

Methods This study investigated CD4 T cell immune activation based on intracellular IFN- γ and Ki-67 expression, after ex-vivo cryptococcal antigen stimulation of whole blood samples taken from HIV-1 positive adult patients infected with or without cryptococcal meningitis, initiated on ART.

Results In the CM positive group at pre-ART visit; stimulation with *C. neoformans* crude cell wall (CW) induced a significant increase in CD4 IFN- γ production ($p < 0.05^{***}$), as compared to *C. neoformans* glucuronoxylomannan (GXM) polysaccharide antigen ($p < 0.05^*$), whilst *C. neoformans* mannoprotein (MP) stimulation failed to induce greater than baseline IFN- γ expression. The effector memory T cell subset was the major contributor to the IFN- γ elevation exhibited in CW stimulated samples. Interestingly, T cell responses to CW were found to be significantly higher in the CM positive group compared to the CM negative group ($p < 0.05^*$). Furthermore, stimulation with CW and GXM exhibited higher frequency of terminally differentiated effector memory T cells (TDEMS) compared to either negative control or MP stimulation.

Conclusion Immune activation of CD4 T cells can be achieved by *C. neoformans* CW rather than purified MP antigen, by inducing the effector memory subset to produce IFN- γ .

P5.075 BIOPLEX® 2200 HIV AG-AB: AN AUTOMATED SCREENING METHOD PROVIDING DISCREET DETECTION OF HIV-1 P24, HIV-1 ANTIBODY, AND HIV-2 ANTIBODY

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W Link, R del Rosario, M Leos, A Carillo, D King, M Baumeister. *Bio-Rad Laboratories, Benicia, CA, United States*

Background Develop an automated HIV assay with 4th generation sensitivity that can report antibody and antigen results individually, and distinguish HIV-1 from HIV-2 positives.

Methods The BioPlex 2200 HIV Ag-Ab assay uses multiplex flow immunoassay to detect HIV-1 p24 antigen, HIV-1 antibody (Groups M and O), and HIV-2 antibody in a single reaction vessel using a mixture of four populations of dyed microparticles. Each population is coated with a different HIV antigen or with p24 antibody. Results for each marker can be reported individually, and antibody-reactive specimens can be typed as HIV-1 or HIV-2. Specimens with similar levels of HIV-1 and HIV-2 antibody reactivity are reported as reactive but undifferentiated.

Results of in-house testing: To assess specificity, 5239 samples of unknown risk were tested resulting in specificity of 99.83%. To assess sensitivity, known positive specimens (209 HIV-1 Group M, 21 HIV-1 Group O, and 177 HIV-2) were tested and all were reactive. All HIV-1 samples (209 Group M and 21 Group O) were correctly identified as HIV-1, and 153 of 177 HIV-2 samples were correctly identified as HIV-2. Of 24 that were undifferentiated, 18 could not be typed by Orgenics Immunocomb®. Among 26 commercial seroconversion panels, BioPlex 2200 detected HIV-1 infection one donation sooner than Abbott Architect Combo HIV (4th generation) in three panels. In one panel, BioPlex 2200 missed one donation positive by Architect. Both tests gave equivalent results for 22 of the 26 panels.

Conclusion The BioPlex 2200 HIV Ag-Ab assay, which is currently in development, is highly sensitive and specific, and can also provide detailed screening results that will assist in identifying specimens from primary infection and HIV-2 positives and guide selection of supplemental testing.

P5.076 DEEP MYCOSES IN PATIENTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN MONTEVIDEO, URUGUAY

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M Carbia, A Otero, B Ana Laura, B Raquel. *Clinical Hospital. Medicine School, Montevideo, Uruguay*

Background Deep fungal infections have been a major cause of morbidity and mortality in HIV-infected host, gaining importance due to their severity and poor prognosis. Because instituted antiretroviral therapy in patients infected with HIV in the last decade, it is expected that the profile of the deep mycoses in our country has changed, despite the increase in cases of HIV infection registered. The objective of this study was to report the frequency of deep mycosis in a population of HIV-positive patients in Montevideo, Uruguay.

Method The clinical fungal isolates obtained from biological samples, in HIV positive patients, processed in Section Mycology, Laboratory of Pathology University Hospital, between 2008 and 2012. The diagnosis of deep mycoses, was established by conventional mycological study (direct examination and culture). This was complemented by detection of antigen of *Cryptococcus* spp. in CSF samples, and direct immunofluorescence for *Pneumocystis jirovecii* in bronchoalveolar-lavage specimens.

Results Of 479 studies in HIV-positive patients, 248 were of cerebrospinal fluid, 83-bronchioloalveolar washes, 42 biopsies, 36 blood, 24 bone marrow, skin 20, and 26 belonged to other locations. 89 samples were found positive, of which 76 were from *Cryptococcus* spp.; 6 to *Histoplasma capsulatum*, *Pneumocystis jirovecii* 6 and 1 to *Candida albicans* isolated from the peritoneal fluid. Discussion: The frequency of deep mycoses was 18.6% of the samples studied. Cryptococcosis and meningo-encephalic presentation was the most frequent (75% of samples positive for *Cryptococcus* spp.), histoplasmosis, and pneumocystosis represented 7% each of the total positive samples. The deep mycoses remains so similar to previous periods in our country, persisting as a real problem in this population. The distribution of etiologic agents remained significantly unchanged as *Cryptococcus* spp. main exponent, but still keeps a significant decrease in the frequency and *Pneumocystis jirovecii*, *Histoplasma capsulatum*, and the absence of cases of aspergillosis.

P5.077 NUCLEIC ACID AMPLIFICATION TEST (NAAT) DIAGNOSTICS COMBINED WITH DELAYED NEISSERIA GONORRHOAE CULTIVATION OF NAAT POSITIVE SAMPLES USING THE ESWARE™ SYSTEM - THE SOLUTION FOR FUTURE GONOCOCCAL ANTIMICROBIAL SUSCEPTIBILITY SURVEILLANCE?

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^{1,2,3}C M Wind, ^{1,2,3}H J C de Vries, ⁴M Unemo, ^{5,6}A P van Dam. ¹STI Outpatient clinic, Cluster Infectious Diseases, Municipal Health Service Amsterdam, Amsterdam, The Netherlands; ²Dept. of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ³Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands; ⁴WHO Collaborating Centre for Gonorrhoea & Other STIs, National Reference Laboratory for Pathogenic Neisseria, Dept. of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden; ⁵Public Health Laboratory, Cluster Infectious Diseases, Municipal Health Service Amsterdam, Amsterdam, The Netherlands; ⁶Dept. of Medical Microbiology, Onze Lieve Vrouwe Gasthuis general hospital, Amsterdam, The Netherlands

Background Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* (Ng) is a major public health problem worldwide. Nucleic acid amplification tests (NAATs) have rapidly replaced cultivation for detection of Ng. We have evaluated the ESware system for NAAT diagnostics combined with delayed Ng cultivation of NAAT positive samples for gonococcal AMR surveillance.

Methods Based on clinical indications, a urethral, cervical or anal swab was collected from patients with purulent discharge. Gold standard for diagnosis was the APTIMA Combo 2 assay (Gen-Probe). In another study, swabs from urine (UR) and urine sediment (US) were collected if Gram-negative diplococci were observed in direct smears. Flocked swabs were stored in ESware Liquid Amies (Copan) at room temperature (RT) and 4°C and cultured after 1, 24 and 48 hours.

Results From 35 patients with Ng positive NAAT, we obtained 34 (97%) Ng cultures from ESwab samples stored for 1 hour at RT. Storage for 24 hours at 4°C and RT resulted in 32 (91%) cultures. Storage for 48 hours at 4°C yielded 25 (71%), and at RT only 13 (37%, $p = 0.007$) cultures. Fourteen urine samples resulted in 13 (UR) respectively 14 (US) cultures after storage for 1 hour at RT. Storage for 24 hours at 4°C and RT resulted in 11 and 7 (UR), respectively 12 and 10 (US) cultures. Storage for 48 hours at 4°C and RT gave 3 and 1 (UR), respectively 5 and 2 (US) cultures.

Conclusion Delayed Ng cultivation from the ESwab system was successful after storage at 4°C for 24 hours in 91% and for 48 hours in 71% of cases. The ESwab system for NAAT diagnostics combined with delayed Ng cultivation of positive NAAT samples appears highly effective for future sustainable and essential gonococcal AMR surveillance. This approach is now being validated in routine practise.

P5.078 FALSE-POSITIVE NEISSERIA GONORRHOEAE RESULTS IN URINE SAMPLES USING A HIGHLY SENSITIVE NAAT TESTS: THE SAMPLING SITE AS A SOURCE OF CONTAMINATION?

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^{1,2}A P Van Dam, ¹K Adams, ¹I Linde, ¹S Bruisten, ¹A C G L Speksnijder, ³P Van Leeuwen, ^{3,4}H J C De Vries. ¹Public Health Laboratory, Cluster Infectious Diseases, Municipal Health Service, Amsterdam, The Netherlands; ²Onze Lieve Vrouwe Gasthuis, dept of Medical Microbiology, Amsterdam, The Netherlands; ³STI outpatient clinic, Cluster Infectious Diseases, Municipal Health Service, Amsterdam, The Netherlands; ⁴Department of Dermatology, Academic Medical Center, Amsterdam, The Netherlands

Introduction False-positive results due to contamination of NAATs have been described. Apart from the laboratory, also the area where samples from patients are collected can be the source of the contamination.

Methods and results: In a 46 days period, 62 (7.3%) of male patients visiting the STI outpatient clinic with a low risk for gonorrhoea showed a positive NAAT (AC 2, Hologic-GenProbe) for *Neisseria gonorrhoeae* (NG) in urine. This was only 0.8% in the previous 6 months. The prevalence of positive NAAT results for *Chlamydia trachomatis* (CT) remained unchanged. Culture was positive in only 2/24 NG-NAAT-positive patients whose cultures were available. The prevalence of NG among high-risk patients as determined by culturing, and the positive NG-NAAT results from vaginal, rectal and pharyngeal swabs from the STI clinic and from urines received from other practises remained unchanged.

All 5 environmental swabs from the male bathroom and all 4 swabs from transport trays were positive in NG-NAAT, but only 1 of these 9 was positive for CT. Swabs from trays from the laboratory, routinely cleaned with chlorine, were negative. An audit showed that some clients do not deliver their urine samples in a hygienic way and employees who transferred urine into Aptima tubes might have touched the seal of these tubes.

The pseudo-outbreak ended after daily cleaning of bathrooms and trays with chlorine and strictly following anti-contamination guidelines. Afterwards only 0.2% of low-risk male patients had a positive NG-NAAT in urine. Thirty-seven patients who had been treated for gonorrhoea were informed about the possible incorrect diagnosis.

Conclusion This pseudo-outbreak was most likely a consequence of external contamination of trays and test tubes with nucleic acids from the sampling site, in combination with inadequate handling of tubes during pipetting.

P5.079 LABORATORY DIAGNOSIS OF NEUROSYPHILIS IN PATIENTS CO-INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND NEGATIVE-HIV PATIENTS IN MONTEVIDEO-URUGUAY

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R Balleste, N Rodriguez, N Garcia, P Lopez, C Buzzi. *Clinical Hospital, Medicine Faculty, Republic University, Montevideo, Uruguay*

Background Syphilis laboratory diagnosis, is made through the dosage of circulating antibodies in blood, but is not enough when neurological involvement is suspected.

A positive Venereal Disease Research Laboratory test (VDRL) result in cerebrospinal fluid (CSF) establishes the diagnosis of neurosyphilis, but it's negativity does not rule it out, therefore the need to use other immunological tests arises. The aim of our study was to evaluate the immunological tests performance used for neurosyphilis diagnosis and compare them between HIV infected and uninfected patients.

Methods We studied 37 patients (17 positive HIV and 20 negative HIV) from 2005 to 2012. We selected patients with positive Treponema Pallidum Hemagglutination Assay (TPHA) result in serum and CSF. Those patients underwent VDRL and, IgG and Albumin dosage in serum and CSF. TPHA-index and ITpA-index were calculated, intrathecal IgG production and indemnity of the blood brain barrier were evaluated through "Reiber's" diagram. Results: Neurosyphilis was diagnosed in 21 patients (6 positive HIV and 15 negative HIV): 18 reactive CSF VDRL, 13 TPHA in CSF > 1/320, 13 Index TPHA/Albumin > 70, 24 ITpA index ≥ 2 , in 3 patients neurosyphilis diagnosis was established only by high TPHA titers and high TPHA-index (2 positive HIV and 1 negative HIV). 14 patients had Intrathecal IgG synthesis and 10 had blood-brain barrier disruption.

Conclusions CSF VDRL made neurosyphilis diagnosis in 86% of patients, with a high concordance between CSF VDRL and CSF TPHA titer > 1/320 and TPHA-index > 70; the immunological tests performance was similar in HIV positive and HIV negative patients. Neurosyphilis diagnosis was established with the CSF/serum indexes and TPHA titers in 14% of the studied population. This highlights the importance of including the indexes in the routine diagnosis of neurosyphilis. Intrathecal IgG synthesis and disruption of the blood-brain barrier predominated in patients with neurosyphilis.

P5.080 COMPARISON OF AN IN-HOUSE POLYMERASE CHAIN REACTION AND DIRECT FLUORESCENT ANTIBODY ASSAY FOR DETECTION AND TYPING OF HERPES SIMPLEX VIRUS IN CLINICALLY SUSPECTED GENITAL HERPES

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P Bhalla, V Patwardhan. *Maulana Azad Medical College, New Delhi, India*

Background Molecular assays based on PCR have become an important tool for the detection of herpes simplex virus-1&2 DNA in clinical specimens. Detection and typing of HSV can also be done by a monoclonal antibody based DFA. The present study was undertaken to standardise an in-house PCR for detection and typing of Herpes Simplex Virus (HSV) and compare it with Direct Fluorescent Antibody (DFA) test.

Methods 44 patients with genital herpes attending the STD clinic were studied. Specimens collected from genital lesions were placed in Viral transport medium (VTM) and stored at -70°C till tested. DNA extraction was done using QiaAmp DNA mini kit (Qiagen, USA), PCR was carried out in GeneAmp PCR system 9700 (Applied Biosystems). Post PCR analysis of PCR product was done by electrophoresis using 2% agarose gel. DFA (BioRad) was also done for identification and typing of HSV-1&2.

Results By DFA, 4 specimens were positive for HSV 1, 19 were positive for HSV 2 while 7 were positive for both. By PCR, 5 were HSV-1 positive, 18 were HSV-2 positive while 6 were positive for both HSV-1 & 2. (κ for HSV-1 = 0.879, HSV-2 = 0.63.) One HSV- 1 and 3 HSV-2 cases was positive by PCR but not by DFA. Four specimens that were positive by DFA but negative by PCR