QUALITY ASSURANCE FOR RPR TESTING IN FIRST ANTENATAL ATTENDEES

BY

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THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE

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A Dissertation submitted to the University of Zambia in Partial Fulfillment

of the Requirement of the Degree of Master of Public Health

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2005

TABLE OF CONTENTS

LIST OF TABLES

DEDICATION

This work is dedicated to my late wife Agness Nyauma Kapanda. May Her Soul Rest In Eternal Peace.

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2005

DECLARATION

I Suzgo Clement Kapanda hereby declare that this dissertation represent my own work and has not been presented either wholly or in part for a degree in the University of Zambia or any other University.

Signed:_____ Date 26/05/06

Candidate

CERTIFICATE OF COMPLETION OF DISSERTATION

I/We.....having supervised and read this dissertation, am/are satisfied that this is the original work of the author under whose name it is being presented. I/We confirm that the work has been completely satisfactory and is ready for presentation to the examiners. (Delete sections that are not applicable).

Supervisor.....Prof. K. S. BARBOZA.....

Signature.....Date.....6/6/06

HEAD...Dr. G. B. Sivarajan.....Date.....05.06.06

Signature.....

Department.....

APPROVAL

This dissertation of Suzgo Clement Kapanda is approved as partial fulfillment of the requirements for the award of the Master of Public Health by the University of Zambia.

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ABSTRACT

Reported here are findings of the RPR results tested by nurses and laboratory technicians. The involvement of nurses in testing for RPR was aimed at increasing the coverage for women attending antenatal clinics during the outreach programs. There were no laboratory facilities in such places beside the long distances. Nurses in these outreach programs did not use basic equipment such as shakers and centrifuges.

A total of 990 samples were screened of which nurses did 493 and the other 497 were done by laboratory technicians. Both nurses and laboratory technicians reported some amount of false positive results, 11/58 (18.9%) and 9/47 (20%) respectively. Total positive from all the sites was found to be 10.8% (106/990), while upon retesting using the same test (RPR) was 9.7%. Later on TPHA confirmatory test revealed 8.9% (88/990). Even though statistically the difference is minimal, the fact cannot be ignored that there is some discrepancy in the level of training between the nurses, laboratory technician and those at the reference laboratory.

This study also shows that not using basic equipment such as a shaker and a centrifuge did not affect the result.

False positive results, which may be technical or biological, may be not infrequently encountered when tests employing non-treponema antigens are used. Thus an initial positive result may not necessarily mean that the client has a syphilis infection before confirmation with specific tests such as TPHA.

With such high prevalence of reactive syphilis (8.9% confirmed), screening should be aimed at covering/reaching out to all pregnant women to avoid the adverse outcome of undetectable and untreated syphilis such as congenital syphilis. If all women attended ANC the real picture of RPR positivity and negativity could be estimated. In order to ensure quality of RPR test, random TPHA needs to be done. If the difference in the accuracy of RPR tests between the nurses and laboratory technicians is more, then there is need to carry out training program to strengthen their ability.

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I am greatly indebted to Dr. Evans Mpabalwani who persistently encouraged me to do this work. He was so keen to see to it that am doing something towards this presentation. Am so grateful and cherish his encouragement.

Lastly but not the least to my sponsors of this work, Bionor Laboratory Diagnosis (Norway) in general, and Mr. Birger Sorensen in particular for giving me the funds.

LIST OF ABBREVIATIONS

ANC	Antenatal clinic
CHW	Community health worker
HIV	Human immunodeficiency virus
RPR	Rapid plasma reagin
STI	Sexually transmitted infection
TPHA	Treponema pallidum heamagglutination assay
UNICEF	United Nations Children Fund
VDRL	Venereal Diseases Research Laboratory

CHAPTER ONE

1.0 INTRODUCTION

Syphilis is increasingly a disease of public health importance worldwide. It is a complex sexually transmitted infection (STI) caused by the bacterium *Treponema pallidum*. Syphilis is usually transmitted by sexual contact or kissing.

During pregnancy the infection has serious consequences for the unborn baby. It may lead to spontaneous abortion, prematurity, stillbirth and congenital syphilis (Schulz et al, 1987). Study conducted by Baboo et al (1992) showed 17% antenatal attendees in Lusaka to be Rapid Plasma Reagin (RPR) positive. Hira et al (1990) found that 8% of infants examined at UTH had congenital syphilis. An infected pregnant woman has about a 40% chance of having a stillbirth (syphilitic stillbirth), or giving birth to a baby who dies shortly after birth. A baby born to a mother with either untreated syphilis or syphilis treated after the 34th week of pregnancy has a 40% - 70% chance of being infected with syphilis (congenital syphilis). An infected baby may be born without symptoms but may develop them within a few weeks, if not treated immediately. These signs and symptoms can be very serious and include skin sores, a very runny nose, which is sometimes

bloody (and infectious), slimy patches in the mouth, inflamed arm and leg bones, a swollen liver, anemia, jaundice, or a small head. Untreated babies may become retarded or may have seizures. About 12% of infected newborns will die because of the disease.

The consequences of syphilis during pregnancy can easily be prevented by early accurate laboratory diagnosis and treatment during the antenatal period. Routine serologic screening for syphilis is recommended for all pregnant women and for persons at increased risk of infection. Nontreponemal tests are used to screen pregnant women (and patients) for the presence of nonspecific reagin antibodies that appear and rise in titer following infection. The *Treponema pallidum* can be detected by examining material from infectious sores under a microscope. Shortly after infection occurs, the body produces syphilis antibodies that are detected with a blood test. A syphilis blood test is accurate, safe, and inexpensive. A low level of antibodies will persist in the blood for months or years after the disease has been successfully treated, and antibodies can be found by subsequent blood tests. Because untreated syphilis in a pregnant woman can infect and possibly kill her developing baby, every pregnant woman should have a RPR blood test for syphilis.

In Zambia there has been a vigorous campaign initiated by the Ministry of Health to sensitize all pregnant women to attend antenatal clinics. Among the tests done routinely at the first antenatal visit is the syphilis test. A program has been put in place to reach out to mothers who live long distances from the health institutions. In some rural health centres nurses/midwives do the antenatal clinics during the outreach activities and also do the RPR screening.

Mobilization of these pregnant women to attend the antenatal clinic (ANC) is done through the community health workers (CHW). These CHW sensitize the pregnant women on the need to be screened for syphilis during pregnancy. The community health workers provide health education on all aspects of keeping healthy during pregnancy.

With limited human resources (laboratory technicians) and facilities, many pregnant women were not being screened for syphilis. Like other laboratory investigations, syphilis screening requires trained personnel and use of basic laboratory equipment.

Government through its line ministry of Health saw the need for *nurses/midwives who directly attend to pregnant women to acquire basic* skills in screening for syphilis. This initiative has increased syphilis screening for all pregnant women attending antenatal clinics.

Quality and accuracy of results cannot be compromised. It is imperative though that those who perform these investigations should generate quality and acceptable results. This study therefore looks at the quality of results generated by both nurses/midwives and the laboratory technicians.

CHAPTER TWO

2.0 STATEMENT OF THE PROBLEM

Screening for syphilis among pregnant women was generally very low, 34% (UNICEF, 1995). One of the contributing factors to this low coverage was the low number of laboratory technicians in the health institutions. Many health institutions not only didn't have laboratory technicians, but also had no basic laboratory facilities. UNICEF, in conjunction with the Ministry of Health, expressed concern at the low coverage of syphilis testing in pregnant women in Zambia. It is for this reason that nurses/midwives were trained to do syphilis screening using RPR in pregnant women. This initiative to involve the nurses do RPR has increased the percentage of women screened from a low 34% to a high 92% with the test results being fairly reliable (UNICEF, 1995). The nurses trained to do RPR in turn have trained other nurses. They carry out syphilis testing in the field (during outreach programs) without using the basic equipment e.g. a shaker and/or a centrifuge.

Inaccurate test results for syphilis may be generated and can cause an individual to experience serious social and medical consequences. Being a sexually transmitted disease it carries a stigma of promiscuity especially on

the spouse. With current levels of HIV infection one may be suspected of equally being infected with HIV, which leads to AIDS. And where counseling is not available, such stigmatization between the couples would bring a lot of suffering.

False positive results using RPR subjects an individual to receiving drugs that they may not need. This is wastage of resources and can create unnecessary resistance to the particular drug. Drugs are very expensive and not readily available at the clinics. This creates an economic burden on the individuals. A false negative result will deny the client prompt medication which may lead to adverse effects on both the mother and the unborn baby.

This study aims at investigating the accuracy and quality of RPR test results done in the clinics by nurses/midwives and laboratory technicians in the first antenatal attendees. This would help estimate the quality of RPR provided in the selected sites covered by this study.

CHAPTER THREE

3.0 LITERATURE REVIEW

There are two theories concerning the origins of syphilis: the Columbian theory and the Environmental theory. In the former, it is believed that syphilis was epidemic in the fifteenth-century Europe, and early stages apparently were often unusually severe by contemporary standards. The rapid spread and considerable effects of the disease throughout Europe in the last decade of the fifteenth century caused it to be termed the Great Pox in contrast to another scourge, small pox. The disease received its present name from the poem by Fracastoro in 1530 about afflicted shepherd, Syphilis (Willcox, 1964).

The European epidemic of the fifteenth century coincided with the return of Columbus from America in 1493, causing many to assume that the disease was acquired from natives in West Indies and carried it back to nonimmune populations in Europe. On the other hand there are biblical and ancient Chinese writings that are consistent with the description of late cutanous syphilis, although other illnesses such as tuberculosis or leprosy could have caused similar description to be written. These and other considerations led some to speculate that venereal syphilis did not arise suddenly in Europe

after 1493 but may have been endemic already, only to become more wide spread and severe as a consequences of the wars that coincided the return of Columbus and his men (Holmes et al, 1999)

The Environmental theory suggests that *T. pallidum*; the causative agent of venereal syphilis is really the same species as *T. pallidum* subspecies *pertenue*, the causative agent of yaws; *T. pallidum* spp *carateun,* the causative agent of pinta and *T. pallidum* spp *bosnia,* the causative agent of nonvenereal syphilis. According to this hypothesis, all treponematoses are merely variants of a single disease, the expression of which has been modified by environmental factors especially temperature.

3.1 THE INFECTIOUS AGENT

Treponema pallidum, subspecies *pallidum,* was identified in 1905 by Schauddin and Hoffman (Smibert, 1984). It is an obligate human parasite. There are no reservoirs for this organism in animals or the environment. Nearly all cases of venereal syphilis are acquired by direct sexual contact with lesions of an individual who has active primary or secondary syphilis. Transmission of syphilis occurs in approximately one-half of such contacts.

Syphilis is one of the treponematoses caused by *T. pallidum*, or by organisms morphologically indistinguishable. It can be either venereal or non-venereal, the latter being endemic or sporadic. Other treponematoses, also non-venereal, include yaws, a disease confined to the humid tropical belt, and pinta (the "blue stain disease"), which is encountered in Central and South America. Both are non-venereal contagious diseases predominantly of children the epidemic of which resembles endemic syphilis. It is usually spread by the conveyance of infectious matter from skin, nose, or mouth, the fingers being important agents. Kissing, inanimate objects as communal eating and drinking vessels and possibly flies (Willcox, 1964) also play their part. Endemic syphilis is essentially a disease of hot dry countries and used to be endemic in rural communities of Middle East, Bosnia, Bulgaria and Botswana from where it is practically eradicated.

The causative organisms of the treponematoses are spirochaetes of the genus Treponemata. The name "Spirochaete" was first given by Ehrenberg in 1838 to large, free-living flexible coiled organism found in fresh and marine water. The spirochaetes are actively motile, not flagellate, but by means of a

screw like rotation of the organism. The four main group of the spirochaete include *spirochaeta, cristispira, leprospira* and *Treponemata.*

Treponemata are widely distributed in nature. In man they are found in mouth, alimentary tract, bronchi, around the urethral orifice, in the vagina, in certain ulcerating conditions of the skin, in dirty general lesion and in many of the lesion of syphilis, yaws and pinta. The four principal pathogenic treponemata are T. *cuniculi*, which is responsible for a venereal disease in rabbits, T. *pallidum*, the cause of human syphilis and T. *pertenue* and T. *carateum*, the causative organisms of yaws and pinta respectively. These four are morphologically similar. The final confirmation of T. *pallidum* as the causative organism and indeed its discovery is attributed to Schauddin and Hoffmann (1904—1905) who did this without the aid of the darkfield microscope.

3.2 STRUCTURE

T. pallidum subspecies pallidum is 6-20um in length with a diameter of 0.10 to 0.18um, which places it below the resolution of the light microscope. It has a regular tight spiral with a coil wavelength of 1.1um and amplitude of 0.2-0.3um. It also has an outer membrane (Radolf et al, 1995), an inner (cytoplasmic) membrane, and a thin cell wall composed of peptidoglycan

(Blamo, 1994). The outer membrane lack lipopolysaccharide, and this make them more susceptible to disruption by routine physical manipulation.

3.3 EPIDEMIOLOGY OF SYPHILIS IN PREGNANCY

Syphilis remains a common complication of pregnant women, despite the availability of cheap, accurate diagnostic tests and continued sensitivity of *T. pallidum* to penicillin. Before antibiotics were introduced syphilis was estimated to be responsible for as many as 40% of stillbirth (Dippe, 1944). Presently, in developed countries, syphilis infrequently causes stillbirth but remains a common cause in developing countries. Congenital syphilis still remains a major challenge in developing countries (Brunham et al, 1995). In past studies done in Addis Ababa, Ethiopia, and in Durban, South Africa, congenital syphilis was fifth and fourth leading cause of prenatal death respectively. In a study of pregnant women in Zambia, serological tests for syphilis were reactive in 43% of women who delivered stillbirth babies, 19% who aborted, and 13% of the normal women attending their first antenatal visit (Ratnam et al, 1981).

In the surveys in sub-Saharan Africa that estimated rates of active syphilis (positive results for both *T. pallidum* hemagglutination antigen (TPHA) and

RPR or Venereal Diseases Research Laboratory (VDRL) tests) in pregnant women, the median reported prevalence was 6% with areas in Kenya, Cameroon, Tanzania, Gabon and Malawi all reporting rates greater than 10% (Stanecki et al, 1995). With such seroprevalence in excess of 10% one can safely say that syphilis during pregnancy is prevalent throughout much of Africa.

Studies show that the prevalence rate is higher in African countries compared to the developed countries. For instance, in Mozambique the reported prevalence varies between 1.6-15%, in Zambia it is between 13-15% while in the USA the overall prevalence of syphilis in 1990 was 0.02% (Schulz et al, 1987). Manning et al (1985) found that the prevalence of syphilis in antenatal mother to be between 5.5 – 11% in South Africa.

The contributing factors to high prevalence rate in Africa are low social – economic standards, associated with unemployment, drug misuse, prostitution and family disruption which promote the spread of syphilis particularly in urban areas (Ban et al, 1995). Dada et al (1998) further implicates illicit drug use and the exchange of drugs for sex as being

responsible for the dramatic rise of primary and secondary syphilis cases in young women in the late 1980s and 1990s.

The most common outcome of syphilis during pregnancy is probably spontaneous abortion during the second and third trimester. The precise magnitude of this problem is difficult to measure, as most women do not go for prenatal care until the third trimester (Ratnam et al, 1985). This is further complicated by non-compliance to treatment. In Ethiopia, almost 5% of all pregnancies are lost each year as a result of syphilis-induced abortions (Bishaw T et al, 1983). In another study it was found that pregnant women who were seroreactive to syphilis were five times more likely to have a spontaneous abortion or stillbirth than women who were seronegative (Larsony et al, 1970). Infants born to mothers with primary or secondary syphilis up to 50% will be premature, still born or die in the neonatal period (Dada et al, 1998). It has been observed that most of these children are born with congenital disease that may not be apparent for years.

Quantifying the morbidity due to syphilis is however difficult. Some of the reasons include:

- poor documentation and reporting of STIs at health institutions

- patients may seek treatment from providers e.g. traditional healers or private clinics who do not normally report STIs.
- lack of technical and material resources to conduct community STIs morbidity surveys.
- social stigma and cultural barriers which can impede access to STIs services or participation in community surveys (Matondo et al 1998).

It is estimated that spontaneous abortion rate among pregnant African women with syphilis is as high as 50%. A case-control study done in Zambia demonstrated a 28-fold increased risk in stillbirth among women with high titer RPR card test seroreactivity (Watts et al, 1984). Another 20-30% of total prenatal infants' deaths were due to congenital syphilis in Zambia (Hira, 1984). In another study done at the University Teaching Hospital, revealed that 1% of the babies delivered had signs of congenital syphilis at birth and as many as 6.5% were seroreactive at birth and thus considered at risk (Bhat et al, 1982). Hira et al (1983) also found a 2.9% seroreactivity among infants less than 6 months of age.

One of the health concerns in the antenatal attendees is the prenatal transmission of syphilis. There are three ways in which syphilis is transmitted. These include sexual, which maybe heterosexual or

homosexual, prenatal transmission and through blood (blood transfusion), unsterile needles and other implements (Matondo et al, 1998).

3.4 PATHOGENESIS

Placental infection with *T. pallidum* occurs during maternal spirochetemia, which is intense until resolution of the secondary stage. After resolution of the initial and secondary stages, the prevalence and intensity of spirochetemia are unknown except during secondary relapses. Once the infection reaches the placenta, fetal infection usually follows. Fetal infection with *T. pallidum* is not detectable until after 18 weeks of gestation. Although a mother can transmit syphilis to her infant at the time of delivery (Sanchez et al, 1991), the vast majority of cases are believed to arise from *in utero* infection. The finding of spirochetes in the placenta and umbilical in association with typical histopathology changes supports transplacental invasion during maternal spirochetemia as the major route of transmission. Alternatively, *T. pallidum* may gain access to the fetal circulation by first traversing the fetal membranes and infecting the amniotic fluid (Wendel et al, 1993; 1989; Nathan et al, 1993; 1997). The risk of congenital syphilis is directly related to the stage of maternal syphilis during pregnancy. It is extremely high during the first four years of infection, when spirochetemia

is common, and then decreases during late syphilis, when spirochetemia becomes a rare event.

3.5 CLINICAL PRESENTATION

Intrauterine growth retardation, a commonly cited feature of congenital syphilis is thought to reflect inadequate nutrition of the fetus as a result of syphilis placentitis (Ingall et al, 1994; Budell, 1976; Rathbun, 1983). Recent controlled studies, however, strongly support the association between intrauterine infections with T. *pallidum* and prematurity, low birth weight, and small size for gestation age (Stoll et al 1993; Reyes et al, 1993). The clinical manifestations of early congenital infection are a consequence of active infection with *T. pallidum* and the resultant inflammatory response induced in various organs and tissues. Most infected infants are entirely asymptomatic at birth (Nabarro, 1954; Ingraham, 1935; Brown and Moore, 1963; Mamunes et al, 1970; Bwibo, 1971). Neonates born with manifest syphilis are often severely affected and carry a worse prognosis.

3.6 PREVENTION

Congenital syphilis is entirely preventable with appropriate case detection and treatment programs. The provision of adequate prenatal care to all

pregnant women remains the foundation for prevention of congenital syphilis. The majority of women diagnosed with syphilis are asymptomatic, underscoring the need for routine serological screening of all pregnant women as early as possible. Pregnant women in high risk groups should have repeat serologic screening for syphilis in the third trimester in addition to their first visit.

High rates of positive RPR test indicate an increase in syphilis in pregnancy and may reflect increased prevalence rate of syphilis in general society. It justifies greater efforts in sensitizing communities on the ever-increasing morbidity due to STI and its implication on the spread for Human Immunodeficiency Virus (HIV). Falsely high positive rates on the other hand may create false alarm, not just for maternal syphilis but also for other STIs especially the threat of HIV. Such alarms have a negative influence on efforts to popularize preventive measures. On the other hand high rates of false positive results result in a waste of antibiotic and reduce cost-effectiveness of the program.

3.7 SOME ZAMBIAN LOCAL LANGUAGE NAME FOR SYPHILIS

Syphilis is found everywhere in Zambia. Most major languages in the country have a name for it:

1. **IciBemba-*Akaswende*** covering Northern, Luapula and parts of Central and Copperbelt Provinces
2. **ChiNyanja-*Chizonono*** covering Eastern, Lusaka and parts of Central Provinces
3. **ChiTonga-*Kansweende*** covering Southern, and parts of Lusaka and Central Provinces
4. **SiLozi-*Kutuku bwa sihule*** covering Western and parts of Southern Provinces
5. **KiKaonde-*Kasete*** covering Northwestern and part of central Provinces
6. **Lunda-*Kasong`o*** covering Northwestern Province
7. **Luvale-*Kasong`o*** covering parts of both North-Western and Western Provinces

3.8 LABORATORY DIAGNOSIS OF SYPHILIS

Recent data from the surveys in Zambia shows that the prevalence of syphilis in pregnant women varies from 6–22 % (Evaluation Report 1998). There is a discrepancy result reported on the sero–prevalence of syphilis among pregnant women in Zambia. Confirmed results using TPHA at the reference laboratory has reported a range of 13-15% positivity (1994 Sentinel Surveillance). In the sentinel surveillance of 1999, the range of false positive was from 0.7%-40%.

The diagnosis of syphilis depends on the dark field (dark ground) tests in the primary stages, dark field and serum in the secondary stages and serum tests, clinical finding and the history of the case in the latent stages of the disease.

There are many types of tests for syphilis. These tests are not directly for *T. pallidum*, but detect antibodies to it, which appear in the serum (and sometimes the cerebro-spinal fluid) of syphilitic persons. The tests depend on the observation of an antigen-antibody reaction conducted in *vitro*. Both non-treponemal and treponemal antigens are used for the purpose and different antibodies are demonstrated by these means. The antibodies detected by non-treponemal antigens being auto-antibodies to a slimy layer

on the treponema provided by the host and those detected by treponema antigens being provided by the treponema itself. Tests that are based on non-preponemal antigens detect antibody *reagin*. There are two types of tests that determine the presence of the *reagin*: complement-fixation and flocculation procedures.

In complement-fixation, the antigen-antibody reaction is demonstrable by the use of an ingenious indicator system e.g. in the Wasserman reaction. In cases of flocculation, the reaction is apparent from a visible aggregation of particles as seen in VDRL tests. Today the cardiolipin antigens are used for the serological diagnosis of syphilis.

3.9 PRINCIPLE OF RPR

RPR is a microscopic nontreponemal flocculation card test used to screen for syphilis (Portnoy et al, 1962). The antigen is prepared from a modified VDRL antigen suspension containing choline chloride to eliminate the need to heat inactivate serum ethylenediaminetetraacetic acid (EDTA) to enhance the stability of the suspension, and finally divided charcoal particles as a visualizing agent.

The test measures IgM and IgG antibodies to lipoidal materials released from damaged host cells as well as to lipoproteinlike material, and possibly cardiolipin released from the treponemes (Mathew et al, 1979; Belisle et al, 1980). These are antibodies that are produced not only as a consequence of syphilis and other treponemal diseases, but also in response to nontrponemal diseases of acute and chronic nature in which tissue damage occurs (Catherall, 1972). If antibodies are present, they combine with lipid particles of the antigen, causing them to agglutinate. The charcoal particles coagglutinate with the antibodies and show up as black clumps against the white card. If not present the test mixture is uniformly gray. Without some other evidence for the diagnosis of syphilis, a reactive non treponemal test does not confirm *T. pallidum* infection.

3.10 TREPONEMA TESTS

Treponema tests e.g. TPHA and FTA-AB use treponema antigens to detect anti-treponema antibodies in the blood (Tomizawa et al 1996). They are more specific than non- treponema tests. When diluted reactive samples are mixed with sensitized erythrocytes, antibody to the sensitizing antigen causes agglutination of the cells. A characteristic pattern of cells forms in the bottom of a microtitration plate well if positive, while cells form a

compact button in the well when antibodies are absent. They provide a definite diagnosis of syphilis infection. They remain positive for life even after an individual has received adequate treatment. These tests do not, however, discriminate between past, or active and/or treated infection.

Other tests employing the principles of flocculation include the Price Precipitation Reaction, Sigma and Meinicke to name a few.

CHAPTER FOUR

4.0 OBJECTIVE

GENERAL OBJECTIVE

Investigate how accurate are the RPR test results in antenatal screening for syphilis.

4.1 SPECIFIC OBJECTIVES

1. To determine the reproducibility of the RPR tests results from the centres doing antenatal screening for syphilis in the first attendees.
2. To compare the RPR results obtained by the laboratory technicians and nurses/midwives involved in testing for syphilis.
3. To compare whether or not using basic equipment such as a shaker (rotator), centrifuge has an effect on the result.
4. Make the necessary recommendations to the relevant authorities on the findings.

CHAPTER FIVE

5.0 METHODOLOGY

5.1 Sampling method

Ten different centres/sites were selected where antenatal screening for syphilis using RPR was conducted. Five of these centers (Kapata clinic, Kapiri Mposhi clinic, Lubuto clinic, Malamba clinic and Luangwa clinic) were picked because they had nurses/midwives who were performing the RPR test in antenatal attendees. Three of these sites (Kapiri Mposhi, Kapata and Luangwa) had an outreach program where the nurses went out in the community and carried out syphilis testing on pregnant women there. All the sites where nurses/midwives performed RPR tests did not use a shaker and a centrifuge. The other five (Chipata General Hospital, Kitwe Central Hospital, Livingstone General Hospital, Ndola Central Hospital and Monze Mission Hospital) had laboratory technicians who performed syphilis screening beside other routine tests. Thus this selection was based on which clinic has nurses doing the RPR tests and also accessibility to the reference laboratory.

An introduction letter to enable this work be carried out at the respective institutions was provided by the Head of Department of Community Medicine. See appendix 10.2

5.2 Sample size

Each site was assigned to test about 100 samples from their routine first antenatal attendees. This sample size was generated on the computer using an EPI-INFO sample size calculation. 95 samples per site were estimated giving a total of 950 samples from ten sites. However extra 40 samples collected were also included.

5.3 Method

The samples were collected from all pregnant women who were due for a syphilis screening on that particular visit using venous blood. This was a convenient sample. 2ml of blood was collected and put in the plain container. The technicians at the centres tested the samples collected as routine. During the outreach, nurses tested the samples in the field.

The kit used in all these centres was Immunitrep RPR by Omega Diagnostics Limited. This involved mixing of one drop of the serum and a drop of the carbon antigen and then either rotates it on a shaker or does it manually for about eight minutes. Results were read visually for either agglutination or none. A portion of each sample tested at the site was kept for further testing at the reference laboratory. All the clients with the initial

reactive samples were treated for syphilis even before the samples were further tested and/or confirmed.

All the reactive samples from these testing sites were retested using RPR (as at the sites) and confirmed by TPHA at the reference laboratory. This TPHA (Omegas Immunitrep) test is a specific, sensitive passive haemaglutination test for the detection of antibodies to *Treponema pallidum*. In addition 10% of all negative samples were also tested. This was to ensure that there are no false negatives.

A physical check on the laboratories where the RPR tests are done was carried out. The check focused on the assessment of the equipment (storage of the reagents, shakers), and how specimens were being handled before and during the testing.

5.4 Data analysis

The data was analysed using SPSS 9.0 to find out whether there are any significant differences in the results obtained by nurses/midwives in the field and the laboratory technicians at the sites. Kappa which measures

agreement between the evaluation of two raters when both rating the same object will be used.

5.5 ETHICAL APPROVAL

This work was approved by the University of Zambia, School of Medicine Research Ethics Committee. Permission was also obtained at the respective sites to collect samples from the potential clients. No informed consent from clients was required as the study took advantage of the routine testing of syphilis in first attendees. Letter of approval from the Directorate in the Appendix No 10.1

CHAPTER SIX

6.0 RESULTS

A total of 990 samples were screened from the sites, of these 493 were screened by nurses/midwives. A proportion of this sample (293) was done during the outreach for antenatal. All the samples done by nurses did not use a mechanical rotator. They were rotated using their hands. Laboratory technicians with the aid of a mechanical rotor tested the other 497. The initial screening found 58 (11.8%) positive using RPR. The same samples were screened at the Reference centre using RPR. 52 (10.5%) samples were found positive. All the positive samples, including those from the initial screening at the site were further subjected to a confirmatory test using TPHA.

Upon confirmation 9.5% positivity was registered. Eleven samples out of 58 (18.9%) were negative (false positive) upon confirmation at the reference laboratory. Similar results were obtained from the sites where laboratory technicians performed the tests. Confirmation results yielded a 20% (10/49) false positive.

Upon retesting with RPR at the reference centre, 44 samples (8.9%) were found positive. Pulled positive rate at all the sites was determined to be 10.8% (106/990), while retesting using the same test (RPR) was 9.7% (96/990). The overall confirmed results using TPHA at the reference centre found to be 8.9%, 88 out of 990.

Table 1 shows the results obtained between nurses and laboratory technician when re-screened at the reference centre. The results by the nurses and laboratory technicians from the field were reproducible. The sensitivity for the results of the nurses was calculated to be 98.6%, with a specificity of 100%. The Kappa value was 0.94, giving a p value of <0.001. Similar results were obtained among laboratory technician. A sensitivity and specificity of 98.8% and 100% was found for the laboratory technician respectively, with a Kappa value of 0.94. This was also highly significant at p=<0.001.

Table 1: Reproducibility of RPR results by nurses and lab technicians

			Reference Laboratory		Total
Type of personnel			+ve	-ve	
Nurses	+ve	Count	52.0		52.0
		% within RPR	100.0		100.0
		% within RPR	89.7		10.5
		% of Total	10.5		10.5
	-ve	Count	6.0	435.0	441.0
		% within RPR	1.4	98.6	100.0
		% within RPR	10.3	100.0	89.5
		% of Total	1.2	88.2	89.5
Total		Count	58.0	435.0	493.0
		% within RPR	11.8	88.2	100.0
		% within RPR	100.0	100.0	100.0
		% of Total	11.8	88.2	100.0
Lab Techs	+ve	Count	44.0		44.0
		% within RPR	100.0		100.0
		% within RPR	89.8		8.9
		% of Total	8.9		8.9
	-ve	Count	5.0	448.0	453.0
		% within RPR	1.1	98.9	100.0
		% within RPR	10.2	100.0	91.1
		% of Total	1.0	90.1	91.1
Total		Count	49.0	448.0	497.0
		% within RPR	9.9	90.1	100.0
		% within RPR	100.0	100.0	100.0
		% of Total	9.9	90.1	100.0
		Value	**Asymp. Std. Error(a)**	**Approx. T(b)**	**Approx. S**
Nurses Measure of Agreement	Kappa	.939	.025	20.880	‹(
N of Valid Cases		493			
Lab Techs Measure of Agreement	Kappa	.941	.026	21.009	‹(
N of Valid Cases		497			

a Not assuming the null hypothesis.

b Using the asymptotic standard error assuming the null hypothesis.

Table 2 below shows the results obtained by nurses were similar to those obtained by laboratory technicians (Kappa = 0.94, $p<0.001$)

Table 2: Agreement between results obtained by nurses and laboratory technicians on RPR results

			Laboratory technicians		Total
			+ve	-ve	
Nurses	+ve	Count	96.0	11.0	107.0
		% within RPR	89.7	10.3	100.0
		% within RPR	100.0	1.2	10.8
		% of Total	9.7	1.1	10.8
	-ve	Count		883.0	883.0
		% within RPR		100.0	100.0
		% within RPR		98.8	89.2
		% of Total		89.2	89.2
Total		Count	96.0	894.0	990.0
		% within RPR	9.7	90.3	100.0
		% within RPR	100.0	100.0	100.0
		% of Total	9.7	90.3	100.0
		Value	**Asymp. Std. Error(a)**	**Approx. T(b)**	**Approx. Sig.**
Measure of Agreement	Kappa	.940	.018	29.619	<0.001
N of Valid Cases			990		

a Not assuming the null hypothesis.

b Using the asymptotic standard error assuming the null hypothesis.

The test results were computed to find out whether there were any differences in the results between those obtained by the nurses and the laboratory technician on one hand and TPHA results. There was a perfect agreement between the two groups (Kappa = 0.88, $p<0.001$ for nurses and Kappa =).90, $p<0.001$ for laboratory technicians). Table 3 overleaf.

ble 3: Confirmation with TPHA of results obtained by the nurses and lab hs

				TPHA at Reference labortory		Total
ype of personnel				+ve	-ve	
urses	RPR	+ve	Count	47	11	58
			% within RPR	81.0	19.0	100.0
			% within TPHA	100.0	2.5	11.8
			% of Total	9.5	2.2	11.8
		-ve	Count		435	435
			% within RPR		100	100
			% within TPHA		97.5	88.2
			% of Total		88.2	88.2
	Total		Count	47	446	493
			% within RPR	9.5	90.5	100.0
			% within TPHA	100.0	100.0	100.0
			% of Total	9.5	90.5	100.0
ab Techs	RPR	+ve	Count	41	8	49
			% within RPR	83.7	16.3	100.0
			% within TPHA	100.0	1.8	9.9
			% of Total	8.2	1.6	9.9
		-ve	Count		448	448
			% within RPR		100	100
			% within TPHA		98.2	90.8
			% of Total		90.1	90.1
	Total		Count	41	456	497
			% within RPR	8.2	91.8	100
			% within TPHA	100.0	100.0	100
			% of Total	8.2	91.8	100
Type of personnel			**Value**	**Asymp. Std. Error(a)**	**Approx. T(b)**	**Approx. Sig**
Nurses	Measure of Agreement	Kappa	.883	.035	19.739	<0..0
	N of Valid Cases		493			
Lab Techs	Measure of Agreement	Kappa	.902	.034	20.213	<0..0
	N of Valid Cases		497			

Not assuming the null hypothesis.

Using the asymptotic standard error assuming the null hypothesis.

The overall results confirmed at the reference laboratory using TPHA found a sensitivity of 100% while the specificity was calculated at 97.8 %, table 4 below.

Table 4: Overall results of confirmed TPHA at the reference laboratory

			TPHA at Reference Laboratory		Total
			+ve	-ve	
RPR	+ve	Count	88	19	107
		% within RPR	82.2	17.8	100.0
		% within TPHA	100.0	2.1	10.8
		% of Total	8.9	1.9	10.8
	-ve	Count		883	883
		% within RPR		100.0	100.0
		% within TPHA		97.9	89.2
		% of Total		89.2	89.2
Total		Count	88	902	990
		% within RPR	8.9	91.1	100.0
		% within TPHA	100.0	100.0	100.0
		% of Total	8.9	91.1	100.0
		Value	**Asymp. Std. Error(a)**	**Approx. T (b)**	**Approx. Sig.**
Measure of Agreement	Kappa	.892	.024	28.232	<0.001
N of Valid Cases		990			

a Not assuming the null hypothesis.
b Using the asymptotic standard error assuming the null hypothesis.

All the 10% (90) negative samples pulled from the two groups came out negative on both re-screening and confirmation on TPHA i.e. no false negatives.

The physical inspection of the sites revealed that all the sites had a working refrigerator where the reagents were kept. During outreach activities, the reagents were carried in cool boxes with ice packs. This was aimed at maintaining the minimum temperature for the reagents. During the outreach, nurses did not use centrifuges to spin the samples. They left the sample to clot and serum separated before they could perform the tests. This would take about twenty minutes.

CHAPTER SEVEN

7.0 DISCUSSION

The results of RPR obtained by nurses and the laboratory technicians show a nearly perfect agreement. This may illustrate the simplicity in performing the test. It does not require the high technical proficiency as in other tests e.g. dark field microscopy in the identification of the *Treponema pallidum*. This (dark field) is the only test that specifically establishes the diagnosis of syphilis by identifying the *Treponema pallidum*. Antibodies to cardiolipin as measured by RPR test is present often at relatively low levels (< 1:16) in about 80% of the clients at the time they come for medical attention for primary syphilis. Cardiolipin is a component of mammalian cells that is incorporated and presumably modified by *T. pallidum* so that the infected host generates antibodies to it. Tests that measure antibodies to surface proteins of *T. pallidum* by hemagglutination assay (TPHA) or MHA-TP) are positive in about 90% of the clients.

The sensitivity of nontreponemal tests varies with the levels of antibodies present during the stages of the infection. In early primary syphilis, when antibody levels may be too low to detect, results may be nonreactive, and the sensitivity of nontreponemal tests is 62-76% (Hart, 1980). Antibodies

rise as disease progresses with titers peaking during secondary syphilis. At this point the sensitivity of the nontreponemal test approaches 100%. In late syphilis titers decline and previously reactive results revert to nonreactive in 25% of the patients. In untreated syphilis test sensitivity averages only 70%.

The specificity of nontreponemal tests is 75-85% in persons with preexisting disease or conditions, and it approaches 100% in persons without them (Feder et al, 1988). Because nontreponemal serodiagnostic tests may be falsely positive, all reactive results in asymptomatic patients should be confirmed with a more specific treponemal test, which has a sensitivity of 84% in primary syphilis and almost 100% for other stages and a specificity of 96% (Larsen et al, 1990).

The results obtained by nurses and laboratory technicians had about the same proportion of false positives (20%). This may be attributed to the interpretation of the tests as to whether there is agglutination or not. Tests of this nature normally pose challenges, as they may also be subjective. To distinguish as to whether the agglutination formed is as a result of the reaction between the antigen-antibody and the carbon antigen added in excess can also be a challenge.

There is also likelihood that samples which are not properly spun (with red blood cells residual) can be seen as having formed an agglutination reaction. In cases were a centrifuge is not used for the separation of serum, enough time should be given to allow for this separation. RPR testing uses serum and is not affected as to when the test is done as long as the serum is stored in the refrigerator.

RPR tests pose certain limitations. A prozone reaction may be encountered occasionally. In this reaction, complete or partial inhibition of reactivity occurs with undiluted serum. Maximum reactivity is obtained only with diluted serum. Using the quantitative procedure should retest all test specimens producing any degree of roughness or reactivity with the RPR test antigen in the qualitative test.

The RPR test may be reactive in persons from areas where *yaws, pinta* or nonvenereal syphilis is endemic. Generally residual titers from these infections will be <1:8 (Gershman et al 1992).

Biological false-positives occur occasionally with cardiolipin antigens, mainly in specimens from persons who abuse drugs, who have diseases such

as lupus erythematosus, malaria, leprosy or viral pneumonia, or who have recently been vaccinated.

Nontreponemal test titers of persons who have been treated in latent or late stages of syphilis or who have become resistant do not decrease as rapidly as do those of the person in the early stages of their infection (Fiumara et al, 1979).

False positive results, which maybe technical or biological may not infrequently be encountered when tests employing non-treponema antigen are used. It has been estimated that 1 in 3,000 normal persons (Wilcox, 1964) may carry reagin or a similar substance in the blood, temporarily or permanently, in sufficient quantities to show false-positive results to standard serum tests for syphilis. It is thus important that all samples that will test positive on screening should be retested with a more specific test such as TPHA. It is further estimated that a proportion of the population, possibly 20% have some anomaly of the serum which permits reagin sometimes to increase in the blood in response to diseases other than syphilis (Wilcox, 1964). Diseases in which biological false-positive serum reactions to syphilis may occur to reagin include spirochaetal infections as

in *relapsing fever*, bacterial infections as in *leprosy*, advanced *tuberculosis* and occasionally pneumonia. Protozoal conditions such as *malaria* and *tryponosomiasis* may also cause reagin reaction.

CHAPTER EIGHT

8.0 CONCLUSION

8.1 The results obtained by the nurses and the laboratory technicians from the field were reproducible at the reference laboratory.

8.2 Nurses/midwives who were trained to do RPR tests in antenatal attendees obtained similar results (90.2%) to those of the laboratory technicians (91.8%).

8.3 Despite not using the rotator for shaking the cards and the centrifuge to separate serum there was no statistical difference in the results obtained by nurses compared to the laboratory technicians using the basic equipment.

8.1 RECOMMENDATIONS

8.1.1 Where there are no laboratory technicians to do the screening for syphilis in pregnant women, trained nurses in this technique can do the screening.

8.1.2 With such prevalence of reactive syphilis (8.9%), screening should be aimed at covering all pregnant women to avoid the adverse outcome of undetected and untreated syphilis.

8.1.3 Those who test positive to syphilis need to be counseled to reveal the identity of the partner who may be other than their spouse. Whatever the case they need to be treated at an early stage to prevent further spread of the disease which can lead to HIV infection.

8.1.4 Screening for syphilis should not just be done during the first visit, but also in the second and third trimester of the pregnancy, as there is a possibility that they can be infected at a later stage during the pregnancy.

8.1.5 With a minimum of a fridge for the storage of RPR reagents, syphilis testing can be carried out without a shaker and a centrifuge by nurses having received basic training in RPR testing

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Waiting Publication

45. Baboo K.S. Sukwa T. (1995), *Outcome of RPR in Health centers in the City of Lusaka.* Report submitted to Zambia Medical Journal. Unpublished in Scientific Journal

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10th September, 1999

Suzgo C Kapanda
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Dear Ms Kapanda

On behalf of the Board of Graduate Studies, I am Pleased to inform you that your research proposal entitled: ***"Quality Assurance for RPR Testing in first Antenatal Attendants"*** was approved by the board.

This completes all the requirements for part one of the Masters programme and you can proceed to part two of the programme. Your supervisor is Dr K S Baboo and your Co-Supervisor is Dr P Matondo.

Congratulations!

Yours sincerely

Geoffrey Lungwangwa (PhD)
DIRECTOR

Cc Dean School of Medicine
Assistant Dean (PG) – Medicine
Head – Community Medicine
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24th May 2000

TO WHOM IT MAY CONCERN

RE: KAPANDA SUZGO: MPH STUDENT

This is to certify that Mr S Kapanda, is a student doing a Masters degree programme in Public Health in the School of Medicine, University of Zambia.

His research work entitled: "Quality Assurance for RPR Testing in first Antenatal Attendants" has been approved by the Board of Graduate Studies of the School of Medicine.

Kindly allow him to carry out this work at your institution.

Yours sincerely

Dr L Chiwele
HEAD
DEPARTMENT OF COMMUNITY MEDICINE