Evaluation of the SpeeDX Real-Time PCR assay for *Mycoplasma genitalium* and Azithromycin Resistance Compared to Two Real-Time Research PCR Assays and Aptima Combo 2

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Background and Objective

Mycoplasma genitalium (MG) is a sexually transmitted infection (STI) associated with cervicitis and pelvic inflammatory disease (PID) in women and urethritis in men.

As with other bacterial STIs, there is growing concern that MG is developing antibiotic resistance resulting in treatment failures.

The objective of this study was to conduct a performance evaluation of the SpeeDX assay, a real-time PCR capable of detecting MG while testing for 23S rRNA mutations associated with Azithromycin resistance.

We conducted the performance evaluation both retrospectively and prospectively.

Methods: Retrospective Study

For the retrospective study we tested vaginal samples (N=289) with the following composition:

- 59 samples consisted of a vaginal swab placed in PBS
- 230 samples consisted of a vaginal swab placed in Amies media.

For the retrospective study we utilized the SpeeDX assay as well as real-time PCR assay for the MG 16S rRNA gene and the MG pdhD gene for comparison purposes. The gold standard utilized was two of three assays in agreement for a true positive.

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Methods: Prospective Study

These samples came from a study to determine the prevalence of MG among a cohort of young urban pregnant and non-pregnant women and the associated baseline clinical risks.

For the prospective study, we tested dry vaginal swabs (N=176) resuspended in water and utilized the SpeeDX assay as well as real-time PCR assay for the MG 16S rRNA gene and the MG pdhD gene for comparison purposes. These samples were also tested with the Aptima Combo-2 MG assay. The gold standard utilized was three of four assays in agreement for a true positive.

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For both studies we also sought to assess the rate of 23S mutations is positive samples associated with MG Azithromycin resistance.

Results: Retrospective Study (N=289)

Assay	Sensitivity*	Specificity	Kappa [95% CI]
SpeeDX	92% (23/25)	99% (262/264)	0.91 [0.813-0.997]
16S PCR	84% (21/25)	100% (264/264)	0.91 [0.813-0.997]
pdhD PCR	96% (24/25)	99% (261/264)	0.91 [0.883-0.997]

*True positive defined by two of three assays in agreement with each other.

Results: Prospective Study (N=176)

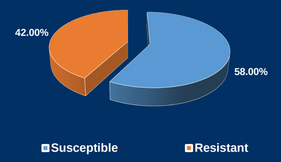
Assay	Sensitivity*	Specificity	Kappa [95% CI]
SpeeDX	91% (10/11)	99% (164/165)	0.9 [0.77-1]
16S PCR	100% (11/11)	100% (165/165)	1 [1-1]
pdhD PCR	100% (11/11)	100% (165/165)	1 [1-1]
Aptima	91% (10/11)	95% (156/165)	0.64 [0.43-0.84]

*True positive defined by three of four assays in agreement with each other.

Results: Composition of Azithromycin Resistance

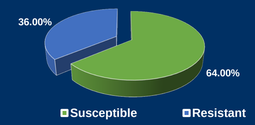
The following charts illustrate the distribution between Azithromycin susceptible and Azithromycin resistant samples in the studies performed.

Percentage of Azithromycin resistance in positives from both studies

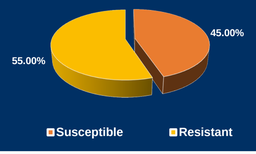


Results: Composition of Azithromycin Resistance

Percentage of Azithromycin resistance in positives from the retrospective study



Percentage of Azithromycin resistance in positives from the prospective study



Limitations

- Small number of positives for evaluation in the prospective study
- No Aptima Combo 2 results for retrospective study for comparison
- Time to result for SpeeDX assay is over 2 hours including extraction time

Conclusions

The SpeeDX assay performed well compared to other assays for both studies, with a mean sensitivity and specificity of 91.5% and 99% respectively, and was able to detect 23S mutations associated with Azithromycin resistance.

Azithromycin resistance was high across both studies at 36% and 45%, respectively.

Implications: The SpeeDX assay could be useful for surveillance efforts where MG is high in incidence and Azithromycin treatment failures are reported/expected.