

Congresso NEWMICRO

Biotechnologie e diagnostica molecolare
nella diagnosi delle malattie infettive



19/21 GENNAIO 2011

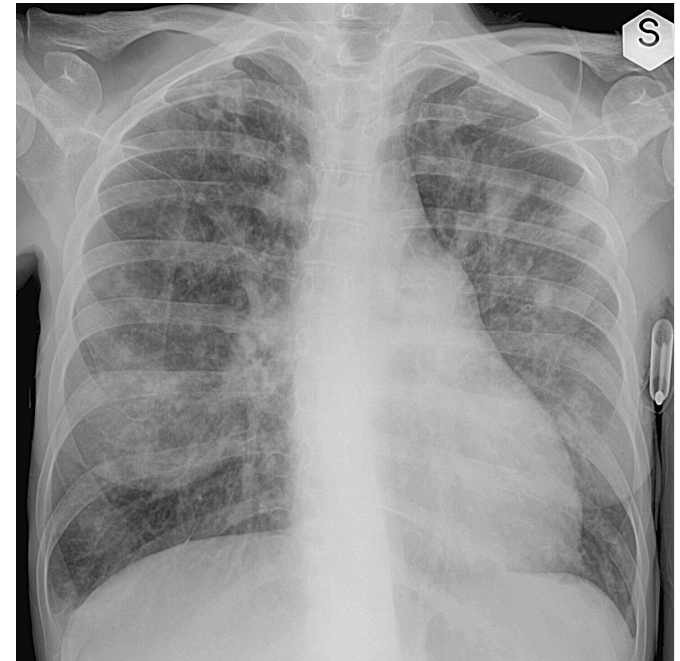
Le tecniche molecolari nella diagnosi di infezione:
proposte di protocolli diagnostici

Le tecniche molecolari nella diagnosi delle infezioni respiratorie

Alessandro Camporese, Maria Luisa Modolo
Microbiologia e Virologia - Pordenone

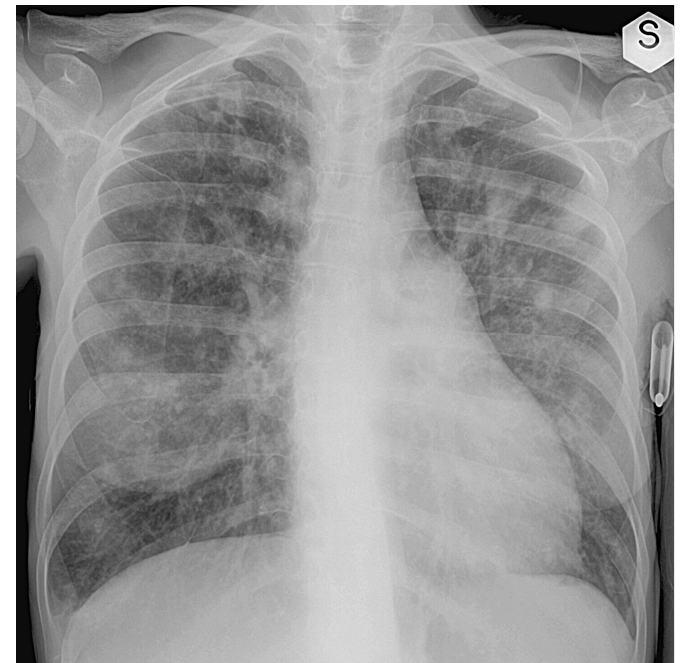
Acute respiratory infections are a leading cause of acute illnesses worldwide.

Herman Goossens BMJ 2006



While **upper respiratory** tract infections are very frequent but seldom life-threatening, **lower respiratory tract infections (LRTI)** are responsible for more severe illnesses.

Herman Goossens BMJ 2006



Lower respiratory tract infections are among **the most common** infections of **adults and children.**

Murdoch DR. APMIS 112: 713–27, 2004

The **populations most at risk** for developing a fatal respiratory disease are the **very young**, the **elderly**, and the **immunocompromised**.

LRTI: a challenge for diagnostic microbiology

The **number of pathogens** involved in lower respiratory tract infections (LRTI), with various susceptibilities to antimicrobials, is large constituting **an enormous challenge** for diagnostic microbiology.

LRTI: a challenge for diagnostic microbiology

During recent years a considerable number of previously unknown respiratory agents were discovered whose in vitro culture is very slow or even unrealized.

Etiological diagnosis: the real problem

While the clinical diagnosis of LRTI is usually relatively straightforward, determining the etiological diagnosis can be much more difficult due to the limitations of conventional diagnostic tests.

Etiological diagnosis: the real problem

At present there is still a great deficit in the etiologic diagnosis of LRTI.

In most studies more than 50% of cases remain without an etiologic diagnosis.

Etiological diagnosis: the real problem

In general, in only 50% of cases is an etiologic agent detected.

Documented infection is uncommon in community-managed infection and is usually only defined in 25 to 50% of hospital-managed infections.

At the beginning of the 21st century, a high proportion of diagnostic tests are still performed according to methodologies pioneered by Pasteur at the end of the 19th century, i.e. methods based on culture, microscopy, and serology.

Bissonnette L and Bergeron MG. CMI 2010.



Infectious diseases diagnosis and empirical approach

In the 1960s and 1970s, physicians embraced an **empirical approach** to the management of many infectious diseases, favouring **overuse of antibiotics**.



Genomics to combat Resistance against
Antibiotics in Community-acquired LRTI in Europe

In Europe **90-95%** of antibiotic use occurs **outside** hospitals, and community acquired **lower respiratory tract infections** are **the leading reason** for prescribing antibiotics.

The overall **objective of GRACE** is to combat antimicrobial resistance through integrating centres of excellence for studying the **application of genomics to community-acquired lower respiratory tract infections (CA-LRTI)**.

<https://www.grace-lrti.org>



Genomics to combat Resistance against
Antibiotics in Community-acquired LRTI in Europe

The goals of GRACE is to enroll 3,000 LRTI patients and matched controls to **study the etiology** of LRTI in the community **by applying conventional and NAATs** to detect the causative organisms.

GRACE should therefore help identifying the **optimal microbiological method** for diagnosis of CA-LRTI.



Genomics to combat Resistance against
Antibiotics in Community-acquired LRTI in Europe

Traditional bacterial culture and serological testing for detection of pneumonia microorganisms have low sensitivity, are time-consuming, take several days and focus only on a few of the large number of aetiologic agents.

To evaluate the usefulness of NAAATs in the diagnosis of acute respiratory infection the objectives of an etiologic diagnosis in this condition should be remembered.

Objectives of an etiologic diagnosis

1. To **avoid empirical start of antibiotic treatment** and to allow narrow spectrum targeted antibiotic treatment;
2. to allow appropriate **use of antiviral** drugs;
3. to allow **cohorting of patients** in case of hospitalization, preventing nosocomial spread;
4. to provide more accurate **epidemiological information** to formulate preventive and therapeutic recommendations;
5. to **decrease duration of hospital stay** and to reduce management **costs**.

To answer the **first three** objectives, diagnosis **should be available rapidly**, preferably within about 4 h.

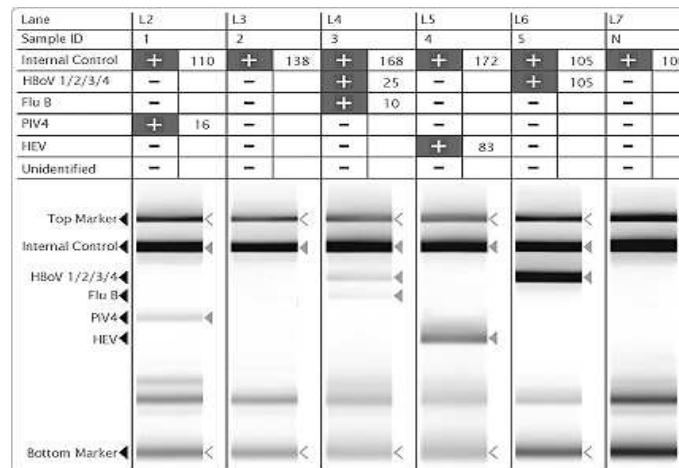
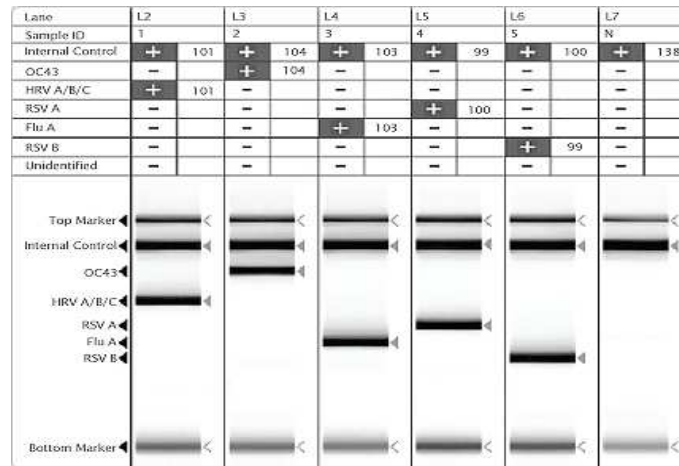
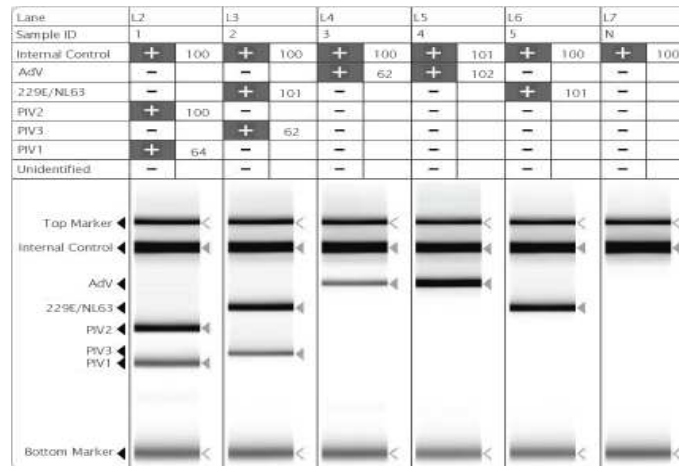
The **traditional NAATs are unable** to fulfill this requirement, but recent technical progress has brought NAATS to age, through the development of **multiplex PCR and real time (RT) PCR**, coupled with **automatic NA extraction**.

Seeplex® RV 15 ONE STEP

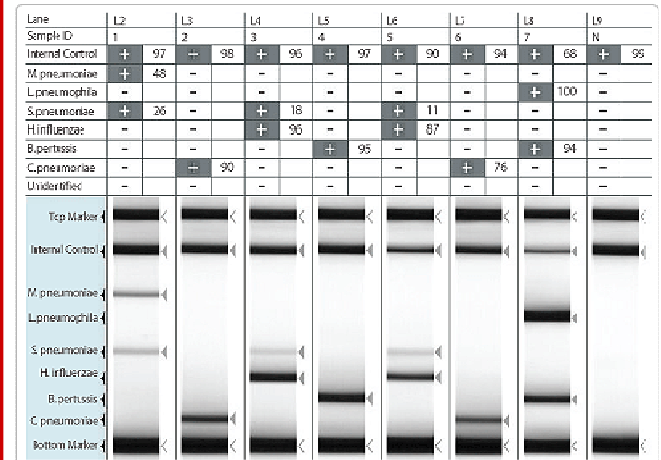
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Adenovirus A/B/C/D/E
Coronavirus 229E/NL63

Coronavirus OC43
Rhinovirus A/B/C
Influenza A virus
RSV A
RSV B

Bocavirus 1/2/3/4
Influenza B virus
Parainfluenza virus 4
Enterovirus



Dual Priming Oligonucleotide technology (Seegene)



M. pneumoniae
C. pneumoniae
L. pneumophila
S. pneumoniae
H. influenzae
B. pertussis

Reevaluation of serological tests

The availability of the very sensitive NAATS has in recent years also put the often-used **serological tests in their right perspective.**

Serologic tests **can never offer an early diagnosis** and are therefore rather an epidemiological than a diagnostic tool.

Optimization of laboratory strategy

Strategies will have to be developed **adapting** the evolution of the **technology** of the NAATs, the **population** of patients served (children, elderly, and immunocompromised patients) the **resources** available (infrastructure, staff, full-time service or service limited during some hours of the day, or some days of the week), the **number and nature of the agents** that can be covered.

L'organizzazione e le competenze

Nella dichiarazione congiunta di Copenhagen (1993), l'*European Council of Legal Medicine* (ECLM) e l'*Organizzazione Mondiale della Sanità* (OMS) definiscono che la Medicina di Laboratorio è rappresentata da **5 discipline**: *Clinical Chemistry, Clinical Haematology, Clinical Immunology, Clinical Microbiology e Clinical Pathology.*

LRTI guidelines...?

MICROBIOLOGIA MEDICA, Vol. 24 (4), 2009

CONSENSUS ON MICROBIOLOGICAL DIAGNOSIS OF PNEUMONIA

Le Polmoniti: proposta di un percorso condiviso per la diagnosi microbiologica

Claudio Farina

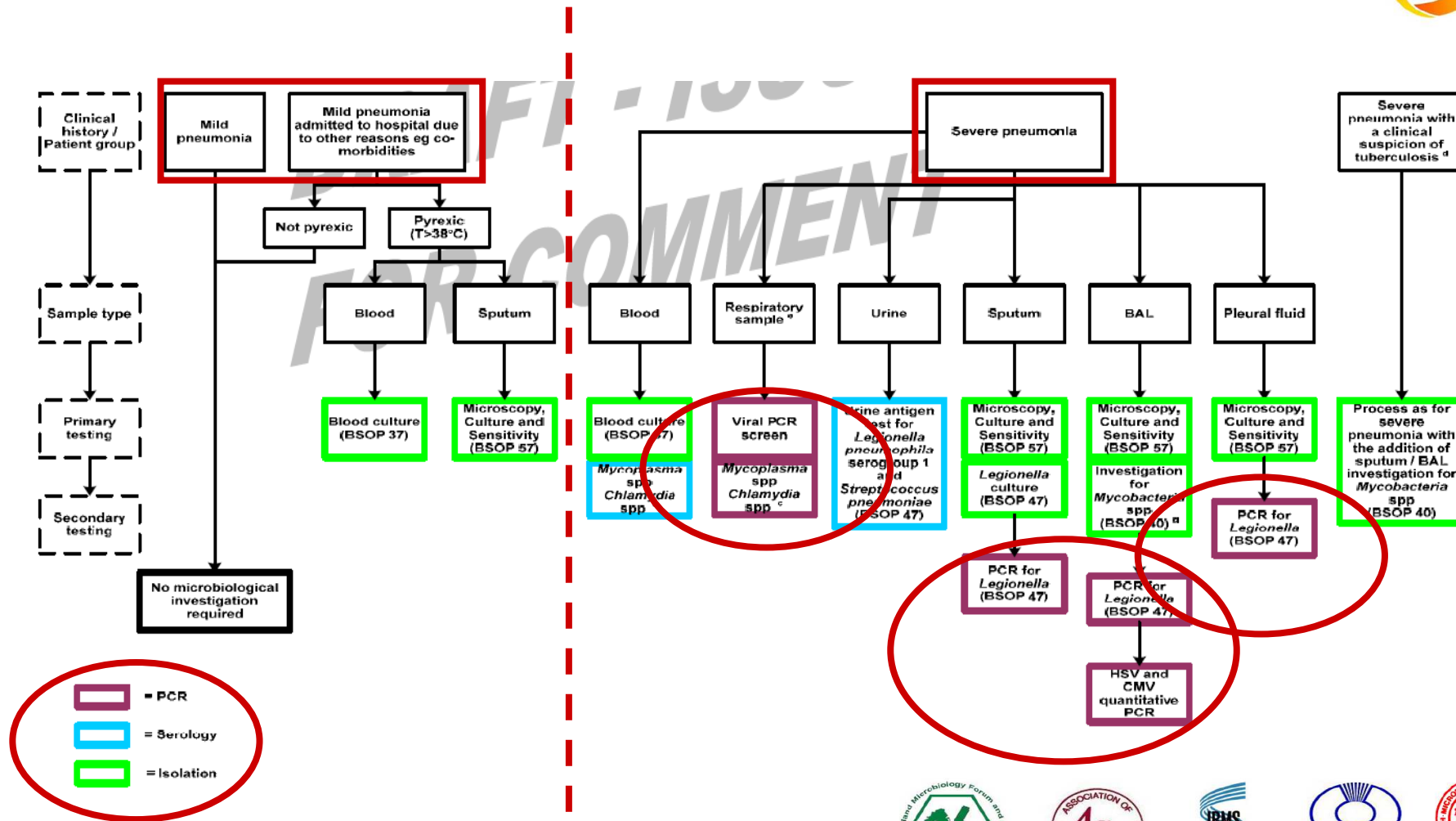
UOC Microbiologia e Virologia, Azienda Ospedaliera "Ospedale San Carlo Borromeo", Milano

con la collaborazione di:

Stefano Andreoni^{4,18}, Giancarlo Basaglia^{3,31}, Maria Rosaria Capobianchi^{7,22}, Edoardo Carretto^{10,16}, Bruno del Prato^{9,25}, Maria Laura Garlaschi^{5,20}, Giovanni Pietro Gesu^{1,15}, Massimo Giusti^{11,27}, Martin Langer^{8,14,24}, Francesco Nicola Lauria^{12,32}, Esther Manso^{4,19}, Piero Marone^{2,16}, Pierluigi Nicoletti^{2,23}, Paola Pauri^{6,21}, Alberto Podestà^{13,29}, Gaetano Privitera^{14,30}, Vittorio Sambri^{10,26}, Pierluigi Viale^{12,28}, Egidio Franco Viganò^{3,17}

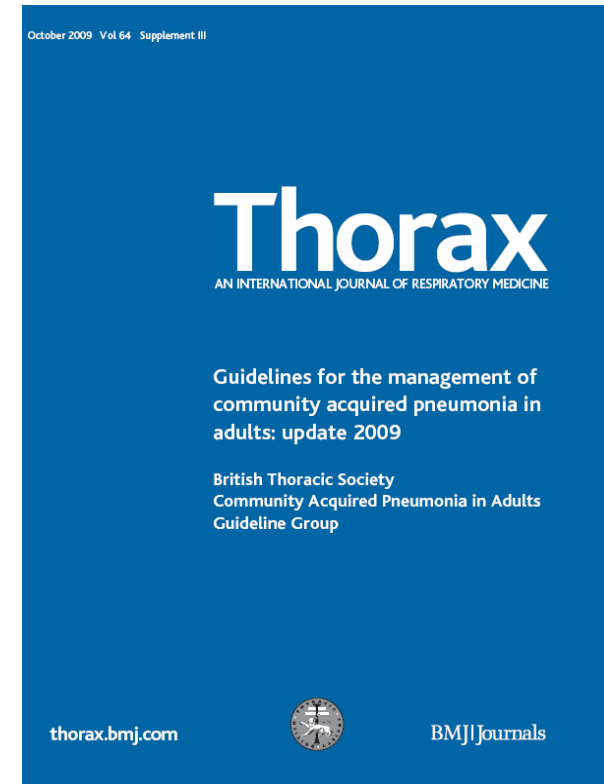
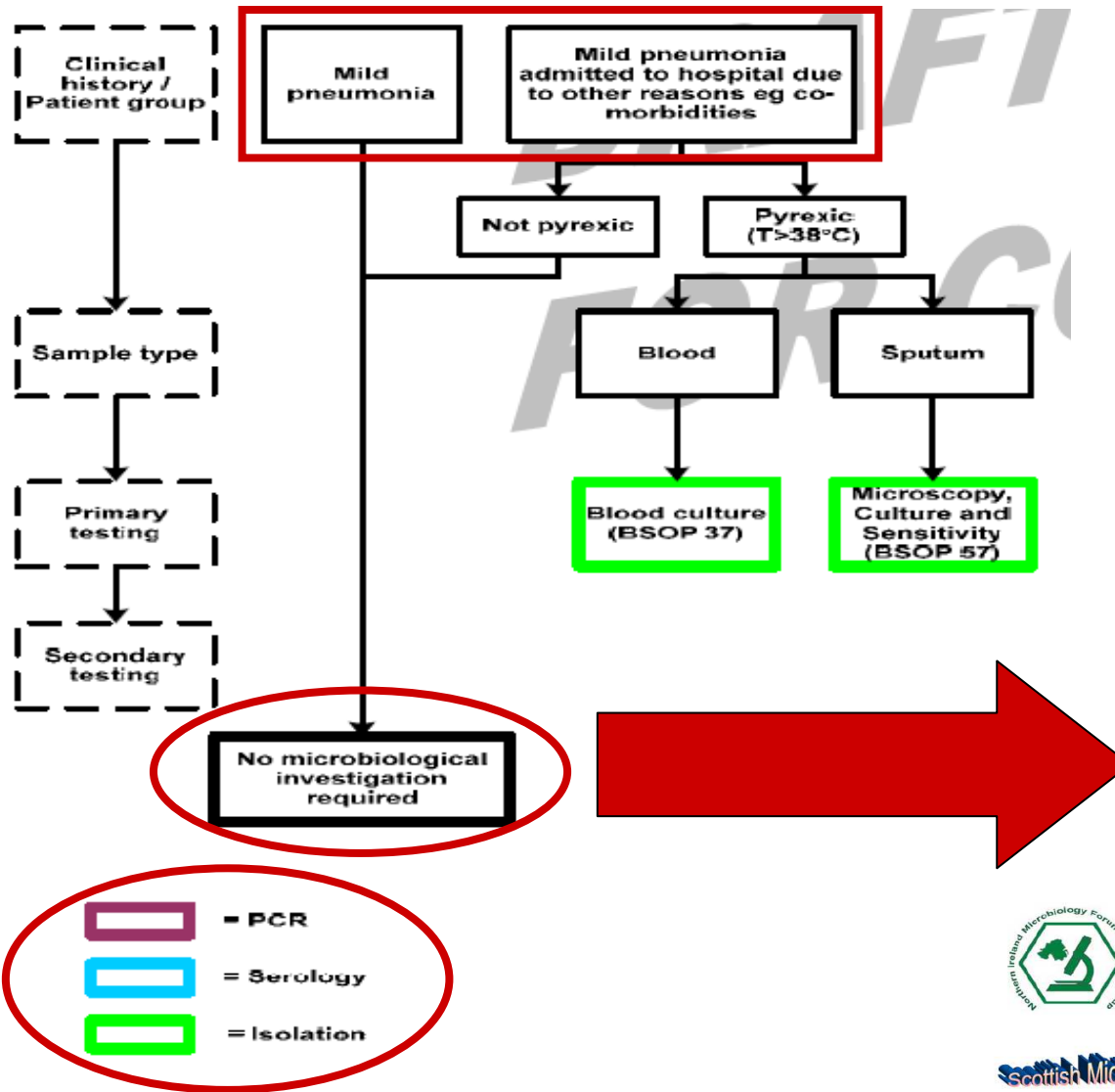
Pneumonia in immuno-competent adults

National Standard Method



Pneumonia in immuno-competent adults

National Standard Method



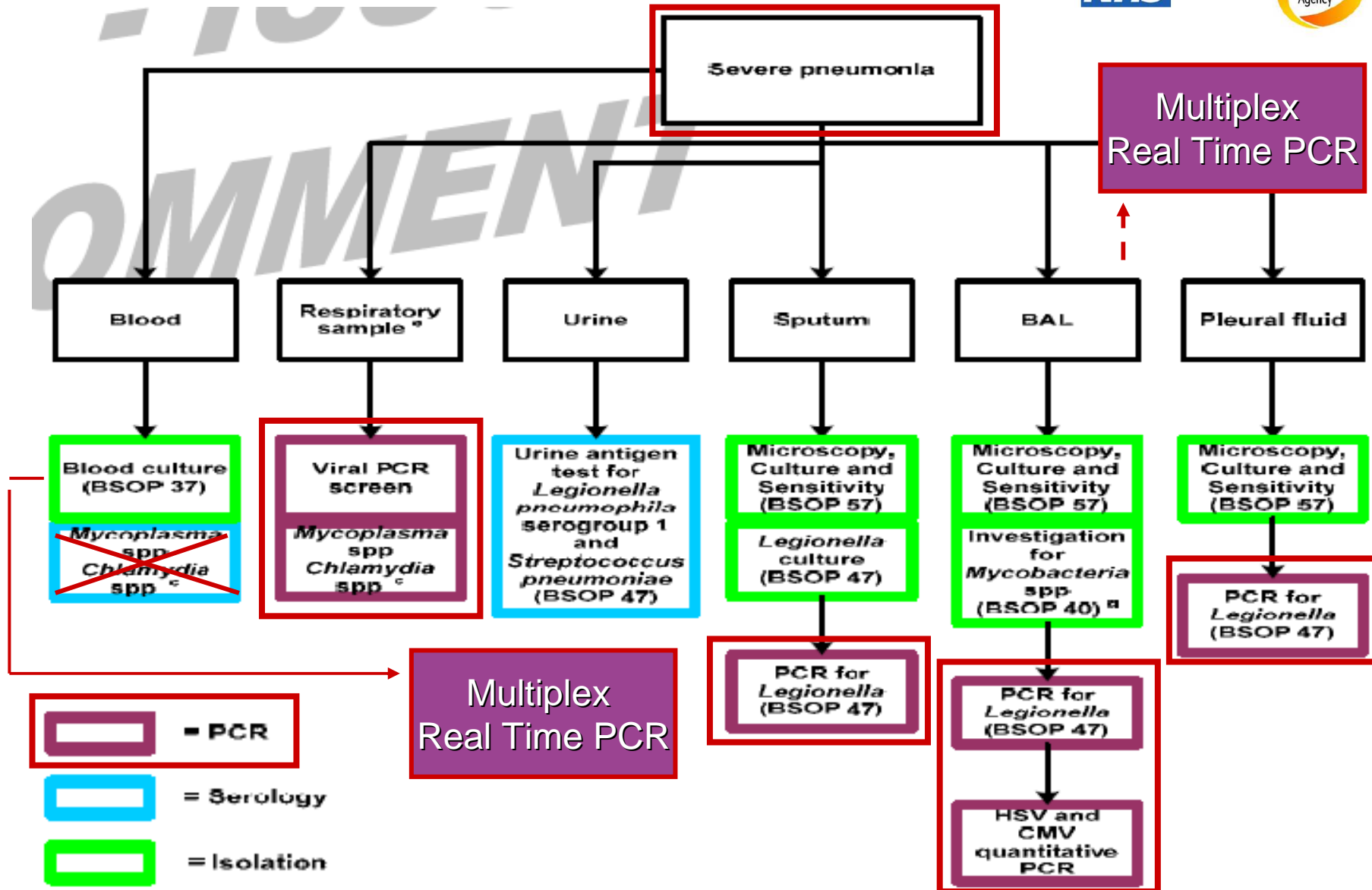


Genomics to combat **R**esistance against
Antibiotics in **C**ommunity-acquired LRTI in **E**urope

<https://www.grace-lrti.org>

Pneumonia in immuno-competent adults

National Standard Method



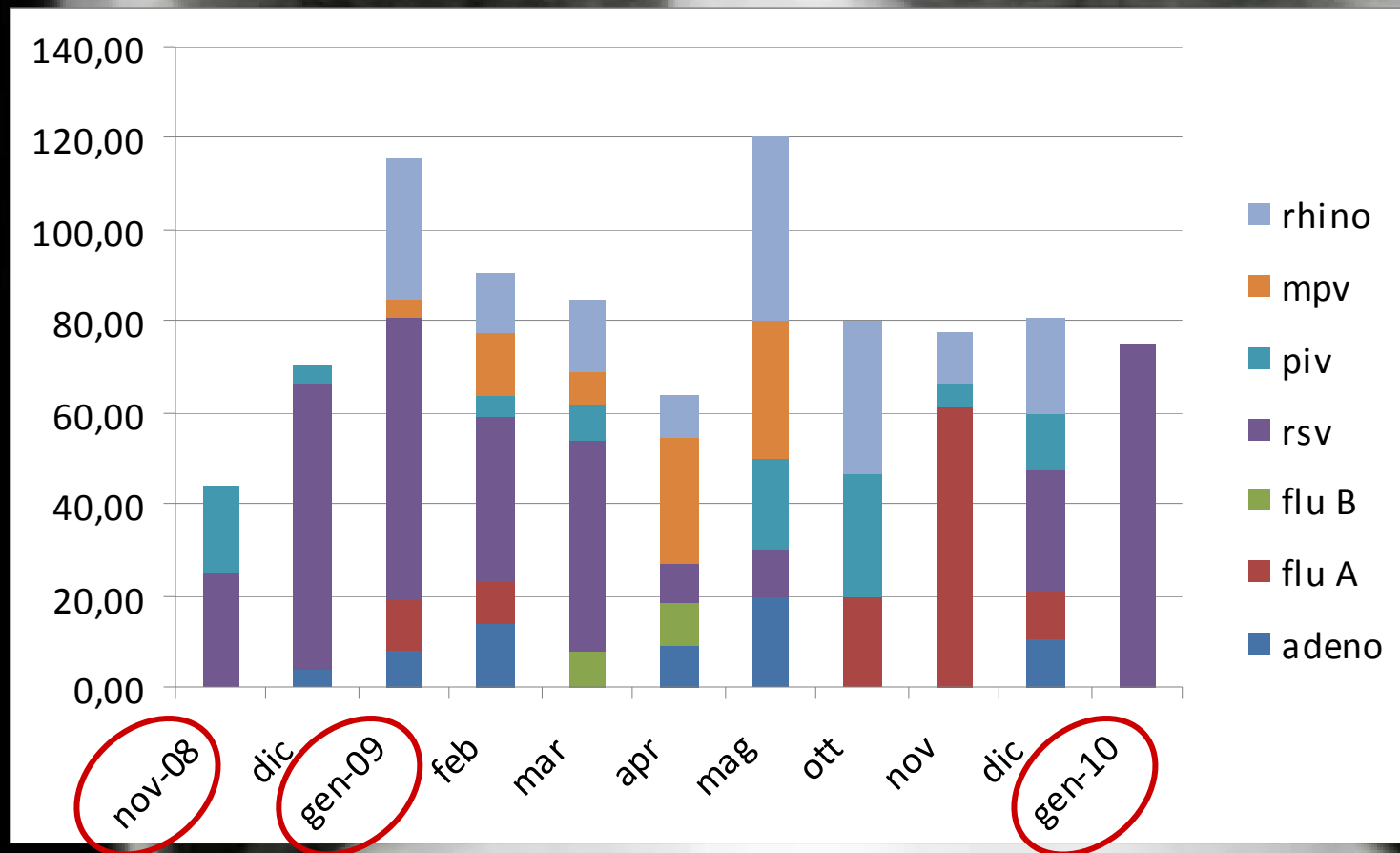


LRTI in infants and young children

An acute respiratory tract infection is one of the common causes for hospital admissions of children.

Kyoung Ho Roh et al. *Annals of Clinical & Laboratory Science*, 2008

Eziologia infezioni respiratorie ad eziologia virale in età pediatrica a Pordenone: novembre 2008-gennaio 2010



Influenza virus type A and B, parainfluenza virus type 1, 2, 3, respiratory syncytial virus (RSV) type A and B, and adenovirus are major causes of lower respiratory tract infections in infants and young children under 5 yr old.

Human metapneumovirus, also identified in children with respiratory infection, rhinovirus, and coronavirus are known as causative agents of the common cold.

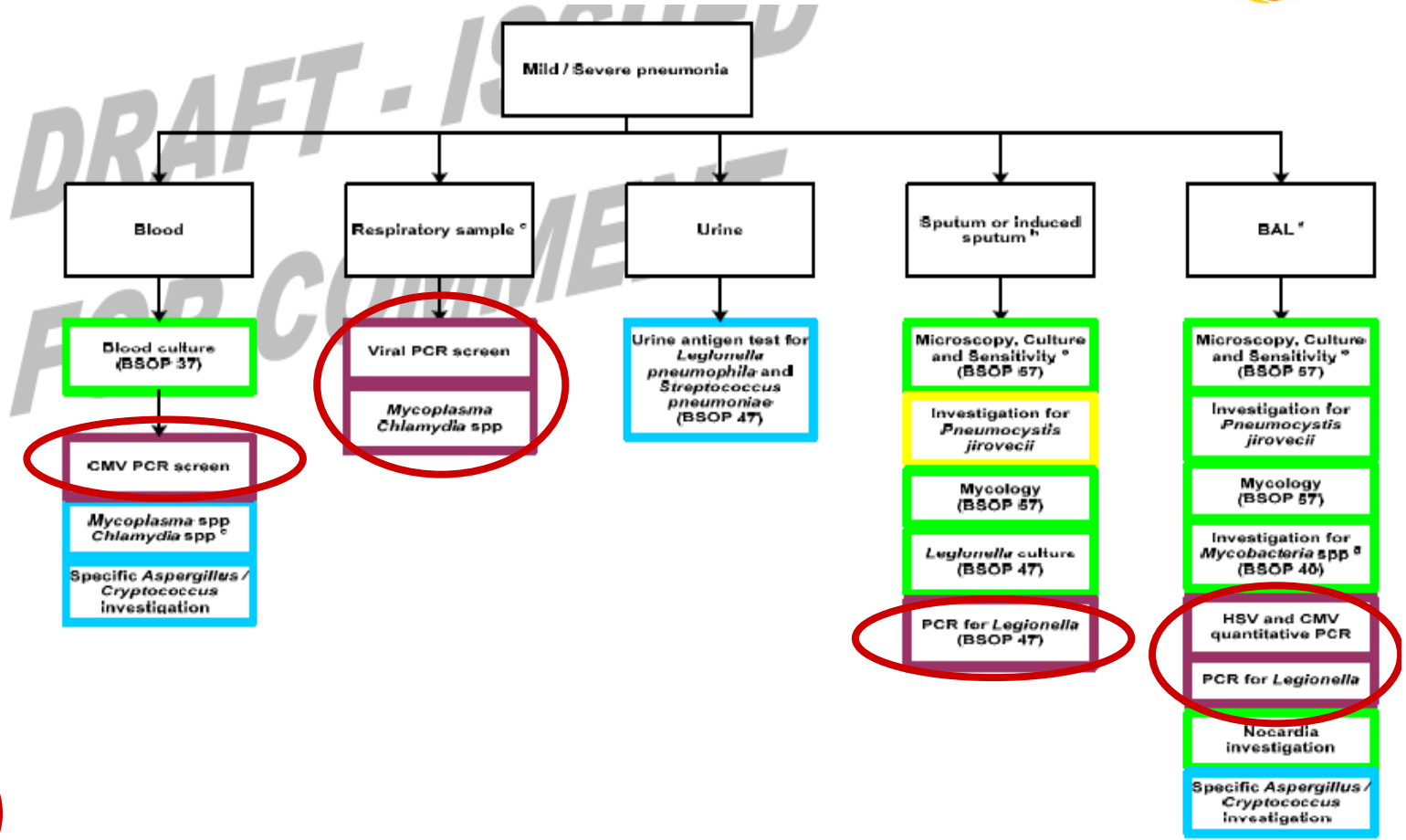
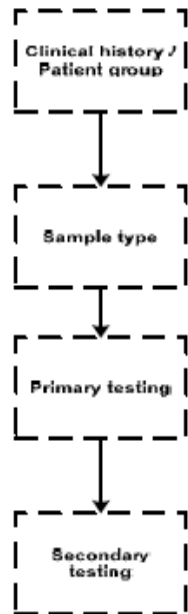
The availability of a **rapid viral diagnostic assay** will enable physicians to make **more accurate treatment decisions**, **reduce unnecessary antimicrobial agent use**, and **shorten hospital stays** for patients.

Kyoung Ho Roh et al. *Annals of Clinical & Laboratory Science*, 2008



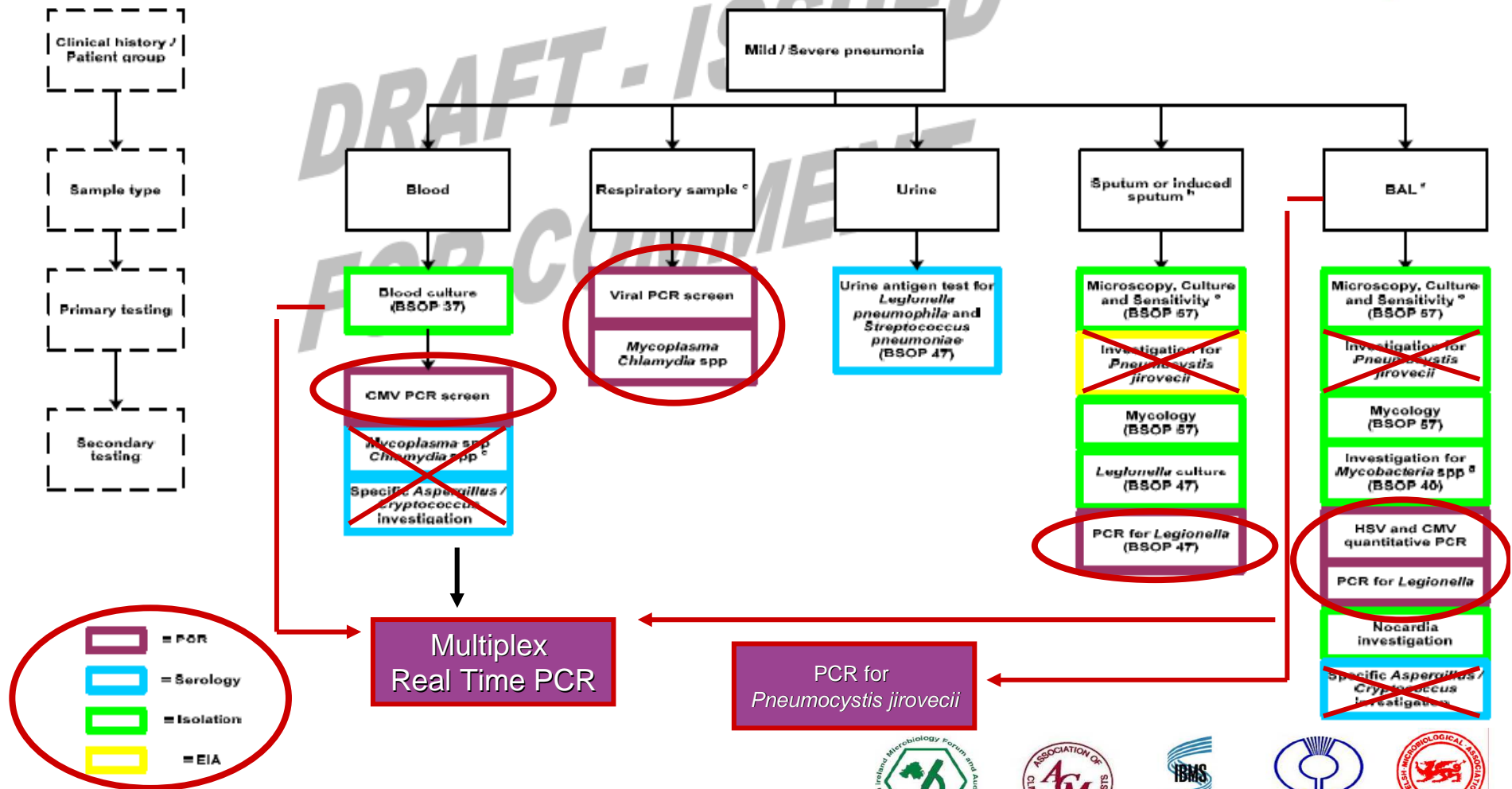
Pneumonia in immunocompromised adults

National Standard Method



Pneumonia in immunocompromised adults

National Standard Method



Optimal recovery of respiratory pathogens

For the molecular diagnosis of respiratory infections the preferred clinical specimens are **nasopharyngeal aspirates (NPA)** and **sputum** as well as **bronchoalveolar lavage** specimens, if available.

The **superiority of NPA** for the detection of all **viruses** was clearly illustrated in the study by Gruteke et al. (2004).

Prospects

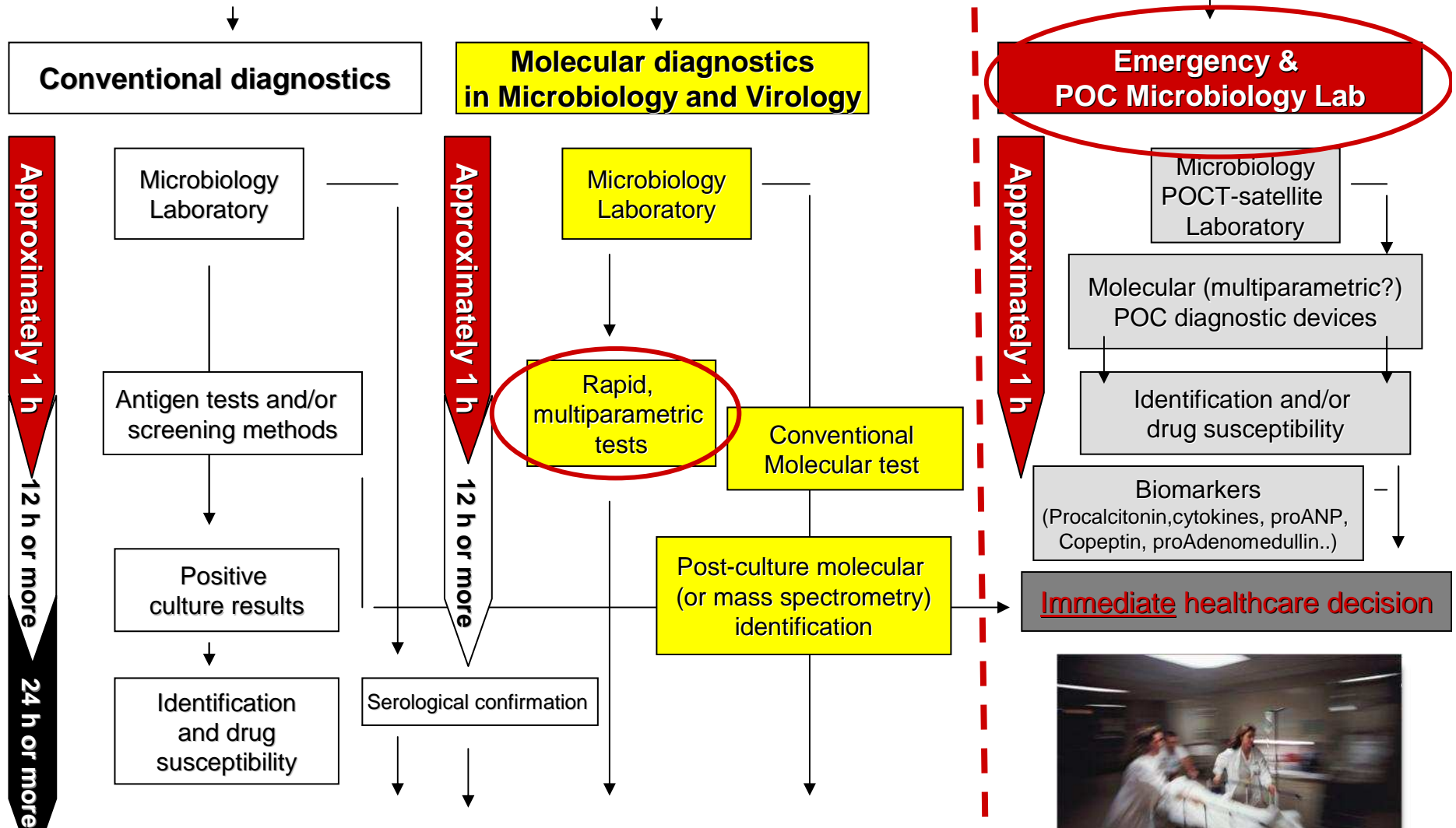
Where available, PCR tests are **an extremely useful addition** to the diagnostic armamentarium and have the advantage of being **rapid** (relevant on occasions **for both clinical and infection control purposes**) and sensitive, and so are to be preferred over serological tests.

Prospects

When molecular tests can detect within hours (or even minutes) many pathogens in nasopharyngeal swabs, throat swabs, nasopharyngeal aspirates, and sputum, more focused and efficient management of patients with LRTI will become possible.

Entrance into the diagnostic cycle (0–6 hours):

patient arrival, triage, primary evaluation, questionnaire and physical examination by physician, presumptive diagnosis, physician laboratory analysis request(s), clinical sampling, transfer to laboratories, etc.



Results of analyses and healthcare decision process (hours to days):
 transmission of results, interpretation, patient management, therapeutic intervention, confirmatory testing, treatment adjustment, etc.

Community acquired pneumonia in primary care

Doctors cannot target antibiotics and reduce resistance until new diagnostic tests prove feasible and affordable at the point of care

Herman Goossens *BMJ* 2006

Prospects

A change in culture
without culture...

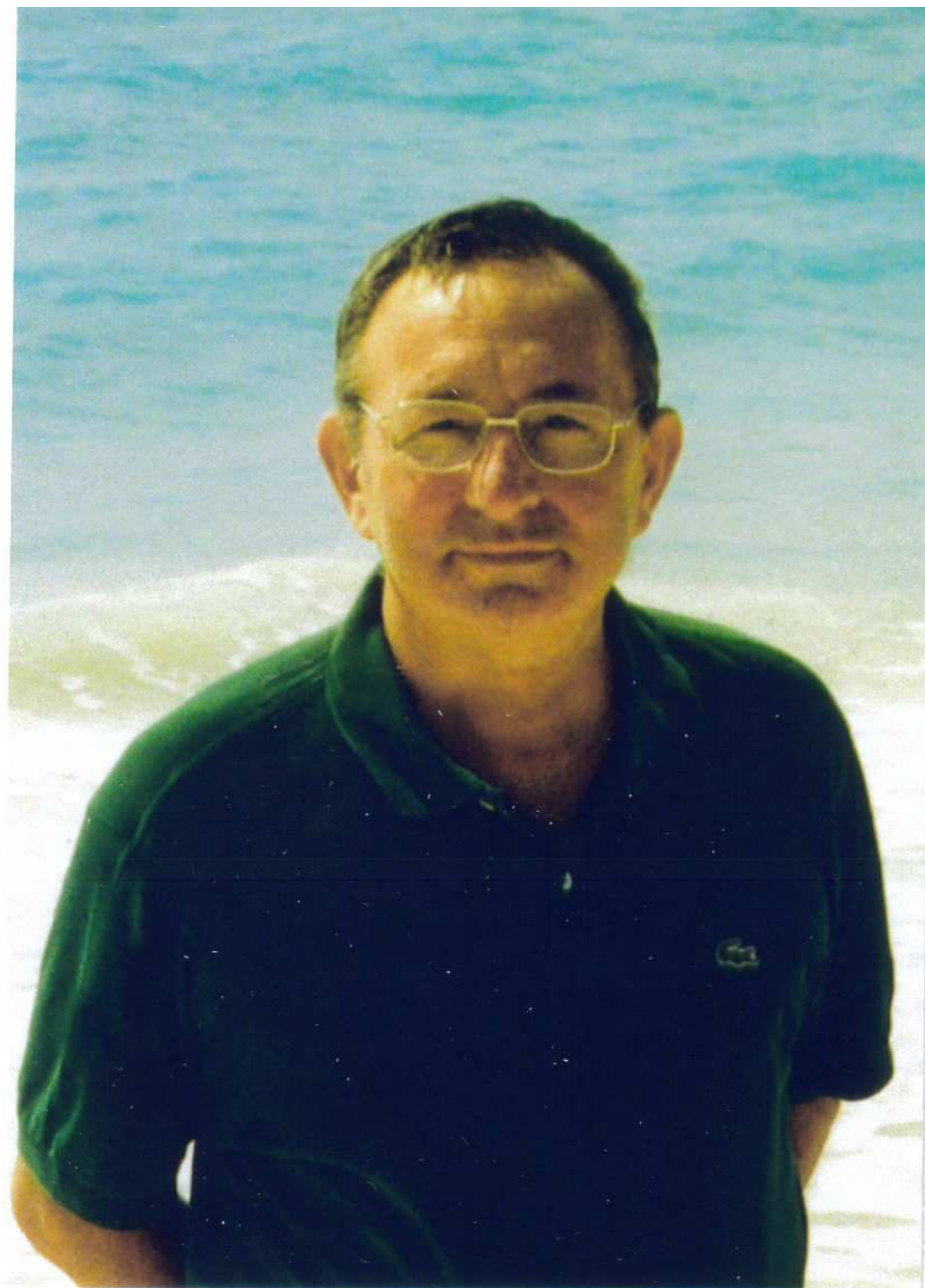
Bissonnette L and Bergeron MG. CMI 2010.

Prospects

The process of diagnosing infectious diseases is **gradually entering an era** where a physician is in the position to obtain valuable **information on a time-scale comparable** to those in other fields of diagnostic medicine.

Bissonnette L and Bergeron MG. CMI 2010.





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GRAZIE PER L'ATTENZIONE

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