

most clinically important genetic markers conferring resistance to antibiotics used for the treatment of gonorrhoea.

**Methods** We designed a fluorescent dye real-time PCR assay combined with high resolution melting (HRM) analysis. Several triplex and duplex reactions included target sequences for: two NG-specific genes (*porA* and *opa*); targets specific for the *penA* mosaic XXXIV (A501, G545S), associated with reduced cephalosporin susceptibility; single nucleotide polymorphisms conferring resistance to ciprofloxacin (GyrA S91F), azithromycin (23S rRNA A2059G and C2611T) and spectinomycin (16S rRNA C1192T and 5S rRNA T24P). We tested >50 characterised NG isolates, including: clinical isolates resistant to ciprofloxacin, azithromycin, spectinomycin, ceftriaxone (strain F89), strains with reduced cephalosporin susceptibility, the wild-type reference strain ATCC49226, and commensal *Neisseria* spp.

**Results** HRM analysis correctly identified: all NG strains and all mutations, except for the A501P mutation in strain F89. However, cross-reactions with commensal *Neisseria* spp. occurred for: *penA* mosaic XXXIV ( $n = 3$  species) and 23S rRNA ( $n = 3$  species).

**Conclusion** Our multiplex PCR assay accurately identified NG and detected the most frequent mutations associated with antimicrobial resistance in cultured isolates. The assay provides results significantly quicker than current culture-based methods. The analytical sensitivity and specificity of the assay for use with urethral, rectal, pharyngeal and vaginal specimens should be evaluated in the near future.

#### 005.4 MULTIPLEX ASSAY FOR SIMULTANEOUS DETECTION OF MYCOPLASMA GENITALIUM AND MACROLIDE RESISTANCE USING PASS MNAZYME QPCR

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**Introduction** Treatment of *M. genitalium* (Mg) infection with single dose 1 g macrolide antibiotic, azithromycin, is routinely utilised in clinical practice however this has been associated with emergence of macrolide resistance and ineffective cure rates. Mutations at two positions, 2058 and 2059 (E. coli numbering) in the Mg 23S rRNA gene have been associated with macrolide resistance. Simultaneous detection of Mg and mutations associated with azithromycin failure could be used to offer rapid delivery of effective second line regimens. This study evaluates a combined diagnostic-resistance assay for potential use in clinical settings.

**Methods** Clinical samples diagnosed with Mg were evaluated with a combined-diagnostic resistance assay that employs novel "Primer Assisted Sequence Switching" (PASS) primers coupled with Multi-component Nucleic Acid enzyme" (MNAzyme) detection. PASS primers selectively amplify target sequences resulting in enhanced specificity for mutant over wild-type and MNAzymes allow for efficient detection and discrimination of multiple mutations simultaneously. Multiplexed PASS MNAzyme qPCR was evaluated by comparison to previously screened clinical samples for Mg (MgPa gene) and 23S mutations using Sanger sequencing and HRM analysis.

**Results** Using artificial templates, this assay was able to detect mutation templates ranging from 10–10,240 copies/reaction, with an associated Mg detection limit comparable to existing

assays used in routine diagnostics. Preliminary screening of DNA from 24 clinical samples revealed Mg detection range of 3–300,000 copies/reaction. This assay was able to detect the correct mutation in 21/24 cases (87.5%), however was not readily able to assign a mutation at the lowest concentrations tested, an issue present in all rapid mutation screening tests for Mg.

**Conclusion** Multiplexed PASS MNAzyme qPCR offers the ability for simultaneous detection of Mg and macrolide resistance mutations. This assay offers considerable advantages in clinical settings with rapid identification of macrolide resistant strains and the ability to implement effective second line agents without delay.

**Disclosure of interest statement** SpeeDx is the developer and manufacturer of the assay evaluated in this study.

#### 005.5 TREPONEMA PALLIDUM STRAIN-TYPES IN AUSTRALIA AND ASSOCIATION WITH MACROLIDE RESISTANCE

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**Introduction** Greater understanding of the molecular epidemiology of *Treponema pallidum* has the potential to enhance control measures. The aim of this study was to determine the strain-types of *T. pallidum* in Sydney, Australia, and investigate clinical and epidemiological associations.

**Methods** Stored *T. pallidum* DNA from samples between 2004–2011 were categorised into strain-types using analysis of the acidic repeat protein (arp), *T. pallidum* repeat sub-family (tpr) genes, and sequence analysis of the TP0548 gene as described by Marra (2010). Associations between strain-type, the macrolide resistance mutation A2058G, and clinical and demographic characteristics were further investigated.

**Results** 194 samples from 187 patients were successfully strain-typed into at least 19 separate strains. The predominant strains were 14d/g (91/194; 47%), and 14d/f (18/194; 9%). 14d/g remained the commonest strain throughout the study period, and was associated with the A2058G mutation (90/91 vs 70/103 non-14d/g strains: OR 42.4; 95% CI 5.7–317.9  $p < 0.001$ ). Clinical information was available for 91 samples. 90 were male, of whom 89 reported sex with other men. 21/48 (44%) strains from HIV negative patients were strain 14d/g vs 20/43 (47%) from those HIV positive (OR 1.1; 95% CI 0.5–2.5  $p = 0.79$ ). When acquisition was reported as being outside Australia 2/13 (15%) cases were strain 14d/g vs 39/78 (50%) of those reporting sex only within Australia (OR 0.18; 95% CI 0.04–0.87  $p = 0.033$ .) Both cases of neurosyphilis were attributable to TP0548 gene sequence "f".

**Conclusion** This is the first time that enhanced strain-typing has been used to define the epidemiology of *Treponema pallidum* infections in the Asia-Pacific region. The most common strain (14d/g) was associated with macrolide resistance and acquisition within Australia. Despite the diversity of strains, the lack of association with HIV status suggests sexual networks between HIV negative and HIV positive men overlap.

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