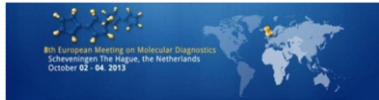




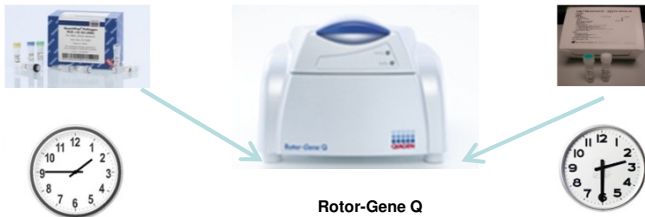
# COMPARISON OF THE QUANTIFAST PATHOGEN RT-PCR® (QIAGEN) AND TAQMAN® RNA AMPLIFICATION KIT (ROCHE) FOR THE DETECTION OF RESPIRATORY PATHOGENS ON THE ROTOR-GENE Q PLATFORM

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## Objectives

- A "classical" one-step RT-PCR takes about 2,5 hours and a "fast" RT-PCR takes about 45 minutes less. Does this have an impact on the accuracy or sensitivity?
- The "Fast" mastermix (MMX) (**Quantifast Pathogen RT-PCR®, Qiagen**) will be compared with the "classical" RT-PCR-MMX (**TaqMan® RNA Amplification Kit, Roche**).



## Methods

- 47 RNA samples (12 patients, 33 external quality controls and 2 Vircell RSV controls) were analysed by an in-house multiplex PCR for the detection of influenza A/B, and one multiplex PCR for respiratory syncytial virus (RSV) A/B and human metapneumovirus (hMPV). Primers and probes are chosen from the literature and are checked against reference sequences. The same oligo concentration and RNA was used in the different reactions. The PCR profile was done as proposed by the supplier.

## Results (1)

- Accuracy: 5 negative, 14 RSV positive (6 RSVA, 7 RSVB), 10 hMPV positive, 12 influenza A and 6 influenza B positive samples were tested. All negative samples were negative. All results for RSV were concordant. 2 weak positive hMPV samples were missed with the TaqMan® MMX. Although all positive influenza samples were positive with both MMX, with the Quantifast Pathogen MMX 4 out of 12 influenza A and 1 out of 6 influenza B positive samples showed nonspecific signals in the other channel (i.e. false positive result - influenza A and B are detected in 2 different channels, green en yellow respectively).
- Analytical sensitivity: For RSV the mean Cq (quantification cycle) value was about 0.9 lower with the Quantifast MMX, but for the weak positive RSV A Vircell control (ATCC VR26, about 150 copies/PCR) the Cq value was 8 cycles higher. For hMPV and influenza A the mean Cq value was about 1.7 and 0.6 respectively lower with the Quantifast®. For influenza B the mean Cq value was about 0.8 higher with the Quantifast MMX.

| INFLUENZA A    | Type         | Cq Qiagen | Cq Roche | Difference | Remarks                      |
|----------------|--------------|-----------|----------|------------|------------------------------|
| INSTAND 370019 | H3N2         | 20,28     | 19,9     | 0,38       | False positive InfB Cq 33,67 |
| INSTAND 370020 | H5N1         | 27,14     | 29,27    | -2,13      | False positive InfB Cq 33,43 |
| INSTAND 370023 | H1N1         | 19,6      | 19,68    | -0,08      |                              |
| INSTAND 370007 | (H1N1) pdm09 | 22,67     | 22,81    | -0,14      |                              |
| INSTAND 370009 | H1N1         | 22,06     | 21,4     | 0,66       |                              |
| INSTAND 370011 | H5N1         | 25,86     | 27,94    | -2,08      | False positive InfB Cq 33,96 |
| INSTAND 370012 | H3N2         | 20,91     | 20,71    | 0,2        |                              |
| INSTAND 370014 | H1N1         | 20,17     | 20,37    | -0,2       | False positive InfB Cq 34,00 |
| INSTAND 370015 | H5N1         | 26,79     | 29,85    | -3,06      |                              |
| INSTAND 370016 | H3N2         | 19,95     | 20,32    | -0,37      |                              |
| INSTAND 370018 | (H1N1) pdm09 | 22,24     | 22,32    | -0,08      |                              |
| 212-009502     |              | 20,48     | 21,07    | -0,59      |                              |
| INSTAND 370008 | negative     | negative  | negative | *          |                              |
| INSTAND 370017 | negative     | negative  | negative | *          |                              |
| INSTAND 370022 | negative     | negative  | negative | *          |                              |

## Results (2)

| INFLUENZA B    | Cq Qiagen | Cq Roche | Difference | Remarks                      |
|----------------|-----------|----------|------------|------------------------------|
| INSTAND 370024 | 21,61     | 20,63    | 0,98       | False positive InfA Cq 37,83 |
| INSTAND 370010 | 21,67     | 24,22    | -2,55      |                              |
| INSTAND 370013 | 21,6      | 20,71    | 0,89       |                              |
| 212-009714     | 24,36     | 24,27    | 0,09       |                              |
| 211-004079     | 24        | 22,4     | 1,6        |                              |
| 211-004018     | 15,25     | 11,49    | 3,76       |                              |
| INSTAND 370008 | negative  | negative | *          |                              |
| INSTAND 370017 | negative  | negative | *          |                              |
| INSTAND 370022 | negative  | negative | *          |                              |

| hMPV           | Cq Qiagen | Cq Roche | Difference |
|----------------|-----------|----------|------------|
| vr11/0267      | 23,88     | 33,37    | 9,49       |
| vr11/0088      | 18,14     | 18,4     | 0,26       |
| 4296518        | 32,75     | N        | *          |
| QCMD hMPV11-01 | 30,43     | 31,81    | 1,38       |
| QCMD hMPV11-02 | 38,39     | N        | *          |
| QCMD hMPV11-03 | 27,49     | 28,53    | 1,04       |
| QCMD hMPV11-05 | 32,62     | 30,41    | -2,21      |
| QCMD hMPV11-06 | 27,59     | 28,49    | 0,9        |
| QCMD hMPV11-07 | 34,05     | 36,93    | 2,88       |
| QCMD hMPV11-08 | 35,19     | 35,17    | -0,02      |
| QCMD hMPV11-04 | negative  | negative | *          |

| RSV                  | Type     | Cq Qiagen | Cq Roche | Difference |
|----------------------|----------|-----------|----------|------------|
| vr10/0194            | RSV A    | 18,43     | 18,27    | 0,16       |
| 0081/0144            | RSV A    | 25,48     | 23,26    | 2,22       |
| Vircell RSV A 1/1000 | RSV A    | 36,64     | 28,29    | 8,35       |
| QCMD RSV11-03        | RSV A    | 28,95     | 29,59    | -0,64      |
| QCMD RSV11-06        | RSV A    | 35,32     | 37,96    | -2,64      |
| QCMD RSV11-08        | RSV A    | 32,27     | 33,68    | -1,41      |
| 0091/2752            | RSV B    | 17,6      | 18,32    | -0,72      |
| 18854618-9100        | RSV B    | 23,21     | 25,02    | -1,81      |
| Vircell RSV B 1/1000 | RSV B    | 27,97     | 26,12    | 1,85       |
| QCMD RSV11-01        | RSV B    | 29,32     | 31,71    | -2,39      |
| QCMD RSV11-02        | RSV B    | 26,7      | 28,64    | -1,94      |
| QCMD RSV11-04        | RSV B    | 29,87     | 31,9     | -2,03      |
| QCMD RSV11-07        | RSV B    | 31,89     | 34,54    | -2,65      |
| 4245908              | RSV      | 14,07     | 13,4     | 0,67       |
| QCMD RSV11-05        | negative | negative  | negative | *          |

## Conclusion

Although the Quantifast Pathogen RT-PCR® kit seems to be more sensitive in most cases, it is not compatible with our primer-probe set for influenza (non-specific signal). It is clear that with a "Fast" RT-MMX one has to check the sequences of the oligos used for the test again and adapt them when needed to avoid non-specific PCR products. The TaqMan® Amplification kit will stay our choice of method.

References: [www.Virolog.nl](http://www.Virolog.nl); "Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions" R.N. Gunson et al. Journal of Clinical Virology 33 (2005) 341-344