Diagnosis of influenza following the first wave of pandemic (H1N1) 2009 influenza

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Aetiological confirmation of respiratory tract infections in individual patients facilitates appropriate antimicrobial use and infection control procedures. From a public health perspective, influenza confirmation allows assessment of community attack rates and the efficacy of vaccination programs, while assisting in modelling for pandemic preparedness planning. Rapid antigen and immunofluorescent antigen tests are relatively insensitive in detecting pandemic (H1N1) 2009 influenza, and influenza subtype-specific nucleic acid amplification tests should be used as the 'gold standard' for diagnosis. Pathogen-specific serological testing aids the retrospective diagnosis of infection. Although some challenges surrounding diagnostic testing during the first pandemic wave have been resolved, others remain; this may test laboratories again in the second and subsequent pandemic waves.

Pandemic (H1N1) 2009 influenza (hereafter H1N1 09), the first influenza pandemic of the 21st century and the first in 41 years, provided a significant challenge to the community and health authorities. Although a range of laboratory methods is available to diagnose influenza [1], the rapid spread of the pandemic required the development of high-throughput H1N1 09-specific laboratory tests.

The role and use of diagnostic testing varied during the different phases of the pandemic. In several states, including New South Wales (NSW), a decision was made to initially concentrate diagnostic services in reference laboratories. Sensibly, this expanded as the pandemic unfolded to include other laboratories with nucleic acid testing expertise. Some clinicians have subsequently questioned the merits of this initial approach, as there were occasionally significant delays in turnaround times between specimen collection to notification of results [2,3]. There were multiple reasons for the delays. The main one was the overwhelming number of tests requested for surveillance of disease prior to significant community transmission, followed by testing individuals with clinically mild disease, during the

DELAY and CONTAIN phases of the pandemic respectively. Other issues that arose include long transit times between the point of collection and receipt in the reference laboratory, inappropriately collected specimens, request forms that were incorrectly filled out or missing and the lag between result validation to notification. Despite the recommendation that laboratory testing be confined to those with, or at risk of, severe influenza infection during the PROTECT phase (declared on 17 June 2009), high numbers of specimens requesting H1N1 09 testing were still being received in the laboratory. Diagnostic testing was also complicated by the fact that the peak of H1N1 09 activity coincided with the peak activity of other winter respiratory viruses, including respiratory syncytial virus (RSV) and seasonal influenza.

Nucleic acid amplification tests (NAAT) were used as the definitive test for H1N1 09 as there was decreased sensitivity of rapid antigen tests (RAT) and direct immunofluorescent antigen (IFA) tests in detecting H1N1 09 (17.8%–53.4% and 46.7% respectively)[4-6]. The sensitivity of both RAT and IFA are affected by patient age and sample type; improved RAT sensitivity was noted in the paediatric population (66.2% in respiratory swabs and 84.1% in nasopharyngeal aspirates), suggesting that RAT may be a 'reasonable' test to exclude H1N1 09 in nasopharyngeal swabs collected from young children [7]. Importantly, it was observed that in patients with severe influenza requiring mechanical ventilation, it was imperative to test lower respiratory tract samples using NAAT due to the reduced sensitivity of antigen tests (RAT and IFA) on upper respiratory tract samples (100% versus 25%; sensitivity of NAAT on upper respiratory tract samples was 81%)[8]. In this group of patients, at least 23% had bacterial and/or viral co-infections (Blyth, C.C., unpublished data). Suspected bacterial co-infections were confirmed with positive cultures (blood, bronchoalveolar lavages and/or endotracheal aspirates) or urinary antigen detection tests, while multiplex-tandem reverse-transcriptase PCR allowed simultaneous detection of other respiratory viruses. The diagnosis of bacterial co-infections helps optimise antimicrobial use in this population following initial empirical therapy with broad-spectrum antibiotics [9]. The

co-circulation of H1N1 09 and other respiratory viruses increases the likelihood of dual infections.

Despite the development of new H1N1 09-specific RAT that have improved the sensitivity of H1N1 09 detection to 77% [10], NAAT remains the test of choice in diagnosing acute H1N1 09. H1N1 09-specific serology may also assist the retrospective diagnosis of severe influenza in NAAT negative cases [11]. Although viral cultures for influenza were performed during the pandemic, it is no longer the 'gold standard' for diagnosing influenza infection as it is less sensitive compared to NAAT, has longer turnaround times and requires specialised staff and laboratory equipment [1].

The true incidence of H1N1 09 during the first pandemic wave in Australia remains uncertain. In 2009, there were 37,636 confirmed cases and 191 deaths from H1N1 09 in Australia. Of the 931 confirmed cases of influenza in 2010 (up to 18 June), 86 (9.2%) were H1N1 09, although 708 (76%) of untyped influenza A samples were also likely to be H1N1 09 [12]. However, this underestimates the true community attack rate as laboratory confirmation of suspected influenza was not universal, particularly during the PROTECT phase. Seroprevalence studies using haemagglutination inhibition (HI) tests undertaken after the pandemic have suggested an overall community rate of 28.4% in NSW [13]. This varied according to different age groups, with higher seroprevalence rates observed in the younger population. However, if one assesses the differences between pre-pandemic and post-pandemic HI seroprevalence, then the NSW community rate was ~15% overall. The ideal way to determine true H1N1 09 seroprevalence is to demonstrate a fourfold or greater rise in HI antibody levels between pre- and post-pandemic wave sera examined in parallel; however, such specimens are rarely available in retrospective studies.

As of 18 June 2010, RSV, rhinovirus and picornavirus were the most common respiratory viruses detected in Australian samples tested for suspected influenza this year [12]. It is unknown if H1N1 09 will be the predominant viral respiratory tract infection again this winter. Prior H1N1 09 infection (or vaccination) will likely provide some protection in second or subsequent pandemic waves; hence the overall activity of H1N1 09 should be less in 2010. At the time of writing, although seasonal influenza B is the predominant influenza virus circulating worldwide, it has only accounted for 9.1% of typed influenza cases in Australia this year [12].

The World Health Organisation (WHO) has reported only 298 oseltamivir-resistant (but zanamavir susceptible) H1N1 09 viruses worldwide [12], with all but one virus possessing the H275Y mutation, confirmed by either neuraminidase (NA) gene reverse-transcriptase PCR, sequencing of the NA gene product, or by phenotypic testing to determine the oseltamivir IC_{50}. There have been at least 11 cases of oseltamivir-resistant H1N1 09 detected in Australia since the beginning of the pandemic [12,14]. Resistant viruses have been isolated from immunocompromised patients (solid organ transplants or haematological malignancies) receiving prolonged oseltamivir treatment or primary prophylaxis [15-17]. This year, it will be important to continue monitoring for oseltamivir-resistant H1N1 09 and seasonal influenza A/H1N1 viruses, particularly the latter as it was not included in the 2010

trivalent influenza vaccine because there were only sporadic cases in 2009 and so far in 2010 (up to 18 June).

In conclusion, it is essential to identify pandemic influenza in the presence of co-circulating respiratory viruses due to its significant impact on clinical medicine and public health. Good sample collection, together with rapid and reliable testing methodologies, remains the cornerstone for accurate diagnosis.

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Biography

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