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

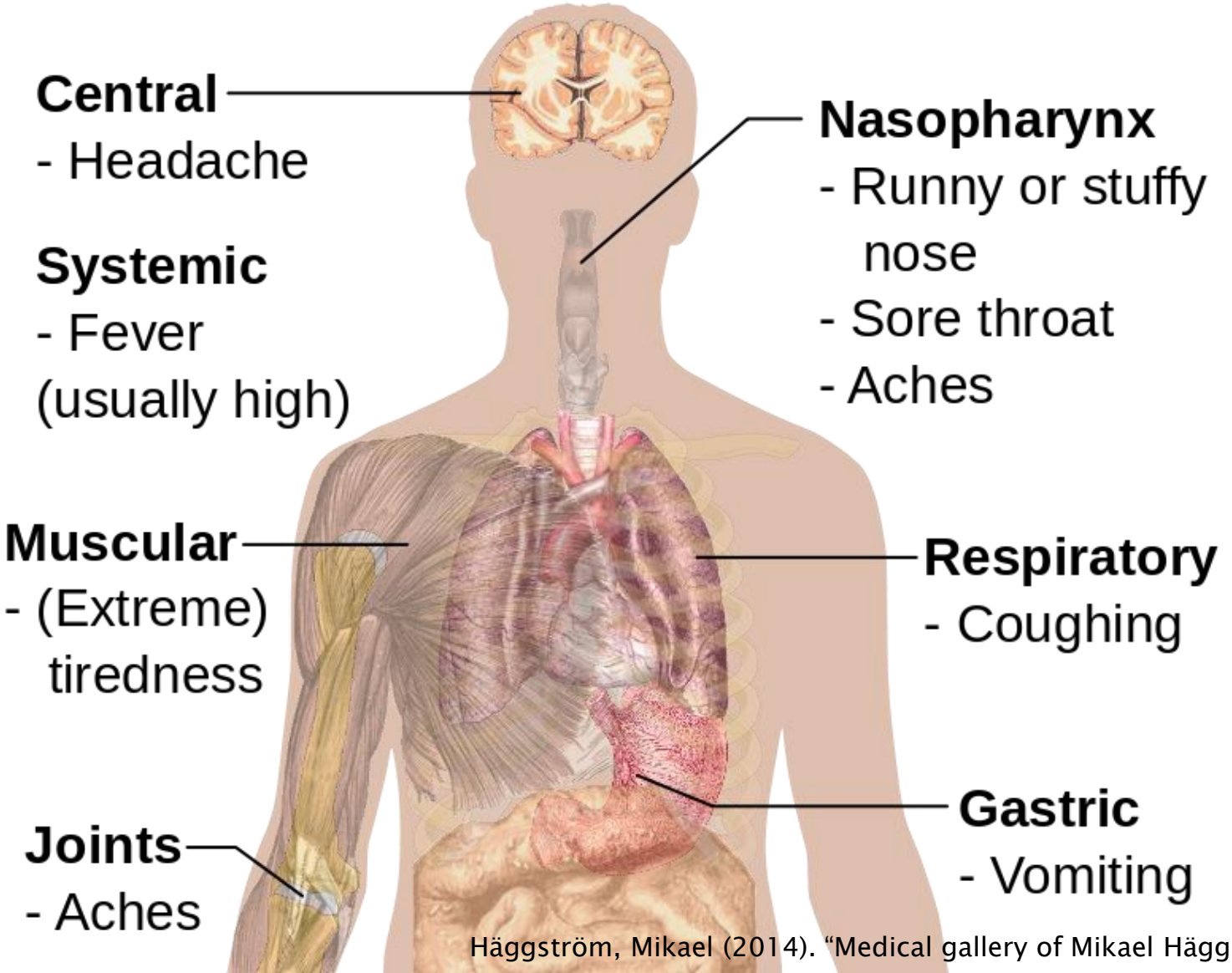
Influenza A

- 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



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!

Treatment for influenza

- 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.

https://www.cdc.gov/flu/treatment/index.html

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

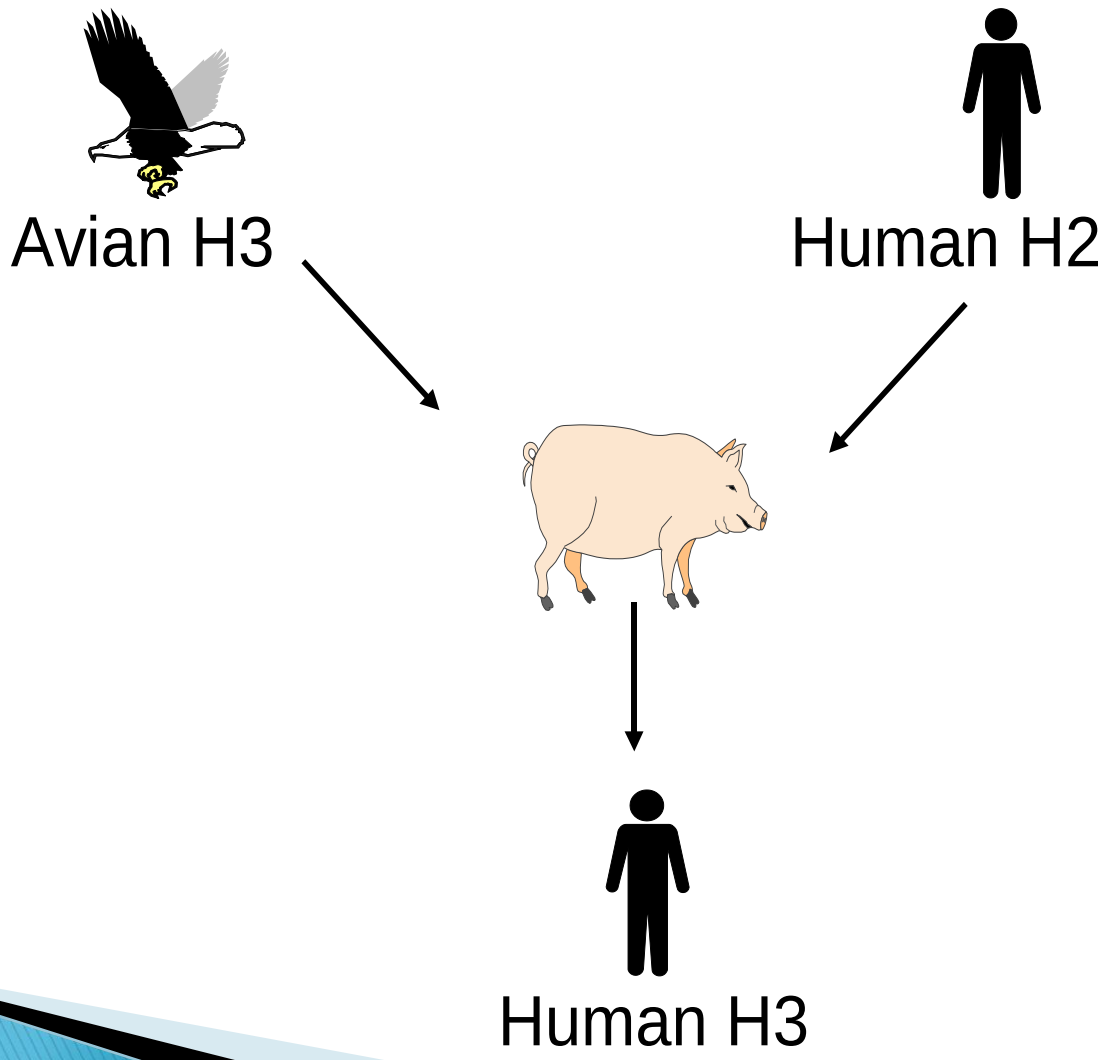
- More typical, yearly change in influenza virus
- Reason why a new vaccine formulation is needed every year, even for the "same" virus

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?



HEALTH

FEB 20 2014, 1:53 PM ET

Flu Killing More Young Adults This Year, CDC Says

Public health officials are encouraging people to get flu shots as the seasonal virus hits young adults hard this year. Justin Sullivan / Getty Images

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

DATA: Influenza Division program impact report 2015-2016, https://www.cdc.gov/flu/about/disease/2015-16.htm.

NCIRDig-807 | 12/08/16

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

get **vaccinated**
www.cdc.gov/flu

Flumist isn't working

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

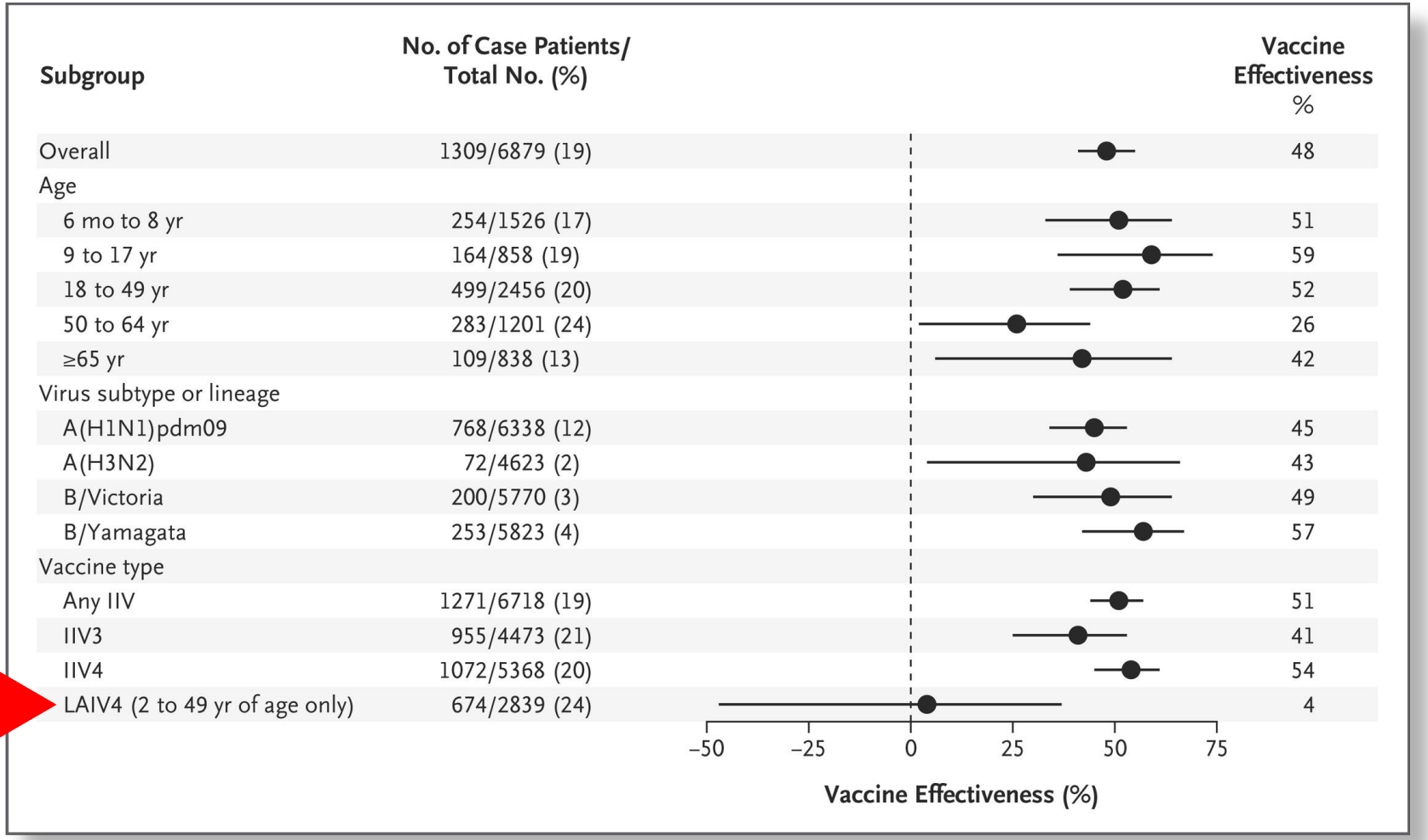
Influenza Vaccine Effectiveness in the United States during the 2015–2016 Season

Michael L. Jackson, Ph.D., Jessie R. Chung, M.P.H., Lisa A. Jackson, M.D., C. Hallie Phillips, M.Ed., Joyce Benoit, B.S.N., Arnold S. Monto, M.D., Emily T. Martin, Ph.D., Edward A. Belongia, M.D., Huong Q. McLean, Ph.D., Manjusha Gaglani, M.D., Kempapura Murthy, M.P.H., Richard Zimmerman, M.D., Mary P. Nowalk, Ph.D., Alicia M. Fry, M.D., and Brendan Flannery, Ph.D.

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.



Jackson ML et al. N Engl J Med 2017;377:534–543.

nature medicine

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

E Kaitlynn Allen[1], Adrienne G Randolph[2], Tushar Bhangale[3], Pranay Dogra[1], Maikke Ohlson[4], Christine M Oshansky[1,9], Anthony E Zamora[1], John P Shannon[1], David Finkelstein[5], Amy Dressen[3], John DeVincenzo[6,7], Miguela Caniza[8], Ben Youngblood[1], Carrie M Rosenberger[4] & Paul G Thomas[1]

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

https://commons.wikimedia.org/wiki/File:Aortic_Karboniks-1_bileafter_prosthetic_heart_valve.jpg
http://www.mwdental.com/media/catalog/product/cache/1/thumbnail/1000x1000/9df78eab33525d08d6e5fb8d27136e95/4/4/440-0500_6.jpg

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)?

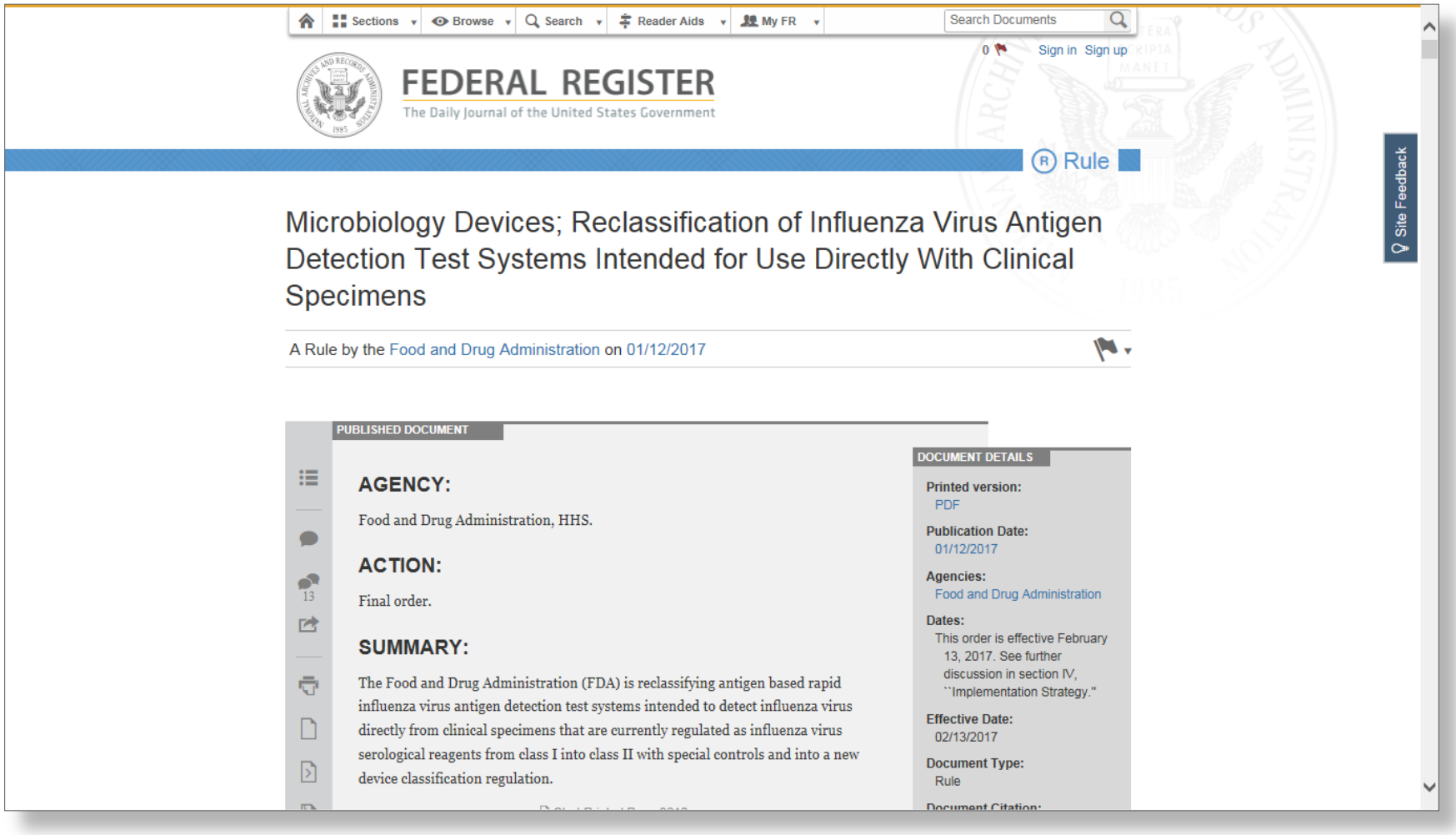
These tests were classified as Class I devices

- General controls were considered sufficient

FDA has re-classified them to Class II

- Both general and special controls must now be followed

FDA decision:



Sections Browse Search Reader Aids My FR Search Documents

0 Sign in Sign up

FEDERAL REGISTER

The Daily Journal of the United States Government

Rule

Site Feedback

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

PUBLISHED DOCUMENT

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.

DOCUMENT DETAILS

Printed version:
PDF

Publication Date:
01/12/2017

Agencies:
Food and Drug Administration

Dates:
This order is effective February 13, 2017. See further discussion in section IV, ``Implementation Strategy.''

Effective Date:
02/13/2017

Document Type:
Rule

Document Citation:

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

https://www.federalregister.gov/d/2017-00199/p-19

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."

https://www.federalregister.gov/d/2017-00199/p-94

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**

CDC. MMWR Recommendations and Reports. November 11, 2005 / 54(RR13);1-25

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

CDC. MMWR Recommendations and Reports. November 11, 2005 / 54(RR13);1-25

Influenza testing methodologies

Method[1]	Types Detected	Acceptable Specimens[2]	Test Time	CLIA Waived[3]
Rapid Influenza Diagnostic Tests[4] (antigen detection)	A and B	NP[5] swab, aspirate or wash, nasal swab, aspirate or wash, throat swab	<15 min.	Yes/No
Rapid Molecular Assay [influenza viral RNA or nucleic acid detection]	A and B	NP[5] swab, nasal swab	<20 minutes[6]	Yes/No[6]
Immunofluorescence, Direct (DFA) or Indirect (IFA) Florescent Antibody Staining [antigen detection]	A and B	NP[4] swab or wash, bronchial wash, nasal or endotracheal aspirate	1–4 hours	No
RT-PCR[7] (singleplex and multiplex; real-time and other RNA-based) and other molecular assays [influenza viral RNA or nucleic acid detection]	A and B	NP[5] swab, throat swab, NP[5] or bronchial wash, nasal or endotracheal aspirate, sputum	Varies (1 to 8 hours, varies by the assay)	No
Rapid cell culture (shell vials; cell mixtures; yields live virus)	A and B	NP[5] swab, throat swab, NP[5] or bronchial wash, nasal or endotracheal aspirate, sputum; (specimens placed in VTM[8])	1–3 days	No
Viral tissue cell culture (conventional; yields live virus)	A and B	NP[5] swab, throat swab, NP[5] or bronchial wash, nasal or endotracheal aspirate, sputum (specimens placed in VTM)		

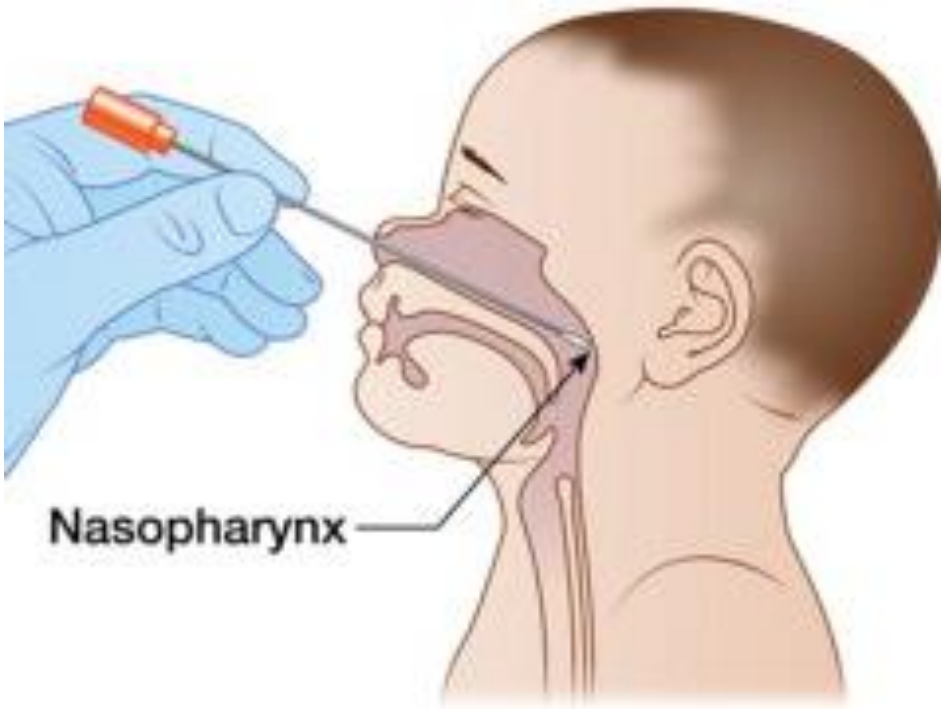
CDC. Rapid Diagnostic Testing for Influenza: Information for Clinical Laboratory Directors
https://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm#table2

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

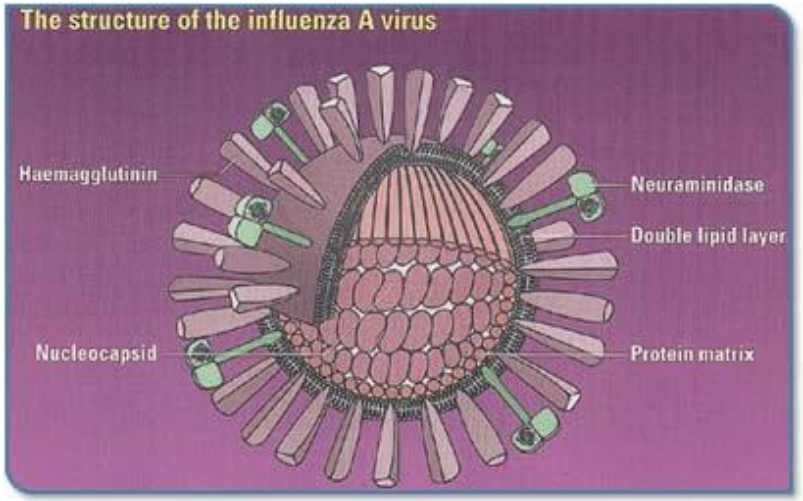
- Ideally, collected within 3 days of symptom onset
- Use sterile Dacron/nylon swab, Insert into the posterior pharynx and tonsillar areas, remove swab and place into viral transport medium
- Test ASAP or keep specimen at 4°C

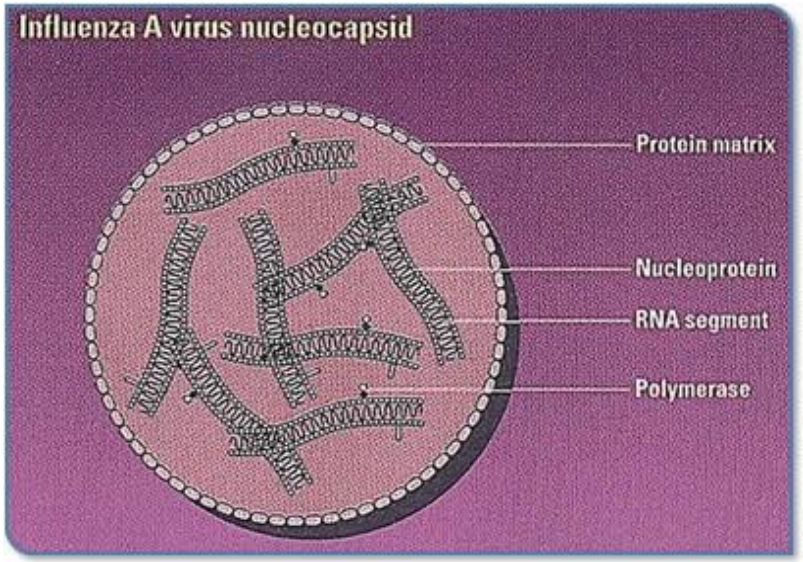


C. Satzke et al. / Vaccine 32 (2014) 165–179

RIDT targets

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





RIDT pros and cons

Pros	Cons
Rapid results Simple to use Several designated as CLIA waived (POCT eligible)	Poor sensitivity ◦ False negatives common Bad at detecting novel viral strains

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

Pros	Cons
Can amplify genome	Typically costs more
Highly sensitive and specific	Takes longer

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?