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Prevalence of Syphilis in Pregnancy using a Rapid Treponemal Specific Test in a Low Resource Setting

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ABSTRACT

Background: *The rapid treponemal specific test such as immunochromatographic (ICS) test is being recommended as a point of care (POC) test as alternative to the non-specific Treponemal test such as Venereal Disease Research Laboratory (VDRL) commonly used in most developing countries.*

Objectives: *To determine the yields of the ICS test for syphilis among pregnant women. Method: This was a cross-sectional study. One hundred and sixty consecutive pregnant women who satisfied the inclusion criteria were recruited into the study. Blood samples obtained from respondents were tested for the presence of Treponema pallidum antibodies using both, Diaspot®syphilis ultra-rapid test kit and MAST-TPHA™ TPHA (Treponema pallidum Haemaglutination Assay) test.*

Results: *Out of the 160 pregnant women studied, 4 (2.5%) were sero-reactive for syphilis using the Treponema pallidum haemmaglutination assay (TPHA) while only 2 (1.25%) were sero-reactive to syphilis using the (immunochromatographic screening) ICS method of screening. The sensitivity of the ICS method in detecting syphilis infection was 50% (95% CI: 6.76% – 93.24%) whereas it was 100% specific for syphilis (95% CI: 97.66% -100%). The Positive Predictive Value of the ICS for syphilis screening was 100% while its Negative Predictive value was 98.73% (95% CI: 96.70% – 99.52%). There was no statistically significant difference in the yield of a positive syphilis serology test result comparing the rapid immunochromatographic screening test with the TPHA (P= 0.479).*

Conclusion: *The rapid immunochromatographic screening test (ICS) for syphilis is highly specific and compares favorably with TPHA test in excluding syphilis infection but has a lower sensitivity.*

Keywords
Syphilis, Pregnancy, Immunochromatographic test, Haemmaglutination assay.

Background
Syphilis is a human infection of global public health importance. It is a chronic and progressive disease that can be associated with significant morbidity and mortality without treatment especially when transmitted vertically from mother to child or in patients with advanced tertiary disease [1]. acquired through blood transfusion and infected needles [2-4]. *Treponema pallidum,* the causative organism of syphilis, is able to cross the placenta in pregnant women and result in fetal infection [5-7]. Globally, about 340 million cases of curable new sexually transmitted infection occur

every year with syphilis accounting for an estimated 11 million cases [4,8-11]. The World Health Organization (WHO) estimates that untreated syphilis in pregnancy resulted in approximately 521,000 adverse perinatal outcomes globally in 2008, including an estimated 212,000 stillbirths, 92,000 neonatal deaths, 65,000 preterm/low birth weight infants and 152,000 syphilis infected newborns [12-14].

The serological tests which remain the mainstay for the diagnosis of syphilis fall into two categories namely: Non Treponemal tests for screening (e.g. Venereal Disease Laboratory (VDRL) test and Rapid plasma reagin (RPR) and Treponemal specific tests for confirmation (e.g *Treponema pallidum* Heamagglutination Assay (TPHA), Fluorescent treponemal antibody absorption test (FTA – ABS), *Treponema pallidum* particle agglutination (TPPA) and various enzyme linked immunosorbent assays) [2,15]. In recent times, there has been implementation of point of care Treponemal specific antibodies rapid tests to enable early diagnosis and treatment of maternal syphilis especially in resource constraint settings. Solid phase immunochromatographic screening (ICS) assay is a Treponemal specific antibody test, which allows qualitative detection of all isotypes (IgG, IgM and IgA) against T. palladium. They are rapid, cheap and commercially available point of care tests that can be performed in rural settings and has the clear benefit of a rapid result facilitating immediate treatment at the initial visit. The reported sensitivities and specificities vary from 75 – 100% and 93 – 10% respectively [16-18].

The traditional approach to the sero-diagnosis of syphilis has been the use of Non – Treponemal specific screening assay followed by the performance of a Treponemal specific confirmatory test if the initial Non – Treponemal screening test was reactive [1]. More recently, with the increasing availability of automated, easy – to – perform and rapid Treponemal – specific antibody test with higher sensitivities and specificities, a reverse screening sequence (i.e. screening with a treponemal specific antibody assay first) is being advocated especially in populations with low prevalences for syphilis [1,19]. The use of Treponemal specific antibody tests as a screening tool increases disease detection rate especially in late latent and early primary states of infection when the Non – Treponemal antibody tests may be non – reactive. However, a drawback to this approach is said to be the possibility of an increase in false positive results. Although, a perfect reference standard does not exist, *Treponema pallidum* Haemaglutination Assay (TPHA) is one of the conventional tests used as a reference standard in determining the test performance of other syphilis diagnostic tests. It is a widely used Treponemal specific test for syphilis diagnosis [12,20].

This study was therefore designed to determine the true seroprevalence of syphilis among the Obstetric population using immunochromatographic test (Rapid Treponemal specific test) and Treponemal Pallidium Haemaglutination (TPHA) as a reference (gold) standard to define the true positive and true negative cases.

Methods

This cross-sectional study was carried out over a period of five months from May to September, 2017. Approval for the study was obtained from the Ethical review board of Federal Teaching Hospital, Ido Ekiti. The study included all pregnant women who presented for antenatal care at any gestational age who gave their consent, while patients who were previously sero-reactive or treated for syphilis were excluded. Other exclusion criteria were ongoing febrile illness, known retroviral positive patients and those who declined participation in the study. The calculated sample size was 160 based on a prevalent rate of 10 percent and deliberate oversampling of 15 percent to account for attrition. This gave a statistical power of 80%, P value of 0.05 at 95% confidence interval level. One hundred and sixty consecutive pregnant women who presented for care at the antenatal clinic and had satisfied the inclusion criteria were recruited. A brief relevant history was obtained from all pregnant women recruited into this study when they presented for antenatal care. The age, parity, occupation and level of education were recorded on the proforma. Presence of risk factors for syphilis infection such as the previous history of multiple sexual partners, blood transfusion, past history of genital ulcer in a patient or her sexual partner, previous history of treatment for sexually transmitted diseases and history suggestive of congenital syphilis infection in previous deliveries were also sought. They subsequently underwent tests for syphilis detection using both Diaspot®syphilis ultra-rapid screening kit and MAST-TPHA™ (*Treponema pallidum* Haemagglutination Assay) kits according to the manufacturer's instructions.

Laboratory Procedures

Venous blood (5mL was collected from the ante-cubital vein of each woman into plain sterile blood collection tubes by aseptic technique. The blood was allowed to retract and then centrifuged, to obtain the serum. Serum samples from all patients were subjected to syphilis testing using both DiaSpot® Syphilis Ultrarapid test strips manufactured by the DiaSpot® Inc. USA and MAST-TPHA™ (*Treponema pallidum* Haemagglutination Assay) HA 101-200 tests kits manufactured by MAST Group Ltd, UK.

Quality Control

Measures were taken to ensure that the results obtained from this study are accurate. The procedures were carried out strictly in accordance with the manufacturer's instructions. Every tenth sample was retested to verify the results obtained.

Results

A total of one hundred and sixty eligible pregnant women were recruited for the study. Data were complete and available for the sampled study population. Table 1: shows the socio-demographic characteristics of the respondents. The mean age was 30.91± 4.08years.

Table 1: Socio-demographic characteristics of the respondents.

Variables	N = 160 Frequency (%)
Age (years)	
< 20	1 (0.6)
20 – 24	6 (3.8)
25 – 29	58 (36.2)
30 – 34	63 (39.4)
≥ 35	32 (20.0)
Occupation	
Unemployed	30 (18.8)
Self Employed	66 (41.2)
Civil servant	64 (40.0)
Educational Status	
Primary/Arabic	5 (3.1)
Secondary	38 (23.8)
Tertiary	117 (73.1)
Marital Status	
Single	5 (3.1)
Married	155 (96.9)

Majority of the women were married (96.9%). Most of them were self-employed (41.2%) while the unemployed accounted for 18.8% of the studied population. All the women had some form of education, tertiary level of education ranking highest (73.1%). The Obstetric and clinical characteristics of the respondents are as shown in Table 2. Majority of the respondents were multigravidae (83.8%) while the mean gravidity was 2.84 ±1.43. The parity of the respondents ranges from 0 to 6 with a mean parity of 1.38 ± 1.17. Majority of the respondents (65%) had their first antenatal visit in the second trimester. Only 11.2% booked their pregnancies at the first trimester (estimated gestational age less than 14 weeks). The mean gestational age at booking was 22.15 ± 6.98weeks. Outcomes of previous pregnancy and deliveries showed that 12.5% of respondents had a previous history of spontaneous abortion, 9.4% had the previous history of stillbirth or perinatal mortality. A respondent (0.6%) had a history of congenital anomaly in previous delivery while 9 (5.6%) were unsure of congenital anomaly in previous delivery with the neonate having suffered perinatal death.

Table 2: Obstetric and clinical data of participants.

Variable	N – 160 Frequency (%)	
Gravidity		
Primigravidae	26 (16.2)	
Multigravidae	134 (83.8)	
Mean ± SD		2.84 ± 1.43
Median (Range)		2.0 (1.0 – 8.0)
Parity		
Mean ± SD		1.38 ± 1.17
Median (Range		1.0 (0.0 – 6.0)
No of Children Alive		
Mean ± SD		1.27 ± 1.13
Median (Range)		1.0 (0.0 – 5.0)
Estimated Gestational Age at Presentation (weeks)		
< 14 weeks	18 (11.2)	
14 – 26 weeks	104 (65.0)	
27 – 40 weeks	38 (23.8)	
Mean ± SD		22.15 ± 6.98
Median (Range)		21.86 (7.00 – 40.43)
History of Spontaneous Abortions		
Yes	20 (12.5)	
No	140 (87.5)	
History of Perinatal death or stillbirth		
Yes	15 (9.4)	
No	145 (90.6)	
History of Congenital Anomaly in previous deliveries		
Yes	1 (0.6)	
No	150 (93.8)	
Not Sure	9 (5.6)	

Figure 1 shows the risk factors for syphilis identified in respondents. Two of the respondents had a previous history of genital ulcers, 9 had a history of blood transfusion while 17 had a history of multiple sexual partners in the last one year.

Figure 1: Risk factors identified among respondents (MSP – Multiple sexual partners).

The relationship between the socio-demographic characteristics of the respondents, risk factors identified and syphilis infection using the TPHA serology result was shown in Table 3. Among the respondents, 2 (1.3%) in the age group 25-29 years were seroreactive to syphilis infection while 1 (0.6%) seroreactive respondent was found among those aged 30-34 years. The only respondent (0.6%) less than 20 years in this study was also sero-reactive to syphilis. There was a significant association between maternal age and the risk of maternal infection (p=0.025). Pregnant women aged less than 20 years and those between 25-29 years are more likely to have syphilis infection. Although, all the seroreactive respondents (2.5%) were married, there was no significant association between marital status and syphilis infection (p=1.000). History of genital ulcer was obtained from 2 (1.3%) of the respondents but they were not sero-reactive to

syphilis and thus, not statistically significant (p=1.000). No statistically significant association was found between the previous history of blood transfusion, history of multiple sexual partners in the preceding one year and seroreactivity to maternal syphilis infection (p>0.05%).

Table 3: Association between the socio-demographic characteristics of respondents, risk factors and syphilis infection.

Variable	TPHA test N – 160			
	Non-Reactive (%)	Reactive (%)	X²	p-value
Age (years)				
<20	0 (0.0)	1 (0.6)	10.182‡	0.025
20 – 24	6 (3.8)	0 (0.0)		
25 – 29	56 (35.0)	2 (1.3)		
30 – 34	62 (38.8)	1 (0.6)		
≥35	32 (20.0)	0 (0.0)		
Marital Status				
Single	5 (3.1)	0 (0.0)	0.132‡	1.000
Married	151 (94.4)	4 (2.5)		
Level of Education				
Primary	4 (2.5)	1 (0.6)		
Secondary	37 (23.1)	1 (0.6)	4.849‡	0.111
Tertiary	115 (71.9)	2 (1.3)		
Occupation				
Unemployed	28 (17.5)	2 (1.3)		
Self Employed	66 (41.3)	0 (0.0)	3.879‡	0.106
Civil Servant	62 (38.8)	2 (1.3)		
Estimated Gestational Age at Presentation (weeks)				
First Trimester	16 (10.3)	0 (0.0)		
Second Trimester	99 (63.5)	3 (1.9)	0.301‡	1.000
Third Trimester	37 (23.7)	1 (0.6)		
History of Genital Ulcers				
Yes	2 (1.3)	0 (0.0)	0.052‡	1.000
No	154 (96.3)	4 (2.5)		
Past History of blood transfusion				
Yes	9 (5.6)	0 (0.0)	0.245‡	1.000
No	147 (91.9)	4 (2.5)		
History of Multiple sexual partners in last 12 months				
Yes	15 (9.4)	2 (1.3)	6.698‡	0.056
No	141 (88.1)	2 (1.3)		
X²= Chi Square	‡ = Fischer's Exact			

Table 4 is a 2x2 contingency of results obtained from both the immunochromatographic screening test and the TPHA using the TPHA as the standard test. A total of 4 respondents were seroreactive to syphilis using the TPHA. The remaining 156 respondents who were not reactive to syphilis using the TPHA formed the True Negative population. While using the ICS, only 2 respondents were sero-reactive to syphilis. The seroreactive samples using the ICS were true positive results, while the remaining 2 sero-reactive samples missed by ICS were the False Negative. There were no False Positive results obtained. There was no statistically significant difference in the yield of a positive syphilis serology test result comparing the rapid immunochromatographic screening test with the TPHA (P=0.479).

Table 4: 2x2 Contingency of test results.

ICS Test	TPHA			McNemar Test	p-value
	Positive	Negative	Total		
Positive	2	0	2	0.500	0.479
Negative	2	156	158		
Total	4	156	160		

Table 5 shows the result of syphilis screening of the 160 respondents using both TPHA and the ICS. The seroprevalence of syphilis infection was 2.5% using the TPHA (standard) method. Out of the 160 pregnant women studied, 4 (2.5%) were seroreactive for syphilis using the TPHA while only 2 (1.25%) were sero-reactive to syphilis using the ICS method of screening. The sensitivity of the ICS method in detecting syphilis infection was 50% whereas it was 100% specific for syphilis. The Positive Predictive Value of the ICS for syphilis screening was 100% while its Negative Predictive value was 98.73%.

Table 5: Sensitivity, Specificity and Predictive values.

Variable	TPHA Test		95% C.I.	ICS Test
Reactive	4			2
Non – Reactive	156			158
True Positive (TP)		2		
False Positive (FP)		0		
True Negative (TN)		156		
False Negative (FN)		2		
Sensitivity (%)		50.00%	6.76% – 93.24%	
Specificity (%)		100.00%	97.66% – 100.00%	
Positive Predictive Value (%)		100.00%		
Negative Predictive Value (%)		98.73%	96.70% – 99.52%	
Prevalence		2.50%	0.69 – 6.28%	

Discussion

Serological tests are important tests for the diagnosis of all stages of syphilis, and are the only means of identifying infection in asymptomatic individuals and in patients whose lesions cannot be tested for *T. pallidum* [15,17]. Treponemal specific methods of syphilis detection was carried out in this study using both the rapid immunochromatographic screening test (ICS) and *Treponemal pallidum* Haemagglutination assay (TPHA) to determine the seroprevalence of syphilis infection among our obstetric population. In addition, the test performance of the ICS was equally determined. Serological testing of syphilis using the TPHA is characterized by its high sensitivity and specificity [21-23]. Using this method, the seroprevalence of syphilis infection in this study was 2.5%, which was higher than the seroprevalence of 1.25% obtained from the same study population using the rapid immunochromatographic screening test. Serological screening for syphilis in antenatal patients in different parts of the world showed that the prevalence of the disease vary [8,24]. The seroprevalence of 2.5% obtained in this study is higher than the 0.3% reported as the national average for syphilis in pregnant women in Nigeria in 2004, in which 27708 pregnant women were screened for syphilis using rapid plasma reagin with positive test confirmed with TPHA [25]. Similarly, it is also higher than syphilis seroprevalence of 0.7% obtained during 2014 sentinel survey [26]. In addition,

higher than the median seroprevalence of 1.6% among antenatal attendees reported by the WHO for the African Region in 2014. However, the observed prevalence is within the reported range of 0 to 11.3% in the sub-region [27]. The seroprevalence of 2.5% obtained in this study using the TPHA is similar to the rate of 2.97% reported by Taiwo et al. [28] among the antenatal population in Osogbo following a TPHA confirmatory test of an initially positive RPR screening test. Adesina, in a study done in Ibadan, reported a lower syphilis seroprevalence of 0.13% among the antenatal attendees [29]. Ogiogwa reported a low seroprevalence of 0.3% among pregnant women in Abeokuta using the ICS as a confirmatory test following a positive VDRL (non-treponemal test) [30]. Also, in a study done among pregnant women in Ebonyi, reported a low syphilis seroprevalence of 0.5% using the ICS test [31]. The low syphilis sero-prevalence observed in these studies were however not confirmed nor refuted by confirmatory tests such TPHA as was done in this study in which a higher seroprevalence of 2.5% was obtained with the TPHA test refuting the low syphilis seroprevalence of 1.25% obtained with the ICS test. Ojo in Osogbo using a non-treponemal-specific test (VDRL) reported a higher prevalence of 10% and the high syphilis seroprevalence obtained in the study was not confirmed with a treponemal-specific test [24]. In Yenogoa, Buseri also reported a higher syphilis seroprevalence of 5% among pregnant women, which was confirmed with TPHA [32]. TPHA was not used on all studied populations in these studies but rather as a confirmatory test, unlike in the current study where it was used on all samples obtained. A similar syphilis seroprevalence of 2.5% was obtained in a study carried out among pregnant women in Tanzania while a higher prevalence of 10.6% was found in HIV Negative women in a study carried out in Cameroon [33,34]. The differences in seroprevalence of *Treponema pallidum* infection in the different populations of pregnant women may not be unconnected with the differences in predisposing factors, cultural practices, sexual practices, access to improved sexually transmitted disease management and the degree of public awareness, as well as method of diagnosis [35]. The reported disease rates may be more reflective of the availability of laboratory diagnosis than actual disease rates; countries that lack universal access to laboratory diagnosis may report low case rate even in a setting of the high burden of disease [27]. Non-treponemal tests have reduced sensitivity to primary and latent syphilis, as well as the potential for false negative results due to prozone phenomenon in secondary syphilis [29,36]. These may be responsible for the low syphilis seroprevalence reported in the studies by Adesina and Ogiogwa [29,30]. Also reporting prevalence based solely on screening by non-treponemal test results in high rates of false positives due to cross-reactivity [27], as this may be responsible for the wide difference in the syphilis seroprevalence in this study compared with what was obtained by Ojo in Osogbo [24]. In this study, women aged 24-29 years were the most seropositive for syphilis infection and a statistical significance association was found between maternal age and syphilis infection (p=0.025). Similar to this study, Yakasai in Kano and Ojo in Osogbo, reported the highest syphilis seroprevalence among women aged 20-30 year [24,37]. In the study conducted by Igiogwa

et al. in Abeokuta [30], majority of the seropositive cases were among pregnant women aged 36-40years, whereas the disease prevalence was low among those aged 26-30 years even though they constituted the highest obstetric population [30] unlike what was observed in this study. A case-control study carried out by Zhou in China found no significant association between age, occupational status and syphilis seropositivity. It however identified unmarried status, unemployment and self-employment as being significantly associated with syphilis infection unlike in this study in which employment as well as marital status had no significant association with syphilis infection. The association between the socio-demographic characteristics of the respondents and syphilis infection in the study by Zhou were controlled for unlike in this study [38]. The significant association between maternal age and syphilis infection in this study may be related to the educational achievement of the respondents as the majority of them had a tertiary level of education and might have delayed their sexual activity until this age or had their maximal sexual activity by this age due to their educational quest. Syphilis seropositivity was also highest among those with tertiary education in this study although, the association was not significant. This study compared the test performance of the ICS as a method of syphilis screen with TPHA which is a gold standard test used often as a confirmatory test based on its known sensitivity of greater than 95% and specificity of greater than 99% [38]. In this study, ICS showed 2 (50%) positive results out of the 4 samples sero-reactive to syphilis using TPHA given the sensitivity of 50% (95% CI: 6.76% – 93.24%). Its specificity however was 100% (95% CI: 97.66% – 100.00%) as the two positive results were True positive and there was no False positive result. The difference in the sensitivity of the two methods used in this study was however not statistically significant (p> 0.05). Studies carried out testing the sensitivity and specificity have shown some variability in the sensitivity of the rapid immunochromatographic screening (POC) tests for syphilis diagnosis [12,17,39]. The World Health Organization in a study compared the performance of 6 rapid syphilis diagnostics (kits) with a combined reference standard of TPHA/TPPA [17]. The sensitivity of ICS as a method of syphilis detection was reported to vary between 84.1% - 97.7% with specificity between 92.8% - 98% among the different diagnostic kits tested with the highest sensitivity observed with the Abbott Determine Syphilis TP [17]. The variability observed in the sensitivity of the various rapid kits tested in the study was noted to be significant across some kits. There were lot-to- lot as well as operator- to- operator variation in the results obtained using the test kits and the degree of this variation differs across various diagnostic kits. The reported sensitivity was higher than the sensitivity of 50% observed in this study using the Diaspot®syphilis ultra-rapid test kit. The specificity however was similar to what was obtained in this study. Overall, the rapid tests were adduced to be highly sensitive and specific [17]. The low sensitivity observed in this study may be as a result of lot-lot-lot variation in the test kits and the relatively small sample size. Montoya et al. in a study carried out in Mozambique, evaluating the diagnostic accuracy of the ICS reported inter-laboratory variation in the sensitivity of the ICS between tests

conducted at the reference laboratory and the health facilities using the same ICS kit of the same lot with the reference standard of TPHA. The higher sensitivity of 95.3% was reported at the reference laboratory while at the health facilities the sensitivity obtained was 84.1% [40]. The sensitivity of the ICS was noted to have decreased to 78% by the end of the study among samples analyzed in the health facility. The sensitivity of ICS decreased significantly in the presence of malaria. Overall, the diagnostic accuracy of ICS was noted to compare favorably with that of TPHA [40]. The sensitivity of ICS in the Mozambique study was higher than the 50% sensitivity obtained in this study. This study was carried out in a malaria endemic population; however, malaria co-infection, which was reported to decrease ICS sensitivity, was not ruled out. The specificity of ICS however was high, similar to what was obtained in this study. A study done in India by Kashyap compared the performance of an ICS test with VDRL and TPHA on sera obtained from the antenatal mother. The sensitivity, specificity, positive predictive and negative predictive values of ICS were reported as 75%, 100%, 100% and 99.4% respectively [16]. The test performance of ICS in the study was similar to the results obtained in this study except for the lower sensitivity of ICS (50%) in this study. There was no statistically significant difference in the test performance of ICS as a method of syphilis screening similar to this study [16]. The lack of statistically significant difference despite the low sensitivity may be due to the relatively small sample size in the two studies. The numbers of respondents in the study by Kashyap were 200 antenatal mothers [16]. Similar to the minimum sample size of 160 respondents screened in this study. The variation in the sensitivity results obtained across studies may be because of the lower sensitivity of the rapid ICS tests in early primary syphilis where TPHA shows a better sensitivity [16]. The studies reported above were also carried out at varying gestational ages, this present study included patients across all the gestational ages. In addition, most rapid tests utilize one or more recombinant antigens in formats that confer varying test sensitivity and specificity [17]. The test performance of the ICS can be affected by exposure to high temperature or humidity during transportation and storage. The optimal temperature for storage was however ensured in our laboratory. There are no local studies in Nigeria evaluating the sensitivity, specificity and the predictive values of the ICS as a method of syphilis screening.

Conclusion
The seroprevalence of syphilis in our obstetric population is low based on the prevalence of 2.5% obtained from this study using the TPHA technique. Although there was no statistically significant difference in yield between the immunochromatographic screening test and the *Treponema pallidum* haemagglutination assay, the sensitivity of the ICS test was low and may not adequately show the true disease prevalence of syphilis infection in our Obstetric population. While TPHA is widely as a gold standard as a confirmatory test, it also has some drawbacks in that its sensitivity may be low in early active or in late latent cases. In such cases other Treponemal specific tests such as FTA-ABS are recommended, which however was not done in this study. We therefore recommend that future studies should look into overcoming the limitations of this study, which are mainly small sample size, and not carrying

out of FTA-ABS test on the samples.

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