Comparison of two commercial influenza RT-PCR assays by analyzing seasonal respiratory samples from India and the recommended influenza vaccine strains for the season 2019/2020 (recommended by WHO)

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Introduction

Influenza is an acute respiratory illness and imposes a considerable burden worldwide. Influenza affects the upper and/or lower respiratory tract and is caused by influenza virus, usually of type A or B. Influenza circulates continuously, causing seasonal epidemics in temperate regions and year-round epidemics in some tropical regions. Influenza A viruses may also cause pandemics characterized by rapid dissemination of a new, virulent influenza A subtype to which there is little or no existing immunity (www.WHO.int).

Due to the constant changing of circulating influenza viruses, influenza vaccine composition is adapted annually (for both the Northern Hemisphere and the Southern Hemisphere) to more closely match currently circulating virus strains.

Material and Methods

Influenza virus strains which were recommended by WHO for vaccination for the season 2019/2020 were ordered at NIBSC (National Institute for Biological Standards and Control). Unfortunately, Influenza virus Kansas/14/2017 (H3N2) was not available for testing.

All sera were reconstituted as recommended in UTM™ Viral Transport Media (Copan). A pre-dilution (10E-5) was made for further testing.

From all five pre-diluted strains nucleic acids were extracted using the MagnaPure Pure 96 DNA and Viral NA Small Volume Kit on the Magna Pure 96 System (Roche), following the protocol “Pathogen Universal 200 3.1.”

Real-time PCR was performed using the RealStar® Influenza Screen & Type RT-PCR Kit 4.0 and with the RT-PCR Pandemic H1N1/09 Assay Set version 2.0 (Applied Biosystems®) on the CFX96 (BioRad instrument). A total of 70 individual bronchial secretion samples from patients with typical influenza symptoms were collected from individual patients in India during the influenza season 2018. The samples were previously tested and analysed with the RT-PCR Pandemic H1N1/09 Assay Set version 2.0. RNA was extracted as described above.

Results

Testing of influenza virus vaccine strains:

All five strains were detected and typed correctly according to the manufacturers claims. The RealStar® Influenza Screen & Type RT-PCR Kit 4.0 detected and typed all five influenza A and B strains. The RT-PCR Pandemic H1N1/09 Assay Set version 2.0 is designed only for detection of influenza A virus and detected and typed all of these correctly.

Testing of clinical samples (respiratory samples from the influenza season 2018) from India:

70 individual bronchial secretion samples from patients in India were pre-tested with the RT-PCR Pandemic H1N1/09 Assay Set version 2.0, and re-tested with RealStar® Influenza Screen & Type RT-PCR Kit 4.0. Results are shown in Table 2 and 3.

Table 1: RT-PCR results for influenza virus strains by RealStar® Influenza Screen & Type RT-PCR Kit 4.0 and RT-PCR Pandemic H1N1/09 Assay Set version 2.0

Table 2: Influenza-positive and -negative results by RealStar® Influenza Screen & Type RT-PCR Kit 4.0 and RT-PCR Pandemic H1N1/09 Assay Set version 2.0

Result Summary: The two assays show comparable results for the detection of influenza A (H1N1) pdm09 of both, vaccine strains for influenza A and the respiratory samples from India. Discordant results for (H1N1) pdm09 may be due to different sensitivities of the two assays. The Pandemic H1N1/09 Assay Set version 2.0 does not detect influenza B and therefore missed 2 influenza B positives from the respiratory samples of India.

Conclusion

In countries with need for detection of influenza A, influenza A (H1N1) pdm09 and influenza B virus simultaneously, the RealStar® Influenza Screen & Type RT-PCR Kit 4.0 covers this demand with a high sensitivity and specificity. The advantage of detection and typing for all strains / types in one PCR reaction in comparison to four reactions by the Pandemic H1N1/09 Assay Set version 2.0 leads to a faster and less error-prone result analysis with the RealStar® Influenza Screen & Type RT-PCR Kit 4.0.

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