Abstract P3-S6.03 Table 1 Seroreversion TT Congenital Syphillis

Case*	Maternal stage/GA at treatment	Neonatal/infant diagnostic features	Infant age at treatment (GA at birth)	Infant age /final serologic results
1	Primary/postpartum	Abnormal CSF (4)	Birth (34 weeks)	7 months/RPR NR, TPPA NR, FTA-ABS NR
2	Primary/postpartum	Abnormal CSF (3) Abnormal long bone radiographs Intraventricular haemorrhage Fetal hydrops	Birth (unknown)	14 months/RPR NR, TPPA NR
3	Primary/postpartum	Abnormal CSF (3)	Birth (38 weeks)	10 months/syphilis EIA negative
4	Early latent/postpartum	Abnormal CSF (3)	Birth (30 weeks)	5 months/syphilis EIA negative
5	Early latent/34 weeks GA	Abnormal CSF (3) Abnormal long bone radiographs	Birth (37 weeks)	12 mos/syphilis EIA negative
6	Primary/postpartum	Abnormal CSF (3)	Birth (38 weeks)	18 months/syphilis EIA negative
7	Early latent/postpartum	Abnormal CSF (4)	Birth (36 weeks)	19 months/syphilis EIA negative
8	Secondary/28 weeks GA	Abnormal CSF (4) Intrauterine anaemia, hydrops, cardiomegaly, ascites. Positive syphilis PCR from intrauterine fetal blood	Birth (36 weeks)	13 months/syphilis EIA negative

CSF, cerebrospinal fluid; GA, gestational age; NR, non reactive; R, reactive; EIA, enzyme immunoassay; RPR, rapid plasma reagin; TPPA,*Treponema pallidum* particle agglutination; FTA-ABS, fluorescent treponemal antibody absorbed; PCR, polymerase chain reaction.
*Number of CSF abnormalities (elevated WBC, RBC or protein, low glucose, reactive VDRL).

under 18 month serologic follow-up, one had persistently reactive TT (21 months) and four had reactive TT at the end of their follow-up period (ages 11, 12, 13 and 15 months). 3/5 cases with persistently reactive TT were treated with 9–10 days of intravenous penicillin within 0–2 days of birth, 1 at 3 months of age and 1 at 8 months of age. In 4/5 of these cases, the RPR had reverted to non reactive at the end of the follow-up period while in the 5th case (treated at 8 months), the RPR declined from a titre of 1:4096 dilutions at birth to 1:64 dilutions at 11 months of age. The remaining eight cases had negative TTs, as summarised in the table. All were treated with 10 days of intravenous penicillin (except case #2 treated with 9 days) see Abstract P3-S6.03 table 1.

Conclusions As with early treatment of primary syphilis cases, seroreversion of TT can occur in cases meeting clinical and laboratory criteria for congenital syphilis. Seroreversion was observed with older TT such as TPPA and FTA-ABS as well as the newer syphilis EIA.

P3-S6.04 USE OF A POINT OF CARE TEST DEVICE TO DETECT SYPHILIS IN A STD CLINIC

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Background Diagnosis of syphilis is problematic and an accurate rapid point of care (POC) test could be useful in busy STD clinics. There are no FDA Cleared POC tests for Syphilis serology in the USA.

Objective To determine the performance of a new, rapid point of care (POC) test, Syph-Check, which is not yet FDA cleared, for the serological diagnosis of Treponema pallidum in female and male STD patients.

Methods Men and women >18 yr visiting the Baltimore City Health Department STD clinic were consented to enrol in a trial to determine the accuracy of a new, innovative POC test for syphilis (Veda, manufactured in France) that used a cassette format to test syphilis serology. The Syph-Check One-Step Syphilis test is a point of care, rapid immunoassay screening test for qualitative detection of IgG and IgM antibodies to *Treponema pallidum* in finger stick blood, plasma, and serum. This product can be used as an initial screening test or as a confirmatory diagnostic test, but is not FDA cleared for use in screening blood or plasma donors. The assay was performed in the STD clinic, required only 20 min to perform, and required no instrumentation. RPR and TPPA tests were performed to determine the sensitivity and specificity of the Syph-Check POC test.

Results 194 men and 205 women were enrolled. Of the 399 samples tested, 33 were positive and 366 were negative by the Syph-Check. There were 14 positives and 385 negatives by RPR confirmatory testing. Overall sensitivity compared to RPR testing was 85.7% (95% CI 60.3% to 97.5%) and specificity was 94.5% (95% CI 91.9% to 96.5%). There were 32 positives and 367 negatives by TPPA confirmatory testing. Overall sensitivity compared to TPPA was 43.8% (95% CI 27.5% to 61.1%) and specificity was 94.8% (95% CI 92.2% to 96.8%).

Conclusions The Syph-Check POC test was moderately accurate compared to the RPR test, but not as sensitive compared to TPPA. A more accurate POC test for syphilis could be useful for clinicians to test clinic patients and provide immediate screening results for syphilis to patients during an office or clinic visit.

P3-S6.05 COMPARING THE ANALYTICAL SENSITIVITIES OF SIX TREPONEMAL TESTS

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Background Traditional syphilis testing consists of screening with a non-treponemal test (RPR) and confirmation with a treponemal test (TP-PA, FTA-ABS, EIA, CIA, etc). Recently, that testing algorithm has been reversed due to efforts to reduce labour costs and the availability of automatable tests (EIA, CIA). Large numbers of discordant test results (treponemal +, non-treponemal–) can be obtained using the reverse algorithm and can be due to (1) treated cases of syphilis, (2) a false-positive treponemal test, or (3) a case of early primary syphilis that has yet to seroconvert. Those sera need to be confirmed with a second treponemal test to eliminate false positive specimens. The dilemma arises which treponemal test is best suited for confirmation, and secondly what are the relative analytical sensitivities of available treponemal tests used for both screening and confirmation.

Methods Two hundred randomised TP-PA positive, cleanascite treated samples (GADPH) were serially diluted with normal human sera to determine the analytical endpoint and sensitivity of six commonly used treponemal tests (TP-PA, FTA-ABS, TrepSure, TrepChek, TrepID, and LIAISON). All dilutions were treated as neat sera in each test, and the tests were performed according to the manufacturer's instructions.

Results The FTA-ABS was used as the reference since it has been considered the Gold Standard" among treponemal tests. The analytical sensitivity of the six tests fell into three statistically different groups (from lowest to highest): (1) the FTA-ABS, the TP-PA, and the TrepChek, (2) the LIAISON and the TrepID, and (3) the TrepSure. In addition, there were 24 sera that were found non-reactive by FTA-ABS and nine found non-reactive by TrepChek that were reactive by at least two other treponemal tests.
Conclusions These results highlight significant differences in the analytical sensitivity of various treponemal tests and could explain some discordant results between treponemal tests used to confirm screening EIAs and CIAs. First, the FTA-ABS should no longer be considered the "Gold Standard". Second, low titre sera could appear non-reactive if relatively insensitive tests are used for the either screening or confirmatory tests. Third, the question arises, "Should initial screening tests be confirmed by treponemal tests with lower analytical sensitivity?" Future patient studies could verify these results, so that a more accurate standard protocol for syphilis testing can be established.

P3-S6.06 DOES THE CONCEPT OF EARLY AND LATE LATENT SYPHILIS HOLD WELL IN TODAY'S SCENARIO?

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Background Latent syphilis refers to the asymptomatic stage in the natural evolution of syphilis in a patient not treated or inadequately/inappropriately treated for syphilis. Latent syphilis has been categorised as early latent and late latent for therapeutic purposes. Indiscriminate and inadequate over the counter use of antibiotics (happenstance) and HIV co-infection in present day scenario tends to change natural evolution of syphilis. Even though some patients remain asymptomatic without any historical evidence of clinical manifestation of syphilis, serologically they continue to reflect the ongoing infection process; the so called syphilis of unknown duration.
Objective To determine the sero-epidemiological characteristics of patients with syphilis of unknown duration registered to a sexually transmitted disease (STD) clinic in an Institute setting.
Patients and Methods Clinic records of patients registered to the STD clinic between 2006 and 2010 were retrieved. For study purposes, syphilis of unknown duration was defined as positivity with *Treponema Pallidum* Haemagglutination assay (TPHA) irrespective of Venereal Disease Research Laboratory (VDRL) titre in patients who did not have any clinical sign of syphilis including neurological and cardiac, during clinic entry; had no features to suggest syphilis in the past and had not been treated with parenteral penicillin.
Results Of the 76 patients registered during the study period, 52 (68%) satisfied the diagnosis of syphilis of unknown duration. Age of patients was between 20 and 56 years (mean 29.76, standard deviation 8.32). Males outnumbered females by a ratio of 2.25:1. Majority (33, 63.4%) of the patients were direct walk-in with positive serology results done in private laboratories, mostly in patients who are habituated to unprotected extramarital sexual intercourse. Others were referred from obstetric clinics (15.4%), private practitioners (15.4%), HIV clinic (1.9%), transfusion medicine (1.9%) and urology clinic (1.9%). Majority patients (31, 59.61%) showed low titre VDRL (upto 1:8) positivity while 5 (9.6%) were VDRL non-reactive.
Conclusion In majority of syphilis patients, duration of latency could not be determined due to lack of history of clinical lesions of early syphilis or reliable history of time of acquisition of infection. Consequently, the categorisation of latent syphilis to early and late phases in present day scenario appears redundant.

P3-S6.07 ABSTRACT WITHDRAWN

P3-S6.08 DETECTION OF THE 23S RRNA POINT MUTATIONS (A2058G AND A2059G) ASSOCIATED WITH AZITHROMYCIN RESISTANCE IN TREPONEMA PALLIDUM USING A TAQMAN-BASED REAL-TIME TRIPLEX-PCR ASSAY

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Background To develop a TaqMan-based real-time allelic discrimination assay for the simultaneous detection of two point mutations (A2058G and A2059G) in the 23S rRNA gene of *T pallidum* that have been associated with azithromycin treatment failures.
Methods Initially, two TaqMan-based real-time duplex PCR assays were used to detect the A2058G and A2059G point mutations within the 23S rRNA gene of *T pallidum*. Genotyping results from these assays were then compared to a previously described real-time PCR assay using fluorescence resonance energy transfer (FRET) probes and melting curve analysis that is specific for the detection of the A2058G point mutation. Subsequently, a real-time triplex PCR was developed to distinguish the A2058G from the A2059G point mutation in a single assay and the results were confirmed by pyrosequencing.
Results Sixteen of 67 (23.9%) *T pallidum*-positive specimens collected from patients with genital ulcer disease in the US were found to have the A2059G point mutation. These strains were previously characterised as having azithromycin-susceptible genotypes (no point mutations in the 23S rRNA gene). The A2059G mutation was confirmed by a real-time duplex PCR assay containing the TaqMan probe specific for the mutation and by pyrosequencing. None of the *T pallidum* strains examined to date had both point mutations. The real-time triplex PCR assay was able to differentiate azithromycin-susceptible genotypes from resistant genotypes containing either the A2058G or A2059G point mutation in a single assay. The limit of detection of the assay was 1–10 copies using 23S rRNA gene fragments that were cloned into a plasmid.
Conclusions The previously reported real-time PCR detection platforms were designed to detect only the A2058G point mutation and were unable to differentiate *T pallidum* strains with susceptible genotypes from resistant genotypes with the A2059G mutation. The new TaqMan-based real-time allelic discrimination assay offers an alternative to the previously described PCR/RFLP method to rapidly detect both point mutations associated with azithromycin resistance in *T pallidum*. The prevalence of T. pallidum strains that harbour point mutations in the 23S rRNA gene associated with resistance to azithromycin might be much higher than previously estimated.

P3-S6.09 ABSTRACT WITHDRAWN

P3-S6.10 ANALYSIS OF SYSTEMIC AND CUTANEOUS IMMUNE RESPONSES HELPS EXPLAIN THE DUALITY OF IMMUNE EVASION AND RECOGNITION IN SECONDARY SYPHILIS

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Background Despite the robust adaptive immune responses associated with secondary syphilis (SS), which include the presence of high titres of anti-*T pallidum* (Tp) antibodies with opsonizing