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Profile of the Alere i Influenza A & B assay: a pioneering molecular point-of-care test

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Abstract

Introduction: The Alere i Influenza A & B assay incorporates the Nicking Enzyme Amplification Reaction technique on the Alere i instrument to detect and differentiate influenza virus (Flu) A and B nucleic acids in specific specimens.

Areas Covered: The Alere i Influenza A & B assay was cleared by the US Food and Drug Administration for use with nasal swabs (NS) and nasopharyngeal swabs, either directly or in viral transport medium. Notably, direct use on NS was the first ever CLIA-waived nucleic acid-based test. Previously published evaluations have reported sensitivities and specificities of 55.2–100% and 62.5–100% for Flu A and 45.2–100% and 53.6–100% for Flu B, respectively.

Expert Commentary: The Alere i Influenza A & B assay provides a rapid and simple platform for detection and differentiation of Flu A and B. Efforts are expected to further improve sensitivity and user-friendliness for effective and widespread use in the true point-of-care setting.

Keywords

Influenza viruses A and B; isothermal nucleic acid amplification technology; nasal swabs; nasopharyngeal swabs; nicking enzyme amplification reaction; rapid molecular diagnosis

1. Introduction

Influenza is a highly contagious, viral respiratory illness caused by influenza viruses (Flu) A and B. It is transmitted through coughing, sneezing or touching contaminated surfaces and circulates in all parts of the world. Clinical signs and symptoms range from mild to deadly.

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Declaration of interest

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Severe complications include viral pneumonia, secondary bacterial pneumonia, sinus infections and worsening of underlying diseases, especially in immunocompromised patients, pregnant women, children aged 6~59 months and the elderly [1,2]. Commercially available influenza antivirals have been demonstrated to reduce symptoms, risk of complications and severe outcomes in hospitalized patients [3]. Most antiviral drugs are effective in treating influenza if given within 24 to 48 hours of onset of symptoms [4,5], appropriate treatment and accurate and real-time influenza surveillance and infection control of influenza depend on an accurate and timely diagnosis [6,7].

Much effort has been expended to develop accurate and rapid laboratory methods to detect and characterize influenza viruses for clinical management and infection control. Although several tests are commercially available to detect and differentiate Flu A and B, they have different sensitivities, specificities, turnaround times and costs that play a crucial role in the selection of a diagnostic test for clinics [8–10]. After the onset of flu, the virus can be detected in nasopharyngeal swabs (NPS), NP aspirate or wash and nasal swabs (NS), but the sensitivity and specificity might vary by the type of laboratory facility and technique available, the type of specimen tested and the time between onset of illness and specimen collection. There are numerous commercial assays available, including direct antigen detection [11,12], viral culture [13], serology [14], and molecular assays [8–10,15] (Table 1).

While cell culture remains the gold standard for detection and characterization of Flu A and Flu B, molecular methods have gradually become the mainstay in daily clinical virology laboratory services due to their rapidity and accuracy [7–10,15]. However, classical molecular methods incorporating PCR are laborious, technically demanding and do not provide a quick turnaround time from sample collection to results in outpatient clinics and emergency departments [10,16,17]. Non-PCR nucleic acid amplification-based techniques such as transcription-mediated amplification [18], nucleic acid sequence based amplification [19], single primer isothermal amplification [20], helicase-dependent amplification [21], and loop mediated isothermal amplification [22], have all been developed for detection of Flu. Use of a rapid and accurate diagnostic method for Flu such as these can enhance clinical management of influenza patients, prevent nosocomial outbreaks and help reduce the overuse of antibiotics [23,24].

The Alere i Influenza A & B assay uses an isothermal amplification platform to detect and differentiate Flu A and Flu B in approximately 15 minutes [13,25–27]. The assay was launched in 2014, received US Food and Drug Administration (FDA) clearance in 2014 and was granted Clinical Laboratory Improvement Amendments (CLIA) waiver (for NS only) on January 2015 as the first CLIA-waived point-of-care test (POCT) in the molecular microbiology field. An updated version, Alere i Influenza A & B 2 assay, was cleared in 2017 with claimed improved sensitivity and shortened turnaround time (https://www.accessdata.fda.gov/cdrh_docs/pdf17/K171792.pdf).

2. General Principles and Methods

The Alere i Influenza A & B assay, which is performed on the Alere i instrument, incorporates the Nicking Enzyme Amplification Reaction (NEAR) technique to detect and differentiate influenza viruses A and B [27,28]. Based on the NEAR technique, the templates are designed to target the Flu A-specific PB2 segment and the Flu B-specific PA segment. Fluorescently-labeled molecular beacons are used to identify each of the specifically amplified RNA targets. As presented in Figure 1, the recognition region of the template (T2) binds to the complementary target region and is extended by the polymerase along the target and then another identical template binds to the same target, displacing the first template as it is extended (1a-3a). A different template (T1) binds to the complementary target region, is extended and creates a double-stranded nicking site, which is then cut by a Nicking Enzyme (4a-6a). Repeated nicking, polymerization and strand displacement result in the rapid synthesis of amplification products (B). Due to the simplicity of the reaction, it is easy to replicate DNA at a constant temperature ranging from 55°C to 59°C and 20-mer to 30-mer products can be amplified 10^8 to 10^{10} -fold from genomic DNA in 2.5 to 10 minutes. Moreover, the method is able to amplify RNA without a separate reverse transcription step. Molecular beacons are coupled to detect the fluorescence signals that are optically filtered by a confocal lens and mirror arrangement. The results are calculated automatically and available on the user interface or can be exported for printing.

There are three parts to the Alere i instrument, including the Transfer Cartridge, Sample Receiver and Test Base. The Alere i Influenza A & B kit contains all of the components required to carry out an assay for Flu A and Flu B as well as Positive and Negative Control Swabs for monitoring. The sample is added to the Sample Receiver and transferred to the Test Base via the Transfer Cartridge. Heating, mixing, initiating target amplification and detection are performed in the instrument and the results will be reported automatically in 15 minutes (Figure 1). The current protocol requires the operator to stay for the 5-minute heating time otherwise the instrument will time out with no result reported if the 10-minute window is missed. Visibly bloody samples must not be used with Alere i Influenza A & B test as human genomic DNA (gDNA) inhibits the NEAR technology. NS and NPS specimens should be tested as soon as possible although the specimens can be stored in refrigerator for up to 24 hours after collection. If immediate testing cannot be performed, most viral or universal transport media (except brain-heart infusion broth) are acceptable for use in the Alere i assay; however, the CLIA complexity of the test is considered moderate if performed from transport media. Ability to accept specimens in transport media provides clinicians and laboratorians with more flexibility for viral testing, but dilution in transport media may decrease the test sensitivity [13,25,27,29].

3. Analytical Performance

3.1. Analytical sensitivity

The limits of detection (LODs) for the Alere i Influenza A & B assay were determined by evaluating limiting dilutions of three titrated strains of Flu A (A/H1N1, A/H3N2 seasonal and A/H1N1 pandemic) and two titrated strains of Flu B virus. The dilutions of each viral strain was tested for detection by Alere i Influenza A & B in both swabs and in VTm. The

LOD was determined to be the lowest virus concentration that was detected 95% of the time. The LOD for the three strains of Flu A were 10^2 to 10^5 TCID₅₀/mL, while those for the two strains of Flu B were 10^2 to 10^3 TCID₅₀/mL, which is higher than that of the cobas Liat Influenza A/B Assay (10^{-3} to 10^{-1} TCID₅₀/mL) (Table 2). The analysis revealed that the Alere i Influenza A&B assay LODs using simulated nasal swab matrix (i.e., Universal Transport Medium, UTM) were similar to those using natural nasal swab matrix (https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163266.pdf, https://www.accessdata.fda.gov/cdrh_docs/pdf17/K171792.pdf).

3.2. Analytical reactivity

An analytical reactivity study evaluated the ability of the Alere i Influenza A&B assay to detect Flu strains representing temporal and geographical diversity. The Alere i Influenza A&B assay was evaluated for reactivity with 17 Flu A strains and 9 Flu B strains, including: 1 in the H7N9 group, 4 in the H1N1 group, 3 in the pH1N1 group, 8 in the H3N2 seasonal group, 4 in the Victoria lineage and 5 in the Yamagata lineage. The Alere i Influenza A&B assay detected all strains tested and had excellent performance for Flu B detection, but the performance characteristics of this device with clinical specimens that are positive for the Flu A/H1N1, A/H7N9 and A/H3N2v viruses have not been published in recent years (https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163266.pdf, https://www.accessdata.fda.gov/cdrh_docs/pdf17/K171792.pdf).

3.3. Analytical specificity

In order to demonstrate the absence of cross-reactivity with other common human respiratory pathogens, the Alere i Influenza A & B assay was tested with 53 microorganisms including 37 bacteria (*Acinetobacter calcoaceticus*, *Bacteroides fragilis*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Corynebacterium diphtheria*, *Enterococcus faecalis*, *Escherichia coli*, *Gardnerella vaginalis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Lactobacillus casei*, *L. plantarum*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *M. intracellulare*, *M. tuberculosis*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *N. meningitidis*, *N. sicca*, *N. subflava*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus* Group A, B, C, F, and G, *S. mutans*, *S. pneumoniae*, *S. salivaris*, and *S. sanguis*), 15 viruses (adenovirus 1 and 7, cytomegalovirus, coronavirus 229E, OC43, enterovirus/Coxsackievirus B4, Epstein-Barr virus, human metapneumovirus, parainfluenza virus 1, 2, 3, measles, mumps, respiratory syncytial virus, rhinovirus) and 1 yeast (*Candida albicans*). The assay reported negative results at concentrations ranging from 10^8 to 10^{10} cells/ml (bacteria) or 10^4 to 10^8 TCID₅₀/ml (virus) in triplicate, and no cross-reactivity was found with these microorganisms (https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163266.pdf, https://www.accessdata.fda.gov/cdrh_docs/pdf17/K171792.pdf).

Substances that are naturally present in respiratory specimens or swabs (e.g., mucin, whole blood, chemical material, etc.) were evaluated at certain concentrations and were found to have no effect on the test performance with the exception of the live nasal influenza vaccine (FluMist), which may test positive for up to three days after vaccination. Patients who receive the FluMist vaccine may have false-positive results for many influenza assays, such

as rapid antigen, PCR and culture for three or more days after vaccination. The assay cross-reactivity does not occur with the inactivated injectable Flu vaccine.

4. Clinical Validation

A multi-center clinical trial was performed to assess the clinical performance characteristics of the Alere i Influenza A&B assay at a total of eight sites during the flu season between the years of 2012–2013. Patients with flu-like symptoms were enrolled and direct nasal swabs were collected at these investigational sites. All samples were tested using the Alere i instrument with viral culture as the comparator. Discrepant results between Alere assay and culture were resolved using an FDA-cleared RT-PCR assay. The sensitivity and specificity were 97.9% (95% CI: 92.6%–99.4%) and 86.2% (95% CI: 82.8%–89.0%), respectively, for detection of Flu A virus and 92.5% (95% CI: 84.6%–96.5%) and 96.5% (95% CI: 94.5%–97.8%), respectively, for detection of Flu B virus [13] (https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163266.pdf, https://www.accessdata.fda.gov/cdrh_docs/pdf17/K171792.pdf).

Several clinical evaluation studies on the Alere i Influenza A & B test have been published. The Alere assay showed higher sensitivity and specificity in children than in adults [23,25]. The Alere i Influenza A & B test is intended to be used for both direct NS specimens (CLIA waived) and for NS and NPS eluted in VTM (CLIA moderate complexity). Bell et al used the direct NS samples and NS in VTM and demonstrated similar sensitivity for detection of influenza A and influenza B, 97.8% and 91.8%, respectively, but lower specificity (85.6% and 96.3%, respectively) [13,25]. Sensitivities were reported lower when VTM specimens were used [24,26,27,29–36]. The Alere assay showed sensitivities of 87.2%, 92.5%, and 97.4% for Flu A H1, H3, and Flu B, respectively, but only 25.0% for untypable Flu A. The assay did show poorer sensitivity with samples determined to have low Flu A titers determined by higher Ct values [27]. Nolte et al [29] showed the sensitivities of the Alere i Influenza A & B test were 71.3% for Flu A and 93.3% for Flu B, with specificities of 100% for both viruses. In a study by Young et al on adult NPS in VTM, the sensitivity and specificity of the Alere i Influenza A & B assay for the detection of influenza A and B was 63.8% (95% CI: 49.5%, 76.0%) and 97.5% (95% CI: 87.1%, 99.6%), respectively, which were significantly lower than the cobas Liat Influenza A/B assay [36]. A recent meta-analysis showed that the overall pooled sensitivity of the Alere was only about 85% for Flu A and B, which was significantly lower than the cobas Liat assay [7]. The poor sensitivity of the Alere i test for detection of influenza A virus was likely due to a study set that further diluted in VTM and went through multiple freeze and thaw cycles [27,29]. Studies using different off-label specimen types such as throat swabs (TS) and saliva have been reported [37,38].

The Reproducibility Study site-to-site qualitative results showed 100% (95% CI: 95.9%–100%) agreement for the Flu A Moderate Positive (MP, 2–3 X the respective LOD), Low Positive (LP, 1 X the respective LOD) and True Negative (TN), 70% (95% CI: 59.9%–78.5%) for High Negative (HN, less than the expected LOD) category; 100% (95% CI: 95.9%–100%) for the Flu B MP and TN, 92.2% (95% CI: 84.8%–96.2%) and 90.0% (95% CI: 82.1%–94.6%) for LP and HN categories, respectively (https://www.accessdata.fda.gov/cdrh_docs/pdf17/K171792.pdf).

www.accessdata.fda.gov/cdrh_docs/pdf16/K163266.pdf, https://www.accessdata.fda.gov/cdrh_docs/pdf17/K171792.pdf). Excellent reproducibility was revealed in several clinical studies [25,27,34,35].

5. Interpretation and Precautions

The Alere i Influenza A&B is a qualitative test used only in conjunction with the Alere i instrument for in vitro diagnosis. The performance characteristics may vary according to the specimen types, circulating virus genotypes/subtypes, proper sample collection, sample storage and transport. Influenza A/H3, A/H1N1 and B are the major types and subtypes covered by the instrument. The performance characteristics of this device for emerging influenza A subtypes have not been determined.

False negative results may occur if a specimen is improperly handled or if inadequate levels of virus are present in the specimen. Positive and negative predictive values may vary depending on virus prevalence and the patient population tested. Negative results reported by Alere i do not need to be confirmed, but negative results should not be used as the sole basis for flu diagnosis and treatment. Results from this assay must be correlated with the clinical symptoms, epidemiological data and other tests available to the clinician evaluating the patient. Detection of the target nucleic acid regions does not imply the influenza virus is infectious or the pathogen causing clinical symptoms.

6. Conclusions

The Alere i Influenza A & B assay is a type of rapid molecular diagnostic test that incorporates the NEAR technology to detect and differentiate influenza viruses A and B. It combines speed, accuracy and ease of use. Rapid and accurate detection and identification of Flu A and Flu B can strengthen influenza surveillance, increase laboratory capacity for the diagnosis of influenza.

7. Expert Commentary

The Alere i Influenza A & B test was FDA-cleared for use with NS directly and NS/NPS in VTM, CLIA-waived for use with NS directly only and CLIA moderate for swabs in VTM. The sensitivity of current version of Alere i Influenza A & B is lower than that of other molecular assays (e.g., Liat) [29,36] especially when VTM specimens are used.

The accuracy of an influenza diagnostic test is determined by the sensitivity and specificity of the test and the influenza prevalence in the community. Currently available assays include: rapid influenza antigen detection tests, direct immunofluorescent assays and nucleic acid amplification tests (NAATs) [7,8,10–12]. Rapid antigen tests can produce results in just 15–30 minutes and are simple to perform; however, false negative results are a major concern with these tests. Immunofluorescent tests are inexpensive and provide fast results but are not as sensitive as NAATs and require skilled laboratory staff. NAATs, such as PCR, are sensitive with turnaround times of several hours. Understanding the performance characteristics of different methods can help guide decisions for clinicians and medical institutions to select an appropriate test across different patient populations. Fast and

accurate tests help clinical practices make therapeutically optimal and cost-effective decisions for prompt administration of antiviral therapy, infection control and surveillance, further diagnostic testing and antibiotic therapy, as appropriate.

The Alere i Influenza A & B assay is suitable for POCT due to its speed and accuracy, with a rapid turnaround time within 15 min, and one or two instruments can satisfy the routine service needs of most healthcare settings for outpatients. But during influenza outbreaks or epidemics, a high throughput solution that can handle large batches of samples at a time is required. Additionally, both Flu A and B and respiratory syncytial virus (RSV) are among the leading causes of respiratory illness during the respiratory virus season and are responsible for nosocomial transmission in the healthcare setting [42,43]. To design and implement a combo device to simultaneously detect and differentiate Flu A, B, and RSV allows for prompt patient care including efficient antiviral treatment as well as effective patient cohorting and isolation which, in turn, reduces the use of antibiotics and lowers the cost burden [42–44].

8. Five-Year View

The Alere i Influenza A & B test was the first FDA-cleared, CLIA-waived molecular device which was designed for POCT. It has the ability to report a positive result in as little as 15 minutes and provides greater convenience in reagent storage. The platform menu has been extended to include test cartridges for the detection of Group A *Streptococcus* (Alere i Strep A) [30,39] and respiratory syncytial virus (Alere i RSV) [40,41]. This menu expansion will continue over the next five years and beyond. Efforts are expected to be spent on improving sensitivity and specificity, especially for specimens diluted in VTM. Operating procedures are expected to be optimized to make the platform even simpler and more user-friendly.

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Key Issues

- The Alere i Influenza A & B test is the first FDA-cleared POCT molecular assay which provides detection and differentiation of Flu A and Flu B within 15 minutes.
- It was FDA-cleared for use with both swabs directly and swabs in VTM.
- CLIA-waived status was granted on direct swab use only.
- Test sensitivities are lower when VTM specimens were used, which was probably due to the further specimen dilution in VTM.
- In addition to Flu A and Flu B, the system menu has been expanded to test for other pathogens including Group A *Streptococcus* and RSV.

Figure 1. Procedure and the mechanism of the Alere i Influenza A&B test system.
Modified and reused from Nie et al [27].

Table 1.**Influenza Virus Testing Methods for Respiratory Specimens**

Method	Characteristics	Test time	Application	Reference
Antigen EIA	Rapid, poor sensitivity, some are CLIA-waived	<30 min	Diagnosis (detection)	[12]
Antigen IFA	Good sensitivity and specificity, subjective interpretation	1 to 4 h	Diagnosis (detection)	[11]
Tube and shell vial culture	Good standard, time consuming	1 to 7 days	Diagnosis (detection, differentiation, typing and characterization) and research	[13]
Serology	Retrospective, cross-reaction	2 to 8 h	Epidemiology and research	[14]
NAAT, monoplex	High sensitivity and specificity, potential quantification	1 to 8 h	Diagnosis (detection, differentiation, and limited typing) and research	[25,27]
NAAT, multiplex	High sensitivity and specificity, covering other pathogens, FilmArray RP EZ is CLIA-waived	1 to 8 hr	Diagnosis (detection, differentiation, and limited typing) and research	[16,45]
NAAT, POCT	Rapid, good sensitivity and specificity, some are CLIA-waived (see Table 2)	15–30 min	Diagnosis (detection and limited differentiation) and research	[27,29,31]

Table 2.

Overview of FDA-cleared Flu molecular assays

Products	Amplification principles	Acceptable specimens	Test time	Remarks
Alere i Influenza A & B	NEAR	NS/NPS (Direct), NS/NPS (in VTM)	15 min	CLIA-waived for direct swabs and moderate for swabs in VTM; integrated
Cepheid Xpert Flu, Flu/RSV XC and Flu/RSV Xpress	Real-time PCR	NPS (in VTM), nasal aspirates, and nasal washes	45 min – 1 h	CLIA-moderate complexity; combo kits cover RSV; integrated
Roche cobas Influenza A/B and Flu/RSV	Real-time PCR	NPS	20 min	CLIA-waived; combo kits cover RSV; integrated
Hologic Panther Fusion Flu A/B/RSV	Real-time PCR	NPS	3 h	CLIA-high complexity; cover RSV as well
GenMark eSensor and ePlex RVP	Multiplex PCR	NPS (in VTM)	8 h/High	CLIA-high complexity; multiplex panels
BioFire FilmArray RP, RP EZ, RP2	Multiplex and nested PCR	NPS (in VTM)	45 min – 1 h	CLIA-waived for EZ and moderate for the rests; multiplex panels
Focus Simplexa Flu A/B & RSV (Direct)	Multiplex PCR	NPS (in VTM)	1 – 4 h	CLIA-moderate (Direct) to high (original) complexity;
Quidel Influenza A+B, Lyra, Solana	Real-time PCR (Lyra); HDA (Solana)	NPS and NS (in VTM)	4 – 6 h	CLIA-moderate complexity
Nanosphere Verigene RV, RV+, and RP <i>Flex</i>	Multiplex PCR	NPS (in VTM)	4 h	CLIA-moderate complexity
Luminex x-TAG RVP, RVP Fast, and NxTAG RP	Multiplex PCR	NPS (in VTM)	6 – 8 h	CLIA-high complexity
Luminex Aries Flu A/B & RSV	Real-time PCR	NPS (in VTM)	6 h	CLIA-moderate complexity; combo kits cover RSV; integrated

Abbreviations: POCT, point of care testing; NEAR, nicking endonuclease amplification reaction; NS, nasal swabs; NPS, nasopharyngeal swabs; VTM, viral transport medium; CLIA, clinical laboratory improvement act; RSV, respiratory syncytial virus.

Table 3.

Summary of Alere i Influenza A & B clinical validation studies

Authors	Referee standard	Sample type/size	Sensitivity (%)	Specificity (%)	Remarks
Nie et al, 2014 [27]	FilmArray RP	360 NPS in VTM	Flu A, 73.2; Flu B, 97.4	100.0	Low sensitivity was noticed in FilmArray Flu A+, low Ct, untypable specimens.
Bell et al, 2014 [13]	Culture	545 direct NPS	Flu A, 97.8; Flu B, 91.8	Flu A, 85.6; Flu B, 96.3	Multi-center data for FDA clearance.
Bell and Selvarangan, 2014 [25]	Culture and ProFlu	236 NPS in VTM	Flu A, 88.8–93.3; Flu B, 94.5–98.3	100.0	Relatively good sensitivity for Flu A was indicated.
Chapin et al, 2015 [26]	Xpert Flu A/B assay	291 NPS in UTM	Flu A, 93.8; Flu B, 91.8	Flu A, 62.5; Flu B, 53.6	Validating performance parameters with UTM may be useful to diagnosis of coinfections
Hurtado et al, 2015 [46]	In-house real-time PCR	98 NPS in UTM	Flu A, 93.8%; Flu B, 94.1%	100.0	Five invalid results were revealed
Hazelton et al, 2015 [33]	RT-PCR	202 NPS in UTM	Flu A, 77.8; Flu B, 75.0	Flu A, 100; Flu B, 99.0	PPV for Flu A, 100%; Flu B, 81.8%; NPV for Flu A, 95.4%; Flu B, 98.4%
Jokela et al, 2015 [34]	Xpert Flu A/B and in house real-time RT-PCR	140 NPS in UTM	Flu A, 80.0; Flu B, 45.2	Flu A, 98.1; Flu B, 98.2	PPV for Flu A, 93.3%; Flu B, 87.5%; NPV for Flu A, 93.6%; Flu B, 86.3%
Nguyen Van et al, 2015 [35]	Xpert Flu/RSV	96 NS in VTM	Flu A, 93.8; Flu B, 100	100.0	PPV for Flu A, 100%; Flu B, 100%; NPV for Flu A, 97.0%; Flu B, 100%;
Chiarella et al, 2016 [32]	Real time RT-PCR	119 NPS in VTM	Flu A, 66.0; Flu B, 53.3	Flu A, 98.5; Flu B, 96.0	PPV for Flu A, 96.9%; Flu B, 66.7%; NPV for Flu A, 80.5%; Flu B, 93.1%; five invalid results were revealed
Nolte et al, 2016 [29]	Real-time RT-PCR	126 NPS in VTM, 2 NS, and 1 BAL	Flu A, 71.3; Flu B, 93.8	100.0	The poor sensitivity for Flu A was likely due to a study set that included many low-positive samples
Busson et al, 2017 [23]	Real-time RT-PCR	932 NPS in VTM, NPA, or BAL	Flu A, 91.4; Flu B, 54.5	Flu A, 96.7; Flu B, 96.8	
Davis et al, 2017 [47]	Real-time RT-PCR	589 throat swabs	Flu A, 75; Flu B, 83	Flu A, 98; Flu B, 98	PPV, 81.2%; NPV, 95.6%
Young et al, 2017 [36]	RT-PCR	87 NPS in VTM	Flu A, 55.2; Flu B, 72.2	Flu A, 98.3; Flu B, 97.1	
Chen et al, 2017 [31]	Real-time RT-PCR	22 NPA, 112 NPS in VTM	Flu A, 97.4; Flu B, 81.5	Flu A, 100; Flu B, 99.1	PPV for Flu A, 100%; Flu B, 95.7%; NPV for Flu A, 96.6%; Flu B, 95.5%;
I. Riazzo et al, 2017 [48]	In-house RT-PCR	31 NPA, 3 BAL, 36 NPS in VTM	Flu A, 59.1; Flu B, 83.3	100.0	PPV for Flu A and Flu B, 100%; NPV for Flu A, 84.2%; Flu B, 92.0%;

Abbreviations: POCT, point of care testing; NEAR, nicking endonuclease amplification reaction; NS, nasal swabs; NPS, nasopharyngeal swabs; NPA, nasopharyngeal aspirate; VTM, viral transport medium; UTM, universal transport medium; RT, reverse transcriptase; PPV, positive predictive value; NPV, negative predictive value.

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