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 meningoitis, 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.

**Poster presentations**

**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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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 157 of 177 HIV-2 samples were correctly identified as HIV-2. Of 24 that were undifferentiated, 18 could not be typed by Orgenics Imunocomb®. 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 prophylactic treatment.

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


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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.

Methods 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 jiroveci 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 meningocencephalic 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 aspergillosis.

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


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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 ESwab 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 ESwab Liquid Amies (Copen) 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 (UK) 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 (UK), 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 practice.


Introduction False-positive results due to contamination of NAATs have been described. Apart from the laboratory, also the area where samples from patients can be collected is 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 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 nuclic acids from the sampling site, in combination with inadequate handling of tubes during pipetting.


Background Molecular assays based on PCR have become an important tool for the detection of herpes simplex virus-1or2 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 herps 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. (kappa 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...