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Background

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) is being used with great success for the identification of microorganisms directly from positive blood cultures. The novel rapidBACpro® II preparation kit (Nittobo Medical Co, Tokyo, Japan) contains cationic copolymer technology that facilitates bacterial aggregation via copolymerization resulting in macroscopically visible aggregates. Here we present a modified workflow of the novel rapidBACpro® II pretreatment kit and compare it with the standard rapidBACpro® II workflow. Last we compare the modified workflow against the rapid extended direct transfer (eDT) Sepsityper workflow using the Sepsityper pretreatment kit (Bruker Daltonik, Bremen, Germany).

Materials/Methods

In parallel we analyzed 200 positive blood cultures (BD BACTEC) using the standard rapidBACpro® II and the Sepsityper workflows according to the manufacturers instructions. The samples were analyzed in duplicate and score values recorded. Additionally, we modified the standard rapidBACpro® II protocol picking a small portion of the produced pellet after the washing step (step 3) instead of after the extraction step (step 4). The material was smeared on MSP-target plate in duplicate spots, covered with 100% formic acid, dried, covered with HCCA-matrix, dried and analyzed with a MALDI Biotyper system (Bruker Daltonik). Score values were recorded and compared with score values using the standard protocol. As for the Sepsityper kit the rapid extended direct transfer workflow was used starting with picking a small portion of the pellet after the washing step in the procedure.

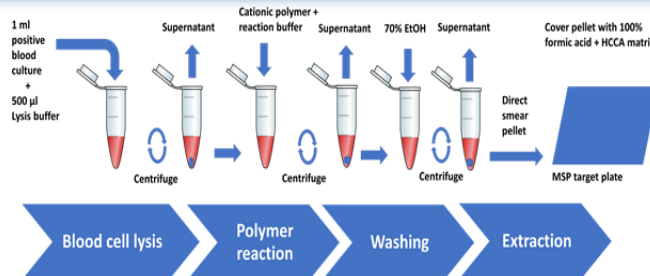


Figure: Workflow of the modified rapidBACpro® II protocol.

rapidBACpro II					
Average score	Modified:	2,12	Standard protocol:	2,14	
	Total samples	200	%	Total samples	200
	Above 1.7 (accepted)	158	79	Above 1.7 (accepted)	175
	Below 1.7 (orange)	17	8,5	Below 1.7 (orange)	12
	No peak (red)	18	9	No peak (red)	6
					3
Sepsityper					
Average score	eDT protocol	1,92	Standard protocol:	2,09	
	Total samples	200	%	Total samples	200
	Above 1.7 (accepted)	127	63,5	Above 1.7 (accepted)	152
	Below 1.7 (orange)	29	14,5	Below 1.7 (orange)	22
	No peak (red)	37	18,5	No peak (red)	19
					9,5

Table: Performance of the modified rapidBACpro® II protocol.

Results

Comparing the results with the standard routine diagnostics 188 of the 200 positive blood cultures covering 35 different species were correctly identified using the rapidBACpro® II preparation kit. The identification threshold was set to a score value over 1.7. The modified protocol identified 90% of the gramnegative bacteria and 80% of the grampositive bacteria with an average score value of 2,28 and 2,01 respectively. The standard rapidBACpro® II protocol identified 97% of the gramnegatives and 90% of the grampositives with an average score value of 2,27 and 2,08 respectively. The only yeast isolate was identified by both rapidBACpro® II protocols. There were two cases of positive blood cultures with more than one bacterial species. The rapidBACpro® II protocols were only able to identify one of the species in each sample. The rapid eDT Sepsityper workflow identified 69% of the gramnegatives and 64% of the grampositives with an average score value of 2,120 and 2,077 respectively. The protocol was able to identify both microbes in one of the positive blood cultures with more than one bacterial species.

Conclusions

The modified rapidBACpro® II protocol performs slightly poorer than the standard rapidBACpro® II protocol but with a shorter turn-around-time making it more valuable to use in a routine diagnostic setting. The eDT Sepsityper protocol has the same turn-around-time as the modified rapidBACpro® II protocol but the score values are lower.

Disclosures

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