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Evaluation of the Alere i Influenza A&B Nucleic Acid Amplification Test by Use of Respiratory Specimens Collected in Viral Transport Medium

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The Alere i Influenza A&B assay is a newly developed rapid molecular assay which has the potential to generate results within 15 min from sample collection. In this study, we evaluated the Alere i Influenza A&B assay by using salvaged frozen respiratory specimens that were collected in viral transport medium from children ages 10 months to 19 years. Alere i Influenza A&B assay test results were compared with viral culture and ProFlu$^+$ real-time reverse transcription-PCR (RT-PCR) assay results. We found that the overall sensitivity and specificity of the Alere i Influenza A&B assay were 93.3% and 94.5% for the detection of influenza A virus and 100% and 100% for the detection of influenza B virus, respectively, compared to viral culture. In comparison to ProFlu$^+$ real-time RT-PCR, overall sensitivity and specificity of the Alere i Influenza A&B assay for the detection of influenza A virus were 88.8% and 98.3% and 100% and 100% for detecting influenza B virus. Overall, the Alere i Influenza A&B assay performed well compared to either virus cell culture or RT-PCR.

Influenza viruses cause a significant number of infections each year during respiratory illness seasons. Individuals with increased risks for influenza virus infection include children, the elderly, and those with compromised immune systems resulting from other ailments (1–5). The illnesses caused by these viruses continue to result in a considerable economic impact (4, 6). Treatment options such as influenza antiviral medications generally need to be administered within 24 to 72 h from the onset of symptoms for adequate efficacy (7). Therefore, rapid diagnosis is key for such therapies.

Several laboratory methods are available for detecting influenza viruses and aiding in the diagnosis of influenza virus infections, most of which will distinguish between influenza A and influenza B viruses. Commonly used methods in the lab include viral culture (8), direct fluorescent antibody (DFA) staining, immunochromatographic virus antigen detection-based assays (9–11), and nucleic acid amplification assays (1, 12, 13). Traditionally, viral culture is considered the gold standard for detection of influenza virus infection, and shell vial cultures using cocultured cell lines (R-mix) have been used to increase the rapidity of culture results. However, these results are not available in a timely fashion to impact clinical decisions in an outpatient setting. DFA assays have performed well and have improved the result turnaround time (TAT) (14, 15). Unfortunately, these assays are laborious, subjective, technically demanding, and generally exhibit lower sensitivity when used alone rather than in conjunction with viral culture (16–18). Real-time reverse transcription-PCR (RT-PCR) is becoming increasingly accepted as a gold standard for detection of influenza viruses, but it is technically demanding, laborious, and expensive; all these factors limit the usefulness of the technique in an outpatient setting. Antigen detection methods are available for rapid diagnosis of viral infections from respiratory specimens. These assays show benefit in Emergency Departments and outpatient settings due to their ease of use and rapid TAT, generally 30 min or less. For these reasons, many clinical laboratories employ rapid antigen-based assays as their first-line diagnostic test for influenza virus infections. Though generally exhibiting very high specificity and positive predictive values, the major limitations of currently available rapid antigen tests involve their low and widely variable sensitivity (19).

A current need in diagnostic microbiology laboratories is for a rapid, molecular-based assay with high sensitivity and quick TAT for detection of influenza virus. Isothermal nucleic acid amplification allows nucleic acid amplification in a very narrow temperature range, eliminating the need for expensive thermal cyclers and allowing for results to be obtained very quickly. The Alere i Influenza A&B nucleic acid amplification test is a simple-to-use, automated test for influenza A and B viruses that is intended to provide the sensitivity of a molecular test with the quick results that a traditional rapid antigen test provides. The Alere i Influenza A&B test can provide results within 15 min of initiating the test. Specimen preparation, lysis, and nucleic acid amplification are all accomplished with minimal hands-on time.

The aim of the current study was to evaluate the performance characteristics of the Alere i Influenza A&B assay in comparison to viral culture and a real-time RT-PCR assay for influenza virus. Previously characterized frozen respiratory specimens obtained from children were used in this evaluation study.

(These study findings were presented at the Pan American Society for Clinical Virology Annual Meeting 2013.)

MATERIALS AND METHODS

Clinical specimens. This study utilized a total of 236 salvaged frozen respiratory specimens collected in 3 ml of universal transport medium

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TABLE 1 Performance of the Alere i Influenza A&B assay versus viral cell culture

Virus	Culture specimen source	TP	FP	TN	FN	Total no. of samples	% sensitivity (95% CI)[a]	% specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Influenza A virus	MTS	74	3	74	5	156	96.1 (88.3–99.0)	93.7 (85.2–97.6)	93.7 (85.2–97.6)	96.1 (88.3–99.0)
	NPW/A	24	4	47	2	77	92.3 (73.4–98.7)	92.2 (80.3–97.5)	85.7 (66.4–95.3)	95.9 (84.9–99.3)
	Total	98	7	121	7	233	93.3 (86.3–97.0)	94.5 (88.6–97.6)	93.3 (86.3–97.0)	94.5 (88.6–97.6)
Influenza B virus	MTS	31		124		155	100 (86.3–100)	100 (96.3–100)	100 (86.3–100)	100 (96.3–100)
	NPW/A	27		50		77	100 (84.5–100)	100 (91.1–100)	100 (84.5–100)	100 (91.1–100)
	Total	58		174		232	100 (92.3–100)	100 (97.3–100)	100 (92.3–100)	100 (97.3–100)

[a] CI, confidence interval.

(UTM). The specimens were previously collected for clinical testing from August 2009 through June 2012 at Children's Mercy Hospitals and Clinics in Kansas City, MO. The specimen collection consisted of 158 midturbinate swabs (MTS) and 78 nasopharyngeal wash/aspirates (NPW/A) collected in 3 ml UTM from children aged 10 months to 19 years. The historical results were obtained through a combination of rapid antigen testing (180 specimens) and RT-PCR (56 specimens). Included in the study were 116 historically positive influenza A virus specimens, 60 historically positive influenza B virus specimens, and 60 historically negative specimens. Each specimen was thawed, deidentified, and given a unique study identifier prior to enrollment into the study.

Alere i Influenza A&B nucleic acid amplification testing. Specimens were tested with the Alere i Influenza A&B assay within 24 h of thawing. Briefly, the test base is inserted into the appropriate color-coded receptacle, followed by placing the sample receiver into the corresponding color-coded receptacle. The sample receiver and buffer inside are then heated 3 minutes. Following the heating step, 200 μl of thawed specimen in UTM is directly added to the buffer in the sample receiver. The transfer cartridge is then used to transfer sample to the test base. Once this step has been confirmed by the user, the test proceeds and results are available within 10 min. The instrument reports results as either positive or negative for both influenza A and influenza B viruses. All instructions are reported via animated graphics on the instrument display. For the study, positive and negative quality control swabs were processed every day before testing of study specimens.

Respiratory viral culture. Viral culture was performed using R-Mix shell vials from Diagnostic Hybrids (Athens, OH). The viral cultures were stained with Light Diagnostics SemiFluor FluA/FluB duet antibody from EMD Millipore (Billerica, MA) on day 2 of culture.

Nucleic acid extraction. Total nucleic acid was extracted from 200 μl of each specimen along with positive and negative extraction controls with the NucliSENS easyMAG automated extractor from bioMérieux (Durham, NC). Extractions were carried according to the manufacturer's instructions. All nucleic acid extractions were performed within 24 h of thawing a specimen. Samples were extracted in batches daily and stored frozen at −80°C until real-time RT-PCR testing.

Real-time RT-PCR. RT-PCR was performed with the Prodesse ProFlu$^+$ assay for detection of influenza A virus, influenza B virus, and respiratory syncytial virus (Gen-Probe, San Diego, CA). The assay was performed on all tested specimens according to the manufacturer's instructions along with recommended controls on the Cepheid SmartCycler II real-time instrument with Dx software version 1.7b.

Statistical analyses. Results obtained with the Alere i Influenza A&B assay were compared to those obtained via viral cell culture and RT-PCR reference methods to characterize the assay's performance. The statistical analyses included all specimens that yielded a valid test result for both the Alere i Influenza A&B assay and the comparator methods. Reportable data were summarized in two-by-two data tables in which the number of specimens in each of the four result categories were listed: true positive (TP), true negative (TN), false positive (FP), and false negative (FN). Sensitivity, specificity, positive predictive value (NPV), and negative predictive value (NPV) were determined. Confidence intervals at 95% were calculated by using a modified Wald method (20).

RESULTS

This study tested 236 salvaged and previously frozen respiratory specimens by use of the Alere i Influenza A&B rapid molecular assay. The overall performance of the test compared with viral cell culture is shown in Table 1. For each test, the assay generated separate results for influenza A and influenza B viruses. Importantly, the validity of each result does not depend on the other (i.e., an invalid influenza A virus result does not guarantee an invalid influenza B virus result, and vice versa). Over the course of the study, there were invalid results obtained for 6 specimens. One specimen was invalid for both influenza A and influenza B viruses. The other 5 generated an invalid result for either influenza A or influenza B viruses, but not both, resulting in invalid rates of 1.3% for influenza A virus (3/236) and 1.7% for influenza B virus (4/236). Importantly, in half of the specimens that yielded an invalid result, the true result, compared with both RT-PCR and viral culture, was confirmed. The sensitivity for the detection of influenza A virus when testing MTS specimens was 96.1%, while for NPW/A specimens the sensitivity was 92.3%, resulting in an overall sensitivity of 93.3% compared to viral culture. Specificities were 93.7% and 92.2% for MTS and NPW/A, respectively, for an overall specificity of 94.5% in detection of influenza A virus. As described below and shown in Table 2, more than 50% of the FP results (compared with culture) affecting the specificity were confirmed as TP by real-time RT-PCR, resulting in a higher true specificity. For influenza B virus detection, both sensitivity and specificity were 100% for all specimen types in comparison to viral culture.

When using real-time RT-PCR as the comparator method, detection of influenza A virus by use of the Alere i Influenza A&B assay resulted in sensitivity of 89.4% for MTS specimens and 87.1% for NPW/A specimens, for a combined sensitivity of 88.8% for all specimens combined. The corresponding specificities were 98.6% for MTS, 97.8% for NPW/A, and 98.3% overall. For the detection of influenza B virus, again, sensitivity and specificity were 100% for both MTS and NPW/A specimens. For those influenza A virus specimens that were Alere i Influenza A&B test positive ($n = 103$), the median threshold cycle (C_T) value was 24.1, with a range of 19.4 to 30.4 (Fig. 1). The interquartile ranges (IQR) were as follows: first IQR (Q1), 22.05; Q3, 26.25. For the influenza A virus specimens that were negative by the Alere i Influenza A&B test ($n = 13$), the median C_T value was 32.7, with a range of 29.4 to 35.9. The IQR were as follows: Q1, 31.2; Q3, 33.5. The difference in C_T values between the groups was significant ($P < 0.001$).

TABLE 2 Performance of the Alere i Influenza A&B assay versus real-time RT-PCR

Virus	Specimen source	TP	FP	TN	FN	Total no. of samples	% sensitivity (95% CI)[a]	% specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Influenza A virus	MTS	76	1	70	9	156	89.4 (80.4–94.7)	98.6 (91.3–99.9)	98.7 (92.0–99.9)	88.6 (79.0–94.3)
	NPW/A	27	1	45	4	77	87.1 (69.2–95.8)	97.8 (87.0–99.9)	96.4 (79.8–99.8)	91.8 (79.5–97.4)
	Total	103	2	115	13	233	88.8 (81.3–93.7)	98.3 (93.3–99.7)	98.1 (92.6–99.7)	89.8 (82.9–94.3)
Influenza B virus	MTS	31		124		155	100 (86.3–100)	100 (96.3–100)	100 (86.3–100)	100 (96.3–100)
	NPW/A	27		50		77	100 (84.5–100)	100 (91.1–100)	100 (84.5–100)	100 (91.1–100)
	Total	58		174		232	100 (92.3–100)	100 (97.3–100)	100 (92.3–100)	100 (97.3–100)

[a] CI, confidence interval.

DISCUSSION

One of the keys to managing outbreaks of influenza during any respiratory disease season is accurate and rapid diagnosis. Unfortunately, antigen-based rapid tests are significantly lacking in sensitivity compared with molecular-based assays, such as real-time PCR (21–23). Additionally, traditional rapid antigen assays depend on user interpretation, which may vary from operator to operator. In this study, we evaluated the Alere i Influenza A&B assay, an isothermal molecular test for the qualitative detection of influenza A and B viruses. We found, using viral culture as the gold standard, that the overall sensitivity of the Alere i Influenza A&B assay was 93.3% for the detection of influenza A virus and 100% for the detection of influenza B virus. The specificities were 94.5% and 100% for influenza A and B viruses, respectively. In comparison to PCR, the overall sensitivity of the Alere i Influenza A&B assay for the detection of influenza A virus was 88.8% and 100% for detecting influenza B virus. Specificity was 98.3% for influenza A virus and 100% for influenza B virus. The PCR cutoff for the detection of influenza A and B viruses for specimens collected in 3 ml of VTM and analyzed via the Prodesse ProFlu$^+$ assay was determined to be a C_T of ~30. For the Alere i Influenza A&B assay specimens that were negative, the C_T range was 29.4 to 35.9 (IQR: Q1, 31.2; Q3, 33.5). For the Alere i Influenza A&B assay specimens that were positive, the C_T range was 19.1 to 30.4 (Q1, 22.05; Q3, 26.25). It is also interesting that the Alere i Influenza A&B assay performed equally well with both the midturbinate swabs and nasopharyngeal aspirates for both influenza A and influenza B virus detection.

A formal evaluation for detection of a broad spectrum of known influenza virus subtypes was not completed as a part of this study, but comparison of the Alere i Influenza A&B assay results from a subset of specimens previously characterized by use of molecular testing as pH1N1 ($n = 56$) demonstrated a sensitivity of 80% (44/55) (data not shown). One specimen with a historical pH1N1 result was not confirmed in the recent study with the Alere i Influenza A&B assay or the ProFlu$^+$ assay. The performance levels of antigen tests and some molecular tests have been found to be affected by some influenza virus strains, usually the novel and emerging virus types (24, 25). It is possible that the Alere i Influenza A&B assay is affected by novel strains; hence, a focused investigation with clinical specimens containing various influenza virus subtypes will be important.

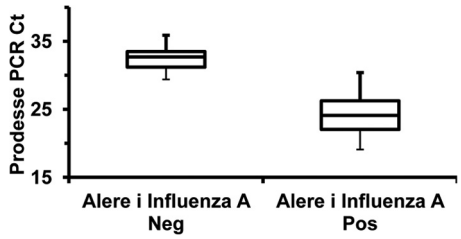

FIG 1 Prodesse ProFlu$^+$ PCR C_T values for specimens positive or negative in the Alere i Influenza A&B assay (116 positive PCR specimens).

Isothermal amplification assays have recently been reported in the literature for detection of several pathogens, including influenza viruses. Multiple variations of this technology, such as loop-mediated isothermal amplification assay (LAMP), have been designed for isothermal conditions. Several reverse transcription LAMP assays developed for detection of emerging highly pathogenic avian influenza virus of the H5N1 strain have recently been reviewed (26). Another LAMP assay for detection of influenza A and B viruses demonstrated a combined sensitivity and specificity of 97.9% and 100% for influenza A and influenza B viruses. This assay had an associated off-board sample preparation step of 10 min, which is near the total assay time for the Alere i Influenza A&B nucleic acid amplification test. Total assay time for this method was 40 min (27). A simple amplification-based assay (SAMBA) duplexed with visual detection of nucleic acid on a dipstick displayed a sensitivity of 100% for detection of both influenza A and B viruses, with specificities of 97.9% for influenza A virus and 100% for influenza B virus, compared with a lab-developed real-time RT-PCR (28). The TAT for the SAMBA was 135 min, making it unsuitable for "STAT" rapid testing. In contrast, the Alere i Influenza A&B assay was designed as a simple and rapid nucleic acid amplification test for influenza virus detection directly from nasal swabs collected and tested in point-of-care (POC) settings. In a recent prospective multicenter clinical study, the clinical performance of the Alere i Influenza A&B assay was evaluated. In comparison with viral cell culture, the overall sensitivity and specificity of the Alere i Influenza A&B assay direct nasal swab testing were 97.8% and 85.6% for the detection of influenza A virus and 91.8% and 96.3% for the detection of influenza B virus, respectively. Following resolution of discrepant results by real-time RT-PCR, the sensitivity and specificity of the Alere i Influenza A&B assay improved to 99.3% and 98.1% for influenza A virus and 97.6% and 100% for influenza B virus, respectively (29).

In this study, we demonstrated that the Alere i Influenza A&B assay works equally well with MTS or NW/A transported in 3 ml of universal transport medium. The sensitivity of this assay seems to be slightly reduced when results are compared with culture when testing direct foam-tipped nasal swabs (97.8%) versus dilution of the MTS and NW/A specimen in 3 ml UTM (93.3%).

In summary, the Alere i Influenza A&B assay requires minimal hands-on time, and results can be obtained within 15 min of specimen collection. The rapid TAT compares very favorably to viral culture (which requires days) and to traditional real-time PCR methods (which require hours) that would require an additional extraction step. The Alere i Influenza A&B assay instrument is relatively small and portable, requires minimal training, and can be ideally placed near patient settings for the most minimal TAT for results. The performance of the Alere i Influenza A&B assay, compared with the gold standard methodologies, such as culture and RT-PCR, coupled with a much quicker TAT from sample collection to result, makes it an excellent choice for POC testing. Additional studies evaluating the performance of the Alere i Influenza A&B assay in direct comparison with other molecular tests in the POC setting and rapid antigen tests are needed to fully understand its potential among the commercially available rapid influenza virus assays for the POC setting.

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REFERENCES

1. **Falsey AR, Cunningham CK, Barker WH, Kouides RW, Yuen JB, Menegus M, Weiner LB, Bonville CA, Betts RF.** 1995. Respiratory syncytial virus and influenza A infections in the hospitalized elderly. J. Infect. Dis. **172:**389–394. http://dx.doi.org/10.1093/infdis/172.2.389.
2. **Neuzil KM, Wright PF, Mitchel EF, Jr, Griffin MR.** 2000. The burden of influenza illness in children with asthma and other chronic medical conditions. J. Pediatr. **137:**856–864. http://dx.doi.org/10.1067/mpd.2000.110445.
3. **Pitman RJ, Melegaro A, Gelb D, Siddiqui MR, Gay NJ, Edmunds WJ.** 2007. Assessing the burden of influenza and other respiratory infections in England and Wales. J. Infect. **54:**530–538. http://dx.doi.org/10.1016/j.jinf.2006.09.017.
4. **Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, Fukuda K.** 2003. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA **289:**179–186. http://dx.doi.org/10.1001/jama.289.2.179.
5. **Welliver RC.** 2003. Respiratory syncytial virus and other respiratory viruses. Pediatr. Infect. Dis. J. **22:**S6–S10. http://dx.doi.org/10.1097/01.inf.0000053880.92496.db.
6. **Molinari NA, Ortega-Sanchez IR, Messonnier ML, Thompson WW, Wortley PM, Weintraub E, Bridges CB.** 2007. The annual impact of seasonal influenza in the US: measuring disease burden and costs. Vaccine **25:**5086–5096. http://dx.doi.org/10.1016/j.vaccine.2007.03.046.
7. **Moscona A.** 2005. Neuraminidase inhibitors for influenza. N. Engl. J. Med. **353:**1363–1373. http://dx.doi.org/10.1056/NEJMra050740.
8. **Gardner PS.** 1970. Rapid diagnostic techniques in clinical virology. Mod. Trends Med. Virol. **2:**15–50.
9. **Borek AP, Clemens SH, Gaskins VK, Aird DZ, Valsamakis A.** 2006. Respiratory syncytial virus detection by Remel Xpect, Binax Now RSV, direct immunofluorescent staining, and tissue culture. J. Clin. Microbiol. **44:**1105–1107. http://dx.doi.org/10.1128/JCM.44.3.1105-1107.2006.
10. **Ohm-Smith MJ, Nassos PS, Haller BL.** 2004. Evaluation of the Binax NOW, BD Directigen, and BD Directigen EZ assays for detection of respiratory syncytial virus. J. Clin. Microbiol. **42:**2996–2999. http://dx.doi.org/10.1128/JCM.42.7.2996-2999.2004.
11. **Ribes JA, Seabolt JP, Overman SB.** 2002. Performance characteristics of VIDAS and Directigen respiratory syncytial virus (RSV) antigen detection assays and culture for the identification of RSV in respiratory specimens. J. Clin. Microbiol. **40:**1818–1820. http://dx.doi.org/10.1128/JCM.40.5.1818-1820.2002.
12. **van Elden LJ, van Loon AM, van der Beek A, Hendriksen KA, Hoepelman AI, van Kraaij MG, Schipper P, Nijhuis M.** 2003. Applicability of a real-time quantitative PCR assay for diagnosis of respiratory syncytial virus infection in immunocompromised adults. J. Clin. Microbiol. **41:**4378–4381. http://dx.doi.org/10.1128/JCM.41.9.4378-4381.2003.
13. **Weinberg GA, Erdman DD, Edwards KM, Hall CB, Walker FJ, Griffin MR, Schwartz B.** 2004. Superiority of reverse-transcription polymerase chain reaction to conventional viral culture in the diagnosis of acute respiratory tract infections in children. J. Infect. Dis. **189:**706–710. http://dx.doi.org/10.1086/381456.
14. **Storch GA.** 2003. Rapid diagnostic tests for influenza. Curr. Opin. Pediatr. **15:**77–84. http://dx.doi.org/10.1097/00008480-200302000-00013.
15. **Gavin PJ, Thomson RBJ.** 2003. Review of rapid diagnostic tests for influenza. Clin. Appl. Immunol. Rev. **4:**151–172.
16. **Leland DS, Ginocchio CC.** 2007. Role of cell culture for virus detection in the age of technology. Clin. Microbiol. Rev. **20:**49–78. http://dx.doi.org/10.1128/CMR.00002-06.
17. **Mahony JB.** 2008. Detection of respiratory viruses by molecular methods. Clin. Microbiol. Rev. **21:**716–747. http://dx.doi.org/10.1128/CMR.00037-07.
18. **Zavattoni M, Percivalle E, Cattaneo E, Revello MG, Torsellini M, Gerna G.** 2003. Optimized detection of respiratory viruses in nasopharyngeal secretions. New Microbiol. **26:**133–140.
19. **Peaper DR, Landry ML.** 2014. Rapid diagnosis of influenza: state of the art. Clin. Lab Med. **34:**365–385. http://dx.doi.org/10.1016/j.cll.2014.02.009.
20. **Clopper CJ, Pearson ES.** 1934. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika **26:**404–413. http://dx.doi.org/10.1093/biomet/26.4.404.
21. **Miernyk K, Bulkow L, DeByle C, Chikoyak L, Hummel KB, Hennessy T, Singleton R.** 2011. Performance of a rapid antigen test (Binax NOW® RSV) for diagnosis of respiratory syncytial virus compared with real-time polymerase chain reaction in a pediatric population. J. Clin. Virol. **50:**240–243. http://dx.doi.org/10.1016/j.jcv.2010.11.011.
22. **Aslanzadeh J, Zheng X, Li H, Tetreault J, Ratkiewicz I, Meng S, Hamilton P, Tang YW.** 2008. Prospective evaluation of rapid antigen tests for diagnosis of respiratory syncytial virus and human metapneumovirus infection. J. Clin. Microbiol. **46:**1682–1685. http://dx.doi.org/10.1128/JCM.00008-08.
23. **Gröndahl B, Puppe W, Weigl J, Schmitt HJ.** 2005. Comparison of the BD Directigen Flu A+B kit and the Abbott TestPack RSV with a multiplex RT-PCR ELISA for rapid detection of influenza viruses and respiratory syncytial virus. Clin. Microbiol. Infect. **11:**848–850. http://dx.doi.org/10.1111/j.1469-0691.2005.01223.x.
24. **Ginocchio CC, Zhang F, Manji R, Arora S, Bornfreund M, Falk L, Lotlikar M, Kowerska M, Becker G, Korologos D, de Geronimo M, Crawford JM.** 2009. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J. Clin. Virol. **45:**191–195. http://dx.doi.org/10.1016/j.jcv.2009.06.005.
25. **Centers for Disease Control and Prevention.** 2009. Performance of rapid influenza diagnostic tests during two school outbreaks of 2009 pandemic influenza A (H1N1) virus infection—Connecticut, 2009. MMWR Morb. Mortal. Wkly. Rep. **58:**1029–1032.
26. **Sakurai A, Shibasaki F.** 2012. Updated values for molecular diagnosis for highly pathogenic avian influenza virus. Viruses **4:**1235–1257. http://dx.doi.org/10.3390/v4081235.
27. **Mahony J, Chong S, Bulir D, Ruyter A, Mwawasi K, Waltho D.** 2013. Multiplex loop-mediated isothermal amplification (M-LAMP) assay for the detection of influenza A/H1, A/H3 and influenza B can provide a specimen-to-result diagnosis in 40 min with single genome copy sensitivity. J. Clin. Virol. **58:**127–131. http://dx.doi.org/10.1016/j.jcv.2013.06.006.
28. **Wu LT, Thomas I, Curran MD, Ellis JS, Parmar S, Goel N, Sharma PI, Allain JP, Lee HH.** 2013. Duplex molecular assay intended for point-of-care diagnosis of influenza A/B virus infection. J. Clin. Microbiol. **51:**3031–3038. http://dx.doi.org/10.1128/JCM.00740-13.
29. **Bell J, Bonner A, Cohen DM, Birkhahn R, Yogev R, Triner W, Cohen J, Palavecino E, Selvarangan R.** 2014. Clinical evaluation of the Alere i influenza A&B assay, a point-of-care rapid nucleic acid amplification test for detection of influenza. J. Clin. Virol. **61:**81–86. http://dx.doi.org/10.1016/j.jcv.2014.06.001.