22nd ECCMID London, United Kingdom 31 March – 3 April 2012
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

P1809

VALIDATION OF COBAS® TAQMAN® CT TEST V2.0 (ROCHE) ON THE ROTOR GENE Q PLATFORM

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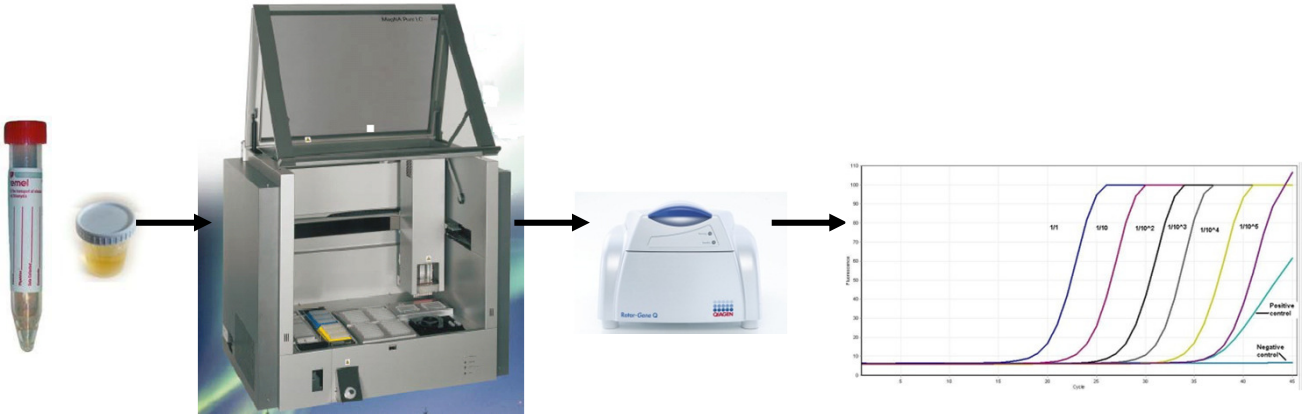
Introduction and Purpose

Validation of the COBAS® TaqMan® CT Test real-time PCR assay, de facto designed to be used with the COBAS® TaqMan® 48 Instrument (Roche), on the Rotor Gene Q platform (Qiagen) for detection of *Chlamydia trachomatis* in genital swabs and urine specimens.

Methods

200 µl M4RT® (Remel) transport medium (swabs)/urine was used for extraction using the MagNA Pure platform (Roche, DNA I High Performance protocol). Elution in 110 µl elution buffer. The proposed reaction volume was reduced from 100 µl (50 µl DNA + 50 µl mastermix) to 50 µl (25µl DNA + 25 µl mastermix). The assay was checked for analytical sensitivity, specificity, accuracy and precision following the Belgian guidelines (Raymaekers et al., Acta Clinica Belgica, 2011).



From sample to result: DNA extraction using the MagNA Pure LC instrument, Real-time PCR on the Rotor Gene Q with amplification plots of a 10 fold dilution serie

Results

Analytical sensitivity
M4RT® transport medium was spiked with the positive control of the kit and a negative urine specimen was spiked with a Vircell DNA control (Serovar L2) to determine the limit of detection (LOD with a 95% hit rate). The lowest concentration was 860 and 880 copies/ml M4RT and urine respectively, correlating with 40 copies/PCR. This met our validation criteria and is comparable with other commercial kits (1000 copies/ml).

Specificity
Although the specificity was sufficiently documented by the manufacturer, the specificity was checked against 43 negative samples. There was no cross reaction found with any of these organisms (including *Citrobacter freundii, Staphylococcus aureus, Neisseria gonorrhoeae, Escherichia coli, Enterobacter aerogenes, Chlamydia pneumoniae*).

Accuracy
77 specimens were tested (36 positive and 43 negative samples). The panel composition consisted of 50 patients tested against a reference method (AMPLICOR® CT Test/ BD ProbeTec™) and 27 external quality controls. The external quality controls included the Swedish variant, Serovars J, E and L2. There was a 100% agreement.

Precision
Two pools of 0.4 and 15 DNA copies/µl elution buffer were analysed in double during 10 days. The standard deviations (SD) for the weak positive and positive sample were 0.66 and 0.84 Cq. This met our validation criteria of SD < 1 Cq.

Conclusions

The COBAS® TaqMan® CT Test met all our validation criteria and was implemented in our routine diagnostic laboratory. The kit can be used in combination with the Rotor Gene Q platform and costs can be saved by reducing the reaction volume without any loss of sensitivity.

Acknowledgement: we appreciate the effort of the laboratory technicians V. Allegaert and M. Blomme, for their contributions.