Fast and Cost-Effective Pathogen Diagnosis on an Automated DNA/Protein Diagnostics Platform Based on Cylindrical Microarrays (hybcells)

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Objectives

Polymerase Chain Reaction is commonly used to detect microbial nucleic acids in clinical samples. Current microbiology practice takes days to find pathogens in clinical samples, which is sometimes too slow to provide prompt and specific therapy for the patient especially concerning fungal pathogens. On the other hand these pathogens are sometimes weakened due to antimicrobial therapy so that their growth is even decelerated or suspended. A PCR based method would also solve this issue.

Cube Dx' hybcell

Methods

Samples from clinical material were parted into three parts. The first part was used for bacteriological culture, followed by MALDI-TOF, the second part for manual DNA extraction with SeptiFast Prep Kit® and Lys Kit® (Roche) and testing with SeptiFast® (Roche). The third part was used for automated extraction with Select NA Blood Pathogen Kit® on Select NA® (Molzym) and further processed with hybcell Pathogen DNA plexA® (Cube Dx).

Bacterial type strains			Fungi		
Gram positiv bacteria	Enterococcus	faecalis	Ascomycota	Saccharomyces	Saccharomyces cerevisiae
	Streptococcus	anginosus		Candida	Candida albicans
		agalactiae, dysgalactiae			Candida dubliniensis
		pneumoniae			Candida glabrata
		pyogenes			Candida parapsilosis
	Staphylococcus	aureus			Candida tropicalis
		epidermis		Pichia	Issatchenkia orientalis (Candida krusei)
Gram negativ bacteria	Enterobacter	aerogenes		Aspergillus	Aspergillus flavus
		cloacae			Aspergillus clavatus
	Escherichia	coli			Aspergillus fumigatus
	Klebsiella	oxytoca		Trichophyton	Trichophyton sp.
		pneumoniae	Basidiomycota	Filobasidiella	Cryptococcus sp.
	Proteus	mirabilis			
	Pseudomonas	aeruginosa			

Panel of bacteria and fungi. Only potent pathogens are considered, multiple pathogens are detected.

Cube Dx' hyborg

Results

Nr.	Clinical material	hybcell	Septifast	Blood culture
1	Synovial fluid	neg	neg	neg
2	Synovial fluid	*Strep. epidermidis*	neg	neg
3	Synovial fluid	neg	neg	neg
4	Synovial fluid	neg	neg	neg
5	Synovial fluid	*Staph. aureus*	*Staph. aureus*	*Staph. aureus*
6	Synovial fluid	*Strep. anginosus*	Strep. ssp.	*Strep. intermediusStrep. constellatus*
7	Synovial fluid	*Staph. aureus*	*Staph. aureus*	neg
8	Synovial fluid	neg	neg	neg
9	Synovial fluid	neg	neg	neg
10	Synovial fluid	neg	neg	neg
11	Synovial fluid	neg	neg	neg
12	Synovial fluid	neg	-	neg
13	Synovial fluid	*Strep. pneumoniae*	*Strep. ssp.*	*Strep. mitis*
14	Synovial fluid	neg	neg	neg
15	Synovial fluid	*C. albicans*	*C. albicans*	neg
16	Synovial fluid	neg	neg	neg
17	Synovial fluid	neg	neg	neg
18	Synovial fluid	neg	CONS	neg
19	Synovial fluid	neg	neg	neg
20	Synovial fluid	*Staph. aureus*	*Staph. aureus*	*Staph. aureus*
21	Synovial fluid	neg	*Staph. aureus*	neg
22	Culture	Bacteria	neg	*Scopulariopsis ssp.*
23	EDTA-Blood	neg	neg	neg
24	EDTA-Blood	*Staph. aureus*	*Staph. aureus*	*Staph. aureus*
25	EDTA-Blood	neg	neg	neg
26	EDTA-Blood	neg	neg	neg
27	EDTA-Blood	neg	neg	neg
28	EDTA-Blood	neg	neg	neg
29	EDTA-Blood	neg	*CONS*	*E. coli*
30	EDTA-Blood	*Strep. epidermidis*	neg	*E. coli*
31	Tissue	*nter. faecalisAspergillus fumigatus*	-	*Entr. faeciumPseud. aeruginosa*
32	Liquor	*Staph. aureus*	*Staph. aureus*	*Staph. aureus*
33	Liquor	neg	*CONS*	neg
34	Paraffin embedded material	Bacteria	neg	-
35	Paraffin embedded material	neg	neg	-
36	Swab (liver)	neg	*C. krusei*	-
37	Swab (lung)	neg	*CONS*	*Enter. faeciumC. krusei*
38	Swab (lung)	*Strep. ssp.*	*CONS*	-
39	Swab (lung)	*Strep. equines*	*CONS*	-
40	Swab (lung)	*Strep. equines*	*CONS*	-
41	Swab (liver)	neg	*C. krusei*	*Stenotrophomonas maltophilaEnter. faecium*
42	Swab (spleen)	*Strep. epidermidis*	neg	-
43	Swab (spleen)	neg	neg	-
44	Liquor	neg	*CONS*	neg
45	Mtral valve	*Staph. aureus*	*Staph. aureus*	-
46	Mtral valve	*Enter. faecalis*	*Enter. faecalis*	-

Clinical Results

46 samples with clinically relevant material like EDTA blood, lung swabs (posthumous), liquor, mitral valves, orthopedic material and paraffin embedded tissue were included in this study. The detection rate of PCR was higher with both methods than that of culture. Results were similar in both methods. Turnaround time for both molecular Dx methods is 6 hours. Due to the automated extraction, hands on time is two hours shorter with hybcell and Select NA (Cube Dx/Molzym).

Conclusion

With its simple workflow and user-friendly format, Cube Dx/Molzym has developed a highly sensitive and specific detection method for human pathogens. This technology is an essential step toward routine diagnostics and is ideally suited to address current issues and provides fast and cost effective workflow.

	Cube Dx	Roche
Minimum turnaround time	6 hours	6 hours
Hands-on time	0.5 hours	2 hours
Simplicity / useability	++	+