

"Infezioni delle basse vie respiratorie (LRTI): aspetti clinici e diagnosi microbiologica "

I percorsi diagnostici microbiologici nel paziente immunocompetente

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Il tipo di patogeni coinvolti nelle LRTI è ampio e con varie sensibilità agli antibiotici e costituisce una grande sfida per la diagnostica microbiologica



K. Loens. JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009,

* There have been a large number of publications looking at the possibility of predicting the aetiological agent from the clinical features at presentation; however, while certain symptoms and signs are more common with specific pathogens, none allow accurate differentiation.

There are no characteristic features on the chest radiograph in CAP that allow confident prediction of the causative organism

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009 1. Identification of pathogens and antibiotic sensitivity patterns permits selection of optimal antibiotic regimens.

- 2. Targeted and narrow-spectrum antibiotic therapy limits drug costs, the threat of antibiotic resistance and adverse drug reactions such as C difficile-associated diarrhoea.
- 3. Specific pathogens have public health or infection control significance, including legionella, psittacosis, C burnetii, influenza A and multiresistant organisms.

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009 Microbiological investigations allow monitoring of the spectrum of pathogens causing CAP over time.

Without the accumulated information available from these culture results, trends in antibiotic resistance are more difficult to track, and empirical antibiotic recommendations are less likely to be accurate.

IDSA/ATS Guidelines for CAP in Adults • CID 2007:44 (Suppl 2) • S27

Local reccomandations for empiric antibiotic therapy

The Importance of Initial Empiric Antibiotic Selection

■ Adequate init. antibiotic ■ Inadequate init. antibiotic



(Luna CM, Vujacich P, Niederman MS et al. Chest 1997;111:676-685)

(Kollef MH and Ward S. Chest 1998;113:412-20)

(Clec'h C, Timsit J-F, De Lassence A et al. Intensive Care Med 2004;30:1327-1333)

Almost all of the major decisions regarding management of CAP, including diagnostic and treatment issues, revolve around the initial assessment of severity.

IDSA/ATS Guidelines for CAP in Adults • CID 2007:44 (Suppl 2) • S27





Score per determinare gravità delle CAP

> PSI

- Molto popolare
- Predittivo della mortalità
- Necessari più di 20 parametri
- Difficile da applicare fuori dall'ospedale
- CRB 65 (confusione, atti Respiratori > 30/min, ipotensione, età > 65)
 - Più utile e comodo in comunità



British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009

CAP severity assessment should be based in three key points:
✓ a pneumonia-specific score,
✓ biomarkers,
✓ and clinical judgment.

J.M.Pereira. Semin Respir Crit Care Med. 2012 Jun;33(3)



The American Journal of Emergency Medicine

www.elsevier.com/locate/ajem

Brief Report

The value of procalcitonin level in community-acquired pneumonia in the $\text{ED}^{\div,\,\div\,\div}$

Jeong Ho Park MD, Jung Hee Wee MD, Seung Pill Choi MD, PhD*, Sang Hoon Oh MD



appropriatezza

It has always been recognized that the use of a diagnostic test is an intervention. A diagnostic test should be requested only when a question is being posed and when there is evidence that the result will provide an answer to the question.

C. P. Price. Clin Chem 46, No. 8, 2000

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



mic | S 2 | Issue no: 1 | Issue date: 20.07.11 | Page: 8 of 12

rds for Microbiology Investigations | Issued by the Standards Unit, Health Protection Agency

General investigations are not necessary for the majority of patients with CAP who are managed in the community.

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009

For patients with low severity CAP the extent of microbiological investigations should be guided by clinical factors (age, comorbid illness, severity indicators), epidemiological factors and prior antibiotic therapy. [A]

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009





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.

Syndromic | S 2 | Issue no: 1 | Issue date: 20.07.11 | Page: 7 of 12

UK Standards for Microbiology Investigations | Issued by the Standards Unit, Health Protection Agency

Gram Microscopy

- Gram stains on sputum specimens may be used for determining the quality of the specimen
- It may not be appropriate to identify organisms if gross contamination with oropharyngeal flora is evident.
 The main limitation is the difficulty good-quality, purulent s pneumonia patients or pat

K. Loens. JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009 Bacteriology | B 57 | Issue no: 2.4 | Issue date: 02.08.12 | Page: 13 of 29 UK Standards for Microbiology Investigations | Issued by the Standards Unit, Health Protection Agency



If the patient is immuno-competent, report poor quality or salivary specimens

Gram Microscopy

- Gram stain can also be used to predict the likely pathogens by their characteristic appearance
- Care must be taken in interpreting a Gram-stained sputum smear as the use of antimicrobials may render organisms, which are visible in the smear, non-viable.
- All aspects of specimen appearance, Gram stain and culture together with the clinical condition of the patient need to be considered.

Bacteriology | B 57 | Issue no: 2.4 | Issue date: 02.08.12 | Page: 13 of 29 UK Standards for Microbiology Investigations | Issued by the Standards Unit, Health Protection Agency





Culture remains a cornerstone of the diagnostic techniques, as it can provide information about antibiotic susceptibility.

K. Stralin. International Journal of Antimicrobial Agents 31 (2008)

Microbiological Testing and Outcome of Patients With Severe Community-Acquired Pneumonia*

Jordi Rello, MD, PhD; Maria Bodi, MD; Dolors Mariscal, MD; Marta Navarro, MD; Emili Diaz, MD; Miguel Gallego, MD; and Jordi Valles, MD, PhD

The result of microbiologic investigations in the clinical setting of critically ill patients led to a change in therapy in approximately 40% of cases (and in 75% of patients in whom the etiology was identified).

CHEST / 123 / 1 / JANUARY, 2003

A sterile culture from the lower respiratory tract of an intubated patient, in the absence of a recent change in antibiotic therapy, is strong evidence that pneumonia is not present, and an extrapulmonary site of infection should be considered.

ATS/IDSA. Am J Respir Care Med. 2005.

Samples of lower respiratory tract secretions should be obtained from all patients with suspected HAP, and should be collected before antibiotic changes. Samples can include an endotracheal aspirate, bronchoalveolar lavage sample, or protected specimen brush sample (Level II).

Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia. Am J Respir Crit Care Med Vol 171. 2005



The specificity of bronchoscopy for the diagnosis of LRTI is not high because of contamination with the upper airway flora

Diagnostic accuracy is improved by the use of a protected specimen brush (PSB) and BAL.



K. Loens. JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009,

The diagnostic threshold to discriminate infection from colonization varies with the technique used.

Sample	tio-tuO	Sensitivity	Specificity
Brush (PSB)	≥ 10 ³	33 – 100%	50 –100%
BAL	≥ 10 ⁴	42 –93%	45 –100%
Endotracheal aspirate more representative samples than the PSB, which samples only a single bronchial segment	≥ 10 ⁶	38-82%	72-85%

ATS/IDSA. Am J Respir Care Med. 2005

K. Loens. JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009

Feature or Organism	Patients Who Received Invasive Management (n = 204)	Patients Who Received Clinical Management (n = 209)
Negative culture, n (%) Monomicrobial pneumonia, n (%) Polymicrobial pneumonia, n (%) Total number of pathogens, n Bacilli, n (%) Pseudomonas aeruginosa Haemophilus influenzae Escherichia coli Acinetobacter baumannii Enterobacter species Proteus species Serratia marcescens Klebsiella species Citrobacter species Morganella morganii Moraxella species Stenotrophomonas maltophilia Corynebacterium Alcaligenes xylosoxidans	14 (55.9) 65 (31.9) 25 (12.3) 121 27 (22.3) 9 (7.4) 6 (5.0) 6 (5.0) 4 (3.3) 3 (2.5) 3 (2.5) 3 (2.5) 2 (1.7) 1 (0.8) 1 (0.8) 1 (0.8) 0 0 0	30 (14.4) 84 (40.2) 95 (45.5) 312 57 (18.3) 12 (3.8) 23 (7.4) 11 (3.5) 12 (3.8) 14 (4.5) 7 (2.2) 11 (3.5) 7 (2.2) 3 (1.0) 1 (0.3) 4 (1.3) 4 (1.3) 1 (0.3)
Staphylococcus aureus Streptococcus species Neisseria species Streptococcus pneumoniae Coagulase-negative staphylococci Enterococcus species Fungi, n (%)	20 (16.5) 19 (15.7) 7 (5.8) 3 (2.5) 3 (2.5) 1 (0.8) 5 (4.1)	40 (12.8) 28 (9.0) 6 (1.9) 10 (3.2) 17 (5.4) 6 (1.9) 38 (12.2)

Clinical management: qualitative cultures of endotracheal aspirates

Invasive management: quantitative cultures of protected specimen bronchoalveolar lavage samples

a strategy based on quantitative bronchoscopic specimen

cultures has beneficial effects: improved early survival, fewer early organ failures, and less antibiotic use.

Fagon JY et al for the VAP I rial Group. Ann Intern Ivieu. 2000

Blood cultures

- Two blood cultures should be obtained as early as possible in the disease and before any antibiotic treatment is started.
- A direct correlation was found between the severity of pneumonia and blood culture positivity rate
- S. pneumoniae is identified in approximately 60% of positive blood cultures and Haemophilus influenzae in various percentages from 2 to 13%.

- Waterer, G. W. et al., Respir. Med. 2001; 95:78-82
- K. Loens. JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009.

Multiplex PCR

The SeptiFast test is more sensitive in the detection of relevant blood pathogens in VAP than the blood culture.

VALUE OF LIGHTCYCLER SEPTIFAST® IN DETECTION OF VENTILATOR-ASSOCIATED PNEUMONIA A. Kalenka, J. Timm, S. Schmid, G. Beck.- ESICM -Vienna 2009



S. pneumoniae

Rapid immunochromatographic test detecting the group C polysaccharide cell wall antigen common to all pneumococcal strains in urine and other biological fluids

It can give positive results in healthy children with carriage of pneumococci and of other closely related Streptococcus species

Thus, it is necessary to use this test in conjunction with other diagnostic modalities.



Sensitivity: 77%–88% Specificity: 67%–100%

A. J. Blaschke. CID 2011:52 (Suppl 4)

Persistence of S. pneumoniae urinary antigen excretion after pneumococcal pneumonia



Fig. 1 Distribution of positive (*black*) and negative (*white*) results of *S. pneumoniae* urinary antigen detection in NCU samples collected during the study period

F. Andreo. Eur J Clin Microbiol Infect Dis (2009) 28:197-201



Investigations for legionella pneumonia are recommended for all patients with high severity CAP, for other patients with specific risk factors and for all patients with CAP during outbreaks.

This assay principally detects infection with L pneumophila serogroup 1.

Antigenuria can be detected as early as 1 day after onset of symptoms and persists for days to weeks.



Table 1. Diagnostic tests for Legionella infection.

Legionella PCR combined with urinary antigen testing is likely to be the best initial testing strategy that will detect all Legionella species and provide results within a time frame that will affect clinical management.

D. R. Murdoch. MEDICAL MICROBIOLOGY • CID 2003:36

Atypical pathogens

- Mycoplasma pneumoniae: younger patients, prior antibiotics, less multisystem involvement.
- Chlamydophila pneumoniae: longer duration of symptoms
 before hospital admission, headache
- M. pneumoniae, and to a lesser extent C. pneumoniae, may precipitate an attack of asthma or exacerbate existing asthma.
- Some patients who have recently had *M. pneumoniae* CAP develop post-CAP asthma which may be permanent.

Atypical pathogens

- The outpatient setting is the area where atypical pathogens are quantitatively more important than their typical CAP counterparts.
- The atypical pneumonias require a different therapeutic approach than that for typical CAPs.
- The treatment of *M. pneumoniae* and *C. pneumoniae* CAP is important, not because of the severity of the illness, but if for no other reason, to decrease communicability and to decrease post-CAP asthma

B. A. Cunha. Clin Microbiol Infect 2006; 12 (Suppl. 3): 12–24

Atypical pathogens

Given the high sensitivity and specificity of nucleic acid amplification techniques (NAATs), **NAATs are the preferred diagnostic procedures** for the diagnosis of *M. pneumoniae* and *C. pneumoniae*

The best specimen for *M. pneumoniae* and *C. pneumoniae* detection are nasopharyngeal aspirate or nasopharyngeal swab since inhibitors in sputum occur frequently and may be difficult to eliminate

B. A. Cunha. Clin Microbiol Infect 2006; 12 (Suppl. 3).

Panel: Viruses linked to community-acquired pneumonia in children and adults

- Respiratory syncytial virus
- Rhinovirus
- Influenza A, B, and C viruses
- Human metapneumovirus
- Parainfluenza viruses types 1, 2, 3, and 4
- Human bocavirus*
- Coronavirus types 229E, OC43, NL63, HKU1, SARS
- Adenovirus
- Enteroviruses
- Varicella-zoster virus
- Hantavirus
- Parechoviruses
- Epstein-Barr virus
- Human herpesvirus 6 and 7
- Herpes simplex virus
- Mimivirus
- Cytomegalovirus†
- Measles†

*Mostly in children. †Mostly in developing countries.

	Suggests viral cause	Suggests bacterial cause
Age	Younger than 5 years	Adults
Epidemic situation	Ongoing viral epidemic	
History of illness	Slow onset	Rapid onset
Clinical profile	Rhinitis, wheezing	High fever, tachypnoea
Biomarkers		
Total white-blood cell count	<10×10° cells per L	>15×10° cells per L
C-reactive protein concentration in serum	<20 mg/L	>60 mg/L
Procalcitonin concentration in serum	<0-1 µg/L	>0-5 µg/L
Chest radiograph findings	Sole interstitial infiltrates, bilaterally	Lobar alveolar infiltrates
Response to antibiotic treatment	Slow or non-responsive	Rapid

Table 1: Variables used to distinguish viral from bacterial pneumonia

No clinical algorithm exists to discern clearly the cause of

pneumonia.

Respiratory viruses usually follow seasonal patterns of activity and are most likely to cause pneumonia during those times.



Figure 1: Age-specific incidence of community-acquired pneumonia Error bars= 95% CIs. Modified from reference 8 with permission of 0xford. University Press.

Perché diagnosi di infezione virale?

The knowledge of which virus or viruses is/are present and who has had close contact may guide for prophylaxis or use of antiviral agents.

- If a child with chronic cardiac disease has an increased risk of catching RSV, then palivizumab can be administered to prevent more severe complications from RSV infection.
- The treatment of the influenza virus infection increase survival in persons who are at high risk for complications and mortality during influenza.

It is clear that rapid and accurate diagnosis is central to such therapy decisions.

K. TempletonJ ournal of Clinical Virology 40 Suppl. 1 (2007) S2-S4

Optimal samples in viral pneumonia

- In children, for detection by PCR of respiratory viruses nasopharyngeal aspirates are generally deemed the specimen of choice because both nasal and nasopharyngeal mucus samples are gathered.
- In adults, transnasal nasopharyngeal flocked swabs also have high virus detection rates.
- UTM-RT medium are well ecimens have obvious suited for the detection of respiratory viruses by PCR. blishing the cause of mey come from the site of

O. Ruuskanen et al. www.thelancet.com Vol 377 April 9, 2011

Pathogen	Sample ranking	Method	Age (yr)	Total no. of specimens/ no. of patients	Reference	
M. pneumoniae	Sputum > TW > NPS > OPS	PCR	20-93	552/144	31	
	OPS > NPS	PCR	NSp	132/66	43	
	OPS > BAL > sputum	PCR	NSp	325/197	49	
	Sputum > OPS	Gene-probe test	>18	160	57	
	Sputum > NPA	Ag-EIA	>18	102/51	56	
	Sputum > OPS	Culture, PCR, NASBA	NSp	302/180	61, 62	
	NPS – OPS	PCR	NSp	63	69	
	Sputum > NPA = OPS	PCR	22-29	96/32	79	
	OPS > NPA	PCR	NSp	102	82	
C. pneumoniae	NPS > TS	Culture, PCR	3-12	260	10	
	Sputum > NPS - OPS	PCR, culture	3-79	319/129	12	
	OPS > NPS	PCR	NSp	132/66	43	
	Sputum > NPA > OPS	PCR	NSp	105/35	58	
	NPS > OPS > sputum	PCR	20-93	468/156	103	
RSV	PFS - NPA	DFA	0-5	910/455	1	
	NPA > NPFS	PCR	0 - 18	338/169	19	
	NPA > NPFS	DIF	0 - 18	338/169	19	
	NPA – NPS	Culture, IFA	0-16	250/125	38	
	NPA > NPS	DIF	0-2	366/183	64	
	NPA > NPS	Culture, Ag-EIA, FAT	0-18	242/121	67	
Adenovirus	PFS = NPA	DFA	0-5	910/455	1	
Parainfluenza virus 1, 2, or 3	PFS = NPA	DFA	0-5	910/455	1	
Picornaviruses	Sputum > NS > OPS	Culture	5-15	66/22	50	
influenza virus	PFS - NPA	DFA	0-5	910/455	1	
	NPS > NS > NPA	Quidel quickvue	0 - 18	366/122	2	
	NPFS - NPA	PCR	0-18	338/169	19	
	NPA > NPFS	DIF	0 - 18	338/169	19	
	Sputum > NA > NPS > OPS	FLU OIA test	0-76	403/184	24	
	NA > sputum > NPS > OPS	Culture	0-76	403/184	24	
	NPS > NPA > OPS	PCR, Directigen Flu A+B	61-97	85/47	44	
	NPS > OPS	Binax Now, Directigen Flu A+B, DIF	NSp	521/448	93	
All viruses	NPA>NS - OPS	PCR	0-16	221/178	46	
	NFS = NA >unpreserved saline	PCR	0-1.5	543/181	105	
	NPA > NS	PCR culture DIF	<5	950/475	07	

^a BAL, bronchoalveolar lavage; DIF, direct immunofluorescence; DFA, direct fluorescent antibody assay; Ag-EIA, antigen enzyme immunoassay; IFA, indirect fluorescent antibody test; FAT, fluorescent antibody test; NA, nasal aspirate; NASBA, nucleic acid sequence-based amplification; NPA, nasopharyngeal aspirate; NPFS, nasopharyngeal flocked swab; NFS, nasal flocked swab; NPS, nasopharyngeal swab; NW, nasal wash; NSp, not specified; NS, nasal swab; OPS, oropharyngeal swab; PFS, pernasal flocked swab; TW, throat wash. (Adapted from reference 111 with permission of the publisher.)

Lane	L2		L3		L4		L5		L6		L7			Lane	L2		L3		L4		LS		L6		L7	
Sample ID	1		2		3		4		5		N			Sample ID	1		2		3		4		5		N	
Internal Control	+	100	+	100	+	100	+	101	+	100	+	100		Internal Control	+	101	+	104	+	103	+	99	+	100	+	138
AdV			25		+	62	+	102						OC43			+	104							-	
229E/NL63	<u> </u>		+	101	-		-		+	101	<u></u>			HRV A/B/C	+	101			-		-		-		-	
PIV2	+	100	· - ·)		-		-		-		-			RSV A	- 1		-		-		+	100	- 1		- 1	
PIV3			+	62	1.00		-							Flu A	1.0		1.7		+	103	-				1.7	
PIV1	+	64	-		-		-		- 1		-			RSV B	-		-		-		-		+	99	-	
Unidentified			<u></u>		-		-				-			Unidentified					-		-				-	
Top Marker Internal Control AdV 229E/NL63 PIV2 PIV3 PIV1					_		_				-	<		Top Marker Internal Control OC43 HRV A/B/C RSV A Flu A RSV B					-	< <	-		_			< <
Bottom Marker ↓ Parain Parain Parain Adeno Corona	flue flue flue viru avir	enz enz enz is A us	a vi a vi a vi \/B/ 229	irus irus irus /C/I 9E/	5 1 5 2 5 3 D/E NL(63		K		Cor Rhi Influ RS ¹ RS ¹	rona nov uen V A V B	avir /iru iza	rus OC43 s A/B/C A virus	Eattorn Marker		110	L3 2 + - + -	138		< 168 25 10		L 172 83	6 5 + - -	L2 N 105	7	00
Multiplex PCR Single gene target PCR					Management of the					Unidentified Top Marker Internal Control HBoV 1/2/3/4 Flu B	-	-< 4	- 1	< <	-	~ ~ ~	-	<	-	-< -	-	<				
								8 	Boc nflu Met Par Ent	avi uen api ain ero	rus za neu flue viru	B v B v Imc enza	2/3/4 ⁄irus ovirus a virus 4	MPV PIV4 HEV Bottom Marker		4	_	<		<		4		<		<





Detection of several viruses

- For childhood pneumonia, two or three viruses have been detected in 10–20% of children.
- Specifically, human bocavirus is detected frequently in association with other respiratory viruses
- Detection of a virus in the nasopharynx could represent coincidental upper-respiratory infection or a pneumonia pathogen?
- severe pneumonia than with No colonisation for virus admission were looked at In one study, viral co-infe

rales of

ne

Bacterial co-infection in influenza A H1N1 Pneumonia

Table 2 Bacterial of	o-infection in stu	dy populations	а •					
Pathogen	Number of patients ^b $(n = 42)$	Blood culture (n = 38)	Sputum culture (n = 16)	Urinary antigen (n = 39)	BAL/BAS ($n = 9$)	Pleural effusion culture (n = 3)	Serology (n = 30	
S. pneumoniae	26 (62)	3 (7.8)	7 (43.7)	24 (61.5)	3 (33.3)	-	-	
S. pyogenes	1 (2)	<u></u>	1 (6.3)	-	-	1 (33.3)	-	
S. aureus	2 (5)	-	2 (12.5)			-	-	
M. pneumoniae	3 (7)	-	-	-	-	-	3 (10)	
M. catarrhalis	1 (2)	÷2	1 (6.3)	-	-		-	
C. burnetti	1 (2)	-	-	-	-	÷+	1(3.3)	
E. coli	1 (2)	<u> </u>	<u></u>	-	1 (11.1)	<u></u>	_	
P. aeruginosa	6 (14)	-	4 (25.0)	-	5 (55.5)	-	-	
Fusobacterium sp.	1 (2)	1(2.6)	-	-	-		-	

^a Data are presented as number (percentage).

^b Total number of patients for each etiologic agent.

C. Cilloniz et al. Journal of Infection (2012) 65, 223e230





G.W. Waterer. Am J Respir Crit Care Med. 2011

