DETECTION OF MYCOPLASMA GENITALIUM MACROLIDE RESISTANCE USING THE OPEN CHANNEL OF THE PANTHER FUSION® SYSTEM

Robert Kulis-Horn, Klaus Jansen, Jorgen Jensen, Carsten Tiemann, Krone Laboratory, Molecular Diagnostics, Bad Salzuflen, Germany; Robert Koch Institute, Infectious Disease Epidemiology, Berlin, Germany; Statens Serum Institut, DK, Denmark; Krone Laboratory/LABCON-OWL, Molecular Diagnostics, Bad Salzuflen, Germany.

Background: Current epidemiological studies demonstrate a high prevalence of Mycoplasma genitalium (MG) infections in high-risk groups, especially MSM. Owing to the widespread macrolide resistance the European Guideline on MG infections recommend complementing the molecular detection of MG with an assay capable of detecting macrolide resistance-associated mutations. Macrolide resistance is caused by a single nucleotide polymorphism (SNP) in region V of the 23S rRNA gene. Two nucleotide positions in the 23S rRNA gene have been associated with azithromycin resistance: A2058 and A2059.

Methods: The Aptima M. genitalium Assay® was used for the initial detection on the Panther® system. A hybridization probe real-time PCR on the Open Channel® of the Panther Fusion® system for automated reflex testing after MG positive results was established and validated. The Open Channel® of the instrument allows the use of custom primers and probes on the Panther Fusion® system. Minor groove binder (MGB) hybridization probes were used for accurate and reliable SNP discrimination at position 2058/2059. The workflow enables an automated analysis process including DNA extraction, PCR setup, and results interpretation. Using the open channel 300 samples could be genotyped within an 8 h working day.

Results: 60 MG positive clinical samples were tested. The laboratory-developed test (LDT) was able to detect the wild type variant in 20 samples and the A2058G/A2059G mutations in 22 samples. All results were confirmed by amplicon sequencing and commercially available test system. 18 (30%) samples did not show any typing result either using different LDT or commercial test system.

Conclusion: MG positive samples can be typed for macrolide resistance using our LDT on the same platform during one run. The combination of a MG high-throughput test followed by macrolide resistance testing improves the efficiency of large-scale epidemiological resistance surveillance. However, highly sensitive TMA assay might result in a significant number of non-typable samples. Further studies are needed to improve the sensitivity and explanatory power of MG resistance testing.

Disclosure: No significant relationships.

CLINICAL EVALUATION OF THREE COMMERCIAL PCR ASSAYS FOR THE DETECTION OF MYCOPLASMA GENITALIUM AND MACROLIDE RESISTANCE

Chloé Le Roy, Cécile Bébèar, Sabine Pereyre, University of Bordeaux, Bordeaux, France; University of Bordeaux, USC-EA 3671 Mycoplasmal and Chlamydial Infections in Humans, Bordeaux, France.

Background: Because macrolide resistance is increasing worldwide in Mycoplasma genitalium (MG), it is recommended to detect macrolide resistance-associated mutations in MG-positive specimens. Some new commercial kits detect macrolide resistance at the same time as MG detection. The aim the study was to prospectively evaluate the clinical performance of three commercial kits for the detection of MG and macrolide resistance.

Methods: Two hundred MG-positive urogenital specimens detected using an in-house real-time PCR are prospectively collected at the Bacteriology Department of Bordeaux University Hospital (France). After DNA extraction of the specimens using the MagNa Pure 96 (Roche), the specimens are submitted to four assays: the SpecDx ResistancePlus MG assay, the Diagenode S-DiaMGRES assay, the PathoFinder Real accurate TVMGRES and the in-house FRET qPCR assay and 23S rRNA sequencing used as reference. The internal controls of each kit were added in the specimen before extraction and the absence of amplification inhibition associated with the addition of the three internal controls was previously checked.

Results: To date, 114 MG-positive specimens have been analyzed. The clinical sensitivity for MG detection was similar for the three commercial kits, ranging between 95.5 and 98.2%. To compare the macrolide resistance detection performance, 90 specimens that have been found MG-positive using the three kits and that could be amplified using the in-house FRET qPCR assay were retained. Therefore, compared