

# Influenza Testing Methods

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# Overview

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- Two diagnostic tests that detect influenza viruses in respiratory specimens
  - Molecular Assays
  - Antigen Detection Tests
- Molecular Assays
  - Rapid molecular assays
  - Reverse transcription polymerase chain reaction (RT-PCR)
  - Other nucleic acid amplification tests
- Antigen Detection Tests
  - Rapid influenza diagnostic tests
  - Immunofluorescence assays
- Viral Cultures used for public health purposes
- Sensitivity and specificity vary among tests (CDC, 2018)

# Influenza Virus Testing Methods

|   |   | Table 1: Influenza Virus Testing Methods   |                |   |  |                          |
|---|---|--|----------------|---|--|--------------------------|
|   |   | Method <sup>1</sup>  | Types Detected | Acceptable Specimens <sup>2</sup>   | Test Time                                  | CLIA Waived <sup>3</sup> |
| Symptoms & Diagnosis                                  | + | Rapid Influenza Diagnostic Tests <sup>4</sup> (antigen detection)  | A and B        | NP <sup>5</sup> swab, aspirate or wash, nasal swab, aspirate or wash, throat swab   | <15 min.                                   | Yes/No                   |
| Treatment   | + | Rapid Molecular Assay [influenza viral RNA or nucleic acid detection]  | A and B        | NP <sup>5</sup> swab, nasal swab  | 15-30 minutes <sup>6</sup>                 | Yes/No <sup>6</sup>      |
| Schools, Businesses & Travelers                       | + | Immunofluorescence, Direct (DFA) or Indirect (IFA) Florescent Antibody Staining [antigen detection]  | A and B        | NP <sup>4</sup> swab or wash, bronchial wash, nasal or endotracheal aspirate  | 1-4 hours                                  | No                       |
| Flu Activity & Surveillance                           | + | RT-PCR <sup>7</sup> (singleplex and multiplex; real-time and other RNA-based) and other molecular assays [influenza viral RNA or nucleic acid detection]   | A and B        | NP <sup>5</sup> swab, throat swab, NP <sup>5</sup> or bronchial wash, nasal or endotracheal aspirate, sputum  | Varies (1 to 8 hours, varies by the assay) | No                       |
| FluVaxView  |   | Rapid cell culture (shell vials; cell mixtures; yields live virus)   | A and B        | NP <sup>5</sup> swab, throat swab, NP <sup>5</sup> or bronchial wash, nasal or endotracheal aspirate, sputum; (specimens placed in VTM <sup>8</sup> ) | 1-3 days                                   | No                       |
| Health Professionals                                  | - | Viral tissue cell culture (conventional; yields live virus)  | A and B        | NP <sup>5</sup> swab, throat swab, NP <sup>5</sup> or bronchial wash, nasal or endotracheal aspirate, sputum (specimens placed in VTM <sup>8</sup> )  | 3-10 days                                  | No                       |
| Health Care Workers Need A Flu Vaccine                |   | <sup>1</sup> Serologic (antibody detection) testing is not recommended for routine patient diagnosis and cannot inform clinical management. A single acute serum specimen for seasonal influenza serology is uninterpretable and should not be collected. Serological testing for detection of antibodies to seasonal influenza viruses is useful for research studies and |                |   |  |                          |
| CDC Updates for Health Care Providers                 |   |  |                |   |  |                          |
| ACIP Recommendations                                  | + |  |                |   |  |                          |
| Vaccination   | + |  |                |   |  |                          |
| Vaccine Effectiveness Studies                         | + |  |                |   |  |                          |
| Information for Clinicians on Influenza Virus Testing | + |  |                |   |  |                          |
| Information for Laboratories                          | - |  |                |   |  |                          |

# **Rapid Influenza Diagnostic Tests (RIDTs)**

- Antigen detection assays that detect viral antigens in 10-15 minutes with 50-70% sensitivity and high specificity
- Some tests approved for outpatient settings and some require clinical laboratory settings
- Detection of the influenza virus antigen does not necessarily indicate detection of infectious virus or on-going influenza viral replication
- None of the RIDTs provide information about influenza A virus subtypes
- Types of specimens vary depending on test
- False negatives are common - recommend using molecular assays to determine if positive (CDC, 2018)

# FDA-Cleared Rapid Influenza Diagnostic Test

|  |   |   |  |                      |  |                          |
|--|---|---|--|----------------------|--|--------------------------|
| Symptoms & Diagnosis   | + | Table 2: Available FDA-Cleared Rapid Influenza Diagnostic Tests (Antigen Detection Only)                                |  |                      |  |                          |
| Treatment  | + | These tests provide results in 10-15 minutes and differentiate between influenza A and B                                |  |                      |  |                          |
| Schools, Businesses & Travelers  | + | Manufacturer  | Product  | Platform/Instrument  | Approved Specimens <sup>1</sup>                  | CLIA Waived <sup>2</sup> |
| Flu Activity & Surveillance  | + | Alere   | Binax Now Influenza A & B Card 2   | Alere Reader         | NPS, NS direct                                   | Yes                      |
| FluVaxView   |   | Becton Dickinson & Co.  | BD Veritor™ Flu A + B  | BD Veritor Reader    | NPS, NS direct                                   | Yes                      |
| Health Professionals   | - | Quidel Corp.  | Sofia® Influenza A + B FIA   | Sofia FIA Analyzer   | NS, NPS, NPA, NPW direct, NP, NPA, NPW in VTM    | Yes                      |
| Health Care Workers Need A Flu Vaccine   |   | Quidel Corp.  | Sofia® Influenza A + B FIA   | Sofia 2 FIA Analyzer | NS, NPS, NPA, NPW direct, NP, NPA, NPW in VTM    | Yes                      |
| CDC Updates for Health Care Providers  |   | Quidel Corp.  | QuickVue® Influenza A + B  | N/A                  | NPS, NS direct                                   | Yes                      |
| ACIP Recommendations   | + | Princeton BioMeditech Corp.   | BioSign® Flu A & B<br>LABSCO Advantage Flu A & B<br>LifeSign LLC Status Flu A & B<br>OraSure QuickFlu Rapid A + B<br>Polymedco Poly stat Flu A & B<br>Sekisui Diagnostics OSOM® ULTRA Flu A & B<br>Meridian BioScience ImmunoCard STAT Flu A&B<br>McKesson Consult Diagnostics Influenza A&B | N/A                  | NS, NPS direct (waived)<br>NPA, NPW (not waived) | Yes                      |
| Vaccination  | + | Becton Dickinson & Co.  | BD Veritor™ Flu A + B  | BD Veritor Reader    | NPW, NA, NPS in VTM                              | No                       |
| Vaccine Effectiveness Studies  | + | Remel/Thermo Fisher   | XPECT™ Flu A & B   | N/A                  | Nasal wash                                       | No                       |
| Information for Clinicians on Influenza Virus Testing                          | - | 1 Available FDA cleared tests as of February 7, 2018. List may not include all available test kits approved by the FDA. |  |                      |  |                          |
| Overview of Influenza Testing Methods  |   |   |  |                      |  |                          |
| Information on Collection of Respiratory Specimens for Influenza Virus Testing |   |   |  |                      |  |                          |
| Clinical Signs & Symptoms of Influenza   |   |   |  |                      |  |                          |

<https://www.cdc.gov/flu/professionals/diagnosis/table-ridt.html>

# RT-PCR

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- Identify the presence of influenza viral RNA or nucleic acids in respiratory specimens with high sensitivity and specificity
- Identify influenza virus infection as a cause of respiratory outbreaks in institutions (e.g., nursing homes, chronic care facilities, and hospitals)
- Designed to accurately identify influenza A and B viral or nucleic acids by using conserved gene targets
- False negatives results can occur due to improper or poor clinical specimen collection or from poor handling of a specimen after collection and before testing (CDC, 2018)

*Nucleic Acid Detection*

# FDA-Cleared Nucleic Acid Detection Based Tests for Influenza Viruses

Table 3. FDA-cleared Nucleic Acid Detection Based Tests for Influenza Viruses<sup>1</sup>

|  | Complexity <sup>4</sup> | Manufacturer                | Product                          | Platform and/or Instrument | Influenza Virus Types Detected | Influenza Virus Subtypes Detected | Other Respiratory Viruses Differentiated   | Approved Specimens <sup>2</sup> | Test Time <sup>3</sup>       |
|--|-------------------------|-----------------------------|----------------------------------|----------------------------|--------------------------------|-----------------------------------|--|---------------------------------|------------------------------|
| Symptoms & Diagnosis +   | CLIA waived             | Alere                       | Alere i Influenza A & B          | Alere™i Platform           | Influenza A and B              | None                              | None   | NS direct                       | <15 min                      |
| Treatment +  | CLIA waived             | BioFire, Inc. BioMerieux    | FilmArray® Respiratory Panel EZ  | FilmArray 2.0 EZ           | Influenza A and B              | H1, H1pdm09, H3                   | Adenovirus, Coronavirus, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Parainfluenza Virus, Respiratory Syncytial Virus | NPS in VTM                      | 1-2 hr                       |
| Schools, Businesses & Travelers +  | CLIA waived             | Cepheid                     | Xpert Xpress Flu                 | GeneXpert Xpress           | Influenza A and B              | None                              | None   | NPS, NS in VTM                  | approximately 30 min or less |
| Flu Activity & Surveillance +  | CLIA waived             | Mesa Biotech, Inc           | Accula Flu A/Flu B               | Accula Dock                | Influenza A and B              | None                              | None   | NS direct                       | approximately 30 min         |
| FluVaxView   | CLIA waived             | Roche Molecular Diagnostics | Cobas® Influenza A/B Assay       | Cobas® Liat® Analyzer      | Influenza A and B              | None                              | None   | NPS in VTM                      | approximately 20 min         |
| Health Professionals -   | CLIA waived             | Roche Molecular Diagnostics | Cobas® Influenza A/B & RSV Assay | Cobas® Liat® Analyzer      | Influenza A and B              | None                              | Respiratory Syncytial Virus  | NPS in VTM                      | approximately 20 min         |
| Health Care Workers Need A Flu Vaccine   | Moderate                | Alere                       | Alere i Influenza A & B          | Alere™i Platform           | Influenza A and B              | None                              | None   | NS in VTM                       | <15 min                      |
| CDC Updates for Health Care Providers  | Moderate                | Alere                       | Alere i                          | Alere™i Platform           | Influenza A                    | None                              | None   | NS, NPS                         | <15 min                      |
| ACIP Recommendations +   |                         |                             |                                  |                            |                                |                                   |  |                                 |                              |
| Vaccination +  |                         |                             |                                  |                            |                                |                                   |  |                                 |                              |
| Vaccine Effectiveness Studies +  |                         |                             |                                  |                            |                                |                                   |  |                                 |                              |
| Information for Clinicians on Influenza Virus Testing -                        |                         |                             |                                  |                            |                                |                                   |  |                                 |                              |
| Overview of Influenza Testing Methods  |                         |                             |                                  |                            |                                |                                   |  |                                 |                              |
| Information on Collection of Respiratory Specimens for Influenza Virus Testing |                         |                             |                                  |                            |                                |                                   |  |                                 |                              |
| Clinical Signs & Symptoms of Influenza   |                         |                             |                                  |                            |                                |                                   |  |                                 |                              |

<https://www.cdc.gov/flu/professionals/diagnosis/table-nucleic-acid-detection.html>

*Nucleic Acid Detection*

# FDA-Cleared Nucleic Acid Detection Based Tests for Influenza Viruses

|  |          |                          |                                    |                                 |                   |                 |   |                             |                              |
|--|----------|--------------------------|------------------------------------|---------------------------------|-------------------|-----------------|---|-----------------------------|------------------------------|
| Symptoms & Laboratory Diagnosis  | Moderate | Alere                    | Alere i<br>Influenza A & B         | Alere i Platform                | Influenza A and B | None            | None  | NPS, NPS direct, NPS in VTM | < 15 min                     |
| Information for Clinicians on Rapid Diagnostic Testing for Influenza   | Moderate | BioFire, Inc. BioMerieux | FilmArray Respiratory Panel        | FilmArray® FilmArray® Torch     | Influenza A and B | H1, H1pdm09, H3 | Adenovirus, Coronavirus (HKU1, NL63, 229E, OC43), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Parainfluenza Virus (1, 2, 3, 4) Respiratory Syncytial Virus           | NPS in VTM                  | 1-2 hr                       |
| Rapid Influenza Diagnostic Tests (RIDTs)   |          |                          |                                    |                                 |                   |                 |   |                             |                              |
| Guidance: Use of Rapid Diagnostic Test   |          |                          |                                    |                                 |                   |                 |   |                             |                              |
| Information on Rapid Molecular Assays, RT-PCR, and other Molecular Assays for Diagnosis of Influenza Virus Infection | Moderate | BioFire, Inc. BioMerieux | FilmArray Respiratory Panel 2      | FilmArray® FilmArray® Torch     | Influenza A and B | H1, H1pdm09, H3 | Adenovirus, Coronavirus (HKU1, NL63, 229E, OC43), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Parainfluenza Virus (1, 2, 3, 4) Respiratory Syncytial Virus           | NPS in VTM                  | 30-60 min                    |
| <b>Nucleic Acid Detection Based Tests</b>  |          |                          |                                    |                                 |                   |                 |   |                             |                              |
| Medical Office Telephone Evaluation  |          |                          |                                    |                                 |                   |                 |   |                             |                              |
| UPDATE: Parotitis and Influenza  | Moderate | BioFire, Inc. BioMerieux | FilmArray Respiratory Panel 2 plus | FilmArray® 2.0 FilmArray® Torch | Influenza A and B | H1, H1pdm09, H3 | Adenovirus, Coronavirus (HKU1, NL63, 229E, OC43), Human Metapneumovirus, Human Rhinovirus/Enterovirus, MERS-CoV, Parainfluenza Virus (1, 2, 3, 4) Respiratory Syncytial Virus | NPS in VTM                  | 30-60 min                    |
| FAQ Parotitis and Influenza  |          |                          |                                    |                                 |                   |                 |   |                             |                              |
| Algorithm: Interpreting Influenza Testing Results When Influenza is Circulating                                      |          |                          |                                    |                                 |                   |                 |   |                             |                              |
| Algorithm: Interpreting Influenza Testing Results When Influenza is NOT Circulating                                  | Moderate | Cepheid                  | Xpert Xpress Flu                   | GeneXpert                       | Influenza A and B | None            | None  | NPS in VTM                  | approximately 30 min or less |
| Guide: Considering Influenza Testing   | Moderate | Cepheid                  | Xpert Xpress Flu/RSV               | GeneXpert                       | Influenza A and B | None            | Respiratory Syncytial Virus   | NPS in VTM                  | approximately 30 min or less |

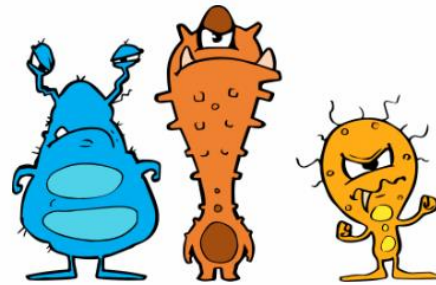
<https://www.cdc.gov/flu/professionals/diagnosis/table-nucleic-acid-detection.html>



# BioFire<sup>®</sup> Respiratory Panel

- Nucleic acid detection based test used by MDH Public Health Lab (personal communication, 2018)
- Delivers results on 17 viral and 3 bacterial targets in about 1 hour including

- Influenza A
- Influenza A/H1
- Influenza A/H3
- Influenza A/H1-2009
- Influenza B (BioFire Diagnostics, 2018)



Source: BioFire Diagnostics



# **Other Influenza Testing Methods**

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## **Viral Culture**

- Results do not yield timely results to inform clinical management
- Used for public health surveillance

## **Serologic Testing**

- Results do not yield timely results to help with clinical decision-making
- Used for research and public health investigations (CDC, 2018)

# References

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- BioFire Diagnostics. (2018). *BioFire® FilmArray® panels: Comprehensive panels and better diagnostics*. Retrieved from <https://www.biofiredx.com/products/the-filmarray-panels/>
- Centers for Disease Control and Prevention (CDC). (2018). *Influenza (flu)*. Retrieved from <https://www.cdc.gov/flu/index.htm>
- E. Luckman, personal communication [email]. September 11, 2018.