3° CONGRESSO NEWMICRO

...The need for speed: il laboratorio di Microbiologia e le Urgenze Infettive

Le nuove tecnologie per la gestione dell' urgenza/emergenza in microbiologia: le polmoniti

A. Camporese
SOC Microbiologia Clinica e Virologia
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J. Pers. Med. **2012**, *2*, 50-70; doi:10.3390/jpm2020050

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Journal of Personalized Medicine

ISSN 2075-4426

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Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

One of the rationales for implementing rapid molecular microbiology in clinical settings is to compensate for the extended period of time, *i.e.*, at least 24 hours that is required by culture-based microbiology to deliver a putative microbe identification.

J. Pers. Med. **2012**, *2*, 50-70; doi:10.3390/jpm2020050

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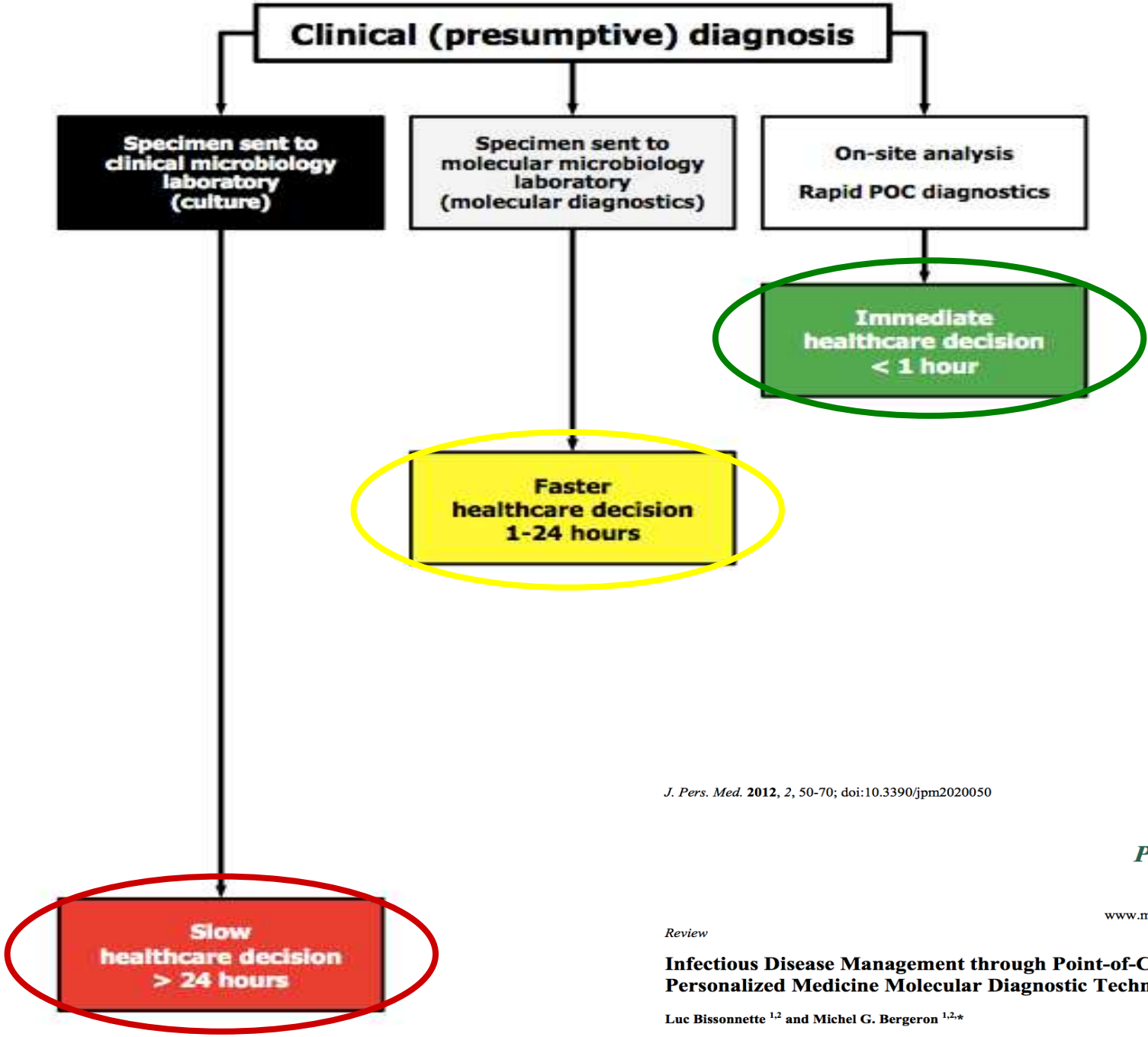
Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

The implementation of rapid point-of-care (POC) microbiology shall decrease the length of the diagnostic cycle in order to accelerate infectious disease management.



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Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

Nucleic acid-based POC tests

The definition of a POC test has progressively broadened, to include tests not formally designed for bedside use, but whose automation has allowed the transfer of complex technology from classical laboratories to sites of diagnosis and treatment.

Clerc O and Greub G. Clin Microbiol Infect 2010.

Integrated fluidic lab-on-a-chip devices for molecular diagnostics

The first technological platform illustrating the feasibility of the concept is undoubtedly the GeneXpert platform of Cepheid, on which several tests for infectious diseases have been implemented.

Bissonnette L and Bergeron MG. CMI 2010.

J. Pers. Med. **2012**, *2*, 50-70; doi:10.3390/jpm2020050

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Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

The management of respiratory infections can be accelerated by multiparametric detection platforms, that can interrogate a sample for the presence of a disease-causing microorganism known to be part of a syndrome-associated microbial panel, instead of performing multiple tests which would increase the cost.

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Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

Time and distance are essential features onto which technology experts and healthcare system authorities should focus to shorten the diagnostic cycle and make molecular POC testing a reality.

J. Pers. Med. **2012**, *2*, 50-70; doi:10.3390/jpm2020050

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Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

Time…

Point-of-care testing can be defined as patient specimens assayed at or near the patient with the assumption that test results will be available instantly or in a very short timeframe to assist caregivers with immediate diagnosis and/or clinical intervention .

J. Pers. Med. **2012**, *2*, 50-70; doi:10.3390/jpm2020050

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Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

Distance…

Bedside testing might constitute the ultimate goal, but the development of near POC laboratories would certainly shorten the diagnostic cycle and increase the efficacy of infectious diseases management by improving access to highly efficient nucleic acid-based tests.

J. Pers. Med. **2012**, *2*, 50-70; doi:10.3390/jpm2020050

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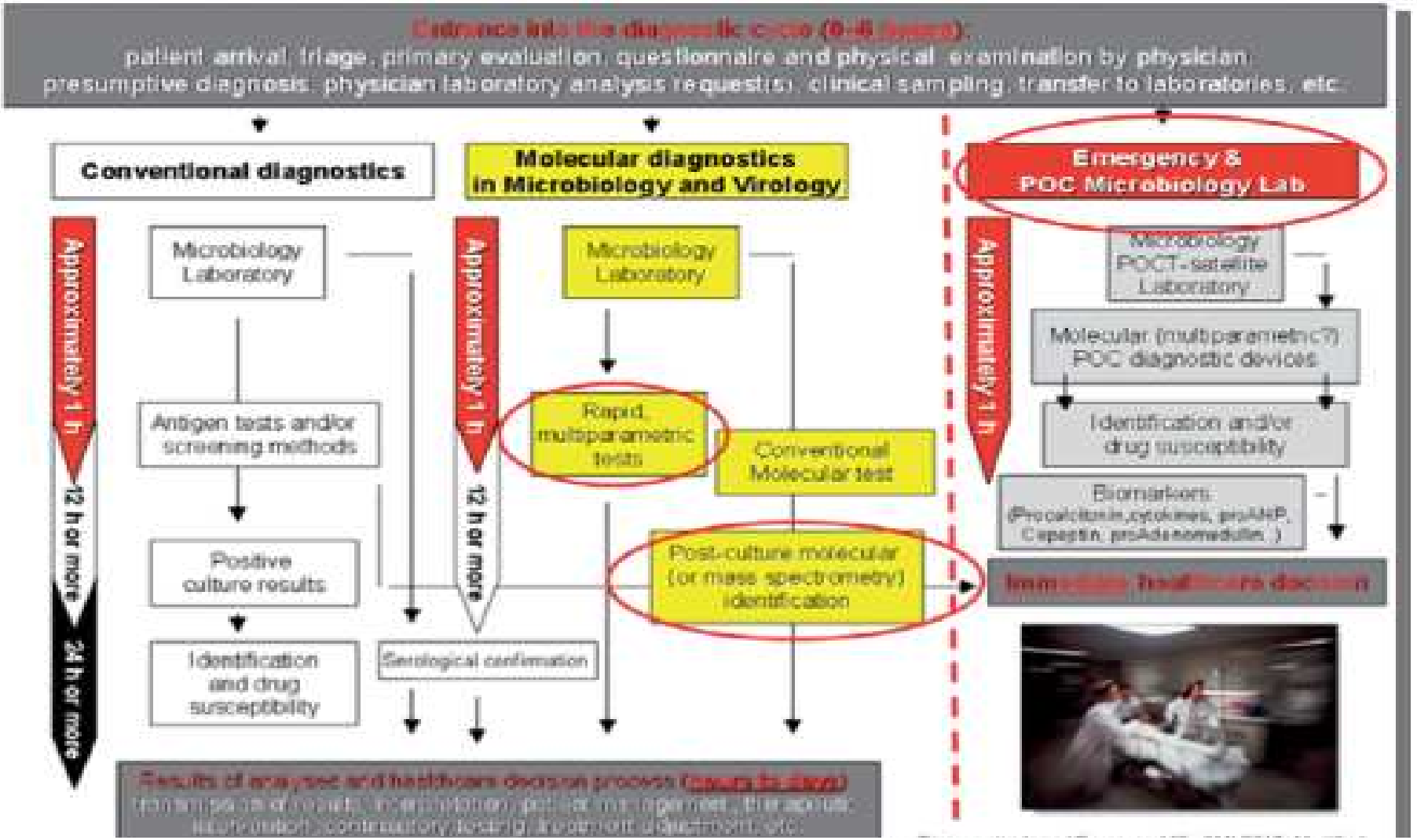
Review

Infectious Disease Management through Point-of-Care Personalized Medicine Molecular Diagnostic Technologies

Luc Bissonnette [1,2] and Michel G. Bergeron [1,2,*]

Near POC microbiology laboratories should be equipped and certified to offer a comprehensive menu of commercially-available molecular diagnostics tests in hospital departments where the impact and cost-effectiveness will be greater.

Figura 4. Sintesi dei possibili assetti analitici e organizzativi del laboratorio di microbiologia e virologia del prossimo futuro, con la presenza di *Emergency & POC Microbiology Lab*, espressamente dedicati all'area clinica dell'emergenza.



Camporese A. Trends Med 2011; 11(1):25-30.

Rapid Diagnostics and Appropriate Antibiotic Use

Louis B. Rice

Louis Stokes Cleveland Veterans Affairs Medical Center and Case Western Reserve University School of Medicine, Cleveland, Ohio

CID 2011:52 (Suppl 4)

Although it is often posited that better real-time information is required to improve physician antimicrobial-prescribing practices, physicians often fail to use tests already available or ignore their results.

Maschio, diabetico, 61 anni
Insufficienza respiratoria grave

Ore 9,47: emogas

Ore 10,07:
Emocromo
Profilo biochimico

Ore 11,46:
CRP = 28,3
PCT = 33,9
TAT : < 1 h

Ore 12,16:
3 emocolture
Septifast

Ore 12,16:
Sierologia per virus + atipici

Ore 14,21:
PCR per
virus respiratori

Ore 08,31:
Legionella Ag = negativo
S.pneumoniae Ag = negativo

TAT : < 1 h (+25h)

Ore 13,21:
Esito Septifast = *S.pneumoniae*

TAT : 24 h

Ore 14,13:
PCR screening virus respiratori = Influenza A

TAT : 25 h

Ore 15,05:
Esito microscopico emocoltura = *cocchi gram + strepto like*

TAT : 26 h

Ore 16,02:
GeneXpert 2009 H1N1 = positivo

TAT : <2 h (+28h)

Ore 08,16:
Identificazione sierologica Ag capsulare di *S.pneumoniae* = positiva

TAT : 43 h

Identificazione con spettrometria di massa da flacone positivo o da coltura

TAT : 27 h

Identificazione biochimica e antibiogramma diretto da flacone positivo

TAT : 44 h

Ore 08,18:
ID biochimica da emo = *S.pneumoniae*

Esito antibiogramma

TAT : 67 h

Ore 12,16:
Sierologia per virus + atipici = Negativa

TAT : 71 h

TAT: <1 h – 71 h

Gestione polmoniti in emergenza/urgenza

Emergenza (1-3 ore)

- Procalcitonina e/o CRP (sangue)
- *S.pneumoniae* Ag (urina)
- Legionella ser.1 Ag (urina)
- RSV Ag (naso/far)
- Influenza A/B (naso/far)
- GeneXpert Flu A/B with H1N1 (naso/far)
- FilmArray PCR *respiratory panel* (21 virus e batteri) (naso/far)
- Nanosphere Verigene Respiratory Virus XP (15 virus e 1 resistenza) (naso/far)

Urgenza (entro 24 ore??)

- Seegene Seeplex PneumoBacter (6 batteri) (BAL)
- Seegene Anyplex II RV16 (16 virus) (naso/far e/o BAL)
- Septifast (sangue e BAL)
- Altre PCR specie specifiche o *clinical related* (naso/far e/o BAL)
- Spettrometria di massa (da emocoltura e/o da isolamento)

Gestione polmoniti in emergenza/urgenza

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- Spettrometria di massa (da emocoltura e/o da isolamento)

PCT levels may assist in the interpretation of molecular diagnostic tests when there is molecular evidence of the presence of both a viral and a bacterial pathogen.

PCT release is attenuated in response to viral infection and thus the measurement of PCT has been proposed as a method to distinguish viral vs bacterial disease.

IDSA. CID 2011.

Early diagnosis of lower respiratory tract infections (point-of-care tests)

Patrick G.P. Charles

Current Opinion in Pulmonary Medicine 2008, 14:176–182

Table 1 Point-of-care tests for respiratory tract infections

Organism and test	Specimen	Type of test	Sensitivity (%)	Specificity (%)	Time (min)
Influenza					
QuickVue A + B	Nasal swab, NPA, nasal washings	EIA	67–95	76–100	10
BinaxNOW influenza A & B	NPA, nasal washings, nasal swab	ICT	62–82	92–100	15
ZstatFlu	Throat swab	EIA	50–96	77–98	30
ZstatFlu-II	Throat swab, NPA	CLT	50–88	83–100	30
Respiratory syncytial virus					
BinaxNow RSV test	NPA, nasal washings, nasal swab	ICT	70–93	89–100	15
SAS RSV test	NPA, nasal swab	ICT	83	91	10
Clearview RSV test	NPA, nasal washings, nasal swab	LFIA	93	96	15
S. pneumoniae					
BinaxNOW *S. pneumoniae* antigen test	Urine	ICT	70–92	>90	15
Legionella					
BinaxNOW *Legionella* antigen test	Urine	ICT	80	97–100	15

CLT, chemiluminescent test; EIA, enzyme immunoassay; ICT, immunochromatographic test; LFIA, lateral flow immunoassay; NPA, nasopharyngeal aspirate; RSV, respiratory syncytial virus; *S. pneumoniae, Streptococcus pneumoniae*

The IDSA and the Food and Drug Administration (FDA) cosponsored a workshop on molecular diagnostic testing for respiratory tract infections on November 2009, with participation by the FDA, industry, authorities in microbiology, statisticians, and others.

Camporese A. *Clinical Laboratory International. Feb/March 2013.*

1 May 2011
Volume 52
Supplement 4

Clinical Infectious Diseases

Workshop on Molecular Diagnostics for Respiratory Tract Infections

An Unmet Medical Need: Rapid Molecular Diagnostics Tests for Respiratory Tract Infections

Infectious Diseases Society of America[a]

CID 2011:52 (Suppl 4)

The Infectious Diseases Society of America (IDSA) perceives a need to develop and implement modern molecular technologies to advance microbiological diagnostic testing.

An Unmet Medical Need: Rapid Molecular Diagnostics Tests for Respiratory Tract Infections

Infectious Diseases Society of America[a]

CID 2011:52 (Suppl 4)

For maximal clinical impact, the test should ideally be available on-site 24 h/day, 7 days/week, with results available within no more than a few hours.

Developing Molecular Amplification Methods for Rapid Diagnosis of Respiratory Tract Infections Caused by Bacterial Pathogens

Fred C. Tenover
Cepheid, Sunnyvale, California

Clinical Infectious Diseases 2011;52(S4):S338–S345

Development of an assay that is rapid but unavailable on evening or night shifts or on weekends because of its technical complexity

limits the medical value of the test.

Developing Molecular Amplification Methods for Rapid Diagnosis of Respiratory Tract Infections Caused by Bacterial Pathogens

Fred C. Tenover
Cepheid, Sunnyvale, California

Clinical Infectious Diseases 2011;52(S4):S338–S345

The key challenge for industry is to develop assays that are not only rapid but also readily accessible and perceived by either laboratories or health care systems as cost-effective.

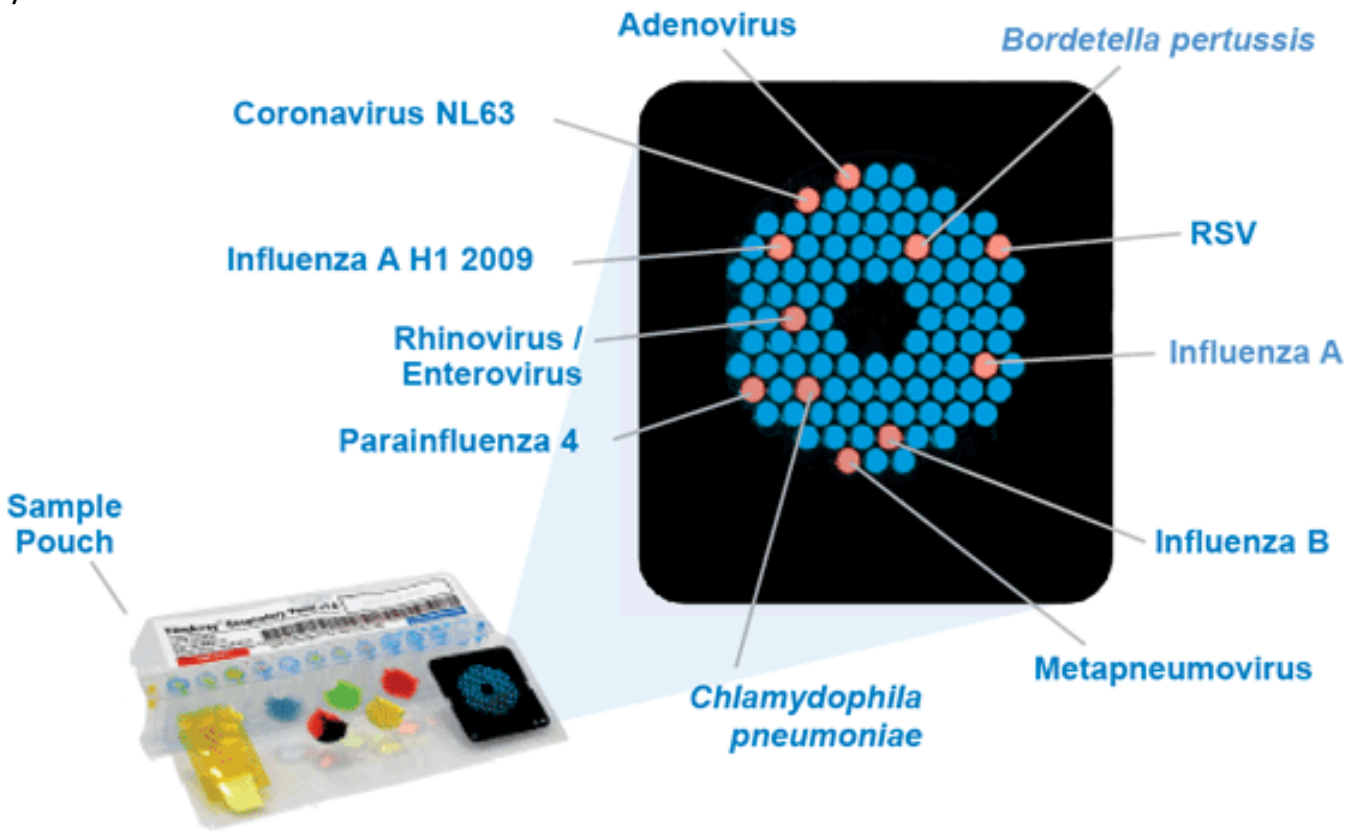
Xpert® FLU
(Flu A, Flu B, with 2009 H1N1)

FilmArray (Idaho Technology Inc., Salt Lake City, USA),
is a all-in-one technology that enables the rapid detection
of 21 viral and bacteria respiratory pathogens in a single test.

Camporese A. *Clinical Laboratory International. Feb/March 2013.*

The FilmArray integrates sample preparation, amplification, detection, and analysis into one simple system that requires 2 minutes of hands on time and has a total run time of about 1 hour.

http://www.biofiredx.com/



JOURNAL OF CLINICAL MICROBIOLOGY, July 2011, p. 2449–2453 Vol. 49, No. 7
0095-1137/11/$12.00 doi:10.1128/JCM.02582-10

Comparison of Two Multiplex Methods for Detection of Respiratory Viruses: FilmArray RP and xTAG RVP▽†

Kenneth H. Rand,[1]* Howard Rampersaud,[2] and Herbert J. Houck[1]

Department of Pathology, Immunology and Laboratory Medicine, University of Florida,[1] and Shands Hospital at the University of Florida,[2] Gainesville, Florida 32610

TABLE 4. Sensitivity and specificity of the FilmArray RP (FA) and xTAG RVP (xTAG) versus PCR-confirmed results

Virus	No. detected				Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	FA^+, $xTAG^+$	FA^+, $xTAG^-$	FA^-, $xTAG^+$	FA^-, $xTAG^-$	FA	xTAG	FA	xTAG	FA	xTAG	FA	xTAG
Influenza A virus	32	0	1	167	97	100	100	100	100	100	99.4	100
Influenza B virus	7	0	0	193	100	100	100	100	100	100	100	100
RSV	37	8	0	155	100	82.2	100	100	100	100	100	95.1
Parainfluenza virus	15	1	0	184	100	93.8	100	100	100	100	100	99.5
Rhinovirus/enterovirus	39	4	2	155	95.6	91.1	100	100	100	100	98.7	97.5
Adenovirus	9	0	1	190	90	100	100	100	100	100	99.5	100
Metapneumovirus	6	1	0	193	100	85.7	100	100	100	100	100	99.5

Verigene System (Nanosphere) enables simple, cost-effective, and highly sensitive nucleic acid testing on a single platform.

Respiratory Virus XP enables the rapid (<2.5 h) detection of 15 virus: Influenza A, Flu A-2009 H1N1, Flu A-H3, Flu A-H1 Influenza B, RSV A/B, Parainfluenza 1-4, Human Metapneumovirus, Adenovirus (A-F), Rhinovirus (A/B), Enterovirus (A/D) and oseltamivir resistance in a single test.

http://www.nanosphere.us

Gestione polmoniti in emergenza/urgenza

Emergenza (1-3 ore)

- Procalcitonina e/o CRP (sangue)
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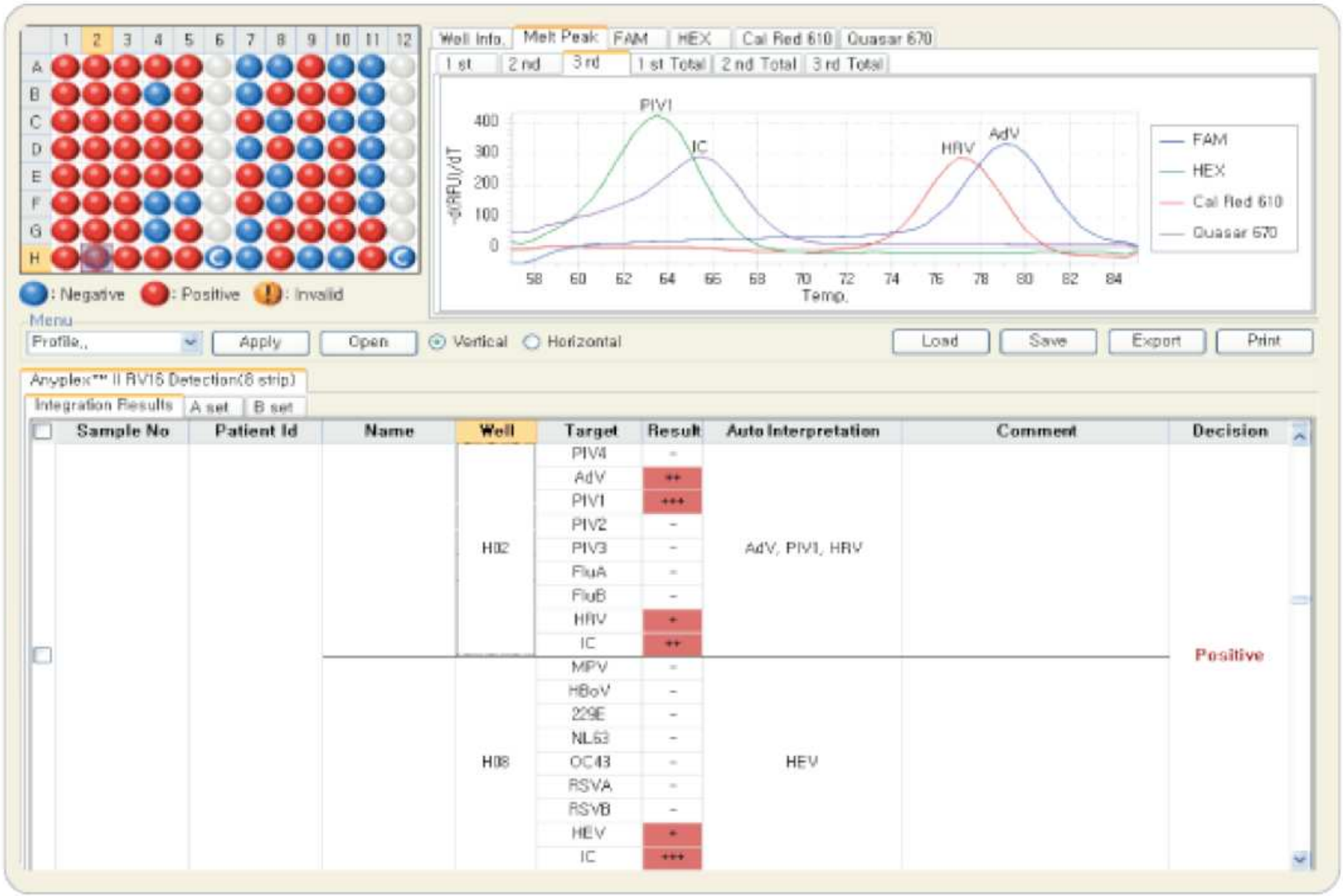

Anyplex™ II RV16 (Seegene, Seoul, Korea) enables detection of 16 respiratory viruses in a single real-time PCR.

Camporese A. *Clinical Laboratory International. Feb/March 2013.*

Seeplex® PneumoBacter ACE Detection, an innovative DPO™ based multiplex PCR for simultaneous detection of 6 relevant bacteria causing respiratory tract infections.

Camporese A. *Clinical Laboratory International. Feb/March 2013.*

Recent advances and perspectives in the molecular diagnosis of pneumonia

by Dr A. Camporese

The application of quantitative molecular tests for the detection of key pathogens, such as *S.pneumoniae*, defining a threshold for classification, such as a colonizer or as an invasive pathogen, might be relevant in LRTI patients, mainly in whom antibiotic therapy has been initiated, and might be a useful tool for severity assessment.

Recent advances and perspectives in the molecular diagnosis of pneumonia

by Dr A. Camporese

There is a significant gap in our knowledge as to how well molecular tests for bacterial pathogens would perform on expectorated sputum samples, compared with performance on BALs or protected brush samples from the same patient collected within a similar period.

Recent advances and perspectives in the molecular diagnosis of pneumonia

by Dr A. Camporese

This knowledge gap is also a barrier to test development, because a molecular test that cannot be performed on expectorated sputum (given all the problems with specimen quality) may not have broad enough appeal among physicians to make it a financially viable product (from the industry perspective).

Recent advances and perspectives in the molecular diagnosis of pneumonia

by Dr A. Camporese

In this age of multidrug resistance, expanding the target selection to include key antimicrobial resistance genes that would alter existing therapy or guide empirical therapy, should also be considered.

	CAP suitable targets	HAP/VAP suitable targets	Other targets in case of specific clinical suspicions and/or in immunocompromised patients
Microbial targets	*Streptococcus pneumoniae*^	*Staphylococcus aureus*^	*Mycobacterium spp.*
	Haemophilus influenzae	*Pseudomonas aeruginosa*^	*Bordetella pertussis*
	Moraxella catarrhalis	*Enterobacteriaceae*^	CMV
	Chlamydophila spp.	*Acinetobacter spp.*^	*HSV*
	Mycoplasma pneumoniae	*Stenotrophomonas maltophilia*^	*Pneumocystis jirovecii*
	Legionella pneumophila	Respiratory viruses*	*Aspergillus fumigatus* and *Candida spp.*^
	Staphylococcus aureus		
	Respiratory viruses*		
Resistance targets	*mec*A gene[a]	*mec*A gene[a]	
	bla_{TEM} gene[b]	bla_{TEM} gene[b]	
		bla_{VIM}, bla_{IMP} genes[c]	
		bla_{KPC} gene[c]	
		bla_{OXA} genes[d]	

**Respiratory viruses: Influenza A virus (with 2009 H1N1 virus), Influenza B virus, Metapneumovirus, Adenovirus A/B/C/D/E, Coronavirus 229 E/NL 63, Enterovirus, Parainfluenza virus 1/2/3/4, Respiratory syncytial virus (RSV) A/B, Rhinovirus A/B/C, Coronavirus OC43, Bocavirus 1/2/3/4.*

*^Targets already feasible with LightCycler Septi*Fast*™ (off label) on BAL samples.*

[a] Mediates resistance to all b-lactam agents with the exception of the novel anti–methicillin-resistant S. aureus cephalosporins; [b] mediates resistance to penicillins and first-generation cephalosporin; [c] mediates resistance to cephalosporins and carbapenems, but not resistance to monobactams. [d] Some OXA b-lactamases can mediate resistance to carbapenems.

Table 1: Potential and/or currently feasible targets for multiplex or individual molecular assays in immunocompetent and/or immunocompromised patients with CAP, HAP, or VAP.

Camporese A. *Clinical Laboratory International. Feb/March 2013.*

Intensive Care Med (2011) 37:1069–1076
DOI 10.1007/s00134-011-2245-x

REVIEW

Mathias W. Pletz
Nele Wellinghausen
Tobias Welte

Will polymerase chain reaction (PCR)-based diagnostics improve outcome in septic patients? A clinical view

The presence of microbial DNA in the bloodstream is a significant and prognostic event even if the accompanying blood culture remains negative.

EXPERT REVIEWS

New technology for rapid molecular diagnosis of bloodstream infections

Expert Rev. Mol. Diagn. 10(4), 399–415 (2010)

In patients with pneumococcal pneumonia, bacterial DNA in the blood is associated with the likelihood of death, the risk of septic shock and the likely need for mechanical ventilation.

Fig. 1.
DNAemia in patients with strong clinical suspect of sepsis.

Microrganism-DNA detected	N°	(SeptiFast+/BC+)	(SeptiFast+/BC-)	(BC not requested)
Asper.fumigatus	2	1	1	0
Candida albicans	7	1	1	5
Candida tropicalis	3	0	0	3
Coagulase Negative Staphylococci	2	1	0	1
Escherichia coli	67	41	16	10
Klebsiella pneumoniae/oxytoca	11	4	4	3
Enterobacter cloacae/aerogenes	6	3	1	2
Enterococcus faecalis	4	0	4	0
Proteus mirabilis	2	1	1	0
Pseudomonas aeruginosa	7	4	0	3
Serretia marcescens	1	0	0	1
Staphylococcus aureus	20	9	5	6
Streptococcus spp.	11	5	1	5
Stenotrophomonas maltophilia	1	1	0	0
Streptococcus pneumoniae	15	7	5	3
Staph.aureus/Kleb.spp	1	0	0	1
Staph.aureus/Str.pneum.	1	0	0	1
Staph.aureus/Ps.aeruginosa	1	0	0	1
Kleb.spp/Enterobacter spp.	1	0	0	1
E.coli / Kleb.spp	3	1	1	1
E.coli/ Ent.faecium	1	1	0	0
E.coli/Ps.aeruginosa	1	1	0	0
E.coli/Serr. marcescens	1	1	0	0
E.coli/Str.spp	1	1	0	0
tot	170	83	40	47

Severe LRTI

1

6

1

3

4

Microbial DNAemia: a promising biomarker of sepsis?

M. Avolio, P. Diamante, R. De Rosa, P. Stano, M. L. Modolo, and A. Camporese

SOC Microbiologia Clinica e Virologia, Dipartimento di Medicina di Laboratorio
Azienda Ospedaliera "S. Maria degli Angeli", Pordenone.

Recent advances and perspectives in the molecular diagnosis of pneumonia

by Dr A. Camporese

Some multiplex assays for respiratory tract disease already include many targets, but in designing new assays, will be critical to understand whether an assay for a determinated number of bacterial pathogens will meet physicians' needs and provide adequate data for initiating or altering anti infective therapy.

Recent advances and perspectives in the molecular diagnosis of pneumonia

by Dr A. Camporese

Developing new molecular tests for other bacterial respiratory pathogens, detection of new key antimicrobial resistance genes in unprocessed samples, and determination of the microbial load by quantitative multi-pathogen tests will be some of the future challenges of molecular diagnosis in CAP/HAP/VAP.

3° CONGRESSO NEWMICRO

...The need for speed: il laboratorio di Microbiologia e le Urgenze Infettive

Vi ringrazio per l'attenzione

A. Camporese
SOC Microbiologia Clinica e Virologia
Azienda Ospedaliera S.Maria degli Angeli, Pordenone