Journal of Clinical Virology 45 (2009) 146–149

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Clinical performance of the 3M Rapid Detection Flu A+B Test compared to R-Mix culture, DFA and BinaxNOW Influenza A&B Test

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ARTICLE INFO

Article history:
Received 23 March 2009
Accepted 25 March 2009

Keywords:
Rapid influenza test
Influenza A
Influenza B
3M Rapid Detection Flu A+B

ABSTRACT

Background: The rapid diagnosis of influenza allows for prompt patient management and the initiation of appropriate infection control measures to reduce spread in healthcare settings.
Objective: To evaluate the clinical performance of the 3M Rapid Detection Flu A+B Test (3MA+B) as compared to R-Mix cell culture, direct immunofluorescence assay (DFA) and the BinaxNOW A&B Influenza Test (BinaxNOW).
Study design: Five hundred fresh respiratory samples, collected from patients aged 5 days to 99 years with respiratory symptoms, were tested by R-Mix culture, DFA, 3MA+B and BinaxNOW. Analytical sensitivity of 3MA+B was compared to BinaxNOW using replicates of serially diluted clinical samples positive for influenza A or B.
Results: Sensitivity, specificity, PPV and NPV for the detection of influenza A and B, respectively, were for R-Mix (96.9%, 100%, 100%, 99.3%; 98.1%, 100%, 100%, 99.8%), DFA (80.4%, 99.2%, 96.1%, 95.3%; 74%, 100%, 100%, 97%), 3MA+B (70.1%, 99.8%, 98.6%, 93%; 86.5%, 98.7%, 88.2%, 98.4%) and BinaxNOW (46.4%, 100%, 100%, 88.6%; 34.6%, 100%, 100%, 93%). R-Mix, DFA and 3MA+B were significantly ($P \leq 0.0001$) more sensitive than BinaxNOW for the detection of both influenza A and B. The analytical sensitivity of 3MA+B was greater than BinaxNOW. Excessive blood in samples may cause 3MA+B false positive influenza B results.
Conclusions: The 3MA+B provided superior results compared to BinaxNOW. The 3MA+B Reader eliminated user misinterpretation and provided quality control and result documentation. The improved sensitivity and easy of use makes 3MA+B an effective first line triage test for emergency departments, clinics and rapid response laboratories.

1. Introduction

Influenza is a significant cause of morbidity and mortality, particularly in the young and elderly.[1] The rapid diagnosis of influenza permits the initiation of antiviral therapy within a beneficial time frame,[2] can result in discontinuation of inappropriate antibiotics[3–5] and prompts infection control measures to reduce spread in healthcare settings.[3,4,6,7] Since the diagnosis of influenza can be difficult when based solely on clinical symptoms,[3,8,9] rapid (15 min) immunochromatic tests for influenza A/B are commonly used as first line diagnostic tests.[3,4,6,7,10] Although direct fluorescent antibody (DFA) tests, viral culture and molecular methods are generally more sensitive and identify a wider range of viruses, these tests are not offered by many laboratories, require more expertise and results can take from 2 h (DFA) to 14 days for traditional culture.[11]

During the 2006–2007 influenza season our laboratory received complaints of poor performance of the BinaxNOW A+B Test (BinaxNOW) (Inverness, Waltham, MA) since a significant number of samples BinaxNOW negative were positive by DFA, R-Mix culture (Diagnostic Hybrids [DHI], Athens, OH) and/or Luminex Respiratory Virus Panel (RVP) (Luminex Molecular Diagnostics, Toronto, CA). Therefore, we evaluated the 3M Rapid Detection Flu A+B Test (3MA+B) (3M Medical Diagnostics, St. Paul, MN) for use in our rapid response laboratories. 3MA+B is a United States Food and Drug Administration (US FDA) cleared qualitative immunochromatographic cartridge test, with automated reading, for the differential determination of influenza A and B in nasal wash/aspirate and nasopharyngeal (NP) aspirate/swab specimens (Fig. 1). The reader printout lists the target specific result, kit lot number, expiration

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doi:10.1016/j.jcv.2009.03.018

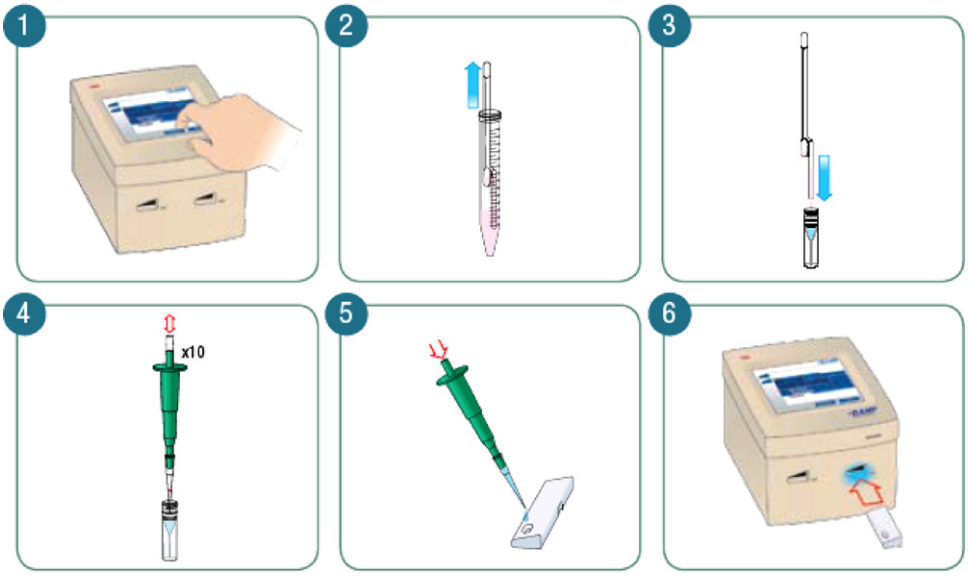

Fig. 1. Test procedure for the 3M Rapid Detection Flu A+B Test. (1) Touch screen Rapid Detection Reader. Sample (2) is added to buffer (3) and mixed with an assay tip (4) that contains fluorescent-dyed particles, coated with anti-influenza A and anti-influenza B antibodies directed against nucleoprotein antigens. Sample is transferred into the well of the Test Cartridge (5), which is inserted into the 3M Rapid Detection Reader (6). Sample migrates along the strip and anti-influenza A and anti-influenza B antibodies bind to any influenza A or B antigens, respectively, if present. Influenza-bound particles are captured at either the influenza A or B detection zone, and excess particles are captured at the internal standard (IS) zone. The reader measures the fluorescence emitted by the complexes at the influenza A, B and IS detection zones and calculates a ratio between the influenza A, B and the IS zone readings. The instrument will flag the test as invalid if the sample fails to migrate through the cartridge or the IS signal is low.

date, test date and time, sample and user ID, test port serial number. Kit positive and negative controls, patient results and internal instrument function checks are stored in the reader for easy reference.

A study by Dale et al. reported that 3MA+B was superior to BinaxNOW and Directigen EZ (Becton Dickinson, Sparks, MD) and equivalent to QuickVue Flu (Quidel, San Diego, CA).[12] Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 3MA+B for influenza A detection were 75%, 98%, 88% and 95%, respectively. Due to a limited number of influenza B(+) samples, similar analysis was not performed. We further studied the performance of 3MA+B in comparison to BinaxNOW, DFA and R-Mix culture. Differences in our study from the Dale study included a larger data set of specimens (500 vs. 249), more pediatric specimens (41% vs. 15%); inclusion of different specimen types (nasopharyngeal [NP] aspirates and washes); comparison to R-Mix culture; and a sufficient number of influenza B(+) specimens, that permitted the determination of sensitivity, specificity, PPV and NPV.

2. Materials and methods

2.1. Sample collection

Five hundred fresh (<24 h) respiratory specimens (nasal washes [$n = 45$], NP aspirates [$n = 55$], NP flocked swabs [Copan, Murrieta, CA] in Universal Transport Media [UTM, DHI] [$n = 369$], nasal swabs [$n = 27$] and tracheal aspirates [$n = 4$]) were randomly selected. Specimens were from symptomatic patients who ranged in age from 5 days to 99 years (≤2 years, $n = 105$; 3–5 years, $n = 35$; 6–10 years, $n = 30$; 11–17 years, $n = 33$; 18–59 years, $n = 161$, >60 years, $n = 136$).

2.2. Detection of influenza A and influenza B

3MA+B and BinaxNOW were performed according to manufacturers' instructions. Specimens were tested by DFA (D3 Ultra Respiratory Virus Panel, hMPV immunofluorescence reagents [DHI]) and R-Mix culture according to laboratory procedures.

Table 1
Sensitivities, specificities, positive predictive values and negative predictive values for the detection of influenza A and influenza B by method.

	%Sensitivity (95% CI)[a]	%Specificity (95% CI)	%PPV[b] (95% CI)	%NPV[c] (95% CI)
Influenza A				
DFA	80.4 (71.2–87.3)	99.2 (97.7–99.7)	96.1 (89.2–98.7)	95.3 (92.8–97.0)
R-Mix	96.9 (91.3–98.9)	100 (99.1–100)	100 (96.1–100)	99.3 (97.9–99.7)
Binax	46.4* (36.8–56.3)	100 (99.1–100)	100 (92.1–100)	88.6 (85.3–91.2)
3MA+B	70.1 (60.4–78.3)	99.8 (98.6–99.96)	98.6 (92.2–99.7)	93.0 (90.5–95.3)
Influenza B				
DFA	74.0 (60.4–84.1)	100 (99.1–100)	100 (90.6–100)	97.0 (94.8–98.2)
R-Mix	98.1 (89.9–99.7)	100 (99.2–100)	100 (93.0–100)	99.8 (98.7–99.96)
Binax	34.6* (23.2–48.2)	100 (99.2–100)	100 (82.4–100)	93.0 (90.3–94.9)
3MA+B	86.5 (74.7–93.3)	98.7 (97.1–99.4)	88.2 (76.6–94.5)	98.4 (96.8–99.2)

[a] 95% CI, 95% confidence interval.
[b] PPV, positive predictive value.
[c] NPV, negative predictive value.
* Significant difference between BinaxNOW and 3MA+B ($P \leq 0.0001$).

Studies were performed with Institutional Review Board approval. A sample was considered influenza A and/or influenza B positive(+) if R-Mix culture(+) and/or a minimum of 2 rapid tests(+) (DFA, BinaxNOW and/or 3MA+B). Discordant(+) results were arbitrated by testing an aliquot with the ProFlu-1 Assay (Prodesse, Waukesha, WI) after nucleic extraction using the NucliSENS easyMAG (bioMérieux, Durham, NC). Results were considered true(+) if the target was detected by ProFlu-1.

2.3. Comparison of analytical sensitivity of 3MA+B and BinaxNow

Comparison of the analytical sensitivity of 3MA+B and BinaxNOW was done by serially diluting (range 1:10–1:400) in UTM a patient sample influenza A(+) and a sample influenza B(+). Five replicates of each dilution were tested with both 3MA+B and BinaxNOW, when indicated. BinaxNOW uses 100 μl of neat sample. The 3MA+B uses 150 μl of neat sample added to 150 μl of buffer, from which 75 μl is tested in the cartridge.

2.4. Statistical analysis

Sensitivity, specificity, PPV and NPV were calculated using standard formulas and the significance between the values determined using the McNemar's test. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical comparison of 3MA+B, BinaxNOW, DFA and R-Mix culture

Results were available for all 4 methods for 463/500 specimens. The remaining 37 specimens did not contain a sufficient quantity (QNS) of cells for DFA. Of the 37 QNS samples, 3 were influenza A(+) and 1 influenza B(+) with 3MA+B. Influenza A was detected in 19.4% of the specimens (43.3%: <18 years; 56.7%: ≥18 years) and influenza B in 10.4% of the specimens (26.9%: <18 years; 73.1%: ≥18 years). For influenza A, R-Mix and DFA were more sensitive (96.9%, 80.4%, respectively) than 3MA+B (70.1%) and the differences were significant ($P < 0.0001$ and $P = 0.03$, respectively) (Table 1). R-Mix, DFA and 3MA+B were all significantly ($P < 0.0001$) more sensitive than BinaxNOW (46.4%). There were no significant differences in assay specificities.

For influenza B, the difference in the sensitivity of R-Mix (98.1%) and 3MA+B (86.5%) approached significance ($P = 0.07$) as did the difference between 3MA+B and DFA (74%, $P = 0.065$). R-Mix, DFA and 3MA+B were all significantly ($P < 0.0001$) more sensitive than BinaxNOW (34.6%). Specificity of 3MA+B for influenza B was less ($P = 0.03$) than all three other assays.

3MA+B performed slightly better with pediatric than adult samples for detecting influenza A (72.1% vs. 66.7%) and influenza B (93.3% vs. 85%). Influenza A and B were detected in all specimen types with the exception of the 4 tracheal specimens. There were no significant differences in the detection of influenza A or B by BinaxNOW and 3MA+B when testing nasal washes and NP aspirates. However, when testing NP swabs there were significant differences ($P \leq 0.05$) in the detection of influenza A and B for both pediatric and adults samples, with BinaxNOW less sensitive (A = 37.9%, 47.2%; B = 16.7%, 39.5%, respectively) than 3MA+B (A = 72.4%, 66.0%; B = 91.7%, 84.2%, respectively).

Discordant 3MA+B(+) results included 3 B(+) and 1A(+)B(+) by 3MA+B only, and 2 A(+)B(+) that were A(+)B(−) by culture and DFA. Discordant DFA(+) results included 5 A(+) by DFA only. There were no discordant(+) results for BinaxNOW. Of the 6 discordant 3MA+B, 1 B(+) was confirmed by ProFlu-1. Three of five 3MA+B influenza B false(+) samples contained excessive blood which may have caused a high fluorescent background signal. Of the 5 discordant DFA(+) results, 2 A(+) were confirmed by ProFlu-1.

3.2. Comparison of the analytical sensitivity of 3MA+B and BinaxNow

Since the clinical sensitivity of 3MA+B was greater than BinaxNOW, a comparison of the analytical sensitivity of each assay was performed. Despite the fact that the final specimen test volume for the 3MA+B (after buffer dilution) was 75% less than that of BinaxNOW (neat sample), 3MA+B demonstrated an approximate 15-fold and 20-fold increase in sensitivity for detecting influenza A and B, respectively, over BinaxNOW (Table 2).

4. Discussion

The 3MA+B demonstrated significantly better sensitivity for detecting influenza A and B as compared to BinaxNOW and as reported by Dale et al.[12] BinaxNOW sensitivity was remarkably decreased from when the laboratory first evaluated the assay in 2005 and may relate to differences in circulating influenza strains.[13] All specimen types performed well in this study, but caution should be used with bloody samples as false influenza B(+) might occur. Although rapid antigen tests generally require little technical expertise, they do require subjective user interpretation. The 3MA+B Reader determined the result, reducing the chance of user misinterpretation leading to inaccurate reporting. In addition, the laboratory has permanent documentation of instrument and kit QC and patient results.

Table 2
Comparison of the analytical sensitivity of 3MA+B and BinaxNOW for the detection of influenza A and influenza B.

	Dilutions of samples positive for influenza A and influenza B						
	1:10	1:20	1:50	1:100	1:200	1:300	1:400
Test	No pos/tested[a]	No pos/tested	No pos/tested	No pos/tested	No pos/tested	No pos/tested	No pos/tested
Influenza A							
3MA+B	5/5	5/5	5/5	5/5	5/5	3/5	0/0
Binax NOW	5/5 w[b]	5/5 vw[c]	0/5	0/5	ND[d]	ND	ND
Influenza B							
3MA+B	5/5	5/5	5/5	5/5	4/5	0/5	0/5
Binax NOW	5/5 w	0/5	0/5	ND	ND	ND	ND

[a] No pos/tested, number of samples with a positive result/number of replicates tested.
[b] w, weak reaction lines.
[c] vw, very weak reaction lines.
[d] ND, not done.

Conflict of interest

C.C. Ginocchio: Research funding for this study was provided by 3M Company (Saint Paul, MN).

Acknowledgements

This study was funded by a research grant from 3M Company (Saint Paul, MN) to CCG. We sincerely thank Dan J. Morse, Clinical Research 3M Center, for statistical analysis.

References

1. Thompson WW, Shay DK, Weintraub E, Brammer L, Cox NJ, Anderson LJ, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;**289**(January (2)):179–86.
2. Blumentals WA, Schulman KL. Impact of oseltamivir on the incidence of secondary complications of influenza in adolescent and adult patients: results from a retrospective population-based study. *Curr Med Res Opin* 2007;**23**(December (12)):2961–70.
3. D'Heilly SJ, Janoff EN, Nichol P, Nichol KL. Rapid diagnosis of influenza infection in older adults: influence on clinical care in a routine clinical setting. *J Clin Virol* 2008;**42**(June (2)):124–8.
4. Harper SA, Fukuda K, Uyeki TM, Cox NJ, Bridges CB. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2005;**54**(July (RR-8)):1–40.
5. Low D. Reducing antibiotic use in influenza: challenges and rewards. *Clin Microbiol Infect* 2008;**14**(April (4)):298–306.
6. Barenfanger J, Drake C, Leon N, Mueller T, Troutt T. Clinical and financial benefits of rapid detection of respiratory viruses: an outcomes study. *J Clin Microbiol* 2000;**38**(August (8)):2824–8.
7. Fasley AR, Murata Y, Walsh EE. Impact of rapid diagnosis on management of adults hospitalized with influenza. *Arch Intern Med* 2007;**167**(February (4)):354–60.
8. Boivin G, Hardy I, Tellier G, Maziade J. Predicting influenza infections during epidemics with use of clinical case definition. *Clin Infect Dis* 2000;**31**(November (5)):1166–9.
9. Monto AS, Gravenstein S, Elliott M, Colopy M, Schweinle J. Clinical signs and symptoms predicting influenza infections. *Arch Intern Med* 2000;**160**(November (21)):3243–7.
10. Noyola DE, Demmler GJ. Effect of rapid diagnosis on management of influenza A infections. *Pediatr Infect Dis J* 2000;**19**(April (4)):303–7.
11. Leland D, Ginocchio CC. Role of cell culture for virus detection in the age of technology. *Clin Microbiol Rev* 2007;**20**(January (1)):49–78.
12. Dale SE, Mayer C, Mayer MC, Menegus MA. Analytical and clinical sensitivity of the 3M Rapid Detection Influenza A+B Assay. *J Clin Microbiol* 2008;**46**(November (11)):3804–7.
13. Weinberg A, Mettenbrink CJ, Ye D, Yang C-F. Sensitivity of diagnostic tests for influenza varies with the circulating strains. *J Clin Virol* 2005;**33**(June (2)):172–5.