Syndromic Testings for Diagnosis of Pulmonary Infectious Diseases – Does it bring new hopes?

Jung-Yien Chien, MD, PhD.

Division of Pulmonary and Critical Care Medicine

National Taiwan University Hospital

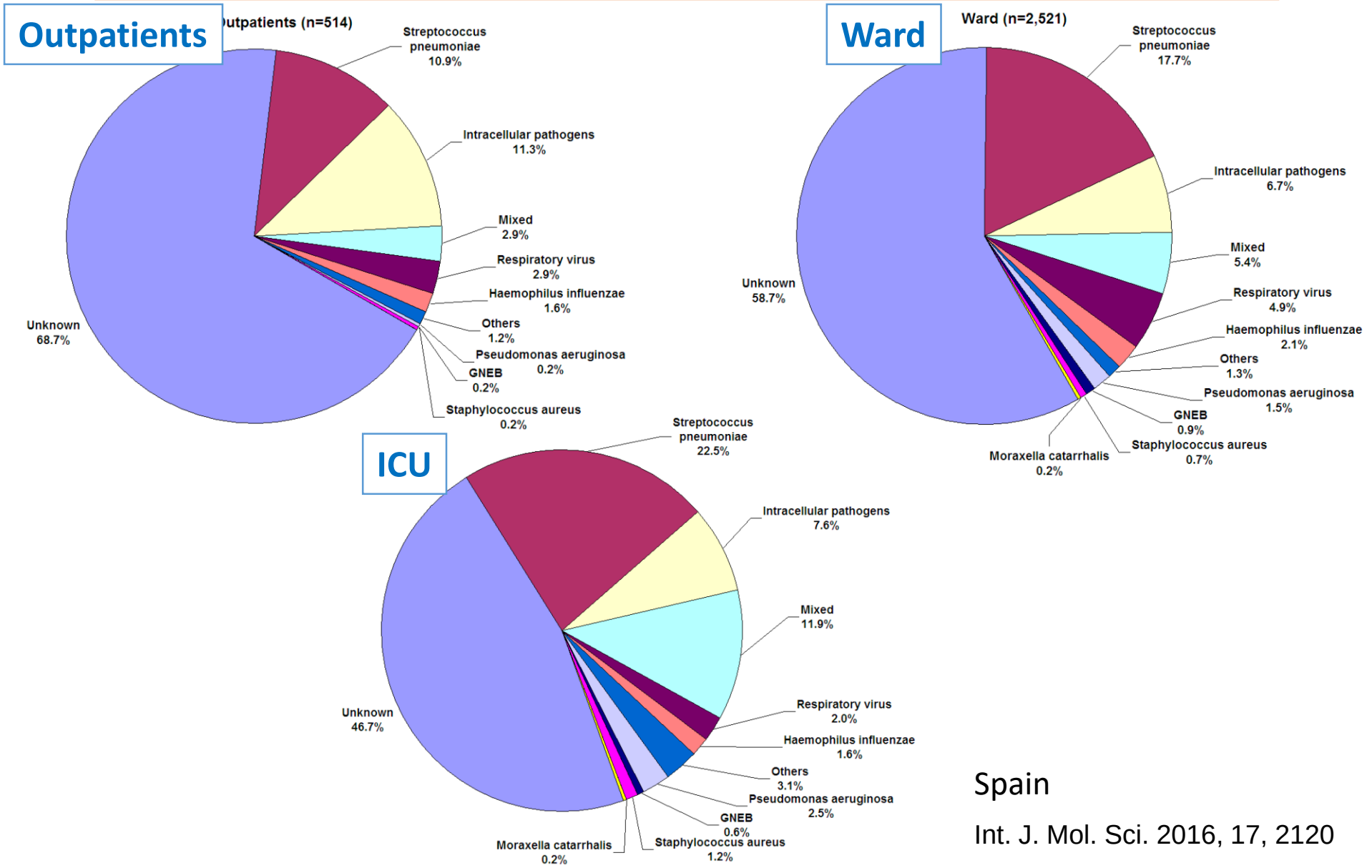
Conventional microbiological diagnosis in Pneumonia

- Sputum culture
- Urinary antigen test
  - *Streptococcus pneumoniae*
  - *Legionella pneumophila*
- Blood culture
- Serological investigation
  - Atypical bacterial pathogens

- **An etiology was only identified in 53–75% samples (mostly *S. pneumoniae*, *Mycoplasma pneumoniae* and *Haemophilus influenza*)**

Torres et al. ERJ 2016 Dec;48(6):1764-1778

Pathogens among CAP

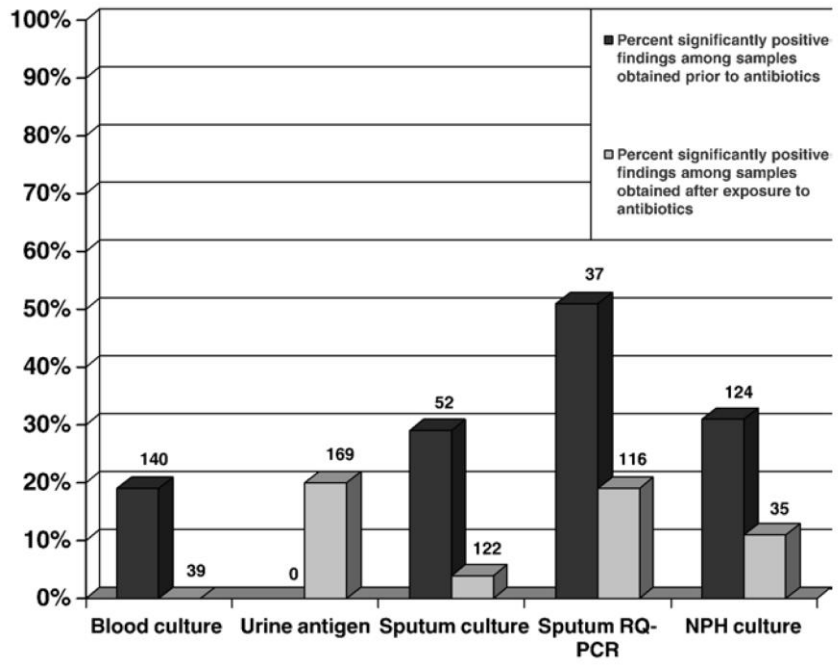


Spain

Int. J. Mol. Sci. 2016, 17, 2120

Polymerase chain reaction(PCR)

- Does not require viable bacteria
- Less influenced by antimicrobial therapy
- Sputum PCR is a more sensitive method than sputum culture, especially in those previously treated with antibiotics.

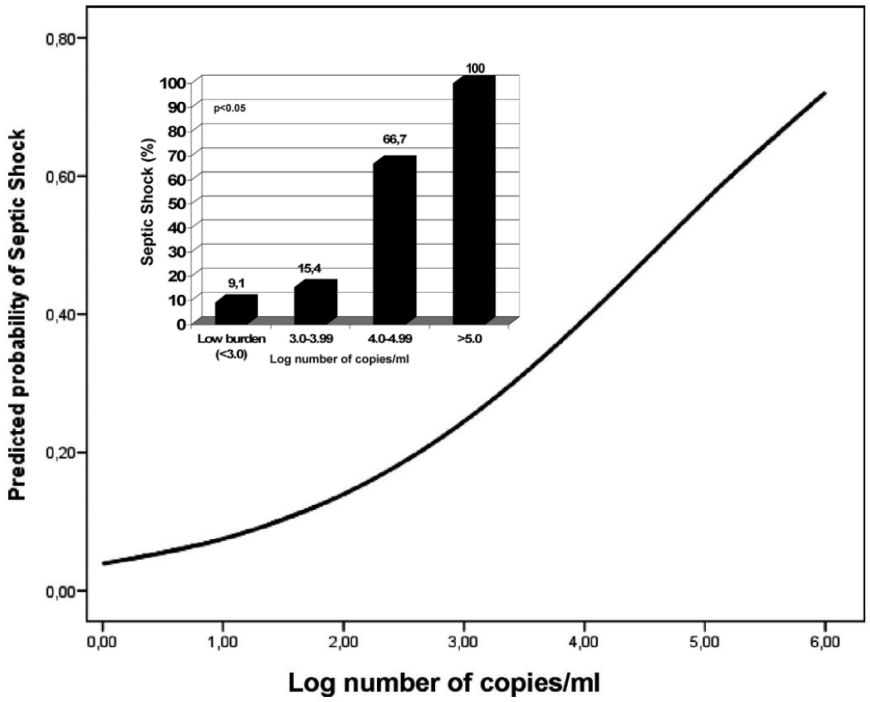


In one study, in patients with CAP who received antibiotic treatment before hospital admission, PCR sensitivity was 7.0 times higher than that of culture (p=0.043)

N. Johansson et al. / Diagnostic Microbiology and Infectious Disease 60 (2008) 255–261

Quantitative PCR assays

- Help to tell colonization from infection
- Predict disease presentation and severity
- Pneumococcal serotyping



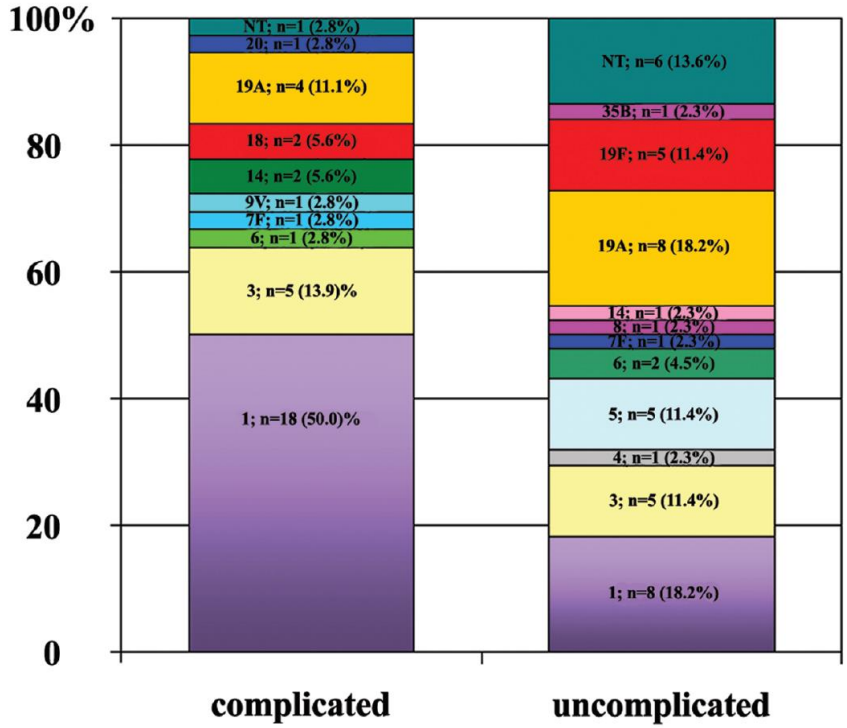
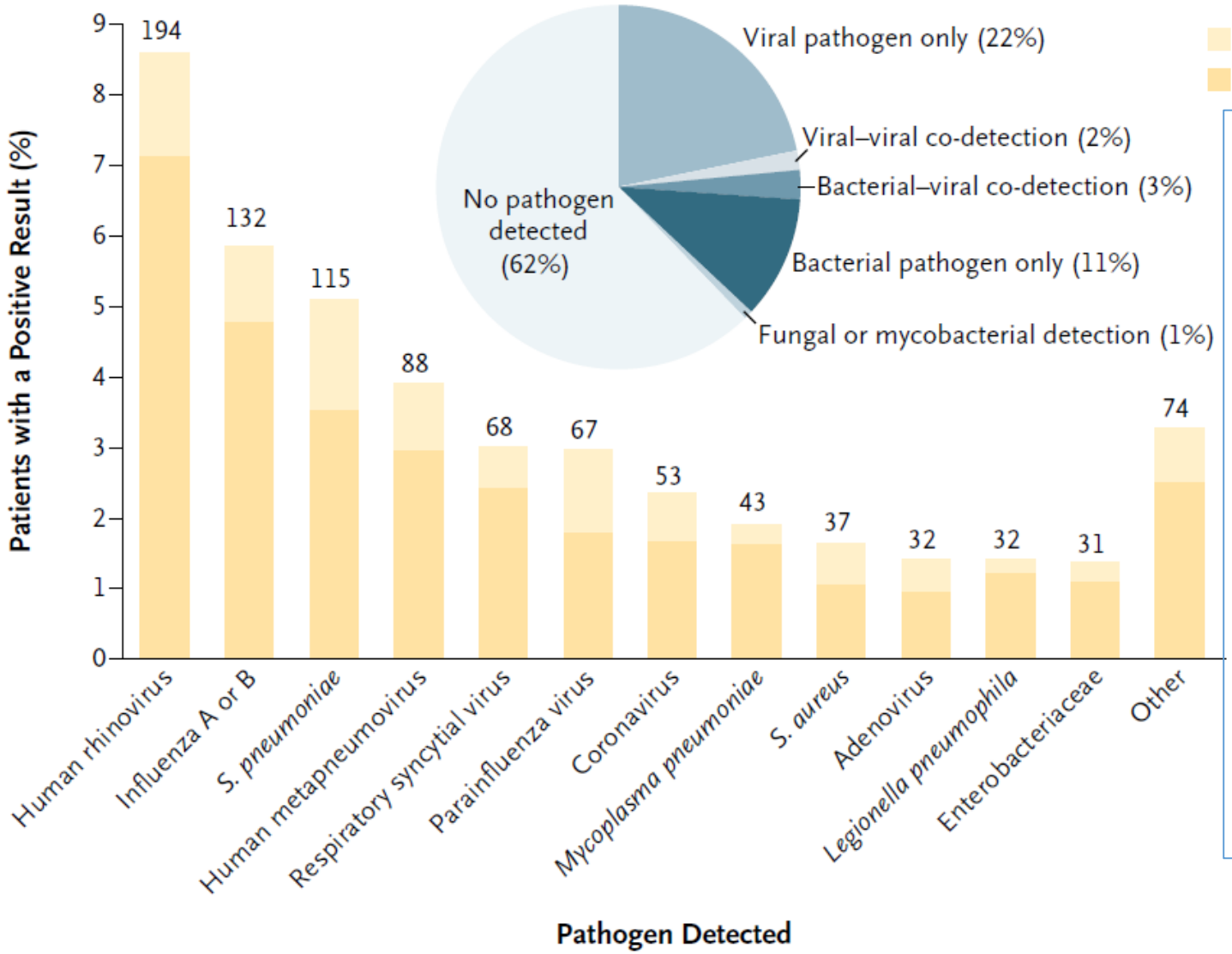


FIGURE 1. Predicted probability of septic shock as a function of *S pneumoniae* bacterial load detected by quantitative rt-PCR.

CHEST 2009; 136:832–840
Clinical Infectious Diseases 2010; 51(9):1042–1049

Pathogens Detected in U.S. Adults with Community-Acquired Pneumonia Requiring Hospitalization



- A pathogen was detected: **38%**
- Respiratory viruses : **23%**
- One or more bacteria: **11%**
- Both bacterial and viral pathogens: **3%**
- Fungi or mycobacteria: 1%

✓ **Most commonly detected pathogens**

- Human rhinovirus: **9%**

Jain et al. N Engl J Med 2015; 373: 415–427

Diagnosis of viral and atypical pneumonia

- Influenza
  - **Rapid Influenza Diagnostic Tests** (RIDT, antigen immunoassays)
    - Low sensitivity (40-70%)
  - **Immunofluorescence antigen assays**
    - More sensitive (50–85%)
  - **PCR**
    - high sensitivity and specificity
    - detect all influenza A subtypes
    - individual subtypes using specific primers
    - negative results may occur with nasopharyngeal samples
    - not provide information regarding infectiousness and dead virus RNA fragments
    - Semi-quantitative assays for virologic response

Torres et al. ERJ 2016 Dec;48(6):1764-1778

Diagnosis of viral and atypical pneumonia

- **Multiplex PCR**
  - Influenza
  - RSV
  - Human metapneumovirus
  - Parainfluenza virus
  - Rhinovirus
  - *M. pneumoniae*
  - *Chlamydophila pneumoniae*

- **Molecular-based point-of-care tests (loop-mediated isothermal amplification (LAMP)**
  - available for detecting influenza and other viruses at the bedside
  - accuracy comparable to conventional laboratory PCR assays

Torres et al. ERJ 2016 Dec;48(6):1764-1778

Multiplex PCR

- Syndromic testing
- Rapid (some <60 minutes)
- Broad coverage
  - Viral
  - Bacterial

Syndromic Approach

- Syndromic approach is a symptom-driven diagnostic method that combines a broad grouping of probable pathogenic causes into a single, rapid test.

With Syndromic Testing…Informed Therapy	Without Syndromic Testing…Educated Guess
• Increases the probability of identifying a pathogen using the right test, the first time	• The infectious cause remains unknown
• Eliminates guesswork and costs of additional testing	• The right test is not ordered
• Improves antibiotic stewardship	• The wrong tests or no test may be ordered
• Provides proper care management on admission, isolation, cohorting and treatment therapy	• Costs associated with additional testing
	• Patient care is compromised
	• Risk of adverse outcomes and patient dissatisfaction

The Right Test, The First Time.

Respiratory Panels for Multiple Pathogen Detection

	Virus	Bacteria	Time to results
FilmArray	17	3	<1
Verigene	13	3	~2-3
x-TAG RVP	12		~8
x-TAG RVP Fast	8		~6
NxTAG-RPP	18	2	~4
eSensor RVP	14		~6
ePlex			~1.5

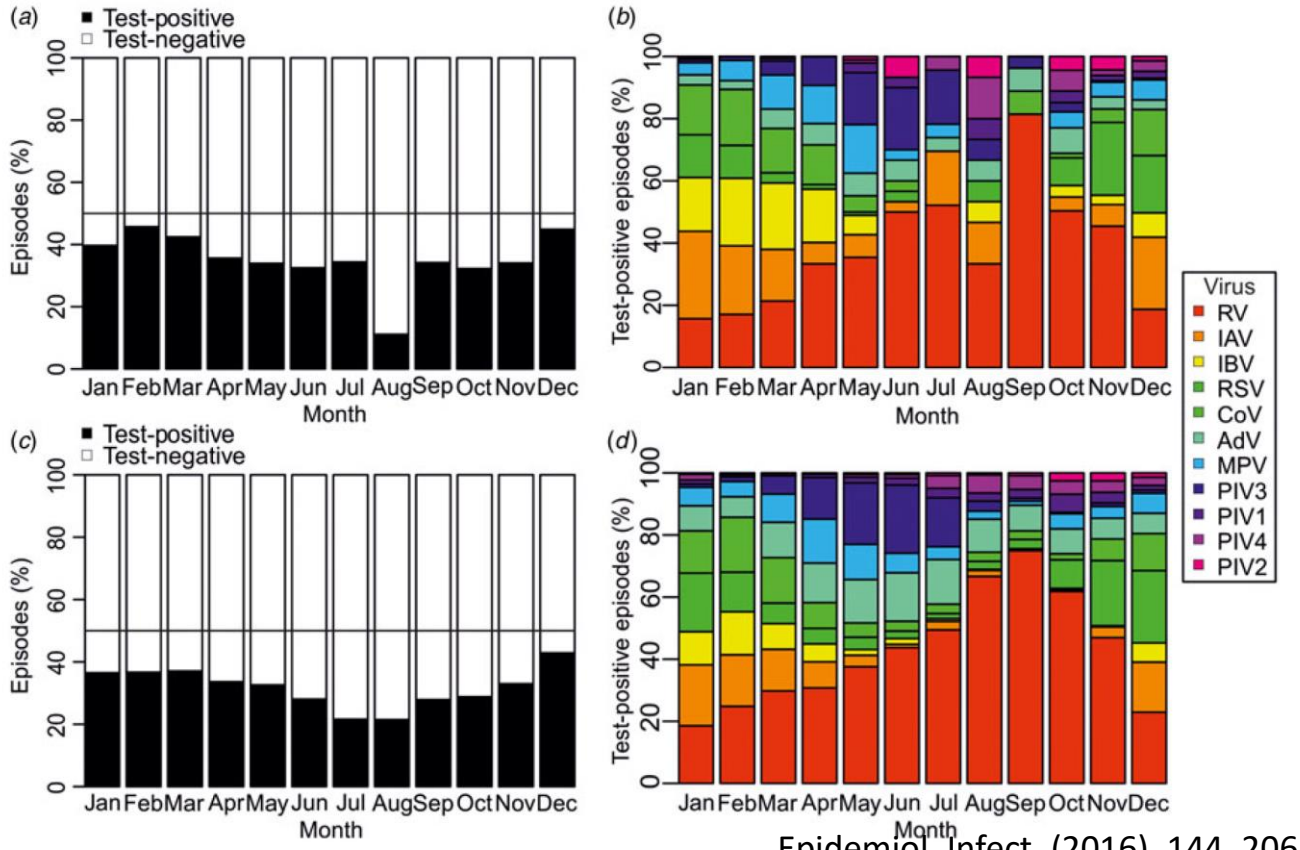
Ramanan et al. Clinical Microbiology Reviews 2018;31:e00024-17

Clinical benefits

- Allow the epidemiology of certain pathogens to be better defined.
  - Multiplex molecular testing identified that infection with human coronavirus was more common during the influenza season than previously recognized

General Practitioners

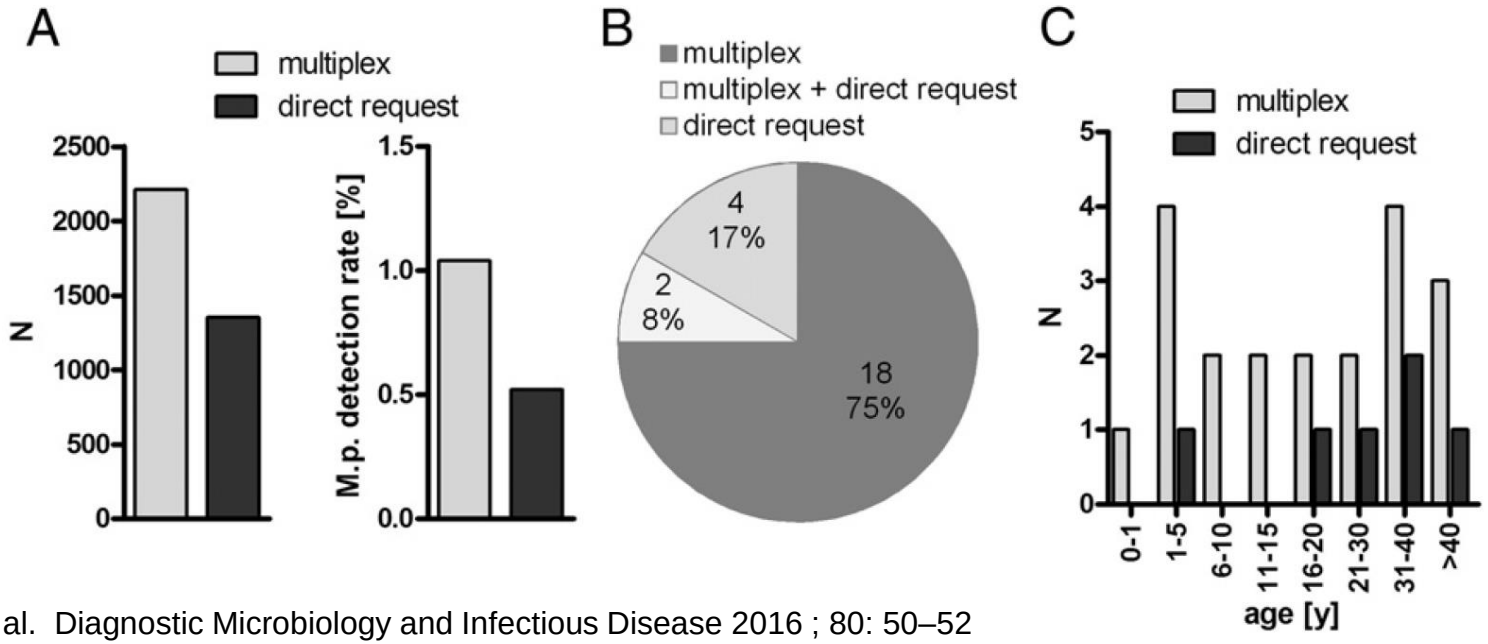
Hospital inpatients and outpatients



Epidemiol. Infect. (2016), 144, 2064–2076.

Clinical benefits

- Diagnosis of some infections that have been commonly missed due to a lack of clinical suspicion or available routine testing.
  - For example, one study reported that 75% of *Mycoplasma pneumoniae* infections were detected unexpectedly by the use of multiplex PCR (48). This is important because it is an treatable finding

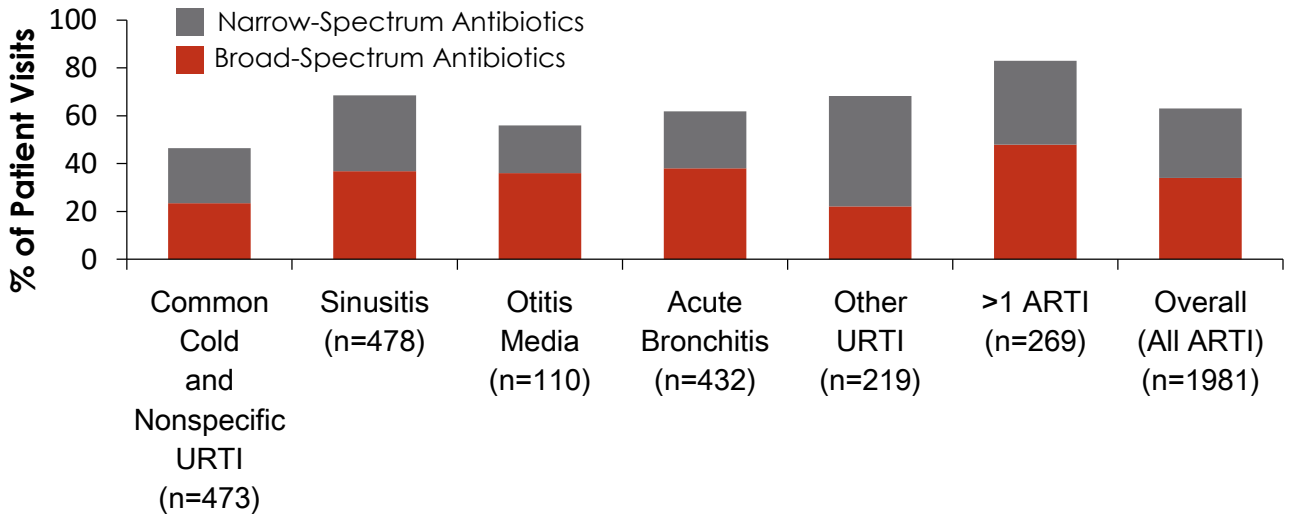


A. Dalpke et al. Diagnostic Microbiology and Infectious Disease 2016 ; 80: 50–52

An Accurate Diagnosis Reduces Overprescribing of Antibiotics and Prevents Antibiotic Resistance

- Each year, more than 10,000,000 courses of antibiotics are prescribed for viral conditions.[2]
- An estimated 55% of antibiotic prescriptions for RTIs are unnecessary.[3]

Antibiotic Prescriptions for Adults With Nonpneumonic RTIs, by Diagnosis[3]

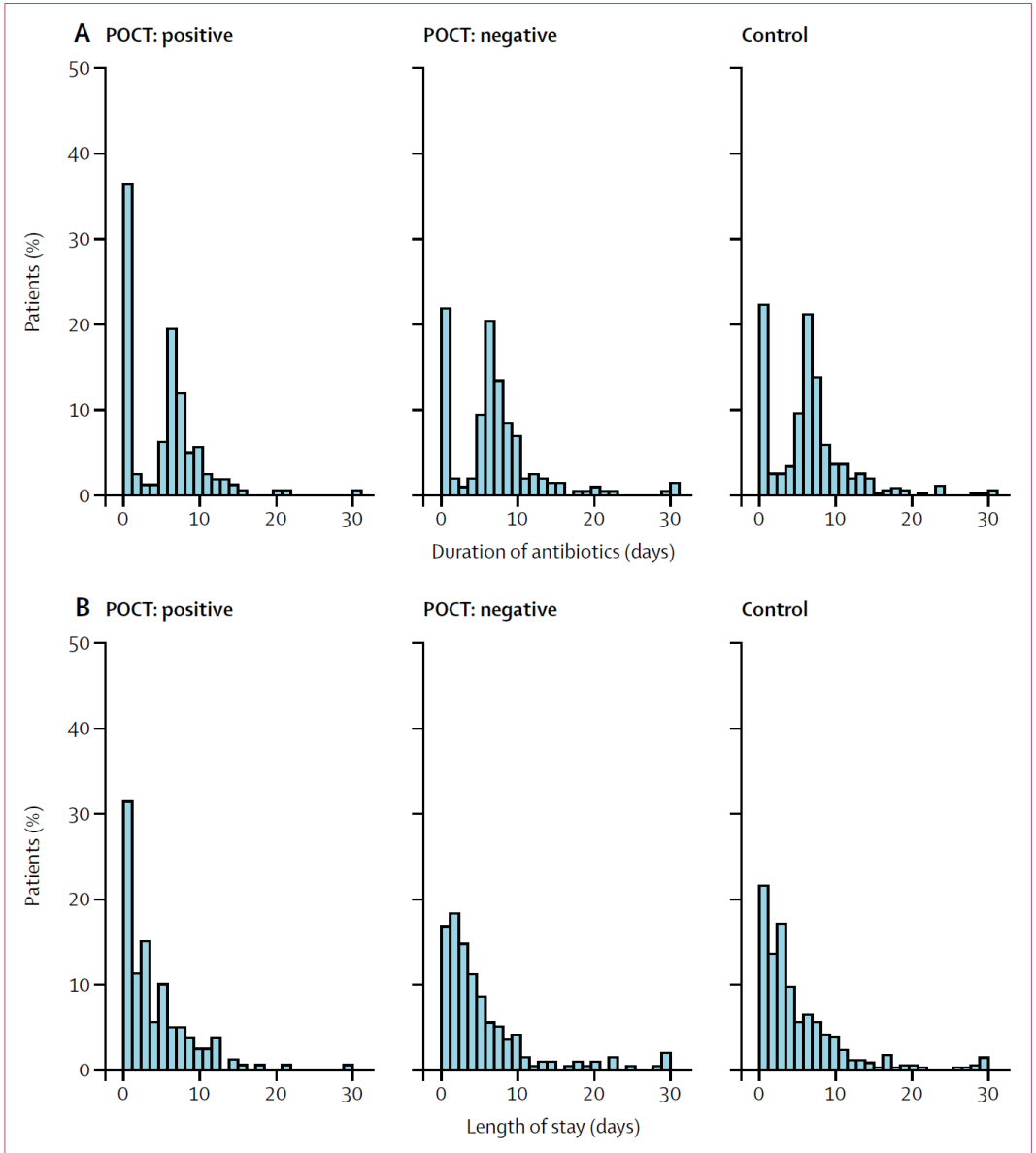


RTI=respiratory tract infection; URTI=upper respiratory tract infection; ARTI=acute respiratory tract infection.

1. Smolinski M et al, eds. Microbial Threats to Health: Emergence, Detection, and Response. Washington, DC: The National Academies Press; 2003.
2. CDC. www.cdc.gov/getsmart/community/improving-prescribing/program-development-eval/program-development/. Accessed February 26, 2016.
3. Steinman MA et al. JAMA. 2003;289(6):719-725.

Point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness

- Routine use of molecular POCT for respiratory viruses did not reduce the proportion of patients treated with antibiotics.
- However, more patients in the POCT group **received single doses or brief courses of antibiotics** than did patients in the control group.



Lancet Respir Med 2017; 5: 401–11

Clinical benefits

- Potentials to deescalate antibiotics if a viral pathogen(s) is detected
- Decrease the use of invasive sample collection procedures
- Allow informed decisions to be made regarding infection control measures and timely outbreak investigations
  - For example, an enterovirus D68 outbreak in 2014

Severe Respiratory Illness Associated with Enterovirus D68 — Missouri and Illinois, 2014

Claire M. Midgley, PhD[1,2], Mary Anne Jackson, MD[3], Rangaraj Selvarangan, PhD[4], George Turabelidze, MD[5], Emily Obringer, MD[6], Daniel Johnson, MD[6], B. Louise Giles, MD[6], Ajanta Patel, MD[6], Fredrick Echols, MD[7], M. Steven Oberste, PhD[2], W. Allan Nix[2], John T. Watson, MD[2], Susan I. Gerber, MD[2] (Author affiliations at end of text)

Journal of Clinical Microbiology 2013; 51:1528–1533

Syndromic panels: Limitations

- Do not allow customized ordering
- Higher cost than conventional laboratory methods
  - However, may be cost effective if multiple routine methods would have been ordered
  - May reduce downstream costs (i.e., hospital stay)
- More information does not always equate to improved patient management/outcomes
  - Example: How should a positive result for rhinovirus be interpreted in an immunosuppressed host?

Journal of Clinical Microbiology 2013; 51:1528–1533

Syndromic panels: Limitations

- **Probably lower sensitivity for the detection of certain pathogens**
  - FilmArray assay was noted to have modest sensitivities for the detection of adenovirus (57%), influenza A virus H1/2009 (73%), and influenza B virus (77%)

Virus	No. of true-positive specimens (n = 300 specimens tested)	% Sensitivity (95% CI) of:			
				xTAG	
		FilmArray RP	eSensor RVP	RVPv1	RVP fast
AdV	35	57.1 (40.8, 72.0)	100 (88.2, 100)	74.3 (57.8, 86.0)	82.9 (66.9, 92.3)
Influenza virus					
A	30	86.2[a] (68.8, 95.1)	100 (86.5, 100)	100 (86.5, 100)	86.7 (69.7, 95.3)
A H1/09	16	73.3[a] (47.6, 89.5)	100 (77.3, 100)	100 (77.3, 100)	81.3 (56.2, 94.2)
A H3	14	100 (74.9, 100)	100 (74.9, 100)	92.9 (66.5, 99.9)	78.6 (51.7, 93.2)
B	22	77.3 (56.2, 90.3)	100 (82.5, 100)	95.5 (76.5, 99.9)	45.5 (26.9, 65.4)
MPV	26	96.2 (79.6, 99.9)	100 (84.8, 100)	100 (84.8, 100)	100 (84.8, 100)
PIV					
1	14	100 (74.9, 100)	100 (74.9, 100)	100 (74.9, 100)	NA[b]
2	13	92.3 (64.6, 99.9)	100 (73.4, 100)	100 (73.4, 100)	NA
3	13	100 (73.4, 100)	100 (73.4, 100)	100 (73.4, 100)	NA
RSV					
A	22	86.4 (65.8, 96.1)	100 (82.5, 100)	86.4 (65.8, 96.1)	86.4 (65.8, 96.1)
B	14	100 (74.9, 100)	100 (74.9, 100)	92.9 (66.5, 99.9)	85.7 (58.8, 97.2)
RhV/EV	43	83.7 (69.7, 92.2)	90.7 (77.8, 96.9)	93.0 (80.7, 98.3)	93.0 (80.7, 98.3)

Journal of Clinical Microbiology 2013; 51:1528–1533

Syndromic panels: Limitations

- **Significance of the detection of multiple targets in these multiplex panels remains unclear**
  - a coinfection rate of 10%.

Viral combination[b]	No. of coinfections detected by: FilmArray RP	eSensor RVP	xTAG RVPv1	xTAG RVP fast	No. of true-positive results
AdV + InfA H1/2009	0	1	0	0	1
AdV + MPV	0	1	0	1	1
AdV + PIV2	1	1	1	NA[a]	1
AdV + RhV/EV	4	6	5	5	6
AdV + RSV A	1	4	2	3	4
InfA H1/2009 + RhV/EV	1	1	1	1	1
InfA H1/2009 + RSV B	1	2	2	2	2
MPV + RhV/EV	1	1	1	1	1
PIV1 + RhV/EV	2	1	1	NA	2
PIV2 + RhV/EV	2	4	3	NA	4
PIV3 + RhV/EV	0	1	0	NA	1
RSV A + RhV/EV	5	5	5	4	5
RSV B + RhV/EV	1	1	1	0	1
AdV + MPV + RSV A	0	1	0	0	1
Total	19	30	22	17	31

- Most coinfections involved enterovirus (EV) and Rhinovirus
- Cross-reaction ?

Journal of Clinical Microbiology 2013; 51:1528–1533

Syndromic panels: Limitations

- Positive results may not distinguish between colonization and active infection
  - Prolonged shedding of microorganisms or nucleic acid in immunocompromised patients without necessarily causing clinical disease.
  - Laboratory results should be interpreted in the context of clinical findings.

- Miss coinfection with bacteria or fungi

Journal of Clinical Microbiology 2013; 51:1528–1533

Syndromic panels: Limitations

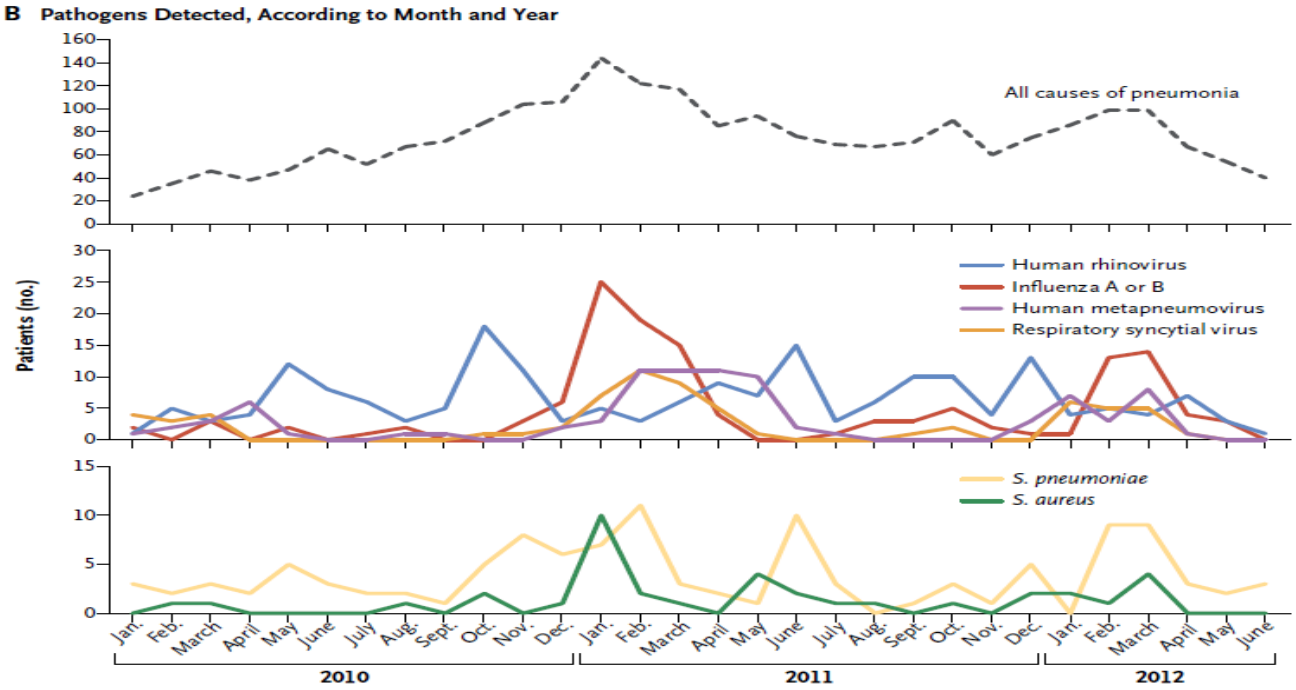
- Nasopharyngeal specimen collection may miss lower respiratory tract infection in critically ill patients
  - additional testing of BAL fluid samples.

- These panels do not offer exhaustive testing and do not detect some virus
  - Cytomegalovirus (CMV)
  - Middle East respiratory syndrome coronavirus (MERS-CoV)
  - Severe acute respiratory syndrome-associated coronavirus (SARS-CoV)
  - Hantavirus

Journal of Clinical Microbiology 2013; 51:1528–1533

Issues to consider prior to ordering a syndromic respiratory panel

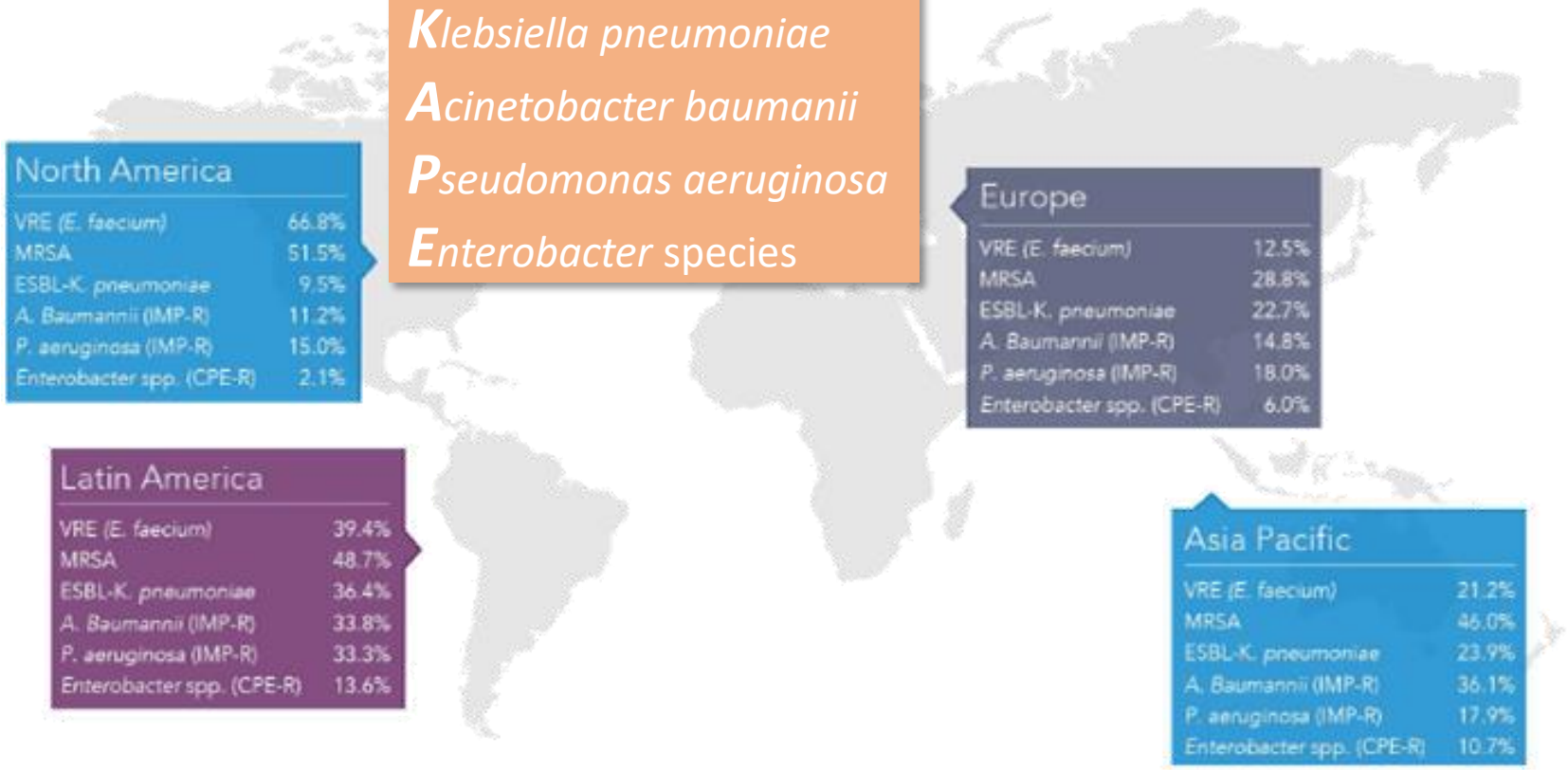
- Is the patient otherwise healthy or immunosuppressed/critically ill?
- Will I manage my patient differently based on the results of this test (e.g., What if I get a positive)?
- Is targeted testing (e.g., influenza) prudent due to the clinical presentation and/or seasonality?



N Engl J Med 373;5 nejm.org July 30, 2015

Antibiotic-resistant pathogens: "ESKAPE"
Hospital-wide resistance rates a global problem

***E**nterococcus faecium*
***S**taphylococcus aureus*
***K**lebsiella pneumoniae*
***A**cinetobacter baumanii*
***P**seudomonas aeruginosa*
***E**nterobacter* species



North America

VRE (*E. faecium*)	66.8%
MRSA	51.5%
ESBL-*K. pneumoniae*	9.5%
A. Baumannii (IMP-R)	11.2%
P. aeruginosa (IMP-R)	15.0%
Enterobacter spp. (CPE-R)	2.1%

Europe

VRE (*E. faecium*)	12.5%
MRSA	28.8%
ESBL-*K. pneumoniae*	22.7%
A. Baumannii (IMP-R)	14.8%
P. aeruginosa (IMP-R)	18.0%
Enterobacter spp. (CPE-R)	6.0%

Latin America

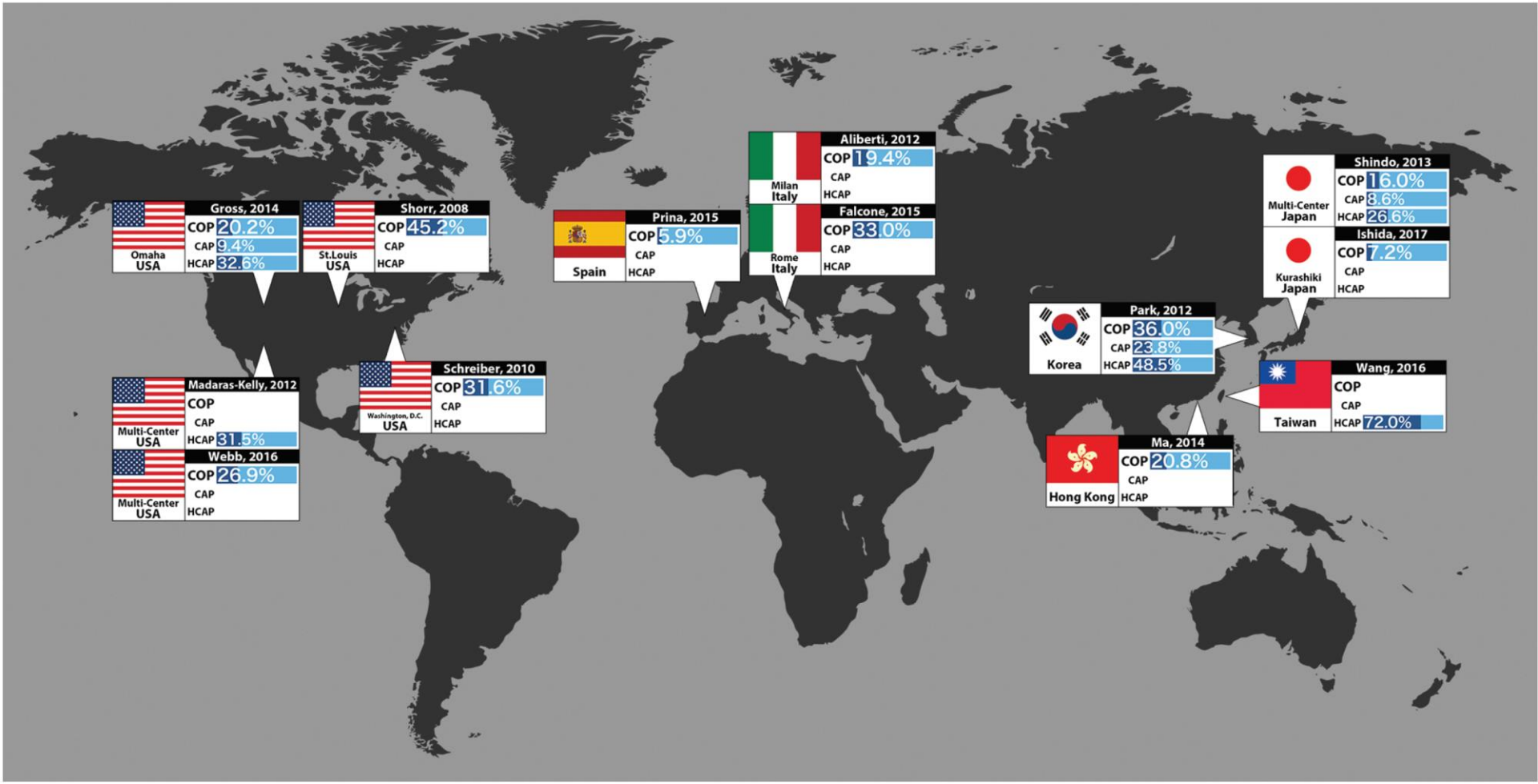
VRE (*E. faecium*)	39.4%
MRSA	48.7%
ESBL-*K. pneumoniae*	36.4%
A. Baumannii (IMP-R)	33.8%
P. aeruginosa (IMP-R)	33.3%
Enterobacter spp. (CPE-R)	13.6%

Asia Pacific

VRE (*E. faecium*)	21.2%
MRSA	46.0%
ESBL-*K. pneumoniae*	23.9%
A. Baumannii (IMP-R)	36.1%
P. aeruginosa (IMP-R)	17.9%
Enterobacter spp. (CPE-R)	10.7%

2004-2013 data. Available at: www.testsurveillance.com

Prevalence of Drug-resistant pathogens increasing among HAP



Respirology (2017) 22, 1536 – 1546

Carbapenem resistant GNB

	Medical cenetr		Local hospital	
CR-GNB	2006	2017	2006	2017
Carbapenem resistant *Acinetobacter baumannii* (CRAB)	33.4%	71,9%	39.7%	70.7%
Carbapenem resistant *Enterobacteriaceae* (CRE)	0.7%	16.1%	2.2%	14.1%
Carbapenem resistant *Escherichia coli* (CR *E. Coli*)	0.2%	3.9%	0.5%	2.7%
Carbapenem resistant *Klebsiella pneumoniae* (CRKP)	1.7%	29.9 %	3.6%	22.3%
Carbapenem resistant *Pseudomonas aeruginosa* (CRPA)	13%	19.8%	14.9%	15.1%

Diagnosis of pneumonia caused by potentially multidrug-resistant bacteria

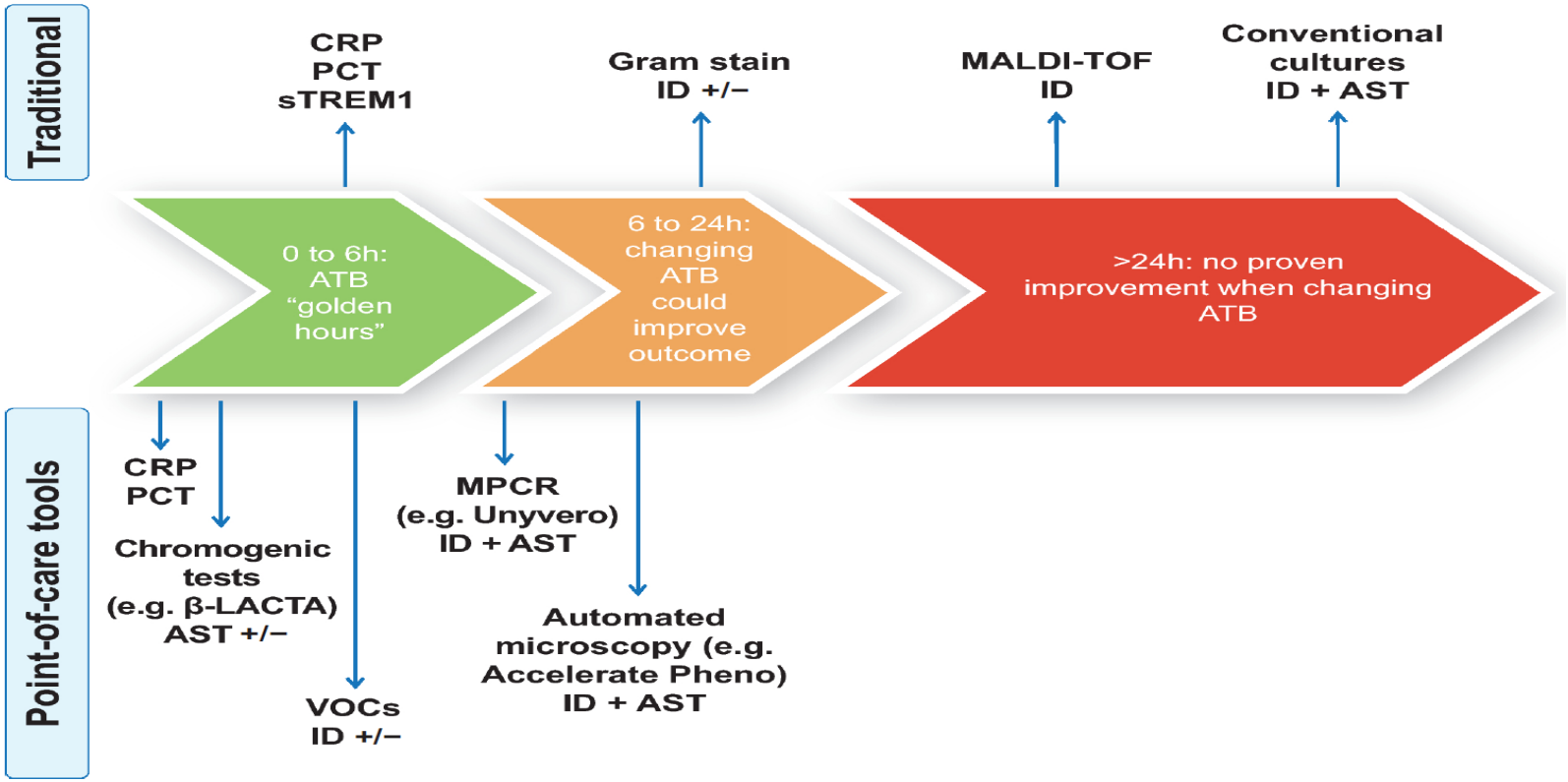
- Most frequent multidrug-resistant bacteria involved in pneumonia
  - MRSA
  - *P. aeruginosa*
  - *Acinetobacter baumannii*
  - *Enterobacteriaceae*

- Reference diagnostic techniques
  - Gram stain > Culture > MALDI-TOF > Susceptibility
    - Difficulty of differentiating between colonization and infection
    - Requires a minimum of 2 days
    - Low sensitivity, if sample is taken after the start of antibiotic treatment

Torres et al. ERJ 2016 Dec;48(6):1764-1778

Rapid point-of-care testing

- Automated microscopy as a POCT tool for VAP
- Multiplex PCR (MPCR)
- Exhalome analysis
- Rapid chromogenic tests



Ann Transl Med 2017;5(22):451

Automated microscopy

- Based on fluorescent in situ hybridization (FISH), allowing for both rapid pathogen identification and antibiotic susceptibility test (AST)
  - Sensitivity of 100%
  - Specificity of 97%
  - Antibiotic susceptibility testing (AST) in less than 12 h

	≥ 1 × 10^4 CFU/mL	
	Positive	Negative
Positive	7	2
Negative	0	64
	Sensitivity=100%	Specificity=97%

Am J Respir Crit Care Med 2015;191:566-73

Multiplex PCR

- Simultaneously identify and quantify multiple respiratory pathogens
- Detection of drug-resistance genes in about 6 h
- Prior antimicrobial therapy does not influence PCR diagnostic accuracy

Torres et al. ERJ 2016 Dec;48(6):1764-1778
J Clin Microbiol 2014;52:2487-92

Multiplex PCR

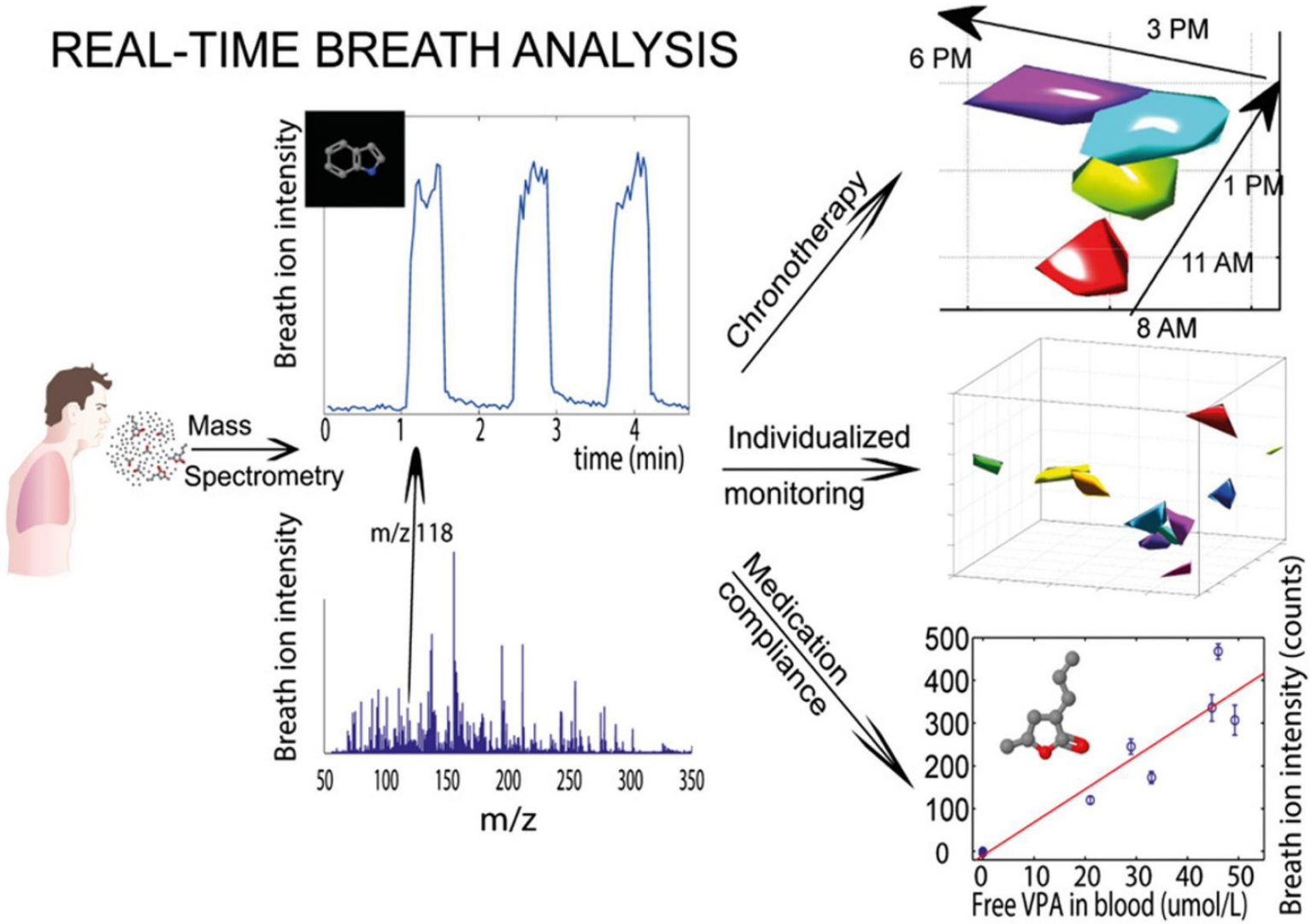
- Using the **Unyvero** MPCR, results were obtained on average in 6.5 h (4.7–18.3 h), vs. 71 h (37.2–217.8 h) for conventional methods
- Sensitivity of 89.2% and specificity of 97.1%

- Only half of the results were concordant with conventionally obtained results.
- Discordance was also of a concerning level, probably because of the detection or resistance-related genes from resident species of the airways.

- Test failure in about 40% of samples

Torres et al. ERJ 2016 Dec;48(6):1764-1778
J Clin Microbiol 2014;52:2487-92

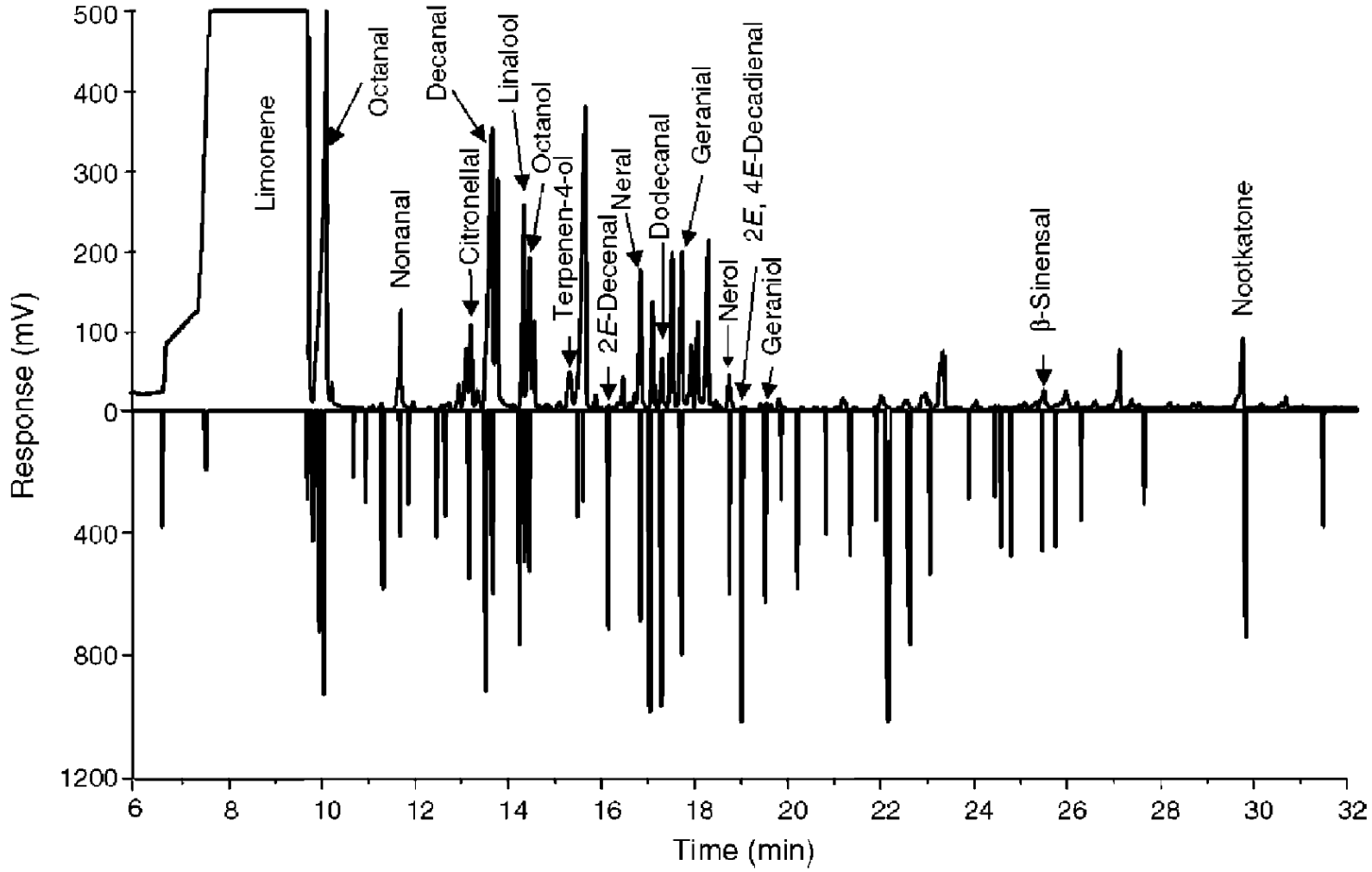
Exhalome analysis (VOC)



Chest 2013;144:746

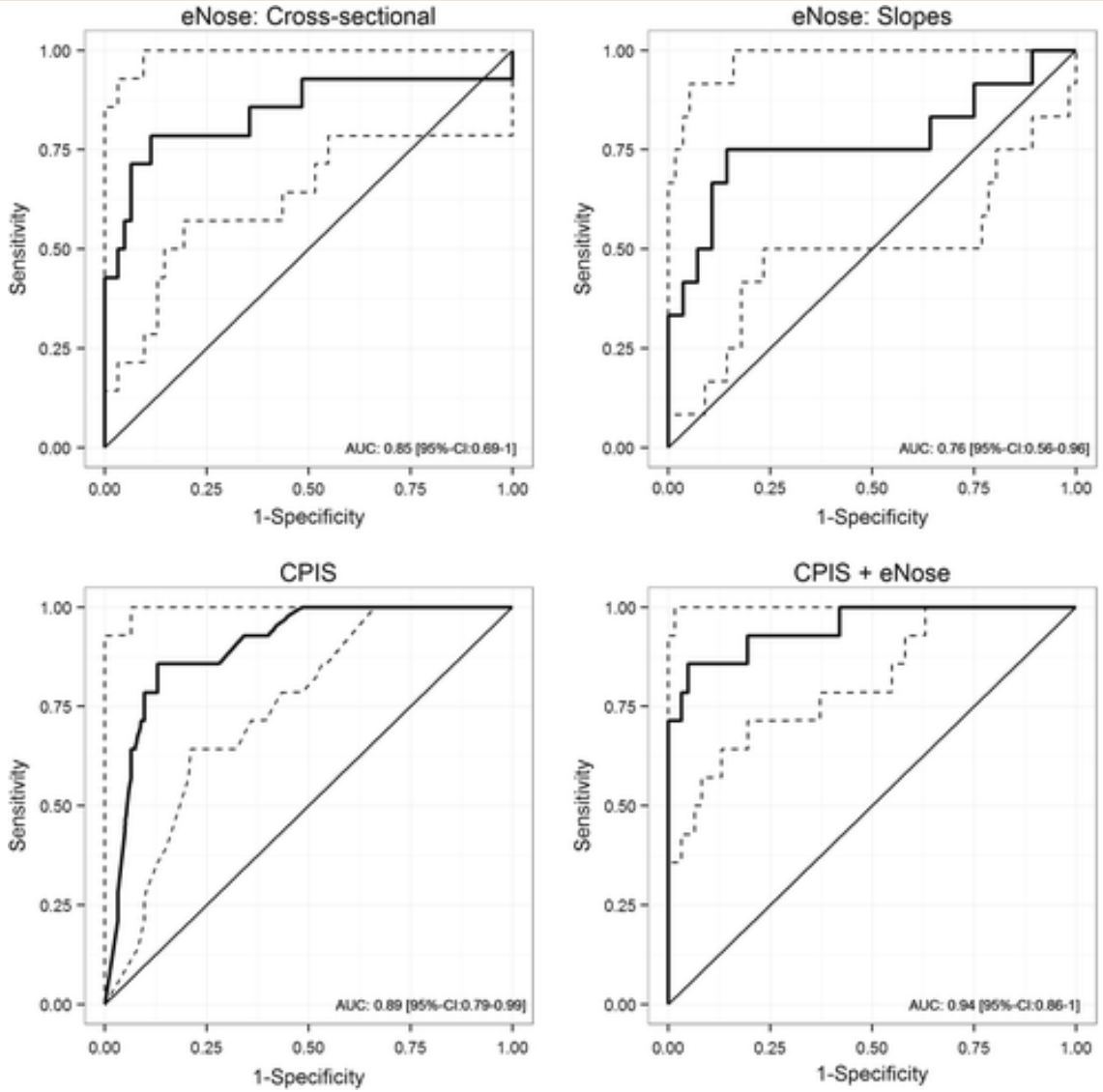
Electronic nose (eNose)

- VOC fingerprint



Chem. Rev. **2008,** 108, 705–725

eNose for detect VAP

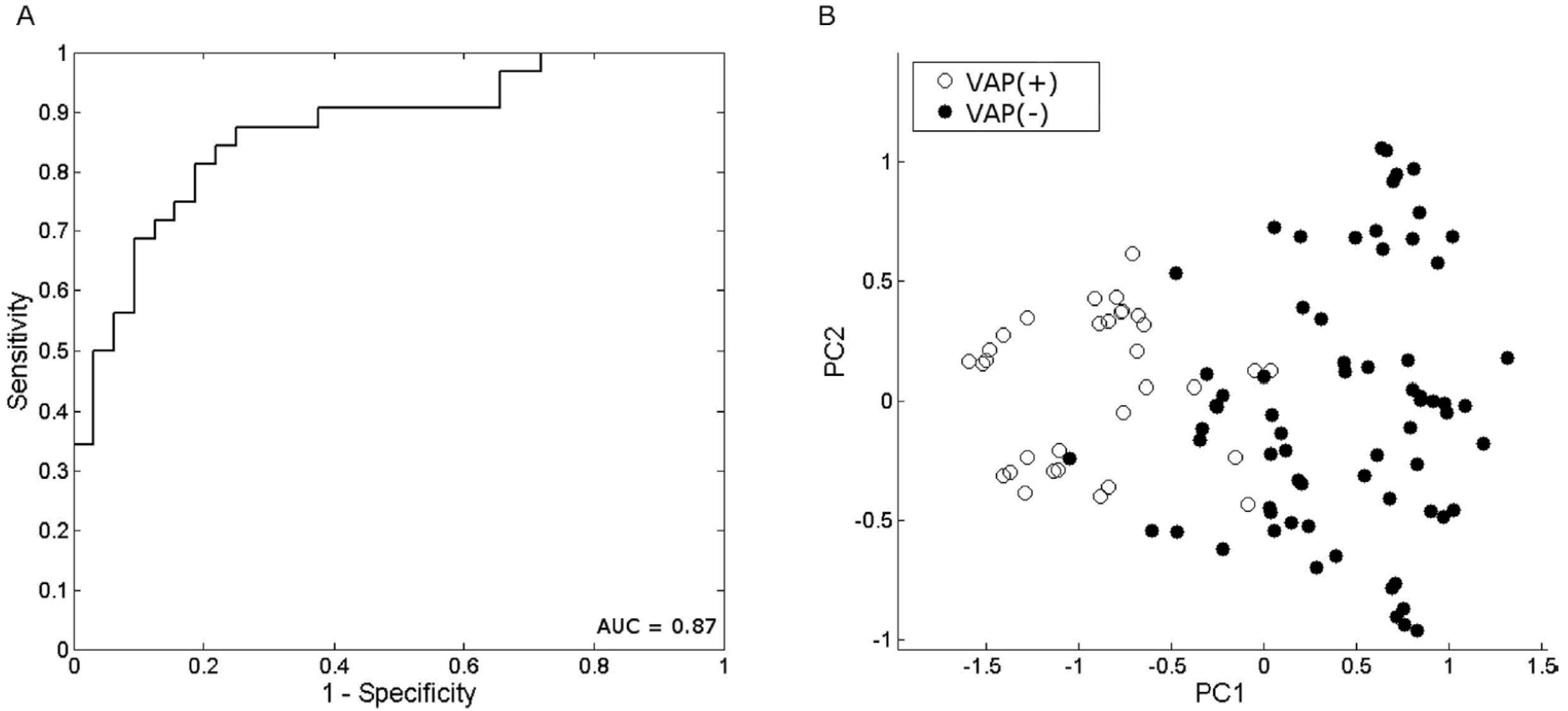


An area under the curve (AUC) of receiver operating characteristic (ROC) curve of 0.85, not that different from that of the clinical pulmonary infection score (CPIS) with a significant improvement when combining both diagnostic tools (AUC=0.94; 95% CI, 0.86–1.00).

Bos et al. Intensive Care Med 2014;40:761-2.

VOCs for detect VAP

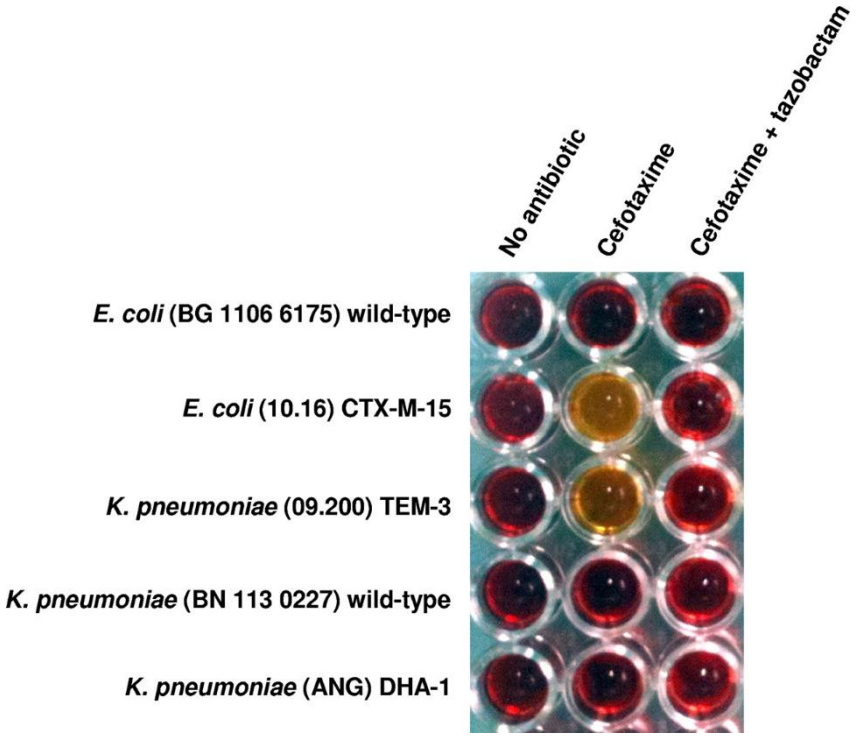
- A sensitivity of 75.8%±13.8% and a specificity of 73.0%±11.8% (ROC AUC, 0.87) in less than an hour



Schnabel *et al.* Sci Rep 2015;5:17179

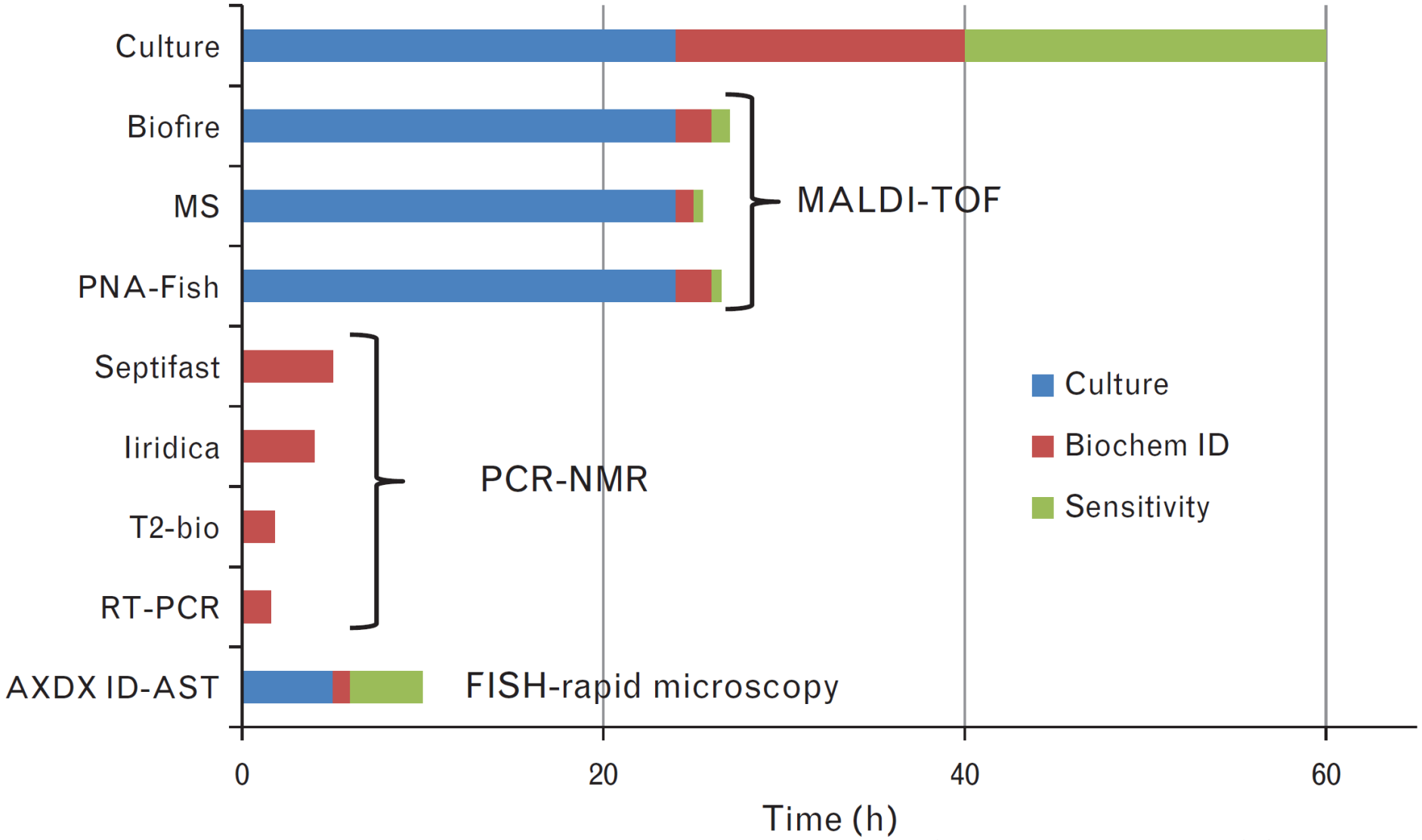
Rapid chromogenic tests

- Detect ESBL and/or carbapenemase production in 30 to 120 min, with a sensitivity ranging from 80% to 95% and a specificity between 71% and 100% (52).



Clin Microbiol 54:423–427

Assay sample-to-result times



Curr Opin Infect Dis. 2016 Apr;29(2):197-204

Pneumonia Panel in FDA Reviewing

35 targets

Bacteria
Semi-Quantitative Bacteria

Acinetobacter calcoaceticus-baumannii complex
Serratia marcescens
Proteus spp.
Klesiella pneumoniae group
Enterobacter aerogenes
Enterobacter cloacae
Escherichia coli
Haemophilus influenzae
Moraxella catarrhalis
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pneumoniae
Klebsiella oxytoca
Streptococcus pyogenes
Streptococcus agalactiae

Atypical Bacteria
Qualitative Bacteria

Legionella pneumoniae
Mycoplasma pneumoniae
Chlamydia pneumoniae

Viruses

Influenza A
Influenza B
Respiratory Syncytial Virus
Human Rhinovirus/ Enterovirus
Human Metapneumovirus
Parainfluenza virus
Adenovirus
Coronavirus
Middle East Respiratory Coronavirus

Antimicrobial Resistance Genes

mecA/C and MREJ
KPC
NDM
Oxa48-like
CTX-M
VIM
IMP

Fungi

Cryptococcus spp.

Sample : Sputum / BAL

Large Multiplex Panels

Pros	Cons
• Convenience • Rapid turnaround time to results • Guide treatment • Impact isolation practices • Patient satisfaction • Identify outbreaks • Epidemiologic studies	• Cost • Not tailored to the individual patient • Nucleic acid detection ≠ viable organism • Detects asymptomatic carriers, prolonged shedding, or latent/reactivated viruses • May still need culture, additional PCRs, antigens, and/or stool O&P • Potential for contamination and false positive results

Clinical Infectious Diseases 2016;63(10):1361–7

Summary

- Syndromic panels offer a rapid (some <60 min) and broad (some ~20 targets) approach to testing for causes of respiratory infection
- Promising techniques are becoming available and could be candidates for drug-resistance microorganism detection tools
- Since the majority of studies shows the potential advantages of molecular techniques, such as improved sensitivity and improved speed in establishing a microbiological diagnosis, more studies are needed to evaluate their performance in daily practice.

Thank for your attention!