Comparison of Two Rapid Influenza A/B test Kits with Reference Methods Showing High Specificity and Sensitivity for Influenza A Infection

Susanne Booth[1], Cristina Baleriola[2] and William D Rawlinson[3]

[1] Clinical Trials Scientist, Virology Division, Department of Microbiology, South Eastern Area Laboratory Services, Prince of Wales Hospital, Randwick NSW 2031.

[2] Clinical Projects Coordinator, Virology Division, Department of Microbiology, South Eastern Area Laboratory Services, Prince of Wales Hospital, Randwick NSW 2031.

[3] Professor, Virology Division, Department of Microbiology, South Eastern Area Laboratory Services, Prince of Wales Hospital, Randwick NSW 2031 and Schools of Biotechnology and Biomolecular Sciences and School of Medical Sciences, University of NSW, Sydney NSW 2052, Australia

Corresponding author:

Professor William Rawlinson

Virology Division, Department of Microbiology

South Eastern Area Laboratory Services

Prince of Wales Hospital, Randwick 2031, Australia

Telephone: +612 9382 9113

Fax: +612 9398 4275

Email: W.Rawlinson@unsw.edu.au

Shorten title: Comparison of two rapid Influenza A/B test kits

Abstract

The rapid detection of influenza viruses is important for forming preventative strategies, directing initiation of antiviral therapy, detecting potential avian influenza viruses and excluding influenza-like pathogens such as SARS. The Immuno*Card* STAT! Flu A&B Plus test (Meridian Bioscience) is a new point of care (POC) test utilizing influenza-specific monoclonal antibodies for rapid diagnosis. The performance of this assay was compared to the established point of care Binax NowFlu A & NowFluB test, and the reference diagnostic standards of viral culture, indirect immunofluorescence (IFA) and RT-PCR where appropriate. Testing of nasopharyngeal aspirates (NPA) from children, throat swabs and nasal swabs from adults indicated Immuno*Card* STAT! specificity of 98% and 100% for Influenza A and B respectively in 224 specimens. The Binax test showed specificity of 99% respectively 100% for Influenza A and B. Sensitivity results were identical for both rapid detection kits (80% and 47% for Flu A and B respectively). Overall results were very similar for both testing devices with the advantage of Immuno*Card* STAT! Flu A&B Plus test detecting Influenza A and B with sharp and easy to read results.

Key Words: Flu, Rapid detection, Point of Care diagnosis

Influenza is a major cause of illness in adults and children causing significant morbidity and mortality especially in the elderly and patients with chronic disease. Given that the vaccine and neuraminidase inhibitors are influenza specific, accurate and timely diagnosis of influenza is critical as it impacts significantly upon treatment [Baleriola, 2003 et al; Rawlinson, 2001]. Furthermore, after the SARS epidemic rapid diagnosis is more important than ever before as it enables excluding other influenza-like pathogens or detecting potential avian influenza viruses [Lee and Krilov, 2005]. Rapid diagnosis has also proved to be useful for informing preventative strategies and controlling influenza epidemics in geriatric institutions [Monto et al, 2001].

The "gold standard" for the diagnosis of influenza is tissue culture isolation, which takes from 2 to 14 days. Detection of virus-infected cells by indirect immunofluorescence staining (IFA) is widely used but its completion requires approximately 2 hours. Currently several rapid POC kits have been developed that can be easily performed in less than 30 minutes [Ruest et al, 2003; Ryan-Poirier et al, 1992; Rawlinson et al, 2004] and most of them have been compared with culture and IFA [Dunn et al, 2003; Cazacu et al, 2004; Waner et al, 1991] and occasionally with PCR.

This study aimed to evaluate the diagnostic performances of two new antibody-based rapid Influenza A/B test kits: the Immuno*Card* STAT! Flu A&B Plus with the Binax NowFlu A & NowFluB for the rapid detection of Infuenza A and B viruses and the results were compared with those of culture, IFA and PCR where appropriate.

165 Nasopharyngeal aspirates (NPA) and 59 throat and nasal swabs from patients with respiratory symptoms suggestive of influenza infection were collected between May and November, 2004. The sample population included adults from the community, adults from Prince of Wales Hospital, and children (age range 1 to 16) presenting to the Emergency Department at Sydney Children's Hospital. One throat swab and one nasal swab were collected from each adult patient and one NPA from each child. The specimens were processed for testing in the Virology Diagnostic Laboratory and subsequently the specimens were tested on rapid POC tests. Specimens were kept at 4^{o}C and tested within 24 hours, then stored frozen at –20^{o} C. Testing was performed in batches of varying numbers of specimens and on different days.

Each specimen was processed by adding 2ml of 0.9% sterile saline (Baxter Healthcare, Australia) then divided into aliquots for immediate IFA staining and viral culture. Each specimen was further divided into a 0.5ml aliquot for Rapid POC testing using Immuno*Card* STAT! Flu A&B Plus (Meridian Biosciences Inc, Cincinnati, OH) and Binax NowFlu A & NowFlu B tests (Binax Inc, Portland, ME). Preliminary screening for influenza virus A and B, adenovirus, parainfluenza 1, 2, 3 and respiratory syncytial virus was done on all specimens using a commercial monoclonal antibody based indirect immunofluorescence assay (IFA) (Bartels, Inc., Issaquah, WA). Viral cultures were performed in accordance with standard virological techniques [Dowdle et al, 1979]. Specimens were inoculated onto shell vial cultures of Madin-Darby canine kidney (MDCK) cells [Reina et al, 1997]. After incubation for 5-7 days, infection is confirmed by staining using the same commercial monoclonal antibody based indirect immunofluorescence assay

(Bartels, Inc.). If a specimen proved to be positive for Influenza A or B only after viral culture, the specimen was defrosted and POC tested retrospectively.

The Immuno*Card* STAT! Flu A&B Plus POC testing was performed according to the kit procedure manual. The test is a lateral-flow immuno-chromatographic assay, which uses monoclonal antibodies specific for influenza A and B nucleoprotein as both the capture and detection antibodies. The patient specimens are diluted approx 1:2 in a sample buffer and added to the sample port of the device. As the sample flows through the device the influenza A or B antigen in the diluted sample binds to the corresponding monoclonal antibody-colloidal gold complex and yields a visible pink-red line upon contact with the lined capture antibody. If no antigen is present, no complex is formed and no pink line will appear. The adequate flow of the specimen is tested with a control line of goat anti-mouse antibody. If no pink red control line is visible the test is considered invalid. Binax NowFlu A & NowFluB card testing was performed in accordance to the manufacturer's instructions. Each testing batch included manufacturer's positive and negative controls.

18 specimens were analysed using a nested reverse transcriptase PCR, which detected both influenza A, and B. Specimens tested using PCR were selected where there was a discrepancy between IFA staining and viral culture results and the Immuno*Card* STAT! Flu A&B Plus tests. Specimens were thawed for 5-10 minutes at room temperature and RNA was extracted using a Qiagen QIAamp viral RNA extraction kit. Two separate PCR reactions were done for Influenza A and B. Influenza viruses were detected by adding 10µl of the extracted RNA to a 30µl mix, of Qiagen OneStep RT-PCR kit containing Reverse Transcriptase and the outer primers targeting the

matrix protein genes of influenza virus A and B [Zhang and Evans, 1991]. Two-step amplifications were performed in a Perkin Elmer thermal cycler. For the first PCR, after an initial pre-incubation at 42^0 C for 60 min and 94^0 C for 2 min, there were 25 cycles of denaturation (94^0 C for 1min) annealing (55^0 C for 90 sec) and extension (72^0 C 3min), followed by a hold at 4^0 C. 2µl of amplicons from this first PCR were added to a mixture of 48µl of AmpliTaq Gold PCR MasterMix (Applied Biosystem) containing the inner primer pair and subjected to 25 cycles using identical cycling conditions. The product was visualized using a 2.5% agarose gel and ethidium bromide staining.

Calculations of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were done according to Baron (2001). Differences between both POC tests were statistically analysed using the Chi-square test ($p > 0.05$).

All of the 224 specimens were tested by both IFA and culture, although for the purpose of this study specimens were considered positive for influenza A or B if either IFA or culture results were positive.

Of the 224 specimens tested (Table I), 35 specimens gave positive results for Influenza A and 15 specimens for Influenza B by both IFA and Culture; 23 specimens were positive for Influenza A and 10 specimens were positive for Influenza B only by IFA results; Finally, 29 specimens were positive for Influenza A and 14 for Influenza B by culture results only. Viral culture was more sensitive than IFA as 10 Influenza A and B results showed positive results on viral culture and point of care tests but were

negative on IFA. Overall a total of 87 Influenza A and 39 Influenza B specimens were identified.

Of the 87 Influenza A identified by IFA and/or culture, 85 specimens gave positive results with the Binax NowFluA kit and 94 with the Immuno*Card.* The positive predictive values for Influenza A virus (PPV) for both assays were 97 and 88% respectively and the negative predictive values (NPV) were 96% in both. The specificity values were 99 and 98% and the sensitivity was 80% for both assays.

Of the 39 Influenza B identified by IFA and /or culture, 24 gave positive results with the Binax NowFlu B kit and 21 with the Immuno*Card.* The PPV for the above mentioned assays were 88 and 100% respectively and the NPV was 96 % for both POC kits. Specificity values for the detection of Influenza B were 100% using both the Binax NowFlu B kit the Immuno*Card.* However, both kits showed low sensitivity values (47%).

Some-cross reactivity was observed in three specimens that tested positive RSV by culture and IFA. However, when testing with both POC tests showed 2 positive Influenza A and one positive Influenza B results; one positive Adenovirus result in viral culture and IFA also tested faint positive for Influenza A in both POC tests. In another instance, one Immuno*Card* STAT! Flu A&B plus card tested faint positive for Influenza A without any other positive result by culture or IFA. The false negative rate for Influenza A was 3% in both detection kits and 3.5 % for Influenza B also in both kits.

18 specimens were analysed using nested PCR, which detected both Influenza A, and B [Zhang and Evans, 1991]. Specimens tested using PCR were selected where there were discrepancies between IFA staining and viral culture results and the POC tests. 2 positive POC tests, but negative culture results were confirmed as positives with a positive RT-PCR result. 8 cases of negative POC testing but positive culture results were confirmed by positive PCR results, though this result was related to the type of specimen used, as they were all swabs. 2 swab specimens were negative with PCR and negative POC but positive for culture. In 2 cases we obtained positive POC results but all other tests were negative (culture /IFA/ PCR). These latter specimens tested were all NPAs.

The overall performance of both POC tests was alike. Positive Influenza A and B detections were surprisingly similar in both tests. This was reflected in the NPVs which were practically identical. Minor discrepancies were found in the negative detections of Influenza A and B (i.e. false-positive rate). However no significant difference could be detected using the Chi-square test ($p>0.05$). The PPVs indicate that the Binax tests is slightly more reliable in preventing false positives for Influenza A and the Immuno*Card* slightly more reliable in yielding true positive Influenza B results.

Although positive results by rapid methods correlate well with actual infection, rapid POC kits typically show poor sensitivity, ranging between 39 and 96% depending on the reference method used [Ruest et al, 2003; Ryan-Poitier et al, 1992; Rawlinson et al, 2004; Dunn et al, 2003; Cazacu et al, 2004; Waner et al, 1991]. Confirmatory testing by reference methods is recommended, although this is likely to become less

necessary during confirmed outbreaks and as point of care tests have improved sensitivities. Besides the similar diagnostic performance exhibited by both tests, the Immuno*Card* STAT! Flu A&B Plus test has the advantage of detecting Influenza A and B with sharp and easy to read results in comparison to the established POC test, where colour change is less clear. Furthermore, the Immuno*Card* STAT! Flu A&B Plus requires minimal sample manipulation, being ideal for preliminary and rapid screening tests for Influenza A and B.

References

Baleriola C, Waliuzzaman Z, Rawlinson WD. 2003. Influenza - Diagnosis, Treatment and Vaccination. Home Health Care Consultant. January p 17-23.

Baron JA. (2001). Clinical epidemiology. In: Teaching Epidemiology eds. Olsen J. Saracci R. and Trichopoulos D. Oxford: Oxford University Press p 237-249.

Cazacu AC, Chung SE, Greer J, Demmler GJ. 2004. Comparison of the Directigen Flu A+B membrane enzyme immunoassay with viral culture for rapid detection of Influenza A and B viruses in respiratory specimens. J Clin Microbiol 42: 3707-3710.

Dowdle WA, Kendal AP, Noble GR.1979. Influenza viruses. In: Leanette EH, Schmidt NJ editors, Diagnostic procedures for: Viral, rickettsial and chlamydial infections, 5th edn. Washington DC: APHA p585-609.

Dunn JJ, Gordon C, Kelley C, Carroll C. 2003. Comparison of the Denka-Seiken INFLU A.B-Quick and BD Directigen Flu A+B Kits with direct fluorescent-antibody staining and shell vial culture methods for rapid detection of Influenza viruses. J Clin Microbiol 41: 2180-2183.

Lee PJ, Krilov LR. 2005. When animal viruses attack: SARS and avian influenza. Pediatr Ann 34: 42-52.

Monto AS, Hornbuckle K, Ohmit SE. 2001. Influenza vaccine effectiveness among elderly nursing home residents: a cohort study. Am J Epidemiol 154: 155-160.

Rawlinson WD, Waliuzzaman ZM, Fennell M, Appleman JR, Shimasaki CD, Carter IW. 2004. New point of care test is high specific but less sensitive for influenza virus A and B in children and adults. J Med Virol 74: 127-131.

Rawlinson WD. 2001. Antiviral agents for influenza, hepatitis C and herpesvirus, enterovirus and rhinovirus infections. MJA 175: 112-116.

Reina J, Fernandez B, Blanco I, Munar M. 1997. Comparison of Madin-Darby canine kidney cells MDCK with a green monkey continuous cell line (Vero) and human lung embryonated cells (MRC-5) in isolation of influenza A virus from nasopharyngeal aspirates by shell vial culture. J Clin Microbiol 35:1900-1901.

Ruest A, Michaud S, Deslandes S, Frost E. 2003. Comparison of the Directigen Flu A+A test, the QuickVue Influenza test and clinical case definition to viral culture and reverse transcription-PCR for rapid diagnosis of Influenza virus infection. J Clin Microbiol 41: 3487-3493.

Ryan-Poirier K, Katz J, Webster RG, Kawaoka Y. 1992. Application of Directigen Flu A for the detection of Influenza A virus in human and non-human specimens. J Clin Microbiol 30: 1072-1075.

Waner JL, Todd SJ, Shalaby H, Murphy P, Wall LV. 1991. Comparison of Directigen FLU-A with viral isolation and direct immunofluorescence for the rapid detection and identification of Influenza A virus. J Clin Microbiol 29: 479-482.

Zhang W. and Evans, DH. 1991. Detection and identification of human influenza viruses by polymerase chain reaction. J Virol Meth 33: 165-189.