



2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance



Impatto delle nuove tecnologie nell'ottimizzazione della diagnostica microbiologica diretta e indiretta

A. Camporese

SOC Microbiologia e Virologia

Azienda Ospedaliera S.Maria degli Angeli, Pordenone

Optimization of laboratory strategy

Strategies should be developed adapting

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

Serological versus molecular diagnosis

Recent **advances** in **biotechnology** and **molecular biology** have played a significant role in the **development** of **rapid, specific and sensitive** assays for **direct diagnosis**.

Tonukari NJ. <http://www.academicjournals.org>



Reevaluation of serological tests

The availability of the very sensitive NAATS has in recent years 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.

A black and white micrograph showing numerous small, dark, circular bacteria (likely Leptospira) scattered across the field. Two larger, elongated, and highly motile bacteria are highlighted with a white outline, showing their characteristic corkscrew shape and flagella. The background is a dark, textured surface.

Laboratory diagnosis of
leptospirosis

Laboratory diagnosis of leptospirosis

PCR has many advantages in the early diagnosis of leptospirosis, and can easily be used for diagnosing leptospirosis.

PCR can detect leptospiral **DNA about 1 or 2 days post-infection**, while antibody and culture can be detected from blood after 7 days and 7-30 days post-infection, respectively.

Wangroongsarb P, et al. *J Trop Med Parasitol* 2005.





UK National Standard Methods for Microbiology Investigations

<http://www.hpa.org.uk>



GIG
CYMRU
NHS
WALES

Iechyd Cyhoeddus
Cymru
Public Health
Wales



Molecular microbiology, pathology oriented

- Pneumonia in immunocompetent adults
- Pneumonia in immunocompromised adults
- Encephalitis
- Sexually Transmitted Infections (STIs)

LRTI 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.

LRTI: a challenge for diagnostic microbiology

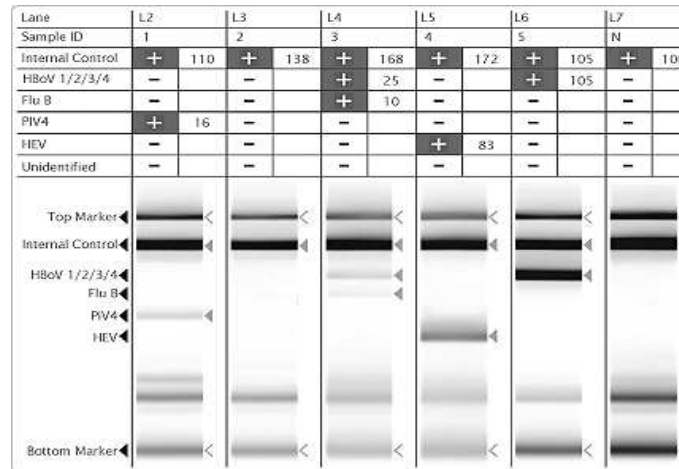
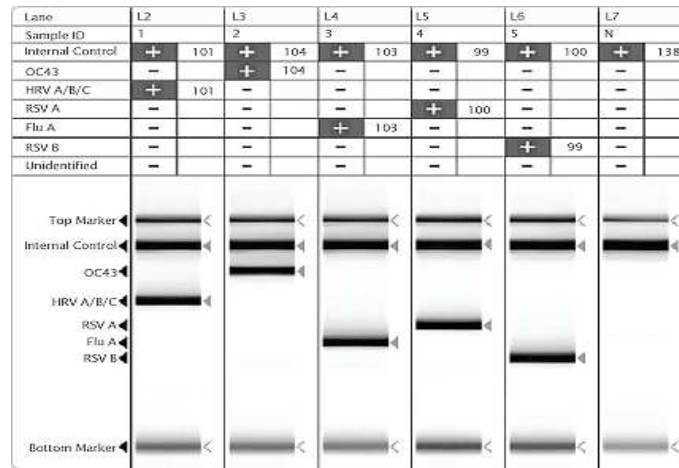
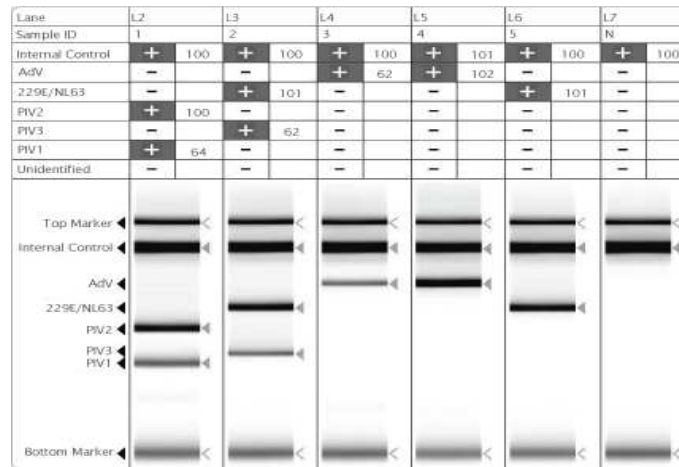
During recent years a considerable number of previously unknown respiratory agents were discovered whose in vitro culture is very slow or even unrealized.

Seeplex® RV 15 ONE STEP

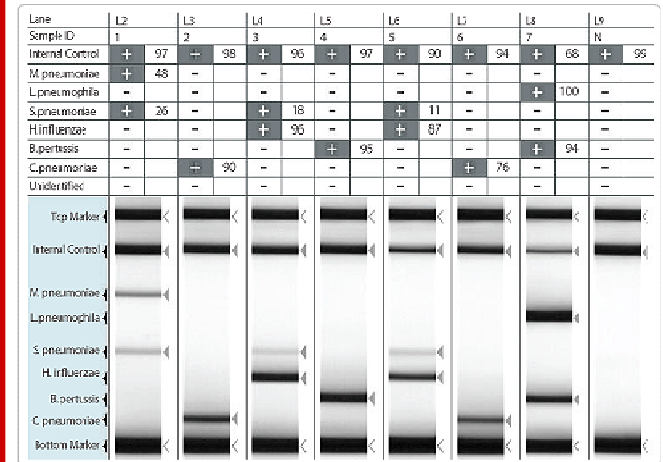
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Adenovirus A/B/C/D/E
Coronavirus 229E/NL63

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

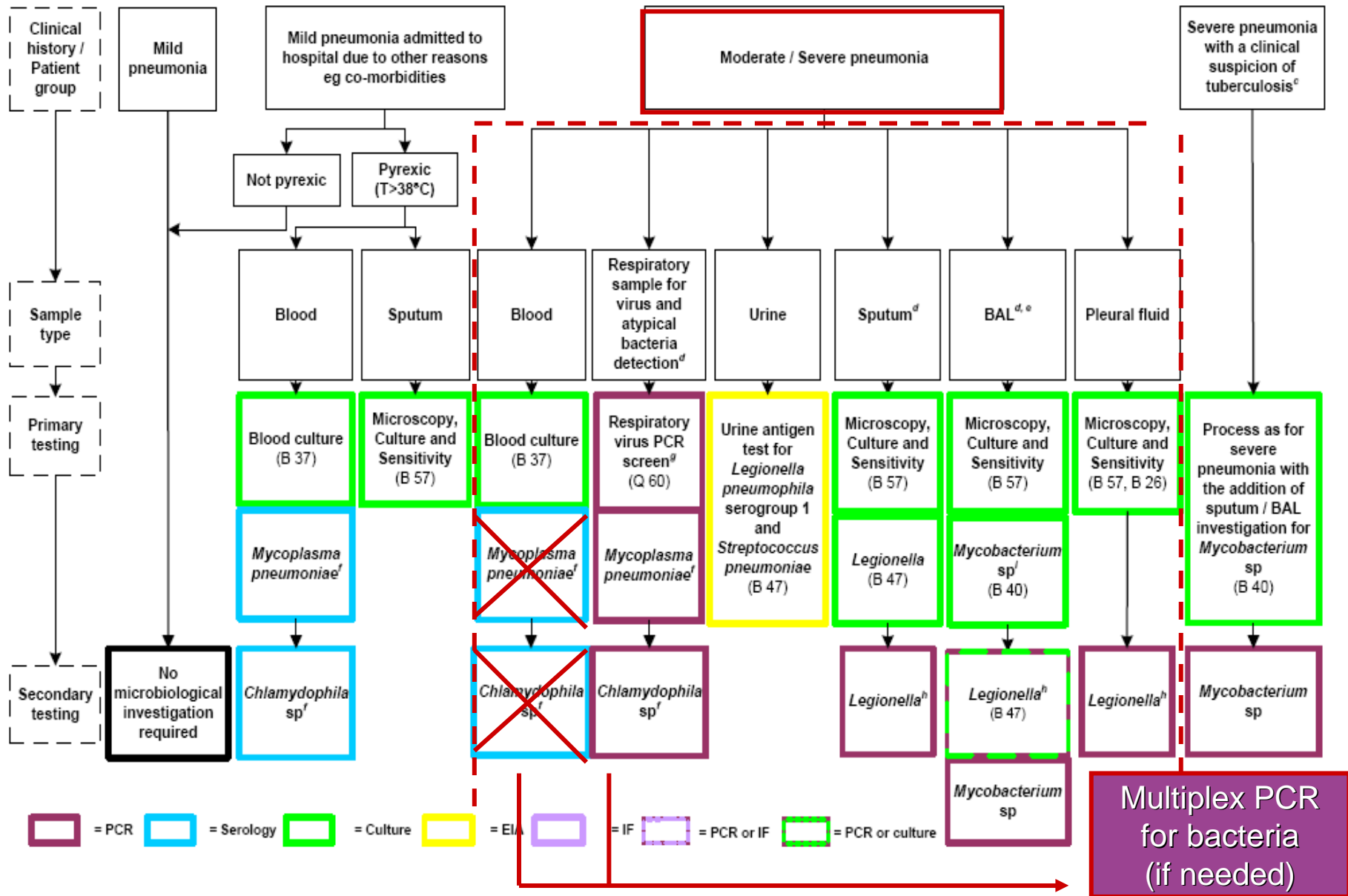


Dual Priming Oligonucleotide technology (Seegene)

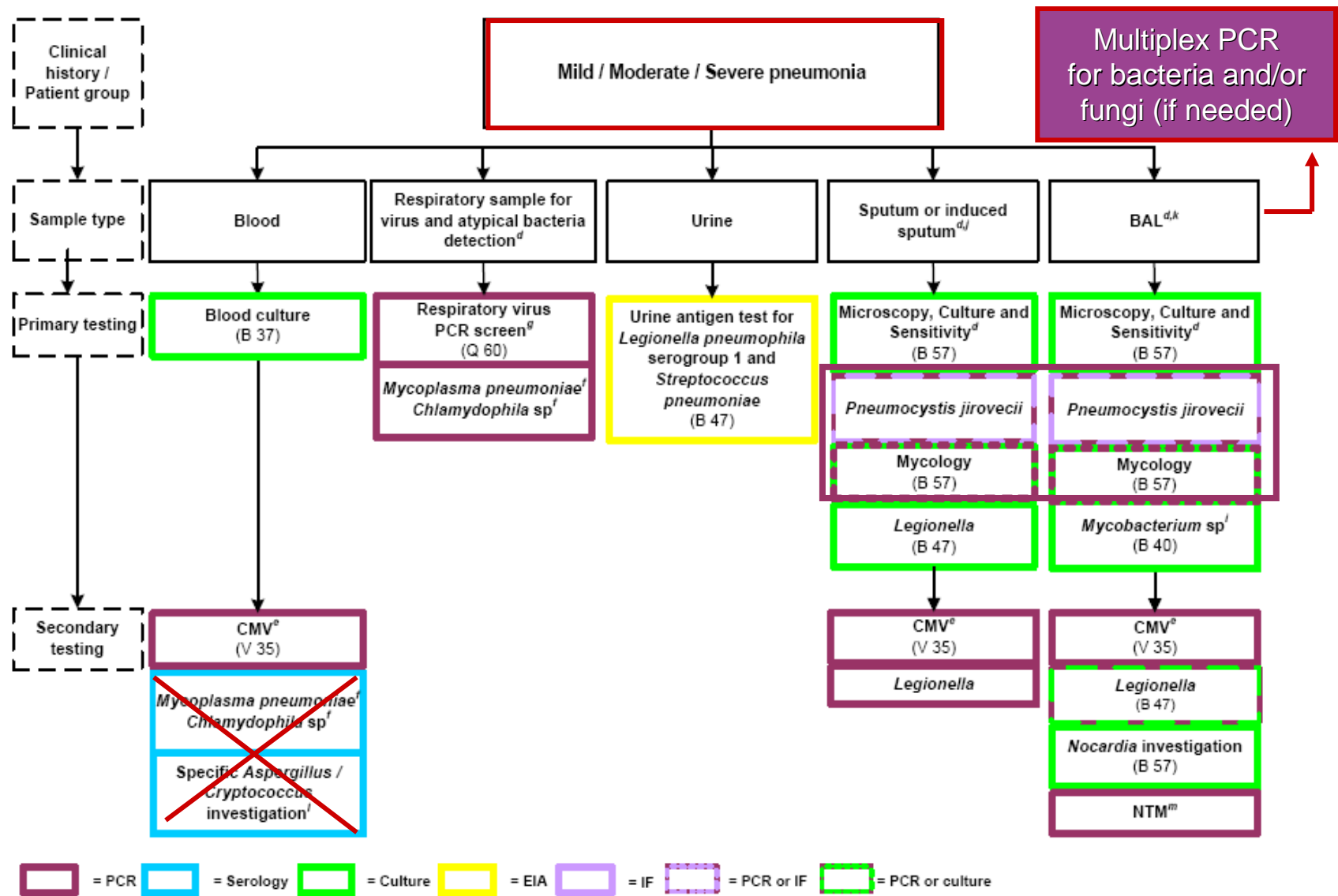


M. pneumoniae
C. pneumoniae
L. pneumophila
S. pneumoniae
H. influenzae
B. pertussis

Pneumonia in immunocompetent adults^{1-8, b}



Pneumonia in immunocompromised adults^{1-8, b}

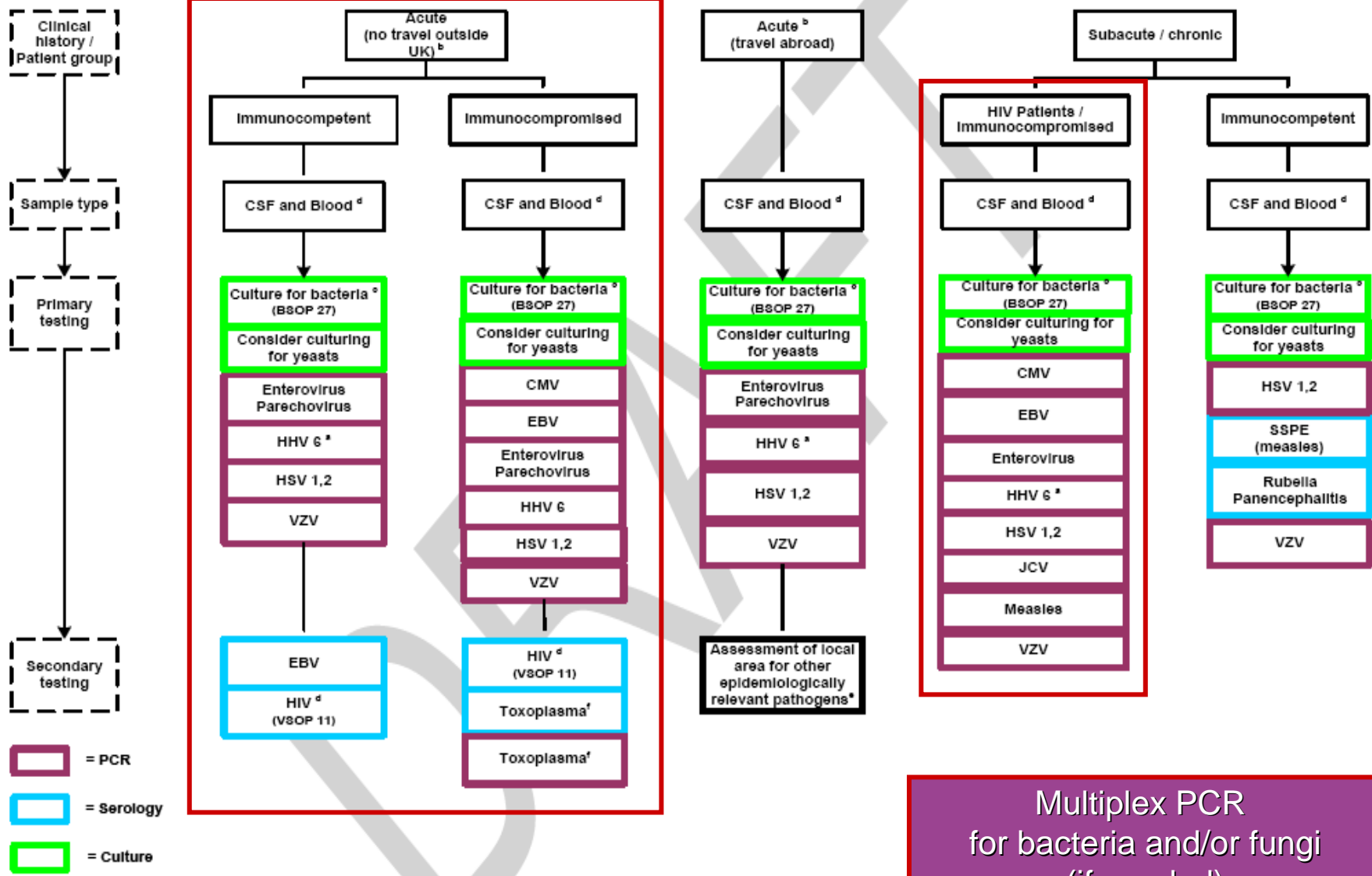


The encephalitis syndrome

Encephalitis has over 100 causes including viral infections (the majority), infection associated with other microorganisms, and immune-mediated conditions.

<http://www.hpa.org.uk>

Encephalitis Flowchart



Multiplex PCR for bacteria and/or fungi (if needed)

STIs: the problem

An estimated **340 million new cases** of sexually transmitted infections (STIs) are **acquired annually throughout the world**, making these infections an important **public health and economic concern**.

McGowin CL, Anderson-Smits C. PLoS Pathog 2011.



New views through the prism of molecular microbiology...

The rapidly advancing technology of modern molecular microbiology has greatly improved our understanding of the epidemiology of sexually transmitted infections and the etiology and pathogenesis of the diseases they cause.

New views through the prism of molecular microbiology...



3.5 hours

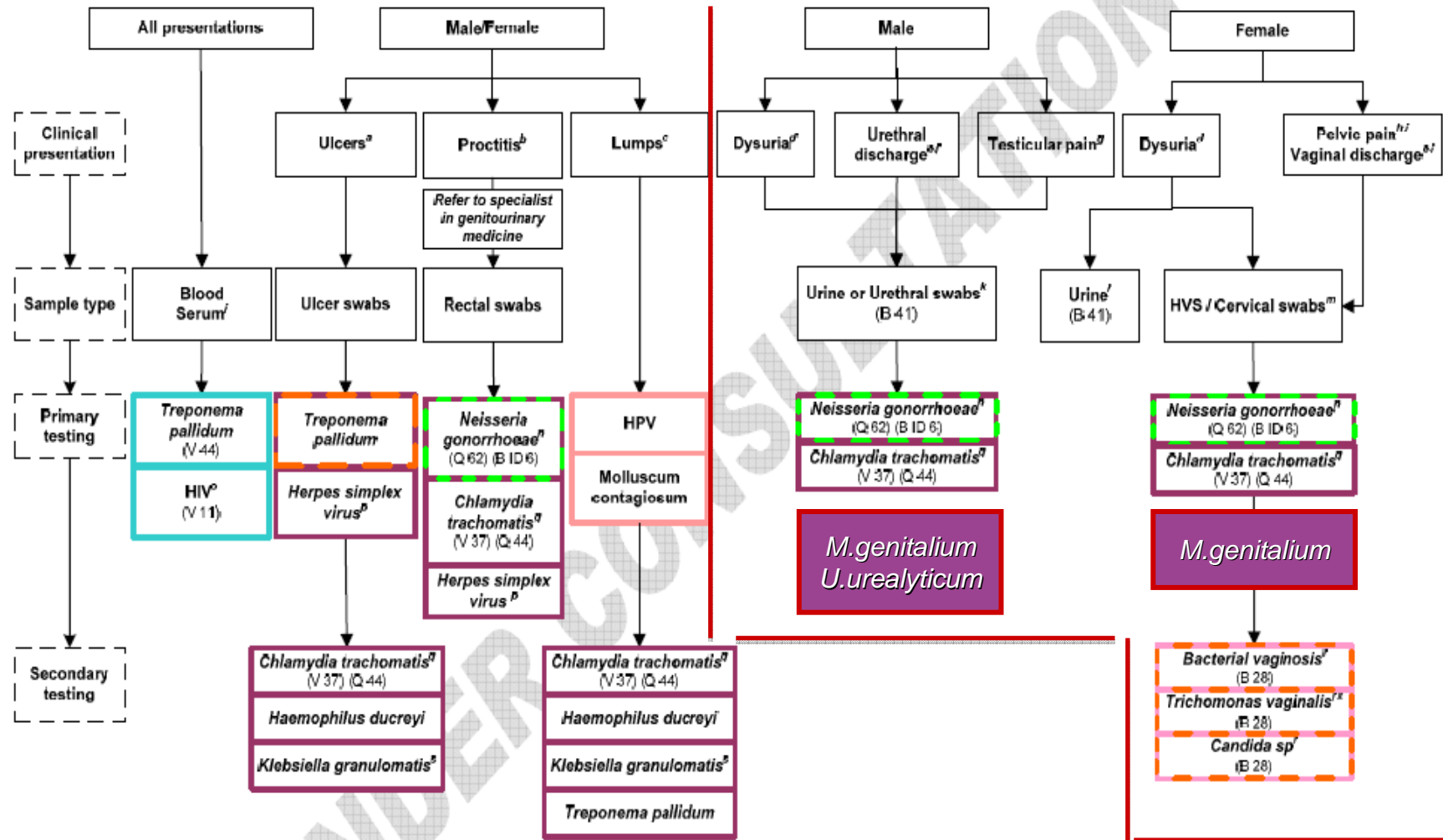


Chlamydia trachomatis
Neisseria gonorrhoeae
Mycoplasma genitalium
Mycoplasma hominis

Trichomonas vaginalis
Ureaplasma urealyticum
Ureaplasma parvum

Sexually Transmitted Infections Flowchart

Cervicitis and urethritis



 = PCR
 = Culture
 = PCR or culture
 = Serology
 = Clinical diagnosis
 = Microscopy
 = Microscopy and/or Clinical diagnosis

Cervicitis: etiology

- When an etiologic organism is isolated in the presence of cervicitis, it is typically *C.trachomatis* or *N.gonorrhoeae*.
- Cervicitis also can accompany trichomoniasis and genital HSV.
- Moreover, *M. genitalium* should be considered an etiologic agent of cervical inflammation and upper tract disease syndromes, including PID.

Urethritis: etiology

- ❑ Nongonococcal urethritis (NGU) is caused by *C.trachomatis* in 15%–40% of cases and *M.genitalium* in 15%–25% of cases.
- ❑ *T. vaginalis*, HSV, and adenovirus also can cause NGU, but the real contribution is difficult to estimate.
- ❑ Enteric bacteria have been identified as an uncommon cause of NGU.

Mycoplasmas infections

Cervicitis:

❑ *M. genitalium* should be considered an etiologic agent of cervicitis and upper genital tract disease syndromes, including PID.

Urethritis:

❑ *M. genitalium* is a cause of a significant number of NGU cases (15-25%).

❑ *U. urealyticum* “may be” associated with NGU.

❑ **Other *Mycoplasma* species** as etiologic agents of NGU are inconsistent.

The **genus *Ureaplasma*** contains two species, ***U. urealyticum*** and ***U. parvum***. Only *U. urealyticum* may be associated with NGU.

Workowski KA et al. CDC Sexually Transmitted Diseases Treatment Guidelines. MMWR 2010; 17 (59): 1-110.

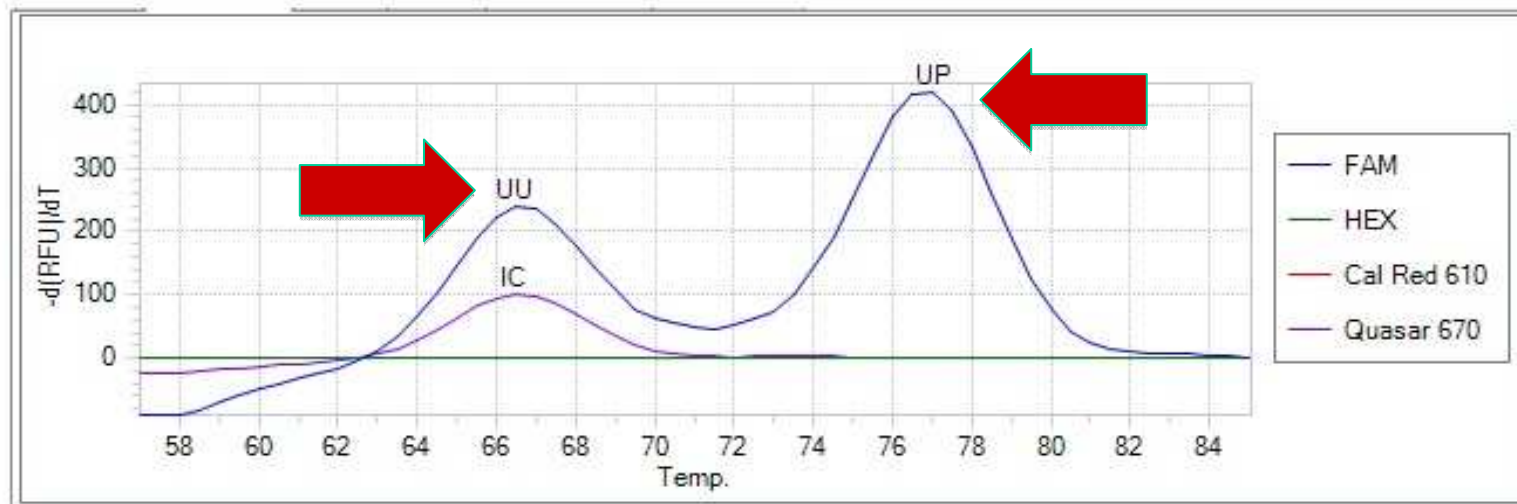


M. parvum o *U.urealyticum*?

Dal confronto tra risultato colturale e molecolare:

- ❑ 70% *Mycoplasma parvum*
- ❑ 15% *Ureaplasma urealyticum*
- ❑ 15% *M. parvum* + *U.urealyticum*

Camporese A et al. Dati non pubblicati.



Microbiology optimization:

New Views through the Prism of Modern Molecular Microbiology

David H. Martin, MD

Current Infectious Disease Reports 2008, 10:128–132



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**Vi ringrazio per
l'attenzione**

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