2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Il ruolo del laboratorio di Microbiologia

Giordano Dicuonzo

Università Campus Bio-Medico

ROMA

Sono importanti il laboratorio di microbiologia e la microbiologia clinica?

Ho pensato di dare una risposta quantitativa e di cui sono sicuro

I dimessi che hanno sofferto infezione i morti infetti nel mio ospedale

Pazienti con infezione/Dimessi per anno

	2009 Conta Infetti	2009 Tot. Dimessi	2009%	2010 Conta Infetti	2010 Tot. Dimessi	2010%	2011 Conta Infetti	2011 Tot. Dimessi	2011%
Policlinico	1806	13329	**14%**	3322	14245	**23%**	3695	15750	**23%**
Anestesia e Rianimazione	4	21	19%	13	18	72%	10	10	100%
Cardiologia	39	1422	2.70%	46	1399	3.30%	48	1695	2.80%
Chirurgia cardiovascolare	48	298	16%	48	430	11%	59	574	10%
Chirurgia generale	92	811	11%	160	828	19%	179	1158	15%
Chirurgia ricostruttiva	51	597	8.50%	84	461	18%	87	537	16%
Ematologia	72	322	**22%**	141	292	**48%**	160	232	**69%**
Endoscopia digestiva	24	419	**5.70%**	38	462	**8.20%**	60	549	**11%**
Fisiatria	46	198	**23%**	99	297	**33%**	82	258	**32%**
Gastroenterologia	54	566	**9.50%**	56	383	**15%**	61	319	**19%**
Geriatria	315	753	**42%**	504	954	**53%**	501	975	**51%**
Ginecologia	37	787	4.70%	66	869	7.60%	56	1050	5.30%
Medicina clinica	235	792	**30%**	557	978	**57%**	726	1180	**62%**
Medicina interna	230	644	**36%**	527	919	**57%**	560	1034	**54%**
Neurologia	111	605	18%	154	619	25%	244	657	37%
Oncologia	209	3054	**6.80%**	354	3445	**10%**	300	3119	**9.60%**
Ortopedia	24	675	**3.60%**	54	448	**12%**	86	808	**11%**
Otorinolaringoiatria	4	478	0.84%	6	454	1.30%	7	500	1.40%
Senologia	2	243	0.82%	4	211	1.90%	3	218	1.40%
Urologia	205	628	**33%**	405	751	**54%**	437	834	**52%**

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Deceduti infetti /Dimessi per anno

	2009 Conta Deceduti	2009 Tot. Dimessi	2009%	2010 Conta Deceduti	2010 Tot. Dimessi	2010%	2011 Conta Deceduti	2011 Tot. Dimessi	2011%
Policlinico	111	12518	0.89%	155	13460	1.20%	171	14912	1.10%
Anestesia e Rianimazione	3	21	14%	6	18	33%	3	10	30%
Cardiologia	4	1422	0.28%	0	1399	0%	3	1695	0.18%
Chirurgia cardiovascolare	8	298	2.70%	2	430	0.47%	4	574	0.70%
Chirurgia generale	11	811	1.40%	11	828	1.30%	7	1158	0.60%
Ematologia	10	322	3.10%	11	292	3.80%	10	232	4.30%
Endoscopia digestiva	1	419	0.24%	2	462	0.43%	7	549	1.30%
Gastroenterologia	3	566	0.53%	0	383	0%	2	319	0.63%
Geriatria	21	753	2.80%	31	954	3.20%	38	975	3.90%
Ginecologia	0	787	0%	2	869	0.23%	2	1050	0.19%
Medicina clinica	4	792	0.51%	10	978	1%	19	1180	1.60%
Medicina interna	15	644	2.30%	30	919	3.30%	39	1034	3.80%
Neurologia	0	605	0%	3	619	0.48%	4	657	0.61%
Oncologia	29	3054	0.95%	42	3445	1.20%	29	3119	0.93%
Ortopedia	1	675	0.15%	1	448	0.22%	0	808	0%
Otorinolaringoiatria	1	478	0.21%	0	454	0%	0	500	0%
Radioterapia	0	0		0	0		1	0	
Senologia	0	243	0%	1	211	0.47%	1	218	0.46%
Urologia	0	628	0%	3	751	0.40%	2	834	0.24%

Deceduti/Dimessi per anno con evidenza di resistenze

	2009 Conta Deceduti	2009 Tot. Dimessi	2009%	2010 Conta Deceduti	2010 Tot. Dimessi	2010%	2011 Conta Deceduti	2011 Tot. Dimessi	2011%
Policlinico	54	11843	0.46%	63	13012	0.48%	55	14104	0.39%
Anestesia e Rianimazione	3	21	**14%**	3	18	**17%**	2	10	**20%**
Cardiologia	3	1422	0.21%	0	1399	0%	0	1695	0%
Chirurgia cardiovascolare	6	298	2%	0	430	0%	3	574	0.52%
Chirurgia generale	9	811	1.10%	8	828	0.97%	3	1158	0.26%
Ematologia	4	322	1.20%	6	292	2.10%	4	232	1.70%
Endoscopia digestiva	1	419	0.24%	1	462	0.22%	4	549	0.73%
Gastroenterologia	1	566	0.18%	0	383	0%	1	319	0.31%
Geriatria	8	753	1.10%	12	954	1.30%	14	975	1.40%
Ginecologia	0	787	0%	2	869	0.23%	0	1050	0%
Medicina clinica	0	792	0%	3	978	0.31%	3	1180	0.25%
Medicina interna	5	644	0.78%	11	919	1.20%	10	1034	0.97%
Neurologia	0	605	0%	3	619	0.48%	1	657	0.15%
Oncologia	13	3054	0.43%	10	3445	0.29%	8	3119	0.26%
Otorinolaringoiatria	1	478	0.21%	0	454	0%	0	500	0%
Senologia	0	243	0%	1	211	0.47%	1	218	0.46%
Urologia	0	628	0%	3	751	0.40%	1	834	0.12%

Deceduti per tipo infezione

	% 2009	% 2010	% 2011
Totale Deceduti infetti	**0.89%**	**1.2%**	**1.1%**
BATTERIEMIA-SEPSI	0.49%	0.89%	0.78%
URINARIA	0.44%	0.71%	0.74%
RESPIRATORIO	0.29%	0.33%	0.36%
SITO CHIRURGICO	0.34%	0.38%	0.42%

Deceduti/Dimessi per anno con batteriemia-Sepsi

	2009 Conta Deceduti	2009 Tot. Dimessi	2009%	2010 Conta Deceduti	2010 Tot. Dimessi	2010%	2011 Conta Deceduti	2011 Tot. Dimessi	2011%
Totale	54	11122	0.49%	110	12347	0.89%	104	13386	0.78%
Anestesia e Rianimazione	1	21	**4.80%**	6	18	**33%**	2	10	**20%**
Cardiologia	2	1422	0.14%	0	1399	0%	2	1695	0.12%
Chirurgia cardiovascolare	3	298	1%	1	430	0.23%	4	574	0.70%
Chirurgia generale	6	811	0.74%	10	828	1.20%	5	1158	0.43%
Ematologia	6	322	**1.90%**	11	292	**3.80%**	8	232	**3.40%**
Endoscopia digestiva	0	419	0%	1	462	0.22%	6	549	1.10%
Gastroenterologia	3	566	0.53%	0	383	0%	1	319	0.31%
Geriatria	8	753	**1.10%**	19	954	**2%**	17	975	**1.70%**
Ginecologia	0	787	0%	1	869	0.12%	2	1050	0.19%
Medicina clinica	0	792	0%	7	978	0.72%	7	1180	0.59%
Medicina interna	9	644	**1.40%**	24	919	**2.60%**	26	1034	**2.50**%
Neurologia	0	605	0%	2	619	0.32%	3	657	0.46%
Oncologia	16	3054	0.52%	26	3445	0.75%	19	3119	0.61%
Urologia	0	628	0%	2	751	0.27%	2	834	0.24%

Deceduti/Dimessi per anno con infezione delle vie urinarie

	2009 Conta Deceduti	2009 Tot. Dimessi	2009%	2010 Conta Deceduti	2010 Tot. Dimessi	2010%	2011 Conta Deceduti	2011 Tot. Dimessi	2011%
Policlinico	55	12518	0.44%	96	13460	0.71%	110	14912	0.74%
Anestesia e Rianimazione	3	21	**14%**	3	18	**17%**	3	10	**30%**
Cardiologia	3	1422	0.21%	0	1399	0%	3	1695	0.18%
Chirurgia cardiovascolare	3	298	1%	2	430	0.47%	4	574	0.70%
Chirurgia generale	4	811	0.49%	5	828	0.60%	5	1158	0.43%
Ematologia	6	322	**1.90%**	10	292	**3.40%**	8	232	**3.40%**
Endoscopia digestiva	0	419	0%	2	462	0.43%	6	549	1.10%
Gastroenterologia	0	566	0%	0	383	0%	1	319	0.31%
Geriatria	15	753	**2%**	20	954	**2.10%**	30	975	**3.10%**
Ginecologia	0	787	0%	2	869	0.23%	1	1050	0.10%
Medicina clinica	2	792	0.25%	8	978	0.82%	12	1180	1%
Medicina interna	8	644	**1.20%**	18	919	**2%**	22	1034	**2.10%**
Neurologia	0	605	0%	3	619	0.48%	4	657	0.61%
Oncologia	10	3054	0.33%	19	3445	0.55%	8	3119	0.26%
Ortopedia	0	675	0%	1	448	0.22%	0	808	0%
Otorinolaringoiatria	1	478	0.21%	0	454	0%	0	500	0%
Radioterapia	0	0		0	0		1	0	
Senologia	0	243	0%	1	211	0.47%	1	218	0.46%
Urologia	0	628	0%	2	751	0.27%	1	834	0.12%

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Deceduti/Dimessi per anno con infezione delle vie respiratorie profonde

	2009 Conta Deceduti	2009 Tot. Dimessi	2009%	2010 Conta Deceduti	2010 Tot. Dimessi	2010%	2011 Conta Deceduti	2011 Tot. Dimessi	2011%
Policlinico	29	10132	0.29%	37	11188	0.33%	44	12113	0.36%
Anestesia e Rianimazione	2	21	9.50%	5	18	28%	2	10	20%
Cardiologia	2	1422	0.14%	0	1399	0%	2	1695	0.12%
Chirurgia cardiovascolare	5	298	1.70%	1	430	0.23%	2	574	0.35%
Chirurgia generale	3	811	0.37%	5	828	0.60%	4	1158	0.35%
Ematologia	3	322	0.93%	5	292	1.70%	3	232	1.30%
Endoscopia digestiva	0	419	0%	1	462	0.22%	3	549	0.55%
Gastroenterologia	2	566	0.35%	0	383	0%	0	319	0%
Geriatria	6	753	0.80%	8	954	0.84%	8	975	0.82%
Ginecologia	0	787	0%	0	869	0%	1	1050	0.10%
Medicina clinica	1	792	0.13%	3	978	0.31%	5	1180	0.42%
Medicina interna	4	644	0.62%	3	919	0.33%	7	1034	0.68%
Oncologia	1	3054	0.03%	6	3445	0.17%	6	3119	0.19%
Senologia	0	243	0%	0	211	0%	1	218	0.46%

Deceduti/Dimessi per anno con infezione del sito chirurgico

	2009 Conta Deceduti	2009 Tot. Dimessi	2009%	2010 Conta Deceduti	2010 Tot. Dimessi	2010%	2011 Conta Deceduti	2011 Tot. Dimessi	2011%
Policlinico	40	11797	0.34%	48	12795	0.38%	59	14194	0.42%
Anestesia e Rianimazione	3	21	14%	4	18	22%	2	10	20%
Cardiologia	1	1422	0.07%	0	1399	0%	0	1695	0%
Chirurgia cardiovascolare	5	298	1.70%	0	430	0%	3	574	0.52%
Chirurgia generale	8	811	0.99%	8	828	0.97%	6	1158	0.52%
Ematologia	2	322	0.62%	3	292	1%	3	232	1.30%
Endoscopia digestiva	1	419	0.24%	1	462	0.22%	3	549	0.55%
Gastroenterologia	2	566	0.35%	0	383	0%	0	319	0%
Geriatria	5	753	0.66%	7	954	0.73%	8	975	0.82%
Ginecologia	0	787	0%	1	869	0.12%	1	1050	0.10%
Medicina clinica	1	792	0.13%	6	978	0.61%	14	1180	1.20%
Medicina interna	2	644	0.31%	7	919	0.76%	11	1034	1.10%
Neurologia	0	605	0%	1	619	0.16%	2	657	0.30%
Oncologia	9	3054	0.29%	9	3445	0.26%	5	3119	0.16%
Ortopedia	1	675	0.15%	0	448	0%	0	808	0%
Urologia	0	628	0%	1	751	0.13%	1	834	0.12%

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

"I feel the need…the need for speed." This is a sentiment felt by many infectious diseases clinicians and clinical microbiologists, particularly when confronted with a patient who is seriously ill, getting progressively worse, and for whom an infectious etiology has yet to be identified. The need for speed also is a factor when (1) a physician awaits the results of standardized antimicrobial susceptibility testing for an infectious agent that has been isolated from a patient whose condition is deteriorating clinically (despite receipt of empirical therapy) and (2) an infection control practitioner awaits the results of tests to determine whether a patient is colonized or infected with methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant enterococci (VRE) and requires isolation.
It is in these types of situations that molecular diagnostic methods can provide the data needed more rapidly and, in many cases, more cost effectively than traditional culture methods.

Rapid Detection and Identification of Bacterial Pathogens Using Novel Molecular Technologies: Infection Control and Beyond

Fred C. Tenover   Clinical Infectious Diseases 2007; 44:418–23

Assuring the Quality of Clinical Microbiology Test Results

Michael L. Wilson **Clinical Infectious Diseases 2008;47:1077–82**

Table 1. Select issues covered under laboratory accreditation.

Management and personnel qualifications
Life safety
Water quality
Informatics
Record retention
Proficiency testing
Quality control
Competency testing
General safety

Aggiungiamo: epidemiologia e controllo infezioni ospedaliere e nel territorio
adeguate indicazioni per la terapia antibiotica (resistenze, sensibilità, farmacocinetica)

Dobbiamo dare la stessa qualità delle risposte ma con tempi compatibili con una autentica modifica di comportamento del clinico nei confronti del paziente, e cioè al massimo in alcune ore
Alcune ore è una frase non netta

Mi butto: diciamo 5 ore? (in realtà non è buttarsi, ma una risposta reale)

In cinque ore dobbiamo fornire la diagnosi microbiologica e l'antibiogramma

Ora ciò è possibile.

Cominciamo con la sindrome credo più importante

La sepsi correlata a batteriemia

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Journal of Clinical Microbiology, Oct. 2010, p. 3510–3516 Vol. 48, No. 1
0095-1137/10/$12.00 doi:10.1128/JCM.00147-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Multiplex Blood PCR in Combination with Blood Cultures for Improvement of Microbiological Documentation of Infection in Febrile Neutropenia▽

F. Lamoth,[1] K. Jaton,[2] G. Prod'hom,[2] L. Senn,[1] J. Bille,[2] T. Calandra,[1] and O. Marchetti[1*]

The frequent lack of microbiological documentation of infection by blood cultures (BC) has a major impact on clinical management of febrile neutropenic patients, especially in cases of unexplained persistent fever. We assessed the diagnostic utility of the LightCycler SeptiFast test (SF), a multiplex blood PCR, in febrile neutropenia. Blood for BC and SF was drawn at the onset of fever and every 3 days of persistent fever. SF results were compared with those of BC, clinical documentation of infection, and standard clinical, radiological, and microbiological criteria for invasive fungal infections (IFI). A total of 141 febrile neutropenic episodes in 86 hematological patients were studied: 44 (31%) microbiologically and 49 (35%) clinically documented infections and 48 (34%) unexplained fevers. At the onset of fever, BC detected 44 microorganisms in 35/141 (25%) episodes. Together, BC and SF identified 78 microorganisms in 61/141 (43%) episodes ($P = 0.002$ versus BC or SF alone): 12 were detected by BC and SF, 32 by BC only, and 34 by SF only. In 19/52 (37%) episodes of persistent fever, SF detected 28 new microorganisms (7 Gram-positive bacterial species, 15 Gram-negative bacterial species, and 6 fungal species [89% with a clinically documented site of infection]) whereas BC detected only 4 pathogens (8%) ($P = 0.001$). While BC did not detect fungi, SF identified 5 *Candida* spp. and 1 *Aspergillus* sp. in 5/7 probable or possible cases of IFI. Using SeptiFast PCR combined with blood cultures improves microbiological documentation in febrile neutropenia, especially when fever persists and invasive fungal infection is suspected. Technical adjustments may enhance the efficiency of this new molecular tool in this specific setting.

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2009, p. 2405–2410 Vol. 47, No. 8
0095-1137/09/$08.00+0 doi:10.1128/JCM.00491-09
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

Utility of a Commercially Available Multiplex Real-Time PCR Assay To Detect Bacterial and Fungal Pathogens in Febrile Neutropenia∇

Marie von Lilienfeld-Toal,[1,2]†* Lutz E. Lehmann,[3]† Ansgar D. Raadts,[3] Corinna Hahn-Ast,[1] Katjana S. Orlopp,[1] Günter Marklein,[4] Ingvill Purr,[4] Gordon Cook,[2] Andreas Hoeft,[3] Axel Glasmacher,[1] and Frank Stüber[5]

Infection is the main treatment-related cause of mortality in cancer patients. Rapid and accurate diagnosis to facilitate specific therapy of febrile neutropenia is therefore urgently warranted. Here, we evaluated a commercial PCR-based kit to detect the DNA of 20 different pathogens (SeptiFast) in the setting of febrile neutropenia after chemotherapy. Seven hundred eighty-four serum samples of 119 febrile neutropenic episodes (FNEs) in 70 patients with hematological malignancies were analyzed and compared with clinical, microbiological, and biochemical findings. In the antibiotic-naïve setting, bacteremia was diagnosed in 34 FNEs and 11 of them yielded the same result in the PCR. Seventy-three FNEs were negative in both systems, leading to an overall agreement in 84 of 119 FNEs (71%). During antibiotic therapy, positivity in blood culture occurred only in 3% of cases, but the PCR yielded a positive result in 15% of cases. In six cases the PCR during antibiotic treatment detected a new pathogen repetitively; this was accompanied by a significant rise in procalcitonin levels, suggestive of a true detection of infection. All patients with probable invasive fungal infection (IFI; *n* = 3) according to the standards of the European Organization for Research and Treatment of Cancer had a positive PCR result for *Aspergillus fumigatus*; in contrast there was only one positive result for *Aspergillus fumigatus* in an episode without signs and symptoms of IFI. Our results demonstrate that the SeptiFast kit cannot replace blood cultures in the diagnostic workup of FNEs. However, it might be helpful in situations where blood cultures remain negative (e.g., during antimicrobial therapy or in IFI).

TABLE 2. Blood culture and PCR results in the first 24 h of the 119 FNEs with 61 isolates total

Pathogen	No. of FNEs with positive result by:			
	Either method	Both methods	PCR only	Blood culture only
None		73		
Gram-positive bacteria				
Staphylococcus species (CoNS)	16	1	5	10
Staphylococcus aureus	8	4	4	0
Streptococcus species	7	0	0	7
Streptococcus pneumoniae	1	0	1	0
Enterococcus faecalis	1	0	1	0
Enterococcus faecium	3	1	0	2
Gram-negative bacteria				
Escherichia coli	7	2	2	3
Stenotrophomonas maltophilia	2	1	1	0
Klebsiella pneumoniae/oxytoca	2	0	0	2
Pseudomonas aeruginosa	5	2	1	2
Fungi				
Candida albicans	1	0	1	0
Aspergillus fumigatus	2	0	2	0
Candida glabrata	1	0	0	1
Not included in PCR list				
Corynebacterium species	2	0	0	2
Fusobacterium species	1	0	0	1
Morganella morganii	2	0	0	2

TABLE 3. Results of blood cultures and PCR during antimicrobial therapy

Pathogen	No. (%) of positive results by[c]:		No. of FNEs (no. initially positive)
	Blood culture	PCR	
None	113 (97)	565 (84)	
Staphylococcus aureus	0	32 (4.8)	19 (6)
Staphylococcus species (CoNS)	2 (1.8)[a]	13 (1.9)	9 (2)
Enterococcus faecium	0	14 (2.0)	6 (1)
Enterococcus faecalis	0	3 (0.4)	1 (1)
Escherichia coli	0	6 (0.9)	4 (3)
Pseudomonas aeruginosa	0	14 (2.0)	3 (1)
Stenotrophomonas maltophilia	1 (0.9)[b]	4 (0.6)	3 (2)
Candida krusei	0	1 (0.1)	1 (0)
Candida albicans	0	8 (1.2)	2 (0)
Aspergillus fumigatus	0	9 (1.3)	5 (1)

[a] One sample yielded a positive result with the same isolate in the accompanying PCR.
[b] Follow-up result of previously positive blood culture; the accompanying PCR yielded the same isolate.
[c] The numbers of samples taken on febrile days were 116 for blood culture and 665 (with 669 results) for PCR.

Correlazione tra procalcitonina plasmatica e risultati della PCR

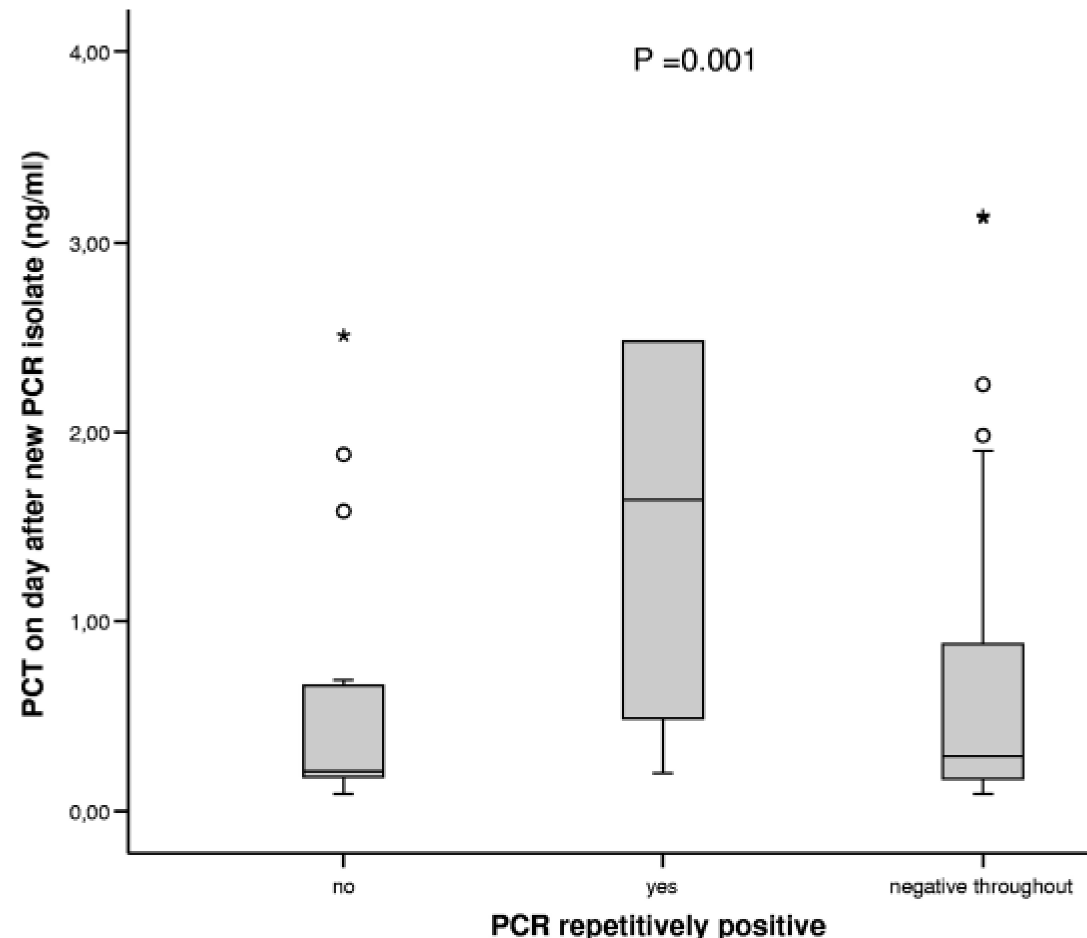


FIG. 1. PCT levels in the group of patients with repetitively positive PCR results in follow-up (*n* 6; median, 1.64 ng/ml; IQR, 0.49 to 2.48 ng/ml) compared to the group with only one positive PCR sample (*n* 22; median, 0.24 ng/ml; IQR, 0.19 to 0.69 ng/ml) or with negative PCR/blood culture results throughout the FNE (*n* 50; median, 0.29 ng/ml; IQR, 0.17 to 0.88 ng/ml; *P* 0.001, Kruskal-Wallis test). Horizontal lines within bars represent medians; bars represent IQRs; whiskers represent confidence intervals; circles and asterisks represent outliers.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2010, p. 26–33 Vol. 48, No. 1
0095-1137/10/$12.00 doi:10.1128/JCM.01447-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Multiplex PCR To Diagnose Bloodstream Infections in Patients Admitted from the Emergency Department with Sepsis∇

Ephraim L. Tsalik,[1] Daphne Jones,[2] Bradly Nicholson,[2] Lynette Waring,[6] Oliver Liesenfeld,[6] Lawrence P. Park,[1] Seth W. Glickman,[3] Lauren B. Caram,[1] Raymond J. Langley,[5] Jennifer C. van Velkinburgh,[5] Charles B. Cairns,[3] Emanuel P. Rivers,[4] Ronny M. Otero,[4] Stephen F. Kingsmore,[5] Tahaniyat Lalani,[7] Vance G. Fowler,[1] and Christopher W. Woods[1]*

Sepsis is caused by a heterogeneous group of infectious etiologies. Early diagnosis and the provision of appropriate antimicrobial therapy correlate with positive clinical outcomes. Current microbiological techniques are limited in their diagnostic capacities and timeliness. Multiplex PCR has the potential to rapidly identify bloodstream infections and fill this diagnostic gap. We identified patients from two large academic hospital emergency departments with suspected sepsis. The results of a multiplex PCR that could detect 25 bacterial and fungal pathogens were compared to those of blood culture. The results were analyzed with respect to the likelihood of infection, sepsis severity, the site of infection, and the effect of prior antibiotic therapy. We enrolled 306 subjects with suspected sepsis. Of these, 43 were later determined not to have infectious etiologies. Of the remaining 263 subjects, 70% had sepsis, 16% had severe sepsis, and 14% had septic shock. The majority had a definite infection (41.5%) or a probable infection (30.7%). Blood culture and PCR performed similarly with samples from patients with clinically defined infections (areas under the receiver operating characteristic curves, 0.64 and 0.60, respectively). However, blood culture identified more cases of septicemia than PCR among patients with an identified infectious etiology (66 and 46, respectively; $P = 0.0004$). The two tests performed similarly when the results were stratified by sepsis severity or infection site. Blood culture tended to detect infections more frequently among patients who had previously received antibiotics ($P = 0.06$). Conversely, PCR identified an additional 24 organisms that blood culture failed to detect. Real-time multiplex PCR has the potential to serve as an adjunct to conventional blood culture, adding diagnostic yield and shortening the time to pathogen identification.

Sample processing. Blood was collected for culture by the use of sterile technique. The volume inoculated was not monitored and was subject to user variability. At the Veterans Affairs Medical Center, the BacT/Alert system (bioMérieux, Marcy l'Etiole, France) was used. At the Duke University Medical Center, the BacT/Alert system was used along with the BD Bactec system (Becton Dickinson and Company, Franklin Lakes, NJ). DNA was extracted by use of a Septi*Fast* Lys kit on a MagNA Lyser platform (Roche Diagnostics), followed by silica matrix purification. We performed multiplex PCR using the LightCycler Septi*Fast* M^GRADE test, version 2.0 (Septi*Fast* test; Roche, Basel, Switzerland), an in vitro nucleic acid amplification test for the detection and identification of DNA from bacterial and fungal microorganisms (see the Septi*Fast* master list in Table 1). Internal controls for the amplification step are included with the assay. Gram-positive and gram-negative organisms were targeted via the internal transcribed spacer between the 16S and 23S rRNA genes, whereas fungi were amplified by using the 18S and 5.8S rDNA sequence.

At the time of the initial ED visit, 1.5 ml of whole blood was drawn from each subject and stored at −70°C at Duke University. Blood for experimental analysis, including blood culture, was typically obtained within 120 to 180 min after the initial clinical assessment. The frozen samples were later thawed and processed according to the manufacturing guidelines and as published previously (19), and negative and reagent controls were included. Septi*Fast* identification software (SIS; version 1.1) was used to identify the organisms on the Septi*Fast* master list by melting curve analysis. All tests were performed and all results were interpreted by a single research laboratory technician (D.J.) blinded to the hypothesis. The processing time totaled about 6.5 h and consisted of 30 min for sample thawing, 90 min for DNA extraction, 45 min for PCR setup, and 3.5 h for the PCR itself, followed by 5 min for automated software analysis.

TABLE 3. Assay performance as a function of infection status, excluding data for contaminants[a]

Assay result	No. of subjects in which infection was:		No. of subjects with the following blood culture result:	
	Present	Absent	Positive	Negative
Blood culture positive	66	0		
Blood culture negative	197	43		
PCR positive	53	0	40	13
PCR negative	210	43	26	227
Blood culture and/or PCR positive	79	0		
Blood culture and PCR negative	184	43		

[a] Blood culture and PCR results are presented separately, followed by performance of a combined blood culture and PCR strategy. The performance of PCR versus that of blood culture as the reference standard is also presented.

No. (%) of subjects in the following infection category:

- 1 (confirmed) 127 (41.5)
- 2 (probable) 94 (30.7)
- 3 (possible) 42 (13.7)
- 4 (negative) 4 (1.3)
- 5 (negative) 39 (12.7)

No. (%) of subjects with infection at the following site:

- Lung 55 (18.0)
- Urinary tract 46 (15.0)
- Skin 41 (13.4)
- Intra-abdominal 25 (8.2)
- Intravascular catheter 16 (5.2)
- Other[b] 32 (10.5)
- Unknown[c] 91 (29.7)

TABLE 5. Performance of blood culture versus performance of PCR stratified by infection category

Infection category (no. of samples)[a]	Blood culture result	No. of samples with the following PCR result:		% Concordance (kappa statistic value)
		Positive	Negative	
Category 1, definite (127)	+	40	26	74.8 (0.50)
	–	6	55	
Category 2, probable (94)	+	0	0	94.7 (0)
	–	5	89	
Category 3, possible (42)	+	0	0	95.2 (0)
	–	2	40	
Category 4, negative (4)	+	0	0	100 (0)
	–	0	4	
Category 5, negative (39)	+	0	0	100 (0)
	–	0	39	

[a] For category 1, $P = 0.0004$ by McNemar's test for agreement between blood culture and PCR stratified by infection category. McNemar's test could not be performed for categories 2 to 5 due to the absence of positive blood cultures.

No. (%) of subjects with the following:
- Noninfected SIRS positive ... 43 (14.1)
- Sepsis ... 184 (60.1)
- Severe sepsis ... 42 (13.7)
- Septic shock ... 37 (12.1)

TABLE 6. Performance of blood culture versus that of PCR stratified by severity of sepsis

Sepsis severity (no. of samples)[a]	Blood culture result	No. of samples with the following PCR result:		% Concordance (kappa statistic value)
		Positive	Negative	
Noninfected SIRS positive (43)	+	0	0	100 (0)
	−	0	43	
Sepsis (184)	+	23	17	85.9 (0.55)
	−	9	135	
Severe sepsis (42)	+	9	5	83.3 (0.60)
	−	2	26	
Septic shock (37)	+	8	4	83.8 (0.61)
	−	2	23	

[a] For the sepsis group, $P = 0.12$; for the severe sepsis group, $P = 0.26$; for the septic shock group, $P = 0.41$. Statistical analysis for the noninfected SIRS-positive group is not available due to the absence of positive blood cultures.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, June 2011, p. 2252–2258 Vol. 49, No. 6
0095-1137/11/$12.00 doi:10.1128/JCM.02460-10
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Multiplex PCR Allows Rapid and Accurate Diagnosis of Bloodstream Infections in Newborns and Children with Suspected Sepsis▽†§

Barbara Lucignano,[1]‡ Stefania Ranno,[1]‡ Oliver Liesenfeld,[2] Beatrice Pizzorno,[3]
Lorenza Putignani,[1]* Paola Bernaschi,[1]* and Donato Menichella[1]

Sepsis is a major health problem in newborns and children. Early detection of pathogens allows initiation of appropriate antimicrobial therapy that strongly correlates with positive outcomes. Multiplex PCR has the potential to rapidly identify bloodstream infections, compensating for the loss of blood culture sensitivity. In an Italian pediatric hospital, multiplex PCR (the LightCycler SeptiFast test) was compared to routine blood culture with 1,673 samples obtained from 803 children with suspected sepsis; clinical and laboratory information was used to determine the patient infection status. Excluding results attributable to contaminants, SeptiFast showed a sensitivity of 85.0% (95% confidence interval [CI] = 78.7 to 89.7%) and a specificity of 93.5% (95% CI = 92.1 to 94.7%) compared to blood culture. The rate of positive results was significantly higher with SeptiFast (14.6%) than blood culture (10.3%) ($P < 0.0001$), and the overall positivity rate was 16.1% when the results of both tests were combined. *Staphylococcus aureus* (11.6%), coagulase-negative staphylococci (CoNS) (29.6%), *Pseudomonas aeruginosa* (16.5%), and *Klebsiella* spp. (10.1%) were the most frequently detected. SeptiFast identified 97 additional isolates that blood culture failed to detect (24.7% *P. aeruginosa*, 23.7% CoNS, 14.4% *Klebsiella* spp., 14.4% *Candida* spp.). Among specimens taken from patients receiving antibiotic therapy, we also observed a significantly higher rate of positivity of SeptiFast than blood culture (14.1% versus 6.5%, respectively; $P < 0.0001$). On the contrary, contaminants were significantly more frequent among blood cultures than SeptiFast ($n = 97$ [5.8%] versus $n = 26$ [1.6%]), respectively; $P < 0.0001$). SeptiFast served as a highly valuable adjunct to conventional blood culture in children, adding diagnostic value and shortening the time to result (TTR) to 6 h.

Una mia esperienza: multiplex PCR nella sepsi

Gram-positive cocci (81)

- Clusters (61)
  - *Coagulase +* *SA442 +* (18) → *S. aureus* → *mecA* + / -
    - + → MRSA
    - - → MSSA
  - *Coagulase -* *SA442 –* (43) → CoNS
    - *mecA +*
    - *mecA -*
- Chains (20)
  - *Coagulase -* *SA442 –* (20) → 23S rRNA + (20) → *Enterococcus spp (12)*, *Streptococcus spp (8)*

Gram-positive rods (2)

- *Coagulase -* *SA442 –* (2) → 23S rRNA + (2) → *Bacillus spp (1)*, *L. Monocytogenes (1)*

Gram-negative rods and coccobacilli (124)

- *Oxidase*
  - + (23)
    - *rpoC +* (3) → *Moraxella spp (1)*, *Ochrobactrum antrophi (2)*
    - *rpoC –* (20) → *P. Aeruginosa (19)*, *S. Paucimobilis (1)*
  - - (101) → *rpoC +* (101)
    - *E. coli (65)*
    - *K. pneumoniae (11)*
    - *E. cloacae (6)*
    - *P. mirabilis (3)*
    - *S. marcescens (5)*
    - Other *Enterobacteriaceae* *(8)*
    - *S. maltophilia (2)*
    - *B. uniformis (1)*

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

Clinical Infectious Diseases 2009; 49:543–51

Piseth Seng,[a] Michel Drancourt,[a] Frédérique Gouriet, Bernard La Scola, Pierre-Edouard Fournier, Jean Marc Rolain, and Didier Raoult

Background. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry accurately identifies both selected bacteria and bacteria in select clinical situations. It has not been evaluated for routine use in the clinic.

Methods. We prospectively analyzed routine MALDI-TOF mass spectrometry identification in parallel with conventional phenotypic identification of bacteria regardless of phylum or source of isolation. Discrepancies were resolved by 16S ribosomal RNA and *rpo*B gene sequence–based molecular identification. Colonies (4 spots per isolate directly deposited on the MALDI-TOF plate) were analyzed using an Autoflex II Bruker Daltonik mass spectrometer. Peptidic spectra were compared with the Bruker BioTyper database, version 2.0, and the identification score was noted. Delays and costs of identification were measured.

Results. Of 1660 bacterial isolates analyzed, 95.4% were correctly identified by MALDI-TOF mass spectrometry; 84.1% were identified at the species level, and 11.3% were identified at the genus level. In most cases, absence of identification (2.8% of isolates) and erroneous identification (1.7% of isolates) were due to improper database entries. Accurate MALDI-TOF mass spectrometry identification was significantly correlated with having 10 reference spectra in the database ($P = .01$). The mean time required for MALDI-TOF mass spectrometry identification of 1 isolate was 6 minutes for an estimated 22%–32% cost of current methods of identification.

Conclusions. MALDI-TOF mass spectrometry is a cost-effective, accurate method for routine identification of bacterial isolates in <1 h using a database comprising ≥10 reference spectra per bacterial species and a ≥1.9 identification score (Brucker system). It may replace Gram staining and biochemical identification in the near future.

Bacterial identification is routinely based on phenotypic tests, including Gram staining, culture and growth characteristics, and biochemical pattern [1]. Although some of these tests are performed within minutes, com-

plete identification is routinely achieved within hours in the best cases or days for fastidious organisms. Such conventional, time-consuming procedures hamper proper treatment of patients with respect to antibiotic and supportive treatments. Rapid and accurate identification of routinely encountered bacterial species is therefore warranted to improve the care of patients with infectious diseases.

Bacterial identification based on peptidic spectra obtained by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was proposed >30 years ago [2–4]. It has only recently been used as a rapid, inexpensive, and accurate method for identifying isolates that belong to certain bacterial phyla (Figure 1). It has also proved useful for identifying bacteria isolated in selected clinical situations, such as cystic fibrosis [5]. However, previous studies did not evaluate the effectiveness of MALDI-TOF mass spectrometry identification for routine use in the clinics, because they included bacterial isolates gathered from past collections and grown in conditions selected for the study [6] or incorporated isolates subcultured in selected growth conditions prior to MALDI-TOF mass spectrometry analysis [7].

Table 1. Concordance between Conventional Routine Identification (Vitek; bioMérieux) and Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry Identification (Brucker Mass Spectrometer and Database Complemented with Local Database)

	Routine phenotypic identification, no. of isolates				
MALDI-TOF identification	Species identification	Genus identification	No identification	Misidentification	Total
Species identification	1392	0	4	1	1397
Genus identification	185	0	2	2	189
No identification	18	0	26	2	46
Misidentification	27	0	0	1	28
Total	1622	0	32	6	1660

Phenotype erroneous identifications. The current methods of identification failed for 32 isolates (1.9%), which were all anaerobes (Table 2). Phenotypic identification was erroneous for 28 isolates (1.7%). One isolate phenotypically identified as *Streptococcus mitis* was identified as *Actinomyces* species by MALDI-TOF mass spectrometry and was confirmed to be *Actinomyces naeslundii* by 16S rRNA gene sequencing. One isolate phenotypically identified as *Aerococcus viridans* was identified as *S. parasanguinis* by MALDI-TOF mass spectrometry and as *S. infantis* by partial *rpo*B gene sequencing. One isolate phenotypically identified as *Gemella morbilorum* was identified as *Streptococcus* species by MALDI-TOF mass spectrometry and was confirmed to be *Streptococcus sanguinis* by partial *rpo*B gene sequencing. One *Corynebacterium* group G isolate was identified as *Lactobacillus* species by MALDI-TOF mass spectrometry and was confirmed to be *Lactobacillus zeae* by 16S rRNA gene sequencing. One isolate phenotypically identified as *Staphylococcus epidermidis* was identified as *Propionibacterium* species by MALDI-TOF mass spectrometry and as *S. epidermidis* by *rpo*B sequencing.

Table 3. Delays, Costs, and Level of Training for Isolate Identification Methods

Method	Delay, minutes	Cost, €[a]	Level of training
Manual			
Gram staining	6	0.6	Medium to high
API system identification (bioMérieux)	1080–2880	4.6–6.0	Medium
Antibiotic susceptibility test	1080–2880	6.6–7.4	Medium
Phoenix system identification and susceptibility test (BD Diagnostics)	300–1200	12.65	Medium
Vitek system (bioMérieux)			
Identification	300–480	5.9–8.23	Medium
Identification and susceptibility test	300–480	10.38–12.71	
MALDI-TOF	6–8.5	1.43	Low to medium

NOTE. MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.

[a] Costs have been tabulated based on December 2008 price list of the providers in France

Detection of microorganisms in blood specimens using matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a review

Clin Microbiol Infect 2010; **16:** 1620–1625

M. Drancourt

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) initiated a revolution in the identification of organisms grown on solid medium, including bacteria and fungi. Rapid identification of organisms responsible for septicaemia, which are typically grown in broth, is now expanding the field of application. Despite the fact that there are fewer than ten reports in the literature, published data indicate that MALDI-TOF MS yields accurate identification of blood-borne organisms in ≥80% of cases for inocula of $>10^7$ organisms/mL. A major current limitation is failure to accurately identify *Streptococcus pneumoniae* among viridans steptococci. Identification is achieved in <2 h, sharply reducing the turn-around time for communication of identification to the clinician. Further progress in handling protocols and automation, and extraction of antibiotic resistance data from the MALDI-TOF MS spectra, will further push this emerging approach as the standard one in the laboratory diagnosis of septicaemia, paving the way to a [illegible] further clinical situations and clinical specimens.

TABLE 1. Performance in identifying bacteria in positive blood culture broth

Nature of specimen (*n* = number of specimens)	Organism	Percentage of interpretable spectra (%)	Percentage of correct identification: genus level (%)	Percentage of correction identification: species level (%)	Major identification failures
Positive blood culture broth (*n* = 599)	Bacteria	94	76	76	*Streptococcus* spp.
Positive blood culture broth (*n* = 126)	Bacteria	97	79	57	*Streptococcus pneumoniae*
Positive blood culture broth (*n* = 179)	Bacteria	100[a]	80[a]	80[a]	*S. pneumoniae*
Spiked bottles (*n* = 33)					*Propionibacterium acnes*
Spiked bottles (*n* = 312)	Bacteria	98	98	89	*S. pneumoniae*
Positive blood culture broth (*n* = 388)	*Candida* spp.	96	98	91	*S. pneumoniae*
Positive blood culture broth (*n* = 304)	Bacteria	94.7	87	87	Uncommon species
Spiked bottles (*n* = 48)	*Candida* spp.	100	100	100	–
Positive blood culture broth (*n* = 1)	*Candida albicans*	100	100	100	–

[a]Including spiked bottles.

Comparison of the MALDI Biotyper System Using Sepsityper Specimen Processing to Routine Microbiological Methods for Identification of Bacteria from Positive Blood Culture Bottles

Blake W. Buchan, Katherine M. Riebe and Nathan A. Ledeboer

J. Clin. Microbiol. 2012, 50(2):346. DOI: 10.1128/JCM.05021-11.
Published Ahead of Print 7 December 2011.

Bloodstream infections are a leading cause of admissions to hospital intensive care units and carry a high mortality rate. Clinical outcome can be greatly improved by early effective antibiotic therapy; therefore, broad-spectrum antimicrobial therapy is often initiated when there is a clinical suspicion of bloodstream infection. Unfortunately, this method may not always be effective when dealing with inherently resistant organisms and can also result in iatrogenic infection and the development of resistant isolates. Rapid identification of the infecting organism may aid in choosing appropriate antimicrobial therapy, thereby reducing these potential adverse events. We compared the matrix-assisted laser desorption ionization (MALDI) Biotyper system with Sepsityper specimen processing (Bruker Daltonics, Billerica, MA) to routine methods for the identification of microorganisms from 164 positive blood cultures. The MALDI Biotyper/Sepsityper identified 85.5% of bacterial isolates directly from positive monomicrobial blood cultures with 97.6% concordance to genus and 94.1% concordance to species with routine identification methods. Gram-negative isolates were more likely to produce acceptable confidence scores (97.8%) than Gram-positive isolates (80.0%); however, genus and species concordance with routine identification methods were similar in both groups. Reanalysis of collected spectra using modified blood culture-specific parameters resulted in an improved overall identification rate fo positive bacteria (89.0%). Median times to identification using the MALDI Biotyper/Sepsityper were 23 to 83 h faster th tine methods for Gram-positive isolates and 34 to 51 h faster for Gram-negative isolates.

Empiric broad-spectrum antibiotic therapy is initiated when there is clinical suspicion of sepsis. While effective, broad-spectrum therapy is associated with adverse events, including *Clostridium difficile* disease, and potentially contributes to the selection of more resistant microorganisms (14, 27, 30). Earlier de-escalation of broad-spectrum therapy may reduce these risks. Therefore, rapid identification of the etiologic agents of bacteremia and septicemia is an important component to management of the infection and can affect clinical outcome. The MALDI Biotyper/Sepsityper can be used to directly analyze positive blood cultures in real time and provide definitive species identification within 20 min. In the current study, the use of MALDI Biotyper/Sepsityper reduced the time to positive identification of isolates by as much as 130 h (*Gemella* spp.) and minimally by approximately 24 h (*S. aureus*, *Streptococcus* group A and group B). Combined with predictable antibiotic resistance profiles and effective real-time antibiograms, these reduced times to bacterial identification could aid in guidance of antibiotic therapy in patients with bacteremia.

A major advantage of MALDI-TOF-based identification is a departure from reliance on physical, biochemical, and metabolic characteristics in favor of protein profile analysis. This allows better discrimination of species that have similar biochemical/metabolic profiles, such as CoNS. Accurate identification of CoNS can be helpful in differentiating a true infection from contamination. Identification based on the protein profile may also be advantageous when working with biochemically inert bacteria or organisms isolated from patients undergoing antibiotic therapy. Pretreated isolates often produce poor or inconsistent biochemical/metabolic profiles, making identification by routine methods difficult. Identification using MALDI-TOF relies primarily on ribosomal protein profiles which are of high abundance and are unaffected by many classes of antibiotics (22). Finally, automated identification systems require correct interpretation of Gram stain results for selection of an appropriate identification panel. Misinterpretation of the Gram stain can lead to a failed identification, which further extends turnaround time.

Characterization of discordant results supported the MALDI Biotyper identification in 3 of 7 cultures that generated acceptable confidence scores (>1.7). Three of the four incorrect identifications by MALDI were viridans group streptococci misidentified as *S. pneumoniae*. The inability of the MALDI Biotyper to distinguish between *S. pneumoniae* and viridans group streptococci (specifically the mitis group) is due to the high similarity in protein profiles generated by these strains. This has been previously reported and is acknowledged by the manufacturer in the product insert (16, 18, 28). The remaining incorrect MALDI identification was an *E. cloacae* isolate identified as *E. asburiae* by the MALDI Biotyper. *E. asburiae* is closely related to *E. cloacae* and resides within the *E. cloacae* complex. Species within this complex are difficult to distinguish using biochemical tests and are often misidentified using automated systems (19, 31).

The MALDI Biotyper was unable to identify multiple organisms in polymicrobial cultures; however, when a high confidence score identification was obtained, the identified organism was present.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, May 2010, p. 1542–1548 Vol. 48, No. 5
0095-1137/10/$12.00 doi:10.1128/JCM.02485-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Real-Time Identification of Bacteria and *Candida* Species in Positive Blood Culture Broths by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry∇

Agnès Ferroni,[1*] Stéphanie Suarez,[1] Jean-Luc Beretti,[1] Brunhilde Dauphin,[2] Emmanuelle Bille,[1,3] Julie Meyer,[3] Marie-Elisabeth Bougnoux,[1,3] Alexandre Alanio,[1] Patrick Berche,[1,3] and Xavier Nassif[1,3]

Delays in the identification of microorganisms are a barrier to the establishment of adequate empirical antibiotic therapy of bacteremia. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF–MS) allows the identification of microorganisms directly from colonies within minutes. In this study, we have adapted and tested this technology for use with blood culture broths, thus allowing identification in less than 30 min once the blood culture is detected as positive. Our method is based on the selective recovery of bacteria by adding a detergent that solubilizes blood cells but not microbial membranes. Microorganisms are then extracted by centrifugation and analyzed by MALDI-TOF–MS. This strategy was first tested by inoculating various bacterial and fungal species into negative blood culture bottles. We then tested positive patient blood or fluid samples grown in blood culture bottles, and the results obtained by MALDI-TOF–MS were compared with those obtained using conventional strategies. Three hundred twelve spiked bottles and 434 positive cultures from patients were analyzed. Among monomicrobial fluids, MALDI-TOF–MS allowed a reliable identification at the species, group, and genus/family level in 91%, 5%, and 2% of cases, respectively, in 20 min. In only 2% of these samples, MALDI-TOF MS did not yield any result. When blood cultures were multibacterial, identification was improved by using s databases based on the Gram staining results. MALDI-TOF–MS is currently the fastest technique to acc identify microorganisms grown in positive blood culture broths.

The sensitivity of direct identification from positive BacT/ALERT™ (bioMérieux) blood culture bottles by matrix-assisted laser desorption ionization time-of-flight mass spectrometry is low

F. Szabados, M. Michels, M. Kaase and S. Gatermann

The rate of the MALDI TOF Msbased identification in the present study from positive BacT/ALERT (bioMe´rieux, Marcy l'Etoile, France) blood culture bottles was 30%, which is far below the previously reported sensitivities using the BACTEC (Becton Dickinson, Franklin Lakes, NJ, USA) system. We also found evidence that the Biotyper algorithm did not identify a second pathogen in cases of positive BacT/ALERT blood culture bottles containing two different species.

Unresolved Issues

The identification of viridans *Streptococcus* spp. organisms in blood culture bottles remains problematic in most studies [16,17], in agreement with previous observations made with the use of agar medium-grown colonies [13]. Pooling of data from currently published reports indicates that 16/20 (80%) *S. pneumoniae* strains were not identified solely by MALDI-TOF MS. The lack of an appropriate database may partially explain this fact, which also illustrates difficulties in the taxonomy of this particular group of organisms. Therefore, complementation of the MALDI-TOF MS identification with an agglutination test is mandatory in the case of viridans *Streptococcus* sp. identification.

Identifying mixed organisms in the same blood culture specimen remains problematic. In one study, 15 mixed bacteria bottles were tested, and yielded one or two bacteria; the authors observed that use of a specific database improved the identification score [19]. Most studies report that only one of the two organisms is identified by MALDI-TOF MS [17,20]; moreover, mixed flora may result in a lack of identification or false species identification [16].

The optimal protocol for blood culture broth processing remains to be determined and evaluated; every one of the six major available studies listed its own protocol. Companies will probably develop and sell their own solutions for red cell lysis and protein extraction, thus standardizing the blood specimen processing and paving the way towards an automated process. Also, the optimization of laboratory processes, combined with the use of last-generation mass spectrometers and software, would further reduce the turn-around time for identification of blood-borne organisms. Mass spectrometers are, indeed, amenable to integration into an semi-automated or fully automated laboratory.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Identification of Bacteria in Blood Culture Broths Using Matrix-Assisted Laser Desorption-Ionization Sepsityper™ and Time of Flight Mass Spectrometry

Jen Kok[1,2]*, Lee C. Thomas[1], Thomas Olma[1], Sharon C. A. Chen[1], Jonathan R. Iredell[1,2]

PLoS ONE 6(8): e23285. doi:10.1371/journal.pone.0023285

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) is a novel method for the direct identification of bacteria from blood culture broths. We evaluate for the first time, the performance of the MALDI Sepsityper™ Kit and MS for the identification of bacteria compared to standard phenotypic methods using the manufacturer's specified bacterial identification criteria (spectral scores ≥1.700–1.999 and ≥2.000 indicated identification to genus and species level, respectively). Five hundred and seven positive blood culture broths were prospectively examined, of which 379 (74.8%; 358 monomicrobial, 21 polymicrobial) were identified by MALDI-TOF MS; 195 (100%) and 132 (67.7%) of 195 gram-positive; and 163 (100%) and 149 (91.4%) of 163 gram-negative organisms from monomicrobial blood cultures were correctly identified to genus and species level, respectively. Spectral scores <1.700 (no identification) were obtained in 128/507 (25.2%) positive blood culture broths, including 31.6% and 32.3% of gram-positive and polymicrobial blood cultures, respectively. Significantly more gram-negative organisms were identified compared to gram-positive organisms at species level ($p<0.0001$). Five blood cultures were misidentified, but at species level only; including four monomicrobial blood cultures with *Streptococcus oralis/mitis* that were misidentified as *Streptococcus pneumoniae*. Positive predictive values for the direct identification of both gram-positive and gram-negative bacteria from monomicrobial blood culture broths to genus level were 100%. A diagnostic algorithm for positive blood culture broths that incorporates gram staining and MALDI-TOF MS should identify the majority of pathogens, particularly to genus level.

Altra possibile soluzione: microarray

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study

Lancet 2010; 375: 224–30

Päivi Tissari, Alimuddin Zumla, Eveliina Tarkka, Sointu Mero, Laura Savolainen, Martti Vaara, Anne Aittakorpi, Sanna Laakso, Merja Lindfors, Heli Piiparinen, Minna Mäki, Caroline Carder, Jim Huggett, Vanya Gant

Summary

Background New DNA-based microarray platforms enable rapid detection and species identification of many pathogens, including bacteria. We assessed the sensitivity, specificity, and turnaround time of a new molecular sepsis assay.

Methods 2107 positive blood-culture samples of 3318 blood samples from patients with clinically suspected sepsis were investigated for bacterial species by both conventional culture and Prove-it sepsis assay (Mobidiag, Helsinki, Finland) in two centres (UK and Finland). The assay is a novel PCR and microarray method that is based on amplification and detection of *gyrB*, *parE*, and *mecA* genes of 50 bacterial species. Operators of the test assay were not aware of culture results. We calculated sensitivity, specificity, and turnaround time according to Clinical and Laboratory Standards Institute recommendations.

Findings 1807 of 2107 (86%) positive blood-culture samples included a pathogen covered by the assay. The assay had a clinical sensitivity of 94·7% (95% CI 93·6–95·7) and a specificity of 98·8% (98·1–99·2), and 100% for both measures for meticillin-resistant *Staphylococcus aureus* bacteraemia. The assay was a mean 18 h faster than was the conventional culture-based method, which takes an additional 1–2 working days. 34 of 3284 (1·0%) samples were excluded because of technical and operator errors.

Interpretation Definitive identification of bacterial species with this microarray platform was highly sensitive, specific, and faster than was the gold-standard culture-based method. This assay could enable fast and earlier evidence-based management for clinical sepsis.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

	HUSLAB (n=2439)	UCLH (n=879)	Total (N=3318)
Positive blood-culture specimens	1535 (63%)	572 (65%)	2107 (64%)
Samples with bacteria covered by assay	1278 (52%)	392 (45%)	1670 (50%)
Samples with pathogen not covered by assay	195 (8%)	109 (12%)	304 (9%)
Polybacterial samples covered by assay	62 (3%)	71 (8%)	133 (4%)
Negative blood-culture specimens	904 (37%)	307 (35%)	1211 (36%)

HUSLAB=Helsinki University Hospital Laboratory. UCLH=University College London Hospitals.

Table 1: **Number of blood-culture specimens tested**

	Positive sepsis assay (n; result)	Negative sepsis assay (n; result)	Sensitivity (95% CI)‡	Specificity (95% CI)‡
Reference method positive	1696; true positive	94; false negative	94·7% (93·6–95·7)§	--
Reference method negative or positive*	18; false positive†	1476; true negative	--	98·8% (98·1–99·2)§

*Blood-culture-positive samples, including pathogens not covered by the assay. †False positives also included specimens identified as a different species by the assay. ‡Sensitivity, specificity, and 95% CIs were calculated according to Clinical and Laboratory Standards Institute recommendations. §Sensitivity and specificity for all the samples including the 34 excluded samples (3318) were 94·6% and 96·2%, respectively.

Table 4: **Accuracy of sepsis assay analysed with all blood-culture specimens (3284)**

	Number of specimens	Specimens (% of all tested specimens)*	BC-positive specimens (% BC-positive specimens)*	BC-negative specimens (% BC-negative specimens)*
False positives of sepsis assay				
Polybacterial finding	11	0·3%	0·5%	--
Cross-hybridisation	3	0·1%	0·1%	--
Coagulase-negative staphylococci reported for *S epidermidis* findings†	3	0·1%	0·1%	--
BC negative reported for *B fragilis* finding	1	0·1%	0·1%	--
False negatives of sepsis assay				
Taxon identification‡	6	0·2%	0·3%	--
Positive only for *mecA*‡	5	0·2%	0·2%	--
Limitations in sensitivity	23	0·7%	1·1%	--
Inaccuracy in a polybacterial finding§	60	1·8%	2·8%	--
Samples excluded from sensitivity and specificity calculations				
BC positive samples				
Software failure	2	0·1%	0·1%	--
Contamination or other‖	6	0·2%	0·3%	--
BC negative samples				
Contamination or other‖	26	0·8%	--	2·2%

Discrepancies categorised by cause. BC+=blood-culture positive. BC–=blood-culture negative. . *Total number of tested specimens was 3318, of BC-positive specimens was 2107, and of BC-negative specimens was 1211. †Coagulase-negative staphylococci identified instead of *Staphylococcus epidermidis*. ‡Inaccuracy in identification; Enterobacteriaceae family reported instead of species identification, or positive *mecA* reported without an identification of *S epidermidis* (one sample), or coagulase-negative staphylococcus included in the assay panel (four). §Not all causative agents were identified. ‖Sampling errors or unknown reason.

***Table 5*: Discrepancies between Prove-it sepsis assay and reference method for identification of bacteria from blood culture**

Guidelines on Blood Cultures

J Microbiol Immunol Infect 2010;43(4):347–349

Michael Lloyd Towns[a], William Robert Jarvis[b], Po-Ren Hsueh[c]*

Just over one-third of sepsis patients have positive blood cultures, mainly due to inadequate sampling volumes (50% of adults have < 1.0 CFU/mL blood) and the prior use of antibiotics. However, 20–30% of sepsis patients are given inappropriate empirical antibiotics. The Clinical and Laboratory Standards Institute guidelines recommend paired culture sets to help discriminate between contaminant organisms and true pathogens; four 10-mL bottles (2 sets) should be used for the initial evaluation to detect about 90–95% of bacteremias and six 10-mL bottles (3 sets) should be used to detect about 95–99% of bacteremias. It has also been shown that the positivity rate increased by 15–35% with resin-based media in patients on antibiotics. For diagnosing catheter-related bloodstream infections, differential time-to-positivity is one method recommended to help determine whether the catheter is truly the source of infection. The proper training of personnel with regard to drawing an appropriate blood volume and the importance of clear labeling of culture bottles is also of critical importance. Furthermore, if the contamination rate is relatively high, hiring dedicated staff who are well-trained in order to get a lower blood culture contamination rate may be cost-effective. It is because high false-positive blood culture rates due to contamination are associated with significantly increased hospital and laboratory charges.

Altra sindrome:

Clostridium difficile diarrea/colite pseudomembranosa ecc.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2010, p. 124–130 Vol. 48, No. 1
0095-1137/10/$12.00 doi:10.1128/JCM.00734-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Evaluation of *tcdB* Real-Time PCR in a Three-Step Diagnostic Algorithm for Detection of Toxigenic *Clostridium difficile*▽

Ann M. Larson,[1,2] Angela M. Fung,[3] and Ferric C. Fang[1,2,3]*

***Clostridium difficile* is the most common infectious cause of diarrhea in hospitalized patients. The optimal approach for the detection of toxigenic *C. difficile* remains controversial because no single test is sensitive, specific, and affordable. We have developed a real-time PCR method (direct stool PCR [DPCR]) to detect the *tcdB* gene encoding toxin B directly from stool specimens and have combined it with enzyme immunoassays (EIAs) in a three-step protocol. DPCR was performed on 699 specimens that were positive for *C. difficile* glutamate dehydrogenase (GDH) by Wampole C Diff Quik Chek EIA (GDH-Q) and negative for toxins A and B by Wampole Tox A/B Quik Chek EIA (AB-Q), performed sequentially. The performance of this three-step algorithm was compared with a modified "gold standard" that combined tissue culture cytotoxicity (CYT) and DPCR. A separate investigation was performed to evaluate the sensitivity of the GDH-Q as a screening test, and toxigenic *C. difficile* was found in 1.9% of 211 GDH-Q-negative specimens. The overall sensitivity, specificity, and positive and negative predictive values, respectively, were as follows for an algorithm combining GDH-Q, AB-Q, and DPCR: 83.8%, 99.7%, 97.1%, and 97.9%. Those for CYT alone were 58.8%, 100%, 100%, and 94.9%, respectively. In comparison, the sensitivity and specificity of DPCR were estimated to be 97.5% and 99.7%, respectively, using the same modified gold standard. Neither CYT nor toxin EIA was sufficiently sensitive to exclude toxigenic *C. difficile*, and combining EIAs with CYT in a three-step algorithm failed to substantially improve sensitivity. DPCR is a sensitive and specific method for the detection of toxigenic *C. difficile* that can provide same-day results at a cost-per-positive test comparable to those of other methods. A three-ste[illegible] algorithm in which DPCR is used to analyze GDH EIA-positive, toxin EIA-negative specimens provides [illegible] convenient and specific alternative with rapid results for 87.7% of specimens, although this approach is le[illegible] sensitive than performing DPCR on all specimens.**

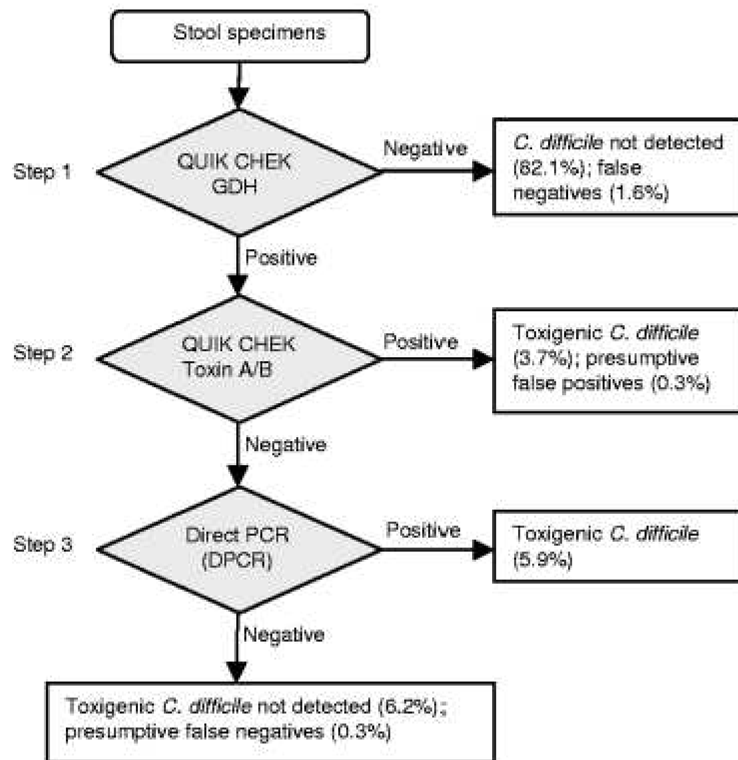


FIG. 1. Three-step algorithm for detection of toxigenic *C. difficile*. This algorithm provides rapid, sensitive, specific, and cost-effective detection of toxigenic *C. difficile*. The Quik Chek EIAs (Wampole/TechLab, Blacksburg, VA) are C Diff Quik Chek for glutamate dehydrogenase antigen (GDH) and Tox A/B Quik Chek for toxins A and B. DPCR detects the *tcdB* gene.

Clearly, GDH-Q is insufficiently specific and CYT and AB-Q are insufficiently sensitive to be relied upon as assays for toxigenic *C. difficile*, whether used alone or in combination with each other, although EIA testing is the least expensive method for the laboratory budget. The incorporation of DPCR into an algorithm using EIAs as screening steps increases overall costs of testing but not the cost per case detected (Table 3). The increased number of cases detected (approximately 182 specimens per year positive by three-step EIA/DPCR but negative by EIA) may compensate for higher labor and reagent costs by reducing the nosocomial spread of CDI. Infected patients are an important reservoir of *C. difficile* in institutional settings, and person-to-person transmission has been demonstrated in 10 to 20% of cases of hospital-associated CDI (8, 56). Emerging 027/NAP1 epidemic strains may have even greater transmissibility due to enhanced toxin and spore production (2).

The choice of real-time PCR methods for direct detection from stools now includes the Xpert *C. difficile* test (Cepheid, Sunnyvale, CA), the ProGastro Cd assay (Prodesse, Waukesha, WI), and the BD GeneOhm Cdiff assay (BD Diagnostics, San Diego, CA), with published GeneOhm sensitivity values of 83.6 and 93.9% and NPVs of 97.1% to 99.2%, when compared to toxigenic culture (7, 54), although unresolved GeneOhm results were as high as 7.3% in one study (7). Our observations confirm that DPCR is a sensitive, specific, and cost-effective method for the detection of toxigenic *C. difficile*. A multistep algorithm consisting of a GDH EIA screen fol specific toxin EIA screen and direct real-time PC convenient, rapid, and specific alternative strategy, trade-off of some reduction in sensitivity.

Effective Utilization of Evolving Methods for the Laboratory Diagnosis of *Clostridium difficile* Infection

Anna M. Kufelnicka[1] and Thomas J. Kirn[1,2]

Clinical Infectious Diseases 2011;52(12):1451–1457

Table 1. Advantages and Disadvantages of *Clostridium difficile* Assays

Assay	Method/target	Advantages	Disadvantages
Culture	Organism	High sensitivity (often considered to be the gold standard)	Turn-around time >7 days Labor intensive Lacks specificity; does not distinguish between toxigenic and nontoxigenic strains Isolates must be further tested for the presence of toxin(s) or toxin genes (toxigenic culture)
Cell cytotoxicity neutralization assay	Functional assay for *C. difficile* toxin B (TcdB)	Moderate-to-high sensitivity High specificity	48-72 h turn-around time Subjective interpretation; requires skilled technicians/ technical expertise Labor intensive
Enzyme immunoassays (EIA), *C. difficile* toxin A (TcdA)	Toxin A detection	Easy to perform Rapid turn-around time Inexpensive High specificity	Low sensitivity Misses TcdA-/TcdB+ isolates
EIA, TcdB or TcdA/B	Toxin A/Bdetection	Easy to perform Rapid turn-around time Inexpensive High specificity	Lower sensitivity
EIA, glutamate dehydrogenase	Common antigen detection	High sensitivity Good screening test	Low specificity and does not distinguish between toxigenic and nontoxigenic strains Positive specimens must be further tested for the presence of toxin(s) or toxin genes
Nucleic acid amplification tests	Toxin gene(s) detection	High sensitivity and specificity (new gold standard?) Short turn-around time Some easy to perform and minimal hands-on time	Expensive when used to test all samples Detection of asymptomatic colonization a possible concern Some require significant molecular expertise

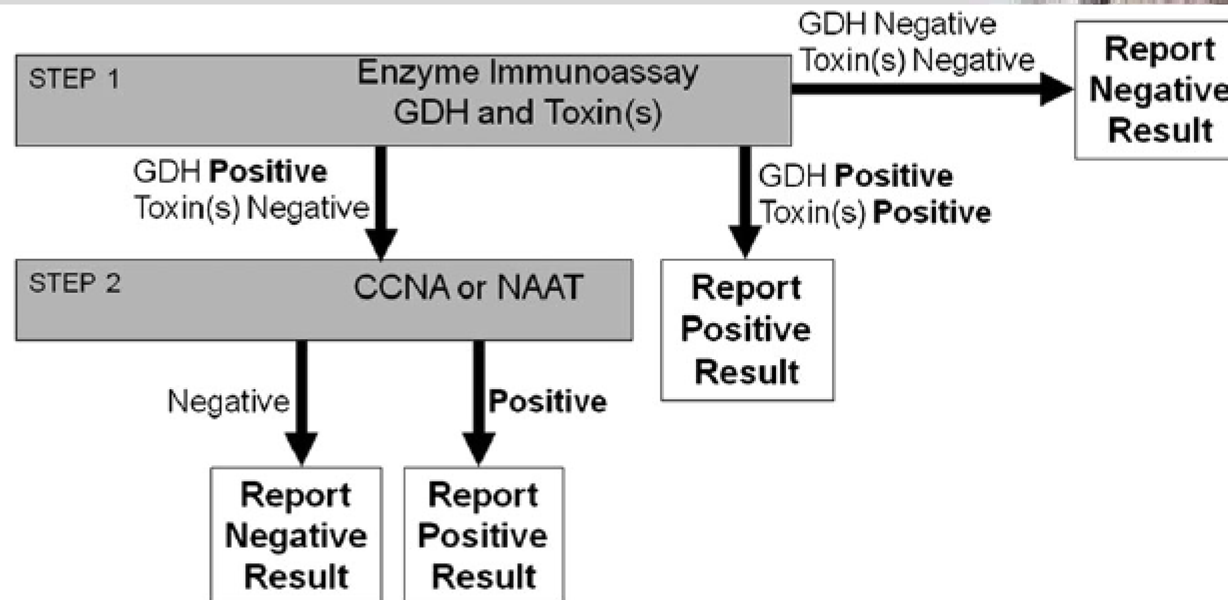


Figure 1. Typical diagnostic algorithm for the detection of toxigenic *Clostridium difficile* in stool specimens. Rapid, inexpensive immunoassays that detect glutamate dehydrogenase (GDH) (and may also include toxin detection as well; Step 1) are followed by more-laborious and/or more-expensive approaches that often demonstrate better performance characteristics (Step 2). Specimens with negative results in the first step (which often represent >80% of specimens) and those that are GDH positive as well as *C. difficile* toxin A (TcdA) and/or *C. difficile* toxin B (TcdB) positive may be reported after the first step, allowing most laboratories to achieve a very favorable turn-around time for the bulk of specimens. Those that are positive for GDH (and negative for toxin(s) if tested) are further assessed using a nucleic acid amplification test (NAAT) or cell cytotoxicity neutralization assay (CCNA). Although NAATs are often more expensive than CCNAs, the performance and turn-around time for NAATs are superior to those for CCNAs.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2010, p. 4519–4524 Vol. 48, No. 12
0095-1137/10/$12.00 doi:10.1128/JCM.01648-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Evaluation of the Cepheid Xpert *Clostridium difficile* Epi Assay for Diagnosis of *Clostridium difficile* Infection and Typing of the NAP1 Strain at a Cancer Hospital[∇]

N. Esther Babady,[1*] Jeffrey Stiles,[1] Phyllis Ruggiero,[1] Perminder Khosa,[2] David Huang,[2] Susan Shuptar,[1] Mini Kamboj,[2] and Timothy E. Kiehn[1]

***Clostridium difficile* is the most common cause of health care-associated diarrhea. Accurate and rapi diagnosis is essential to improve patient outcome and prevent disease spread. We compared our two-ste diagnostic algorithm, an enzyme immunoassay for glutamate dehydrogenase (GDH) followed by the cytotoxi neutralization test (CYT) with a turnaround time of 24 to 48 h, versus the Cepheid Xpert *C. difficile* Epi assay a PCR-based assay with a turnaround time of <1 h. In the first phase of the study, only GDH-positive stoo samples were tested by both CYT and Xpert PCR. Discordant results were resolved by toxigenic culture. In th second phase, all stool samples were tested by GDH and Xpert PCR. Only GDH-positive stools were furthe tested by CYT. Genotypic characterization of 45 Xpert PCR-positive stools was performed by sequencing of th *tcdC* gene and PCR ribotyping. In phase 1, the agreement between the GDH-CYT and the GDH-Xpert PCR wa 72%. The sensitivities and specificities of GDH-CYT and GDH-Xpert PCR were 57% and 97% and 100% an 97%, respectively. In phase 2, the agreement between GDH-CYT and Xpert PCR alone was 95%. As in phas 1, sensitivity of the Xpert PCR was higher than that of the GDH-CYT. The correlation between PCR-ribotyping sequencing, and Xpert PCR for detection of NAP1 strains was excellent (>90%). The excellent sen[...]n specificity and the rapid turnaround time of the Xpert PCR assay as well as its strain-typing capa[...]k it an attractive option for diagnosis of *C. difficile* infection.**

TABLE 1. Comparison of Xpert *C. difficile* PCR assay and cytotoxicity neutralization assay results for GDH-positive stool samples

GDH-Xpert PCR result	GDH-CYT result		Total
	Positive	Negative	
Positive	24	19	43
Negative	1	29	30
Total	25	48	73

TABLE 4. Comparison of Xpert *C. difficile* PCR results with those based on the GDH-CYT algorithm (unique specimens only)

Xpert PCR result	GDH-CYT result		Total
	Positive	Negative	
Positive	43	35	78
Negative	1	329	330
Total	44	364	408

TABLE 2. Comparison of Xpert *C. difficile* PCR assay results with the GDH-CYT assay algorithm, regardless of GDH result for stool sample

Xpert PCR result	GDH-CYT result		Total
	Positive	Negative	
Positive	33	27	60
Negative	1	426	427
Total	34	453	487

TABLE 3. Results of further analysis of Xpert *C. difficile* PCR and toxigenic culture discordant findings

Sample no.	Finding based on: GDH-CYT	Xpert PCR	Toxigenic culture	Organism(s)	Comment	Conclusion
1	–	+	–	*C. innocuum, C. beijerinckii/diolis*	Previous *C. difficile* positive (PCR and toxigenic culture)	Residual DNA
2	–	+	–	*C. innocuum*	Previous *C. difficile* positive (GDH-CYT and PCR)	Residual DNA
3	–	+	–	*C. innocuum*	Previous *C. difficile* all negative	False[illegible]R
4	–	+	–	No growth	Subsequent positive (PCR and CYT)	True [illegible]
5	–	+	–	No growth	Previous *C. difficile* all negative	False[illegible]R
6	–	+	–	*C. innocuum*	Previous *C. difficile* all negative	False[illegible]R
7	–	+	–	No growth	Same as sample 5	False-positive PCR

TABLE 5. Comparison of Xpert *C. difficile* PCR results with those of PCR-ribotyping and sequencing for detection of NAP1 strains

PCR-ribotyping or sequencing result	Xpert *C. difficile* Epi PCR result	
	NAP1	Not NAP1
PCR-ribotyping		
NAP1	11	1
Not NAP1	2	31
Total	13	32
Sequencing		
NAP1	13	3
Not NAP1	0	29
Total	13	32

The difference between the three methods was not significantly different (*P* 0.749) as determined by one-way ANOVA.

a testing algorithm comprising the GDH EIA followed by the Xpert PCR would represent a sensitive and specific option for *C. difficile* testing. Our findings are different from those reported by Novak-Weekly etal. (21), who showed a lower assay sensitivity when the Xpert PCR was used following an initial screen with the GDH EIA. Because those authors used toxigenic culture to test all stools,the observed differences between the two studies might be due to a difference in testing methodology.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2010, p. 4347–4353 Vol. 48, No. 12

POINT-COUNTERPOINT

What Is the Current Role of Algorithmic Approaches for Diagnosis of *Clostridium difficile* Infection?∇

With the recognition of several serious outbreaks of *Clostridium difficile* infection in the industrialized world coupled with the development of new testing technologies for detection of this organism, there has been renewed interest in the laboratory diagnosis of *C. difficile* infection. Two factors seem to have driven much of this interest. First, the recognition that immunoassays for detection of *C. difficile* toxins A and B, for many years the most widely used tests for *C. difficile* infection diagnosis, were perhaps not as sensitive as previously believed at a time when attributed deaths to *C. difficile* infections were showing a remarkable rise. Second, the availability of FDA-approved commercial and laboratory-developed PCR assays which could detect toxigenic strains of *C. difficile* provided a novel and promising testing approach for diagnosing this infection. In this point-counterpoint on the laboratory diagnosis of *C. difficile* infection, we have asked two experts in *C. difficile* infection diagnosis, Ferric Fang, who has recently published two articles in the *Journal of Clinical Microbiology* advocating the use of PCR as a standalone test (see this author's references 12 and 28), and Mark Wilcox, who played a key role in developing the IDSA/SHEA guidelines on *Clostridium difficile* infection (see Wilcox and Planche's reference 1), along with his colleague, Tim Planche, to address the following question: what is the current role of algorithmic approaches to the diagnosis of *C. difficile* infection?

Ways to combine tests

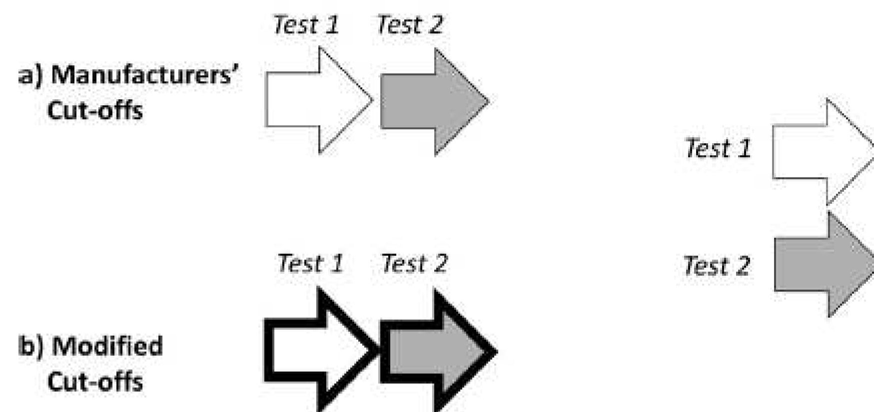


FIG. 1. Schema of the possible methods of combining *C. difficile* tests.

algorithm sensitivity = $sens_{test1} \times sens_{test2}$ and algorithm specificity = $spec_{test1} + [spec_{test\ 2} \times (1 - spec_{test1})]$, where "sens" is sensitivity and "spec" is specificity.

COUNTERPOINT

"Fast is fine but accuracy is everything."
—Wyatt Earp

Molecular assays are the simplest approach to *C. difficile* diagnosis. A prompt and accurate diagnosis of CDI saves money (7) and almost certainly saves lives as well. Although the cost of molecular testing is a concern for many laboratories, the rapid detection of additional CDI cases can offset laboratory expenses by reducing hospitalization and *C. difficile* transmission, along with the need for repeat testing (12, 32). Molecular assays for CDI combine sensitivity, specificity, speed, and convenience in a single test (Table 1). Prevention of *C. difficile* infections and the treatment of relapsing or fulminant colitis remain *tres difficile*. But with molecular methods, the diagnosis of *C. difficile*, *c'est simple*!

TABLE 1. Diagnostic assays for toxigenic *Clostridium difficile*[a]

Assay	Sensitivity	Specificity	Speed	Convenience	Economy
Cytotoxin neutralization	++	++++	++	++	+++
Toxigenic culture	++++	++++	+	+	+++
Toxin EIA	+	++++	++++	++++	++++
GDH-based algorithm	+++	++++	++/++++	+++	+++
Molecular assay (PCR, LAMP)	++++	++++	+++	+++	++

[a] Each feature is rated from least (+) to most (++++) desirable.

Clostridium difficile testing algorithms: What is practical and feasible?

Monica L. Schmidt [a], Peter H. Gilligan [a,b,c,*]

Anaerobe 15 (2009) 270–273

There has been renewed interest in the laboratory diagnosis of *Clostridium difficile* infections due in large measure to the increase in both numbers and severity of cases of this disease. For the past two decades, enzyme-immunoassays (EIAs) for the detection of first *C. difficile* toxin A and then toxins A and B have been the most widely used diagnostic test for diagnosis of *C. difficile* infections. Recently this diagnostic approach has been called into question by the recognition that a screening test which detects glutamate dehydrogenase, a cell wall antigen of *C. difficile*, was significantly more sensitive than toxins A and B EIAs making it an effective screening test for *C. difficile* infection. Although sensitive, GDH lacks specificity and so if this test was utilized, a confirmatory test to differentiate false positives from true positives was needed. Studies to date have used cytotoxin neutralization or toxigenic culture as confirmatory tests but both of these have their limitations. A testing algorithm using rapid immunochromatographic devices for detection of GDH and toxins A and B as screening tests will give an accurate test result in approximately 90% of specimens within one hour when using cytotoxin neutralization as a reference method. For the other 10% of specimens, a third test would be needed in the algorithm. This test could be cytotoxin neutralization, toxigenic culture, or PCR for toxin or toxin operon genes.

The 'Gold Standard'

Which method is the gold standard for *C. difficile* diagnosis? The detection of *C. difficile* toxins A or B by EIA or CTN, used in combination with toxigenic culture, is the recommendation from the Society for Healthcare Epidemiology of America (SHEA). Toxin production by clinical isolates must be demonstrated if culture is used diagnostically since 20–25% of strains may be non-toxigenic and are not believed to be capable of causing disease [7]. The Infectious Disease Society of America recommends testing for both toxins A and B but offers no specific recommendation on how this should be accomplished [8].

What then is the gold standard for *C. difficile* infection? There are probably three possible approaches that are reasonable. Cytotoxin neutralization, because of its high positive predictive value and good sensitivity, is one possibility. A second could be to use toxigenic culture which is likely to have higher sensitivity but perhaps a lower positive predictive value. A third would be to use cytotoxin neutralization as the initial reference method and for discrepant results, use toxigenic culture as a discriminator [18]. The important point is the reported performance of toxin A/B EIAs or ICD tests for glutamate dehyrogenase (GDH) and/or toxin A/B is likely to be dependent on the reference method used and all curre[illegible] methods have shortcomings.

Use of diagnostic algorithms

Table 3

Comparison GDH as screening test for detection of *Clostridium difficile* with CTN as the reference method.

	Sens (%)	Spec (%)	PPV[a] (%)	NPV[b] (%)
Ticehurst et al.[c]	98%	89.3%	53%	99.7%
Gilligan[d]	100%	90%	53%	100%

[a] Positive predictive value.
[b] Negative predictive value.
[c] From Ref. [19].
[d] From Ref. [23].

Performance characteristics of the Meridian Toxin A/B ICA, TechLab Toxin A/B ICA and Remel Toxin A/B when compared to CTN as the reference method.

Test and result PPV (%)	CTN result		Sensitivity (%)	Specificity (%)	NPV (%)
	Positive	Negative			
TechLab Toxin A/B[a]					
Positive 100%	22	0	68.8%	100%	71.4%
Negative	10	25			
Meridian Toxin A/B					
Positive 100%	23	0	69.7%	100%	71.4%
Negative	10	25			
Remel Toxin A/B					
Positive 100%	25	0	75.8%	100%	75.8%
Negative	8	25			

[a] 57 total specimens tested due to one sample with insufficient quantity.

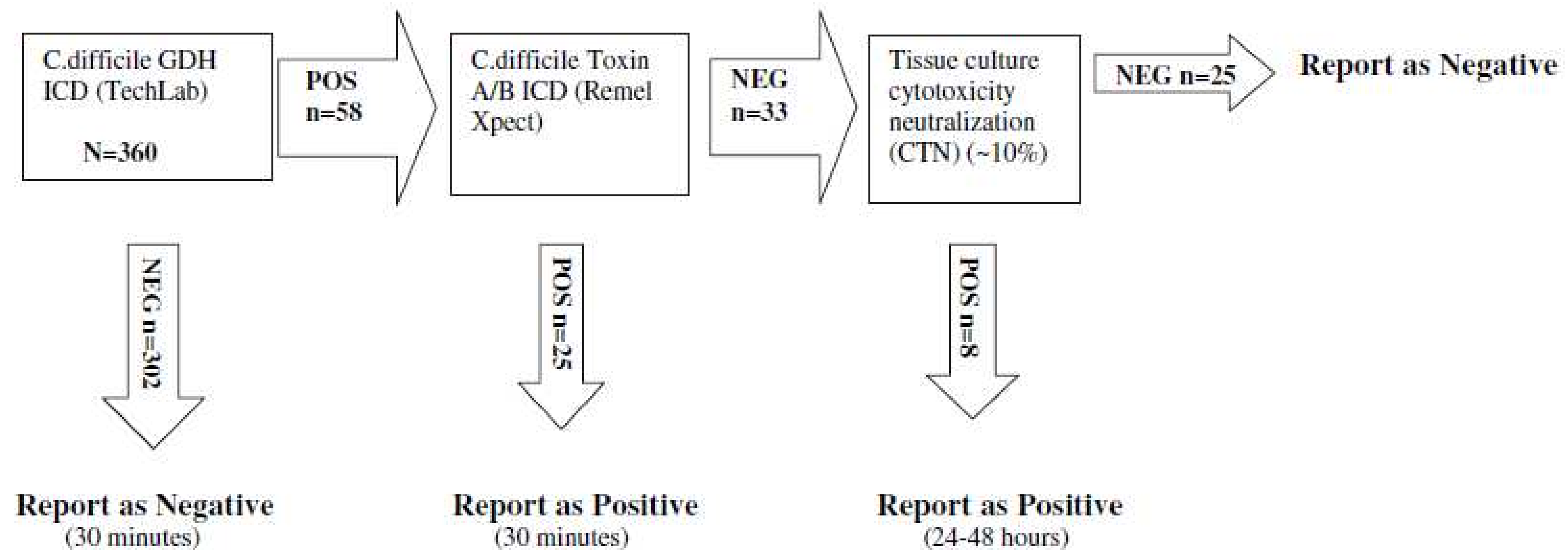


Fig. 1. Proposed three-step algorithm with Remel Xpect *C. difficile* toxin A/B ICD.

The PCR
assay for toxin or toxin operon genes is a simple cartridge assay but the cost is expensive and reimbursement may fall short. One option would be for the small community hospitals and long-term care facilities perform the combination GDH–Toxin A/B ICD then send out the 8–10% of discordant samples to a reference laboratory for PCR testing. Even with the potential for PCR to change our approach to C. difficile diagnosis, there may continue to be a place for the algorithmic approach to diagnosing C. difficile disease.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

The role of glutamate dehydrogenase for the detection of *Clostridium difficile* in faecal samples: a meta-analysis

Journal of Hospital Infection 77 (2011) 1–6

N. Shetty*, M.W.D. Wren, P.G. Coen

Clostridium difficile causes a serious, occasionally fatal, hospital-acquired infection. The laboratory diagnosis of *C. difficile* infection (CDI) needs to be accurate to ensure optimal patient management, infection control and reliable surveillance. Commercial enzyme-linked immunosorbent assays for *C. difficile* toxins have poor sensitivity when compared with cell culture cytotoxin assay (CTA) and toxigenic culture (TC). We performed a meta-analysis of the role of glutamate dehydrogenase (GDH) in diagnosis of CDI. We analysed 21 papers, of which eight were excluded. We included publications of original research that used a 'gold standard' reference test (either CTA or TC). We also included publications that used culture without toxin testing of the isolate as a reference test even though this is not recognised as a gold standard. Exclusion criteria were failure to use a gold standard reference test and where the index test was used as the gold standard. Significant heterogeneity between study results justified the summary receiver operating characteristic (SROC) analysis. The meta-analysis demonstrated high diagnostic accuracy of GDH for the presence of *C. difficile* in faeces; when compared with culture it achieved a sensitivity and specificity of >90%. The SROC plot confirmed this finding. As a surrogate for toxigenic strains the GDH yields a specificity of 80–100% with a false positivity rate of ~20%, as it detects toxigenic and non-toxigenic strains of the organism. However, GDH test has high sensitivity and negative predictive value and would be a powerful test in a dual testing algorithm when combined with a test to detect toxin.

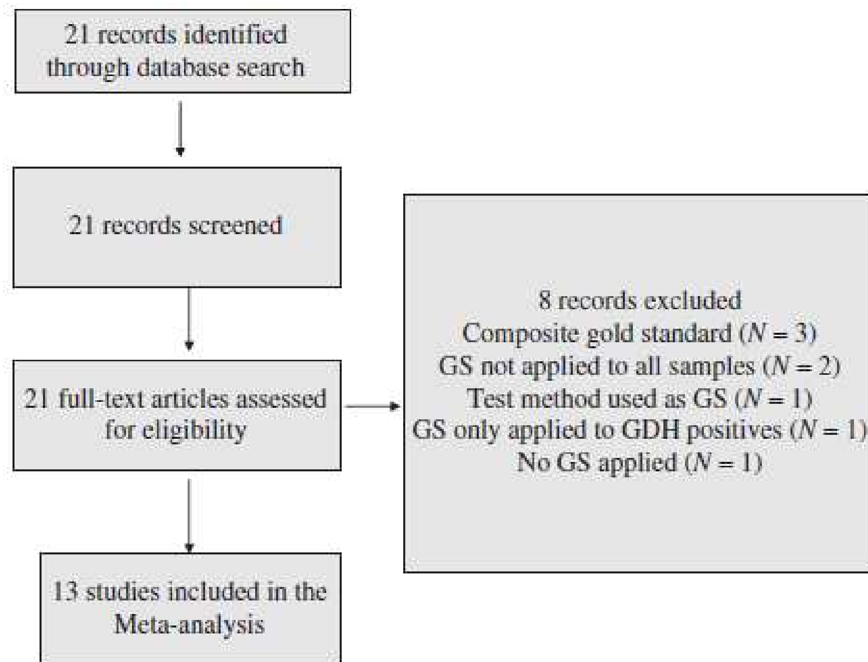


Figure 1. Selection process summary and reasons for exclusion. GS, gold standard; GDH, glutamate dehydrogenase.

The SROC analysis also shows the high diagnostic sensitivity and low false positivity rate of GDH when compared with culture. To use it as a surrogate for toxin-producing strains, a faecal sample tested by the GDH test must also be subjected to a more specific second test to detect toxin.

There is still much debate over which second test to use; CTA, TC, or a molecular test for the *tcd*B gene. Each has its advantages and disadvantages.[10] In addition, samples with a positive GDH test but a negative confirmatory toxin test may require a third test to resolve the discrepant result. This will inevitably happen; it may occur as a result of colonisation with a non-toxigenic strain or the faeces may contain a toxigenic isolate but no toxin is detected because the isolate is making no contribution to diarrhoea in the patient. Further prospective work is required to resolve these issues.

This paper shows that the GDH test has excellent concordance when compared with culture for the presence of the organism in faeces. Based on the data reported in this study, coupled with our own experience in the laboratory, we support the use of a dual test approach for testing for CDI to increase diagnostic accuracy. Our recommendation would be to use the GDH test as part of a diagnostic algorithm for CDI coupled with a reliable test for toxin. Discrepant results need to be confirmed by a recognised gold standard reference test for faecal toxin or the toxin gene.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Positive predictive value

1
0.8
0.6
0.4
0.2
0

0
0.2
0.4
0.6
0.8
1

C. difficile prevalence

Figure 2. Positive predictive value of the glutamate dehydrogenase test plotted against local *C. difficile* prevalence (error bars are 95% confidence intervals) with logistic regression fit (line).

UNIVERSITÀ CAMPUS BIO-MEDICO DI ROMA

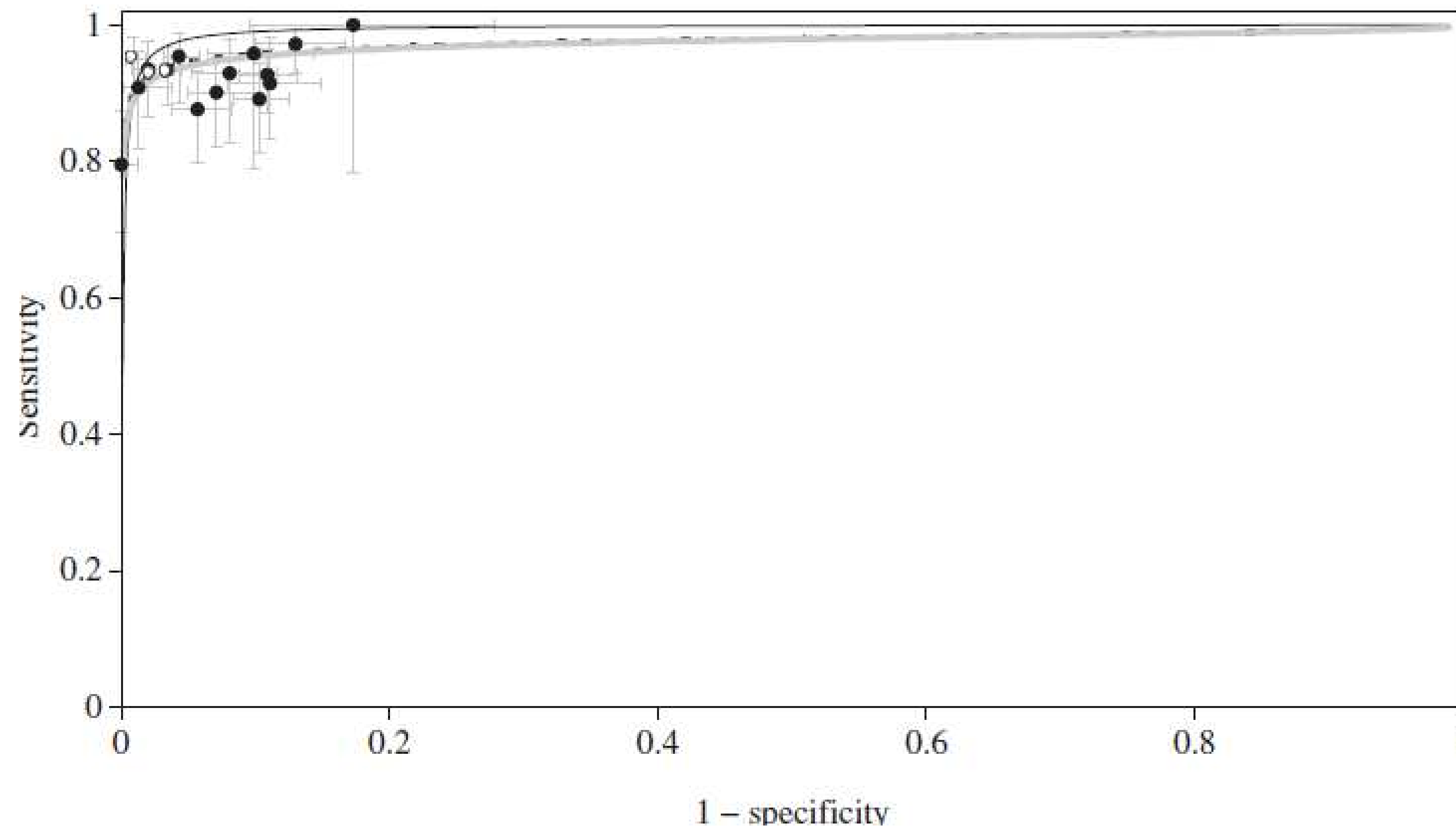
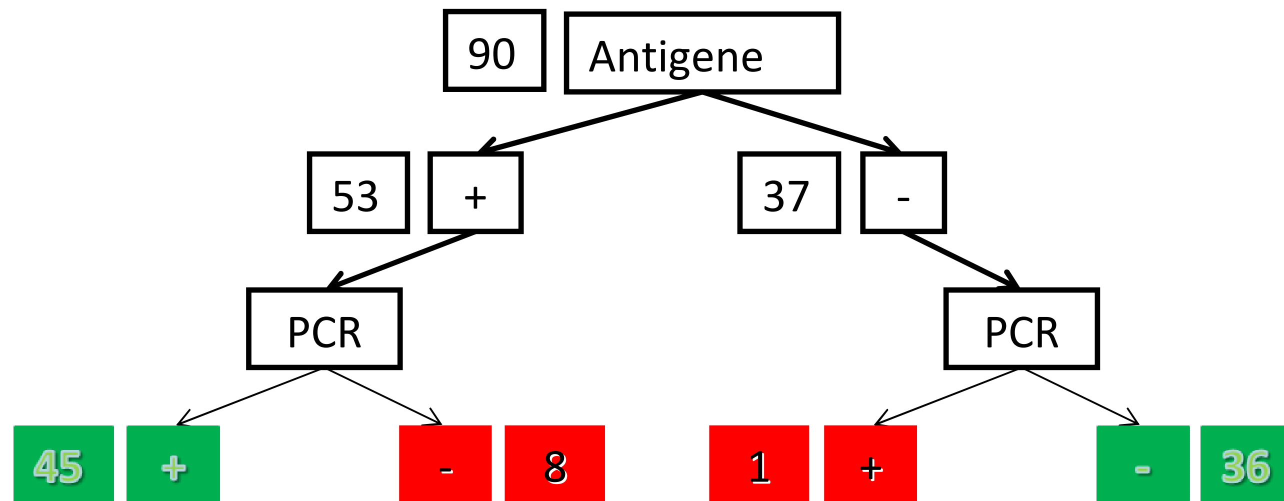


Figure 4. Summary receiver operating characteristic curve (data and parametric model fit): for sensitivity of glutamate dehydrogenase versus false positives. Hollow markers are from studies based on the culture 'gold standard' and show high sensitivity and low false positivity. Solid circles are based on cell culture cytotoxin assay (CTA) and t (TC) as reference standard, demonstrating up to 20% false positive rate. Grey line (unweighted analysis, all data), black line (weighted, cluster linear regression analy (weighted, cluster linear regression analysis, only for CTA and TC studies). Error bars are 95% confidence intervals.

Una mia esperienza sulla diagnostica di infezione da *C. difficile*

IMPEGNO ECONOMICO PER IL CAMBIAMENTO

- Tossina A e B nelle Feci: 28 € cad.
- Antigene Comune 9 € cad.
- PCR 77 € cad.



81 Concordanti

9 Discordanti

8 Ag+ PCR –

1 Ag- PCR +

Tutti in campioni ripetuti

III esempio : infezioni a coltura negativa

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Broad-Range 16S rRNA Gene Polymerase Chain Reaction for Diagnosis of Culture-Negative Bacterial Infections

Silvana K. Rampini, Guido V. Bloemberg, Peter M. Keller, Andrea C. Buchler,Gunter Dollenmaier, Roberto F. Speck, and Erik C. Bottger

Background. Broad-range 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) is used for detection and identification of bacterial pathogens in clinical specimens from patients with a high suspicion for infection. However, prospective studies addressing the impact and clinical value of broad-range bacterial 16S rRNA gene amplification for diagnosis of acute infectious diseases in nonselected patient populations are lacking.

Methods. We first assessed the diagnostic performance of 16S rRNA gene PCR compared with routine bacterial culture. Second, we addressed prospectively the impact and clinical value of broad-range PCR for the diagnosis of acute infections using samples that tested negative by routine bacterial culture; the corresponding patients' data were evaluated by detailed medical record reviews.

Results. Results from 394 specimens showed a high concordance of >90% for 16S rRNA gene PCR and routine bacterial culture, indicating that the diagnostic performance of PCR for acute bacterial infections is comparable to that of bacterial culture, which is currently considered the gold standard. In thisprospective study, 231 specimens with a negative result on routine bacterial culture were analyzed with PCR, and patients' clinical data were reviewed. We found that broad-range 16S rRNA gene PCR showed a sensitivity, specificity, positive predictive value, and negative predictive value of 42.9%, 100%, 100%, and 80.2% for culture-negative bacterial infections.

Conclusions. This study defines the role of 16S rRNA gene PCR for diagnosis of culture-negative b[...] infections. Our data show that 16S rRNA gene PCR is particularly useful for identification of bacterial patho[...] patients pretreated with antibiotics.

Clinical Infectious Diseases 2011:53(12):1245–51

Table 1. Broad-Range 16S rRNA Gene PCR Compared With Conventional Culture

		Culture	
		+	–
PCR	+	86 (21.8%)	18 (4.6%)
	–	19 (4.8%)	271 (68.8%)

A total of 394 clinical specimens were included in the study. For bacterial identification see Table 2.

Abbreviations: PCR, polymerase chain reaction; –, negative; +, positive.

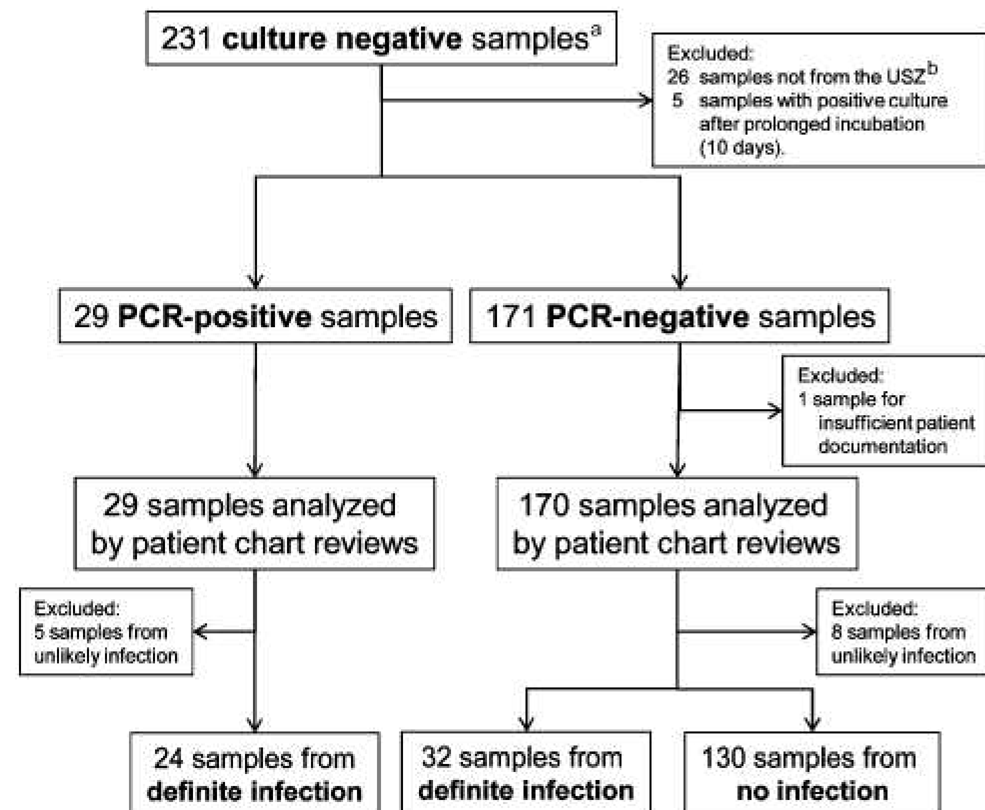


Figure 1. Enrollment of specimens in the clinical study. [a]Culture negative for 3 days. [b]USZ, University Hospital Zürich. PCR, chain reaction.

Table 3. Summary of Culture-Negative, Polymerase Chain Reaction-Positive Specimens in the Clinical Study

Clinical specimen		Bacteria observed by microscopy (+, ++, +++[a])	Leukocytes observed by microscopy (+, ++, +++[b])	Bacterial species identified by 16S rRNA gene PCR	Days after end of antibiotic therapy/days undergoing antibiotic therapy[c]
1	Aortic valve	nd	+	*Streptococcus mutans*	0/32
2	Mitral valve	nd	++	*Enterococcus faecalis*	0/54
3	Deep wound (swab)	++ (Gram-positive cocci)	+++	*Parvimonas micra*	0/1
4	Cerebrospinal fluid	++ (Gram-positive cocci)	+++	*Staphylococcus aureus*	0/2
5	Sternal wound (swab)	nd	+++	*Staphylococcus epidermidis*	0/3
6	Cerebrospinal fluid	+++	nd	*S. epidermidis*	0/16
7	Mitral valve	nd	+	*S. aureus*	0/48
8	Abscess (brain)[d]	nd	nd	*Fusobacterium nucleatum* *Porphyromonas endodontalis*	0/7
9	Abscess (brain)[d]	nd	nd	*P. endodontalis*	0/7
10	Aspirate (shoulder)	nd	+++	*Streptococcus dysgalactiae*	20/39
11	Abscess (brain)	+ (Gram-positive cocci)	+++	*Streptococcus intermedius*	0/12
12	Aortic valve	+++ (Gram-positive cocci)	+	*Streptococcus* sp (*Streptococcus mitis* group)	0/2
13	Pleural effusion	nd	+++	*P. endodontalis*	0/28
14	Sternal wound (swab)	nd	+	*S. epidermidis*	0/49
15	Aspirate (knee)	nd	+++	Enterobacteriacea	0/5
16	Tissue	nd	+++	*S. aureus*	0/3
17	Tissue	nd	+++	*S. aureus*	0/3
18	Abscess (psoas muscle)	nd	++	*Coxiella burnetii*	0/1
19	Tissue (aneurysma)	nd	+	*C. burnetii*	0/7
20	Aortic valve	nd	+++	*Streptococcus* sp. (*S. mitis* group)	21/42
21	Mitral valve	nd	+	*S. mitis*	0/25
22	Sternal wound (swab)	nd	+	*Ureaplasma urealyticum*	0/4
23	Sternal wound (swab)	nd	+	*U. urealyticum*	0/4
24	Sternal wound (swab)	nd	++	*U. urealyticum*	0/4

Abbreviations: nd, not detected; PCR, polymerase chain reaction; pos, positive; rRNA, ribosomal RNA.

[a] Bacterial cell count in ten 1000-fold visual fields: +, <5; ++, 5–25; +++, >25.

[b] Leukocyte count in ten 100-fold magnified fields: +, 1–9; ++, 10–25; +++, >25.

[c] 0/32 indicates that the specimen was obtained while the patient was under antibiotic therapy and had received antibiotic therapy for the past 32 days; 20/39 indicates that the specimen was retrieved 20 days after an antibiotic therapy of 39 days; 0/1 indicates that the specimen was retrieved at the start of antibiotic therapy.

[d] This case was recently published in detail [17].

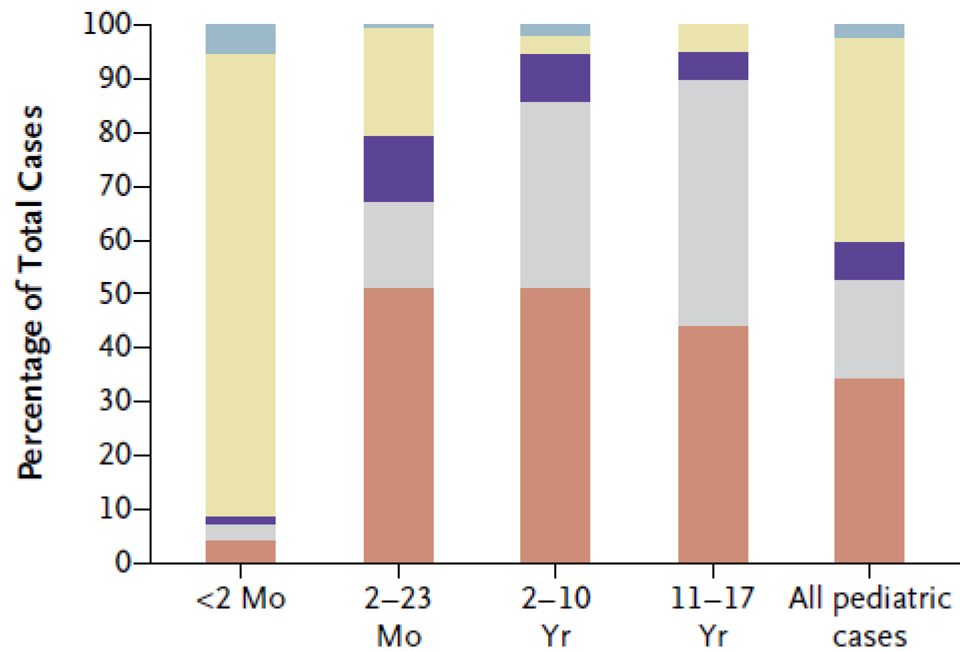
In summary, this study shows that there is a high concordance between broad-range 16S rRNA gene PCR and culture in pathogen detection and that broad-range PCR is particularly valuable for patients under antibiotic therapy. On the basis of our data, we propose that broad-range PCR of samples from primary sterile body sites should be done for patients with a high clinical suspicion of infection and negative culture results. The suspicion for infections with difficult-to-culture pathogens justifies a broad-range PCR on its own, in particular when a species-specific molecular test is not available. Implementing an algorithm that excludes culture-positive samples is both diagnostically and economically feasible.

Altra sindrome : meningite batterica

Neisseria meningitidis, *Haemophilus influenzae*, and *Streptococcus pneumoniae* are important causes of meningitis and other infections, and rapid, sensitive, and specific laboratory assays are critical for effective public health interventions. Singleplex real-time PCR assays have been developed to detect *N. meningitidis ctrA*, *H. influenzae hpd*, and *S. pneumoniae lytA* and serogroup-specific genes in the *cap* locus for *N. meningitidis* serogroups A, B, C, W135, X, and Y. However, the assay sensitivity for serogroups B, W135, and Y is low. We aimed to improve assay sensitivity and develop multiplex assays to reduce time and cost. New singleplex real-time PCR assays for serogroup B *synD*, W135 *synG*, and Y *synF* showed 100% specificity for detecting *N. meningitidis* species, with high sensitivity (serogroup B *synD*, 99% [75/76]; W135 *synG*, 97% [38/39]; and Y *synF*, 100% [66/66]). The lower limits of detection (LLD) were 9, 43, and 10 copies/reaction for serogroup B *synD*, W135 *synG*, and Y *synF* assays, respectively, a significant improvement compared to results for the previous singleplex assays. We developed three multiplex real-time PCR assays for detection of (i) *N. meningitidis ctrA*, *H. influenzae hpd*, and *S. pneumoniae lytA* (NHS assay); (ii) *N. meningitidis* serogroups A, W135, and X (AWX assay); and (iii) *N. meningitidis* serogroups B, C, and Y (BCY assay). Each multiplex assay was 100% specific for detecting its target organisms or serogroups, and the LLD was similar to that for the singleplex assay. Pairwise comparison of real-time PCR between multiplex and singleplex assays showed that cycle threshold values of the multiplex assay were similar to those for the singleplex assay. There were no substantial differences in sensitivity and specificity between these multiplex and singleplex real-time PCR assays.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance



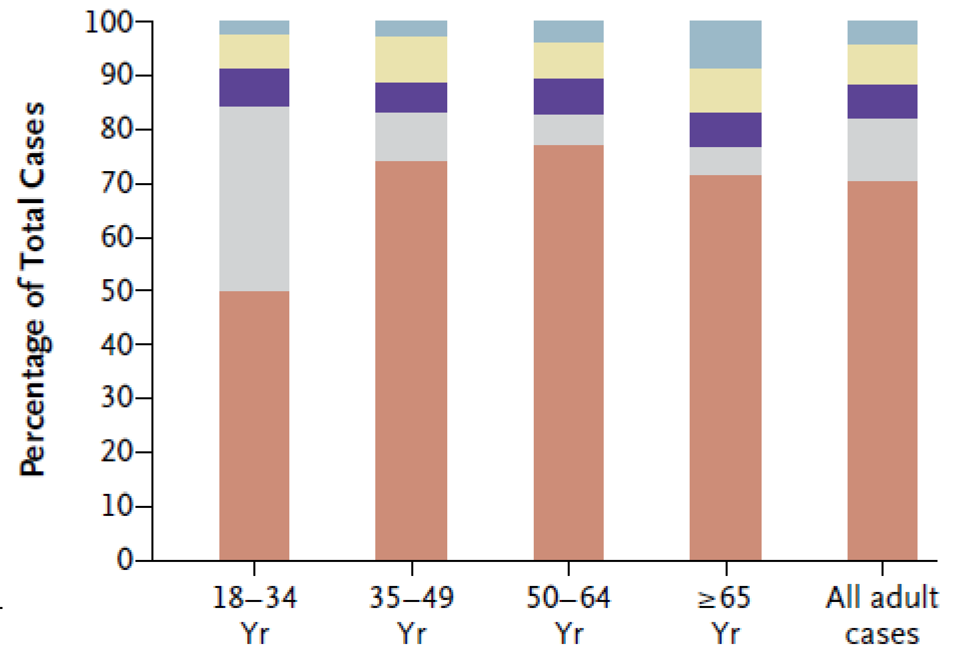


TABLE 4 Sensitivities and specificities of the multiplex and singleplex real-time PCR assays for detection of meningitis pathogens from clinical specimens

Specimen group (reference standard)	Assay (type)[a]	No. positive/no. tested	Sensitivity (95% CI)	No. negative/no. tested	Specificity (95% CI)
Lackland (*fucK* PCR)	*H. influenzae hpd* (M)	206/244	84.4 (79.3–88.7)	217/243	89.3 (84.7–92.9)
	H. influenzae hpd (S)	206/244	84.4 (79.3–88.7)	218/243	89.7 (85.2–93.2)
Brazil (CIE[b])	*N. meningitidis ctrA* (M)	72/75	96.0 (88.8–99.2)	8/24	33.3 (15.6–55.3)
	N. meningitidis ctrA (S)	72/75	96.0 (88.8–99.2)	9/24	37.5 (18.8–59.4)
	Serogroup C *synE* (M)	71/75	94.7 (86.9–98.5)	17/24	70.8 (48.9–87.4)
	Serogroup C *synE* (S)	72/75	96.0 (88.8–99.2)	17/24	70.8 (48.9–87.4)
South Africa (composite[c])	*N. meningitidis ctrA* (M)	56/76	73.7 (62.3–83.1)	43/44	97.7 (88.0–99.9)
	N. meningitidis ctrA (S)	58/76	76.3 (65.2–85.3)	37/44	84.1 (69.9–93.4)
	H. influenzae hpd (M)	16/21	76.2 (52.8–91.8)	98/99	99.0 (94.5–100.0)
	H. influenzae hpd (S)	18/21	85.7 (63.7–97.0)	91/99	91.9 (84.7–96.4)
	S. pneumoniae lytA (M)	16/17	94.1 (71.3–99.8)	102/103	99.0 (94.7–100.0)
	S. pneumoniae lytA (S)	16/17	94.1 (71.3–99.8)	96/103	93.2 (86.5–97.2)

[a] M, multiplex; S, singleplex.

[b] One specimen was not tested by CIE and was therefore excluded from this analysis.

[c] Combined laboratory tests (culture and/or latex agglutination) were used as the composite reference standard, where a positive result was defined as culture or latex agglutination positive (negative otherwise). One specimen was not tested by culture or latex agglutination and was therefore excluded from this analysis.

Altra sindrome: meningoencefalite virale

Table 1. Positive results of viral PCR of CSF samples.

Virus	No. of patients with positive PCR results	
	⩾16 years of age (n = 59)	<16 years of age (n = 50)
Enterovirus	24	41
Herpes simplex virus type 1	4	1
Herpes simplex virus type 2	10	1
Varicella zoster virus	10	5
Mumps	4	1
Epstein-Barr virus	7	0
Human herpes virus 6	0	1

Table 3. Laboratory results for patients with enterovirus, herpes simplex type 2 (HSV-2), or varicella zoster virus (VZV) infection.

Laboratory result	Patients with enterovirus infection (n = 22)	Patients with HSV-2 infection (n = 8)	Patients with VZV infection (n = 8)
CSF			
Leukocyte count, $\times 10^6$ leukocytes/L[a]	51 (0–1298)	240 (180–2200)	207 (6–450)
Lymphocyte percentage[b]	91 (5–100)	100 (80–100)	100 (90–100)
Protein level, mg/L[c]	640 (100–875)	1205 (611–3704)	974 (581–2616)
CSF:serum glucose ratio[d]	0.6 (0.26–0.76)	0.48 (0.47–0.67)	0.55 (0.4–0.73)
Peripheral blood			
Leukocyte count, $\times 10^9$ leukocytes/L[e]	6.6 (3.6–12.2)	7.9 (5.5–13.9)	7.7 (5.6–15.6)
C-reactive protein level, mg/L[f]	15 (2.1–112.3)	4.6 (2–28)	6.2 (2–22.9)

NOTE. Data are median value (range).

[a] $P<.01$ for the comparison between patients with enterovirus and patients with HSV-2 by 2-tailed Mann-Whitney U test.

[b] Data are for 15 patients with enterovirus infection and 7 patients with VZV infection.

[c] $P<.001$ for the comparison between patients with enterovirus infection and patients with HSV-2 infection, and $P<.01$ for the comparison between patients with enterovirus infection and patients with VZV infection, by 2-tailed Mann-Whitney U test.

[d] Data are for 16 patients with enterovirus infection and 6 patients with VZV infection.

[e] Data are for 21 patients with enterovirus infection and 7 patients with HSV-2 infection.

[f] Data are for 7 patients with HSV-2 infection.

Altra sindrome: Endocardite a coltura negativa

High Frequency of Tropheryma whipplei in Culture-Negative Endocarditis

Walter Geißdörfer, Verena Moos, Annette Moter, Christoph Loddenkemper, Andreas Jansen, René Tandler, Andreas J. Morguet, Florence Fenollar, Didier Raoult, Christian Bogdan and Thomas Schneider
J. Clin. Microbiol. 2012, 50(2):216. DOI: 10.1128/JCM.05531-11.
Published Ahead of Print 30 November 2011.

"Classical" Whipple's disease (cWD) is caused by *Tropheryma whipplei* and is characterized by arthropathy, weight loss, a diarrhea. *T. whipplei* infectious endocarditis (TWIE) is rarely reported, either in the context of cWD or as isolated TWIE w out signs of systemic infection. The frequency of TWIE is unknown, and systematic studies are lacking. Here, we performe observational cohort study on the incidence of *T. whipplei* infection in explanted heart valves in two German university ce Cardiac valves from 1,135 patients were analyzed for bacterial infection using conventional culture techniques, PCR ampli tion of the bacterial 16S rRNA gene, and subsequent sequencing. *T. whipplei*-positive heart valves were confirmed by speci PCR, fluorescence *in situ* hybridization, immunohistochemistry, histological examination, and culture for *T. whipplei*. Ba endocarditis was diagnosed in 255 patients, with streptococci, staphylococci, and enterococci being the main pathogens. *T. whipplei* was the fourth most frequent pathogen, found in 16 (6.3%) cases, and clearly outnumbered *Bartonella quintana*, *iella burnetii*, and members of the HACEK group (*Haemophilus* species, *Actinobacillus actinomycetemcomitans*, *Cardioba rium hominis*, *Eikenella corrodens*, and *Kingella kingae*). In this cohort, *T. whipplei* was the most commonly found patho associated with culture-negative infective endocarditis.

Total 2000-2007; **1135 patients with valve replacement surgery**

Cohort I Erlangen 2000-2004	Cohort II Erlangen 2004-2007	Cohort III Berlin 2001-2006
878 unselected consecutive patients	**128 patients with suspected/possible IE**	**129 patients with suspected/possible IE**

↓

Gram-stain, culture, pan-bacterial PCR and sequencing

↓

Cohort I	Cohort II	Cohort III
84 valves with detection of microorganisms	**68 valves** with detection of microorganisms	**103 valves** with detection of microorganisms including **66 valves** confirmed by fluorescence *in situ* hybridisation (FISH)
6 patients with TWIE	**5 patients with TWIE**	**5 patients with TWIE**
T. whipplei-specific PCR (with *T. whipplei*-positive PCR/sequencing) confirmed all 11 cases		*T. whipplei*-specific PCR (129 samples) confirmed all 5 cases

↓

16 TWIE patients

FIG 1 Details of the study cohorts.

Una sindrome in cui neanche le nuove metodiche danno per ora un risultato accettabile: le infezioni respiratorie profonde

Diagnostic Techniques and Procedures for Establishing the Microbial Etiology of Ventilator-Associated Pneumonia for Clinical Trials: The Pros for Quantitative Cultures

Clinical Infectious Diseases 2010;51(S1):S88–S92

Jean Chastre, Jean-Louis Trouillet, Alain Combes, and Charles-Edouard Luyt

POTENTIAL LIMITATIONS OF QUANTITATIVE TECHNIQUES

Four studies using a protocol based on postmortem lung biopsies have suggested that, in the presence of prior antibiotic treatment, many patients with histopathologic signs of pneumonia have no or only minimal growth on bronchoscopic specimens cultures, casting some doubt on the ability of invasive techniques for diagnosing VAP [13, 34–36]. For example, in a study involving 30 patients who died during receipt of mechanical ventilation after having received prior antibiotic treatment, Torres et al [37] found that quantitative bacterial cultures of PSB and BAL fluid specimens had low sensitivity (36% and 50%, respectively) and low specificity (50% and 45%, respectively) and could not differentiate between the histologic absence or presence of pneumonia.

Unfortunately, several methodological constraints specific to the evaluation of procedures used in the diagnosis of bacterial pneumonia were not respected in these studies [13, 34–37]. None of them excluded patients who had received new anti-

Although a general consensus has emerged on the use of 1×10^3 cfu/mL as the cutoff for culture of PSB specimens and 1×10^4 cfu/mL for culture of BAL fluid specimens, concern has been raised about the reproducibility of these results, particularly near the diagnostic thresholds. Three groups [41–43] have concluded that, although in vitro repeatability is excellent and in vivo qualitative recovery is 100%, quantitative results are more variable. For 14%–17% of patients, results of culture of replicate samples decreased on both sides of the 1×10^3 cfu/mL threshold, and results varied by >1 $\log^{10}$ for 59%–67% of samples. Finally, many microbiology laboratories may not be

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

The Argument against Using Quantitative Cultures in Clinical Trials and for the Management of Ventilator-Associated Pneumonia

Clinical Infectious Diseases 2010;51(S1):S93–S99

Michael S. Niederman

Quantitative cultures have been proposed as the most accurate way to both establish the presence of ventilator-associated pneumonia (VAP) and define the etiologic pathogen. Although the clinical diagnosis of VAP has been much maligned, it may be very accurate, particularly if it is objectively defined by calculating the Clinical Pulmonary Infection Score and if the score incorporates a Gram stain of a lower respiratory tract sample. After the clinical diagnosis of VAP is made, a culture is needed to identify the etiologic pathogen, but this culture does not need to be quantitative or bronchoscopic. Quantitative culture-based diagnosis may not be more accurate than clinical diagnosis, and quantitative cultures have a number of methodologic limitations that can cause both false-positive and false-negative results. Finally, a number of studies have suggested that clinical management without quantitative cultures may be accurate and that outcomes, such as mortality and change in antibiotics to a focused regimen, are not improved by the use of quantitative cultures. In clinical

Altra sindrome :Ascesso cerebrale

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, June 2010, p. 2250–2252 Vol. 48, No. 6
0095-1137/10/$12.00 doi:10.1128/JCM.01922-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Detection of a Mixed Infection in a Culture-Negative Brain Abscess by Broad-Spectrum Bacterial 16S rRNA Gene PCR[▽]†

Peter M. Keller,[1]§ Silvana K. Rampini,[1,2]§ and Guido V. Bloemberg[1]*

TABLE 1. Analysis of patient samples taken from the cerebral abscess

Sample	Microscopy results		Culture result[c]	Identification by bacterial 16S rRNA gene PCR analysis	No. of mismatches/ total no.	% Homology
	Leukocytes[a]	Microorganisms[b]				
I	>25 leukocytes	Negative	Negative	*Fusobacterium nucleatum*	1/499	99.8
				Porphyromonas endodontalis	1/507	99.8
II	Negative	Negative	Negative	ND[d]		
III	Negative	Negative	Negative	*Fusobacterium nucleatum*	1/499	99.8
				Porphyromonas endodontalis	1/5071	99.8

[a] Average of 10 visual fields at a 100× magnification.
[b] Average of 10 visual fields at a 1,000× magnification.
[c] Samples were streaked on seven different agar media (Columbia blood agar, MacConkey agar, CNA blood agar, Crowe agar, brucella agar, kanamycin-vancomycin agar, and phenylethyl alcohol agar) and incubated under aerobic and anaerobic conditions as described in Materials and Methods.
[d] ND, not done due to insufficient sample volume.

JOURNAL OF CLINICAL MICROBIOLOGY, June 2010, p. 2268–2270 Vol. 48, No. 6
0095-1137/10/$12.00 doi:10.1128/JCM.00166-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Multicenter Evaluation of a New Shortened Peptide Nucleic Acid Fluorescence *In Situ* Hybridization Procedure for Species Identification of Select Gram-Negative Bacilli from Blood Cultures▽

Margie Morgan,[1] Elizabeth Marlowe,[2] Phyllis Della-Latta,[3] Hossein Salimnia,[4]
Susan Novak-Weekley,[2] Fann Wu,[3] and Benjamin S. Crystal[5]*

A shortened protocol for two peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) assays for the detection of Gram-negative bacilli from positive blood cultures was evaluated in a multicenter trial. There was 100% concordance between the two protocols for each assay (368 of 368 and 370 of 370 results) and 99.7% (367 of 368 and 369 of 370 results) agreement with routine laboratory techniques.

Enhanced Diagnosis of Pneumococcal Meningitis with Use of the Binax NOW Immunochromatographic Test of *Streptococcus pneumoniae* Antigen: A Multisite Study

Jennifer C. Moïsi, Samir K. Saha, Adegoke G. Falade, Berthe-Marie Njanpop-Lafourcade, Joseph Oundo, Anita K. M. Zaidi, Shirin Afroj, R. A. Bakare, Julie K. Buss, Razzaq Lasi, Judith Mueller, A. A. Odekanmi, Lassana Sangaré, J. Anthony G. Scott, Maria Deloria Knoll, Orin S. Levine, and Bradford D. Gessner

GAVI Alliance's PneumoADIP Surveillance Network

Clinical Infectious Diseases 2009;48:S49–56

Background. Accurate etiological diagnosis of meningitis in developing countries is needed, to improve clinical care and to optimize disease-prevention strategies. Cerebrospinal fluid (CSF) culture and latex agglutination testing are currently the standard diagnostic methods but lack sensitivity.

Methods. We prospectively assessed the utility of an immunochromatographic test (ICT) of pneumococcal antigen (NOW *Streptococcus pneumoniae* Antigen Test; Binax), compared with culture, in 5 countries that are conducting bacterial meningitis surveillance in Africa and Asia. Most CSF samples were collected from patients aged 1–59 months.

Results. A total of 1173 CSF samples from suspected meningitis cases were included. The ICT results were positive for 68 (99%) of the 69 culture-confirmed pneumococcal meningitis cases and negative for 124 (99%) of 125 culture-confirmed bacterial meningitis cases caused by other pathogens. By use of culture and latex agglutination testing alone, pneumococci were detected in samples from 7.4% of patients in Asia and 15.6% in Africa. The ICT increased pneumococcal detection, resulting in similar identification rates across sites, ranging from 16.2% in Nigeria to 20% in Bangladesh. ICT detection in specimens from culture-negative cases varied according to region (8.5% in Africa vs. 18.8% in Asia; $P < .001$), prior antibiotic use (24.2% with prior antibiotic use vs. 12.2% without; $P < .001$), and WBC count (9.0% for WBC count of 10–99 cells/mL, 22.1% for 100–999 cells/mL, and 25.4% for ⩾1000 cells/mL; $P < .001$ by test for trend).

Conclusions. The ICT provided substantial benefit over the latex agglutination test and culture at Asian sites but not at African sites. With the addition of the ICT, the proportion of meningitis cases attributable to pneumococci was determined to be similar in Asia and Africa. These results suggest that previous studies have underestimated the proportion of pediatric bacterial meningitis cases caused by pneumococci.

Altra sindrome: esempio infezioni delle vie urinarie

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

JOURNAL OF CLINICAL MICROBIOLOGY, June 2010, p. 2110–2115 Vol. 48, No. 6
0095-1137/10/$12.00 doi:10.1128/JCM.02215-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Direct Identification of Urinary Tract Pathogens from Urine Samples by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry$^{\nabla}$

Laura Ferreira,[1]† Fernando Sánchez-Juanes,[1]† Magdalena González-Ávila,[2] David Cembrero-Fuciños,[3] Ana Herrero-Hernández,[2] José Manuel González-Buitrago,[1,4]‡ and Juan Luis Muñoz-Bellido[2,5]‡*

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been suggested as a reliable method for bacterial identification from cultures. Direct analysis of clinical samples might increase the usefulness of this method, shortening the time for microorganism identification. We compared conventional methods for the diagnosis of urinary tract infections (UTIs) and identification of the urinary tract pathogens (automated screening, plate cultures, and identification based on biochemical characteristics) and a fast method based on conventional screening and MALDI-TOF MS. For this latter method, 4 ml of urine was centrifuged at a low-revolution setting (2,000 × *g*) to remove leukocytes and then at high revolutions (15,500 × *g*) to collect bacteria. The pellet was washed and then applied directly to the MALDI-TOF MS plate. Two hundred sixty urine samples, detected as positive by the screening device (UF-1000i), were processed by culture and MALDI-TOF MS. Twenty samples were positive in the screening device but negative in culture, and all of them were also negative by MALDI-TOF MS. Two-hundred thirty-five samples displayed significant growth of a single morphological type in culture. Two-hundred twenty of them showed bacterial growth of >10^{5} CFU/ml. Microorganism identifications in this group were coincident at the species level in 202 cases (91.8%) and at the genus level in 204 cases (92.7%). The most frequent microorganism was *Escherichia coli* (173 isolates). MALDI-T MS identified this microorganism directly from the urine sample in 163 cases (94.2%). Our results show t MALDI-TOF MS allows bacterial identification directly from infected urine in a short time, with high accuracy, a especially when Gram-negative bacteria with high bacterial counts are involved.

il caso clamoroso: (rispetto al tempo): tubercolosi polmonare

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

N Engl J Med 2010;363:1005-15.

BACKGROUND

Global control of tuberculosis is hampered by slow, insensitive diagnostic methods, particularly for the detection of drug-resistant forms and in patients with human immunodeficiency virus infection. Early detection is essential to reduce the death rate and interrupt transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect.

METHODS

We assessed the performance of Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), with fully integrated sample processing in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis. Eligible patients in Peru, Azerbaijan, South Africa, and India provided three sputum specimens each. Two specimens were processed with *N*-acetyl-L-cysteine and sodium hydroxide before microscopy, solid and liquid culture, and the MTB/RIF test, and one specimen was used for direct testing with microscopy and the MTB/RIF test.

RESULTS

Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The test was specific in 604 of 609 patients without tuberculosis (99.2%). Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by 12.6 percentage points and a third by 5.1 percentage points, to a total of 90.2%. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria. Sequencing resolved all but two cases in favor of the MTB/RIF assay.

CONCLUSIONS

The MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time. (Funded by the Foundation for Innovative New Diagnostics.)

Comparison of Xpert MTB/RIF with Other Nucleic Acid Technologies for Diagnosing Pulmonary Tuberculosis in a High HIV Prevalence Setting: A Prospective Study

Lesley E. Scott[1]*, Kerrigan McCarthy[2], Natasha Gous[1], Matilda Nduna[3], Annelies Van Rie[4], Ian Sanne[5], Willem F. Venter[2], Adrian Duse[3,6], Wendy Stevens[1,3]

PLoS Med 8(7): e1001061. doi:10.1371/journal.pmed.1001061

Conclusions: The Xpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis* over existing AFB smear microscopy and other molecular methodologies in an HIV- and TB-endemic region. Its place in the clinical diagnostic algorithm in national health programs needs exploration.

Editors' Summary

What Do These Findings Mean? Although these findings are likely to be affected by the study's small size, they suggest that Xpert MTB/RIF may provide a more accurate rapid diagnosis of TB than smear microscopy and other currently available NAAT tests in regions where HIV and TB are endemic (i.e., always present). Indeed, the reported accuracy of Xpert MTB/RIF for TB diagnosis—85% sensitivity and 97% specificity—has the potential to save more than 400,000 lives per year. Taken together with the results of other recent studies (including an accompanying article by Lawn et al. that investigates the use of Xpert MTB/RIF for screening for HIV-associated TB and rifampicin resistance), these findings support the WHO recommendation that Xpert MTB/RIF, rather than smear microscopy, should be the initial test in HIV-infected individuals who might have TB.

Ma l’antibiogramma?

Meglio dire:

Identificazione di resistenze agli antibiotici
Determinazione della sensibilità agli antibiotici

A Real-Time PCR Antibiogram for Drug-Resistant Sepsis

John R. Waldeisen, Tim Wang, Debkishore Mitra, Luke P. Lee* PLoS ONE 6(12): e28528. doi:10.1371/journal.pone.0028528

Current molecular diagnostic techniques for susceptibility testing of septicemia rely on genotyping for the presence of known resistance cassettes. This technique is intrinsically vulnerable due to the inability to detect newly emergent resistance genes. Traditional phenotypic susceptibility testing has always been a superior method to assay for resistance; however, relying on the multi-day growth period to determine which antimicrobial to administer jeopardizes patient survival. These factors have resulted in the widespread and deleterious use of broad-spectrum antimicrobials. The real-time PCR antibiogram, described herein, combines universal phenotypic susceptibility testing with the rapid diagnostic capabilities of PCR. We have developed a procedure that determines susceptibility by monitoring pathogenic load with the highly conserved 16S rRNA gene in blood samples exposed to different antimicrobial drugs. The optimized protocol removes heme and human background DNA from blood, which allows standard real-time PCR detection systems to be employed with high sensitivity (<100 CFU/mL). Three strains of E. coli, two of which were antimicrobial resistant, were spiked into whole blood and exposed to three different antibiotics. After real-time PCR-based determination of pathogenic load, a $\Delta C_t<3.0$ between untreated and treated samples was found to indicate antimicrobial resistance ($P<0.01$). Minimum inhibitory concentration was determined for susceptible bacteria and pan-bacterial detection was demonstrated with 3 Gram-negative and 2 Gram-positive bacteria. Species identification was performed via analysis of the hypervariable amplicons. In summary, we have developed a universal diagnostic phenotyping technique that assays for the susceptibility of drug-resistant septicemia with the speed of PCR. The real-time PCR antibiogram achieves detection, susceptibility testing, minimum inhibitory concentration determination, and identification in less than 24 hours.

2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

Gram-positive cocci (81)

- Clusters (61)
  - *Coagulase + SA442 +* (18) → *S. aureus* → *mecA* + / -
    - + → MRSA
    - - → MSSA
  - *Coagulase - SA442 –* (43) → CoNS
    - *mecA +*
    - *mecA -*
- Chains (20)
  - *Coagulase - SA442 –* (20) → 23S rRNA + (20)
    - *Enterococcus spp (12)*
    - *Streptococcus spp (8)*

Gram-positive rods (2)

- *Coagulase - SA442 –* (2) → 23S rRNA + (2)
  - *Bacillus spp (1)*
  - *L. Monocytogenes (1)*

Gram-negative rods and coccobacilli (124)

- *Oxidase*
  - + (23)
    - *rpoC +* (3)
      - *Moraxella spp (1)*
      - *Ochrobactrum antrophi (2)*
    - *rpoC –* (20)
      - *P. Aeruginosa (19)*
      - *S. Paucimobilis (1)*
  - - (101) → *rpoC +* (101)
    - *E. coli (65)*
    - *K. pneumoniae (11)*
    - *E. cloacae (6)*
    - *P. mirabilis (3)*
    - *S. marcescens (5)*
    - Other *Enterobacteriaceae (8)*
    - *S. maltophilia (2)*
    - *B. uniformis (1)*

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Functional Assay for Rapid Detection of Resistance against β-Lactam Antibiotics

Journal of Clinical Microbiology p. 927–937

Katrin Sparbier,[a] Sören Schubert,[b] Ulrich Weller,[c] Christiane Boogen,[c] and Markus Kostrzewa[a]

J. Clin. Microbiol. **2012, 50(3):927. DOI: 10.1128/JCM.05737-11.**

Resistance against β-lactam antibiotics is a growing challenge for managing severe bacterial infections. The rapid and cost-efficient determination of β-lactam resistance is an important prerequisite for the choice of an adequate antibiotic therapy. β-Lactam resistance is based mainly on the expression/overexpression of β-lactamases, which destroy the central β-lactam ring of these drugs by hydrolysis. Hydrolysis corresponds to a mass shift of +18 Da, which can be easily detected by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). Therefore, a MALDI-TOF MS-based assay was set up to investigate different enterobacteria for resistance against different β-lactam antibiotics: ampicillin, piperacillin, cefotaxime, ceftazidime, ertapenem, imipenem, and meropenem. β-Lactamases are enzymes that have a high turnover rate. Therefore, hydrolysis can be detected by MALDI-TOF MS already after a few hours of incubation of the bacteria to be tested with the given antibiotic. The comparison of the MS-derived data with the data from the routine procedure revealed identical classifi tion of the bacteria according to sensitivity and resistance. The MALDI-TOF MS-based assay delivers the results on the same The approved routine procedures require at least an additional overnight incubation.

Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates

Giovanni Gherardi[a,*], Silvia Angeletti[a], Miriam Panitti[a], Arianna Pompilio[b], Giovanni Di Bonaventura[b], Francesca Crea[a], Alessandra Avola[a], Laura Fico[a], Carlo Palazzo[a], Genoveffa Francesca Sapia[a], Daniela Visaggio[a], Giordano Dicuonzo[a]

We performed a comparative evaluation of the Vitek-2 Compact and Phoenix systems for direct identification and antimicrobi susceptibility testing (AST) from positive blood culture bottles in comparison to the standard methods. Overall, 139 monomicrobial bloo cultures, comprising 91 Gram-negative and 48 Gram-positive isolates, were studied. Altogether, 100% and 92.3% of the Gram-negativ isolates and 75% and 43.75% of the Gram-positive isolates showed concordant identification between the direct and the standard methoc with Vitek and Phoenix, respectively. AST categorical agreements of 98.7% and 99% in Gram-negative and of 96.2% and 99.5% in Gran positive isolates with Vitek and Phoenix, respectively, were observed. In conclusion, direct inoculation procedures for Gram-negative isolatc showed an excellent performance with both automated systems, while for identification of Gram-positive isolates they proved to be le reliable, although Vitek provided acceptable results. This approach contributes to reducing the turnaround time to result of blood culture with a positive impact on patient care.

Farmacocinetica e farmacodinamica degli antibiotici nei pazienti critici

2° CONGRESSO NEWMICRO

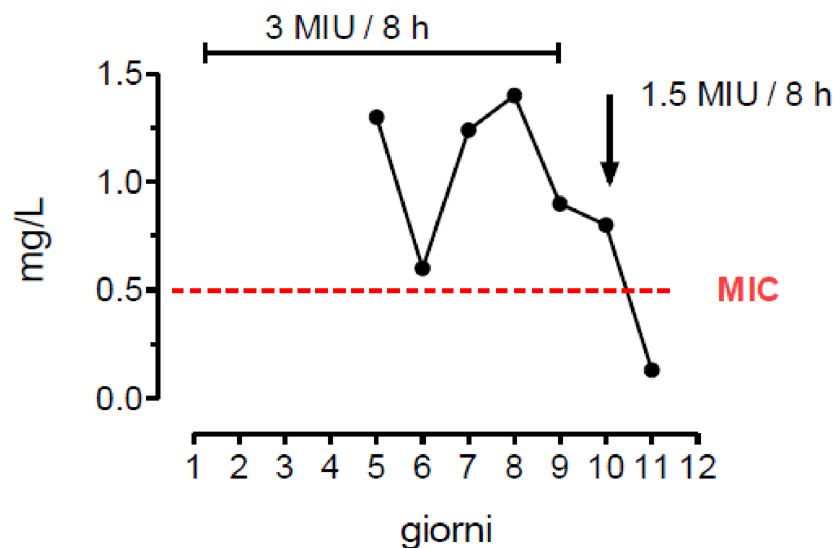
I Laboratori di Microbiologia e la Clinical Governance

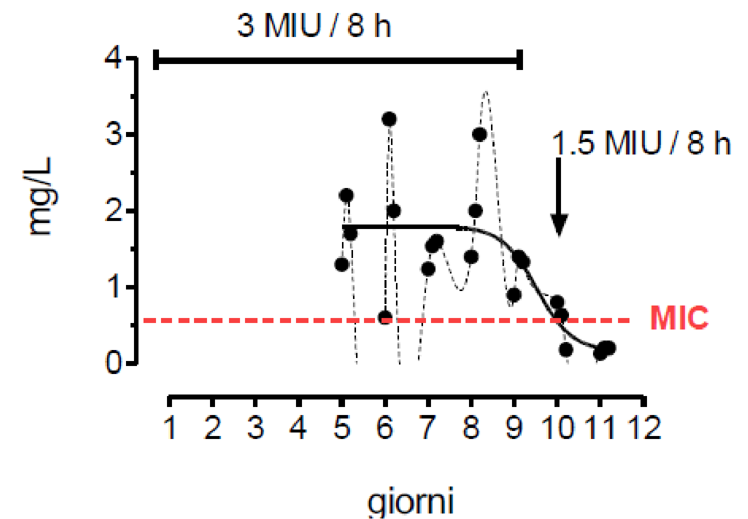
N. Pz	Dosaggi plasmatici consecutivi	Patogeno isolato	MIC (mg/L)	AUC metà ciclo	AUC/MIC metà ciclo	Css metà ciclo	Css metà ciclo sec.Couet $C_{ss,avg} = \frac{CL_{NR}}{CL_R + CL_{NR}} \times \frac{Dose}{\tau \times CL_{coli}}$
1	47	*Acinetobacter baumannii* MDR	<0,5	26	52	1,5 mg/L	1,45 mg/L
2	27	*Klebsiella pneumoniae* MDR	<0,5	29	58	1,5 mg/L	2,3 mg/L
3	18	*Acinetobacter baumannii* MDR	<0,5	59	118	2 mg/L	1,86 mg/L
4	15	*Pseudomonas aeruginosa* MDR	2	12	6	0,5 mg/L	3,7 mg/L
8	44	*Pseudomonas aeruginosa* MDR	2	56	28	2,3 mg/L	4 mg/L

Paziente n.2:

il paziente n.2 è giunto presso l'area di …per il trattamento di raccolte addominali settiche da pregressa pancreatite acuta necrotico-emorragica. Dall'emocoltura è stata isolata una *Klebsiella pneumoniae* MDR sensibile alla colistina (MIC ≤ a 0,5 mg/L).Si è instaurata terapia con 3MI/8h di CMS somministrati ev per 9 gg al termine dei quali la dose viene dimezzata a 1,5 MI/8h.

Il pannello **A** della **FIGURA 11** mostra i valori di colistina pre-infusionali dosati dal quinto all'undicesimo giorno di terapia. I dosaggi pre-infusionali di colistina restano sopra la MIC fino al dimezzamento della dose di CMS infuso.





E' possibile ed è diventato un dovere arrivare ad una diagnosi microbiologica e indicazione sulle resistenze antibiotiche diciamo in un turno di lavoro.

Non vi sono scuse né per noi microbiologi né per chi ci deve dotare di strumenti e personale adeguato.
Così si salvano i pazienti, si collabora efficacemente con i clinici e tutto il personale, e si risparmiano anche denari (quanto costa una giornata di degenza in più??!!)

Ringraziamenti:
Silvia Angeletti
Francesca Crea
Lucia De Florio
Giovanni Gherardi
Massimiliano Vitali

ERROR: undefined
OFFENDING COMMAND: Il

STACK:

(2)
/Title
()
/Subject
(D:20120423134746+02’00’)
/ModDate
()
/Keywords
(PDFCreator Version 0.9.5)
/Creator
(D:20120423134746+02’00’)
/CreationDate
(5317841)
/Author
-mark-