Building Capacity for Laboratory Testing of Respiratory Viruses other than Influenza

Lessons learned from MERS-CoV and Respiratory Syncytial Virus (RSV)

Teresa C. T. Peret, PhD

Respiratory Viruses Branch (proposed)
Division of Viral Diseases
Centers for Disease Control and Prevention

Severe Acute Respiratory Infections Network SARI)
Surveillance in the Americas (SARI)net) Fourth Annual Meeting
Organización Panamericana de la Salud (OPS)
Organización Mundial de la Salud (OMS)

Punta Cana, Dominican Republic May 23 – 25 2017

The opinions in this presentation are those of the presenter and do not necessarily reflect the official position of the Centers for Disease Control and Prevention.
Outline

• MERS-CoV
  • Domestic (US) laboratory response
    • Real-time RT-PCR assays
    • EQA panels
    • Serological assays
  • International laboratory response
    • Deployment Real-time RT-PCR assays
    • Training

• RSV
  • WHO RSV Initiative (Laboratory Components)
Confirmed Cases of MERS-CoV 2012-2017 (12th May 2017)
MERS-CoV Domestic Response (1)

Real-time RT-PCR (rRT-PCR) Assays

- MERS-CoV rRT-PCR assays development
- Validation data submitted to the US FDA > Emergency use authorization (EUA)
- MERS-CoV rRT-PCR assays manufactured by CDC Scientific Resources
- Test deployment to domestic laboratories by the Laboratory Response Network (LRN)
- Testing for MERS-CoV in the US
# CDC MERS-CoV rRT-PCR Assays

## Primers & Probes

<table>
<thead>
<tr>
<th>Assay</th>
<th>Primers &amp; probes&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Sequence (5’ &gt; 3’)</th>
<th>50X working con. (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCV.N2 (CDC ver.1)</td>
<td>For GGC ACT GAG GAC CCA CGT T</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rev TTG CGA CAT ACC CAT AAA AGC A</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe CCC CAA ATT GCT GAG CTT GCT CCT ACA</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>NCV.N3 (CDC ver.1)</td>
<td>For GGG TGT ACC TCT TAA TGC CAA TTC</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rev TCT GTC CTG TCT CCG CCA AT</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe ACC CCT GCG CAA AAT GCT GGG</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>NCV.upE (Corman ver.1)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>For GCA ACG CGC GAT TCA GTT</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rev GCC TCT ACA CGG GAC CCA TA</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe CTC TTC ACA TAA TCG CCC CGA GCT CG</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Primer/probes designed from published MERS-CoV sequence, GenBank accession no, JX869059. TaqMan® probes are labeled at the 5’-end with the reporter molecule 6-carboxyfluorescein (FAM) and at the 3’-end with Black Hole Quencher® 1 (Biosearch Technologies Inc., Novato, CA).

CDC MERS-CoV rRT-PCR Assays
Primer/Probes Positive Control Kit
MERS-CoV Domestic Response (2)

MERS-CoV Propagation in Cell Culture
MERS-CoV (strain Jordan-N3/2012) propagation (γ-IR)

MERS-CoV Proficiency Panels ((PT)
• PT Panels for MERS-CoV rRT-PCR assays
• Two generations:
  • Frozen panel/dry ice (2013-14)
  • lyophilized samples/cold pack (2015-17)

Serological Assays
• A two-phased approach was used for MERS-CoV serology:
  • screening by enzyme immunoassay (EIA)
  • confirmatory testing by immunofluorescence (IFA) and microneutralization (MNT) assays
CDC MERS-CoV rRT-PCR Assays
Proficiency Panel (PT)
MERS-CoV Proficiency Panel (PT)

Commercial Sources

QCMD
Quality Control for Molecular Diagnostics

MERS CoV
QCMD 2015 Middle East Respiratory Syndrome Coronavirus (MERS CoV) EQA Pilot Study

RCPAQAP
RCPA Quality Assurance Programs

WHO & RCPAQAP
PTP for the Detection of MERS-CoV by PCR
**CDC MERS-CoV Serology Assays**

- **ELISA-based assays**
  - Whole MERS-CoV infected cells lysate (γ-IR)
  - Recombinant MERS-CoV Nucleocapsid (N) and S Proteins (second gen)

- **Immunofluorescence assay (IFA)** *
  
  ![Immunofluorescence assay images](image)

- **Microneutralization Assay (MERS-CoV MNt)** **
  - Detection of neutralizing antibodies
  - No large scale screening; technically challenging (BSL-3)
  - Correlation with MERS-CoV N and MERS-IFA assays

* Jennifer Harcourt, DVD, CDC
** Azaibi Tamin, DVD, CDC
MERS-CoV International Response

MERS-CoV rRT-PCR assays and EQA panels deployment

• 49 countries on 5 continents
• Support MERS-CoV surveillance, reference testing and epidemiology studies

• Six regional training courses
  • WHO regional offices
  • 42 countries

• Serological assays
  • International studies
  • Serological testing capacity transferred to the Kingdom of Saudi Arabia
### CDC rRT-PCR MERS-CoV Assay Deployment (May 2017)

**EMRO**
- Bahrain CPHL
- Egypt CPHL
- Iran CPHL
- Iraq CPHL
- Jordan CPHL
- King Faisal U, KSA
- KSA CPHL
- Lebanon CPHL
- Libya CPHL
- Morocco CPHL
- Oman CPHL
- Pakistan CPHL
- Qatar CPHL
- Sudan CPHL
- Syria CPHL
- Tunisia CPHL
- UAE CPHL
- W. Palestine CPHL
- Yemen CPHL

**CDC GDD**
- Bangladesh CPHL
- Bangladesh GDD
- Egypt GDD
- Georgia GDD
- India (NCDC)
- Kenya GDD
- Thailand GDD
- Uganda GDD

**DoD/GEIS**
- Egypt (NAMRU-3)
- Germany (Landstuhl, LRMC)
- Kenya (USAMRU-K)
- South Korea (121st US Military Lab)
- US-CA, San Diego (NHRC)
- US-HI, Tripler (AMC)
- US-MD, USAMRID
- US-MD, Walter Reed (AMC)
- US-MD, Naval Infectious Diseases
- Diagnostic Laboratory (NIDDL)
- US-OH, Wright Patterson
  (USAFSAM)
- US-TX, Brooke (AMC) San Antonio
- US-WA, Madigan (AFB) Seattle
- VA Public Health Reference Lab, CA

**Other – International**
- Armenia CPHL
- Azerbaijan CPHL
- India (NIV-Pune)
- Indonesia (Research Facility)
- Indonesia CPHL
- Kyrgyzstan CPHL NIC
- Macedonia CPHL NIC
- Georgia NCDC NIC
- South Korea CDC/NIH
- The Philippines CPHL
- Turkey CPHL
- Uzbekistan CPHL
- Vietnam, Hanoi (NIHE NIC)
- Vietnam, Ho Chi Minh City (PI-HCMC NIC)

**PAHO**
- Argentina CPHL NIC
- Brazil CPHL NIC
- Chile CPHL NIC
- Canada CPHL NIC
- Mexico CPHL NIC
- Trinidad CARPHA NIC

**Other – Domestic**
- USDA - Ames, IA
- USDA - Plum Island, NY

**APDRS**
- Bangladesh GDD
- Algeria CPHL/Pasteur
- Ethiopia CPHL
- Nigeria CPHL
- Niger CPHL
- Tanzania CPHL

**AFRO**
- USDA - Ames, IA
- USDA - Plum Island, NY

**Courtesy:** Eileen Schneider, DVD, CDC
Global CDC Kit Deployment & Training
MERS-CoV: Lessons Learned

- Flexibility of existing surveillance platforms for respiratory infections
- Need to improve dissemination of data
- Need to improve system of sharing specimens and viruses
- Need early coordinated effort among responding international reference labs in assay design, development and deployment
- Encourage partnerships with qualified reference laboratories that can confirm test results and obtain genomic sequence
- Need prompt and sustained proficiency testing to monitor test performance
MERS-CoV
5 Years Later – We Know More

• Genome sequences deposited in GenBank
  – human and camel derived
  – > 800 sequences from 2012 - 2017

• Published assays

• Preferred specimens: LRT, URT, acute serum

• Serology has matured to include multiple assays
  with more robust performance data
MERS-CoV

Diagnostic Gaps

- Alternative FDA cleared MERS-CoV assays (in process)
- Integrated multi-pathogen assay panels (in process)
- Rapid Tests (POC)
- Tests for veterinary applications
- Generic CoV assays
Surveillance of Respiratory Syncytial Virus on the Global Influenza Surveillance and Response System (GISRS) Platform  
(WHO RSV Initiative)
Implementation of CDC RSV rRT-PCR assay

- Pre-survey questionnaire ✔
- Material transfer agreement (MTA) CDC TTO ✔
- CDC Real-time RT-PCR Protocol
  - ✔ troubleshooting ✔ translation ✔
- Commercial invoices + Import permits
- Qualified reagents >> primers & probes ✔ PC ✔
- EQA/ Proficiency panels (+/- and detection range) ✔
  - ✔ CDC real-time PCR assay
  - ✔ Laboratory Developed Test (LDTs)
- Distribution of real-time reagents and EQA proficiency panels ✔
- Reporting EQA/ Proficiency panels ✔
- Troubleshooting ✔
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)
CDC RSV Real-time RT-PCR Assay
Primers and Probes

<table>
<thead>
<tr>
<th>Assay</th>
<th>Primer/probes</th>
<th>Sequence (5' &gt; 3')</th>
<th>50X (μM)(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory syncytial virus</td>
<td>For</td>
<td>GGC AAA TAT GGA AAC ATA CGT GAA</td>
<td>25</td>
</tr>
<tr>
<td>(ver. 1)</td>
<td>Rev</td>
<td>TCT TTT TCT AGG ACA TTG TAY TGA ACA G</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Probe(^{b})</td>
<td>CTG TGT ATG TGG AGC CTT CGT GAA GCT</td>
<td>2.5</td>
</tr>
<tr>
<td>Human RNase P control (RNP)</td>
<td>For</td>
<td>AGA TTT GGA CCT GCG AGC G</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Rev</td>
<td>GAG CGG CTG TCT CCA CAA GT</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Probe(^{b})</td>
<td>TTC TGA CCT GAA GGC TCT GCG CG</td>
<td>5</td>
</tr>
</tbody>
</table>

- 50X working concentration of primers and probes
- TaqMan® probes are labeled at the 5’-end with the reporter molecule 6-carboxyfluorescein (FAM) and at the 3’-end with the quencher, Black Hole Quencher® 1
CDC RSV Real-time RT-PCR Assay
Primer/Probes & Positive Control Kits

**CDC Respiratory syncytial virus (RSV) and Human RNase P control (RNP) Real-time RT-PCR Primers/Probes**

**CATALOG:** K02513

**EXPIRATION DATE:** 04/2018

**INTENDED USE:** CDC real-time RT-PCR primers/probes are intended for the in vitro qualitative detection of respiratory syncytial virus (RSV) and Human RNase P control (RNP).

**USE LIMITATIONS:** For research use only. Not intended for clinical diagnostic use.

**REAGENTS:**

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>LABEL</th>
<th>START NUMBER</th>
<th>LOT NUMBER</th>
<th>QUANTITY PER VIAL</th>
<th>REMOVAL VOLUME</th>
<th>STATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resuspended Ladder (K02513)</td>
<td>K02513</td>
<td>70102</td>
<td>X71725</td>
<td>0.1 ml</td>
<td>0.2 ml</td>
<td></td>
</tr>
<tr>
<td>Positive Control (K02513)</td>
<td>K02513</td>
<td>70102</td>
<td>X71725</td>
<td>0.1 ml</td>
<td>0.2 ml</td>
<td></td>
</tr>
<tr>
<td>Internal Control (K02513)</td>
<td>K02513</td>
<td>70102</td>
<td>X71725</td>
<td>0.1 ml</td>
<td>0.2 ml</td>
<td></td>
</tr>
</tbody>
</table>

Probes contain 8 AM and 8 HM quencher. Approximate number of tests per kit: 1000.

**REMOVAL VOLUME:** Resuspended ladder and positive control are removed in buffer for 3 minutes. The ladder and positive control are then diluted in 500 μl of water.

**STORAGE CONDITIONS:** Store at room temperature or at 4°C. Do not freeze.

**PROCEDURE/INTERPRETATION/LIMITATIONS:** Refer to the CDC Real-Time RT-PCR Assay for Respiratory Syncytial Virus (RSV) protocol for instructions.
Stock Reagent Preparation – Positive Control

- Read the package insert carefully before set-up
- Store first and second dilutions at -70°C
- Do not reuse a single use aliquot
- Do not use laboratories used for clinical specimens handling and testing to set-up the positive control
**RSV Real-time RT-PCR Assay**

**Master Mix and Plate Set-Up**

8. Mix reaction components by pipetting slowly up and down (avoid bubbles).
9. Add 20 µL of each master mix into each well of a chilled optical plate as in the example below:

![Diagram showing the setup of master mix and plate set-up](image)

- No template reaction mix controls (NTC) used to check for reagent contamination (column 1), sample extracts (S); no template extraction control (NTC) used to check for contamination occurring during extraction or specimen extract handling during set-up (column 1); viral template control (VTC) used to assess assay performance (column 12).
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

- 20 samples:
  - “proxy” assay testing
  - 2 historical RSV strains (A and B)
  - 2 recent RSV strains (A and B)
  - Material for 2 tests
  - Results:
    - +/-, reproducibility, contamination, distinction between A and B, other RSV assays (limited)
December 8th, 2016

Dear colleagues,

An external quality assessment (EQA) panel is being provided to assess your continued competence in performing real-time RT-PCR assays for respiratory syncytial virus (RSV). Each panel consists of 20 mock clinical specimens spiked with cell cultured RSV or uninfected cells. All samples have been gamma irradiated and are biologically inert. Multiple sample panels were evaluated by CDC and the expected Ct values were obtained. Please refer to package insert for explicit instructions for testing the EQA panel.

Please use the attached form for reporting your test results. All test results will be held in strict confidence. Record the test results (Ct values) you obtain for each individual assay on the included report form. Please note some fields have “drop down” menus. Each laboratory will receive their individual results and an aggregate summary of test results will be sent to all laboratories later in an anonymous manner.

If you have any questions regarding the RSV Real-time RT-PCR Assay 2016 EQA, please do not hesitate to contact us.

Sincerely yours,
### CDC RSV Real-time RT-PCR Assay 2016 EQA Panel Report Form

#### General Information

| Facilitator Name: | | | | |
|-------------------|---|---|---|
| Point of Contact: | | | |
| Address: | | | |
| City: | State/Province: | Country: | |
| Postal Code: | | | |
| Email Address: | | | |

#### RNA Extraction Method

| Product Name: | Choose an item: | Other (specify): | |
|--------------|----------------|-----------------|
| Catalog Number: | Lot Number: | | |
| Equipment Name and Model (if applicable): | | | |
| Other Information (please specify): | | | |

#### Real-time RT-PCR Assay (master mix)

| Master Mix: | Choose an item: | Other (specify): | |
|-------------|----------------|-----------------|
| Product Name: | Choose an item: | Other (specify): | |
| Catalog Number: | Lot Number: | | |

#### Real-time PCR Instrument

| Manufacturer: | Choose an item: | Other (specify): | |
|---------------|----------------|-----------------|
| Product Name and Model: | Choose an item: | Other (specify): | |
| Software Name and Version: | | | |

### CDC RSV Real-time RT-PCR Assay currently used by your lab

<table>
<thead>
<tr>
<th>Assay:</th>
<th>Choose an item:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog Number:</td>
<td>Lot Number:</td>
<td></td>
</tr>
<tr>
<td>Equipment Name and Model (if applicable):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Information (please specify):</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Other Remarks:

<table>
<thead>
<tr>
<th>Remarks:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference/Citation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Remarks:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Distribution Copy**

**Effective:** 05/14/2018

**Page:** 1 of 4

**Version:** RSV 001

---
# CDC EQA Proficiency Panel

## RSV EQA Report Form 2

### CDC RSV Real-time RT-PCR Assay 2016 EQA Panel Report Form

<table>
<thead>
<tr>
<th>Remarks</th>
<th>Choose an Item</th>
<th>Choose an Item</th>
<th>Choose an Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Real-time RT-PCR Assay:</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
</tr>
<tr>
<td>Date of EQA Panel Testing:</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
</tr>
<tr>
<td>Submitter’s name:</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
</tr>
<tr>
<td>Submitter’s name performing CDC real-time RT-PCR:</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
</tr>
<tr>
<td>Supervisor’s name:</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
</tr>
<tr>
<td>Supervisor Reviewed:</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
</tr>
<tr>
<td>Supervisor Signature:</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
<td>Choose an Item</td>
</tr>
</tbody>
</table>

### CDC RSV Real-time RT-PCR Assay 2016 EQA Panel Report Form

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>CDC Real-time RT-PCR assay (Ct values)</th>
<th>Real-time RT-PCR assay currently in use by your lab (Ct values)***</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>RSV</td>
<td>Choose an Item</td>
<td></td>
</tr>
</tbody>
</table>

*** If different from the CDC real-time RT-PCR assay.

Please save this file followed by the country name “RSV EQA Panel Report Form, Country Name” per the instructions on page 1 of the document. Email TParad@cdc.gov.
WHO RSV Pilot Proficiency Testing Programs

RSV PT Panel

• 13 countries
• Pre-survey
• RSV EQA Report
  • Real-time Platform: Applied 7500 and BioRad (2)
  • Extraction Platforms: EasyMag (1) Qiagen (12)
  • Real-time RT-PCR chemistry: AgPath and SuperScript III Platinum One-Step qRT-PCR System
• Compilation
• Final Report
## Contrasting MERS & RSV Laboratory Capacity Surveillance

### MERS-CoV

**Objective:** identify any MERS cases; emergency public health response

**Subjects:** any person that meets MERS case definition; testing country-wide; surveillance duration unlimited (?)

**Features:** rare, travel associated; low predictive value positive (PVP) test

**Laboratory:** high sensitivity/specificity tests essential; requires multiple rRT-PCR signatures for MERS-CoV detection and confirmation + confirmation by reference laboratory; should include testing for other respiratory pathogens to “rule in” other etiologies; wide network of qualified laboratories needed to minimize test result turn-around time; EQA programs essential, but restricted availability

### RSV

**Objective:** research to determine seasonality, risk groups, disease burden, etc.

**Subjects:** target children <5 yrs. (or elderly ≥65 yrs.) who meet RSV case definition; usually limited to networks of representative pediatric hospitals (or long-term care facilities); surveillance duration determined by study

**Features:** common, seasonal; high predictive value positive (PVP) test

**Laboratory:** high sensitivity/specificity tests ideal; single rRT-PCR signature for RSV sufficient; rapid tests may be effective; multiple commercial FDA or CE-marked molecular assays available; EQA programs ideal and more readily available