INFEZIONI DELLE BASSE VIE RESPIRATORIE (LRTI): ASPETTI CLINICI E DIAGNOSI MICROBIOLOGICA

I percorsi diagnostici microbiologici nel paziente immunocompromesso

Maria Luisa Modolo

S.C. Microbiologia Clinica e Virologia
A.O. "S. Maria degli Angeli"

Pordenone 14 Dicembre 2012

Immunocompromised host

The term “**immunocompromised host**” describes a patient who is at increased risk for life-threatening infection as a consequence of a **congenital or acquired abnormality of the immune system** .

During the past few decades, **the population of immunocompromised hosts has expanded enormously**,reflecting the increased use of immunosuppressive therapies used in solid-organ and haematopoietic transplantations, cancer and systemic illnesses.
In addition, acquired immunodeficiency syndrome (AIDS) has resulted in the existence of many immunocompromised patients.

The **respiratory system**, which is extensively connected with the environment, is **a favourite target for microorganisms**, **usually pathogenic**, or **otherwise with scarce pathogenic activity** (opportunistic), both exacerbating their virulence in immunocompromised patients

Pneumonia in Immunocompromised Patients

Host Defect	Disorders Or Therapy Associated With Defect*	Likely Pathogens
Defective PMNs		
Neutropenia	Acute leukemia, aplastic anemia, cancer chemotherapy	Gram-negative bacteria, *Staphylococcus aureus*, *Aspergillus* sp, *Candida* sp
Defective chemotaxis	Diabetes mellitus	*S. aureus*, gram-negative aerobes
Defective intracellular killing	Chronic granulomatous disease	*S. aureus*
Defective alternative pathway	Sickle cell disease	*Streptococcus pneumoniae*, *Haemophilus influenzae*
C5 deficiency	Congenital disorder	*S. pneumoniae*, *S. aureus*, gram-negative bacteria
Cell-mediated immunodeficiency		
(T-cell deficiency or dysfunction)	Hodgkin lymphoma, cancer chemotherapy, corticosteroid therapy	Mycobacteria, viruses (herpes simplex virus, cytomegalovirus), *Strongyloides* sp, opportunistic fungi (*Aspergillus*, *Mucor*, *Cryptococcus* spp), *Nocardia* sp, *Toxoplasma* sp
	AIDS	*Pneumocystis jirovecii*, *Toxoplasma* sp, cytomegalovirus, herpes simplex virus, opportunistic fungi (*Aspergillus*, *Mucor*, *Cryptococcus* spp), mycobacteria
Humoral immunodeficiency		
(B-cell deficiency or dysfunction)	Multiple myeloma, agammaglobulinemia	*S. pneumoniae*, *H. influenzae*, *Neisseria meningitidis*
	Selective deficiency: IgA, IgG, IgM	*S. pneumoniae*, *H. influenzae*
	Hypogammaglobulinemia	*P. jirovecii*, cytomegalovirus, *S. pneumoniae*, *H. influenzae*

*Examples.

In the immunocompromised hosts **enhanced susceptibility to a subset of pathogens** depending upon the **nature of the underlying immune defects**:

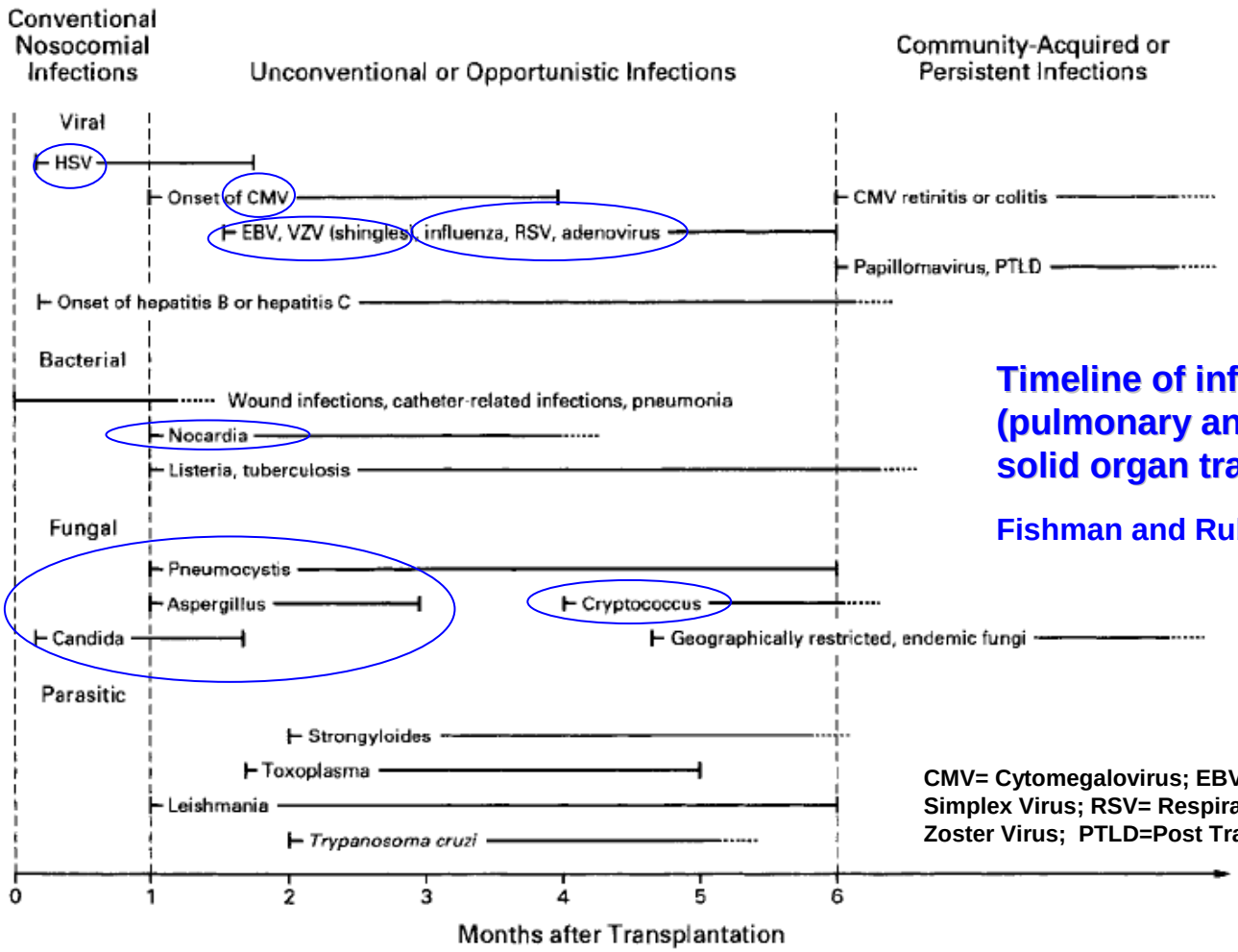
- abnormalities in neutrophils
- T lymphocytes
- B lymphocytes

In clinical practice, mixed patterns of immunodeficiency are frequently present

The etiologic agents of pneumonia in the immunocompromised host consist not only of the same agents that cause pneumonia in the immunocompetent host but also of a large number of opportunistic agents:

- bacterial infections
- viral infections
- fungal infections
- parasitic infections

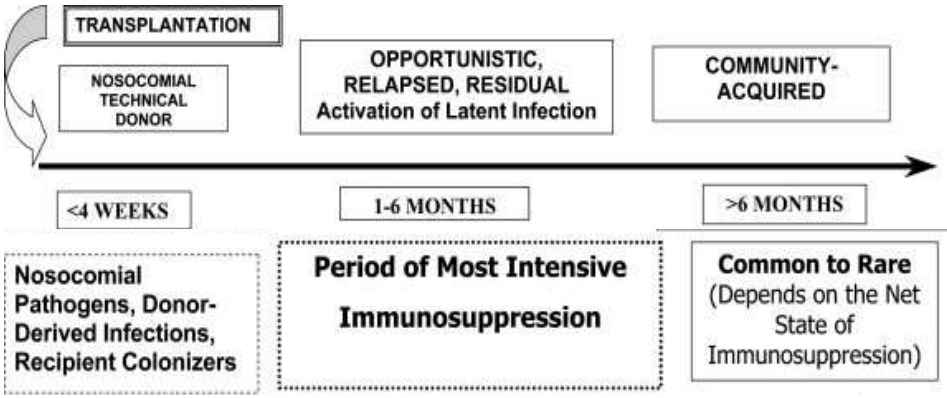
May 2008 by John G. Bartlett



Timeline of infectious complications (pulmonary and nonpulmonary) after solid organ transplantation.

Fishman and Rubin (NEJM;338:1741-1751)

CMV= Cytomegalovirus; EBV= Epstein BarrVirus; HSV= Herpes Simplex Virus; RSV= Respiratory Syncytial Virus; VZV= Varicella Zoster Virus; PTLD=Post Trasplantation lymphoproliferative disorder



COMMON VARIABLES IN IMMUNOSUPPRESSION

- **Antirejection therapy (anti-lymphocyte sera) and new agents for immunosuppression**
- **Neutropenia and lymphopenia**
- **Immunomodulatory viral infections (CMV, HCV, and EBV)**

Fishman J.A. Liver Transplantation
Volume 17, Issue S3, pages S34-S37, 26 OCT 2011

UK Standards for Microbiology Investigations

SMI Syndromic algorithm (S) Pneumonia S2

Pneumonia is defined as the presence of **clinical signs** and **symptoms** of **LRTI**, along with **radiological changes** that are consistent with pneumonia . An **assessment** of **illness severity should be made clinically**

On this basis, this algorithm deals with the investigation of patients presenting with **pneumonia** that is judged to be either **clinically mild or severe**. Pneumonia that may be judged to be **moderate** can still reflect a significant risk of mortality and therefore, **should be investigated as for severe pneumonia**

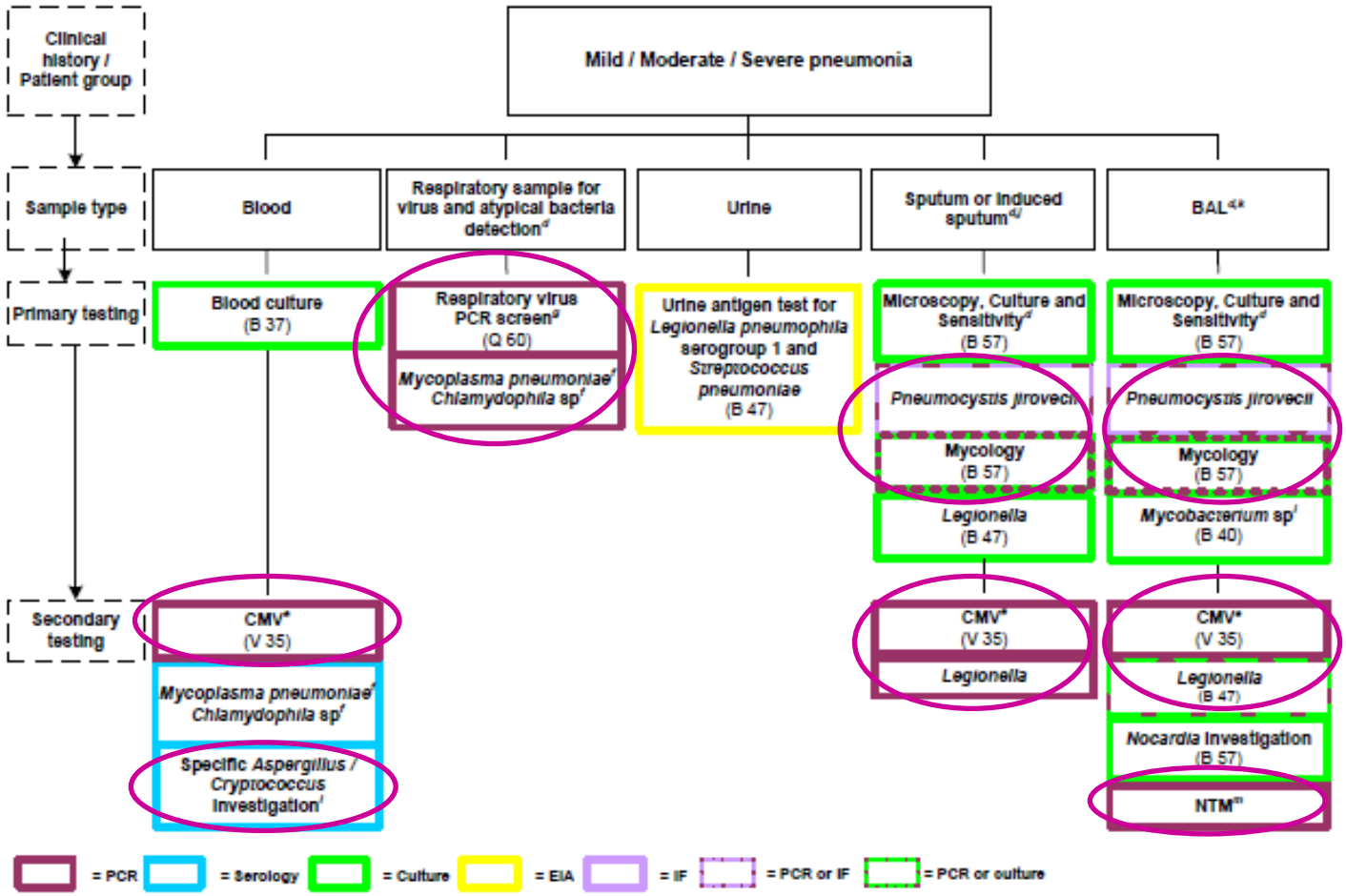
The **collection of diagnostic samples** (respiratory, urine and blood) should be carried out **before the administration of antimicrobials** in order to increase the likelihood of a microbiological diagnosis but initiation of treatment should not be delayed in severe cases.
If this is **not possible**, then samples taken for bacterial diagnosis should be **collected at a maximum of 24 hours** from the start of antimicrobial therapy whenever possible

In patients who are immunocompromised, microbiological investigation should be carried out **to the same extent if they are judged to have mild or severe pneumonia**. This is due to the fact that the **presentation of pneumonia** in this patient group **can be atypical** and the CURB-65a scoring system has not been validated for them. In addition, **progression from mild to severe illness can be rapid**

LRTI nel paziente immunocompromesso

Le differenze fondamentali rispetto all'approccio nel paziente immunocompetente
consistono nella **ricerca maggiormente orientata** all'individuazione
di **virus, batteri , funghi e parassiti**
che caratterizzano il diverso inquadramento etiologico delle LRTI
che insorgono nel paziente immunocompromesso

Pneumonia in immunocompromised adults[1-8, b]



Health Protection Agency

Blood → **Blood culture** → MALDI-TOF-MS / PCR-ESI-MS

Blood → Multiplex PCR (bacterial, fungal infections) / MALDI-TOFMS / PCR-ESI-MS

Fungal infections in immunocompromised hosts_1

Aspergillus spp.

Invasive pulmonary aspergillosis : **increasingly common problem in hospitalised patients**, especially in patients immunocompromised, those receiving systemic corticosteroids and those with prior pulmonary disease

The maiority of cases occurred within the first 90 days

Diagnosis of invasive aspergillosis can be problematic

- *Aspergillus* is cultured from sputum in only 8-34%, from BAL in 45-62% in patients with invasive disease, from respiratory tract cultures 28-55% in organ transplant recipients , with the highest rates of airway colonization after lung trasplantation. Serological studies have historically been unhelpful
- Detection of **serum galactomannan antigen** (a polysaccharide cell wall component of aspergillus) EIA has a sensitivity of 80-96% (HSCT, neutropenia, hematologic disease), 30% in lung transplant for the diagnosis of invasive aspergillosis
- Detection of **galactomannan antigen in BAL**: greater sensitivity and specificity (67-100% and 91-98%)
- **PCR *Aspergillus* DNA in BAL** : sensitivities similar to galactomannan detection. Use of both has been recommended to improve sensibility

Candida spp.

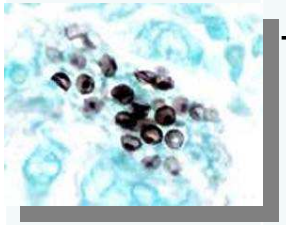
Candida sp. are the **most frequent cause of nosocomial fungal colonization/infection** in solid organ transplant patients causing 98% of fungal infections in a series of patients in a transplant ICU (*C. albicans, C. tropicalis , C. krusei*).

In lung transplanl recipients < 10% of patients colonized with *candida* species develop invasive disease. Pulmonary candidiasis is quite rare, more often in conjunction with disseminated candidiasis. **Proving a «true» *Candida* pulmonary infection can be difficult** . Therefore, the diagnosis **often requires lung biospy**

Singh N., et al. http//:www.antimicrobe.org/new; Kotloff R.M., et al. Am J Respir Crit Care Med 2004 170:22-48
Wheat LJ Transpl Infect Dis 2006;8 :128-139

Fungal infections in immunocompromised hosts_2

Pneumocystis jiroveci



P. *Jiroveci* is the **commonest cause of severe pneumonia** in patients with avanced HIV infection, and defines AIDS. It also occurs in numerous other immunocompromised adults and children, although co-trimoxazole prophylaxis is effective in the majority of cases

The greatest risk of *Pneumocystis jiroveci* pneumonia falls between 2 - 6 post-transplantation months. The risk declines significantly beyond the first year for all groups except lung transplant recipients.

Pneumocystis pneumonia before chemoprophylaxis organ-specific prevalence rates:

- kidney and heart trasplant recipient 4%
- liver transplant patients 11%
- allogenic HSC trasplant recipient 16%
- heart-lung recipients up to 33%
- HIV infected person (before 1995 from **4,9** cases/100 person-years to **0,3** cases/100 person-years after 1998

Laboratory diagnosis

- Lack of a reliable culture system
- Microscopy: cytological stain or immunofluorescence assay (cystis and/or trophic forms)
- **Molecular methods: several PCR/ Realtime PCR ⇒ BAL and sputum most useful clinical samples**

***Pneumocystis* colonization**

Healthy children ⇒10,5%; infant with respiratory syntoms and/or bronchiolitis ⇒15%
Adults most healthy people are not colonized ; patient with respiratory disorder ⇒ 7%-19% ; pregnant women ⇒ 16%; HIV-infected ⇒ 10-69%

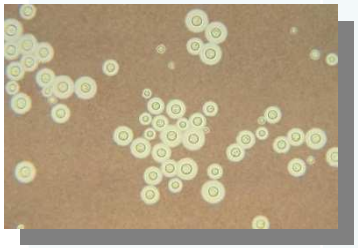
⇒ ⇒ ⇒ Quantitative molecular methods

Huang l., et al Proc Am Thorac Soc 2006;3:655-664 ; Singh N., et al. http//:www.antimicrobe.org/new;
Kotloff R.M., et al. Am J Respir Crit Care Med 2004 170:22-48

Fungal infections in immunocompromised hosts_3

Cryptococcus Neoformans

C. neoformans causes the **vast majority of cryptococcal infections** in immunosuppressed hosts, including patients with AIDS : is the most common fungal pulmonary infection in AIDS patients who have CD4 < 100 cell/mmc (usually coexsit with cryptococcal meningitis).



Cryptococcosis **prevalence rate**

- 19 patients/ 31 patients with cancer- pulmonary involvement (61%).
- SOT 2.8%, the CNS was the most commonly affected site: 55% of patients had CNS infection alone, 6% of patients had pulmonary infection only, and 24% had infection at more than 1 site

Laboratory diagnosis

C. neoformans can be isolated on most **routine media** : detected from **3 to 7 days** after

Standard **blood culture** methods routinely identify *C neoformans* and confirm a diagnosis of cryptococcemia (up to 75% of patients with HIV-1–associated cryptococcal meningitis will have positive blood cultures)

Clinical specimens staining India ink staining (encapsulated yeast cells): yeasts are easily highlighted on routine Gram stains

Antigen detection (capsular polysaccharide)

- **Serum** : especially in patients with AIDS
  In cryptococcal lung disease test highly effective at identifying active disease, it does not discriminate the site of infection. Often require a second diagnostic procedure: biopsy or sputum examination
- **Cerebrospinal fluid** (CSF) in patients who have meningitis or meningoencephalitis detection of is both sensitive and specific (90%, 95%)
- **BAL** in patients with pneumonia (BAL 100%, serum 2/3 of patients,fungal stain only 1/3)

Serology : antibody response no role in diagnosis of cryptococcosis

Opportunistic Fungal Infections, Part 3: Cryptococcosis, Histoplasmosis, Coccidioidomycosis, and Emerging Mould Infections By Michelle A. Barron, MD and Nancy E. Madinger, MD | 18 november 2008; Wheat LJ transpl Infect Dis 2006; 8:128-139

Bacterial infections in immunocompromised hosts

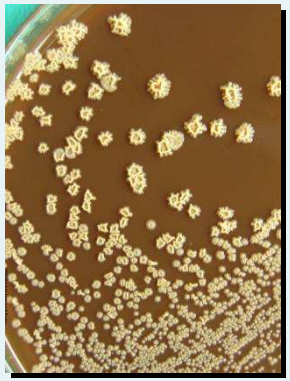
Nocardia spp.

Infections caused by *Nocardia* are **infrequent but challenging** to clinicians:the incidence of *Nocardia* pneumonia **has declined substantiall** with the use of trimethoprim-sulfamethoxazole **prophylaxis**, however, it continues to be reported

- **In the early era** of organ transplantation, *Nocardia* infections were relatively common, with a prevalence of **2–13%** documented in several large series
- **More recent case series** have suggested a lower frequency of infection, on the order of **0.2–2.1%,** mainly **lung** trasplant and **hear**t trasplant recipients

Mortality directly attributable to *Nocardia* infection ranges from **0 to 30%** among the various SOT populations

Infection due to this aerobic, gram-positive filamentous rod **is most common beyond the first month after transplantation**



Laboratory diagnosis is difficult and requires a high index of suspicion

- **Diagnotic specimens** : BAL, lung biopsy, pleural fluid and sputum culture
- **Microscopy** : initial visualization is often not possible with routine stains
- **Standard culture methods** : growth slowly on the standard medium for bacterial cultures ; identification (routine phenotypic testing; a real-time PCR assay (of 16S rDNA)⇒ melting-curve analysis to identify *Nocardia* spp.)
- **Serological methods** : usefulness limited by the variety of species , potential lack of sensitivity in immunocompromised patients

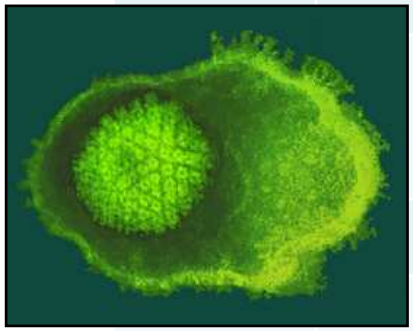
If *Nocardia* is suspected, cultures should be held longer to ensure that this diagnosis is not missed

Corti M., et al. Current Opinion in Pulmonary Medicine. 2009, 15:209-217

Viral infections in immunocompromised hosts-1

CMV

CMV is still the most important virus affecting immunocompromised patients (in the absence of antiviral prophylaxis 20-35%)



<u>CMV pneumonia incidence</u>

- HSCT 10-30% in allogenic recipients (mortality rate 4 ⇒ 0,8%)
- SOT recipients : lung 10-55%; liver 9,2%; heart 0,8-8% , renal <1%
  Lungs are a major organ site of CMV latency and recurrence: higher burden of latent viral CMV genome than other organs
- Lymphoma 82% (mortality rate 30%); non-Hodgkin lymphoma (89%)
- HIV patients connotation of CMV pneumonia is not clear , often not the only pathogen

Vigil KJ., et al 2010; J Intensive Care Medicine 2010; 25(6):307-326

<u>Diagnosis</u>

EDTA - blood samples ⇒ QNAT (QPCR) CMV DNA

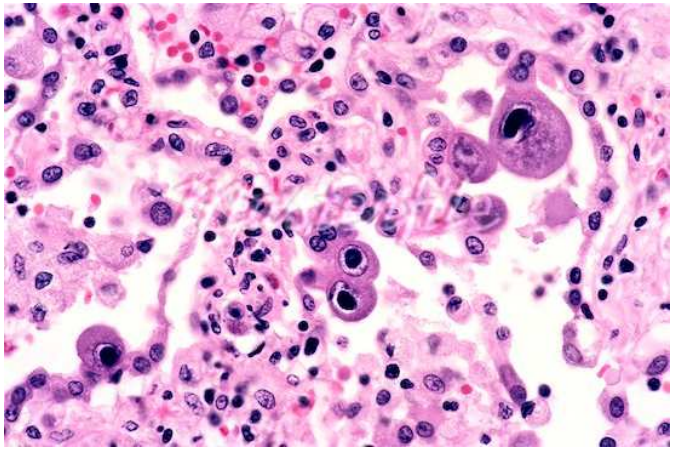
<u>Dynamic determination of CMV-DNA</u> may predict the occurrence of CMV-IP (Interstitial Pneumonia).
Viral loads > 10^4 copies/mL plasma continuing for 3 weeks may serve as a cutoff to predict CMV-IP (Renal transplant recipients)

Ye Q., et al. Trasplant Proc. 2004; 36(10):3036-41.

<u>Monitoring CMV infection</u> in both blood samples and lungs (BAL) may improve preemptive therapy efficacy (Lung trasplant Recipients)

Gerna G., et al. American Journal of Transplantation 2009;9(5)1142-1150

Preemptive Therapy for Systemic and Pulmonary Human Cytomegalovirus Infection in Lung Transplant Recipients



Appears highly advisable to monitor HCMV infection in lungs of LTR for the following reasons:

(i) a high HCMV load in BAL (> 5x 10^5 copies/mL) was clearly associated with HCMV pneumonia

(ii) early HCMV infection in blood has been associated with a greater risk of developing BOS (bronchiolitis obliterans syndrome)(3.0x10^5DNA copies/mL whole blood= cutoff for systemic infection)

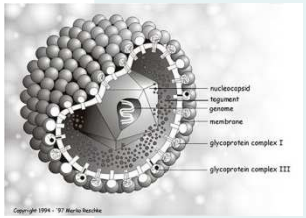
(iii) lung infection may occur in the absence of systemic infection, as reported in other studies and as shown in this study, where two patients with histologically proven HCMV pneumonia had viral load in blood< cutoff for initiating pre-emptive therapy

In this report, for preemptive therapy we selected a cutoff of 1.0 x 10^5DNA copies/mL BAL

G. Gerna1, et al. American Journal of Transplantation
Volume 9, Issue 5, pages 1142-1150, 16 APR 2009

Viral infections in immunocompromised hosts-2

HSV



HSV1 and HSV2 have been reported to cause pneumonia in HSCT recipient, liver trasplant, patients with solid tumors.

However, it is difficult to distinguish between asyntomatic shedding of the virus and disease

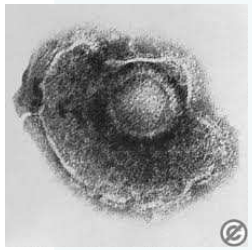
Vigil K.J., et al. Journal of Intensive Care Medicine2010.25(6) 307-326

Quantitative detection of HSV DNA in BAL is a potential diagnostic tool for detection of relevant viral infection of the lower respiratory tract.

Patients with HSV DNA levels ≥ 7.5 log had severe respiratory failure

Gooskens J., et al. J.Med:Virol. 2007 May;79(5):597-604.

VZV



Pneumonia HSCT or SOT recipients (lung trasplant): evidence for the diagnosis of varicella pneumonia viral inclusion bodies on histology

Toby M. Maher. *AJR* 2007; 188:W557–W559

Pneumonia HSCT recipients: visceral VZV disease ≈ 6 months after transplantation

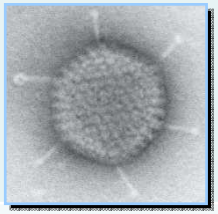
PCR in whole blood ⇒ VZV DNA detection

PCR quantification VZV DNA ⇒ possible usefulness for assessing the effectiveness of treatment

Ishizawa J et al. Int. J.Hemat. 2006: 242-245

HSV1, HSV2 and VZV : rare causes of LRTI. Pneumonia is typically due to reactivation of a latent infection and is now infrequent ↔ prophylaxis

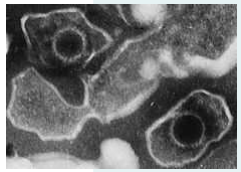
Viral infections in immunocompromised hosts-3

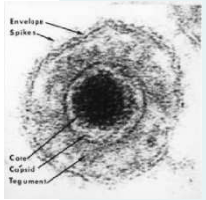
ADENOVIRUS 	Important causa of morbility and mortality in patients immunocompromised , particularly children, neonates and HSCT Immunocompromised hosts may experience reactivation of latent infections Infection usually occurs in the first 3 months post-transplant and may be organ specific (eg. pneumonia) or infection disseminated
HSCT	Adenoviruses 2,5 (species C, often showing a more severe course of illness), 34,35 (sub-species B2), and 31 (species A) have been isolated from HSCT recipients with respiratory diseases (children incidence up to 31%)
SOT	Serotypes 1 and 2 (subgroup C) are more commonly associated with pneumonia
HIV	Adenovirus in 7,4% of patients with CAP, but is almost always associated wtih multiple other pathogens
Diagnosis Respiratory viruses screening	Adenovirus DNA: viral load quantification (qRT-PCR), in serum or plasma marker ⇒ detecting ⇒ monitoring disease progression ⇒ treatment response in patients immunocompromised, particularly in HSCT PCR whole blood ⇒ significant screening method in pediatric HSCT (asyntomatic patients who are at risk for progressive adenoviral disease) No specific threshold although viral loads >1 x 10^6 copie/mL ⇒ increased likelihood of death in HSCT recipients **Statistically significant differences between viral loads of different AV types**

Vigil KJ., et al 2010; J Intensive Care Medicine 2010; 25(6):307-326

Viral infections in immunocompromised hosts-4

EBV





There is a high prevalence of EBV infection in immunocompromised patients, transplant receivers, acquired human immunodeficiency vírus and non-Hodgkin lymphoma.

EBV has a central role in the pathogenesis of Post Trasplantation Lymphoproliferative disorder (PTLD) although not all PTLD is EBV-related.

Remitting-relapsing EBV infection is common in transplant recipients and may reflect ⇒ *relatively excessive immune suppression*

Primary EBV infection presenting as pneumonia in recent HSCT recipient (donor seropositive ⇒ EBV-seronegative transplant recipient)

quantitative PCR for EBV DNA → samples of blood and nasopharyngeal secretions, were highly positive : 123000 copies/mL and 1344 copies/mL, respectively)

Teira P et al. Clin Infect Dis. 2006;43:892-895

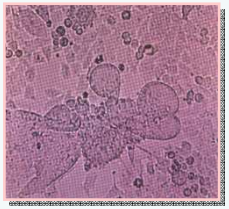
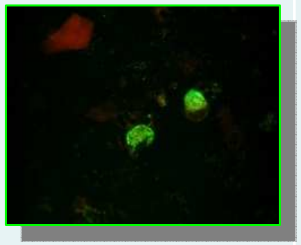
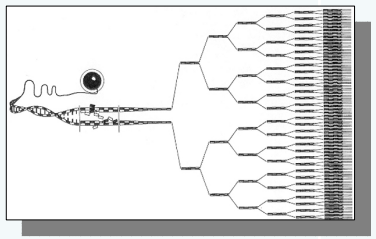
Diagnosis acute EBV infection or PTLD

- **Serologic testing** is not useful
- **Quantitative EBV viral load** testing helpful for the diagnosis and management PTLD
  - serial assays of whole blood may be useful in individual patients
  - specific diagnostic levels of viral loads are not available

EBV assays are not standardized and cannot be easily compared between centers

Camille N. Kotton and Jay A. Fishman*J Am Soc Nephrol* 16: 1758–1774, 2005.

Diagnosis of respiratory viral infections

Serology	**In general is not useful for initial diagnosis and has reduced sensitivity among trasplant recipients**
Virus culture 	• For most of the common DNA /RNA virus except Rhinovirus, Coronavirus.... • Special cell lines and condition are need to grow these viruses and culture tend to be **inefficient** : several cell lines (3-21 days) >> shell vial assay/ fixed mixture of cells + monoclonal/polyclonal antibodies-FITC (24-48 h)
Direct fluorescent assay (DFA) 	**Direct antigen detection in a specimen** (IFA, enzyme-linked immunoassay, immune chromatography) – Can detect several viruses from a single specimen – Good clinical specificity and short turn-around time < 2 h – Sensitivity can be lower than reported in licensing studies **Lack of reagents for some the viruses** (eg. rhinovirus,coronavirus) , **Less sensitivity** in detecting dual infections, **sensitivity** can be substantially **Lower among immunocompromised patient**
Nucleic acid testing 	**Nucleic acid amplification assays** **Most sensitive diagnostic tool** available to **screen for a wide range of pathogen in tandem** ⇒ **multiplex testing platform** ⇒ **microbead-based assay ⇒ microarray and nanotechnologies** **Many have been tested in trasplant population** **PCR is the preferred testing method for immunocompromised patients**

Respiratory virus screening

Viral PCR screen is the same for patients who are immunocompetent and immunocompromised

Respiratory samples for viral PCR screening are ideally lower respiratory tract samples such as an induced sputum, BAL or endo-tracheal aspirate. Where this is not possible, a nose/throat swab is acceptable.

Types of samples	**Upper Respiratory Tract** • nasopharyngeal aspirate • nasopharyngeal swab, nose /throat swabs (combined) preferably flocked for nasal sampling
	Lower Respiratory Tract infections Bronchoalveolar lavage (BAL) is the preferred sample, but is not commonly available Sputum is an acceptable sample although this may present problems in processing due to the nature of the specimen which is frequently mucoid If a cough is non-productive and not severe enough to warrant taking a BAL sample then a sample from the upper respiratory tract may be the only sample available

The quality of the sample is paramount in diagnosing respiratory virus infection

Respiratory viruses - old viruses: Influenza , parainfluenza, RSV, HMPV, Adenovirus

SOT

Greater propensity for these pathogens to involve the lower respiratory tract ⇒ severe illness

SOT populations : the highest rates of infection in lung transplant recipients, up to 21% of whom develop respiratory viral infections

Mortality rates in the range of 0–20% have been reported in association with respiratory viral infections in the various solid organ transplant populations

Seasonality

RSV and **Influenza virus** infections ⇒ epidemics in the winter and spring months
Adenovirus and **Parainfluenza** ⇒ infections throughout the year

HSCT

These pathogens are recovered from up to 1/3 of HSC transplant recipients hospitalized with acute respiratory illnesses : **RSV is most commonly isolated**

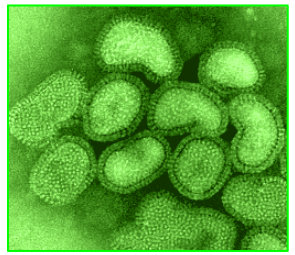
Outbreaks among the HSCT population tend to coincide with community outbreaks

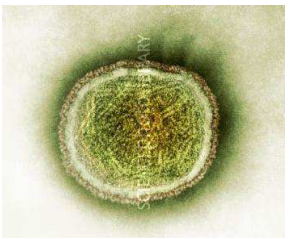
Most patients present initially with **URI** ⇒ **pneumonia** occurs frequently in association with **RSV** and **parainfluenza** infection but considerably less so with **influenza**, although postinfluenza bacterial pneumonias are a concern

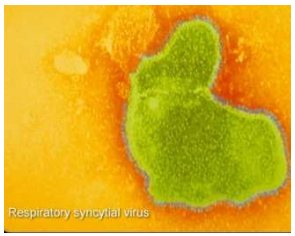
Among patients with **RSV** infection, the risk of pneumonia ≈ 80% for those who are < 1 month posttransplantation or still in the preengraftment stage, but falls to less than 40% for those beyond this critical period

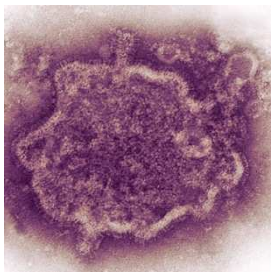
Once pneumonia develops, mortality from untreated RSV infection approximates 80%
Mortality associated with parainfluenza and influenza pneumonia is considerably lower

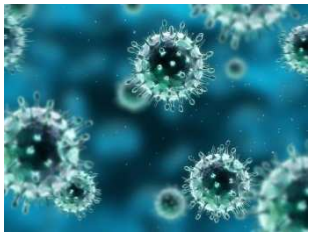
hMPV (2001) : similar epidemiology and clinical course to RSV. Presence of copathogens, particularly RSV ⇒ more severe disease







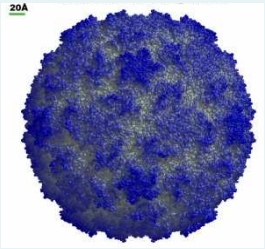




Kotloff R.M., et al. Am J Respir Crit Care Med 2004 170:22-48 ;Ison MG. Antiviral therapy . 2007; 12:627-638
Vigil KJ., et al 2010; J Intensive Care Medicine 2010; 25(6):307-326

Viral infections of LRT : new viruses and the role of diagnosis

Rhinovirus



- Rhinoviruses are the most common cause of URI, detection in asymptomatic persons is relatively common . Recent studies: rhinovirus can replicate at higher temperatures and infect the lower respiratory tract

  ⇒ **genotypes** with **greater capacity** for LRT disease and severe disease

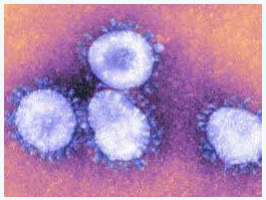
Viral quantification may prove helpful to determine when an isolate is associated with severe disease

- 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

Coinfections with other pathogens ⇒ high morbility and mortality of this infection

---

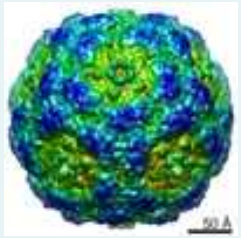
Coronaviruses
No SARS-CoV



- Human coronavirus NL63 (HCoV-NL63,Netherlands 2004) clearly been associated with croup and bronchiolitis and occasionally with pneumonia
- HKU1, NL63, OC43, and 229E were isolated from 2.2% of children hospitalized with respiratory disease
- Coronaviruses are being increasingly found in immunocompromised patients and those with underlying pulmonary disorders, but there is little information on risk of progression to pneumonia

---

HBoV



- Bocavirus isolated in 2005 (DNAss, family *Parvoviridae*) in NPA specimens (also in stool and in serum) from children with respiratory tract infection
- Bocavirus frequently in conjunction with other pathogens and detected for prolonged periods ⇒, viral load were significantly higher in patients with only bocavirus than in those with coinfection

⇒ accumulating data support the role of bocavirus as a true pathogen ⇒ clinical profile and role as a causative agent of respiratory disease **is still not clear**

Gerna G et al. J Med Virol 2009; 81:1498-1507; Pavia AT. Clinical infectious Diseases. 2011;52(S4):S284-S289

The challenge of respiratory virus infections in hematopoietic cell transplant recipients

Table II. Seasonality, symptoms, and diagnosis of respiratory viruses in immunocompetent subjects and HCT recipients [adapted from Nichols *et al* (2008), with permission from American Society for Microbiology].

		Disease manifestations in immunocompetent persons						Disease in HCT recipients			Diagnosis			
Virus	Seasonality	URI	Otitis media	Croup	Bronchiolitis	Pneumonia	AFO and/or wheezing	URI	Pneumonia	AFO	Culture	EIA	FA rapid cultures	PCR
RSV	++	+++	++	+	+++	+++	+++	+++	+++	++	+	+	++	+++
HMPV	++	+++	++	+	++	+++	++	+++	+++	++	Research	NA	Research	+++
PIV 1	+	+++	+	+++	+	++	++	+++	+++	+++	+	NA	+	+++
PIV 2	+	+++	+	++	+	++	++	+++	+++	+++	+	NA	+	+++
PIV 3	−	+++	+	++	++	++	+++	+++	+++	+++	+	NA	+	+++
PIV 4	−	+++	+	+	+	+	+	+++	+++	ND	+	NA	++	+++
Influenza A, B	++	+++	+	+	+	++	+	+++	+++	+	+	+	++	+++
Adenoviruses	+	+++	+	+	++	+	−	+	+++	−	+	++	++	+++
Rhinoviruses	+	+++	+	+	+	+	+++	+++	+	++	+	NA	NA	++
Coronaviruses	+	+++	+	++	+	+	++	ND	ND	ND	Research	NA	NA	+++
Bocavirus	+	+++	ND	+	+	+	+	ND	ND	ND	NA	NA	NA	+++

HCT, haematopoietic cell transplantation; RSV, respiratory syncytial virus; HMPV, human metapneumovirus; PIV, parainfluenza virus; URI, upper respiratory infection; AFO, airflow obstruction; EIA, enzyme immunoassay; FA, fluorescent antibody; PCR, polymerase chain reaction; ND, no data; NA, not available.

Michael Boeckh Br J Haematol 2008;143,455-467

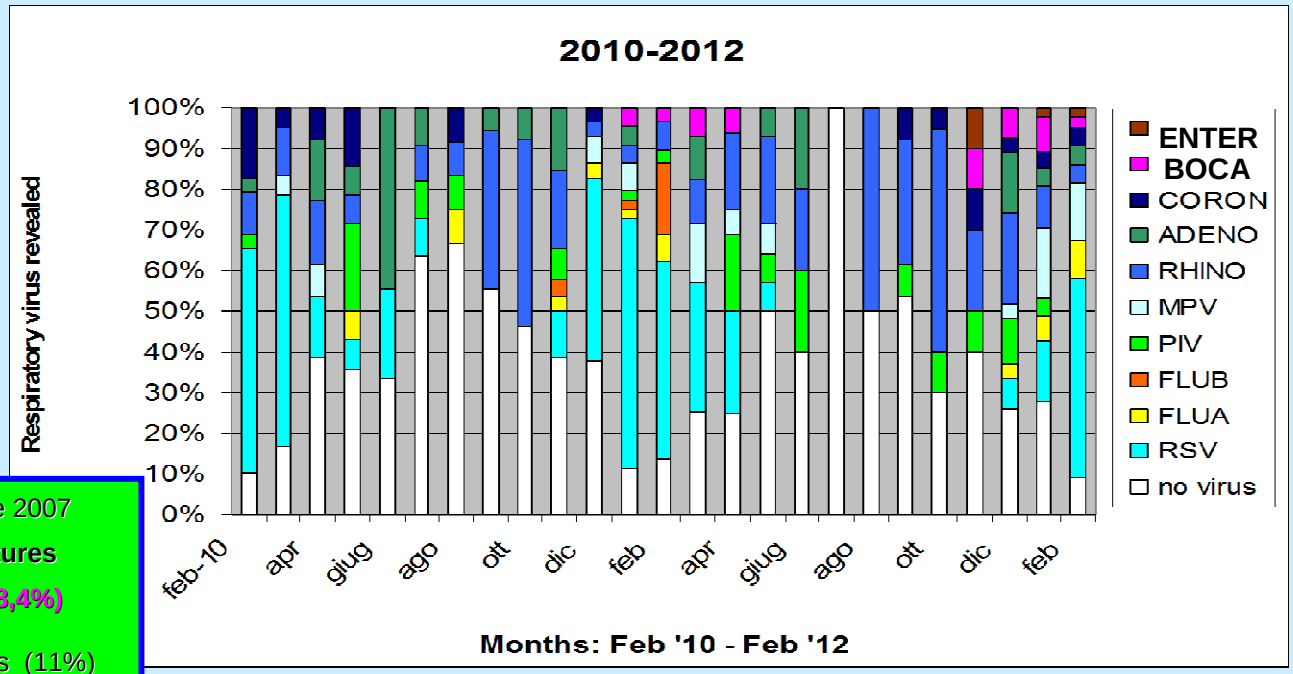
For transplant recipients with respiratory infection the ability to detect the full range of viral pathogens is critical

Improved molecular detection

- RSV, hMPV, PIV and Influenza virus are well known for their potential ⇒ fatal pneumonia
- new viruses hMPV, coronavirus, bocavirus, and rhinovirus

Early identification allow effective cohorting and isolation

Largest potential societal benefit may come from reduction unnecessary use of antibiotics for LRTI in children : respiratory tract infections remain the most common reason for prescribing antibiotics, and each course of antibiotics adds selective pressure



Ottobre 2006 – Aprile 2007

Standard viral cultures

118/202 positive (58,4%)

RSV (42%) > Adenovirus (11%) > Influenza A (1,5%) > (0,5%) PIV 3

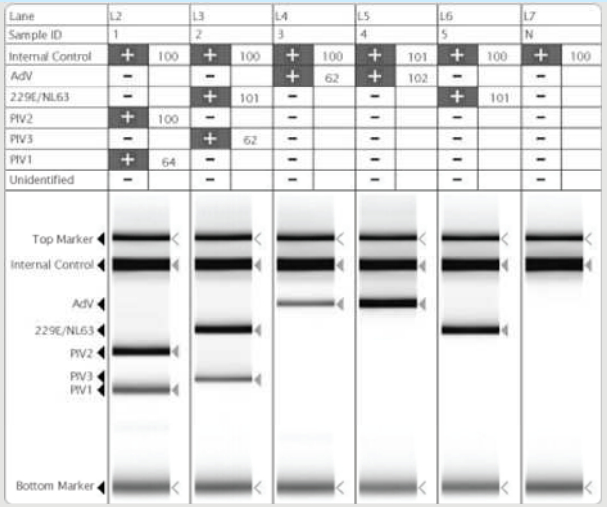
Epidemiology of respiratory virus infections in children, the virus-specific positivity rates, and seasonality for respiratory virus infections, over a 24 months period (February 2010-2012)

- **Multiplex PCR** : 490 specimens tested, 336 (68.6%) were positive for at least one respiratory virus
- RSV A/ B (30%) > RHINO (16%) > AD (7%) > MPV (6%) , PIV (6%) > BOCA (5%) > COR (4%) > IA (3%) > IB (1%) ; Enterovirus (1%). Dual respiratory virus infections (8%), and only 4 triple virus infections

RV12 and **RV15** increased our understanding of the **epidemiology of respiratory viral infections** and assist us in the **diagnosing** the **etiology** of respiratory tract infections **in individual** and **in outbreak situation**

Amplified PCR products using Seeplex® RV15 ACE Detection and result analysis.

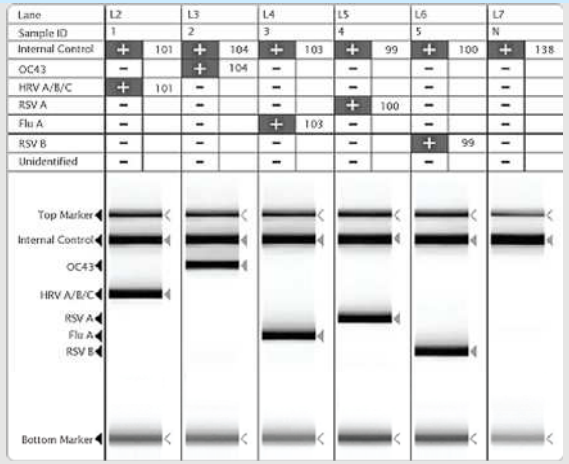
A | A set



Sample ID	Result
1	PIV2, PIV1
2	229E/NL63, PIV3
3	AdV
4	AdV
5	229E/NL63

1~5 : Clinical samples,
N : Negative Control

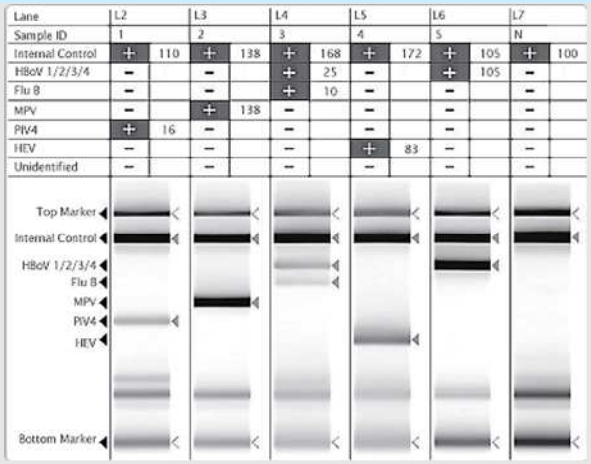
B | B set



Sample ID	Result
1	HRV A/B/C
2	OC43
3	Flu A
4	RSV A
5	RSV B

1~5 : Clinical samples,
N : Negative Control

C | C set



Sample ID	Result
1	PIV4
2	MPV
3	HBoV, Flu B
4	HEV
5	HBoV

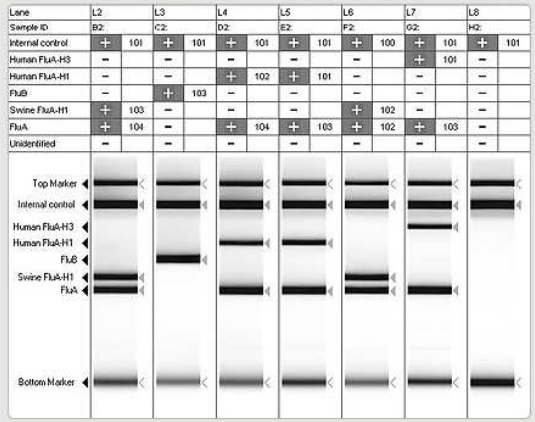
1~5 : Clinical samples,
N : Negative Control

Seeplex® *RV15 OneStep ACE Detection* is designed to test most currently known respiratory viruses by one-step multiplex RT-PCR

Flu A virus; Flu B virus; RSV A, B; MPV; PIV 1-4, Adenovirus A/B/C/D/E; Enterovirus; Bocavirus 1/2/3/4; Coronavirus 229E/NL63-OC43, Rhinovirus A/B/C

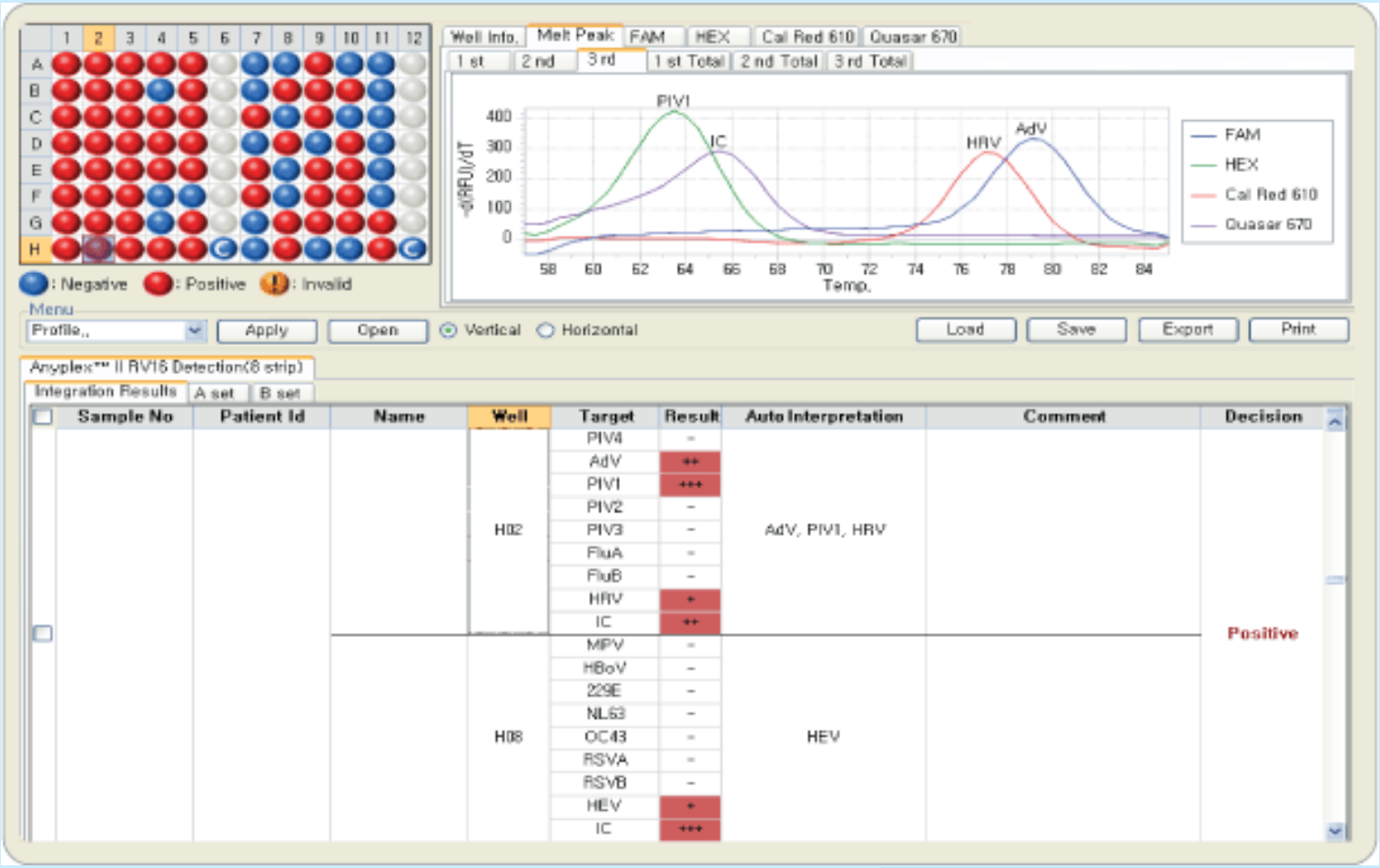
Seeplex® *Influenza A –H1, A-H3, 2009 pandemic H1 / InfluenzaB*

Influenza A/B OneStep Typing



Lane	Result
B2	2009 pandemic H1, FluA
C2	FluB
D2	Human FluA-H1, FluA
E2	Human FluA-H1, FluA
F2	2009 pandemic H1, FluA
G2	Human FluA-H3, FluA

B2~G2 : Clinical samples,
H2 : Negative Control



Multiplex Real-time PCR

Simultaneous detection of 16 respiratory viruses

Panel A

- Adenovirus (AdV)
- Influenza A virus (FluA)
- Influenza B virus (FluB)
- Parainfluenza virus1 (PIV1)
- Parainfluenza virus2 (PIV2)
- Parainfluenza virus3 (PIV3)
- Parainfluenza virus4 (PIV4)
- Rhinovirus A/B/C (HRV)
- Internal control (IC)

Panel B

- Respiratory syncytial virus A (RSV A)
- Respiratory syncytial virus B (RSV B)
- Bocavirus 1/2/3/4 (HBoV)
- Coronavirus 229E (CoV 229E)
- Coronavirus NL63 (CoV NL63)
- Coronavirus OC43 (CoV OC43)
- Metapneumovirus (MPV)
- Enterovirus (HEV)
- Internal control (IC)

LRTI nel paziente immunocompromesso

Le differenze fondamentali rispetto all'approccio nel paziente immunocompetente
consistono nella **ricerca maggiormente orientata** all'individuazione
di **virus, batteri , funghi e parassiti**
che caratterizzano il diverso inquadramento etiologico delle LRTI che insorgono nel paziente immunocompromesso

In questo contesto clinico e immunologico, se si intende garantire una **diagnostica davvero efficiente ed efficace** , non si può prescindere dai **metodi molecolari** , che permettono di rilevare anche **microrganismi altrimenti non diagnosticabili con la medesima efficacia e sensibilità**

A. Camporese
Le Infezioni in Medicina, n. 4, 237-244, 2012