Verification of Seegene Allplex™ assays for *Mycoplasma genitalium* resistance testing

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Introduction

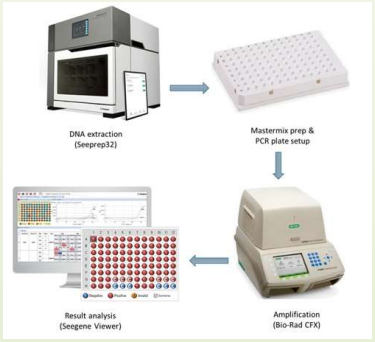
Mycoplasma genitalium (MG) is a sexually transmitted pathogen of growing concern due to increasing antimicrobial resistance, particularly to macrolides and fluoroquinolones. Culture-based diagnosis is impractical because of the organism's fastidious nature, extremely slow growth, and requirement for specialist conditions. Molecular methods provide a rapid and sensitive alternative, enabling both pathogen detection and identification of resistance-associated mutations.

Objectives

- Internal verification of the performance of Seegene Allplex™ MG &AziR and Seegene Allplex™ MG & MoxiR real-time PCR assays.
- Evaluation of the practicality of introducing Seegene assays into routine diagnostics, considering cost, hands-on time, sample throughput, and the clinical need for fluoroquinolone resistance testing.

Methods

- DNA extraction using Seeprep32 platform and STARMag ProPrep kit (tube type)
- Manual preparation of Mastermixes and PCR plate set up
- Amplification on BioRad CFX96 Dx System and result analysis by Seegene Viewer Software
- Comparison of results obtained by Seegene assays with:
  - Cepheid SpeeDX ResistancePlus MG Flexible (for macrolides resistance)
  - in-house developed assay used in UKHSA STI laboratory in Colindale (for fluoroquinolones)
- Calculating Segeene assays' diagnostic specificity (Dsp), sensitivity (Dse), accuracy (Da), negative and positive predictive value (NPV and PPV) and establishing level of agreement with reference methods using Gold Standard analysis and Coehn's kappa coefficient (κ value)
- Calculating inter- and intra-precision for each mutation



Results and Discussion

- In total, 31 samples were tested for macrolide (azithromycin) resistance and 5 samples for fluoroquinolone (moxifloxacin) resistance.
- Specimens included clinical samples (urine, vaginal and cervical swabs) and EQA material from Aurevia Labquality. Clinical samples were collected into Roche Cobas PCR media – an UTM routinely used for STI screening by the Immunology and Virology Department ~~in~~ at Cumberland Infirmary.
- The Allplex™ MG & AziR assay demonstrated overall agreement of 97% with the Cepheid assay. The Allplex™ MG & MoxiR assay showed 100% agreement with the in-house assay used in UKHSA STI laboratory (Figure 1).
- Gold Standard analysis of Seegene AllplexTM MG & AziR kit based on the obtained results reveals an very good performance with Dsp=100%, Dse=94.74%, Da=96.77% , NPV=92.31%, PPV=100% and κ=0.97 (Figure 2).
- Intra- and inter-precision of both assays, calculated for each individual mutation confirms assays' excellent performance; with %CV below 10% (Figure 3).

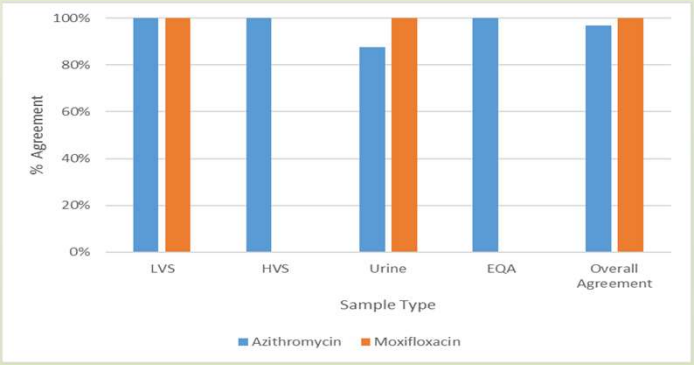


Figure 1 Seegene Allplex™ assays agreement with a reference method by sample type.

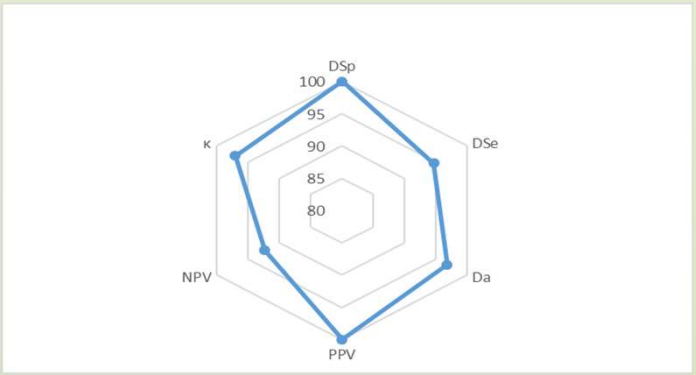


Figure 2 Seegene Allplex™ MG & AziR performance metrics for Gold Standard analysis: Dsp=100%, Dse=94.74%, Da=96.77% , NPV=92.31%, PPv=100% and κ=0.97

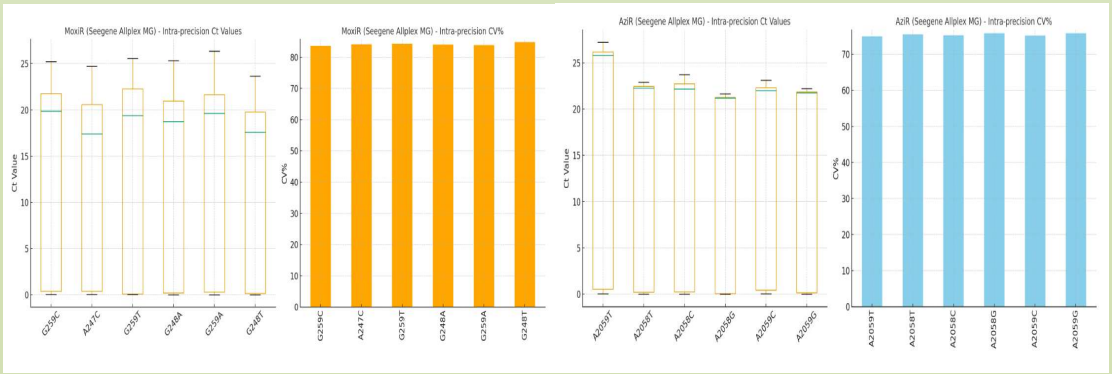


Figure 3 Inter- and intra-precision for both Seegene Allplex™ MG assays calculated for each detected mutation.

- Due to very limited number of specimens containing *M. genitalium* strains resistant to fluoroquinolones, the statistical analysis of Seegene Allplex™ MG & MoxiR could not be completed.
- Not all point mutations could be found in the clinical samples available for this verification.
- The diagnostic sensitivity for azithromycin resistance, especially in urine samples, may be lower than 95%.

Conclusions

Based on this verification, Seegene Allplex™ MG & AziR and MG & MoxiR kits could serve as an excellent alternative to currently used assay. The advantages of using Seegene kits include: testing for resistance against fluoroquinolones as well as macrolides, providing a broader picture of organism's sensitivity patterns with the same sample and testing bigger batches of samples (as oppose to a single sample per cartridge). However, several factors must be considered before routine implementation, including sensitivity, hands-on time, workflow complexity, cost, consumables management, and interpretive skill requirements. As the study was conducted on an admittedly small sample batch, it is recommended that validation is performed with a larger sample set prior to implementation into routine use.

Bibliography

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