

# Expanding Surveillance using Influenza Platform

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

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Division of Viral Diseases  
Centers for Disease Control and Prevention

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

Punta Cana, Dominican Republic May 23 – 25 2017

## ***Outline***

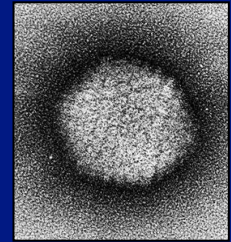
- Implementation of Molecular Diagnostic Testing for Respiratory Viruses other than Influenza (ORV)
- Real-time ORV RT-PCR Assays
- WHO RSV Initiative (Laboratory Components)



# 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

- Dare RK, Fry AM, Chittaganpitch M, et al. **Human coronavirus** infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis* 2007;196:1321-28.
- Fry AM, Chittaganpitch M, Baggett HC, et al. The burden of hospitalized lower respiratory tract infection due to **respiratory syncytial virus** in rural Thailand. *PLoS One* 2010;5:e15098.
- Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of **human adenovirus** DNA by real-time PCR. *Med Virol* 2003;70:228-39.
- Lu X, Holloway B, Dare RK, et al. Real-time reverse transcription-PCR assay for comprehensive detection of **human rhinoviruses**. *J Clin Microbiol* 2008;46:533-39.
- Lu X, Chittaganpitch M, Olsen SJ, et al. Real-time PCR assays for detection of **bocavirus** in human specimens. *J Clin Microbiol* 2006;44:3231-5.
- Morgan OW, Chittaganpitch M, Clague B, et al. Hospitalization due to **human parainfluenza virus-associated** lower respiratory tract illness in rural Thailand. *Influenza Other Respi Viruses*. 2012 Jun 21. doi: 10.1111/j.1750-2659.2012.00393.x. [Epub ahead of print]



# RSV Real-time RT-PCR Assay

## CDC RSV Publications

- Fowlkes A, Giorgi A, Erdman D, et al. Viruses associated with acute respiratory infections and influenza-like illness among outpatients from the Influenza Incidence Surveillance Project, 2010-2011. *J Infect Dis.* 2014;209:1715-25.
- Fry AM, Chittaganpitch M, , Peret, TCP et al. The burden of hospitalized lower respiratory tract infection due to **respiratory syncytial virus** in rural Thailand. *PLoS One* 2010;5:e15098.
- Haynes AK, Manangan AP, Iwane MK, et al. **Respiratory syncytial virus** circulation in seven countries with Global Disease Detection Regional Centers. *Infect Dis.* 2013;208 Suppl 3:S246-54. doi: 10.1093/infdis/jit515.
- Jain S, Williams DJ, Arnold SR, Ampofo K, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. *N Engl J Med.* 2015;372:835-45.
- Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. *N Engl J Med.* 2015; 373:415-427.



**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

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Centers for Disease Control and Prevention

Instructions for Use



Non-Influenza Respiratory Viruses rRT-PCR Assays  
Instructions for Use - ver.009.2015

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## Real-Time RT-PCR Assay for Respiratory Syncytial Virus (RSV)

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Centers for Disease Control and Prevention

Instructions for Use



Respiratory Syncytial Virus rRT-PCR Assay  
Instructions for Use - ver.003.2016

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# ***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)

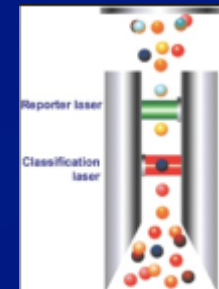
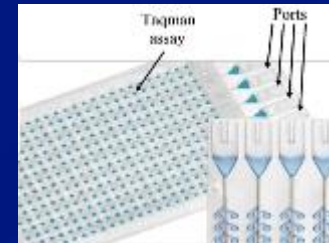
### **Real-time PCR Platforms**

- 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>

RT-PCR and hybridization

FilmArray Respiratory Panel (v.1 & V.2)

[www.biofire.com](http://www.biofire.com) [www.filmarray.com](http://www.filmarray.com)

Biofilm array (pouch)

Prodesse ProAssay

[www.gen-probe.com](http://www.gen-probe.com)

Multiplex and singleplex RT-PCR melting curve analysis

Verigene Respiratory Virus Plus

[www.nanosphere.us](http://www.nanosphere.us)

Multiplexed nucleic acid test gold nano-particle technology

x-TAG RVP & RVP Fast

[www.luminexcorp.com](http://www.luminexcorp.com)

Multiplex RT-PCR microsphere suspension array

FTD Respiratory 21

[www.fast-trackdiagnostics.com](http://www.fast-trackdiagnostics.com)

Multiplex real-time PCR

Respiratory Multi Well System MWS r-gene® range

<http://www.biomerieux-diagnostics.com>

duplex amplification - real-time PCR Taqman technology

Respiratory-V Cassette

[www.icubate.com](http://www.icubate.com)

Amplicon rescued multiplex RT-PCR

TaqMan Array Cards (TAC)

<https://www.lifetechnologies.com>

Low density real-time PCR



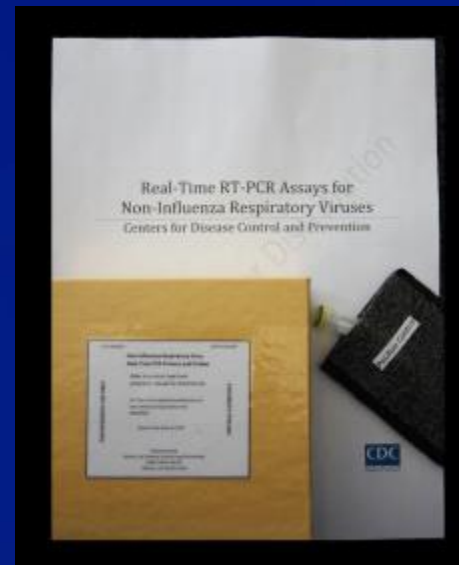
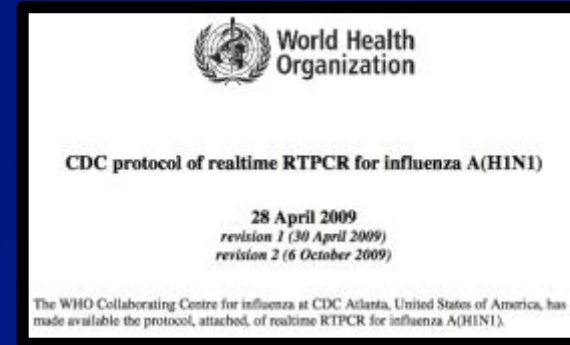
# CDC Real-time RT-PCR Assays

## Primer/probe/+ control kits

- Pre-produced and packaged
- Quality assessment
- Respiratory Viruses \*

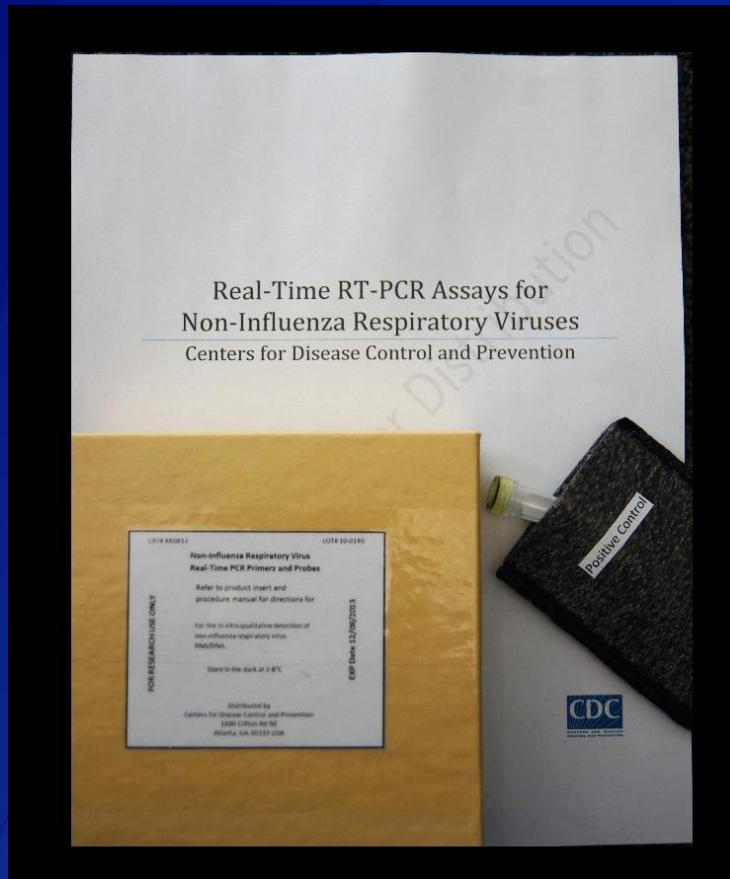
  - RSV
  - Human metapneumovirus
  - Parainfluenza 1-3
  - Adenovirus
  - Rhinovirus
  - RNase P

\* Other pathogens available for special studies





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



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
Centers for Disease Control and Prevention (CDC)  
Atlanta, GA 30333

**"DO NOT DISCARD: Important product-specific information"**

**CDC Non-Influenza Respiratory Virus RT-PCR Positive Control**

LOT: TX102710

**INTENDED USE:** For use as positive control for CDC real-time RT-PCR assays for detection of non-influenza respiratory viruses, including respiratory syncytial virus, human parainfluenza viruses 1, 2, 3, 4, human metapneumovirus, adenovirus, human coronavirus 229E, OC43, NL63, B HCoV1, and rhinovirus, as well as human rhinovirus C (rhinovirus C).

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

**REAGENT:** Precipitated and dried RNA transcript compatible only with CDC non-influenza respiratory virus real-time RT-PCR assays. The amount provided is concentrated and must be diluted to achieve working concentration. Rehydration volume: 100 µL.

**PRECAUTIONS:** This reagent should be handled in an approved nucleic acid handling area to avoid contamination of laboratory equipment and reagents that could cause false positive results. This product is an in vitro transcript and is non-infectious. However, this particular product should be handled in accordance with Good Laboratory Practices. Store reagent at appropriate temperatures (see below) and hold on when thawed.

**STORAGE CONDITIONS:** Upon receipt, immediately store at -20°C or below. After rehydration, store at -70°C.

**PROCEDURE/INVENTORY LIMITATIONS:**

- Positive control material must be diluted and aliquoted before use.
- Centrifuge the tube at max speed for 20 sec to remove pellet (pellet is a bright pink color) at the bottom.
- Add 1000 µL of cold RNA Storage Solution (Antisense) or nuclease free TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and mix gently three spin down liquid. Pellet is in solution when no pink precipitate is visible.
- To ensure complete resuspension, hold tube on ice for 20 min before handling further.
- Aliquot in 100 µL volumes and store at -70°C. These aliquots will be your stock concentrations.
- To obtain a working concentration, dilute 100 µL of the stock concentrate into 10 mL of cold RNA Storage Solution or nuclease free TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and mix gently.
- Dispense working dilution into single use aliquots and store at -70°C.
- Thaw a single working dilution aliquot for each experiment. Discard any unused portion of the aliquot. Do not refreeze.
- Add 5 µL of positive control to each specific positive control reaction
- Expected Ct values < 35.
- A running record of positive control Ct values should be kept. If records show a trend of rising Ct values for one or more assays, the positive control may need to be replaced by a freshly diluted stock.

**REAGENT COMMENT/QUESTIONS:** Please send comments/questions by email to [cdc@cdc.gov](mailto:cdc@cdc.gov)

**DISTRIBUTION**  
Distributed by the Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, Atlanta, GA 30333 USA.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
Centers for Disease Control and Prevention (CDC)  
Atlanta, GA 30333

**"DO NOT DISCARD: Important product-specific information"**

**CDC Non-Influenza Respiratory Virus Real-time RT-PCR Primers/Probes**

CATALOG: KK0813

LOT: 10-0149

EXPIRATION DATE: 12/09/13

**INTENDED USE:** CDC real-time RT-PCR primer/probes are intended for the in vitro qualitative detection of the non-influenza respiratory viruses listed below.

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

**REAGENTS:**

DESCRIPTION	LABEL	PART NUMBER	LOT NUMBER	QUANTITY PER VIAL	REHYDRATION VOLUME	STATE
<b>Adenovirus</b>						
Forward	Adeno-F	SO3331	10-0040	25 nmol	0.5 mL	lyophilized
Reverse	Adeno-R	SO3332	10-0041	25 nmol	0.5 mL	lyophilized
Probe	Adeno-P	SO3333	10-0042	8 nmol	0.5 mL	lyophilized
<b>Respiratory syncytial virus</b>						
Forward	RSV-F	SO3310	10-0025	25 nmol	0.5 mL	lyophilized
Reverse	RSV-R	SO3320	10-0026	12.5 nmol	0.5 mL	lyophilized
Probe	RSV-P	SO3321	10-0027	2.5 nmol	0.5 mL	lyophilized
<b>Human metapneumovirus</b>						
Forward	HMPV-F	SO3316	10-0022	20 nmol	0.5 mL	lyophilized
Reverse	HMPV-R	SO3317	10-0023	30 nmol	0.5 mL	lyophilized
Probe	HMPV-P	SO3318	10-0024	5.0 nmol	0.5 mL	lyophilized
<b>Rhinovirus</b>						
Forward	Rhine-F	SO3431	10-0048	60 nmol	0.5 mL	lyophilized
Reverse	Rhine-R	SO3438	10-0045	60 nmol	0.5 mL	lyophilized
Probe	Rhine-P	SO3439	10-0046	5 nmol	0.5 mL	lyophilized
<b>Parainfluenza virus 1</b>						
Forward	PIV1-F	SO3322	10-0059	25 nmol	0.5 mL	lyophilized
Reverse	PIV1-R	SO3323	10-0060	25 nmol	0.5 mL	lyophilized
Probe	PIV1-P	SO3324	10-0061	2.5 nmol	0.5 mL	lyophilized
<b>Parainfluenza virus 2</b>						
Forward	PIV2-F	SO3325	10-0062	37.5 nmol	0.5 mL	lyophilized
Reverse	PIV2-R	SO3326	10-0063	37.5 nmol	0.5 mL	lyophilized
Probe	PIV2-P	SO3327	10-0064	7.5 nmol	0.5 mL	lyophilized
<b>Parainfluenza virus 3</b>						
Forward	PIV3-F	SO3328	10-0065	37.5 nmol	0.5 mL	lyophilized
Reverse	PIV3-R	SO3329	10-0066	25 nmol	0.5 mL	lyophilized
Probe	PIV3-P	SO3330	10-0067	10 nmol	0.5 mL	lyophilized

Probes contain 6-FAM reporter and BHQ1 quencher. Approximate number of wells per kit: 1,000.

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# RSV Real-time RT-PCR Assay

## CDC EQA Proficiency Testing Programs

### DO NOT DISCARD: Important Product-Specific Information

**DESCRIPTION:** Proficiency Panel for SARS-CoV Real-time RT-PCR

**CDC/LRN CATALOG NO.:** KT0056

**LOT:** 03-0411

**EXPIRATION DATE:** 04/01/2016

**INTENDED USE:** Evaluation of LRN SARS-CoV real-time RT-PCR assay. Do not reuse. Do not redistribute.

### DO NOT DISCARD: Important Product-Specific Information

**DESCRIPTION:** Proficiency Test Panel for Respiratory Virus Real-time PCR Assays

**LOT:** 101306, 12/26/2006

**INTENDED USE:** Evaluation of respiratory virus real-time PCR assays. Do not reuse. Do not redistribute.

**REAGENTS:** Each panel consists of 10 human plasma samples spiked with cell cultured respiratory viruses (200 µl per sample). All samples have been gamma irradiated, however, processing of samples for PCR should be performed using BSL-2 safety procedures.

**INSTRUCTIONS/STORAGE:** Samples should arrive packed on dry ice. Samples should be immediately transferred to a -70°C freezer and held there until ready to test.

**PROCEDURE:** On day of test, rapidly thaw samples in 37°C water bath. Before opening, briefly spin down any fluid in the vial cap. Nucleic acid extractions should be performed immediately and in numerical order. Remove 200 µl of each sample for extraction; if your extraction protocol requires <200 µl of sample, please indicate volume used on the report form. Perform extraction procedure following manufacturer's instructions. Extracts should be tested immediately by PCR following the CDC procedure. To determine accurate Ct values for the test samples, analyze each target separately. Analyze the amplification plots in the log view instead of the linear view and set the threshold in the middle of the exponential phase of the curve (see Figure). If there are multiple curves, set the threshold at the average midway point through all curves.

- 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)

