IN HOUSE REAL-TIME PCR MULTIPLEX FOR SIMULTANEOUS DETECTION OF HUMAN INFLUENZA A AND B VIRUS AND RESPIRATORY SYNCYTIAL VIRUS

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BACKGROUND

Human Influenza A/B and Respiratory syncytial virus (RSV) infection can lead to severe clinical illness, hospitalization, and death [1,2]

The simultaneous detection of influenza A/B and RSV allows the discrimination of different types of infections, requires less hand-on time, and enables the reduction of analysis costs

Co-infections of SARS-CoV-2 with other viral respiratory infections might influence the morbidity and mortality of patients, which reinforces the importance of developing rapid, reliable and specific assays for viral infections detection and differential diagnosis [3]

AIMS

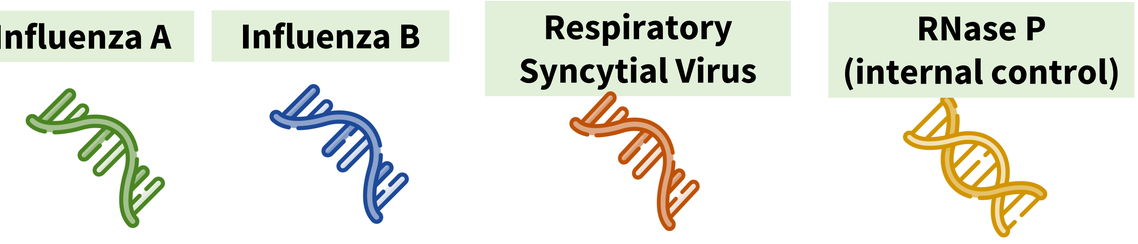
Apply an in-house developed multiplex PCR assay to simultaneously detect Influenza A/B, and RSV

Analyze the prevalence of concurrent infection of these viruses and SARS-Cov-2 in fatal victims

METHODOLOGY

I. PRIMERS, PROBES AND ASSAY DESIGN

Highly conserved regions



II. SAMPLE SELECTION

- 85 nasopharyngeal swabs collected post-mortem
- Positive for SARS-CoV-2 infection

III. NUCLEIC ACIDS EXTRACTION

- Qiagen EZ1 Virus mini kit v2.0
- According to the manufacturer's instructions

IV. AMPLIFICATION AND DETECTION

- One-step multiplex RT-PCR
- Reverse transcription and amplification reaction combined in a single protocol

FAM Influenza A

HEX Influenza B

CALRED 610 RSV

QUASAR 670 RNase P

RESULTS

Identification of one case case of co-infection

50-year-old female

Influenza A POSITIVE

Prevalence of co-infection

1.18%

- **Influenza A POSITIVE**
- **SARS-CoV-2 POSITIVE**

CONCLUSIONS

- Our multiplex PCR assay is a useful option of a high-throughput test, with the simultaneous detection of influenza A/B and RSV viruses
- Co-infection was identified in 1.18% of the present cohort
- A multiplex method for the detection of viral infections can discriminate different types of infections as well as to detect the presence of coinfections, which in turn can reduce transmission and gather data for surveillance purposes

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