EVALUATION OF THE ANALYTICAL PERFORMANCE OF THE MULTIPLEX R-DIAFLU™ KIT (DIAGENODE) FOR THE DETECTION OF INFLUENZA A AND B VIRUSES IN NASOPHARYNGEAL SPECIMENS

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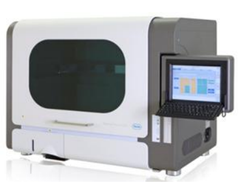
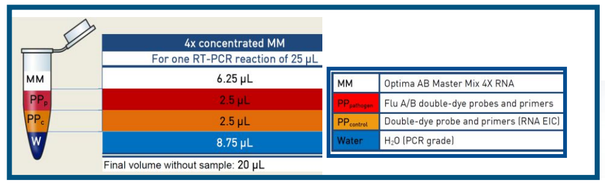
Background

The objective of this study is to evaluate the analytical performance of the multiplex R-DiaFlu™ kit (Diagenode) for the detection of influenza (INF) A and B viruses with other extraction and detection platforms than proposed by the supplier.

Material and Methods

RNA from patients and external quality controls (INSTAND) was extracted using the MagNA Pure LC2 platform (Roche, TNA High Performance protocol). 200 µl sample was used for extraction. 10 µl internal control (IC; RNA-EIC, Diagenode) was added to each sample, checking for inhibition and monitoring the efficiency of the purification. RNA was eluted in 70 µl elution buffer. qPCR was performed using the short protocol (2 h), amplification was detected in the green, yellow and red channel of the Rotor Gene Q (Qiagen). The R-DiaFlu™ kit contains all the reagents necessary for the test. Results were compared to an in-house reference test [1]. The assay was checked for analytical sensitivity, accuracy, linearity and precision following the Belgian guidelines [2].





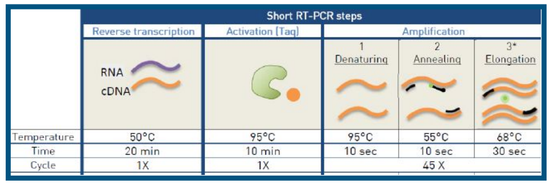


***Fig 1.* Workflow:** extraction on the MagNA Pure LC2 Extraction platform, preparing the mastermix, qPCR on the Rotor Gene Q.

Results

Analytical sensitivity:

A negative Eswab™ (Copan) specimen was spiked with AmpliRun® INFA and INFB RNA control (Vircell) to determine the limit of detection (LOD with a 95% hit rate). The LOD of the in-house test is 2000 copies/ml for INFA. This could not be achieved with the R-DiaFlu™ kit where only 12 out of 20 replicas were positive (mean Cq 37) however the estimated LOD in the package insert is 116 copies/ml.

The lowest concentration of INFB was 1000 copies/ml (mean Cq 36), this corresponds with the estimated LOD (962 copies/ml).

Accuracy:

58 specimens (36 patients and 22 external quality controls) were tested. 21 samples were positive for INFA, 17 for INFB and 20 were negative. There was a 100% agreement. The external quality controls included five different strains of INFA (H1N1, H3N2, H7N9, H3N2 drift variant (2015), (H1N1)pdm2009) and 3 different strains of INFB. One sample which was only INFB positive, was also false positive for INFA with the in-house method. This sample was confirmed with the RespiFinder® RG (PathoFinder). No samples were inhibited.

Linearity:

RNA of two external quality controls was serially diluted. Each of the five dilutions was repeated in duplicate. The efficiency of the reaction was 91% for both pathogens.

Precision:

One positive (mean Cq INFA 24.88; INFB 29.95) and one weak positive (mean Cq INFA 31.72; INFB 37.61) sample of each pathogen were extracted in duplicate on 4 different days. All 16 results were positive. The coefficients of variation were similar as described in the package insert (< 2%).

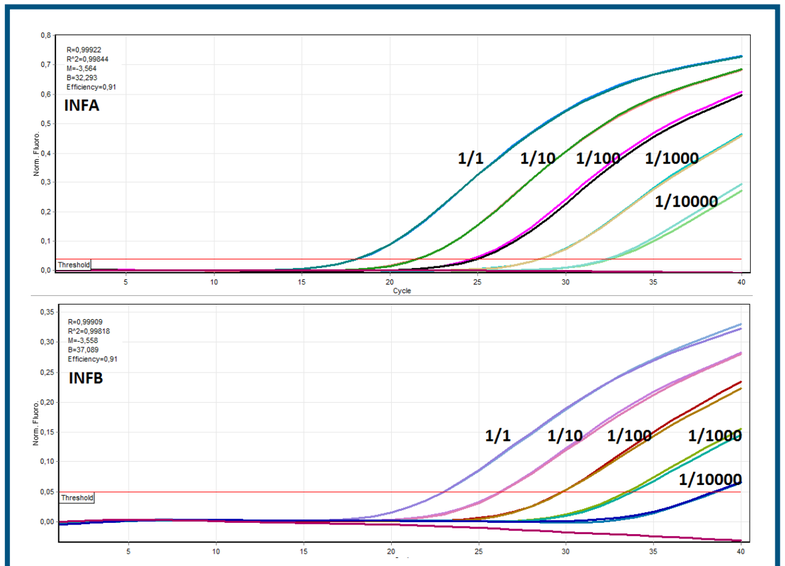


***Fig 2.* Results:** amplification plots of a 10 fold dilution serie.

Conclusion

Although the analytical sensitivity of INFA did not met the predefined criteria in our laboratory setting, the R-DiaFlu™ assay is an easy and accurate method and can be performed on the Rotor Gene Q.

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References: [1] Ward et al., J Clinical Virol, 2004 [2] Raymaekers et al., Acta Clinica Belgica, 2011