Az Damiaan
Dicht bij het leven

EVALUATION OF PELVOCHECK® CT/NG (GREINER BIO-ONE): A NEW DIAGNOSTIC MICROARRAY-BASED KIT FOR THE DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* DNA IN UROGENITAL SPECIMENS

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Background

Pelvic inflammatory disease is the most common and severe complication of some sexually transmitted infections mostly caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). PelvoCheck® CT/NG (Greiner Bio-One) is a new DNA microarray-based test for the detection of a 16S rRNA gene fragment including assay-controls for the monitoring of sampling/extraction, PCR and hybridisation. The assay has been validated using the oCheck® DNA extraction kit (Greiner Bio-One) which is a manual procedure that might be time consuming. In this evaluation the performance of the assay was compared with a validated reference in-house real-time qPCR method [1,2] using an alternative automated extraction platform.

Material and Methods

DNA from 28 patients (13 CT, 5 NG, 2 CT+NG positive and 8 negative) and 20 external quality controls (QCMD-INSTAND) was extracted using the MagNA Pure LC2 platform (Roche, DNA I High Performance protocol).
200 µl sample (swab in M4RT® transport medium (Remel)/urine) was used for extraction. DNA was eluted in 110 µl elution buffer and stored at -20° C until analysis. PCR and detection was performed according to the instructions of the manufacturer.
The assay was checked for accuracy, specificity, sensitivity and reproducibility, following the Belgian guidelines [3].

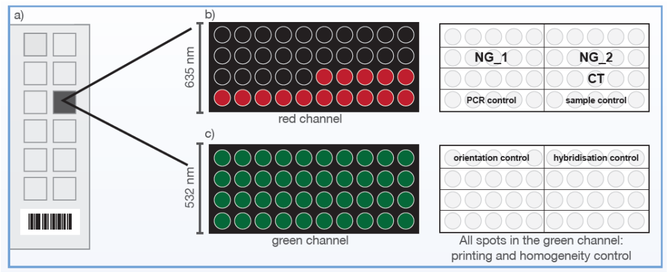
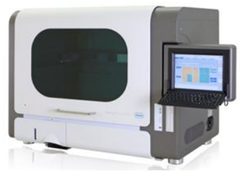


Fig 1: Design of the Pelv**oCheck**® CT/NG chip. (a) Schematic drawing of the chip with 12 independent compartments containing each one microarray. (b) and (c) Microarray images displayed by the CheckReport™Software for the two different excitationwavelengths used for scanning and schematic drawings of the chip layout. Pathogen-specific probes and on-chip controls are indicated.

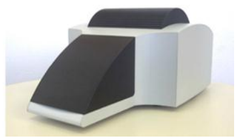
Urogenital specimen

PelvoCheck® CT/NG assay



MagNA Pure LC2 DNA extraction platform (Roche)

GeneAmp 9700 PCR System (Applied Biosystems)



CheckScanner™

Fig 2: Workflow from sample to result. The CheckScanner™ reads the Pelv**oCheck**® DNA chip and the CheckReport™Software interprets the Signal to Noise Ratio (SNR) for each probe.

Results

Patients:
25 out of 28 patients were concordant with the reference method. The three missed samples had a Cq (Quantification Cycle) value over 35 for CT.
The aberrant NG strain described by Geeraerts et al. [4], was correctly detected. Human DNA was detected in all samples.

External quality controls:
15 out of 20 external quality controls were concordant with the reference method. Three weak CT and two weak NG positive samples all with a Cq value over 35 were missed. Two were QCMD core samples for CT (424 copies/ml and 8620 copies/ml). The NG strain harbouring an Neisseria meningitis porA gene, the CT Swedish new variant, the serovars L and F, were all correctly detected. Negative samples were scored “failed” by the software because of the lack of human DNA in the QCMD panel.

Specificity
Specificity was well documented by the manufacturer. No false positive results were obtained. There was no cross reaction with *Trichomonas vaginalis* and *Mycoplasma genitalium*.

Reproducibility
Three samples (one positive for CT, one positive for NG and one positive for both pathogens) were analysed on three different days all giving the same result.

Conclusion

The PelvoCheck® assay is easy to perform with little hands on time if DNA is extracted by an automatic platform. The assay appears to be analytically slightly less sensitive compared to the reference in-house method. To address this question in more detail further analyses with clinical samples (including very weak positive samples) and quality controls stabilized in a suitable matrix are required and they have to be processed as validated by the manufacturer. The clinical significance of very weak positive samples (Cq > 35) can also be a topic for further discussions.

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References: [1] Tabrizi et al., Sex Transm Dis, 2005 [2] modified COBAS® TaqMan® CT Test v2.0, Roche [3] Raymaekers et al., Acta Clinica Belgica, 2011 [4] Geeraerts et al.,J. Clin Microbiol, 2005