Influenza Testing Methodologies and the FDA Re-classification

Gregory J. Berry, Ph.D., D(ABMM)
Assistant Professor, Pathology and Laboratory Medicine, Hofstra Northwell School of Medicine
Assistant Director, Infectious Disease Diagnostics, Northwell Health Laboratories
Objectives

- Review FDA Influenza Reclassification Requirements
- Introduce the different types of influenza tests available
- Explain the pros and cons of each type of influenza testing
- Describe which influenza tests are compliant with new FDA guidelines
Influenza virus

- RNA viruses
- Belong to the Orthomyxoviridae family
  - Several members of this family
  - Only influenza A and B cause human epidemics
- Have been causing human pandemics for a few hundred years
Influenza A&B in the U.S.

- 5–20% of population is affected each year

- Approximately 36,000 deaths each year with more than 200,000 hospitalizations
  - Ranges from 4,000–50,000 deaths per year

- Most deaths are in elderly
  - But can also occur in healthy individuals (2009 H1N1)
Influenza A vs. B

Influenza A

- Can cause disease in a wide variety of animals
- More severe disease than B
- Divided into subtypes based on two surface proteins:
  - Hemagglutinin (H) – ~13 types
    - Allows virus to bind to cells for infection
  - Neuraminidase (N) – 9 types
    - Allows new viruses to escape from cells

Influenza B

- Causes a milder flu, usually in the spring months
- Broken down into lineages
  - e.g. B/Yamagata, B/ Victoria
Symptoms of influenza

**Central**
- Headache

**Systemic**
- Fever
  (usually high)

**Muscular**
- (Extreme) tiredness

**Joints**
- Aches

**Nasopharynx**
- Runny or stuffy nose
- Sore throat
- Aches

**Respiratory**
- Coughing

**Gastric**
- Vomiting

Häggström, Mikael (2014). “Medical gallery of Mikael Häggström 2014”. 
Spread of influenza

- Spread person-to-person
- Droplets spread when coughing, sneezing, talking
  - Can spread about 6 feet away
- Touching contaminated surfaces and then touching nose, mouth
Avoiding spread of influenza

- Wash hands!
- Surgical mask
- Vaccination

Remember: You can spread flu one day before you are symptomatic!
Most people do not need treatment

Antiviral drugs can be used for treatment in those at high risk for flu complications
- Shorten disease duration by 1–2 days
- Could prevent certain complications

People at high risk of flu complications:
- Young children,
- Adults ≥ 65 years of age
- Pregnant women
- Those with certain medical conditions—
  - Asthma, diabetes, heart disease, immunosuppressed, etc.
Identification of pneumococcal infection is critical

- ~25% of influenza–related deaths have a secondary bacterial pneumonia\(^1\)
- Post-mortem lung tissue from 1918 pandemic showed that most patients also had bacterial infection\(^2\)
- 1957 pandemic showed that 2/3 of deaths were associated with bacterial pneumonia\(^3\)
How does the flu virus change?

**ANTIGENIC “DRIFT” VS. “SHIFT”**

**Drift** – small genetic changes over time
- More typical, yearly change in influenza virus
- Reason why a new vaccine formulation is needed every year, even for the “same” virus

**Shift** – major change resulting in a new hemagglutinin and/or neuraminidase
- Leads to a new virus to which people’s immune system is naïve
  - Can lead to influenza pandemics
- Occurred in 2009 – Novel H1N1

Changes can and will affect the performance of influenza tests
How does antigenic shift happen?

Avian H3

Human H2

Human H3
Flu Killing More Young Adults This Year, CDC Says

Influenza is killing more young and middle-aged adults this year than usual, in part because they're less likely to be vaccinated, federal health officials said Thursday.

More than 60 percent of those killed or put into the hospital by flu so far this season have been aged 18 to 64, the Centers for Disease Control and Prevention says. And 50 children have died of flu so far.

The good news is the vaccine is pretty effective for a flu vaccine, with a 61 percent effectiveness rate, CDC and other experts found.
The benefits of flu vaccination 2015-2016

The estimated number of flu illnesses prevented by flu vaccination during the 2015-2016 season:

5 million
as many people use Denver International Airport in one month

The estimated number of flu medical visits prevented by vaccination during the 2015-2016 season:

2.5 million
equal to the population of Portland, Oregon

The estimated number of flu hospitalizations prevented by vaccination during the 2015-2016 season:

71,000
enough to fill every registered hospital bed in the state of Texas


get vaccinated
www.cdc.gov/flu
ACIP Says No to LAIV for 2017-18 Flu Season

Group Updates Influenza Vaccine Recommendations for Pregnant Women

June 30, 2017 07:35 am Chris Crawford – Just as it did for the 2016-2017 influenza season, the CDC’s Advisory Committee on Immunization Practices (ACIP) has again decided to recommend against use of live attenuated influenza vaccine (LAIV, Flumist) for the 2017-2018 flu season because of the vaccine’s reduced efficacy. This decision was made during the ACIP’s June 21-22 meeting.

Data presented at the meeting showed that last year’s exclusion of LAIV didn’t affect vaccine coverage numbers for the 2016-2017 season compared with the previous season, with the flu vaccine shown to be 42 percent effective in preventing infection from A and B viruses in patients of all ages.

For 2016-2017, influenza A accounted for 70 percent of circulating flu strains, with influenza B making up the other 30 percent.
Influenza Vaccine Effectiveness in the United States during the 2015–2016 Season


ABSTRACT

BACKGROUND
The A(H1N1)pdm09 virus strain used in the live attenuated influenza vaccine was changed for the 2015–2016 influenza season because of its lack of effectiveness in young children in 2013–2014. The Influenza Vaccine Effectiveness Network evaluated the effect of this change as part of its estimates of influenza vaccine effectiveness in 2015–2016.
<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of Case Patients/ Total No. (%)</th>
<th>Vaccine Effectiveness %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td>1309/6879 (19)</td>
<td>48</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mo to 8 yr</td>
<td>254/1526 (17)</td>
<td>51</td>
</tr>
<tr>
<td>9 to 17 yr</td>
<td>164/858 (19)</td>
<td>59</td>
</tr>
<tr>
<td>18 to 49 yr</td>
<td>499/2456 (20)</td>
<td>52</td>
</tr>
<tr>
<td>50 to 64 yr</td>
<td>283/1201 (24)</td>
<td>26</td>
</tr>
<tr>
<td>≥65 yr</td>
<td>109/838 (13)</td>
<td>42</td>
</tr>
<tr>
<td><strong>Virus subtype or lineage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A(H1N1)pdm09</td>
<td>768/6338 (12)</td>
<td>45</td>
</tr>
<tr>
<td>A(H3N2)</td>
<td>72/4623 (2)</td>
<td>43</td>
</tr>
<tr>
<td>B/Victoria</td>
<td>200/5770 (3)</td>
<td>49</td>
</tr>
<tr>
<td>B/Yamagata</td>
<td>253/5823 (4)</td>
<td>57</td>
</tr>
<tr>
<td><strong>Vaccine type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any IIIV</td>
<td>1271/6718 (19)</td>
<td>51</td>
</tr>
<tr>
<td>IIIV3</td>
<td>955/4473 (21)</td>
<td>41</td>
</tr>
<tr>
<td>IIIV4</td>
<td>1072/5368 (20)</td>
<td>54</td>
</tr>
<tr>
<td>LAIV4 (2 to 49 yr of age only)</td>
<td>674/2839 (24)</td>
<td>4</td>
</tr>
</tbody>
</table>

Vaccine Effectiveness (%)
SNP-mediated disruption of CTCF binding at the *IFITM3* promoter is associated with risk of severe influenza in humans

E Kaitlynn Allen¹, Adrienne G Randolph², Tushar Bhangale³, Pranay Dogra¹, Maikke Ohlson⁴, Christine M Oshansky¹,⁹, Anthony E Zamora¹, John P Shannon¹, David Finkelstein⁵, Amy Dressen³, John DeVincenzo⁶,⁷, Miguela Caniza⁸, Ben Youngblood¹, Carrie M Rosenberger⁴ & Paul G Thomas¹

Previous studies have reported associations of *IFITM3* SNP rs12252 with severe influenza, but evidence of association and the mechanism by which risk is conferred remain controversial. We prioritized SNPs in *IFITM3* on the basis of putative biological function and identified rs34481144 in the 5' UTR. We found evidence of a new association of rs34481144 with severe influenza in three influenza-infected cohorts characterized by different levels of influenza illness severity. We determined a role for rs34481144 as an expression quantitative trait locus (eQTL) for *IFITM3*, with the risk allele associated with lower mRNA expression. The risk allele was found to have decreased IRF3 binding and increased CTCF binding in promoter-binding assays, and risk allele carriage diminished transcriptional correlations among *IFITM3*-neighboring genes, indicative of CTCF boundary activity. Furthermore, the risk allele disrupts a CpG site that undergoes differential methylation in CD8⁺ T cell subsets. Carriers of the risk allele had reduced numbers of CD8⁺ T cells in their airways during natural influenza infection, consistent with IFITM3 promoting accumulation of CD8⁺ T cells in airways and indicating that a critical function for IFITM3 may be to promote immune cell persistence at mucosal sites. Our study identifies a new regulator of *IFITM3* expression that associates with CD8⁺ T cell levels in the airways and a spectrum of clinical outcomes.
What is a medical device (as per FDA)?

“an instrument, apparatus...intended for use in the diagnosis of disease or other conditions...”

Can range from dental floss to prosthetic heart valve
FDA classification of a medical device:

- Based on the risks associated with the device
- One of three categories—Class I, Class II, and Class III

**Class I devices**
are deemed to be low risk and are therefore subject to the least regulatory controls (general controls).
e.g., dental floss

**Class II devices**
are higher risk devices than Class I and require greater regulatory controls to provide reasonable assurance of the device’s safety and effectiveness (general and special controls).
e.g., condoms

**Class III devices**
are generally the highest risk devices and are therefore subject to the highest level of regulatory control. Class III devices must typically be approved by FDA before they are marketed (pre-market approval.)
e.g., replacement heart valves

https://www.fda.gov/aboutfda/transparency/basics/ucm194438.htm
General vs. special controls

General controls apply to all medical devices (unless exempt)

- Sufficient for low risk (Class I) devices
- Include protections regulating adulteration/misbranding, registration, listing with FDA, Good Manufacturing Practices, proper labeling, and reporting adverse reactions, etc.

Special Controls are required when general controls alone are not sufficient (Class II)

- Include guidelines, performance standards, special labeling, etc.
What changed with rapid influenza virus antigen detection tests (RIDTs)?

These tests were classified as Class I devices
- General controls were considered sufficient

FDA has re-classified them to Class II
- Both general and special controls must now be followed
Microbiology Devices; Reclassification of Influenza Virus Antigen Detection Test Systems Intended for Use Directly With Clinical Specimens

A Rule by the Food and Drug Administration on 01/12/2017

AGENCY:
Food and Drug Administration, HHS.

ACTION:
Final order.

SUMMARY:
The Food and Drug Administration (FDA) is reclassifying antigen based rapid influenza virus antigen detection test systems intended to detect influenza virus directly from clinical specimens that are currently regulated as influenza virus serological reagents from class I into class II with special controls and into a new device classification regulation.
Why the change with RIDTs?

During the H1N1 influenza pandemic of 2009, questions were raised about the sensitivity of RIDTs
  ◦ Lower sensitivity than package insert

Concerns raised about the overall quality of influenza testing

Overall goal: lower the number of misdiagnosed influenza infections by increasing the number of devices that can reliably detect the influenza virus
Why have rapid antigen tests been re-classified?

“A false negative result may lead to failure to provide a correct diagnosis and the appropriate treatment of infection caused by influenza virus and may contribute to unnecessary treatment for another suspected condition. A false negative result will also provide incorrect epidemiological information leading to failure to initiate appropriate corrective measures to control and prevent additional infections.”

“A false positive result on the other hand may lead to delayed treatment of a respiratory infection caused by another etiologic agent, which could potentially result in a more serious patient outcome. A false positive result will also provide incorrect epidemiological information on the presence of influenza in a community, which may result in unnecessary patient isolation or contact limitations and in unnecessary close contact investigations.”

http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/MicrobiologyDevicesPanel/UCM356185.pdf
Minimum acceptance criteria

**Sensitivity**

**Flu A** Point estimate of 90% with 80% lower bound of the 95% confidence interval  
**Flu B** Point estimate of 80% with 70% lower bound of the 95% confidence interval

**Specificity**

All influenza detection devices should demonstrate specificity with a lower bound of the 95% confidence interval exceeding 90% for both, Flu A and Flu B.

b. **When compared to a molecular comparator method:**

**Sensitivity**

**Flu A** Point estimate of 80% with 70% lower bound of the 95% confidence interval  
**Flu B** Point estimate of 80% with 70% lower bound of the 95% confidence interval

**Specificity**

All influenza detection devices should demonstrate a specificity estimate with a lower bound of the 95% confidence interval exceeding 90% for both, influenza A and influenza B.
What is the timeline for this change?

Rule was published 01/12/2017

- Effective Date: 02/13/2017

For antigen-based RIDTs legally marketed prior to 2/13/2017:

- Manufacturers must obtain a new 510(k) clearance and demonstrate compliance with the special controls included in the new clinical performance standards before marketing their changed or new devices

FDA will allow for a one year transition before enforcement of new rule (January 12, 2018)
Enforcing compliance

“For antigen based RIDTs that have been legally marketed prior to February 13, 2017, FDA does not intend to enforce compliance with the special controls until January 12, 2018. If a manufacturer markets such a device after January 12, 2018, and that device does not comply with the special controls, then FDA would consider taking action against such a manufacturer under its usual enforcement policies.”
What does this change mean for you?

Tests that are not compliant with new regulations can be purchased up until January 12th, 2018
  - Cannot be sold by manufacturers after this date

Purchased tests can be used by customers until their expiration date.
  - Using these kits is NOT a violation
  - No changes for the 2018 flu season are necessary if enough kits are purchased for the duration of the season before 1/12/2018

Clinics should begin to look into new assays that are compliant with new regulations with next season in mind
CLIA regulations for testing

- All laboratory testing in the U.S. falls under the jurisdiction of Clinical Laboratory Improvement Amendments of 1988 (CLIA)
- Administered by CMS and is implemented through three federal agencies—CDC, CMS, and the Food and Drug Administration (FDA)
- The three categories of testing for CLIA purposes are **waived, moderate complexity** (including the provider-performed microscopy procedures [PPMP] subcategory), and **high complexity**
CLIA regulations for testing

- Waived, moderate complexity, and high complexity designation based on ease of use
- CLIA requires that waived tests must be simple and have a low risk for erroneous results
- Tests classified as moderately complex must be performed in a clinical laboratory
## Influenza testing methodologies

<table>
<thead>
<tr>
<th>Method</th>
<th>Types Detected</th>
<th>Acceptable Specimens</th>
<th>Test Time</th>
<th>CLIA Waived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid Influenza Diagnostic Tests&lt;sup&gt;4&lt;/sup&gt; (antigen detection)</td>
<td>A and B</td>
<td>NP&lt;sup&gt;5&lt;/sup&gt; swab, aspirate or wash, nasal swab, aspirate or wash, throat swab</td>
<td>&lt;15 min.</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Rapid Molecular Assay [influenza viral RNA or nucleic acid detection]</td>
<td>A and B</td>
<td>NP&lt;sup&gt;5&lt;/sup&gt; swab, nasal swab</td>
<td>&lt;20 minutes&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Yes/No&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Immunofluorescence, Direct (DFA) or Indirect (IFA) Florescent Antibody Staining [antigen detection]</td>
<td>A and B</td>
<td>NP&lt;sup&gt;4&lt;/sup&gt; swab or wash, bronchial wash, nasal or endotracheal aspirate</td>
<td>1–4 hours</td>
<td>No</td>
</tr>
<tr>
<td>RT–PCR&lt;sup&gt;7&lt;/sup&gt; (singleplex and multiplex; real-time and other RNA–based) and other molecular assays [influenza viral RNA or nucleic acid detection]</td>
<td>A and B</td>
<td>NP&lt;sup&gt;5&lt;/sup&gt; swab, throat swab, NP&lt;sup&gt;5&lt;/sup&gt; or bronchial wash, nasal or endotracheal aspirate, sputum</td>
<td>Varies (1 to 8 hours, varies by the assay)</td>
<td>No</td>
</tr>
<tr>
<td>Rapid cell culture (shell vials; cell mixtures; yields live virus)</td>
<td>A and B</td>
<td>NP&lt;sup&gt;5&lt;/sup&gt; swab, throat swab, NP&lt;sup&gt;5&lt;/sup&gt; or bronchial wash, nasal or endotracheal aspirate, sputum; (specimens placed in VTM&lt;sup&gt;8&lt;/sup&gt;)</td>
<td>1–3 days</td>
<td>No</td>
</tr>
<tr>
<td>Viral tissue cell culture (conventional; yields live virus)</td>
<td>A and B</td>
<td>NP&lt;sup&gt;5&lt;/sup&gt; swab, throat swab, NP&lt;sup&gt;5&lt;/sup&gt; or bronchial wash, nasal or endotracheal aspirate, sputum (specimens placed in VTM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Method
2. Types Detected
3. Acceptable Specimens
4. Test Time
5. CLIA Waived

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<sup>1</sup> CDC. Rapid Diagnostic Testing for Influenza: Information for Clinical Laboratory Directors

https://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm#table2
Point–of–care testing (POCT)

- These are CLIA waived tests that can be performed by facilities with a Certificate of Waiver
- Increasingly larger portion of patient testing
- Huge advantage of rapid answer for treatment decisions
- QUALITY is key
Specimen collection

- Ideally, collected within 3 days of symptom onset
- Use sterile Dacron/nylon swab, Insert into the posterior pharynx and tonsillar areas, remove swab and place into viral transport medium
- Test ASAP or keep specimen at 4°C
RIDT targets

- Immunoassays—identify influenza A&B viral nucleoprotein antigens
- The nucleoproteins are conserved throughout a given species
- Qualitative resulting
# RIDT pros and cons

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid results</td>
<td>Poor sensitivity</td>
</tr>
<tr>
<td>Simple to use</td>
<td>False negatives common</td>
</tr>
<tr>
<td>Several designated as CLIA waived (POCT eligible)</td>
<td>Bad at detecting novel viral strains</td>
</tr>
</tbody>
</table>
CLIA waived RIDT options
BD Veritor™ System for Rapid Detection of Flu A+B

Chromatographic immunoassay with automated reader design

- **Run time:** 10 minutes
- **Specimen types:** Nasopharyngeal swabs in transport media and nasopharyngeal wash aspirates
Binax NOW® Influenza A&B Test

Chromatographic immunoassay with automated reader design

- **Run time:** ~15 minutes
- **Specimen types:** Nasopharyngeal (NP) swab and nasal swab specimens
Alere™ Influenza A&B Test

Dipstick design with visual read

- Run time: 10 minutes
- Specimen types: Nasal swab specimens
QuickVue® Influenza A/B Test

Dipstick design with visual read

- **Run time:** ~10 minutes
- **Specimen types:** Nasal swab, nasopharyngeal swab, nasal aspirate/nasal wash
SAS™ FluAlert

Lateral flow immunoassay with visual read

- **Run time:** 15 minutes
- **Specimen types:** Nasal aspirates/washes

Note: detects flu A and B separately
Sofia® Analyzer and Influenza A+B FIA

Lateral flow immunoassay + fluorescence with automated reader

- **Run time:** 15 minutes
- **Specimen types:** Nasal swab, nasopharyngeal swab and nasopharyngeal aspirate/wash specimens
Molecular tests for flu

- Traditionally designated by CLIA as moderate/high complexity and have been performed in the clinical laboratories
  - Only rapid antigen influenza testing was available as CLIA waived

- CLIA waived tests for influenza have become available in the past two years
CLIA waived molecular tests for flu

- January 8th, 2015: First CLIA waived test for influenza A and B (Alere i Influenza A&B)
- Followed by the Roche cobas Influenza A/B
- Both of these tests are classified as class II, so they are already compliant
### Molecular flu testing pros and cons

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can amplify genome</td>
<td>Typically costs more</td>
</tr>
<tr>
<td>Highly sensitive and specific</td>
<td>Takes longer</td>
</tr>
</tbody>
</table>
CLIA waived molecular influenza testing options
Alere™ i Influenza A&B

- FDA–cleared for use with both nasal swabs (direct) and NP or nasal swabs in VTM
  - CLIA–waived for use with nasal swabs (direct) only
- < 15 minutes to results
- Footprint: 8.15”W x 5.71”H x 7.64”D
- 1.4 lbs / 3 kg
Roche® cobas® Influenza A/B

- CLIA–waived by FDA for use with nasopharyngeal swabs only
- 20 minutes to results
- Footprint: 4.5”W x 9.5”H x 7.5”D
- Weight 8.3 lbs
Review: tests that are (or will be) compliant

- Any test already classified as a Class II device:
  - Rapid molecular influenza tests

- Certain RIDTs that have already met, or applied for and were granted Class II status

- Any test that obtains a new 510(k) clearance to be Class II
Summary

- RIDTs that are currently classified as I will no longer be sold after January 12th, 2018
- Tests purchased before January 12th, 2018 can be used by customers until their expiration date
- There are several tests already on the market (both rapid antigen and molecular options) that are already compliant with new regulations

Speak with your influenza test manufacturer to ensure you are prepared for the influenza season.
Thank you!

Questions?