Performance of the Molecular Alere™ i Influenza A&B Test Compared to Xpert® Flu A/B Assay

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Article type: Short Form Paper

Running head: Performance of Alere™ i Rapid Molecular Influenza Test

Key words: Influenza viruses A and B, isothermal nucleic acid amplification reaction, point-of-care testing

Manuscript word count: 1,435 (only text, not counting cover page, abstract, tables, references)

Abstract word count: 75

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Abstract

Performances of rapid molecular point-of-care use platforms for influenza are lacking. We validated nasopharyngeal (NP) flocked specimens in universal transport medium (UTM) and evaluated clinical sensitivity and specificity of Alere™ i Influenza A&B versus Xpert® FluA/B. Alere™ i Influenza A&B had an overall sensitivity and specificity of 93.8% and 62.5% for influenza A, and 91.8% and 53.6% for influenza B, respectively. Poor specificity was due to influenza samples determined positive for both type A and B.
Rapid and accurate diagnoses of influenza prompt necessary infection control, public health notification, tracking, and accurate administration of antiviral therapy. During the pandemic H1N1 outbreak of 2009, performance of rapid antigen detection tests for influenza were shown to be inferior when compared to molecular methods, with sensitivity ranges of 10% to 70%, respectively (1-3). Rapid molecular testing was not available for many hospitals, clinics, and physician offices due to either cost of equipment, reagents, and/or use of complex molecular diagnostics requiring skilled technologists to perform testing, with slow turn-around time to results (2, 4, 5). Recently, the Alere™ i Influenza A&B assay (Alere, Scarborough, ME) became a FDA-cleared molecular test for detection of influenza viruses A and B.

The Alere™ i Influenza A&B (Alere™ i system) is a rapid, semi-automated in vitro diagnostic test for the detection and differentiation of influenza A and influenza B with objective results available in less than 15 minutes. The Alere™ i system incorporates isothermal nucleic acid amplification technology using primers and fluorescent probes specific for amplification of RNA targets for influenza A and B on samples of patients presenting with influenza-like illness (ILI). The test is coupled with an Alere™ i instrument and three test components: Sample Receiver, containing elution buffer, Test Base with two sealed reaction tubes containing a lyophilized pellet containing reagents for amplification of target RNA, and a Transfer Cartridge for transferring the eluted sample to test base. The Alere™ i Influenza A&B is intended for direct nasal swab specimen testing for influenza A and B viral infections in conjunction with clinical and epidemiological risk factors. Similar to the earlier IQuum Liat™ Influenza A/B assay (Roche Diagnostics, Indianapolis, IN), the Alere™ i system was developed to address the unmet clinical need for rapid point-of-care testing of influenza at clinical sites with low test
volume and limited easy to use molecular technology. Performance characteristics of such devices are lacking, as well as comparisons to traditional PCR methods.

The Alere™ i Influenza A&B assay is equipped with sterile foam tipped applicator swabs (Puritan Medical Products LLC, Guilford, ME) for fresh specimen collection, however rayon or flocked nasal swabs have been validated for use by Alere. Specimen transportation and storage has also been validated for media such as saline, VirCell, and Universal Transport Media (UTM) in leak-proof containers with a suggested 0.5 to 3.0 mL dilution range to maximize sensitivity.

The objective of this study was to evaluate the clinical sensitivity and specificity of the Alere™ i Influenza A&B versus PCR results from residual frozen swab specimens eluted in UTM from Rhode Island Hospital (RIH) patients. Evaluation of NP UTM specimens is appropriate as it is a common respiratory sample collection method for many laboratories, as it allows for subsequent testing without the need to recollect from the patient(6, 7).

A total of 291 previously tested respiratory specimens from two influenza seasons, November 2012 to March 2013 and February 2014 to April 2014, were selected to be evaluated on the Alere™ i system. Figure 1 shows the calendar time-line and distribution of influenza A and B isolates tested. Patient specimen distribution was 43% male and 57% female, with 21% pediatric (range 3 weeks to 17 years) and 79% adult patients (18 to 96 years). Per standard of care at RIH, a rapid influenza A/B test request is performed using NP flocked swab in 1 mL of UTM (BD™ Universal Viral Transport Combo Kit, BD, Sparks, MD) from patients presenting with influenza-like illness (ILI) and subsequently tested using the Xpert® Flu A/B assay (Cepheid, Sunnydale, CA). Study labeled remnant UTM respiratory samples stored at -70°C, were thawed at room temperature, and 200 uL were tested on the Alere™ i system. Discrepant specimens were stored at 4°C and tested within a week using the xTAG® RVP (Luminex,
Austin, TX). Cohen’s Kappa coefficient was computed to assess the agreement in results obtained with our laboratories current clinical standard, Xpert® Flu A/B assay, and the Alere™ i system. True positive influenza specimens were those that had agreement between two positive test results. Specimen positive for both influenza A and B, or incorrectly typed by the Alere™ i system that were historically influenza A positive on the Xpert® FluA/B and confirmed influenza A positive by xTAG®, were determined Alere™ i influenza B false positive and vice versa. STATA/SE 12.1 (College Station, TX) was used to calculate sensitivity, specificity, with confidence intervals (CI) set at 95%, and Cohen’s Kappa coefficient. The study was approved by the Lifespan/Rhode Island Hospital Institutional Review Board (IRB).

The Alere™ i system reported 180 influenza A, 45 influenza B, and 15 negative results, after discrepant analysis (Table 2). Thirteen samples were eliminated, 4 invalid Alere™ i results (1.4%, 4/283 invalid rate) due to internal control failure, and 9 resulted indeterminate in more than one molecular assay (limited volume when retested on xTAG® RVP), leaving 278 samples for final analysis. We observed a Kappa coefficient of 0.36 denoting a significant 86.33% agreement between diagnostic methods (p<0.0001). Determination of the 38 discrepant samples is displayed in Table 1. After resolution by xTAG® RVP, 22 of 38 (58%) discrepant samples yielded positive results for influenza but yielded an incorrect type on the Alere™ i system. Of the incorrectly typed, 17 of 22 (77%) were dual positives for influenza A and B. The Alere™ i Influenza A&B had an overall sensitivity and specificity of 93.8% and 62.5% for influenza A, and 91.8% and 53.6% for influenza B, respectively, after discrepant resolution with PCR.

Limitations of this study include use of frozen specimens, selection of positive specimens for the majority of testing, which hampered our ability to infer the true PPV and NPV of this test, and inability to compare directly with another point of care molecular platform, like the IQuum.
Liat™ Influenza A/B test (Roche Diagnostics, Indianapolis, IN). Because previously reported issues with waived influenza tests have been poor sensitivity, and data reported from other investigators with Alere™ i molecular test showed excellent specificity, we focused our testing on the ability to detect positive cases. Previously, our team assessed the performance of the IQuum Liat™ Influenza A/B assay to the xTAG® RVP and Xpert® Flu A/B and results indicated a 91.5% agreement with 54/59 NP samples being concordant with mentioned molecular platforms (KC. Chapin and R. Dickenson, presented at the 30th Annual Clinical Virology Symposium, Daytona Beach, FL, April 2014) (8). Strengths of the study include the use of specimens from two consecutive seasons and influenza type distribution, inclusion of pediatric and adult patients samples, as well as the first report of the Alere™ i system compared to the CLIA moderate complexity Xpert® Flu A/B assay. In comparing both molecular diagnostics, the Alere™ i system requires less technician time, minimal capital equipment outlay, and allows greater flexibility for personnel performing the assay in a point-of-care environment.

This retrospective study evaluating the performance of the Alere™ i Influenza A&B on previously tested influenza NP samples in UTM, showed decreased specificity for influenza A and influenza B when compared to the Xpert® Flu assay. Although proper specimen handling, control testing, and decontamination was followed in our lab, we had a high number of dual positive influenza results from the Alere™ i system, which were not previously reported in other studies using the novel system. The package insert provided prior to FDA approval did not call for a repeat testing of such samples and this subsequently has been added. Nei et al 2014 and Bell and Selvarangan 2014 reported high sensitivity and specificity ranges of 87.2% - 93.3% and 93.3%-100% for influenza A, and 97.4%-100% and 100% for influenza B, respectively. Nei et al
evaluated the Alere™ i system with the FilmArray™ RP (BioFire Diagnostics, Salt Lake City, UT) using frozen NP swabs in viral transport media, using the Prodesse™ ProFlu+™ assay (Gen-Probe, San Diego, CA) for discrepant analysis (9). Bell and Selvarangan reported similar findings comparing the Alere™ i system to viral culture results, with discrepant analysis by the Prodesse™ ProFlu+™ in their pediatric study (10). Validating performance parameters with UTM means continued use of a common collection system currently in place in our laboratory and the availability of additional specimen without having to recollect for subsequent testing, which may be necessary to clarify respiratory diagnoses, such as co-infections or address specific infection control requirements (1, 2, 11). Overall, the Alere™ i Influenza A&B assay provided rapid results in less than 15 minutes with two minutes of hands on time and a high sensitivity for detection of influenza, making it a viable point-of-care molecular diagnostic. The Alere™ i system is currently pending consideration for CLIA waiver:


**Conflicts of interest:** None

**Acknowledgements:** We would like to thank the Rhode Island Hospital Microbiology Laboratory for providing initial PCR data and helping conduct discrepant analysis.

**Funding:** This study was funded in part by Alere Scarborough Inc.
174 References


Figure 1 Distribution of positive influenza cases at RIH for two influenza seasons, November 2012 to March 2013 and February 2014 to April 2014, tested on Xpert® Flu A/B.
Table 1. Breakdown and final resolution of 38 discrepant results between Alere™ i Influenza A&B and Xpert® Flu A/B Assay

<table>
<thead>
<tr>
<th>Influenza Type</th>
<th>(n) detected: Alere vs Xpert</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu A</td>
<td>180 9 15 12</td>
<td>93.8 (89.3 – 96.7)</td>
<td>62.5 (40.6 – 81.2)</td>
</tr>
<tr>
<td>Flu B</td>
<td>45 13 15 4</td>
<td>91.8 (80.4 – 97.7)</td>
<td>53.6 (33.9 – 72.5)</td>
</tr>
</tbody>
</table>

Table 2. Performance of the Alere™ i Influenza A&B nucleic acid amplification test vs Xpert® Flu A/B

<table>
<thead>
<tr>
<th>Alere (38)</th>
<th>Xpert</th>
<th>xTAG</th>
<th>Resolution</th>
<th>Determination of Alere Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu A (3)</td>
<td>Flu B (3)</td>
<td>Flu B (3)</td>
<td>Positive Flu B</td>
<td>(3) FP Flu A</td>
</tr>
<tr>
<td>Flu B (2)</td>
<td>Flu A (2)</td>
<td>Flu A (2)</td>
<td>Positive Flu A</td>
<td>(2) FP Flu B</td>
</tr>
<tr>
<td>Flu B (6)</td>
<td>Flu B (6)</td>
<td>Flu B (6)</td>
<td>Positive Flu B</td>
<td>(6) FP Flu A</td>
</tr>
<tr>
<td>Negative (16)</td>
<td>Flu A (12)</td>
<td>Flu A (12)</td>
<td>Positive Flu A</td>
<td>(12) FN Flu A</td>
</tr>
<tr>
<td></td>
<td>Flu B (4)</td>
<td>Flu B (4)</td>
<td>Positive Flu B</td>
<td>(4) FN Flu B</td>
</tr>
</tbody>
</table>