Expanding Surveillance using Influenza Platform

Laboratory Implementation of Molecular Testing of Respiratory Viruses other than Influenza (ORV)

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Organización Panamericana de la Salud (OPS)
Organización Mundial de la Salud (OMS)

Punta Cana, Dominican Republic May 23 – 25 2017
Outline

• Implementation of Molecular Diagnostic Testing for Respiratory Viruses other than Influenza (ORV)

• Real-time ORV RT-PCR Assays

• WHO RSV Initiative (Laboratory Components)
Other Respiratory Viruses
Benefits of Expanded Laboratory Surveillance

• Complements influenza surveillance
• Establish seasonality (inform clinical practice and lab testing)
• Disease burden and risk data in anticipation of vaccines (RSV)
• Broaden capacity for outbreak investigations
• Help pinpoint outbreaks of unknown etiology
• Reference site for confirmatory testing and training
• Respiratory outbreak investigations
  - Understanding of the disease, risk groups and transmission
  - Improve threat response (e.g. SARS-CoV and MERS-CoV)
Other Respiratory Viruses

CDC Real-time RT-PCR Assay Publications


**RSV Real-time RT-PCR Assay**

**CDC RSV Publications**


Expanding Laboratory Capacity
Other Respiratory Viruses (ORV)
Laboratory Implementation of rRT-PCR assays for ORV

- Pre-survey questionnaire
- Laboratory assessment / training
- Protocols
- Qualified reagents (primers & probes)
- Distribution of reagents
- PT panels (+/- and Limit of Detection LOD)
- Archival samples
- Conference calls/troubleshooting
- Sharing data/results
- Reporting
  - US NREVSS >> FluView
  - MoH > PAHO / WHO
Laboratory Implementation RSV rRT-PCR assay

WHO RSV Initiative (GISRS)

- Pre-survey questionnaire
- Protocols
  - Troubleshooting / translation
- Qualified reagents >> primers & probes, positive control
- Commercial invoices and Import permits
- RSV Proficiency panels (+/- and detection range)
  - CDC real-time PCR assay
  - Commercial and Laboratory Developed Test (LDT)
- Distribution of real-time reagents and RSV proficiency panel
- Country Report Proficiency panel to CDC
- Troubleshooting
- Country and WHO communications
- Reporting: NICs > MoH > PAHO > FluNet
CDC Real-time RT-PCR Assay
Protocols

Real-Time RT-PCR Assays for
Non-Influenza Respiratory Viruses
Centers for Disease Control and Prevention

Instructions for Use

Real-Time RT-PCR Assay for
Respiratory Syncytial Virus (RSV)
Centers for Disease Control and Prevention

Instructions for Use
**RSV Real-time RT-PCR Assay**

**Collection of Clinical Specimens**

**CDC Real-time RT-PCR Assay for Respiratory Syncytial Virus (RSV)**

- Respiratory samples: nasopharyngeal swab or oropharyngeal swabs
- Nasopharyngeal aspirates or washes
- Tracheal aspirate and bronchoalveolar lavage
- Sputum

**WHO Laboratory Testing**

**Collection of Clinical Specimens**

- Infants and young children: nasopharyngeal swab or nasal swab
  >> mid-turbinate of the nose, high recovery of respiratory viruses
- Young children: nasopharyngeal aspirates
- Older children, adolescents, adults: combined nasal and throat swabs
- Lower respiratory specimens: tracheal aspirate and bronchoalveolar lavage
- Sputum: older adults and elderly
RSV Real-time RT-PCR Assay

Nucleic Acid Extraction, Real-time Platforms and Enzyme Kits

**Extraction**
- QIAamp® MinElute® Virus Spin (QIAGEN)
- NucliSENS® EasyMag® and minimag® (bioMérieux)
- MagNA Pure Compact System (Roche Applied Science)

**qRT-PCR Enzyme Kits**
- SuperScript III Platinum One-Step qRT-PCR System (Life Technologies)
- Quanta qScript™ One-step qRT-PCR Low Rox (Quantabio / QuantaBioSciences)

**Real-time PCR Platforms**
- 7500 Standard or Fast Dx Real-Time PCR System (Applied Biosystems)
- Mx3000P QPCR System (Agilent Technologies)
- iCycler IQ5, CFX96 (Bio-Rad Laboratories)
RSV RT-PCR Assays

• Singleplex Assays (discrete reactions)

• Multiple Singleplex Assay Arrays (e.g. TaqMan Array Card)

• Multiplex Assays (combined reactions)
  - highly multiplexed (e.g. x-TAG)
  - limited multiplexed (e.g., rt-PCR)
Commercial Multiplex Respiratory Pathogen Assays

eSensor Respiratory Viral Panel
http://www.genmarkdx.com

FilmArray Respiratory Panel (v.1 & V.2)
www.biofiredx.com  www.filarray.com

Prodesse ProAssay
www.gen-probe.com

Verigene Respiratory Virus Plus
www.nanosphere.us

x-TAG RVP & RVP Fast
www.luminexcorp.com

FTD Respiratory 21
www.fast-trackdiagnostics.com

Respiratory Multi Well System MWS r-gene® range
duplex amplification - real-time PCR Taqman technology
http://www.biomerieux-diagnostics.com

Respiratory-V Cassette
www.icubate.com

TaqMan Array Cards (TAC)
Low density real-time PCR
https://www.lifetechnologies.com
**CDC Real-time RT-PCR Assays**

Primer/probe/+ control kits
- Pre-produced and packaged
- Quality assessment
- Respiratory Viruses *
  - RSV
  - Human metapneumovirus
  - Parainfluenza 1-3
  - Adenovirus
  - Rhinovirus
  - RNase P

* Other pathogens available for special studies
RSV Real-time RT-PCR Assay

Primer/Probes & Positive Control Kits
RSV Real-time RT-PCR Assay

CDC EQA Proficiency Testing Programs

- Matrix spiked with inactivated viruses
- Challenge/assessment of nucleic acid extraction and amplification
- Lyophilization/freeze-drying
- Reduced shipping cost
- Any suitable courier

Twenty samples:
- “proxy” assay testing
- 2 historical RSV strains (A and B)
- 2 recent RSV strains (A and B)
- Results:
  Positive/negative, reproducibility, contamination, other RSV assays
Other Respiratory Viruses
Challenges/Questions of Expanded Molecular Testing

• Who should test (NICs only? Regional laboratories? Testing Decentralization?)
• What to test for (viral targets to be included)
• Testing algorithm (all specimens; all specimens negative for influenza; selected specimens)
• Test methodology (CDC; commercial; laboratory-developed test)
• Costs (instrument; reagent/consumables; time)
• Test quality (qualified reagents; updated sequences)
• Certification/external proficiency testing
• Reporting results (established platforms)