Journal of Clinical Virology 94 (2017) 86–90

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Diagnostic accuracy of the real-time PCR **cobas**® Liat® Influenza A/B assay and the Alere i Influenza A & B NEAR isothermal nucleic acid amplification assay for the detection of influenza using adult nasopharyngeal specimens

Stephen Young[a,*], Patrick Illescas[a], Joclin Nicasio[a], Joanna Jackson Sickler[b]

[a] TriCore Reference Laboratories, Albuquerque, NM, USA
[b] Roche Molecular Systems, Pleasanton, CA, USA

ARTICLE INFO

Keywords:
Influenza virus infection
Point-of-care
Real-time polymerase chain reaction
cobas® Liat® Influenza A/B assay
Alere i Influenza A & B assay

ABSTRACT

Background: Accurate detection of influenza requires diagnostic testing; however, methods such as RADTs and central laboratory-based tests are limited by low sensitivity and time constraints, respectively.
Objective: To compare the performances of the **cobas**® Liat® Influenza A/B and Alere™ i Influenza A & B point-of-care (POC) assays for detecting influenza A and B viruses using fresh nasopharyngeal specimens with the GenMark Dx® Respiratory Viral Panel as the reference method, a FDA cleared IVD PCR test.
Study design: A total of 87 samples collected in viral transport medium from adults ≥18 years of age were re-tested on both POC assays (based on the reference PCR method, 29 were influenza A and 18 were influenza B virus positive).
Results: The overall sensitivity and specificity of the cobas Influenza A/B for the detection of influenza A and B relative to reference PCR was 97.9% (95% confidence interval [CI] 88.9%, 99.6%) and 97.5% (95% CI: 87.1%, 99.6%), respectively, while the sensitivity of the Alere i Influenza A & B assay relative to the reference PCR method was 63.8% (95% CI: 49.5%, 76.0%) and the specificity was 97.5% (95% CI: 87.1%, 99.6%). The individual sensitivities and specificities of the cobas Influenza A/B assay for influenza A alone and influenza B alone were comparable to those of the reference PCR method (influenza A: sensitivity of 100% [95% CI: 88.3%, 100.0%] and specificity of 98.3% [95% CI: 90.9%, 99.7%]; influenza B: sensitivity of 94.4% [95% CI: 74.2%, 99.0%] and specificity of 100% [95% CI: 94.7%, 100.0%]). For the Alere i Influenza A & B assay, the individual specificities for influenza A and B were comparable to those of the reference PCR method (98.3% [95% CI: 90.9%, 99.7%] and 97.1% [95% CI: 90.0%, 99.2%], respectively), while the individual sensitivities were low relative to reference PCR (55.2% [95% CI: 37.5%, 71.6%] and 72.2% [95% CI: 49.1%, 87.5%], respectively).
Conclusion: The cobas Influenza A/B assay demonstrated performance equivalent to laboratory-based PCR, and could replace rapid antigen tests.

1. Background

Seasonal influenza is associated with a significant healthcare burden in terms of morbidity and mortality and related costs [1–3]. Outbreaks of epidemic influenza are frequent due to the highly transmissible nature of the virus, leading to a surge in patient visits and consequent overcrowding in emergency departments and healthcare facilities during the winter months. Timely diagnosis of influenza can improve patient management emergency departments, and health care facilities [4,5]. Several of the common signs and symptoms of influenza such as fever, muscle aches, headache, fatigue, dry cough, sore throat, and nasal congestion overlap with those of other bacterial and viral infections, making accurate diagnosis and provision of appropriate treatment difficult based on symptoms alone. Rapid antigen detection tests (RADT) for influenza are fast (10–20 min) and available for use at the point-of-care (POC) but limited by low sensitivity. Consequently, additional testing with more sensitive laboratory-based real-time (RT) PCR tests or viral culture to confirm a negative result is recommended by Center for Disease Control and Prevention [6]. The sensitivity of RADTs is lowest in the adult population because of diminished viral titer and shorter duration of viral shedding compared to young children [7]. The limitation of core laboratory-based methods is time. RT-PCR is increasingly preferred due to the faster turn-around time compared to viral culture, but while some diagnostic systems can provide results in

* Corresponding author at: TriCore Reference Laboratories, 1001 Woodward Place, NE, Albuquerque, NM 87102, USA.
E-mail address: Steve.Young@tricore.org (S. Young).

http://dx.doi.org/10.1016/j.jcv.2017.07.012
Received 2 May 2017; Received in revised form 19 July 2017; Accepted 20 July 2017

approximately one hour [8,9], the time required for specimen transport to central laboratories and the reporting of results significantly increases time-to-result for laboratory-based diagnostic tests to 2–48 h [10]. These results will not be available when patients are initially managed in primary care settings and are often not available when clinicians need to make management decisions in urgent care and emergency departments.

Prompt administration of antivirals for influenza is essential as therapy is most effective when initiated within 48 h of symptom onset; as a result, empiric treatment based on POC RADT results/symptoms is common [11,12]. Empiric antibiotic therapy based on negative RADT results and symptoms is also common [13]. These can lead to incorrect anti-infective treatment triage and engender treatment resistance [13,14].

Recently, two rapid molecular assays, the **cobas**® Influenza A/B nucleic acid test for use on the **cobas**® Liat® system (Roche Molecular Systems, Pleasanton, CA, U.S., subsequently referred to as the cobas Influenza A/B assay) and the Alere™ i Influenza A & B assay (Alere Scarborough, Scarborough, ME, U.S., subsequently referred to as the Alere i Influenza A & B assay), have received Clinical Laboratory Improvement Amendments (CLIA) waivers for use in detecting influenza at the POC from the Food and Drug administration (FDA) [15,16]. The cobas Influenza A/B assay uses RT-PCR technology for nucleic acid amplification and detection and discrimination of influenza A and B viral nucleic acids. The assay is CLIA Waived for use with nasopharyngeal swabs eluted in universal transport media [15]. It provides results in 20 min after ~1 min of time to initiate the assay. The system is fully automated requiring operator intervention only to initiate the run. The Alere i Influenza A & B assay uses nicking enzyme amplification reaction (NEAR) technology for amplification and molecular beacons for the detection and discrimination of influenza A and B viral nucleic acids [16]. The assay is moderately complex for use with nasopharyngeal swabs eluted in several transport media and CLIA Waived for use with nasal swabs directly inserted into the sample receiver. It provides results in 15 min and requires several steps to initiate and run the assay requiring ~6 min of hands-on time [17].

Studies have shown that the cobas Influenza A/B assay showed performance comparable to routinely-used viral culture and RT-PCR methods (sensitivities of 96% to 99.2% and 100% for influenza A and B, respectively, and specificity of 100% for both viruses in comparison to RT-PCR and sensitivities of 97.5 and 96.9% for influenza A and B viruses, respectively, and specificity of 97.9% for both viruses in comparison to viral culture) [18–20]. Additional studies found sensitivities of 95% to 97.5% and specificities of 97.9% to 99% for influenza A and B in comparison to RT-PCR [21,22]. The Alere i Influenza A & B assay demonstrated sensitivity and specificity of 97.8% and 85.6%, respectively, for the detection of influenza A, and 91.8% and 96.3% for the detection of influenza B, respectively, in comparison to viral culture [23]. Another study showed better concordance between the Alere i Influenza A & B assay and viral culture; 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 and 100% and 100% for the detection of influenza B virus, respectively [24]. In comparison to a laboratory-developed RT-PCR method, the Alere i Influenza A & B assay has shown comparable specificity while sensitivity has varied from 59.1% to 93.8% for the detection of influenza A and 45.2% to 100% for the detection of influenza B across studies [24–31]. Nie et al. reported that the Alere i Influenza A & B assay demonstrated lower sensitivity for samples with low influenza A virus titers [27]. To date, a single published head-to-head study for these two POC methods has been conducted, which compared their performances in thawed retrospective nasopharyngeal specimens [17]. The study found that the Alere i Influenza A & B assay had a lower sensitivity compared to the cobas Influenza A/B assay in influenza A samples (71.3% vs 100%) using the FilmArray® respiratory panel (BioFire Diagnostics, Inc., Salt Lake City, UT, U.S.) as the reference method.

2. Objectives

This study compared the performances of the cobas Influenza A/B and Alere i Influenza A & B point-of-care (POC) assays for detecting influenza A and B viruses in a laboratory setting. The results were compared with routine laboratory-based RT-PCR using the GenMark Dx® Respiratory Viral Panel (GenMark Diagnostics, Inc., Carlsbad, CA, U.S., subsequently referred to as the GenMark RVP assay) as the reference method. This is the second study to compare the performances of the cobas Influenza A/B and the Alere i Influenza A & B assays and the first study using prospectively collected fresh specimens focused on the adult population.

3. Study design

The study was conducted at Tricore Reference Laboratories (TRL) Albuquerque, NM, U.S. during the flu season of 2015/16. The GenMark RVP assay was used as the reference method. This study was conducted in compliance with the protocol, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, and applicable U.S. FDA regulations. Institutional Review Board (IRB) approval was obtained from the local regulatory body at the participating site. Testing using the two POC assays was performed according to the manufacturers' FDA-cleared package inserts [15,16], with the Alere i Influenza A & B assay executed in line with the moderately complex indication (nasopharyngeal swabs eluted in transport media). Two operators executed the POC assays: one was a laboratory trained technologist, and the second was not a trained laboratory technologists but had experience with laboratory procedures.

3.1. Clinical specimens

Nasopharyngeal swabs eluted in viral transport media (Remel VTM M6™) collected from patients ≥18 years of age and with a respiratory viral panel order were tested by the lab, and residual sample de-identified by an IRB-approved procedure as required by local regulations to ensure blinding. A total of 89 specimens leftover from routine clinical care were selected based on the routine RT-PCR influenza testing result and appropriate remaining sample volume to conduct both POC nucleic acid amplification tests (NAATs); however, only 87 samples were evaluated as two subjects were below 18 years of age. The results from the reference test were blinded to the operators conducting the POC tests. The samples were stored at 2–8 °C for less than 24 h before testing on the two POC assays in parallel. Repeat testing was performed for one sample with the cobas Influenza A/B assay and two samples with the Alere i Influenza A & B assay due to hardware/software errors that invalidated the initial test results.

3.2. Statistical analysis

All data analyses were performed using SAS/STAT® software (SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary, NC, U.S.). The sensitivity, specificity, and overall percent agreement of the cobas Influenza A/B assay and Alere i Influenza A & B assays with the respective two-sided 95% Clopper-Pearson exact confidence intervals (CIs) were calculated in comparison to the reference laboratory-based RT-PCR method. The paired difference in the sensitivities of the cobas Influenza A/B assay versus RT-PCR and Alere i Influenza A & B assay versus RT-PCR was calculated using samples with positive RT-PCR results at the 5% level of statistical significance. Similarly, the paired difference in the specificities of the two tests was also calculated. McNemar's test was used to assess the statistical difference between the sensitivities and specificities of the two POC tests.