MULTI-PEPTIDE ELISAS OVERCOME CROSS-REACTIVITY AND INADEQUATE SENSITIVITY OF CHLAMYDIA TRACHOMATIS AND C. PNEUMONIAE SEROLOGY

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Background Chlamydia spp. serology is compromised by cross-reactivity of classical antigens. For specific detection of anti-trachomatis (Ctr) and anti-C. pneumoniae (Cpn) antibodies, we developed and validated novel peptide ELISAs.

Methods Strongly reactive peptide antigens of 24 Ctr- and 48 Cpn-specific B-cell epitopes of multiple immunodominant chlamydial proteins were used in this study. For specific detection of anti-Ctr and anti-Cpn antibodies, 185 human sera were tested in colorimetric ELISAs with mixtures of 12–24 Ctr or Cpn peptide antigens using polyclonal anti-human IgG-HRP conjugates. For comparative evaluation, these sera were tested with 4 Ctr and 4 Cpn commercial IgG ELISAs.

Results In commercial ELISAs, Ctr and Cpn individual serum reactivity was 54% biased towards positivity for both species (co-positivity), but unbiased in Ctr and Cpn peptide antibody assays. This finding suggested a severe specificity problem (cross-reactivity) of commercial ELISAs, but not peptide assays. Using hyperimmune mouse sera against each of 11 Chlamydia spp., we confirmed that commercial Ctr and Cpn ELISA antigens are cross-reactive among all Chlamydia spp., but Cpn and Ctr peptide antigens react specifically only with antisera against the cognate chlamydial species. By comparison at 90% specificity to a Ctr-peptide composite reference standard (CRS) for human anti-Ctr antibody status, the Ctr mixed peptide assays showed 86–83% sensitivity, significantly higher than the 59–34% sensitivity of 4 commercial anti-Ctr ELISAs. Relative to a Cpn-peptide CRS, the Cpn mixed peptide assay showed 86–80% sensitivity at 90% specificity, significantly higher than the 48–25% sensitivity of 4 commercial anti-Cpn ELISAs.

Conclusion For detection of anti-Ctr and -Cpn antibodies, commercial ELISAs are not suitable due to cross-reactivity. In contrast, mixed peptide assays are accurate with simultaneous high specificity and sensitivity, and reliably determine anti-Ctr and anti-Cpn antibody prevalence. With convenient use for non-specialized laboratories, these peptide ELISAs will improve Ctr and Cpn serodiagnosis.

Disclosure No significant relationships.

PENILE MICROBIOME AND URINARY CYTOKINES OF KENYAN MEN WHO HAVE SEX WITH MEN AND MEN WHO HAVE SEX WITH WOMEN

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Background MSM are disproportionately affected by HIV and STIs compared to men who have sex with women (MSWomen). This may be due in part to different burden of mucosal inflammation. We compared penile microbial composition between MSM and MSWomen and association with mucosal inflammation.

Methods In this cross-sectional study, we enrolled 43 MSM and 43 MSWomen, who were HIV negative and matched on age and circumcision status. The penile microbiome was assessed via meatal swab, with 16S rRNA gene amplicon sequencing. Urinary cytokine concentrations (TNF-α/IL-1β/IL-8/IL-10/IP-10) were measured using Luminex. Random Forest (RF) identified genus-level taxa differing between MSM and MSWomen. Taxa from RF were regressed on cytokine outcomes, with multiple testing correction and information criterion model selection.

Results Men were median age 24 and 77% circumcised. There were substantial differences in educational attainment, employment, alcohol and drug use, condom use, and number of sexual partners, with MSM having greater behavioral risks. Microbiome composition differed markedly between MSM and MSWomen: RF discriminated between MSM and MSWomen with 84% accuracy. Taxa with greatest
discriminating influence were *Lactobacillus*, *Anaerococcus*, and *Staphylococcus*. In crude analysis, cytokines TNF-α/IP-10/IL-10 were elevated among MSWomen (p<0.05, each); IL-8 did not differ by group; IL-1β was higher among MSM (p=0.03). Cytokine concentration increased in response to *Corynebacterium* (IL-8/TNF-α/IP-10/IL-1β), *Gardnerella* (IL-8/IP-10/IL-1β), *Veillonella* (IL-8/IP-10/IL-1β), and *Peptoniphilus* (IL-8/IL-1β). Microbiome composition did not account for the difference in TNF-α, IP-10, or IL-10 between groups; the difference in IL-1β became non-significant after accounting for taxa. Among MSWomen, IL-1β (p=0.01) and IL-8 (p=0.05) were elevated if the female partner had BV.

**Conclusion** To our knowledge, this is the first comparison between MSM and MSWomen of penile microbiome and urin ary cytokines. Future studies should examine whether microbiome and mucosal inflammation differences between MSM and MSWomen cause differential risk of HIV/STI acquisition or differential impact on efficacy of HIV/STI interventions.

**Disclosure** No significant relationships.