable testing is a critical step in ensuring the diagnosis of syphilis and the safety of blood transfusion service. Performance agreement between syphilis tests is not documented well in Ethiopia.

**Objectives:** To determine agreement between Enzyme Linked Immunosorbent Assay with Rapid Plasma Reagin and *Treponemapallidum* Haemagglutination Assay for screening and diagnosis of syphilis.

**Methods:** Laboratory based Cross sectional study was carried out from January 2016 to June 2016 at National Blood Bank Service Addis Ababa, Ethiopia. All positive (One hundred and ninety) and 190 negative samples were used which stored in NBBS (National Blood Bank Service) laboratory from July 2015 to Dec 2015 (total 380 samples). Systematic random sampling method was used to select negative samples. The data was analyzed by SPSS version 20 software. The percentage agreement and κ value were compared; the sensitivity, specificity, positive predictive value and negative predictive value of the ELISA and RPR were calculated. P value less than 0.05 were taken as statistical significant.

**Results:** From 190 ELISA positive sera, 151 (80%) were confirmed as positive by TPHA, 39 (20%) were found to be false positive. 59 (39%) of samples were positive and 92 (61%) were false negative by RPR. From 190 ELISA negative sera all were negative by RPR and TPHA. The sensitivity, specificity, PPV and NPV of ELISA was 99.9%, 85%, 79% and 100% respectively. While RPR was 62%, 99%, 100% and 63% respectively. Percent agreement of ELISA with TPHA was 90% and corresponding kappa value was substantial 0.795. Percent agreement for RPR was 66% with kappa value of 0.375 which was a fair agreement. Chi-square result with p value 0.000 which is statistical significant indicate the association between categorical variables.

**Conclusion** The study demonstrated that ELISA was very sensitive. There was good agreement between ELISA and TPHA. RPR was with high specific and weak sensitive results. There was poor agreement between RPR and TPHA.
1. Introduction

1.1 Background

The etiological agent of syphilis is *Treponema pallidum*. Syphilis has diverse clinical manifestations and shares many clinical features with other treponemal and nontreponemal diseases. Therefore, it is mandatory that the clinical diagnosis is always supported by appropriate laboratory tests and that the test results are interpreted with reference to the patient's history (1).

Serodiagnosis of syphilis can be performed by two types of methods non treponemal assays, such as the Venereal Disease Research Laboratory and rapid plasma reagin assays, and treponemal assays, such as fluorescent treponemal antibody-absorption, the microhemagglutination assay for antibody to *T. pallidum* or the *T. pallidum* hemagglutination assay, and various enzyme-linked immunosorbent assays, which are typically more sensitive and yield more objective results than agglutination and fluorescence assay. The commercially available diagnostic tests for syphilis are mostly based on the use of extracted antigens of *Treponema pallidum*. Pronounced cross-reactivities with other spirochete antigens are often reported, the use of only one type of test is insufficient for an accurate diagnosis (2).

The need for efficiencies in high-volume screening as well as the need to address the work stress of pipetting large numbers of samples, many laboratories have changed their diagnostic approach and now screen using automated or semi automated treponemal antibody assays in which the blood sample is tested using an EIA. Positive samples are then tested with a quantitative non treponemal test like RPR quantitative test. If the test results disagree, the specimen is then tested using a second treponemal test. The TPPA is generally recommended for use as the confirmatory test based on published data suggesting the enhanced sensitivity and specificity of this assay. If the second treponemal test result is positive, this is generally considered to confirm a diagnosis of syphilis, but if the second treponemal test result is negative, a third treponemal test may be helpful and is generally recommended as a tie-breaker. This approach also has limitations, with an increase in the potential for false positives (3).
Currently available treponemal tests include the fluorescent treponemal antibody absorption test (FTA-ABS), *T. pallidum* haemagglutination assay (TPHA) and enzyme immunoassays (EIA). The advantages of treponemal tests include the production of objective results and the facility for automation, making them useful for large blood centers (4).

The manifestations of *Treponema pallidum* infection depend upon time, site, and the immune status of the infected individual. Time relates to the designation of the stages of syphilis as primary, secondary, and tertiary disease. These clinical stages, in turn, reflect the interaction of the infectious agent with the host, and the effects of the immune response on the infection. Site refers to whether the lesions are located in the skin or mucous membranes. Since the growth of *T. pallidum* is dependent upon temperature the internal temperature of the body is too high for optimal growth, the external surfaces are the major combat zones, in which immune effector mechanisms try to defeat large numbers of rapidly proliferating organisms. In contrast, the internal organs feature evidence of the immune response lymphadenopathy or splenomegaly during primary and secondary stages of infection; chronic, smoldering inflammation during the tertiary stage (granulomas) is reflective of an inadequate immune response to persistent infection. In addition, the tertiary stage has characteristic lesions due to nerve damage. The host’s immune status is reflected in the course and pathology of syphilis in its various stages. Of particular importance (5)

National AIDS Control Organisation recommend RPR or non-treponemal test such as Venereal Disease Research Laboratory (VDRL) test used for screening followed by a treponemal test. *Treponema pallidum* hemagglutination (TPHA) test for confirmation in VDRL reactive cases. One advantage of this approach is that it does not detect most adequately treated cases, thus simplifying patient assessment. There are, however, disadvantages with this approach. Screening undiluted specimens with a non-treponemal test alone can yield false negative reactions in the presence of high titres of antibody. Non-treponemal tests also lack sensitivity in late stage infection and screening with a non-treponemal test alone may also yield false positive reactions in various acute and chronic conditions in the absence of syphilis known as biological false-positive (BFP) reactions. A BFP reaction is generally considered as the combination of a reactive reagin, or non-treponemal, test and a nonreactive treponemal test (6).
Dark-Field Microscopy and direct fluorescence are the best ways to identify *T. pallidum* from primary or secondary lesions are the dark field illumination that shows the typical motile spirochetes. Direct immunofluorescence, a method that uses a fluorescin-labeled antitreponeme serum using these techniques it is possible to make the correct diagnosis in almost 100% of untreated primary syphilitic chancre. The direct fluorescent antibody test is the most specific test for the diagnosis of syphilis when lesions are present. The nonspecific nontreponemal reaginic antibody tests VDRL, RPR are helpful as an indication of disease activity and the treponemal tests are considered today the most sensitive and specific despite their limitations and the complexity of interpretation, serological tests play a crucial role in the diagnosis and follow-up of syphilis. New specific and sensitive methods that employ reverse transcriptase PCR have been recently developed in order to detect very low numbers of spirochetes in clinical samples it is highly sensitive, because able to detect as low as 1–10 organisms per specimen with high specificity. However, these methods have not yet reached the stage of widespread clinical application (7).
1.2 Statement of the problem

Traditional, syphilis screening has been performed using a non treponemal test, such as rapid plasma reagin, with screen-reactive samples being tested by a treponemal assay like fluorescent treponemal antibody for confirmation. Many clinical laboratories have implemented a reverse screening algorithm, in which sera are screened using an automated, treponemal test like, enzyme immunoassay. Samples that are reactive by EIA are then tested by RPR to assess disease and treatment status and provide a supplemental marker of infection. The implementation of reverse screening has allowed clinical laboratories to meet increasing demands for syphilis testing by providing an automated screening test that yields an objective interpretation of results (8).

All blood donations are tested, using a serologic test for syphilis, red cell components stored for less than four days or platelets, which are stored at 22°C, can transmit the disease. Occasional cases of transmission from platelets donated by such individuals have been reported (9).

Transfusion services, in addition to their prime responsibility of supplying safe blood to the patient, also have a responsibility toward donor safety by means of donor notification and post-donation counseling. The national guideline mandates screening all the blood donations for transfusion transmissible infections. They are required to refer donors who tested TTIs reactive to the designated Counseling unit for disclosure, counseling and referral (10).

Nontreponemal antigen tests (RPR), are designed to test serum for reagin, a heterogeneous group of antibodies, which combine with a cardiolipin-lecithin antigen. This antigen, usually derived from beef heart and is a normal component of human tissue. Because cardiolipin (which mimics treponemal antigens) is used, approximately two-thirds of reactive samples during testing can have cross-reactivity and are biological false-positives. These BFP can be either due to other type of pathogenic treponemal infections or a strong immunological stimulus e.g., acute bacterial or viral infection, vaccination. Many Study showed high falsel positivity of RPR in blood donors. Treponemal tests (TPHA) detect an antibody that is directed toward pathogenic members of the
genus treponema. For diagnostic purposes thereby, TPHA is used as the gold standard. In USA(11).

The fresh whole blood recipients who survive to forward surgical facilities should be monitored continuously for transfusion reactions. Blood products at more advanced facilities should be component type resuscitation wherever possible. A rapid crossmatch and rapid serology for transfusion-transmitted infections must be done to limit the exposure of the casualty to untested blood. Recipients of FWB who have survived their injuries should be followed with serology at baseline, at 6 and 12 weeks and at 6 months and referred to an infectious disease specialist should they seroconvert(12).

Transmissions of infection through blood components still occur. Therefore, standard operating procedures of blood establishments worldwide include demands/recommendations for such screening. In many parts of the world, the incidence and prevalence of syphilis still remain high in both volunteer and family/replacement blood donors. There are numerous reports in high-risk groups in the literature, both from developed and developing countries, indicating rising (13).

The confirmation of infection with syphilis is often based on the presence of syphilis-specific antibody in a serum or plasma sample detected by treponemal assays. However, a positive treponemal test result does not differentiate between active and past infections. A person with active infection may have both non-treponemal reagins and treponemal antibodies in the blood, while patients with adequately treated past infection may still have a positive treponemal test result but their non-treponemal test findings will be negative or low titer (14).

The clinical diagnosis of syphilis is difficult due to the complexity of manifestations which requires the aid of serological interpretation. The serological diagnosis of syphilis depends on the detection of two distinct antibodies, the non-treponemal and the treponemes themselves. These antibodies can also be present in other diseases and human conditions such as (lupus, malaria, HIV, intravenous drug users, etc). The presence of non-treponemal antibodies is indicative of active infection, thus a reduction in titer can suggest a successful antibiotic therapy (15).
1.3 Rationale of the study

A single treponemal test cannot be solely relied upon for syphilis diagnosis, a combination of treponemal and non-treponemal tests must be used. The finding of this study help to:-

- Scale up screening and diagnosis of syphilis and plays an important role in transfusion medicines which protect unsafe transfusion practices, and for antenatal care to improve the health status of the community.
- Implement donor safety by providing accurate test result for donor notification and post-donation counseling.
- Establish guidelines for syphilis screening and diagnosis that contribute for policy making.
2. Literature review

Hooper N E et al conducted a study in 1994 in Maryland to evaluate *Treponema pallidum* Enzyme Immunoassay as a Screening Test for Syphilis. One thousand samples were tested, final result indicated that sensitivity of the RPR test and the EIA test are 86.1% and 100%, respectively, and that the specificities are 99.4 and 99.9%, respectively. In addition to the improved sensitivity and specificity of the EIA, other potential benefits of this assay lead to believe that this method could serve as a better screening tool than the RPR test (16).

In Brazil a cross-sectional study was conducted by Almeida N et al in 2009 to determine the demographic characteristics and risk behaviors of blood donors with recent and past syphilis and their implications for blood bank testing and deferral strategies. A total of 2,439 interviews were reviewed, the study found out Community donors were also associated with recent syphilis infection compared to replacement donors. In conclusion Blood banks may choose VDRL, RPR, or EIA, to characterize the profile of blood donors for improvements and blood banks may maintain the safety of the blood supply (17).

A retrospective study was conducted consecutively in China in 2010 to assess sensitivity, specificity, and feasibility of a new chemiluminescence immunoassay (CLIA) in the diagnosis of syphilis. At first a retrospective study was conducted, using 245 sera. A prospective study was also performed by testing 2,071 unselected samples. Chemiluminescence immune assay was compared with a nontreponemal test and a treponemal test. There was an agreement of 100% between Chemiluminescence immune assay and treponemal test (TPPA) in the respective study. The percentage of agreement among the 245 sera tested was 100.0%. CLIA showed 99.5% agreement with TPPA by testing 2,071 unselected samples. And CLIA seemed to be more sensitive than TPPA in detecting the samples of primary syphilis. Chemiluminescence immune assay is easy to perform and it may be suitable for blood banks where for large-scale screening is performed (18).

A cross-sectional analysis of patients with results of syphilis serological testing performed in China by Tong ML et al between December 2011 and May 2013. During this period, the syphilis serologic testing for each sample from 24,124 subjects were performed using RPR,
Treponemapallidum particle agglutination (TPPA) and a new automated to analyze 3 syphilis testing algorithms traditional algorithm, reverse algorithm, and the European Centre for Disease Prevention and Control (ECDC) algorithm. The research supported use of the ECDC algorithm, in which syphilis screening begins with a treponemal immunoassay that is followed by a second, different treponemal assay as a confirmatory test. The non treponemal assay is unnecessary for syphilis diagnosis but can be recommended for determining serological activity and recent infection of syphilis (19).

CDC done an assessment to review the testing algorithms used and the test interpretations provided in four laboratories in New York City. Of the 116,822 specimens included in the convenience sample, 6,587 (6%) were initially reactive to the EIA test. When 6,548 of the EIA-reactive specimens were tested with an RPR test, 2,884 (44%) were reactive and 3,664 (56%) were nonreactive to the RPR test. Further testing with FTA-ABS or TP-PA tests on 2,512 of the specimens reactive to the EIA test but nonreactive to the RPR test found 2,079 (83%) specimens reactive to the second treponemal tests (i.e., FTA-ABS or TP-PA). In addition, the one laboratory that performed TP-PA testing on specimens that were reactive to both the EIA and RPR tests found 78 of 80 (98%) specimens were reactive to the TP-PA test (20).

A prospective study was carried out in the Regional Blood Transfusion Centre New Delhi India from June 2008 to May 2010 to determine the prevalence of syphilis infection by Treponemapallidum hemagglutination assay (TPHA) among blood donors to study their correlation with other markers of transfusion transmitted infections so as to establish the utility of TPHA over and above venereal diseases research laboratory test (VDRL). A total of 8,082 serum samples of healthy blood donors included. The result was TPHA sero reactivity to be 4.4% in Delhi's blood donors. The study proposes that testing for syphilis by more sensitive and specific treponemal markers (TPHA) as opposed to VDRL should be used (21).

Naidu N K et al conducted study in India in 2012 to evaluate and compare the non-Treponemal tests and treponemal tests such as Rapid Plasma Reagin test, Venereal Disease Research Laboratory tests, Enzyme-linked immunosorbent assay and Immunochromatographic assays in comparison with Treponemapallidum haemagglutination Assay which was considered as a gold standard for this study. A total of 8,685 samples of voluntary blood donors were tested. 48
(56.4%) of the results on RPR were biological false positive, while 21.9% of results were false negative on RPR. Omega Pathozyme (ELISA) was quite in agreement with TPHA as compared with Treponisa 3.0, RAPHA (ELISA), and RPR. The study concluded that ELISA can be considered as a suitable test for screening of syphilis in a blood center (22).

A cross sectional study was conducted in South Korea in 2011 to determine the Reverse sequence screening for syphilis screening with treponemal tests, followed by confirmation with nontreponemal tests. The study reported that among 153 sera, RPR was nonreactive in 126 (82.4%). Among them, TP-PA was positive in 103 (81.7%), indeterminate (±) in 7 (5.6%), and negative in 16 (12.7%). Out of 16 CIA(+)/RPR(−)/TP-PA(−) sera, INNO-LIA Syphilis Score and/or FTA-ABS were negative on 14 sera. Out of 7 CIA(+)/RPR(−)/TP-PA(±) sera, INNO-LIA Syphilis Score and FTA-ABS were positive/reactive in 6 sera. Results of reverse sequence screening algorithms for syphilis using chemiluminescence immunoassay for initial screening, rapid plasma reagin (RPR) test, and Treponemapallidum particle agglutination assay (TP-PA) for confirmation. RSSS with confirmation by TP-PA on sera with discordant results between chemiluminescence immunoassay and RPR effectively delineated those discordant results and could be successfully adopted for routine checkup for syphilis (23).

Lee J H et al conducted study in 2014 in North Korea to compare the automated nontreponemal reagin (rapid plasma reagin (RPR)) test with the conventional RPR card test for usefulness in clinical applications. A total of 112 serum samples including 59 Treponemapallidum particle agglutination (TPPA)-positive and 53 TPPA-negative specimens were evaluated. The percentage agreement between the two RPR tests was 78.6% (κ 0.565; 95% CI 0.422 to 0.709). Sensitivity and specificity of the automated RPR test relative to the TPPA test was 52.5% (95% CI 39.1% to 65.7%) and 94.3% (95% CI 84.3% to 98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI 75% to 93.9%) and 94.3% (95% CI 84.3% to 98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test (24).

Study done in North Korea in 2016 to compare the results of the traditional syphilis screening algorithm and a reverse algorithm using automated Mediace RPR or Mediace T PLA as first-line screening assays in subjects undergoing a health checkup. Results were analyzed according to
both the traditional algorithm and reverse algorithm. Among the 24,681 samples, 30 (0.1%) were found positive by traditional screening, and 190 (0.8%) by reverse screening. The identified syphilis rate and overall false-positive rate according to the traditional algorithm were lower than those according to the reverse algorithm (0.07% and 0.05% vs. 0.64% and 0.13%, respectively).

A total of 173 discordant samples were tested with TPPA by using the reverse algorithm, of which 140 (80.9%) were TPPA positive. Samples with discordant results on the reverse algorithm were tested with *Treponema pallidum* particle agglutination (TPPA). In conclusion despite the increased false-positive results in populations with a low prevalence of syphilis, the reverse algorithm detected 140 samples with treponemal antibody that went undetected by the traditional algorithm. The reverse algorithm screening test is more sensitive for the detection of syphilis (25).

A retrospective study conducted in Italy at St. Orsola Hospital, University of Bologna, in 2009 evaluated the diagnostic performances of automated immunoassays in comparison with *T. pallidum* hemagglutination test and Western Blot. Sera were obtained from three different groups of subjects. All the sera were tested by ELISA, chemiluminescence immunoassay, TPHA, and homemade WB. The diagnostic performances of the two assays were very similar: ELISA and chemiluminescence immunoassay performed with a sensitivity of 99.2%, whereas the specificity was 98.5 and 98.4%, respectively. Considering the suitability for automation, both immunoassays may represent a good choice as a screening test. However, the use of a confirmatory test, such as TPHA or WB, remains a must in order to avoid false-positive results (26).

In 2010, national survey of blood donors performed in Italy to determine the proficiency of the transfusion service in screening for syphilis, by external quality assessment. The EQA was based on two shipments of serum panels 133 and 118 of the 326 existing TSs participated in the first and second shipments, respectively. Each panel consisted of both positive and negative serum samples. The study found that the interpretation of the results of manual techniques, such as the RPR test, the VDRL test, the TPHA assay, and the TPPA assay, can vary greatly among different TSs and operators. Total Ig enzyme immunoassays (EIAs) are the most sensitive. However, the determination of syphilis on the basis of the results of a single test is not sufficient for an accurate screening and all blood units should thus be assessed by two distinct treponemal tests, that is, a total Ig EIA and the TPHA or the TPPA assay (27).
A retrospective study was performed in Italy at St. Orsola Hospital, University of Bologna, in 2013 to evaluate diagnostic performances of Syphilis IgG and Syphilis IgM tests, in comparison with the performance of a chemiluminescent immunoassay for the detection of IgG and/or IgM anti-*Treponemapallidum* antibodies. Sera were obtained from different subject groups. *T. pallidum* hemagglutination and Western blot assays were used as confirmatory tests. Considering the IgG Western blot assay to be the gold standard. The study suggested Syphilis IgG could represent a suitable choice for high-volume laboratories. Syphilis IgM ELISA could be considered a good addition to IgG testing for uncovering active infections (28).

Study conducted in Turkey by Saral Y. *et al* in 2012 at Rize Education and Research Hospital to review serologic data of syphilis patients to determine diagnostic performance of three different methods. 117 patients suspected of having syphilis. Syphilis was diagnosed serologically and clinically. Three different methods were used for detection of antibodies Rapid Plasma Reagin (RPR), *Treponemal* Chemiluminescence Micro particle Enzyme Immunoassay (CMIA) and *Treponemapallidum* hemagglutination (TPHA). The sensitivity of RPR and CMIA against TPHA was 58% and 98%, respectively. The specificity of RPR and CMIA against TPHA was 0% and 100%, respectively. Automated enzyme immunoassay systems could contribute to reducing errors that depend on the person (29).

Zrein M *et al* conducted study in France in 1995 to compare an enzyme immunoassay using two major *Treponemapallidum* recombinant antigens with *T. pallidum* hemagglutination assay and a nontreponemal Venereal Disease Research Laboratory test. A total of 1,822 normal donor serum samples were tested for cardiolipin and *T. pallidum* antibodies, respectively, by the VDRL assay and EIA. Among these samples, 440 were further tested by TPHA technology. According to this study, the newly developed EIA kit shows 100% sensitivity combined to specificity greater than 99.8% for detecting treponemal immunoglobulin G antibodies in blood bank syphilis screening. This study clearly suggests that ELISyph-rG is a suitable assay for use in blood bank screening that could replace nontreponemal VDRL assays (30).
Study done by Marangoni A et al in 2005 in France compared two different ELISA test and TPHA as reference test for their sensitivity, specificity and agreement. The study revealed the IgG ELISA is higher in its specificity than IgG and IgM ELISA but the sensitivity was equal for both of them in respective of TPHA. The overall percent agreement and kappa value were good agreement (31).

Study done by Pope V et al in Georgia in 2000 compared Serodia Treponemapallidum Particle Agglutination, Captia Syphilis-G, and SpiroTekReagin II Tests with Standard Test Techniques for Diagnosis of Syphilis, 390 serum were tested. The result revealed Agreements of the MHA-TP with the TP-PA test and the Syphilis-G test were 97.4 and 97.7%, respectively. There was 89.2% agreement between the RPR and Reagin II tests. The Reagin II test was more apt to be reactive if the treponemal test was also reactive. The study concluded that either the Serodia TP-PA test or the Captia Syphilis-G test is an appropriate substitute for the MHA-TP and that the SpirotekReagin II test could substitute for the RPR test as a screening test (32).

Study was conducted in Australia The institute of Clinical Pathology and Medical Research, Lidcombe, New South Wales, in 1973 to show biological false positive reactions to reagin tests for syphilis. The Treponemapallidum haemagglutination test was carried out on 274 sera known to show biological false positive reactions to reagin tests for syphilis. The Treponemapallidum immunization (TPI) and fluorescent treponemal antibody absorption (FTA-ABS) tests were non-reactive on all these sera. It was concluded that where reagin and TPHA tests are reactive in a person who has no history or clinical signs of syphilis, the serum should be referred for further test (33).

Hospital based cross sectional study was done in Nigeria from January, 2007 to December, 2008 To determine the seroprevalence of syphilis among voluntary blood donors in North-eastern Nigeria. Five hundred and ninety five consecutively recruited voluntary blood donors were screened for syphilis using Rapid Plasma Reagin (RPR) test. All those positive were confirmed using Treponemapallidum haemagglutination (TPHA) test. Out of the 595 voluntary blood donors screened, the study revealed that only the male donors had syphilis. The study recommends the screening of all prospective blood donors for all transfusion transmissible infections. Strict selection criteria for blood donors to exclude those with multiple sexual partners, and that blood transfusion should be restricted (33).
Study done in Burkina Faso from August 2011 to December 2012 assessed the effectiveness of PCR testing and serological methods in the diagnosis of *Treponemapallidum subsp.* *pallidum* among blood donors. Of the 242 donors tested, 183 plasmas (75.6%) were reactive to RPR, and only 139 (57.4%) were reactive to CMIA. Out of 108 samples detected positive using serological methods, 97 (89.8%) were negative by PCR for *T. pallidum*, and only 11/108 (10.2%) were positive. The results of the study showed the urgent need for extending the molecular diagnosis of pathogens in blood transfusion to *Treponemapallidum*, which will be a more viable alternative to serological testing in the case of countries facing a high prevalence of infectious diseases (34).

Study done in Ghana by Owusu-Ofori AK *et al* in 2011 reported that from the total of 109 blood samples sixteen units were seropositive for syphilis by EIA and TPHA. Of these 7 units were RPR reactive, which indicated a prevalence of recent infections (35).

West B *et al* conducted the study in Gambia in 2002 to assess the rapid plasma reagin (RPR) test performance in the field and to evaluate a new rapid syphilis test (RST) as a primary screen for syphilis. 1325 women of reproductive age were tested for syphilis seropositivity using a RPR and a RST strip. The RST was easier to use and interpret than the RPR test especially where field conditions were difficult. In this setting with a low prevalence of syphilis in the community the chance of someone with a positive test being confirmed as having serologically active syphilis was less than 50% for both tests. The appropriateness of syphilis screening using RPR testing in antenatal clinics and health centers should be questioned that need improvement. (36).

Study conducted in the suburbs of Addis Ababa Ethiopia in 1999 to 2001 to assess the performance of routine syphilis screening during 5 year follow up of Ethiopian factory workers, participating in a cohort study on HIV/AIDS. At least six blood samples were evaluated. Screening performed by the *Treponemapallidum* particle agglutination (TPPA) assay and by the rapid plasma reagin (RPR) test. The 540 cohort participants were classified as having no (70.5%), past (20.6%), prevalent (6.9%), or incident (2.0%) syphilis. Evaluation of routine syphilis screening as performed in a long term cohort study on HIV/AIDS in Ethiopia showed difficulties encountered in syphilis screening programs such as a high percentage of BFP RPR,
inconsistencies in interpretation of the RPR test, and sample. The findings stress the need to develop a syphilis screening assay that is easy to perform and interpret (37).

Cross-sectional study was conducted at Hawassa University Hospital, southern Ethiopia, in 2009 to determine the diagnostic performance of the SD BIOLINE HIV/syphilis Duo rapid test. Serum samples obtained from clients attending the antiretroviral therapy and voluntary counseling and testing centers. Sera were originally collected for the purpose of investigating syphilis epidemiology. In reference to TPHA, the test kit reported 4 false positives and 2 false negative results for syphilis. The κ values were 0.99 for HIV testing and 0.94 for syphilis testing. This revealed that the performance of the SD BIOLINE HIV/syphilis was excellent (38).
3. Objectives

3.1. General Objective

To determine agreement between Enzyme Linked Immunosorbent Assay (ELISA) result with Rapid Plasma Reagin (RPR) and TPHA at National blood bank service in Addis Ababa, Ethiopia.

3.2. Specific Objectives

- To determine the level overall of agreement between ELISA and TPHA.
- To evaluate the level of overall agreement between ELISA and RPR.
- To characterize the level of overall agreement between RPR and TPHA.

4. Hypothesis

There is fair agreement between ELISA - TPHA, ELISA - RPR and RPR - TPHA for the screening and or diagnosis of syphilis among blood donors.
5. Materials and Methods

5.1 Study design
A cross sectional study was conducted to determine agreement of Enzyme Linked Immunosor bent Assay result with TPHA and Rapid Plasma Reagin with TPHA.

5.2. Study Site
The study site was at National Blood bank Service. NBBS was established in 1969 by Ethiopian Red Cross Society located around national stadium. NBBS is organized as agency in 2016. NBBS support 25 regional blood banks technically and distribute supplies and observe the quality of their service. NBBS collect blood and distribute to health facilities in Addis Ababa and around Addis Ababa. The laboratory prepare blood components, typing blood group and store blood and blood components in appropriate temperature until distribution for health institution. NBBS laboratory screen 50,000 blood in average annually for syphilis and other TTIs by using ELISA method and notify the results to blood donors. Syphilis screened by ELISA method and there is no additional test. ELISA is most sensitive test that can incorporate false positive result which is a challenge for notification of the result to donors, further test is needed to assure the result and notify donors who have positive result to their medical follow up.

5.3 Study Period
The study was conducted from Jan 2016 to Jun 2016.

5.4 Population
5.4.1 Source Population
All blood donors’ sample which was previously screened and stored at NBBS laboratory.
5.4.2. Study Population
All serum samples screened by ELISA for syphilis.

5.5 Inclusion and exclusion criteria

5.5.1. Inclusion criteria
- Blood donors’ sample that was screened for syphilis.

5.5.2. Exclusion criteria
- Hemolyzed serum
- Lipemic serum
- Insufficient sample

5.6 Study variable

5.6.1. Dependent variable
- ELISA test result of syphilis
- TPHA test result of syphilis
- RPR test result of syphilis

5.6.2 Independent variable
- Occupation
- Age of donor
- Types of donors (regular or new)
- Educational background
- Vaccination
- History of sexual behavior
5.7. Measurement and Data collection

5.7.1 Sampling Method

All positive Serum samples for syphilis stored from July 2015 to Dec 2015 in NBBS laboratory were used and systematic random sampling was employed to select negative samples create a list of samples, then, randomly decide which number to start a random number generator to select which sample that begin with. The random number generator produces the number then creates an interval was decided to select 190 samples.

5.7.2 Sample size determination

All positive sera were included which was stored from June 2015 to Dec 2015 and systematic random sampling was employed to select 190 negative sera was included.

5.7.3 Data collection procedure

Donor demographic data were collected from donor enrolment form by professional NBBS staffs. Leftover samples for syphilis positive and negative by ELISA test were used for the study. Serum storage and handling was according to Standard operating procedure. ELISA test were done for Syphilis according to kit instruction which is discussed detail in lab analysis.
5.7.4. Laboratory analysis

5.7.4.1 Principles of ELISA

Principle of ELISA (Anti syphilis AbDialab)
The Anti-Syphilis Ab ELISA is a solid phase qualitative enzyme immunoassay based on sandwich principle for the detection of total antibodies to *Treponema pallidum* in human serum or plasma. The micro plate is coated with recombinant antigens for *T. pallidum*. During testing, the specimen and the enzyme conjugated *T. pallidum* antigens are added to the antigen coated micro well plate and then incubated. If the specimen contain antibodies to *T. pallidum*, it will bind to the antigens coated on the micro well plate and simultaneously bind to the conjugated immobilized antigen *T. pallidum* conjugate complexes. If the specimen does not contain antibodies to *T. pallidum* the complex will not be formed. After initial incubation, the micro well plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of *T. pallidum* antibodies present in the specimen Sulphuric acid solution is added to the micro well plate to stop the reaction producing a color change from blue to yellow. The color intensity which corresponds to the amount of *T. pallidum* antibodies present in the specimen is measured with a micro plate reader at 450/630 -700 nm or at 450 nm. (kit insert of Dialab syphilis).

**Interpretation.**

**Calculation of the cut-off value**
Mean of negative control + 0.140

Interpretation of the result

**Non-reactive:** Specimens with absorbance less than the cut off value are considered non-reactive for antibodies to *T. pallidum* and may be considered negative

**Reactive Specimens:** with absorbance greater than or equal to the cut off value are considered initially reactive for antibodies to *T. pallidum*. 


5.7.4.2 TPHA Principle
Plasma tec TPHA reagents are used to detect human serum antibody to *T. pallidum* by means of an indirect haemagglutination (IHA) method. Preserved avian erythrocytes are coated with antigenic components of pathogenic *T. pallidum* (Nichol’s strain). These Test Cells agglutinate in the presence of specific antibodies to *T. pallidum*, and show characteristic patterns in microtitration plates. Any non-specific reactions occurring are detected using the Control Cells, which are avian erythrocytes, not coated with *T. pallidum* antigens. Non-specific reactions may also be absorbed out using these Control Cells. Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter’s treponemes, included in the cell suspension. Test results are obtained in 45-60 minutes and the cell agglutination patterns are both easily read and long lasting.

**Interpretation**
- Examine macroscopically the agglutination pattern of the cells.
- The test result is negative if compact button of cells were observed at the center of well
- Smooth mat of cells covering entire well bottom is positive.

5.7.4.3 Principles for RPR (Linear chemicals)
The RPR carbon antigen is a non treponemal preparation especially developed for the rapid detection and semi-quantitation by coagulation on slide or micro plate of plasma reagin,a group of antibodies directed against tissue components produced by almost every patient infected with *T. pallidum*. The assay also known as rapid plasma regain (RPR) is performed by testing the antigen-an association of lipid complexes and particulate carbon-against unknown samples. The presence or absence of a visible agglutination indicates the presence or absence of circulating antibodies in the samples tested (kit insert of RPR).

**Interpretation**
Non reactive: when carbon particles remain in smooth suspension with no visible aggregates as shown by negative control.

Reactive: Slight but definite to marked and intense visible aggregates are seen.

5.8. Data Quality Control

5.8.1 Preanalytical
Blood samples were collected by well trained nurses, after having donor’s consent. Reagent storage, labeling of samples were managed properly according to Ethiopian National Blood Bank Service standard.

5.8.2 Analytical
Internal quality control was done and well trained technologists performed the test according to the SOP.

5.8.3 Post analytical
All data recording were checked for completeness, the result was recorded with the donor identification number. Positive results were reported to the respective unit (donor counseling unit) to notify the result to the donor, to start medical follow up.

5.9 Data analysis and interpretation
The data obtained from the three tests were entered and analyzed by using statistical package for social science (SPSS version 20) computer software and was interpreted accordingly. The specificity, sensitivity, PPV and NPV of syphilis diagnostic methods were calculated. The percentage of agreement and κ coefficient was calculated to determine the agreement between ELISA / TPHA and TPHA / RPR. The agreement of the results according to their κ values was categorized as near perfect (0.81–1.0), substantial (0.61–0.8), moderate (0.41–0.6), fair (0.21–0.4), slight (0–0.2), or poor (<0) (44) and chi square was analyzed for association of gender and syphilis test result and to test the association between categorical variables.
6. Ethical consideration
Before the research work, ethical approval for the study was obtained from the Department of Research and Ethical Review Committee of the Department of Medical Laboratory. During data collection all blood donors gave their consent that their blood sample can be used for research purpose and no donor name was recorded and left over blood samples were labeled only with donor Id number which was used as unique study code. Confidentiality of the result was maintained and the results were only shared with authorized medical staff. Computerized files were password protected and paper files were locked safely and only accessible to authorized personnel. NBBS counselors notify their test result to donors, to have their medical follow up.

7. Dissemination of the result
The finalized paper will be submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, School of Allied Health Science of Addis Ababa University, so that it can serve as a reference in the library. In addition, a copy of the study will be given to NBBS and respective Blood Banks. The result will also be disseminated through publication in peer reviewed journals and through presenting it in relevant workshops and seminars.
8. Results

8.1 Socio demographic data of the study population

A total of 380 leftover sera samples from 380 blood donors were included from NBBS laboratory from July 2015 to Dec 2015. Out of these, 190 were positive for syphilis and 190 were negative according to the protocol used by the national blood bank laboratory. Among the study group 269 (70.7%) were males and 111 (29.2%) were females (table). Median age was 30 ranging from 18 to 65. Mean age of the donors was 32.9 years. The majority of the donors were in the age group of 25-30 (23.3%) followed by age group 20-24 (21.4%) . The chi-square result with p value 0.000 which is statistical significant indicate the association between categorical variables. (Table 1) Socio demographic characteristics of donors included in agreement study of ELISA, TPHA and RPR at NBBS, from Jan. 2016 to June 2016 Addis Ababa, Ethiopia

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total</th>
<th>Male and female Syphilis positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>19(7.0)</td>
<td>8(7.2)</td>
<td>26(6.2)</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>20-24</td>
<td>56(20.8)</td>
<td>26(23.4)</td>
<td>82(21.4)</td>
<td>12 (3.1)</td>
</tr>
<tr>
<td>25-30</td>
<td>66(24.5)</td>
<td>22(19.8)</td>
<td>88(23.3)</td>
<td>22 (6.5)</td>
</tr>
<tr>
<td>31-34</td>
<td>21(7.8)</td>
<td>8(7.2)</td>
<td>30(7.8)</td>
<td>10 (2.6)</td>
</tr>
<tr>
<td>35-40</td>
<td>35(13.0)</td>
<td>21(18.9)</td>
<td>56(14.8)</td>
<td>28 (7.3)</td>
</tr>
<tr>
<td>41-44</td>
<td>18(6.6)</td>
<td>7(6.4)</td>
<td>25(6.7)</td>
<td>18 (4.7)</td>
</tr>
<tr>
<td>45-50</td>
<td>28(10.4)</td>
<td>12(10.8)</td>
<td>40(10.6)</td>
<td>26 (6.8)</td>
</tr>
<tr>
<td>51-54</td>
<td>11(4.0)</td>
<td>4(3.6)</td>
<td>15(4.4)</td>
<td>10 (2.6)</td>
</tr>
</tbody>
</table>
From the total 380 study group, 151 were sero-reactive and 229 were negative for syphilis in reference to TPHA result,
One hundred ninety positive sera were included which was stored in appropriate temperature the rest 190 ELISA negative sera were selected by simple random technique at the time of the study. From 380 study group 269 were male and 111 were female donors. Of these male donors 111 (73.5 %) were positive and of the 111 female donors 40 (26.5 %) were found to be sero reactive for syphilis. All 190 ELISA negative sera which were retested by TPHA and RPR they were negative (Table 2).

Table 2. Frequency of Syphilis test result used for agreement study based on TPHA analysis from Jan. 2016 to June 2016 Addis Ababa, Ethiopia study
The statistical analysis report was chi square 0.897 with the p value 0.508 and odds ratio 0.802 with the confidence interval of lower bound 0.508 and upper bound 1.267, the analysis indicate that there was no association of gender with syphilis positivity and negativity.

8.2 Agreement between TPHA and ELISA

Among 190 ELISA positive sera 151 (80%) were confirmed positive by TPHA, 39 (20 %) were found to be false positive. Analysis of the sensitivity of the tests indicated that ELISA had the highest sensitivity (99.9%) in reference to TPHA. The overall agreement of ELISA and TPHA was 0.795 which was substantial and percent agreement was 90%.

8.3 Agreement between TPHA and RPR

RPR result was 59(39%) positive and 92 (61%) false negative. From the total of 151 confirmed positive sera by TPHA. Percent agreement of RPR with TPHA was 66% with kappa value of 0.375 which is fair agreement.

8.4 Agreement between ELISA and RPR

Among 190 ELISA positive sera 59 (31.1%) were positive and 131(68.9%) were negative by RPR. The kappa value was 0.321 which is fair agreement and percent agreement was 66%.

8.5 Sensitivity, Specificity, positive predictive value and negative predictive value of test Methods
The sensitivity of and specificity of ELISA were 99% and 85% respectively while the RPR was 62% and 99.6% respectively, the sensitivity and specificity of TPHA was 99% and 99.6% according to manufacturers kit insert. The sensitivity of RPR was lower than ELISA and TPHA.

Positive and negative predictive values of ELISA were 79% and 99% while the RPR were 99.8% and 63% respectively. The statistical data is summarized in Table 2.

**Table 3.** Statistical analysis of test result by three methods used for agreement study ELISA, TPHA and RPR at NBBS, from Jan. 2016 to June 2016 Addis Ababa, Ethiopia.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Kappa value</th>
<th>Percent agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPHA</td>
<td>positive</td>
<td>151</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>229</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>positive</td>
<td>190</td>
<td>99.9 %</td>
<td>85%</td>
<td>79%</td>
<td>100%</td>
<td>0.795</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>190</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPR</td>
<td>positive</td>
<td>59</td>
<td>62%</td>
<td>99.6%</td>
<td>100%</td>
<td>69%</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>321</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9. Discussion

Syphilis is still a public health problem worldwide. Serological testing is a crucial element of any control program for syphilis and, used both in screening for asymptomatic infections and as an assistant to clinical diagnosis and remains the principal tool for the diagnosis of syphilis. This study allows the identification of the test that is adapted to the context of the country. To our knowledge, three of the tests, ELISA, TPHA and RPR, were tested for the first time for their overall agreement.

Treponemal and non treponemal tests are employed in this study. Dialab ELISA is treponemal test, RPR is non Treponemal test and the reference test TPHA is treponemal test. For each test, the laboratory evaluation results were compared to the reference test (TPHA).

Our study revealed that ELISA performed with a sensitivity of 99.9% and specificity 85%. The study conducted by Ebel A et al (1998) the competitive EIA had a sensitivity of 99.5% (40). And Ozolins D et al (2009) also agreed with our study reported that the sensitivity of ELISA IgG, IgM was 100.0 % (42).

Decreased sensitivity of ELISA was demonstrated by Schmidt B L et al, (2000) the ICE Syphilis ELISA was with the sensitivity of 76.9% (42). Concerning specificity of ELISA less specific test was reported by Naidu NK et al. (2012) that employed two types of ELISA, the study demonstrated one of the two ELISAs was with specificity of 50% (22).
The sensitivity of ELISA in our finding is lower than the sensitivity of the company’s report in their kit insert, this might be happened because our study was employed stored serum and TPPA was their gold standard and our gold standard was TPHA.

Increased specificities of ELISA were reported by Ebel A et al (2000), the competitive EIA had a specificity 99.4% (41). Marangoni A et al (2005) who compared two different ELISA test and TPHA as reference test, specificity were 99% (30). Schmidt B L et al, (2000) demonstrate the specificity of the ICE Syphilis and the Enzygnost Syphilis tests were 99.5 and 99.8% (41).

The sensitivity and specificity of RPR in our study were 62% and 99.6% respectively. Ozolins D et al (2009) study was in accordance with our result the sensitivity of RPR (58.8%) (42). And Saral Y et al (2012) also reported that the sensitivity of RPR and CMIA against TPHA was 58% and 98%, respectively (29). Increased sensitivity of RPR was reported by Hooper N E et al (1994) the sensitivity of RPR was (86.1%), Lee J H et al (2014) also demonstrated that the sensitivity of conventional RPR card test were 86.4% (24).

Sensitivity and specificity of ELISA was affected due to the volume and dilution of the serum, ELISAs using a greater volume of serum and/or a lower dilution yielded higher sensitivity. The main influence on sensitivity can also be attributed to the design of the assay. Recombinant proteins that used for the preparation of antigens varied greatly in their recognition by antibodies in sera from individuals with syphilis. Some recombinant antigens were recognized by almost the entire antibody in the sera from individuals with syphilis. High sensitivity could be obtained with ELISAs using a purified \textit{T. pallidum} antigen applied as a coating to the wells of micro titer plate strips and detection of both IgG and IgM are most sensitive treponemal test with a very high specificity as well (42).

In our study, from 190 ELISA positive sera, 151 (80%) were confirmed positive and 39 (20%) were found to be false positive in reference to TPHA. In comparison of true positivity, EIA was with high rate of false positive result. Binnicker MJ et al (2012) demonstrated that among the 140,176 samples tested by EIA/CIA, 4,834 (3.4%) were reactive by the screening test, and of these screen-reactive sera, 2,743 (56.7%) were subsequently nonreactive by RPR. Importantly, these discordant samples 2,743 were tested by TP-PA or FTA, which gave nonreactive results for 833 (31.6%) sera, suggesting a falsely reactive EIA/CIA screening result. These findings suggest
that reverse screening may detect a higher rate of screen-reactive compared to traditional testing by RPR. In addition, that reverse screening may yield an elevated percentage of falsely reactive results (8).

In our study from 151 confirmed positive 59 (39 %) were positive and 92 (61%) were false negative by RPR test. In comparison of true negative, the assays revealed that RPR had a high rate of false negative. Similar study by Lee K et al (2013) demonstrated that among 153 sera with CIA (EIA) screening reactive results, RPR was reactive in 17.6% and nonreactive in 82.4% sera that indicate the rate of false negative in RPR was high. Among the 126 CIA positive but RPR negative sera, TP-PA was positive in 81.7%, indeterminate in 5.6%, and negative in 12.7%. Among the 27 CIA positive and RPR positive sera, TP-PA was positive in 88.9%, indeterminate in 7.4%, and negative in 1 (3.7%) sera (23). The study reported that high false negative by RPR test. CIA and TPPA result was in good agreement.

Non-treponemal tests may give false negative results. False negative results may also occur due to technical errors, such as the antigen being distributed on a sample not previously placed on the entire test area surface. Reagent temperature is also important for sensitivity, especially when testing samples from patients. A false negative result may also be due to the prozone phenomenon. Assessment done by CDC reported that, of the 116,822 specimens (6%) were initially reactive to the EIA test. When 6,548 of the EIA-reactive specimens were tested with RPR test, 2,884 (44%) were reactive and 3,664 (56%) were nonreactive to the RPR test. Further testing with TP-PA tests on 2,512 of the specimens reactive to the EIA test but nonreactive to the RPR test found 2,079 (83%) specimens reactive to the second treponemal tests (TP-PA) (18). The study demonstrated that false negative result of RPR is high which.

Concerning the agreement our study demonstrated that ELISA performs 90% agreement with TPHA and the corresponding kappa value was 0.795 which is substantial agreement with reference test. Study done by Pope V et al (2000) was in accordance with our study they reported that the agreements of the MHA-TP with the TP-PA test and the Syphilis-G test were 97.4 and 97.7%, respectively (32). Binnicker M J et al (2011) demonstrate similar report concerning the agreement of treponemal tests, the overall percent agreement and corresponding kappa values
were as follows: BioPlex syphilis IgG, 98.0% κ = 0.96; TP-PA, 97.0%, κ = 0.93; Trep-Chek EIA, 97.7% κ = 0.95; Trep-Sure EIA, 95.4% κ = 0.90; Trep-ID EIA, 98.4% κ = 0.96; and Vira Blot IgG, 97.0% κ = 0.93 (43).

Percent agreement of RPR with TPHA was 66% and the kappa value was 0.375 and percent agreement and k value of ELISA and RPR were 66% and 0.321 respectively that was fair agreement. Tong ML (2014) demonstrated that in their cohort study, the reverse and ECDC algorithms had higher diagnostic efficacy than the traditional algorithm. The overall percentage agreement and κ value between the reverse and the ECDC algorithms were 99.9% and 0.996, respectively (16).

Positive predictive value of ELISA and RPR in our study were 79% and 100% consecutively and negative predictive value of ELISA and RPR were 100% and 69%. Study done by Karaca Y et al (2010) partial agreement with our study demonstrated that positive and negative predictive values for VDRL and ELISA were 100% ,80.6% and 98.8% , 98.7% respectively (39).

Study done in Ghana by Owusu-Ofori AK et al (2002) reported that from the total of 109 blood samples sixteen units were seropositive for syphilis by EIA and TPHA. Of these 7 units were RPR reactive, which indicated a prevalence of recent infections (35). The study revealed that RPR is used to indicate recent infection and support the idea that, even though RPR was less sensitive and with high false negative rate, it is recommended for the detection of recent infection. EIA results could reflect greater sensitivity for untreated syphilis, a high frequency of treponemal false-positive results. For situations involving positive treponemal EIA results or with negative non treponemal test results, the CDC recommends use of a second treponemal test, such as the TPPA or the FTA-ABS (37).
10. Strength and limitation of the study

10.1 Strength of the study

- The study demonstrated that selecting the reverse algorithm in syphilis serology could have positive effect on the serological diagnosis of syphilis.
- The study provided information on the challenge of syphilis screening and diagnosis.

10.2 Limitation of study

- The finding of the study was based on stored sera from apparently healthy blood donors. Further studies with documented clinical samples are necessary to demonstrate correlation between antibody titer and the stage of the disease.

- Association could not be made between sexual behavior, history of medication, vaccination and the like.
11. Conclusion and Recommendation

11.1 Conclusion

The study demonstrated that ELISA was very sensitive. It has the capacity of detecting low concentration of disease causing agents. There was good agreement between ELISA and TPHA. RPR was with high specific and weak sensitive results. There was poor agreement between RPR and TPHA.

11.2 Recommendation

- Following appropriate algorithm of syphilis helps to screen and diagnose accurately the disease that improve the health status of the community.
- Using EIA as screening and RPR as second test to identify recent infection and TPHA as a confirmatory test is recommended. This is important for establishing of accurate and reliable testing for critical step in ensuring the diagnosis of syphilis and for the safety of blood transfusion service.
- Syphilis test method should be revised according to standard method in order to improve the laboratory service of health institutions especially those which run antenatal care.
12. References


**Annexes**
Annex I. Syphilis ELISA Test Procedure

Procedure

Allow reagents and specimen to reach room temperature (15-30°C)

1. Leave A1 as blank well.
   - Add 50µl of negative control in well B1 and C1 (Blue reagent)
   - Add 50µl of positive control in wells D1 and E1 (Red reagent)
   - Add 50µl specimen to assigned wells starting at F1
2. Add 50µl of conjugate to each well except to the blank well (Red reagent)
3. Incubate in incubator at 37°C ± 2°C for 60 minutes ± 2 minutes.
4. Wash each well five times
5. Add 50µl of substrate solution A to each well (clear Reagent)
   - Add 50µl of substrate solution B each well (clear Reagent) then a blue color should develop in well containing positive specimen.
6. Incubate in incubator at 37°C ± 2°C for 15 minutes ± 1 minute
7. Add 50µl of stop solution to each well then a yellow color should develop in wells containing positive specimen
8. Read at 450/630-700 nm for better results. Within 30 minutes

**Validation requirement and quality control**

- Calculate the mean absorbance of negative Control and positive control

**Item Validation requirement**

Blank well       Blank absorbance should be < 0.050 if read at 450/630-700 and it should be < 0.100 if read at 450nm
Negative control Mean absorbance after subtraction of blank absorbance should be < 0.100
Positive control Mean absorbance after subtraction of blank absorbance should be > 1000

The test result is considered invalid, if the above validation requirement is not met. The test should be repeated.
**Calculation of the cut off value**

Mean of negative control + 0.140

**Interpretation of the result**

**Non-reactive**  Specimens with absorbance less than the cut off value are considered non-reactive for antibodies to *T. pallidum* and may be considered negative.

**Reactive Specimens** with absorbance greater than or equal to the cut off value are considered initially reactive for antibodies to *T. pallidum*. The specimen should be retested in duplicate before final interpretation. Specimens that are reactive in at least one of the retest are presumed to be repeatedly reactive and should be confirmed using confirmatory testing. Specimen that are negative on both retests should be considered non-reactive. Specimen with values within 10% of the cut off value should be retested in duplication for final interpretation.
### Table 4. Worksheet of syphilis ELISA

Worksheet of syphilis ELISA

Effective date: Syphilis -Form number: FRM LAB-02A

Revision number: 00

page 1of 1

Division: Laboratory

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Repeated samples</th>
<th>Date</th>
<th>Lot No.</th>
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</tbody>
</table>

Blank should be: _______________ Negative control (N.C) should be: _______________

Positive control should be (P.C): _______________ Cut off value: _______________

Gray zone: _______________

Reactive sample No: _______________

Repeat Reactives: _______________

Performed by: _______________ Accepted by: _______________ Approved by: _______________

Authorized by: _______________ Dr. Daniel G/Michael Director
Annex II. TPHA Procedure

Qualitative method
Each sample requires 3 wells of a micro titration plate.
1. Add 190µl of diluent to Well 1.
2. Add 10µl serum to Well 1.
3. Using a micropipette, mix contents of Well 1 and transfer 25µl to Wells 2 & ensure that the Test and Control Cells are thoroughly resuspend. Add 75µl of control cells to Well 2. Add 75µl of Test Cells to Well 3.
4. Tap the plate gently to mix the contents thoroughly.
5. Incubate 45-60 minutes at room temperature.
6. Caution! Keep the plate away from heat, direct sunlight and any source of vibration.
7. Read results. Results are stable for 24hrs if the plate is covered and the above precautions are observed.

Note: Kit controls can be run in parallel and are diluted and ready for use.

Quantitative test
Each sample requires 8 Wells of a micro titration plate. Labeled A through to H.
1. Add 25µl of diluent to Wells B to H inclusive.
2. Transfer 25µl of 1:20 serum dilution from screening test to Wells A and B.
3. Take 25µl of diluted serum from Well B and serially dilute from Wells B to H inclusive in 25µl aliquots, discarding 25µl of diluted serum from Well H. Ensure that the test cells are thoroughly resuspended. Add 75µl of Test cells to wells A to H inclusive. This will give a dilution of serum of 1/ 80 in Well A through 1/ 10240 Well H.
4. Shake the plate gently to mix the contents thoroughly.
5. Incubate for 45-60 minutes at room temperature. Caution! Keep the plate away from heat, direct sunlight and any source of vibration.
6. Read results. Results are stable for 24hrs. If the plate is covered.
Table 5. Results reading of test cells and control cells for TPHA.

<table>
<thead>
<tr>
<th>Results</th>
<th>Test cells</th>
<th>Control cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong positive</td>
<td>Full cell pattern covering the bottom of the well.</td>
<td>No agglutination tight button</td>
</tr>
<tr>
<td>Weak Positive</td>
<td>Cell pattern covers approx. 1/3 of well bottom</td>
<td>No agglutination tight button</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Cell pattern shows a distinctly open center</td>
<td>No agglutination tight button</td>
</tr>
<tr>
<td>Negative</td>
<td>Cells settled to a compact bottom, typically with a small clear center</td>
<td>No agglutination tight button</td>
</tr>
<tr>
<td>Non-specific</td>
<td>Positive reaction</td>
<td>Positive reaction</td>
</tr>
</tbody>
</table>

Non-specific absorption

1. Add 100µl of test serum to a small tube then add 400µl of Control Cells. Mix well and stand for 1 hour.
2. Centrifuge for 15 minutes at 1000 rpm and test the supernatant by the qualitative method.

Note: The sample is now at 1/5, this should be taken into account when preparing the dilutions.

If the result is repeatedly non-specific the sample should be tested by another Method E.g. Reagin or FTA-ABS.
Interpretation of Results.

Strong positive reactions may show some folding at the edge of the cell mat. When the Test well is positive, the Control well should be observed. The Control cells should settle to a compact button. They should not be used as a comparison for Non-Reactive serum patterns since the Control Cells will give a more compact pattern than the Test Cells. Agglutination in the Control well indicates the presence of non-specific agglutinins in the sample, the test should be reported as INVALID. A serum that gives this result may be absorbed using the Control Cells as detailed under Non-specific absorption. A doubtful reaction with Test Cells should be reported as INDETERMINATE. This result may indicate a low level of antibody in early primary syphilis or yaws. This sample should be first retested in the qualitative test then a further sample should be tested at a later date to determine whether or not there is a rising titre. It is also advisable to perform a reagin test and/or another confirmation test (FTA-ABS) to complete the profile of the test serum.

Annex III. Rapid Plasma Reagin Test Procedure

Procedure
Qualitative test

1. Bring reagents and samples to room temperature
2. By means of automatic pipette place 50µl of each sample in to separate circle on the card. Use a separate tip for each sample and discard after use .dispense one drop of each of the two serum control in to two additional circles.
3. Gently shake the dispensing vial in vertical position slightly press to remove air bubbles from the needle and the drop obtained is correct.
4. Place the needle in a vertical position perpendicular to the card. Press gently the dispensing vial and deliver 1 drop of antigen to each circle next to the sample to be tasted.
5. Mix the content of each circle with a disposable stirrer and spread over the entire area enclosed by the ring use separate applicators for each mixture.
6. Place the card on a mechanical rotator and rotate at 100 r.p.m. for 8 minutes.
7. Observe microscopically for agglutination under a high intensity lamp or strong day light within a minute after removing the card from the rotator

Reading result

Non reactive Carbon particles remain in smooth suspension with no visible aggregates as shown by negative control

Reactive Slight but definite to marked and intense visible aggregates are seen

Quantitative test for RPR

1. For each specimen to be tasted place with an automatic pipette 50µl of 0.9% saline solution of each of 5 circle on the reaction card Don’t spread diluent.
2. To circle one add 50µl of specimen to the saline solution and using the same tip mix the saline solution with the sample by repeated aspiration and expulsion of the fluid and transfer 50µl of the mixture to the saline solution in the second circle.

3. Continue with the 2-fold serial dilutions in a similar manner up to the fifth circle and discard 50µl from this circle final sample dilutions will be 1:2, 1:4, 1:8, 1:16, 1:32.

4. Test each dilution as describe in steps 3-7 for the qualitative.

   Reading quantitative test

   Reading same as qualitative test the titer of the specimen is reported as the highest dilution that shows reactivity. The next higher dilution should be negative.

   If the highest dilution tested is reactive repeat the test starting with a preliminary 1:16 dilution. Use a 1:50 dilution of negative control in a 0.9% saline solution in a new 2 fold dilution series

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ANNEX IV. Participant Information Sheet

Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences

**Title:** Agreement between Enzyme Linked Immunosorbent Assay (ELISA) with Rapid Plasma Reagin (RPR) and TreponemapallidumHaemAgglutination Assay (TPHA) for diagnosis of syphilis at National blood bank service in Addis Ababa, Ethiopia.
**Introduction:** Serum samples will be provided by NBBS laboratory to perform the study. sera will be leftover stored samples from routine lab tests.

**Purpose:** To conduct a study with objective of Agreement between Enzyme Linked Immunosorbent Assay (ELISA) with Rapid Protein Reagin (RPR) and TreponemapallidumHaemAgglutination Assay (TPHA) for diagnosis of syphilis at National blood bank service in Addis Ababa, Ethiopia.

**Confidentiality:** All information about the sample during this research will be kept confidential. Information about result will be kept in a secured place. Only the principal investigator and responsible staffs will handle the result, NBBS counselors communicate the donors.

**Benefit:** Findings from this study will help in supplying safe blood and blood products for recipients, notifying reliable syphilis test results to blood donors for their medical follow up and to develop syphilis test algorithm in blood banks.

**Participation:** All blood donors agreed that their blood sample could be used for scientific research purpose to improve the safety of the blood supply to patients and the donors’ health and wellbeing. The donors understand that all the information about their blood will be kept confidentially.

---

**Annex V. Blood donor consent form**

![Blood donor consent form]

NATIONAL BLOOD BANK SERVICE

Effective date: BDS-04A2

Document number: FRM SOP

Revision number: 01

Division/service: Blood donor service/Screening unit
SECTION 3: DECLARATION

1. I have read and understood the pamphlet “Basic Brochure on Blood Donation”.

2. To the best of my Knowledge all the information supplied is the truth.

3. I understand that if I have not answered these questions truthfully this could endanger the patient.

4. I consent to my blood being tested to syphilis, hepatitis B, hepatitis C and HIV.

5. I understand I shall be informed of any test result that are important to my health or affect my ability to donate blood.

6. I accept that donation data may be used on occasion for scientific research the objective of which is to improve the safety of the blood supplied to patients and the donors’ health and wellbeing.

7. I confirm that I am 18 years of age or older.

8. I understand that all the information on this form will be kept confidentially.

Donor’s Signature ……………………………… Date ……………………

Remarks: …………………………………………………………………………………………….

FOR OFFICE USE ONLY

Counseled by…………………………………………….Signature

…………………………………………Donor reaction YES……………… NO………….

Authorized by Dr. Daniel G/Michael General Director

NATIONAL BLOOD BANK SERVICE

Effective date: Form number: FRM SOP BDS-04A1

Revision number: 01 page 2 of 2

Division/service: Blood donor service/Screening unit

BLOOD DONOR QUESTIONNAIRE

Appendix II page 2
ክፍል 3: የሸምነትመግለጫ

1. የመጠይቆችን ይታኝ ’ማረጋገር በውጤት ይሸምና የሚለውን ቦታ ይታኝ ጥናት ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈታ ይስ ስር የሚለውን ፈት
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**Annex VII. Blood Donor Enrollment Form**

Name________________ Father Name__________________ Surname_________ 

Title __ Date of Birth(DD/MM/YY) ________ Age__ Sex __ Occupation __________

City____ Sub-city/Region _____ Zone _____ Woreda _____ Keb. _____ H.No _____

Tele (Res)_________ Tele (Off)_________ Cell Phone_________ Email __________

P.O.Box: __________________________

Reg No. ____________________________

Donor code---------------

<table>
<thead>
<tr>
<th>Date</th>
<th>Pack No</th>
<th>Wt.</th>
<th>Hgb.</th>
<th>B/P</th>
<th>Vol.</th>
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<th>ABO</th>
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</table>
Annex VIII. Collaborator’s Curriculum Vitae

I. PERSONAL INFORMATION

Dr Daniel G/Michael Burssa
Sex: Male
Marital status: Married
Nationality: Ethiopian
Date of Birth: October 30, 1978
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II. SUMMARY OF QUALIFICATIONS AND EXPERIENCES

I have Master of public health (Gondar University) and Medical doctorate degree (Gondar college of medical sciences) and I had more than eleven years of work experience as a clinician and public health specialist in different governmental, nongovernmental and private organizations. Currently I am working as the general director of Ethiopian national blood bank service.

III. TRAINING/WORKSHOPS ATTENDED

- American Association of Blood Banks /AABB/ annual workshop organized by AABB, Anaheim, California, USA from October 24-28, 2015.
- 25th Regional Congress of the International society of blood transfusion /ISBT/ in conjunction with the 33rd Annual Conference of the British Blood Transfusion Society London, United Kingdom from June 27 - July 1, 2015
- The Third Worldwide Network for Blood & Marrow Transplantation (WBMT) workshop, South Africa, Cape town, 14th-16th November 2014
Sino-Africa Cooperation Forum on Development and Humanitarian Action and Seminar on Capacity Building of Red Cross & Red Crescent Societies of Sino-Africa Counties, organized by Chinese Red Cross National Training Center, Beijing, China, 19th August-25th August, 2014

Transfusion medicine attachment at John Hopkins University for two weeks, Baltimore, USA


High level policy makers forum on achieving self sufficiency in safe blood and blood products based on Voluntary Non-Remunerated Donation held in Rome on October 8-9, 2013

Experience sharing visit to Malawi blood transfusion Service/ program by leading a higher delegation team from Ethiopian Federal Ministry of Health (FMOH/NBTS), higher official from the 4 bigger regions in Ethiopia, USG-university partners, CDC and WHO from August 26 – September 1, 2013.

Training on Infection Prevention and Long Acting family planning organized by Marie Stopes International Ethiopia.


Training on syndromic management of sexually transmitted infections organized by ARHB

Training on Anti Retroviral Treatment guidelines

Provider initiative HIV / AIDS counseling and testing (PICT) training organized by PHSP-Ethiopia

Financial management training for non-financial managers organized by About associate

Workshop on management of severe malaria organized by Health Bureau of Amhara National Regional State in 2005

Training on management of severe malnutrition organized by UNCEF in 2005
Training on management of TB and HIV/AIDS co infections organized by PHSP-Ethiopia

Report Writing and Best Practice Documentation Training

Training on project Monitoring and Evaluation

IV. WORK EXPERIENCE

Since 2013 until present

I have been working as the general director of Ethiopian national blood bank service since 2013 with the following responsibilities:

- As a management member and Executive committee member of Ethiopian Ministry of Health participate in strategic planning, implementation and evaluation of health sector.
- Oversee and lead the preparation of strategic document, strategic plan, core plan and comprehensive plan of Ethiopian Blood Bank Service
- Oversee all of the technical, operational, and administrative functions of the national Blood Bank (Central).
- Give direction on the day to day activities of NBBS and Regional blood banks (24 regional blood banks)
- Coordination of all organizations and institutions providing blood transfusion services to harmonize approaches, foster joint planning and collaboration, optimize use of resources, avoid duplication and wastage
- Give the overall directions on supervision and work performance of technical staff, and for assuring that all testing and component preparation in the laboratory is performed according to established operating procedures.
- Prepare and follow the implementation of the national blood transfusion service strategic plan
- Organize and facilitate local, regional and national workshops and trainings for different governmental and nongovernmental organizations on blood safety program
- Prepare detailed technical proposal, modules, training manuals, check lists, and budget tracking for the national blood transfusion service and regional blood banks
- Write, edit and finalized draft researches and training reports
- Recruit and train different categories of blood bank professionals
- Enhance partnerships and networking among stakeholders/clients
- Lead and chair the national blood safety technical working groups

From 2006 – 2013

I was working in Mersa health institution with the following responsibilities
- Technical head of the youth friendly clinic
- Giving different clinical services (STI, Family planning, TB, HIV/AIDS) to patients who attend to the clinic
- Giving and coordinating Health Education program focusing on STI, Family planning, TB, HIV/AIDS in the institution
- Coordinating STI, TB/HIV/AIDS program of the clinic in collaboration with PSP
- Designing different projects related to adolescent health (including STI, HIV/AIDS)
- Giving training to low level health workers at the health center about family planning, STI, TB, HIV/AIDS...
- Organizing and leading the RH program that was run by pathfinder international,

**From 2005 – 2006**
I was worked at Efoyta medium private clinic (Haik) as a physician with responsibilities of treating patients at outpatient department and admitted patients. In addition to this I was also acting as the head of the technical medical team in this clinic.

**From 2004 – 2005**
During this period I was working at Haik government health center as a general practitioner.
Major responsibilities:
- Diagnosing and managing patients at OPD and in patient clinics
- Coordinating different health campaign (polio, measles, tetanus ....) at the woreda level
- Giving training to low level health workers at the health center about malaria, TB, HIV/AIDS, polio, measles .......
- Teaching 2nd and 3rd year nursing students during their clinical attachments to the health center
- Acting as the management committee of the health center

**From 2003-2004**
Just immediately after graduation from Gondar medical college I was assigned at one of rural governmental health center, Masha health center with the following responsibilities:
- The head of Masha health center
- Health management committee of the woreda and the health centre
- Diagnosing and managing patients at OPD and in patient clinics
- Coordinating different health campaign (polio, measles, tetanus ....) at the woreda level
- Giving training to low level health workers at the health center about malaria, TB, HIV/AIDS, polio, measles ....

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V. EDUCATION AND QUALIFICATIONS

Medical doctorate degree (MD) from Gondar College of medical sciences (GCMS) in July 2002

MPH (Master of public health) from Gondar University in Sep 2009

The topic of my thesis for partial fulfillment of MPH is Assessment of gender based sexual violence among Habruworeda high school female students, North Ethiopia.

VI. OTHER RELEVANT SKILLS AND TRAININGS

- I am fluent in listening, reading, writing and speaking in both Amharic and English language.
- Diploma in computer sciences
- Driving license

VII. Professional membership

- Vice chairman and governing board member of Ethiopian Hemophilia Association.
- Chairman of National Stem Cell and Bone Marrow transplant committee assigned by minister of health
- Ethiopian representative on the behalf of Federal Ministry of Health in International Transplant Network organized and coordinated by Turkish Transplant Foundation /www.tov.org.trinfo@tonv.org.tr /
- Full member of International society of blood transfusion /ISBT/

VIII REFERENCE

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Declaration
I, the undersigned, declare that this is my own work, and has not been presented for other university, college or institutions for similar degree or purposes. All sources of materials used for the research has duly acknowledged.

Name: Ejigayehu Afework
Place Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia
Signature ______________
Date of submission 24/2/2016

This research paper has been submitted with my approval as university advisor.

Name of advisor Signature
Mintewab Hussein (BSC. MSC) ______________
Jemal Alemu (BSC. MSC) ______________
Place Addis Ababa University Lecturer of Department of Medical Laboratory Sciences

Date of Submission _____________________