MedCrave
Step into the World of Research

Journal of Microbiology & Experimentation

Protocol Article

Open Access

CrossMark

Comparison of multiplex PCR against blood cultures for the identification of microorganisms in a cohort of patients with bloodstream infections

Abstract

Bloodstream Infections (BSI), represent an important cause of both morbidity and mortality worldwide. However, the insufficient sensibility of blood cultures whenever a patient has previously received antibiotic therapy, as well as the presence of slow growing and/or intracellular microorganisms, has generated the need of implementing new diagnostic methods. As a result, in 2012, Biofire Diagnostics, launched FilmArray®, a blood culture identification panel, which is an FDA, CE-IVD, and TGA certified multiplex PCR system that integrates sample preparation, amplification, detection and analysis.

Material and methods: the objective was to discern the sensitivity and specificity of the BioFire FilmArray® Blood Culture Identification (BCID) Panel compared to blood culture, for the identification of microorganisms causing bacteremia and the susceptibility profile in the National Institute of Respiratory Diseases. A total of 42 clinical records of patients with positive blood cultures, who also underwent a FilmArray® test were evaluated.

Results: The FilmArray® panel showed a sensibility of 96.4% and a specificity of 50% when compared with the Gold Standard. The median in hours elapsed from sample reception to result reports by the clinical microbiology service was 31.31 (±19.35) with the FilmArray® versus the 123.42 hours (±71.28) median of the traditional blood culture method. This difference was statistically significant ($p<0.001$).

Conclusion: FilmArray® panel as an emerging diagnostic method, is of significant utility for pathogens identification even in comparison to the traditional method. By curtailing time to pathogen identification, FilmArray® contributes to an earlier establishing of targeted antimicrobial treatment.

Keywords: blood culture, PCR, microorganism, bloodstream infection

Volume 7 Issue 6 - 2019

Becerril-Vargas Eduardo,[1] Corona-Nava Ana Karen,[2] Martínez-Orozco José Arturo,[3] Flores-Pérez Elia María,[2] Jiménez-Martínez María Elena,[2] Mireles-Dávalos Christian Daniel,[2] Sánchez-Tinajero Ángel,[4] Ruiz-Santillán Danna Patricia,[4] Santillán Segura Francisco Javier[5]

[1]Head of the Clinical Microbiology Laboratory, National Institute of Respiratory Diseases, Mexico

[2]Laboratory of Clinical Microbiology, National Institute of Respiratory Diseases, Mexico

[3]Medical Coordinator of the INER Infectology service, Mexico

[4]Surgeon, postgraduate in Chemical-Biological Sciences, Laboratory of Clinical Microbiology, National Institute of Respiratory Diseases, Mexico

[5]Surgeon, Clinical Microbiology Laboratory, National Institute of Respiratory Diseases, Mexico

Correspondence: Sánchez Tinajero Ángel, Surgeon, postgraduate in Chemical-Biological Sciences, Laboratory of Clinical Microbiology, National Institute of Respiratory Diseases, Tlalpan road no. 4502, Delegation Tlalpan, Colonia Section XVI, Mexico City. C.P. 14080, Tel +5255 32436794, Email angelsan11@hotmail.com

Received: October 24, 2019 | **Published:** November 07, 2019

Abbreviations: BSI, blood stream infections; BCID, blood culture identification; INER, Instituto Nacional de Enfermedades Respiratorias

Introduction

Blood Stream Infections (BSI) represent an important cause of morbidity and mortality worldwide. Most treatment decisions in these cases are made taking into consideration the results of blood cultures, which has been the most important diagnostic procedure to identify the causal agent when there is a clinical suspicion of BSI. However, there is a significant delay in results when conventional methods like these are performed.[1] This diagnostic method, based on the isolation of a microorganism and its identification and susceptibility test using standard biochemical techniques, is a process that can generally take from 48 to 72 hrs, and whose performance is variable. If 2 to 4 samples are obtained (40 to 80 ml of blood) before starting antimicrobial treatment, an etiological agent is detected in 80 to 96% of cases.[2] In patients with bacteremia, which frequently causes sepsis and septic shock, an early and appropriate administration of antimicrobial treatments affects directly in the patient's prognosis.[3,4] an inadequate treatment can duplicate mortality, which also increases a 7,6% each hour that therapy adjustment is delayed.[5,6]

Another element to consider is that whenever a patient has received previous antibiotic treatment, blood cultures can present an insufficient sensibility in pathogen identification, as well as when slow growth microorganisms and/or intracellular microorganisms are involved. These situations have generated the need of implementing new diagnostic methods that improve results in different instances of BSI. To this end, in 2012, BioFire Diagnostics launched its blood culture identification panel (BCID) FilmArray®, a qualitative in vitro diagnostic assay based in nucleic acids, that allows for a multiplex PCR analysis with automatic results readings directly from positive blood cultures in an hour[7,8] for identification of bacterial pathogens and yeasts from positive blood cultures, detecting a total of 24 pathogens and 3 resistance genes[9] that include: gram positive bacteria (*Enterococcus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptoccoccus agalactiae*, *pyogenes* y *pneumoniae*), gram negative (*Acinetobacter baumannii*, *Haemophilus influenza*, *Neisseria meningitides*, *Pseudomonas aeruginosa*, *Enterobacter cloacae complex*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*,

Proteus, Serratia marcescens), yeasts (*Candida albicans, glabrata, krusei, parapsilosis y tropicalis*) and antibiotic resistance genes (mecA for meticilin resistance, vanA/B for vancomycin resistance and KPC for carbapenem resistance).[10] This is the first PCR multiplex assay approved by the FDA.[11] In BSI the use of multiplex PCR assays has been associated with reduced empiric broad spectrum antimicrobial therapy, which also reduces time to appropriate antimicrobial therapy.[12]

Material and methods

This study´s objective was to discern the sensibility and specificity of BioFire FilmArray® BCID in relation to blood culture as the Gold Standard for identification of bacteremia causing microorganisms and to identify the profile of susceptibility at Mexico's National Institute of Respiratory Diseases (INER) through an observational, retrospective, transversal study. The study included all clinical records from patients whose blood culture samples were received at the clinical microbiology laboratory at INER, that presented microorganisms growth and where the pathogen was identified through both blood culture and FilmArray® panel. This study was performed at the microbiology department of INER. All clinical records with samples processed after the acquisition of FilmArray® in INER were included, establishing a 12-month analysis period, from January 2017 to January 2018.

Laboratory results were obtained from the BioFire FilmArray® log in the clinical microbiology lab and clinical data was collected from clinical records of patients diagnosed with bacteremia through blood culture, including antimicrobial treatment at the moment of sample procurement, results for both FilmArray® and blood culture as well as and time elapsed between sample delivery at microbiology lab and results reports. All clinical variables were registered in data collection formats, descriptive statistics was performed through median, frequencies and percentages were estimated. Sensibility and specificity were calculated by comparing the positive and negative results by both methods and comparative statistical analysis was performed using Chi square test for dichotomous qualitative variables and a t of Student test to compare medians of quantitative variables, considering as statistical significance a probability (p) of <0.05. All statistical analysis was performed using SPSS 21 statistical package.

Ethical aspects

This study follows the ethical guidelines established for the use of patient information and has been approved by INER's ethical committee.

Results

42 clinical records were evaluated. All patients had a positive blood culture to which a FilmArray® assay was additionally performed (Table 1).

A higher frequency of bacteremia was observed amongst male patients 64% (27/42), age median was 41 years of age with a predominance of patients 60 years or older 26% (11/42). A comorbidity was reported in 65% (27/42) of evaluated patients, with HIV (Human Immunodeficiency Virus) infection being the most frequent, and Type II Diabetes (DM). 24% of all patients were admitted to the Intense Care Unit (ICU). Blood cultures identified 42 microorganisms through the VITEK® 2 system and the FilmArray® system identified a total of 34 microorganisms (Table II), the pathogens not identified by FilmArray® were microorganisms that are not currently included in the system's identification spectrum; *Stenotrophomonas maltophilia, Ochrobactrum anthropi, Pseudomonas putida, Acinetobacter haemolyticus, Aeromonas hydrophila/caviae, Cryptococcus neoformans* and *Aspergillus sp*.

A general sensibility of 71.1% was found for the FilmArray® system and a specificity of 50% in comparison with traditional gold standard. Additionally, when analysis was performed excluding those microorganisms not included in the FilmArray® panel, a sensibility and specificity of 96.4% and 50% respectively were estimated. No mutations were identified in any sample included in the present study.

Median time lapsed in hours from sample reception and results from the clinical microbiology laboratory was 31.31 (±19,35) for the FilmArray® system and 123,42 (±71,28) for the conventional method, the reduced time to results with FilmArray® in comparison with blood culture, was statistically significant (p<0.001) (Table 2).

A 38% (16/42) of patients included in this study died. In 81% (13/16) of these cases, the causal agent of the bacteremia was identified with FilmArray®. No statistical significant difference was found between patients who died with a pathogen detected by FilmArray® and those that were undetected by FilmArray® (P=0.17) (Table 3).

Table 1 Demographic characteristics of patients diagnosed with bacteremia thorugh FilmArray® and blood culture

Basal demographic characteristics	n=42
Average age (years±SD)	40±14.4
0 to 10 years (%)	3 (7)
11 to 20 years (%)	3 (7)
21 to 30 years (%)	8 (19)
31 to 40 years (%)	7 (17)
41 to 50 years (%)	4 (10)
50 to 60 years (%)	6 (14)
>60 years	11 (26)
Gender	
Female (n, %)	15 (36)
Male (n, %)	27 (64)
Service	
Emergency Room	11 (26)
HIV clinic	9 (21)
Tuberculosis clinic	7 (17)
COPD clinic	4 (10)
ICU	4 (10)
Pediatric pneumology	3 (7%)
Others	4 (10)
Comorbidities	
HIV infection	14 (33)
Type II Diabetes	11 (26)
Systemic Hypertension	6 (14)
Cancer	3 (10)

SD, standard deviation; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; HIV, human immunodeficiency virus

Citation: Becerril-Vargas E, Corona-Nava AK, Martínez-Orozco JA, et al. Comparison of multiplex PCR against blood cultures for the identification of microorganisms in a cohort of patients with bloodstream infections. *J Microbiol Exp.* 2019;7(6):250–253. DOI: 10.15406/jmen.2019.07.00269

Table 2 Comparison of detected microorganisms by gold standard method (blood culture) versus FilmArray® panel

Blood culture	FilmArray®	
E. coli (12)	*E. coli* (12)	Gram negative
Pseudomonas aeruginosa (7)	*Pseudomonas aeruginosa* (7)	
Klebsiella pneumoniae (3)	*Klebsiella pneumoniae* (3)	
Enterobacter cloacae complex (1)	*Enterobacter cloacae* complex (1)	
E. cloacae (1)	*Enterobacter cloacae* complex (1)	
Acinetobacter baumannii (1)	*Acinetobacter baumannii* (1)	
Citrobacter freundii (1)	*Enterobacteria* (1)	
Haemophilus influenzae (1)	*Haemophilus influenzae* (1)	
Enterococcus avium (1)	*Enterococcus* (1)	Gram positive
Staphylococcus aureus (1)	*Staphylococcus aureus* (1)	
No growth	*Streptococcus agalactiae* (1)	
Estafilococcus epidermidis (1)	Not Detected	
No growth (0)	*Candida parapsilosis* (1)	Yeasts
Candida albicans (2)	*Candida albicans* (3)	
Aspergillus sp (1)	Not Detected	Outside panel spectrum
Ochrobactrum anthropi 1	Not Detected	
Pseudomonas putida (1)	Not Detected	
Acinetobacter haemolyticus (2)	Not Detected	
Aeromonas hydrophila/caviae (1)	Not Detected	
Gram negative bacillus (1)	Not Detected	
Cryptococcus neoformans (2)	Not Detected	
Stenotrophomonas maltophilia (1)	Not Detected	

Table 3 Difference in time lapse (hours) between sample reception and results with FilmArray® vs blood culture and endpoint (mortality) in patients with microorganisms detected by FilmArray® vs not detected with Film Array® (p<0.05)

	Diagnosis by FilmArray® 8 (n=34)	Conventional diagnosis (Culture) (n=42)	(p)
Median in hours	31,31 (± 19,35)	123,42 (± 71,28)	p<0.001
Deceases patients before 30 days 38% (16/42).	Detected by FilmArray® (n=13)	Not Detected by FilmArray® (n=3)	P=0.17
	81% (13/16)	19% (3/16)	

Discussion

The importance of developing new identification methods for microorganisms different to cultures, originates from the need of curtailing the time to etiological diagnosis and therefore, shorten the time lapse to an effective and directed treatment, avoiding the unnecessary use of antibiotics or its delay and all related risks.[13] The present study compared cultures; the Gold Standard of microbiologic diagnosis, against the FilmArray® identification panel, finding as a focal point, the reduction of the time elapsed between sample reception and a microorganism identification result, which was statistically significant (p<0.001) where FilmArray® had a median of 31.31 hours vs 123.42 hours that had to elapse for a blood culture to report results.

A higher prevalence of bacteremia was observed in male patients (64%). Clinical records revealed a median age of 41 years, with a higher prevalence in patients 60 years or older. A prospective study by Pazos et al. (2001) that analyzed a total of 320 blood cultures reported a median age of 66,9 years (CI 95%: 65-69) and a male predominance (59%). Several authors have revealed similar distributions. This could be attributed amongst other things, to qualitative and quantitative deficiencies of the immune system associated with age, as well as the increase of comorbidities that associates with older age groups. It is known that bacteremia increases the risk of death 14 times higher, with advanced age being a variable related to a poor prognosis.[14]

An HIV infection was one of the main reported comorbidities in patients with suspected bacteremia in 33% of cases, followed by Type II Diabetes with 26%, both of which are associated with a higher risk of bacteremia.[15] Despite a reduction in opportunistic infections rate as a consequence of the introduction of highly active antiretroviral therapy, HIV infection continues to be the main risk factor for

bacteremia with a higher mortality rate at 30 days than patients without an HIV infection.[16] Several studies have proven a higher involvement of Gram positive bacteria amongst blood isolates. In this study, the most commonly identified pathogens were *E.coli*, *P. aeruginosa* and *K. pneumonia*, in accordance with reports by Kirn (2013), Sabatier (2009) and more recently, Pulido (2018), these microorganisms represent most of the time, a true infection and are considered the main cause of secondary bacteremia due to respiratory infections.[15,17,18] such as pneumonia, which was the principal diagnosis (45%) in this study's patients during their hospital admittance at INER, which is considered one of the most important causes of morbidity and mortality in patients with long hospital internments.[18]

Candida albicans was the most frequently involved yeast, however, the presence of a *Candida parapsilosis*, supposes the increment of other species of *Candida* that were formerly mostly nonexistent, which could be attributed, amongst other things, to a wider spread in use of antifungals as prophylaxis. The utility of this diagnostic method, is not restricted to blood samples. Escudero et al. (2019) proposes that even if the FilmArray® panel's instructions do not include it, it can be used in samples that are not blood, such as pus, brain abscesses, synovial fluid or patients with necrotic fasciitis5 It has been used in the diagnosis of infectious gastroenteritis, substituting in occasions, feces culture and parasite examination.[19] As well as virus detection with results equivalent to traditional PCR methods and better than bacteria detecting cultures in cerebrospinal fluid in patients with meningitis/encephalitis.[20] The present study shows that FilmArray®, when compared with the current Gold Standard for BSI pathogen identification, is rapid, sensitive and specific. Further studies analyzing the clinical endpoints of patients diagnosed with BSI through FilmArray® and the consequent reduced time to adjusted antimicrobial therapy in Mexico are required.

Conclusion

On a clinical horizon, where the development of resistance by specific microorganisms to the drugs so far available, undermines daily medical practice, arises the need to propose new diagnostic strategies in order to have a quick and adequate antimicrobial therapy and, therefore, prevent severe infection cases and even development of greater resistance. Once the results of this scientific research were analyzed, the FilmArray® multiplex PCR method demonstrated to be an excellent diagnostic tool, which provides with by a fast, sensitive and specific method for the detection of infectious etiologies, as well to reduce the time for an accurate diagnosis and, therefore, install a faster and more accurate targeted antimicrobial therapy compared to the blood culture.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflicts of interest.

References

1. Bayindir Y, Cuglan S, Hopoglu M, et al. Rapid Detection of Bloodstream Pathogens in Liver Transplantation Patients With FilmArray Multiplex Polymerase Chain Reaction Assays: Comparison With Conventional Methods. *Transplant Proc*. 2015;47(6):1926–1932.
2. Fransesc M. Molecular methods for septicemia diagnosis. *Enferm Infecc Microbiol Clin*. 2017;35(9):586–592.
3. Bearman GM, Wenzel RP. Bacteremias: a leading cause of death. *Arch Med Res*. 2005;36(6):646–659.
4. Bischoff T, Edmond MB, Seifert H, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis*. 2004;39(3):309–317.
5. Coll P, Navarro F. Speeding up antimicrobial susceptibility testing. *Enferm Infecc Microbiol Clin*. 2016;34(6):331–333.
6. Hunfeld KP, Kost G, Lehman L, et al. Molecular diagnosis of sepsis: New aspects and recent developments. *Eur J Microbiol Immunol (Bp)*. 2014;4(1):1–25.
7. Balboa S, Diaz C, Escudero D, et al. Utility of multiplex PCR (FilmArray Blood Culture Identification) in other biological liquids. Detection of *Streptococcus pyogenes* in brain abscess and synovial fluid. *Rev Esp Quimioter*. 2019;32(2):194–197.
8. Almuhayawi M, Altun O, Özenci V, et al. Clinical Evaluation of the FilmArray Blood Culture Identification Panel in Identification of Bacteria and Yeasts from Positive Blood Culture Bottles. *J Clin Microbiol*. 2013;51(12):4130–4136.
9. FilmArray® Blood Culture Identification (BCID) Panel: Instruction booklet. CE-IVD. Biofire Diagnostics, LLC; 2015. 90 p.
10. Delano JP, Rand KH. Direct identification of bacteria in positive blood cultures: comparison of two rapid methods, FilmArray and mass spectrometry. *Diagn Microbiol Infect Dis*. 2014;79(3):293–297.
11. Chen JM, Chen SY, Chien JY, et al. Usefulness of the FilmArray meningitis/encephalitis (M/E) panel for the diagnosis of infectious meningitis and encephalitis in Taiwan. *J Microbiol Immunol Infect*. 2019;52(5):760–768.
12. Alexander DP, Baures TJ, Benefield RJ, et al. Impact of a Multiplex PCR Assay for Bloodstream Infections with and without antimicrobial stewardship intervention at a cancer hospital. *Open Forum Infect Dis*. 2018;5(10):ofy258.
13. Chen H, He P, Li Y, et al. The Clinical Significance of FilmArray Respiratory Panel in Diagnosing Community-Acquired Pneumonia, 2017. *Biomed Res Int*. 2017;2017:7320859.
14. Abel V, Cantón I, Fernández R, et al. Prognostic factors of bacteraemia: a prospective study. An Med Interna. 2001;18(8):23–28.
15. Peredo R, Sabatier C, Valles J. Bacteremia in the critical patient. *Med Intensiva*. 2009;33(7):336–345.
16. Ochoa-Díaz A, Rodríguez R, Sánchez-Pardo S. Bacteriemias en pacientes con VIH en un hospital de tercer nivel en Colombia, 2014-2016. *Med Int Méx*. 2018;34(3):366–372.
17. Kirn TJ, Weinstein MP. Update on blood cultures: how to obtain, process, report, and interpret. *Clin Microbiol Infect*. 2013;19(6):513–520.
18. Fernandez P, Garnacho-Montero J, Gonzalez-Gal V, et al. Application of BioFire FilmArray Blood Culture Identification panel for rapid identification of the causative agents of ventilator-associated pneumonia. *Clin Microbiol Infect*. 2018;24(11):1213.e1–1213.e4.
19. Chenouard R, Kempf M, Kouatchet A, et al. Performance of the extended use of the FilmArray® BCID panel kit for bronchoalveolar lavage analysis. *Mol Biol Rep*. 2019;46(3):2685–2692.
20. Banaei N, Gomez CA, Hitchcock MM. Low Yield of FilmArray GI Panel in Hospitalized Patients with Diarrhea: an Opportunity for Diagnostic Stewardship Intervention. *J Clin Microbiol*. 2018;56(3):e01558–17.