# What's New With PIV, RSV, hMPV and other respiratory acronyms you can't live without

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## Objective: Provide an update on respiratory viruses and the role of laboratory in testing for these organisms.

- Review new viruses detected in respiratory tract in last decade
  - Metapneumovirus
  - Multiple coronaviruses: SARS, 229E, NL63, OC43, HKU1.
  - Human bocavirus
  - Polyomaviruses KI, WU
- Highlight clinical significance of key noninfluenza viruses.
- Summarize results from respiratory viral testing at PHL, OAHPP.
- Discuss rationale for investing resources in identifying respiratory viruses.

# Why Expanding Role for Diagnostic Virology Lab?

- Recent availability of antiviral agents
- Increased pool of immunocompromised
- More focus on epidemiology
- Results in increasing demand for rapid methods, viral load testing, antiviral susceptibility, genotyping.

# Overview of respiratory viruses – key players

- Influenza A, B
- Respiratory Syncytial Virus
- Parainfluenza virus 1-4
- Rhinovirus (and enteroviruses)
- Adenovirus
- More recently described respiratory viruses:
  - Multiple coronaviruses: SARS, 229E, NL63, OC43, HKU1.
  - Human bocavirus
  - Polyomaviruses KI, WU

# Human Metapneumovirus

- Discovered in 2001, causes RTI in all age groups.
- RNA virus,
- hMPV and RSV in *Pneumovirinae* subfamily of the *Paramyxoviridae* family.
- Four major genotypes (A1,A2, B1,B2)
  - 2 major antigenic subgroups (A and B).
- Detected in serum from 1958.

# Human Metapneumovirus: Epidemiology

- Transmission likely by droplet spread.
  - Healthcare associated infections documented
- Annual epidemics late winter, early spring.
  - Coincides/overlaps with RSV season.
  - Sporadic infection year round.
- Incubation period 3-5 days.
- Viral shedding 1 to 2 weeks.
  - Immunocompromised may shed for months.

# Human Metapneumovirus: Clinical Manifestations

- Bronchiolitis, asthma exacerbations, croup.
- URIs with concomitant OM.
- Most healthy children mild URI, some sever LRTI.
- Immunosuppressed at increased risk severe disease.
- All children infected at least once by 5yo.
  - Recurrent infections throughout life.
- Potential antiviral treatment: susceptible to ribavirin in vitro - no controlled clinical studies as yet.

# "Newer" respiratory viruses: Human Metapneumovirus

- Children <5yo with URI, 1982-2001:
  - hMPV RNA in 3% of 2710 URI episodes (5% of 2384 culture neg.)
    - RSV in 6%, influenza in 6%, PIV in 7%.
  - Mean age hMPV+ 20 months
  - 78% from Dec-May.
  - Acute otitis media (AOM) in 50% (similar to influenza, RSV, PIV).
  - 54% febrile (vs 84% with influenza febrile)
  - Williams et al. JID 2006; 193:387–95

- Detected in 2.2% of 405 samples from community patients with ILI negative for influenza/RSV (1.3% of all samples submitted).

# Human Metapneumovirus

**Table 1. Clinical features at presentation of 118 children with human metapneumovirus upper respiratory tract infections.**

| Symptom/sign | Children with symptom/sign, % |
|---|---|
| Fever | 54 |
| Coryza | 82 |
| Cough | 66 |
| Hoarseness | 8 |
| Otalgia | 31 |
| Rhinitis | 79 |
| Conjunctivitis | 3 |
| Pharyngitis | 44 |
| Abnormal tympanic membrane | 63 |

JID 2006:193 (1 February) • Williams et al.

# Human Bocavirus

- DNA virus, family parvoviridae; first identified in 2005 in children with acute RTI.
- Name derives from similarity to **bo**vine parvovirus 1 and **ca**nine minute virus.
- 2 distinct genotypes; no data regarding antigenic variation or distinct serotypes.
- Detection only described in humans.
- Transmission presumed respiratory secretions; fecal-oral also possible.
- Duration of shedding not known.
- Circulates worldwide and throughout the year.

# Human Bocavirus

- Prominent symptoms: cough, rinorrhea, fever.
- Associated with episodes of wheezing.
- Detected in 5% to 10% of all children with acute upper and lower RTI.
- Role as a pathogen confounded by simultaneous detection of other viruses in up to 80%.
  - Has been detected in resp tract, blood and stool of some ill children.
  - Seroconversion documented after symptomatic disease.
- Infection ubiquitous – all children seropositive by 5yo.

# Human Bocavirus

- Laboratory Detection
  - HBoV PCR and serology mostly used by research labs.
  - Now included in commercial multiplex assays.

- Treatment – no specific therapy available.

# Human Coronaviruses 229E, NL63, OC42, HKU1

- Large, enveloped RNA viruses
- 3 antigenic groups
  - I: Human CoVs 229E and NL63 and animal CoVs.
  - II: Human CoV OC43
  - III: avian CoVs.

- Frequent cause of "common cold" and also associated with LRTIs.
- Mostly transmitted by respiratory tract secretions.
- Incubation period 2-5 days.
- Mostly infectious in early days of illness.

# Human Coronaviruses: 229E, NL63, OC42, HKU1

- Distributed worldwide.
- Outbreaks in winter in temperate climates.
- Laboratory detection:
  - Grown in research labs in specialized cell lines.
  - Molecular assays
  - Serology

- Treatment – no proven antiviral therapy.

# Polyomaviruses: KI and WU

- Family polyomaviridae (includes BK and JC viruses)
- KI and WU detected in respiratory secretions of young patients worldwide.
- No direct link between either virus and respiratory infection.

- KI first detected in resp tract sample from ARI in Stockholm
  - Detected in 0 to 2.6% samples form children with ARI; also in asymptomatics.
  - High percentage of older (>3yo) WUPyV or KIPyV–infected patients are immunocompromised.
- WU detection associated with more clinical disease.

# Polyomaviruses: KI and WU

- KI PyV detected in 71 (2.8%) of 2599 resp samples from persons (1d to 88yr of age; 80% <2yo)
- Median age of the KI+ 12 months (mean: 44 months; range: 3 weeks–55 years)
- Another virus detected in 71% of KI positive samples.
  - rhinovirus (24%), RSV(17%); PIV, adenovirus, and HBoV (11% each)
- Detected year round – small peak in July.
- KIPyV patients were less likely than WUPyV patients to have an infiltrate on chest radiograph (31% vs. 50%, *P* 0.041)
- No link detected between KI virus and respiratory disease.

*Hormozdi et al. The Pediatric Infectious Disease Journal* • Volume 29, Number 4, April 2010

# Polyomaviruses: KI and WU

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 15, No. 3, March 2009

- WU detected in 2 (2.6%) and KI in none of 83 pediatric ICU patients
- In 83 control samples (in ICU for nonrespiratory illness), KIPyV was found in 4 (4.8%), WUPyV in 2 (2.4%).

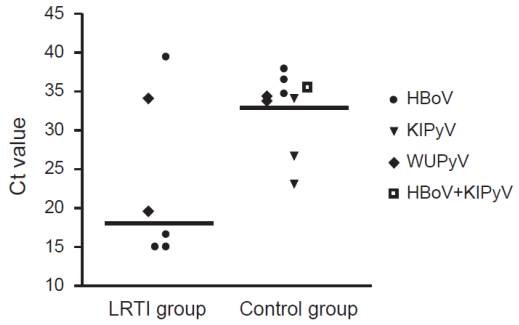
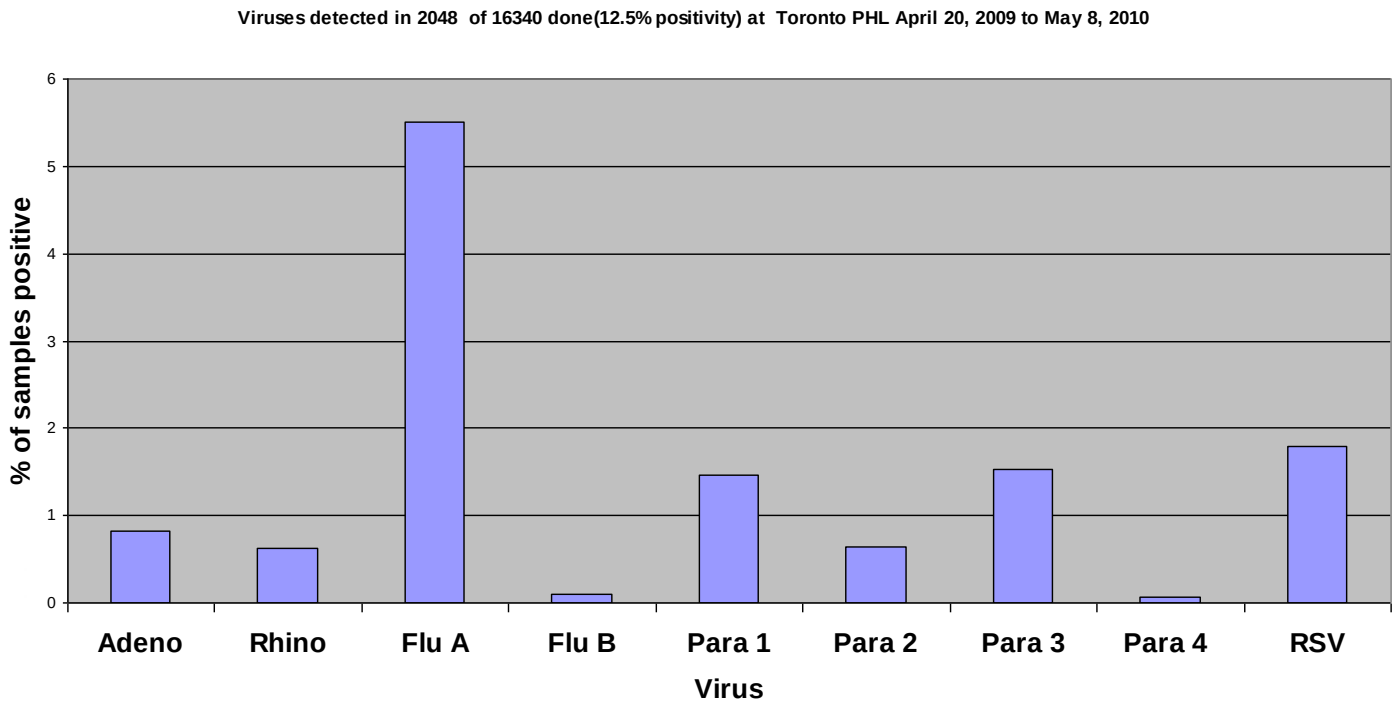



Figure. Cycle threshold (Ct) values of lower respiratory tract infection (LRTI) and control patients with human bocavirus (HBoV), KI polyomavirus (KIPyV), and WU polyomavirus (WUPyV) infections. LRTI patients are those admitted to the Pediatric Intensive Care Unit, Wilhelmina Children's Hospital, University Medical Center Utrecht, the Netherlands; control patients are patients admitted to the Pediatric Intensive Care Unit with other diagnoses. Horizontal bars represent group medians (difference 16.3 Ct, p = 0.09).

# Viruses were detected in 2048 of 16340 cultures done (12.5% positivity) at Toronto PHL, April 29, 2009 to May 8, 2010.



# Multiplex PCR

- Multiple respiratory viruses can cause same clinical syndrome
- Can perform multiplex PCR assays to detect multiple viruses in one reaction.
- Commercial assays to detect up to 18 respiratory viruses in 1 test.

**Development of a Respiratory Virus Panel Test for Detection of Twenty Human Respiratory Viruses by Use of Multiplex PCR and a Fluid Microbead-Based Assay**

J. Mahony, et al. J Clin Micro, Sept. 2007, p. 2965–2970 Vol. 45, No. 9

- Luminex RVP - Detects 20 different respiratory viruses

Seeplex® RV15 ACE

- Influenza A/B virus, RSV ,Parainfluenza virus 1-4, Adenovirus
- Coronavirus 229E/NL63, OC43, Rhinovirus, Enterovirus
- Bocavirus

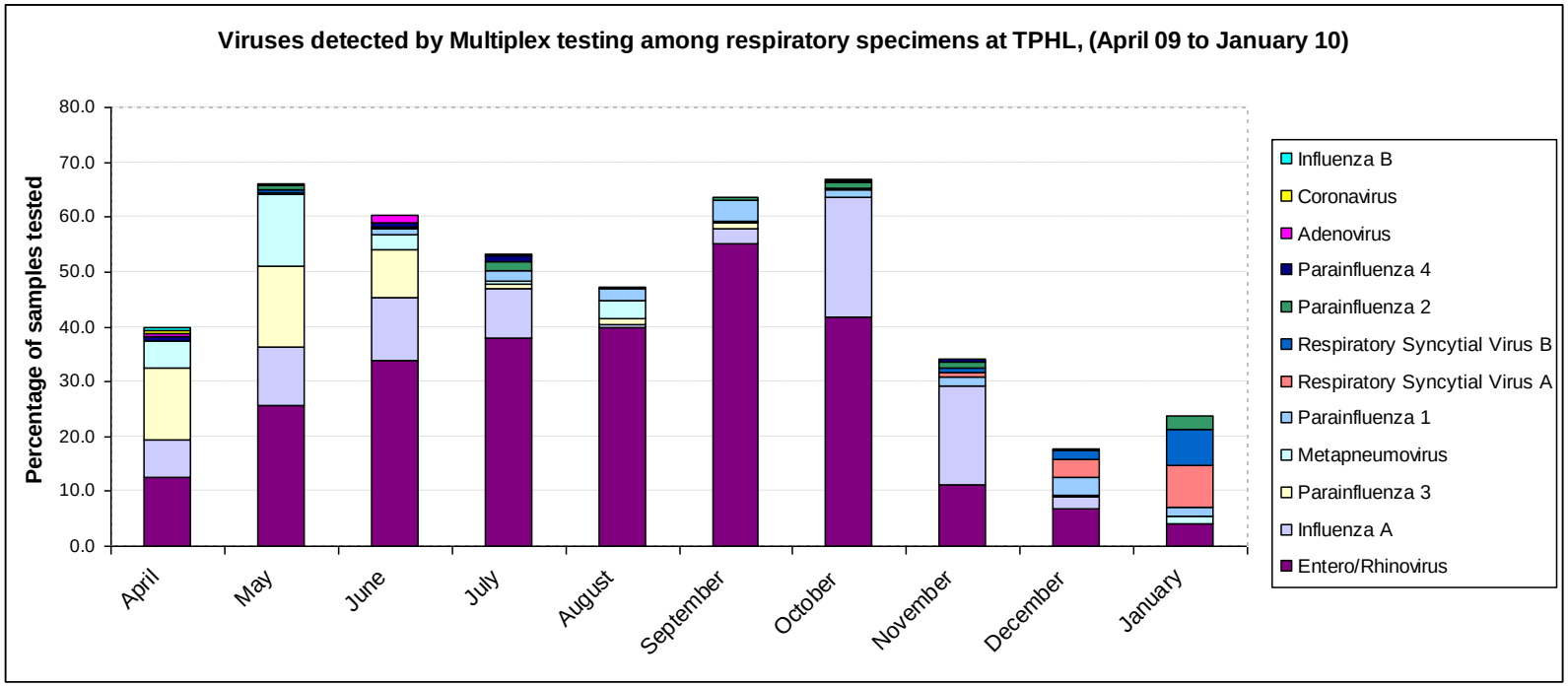
# All multiplex respiratory tests done at PHL April 2009 to January 2010 (ICU, outbreaks, remote communities).

| Overall detected respiratory viruses by time, April 2009 to January 2010 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **April** | **May** | **June** | **July** | **August** | **Septembe** | **October** | **November** | **December** | **January** | **Grand Tota** |
| Entero/Rhinovirus | 20 | 63 | 156 | 97 | 76 | 118 | 278 | 112 | 28 | 8 | **956** |
| Influenza A | 11 | 26 | 52 | 23 | 1 | 6 | 146 | 181 | 9 | 0 | **455** |
| Parainfluenza 3 | 21 | 36 | 40 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | **103** |
| Metapneumovirus | 8 | 32 | 13 | 1 | 6 | 1 | 1 | 0 | 1 | 3 | **66** |
| Parainfluenza 1 | 0 | 1 | 5 | 5 | 4 | 8 | 9 | 17 | 13 | 3 | **65** |
| Respiratory Syncytial Virus A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 14 | 15 | **35** |
| Respiratory Syncytial Virus B | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 10 | 6 | 13 | **32** |
| Parainfluenza 2 | 0 | 2 | 0 | 4 | 0 | 1 | 8 | 10 | 0 | 5 | **30** |
| Parainfluenza 4 | 1 | 0 | 4 | 3 | 1 | 0 | 2 | 5 | 0 | 0 | **16** |
| Adenovirus | 1 | 0 | 6 | 1 | 0 | 0 | 1 | 2 | 1 | 0 | **12** |
| Coronavirus | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1** |
| Influenza B | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** |
| **Total positive** | 64 | 162 | 277 | 136 | 90 | 136 | 446 | 343 | 72 | 47 | **1773** |
| Negatives | 96 | 83 | 182 | 119 | 100 | 78 | 221 | 658 | 335 | 151 | **2023** |
| Total | 160 | 245 | 459 | 255 | 190 | 214 | 667 | 1001 | 407 | 198 | **3796** |

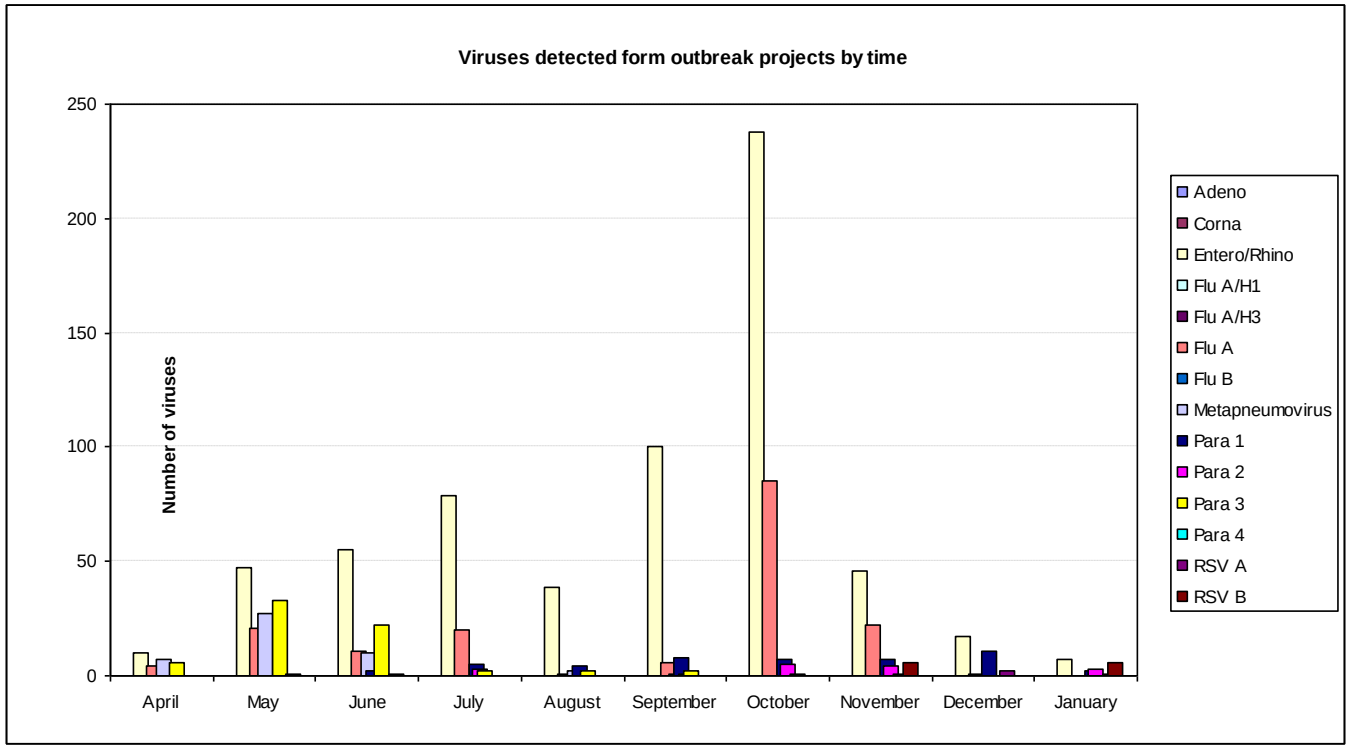
A virus was identified in 47% of samples tested

A. Peci, R Olsha

# All multiplex respiratory tests done at PHL April 2009 to January 2010 (ICU, outbreaks, remote communities).



# Viruses Detected from Outbreaks April 2009 to January 2010



# Respiratory Infections in Institutions during the Second Wave of Pandemic (H1N1) 2009, Ontario, Canada

| FACILITY TYPE | OUTBREAKS TESTED | PROPORTION OF OUTBREAKS TESTED |
|---|---|---|
| CAMPS | 14 | 4.0% |
| CORRECTIONAL FACILITIES | 2 | 0.6% |
| DAY CARES | 5 | 1.4% |
| HOSPITALS | 11 | 3.1% |
| LTCF | 250 | 70.6% |
| PSYCHIATRIC HOSPITALS | 7 | 2.0% |
| SCHOOLS | 37 | 10.5% |
| OTHER | 3 | 0.9% |
| UNKNOWN | 25 | 7.1% |

Alex Marchand-Austin

| ORGANISM DETECTED | OUTBREAKS | PROPORTION OF OUTBREAKS TESTED |
|---|---|---|
| ENTERO/RHINOVIRUS | 214 | 60.5% |
| INFLUENZA B | 1 | 0.3% |
| HUMAN METAPNEUMOVIRUS | 2 | 0.6% |
| PANDEMIC (H1N1) 2009 | 77 | 21.8% |
| PARAINFLUENZA 1-4 | 31 | 8.8% |
| RESPIRATORY SYNCYTIAL VIRUS B | 2 | 0.6% |
| NO ORGANISM DETECTED | 69 | 19.5% |

Entero/Rhinovirus was detected in 169 (68%) outbreaks from LTCFs in contrast to pH1N1 being detected in 19 (7.6%).

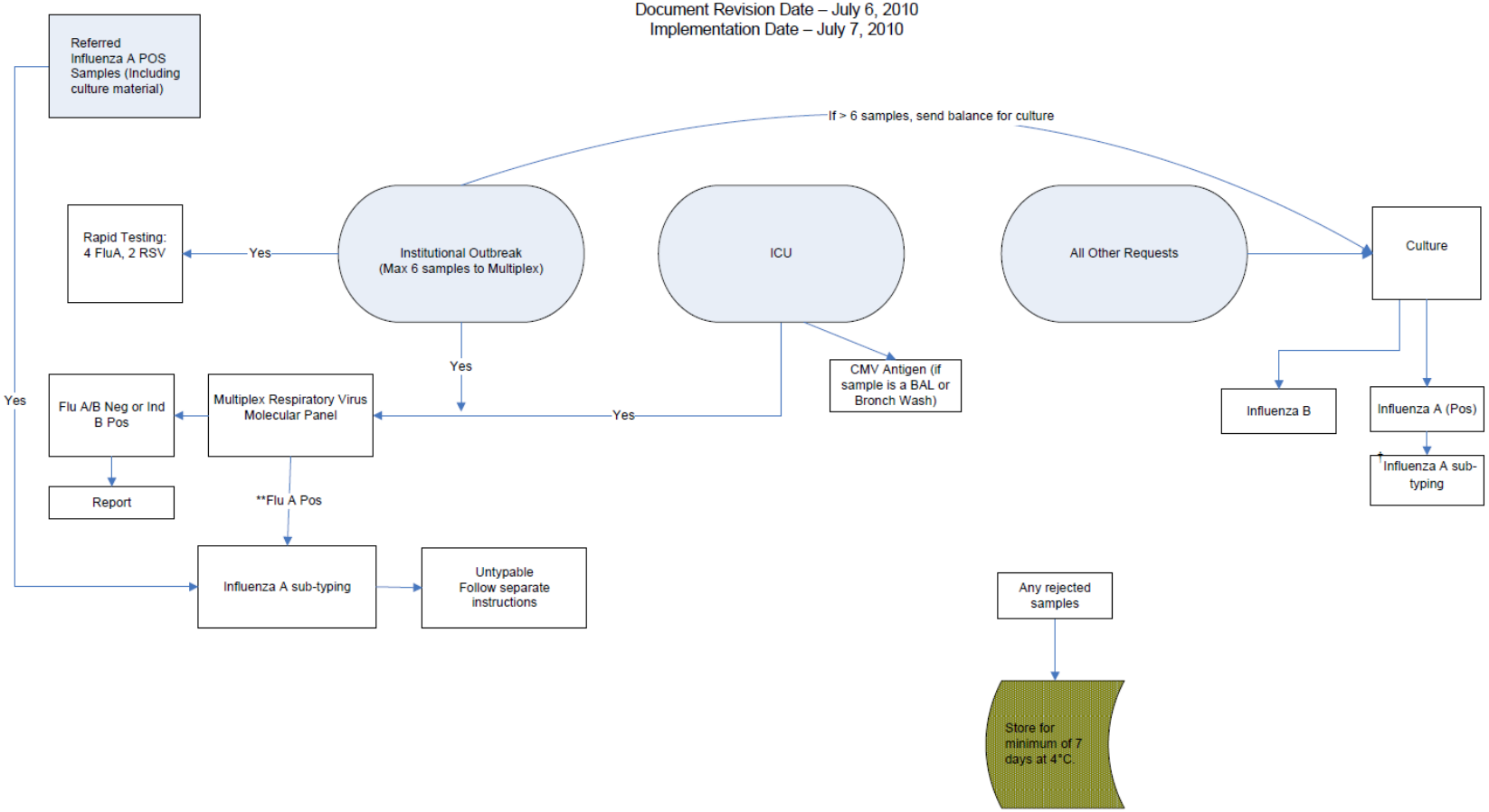
Schools accounted for only 37 (10%) of the outbreaks tested but comprised 37 (44%) of pH1N1 positive outbreaks

# Impact of Molecular Methods on Respiratory Viral Diagnostics

- Much greater sensitivity vs culture and DFA.
  - Better understanding of epidemiology of respiratory viruses.
  - Fewer infections where don't identify a virus
  - Potential impacts on clinical care: less antibacterial therapy, shorter hospital stay, reduced mortality if earlier use of antivirals for influenza.
- Faster turnaround time – greater opportunity to guide therapy.
- Discovery of new viruses in respiratory tract in last decade
  - Metapneumovirus
  - Multiple coronaviruses: SARS, 229E, NL63, OC43, HKU1.
  - Human bocavirus
  - Polyomaviruses KI, WU
- Viral coinfections recognised as a relatively common entity.

# Process for Respiratory Viral Testing

Document Revision Date – July 6, 2010
Implementation Date – July 7, 2010



# Multiplex Respiratory Viral Testing April 2009 to Feb 14, 2010 (outbreaks, ICU, selection of remote communities)

- 1658/4188 (39.6%) samples tested contained a **non**influenza virus.
  - 2087 of these were non outbreak samples (ICU, remote communities).

**Multiplex molecular tests ICU Proxy vs Vaccine Effectiveness Study Samples April 2009 to February 2010**

| | ICU Proxy | | | VE STUDY-APRIL 2009- FEB 2010 | | |
|---|---|---|---|---|---|---|
| **VIRUSES DETECTED** | **Number** | **Tested** | **% Positive** | **Number** | **Tested** | **% Positive** |
| ENTERO/RHINOVIRUS | 366 | 2440 | 15.0 | 222 | 1018 | 21.8 |
| FLU A | 284 | 2440 | 11.6 | 423 | 1018 | 41.6 |
| FLU B | 3 | 2440 | 0.1 | 0 | 1018 | 0.0 |
| RSV A | 108 | 2440 | 4.4 | 12 | 1018 | 1.2 |
| RSV B | 50 | 2440 | 2.0 | 103 | 1018 | 10.1 |
| PARAINFLUENZA* | 141 | 2440 | 5.8 | 46 | 1018 | 4.5 |
| METAPNEUMOVIRUS | 45 | 2440 | 1.8 | 5 | 1018 | 0.5 |
| ADENOVIRUS | 20 | 2440 | 0.8 | 5 | 1018 | 0.5 |
| CORONA OC 43 | 3 | 736 | 0.4 | 2 | 1018 | 0.2 |
| CORONA 229E | 0 | 231 | 0.0 | 3 | 1018 | 0.3 |
| CORONA 229E/NL63 | 1 | 486 | 0.2 | 1 | 1018 | 0.1 |
| CORONA NL63 | 1 | 232 | 0.4 | 0 | 1018 | 0.0 |
| CORONA HKU1 | 3 | 214 | 1.4 | 1 | 1018 | 0.1 |
| Total | 28 | 2440 | ≥40.2 | 12 | 1018 | 80.9 |

- **Respiratory Infection Outbreaks in Institutions Occurring During the Early Stages of the Novel Influenza A (H1N1) Virus Pandemic. A Marchand-Austin, D.J. Farrell, Frances B. Jamieson, N. Lombardi, E. Lombos, S. Narang, H. Akwar, D.E. Low, J.B. Gubbay. Emerging Infectious Diseases 2009 Dec;15(12):2001-3.**

Table 2. Etiologic agents identified by the Luminex Respiratory Virus Panel* from samples submitted by regional health units during outbreaks, Canada†

| Etiologic agent | 2009 spring outbreaks, Ontario, no. (%) | 2009 spring outbreaks GTA,‡ no. (%) | 2008–2009 influenza season outbreaks, GTA,‡ no. (%) |
|---|---|---|---|
| Coronavirus OC43 | 1 (1) | 0 | 18 (23) |
| Coronavirus NL63 | 0 | 0 | 6 (8) |
| Coronavirus 229E | 4 (5) | 0 | 9 (12) |
| Metapneumovirus | 17 (20) | 2 (12) | 21 (27) |
| Respiratory syncytial virus A | 0 | 0 | 5 (6) |
| Respiratory syncytial virus B | 1 (1) | 0 | 17 (22) |
| Influenza A (H3, human) | 11 (13) | 6 (35) | 4 (5) |
| Parainfluenza 1 | 1 (1) | 0 (0) | 1 (1) |
| Parainfluenza 3 | 22 (27) | 7 (41) | 3 (4) |
| Enterovirus/rhinovirus | 31 (37) | 3 (18) | 15§ (19) |
| Pandemic (H1N1) 2009 virus | 1 (1) | 1 (6) | 0 |
| Invalid test¶ | 0 | 0 | 1 (1) |
| None | 6 (7) | 0 | 11 (14) |
| Outbreaks tested | 83 | 17 | 78 |

*Luminex Molecular Diagnostics, Toronto, Ontario, Canada.
†GTA, Greater Toronto area. Spring season is delineated as April 20–June 12; influenza season is delineated as October 1–April 19.
‡Iincludes submissions by Peel, York, and Toronto Public Health Units only.
§Seven of the 15 outbreaks were confirmed as rhinovirus by the Seeplex RV12 detection kit (Seegene, Inc., Seoul, South Korea).
¶Reported when the internal control is not detected during a run.

**Rhinovirus outbreaks in long-term care facilities, Ontario, Canada. J Longtin A Marchand-Austin , A Winter, S Patel, A Eshaghi, F Jamieson, DE Low, JB Gubbay.**

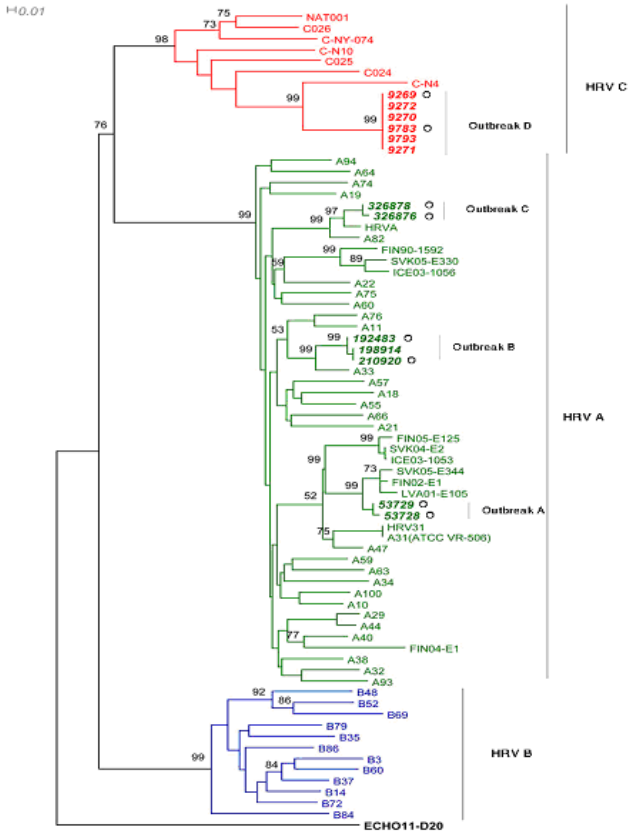
**Emerg Infect Dis. 2010 Sep; [Epub ahead of print]**

Table 1. Viral causes of 297 respiratory illness outbreaks in long-term care facilities, Ontario, Canada, July 1–December 31, 2009

| Virus | Outbreaks, no. (%) |
|---|---|
| Enterovirus/rhinovirus | 174 (59.0) |
| Influenza A | 22 (7.0) |
| Parainfluenza 1 | 18 (6) |
| Parainfluenza 2 | 3 (1.0) |
| Parainfluenza 3 | 3 (1.0) |
| Parainfluenza 4 | 2 (0.7) |
| Metapneumovirus | 2 (0.7) |
| Influenza B | 1 (0.3) |
| Respiratory syncytial virus A | 1 (0.3) |
| Respiratory syncytial virus B | 1 (0.3) |
| Adenovirus | 0 |
| No specimens received | 28 (9.0) |
| Negative | 63 (21.0) |

# Public Health Applications of Molecular Typing

## Molecular typing of 4 rhinovirus outbreaks in LTCFs associated with fatalities.




R. Eshaghi

Molecular typing identified the outbreaks to be due to rhinovirus serotypes A-31, A-33,A-82, and C-N7

# Likely Respiratory Viral Testing Algorithm OAHPP, 2010/11.

**Likely algorithm for respiratory viral testing at OAHPP, 2010/11**

| | FluA/B PCR | Multiplex molecular | Viral Culture | Flu/RSV ICT |
|---|---|---|---|---|
| **Outbreaks** | XX | XX | | XX |
| **ICU** | XX | XX | | |
| **Hospitalized nonICU** | XX | ??? | | |
| **Ambulatory high risk** | XX | | | |
| **Ambulatory low risk** | | | XX | |
| Emergency not-admitted | ?? | | XX | |
| **Ambulatory-VE study** | XX | XX | | |

# Summary

- Viral diagnostics is a dynamic field with potential for significant impact on public health practice. It impacts on:
  - Surveillance of respiratory viruses
  - Understanding epidemiology of respiratory viruses
  - Infection control
  - Antiviral therapy and other clinical management of patients with viral infection.
- New applications of molecular technology are being introduced continuously
  - Moving towards point of care application of advanced diagnostics.

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- **OAHPP: Public Health Laboratory Staff**
  - Virus Detection: Eddie Chong-King and staff
  - Molecular Diagnostics: Joanne Blair and staff
  - Research Staff: Reza Eshaghi, Rachel Higgins
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- **PHAC LLTO**
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