

# A novel FISH test for rapid pathogen identification in positive blood cultures

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**Introduction.** Blood culture (BC) still represents the gold standard for the detection of blood stream pathogens. As soon as growth is detected, a gram stain is performed. Using conventional microbiological techniques it usually takes several hours or even days from the time of microscopy to the pathogen identification. In the present study, a novel commercially available fluorescence in situ hybridization (FISH) test has been validated allowing for rapid bacterial pathogen identification in positive BCs.

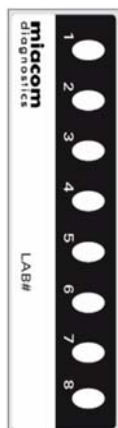
**Methods.** FISH test (Lucesco, Miacom) uses molecular beacons as DNA probes which bind on the rRNA of microbial ribosomes. Analysis was conducted with 152 BCs from different patients using the bottle which became positive first by the BacT ALERT® 3D system (Bio Merieux). Dependent on the gram stain either a gram negative (Bac I) or a gram positive panel (Bac II) were used (Fig.). The time to result was 30 min and the hands-on time only 10 min.

**Results.** Between April and June 2010, 157 isolates (58 gram-negatives and 98 gram-positives) were identified by conventional methods from 152 positive BCs analyzed by FISH. 11/157 microorganisms (7%) were not included in the FISH panels. Of the remaining 146 microorganisms, 140 were identified correctly, 3 were misidentified, 1 was identified only to the family instead to the species level and 2 were not identified at all by FISH (Table). In comparison to conventional methods, the FISH test sensitivity was 95.9% and the specificity was 97.9%. The clinical sensitivity and specificity was 89.2% and 98.1%, respectively.

**Table.** Conventional methods vs. FISH

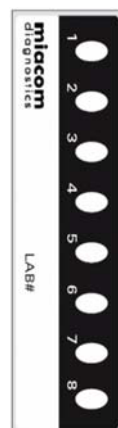
Result by reference methods	n	Result by FISH	n
<b>Gramnegative monocultures</b>			
<i>E. coli</i>	27	<i>E. coli</i>	27
<i>K. pneumoniae</i>	7	<i>K. pneumoniae</i>	4
		<i>S. marcescens</i> + <i>S. maltophilia</i>	1
		<i>E. coli</i>	1
		Enterobacteriaceae	1
<i>K. oxytoca</i>	2	<i>K. oxytoca</i>	1
		Enterobacteriaceae	1
<i>E. cloacae</i>	3	Enterobacteriaceae	3
<i>S. marcescens</i>	4	<i>S. marcescens</i>	4
<i>P. aeruginosa</i>	8	<i>P. aeruginosa</i>	8
<i>S. maltophilia</i>	3	<i>S. maltophilia</i>	3
<i>P. luteola</i>	1	No ID	1
<i>B. holmesii</i>	1	No ID	1
<b>Grampositive monocultures</b>			
<i>S. aureus</i>	15	<i>S. aureus</i>	15
KNS	56	KNS	54
		<i>S. aureus</i>	1
		No ID	1
<i>E. faecalis</i>	5	<i>E. faecalis</i>	4
		<i>E. faecalis</i> + <i>S. agalactiae</i>	1
<i>E. faecium</i>	2	<i>E. faecium</i>	2
<i>S. pneumoniae</i>	2	<i>S. pneumoniae</i>	2
<i>S. agalactiae</i>	1	<i>S. agalactiae</i>	1
<i>Streptococcus spp.</i>	3	<i>Streptococcus spp.</i>	3
<i>C. perfringens</i>	1	<i>C. perfringens</i>	1
<i>A. meyeri</i>	1	No ID	1
<i>P. micra</i>	1	No ID	1
<i>P. acnes</i>	1	No ID	1
<i>Bacillus spp.</i>	3	No ID	3
<b>Polybacterial cultures</b>			
<i>E. cloacae</i> + <i>A. baumannii</i>	1	Enterobacteriaceae	1
<i>S. haemolyticus</i> + <i>S. oralis</i>	1	KNS + <i>Streptococcus spp.</i>	1
<i>S. epidermidis</i> + <i>S. hominis</i>	1	KNS	1
<i>B. stercoris</i> + <i>S. epidermidis</i>	1	No ID	1
<i>Granulicatella spp.</i> + <i>S. epidermidis</i>	1	KNS	1

## Bac I



	Red channel	Green channel
1	Positive control	Negative control
2	<i>Escherichia coli</i> / <i>Shigella spp.</i>	
3	<i>Pseudomonas aeruginosa</i>	
4	<i>Klebsiella pneumoniae</i>	
5	<i>Klebsiella oxytoca</i>	<i>Haemophilus influenzae</i>
6	Enterobacteriaceae	<i>Salmonella spp.</i>
7	<i>Proteus mirabilis</i>	<i>Stenotrophomonas maltophilia</i>
8	<i>Proteus vulgaris</i>	<i>Serratia marcescens</i>

## Bac II



	Red channel	Green channel
1	Positive control	Negative control
2	<i>Staphylococcus spp.</i>	
3	<i>Staphylococcus aureus</i>	
4	<i>Streptococcus spp.</i>	
5	<i>Streptococcus pneumoniae</i>	<i>Clostridium perfringens</i>
6	<i>Streptococcus pyogenes</i>	<i>Enterococcus faecium</i>
7	<i>Streptococcus agalactiae</i>	<i>Enterococcus faecalis</i>
8		

**Fig.** Composition and principle of the Bac I and Bac II slides

**Conclusion.** These data suggest that this novel FISH test accurately identifies blood stream pathogens and may considerably reduce the time to result in clinical use.