Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak

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ABSTRACT

Background: In response to the novel influenza A H1N1 outbreak in the NY City area, 6090 patient samples were submitted over a 5-week period for a total of 14,114 viral diagnostic tests, including rapid antigen, direct immunofluorescence (DFA), viral culture and PCR. Little was known about the performance of the assays for the detection of novel H1N1 in the background of seasonal H1N1, H3N2 and other circulating respiratory viruses. In addition, subtyping influenza A became critical for the identification of high risk and/or hospitalized patients with novel H1N1 infection and for monitoring the spread of the outbreak.
Study design: This study analyzed the performances of the BinaxNOW Influenza A&B test (BinaxNOW), the 3M Rapid Detection Flu A + B test (3MA + B), direct immunofluorescence, R-Mix culture and the Luminex xTAG Respiratory Virus Panel (RVP) for the detection of seasonal influenza, novel H1N1 and other respiratory viruses. RVP was also evaluated for its ability to differentiate seasonal H1N1, H3N2 and novel H1N1.
Results: The sensitivities, specificities, PPVs and NPVs for the detection of novel H1N1, determined by comparing all four-test methods, were: rapid antigen: 17.8%, 93.6%, 77.4%, 47.9%; DFA: 46.7%, 94.5%, 91.3%, 58.9%; R-Mix culture: 88.9%, 100%, 100%, 87.9%; RVP: 97.8%, 100%, 100%, 97.3%. The individual sensitivities of BinaxNOW and 3MA + B as compared to R-Mix culture for the detection of novel H1N1 were 9.6% and 40%, respectively. All unsubtypeable influenza A specimens identified by RVP and tested with the CDC novel H1N1 specific RT-PCR assay were confirmed to be novel H1N1.
Conclusions: Rapid antigen tests, DFA, R-Mix culture and the xTAG RVP test all detected the novel H1N1 strain, but with highly varied sensitivity. The RVP test provided the best diagnostic option as RVP demonstrated superior sensitivity for the detection of all influenza strains, including the novel H1N1, provided accurate influenza A subtyping and identified a significant number of additional respiratory pathogens.

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1. Background

Over the weekend of April 24–26, 2009, high school students from a preparatory school in Queens, NY were evaluated at the Emergency Rooms at Long Island Jewish Medical Center, New Hyde Park, NY and North Shore University Hospital, Manhasset, NY for complaints of flu-like symptoms.[3] Due to recent travel by several students to Cancun, Mexico 1 week previous, there was a high concern for infection with the novel 2009 influenza A (H1N1) strain.[2] Nasopharyngeal swabs were obtained and sent to the hospital laboratories for rapid antigen screening tests for influenza A and influenza B viruses. Initially, 35 specimens that tested rapid antigen positive for influenza A were sent via the New York City Department of Health to the Centers for Disease Control and Prevention (CDC), Atlanta, GA. Testing at CDC confirmed that 28 of the 35 rapid influenza A test samples contained the novel 2009 influenza A (H1N1).

During the first 5 weeks of the novel H1N1 outbreak, from April 24, 2009 to May 27, 2009, a combination of 14,114 viral diagnostic tests were performed on a total of 6090 patients suspected of having influenza. Tests included rapid antigen assays ($n = 4369$) using either the BinaxNOW Influenza A&B test (BinaxNOW) (Inverness, Waltham, MA) or the 3M Rapid Detection Flu A + B test (3MA + B) (3M Medical Diagnostics, St. Paul, MN); direct immunofluorescence (DFA) ($n = 3557$) using D3 Respiratory Virus Reagents (Diagnostic Hybrids [DHI], Athens, OH); R-Mix viral culture (DHI) ($n = 3473$) and the Luminex xTAG Respiratory Virus Panel (RVP) ($n = 2715$) assay (Luminex Molecular Diagnostics, Toronto, Canada). During

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the height of a normal respiratory virus season the laboratories generally perform approximately 400 tests a day. The day prior to the outbreak the number of viral tests performed was 214 as the regular influenza season was ending. The weekend of the initial influenza testing surge a high of 895 viral tests were performed on a single day and during the second surge of the influenza outbreak 3 weeks later, the day high was 970 viral tests performed.

In accordance with hospital testing policies, nasopharyngeal (NP) samples were initially screened with rapid antigen tests at the local hospitals. Due to the suboptimal performance of the rapid antigen tests,[7] samples negative for influenza A or B were referred to the North Shore-LIJ Health System Clinical Virology Laboratory for DFA and R-Mix viral culture. However, after the first several days of the outbreak it was apparent that the laboratory needed to provide influenza A subtyping information and thus be able to quickly identify potential cases of novel H1N1. Therefore, initially all samples from patients at a high risk for exposure to the novel H1N1 (preparatory school students, their siblings, teachers and persons with recent travel to Mexico), patients admitted to the hospital with flu-like illness, or any out-reach patient with an Influenza A-positive rapid antigen test, DFA and/or R-Mix culture were tested with the RVP assay.

Prior to the onset of the influenza epidemic, the RVP assay was being used for selected cases and for virus surveillance research studies, with the intention to fully convert all respiratory virus testing to the RVP assay during the summer months. The Food and Drug Administration (FDA)-cleared version of the RVP assay detects 10 viruses, including adenovirus, human metapneumovirus (hMPV), parainfluenza viruses 1–3, rhinovirus, respiratory syncytial viruses (RSV) A and B, influenza A and influenza B.[6,8,9] In addition to the detection of the influenza A matrix gene, the RVP assay also has the ability to subtype the influenza A hemagglutinin gene as seasonal H1 or H3. Samples positive for the matrix gene but negative for seasonal H1 and H3 are considered influenza A unsubtypeable and are potentially a novel strain of influenza A. The research use-only version of the assay also includes the detection of parainfluenza type 4 and the coronaviruses OC43, NL64, 229E and HKU-1.[6,8,9] Since the RVP assay was able to subtype the influenza A seasonal viruses and detected a broad range of respiratory viruses other than influenza, we made RVP testing broadly available on an immediate basis.

In the 5 weeks following the start of the outbreak the overwhelming number of test requests required continued modifications to the laboratory testing protocols. Testing algorithms that included various combinations of rapid antigen testing, DFA, viral culture and RVP changed frequently to deal with the surge in patient testing, to prioritize testing for admitted patients and to provide the most clinically relevant information for public health officials. Due to the uniqueness of the novel H1N1 strain, the relative performance of the various diagnostic tests for the detection of novel H1N1 was not known during the beginning of the influenza outbreak.

2. Objective

The purpose of this study was to evaluate the performance of rapid antigen testing, DFA, R-Mix culture and the RVP assay for the detection of the novel H1N1 in the background of seasonal H1N1 and H3N2 and other common circulating respiratory viruses.

3. Study design

3.1. Patient population and sample types

Samples from 6090 patients, evaluated for influenza like illness in the hospital setting (emergency department or in-patient) (n = 2888) or as an out-patient (n = 3202) were included in this study. Patients ranged in age from 4 days to 98 years. Sample types tested included flocked nasopharyngeal (NP) swabs (Copan, Murrieta, CA) submitted in universal transport media (UTM, DHI), NP aspirates and NP washes. Samples were stored refrigerated or frozen at $-70\,°C$ until tested. All samples were collected as per standard of care for routine diagnostic testing and informed consent was therefore not required.

3.2. Sample processing

All rapid antigen tests were performed on neat samples. Samples for DFA and R-Mix culture were processed according to standard laboratory procedures. Nucleic acids for testing with the RVP assay and for influenza A subtype confirmation were extracted from a 200 µl aliquot of the respiratory sample using the NucliSENS easy-MAG extraction platform (bioMerieux, Durham, NC) according to the manufacturer's instructions. Nucleic acids were stored at $-70\,°C$ until tested.

3.3. Diagnostic tests

All tests were performed according to the manufacturer's instructions and laboratory validated protocols. From the total number of samples tested, the following number of results were available for determining the performance of the diagnostic tests for the detection of both seasonal and novel H1N1 influenza A viruses: rapid antigen tests (n = 3789) using BinaxNOW (n = 2870) and 3MA + B (n = 919); DFA using D3 Respiratory Virus Reagents (n = 2861); R-Mix viral culture (n = 2726); and RVP assay (n = 2715).

3.4. Confirmation of novel influenza A H1N1

Although an initial set of 35 samples had been sent to the Centers for Disease Control from the very first patients evaluated for novel H1N1 in our system emergency rooms, these samples had not been previously subtyped with the RVP assay in our laboratory. There was a compelling need to evaluate our own RVP subtyping on a subsequent set of samples, early on in our deployment of this assay. Accordingly, a subset of samples identified by RVP as unsubtypeable influenza A (n = 99) that contained sufficient viral titer, were confirmed as novel H1N1 by the New York State Laboratory of Viral Diseases, Wadsworth Center, Albany, NY, as previously described.[5] Additional RVP samples (n = 60) classified a seasonal H1, H3 or unsubtypeable influenza A were tested either at the State Department of Health or in-house with a laboratory validated assay that uses the method developed by the CDC for the confirmation of the novel H1N1 strain.[1]

3.5. Comparative analysis of test methods

Due to the massive influx of test samples and the changing test algorithms during the outbreak, all samples were not tested by all methods. Comparisons were therefore made based upon those test methods used for the evaluation of each specimen. For example, to compare the performance of traditional test methods only, results were available from rapid antigen testing, DFA and R-Mix culture for a set of 1831 samples. Specifically, separate sample sets were used to compare BinaxNOW results with the results of DFA (n = 1860) and R-Mix culture (n = 1352); and to compare 3MA + B test results with those of DFA (n = 448) and R-Mix culture (n = 356).

Analysis of the RVP data is presented separately since original samples tested with RVP were specifically selected based upon a positive rapid antigen, DFA or culture result. Therefore a direct comparison including all samples would be biased in increasing the detection rates of the other assays. Data from an unbiased subset of 288 samples tested by rapid antigen, DFA, R-Mix culture and

RVP was compared and more accurately reflects the comparison between the four test methods.

3.6. Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using standard formulas. Differences in the performance of the various assays were calculated using the McNemer's test. A p-value of <0.05 was considered statistically significant.

4. Results

4.1. Confirmation of novel influenza A H1N1

The seasonal H1 and H3 subtyping accuracy of the RVP assay was previously confirmed by the laboratory using conventional RT-PCR and the CDC designed H1 and H3 specific primers, followed by sequence analysis.[5] In total, 86 seasonal H3N2 and 16 seasonal H1N1 strains identified by RVP were confirmed as the appropriate seasonal subtype.[5] In addition to the set of 99 RVP unsubtypeable influenza A samples confirmed as novel H1N1, testing at the NY State Department of Health and in-house determined that an additional 45 unsubtypeable influenza A strains were the novel influenza A H1N1. Five seasonal H1 and 12 seasonal H3 strains identified by RVP tested negative for novel H1N1. Therefore, based upon our previous published study and the additional in-house data, it was determined that the classification of an influenza A strain by RVP as unsubtypeable was, during the current outbreak, highly predictive for the novel H1N1. Therefore, all unsubtypeable influenza A strains identified in this study will be classified as novel H1N1.

4.2. Detection of seasonal influenza H1N1, H3N2, novel H1N1 and other seasonal respiratory viruses

Overall, influenza A was identified in 1598/6090 (26.2%) of all patients tested. The number of positive results/per total tested by method for the detection of all influenza A strains (seasonal H1N1, H3N2, and novel H1N1) were as follows: 518/3789 (13.7%) for rapid antigen tests; 397/3271 (12.1%) for DFA; 482/2726 (17.7%) for R-Mix culture and 1265/2715 (46.6%) for RVP. Of the 1265 RVP influenza A samples, 1108 (87.6%) were novel H1N1, 151 (11.9%) seasonal H3N2, and 6 (0.5%) seasonal H1N1. The overall prevalence of novel H1N1, seasonal H3N2 and seasonal H1N1 were 40.8%, 5.6%, and 0.2%, respectively. The average age of patients with seasonal H1N1

was 11.6 years (range 8 months to 31 years), seasonal H3N2 was 39.1 years (range 10 months to 97 years) and for novel H1N1 was 13.7 years (range 2 weeks to 97 years).

The prevalence of other respiratory viruses detected in specimens by DFA and/or culture was 9.2% (302/3289) and included adenovirus ($n = 38$), hMPV ($n = 32$), influenza B ($n = 15$), parainfluenza 1 ($n = 40$), parainfluenza 2 ($n = 1$), parainfluenza 3 ($n = 160$) and RSV ($n = 16$). RVP detected one or multiple respiratory viruses in 580 (21.4%) of the influenza A negative specimens, and as a co-infection with influenza A in 86 specimens (Table 1). Other viruses detected included adenovirus ($n = 24$), coronaviruses (NL63: $n = 2$, HKU-1: $n = 6$, 229E: $n = 8$), hMPV ($n = 58$), enterovirus/rhinovirus (E/R) group ($n = 451$), influenza B ($n = 5$), parainfluenza 1 ($n = 34$), parainfluenza 2 ($n = 3$), parainfluenza 3 ($n = 77$), parainfluenza 4 ($n = 18$), and RSV ($n = 16$). E/R group was the most common dual infection seen with influenza A and with the other respiratory viruses. Overall, RVP detected a respiratory virus in 68% of the samples tested.

4.3. Comparison of rapid antigen testing, DFA and R-Mix culture for the detection of all influenza A and novel H1N1

Table 2 presents the sensitivity, specificity, PPV and NPV for data derived from 1831 specimens tested with rapid antigen tests (BinaxNOW and 3MA + B combined), DFA and R-Mix culture. Results are provided for the overall detection of all influenza A subtypes combined and then specifically for novel H1N1. There were 164 samples positive for influenza A (novel H1N1, $n = 123$; seasonal H1N1, $n = 1$; seasonal H3N2, $n = 40$). Overall the sensitivity of the rapid tests was significantly lower ($p \leq 0.0001$) for both the detection of all influenza subtypes (23.8%) and for the novel H1N1 (21.2%) as compared to DFA (50% and 47.2%, respectively) and for R-Mix culture (98.2% and 98.4%, respectively). DFA was significantly less sensitive ($p \leq 0.0001$) than R-Mix culture for the detection of all influenza A subtypes and novel H1N1.

4.4. Comparison of the BinaxNOW Influenza A&B and 3M Rapid Detection Flu A + B tests to DFA and R-Mix culture for the detection of all influenza A and novel H1N1

Table 3 presents the sensitivity, specificity, PPV and NPV for the results of one data set for the BinaxNOW test (top panel) and a second data set with the results obtained with the 3MA + B test (lower panel) as compared to DFA and R-Mix culture. Results are provided for the overall detection of all influenza A subtypes combined and then specifically for novel H1N1. The BinaxNOW test was signifi-

Table 1
Prevalence of respiratory viruses identified by the xTAG RVP assay during the novel H1N1 outbreak.

Virus	Number (%)	Mixed infections
Flu A (UST) novel H1N1	1108 (40.8%)	E/R(57), 229E(3), HKU-1(2), hMPV(2), P1(4), P3(4), P4(3), RSV(1)
Flu A H1N1	6 (0.2%)	Adeno(1), E/R(1)
Flu A H3N2	151 (5.6%)	E/R(6), hMPV(1)
Flu B	5 (0.2%)	P3(2)
Entero/Rhino (E/V)	451 (16.6%)	Flu A(57), H1N1(6), H3N2(6), P1(5), P2(1) P3(12), P4(5), hMPV(6), Adeno(4)
hMPV	58 (2.1%)	E/R(3), RSV(1), H3N2(1)
Parainfluenza 3	77 (2.8%)	E/R(12), Flu B(2), RSV(2)
Parainfluenza 1	34 (1.3%)	E/R(5)
Parainfluenza 4	18 (0.7%)	E/R(2)
Parainfluenza 2	3 (0.1%)	E/R(3)
Adenovirus	24 (0.9%)	E/R(4), H1N1(1)
Coronaviruses	16 (0.6%)	Types: NL63(2), 229E(8), HKU-1(6)
RSV	16 (0.6%)	P3(2), hMPV(1)
Negative	870 (32%)	

UST, unsubtypeable and presumed novel H1N1; Adeno, adenovirus; coronaviruses 229E, NL63 and HKU-1; E/R, enterovirus/rhinovirus group; hMPV, human metapneumovirus; P1, parainfluenza virus 1; P2, parainfluenza virus 2; P3, parainfluenza virus 2; RSV, respiratory syncytial virus. A total of 2715 samples were tested by xTAG RVP assay. Number (%): number identified as positive for virus (percentage of total specimens). Mixed infections: virus (number detected).

Table 2

Comparison of rapid antigen testing, DFA and R-Mix viral culture for the detection of all influenza A subtypes combined and for novel H1N1.

	Sensitivity		Specificity		PPV		NPV	
	Flu A[a] (%)	H1N1[b] (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)
Rapid Ag[c]	23.8	21.2	99.5	99.5	83.0	76.5	93.0	94.5
DFA	50.0	47.2	99.6	99.6	93.2	90.6	95.3	96.2
R-Mix	98.2	98.4	100	100	100	100	99.8	99.9

PPV, positive predictive value; NPV, negative predictive value; DFA, direct fluorescent antibody test. In total, 1831 respiratory samples were tested by all three methods. There were 164 samples positive for influenza A (novel H1N1, $n = 123$; seasonal H1N1, $n = 1$; seasonal H3N2, $n = 40$).

  [a] Comparison for all influenza A positive samples, including seasonal H1, H3 and novel H1N1.

  [b] Comparison for only novel H1N1 positive samples.

  [c] Rapid antigen testing was performed using either BinaxNOW A&B test or the 3M Rapid Detection Flu A + B test.

Table 3

Comparison of BinaxNOW A + B assay and the 3M Rapid Detection Influenza A + B assay to DFA and R-Mix culture for the detection of all influenza A subtypes combined and novel H1N1.

Test	Sensitivity		Specificity		PPV		NPV	
	Flu A[a] (%)	H1N1[b] (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)
Binax versus DFA[c]	29.8	30.6	98.9	98.9	94.9	93.8	95.2	94.1
	98.4	97.1	99.7	99.7	96.1	93.0	99.9	99.9
Binax versus R-Mix[d]	10.4	9.6	100	100	100	100	86.3	88.9
	99.6	99.0	100	100	100	100	99.9	99.9
Test	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)
3MA + B versus DFA[e]	72.2	71.4	98.1	98.1	76.7	72.7	97.6	98.1
	88.9	85.7	99.8	99.8	97.0	97.0	99.0	99.0
3MA + B versus R-Mix[f]	41.2	40.0	97.8	97.8	74.2	73.9	94.0	96.3
	97.1	95.0	100	100	100	100	99.7	99.7

PPV, positive predictive value; NPV, negative predictive value; DFA, direct fluorescent antibody test.

  [a] Comparison for all influenza A positive samples, including seasonal H1, H3 and novel H1N1.

  [b] Comparison for only novel H1N1 positive samples.

  [c] BinaxNOW compared to DFA.

  [d] BinaxNOW compared to R-Mix culture.

  [e] 3MA + B compared to DFA.

  [f] 3MA + B compared to R-Mix culture.

cantly ($p \leq 0.0001$) less sensitive for the detection of all influenza A subtypes combined and for novel H1N1 when compared directly to DFA (29.8% versus 98.4%, 30.6% versus 97.1%, respectively) and when compared directly to R-Mix culture (10.4% versus 99.6%, 9.6% versus 99.0%, respectively). The sensitivity of the 3MA + B test for the detection of all influenza A subtypes combined and for novel H1N1 was not statistically different ($p = 0.18$ and $p = 0.37$, respectively) when compared directly to DFA (72.2% versus 88.9%, 71.4% versus 85.7%, respectively). The sensitivities of 3MA + B for the detection of all influenza A combined (41.2%) and novel H1N1 (40.0%) were significantly less ($p \leq 0.0001$ and $p = 0.006$, respectively) when compared directly to R-Mix culture (97.1% and 95.0%, respectively). Although the two rapid tests were performed on different patient samples and there is not a direct comparison among the two test methods, 3MA + B appears to be the more sensitive test for the detection of seasonal and novel H1N1 influenza A. False positive influenza

A results were identified with both rapid assays, but the specificity and PPV of the 3M test appeared to be less than that of the BinaxNOW assay.

4.5. Comparison of rapid antigen tests, DFA, R-Mix culture and RVP

A subset of 288 samples were tested by rapid antigen, DFA, R-Mix culture and RVP and the results of the four methods were compared. There were 179 samples positive for influenza A (novel H1N1, $n = 132$; seasonal H1N1, $n = 1$; seasonal H3N2, $n = 42$; 4 samples positive by R-Mix only with no subtyping). As shown in Table 4, the sensitivities of rapid antigen tests and DFA were significantly lower ($p \leq 0.0001$) for the detection of all influenza subtypes (20.7% and 48.6%, respectively) and for novel H1N1 (17.8% and 46.7%, respectively) as compared to both R-Mix culture and RVP for the detection

Table 4

Comparison of rapid antigen testing, DFA, R-Mix culture and RVP for the detection of all influenza A subtypes combined and for novel H1N1.

	Sensitivity		Specificity		PPV		NPV	
	Flu A[a] (%)	H1N1[b] (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)	Flu A (%)	H1N1 (%)
Rapid Ag	20.7	17.8	93.6	93.6	84.1	77.4	41.8	47.9
DFA	48.6	46.7	94.5	94.5	93.5	91.3	52.8	58.9
R-Mix	82.7	88.9	100	100	100	100	77.9	87.9
RVP	97.8	97.8	100	100	100	100	99.1	97.3

PPV, positive predictive value; NPV, negative predictive value; DFA, direct fluorescent antibody test. A total of 288 samples were tested by all four methods. There were 4 samples with no influenza A subtyping (positive by R-Mix only) and 175 samples positive for influenza A (seasonal H1N1, $n = 1$; seasonal H3N2, $n = 42$; novel H1N1, $n = 132$).

  [a] Comparison of all influenza A positive samples, including seasonal H1N1, H3N2 and novel H1N1.

  [b] Comparison of only novel H1N1 positive samples.

of all influenza subtypes (82.7% and 97.8%, respectively) and for the detection of novel H1N1 (88.9% and 97.8%, respectively).

5. Conclusions

This study evaluated the performance of multiple influenza A detection methods used during the course of the novel H1N1 outbreak in the New York City area, over a 5-week period. The magnitude of the samples tested and the significant number of novel H1N1 samples included in this study provided a sound basis for this performance evaluation.

Although antigen based tests require little technical skill, are rapid and can be performed in an emergency department setting, the sensitivity and specificity of the assays are questionable. The BinaxNOW assay demonstrated very poor sensitivity for the detection of both seasonal influenza A (10.4%) and novel H1N1 (9.6%) when compared to R-Mix culture (99.6% and 99.0%, respectively). The 3MA + B test demonstrated better sensitivity for the detection of seasonal influenza A (41.2%) and novel H1N1 (40%) when compared to R-Mix culture (97.1% and 95%, respectively). The increased sensitivity of the 3MA + B assay over the BinaxNOW assay was consistent with a previous study from our laboratory that directly compared the two tests for the detection of seasonal influenza A and influenza B.[4] In that study the sensitivity of 3MA + B (70.1%) for the detection of influenza A was significantly greater ($p \leq 0.0001$) than the sensitivity of BinaxNOW (46.4%). In this study, BinaxNOW demonstrated better PPVs for the detection of all influenza A subtypes combined and for novel H1N1 than did the 3MA + B test due more false positive 3MA + B results ($n = 8$) when testing pediatric NP washes from early in the outbreak. In our previous study the 3MA + B demonstrated a high specificity of 99.8% for the detection of influenza A and a PPV of 93%. There was only one false positive influenza A sample detected. The cause of the false positive results in this study is currently not known, but may be related to the type of sample tested, presence of excessive mucous or blood in the samples. Additional studies from our laboratory and by the manufacturer are underway to determine the root cause of the false positive influenza A results. In addition, an extensive head to head comparison of the BinaxNOW and 3MA + B test is being conducted.

DFA identified more seasonal and novel H1N1 influenza A positive samples than the rapid tests. Additional studies are being performed to determine the performance of DFA reagents from other manufacturers for the detection of novel H1N1. R-Mix cells were shown to be an acceptable culture system for the detection of novel H1N1 and, as expected, R-Mix culture provided the highest degree of sensitivity among the traditional test methods. DFA and R-Mix culture had the added benefit of also identifying other common respiratory viruses, including adenovirus, hMPV, parainfluenza viruses 1, 2, 3 and RSV, which were also circulating during the influenza outbreak.

Overall, the RVP assay provided the highest quality of results due to the sensitivity of the assay and the broad scope of viral pathogens detected by the assay. Influenza A viruses were detected in 46.6% of all samples tested by RVP as compared to 13.7% for rapid antigen tests, 12.1% for DFA and 17.7% for R-Mix culture. As demonstrated in our previous study[5] and with the additional samples tested for this study, the RVP assay accurately distinguished the seasonal H1N1, H3N2 and the novel H1N1 influenza A subtypes. The ability to subtype the influenza A virus in the same step as the initial identification proved to be exceptionally useful and saved valuable laboratory resources and time by not having to reflex to a second assay or refer to the Department of Health for the identification of patients infected with novel H1N1. In fact, during the height of the outbreak, our laboratory provided RVP testing to other hospitals outside of our health system and also acted as a first line triage for the local, city and state Departments of Health. In addition, due to the comprehensive nature of the RVP test, the laboratory was also able to identify the virus(es) responsible for numerous other respiratory infections not due to influenza A. Finally, the comprehensive nature of the assay will help us to better understand the epidemiology of novel H1N1 infections and the significance of mixed viral infections.

Moving forward, as the epidemic continues in the NY City area and around the world, it is extremely important to keep our health care providers appraised of the performance characteristics of the diagnostic tests so that appropriate laboratory testing is performed and appropriate clinical decisions can be made based upon accurate and reliable diagnostic tests.

6. Conflict of interest

Acknowledgements

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