Retrospective analysis of laboratory data shows human infections with more than one type or sub-type of Influenza are rare

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- **Introduction and purpose**
- The hypothesis was that co-infection with different types or sub-types of Influenza is unusual.

- The major Influenza A virus subtypes circulating amongst humans are H1N1 and H3N2.
- These subtypes vary from year to year due to genetic drift and shift. Drift is due to inaccurate replication but shift is due to recombination events made possible by co-infection with two or more strains.
- A recombination event might dramatically affect various parameters, such as viral virulence or transmission ability or host immunity as well as the actual subtype. For this reason the frequency of occurrence of mixed infections is interesting.
- There is data suggesting co-infection with variants of the same sub-type is common but our anecdotal experience is that it is not common.
- Our anecdotal experience is based on routine Influenza RT-PCR that has been performed in this laboratory since 2009 with a 4-plex that reports Influenza B and Influenza A (Matrix gene) as well as H1N1 2009 and H3N2, in one tube.
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- **Methods**
- The ability of the RT-PCR system to detect mixtures was tested by mixing RNA from known positive cases of H1N1(2009), H3N2 and Influenza B. Samples were selected, for this experiment, that had given Ct values of 32. RNA was mixed in equal measures in three combinations of two viruses (H3 N2 with H1N1; H3N2 with Inf B; H1N1 with Inf B) and also with all three viruses together.

- A retrospective analysis of Influenza RT-PCR results from routine clinically directed tests between August 2009 and June 2014. Data were obtained from the laboratory information system at Tan Tock Seng Hospital, Singapore; almost all from adults being admitted with an acute infection.

- RNA was extracted with an EasyMag instrument (Biomerieux).
- The normal sample type was a combined nose and throat swab suspended in phosphate buffered saline or a universal transport medium (Copan); other respiratory samples are a tiny minority.
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- **Results**
- The RT-PCR system detected all components in the artificially mixed samples.
- The Ct values for each component were raised by zero to two Ct points in the mixed RNA preparations compared with the original experiments containing only a single target/template.
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- After removal of duplicates, results for 39,510 patients were available. Influenza was detected in 5,825 samples and was not detected in 33,685 cases.
- 1,935 were Influenza A H1N1 (2009)
- 3 were Influenza A H1N1 (seasonal pre-2009).
- 2,309 were Influenza A H3N2.
- 189 were Influenza A with subtype undetermined
- 1,383 were Influenza B.
- **Six samples had more than one type or subtype detected.**
- A total of 6/5825 = 0.1% were mixed, as a proportion of Influenza positive samples; approximately one/year.
- H3N2 and Influenza B were both detected, together, in the same sample in four cases; H1N1 2009 and Influenza B were detected together in two cases.

- **Conclusion**
- Co-infections with different types or sub-types were rare in this group of adults admitted to hospital. This is surprising considering the co-circulation of all three main types/sub-types.
- Co-infections in the literature are often in close knit groups where an outbreak has been investigated. It would be interesting to look at data from the wider community of non-hospitalised cases, including children. It would also be interesting to compare the viral load in single infections with that in co-infections.

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