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

activity, it may take months to years for host defenses to gain control of the infection. Tp's unusual outer membrane architecture is believed to underlie this duality of immune evasion and recognition. To better understand the dynamics of immune evasion and Tp clearance in early syphilis, we studied immune responses of SS patients in peripheral blood (PB) and skin in relation to Tp burdens.

Methods 27 HIV(-) SS patients and 26 healthy controls were enrolled. Circulating monocytes, dendritic cells (DCs) and natural killer (NK) cells were characterised by flow cytometry and qRT-PCR. SS skin biopsies were analysed by immunohistochemistry (IHC) and microarray. Ex vivo opsonophagocytosis assays were performed with live Tp (Nichols) and purified human monocytes.

Results Despite the demonstrated presence of Tp in blood, circulating monocytes exhibited only mild activation by flow cytometry without detectable cytokine production by qRT-PCR. We also observed decreased numbers of circulating IFNg-producing and cytotoxic NK cells along with an emergent CD16+/CD56- NK-cell population. In contrast, skin lesions, which contained abundant Tp by IHC and PCR, contained transcripts for a variety of pro-inflammatory cytokines (IFNg, TNFa, IL1b), monocyte chemotactic factors (CCL2, CCL5 and CXCL10) and macrophage and DC surface activation markers (CD80, CD86). Transcripts for genes associated with Fc-mediated phagocytosis (FcgRI, FcgR3), endosomal TLRs (TLR7-9), and cytotoxic T cells (CD8, granzyme, perforin) also were detected in lesional skin. IHC corroborated the presence of macrophages and CD4 and CD8 T cells, but not NK cells, in the biopsies. Patient sera promoted Tp uptake and monocyte activation, although substantial proportions of Tp were capable of evading phagocytosis.

Conclusions Our results support a model in which the duality of immune evasion/recognition which occurs in SS reflects the relative burdens of Tp in skin and blood as well the presence of Tp populations with differential capacity for binding opsonic antibodies. Macrophage activation due to opsonophagocytosis of a subpopulation of Tp drives the recruitment to skin of immune cells that slowly clear infection. Results also suggest that cytotoxic cellular responses may contribute to elimination of Tp.

Clinical sciences poster session 7: vaginal infections

P3-S7.01 EVALUATION OF A SIMPLE POINT-OF-CARE RAPID TEST FOR DETECTING TRICHOMONAS VAGINALIS AMONG WOMEN IN MYSORE, INDIA

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Background *Trichomonas vaginalis* is one of the easily treatable sexually transmitted infections in the world. Current methods used to diagnose trichomoniasis rely heavily on training and experience of the technician and have low performance reliability. These methods are unsuitable for settings where accessibility to a continuous electrical source may be a challenge. We examined the performance of OSOM Trichomonas rapid point-of-care test (POC) as compared to the gold standard (culture) and the routine method of diagnosis in clinical laboratories (wet-mount microscopy) in Mysore, India.

Methods Sexually active women over age 18 and seeking care at Prerana Reproductive Health Clinic were enrolled into the study from July 2009 to August 2010. Clinician- collected vaginal swabs were evaluated for trichomonads using wet-mount microscopy, InPouchTM culture, and OSOM® Trichomonas Rapid Test in a blinded manner by different investigators.

Results Of the 417 women enrolled, the prevalence of Trichomoniasis diagnosed by culture was 16.3% (95% CI 12.9% to 20.3%). As compared to culture, the sensitivity, specificity, positive predictive value and negative predictive value for wet mount microscopy was 82.4%, 98.9%, 93.3%, 96.6%; for OSOM rapid test it was 88.2%, 99.4%, 96.8%, 97.8% respectively.

Conclusion OSOM Trich rapid test had very good performance with excellent sensitivity, specificity, positive predictive value, and negative predictive value of 88.2%, 99.4%, 96.8%, and 97.8%, respectively. The implementation of OSOM Trichomonas rapid test would significantly reduce the labour and material costs. Furthermore, frequent partner reinfection as a result of wrong or missed diagnosis can be reduced. It will also reduce other complications such as pelvic inflammatory disease and susceptibility to HIV.

P3-S7.02 PERFORMANCE OF A POINT-OF-CARE DIAGNOSTIC FOR BACTERIAL VAGINOSIS AMONG YOUNG REPRODUCTIVE AGE WOMEN IN MYSORE, INDIA

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Background Bacterial vaginosis (BV) remains the most common cause of abnormal vaginal discharge in Indian women of reproductive age and is associated with increased susceptibility to HIV/STI and preterm delivery. Diagnosis of BV in resource-poor settings is often overlooked; there is a need for cheap, rapid, objective point-of-care diagnostic test.

Methods Vaginal swabs were collected from women attending a women's health clinic. Women over the age of 18 with a pH of over 4.5 were invited to participate in the study. BV was diagnosed on the basis of the Nugent score, the Amsel clinical criteria, and results of OSOM BVBlue test independently by study clinician and laboratory personnel who were blinded to the results of the other tests.

Results From August 2009 to May 2010, 313 participants were enrolled. BV prevalence was 45.1% (95% CI 41.5% to 52.8%) according to Nugent score. When compared with the Nugent score, the sensitivity, specificity, positive predictive value, negative predictive value for Amsel clinical criteria was 61.9%, 88.3%, 81.5%, 73.7%; and for BVBlue it was 38.1%, 92.7%, 82.1%, 63.9% respectively. The performance of BVBlue can be increased if it is combined with "Whiff test where the sensitivity increases to 64.4%, sensitivity 85.6%, PPV 79.3% and NPV 73.8%".

Conclusions These results highlight the importance of systematic evaluation of rapid test kits as a low-cost alternative to laboratory diagnosis in resource-constrained settings. The BVBlue test is a simple, rapid, and objective test for the diagnosis of BV and has the potential to facilitate prompt diagnosis and appropriate treatment of BV in the absence of microscopy.

P3-S7.03 THE PREVALENCE OF BACTERIAL VAGINOSIS AMONG YOUNG WOMEN IN URBAN AREAS IN NIGERIA AND ITS MAJOR RISK FACTORS

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Issue Vaginitis is polymicrobial Sexually Transmitted Diseases (STDs), associated with some STD agents, presenting signs/symptoms such as foul smelling vaginal discharge, irritation, and itching. Among the leading infectious agents causing vaginitis among young women in urban communities in Nigeria, *Gardnerella vaginalis*