

significant difference in oligotype populations was observed between URE and SYM (PERMANOVA test $p=0.0072$, $F_{2,182}$), but not between SYM and ASP (PERMANOVA test [JW2] $p=0.0.1797$, $F_{1,601}$).

Conclusion SYM and ASP oligotype populations were dominated by *Lactobacillus* and *Streptococcus* species. Microbiota observed in FVU samples of symptomatic patients with microscopy confirmed urethritis (URE) were distinct to those with symptoms but no urethritis on microscopy (SYM).

P1.39 EVALUATION OF A CHEMILUMINESCENCE ASSAY FOR THE DETECTION OF *TREPONEMA PALLIDUM* ANTIBODIES FOR THE SEROLOGICAL DIAGNOSIS OF SYPHILIS

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Introduction Syphilis is a sexually transmitted disease caused by *Treponema pallidum*, which produces 5.6 million new cases worldwide in 2012 and results in significant morbidity and mortality. Despite the availability of diagnostic tests and affordable treatment, the disease remains a global health problem. Detection of non treponemal and treponemal antibodies is the most reliable method for laboratory diagnosis of syphilis. Recently a chemiluminescence microparticle immunoassay (CMIA) has been introduced for the detection of treponemal antibodies and evaluation of its performance against reference methods is needed.

Methods Sera samples were tested with three syphilis serology tests: Venereal Disease Research Laboratory (VDRL) for non treponemal antibodies, fluorescent treponemal antibody absorption (FTA-ABS) test and CMIA. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and agreement (Cohen's kappa coefficient) of CMIA was calculated against FTA-ABS (reference group) for the diagnosis of syphilis.

Results Sera samples from 80 patients with suspected syphilis were included in this study. According to serology results: 29 syphilis non reactive sera (VDRL, FTA-ABS and CMIA non reactive), 8 VDRL reactive but FTA-ABS and CMIA non reactive sera and 43 syphilis reactive sera including 10 samples with non reactive VDRL test but FTA-ABS and CMIA reactive (past or treated syphilis) and 33 samples with all reactive tests (ongoing syphilis). General agreement between FTA-ABS and CMIA was 91.2% with kappa coefficient 0.82. CMIA clinical sensitivity was 97.7%, clinical specificity was 83.8%, PPV was 87.5% and NPV 96.9%.

Conclusion CMIA has the advantages of automation and avoids the subjectivity of FTA-ABS test. According to our results, CMIA has a good agreement with FTA-ABS but has low specificity. Limitations of this test for the diagnosis of syphilis should be taken into account since confirmatory test like FTA-ABS must still be done along with VDRL test and viewed in the context of clinical presentation

P1.40 PRESENCE OF LANGERHANS CELLS AND ACTIVATION OF T LYMPHOCYTES IN DIFFERENT STAGES OF UTERINE COLO LESIONS ASSOCIATED WITH HUMAN PAPILOMA VIRUS INFECTION

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Cervical cancer is the fourth most common cancer worldwide, and it is associated with human papillomavirus (HPV) infection. In general, most of cervical lesions regress spontaneously, indicating that HPV infection is not sufficient for inducing carcinogenesis. The immune system has a crucial role in HPV infection control, however there is still much to be investigated. Thus, this study aimed to identify the presence of Langerhans cells (LC, CD1a+) and activated T lymphocytes, induced by HPV-infection. The study enrolled 68 women, aged from 18 to 50 years old. From these, 17 were volunteers without cervical lesion, and 51 presenting HPV-related cervical intraepithelial neoplasia (CIN I-III, $n=17$ in each group). HPV-DNA was observed in 46 (90.2%) of 51 CIN volunteers, and all of women from the control group were HPV-negative. We observed an increase of CD45RA+ (naïves) cells and CD45RO+ (activated, memory) cells distribution in the epithelium and basal layer of high-grade lesions (CIN II and/or CIN III) when compared with the control group. In relation to LC, our study showed a reduced number of these cells in low-grade lesions in all of investigated areas when compared with the control group, according to pre-malign lesions evolution. For CCL20 analysis, we observed an increased number of these chemokine-expressing cells in the stroma area, in relation to lesions severity. On the other hand, we observed a decrease of these cells in the epithelium. An increase of the distribution of CCR6+ (CCL20 receptor) cells in the basal layer of the epithelium in high-grade lesions (CIN II and III) occurred when compared with the control group. Thus, we concluded that LC and T lymphocytes migrate in direction to the epithelium area, where viral infection occurs, in association with the different cervical lesions degree. However, the inflammatory cells bordering the epithelial areas, focusing the stroma area, indicate that HPV can influence the lesions microenvironment, changing negatively the inflammatory cells recruitment to the site of infection.

P1.41 A SYSTEMATIC REVIEW OF FIELD PERFORMANCE OF DUAL DIAGNOSTIC TESTS FOR HIV AND SYPHILIS: WHAT IS THE MOST APPROPRIATE "GOLD STANDARD" FOR SYPHILIS INFECTION?

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Introduction Rapid tests (RTs) improve screening and treatment in people without ready access to laboratory testing. Newer RTs linking syphilis and HIV tests on a single device could simplify testing and lower costs. Previous laboratory evaluations of dual HIV/Syphilis RTs suggest high accuracy using trained technicians and characterised sera. However, data from field evaluations would more likely represent "real world" performance.

Methods We searched the medical literature for studies evaluating performance of dual syphilis/HIV RTs against laboratory-based reference tests for syphilis and HIV, and compared performance across studies. For the syphilis component of the RTs, we compared results using laboratory-based treponemal tests (TPPA or TPHA) as the reference and (when available) TPPA+/RPR+ as the reference, considering RPR titers >1:4 to represent active syphilis (vs. previously treated infections).

Results We found 19 studies evaluating dual syphilis/HIV RT performance, of which 7 (37%) were field evaluations studying at least one of three diagnostics: *SD Bioline* HIV/Syphilis Duo Test (n=4); *Chembio* Dual Path Platform HIV-Syphilis Assay (n=2); or *Medmira* Multiplo Rapid TP/HIV Antibody Test (n=1). All used HIV EIA and TPPA or TPHA tests as reference standards; 6 also reported RPR titers. Study populations were pregnant women (n=3), female sex workers (n=1), high-risk men (n=2) and STD clients (n=1), representing a total of 13 915 persons (median study size, 415 participants; range 175 – 9983). Across studies, prevalence of HIV ranged from <1% to 78% (median, 25.3%), and of *T. pallidum* (TP) from <1% to 40.2% (median, 8.2%). RT sensitivity for HIV against EIA ranged from 93.8% to 100% (median, 99.1%), and specificity from 97% to 100% (median, 99.4%). RT sensitivity for TP against TPPA or TPHA ranged from 52.7% to 96.5% (median, 81%), and specificity from 89% to 100% (median, 98.8%), with better performance in study populations with higher TPPA/RPR+ prevalence. Using TPPA+/RPR+ >1:4 as the standard, RT sensitivity ranged from 88.5% to 100% (median, 94.3%).

Conclusion In the few published field evaluations of dual syphilis/HIV RTs, performance of the HIV component was high for all tests studied. Sensitivity of the syphilis component against TPPA was poorer, but was more accurate using probable active syphilis infection as the standard.

P1.42 A NEW MAGNETIC PARTICLE-BASED AGGLUTINATION ASSAY FOR ANTI-CARDIOLIPIN ANTIBODY DETECTION IN SYPHILIS

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Introduction A magnetic particle based assay was developed for the detection of nontreponemal anti-cardiolipin antibodies in sera of suspected syphilis cases. The presence of this group of antibodies in combination with a reactive treponemal test indicates active syphilis. In this study, we aimed to overcome technical difficulties with attaching cardiolipin to solid support. The newly developed assay potentially offers advantages of better result interpretation, accuracy, and minimum equipment need compared to traditional nontreponemal tests in diagnosing syphilis.

Methods To develop the nontreponemal magnetic agglutination assay (NT-MAA), cardiolipin antigen was modified first through a chemical oxidation process. The oxidised antigen was later covalently linked to magnetic particles. To test the beads, serum samples were mixed with cardiolipin-magnetic particle complex, and incubated in round bottom well microplates. The test was interpreted as reactive when agglutination was observed. Non-reactive sample demonstrated a “button” in the centre of a microwell. The NT-MAA was evaluated

using a panel of previously characterised human sera (n=80) and results were compared to rapid plasma reagin (RPR, ASI) and *Treponema pallidum* particle agglutination tests (TP-PA, Fujirebio). A true positive sample was defined as being reactive for both RPR and TP-PA, while a true negative as both RPR and TP-PA non-reactive.

Results Out of 80 sera tested, 48 were found true positive and 32 true negative with the reference tests. In comparison, the NT-MAA, demonstrated a sensitivity and specificity of 100% and 96.8%, respectively.

Conclusion Magnetic particle-based assays offer high flexibility because they work with different assay formats. This exploratory study, describes technical advances for development of nontreponemal test (NT-MAA), and also demonstrated an encouraging performance with the studied samples. Additional evaluation with syphilis samples from defined clinical stages of syphilis will help further validate test performance.

P1.43 ABSTRACT WITHDRAWN

P1.44 MOLECULAR TYPING AND DETECTION OF MACROLIDE RESISTANCE IN *TREPONEMA PALLIDUM* DNA FROM PATIENTS WITH PRIMARY SYPHILIS IN SÃO PAULO, BRAZIL

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Introduction Syphilis is a globally occurring sexually transmitted disease caused by *Treponema pallidum*, a non-cultured *in vitro* bacterium. Molecular typing of *Treponema pallidum* strains isolated from patients are useful for investigating the molecular epidemiologic patterns, diversity of strains and antimicrobial resistance patterns. To date, there was no data on the circulating or prevalent subtype in Brazil. In this study we aimed to determine *T. pallidum* strain diversity and analyse for the mutation associated with macrolide resistance from patients with primary syphilis attended at CSEGPS.

Methods We analysed 24 samples of primary lesion collected from patients attended at CSEGPS between 2013 and 2015. DNA was extracted with DNeasy kit (Qiagen). Standard PCR targeting *tpp47* and *poA* genes was used for screening. Molecular typing was performed by CDC established methods, by determination of the 60 bp repeats within the *arp* gene, and RFLP analysis of *tpr* subfamily II genes (E, G and J). Completed by sequence analysis of a variable region of the *tp0548* gene. The 23S rDNA mutation was analysed by DNA sequencing of PCR product.

Results: *T. pallidum* DNA was detected in samples from 15 patients. Among 12 specimens typed, subtype found were 14d/g (6), 14d/d (5) and 12b/d (1). From 10 samples analysed for 23 rDNA mutation, all showed A2058-G, no mutation was detected at A2059. One case presented a different subtype in re-infection. The first was 14d/g and the second was 14d/d.

Conclusion: *T. pallidum* detected in the samples of patients with primary syphilis are of subtypes 14d/g, 14d/d and 12b/d. The macrolide resistance mutation A2058-G was detected in