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Appendix 1

This appendix was part of the submitted manuscript and has been peer reviewed.
It is posted as supplied by the authors.

Appendix to: Sullivan S, Chilver MB-N, Higgins G, et al. Influenza vaccine effectiveness in Australia: results from the Australian Sentinel Practices Research Network. *Med J Aust* 2014; 201: 109-111. doi: 10.5694/mja14.00106.

Appendix 1. Laboratory methods

Nasal swabs were taken by GPs using pre-prepared kits containing a Sigma Virocult Swab (MW951S) in 2 mL of Σ-Virocult medium (Medical Wire & Equipment, Wiltshire, England). Product information indicates these swabs can maintain the viability of influenza RNA for up to 14 days at ambient temperatures. Samples were sent to SA Pathology in Adelaide, South Australia, via Australia Post's Express post system, allowing for next day delivery from all capital cities. GPs located in tropical climates refrigerated samples prior to posting.

Total nucleic acid was extracted from 200 μL of medium and eluted into 100 μL of elution buffer using the automated Applied Biosystems MagMax Viral RNA Isolation Kit (Cat. AM1836). Eleven different respiratory pathogens including influenza A (matrix gene), influenza A(H1)pdm09 (HA gene), influenza B, respiratory syncytial virus (RSV), parainfluenza viruses 1, 2 and 3, adenovirus, enterovirus, human metapneumovirus, *Mycoplasma pneumoniae* and *Bordetella pertussis* were detected using in-house real time, TaqMan based PCR assays. Amplification was performed using Invitrogen SuperScript III One-Step Quantitative RT-PCR System (Cat. 11732-088) on a Roche LC480 thermocycler in 384 well plates. Interpretation of results was performed using the LC480 2nd Derivative Max algorithm.

Influenza-positive specimens with high viral loads (CT≤30) were forwarded to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne where they were further characterized to identify the virus strain using the haemagglutination inhibition (HI) assay, as previously described [1]. Isolates were identified as antigenically similar to the reference strain if the test samples had a titre that was ≤4-fold different compared with the homologous reference strain. Results were reported against reference antisera raised against A/California/7/2009 (H1N1pdm09), A/Perth/16/2009 (H3N2), B/Wisconsin/1/2010 (Yamagata lineage), and B/Brisbane/60/2008 (Victoria lineage) viruses.

References

1. Hobson, D., et al., *The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses.* J Hyg (Lond), 1972. **70**(4): p. 767-77.