**Sudan University of Science and Technology**
**College of Graduate Studies**

# Seroprevalence and comparison between methods of Syphilis detection among Blood Donors in Khartoum State/Sudan

# الأنتشار والمقارنة بين طرق كشف مرض الزهرى لدى المتبرعين بالدم في ولاية الخرطوم-السودان

A dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. in Medical Laboratory Sciences (Microbiology)

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# Dedication

To my parents

To my brothers and sisters

To my friends

To my teachers

# Acknowledgements

My deep thanks to AllAH, my gratitude to my supervisor Prof. Yousif Fadlallah Hamed Elnil who mad this study possible by his advices and effort. My appreciations to all working staff of Central Blood Bank of Khartoum State for giving me the permission and their cooperation to do this study.

My deep thanks to all blood donors specially those whose samples were collected, also special thanks to all my teachers. Finally my great thanks to anyone who helped me in this study .

# Abstract

This study was conducted among blood donors in Central Blood Bank of Khartoum. On a total of ninety tow samples were collected, during the period from March to May 2018, to demonstrate the frequency of syphilis and to compare between different diagnostic methods for detection of syphilis. All samples were tested from ELISA, ICT and RPR. The seroprevalence of syphilis revealed from ELISA method was 7(7.6 %), which was equal to ICT but was slightly increase in RPR 8 (8.7%). The high frequency of syphilis 3 (42.9%) was found in both age group of (26-35and36-45). There was no positive cases between the ages of (46-55) years. 5 (71.4%) of positive samples were seen among single blood donors, 2 (28.6%) among married donors and no positive cases among divorced blood donors. ICT method showed 100% sensitivity and 100% specificity. RPR showed 100% sensitivity and 98.8% specificity. Results showed that the ICT used in this study was the same when compared with ELISA but the RPR showed less specificity than ELISA .

The study concluded that ICT with ELISA were the best methods for screening blood donors.

# ملخص الدراسة

**اجريت هذه الدراسة علي متبرعي الدم بولاية الخرطوم (بنك الدم المركزي). في الفترة ما بين مارس الي مايو 2018 في 92 عينة دم, وذلك لتحديد مدي انتشار مرض الزهري وسط هذه الفئة وللمقارنة بين عدة اختبارات لفحص مرض الزهري. كل العينات تم فحصها بواسطة تقنية الكروماتوقرفي الممنع وتقنية الاليزا وتقنية الكشف عن المستضد ريأجين .**

**كانت النتائج كلأتي, 7(7.6%) كان لديهم المستضد بواسطة فحص الأليزا وقد اعطت تقنية الكروماتوقرفي الممنع نفس النتائج بينما كانت هناك زيادة قليلة في نسبة وجود المرض عند الفحص بواسطة تقنية الكشف عن المستضد الريأجين8(8.7%). وكان انتشار المرض اكثر في الفئتين العمرية ( 35-26)/ (36 -45) بنسبة3 (42.9%) في كل فئة, بينما نجد انعدام تواجد المرض وسط الفئة العمرية (46 -55) , وايضا كانت نسبة انتشار المرض بين الغير متزوجين5(71.4% ) تفوق نسبة انتشاره بين المتزوجين 2(% 28.6) , ولا توجد حالات وسط المطلقين.**

**اعطت تقنية الكروماتقرفي الممنع حساسية وخصوصية بنسبة100% بينما اعطت تقنية الكشف عن المستضد الريأجين حساسية100% وخصوصية 98.8%.**

**أثبتت هذه الدراسة ان تقنية الكروماتقرفي الممنع المستخدمة في هذه الدراسة لها نفس النتائج مقارنة بتقنية الأليزا بينما أعطت تقنية الكشف عن المستضد خصوصية اقل مقارنة بتقنية الأليزا. اثبتت هذه الدراسة ان تقنية الكروماتوقرفي الممنع والأليزا هي الطرق الافضل لفحص المتبرعين بالدم.**

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## List of abbreviation

| | |
|---|---|
| C.O | Cut-off |
| EIA | Enzyme immune assay |
| ELISA | Enzyme linked immunosorbent assay |
| FTA | Fluorescent treponemal antibodies |
| FTA-ABS | Fluorescent treponemal antibodies absorption |
| FN | False negative |
| FP | False positive |
| HBs Ag | Hepatitis Bs antigen |
| HCV | Hepatitis C antigen |
| HIV | Human immune deficiency virus |
| HRP | Horse reddish peroxidase |
| ICT | Immune chromatography test |
| Ig G | Immunoglobulin G |
| Ig M | Immunoglobulin M |
| MHA-TP | Microtiter hemagglutination *treponema pallidum* |
| OD | Optical density |
| PCR | Polymerase chain reaction |
| RBCs | Red blood cells |
| RPR | Rapid plasma reagin |
| SPSS | Statistical package of social sciences |
| TN | True negative |
| TP | True positive |
| TPHA | *Treponema pallidum* hemagglutination assay |
| TTI | Transfusion transmissible infection |
| TPI | *Treponema palllidum* immobilization |

| | |
|---|---|
| TPPA | *Treponema pallidum* particle agglutination assay |
| VDRL | Venereal disease research laboratory |
| WHO | World health organization |

# CHAPTER ONE

# INTRODUCTION

# 1. Introduction

## 1.1. Background

*Treponema pallidum* is the causative agent of syphilis, the most common sexually transmitted disease (Parij,2012). Syphilis, or lues, is a chronic venereal infection caused by the spirochete *Treponema pallidum.* First recognized in epidemic form in sixteenth-century Europe as the great pox, syphilis has remained an endemic infection in all parts of the world. Although penicillin and public health programs resulted in a gratifying reduction in cases of syphilis from the late 1940 until the late 1970, a significant resurgence of cases of both primary and secondary syphilis has been documented over the past 2 decades. There is a strong racial disparity, with African Americans affected 30 times more than whites in United States (Kumar *et al*; 2003).

World health organization recommends that all blood donations be screened for evidence of infection prior to the release of the blood and its components for clinical or manufacturing use. Screening of all blood donations should be mandatory for human immune deficiency virus HIV, hepatitis B and C and syphilis (WHO, 2017).

It is the responsibility of governments to assure a safe and sufficient supply of blood and blood products for all patients requiring transfusion. Each country should formulate a national blood policy and plan, as part of the national health policy, to define how safe blood and blood products will be made available and accessible to address the transfusion needs of its population, including how blood transfusion services will be organized and managed (WHO, 2009).

The provision of safe and efficacious blood and blood components for transfusion or manufacturing use involves a number of processes, from the selection of blood donors and the collection, processing and testing of blood donations to the testing

of patient samples, the issue of compatible blood and its administration to the patient. There is a risk of error in each process in this “transfusion chain” and a failure at any of these stages can have serious implications for the recipients of blood and blood products. Thus, while blood transfusion can be life-saving, there are associated risks, particularly the transmission of blood borne infections (WHO, 2009).

*T. pallidum* may be found in the blood stream, but levels are variable, and bacteremia is often short lived even in recent contamination. Moreover, the treponemes are relatively fragile and sensitive to cold, storage below +20°C for more than 72 hours destroys the organism and reduces dramatically the infectious risk. Although clearly potentially infectious, the risk of transmission through the transfusion of blood and blood components stored below +20°C is very low (Tagny, 2011).

Serological tests for syphilis contributed greatly to the detection of *T. pallidum* infection in blood donors and especially in those who were not identified during the medical selection. (Tagny, 2011).

Although it had some false positive results, it was a major advancement in the prevention of syphilis because it helped to diagnose the disease before the clinical manifestation and thus prevent its spread (Tagny, 2011).

several labs tests,treponemic or not treponemic exist, among which rapid tests, immunological tests, and genomic. The laboratory assessment of syphilis is generally based on the detection of antibodies against *T. pallidum* antigens in blood by the use of either specific or nonspecific reagents. The detection of genomic particle are more specific but not affordable for most of laboratories (Tagny, 2011).

## 1.2. Rationale

Syphilis is a transfusion transmitted infection (TTI) due to a *spirocheta* called *Treponema pallidum.* The germ is present in the blood of a contaminated blood donor and infects the recipient. The transmissibility of syphilis by blood transfusion has been frequently reported, chiefly based on animal experiments. Cases of syphilis transmitted by blood have been described in literature, with more than a hundred cases since the first description. The main cases reported were shown to occur when donors were in the primary or secondary stage of the disease (Tagny, 2011).

Developing countries are characterized by a difficult epidemiologic, sociological and economic environment which limits the implementation of a high quality of blood safety. Thus, this context requires that tests and algorithms should be selected so that they correspond with the high prevalence of the disease, limited technical know how of the personnel and limited availability of reagents and equipments. The selection criteria of screening strategy must include simple techniques, reliability, sustainability and cost effectiveness. Several blood banks use rapid test technique as it does not required sophisticated lab materials (Tagny, 2011).

Sudan is one of the developing countries ,data from this study could be helpful in detection of the syphilis among healthy blood donors in Central Blood Bank in Sudan in addition to compare between different methods (Enzyme Linked immunosorbent assay ELISA, Immunochromatographic test ICT and Rapid plasma regain RPR for the diagnosis of syphilis.

## 1.3. Objectives

### 1.3.1. General objective

To study Seroprevalence and comparison between methods of Syphilis detection among Blood Donors in Khartoum State/Sudan**.**

### 1.3.2. Specific objectives

A- To determine the frequency of syphilis *(Treponema pallidum*) among blood donors in Central Blood Bank (Khartoum State).

B- To compare between different methods for detection of syphilis (*Treponema pallidum* ) in the study area.

# CHAPTER TWO

# LITERATURE REVIEW

# 2. LITERATURE REVIEW

## 2.1. Historical background

In 1831 Ricord has designed a larger study on syphilis and gonorrhea and succeeded to show that the last occurs only after contact with gonorrohea patients, whilst the former only after contact with syphilis patients (Tampa *et al;* 2014).

It was not earlier than 1905 that Schaudinn (1871-1906) and Hoffman (1868-1959) have discovered the etiologic agent of syphilis, whom they have named *Spirochaeta pallid*, on various syphilis lesions, proving its existence in both fresh and Giemsa coloured specimens. It was them who changed the name of the bacterium subsequently to *Treponema pallidum*.(Tampa *et al;* 2014)

In 1906 Landsteiner introduced the use of the dark-field microscopy method for the detection of the *spirochete* of syphilis. In 1910 the German bacteriologist August Wasserman (1866-1925) came with the first serologic test for syphilis and in 1949 Nelson and Mayer have conceived *Treponema pallidum* immobilization test (TPI) the first specific test for *T.pallidum* (Tampa *et al;* 2014).

## 2.2 Previous Study in Sudan and other different countries

Study about prevalence of syphilis in pregnancy in Addis Ababa was conducted. A total of 410 pregnant women were included in the study, twelve tested positive for syphilis using VDRL giving a seroprevalence of 2.9% (Kebede E and Chamiso B, 2000).

Across-sectional study about syphilis among pregnant women in antenatal clinic of Juba Teaching Hospital,Malakaia National Health Insurance Center and Munuki Primary Health Care Centre in 2010 in Juba Southern Sudan , 231 pregnant women participants,51(22.1%) were positive for syphilis with the rapid

plasma regain test and 79(34.2%) were positive with the *Treponema pallidum* heamagglutination assay.in conclusions There is a high prevalence of syphilis in pregnant women attending the slected health facilities.Treponema pallidum haemagglutination assay can be used as a field test for syphilis due to its high sensitivity and specifity (Emmanuel, 2010).

In Africa, syphilis prevalence rates amongst pregnant women varies from 2.5% in Burkina Faso to 17.4% in Cameroon (WHO 2001).

A study about safety of blood transfusion was conducted in Egypt covering the period between 2006 and 2012 , of 308762 donors ,the overall prevalence of HCV antibodies HBs Ag, human immunodeficiency virus (HIV) and syphilis antibodies were 4.3%,1.22%,0.07%, and 0.13%, respectively (Omram *et al;* 2012).

The rate of primary and secondary syphilis reported in the United States decreased during the 1990 ,in 2000 the rate was the lowest since reporting began in 1941 ,although the rate of primary and secondary syphilis in the United States declined 89.7% during 1990-2000, the rate increased annually during 2001-2009 before decreasing in 2010 and remaining unchanged during 2011. During 2012 , rates again increased (Braxton *et al;* 2012).

### 2.3. *Treponema palidum*

The spirochetes are a large, heterogeneous group of spiral motile bacteria. One family (*Spirochaetaceae*) of the order *Spirochaetales* consists of two genera whose members are human pathogens,*Borrelia* and *Treponema*.The other family *(Leptospiraceae)* include one genus of medical importance *(Leptospiraceae)* (Carroll *et al;* 2016).

### 2.3.1. Typical organisms

*T .Pallidu*m are slender spirals measuring about 0.2 um in width and 5-15 um in length.the spiral coils are regularly spaced at adistance of 1 um from one another. The organisms are actively motile,rotating steadily around their endoflagella even after attaching to cells by their tapered ends.The long axis of the spiral is ordinarily straight but may sometimes bend so that the organism forms acomplete circle for moments at atime,returning then to its normal straight position (Carroll *et al;* 2016).

The spirals are so thin that they are not readily seen unless immunofluorescent stain or dark-field illumination is used.They do not stain well withaniline dyes,but they can be seen in tissues when stained by silver impregnation method (Carroll et al, 2016).

Pathogenic *T. pallidum* has never been cultured continuously on artificial media, infertile eggs,or in the presence of reducing substances, *T. pallidum* may remain motile for 3-6 days at 25°C. In whole blood or plasma stored at 4°C,organisms remainviable for at least 24 hours, which is of potential importance in blood transfusion (Carroll *et al;* 2016 ).

### 2.3.2. Reaction to physical and chemical agents

Drying kills the spirochete rapidly, as dose elevation of the temperature .Treponemes are rapidly immobilized and killed by trivalent arsenical,mercury,and bismuth (contained in drugs of historical interest in the treatment of syphilis). Penicillin is treponemicidal in minute concentrations,but the rate of killing is slow, presumably because of the metabolic inactivity and slow multiplication rate of *T. pallidum* (estimated division time is 30 hours).Resistance to penicillin has not been demonstrated in syphilis (Carrol *et al;* 2016 ).

*T. pallidum* is avery delicate bacterium. It is readily killed by drying or heating at 41–42°C for 60 minutes, at 0–4°C for 1–3 days. They are also readily killed on contact with distilled water, soap, arsenic compounds, and common antiseptics. *T. pallidum* strains can be preserved for laboratory use by many methods. It can be stored frozen in amedium containing 5% glycerol at 70°C or in liquid nitrogen ( Parija, 2012).

### 2.3.3. Genome

The *T. pallidum* genome is acircular chromosome of approximately 1,138,000 base pairs,which is small for bacteria. Most pathogenic bacteria have transposable elements,but *T. pallidum* does not ,which suggests that the genome is highly conserved and may explain its continued susceptibility to penicillin.There are few genes involved in energy production and synthesis of nutrients, indicating that *T. pallidum* obtains these from the host (Carrol et al; 2016 ).

### 2.3.4. Antigenic structure

The fact that *T. pallidum* cannot be cultured in vitro has markedly limited the characterization of its antigens. The outer membrane surrounds the periplasmic space and the peptidoglycan-cytoplasmic membrane complex. Membrane proteins are present that contain covalently bound lipids at their amino terminals. The lipids appear to anchor the proteins to the cytoplasmic or outer membranes and keep the proteins inaccessible to antibodies. The endoflagella are in the periplasmic space. *T. pallidum* has hyaluronidase that breaks down the hyaluronic acid in the ground substance of tissue and presumably enhances the invasiveness of the organism. The endoflagella are composed of three core proteins that ara homologous to other bacterial flagellin proteins plus an unrelated sheath protein.

Cardiolipin is an important component of the treponemal antigens (Carrol *et al;* 2016 ).

### 2.3.5. Transmission and epidemiology

*T.pallidum* is transmitted from spirochete containing lesions of skin or mucous membranes (e.g,genitalia, mouth, and rectum) of an infected person to other persons by intimate contact. It can also be transmitted from pregnant women to their fetuses. Rarely, blood for transfusions collected during early syphilis is also infectious. *T.pallidum* is a human organism only. There is no animal reservoir (Levinson, 2014).

Unprotected sex, promiscuous sex, and intravenous drug use are the major risk factors for syphilis. Doctors, nursing staff, and other healthcare workers are at occupational risk. (Parija, 2012 ).

Syphilis occurs worldwide, and its incidence is increasing. It is one of the leading notifiable diseases in the United States. Many cases are believed to go unreported, which limits public health efforts. There has been a marked increase in incidence of the disease in homosexual men in recent years. (Levinson, 2014).

### 2.3.6. Pathogenesis and Clinical Findings

*T.pallidum* causes veneral (transmitted through sexual contact) syphilis.The clinical presentation of veneral syphilis is varied and complex, often mimicking many other diseases (Forbes *et al;* 2007).

*T.pallidum* produces no important toxins or enzymes. The organism often infects the endothelium of small blood vessels, causing endarteritis. This occurs during all stages of syphilis but is particularly important in the pathogenesis of the brain and cardiovascular lesions seen in tertiary syphilis. In primary syphilis, the spirochetes multiply at the site of inoculation, and a local, nontender ulcer (chancre) usually

forms in 2 to 10 weeks. The ulcer heals spontaneously, but spirochetes spread widely via the bloodstream (bacteremia) to many organs. One to 3 month later, the lesions of secondary syphilis may occur. These often appear as a maculopapular rash, notably on the palms and soles, or as moist papules are called condylomata lata. These lesions are rich in spirochetes and are highly infectious, but they also heal spontaneously. Patchy alopecia also occurs. Constitutional symptoms of secondary syphilis include low-lymph-adenopathy. Pharyngitis, meningitis, nephritis, and secondary stages may not occur, and yet the disease may progress. (Levinson, 2014).

About one –third of these early (primary and secondary) syphilis cases will cure themselves, without treatment. Another third remain latent (on lesions appear, but positive serologic tests indicate continuing infection). The latent period can be divided into early and late stages, the symptoms of secondary syphilis can last for many years, no symptoms occur and patients are not infectious. In the remaining one-third of people, the disease progresses to the tertiary stag. Tertiary syphilis may show granulomas , especially of skin and bones, central nervous system involvement, also known as neurosyphilis, or cardiovascular lesions (e.g, aortitis,aneurysm of the ascending aorta). In tertiary lesions, treponemes are rarely seen. Also *T.pallidum* causes congenital syphilis (Levinson, 2014).

**2.4. Nonvenereal syphilis**

Congenital syphilis and occupational syphilis are example of nonvenereal syphilis (Parija, 2012 ).

## 2.5. Congenital syphilis

It is the most severe outcome of syphilis in humans. The infection occurs by vertical transmission from mother to fetus during pregnancy. If the mother is suffering from secondary syphilis and not treated for the same, a higher proportion of infants are affected compared to untreated early latent syphilis. In more than 40% of untreated maternal infection, *T. pallidum* causes late abortion, still birth, and death. Deaths in neonates may occur due to secondary bacterial infection, fulminant hepatitis, or pulmonary hemorrhage.

The infants suffering from congenital syphilis are born usually without any overt clinical manifestation of the disease. Poor feeding and rhinitis may be the earliest signs of congenital syphilis. Manifestations of the late onset congenital syphilis include neurosyphilis and involvement of the eighth cranial nerve, teeth, and bones (Parija, 2012 ).

## 2.6. Occupational syphilis

It is a condition that may occur in medical and paramedical workers handling a case of secondary syphilis. The lesion develops usually on the palm of infected health workers and may also occur on other exposed body part (Parija, 2012 ).

## 2.7. Syphilitic gumma

It is a solitary, localised, rubbery lesion with central necrosis, seen in organs like liver, testis, bone and brain. In liver, the gumma is associated with scarring of hepatic parenchyma (hepar lobatum) .Histologically, the structure of gumma shows the following features , Central coagulative necrosis resembling caseation but is less destructive so that outlines of necrosed cells can still be faintly seen,

Surrounding zone of palisaded macrophages with many plasma cells, some lymphocytes, giant cells and fibroblasts(Mohan, 2010).

## 2.8. Habitat

*T. pallidum* inhabits the genital tract of infected males and females. Reservoir, source, and transmission of infection *T. pallidum* is a strict human pathogen and does not naturally occur in any animal species. Humans are the only natural hosts. Infected human hosts secreting *T. pallidum* in serous transudates from moist lesions, such as primary chancre, condyloma latum and mucous patch, are the sources of infection (Parija, 2012 ).

## 2.9. Laboratory Diagnosis

### 2.9.1. Direct detection of *T. pallidum*

Darkground microscopy is the traditional method for direct detection of *T.pallidum* in lesion exudates, It can provide rapid results, with identification of the organism by its characteristic morphology and motility. Immunofluorescence using fixed smears of lesion material, or tissue specimens, has several advantages and is of comparable sensitivity ,neither technique differentiates between the pathogenic treponemes, the resluts were non-specific. More recently described monoclonal antibodies are more specific (Gillesspie and Hawkey, 2006).

### 2.9.2. Serology

#### 2.9.2.1. Non-Treponemal or Nonspecific Serologic Tests

These tests involve the use of nontreponemal antigens. Extracts of normal mammalian tissues ( cardiolipin from beef heart) react with antibodies in serum samples from patients with syphilis. These antibodies ,which are a mixture of IgG and IgM, are called reagin antibodies . Flocculation tests are Venereal Disease Research Laboratory VDRL and rapid plasma reagin RPR tests detect the presence of these antibodies. These tests are positive in most cases of primary syphilis and are almost always positive in secondary syphilis. The titer of these nonspecific antibodies decreases with effective treatment, in contrast to the specific antibodies, which are positive for life .False-positive reactions occur in infections such as leprosy, hepatitis B, and infectious mononucleosis and in various autoimmune diseases. Therefore, positive results have to be confirmed by specific tests (Levinson, 2014). Results of nonspecific tests usually become negative after treatment and should be used to determine the response to treatment. These tests can also be falsely negative as a result of the prozone phenomenon. In the prozone phenomenon, the titer of antibody is too high (antibody excess), and no flocculation will occur. On dilution of the serum, however, the test result becomes positive . These tests are inexpensive and easy to perform and therefore are used as a method of screening the population for infection (Levinson, 2014).

The laboratory diagnosis of congenital syphilis is based on the finding that the infant has a higher titer of antibody in the VDRL test than has the mother. Furthermore, if a positive VDRL test result in the infant is a false-positive one because maternal antibody has crossed the placenta, the titer will decline with time. If the infant is truly infected, the titer will remain high. However, irrespective of the VDRL test results, any infant whose mother has syphilis should be treated (Levinson, 2014).

#### 2.9.2.2. *Treponema*-specific tests

The *Treponema* specific tests measure antibodies specific for T. pallidum. These tests use live *T. pallidum* strains (*T. pallidum* immobilization test), or killed *T. pallidum* (*T. pallidum* agglutination test, *T. pallidum* immune adherence test, and fluorescent treponemal antibody test), also *T. pallidum* extracts as antigens (TPHA test and EIA enzyme immunoassa]) (Levinson, 2014).

***T. pallidum* immobilization test:** TPI test was the first specific treponemal test, which was introduced in 1949. This test detects the treponemal antibodies in patient's serum, which immobilize motile virulent *T. pallidum*. The test is performed by incubating live *T. pallidum* strains with test serum in the presence of complement. If the serum contains treponemal antibodies, the treponemes become immobilized, which can be demonstrated under dark ground microscope. The test when introduced was the most specific serological test for diagnosis of syphilis. But because of its complexity and difficulty in maintaining live treponemal strains, this test is no longer used and is replaced by newer tests, such as TPHA and fluorescent treponemal antibody absorption (FTA-Abs) tests(Levinson, 2014).

***T. pallidum* agglutination test**: *T. pallidum* agglutination test uses killed *T. pallidum* suspension inactivated by formalin. The test is performed by mixing the formalin inactivated suspensionof *T. pallidum* with patient's serum. If antibodies are present in the serum, it leads to agglutination of treponemal antigen,which can be demonstrated by dark ground microscopy. However, the test is no longer used, because it is non-specific and is associated with false positive reactions(Levinson, 2014).

***T. pallidum* immune adherence test:** In this test, a suspension of inactivated treponemes is incubated with test serum, complement, and fresh heparinized whole blood from normal individuals. If antibodies are present, treponemes are found to adhere to the erythrocytes. If antibodies are absent, the treponemes do not adhere to the erythrocytes. This test is also not used now adays(Levinson, 2014).

**Fluorescent treponemal antibody test:** FTA test is the mostspecific and popular test used for diagnosis of syphilis. FTA is an indirect immunofluorescence (IIF) test, which uses acetone fixed smears of *T. pallidum* on the slides. The test is performed by adding a drop of test serum to the smear on the slide followed by washing and re incubating the smear with fluorescent labeled antihuman immunoglobulin. The slide is examined under a fluorescence microscope (Levinson, 2014). Demonstration of fluorescent treponemes is suggestive of a positive FTA test.FTA absorption (FTA-Abs) is a modification of FTA test,which shows high sensitivity and specificity. In this test, patient's serum is first absorbed with a sonicated extract of non-pathogenic T. phagedenis to remove group specific antibodies. The test is almost as specific as the TPI test and is considered as a standard reference test in syphilis serology. The FTA-Abs test is positive in 80% primary syphilis, 100% secondary syphilis, and 95% tertiary syphilis. The test is highly specific (92–99%). The test shows occasional false positive reactions in patients with rheumatoid arthritis, systemic lupus erythematosus, cirrhosis, and hypergammaglobulinemia. The IgM FTA-Abs test is another modification of FTA-Abs used to detect serum IgM antibodies in congenital syphilis. This test is used to differentiate seropositivity due to passively transferred IgG maternal antibodies to the fetus from IgM antibodies found in utero in congenital syphilis (Levinson, 2014).

**TPHA test:** TPHA test uses erythrocytes sensitized with asonicated extract of *T. pallidum* as antigen. TPHA is now been modified to perform in microtiter plates and is referred to as microtiter hemagglutination *T. pallidum* (MHA-TP) test. This test is performed by incubating the serial dilution of the patient's serum with erythrocytes sensitized with *T. pallidum* antigen in a microtiter plate. If antibodies are present, hemagglutination of RBCs occur . The serum samples before testing for TPHA are reabsorbed with a diluent containing Reiter's treponeme, rabbit testes, and sheep erythrocytes. TPHA is the most widely used treponemal test

available commercially. It is simple, economical, and does not require any expensive sophisticated equipment (Levinson, 2014).

**Enzyme immunoassay:** EIA uses ultrasonicated *T. pallidum* antigen coated on tubes or ferrous metal beads as solid-phase carrier for antigen. The serum antibodies are detected by an enzymatic reaction. The test is available commercially. All the treponemal tests are not completely specific for syphilis. They also show false positive reactions in patients with other spirochetal diseases, such as leptospirosis, relapsing fever, Lyme disease, pinta, yaws, and rat-bite fever (Parija, 2012).

### 2.9.3. Rapid test

several simple.rapid tests for treponemal antibody are now commercially available .most are in the format of an immunochromatographic strip.An evaluation of these assays was recently published by the WHO sexually Transmitted Diseases Diagnostics Initiative,The rapid assays are potentially suitable for non laboratory use in the developing world,and they may also have arole as point of care tests elsewhere (Gillesspie and Hawkey 2006).

### 2.9.4. Culture

*T. pallidum* does not grow in artificial culture media. *T. pallidum* had been maintained for long time by subculture in animals.*Nichole's strain of T. pallidum*: It is a pathogenic strain, which has been maintained for several decades by serial passage in rabbit testes. This strain was isolated in 1913, originally from the brain of a fatal case of a patient with general paralysis of insane. This strain is most commonly used for diagnostic and research purposes in the laboratory. Strains of *T. pallidum* have been maintained for a short period in cell lines, such as Eagle

and McCoy cell lines supplemented with fetal bovine serum and reducing agent (Parija, 2012).

### 2.9.5. Molecular techniques

Clinical specimens often contain very low numbers of an infecting pathogenic microbe, amplification methods can be used to increase the sensitivity of the test and the likelihood of detection. Such methods remove the need to grow the organism in vitro, aslow and variable process. Polymerase chain reaction PCR is an example of molecular techniques using a variety of targets and test formats with convential or real time PCR (De La Maza *et al;* 1997).

## 2.10. Treatment of syphilis

Primary, secondary, or early latent use penicillin G or tetracycline(2weeks) ,late,latent or benign tertiary use penicillin G benzathine or tetracycline (4 weeks),neurosyphilis or pregnancy or HIV use aqueous crystalline or procaine penicillin G or desensitization and penicillin.(Sastry and Bhatk, 2015).

## 2.11. Prevention

Prevention depends on early diagnosis and adequate treatment, use of condoms, administration of antibiotic after suspected exposure, and serologic follow-up of infected individuals and their contacts. The presence of any sexually transmitted disease makes testing for syphilis mandatory, because several different infections are often transmitted simultaneously. There is no vaccine against syphilis. (Levinson, 2014). Avoiding transmission of *T.Pallidum* in blood The transmission of T.pallidum by blood transfusion can be avoided by storing all donor blood at 2-

6 C for 3 – 5 days and collecting blood from low risk donors or preferably from donors previously serologically screened for syphilis (Cheesbrough 2003).

### 2.12. Other treponemal infections

Three geographically localized treponemal diseases closely mimic syphilis . They include bejel (found in hot , arid areas of Africa, Southeast Asia , and the Middle East), yaws (found in humid, tropical countries), and pinta (found in South and Central America, Mixico,and the phillipin). Unlike syphilis, direct skin contact, crowded living conditions, and poor hygiene contribute to the spread of these diseases. Sexual contact is usually not the mode of transmission, and congenital infections occur rarely if at all. All three diseases are curable with penicillin. (Harvey *et al;* 2007).

### 2.13. Syphilis and Blood Safety

Syphilis is a transfusion-transmitted infection (TTI). The germ is present in the blood of a contaminated blood donor and infects the recipient. The transmissibility of syphilis by blood transfusion has been frequently reported, chiefly based on animal experiments. Cases of syphilis transmitted by blood have been described in literature, with more than a hundred cases since the first description (Tangy, 2011).

The main cases reported were shown to occur when donors were in the primary or secondary stage of the disease. (Syphilis) detection of specific Treponema antigens is possible using methods as passive agglutination, as *T. pallidum* hemagglutination (TPHA) assay or the *T. pallidum* particle agglutination (TPPA) assay, indirect immunofluorescence as the fluorescent treponemal antibody absorbed (FTA-ABS) assay or enzyme immunoassay (EIA) for the detection of specific IgG and IgM or total Ig. Non-treponemal methods are based on non-

treponemal lipid antigens (cardiolipin), using frequently the flocculation technique. Of these, the Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests are the most commonly used. These tests are cheap, fast and more sensitive .They are able to identify the contaminated blood donors few days before the treponemal test and thus useful for acute infection. However, VDRL and RPR cannot be automated and are time-consuming if used for large scale testing. Moreover, they produce more false positive results. These tests are routinely used to screen blood donors. False positives on the rapid tests can be seen in viral infections such as hepatitis, tuberculosis, malaria, or varicella. Thus, non-treponemal tests should be followed up when possible by atreponemal test. The treponemal tests are based on monoclonal antibodies and immunofluorescence they are more specific and more expensive. The tests based on enzyme-linked immunoassays are the more specific and are usually used to confirm the results of simpler screening tests for syphilis (Tangy, 2011).

# CHAPTER THREE

# MATERIALS AND METHODS

# 3. Materials and Methods

### 3.1. Study design

Cross sectional study.

### 3.2. Study area

This study was conducted in Central Blood Bank / Khartoum State.

### 3.3. Study population

Blood donors attending Central Blood Bank.

### 3.4. Study duration

Study was carried out during March to May, 2018.

### 3.5. Sample size

A total of ninety tow subjects (n=92) were enrolled in this study.

### 3.6. Data collection

Personal and clinical data were collected from blood donors by direct interviewing questionnaire from each subject.

### 3.7. Ethical considerations

Permission to carry out the study was obtained from the College of Graduate Studies, Sudan University of Science and Technology.

### 3.8. Sample collection

Under aseptic condition, venous blood (3 mls) were obtained from blood donors, samples were collected by vein puncture using tubes did not contain anticoagulant, serum was collected from whole blood and left to settle for 30 minutes in the rack for blood coagulation and then was centrifuged to get serum specimen supernatant with avoidance of hemolysis. If specimen were not immediately tested they were kept at – 20 °C.

### 3.9. Laboratory Methods

The serological techniques Immunochromatographic test ICT, Rapid plasma regains RPR and Enzyme linked immunosorbent assay ELISA were used for detection of syphilis among blood donors.

#### 3.9.1. Immunochromatography test

##### 3.9.1.1. Principle of the test

The syphilis rapid test cassette (whole blood/serum/plasma) is a qualitative membrane based immunoassay for the detection of treponemal antibodies (IgG and IgM) in whole blood, serum or plasma. In this test procedure, recombinant syphilis antigen is immobilized in the test line region of the test.After specimen is added to the specimen well of the cassette, it reacts with syphilis antigen coated

particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contains treponemal antibodies, a colored line will appear in the test line region, indicating a positive result. If the specimen does not contain treponemal antibodies, a colored line will not always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

### 3.9.1.2. Storage and stability

**Kits:** Stored as packaged in the sealed pouch at room temperature 25 °C. They could be kept at ( 2-30°C ). The kits were used before the expiration date printed on the sealed pouch. The test remained in the sealed pouch until use.

**Specimens:** Serum or plasma stable for 72 hours at 2-8°C. Samples free from contamination and haemolysis. For long term storage, specimens should be kept below -20 °C .

### 3.9.1.3. Procedure of the test

The test cassette was removed from the sealed pouch and placed on a clean and level surface, the dropper was hold (which found with the cassette) vertically and 1 drop of serum was transferred (approximately 40ul) to the specimen area, then 1 drop of buffer was added (approximately 40ul ), the result was read at 5 minutes and not more than 20 minutes.

### 3.9.1.4. Interpretation of the results

According to manufacture instructions as follows:

**Positive:** Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T). The intensity of the color in the test line region (T) will vary depending on the concentration of treponemal antibodies present in the specimen. Therefore, any shade of color in the test line region (T) considered positive.

**Negative:** One colored line appears in the control line region (C). No line appears in the test line region (T).

**Invalid:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reason for control line failure.

### 3.9.1.5. Quality control

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique. Control standards are not supplied with this kit however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

## 3.9.2. Rapid Plasma Regain RPR

### 3.9.2.1. Principle of the test

The Rapid Plasma Reagin or RPR card test is a non treponemal method for the serological detection of syphilis. The antigen a particulate carbon suspension coated with lipid complexes agglutinates in the sera of syphilitic patients. Visible

agglutination in the form of black clumps which can be viewed macroscopically, indicate the presence of such antibodies in the sample tested.

**3.9.2.2. Storage and stability**

**Reagents:** Components stored at 2-8°C . Cards and pipettes may be kept at room temperature.

**Specimens:** Serum or plasma stable for 48 hours at 2-8°C . Samples should be free from contamination and haemolysis. Lipemic will not affect the test results unless it is severe enough to obscure the state of the antigen particles.

**3.9.2.3. Reagent preparation**

According to manufacture instructions as follows:

The carbon antigen was resuspended gently to ensure thorough mixing. The required volume of carbon antigen was transfered to the dispensing bottle. The dispensing bottle was labeled with the antigen lot number, expiry and transfer dates. Once the antigen has been transferred to the dispensing bottle; it is stable for 3 months.

**3.9.2.4. procedure of the test**

According to manufacture instructions as follows:

The reagents and samples were brought to room temperature. 50ul of sample and 1 drop of control were placed into separate circles on the card. The antigen gently resuspended. One drop of free falling antigen were added to each test circle and mixed with the disposable pipette/stirrer and spread over the entire area enclosed by the ring. A new stirrer was used for each sample. The cards rotated at 100 r.p.m for 8 minutes.

### 3.9.2.5. Interpretation of the results

According to manufacture instructions as follow:

**Reactive:** Medium and Large clumps .

**Weakly Reactive**: Small clumps

**Non Reactive:** No clumping or very slight roughness..

### 3.9.2.6. Quality control

Each run of tests should be validated with a positive and negative control.

### 3.9.3. Inzyme Linked Immunosorbent assay ELISA

### 3.9.3.1. Principle of the assay

The detection of anti treponemal antibodies is achieved by antigen sandwich enzyme linked immunosorbent assay, where the microwells are coated with recombinant *Treponema pallidum* antigens expressed in *E.coli*. The sample is incubated in the micro wells together with recombinant treponemal antigens conjugated to HRP. The pre coated antigens, but are expressed in different hosts. In case of presence of anti treponemal in the sampl, during incubation the pre coated and conjugated antigens will be bound to the two variable domains of antibody and the specific antigen antibody immunocomplex is captured on the solid phase.

After washing to remove sample and unbound conjugates, chromogen solution containing TMB and urea peroxidase are added into the wells. In presence of the antigen antibody sandwich complex, the colorless chromogen is hydrolyzed by the bound HRP conjugate to a blue coloured product, which turns yellow upon addition of the stop solution. This color is then read photo metrically and is

directly proportional to the amount of the antibody in the sample. Wells containing samples negative for anti treponemal antibodies remain colorless.

Assay principle scheme: Double antigen sandwich ELISA

Ag(p) + Ab(s) + (Ag)ENZ→ [Ag(p)-Ab(s)-(Ag)ENZ}] →blue→yellow (positive)

.

Ag(p) +(Ag)ENZ→[Ag(p)]→no color (negative).

**Ag(p)** pre coated recombinant anti treponemall antibodies.

**Ab(s)** treponemal antibodies in sample.

**(Ag) ENZ** HRP conjugated recombinant treponemal antigens.

**3.9.3.2. Storage and stability**

**Kits:** Components stored at 2-8°C.

**Specimens:** Serum or plasma stable for 72 hours at 2-8°C if delay occur stored frozen at -20°C or less. Multiple freeze – thaw cycles should be avoided. Samples should be free from contamination and haemolysis.

**3.9.3.3. Reagent preparation**

According to manufacture instructions as follow:

The reagents and samples allowed to reach room temperature (18-30°C) for at least 15 – 30 minutes. The wash buffer concentrate was checked for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. The stock wash Buffer was diluted 1 to 20 with distilled or deionized water. Useing clean vessels to dilute the buffer. For one plate, 50ml of the concentrate was mixed with 950ml of water for a fnal volume of 1000ml diluted wash Buffer.

### 3.9.3.4. procedure of the test

**Numbering wells:** The strips needed was set in strip holder and numbered sufficient number of wells including three negative control, two positive control .

**Adding HRP conjugate:** 100 HRP conjugate were added in to each well.

**Adding sample:** 20ul of positive control, negative control, and specimen were added into their respective wells. Upon addition of the sample the HRP conjugate sample mixture will appear blue. A separate disposable tip was used for each specimen.

**Incubating:** The plate was mixed by tapping gently. And covered over the plate with the plate cover and incubated for 60 minutes at 37°C.

**Washing:** At the end of the incubation, the plate cover was removed and discarded. Each well was washed 6 times with diluted wash buffer. Each time, the microwells allowed soaking for 30-60 seconds. After the final washing cycle, the plate was turned down onto blotting paper or clean towel, and tapped to remove any remaining solution.

**Colouring:** 50ul of chromogen A and 50ul chromogen B solution were dispensed into each well , and mixed by tapping the plate gently. The plate was incubated at 37°C for 15minutes avoided light. The enzymatic reaction between the chromogen solutions and the HRP conjugate produced blue color in positive control and anti treponemal positive sample wells.

**Stopping reaction:** 50ul stop solution were added into each well and mixed gently. Intense yellow color developed in positive control and anti treponemal antibodies positive sample wells.

**Measuring the absorbance:** The reference wavelength were set at 630nm. The cut-off value were calculated and the results evaluated (the absorbance was read within 5 minutes after stopping the reaction.

### 3.9.3.5. Interpretation of the results

According to manufacture instructions as follow:

Each micro plate considered separately when calculating and interpreting results of the assay. The results were calculated by relating each samples optical density (OD) value to the cut – off value ( C.O) of the plate.

**Calculation of cut-off value:** Cut-off value (C.O) = *Nc + 0.18

*Nc = the mean absorbance value for three negative controls.

=0.011+0.009 =0.02/2=0.01

0.01+0.18=0.19

C.O = 0.19

The mean value is calculated using the two values of negative control.

**Negative Results (S/C.O < 1):** samples giving an absorbance less than the cut-off value were considered negative, which indicates that no anti treponemal antibodies have been detected with this anti treponemal ELISA kit, and there are no serological indications for past infection with *Teponema pallidum.*

**Positive Results (S/C.O. > or =1):** Samples giving an absorbance greater than or equal to the cut-off value were considered initially reactive, which indicates that anti treponemal antibodies have been detected with this anti treponemal ELISA kit.

**Borderline (S/C.O = 0.9-1.1):** Samples with absorbance to cut – off ratio between 0.9 and 1.1 are considered. Repeatedly positive samples can be considered positive for ant treponemal antibodies.

### 3.9.3.6. Quality control

The test results are valid if the quality control criteria are verified. The OD value of the positive control must be equal to or greater than 0.800 at 450/630nm. The OD value of the negative control must be less than 0.100 at 450/630nm.

## 3.10. The evaluation of the test results

In order to determine how useful the test is to detect a disease the sensitivity and specificity were calculated. ELISA method was used as gold standard test to determine the prevalence of disease, RPR and ICT tests were interested in determining their sensitivity and specificity.

The sensitivity and specificity were calculated as follow: For people that have the characteristic (as determined by the gold standard which we used ELISA method), the number of people who tested positive and negative were recorded. And same was done with the people that do not have the characteristic (as determined by the gold standard). And ended up with four numbers. People with the characteristic and tested positive were true positive (TP). People with the characteristic and tested negative were the false negatives (FN). People without the characteristic and tested positive were the false positive (FP). People without the characteristic and tested negative were true negatives (TN). To calculate the sensitivity, TP was divided by (TP+FN) and to calculate the specificity, TN was divided by (FP+TN) (wikihow, 2018).

### 3.11. Data analysis

Statistical Package of Social Sciences (SPSS version 18). Computer software was used for data analysis.

# CHAPTER FOUR

# RESULTS

# 4. Results

A total of ninety two blood donors were tested for syphilis .The age range was 18 to 50 years, all of them were males. The seroprevalence of syphilis revealed from ELISA method 7(7.6 %), were it was equal in ICT and increase to 8(8.7 %) in RPR.

## 4.1. Distribution of syphilis among blood donors according to age (result given by ELISA)

The higher frequency of syphilis was found in age group [26-35] 3(42.9%) and age group [36-45] 3(42.9%), and there was no positive cases among the ages of [46-55] years as shown in fig 4.2.

## 4.2. Distribution of syphilis among blood donors according to marital status (result given by EILISA)

As shown in fig.4.3, out of 92 examined samples 5 (71.4%) of positive samples were single blood donors, 2 (28.6)% were married and there were no positive cases among divorced blood donors.

## 4.3. ELISA results

Out of 92 examined blood samples 7 (7.6%) were found positive and 85 (92.4) were found negative for syphilis by ELISA method as shown in fig 4.1.

### 4.4. ICT results

Out of 92 examined blood samples 7 (7.6%) were found positive and 85 (92.4) were found negative for syphilis by ICT method. The same result was found by ELISA.

### 4.5. RPR results

Out of 92 examined blood samples 8 (8.7%) were found positive and 84 (91.3) were found negative for syphilis by RPR method.

### 4.6. Sensitivity and specificity of ICT

ELISA was the gold method
True positive = 7
False negative = 0
False positive = 0
True negative = 85
Sensitivity = TP/ (TP+FN).
Sensitivity = 7/ (7+0) = 1x100 = 100%
Specificity = TN/ (FP+TN)
Specificity = 85/(0+85) = 1x100=100%
ICT method shows 100% sensitivity and 100% specificity.

### 4.7. Sensitivity and specificity of RPR

ELISA was the gold method

True positive = 7

False negative = 0

False positive = 1

True negative = 84

Sensitivity = TP/ (TP+FN).

Sensitivity = 7/ (7+0) = 1x100 = 100%

Specificity = TN/ (FP+TN)

Specificity = 84/ (1+84) = 0.988 x100 = 98.8%

RPR show 100% sensitivity and 98.8% specificity.

# CHAPTER FIVE

# DISCUSSION

# 5. Discussion

This study was conducted to detect the frequency of syphilis among blood donors in Central Blood Bank of Khartoum State in Sudan from March to May 2018. Ninety two samples were tested for syphilis, the age range was 18 to 50 years, all of them were males. The seroprevalence of syphilis revealed 7 (7.6 %) by ELISA which was the gold standard method in this study compare to other study we can find different results. A retrospective analysis study about sero-prevalence of HIV and syphilis infections among blood donors at Kosti Teaching Hospital –White Nile State Sudan was conducted between January 2014 and May 2014, a total number of donors were 1204 all were males, the sero-prevalence for antibodies against HIV and *Treponema pallidum* was positive in 8 (0.7%) and 82 (6.8%) donors, respectively (Bazie *et al;* ,2014).

Another study about prevalence of syphilis infection among adult rural residents of Hassai and Bir Agam area in Red Sea State in Sudan,27 of the 125 samples tested in this study (21.6) were positive for anti *Treponema pallidum* antibodies and 98 of them (78.4%) were negative (Abdalla and Ismail, 2014).

In vitro comparative study, conducted in august 2015 at Central Blood Bank, Gezira State Sudan. 200 samples were collected from blood donors in Central Blood Bank 187 were negative by enzyme linked immuno sorpant assay (ELISA),while they were 191 by immunochromatography test(ICT) ,the positive cases were 13 for ELISA test ,while they were 9 cases for ICT (Abdalla *et al;* 2017). The Gezira State results disagree with this study not only in the prevalence of the disease but also in the specificity and sensitivity of ICT method which show sensitivity 69.2%, specificity 97.9% but in this study the specificity and sensitivity of ICT were equal to ELISA which show 100% sensitivity and 100% specificity, and it may be due to the difference in quality in these test which can differ according to the manufacture. A study about prevalence of syphilis among blood and stem cell donors in Sudi Arabia was conducted. About 240,000 blood donors

were screened, most of the blood donors were males (98.3%), approximately 0.044% of all the blood donors were syphilis positive. No cases were detected as positive among stem cell donors (Elyamamy *et al;* 2016).In this study there was different prevalence but all showed that the hazard was found if using blood for transfusion before testing for this disease.

The RPR result in this study show specificity 98.8%, one of the negative samples by ELISA show positive by RPR and this may be due to different causes. The false positive result by the RPR is related to anti-cardiolipin antibodies produced in other infections eg, narcotic drug abuse , chickenpox, acute malaria and HIV (Cheesbrough, 2003).

## 5.1. Conclusion

ICT and ELISA were the best methods for screening blood donors.High prevalence was observed among the age of [26-45] years and also among single cases.

## 5.2. Recommendations

**1.** More studies are needed with large sample size to support the result which was obtained in this work**.**

**2.** Blood donor should be tested for syphilis before donation.

**3.** ELISA, ICT and RPR can be used for the screening of blood donors, those tests showed high sensitivity according to this study. In the area of Sudan which have poor health service and there is no ability for doing ELISA, ICT and RPR can be the substitution.

**4.** ICT and RPR can use just for screening but to confirm the diagnosis for the patient it is better to use other more advanced techniques like ELISA or more better Polymerase chain reaction PCR.

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# Appendices

# Appendix (1)

## Sudan University of Science and Technology

## College of Graduate Studies

## Microbiology Department

## Questionnaire

## Prevalence of syphilis among blood donors in Kartoum state-Sudan

Date…….  Questionnaire NO…..

1/Age

A/15-25  B/26-35  C/36-45  D/46-55

2/Job

A/Employee  B/Labor  C/Free work  D/Other…….

3/Social State

A/Married  B/Single  C/Divorce  D/Widow

4/Suffering from other STD diseases

A/AIDS  B/Hepatitis B  C/Hepatitis C

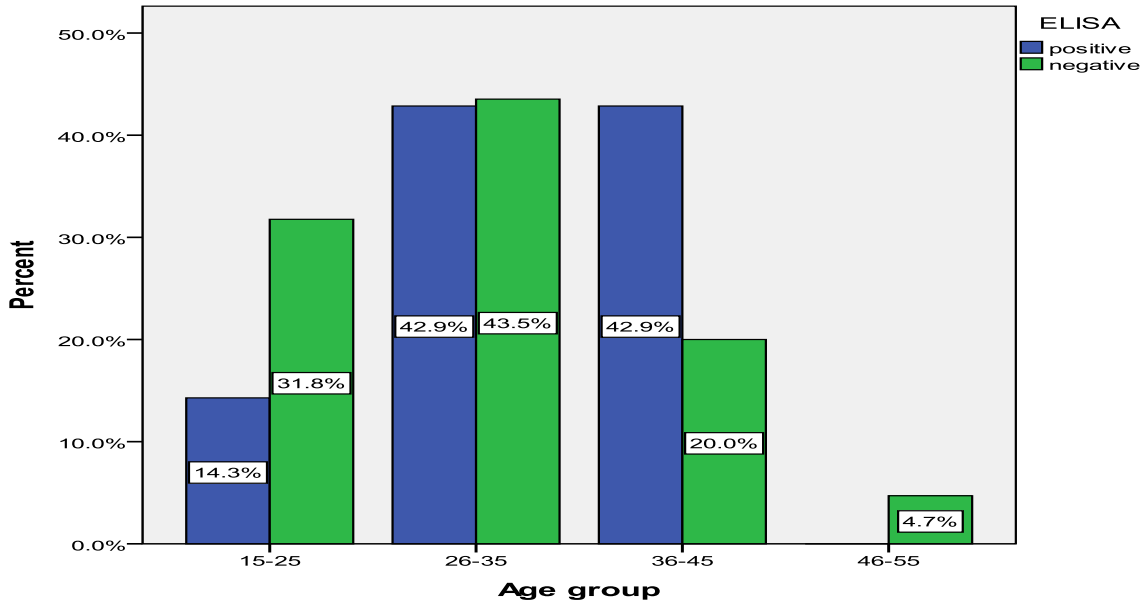


Fig 4.2 Distribution of syphilis among blood donors according to age by ELISA method.

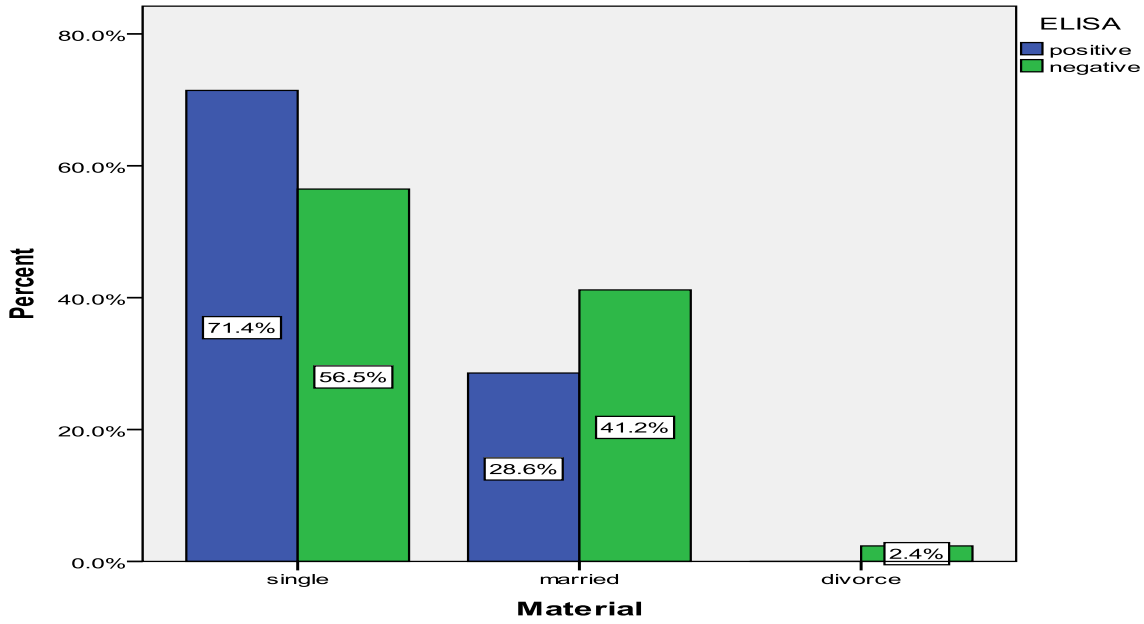


Fig 4.3 Distribution of syphilis among blood donors according to material status by ELISA method.

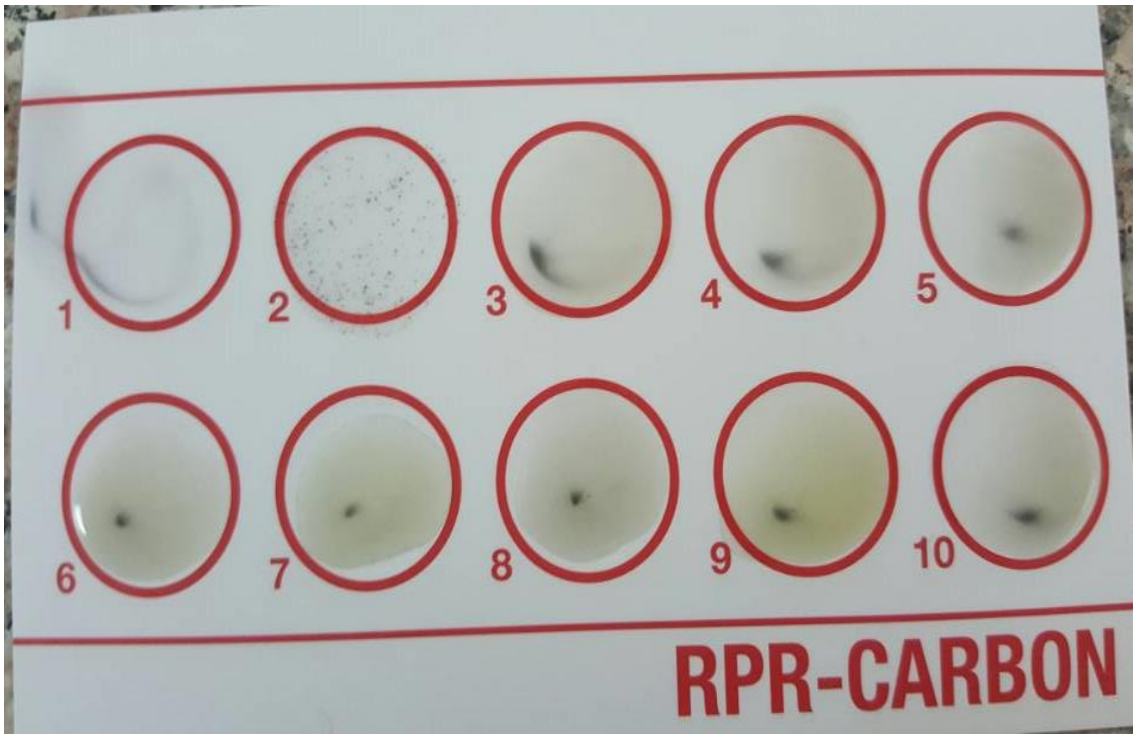


Fig 4.4.RPR card sample NO 1 was control negative and sample NO 2 was control positive, the other samples were all non reactive.

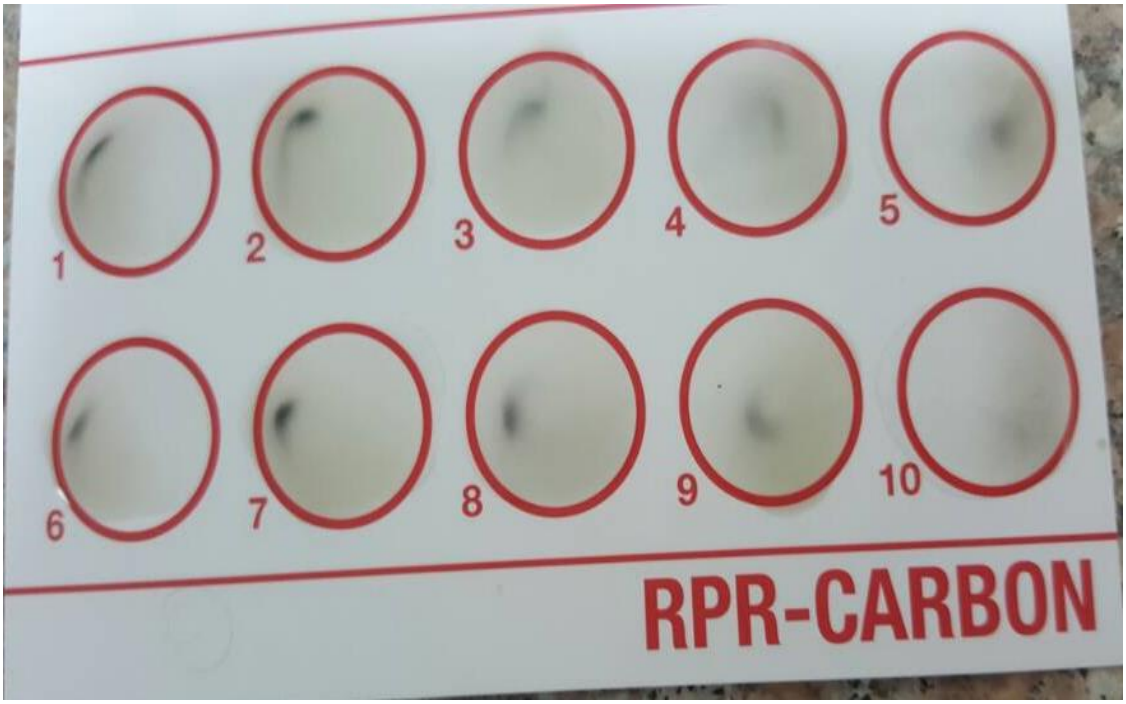


Fig 4.5.RPR card in this card all was non reactive except NO 10 was reactive.

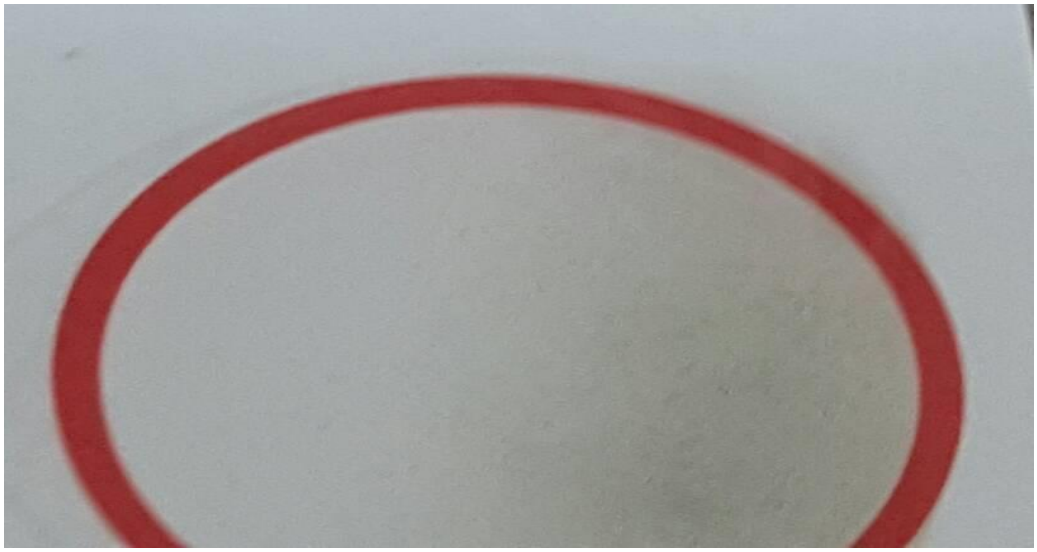

Fig 4.6. RPR reactive reaction.

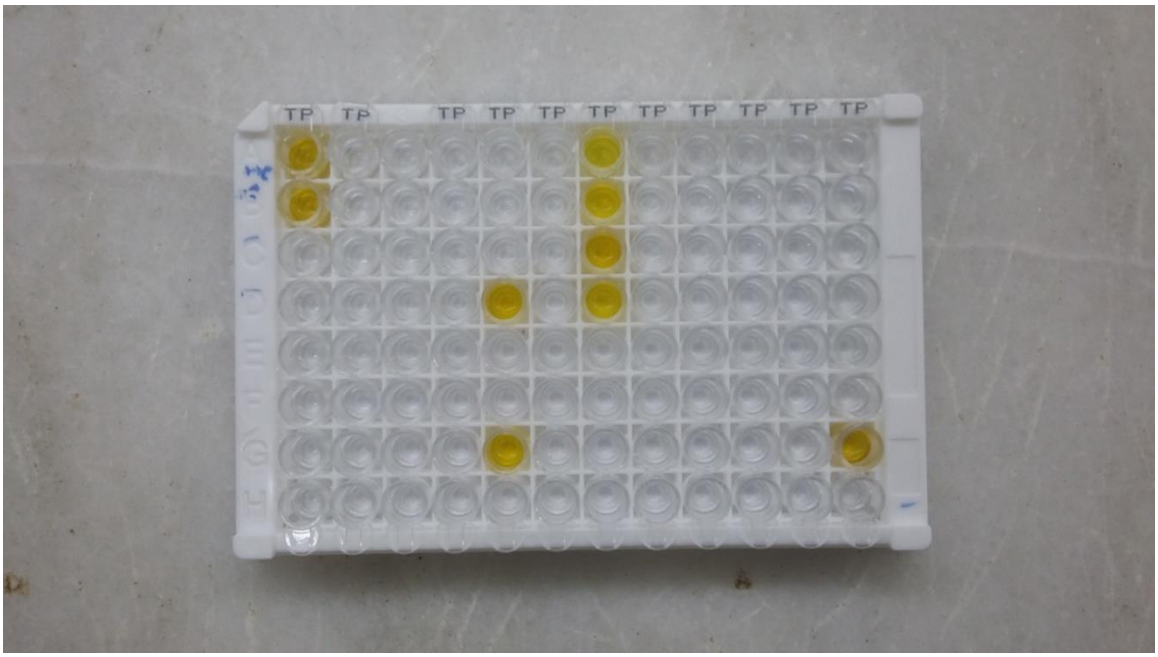


Fig 4.7.ELISA microtiter plate yellow wells indicate positive results the tow upper left was control positive.

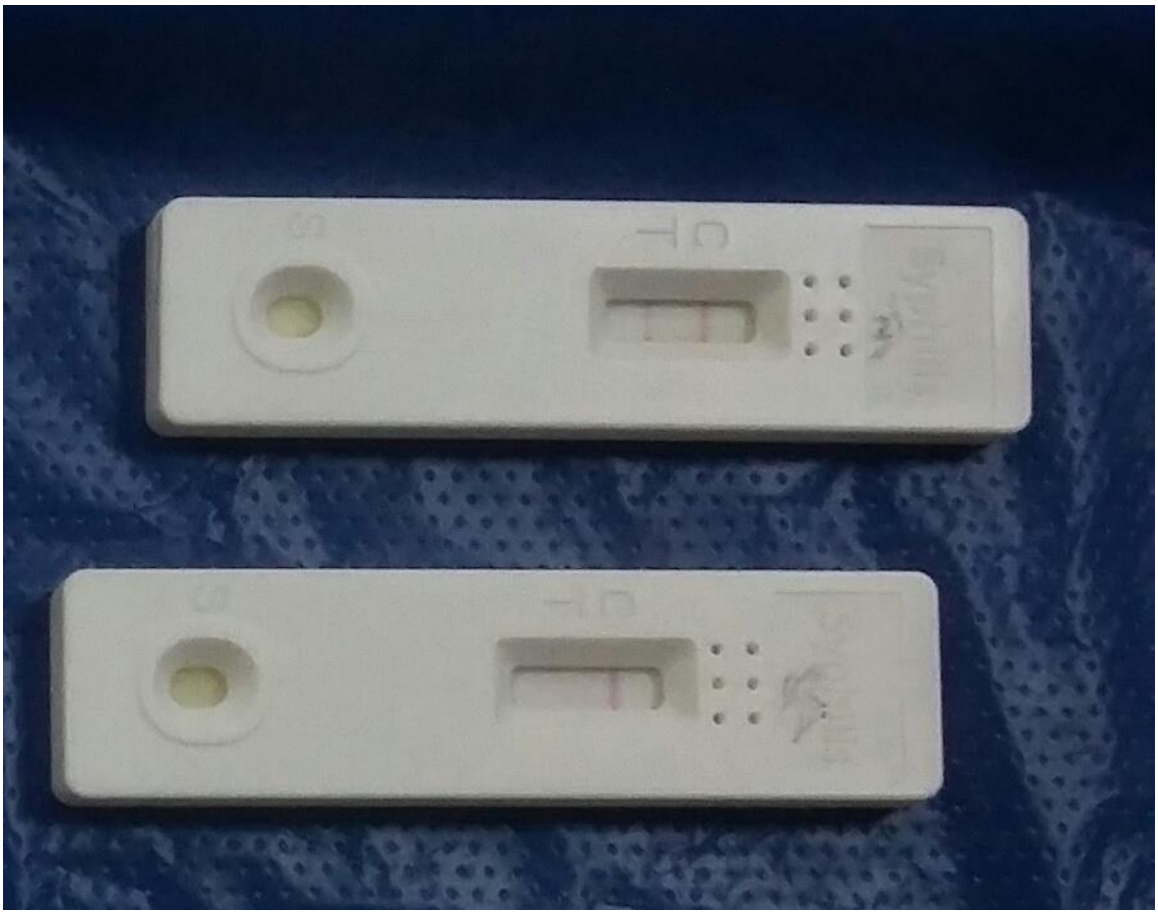

Fig 4.8.ICT rapid test device the upper was positive and the lower was negative.

fortress diagnostics

ISO 13485 accredited company

**BXE0995A**

96 TESTS

STORE AT 2-8°C

**FOR IN-VITRO DIAGNOSTICS USE ONLY**

# Syphilis Treponema pallidum Antibody Elisa

Fortress Syphilis Elisa is an in vitro diagnostic kit for the detection of antibodies to Treponema pallidum in human serum or plasma.

**Intended Use:**

1. For screening of blood donors.
2. Aid in diagnosis and management of clinical conditions of syphilis.

**Principle of the Assay:**

The detection of anti-TP antibodies is achieved by antigen sandwich enzyme linked immunosorbent assay, where the microwells are coated with recombinant Treponema pallidum antigens expressed in E.coli. The sample is incubated in the microwells together with recombinant TP antigens conjugated to HRP. The pre coated antigens express the same epitopes as the HRP conjugate antigens, but are expressed in different hosts. In case of presence of anti-TP in the sample, during incubation the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and the specific antigen-antibody immunocomplex is captured on the solid phase.

After washing to remove sample and unbound conjugates, chromogen solution containing TMB and urea peroxidise are added into the wells. In presence of the antigen-antibody sandwich complex, the colourless chromogen is hydrolysed by the bound HRP conjugate to a blue coloured product , which turns yellow upon addition of the stop solution. This colour is then read photometrically and is directly proportional to the amount of the antibody in the sample. Wells containing samples negative for anti-TP remain colourless.

**Assay principle scheme: Double antigen sandwich ELISA**

**Ag(p) + Ab(s) + (Ag)ENZ →[Ag(p)–Ab(s)–(Ag)ENZ] →blue→yellow (+)**

**Ag(p) +(Ag)ENZ → [Ag(p) ]→ no color (-)**

**Incubation Immobilized Complex Coloring Results**

**60 min. 15min.**

**Ag(p)**–pre-coated recombinant anti–treponema pallidum antibodies;

**Ab(s)**–TP antibodies in sample;

**(Ag)ENZ**–HRP conjugated recombinant TP antigens.

**Kit Contents:**

| Kit Contents; | Volume |
|---|---|
| Microwell Plate 96 Tests | 1 plate ( 12x8/8x12 well strips per plate) |
| Negative Control | 1x0.5ml |
| Positive Control | 1x0.5ml |
| HRP – Conjugate Reagent | 1x13 ml |
| Stock Wash Buffer | 1x50ml (Dilute 1 to 20 with distilled water before use. Once diluted, stable for two weeks at 2-8°C). |
| Chromogen Solution A | 1x7ml (Ready to use and once open, stable for one month at 2-8°C) |
| Chromogen Solution B | 1x7ml (Ready to use and once open, stable for one month at 2-8°C) |
| Stop Solution | 1x7ml |
| Plastic Sealable Bag | 1 Unit |
| Plate Cover | 1 Sheet |
| Package Inserts | 1 Copy |

**Additional Materials And Instruments Required But Not Provided:**

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

**Specimen Collection and transportation:**

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

**Special Instructions for Washing Plates:**

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50 ml of the concentrate with 950ml of water for a final volume of 600ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

**Storage and Stability:**

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C, **do not freeze**. To assure maximum performance of this anti-TP ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

**Precautions and Safety:**

Fortress anti-TP ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Shake the reagent gently before use, and return to the storage temperature immediately after use.
5. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
6. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
7. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
8. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
9. Avoid assay steps interspersed with time interruptions. Assure same working conditions for all wells.
10. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
11. The use of automatic pipettes is recommended.
12. Assure that the incubation temperature is 37° inside the incubator.
13. When adding samples, avoid touching the well's bottom with the pipette tip.
14. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
15. All specimens from human origin should be considered as potentially infectious.
16. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Sirict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
17. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
18. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
19. The Stop solution (2M $H_2SO_4$ ) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
20. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
21. Materials Safety Data Sheet (MSDS) available upon request.
22. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

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**Assay Procedure:**

**Step1 Reagents preparation:** Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.

**Step2 Numbering Wells:** Set the strips needed in strip-holder. and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1),** two Positive control **(e.g. E1, F1)** and one Blank **(e.g. A1**, neither samples nor HRP-Conjugate should be added into the Blank well.

If the results are determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

**Step 3 Adding HRP Conjugate**: Add 100ul HRP Conjugate into each well except the Blank well.

**Step3 Adding Sample:** Add **20µl** of Positive control, Negative control, and specimen into their respective wells. **Upon addition of the sample the HRP conjugate – sample mixture will appear Blue. Use a separate disposable tip for each specimen, Negative control and Positive control to avoid cross-contamination.**

**Step4 Incubating:** Mix the plate by tapping gently. Cover the plate with the plate cover and incubate for **60 minutes at 37°C.** It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

**Step5 Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **6** times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remaining solution.

**Step6 Colouring:** Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, and mix by tapping the plate gently. Incubate the plate at **37°C for 15minutes avoiding light.** The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-TP positive sample wells.

**Step7 Stopping Reaction:** Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intense yellow colour develops in Positive control and anti-TP positive sample wells.

**Step8 Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm.**If a dual filter instrument is used, set the reference wavelength at **630nm.** Calculate the Cut-off value and evaluate the results **(Note:** read the absorbance within **5** minutes after stopping the reaction).

**Interpretation of Results:**

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

**1. Calculation of Cut-off value**

**Cut-off value (C.O.) = *Nc +0.18**

*Nc = the mean absorbance value for three negative controls.

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

**2. Quality control range:**

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm, or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

**3. Interpretations of the results:**

(S = the individual absorbance (OD) of each specimen)

**Negative Results (S/C.O. <1):** samples giving an absorbance less than the Cut-off value are considered negative, which indicates that no anti-TP antibodies have been detected with this anti-TP ELISA kit, and there are no serological indications for past infection with TP.

**Positive Results (S/C.O.≥1):** samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that anti-TP antibodies have been detected with this anti-TP ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for antibodies to anti-TP , therefore there are serological indications for current or past infection with TP. Any blood unit containing antibodies to Treponema pallidum should be immediately discarded.

**Borderline (S/C.O=0.9-1.1):**: Samples with absorbance to Cut-off ratio between 0.9 and 1.10 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for anti-TP antibodies.

**Fortress anti-TP Elisa Performance:**

**Clinical Performance:** The clinical performance of this assay have been evaluated by a panel of samples obtained from 3400 healthy blood donors from 3 blood banks and by a panel of samples from 192 Syphilis positive patients. The evaluation results are given below.

| | - | + | Confirmed positive | Specificity | False Positives |
|---|---|---|---|---|---|
| Donors:3400 | 3392 | 8 | 5 | 99.91% | 3 |

**Analytical Specificity:**

1. No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, HBV and HTLV.
2. No interference from rheumatoid factors up to 2000U/ml observed.
3. No high dose hook effect during clinical testing.
4. The assay performance characteristics are unaffected from elevated concentrations of Bilirubin, haemoglobin and triolein.

| REPRODUCIBILITY | | WITHIN RUN | | BETWEEN RUN | |
|---|---|---|---|---|---|
| SPECIMEN | N | MEAN S/CO | CV | MEAN S/CO | CV |
| WEAK +VE | 10 | 3.35 | 8.4% | 3.23 | 9.0% |
| MODERATE +VE | 10 | 6.75 | 7.0% | 6.40 | 7.5% |
| STRONG +VE | 10 | 10.90 | 4.2% | 10.30 | 4.4% |

**Limitations:**

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is design to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. If, after retesting of the initially reactive samples, the assay results are negative , these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
3. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results cannot be use to measure antigens concentrations.

**Indications of Instability or deterioration of Reagents:**

1. Values of the Positive or Negative controls ,which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

**Reference:**


1. Fraser C.M, et al. Complete genome sequence of Treponema pallidum, the syphilis spirochete. Science 1998; 281:375.
2. Holmes KK, lemon SM, Mardh P, Piot P, Sparling PF, Stamm WE, Wasserheit JM, Weisner PF, Chapters 33-36. In Sexually transmitted diseases, 3rd ed, New York: McGraw-Hill, 1999.

fortress diagnostics

# RPR CARBON ANTIGEN

**PRODUCT CODE: SYRPR025/SYRPR050 SYRPR100/SYRPR500**

CE QUALITY MANAGEMENT SYSTEM ISO 13485 CERTIFIED COMPANY

| SYRPR025 | SYRPR050 | SYRPR100 | SYRPR500 |
|---|---|---|---|
| 25 TESTS | 50 TESTS | 100 TESTS | 500 TESTS |
| STORE AT 2-8°C | | | |
| INSTRUCTIONS FOR USE | | | |
| FOR IN-VITRO DIAGNOSTIC USE ONLY | | | |

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

## RPR CARBON ANTIGEN

### SYPHILIS

**Principle:**
The Rapid Plasma Reagin or RPR Card test is a non-treponemal method for the serological detection of syphilis. The antigen – a particulate carbon suspension coated with lipid complexes – agglutinates in the presence of serum reagins. Reagins are antibodies present in the sera of syphilitic patients. Visible agglutination in the form of black clumps which can be viewed macroscopically, indicate the presence of such antibodies in the sample tested.

**Presentation:**

| Contents | 25 Tests | 50 Tests | 100 Tests | 500 Tests |
|---|---|---|---|---|
| RPR Carbon Antigen | 1 x 0.5ml | 1 x 1.0ml | 1 x 2.0ml | 2 x 5.0ml |
| Positive Control | 1 x 0.5ml | 1 x 0.5ml | 1 x 0.5ml | 1 x 1.0ml |
| Negative Control | 1 x 0.5ml | 1 x 0.5ml | 1 x 0.5ml | 1 x 1.0ml |
| Disp. Needle 16µl | 1 | 1 | 1 | 1 |
| Dispensing Bottle | 1 | 1 | 1 | 1 |
| Disposable Test Cards | 3 | 5 | 10 | 50 |
| Pipette / Stirrers | 25 | 50 | 100 | 500 |

**Composition:**

| | |
|---|---|
| RPR Carbon Antigen | Stabilised Carbon Suspension Coated with a lipid complex. Sodium Azide 0.95g/L. |
| Positive Control | Human Serum Sodium Azide 0.95g/L. |
| Negative Control | Animal Serum Sodium Azide 0.95g/L. |

Although all our components which have been derived from human origin have been tested and found to be negative for the presence of anti-HIV, anti-HCV as well as HbsAg, it is recommended that they be handled cautiously and treated potentially infectious.

**Storage:**
Store components at 2-8°C. Cards and Pipettes may be kept at Room Temperature.

**Samples:**
- Serum or Plasma stable for 48 hours at 2-8°C,
- Samples should be free from contamination and haemolysis.
- Lipemia will not affect the test results unless it is severe enough to obscure the state of the antigen particles.

**Reagent Preparation:**
Resuspend the Carbon Antigen gently to ensure thorough mixing. Transfer the required volume of Carbon Antigen to the dispensing bottle. Label the dispensing bottle with the antigen lot number, expiry and transfer dates. Once the antigen has been transferred to the dispensing bottle, it is stable for 3 months or until expiry date, whichever comes first.

**Additional Equipment:**
Mechanical Rotator set at 100 r.p.m, circumscribing a circle 2.0 cm in diameter.

**Test Procedure: Qualitative Test**
1. Bring the reagents and samples to room temperature.
2. Place 50µl of the sample and 1 drop of the control into separate circles on the card.
3. Resuspend the antigen gently.
4. Add one drop of free falling antigen to each test circle.
5. Mix with the disposabe pipette / stirrer and spread over the entire area enclosed by the ring. Use a new stirrer for each sample.
6. Rotate the cards at 100 r.p.m. for 8 minutes.

**Semi-Quantitative Test:**
1. Using a semi-automatic pipette, add 50µl of saline to circles 2, 3, 4 and 5. Do not spread the saline.
2. Add 50µl of patient sample to circles 1 & 2.
3. Mix the saline and sample in circle 2 by drawing the mixture up and down being careful to avoid the formation of any bubbles.
4. Transfer 50µl from circle 2 to the saline in circle 3.
5. Perform serial dilutions in the same manner until the last circle, disgarding 50µl at the end.
6. Using the pipette / stirrer, spread the diluted samples over the entire area of each circle starting at circle 5 and working backwards to the neat sample in circle 1.
7. Proceed as a qualitative test from step 3.

**RPR Qualitative Test in Microtitre Plates:**
1. Using a flat bottomed microtitration plate, add 50µl of patient sample.
2. Add one drop of carbon antigen.
3. Rotate on a Mechanical Rotator for 20 minutes at 50 r.p.m.
4. Read macroscopically, either over a light box or under a high intensity incandescent lamp above a white surface.

**Quality Control:**
Each run of tests should be validated with a positive and negative control.

**Reading And Interpretation:**
Examine macroscopically for the presence or absence of clumps within a minute of removing the card from the rotator. Readings are scored and reported according to the following criteria:-

| Observed Agglutinations | Reading | Report |
|---|---|---|
| Medium and Large Clumps | R | Reactive |
| Small Clumps | W | Weakly Reactive |
| No Clumping or very slight 'roughness' | N | Non-Reactive |

Reactive sera may be titred. To titre make serial two-fold dilutions in 9g/L saline as described in the Quantitative Procedure earlier on. The serum titre is defined as the highest dilution showing a positive result.

**Performance Characteristics:**
- Cardiolipin-like tests are non-specific for syphilis. All reactive test samples should undergo a further specific serological test i.e. FTA-Abs, TPHA, to confirm the results.
- Conversely, a Non-Reactive result by itself does not rule out the diagnosis of syphilis.
- Biological false positive reactions have been reported in diseases such as infectious mononucleosis, viral pneumonia and toxoplasmosis, pregnancy and autoimmune diseases.
- This test is useful in determining the effectiveness of antibiotic therapy.

**Notes:**
1. At the end of each days testing, the needle should be removed from the dispensing bottle, rinsed with distilled water and air dried.
2. The sensitivity of the test may be reduced at low temperatures. The best results are obtained between 23 and 29°C.
3. It is important to position the dispensing needle vertical to the reaction card, in order to deliver the exact quantity of the antigen.
4. High temperatures may cause test components to dry on card and as a result false positive results may occur. Place the card under a humidifying cover if necessary.

**Reference:**

1. Portnoy j et al. Public Health Report 1962; 77: 654-658
2. McGrew BE et al. Am. J. of Clin. Pathology 1968: 50: 52-55
3. Portnoy J. Public Health Laboratory 1965; 23: 43-47
4. McGrew BE et al. Am. J. of Clin. Pathology 1968:34: 634-639



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CE QUALITY MANAGEMENT SYSTEM ISO 13485 CERTIFIED COMPANY

# Syphilis Rapid Test Cassette (Whole Blood /Serum/Plasma) Package Insert

**REF ISY-402 | English**

*A rapid test for the diagnosis of Syphilis to detect antibodies (IgG and IgM) to Treponema Pallidum (TP) qualitatively in whole blood.Serum or plasma.*
*For professional in vitro diagnostic use only.*

## 【INTENDED USE】

The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM) to *Treponema Pallidum (TP)* in whole blood, serum or plasma to aid in the diagnosis of Syphilis.

## 【SUMMARY】

Treponema Pallidum (TP) is the causative agent of the venereal disease Syphilis. TP is a spirochete bacterium with an outer envelope and a cytoplasmic membrane.[1] Relatively little is known about the organism in comparison with other bacterial pathogens. According to the Center for Disease Control (CDC), the number of cases of Syphilis infection has markedly increased since 1985.[2] Some key factors that have contributed to this rise include the crack cocaine epidemic and the high incidence of prostitution among drug users.[3] One study reported a substantial epidemiological correlation between the acquisition and transmission of the HIV virus and Syphilis.[4]
Multiple clinical stages and long periods of latent, asymptomatic infection are characteristic of Syphilis. Primary Syphilis is defined by the presence of a chancre at the site of inoculation. The antibodies response to the TP bacterium can be detected within 4 to 7 days after the chancre appears. The infection remains detectable until the patient receives adequate treatment.5
The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) utilizes a double antigen combination of a Syphilis antigen coated particle and Syphilis antigen immobilized on membrane to detect TP antibodies (IgG and IgM) qualitatively and selectively in whole blood, serum or plasma.

## 【PRINCIPLE】

The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) is a qualitative membrane based immunoassay for the detection of TP antibodies (IgG and IgM) in whole blood, serum or plasma. In this test procedure, recombinant Syphilis antigen is immobilized in the test line region of the test. After specimen is added to the specimen well of the cassette, it reacts with Syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized Syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contains TP antibodies, a colored line will appear in the test line region, indicating a positive result. If the specimen does not contain TP antibodies, a colored line will not appear in this region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

## 【REAGENTS】

The test contains Syphilis antigen coated particles and Syphilis antigen coated on the membrane.

## 【PRECAUTIONS】

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Do not use test if pouch is damaged.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

## 【STORAGE AND STABILITY】

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use after the expiration date.

## 【SPECIMEN COLLECTION AND PREPARATION】

- The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect **Fingerstick Whole Blood specimens**:
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
  - Add the Fingerstick Whole Blood specimen to the test by using **a capillary tube**:
    - Touch the end of the capillary tube to the blood until filled to approximately80 μL. Avoid air bubbles.
    - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen area of the test cassette.
  - Add the Fingerstick Whole Blood specimen to the test by using **hanging drops**:
    - Position the patient's finger so that the drop of blood is just above the specimen area of the test cassette.
    - Allow 2 hanging drops of fingerstick whole blood to fall into the center of the specimen area on the test cassette, or move the patient's finger so that the hanging drop touches the center of the specimen area. Avoid touching the finger directly to the specimen area.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear non-hemolyzed specimens.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

## 【MATERIALS】

**Materials provided**

- Test Cassettes • Droppers • Buffer • Package insert

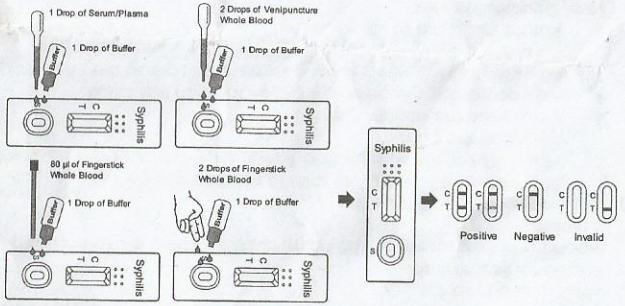
**Materials required but not provided**

- Specimen collection Containers • Centrifuge • Timer
- Lancets • Heparinized capillary tubes and dispensing bulb

## 【DIRECTIONS FOR USE】

**Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.**

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible.
2. Place the cassette on a clean and level surface.
   For **Serum or Plasma** specimen: Hold the dropper vertically and **transfer 1 drop of serum or plasma** (approximately 40 μL) to the specimen area, then **add 1 drop of buffer** (approximately 40 μL),and start the timer, see illustration below.
   For **Venipuncture Whole Blood** specimen: Hold the dropper vertically and **transfer 2 drops of whole blood** (approximately 80 μL) to the specimen area, then **add 1 drop of buffer** (approximately 40 μL), and start the timer. See illustration below.
   For **Fingerstick Whole Blood** specimen:
   - To use a capillary tube: Fill the capillary tube **and transfer approximately 80 μL of fingerstick whole blood specimen** to the specimen area of test cassette, then **add 1 drop of buffer** (approximately 40 μL) and start the timer. See illustration below.
   - To use hanging drops: Allow **2 hanging drops of fingerstick whole blood specimen** (approximately 80 μL) to fall into the specimen area of test cassette, then **add 1 drop of buffer** (approximately 40 μL) and start the timer. See illustration below.
3. Wait for the colored line(s) to appear. **Read results at 5 minutes.** Do not interpret the result after 20 minutes.




## 【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

**POSITIVE:* Two lines appear.** One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).
***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of *TP* antibodies present in the specimen. Therefore, any shade of color in the test line region (T) should be considered positive.

**NEGATIVE: One colored line appears in the control line region (C).** No line appears in the test line region (T).

**INVALID: Control line fails to appear.** Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

## 【QUALITY CONTROL】

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

## 【LIMITATIONS】

1. The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) is for *in vitro* diagnostic use only. The test should be used for the detection of *TP* antibodies in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in *TP* antibodies can be determined by this qualitative test.
2. The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) will only indicate the presence of *TP* antibodies in the specimen and should not be used as the sole criteria for the diagnosis of *TP* infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of *TP* infection.

## 【EXPECTED VALUES】

The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) has been compared with a leading commercial TPPA Syphilis test, demonstrating an overall accuracy greater than or equal to 99.8%.

## 【PERFORMANCE CHARACTERISTICS】

**Sensitivity and Specificity**

The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) has correctly identified specimens of a performance panel and has been compared to a leading commercial TPPA Syphilis test using clinical specimens. The results show that the relative sensitivity of the Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) is >99.9% and the relative specificity is 99.7%.

| Method | | TPPA | | Total Result |
|---|---|---|---|---|
| Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) | Results | Positive | Negative | |
| | Positive | 200 | 1 | 201 |
| | Negative | 0 | 319 | 319 |
| Total Result | | 200 | 320 | 520 |

Relative Sensitivity: >99.9% (95%CI*: 99.4%-100%)
Relative Specificity: 99.7% (95%CI*: 98.3%-100%)
Accuracy: 99.8% (95%CI*: 98.9%-100%)
*Confidence Interval

**Precision**

**Intra-Assay**

Within-run precision has been determined by using 10 replicates of four specimens: a negative, a low positive, a medium positive and a high positive. The negative, low positive, medium positive and high positive values were correctly identified >99% of the time.

**Inter-Assay**

Between-run precision has been determined by 10 independent assays on the same four specimens: a negative, a low positive, a medium positive and a high positive. Three different lots of the Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) have been tested over a 3-day period using negative, low positive, medium positive and high positive specimens. The specimens were correctly identified >99% of the time.

**Cross-reactivity**

The Syphilis Rapid Test Cassette (Whole Blood/Serum/Plasma) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, HCV,HIV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

**Interfering Substances**

The following potentially interfering substances were added to Syphilis negative and positive specimens.

Acetaminophen: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL
Ascorbic Acid: 2g/dL
Creatin: 200 mg/dL
Bilirubin: 1g/dL
Caffeine: 20 mg/dL
Gentisic Acid: 20 mg/dL
Albumin: 2 g/dL
Hemoglobin 1.1 mg/dL
Oxalic Acid: 600mg/dL

None of the substances at the concentration tested interfered in the assay.

## 【BIBLIOGRAPHY】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3. Aral R. Marx. *Crack, sex and STD*, Sexually Transmitted Diseases, 1991; 18:92-101
4. J.N. Wasserheit. *Epidemiological Synergy: Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases*, Sexually Transmitted Diseases 1992; 19:61-77
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**Index of Symbols**

| | | | | | |
|---|---|---|---|---|---|
| | Attention, see instructions for use | Σ | Tests per kit | EC REP | Authorized Representative |
| IVD | For in vitro diagnostic use only | | Use by | | Do not reuse |
| 2°C 30°C | Store between 2-30°C | LOT | Lot Number | REF | Catalog # |
| | Do not use if package is damaged | | | | |


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