Performance of the new Illumigene® Mycoplasma LAMP assay for detection of *M. pneumonia*

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background

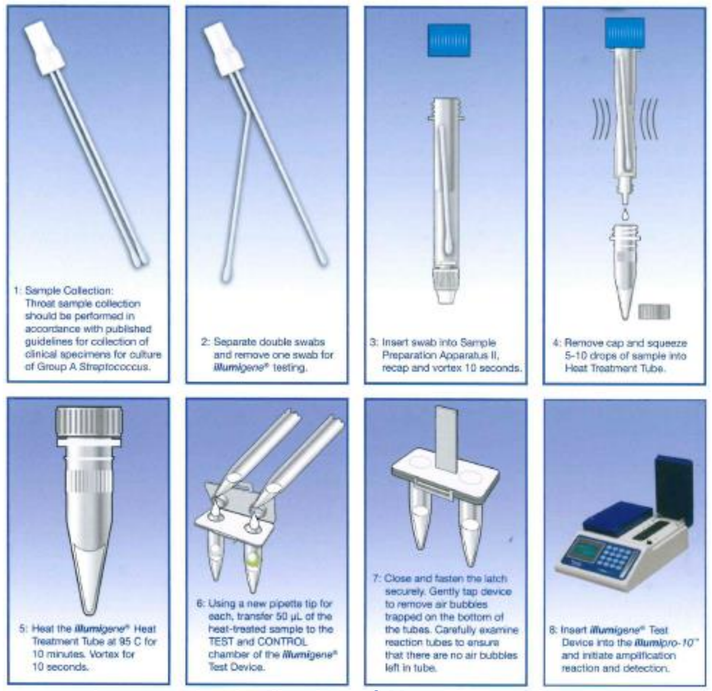
Mycoplasma pneumoniae is a common cause of respiratory tract infections. In Belgium, between 3,000 and 5,000 cases of *M. pneumoniae* infection were registered yearly between 2011 and 2016, mainly based on serology. However, due to suboptimal performance and high inter-test variability of serology assays, interest in better-performing molecular tests has increased lately. The aim of this study was to evaluate the performance of the new Illumigene® Mycoplasma Direct assay using a real-time PCR reference method. This Illumigene® assay is a loop-mediated isothermal amplification (LAMP) test requiring minimal hands-on time and delivering results within the hour.

materials and methods

Dilution series (10^{-3}-10^{-6}) of five titrated *M. pneumoniae* reference strains (PI1428, JAP377, 3996PL, 4972BRA and 6303DES with stock concentration between $1{,}1x10^7$ and $6{,}5x10^7$ colour changing units/mL) were prepared. All dilutions were analysed using the Illumigene® Mycoplasma Direct assay (Meridian Bioscience), performed on the illumipro-10 LAMP amplification and detection system (Meridian Bioscience). The 2016 QCMD *M. pneumoniae* panel (n=10) and *M. pneumonia*-positive respiratory specimens (n=14) were analysed in the same manner. The test performance was evaluated by comparing the results with those generated by an accredited in-house RT-PCR targeting the P1 gene.

strain / dilution	Illumigene®	RT-PCR / repeat RT-PCR
6303DES / 10^{-5}	negative	positive / positive (33,98)
4972BRA / 10^{-5}	negative	positive / positive (32,76)
JAP377 / 10^{-5}	negative	positive / positive (34,31)
JAP377 /10^{-6}	negative	positive / not tested again
3996PL / 10^{-6}	positive	negative / negative (0,00)
PI1428 / 10^{-5}	negative	positive / positive (35,27)
QCMD 3	negative	positive / positive (38,86)

discordant results



preparation steps for the Illumigene® Mycoplasma Direct Assay

results

Sensitivity of the Illumigene® Mycoplasma Direct assay was 83.3%. Five out of six falsely negative samples were 10^{-5}-10^{-6} dilutions of reference strains and one was a QCMD sample. For all of these, RT-PCR yielded only weakly positive reactions with Ct-values around the limit of detection. For reference strain 3996 PL, the Illumigene® assay was positive at the 10^{-6} dilution, whereas RT-PCR was negative. Importantly, full concordance was noted between the Illumigene® assay and RT-PCR using clinical samples.

conclusion

The performance of the Illumigene® assay is comparable to that of our in-house RT-PCR, failing to detect only those samples with low bacterial load. In view of its limited hands-on time and short TAT, the Illumigene® assay can be used as a valid alternative for RT-PCR in settings with limited equipment or expertise in molecular diagnostics.

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