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Evaluation of the Bio-Rad Dx real-time system in combination with the MagNA pure platform (Roche) for the detection of *Mycoplasma genitalium* in urogenital specimens

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Objectives:
To compare the performance of the Bio-Rad CT/NG/MG Dx Assay for the detection of *Mycoplasma genitalium* with a validated reference in-house method (Jensen *et al.*, Journal of Clinical Microbiology, 2004).

Methods

- DNA from patient samples/ external quality controls was extracted using the MagNA Pure platform (Roche DNA I High Performance protocol): 200 µl sample was used for extraction.
- 10 µl of internal control from the kit was added to every sample.
- DNA was eluted in 110 µl elutionbuffer.
- 5 µl DNA was used for the PCR.

The assay was checked for analytical sensitivity, specificity, accuracy, linearity and precision following the Belgian guidelines (Raymaekers *et al.*, Acta Clinica Belgica, 2011).

Results:
Analytical sensitivity:
A negative PreservCyt specimen was spiked with an AmpliRun® *M. genitalium* DNA control (Vircell) to determine the limit of detection (LOD with a 95% hit rate). The lowest concentration was 5000 copies/ml, correlating with 45 copies/PCR. This LOD was about 10 times higher than estimated in the package insert for urine samples. This can be explained that urine samples should be concentrated by centrifugation before lysis. No further purification process is needed afterwards.
The LOD of the in-house test is about 4000 copies/ml.

Specificity:
The specificity was sufficiently documented by the manufacturer, and was not tested again.

Accuracy:
73 specimens (66 patients and 7 external quality controls) were tested. Five out of 37 were missed by the Dx Real-Time System. All these specimens had a high Cq value with the in-house method. The total agreement with the in-house method was 93%.

Linearity:
The AmpliRun® *M. genitalium* DNA control was serial diluted. Each of the five dilutions was repeated in two replicates. The slope was -3,47 and the efficiency of the reaction was 98,68%. This met our criteria.

Precision:
One positive (mean Cq 33,3) and one weak positive (mean Cq 36,6) sample were extracted in triplicate on 3 different days. All 9 results were positive. The standard deviation (SD) and the coefficient of variation (CV%) for the positive sample were similar as described in the package insert (0,4 SD, 1,3 CV% respectively).

Conclusion:
Although the Bio-Rad Dx CT/NG/MG Assay is less sensitive than our in-house method, it is an easy method with few hands on time which can detect 3 pathogens simultaneously in the same reaction (*M. genitalium*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*). As the lysis step, proposed in the instructions for use, is time consuming, the MagNA Pure platform offers a good alternative.