Respiratory infections: which pathogens should we include and when?

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27.04. 2013

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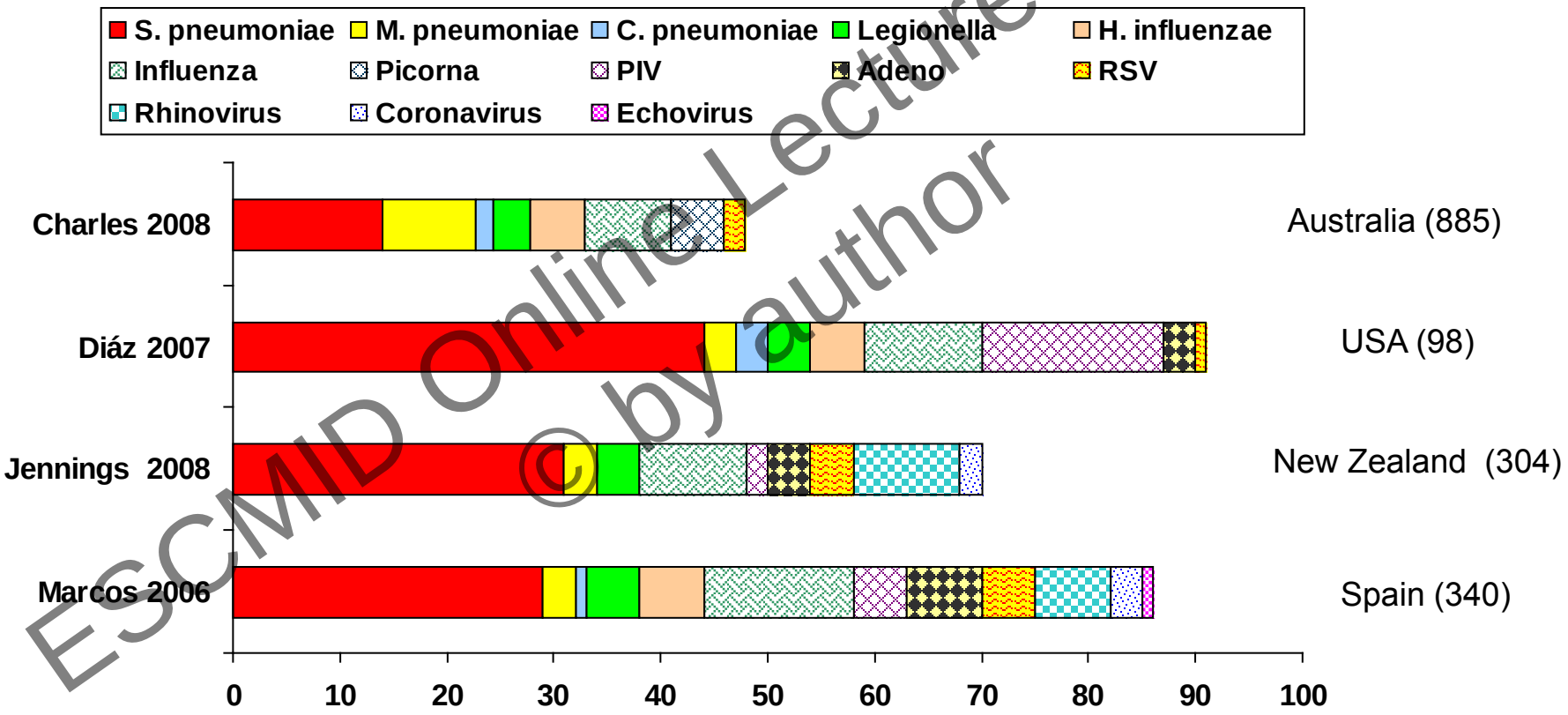
Aetiology of lower respiratory tract infection in the community (%)

Reference	*n*	*S. pn*	*H. infl*	*M. pn*	*C.pn*	Virus
Boldy et al. 1990	42	3.0	3.0	8.0	0	21.0
Creer et al. 2006	80	18.8	6.3	1.2		61.3
Graffelman et al. 2004	145	6.2	9.0	9.0	1.3	39.0
Holm et al. 2007	364	6	4	3	<1	24
Hopstaken et al. 2005	247	2.9	13.8			
Macfarlane et al. 1993	206	30.0	8.0	0.5		8.0
Macfarlane et al. 2001	316	17.1	9.8	7.3	17.4	19.3
GRACE study, 2012	3059	9.1	14.8	2.9	2.2	51.1
Range		**3-30**	**3-15**	**0.5-9**	**0-17**	**8-61**

- ***C. pn* is in some studies reported in a large nr of cases: 0-20%**
- **Early data are largely based on serological analysis only**

Woodhead M et al. Clin. Microbiol.Infect. 2011; 17, E1-E59

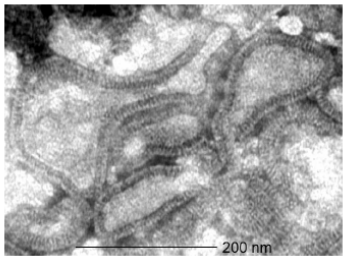
Viral Agents in CAP in Hospitalised Adult Patients



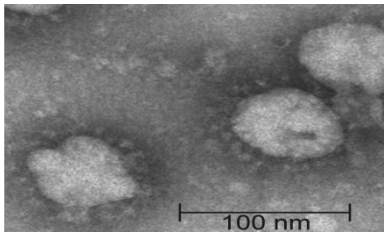
A newly discovered human pneumovirus isolated from young children with respiratory tract disease

2001: hMPV

Bernadette G. van den Hoogen[1], Jan C. de Jong[1], Jan Groen[1], Thijs Kuiken[1], Ronald Groot[2], Ron A.M. Fouchier[1] & Albert D.M.E. Osterhaus[1]



The NEW ENGLAND JOURNAL of MEDICINE

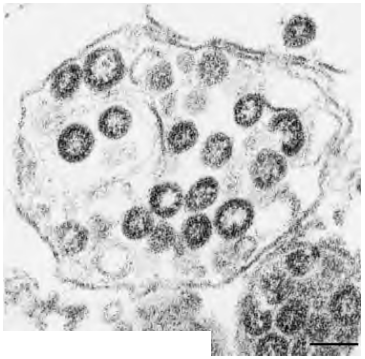


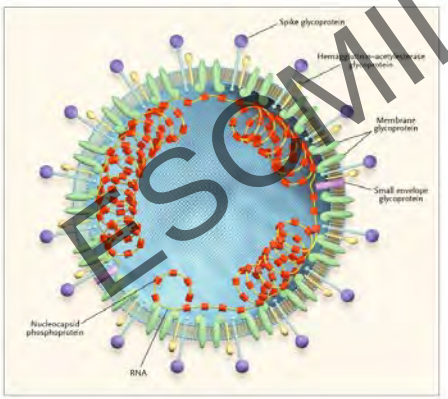
ORIGINAL ARTICLE

2003: HCoV SARS

Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome

Christian Drosten, M.D., Stephan Günther, M.D., Wolfgang Preiser, M.D., Sylvie van der Werf, Ph.D., Hans-Reinhard Brodt, M.D., Stephan Becker, Ph.D., Holger Rabenau, Ph.D., Marcus Panning, M.D., Larissa Kolesnikova, Ph.D., Ron A.M. Fouchier, Ph.D., Annemarie Berger, Ph.D., Ana-Maria Burguière, Ph.D., Jindrich Cinatl, Ph.D., Markus Eickmann, Ph.D., Nicolas Escriou, Ph.D., Klaus Grywna, M.Sc., Stefanie Kramme, M.D., Jean-Claude Manuguerra, Ph.D., Stefanie Müller, M.Sc., Volker Rickerts, M.D., Martin Stürmer, Ph.D., Simon Vieth





nature medicine

2005: HCoV NL 63

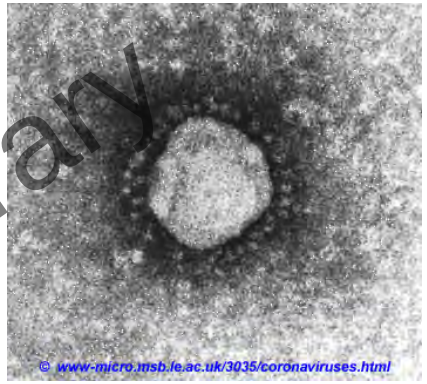
Identification of a new human coronavirus

Lia van der Hoek[1], Krzysztof Pyrc[1], Maarten F Jebbink[1], Wilma Vermeulen-Oost[2], Ron J M Berkhout[2], Katja C Wolthers[1], Pauline M E Wertheim-van Dillen[3], Jos Kaandorp[4], Joke Spaargaren[2] & Ben Berkhout[1]

JOURNAL OF VIROLOGY, Jan. 2005, p. 884–895 Vol. 79,
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.2.884–895.2005

Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia

Patrick C. Y. Woo,[1,2]† Susanna K. P. Lau,[1,2]† Chung-ming Chu,[3] Kwok-hung Chan,[1] Hoi-wah Tsoi,[1] Yi Huang,[1] Beatrice H. L. Wong,[1] Rosana W. S. Poon,[1] James J. Cai,[1] Wei-kwang Luk,[4] Leo L. M. Poon,[1,2] Samson S. Y. Wong,[1,2] Yi Guan,[1,2] J. S. Malik Peiris,[1,2] and Kwok-yung Yuen[1,2]†*



2005: HCoV HKU1

PNAS

Cloning of a human parvovirus by molecular screening of respiratory tract samples

Tobias Allander*†‡, Martti T. Tammi§¶, Margareta Eriksson‖, Annelie Bjerkner*, Annika Tiveljung-Lindell*, and Björn Andersson§

2005: Boca virus

Journal of Clinical Virology
Volume 38, Issue 3 , March 2007, Pages 227-237

2007: rhinovirus C

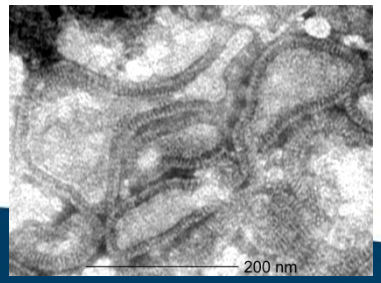
doi:10.1016/j.jcv.2006.12.016 Cite or Link Using DOI

Molecular characterization of a variant rhinovirus from an outbreak associated with uncommonly high mortality

David Kiang[a], , , Shigeo Yagi[a], Katherine A. Kantardjieff[b], Euna J. Kim[b], Janice K. Louie[a] and David P. Schnurr[a]

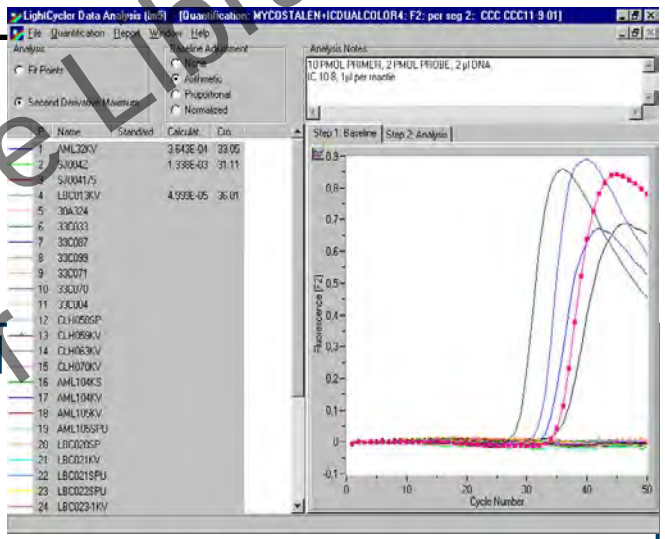
The main respiratory targets in molecular diagnostic tests

- Those in our routine panel:
  - Influenza A (IFVA)
  - Influenza B (IFVB)
  - Parainfluenza (PIV) 1-4
  - Respiratory syncytial virus (RSV)
  - Adenoviruses (ADV)
  - Metapneumovirus (hMPV)



- Those extra assays that many would consider important:
  - Rhinoviruses
  - Enteroviruses
  - Coronaviruses (OC43, 229E, NL63 and HKU1)
  - IFVA sub-typing
  - Bocavirus
  - Atypicals: *M.pn*., *C. pn*., *Leg. pn*., *Bordetella pertussis*

Conventional and Real-Time Mono- and Multiplex NAAT



Author	targets	Species detected
Fan, J et al. 1998	2	RSVA, RSVB
Scheltinga et al. 2005	2	hMPN, RHI
McDonough et al. 2005	4	*M. pn.*, C. *pn.*, *L. pn.*, *B. pertussis*
Gunson et al. 2005	12	IFL A and B, PFL 1,2,3 RHI, hMPN RSVA and B, COR E229, OC 43, NL63 in 4 triplex reactions
Loens et al. 2007	3	*M. pn.*, *C. pn.*, *L. pn*
Choi et al. 2006	12	in 4 multiplex and one monoreaction
Tiveljung et al. 2009	16	in 13 reactions: IFL A and B, RSV A+B, PFL 1+3, PFL 2+ hCoV-229E, ADE, hMPV, RHI, ENT, HCoV-OC43, HCoV-NL63 and HKU, HBoV

leven M, J Clin Virol 2007; 40: 259-76

Commercially available Mono- and Multiplex tests

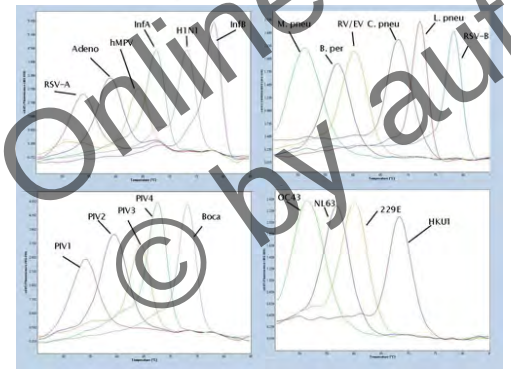
	targets	Species detected
Xpert FluA, Cepheid	2	Influenza A and subtyping
RSV,ASR, Cepheid	2	RSVA, RSVB
ProPneumo-1, Prodesse	2	*M. pneumoniae*, C. *pneumoniae*
RespiFinder plus, Pathofinder	18	IFL A/B, PFL 1-4, RHI, hMPN, RSV A/B, AV, 3 coronaviruses, *M. pn., C.pn., L.pn., Bordetella pertussis*
SeeplexRV, Seegene	19	*S. pneumoniae*, *H. influenzae*, *M. pn., C.pn., L.pn.*, IFL A and B, RSV A/B, PFL 1-3, RHI, 3 coronaviruses, AV, HBoV, EV
xTAG RVP, Luminex	19	IFL A (H1, H3, H5, non-specific) and B, PFL 1-4, RSV A/B, ADE, hMPV, RHI/ENT,SARS-COR, HCoV OC43, HCoV 229E, HCoV NL63 and HKU1

Some commercially Multiplex tests

Cepheid GeneXpert

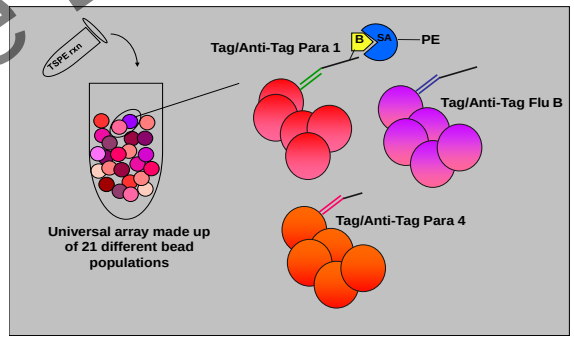
Single use cartridge based
Up to 6 targets
TAT 1.5 to 2/5 hr

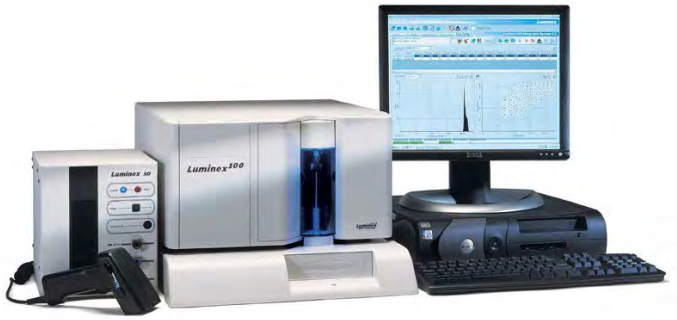
RespiFinder Mx, Pathofinder



Mx amplification and detection by melting curve analysis
Up to 25 targets
TAT 6 hr

Luminex xTAG Universal Bead Array





Liquid microarray based
Up to 20 targets
RVP 10-12 hr to RVP Fast 4-5 hr

Comparison of some mono- and Mx methods for detection resp pathogens

Parameter	Xpert Flu,	RSV Respifinder	xTAG RVP
Amplification platform	Multiplex RT-PCR	Multiplex RT-PCR	Multiplex RT-PCR
Detection format	GeneXpert (solid cartridge)	(real-time)	Luminex (liquid chip)
Nr pathogens covered	5	22	19
No. of reactions needed	2	1	1
Hands-on time (min)[a]	10	70	55
Detection throughput (no. of tests/run)	1	48	96
Test turnaround time (h)[b]	1.5-2	6	10-12

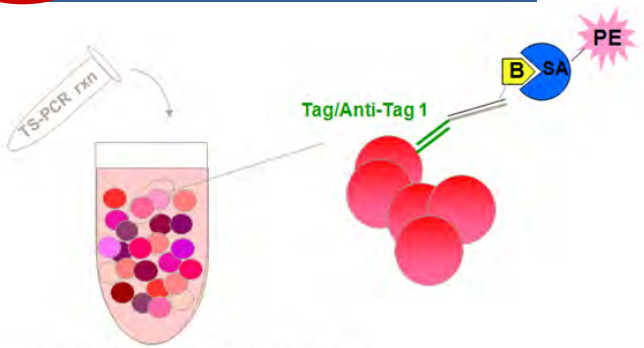
[a] Hands-on and turnaround times include time needed for specimen processing and data analysis
[B] Times are based on a full run, including specimen processing, target amplification, and product identification

Sensitivity and specificity of two different commercial assays

- Evaluation of 2 CE labeled multiplex assays: RespiFinder (Pathofinder) and xTAG RVP (Luminex) on
- Compared with in-house PCRs used in GRACE

Virus	RespiFinder Sensitivity	RespiFinder Specificity	xTAG RVP Sensitivity	xTAG RVP Specificity
INF	84.8%	98.4	72.3	96.8
HCoV	89.1	99.0	32.6	98.7
hMPV	100	98.5	96.6	99.4
HRV	95.2	98.3	88.7	99.6
RSV	88.5	100	71.4	99.1

- Both commercial assays are less sensitive than in-house PCRs used in GRACE
- RespiFinder (Pathofinder) is more sensitive than Luminex assay



When to use which techniques?
Sequential approach or Mx detection?

Some Technical, Scientific and practical hurdles

- For number of newest technologies: limited validation and little proven clinical applications
- Few FDA cleared
- Which pathogen?
- Which clinical specimen?
- Sample preparation
- Some methodologies: low throughput tests
- Some methodologies: need for multiple instruments
- Technically demanding and labor intensive
- Costly

Limited target detection versus multiplex detection

Limited target detection

- Usually ⇧ analytical sens.
- Lower cost
- Often lower TAT
- In outbreak situations
  - SARS Coronavirus
  - Influenza, H1N1
  - RSV, *L. pn*, *M.pn*
- As first approach
  - in high prevalence periods
  - if therapeutic implications
    - Influenza, *Legionella* spp, *Mycoplasma pn.*, *B. pertussis*
- Outside normal lab working hrs

Multiplex detection

- In >90% similar results
- Expensive
- TAT usually > 4-6hours
- For epidemiological studies
  - Prevalence of respiratory etiologies
    - Role of respiratory viruses
- As add-on diagnostic test
  - In severely ill patients
  - In immunocompromised
- For virus discovery studies

Impact of molecular methods on the diagnosis of LRTI

Impact on Influenza H1N1 outbreak management

- Semiautomated and fully automated assays for detection of FluA (including H1N1) and FluB:

  Flu A sensitivities varying between 93% - 98%,

  specificities varying between 99 -100%

Sails AD et al. J Virol Methods 2009, 162: 88-90
Ginocchio CC et al J Clin Virol 2009; 45: 191-195
Casalegno JS et al Clin Microbiol Infect 2009; 16: 326-329
Beck ET, et al J Mol Diagnostics 2010; 12: 74-81

⇨ Automated **high throughput molecular system** allows clinicians and public health officials to **react quickly during outbreaks**

Impact on patient management: Need for speed! TAT is crucial!

- Evaluation of 2 real-time RT-PCR assays (Cepheid, Sunnyvale, USA) on SmartCycler **(TAT < 1hour)**
  - RSV Analyte Specific Reagent (ASR) bead
  - Influenza A/B ASR bead
- Comparison with “in-house” multiplex real-time PCR on +/-100 respiratory samples for Influenza and RSV

- Results: facilitates urgent testing outside batched runs or
  - RSV: sens: 98.2%, spec: 100%
  - Influenza A/B: sens: 96.5%, spec: 100%

⇒ **Compared to “in house” multiplex: significant in ⇓ TAT**

⇒ **facilitates urgent testing outside batched runs or normal working day**

Sails AD et al. J Virol Methods 2009, 162: 88-90

RSV outbreak: an argument for real-time detection!

- Evaluation of rapid antigen testing, culture DFA and RT-PCR in symptomatic residents in a 120-bed LTCF

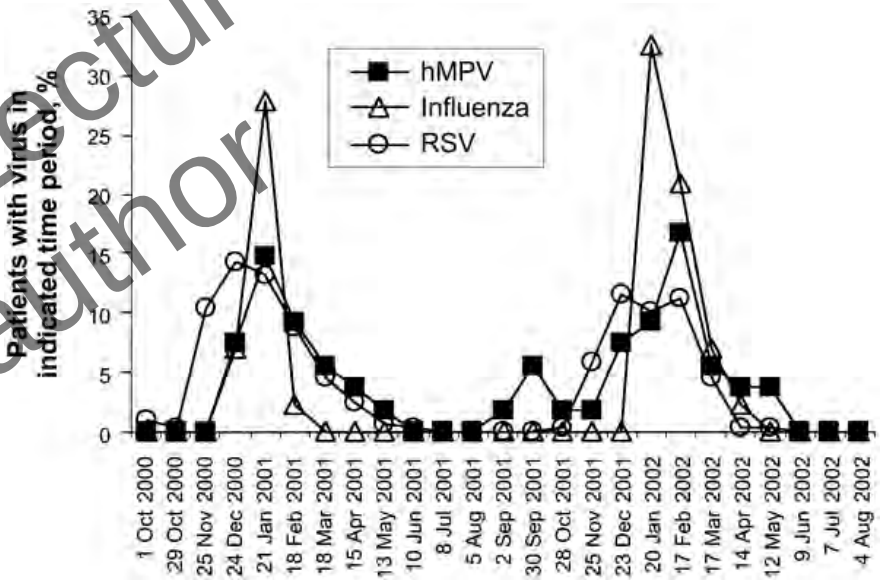
- In 32% (7/22) cases: RSV was detected by RT-PCR
  - None of cases was detected by Antigen testing
  - Only 2 cases (9%) were detected by culture or DFA

⇒ RT-PCR can provide a **more timely and accurate** diagnosis of outbreaks, which **allows for early symptomatic treatment, rational use of antibiotics, and improved infection control.**

Caram BL et al. J Am Geriatr Soc 2009; 57: 482-485

hMPV resembling RSV: Similar but Different?

- RSV: more common than hMPV in infants <6 mo.
- hMPV similar to RSV, majority of hMPV cases occur in young (<5yrs)
- Seasonality with RSV: hMPV later



- co-infection with RSV: More severe?

  Contradictory

  In 1 study, hMPV/RSV coinfection in 70%

  Disease severity and hospitalization appears more common with RSV

Osterhaus A, et al. Lancet. 2003; 361:890-891

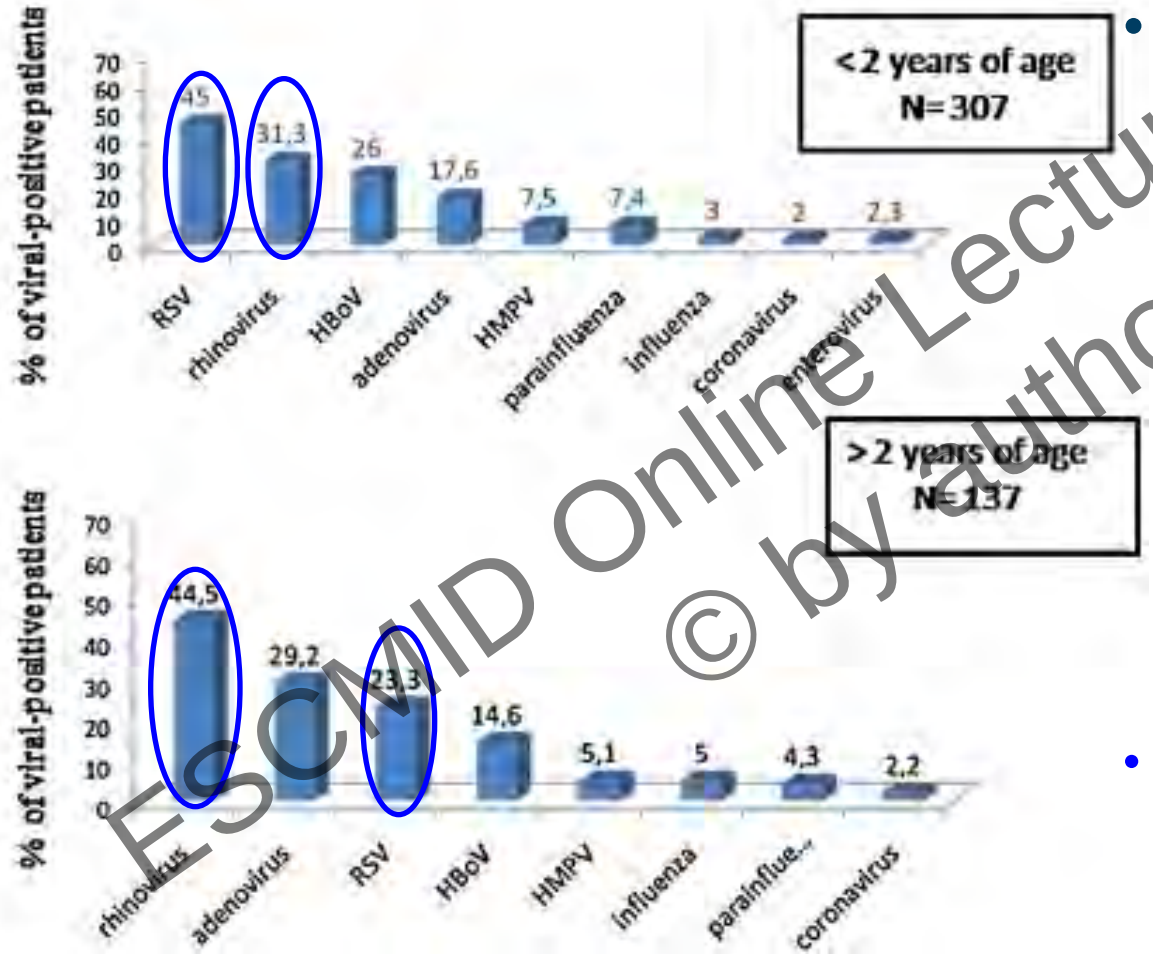
Boivin G, et al. Emerg Infect Dis. 2003;9:634-640

Greensill J, et al. Emerg Infect Dis. 2003; 9: 372-375.

McAdam AJ et al. J Infect Dis 2004; 190: 20-26

Esper F et al. J Infect Dis 2004; 189: 1388-96

Emerging respiratory viruses in children with severe acute wheezing



- viruses detected in 71% of acute wheezing episodes
  - RSV most commonly detected virus: 27%
  - Rhinovirus in 24%
  - Adenovirus 18%
  - Rate of viral detection in infants (77%) than in older children (60%)
- **RSV and rhino most prevalent in wheezing; emerging viruses hBoV and hMPV also important**

Garcia-Garcia ML et al. Pediatric pulmonology 2010; 45: 585-91

Impact of molecular methods on the diagnosis of LRTI

Impact on epidemiological knowledge: Distribution of respiratory pathogens in LRTI

- **From 10/2007 – 12/04/2010**
  - 3102 adult patients with LRTI
  - 2984 controls
  - 16 PCN in 12 countries

⇒ Presence viral etiologic agent by RT-PCR: **53.7%**

⇒ In total: etiology found in **> 70%** in LRTI in community

Significance of etiologic agents?

Target	Patient with LRTI prevalence n/total (%) first visit (n=3059)	follow up visit (n=2566)	*P*-value	Matched Control subject prevalence n/total (%) (n=1678)	*P*-value
Parainfluenza 1-4	73 (2.4)	12 (0.5)	< 0.00001	8 (0.5)	< 0.00001
Rhinovirus	589 (19.3)	112 (4.5)	< 0.00001	72 (4.3)	< 0.00001
Human MPV	126 (4.1)	7 (0.3)	<0.00001	3 (0.2)	< 0.00001
Human AV	38 (1.2)	42 (1.6)	0.26	24 (1.4)	0.68
Bocavirus	13 (0.4)	12 (0.5)	0.96	16 (1.0)	**0.04**
RSV	143 (4.7)	13 (0.5)	< 0.00001	11 (0.7)	< 0.00001
Influenza A/B	300 (9.8)	10 (0.4)	< 0.00001	6 (0.4)	< 0.00001
Coronaviruses	208 (6.8)	70 (2.7)	< 0.00001	27 (1.6)	< 0.00001
Polyomavirus WU	45 (1.5)	54 (2.1)	0.09	39 (2.3)	**0.04**
Polyomavirus KI	27 (0.9)	28 (1.1)	0.51	17 (1.0)	0.77
M. pneumoniae[1]	0/809 (0)	0/653 (0)	NS	0/492 (0)	NS

[1]:Only data of first winter season

How to implement these amplification techniques for the detection of bacterial etiologies?

Individual patient care: Is bacterial quantification useful in molecular testing?

In case of legionellosis

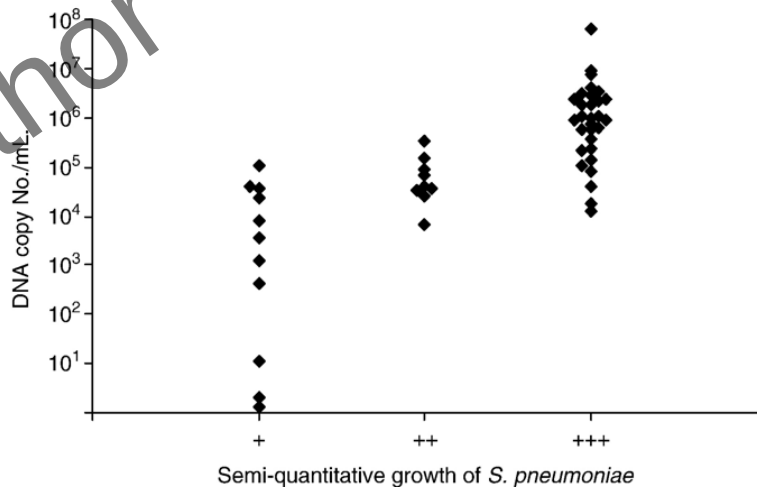
- qRT-PCR useful for predicting disease severity: high bacterial loads significantly associated with higher Fine classes, need for ICU hospitalization

Maurin M et al Clin Microbiol Infect 2010; 16: 379-384

In case of pneumococcal disease

- Cut-off 10^4 DNA copies/ml
  - Sens: 84%
  - Spec: **94%**

Abdeldaim G et al Diagn Microbiol Infect Dis J 2008; 60: 143-50



Q PCR enables differentiation between pathogenicity and colonization

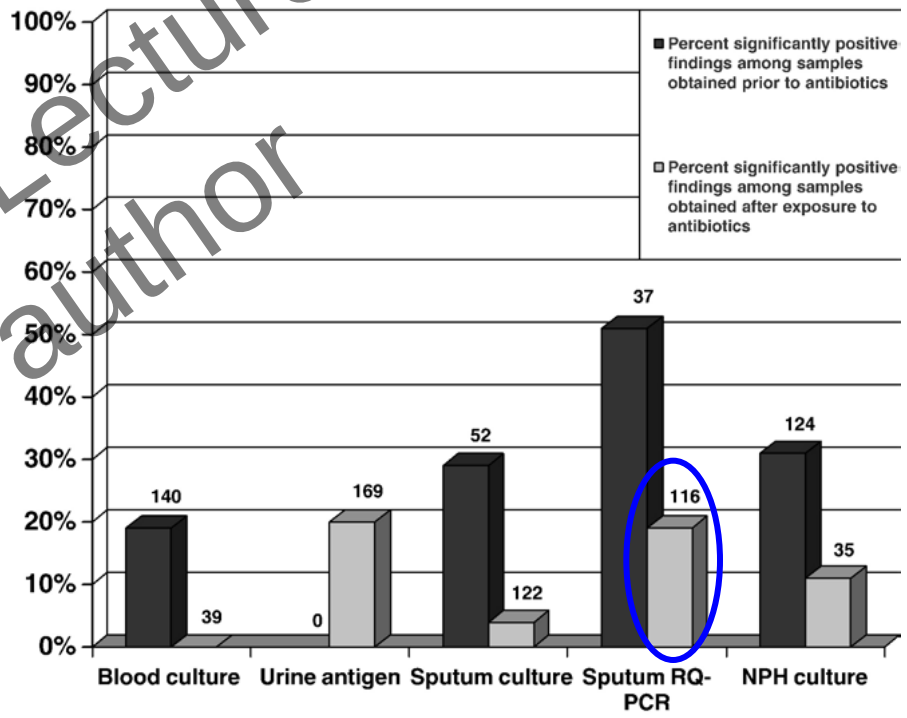
RQ-PCR particularly valuable in patients treated with AB

Johansson N et al Diagn Microbiol Infect Dis J 2008; 60: 255-61

Quantitative PCR for Diagnosis of *S. pneumoniae* Infection

70/184 (38%) patients with *S.pneumoniae*

- 15% by blood culture
- 20% by urinary Ag
- 15% culture positive sputa
- 27% by RQ-PCR
  - 82% of these also detected by other methods
  - 50% of these culture -, most of these treated with AB



⇒ **RQ-PCR particularly valuable in patients treated with AB**

Johansson N et al Diagn Microbiol Infect Dis J 2008; 60: 255-61

Individual patient care: Is viral quantification useful?

In case of PIV, rhinovirus

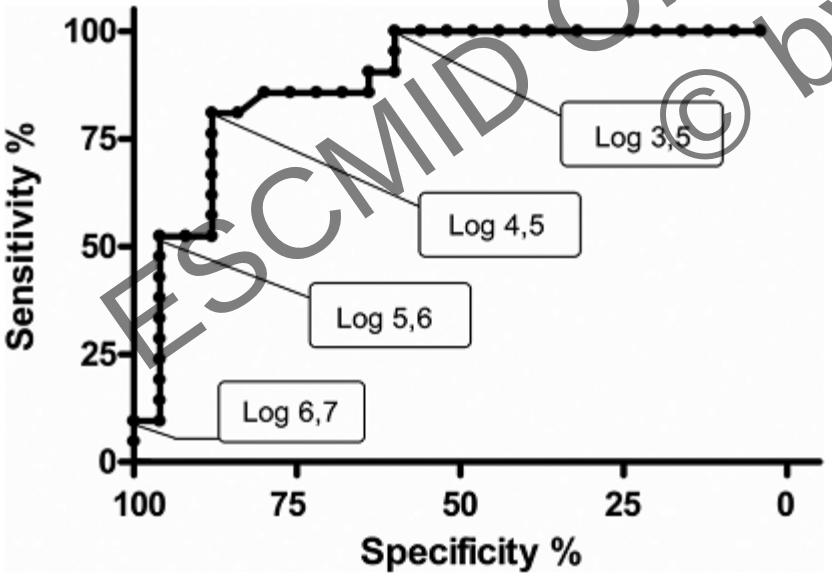
- Total viral load is related to clinical diagnosis in children presenting at emergency room

Utokaparch S et al. Pediatr Infect Dis J 2011; 30: e18-e23

In case of rhinovirus

- At high viral loads (> 10^6 RNA copies/ml): HRVs may cause severe LRTI
- At medium-low viral loads (<10^5 RNA copies/ml): may represent only bystander

Gerna G et al. J Med Virol 2009; 81:1498-1507



- At high viral loads (> $10^{4.5}$ RNA cps/ml): HRVs likely to be the cause of presenting LRTI
- At medium-low viral loads (<$10^{4.5}$ RNA copies/ml): may represent only bystander

- **Q PCR: maybe the next necessary step?**

Jansen R et al. J Clin Microbiol 2011; 49: 2631-36

Impact of molecular detection on diagnosis of acute *M. pneumoniae* infection

- *M. pneumoniae* in 48/164 patients:
  - 45/164 (29%): PCR pos in first week
  - 44/154 (27%): significant ⇧ in IgG or + IgM

    BUT:
    - 21% in 1st week
    - 56% during second week
    - 100% after 3rd week

Nilsson et al., BMC Microbiol 2008; 8: 93-100

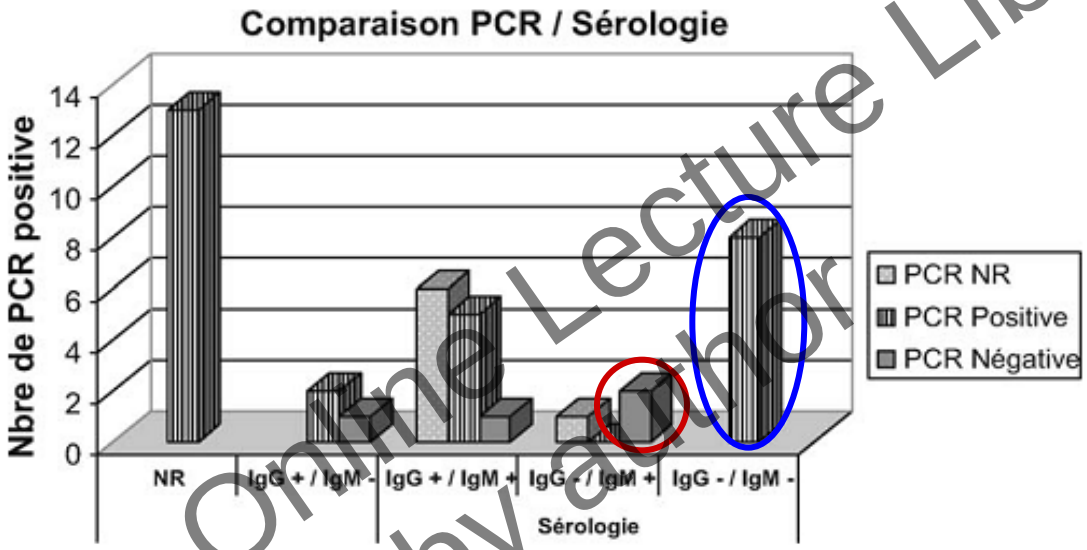
- CAP + LRTI: study in Antwerp: 4 different tests evaluated on 224 patients (205 paired sera)
  - sensitivity of IgM: **10-31%** in first 6 days

    20-42% after more than 16 days

Molecular methods are superior to serology for early diagnosis of *M. pneumoniae* and impact on antibiotic management

Loens K et al., ECCMID 2005

Importance of PCR in the diagnosis of *Mycoplasma pneumoniae* infections



- PCR based detection: most sensitive: 28/32 (87%)
- Sensitivity of serology: 15/26 (58%)
- 7 patients only diagnosed by serology

Dekeyser S et al., Pathol Biol 2011; 83-87

- *M.pn* detected in 2.9% by IgG seroconversion or significant IgG rise
- All 4/374 (1.1%) sputum PCR positive patients also IgG positive

➡ **Combination of PCR and serology detects most cases**

GRACE, unpublished results, 2012

Importance of dual or multiple infections?

Clinical relevance of infection with multiple viruses?

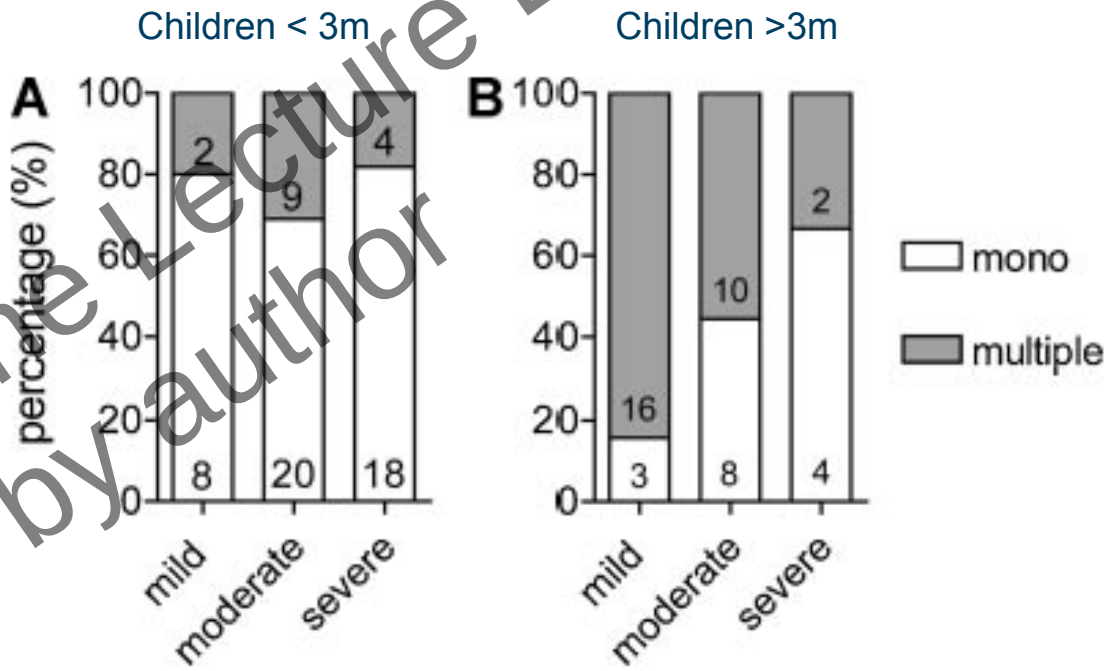
- children < 2yrs old with bronchiolitis:
  - Mild: no supportive treatment
  - Moderate: supplemental oxygen and/or nasogastric feeding
  - Severe: mechanical ventilation
- Mx PCR for 15 viruses on NPA
- Results: overall 211 viruses detected in 142 NPA
  - RSV most commonly detected virus: 73%
  - Rhinovirus in 30%
  - Other respiratory viruses in < 10% of samples

Brand HK et al. Pediatric Pulmonology 2011, 162: 88-90

Importance of infection with multiple viruses?

- Children younger than 3 months: less often infected by multiple viruses compared to children older than 3 months: 25% vs 65%

- Infection with 2 or more viruses: more frequent in children with mild or moderate disease than in those with severe disease



The detection of more than one virus is not associated with increased disease severity in children with bronchiolitis

Co- infections not associated with illness severity in acute febrile RTI

Suryadevara M et al. Clinical Pediatrics 2011, 50: 513-51

Brand HK et al. Pediatric Pulmonology 2011, 162: 88-90

When and where to implement these amplification techniques?

Respiratory Virus Infections in immunocompromised Patients

- Significant viruses in URTI and LRTI in lung transplants:
  - total 27 % patients: Flu > RSV > PI; hMPV 4.3%; rhino 18%

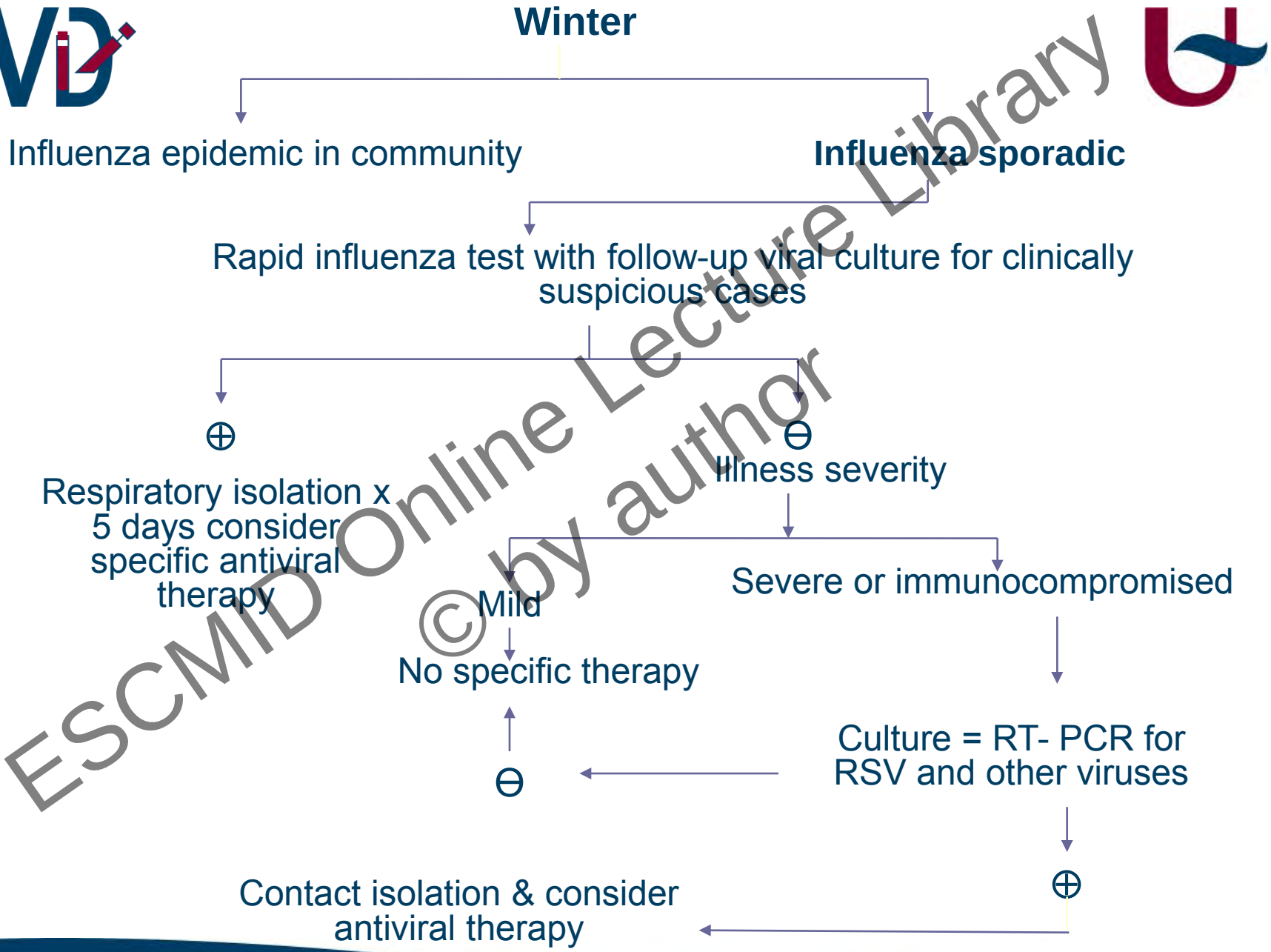
Weinberg A et al. J Clin Virol. 2002; 25: 171-75
Dare R et al. J Clin Microbiol 2007; 45:548-52
Camps Serra M et al. Eur Resp J 2008; 31: 618-24

- Significant cause of pneumonia in hematological cancers
  - total 35 % of patients: RSV > Rhino > Influenza > PIV
  - HCoV increasingly important: 11 %

Van Elden L et al. Clin Infect Dis. 2002; 24: 177
Van Elden L et al. J Infect Dis. 2004; 189: 653

- Acute RTI in elderly and children: up to 40%:
  - mostly rhino, RSV, hMPV, and influenza

Regamey et al 2008, Renwick et al 2007, Dare et al 2007, Jartti et al 2008



Falsey, Walsh CID, 2006; 42: 518