Alere i Isothermal Amplification

Norman Moore, Ph.D.
Director of Scientific Affairs
norman.moore@alere.com
The old thinking... “it’s just the flu”
A bit of history

There are flu epidemics every 1 to 3 years for at least the last 400 years.

Pandemics (worldwide) occur around every 10 to 20 years.
History

Hippocrates described flu back in the 5th century.

Columbus brought a devastating flu on his second voyage to the new world.

Spanish flu of 1918-1919 was the single greatest epidemic in history.

- 50 to 100 million people were killed (3-6% of the world’s population!)
- Another 500 million were infected (1/3rd of the world’s population)
Estimated average global burden of seasonal influenza

- 600 million cases per year
- 3 million cases of severe illness
- 250,000 – 500,000 deaths

Concern is highest in

- The very young, the elderly
- persons with underlying health conditions
- pregnant women
Burden on Healthcare & Industry

Flu causes 30-50% increase in primary care consultations

2-3 fold increase in hospital admissions during epidemics

10% of all sickness absences from work

Impact in industrialised countries:
- US$10-60 million / million population

Lost productivity:
- US$12 billion / annum in US alone
Influenza A versus Influenza B

**Influenza A**
- More severe disease than B
- Can cause disease in a wide variety of animals

**Influenza B**
- Causes a milder flu, usually in the spring months
Treating Respiratory Diseases in the Emergency Department

Is the pathogen bacterial or viral?  Influenza and pneumonia symptoms can overlap dramatically.

Who do you test?  If it is flu season, do you test for other pathogens?

What do you test them for?  Different age groups are linked to different pathogens.

Can treatment be impacted if the appropriate testing is done?  Stop indiscriminate use broad spectrum antibiotics.
### Misuse of Antibiotics Can Lead to Other Medical Issues

<table>
<thead>
<tr>
<th>Respiratory issues can be treated with fluoroquinolone</th>
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<tr>
<td>Disrupts normal intestinal flora</td>
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<td>O27 strain of <em>C. difficile</em> is specifically resistant to fluoroquinolone</td>
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## Diagnostic Methods for Influenza

<table>
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<tr>
<th>Method</th>
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<td>Culture</td>
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<td>DFA</td>
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<tr>
<td>PCR</td>
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<td>Rapid Tests</td>
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Issues with Clinical Samples

- Viral titer is highest in first 48 hours
- Proper sample collection is necessary
- Dilution in transport media
Rapid Tests

Pro

• Tests take minimal time
• Some tests are so simple that they can be CLIA-waived
• Can be used to triage patients
• Positive results can be used to rule out other issues like pneumonia so don’t give unnecessary chest x-ray, antibiotics, etc.

Con

• Performance is not as good as culture, PCR, or DFA
Molecular Assays

**Pro**
- For respiratory specimens, high performance
- Same day results

**Con**
- Turn around time from lab may be extensive, especially if batching specimens
- Expensive
- May require experienced technicians, labs, dedicated equipment, etc.
The need is a test rapid enough to triage patients like a rapid test with molecular results.
What is Alere i?
What is Isothermal Amplification?

Alere™ i is a molecular diagnostic test using isothermal amplification.

Isothermal amplification is not PCR but offers similar sensitivity and specificity.

Uses a constant and low temperature so there is no need for thermal cycler.

Very rapid amplification (many targets amplified in only 5-10 minutes).

Does not require DNA purification step, which adds time, complexity and cost.

Ability to deploy an incredibly sensitive test for infectious diseases at the POC.
NEAR, or **Nicking Enzyme Amplification Reaction**, enables the isothermal unwinding and subsequent amplification of very small amplicons using a set of target-specific templates (primers), a nicking endonuclease and a strand displacing DNA polymerase. Amplicon detection can be performed using a variety of probe formats including molecular beacons or by lateral flow sandwich.
Initial Reaction

- Influenza is RNA virus
  - Perform reverse-transcriptase amplification
  - No purification of the nucleic acid
The primed, duplex amplicon:

- blue recognizes a site on one strand of the target
- brown recognizes a nearly adjacent target on the other strand
- orange is an extra piece on each primer with a “nicking” site (N) and other “stabilizing” sequences
- there is a 10-12 bp “gap” between the “templates” (arrows); this enables use of a target-specific probe for product detection.
- after duplex synthesis, nicking at or near “N” enables strand displacement amplification, providing additional product that can be re-amplified using additional templates (primers).
iNAT Flu A/B Workflow

1. Touch “Run Test”
   This will begin the test process.

2. Enter and confirm patient data

3. Open lid and insert Test Base into holder
   Test Base and holder are color coded.

4. Confirm test
   Touch “Confirm” to proceed.

5. Insert Sample Receiver into holder
   Sample Receiver and holder are color coded.

6. Wait 3 minutes for Sample Receiver to warm up
   Then remove foil from the Sample Receiver.

7. Take nasal swab from patient and insert into Sample Receiver

8. Press Transfer Cartridge onto Sample Receiver
   Listen for click. Blue indicator on top of Transfer Cartridge will appear to confirm sample has been collected.

9. Lift and then attach Transfer Cartridge to Test Base
   Blue indicator on top of Transfer Cartridge will disappear to confirm sample has been dispensed.

10. Close lid and wait 10 minutes for test results
    Attach Test Base/Cartridge onto Sample Receiver and discard after completing test.
Influenza A/B: First Publications

**Clinical Evaluation of the Alere™ I Influenza A&B Assay, a Point-of-Care Rapid Nucleic Acid Amplification Test for Detection of Influenza**

Jeremiah Bell, Aleta Bonner, Daniel M. Cohen, Robert Birkhahn, Ram Yogev, Wayne Triner, Jason Cohen, Elizabeth Palavecino, and Pranganal Selvarajah

**UMKC School of Medicine**

**Children's Mercy Hospitals and Clinics and University of Missouri Kansas City School of Medicine, Kansas City, MO; Vitas, P.A., Belton, TX; Nationwide Children's Hospital, Columbus, OH; New York Methodist Hospital, Brooklyn, NY; Ann & Robert Lurie Children's Hospital of Chicago and Northwestern University Feinberg School of Medicine, Chicago, IL, Albany Medical College, Albany, NY; Wake Forest University Baptist Medical Center, Winston-Salem, NC.**

**Abstract (Revised)**

**Background:** Rapid testing and detection of influenza virus infection in an outpatient setting is crucial for appropriate patient management. Although rapid antigen tests for influenza offer quick results, they lack adequate sensitivity. The rapid antigen test for influenza A (RTA) is not approved for influenza B (RTB) detection.

**Aims:** The aim of the study was to evaluate the performance of the **Alere™ I Influenza A&B Assay** in clinical settings.

**Materials and Methods:** Specimens from patients with suspicion of influenza were collected from 82 to 100 patients at each site. The assay was performed according to the manufacturer's instructions. The sensitivity and specificity of the assay were calculated using the gold standard method of culture and confirmatory testing.

**Results:** The assay showed excellent sensitivity (98.8%) and specificity (100%) for influenza A and B, respectively. The assay was able to detect influenza virus in all patient samples, including those with low virus loads.

**Conclusions:** The **Alere™ I Influenza A&B Assay** is a rapid and sensitive test for the detection of influenza A and B, making it a valuable tool for outpatient settings.

**References:**


**Acknowledgments:** The authors would like to thank the participants and healthcare providers for their contributions to this study.

[http://pasev.ivdnews.net/public/show_abstract/1558](http://pasev.ivdnews.net/public/show_abstract/1558)
EVALUATION OF THE RAPID ALERE™ I INFLUENZA A&B NUCLEIC ACID AMPLIFICATION TEST USING RESPIRATORY SPECIMENS COLLECTED IN VIRAL TRANSPORT MEDIUM

Jeremiah Bell and Rangaraj Selvarangan
Children’s Mercy Hospitals and Clinics, and University of Missouri, Kansas City School of Medicine, Kansas City, MO

ABSTRACT

Background: Molecular nucleic acid-based laboratory tests are among the most sensitive tests available for diagnosis of viral infections. The Alere™ I Influenza A&B assay is a rapid, point-of-care test that provides sensitive and specific identification of influenza A and B in respiratory specimens. Results are available within 15 minutes.

Methods: A total of 256 nasopharyngeal aspirates from children admitted to children’s hospitals were tested using the rapid Alere™ I Influenza A&B assay. A total of 128 specimens were tested using the real-time RT-PCR assay. The results of the Alere™ I Influenza A&B assay were compared to the results of the real-time RT-PCR assay. The Alere™ I Influenza A&B assay was performed using 200 µl of aspirate sample in a 55°C water bath followed by 95°C for 10 minutes. The real-time RT-PCR assay was performed using a thermocycler and 20–30 cycles of PCR amplification.

Results: A total of 256 nasopharyngeal aspirates from children admitted to children’s hospitals were tested using the rapid Alere™ I Influenza A&B assay. A total of 128 specimens were tested using the real-time RT-PCR assay. The results of the Alere™ I Influenza A&B assay were compared to the results of the real-time RT-PCR assay. The Alere™ I Influenza A&B assay was performed using 200 µl of aspirate sample in a 55°C water bath followed by 95°C for 10 minutes. The real-time RT-PCR assay was performed using a thermocycler and 20–30 cycles of PCR amplification.

INTRODUCTION

Several laboratory methods are available for the diagnosis of influenza infections and will distinguish between influenza A and influenza B. Currently, the most useful methods in the clinical laboratory include viral culture (1), direct fluorescent antibody (2), serological assays (3), and nucleic acid amplification techniques (4). These methods are labor-intensive, often requiring up to 14 days to perform. In contrast, molecular nucleic acid-based methods provide rapid and accurate results, often within hours. Molecular nucleic acid-based methods such as polymerase chain reaction (PCR), real-time PCR, and nucleic acid sequence analysis (NASBA) are currently available. These methods are labor-intensive, often requiring up to 14 days to perform. In contrast, molecular nucleic acid-based methods provide rapid and accurate results, often within hours. Molecular nucleic acid-based methods such as polymerase chain reaction (PCR), real-time PCR, and nucleic acid sequence analysis (NASBA) are currently available.
Influenza A/B: First Publications

**Conclusions:**

- Compared well to viral cell culture
- Demonstrates impressive sensitivity
- Combines speed of a RAT with sensitivity of a NAT in POC setting
- Offers the opportunity to improve patient management

Direct NS vs Culture
Flu A: 100% / 97.9%
Flu B: 97.7% / 100%

VTM vs ProFlu+ PCR
Flu A: 88.8% / 98.3%
Flu B: 100% / 100%

[http://pasev.ivdnews.net/public/show_abstract/1558](http://pasev.ivdnews.net/public/show_abstract/1558)
## Advantages of Rapid Flu Tests

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<th>Accurate determination of who needs medication</th>
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<tr>
<td>• Antiviral medication AND antibiotics</td>
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<tr>
<td>• Avoid exacerbation of bacterial &amp; viral resistance</td>
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<td>• In pandemic, can help qualify who gets medication</td>
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<th>Early treatment of high risk patients, to</th>
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<tr>
<td>• Reduce complications</td>
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<td>• Reduce spread</td>
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<td>• Reduce healthcare burden</td>
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<table>
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<th>Cost benefits</th>
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<tbody>
<tr>
<td>• Directed therapy</td>
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<td>• Reduced morbidity and mortality reduces hospital costs</td>
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