EVALUATION OF PNA FISH ASSAYS FOR THE RAPID DIAGNOSIS OF SEPSIS AND OTHER SEVERE INFECTIONS, AND IDENTIFICATION OF *Streptococcus agalactiae* IN THE SCREENING OF PREGNANT WOMEN

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INTRODUCTION AND PURPOSE

Identification of pathogens by conventional methods from liquid culture media requires 24-48 hrs. Peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) represents a new molecular diagnostic tool for the rapid identification of pathogens (bacteria and yeasts) directly from liquid media. The aims of this study were to evaluate the PNA FISH assay in comparison with conventional methods both from positive blood cultures (BC) and other biological fluids from body sites of suspected infection, as well as to evaluate the identification of *Streptococcus agalactiae* from vaginal swabs in pregnant women.

METHODS

In order to evaluate the analytical sensitivity of the different PNA FISH assays (AdvanDx), serial dilutions of reference bacterial strains recognized by the different probes were tested. When the analytical specificity of PNA FISH probes was evaluated several bacterial strains different from the target of the assays were tested.

On the basis of the results of Gram stain microscopy, the PNA FISH assays were performed on 61 positive BC bottles (Bactec 9240, Becton Dickinson) (56 blood samples and 5 biological fluids other than blood) using 4 different panels: "*S. aureus*/CNS PNA FISH" for the identification and differentiation of *Staphylococcus aureus* and other coagulase-negative staphylococci (CNS), "*E. faecalis*/OE PNA FISH" for *Enterococcus faecalis* and other enterococci, "GNR Traffic Light PNA FISH" for *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and "Yeast Traffic Light PNA FISH" for *Candida albicans*/*C. parapsilosis*, *C. tropicalis* and *C. glabrata*/*C. krusei*. All samples arrived at our laboratory for diagnostic purpose and belonged to hospitalized patients with suspected sepsis and/or other severe infections.

For *Streptococcus agalactiae* identification, the "GBS PNA FISH" assay was performed on 25 vaginal swabs belonging to pregnant women near to delivery after 24 hrs incubation in enrichment broth (Todd-Hewitt). The results of all molecular assays were compared to those obtained by the identification with conventional methods.

RESULTS

Analytical sensitivity and specificity. The detection limit for the PNA FISH assays was 10^6 colony-forming units per ml when performed on serial dilutions of reference bacterial strains. The PNA FISH assays performed on 17 bacterial strains different from the probe target of each assay gave negative results on all samples except on the *Shigella sonnei* strain which cross-reacted with *E. coli* probe in the "GNR Traffic Light PNA FISH" assay (Table 1).

Table 1. Results of analytical specificity of the PNA FISH assays on 17 reference bacterial and yeast strains.

Microrganism	Strain origin	PNA FISH results				
		S. Aureus /CNS	E. Faecalis /OE	GNR Traffic Light	Yeast Traffic Light	GBS
A. haemolyticus	NEQAS	-*	-	Negative	-	
H. influenzae	NEQAS	-	-	Negative	-	
S. marcescens	NEQAS	-	-	Negative	-	
L. monocytogenes	NEQAS	Negative	Negative	Negative	-	Negative
Shigella sonnei	ATCC	-	-	Positive	-	
N. gonorrhoeae	NEQAS	-	-	Negative	-	
S. pneumoniae	NEQAS	Negative	Negative	-	-	Negative
S. agalactiae	ATCC	Negative	Negative	-	-	
S. aureus	ATCC	-	-	-	-	Negative
S. epidermidis	Clinical	-	-	-	-	Negative
E. casseliflavus	ATCC	-	-	-	-	Negative
E. faecalis	ATCC	-	-	-	-	Negative
Trichosporon mucoides	ATCC	-	-	-	Negative	
Candida dublinioensis	NEQAS	-	-	-	Negative	
C. lusitaniae	NEQAS	-	-	-	Negative	
C. kefir	ATCC	-	-	-	Negative	
S. cerevisiae	NEQAS	-	-	-	Negative	

* -: Not performed.

Blood samples. Gram stain microscopy on the 56 positive BC bottles inoculated with blood samples, gave positive results for Gram positive cocci (GPC) in 31 cases, for Gram negative rods (GNR) in 19 cases, for GPC in association with GNR in 1 case and for yeasts in 5 cases (Table 2). When the 31 samples containing GPC were tested with the "*S. aureus*/CNS PNA FISH" and the "*E. faecalis*/OE PNA FISH" assays, 14 gave positive results for CNS, 4 for *E. faecalis*, 4 for *S. aureus*, 1 for *S. aureus* in association with *E. faecalis,* 1 for *Enterococcus* sp. other than *E. faecalis* and 7 gave negative results, according to conventional identification methods. Among the 19 BC bottles containing GNR, 13 gave a positive result (9 *E. coli*, 2 *K. pneumoniae* and 2 *P. aeruginosa*, according to conventional methods*)* and 6 were negative by the "GNR Traffic Light PNA FISH" assay. The 6 PNA FISH negative samples contained *K. oxytoca* (2 cases)*, Salmonella typhimurium* (2 cases), *Proteus mirabilis* (1 case) and *Enterobacter cloacae* (1 case).

The sample containing both GPC and GNR by Gram stain microscopy, when tested with the PNA FISH assays, revealed only the presence of *E. faecalis.* By conventional methods *K. oxytoca* was also identified, a species not recognized by the "GNR Traffic Light PNA FISH" probes. All 5 BC bottles containing yeasts submitted to the "Yeast Traffic Light PNA FISH" assay gave positive results: 3 for *C. albicans/C. parapsilosis*, 1 for *C. glabrata/C. krusei* and 1 for *C. tropicalis,* showing 100% agreement with conventional methods.

Other sterile biological fluids. When the PNA FISH assays were tested on 2 peritoneal fluids, 1 cerebrospinal fluid (CSF), 1 bile and 1 liver abscess from patients with suspected severe infections of such body sites, the results agreed with conventional methods in all cases (Table 3).

Overall the PNA FISH assays allowed bacteria identification at least 2 days before the conventional methods: in average 2,8 days (median 2 days, range 2-8 days) for monomicrobial infections and 3,5 days for polimicrobial infections (Table 4) .

Vaginal swabs for screening. When the "GBS PNA FISH" assay was used on 25 vaginal swabs, it showed positive results in 11 cases and negative results in 14 cases, showing 100% agreement with results of conventional methods (Table 5).

"GBS PNA FISH" allowed *S. agalactiae* identification 1 day before the conventional method in 17 cases, and the same day for the positive 8 cases (Table 6). Overall the "GBS PNA FISH" assay was able to identify the presence of *S. agalactiae* in average 1 day before the conventional methods.

Table 2. PNA FISH results on 56 positive blood cultures.

Gram stain microscopy		PNA FISH		Coventional methods	
		Results	N°	Results	N°
GPC	31				
		S. aureus	4	*S. aureus*	4
		CNS	14	CNS[a]	14
		E. faecalis	4	*E. faecalis*	4
		Enterococcus sp.	1	*E. faecium.*	1
		S. aureus + *E. faecalis*	1	*S. aureus* + *E. faecalis*	1
		Negative	7	Other GPC[b]	7
GNR	19				
		Escherichia coli	9	*Escherichia coli*	9
		Klebsiella pneumoniae	2	*Klebsiella pneumoniae*	2
		Pseudomonas aeruginosa	2	*Pseudomonas aeruginosa*	2
		negative	6	Other GNR[c]	6
GPC + GNR	1				
		E. faecalis	1	*E. faecalis* + *K. oxytoca*	1[d]
Yeasts	5				
		C. albicans/C. parapsilosis	3	*C. albicans* *C. parapsilosis*	2 1
		C. glabrata/C. krusei	1	*C. glabrata*	1
		C. tropicalis	1	*C. tropicalis*	1

[a]CNS: coagulase negative staphilococci *Staphylococcus epidermidis (12)*, *S. haemolyticus (1)* and *S. warneri (1)* identified by conventional methods.
[b]*Gemella morbillorum*, *S. gallinarum*, *S. mitis*, *S. pyogenes*, *S. pasteurianus*, *Streptococcus* sp., and *S. zooepidemicus* identified by conventional methods.
[c]*Klebsiella oxytoca (2)*, *Salmonella typhimurium* (2), *Proteus mirabilis* (1) and *Enterobacter cloacae* (1) identified by conventional methods

Table 3. PNA FISH results compared with conventional methods on 5 blood culture bottles containing biological fluids physiologically sterile other than blood.

Material	Gram Stain Microscopy	PNA FISH	Conventional methods
Peritoneal fluid	Yeasts	*C. glabrata/C. krusei*	*C. glabrata*
Peritoneal fuid	GPC + GNR	*E. faecalis* + *E.coli*	*E. faecalis* + *E. coli*
CSF	GPC	CNS	*S. epidermidis*
Bile	GPC + GNR	*Enterococcus* sp.	*E. faecium* + *Enterobacter aerogenes*
Liver abscess	GPC + Yeasts	*Enterococcus* sp.+ *C. albicans/C. parapsilosis*	*E. faecium* + *C. albicans*

Table 4. Comparison of reporting time (in working days from the sample arrival) of PNA FISH assays against conventional methods based on results of 61 positive blood culture bottles.

Type of microrganism	Difference PNA FISH vs conventional method (days)		
	Average	Median	Range (min-max)
Monomicrobial infections	**-2,8**	**-3**	**2-8**
Gram positive cocci	**-2,6**	**-2**	**2-6**
Gram negative rods	**-3**	**-3**	**2-8**
Yeasts	**-2,6**	**-2,5**	**2-4**
Polimicrobial infections	**-3,5**	**-3,5**	**2-4**

Table 5. "GBS PNA FISH" assay results compared with conventional methods on 25 vaginal swabs.

Conventional method	GBS PNA FISH		
	S. agalactiae	Negative	Total
S. agalactiae	**11**	0	**11**
Negative	0	**14**	**14**
Total	**11**	**14**	**25**

Table 6. Comparison of reporting time (in working days from the arrival of the sample) of the "GBS PNA FISH" assay against conventional method based on results of 25 vaginal swabs.

Result	GBS PNA FISH		Conventional method		Difference GBS PNA FISH vs Conventional method	
	1 day	2 days	1 day	2 days	0 day	1 day
POSITIVE	11	-	8	3	8	3
NEGATIVE	14	-	0	14	0	14
Total	25	-	8	17	8	17

CONCLUSIONS. PNA FISH assays showed, even if tested in this study only on a limited number of samples, an excellent efficacy in the rapid identification of main pathogens yielding to a significant reduction on reporting time, leading to a more appropriate patient management and therapy in case of sepsis and severe infections by bacteria and yeasts and a rapid screening for *Streptococcus agalactiae* colonization in pregnant women.

REFERENCES

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