MEDICAL UNIVERSITY OF VIENNA

Comparison of two molecular assays and MALDI-TOF MS Sepsityper for the rapid identification of pathogens from positive blood cultures

P. Starzengruber[1], N. Ankowitsch[1], B. Selitsch[1], B. Willinger[1] and A. Makristathis[1].
1 Division of Clinical Microbiology, Department of Laboratory Medicine, Medical University of Vienna

Objective

Bloodstream infections (BSI) can lead to sepsis, which is a major health problem associated with high mortality. Early diagnosis of the causative pathogen and its resistance profile is of great importance for appropriate antimicrobial treatment. Blood culture (BC) is considered as the gold standard, but can lead to delayed diagnosis due to the need of subcultures. Therefore, molecular diagnostic tests are available that can help to shorten the time to result. **The aim of the present study was to evaluate the accuracy of a new molecular test (Hybcell Pathogens DNA xB), MALDI-TOF MS Sepsityper and a well established multiplex PCR (Biofire FilmArray BCID Panel 2) for the rapid detection of pathogens from positive BC compared to conventional subculturing and MALDI-TOF MS.**

Patients and Methods

The study was conducted at the University Hospital of Vienna from June to November 2021. The performance of three different tests was evaluated in comparison to conventional diagnostics based on MALDI-TOF MS analysis of the isolated pathogen as a reference. 410 positive BC samples were tested using the Sepsityper kit (Bruker Daltonics) and a new singleplex PCR followed by hybridization (Hybcell Pathogens DNA). In addition, 66 of 410 samples were tested by nested multiplex PCR (Biofire FilmArray BCID Panel 2). Only one positive BC bottle was used per patient and episode, provided the pathogen detected was the same within the affected episode.

Blood culture and Sepsityper test
The BC samples were incubated for up to 7 days in a BacT/ALERT 3D analyser (bioMérieux, Marcy l'Etoile, France). If positive, Gram-staining and the Sepsityper test (Bruker Daltonics, Germany) were performed directly from the positive BC followed by subculture on solid culture media to confirm pathogen identification and antimicrobial sensitivity testing.

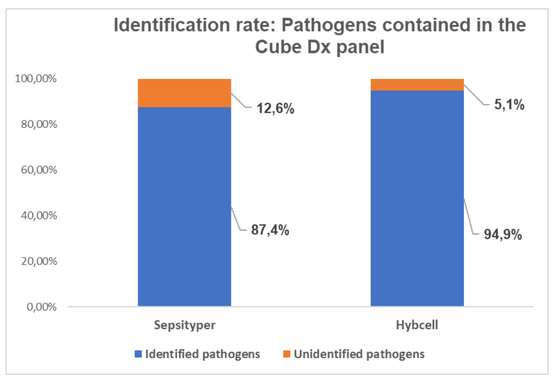
Molecular biological analysis
The samples for molecular testing were processed according to the instructions of the respective manufacturer.

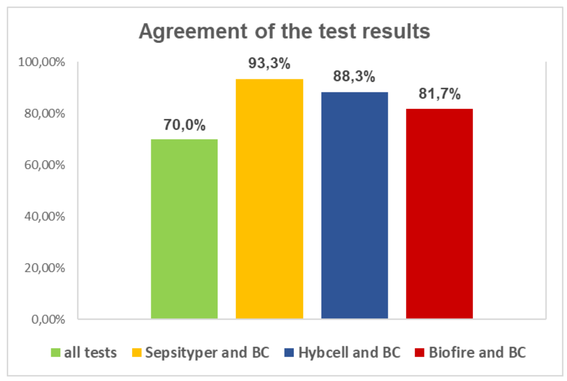
Hybcell Pathogens DNA xB (Cube Dx, Austria) can identify one panbacterial target, four bacterial genera and 28 bacterial species, one panfungal target, two fungal genera and 13 fungal species and the resistance markers vanA, vanB, mecA and mecC. After DNA extraction, four separate PCR reactions are carried out. PCR products are transferred to a microarray and amplicons were identified in the hyborg device by compact sequencing.

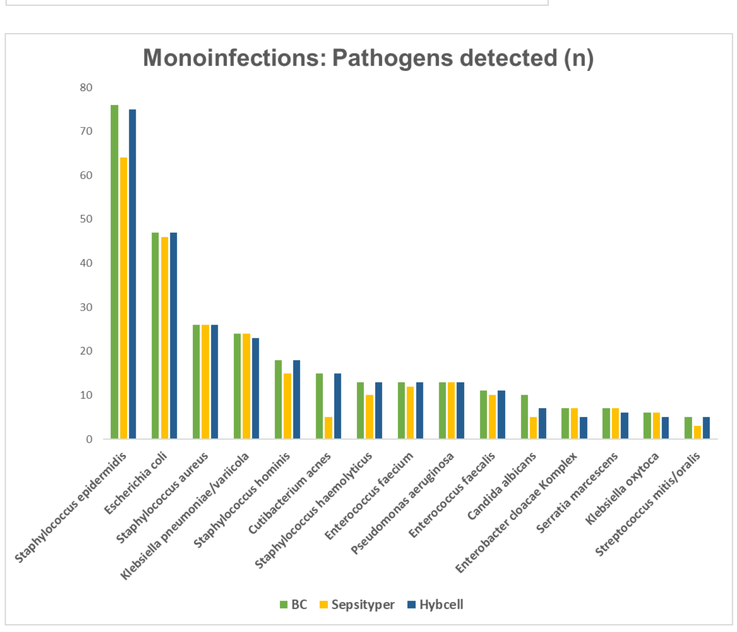
Biofire FilmArray BCID Panel2 (bioMérieux, France), a widely used test with a turnaround time of about 1 hour, is able to detect the 33 most important pathogens or groups of pathogen species and 10 antimicrobial resistance markers.

Results

In 365 monoinfections, the identification rate (IR) by Sepsityper and Hybcell Pathogens DNA in comparison to the reference method BC was 84.9 and 86.9 %, respectively. When only Hybcell Pathogens DNA on-panel microbes were considered, the IR by this test increased to 94.9% and that by Sepsityper to 87.4%. In monoinfections (n=49) analysed by all three tests, the IR by FilmArray BCID, Sepsityper and Hybcell Pathogens DNA was 81.7%, 93.3% and 88.3%, respectively. In the FilmArray BCID on-panel subgroup analysis, the IR by this test reached 100%, that by Sepsityper and Hybcell Pathogens DNA 95.9% and 89.8%, respectively. For polymicrobial infections (n=44), both PCR tests performed better than Sepsityper. Thus, all microorganisms were detected in 20/44 cases (45.5%) by Hybcell Pathogens DNA, but only in 3/44 cases (6.8%) by Sepsityper. Detection of resistance markers (total n=106, mecA n=102, CTX-M-1 n=3, OXA48 n=1) was only possible by PCR, mecA was detected by Hybcell Pathogens DNA in 80.2% of cases. Due to the low case numbers for other resistance markers, further analyses are needed to obtain conclusive results.







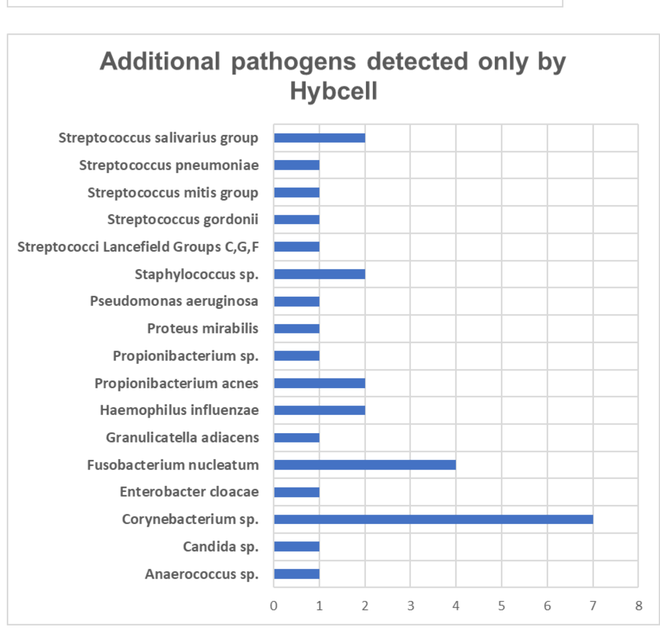


Table 1: List of detected pathogens

Pathogen	BC	Sepsityper		Hybcell	
Yeasts	**n**	**n**	**%**	**n**	**%**
Candida albicans	10	5	50	7	70
Candida dubliniensis	1	1	100	1	100
Candida glabrata	2	1	50	0	0
Candida tropicalis	1	1	100	1	100
Other Gram-positive cocci					
Enterococcus faecalis	11	10	91	11	100
Enterococcus faecium	13	12	92	13	100
Granulicatella adiacens	1	1	100	1	100
Staphylococci					
Staphylococcus aureus	26	26	100	26	100
Staphylococcus capitis	3	3	100	111	99
Staphylococcus epidermidis	76	64	84		
Staphylococcus haemolyticus	13	10	77		
Staphylococcus hominis	18	15	83		
Staphylococcus lugdunensis	2	2	100		
Streptococci					
ß-haemolytic streptococcus group B	2	2	100	1	50
ß-haemolytic streptococcus group C	1	1	100	1	100
ß-haemolytic streptococcus group G	2	2	100	2	100
Streptococcus anginosus group	3	2	67	3	100
Streptococcus gordonii	1	1	100	0	0
Streptococcus mitis/oralis	5	3	60	5	100
Streptococcus pyogenes	1	1	100	1	100
Streptococcus salivarius group	1	1	100	1	100
Streptococcus sanguinis group	3	2	67	3	100
Gram-positive rods					
Corynebacterium jeikeium	1	0	0	1	100
Cutibacterium acnes	15	5	33	15	100
Gram-negative bacteria					
Acinetobacter baumannii complex	2	2	100	2	100
Campylobacter species	2	2	100	2	0
Citrobacter freundii	1	1	100	0	0
Enterobacter cloacae complex	7	7	100	5	71
Escherichia coli	47	46	98	47	100
Klebsiella aerogenes	2	2	100	2	100
Klebsiella oxytoca	6	6	100	5	83
Klebsiella pneumoniae/variicola	24	24	100	23	96
Morganella morganii	2	2	100	1	50
Proteus mirabilis	4	4	100	4	100
Pseudomonas aeruginosa	13	13	10	13	100
Pseudomonas putida group	2	2	100	0	0
Serratia marcescens	7	7	100	6	86
Stenotrophomonas maltophilia	3	3	100	3	100

Conclusion

All three tests proved to be useful additional tools for a faster diagnosis of bloodstream infections. Molecular tests were advantageous in mixed infections and in terms of rapid detection of relevant resistance markers.