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Application Note

Infective Endocarditis – Value of 16S/18S Broad-Range rDNA Diagnosis of Pathogens

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Infective endocarditis (IE) is a serious disease with an incidence of up to 10 episodes per 100,000 person years and a relatively high mortality [up to 20%; 1]. IE is diagnosed by clinical review, echocardiography, vegetation on heart valves and blood culturing [1]. A major limitation is that blood culture stays negative in up to 31% of cases [1] and is limited in the detection of rare pathogens and mixed infections. Culture-negative IE may cause delayed or inappropriate antibiotic treatment with potentially negative clinical outcome.

Broad-range PCR targeting bacterial and fungal rRNA genes together with amplicon sequencing is an evaluated, widely used method of IE pathogen diagnosis [2, 3]. In-house PCR methods, however, are under risk of low sensitivity and false positive results due to DNA contamination of reagents, consumables and other materials used for sample preparation and PCR analysis of heart valves [4]. In this application note clinical results of latest studies are discussed that involved protocols of Molzym's DNA-free reagents and DNA-extraction kits for the direct broad-range PCR and sequencing diagnosis of heart valves for bacterial and fungal pathogens.

Pathogens in IE

A compilation of results from three independent studies was performed comprising of culture and PCR/sequencing results of IE pathogens [6, 8, 9]. The comparative patient-related analysis included combined blood and valve results from culturing (189 patients) and PCR/sequencing (190 patients).

Culture. The positivity of culture diagnosis accounted for 73%. Pathogens at multiple occurrences (8-40 cases) made up the major group of typical aetiologies of IE (66.1% in total), including viridans and ß-haemolytic streptococci, *S. aureus*, coagulase-negative staphylococci (CoNS) and enterococci (Fig. 1, upper graph). Rare pathogens at incidences of 1 to 3 cases (5.8% in total) represented Gram-positive (*P. acnes, G. adiacens, A. defectiva*) and Gram-negative species (*A. actinomycetemcomitans*, *C. burnetii, H. parainfluenzae*, *E. coli*) as well as the yeast *C. albicans*. Two cases of mixed infec-

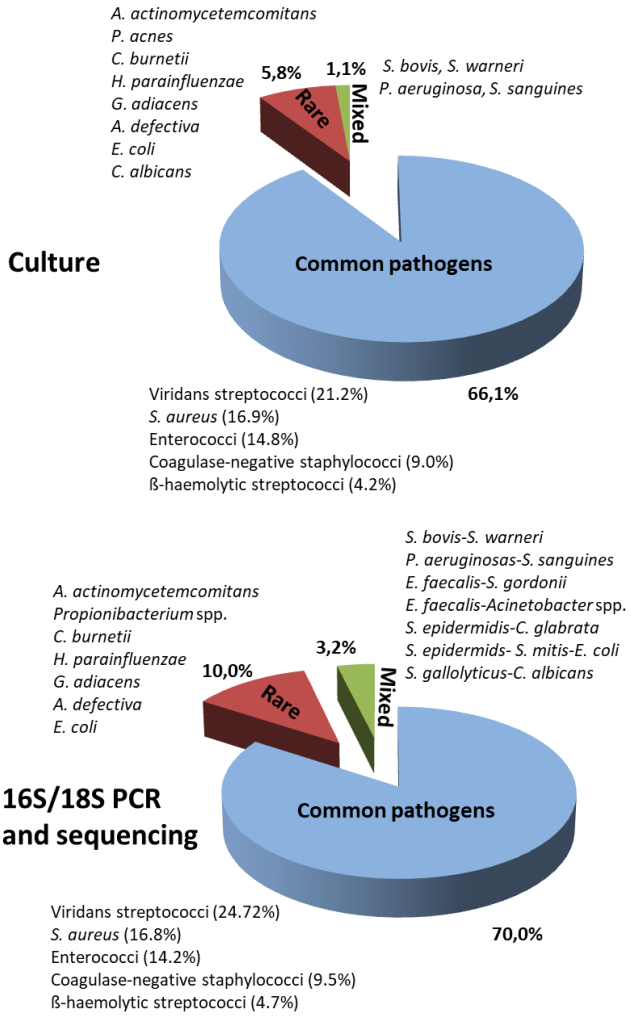

Fig 1: *Pathogens diagnosed in patients with infective endocarditis or aortic valve diseases. The results are the summary of data obtained from direct comparisons of culture and PCR results in three independent studies [6, 8, 9]. The compilation includes results from the analysis of blood and heart valves by culturing (189 patients) and Molzym's 16S and 18S rRNA gene PCR plus sequencing products, SepsiTest™-UMD and MolYsis™ together with Mastermix 16S, respectively (190 patients). The overall positivity of the molecular method (83.2%) was higher compared to culture (73.0%). Percentages are absolute positivity rates.*

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tions by *S. bovis* and *S. warneri* and *P. aeruginosa* and *S. sanguines* were observed, respectively.

PCR and sequencing. With the same cohorts of patients, a higher positivity of 83.2% was determined (Fig. 1, lower graph). With 9 to 47 cases (overall rate: 70%) the same organisms as culture gathered in the main group. The rate of rare pathogens (10%) was 1.7 times higher than culture (5.8%). Again, all culture-determined species were found also by PCR/sequencing (*C. albicans* was in a mixed infection with *S. gallolyticus*; see below). These results emphasize the view that PCR/sequencing reflects culture-determined common and rare pathogens. The rate of mixed infections was 2.9 times higher (3.2%) than culture (1.1%). Importantly, PCR/sequencing uncovered a higher diversity of mixed infections, including combinations of Gram-positive (*S. bovis-S. warneri, E. faecalis-S. gordonii*), Gram-positive with Gram-negative (*P. aeruginosa-S. sanguines, E. faecalis-Acinetobacter* spp., *S. epidermids-S. mitis-E. coli*) and Gram-positive with yeast pathogens (*S. epidermidis-C. glabrata, S. gallolyticus-C. albicans*).

PCR/Sequencing Diagnosis of Heart Valves

On suspicion of IE, blood and resected valves routinely undergo diagnosis by culturing. A comparison of positivity rates of cultured blood samples (median: 62.5%) and PCR/sequencing (65.8%) shows similar values of both methods (Table 1). In contrast, rates of valves by PCR/sequencing (median: 66.7%) were 2.9 times higher than culturing (22.7%). The reasons for culture-negative results are discussed to bear on fastidious growth requirements of certain pathogens and growth inhibition by pre-operative antibiotic treatment of patients [5]. Two studies took the PCR/sequencing results as a basis of change of the antibiotic treatment in 10 to 15% of the patients (Table 1).

Table 1: *Sensitivity of culturing and PCR/sequencing*

		Reference					
Sample	**Method**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**Median**
Blood	**Culture**						
	Positives	n.d.	3	n.d.	25	104	
	Total		28		40	120	
	Rate		10.7%		62.5%	86.7%	62.5%
	PCR						
	Positives	n.d.	17	n.d.	30	n.d.	
	Total		30		40		
	Rate		56.7%		75.0%		65.8%
Valves	**Culture**						
	Positives	9	8	negative	6	31	
	Total	46	26	cohort	40	120	
	Rate	19.6%	30.8%		15.0%	25.8%	22.7%
	PCR						
	Positives	28	20	21	30	105	
	Total	46	30	41	40	120	
	Rate	60.9%	66.7%	51.2%	75.0%	87.5%	66.7%
Antibiotic adjustment according to PCR result		7 15,2%	n.a.	n.a.	n.a.	12 10,0%	

n.d. not done; n.a. not applicable, because this was not the focus of the studies

Summary and Conclusions

Broad-range rDNA PCR and amplicon sequencing is regarded a promising alternative to culture analysis of aetiological agents of IE [1-3, 5-9]. However, there is no standardisation on the grounds of the various methods described in the literature. Besides other factors, a major limitation of in-house methods is DNA contamination of reagents and consumables leading to a high false positive rate. For avoidance, the complete pathway from sample preparation to PCR analysis has to be guided by strict standards of operation. Molzym addresses this problem by the supply of contamination-free products, including CE IVD marked SepsiTest™-UMD. The products have been used in various clinical evaluations of which a selection was taken here to gain a view on their performance with regard to the diagnosis of IE pathogens on heart valves. PCR/sequencing was superior to valve culture in terms of i) notably higher positivity rates, ii) discrimination of highly diverse multiple infections and iii) identification of aetiologies in culture-negative patients. Here, besides common also additional rare IE pathogens were observed, including *Gemella* spp., *Bergeyella* spp., *T. whipplei* and *B. quintana* [5, 7, 10]. Last, PCR/sequencing (approximately 7h) is regarded the faster way to result than culture which can take several days until positivity [5].

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