Molecular diagnosis of sexually transmitted infections

Validation of COBAS® TaqMan® CT Test v2.0 (Roche) on the Rotor Gene Q platform

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Objectives: Validation of the COBAS® TaqMan® CT Test real-time PCR assay, de facto designed to be used with the COBAS® TaqMan® 48 Instrument, 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 (25 µl 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).

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 other organisms.

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 0.56 and 0.84 Cq. This met our validation criteria of SD < 1 Cq.

Conclusion: 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.