Application Note

SepsiTest™ – Culture-Independent Molecular Diagnosis of Bacterial and Fungal Infections of Joints and Bones

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Direct Broad-Range PCR Diagnosis of Pathogens

Culture-independent diagnosis of infections by molecular means is an attractive approach for the identification of fastidious, non-cultivable and antibiotic inhibited microorganisms [10]. PCR assays and sequencing have been widely used to detect and identify bacteria and fungi in various clinical materials by using primers that bind to conserved sites of hypervariable genomic regions. A selection of references as shown in Table 1 stresses the importance of broad-range PCR approaches for the molecular diagnosis of pathogens. The most comprehensive and exact way of identifying infectious agents is sequencing of the amplification product followed by searching of sequence libraries for matching entries. Broad-range PCR has been applied in conjunction with a variety of diseases and clinical material (Table 1).

Broad-range PCR is widely accepted. Rice and Madico [11] state that broad-range PCR is most beneficial in the identification of non-cultivable and antibiotic-inhibited organisms. The diagnostic sensitivities recorded are promising, and findings from PCR analysis are used to confirm as well as supplement blood culture results for non-growing etiologies (Table 1). However, because different target sequences are used, home-made assays suffer from a lack of standardization. Another prominent problem associated with this is contamination of extraction and PCR reagents and consumables with exogenous DNA [1]. Approaches for the decontamination of the reagents are more or less successful.

The SepsiTest™ Molecular Diagnostic System

SepsiTest™ and UMD (Universal Microbe Detection) describe a series of compact kits that are CE-marked for the *in-vitro* diagnosis of pathogenic organisms in clinical material. The kits are based on the amplification of the hypervariable V3/V4 region of the 16S and the V9 region of the 18S rRNA gene sequences of bacteria and fungi, respectively. Positive samples are sequence analyzed in order to identify the detected organisms. The kits allow the rapid, culture-independent diagnosis of a variety of clinical samples, including whole blood, other primary sterile body fluids and tissues (Table 2). All buffers, reagents and consumables for the extraction of microbial DNA and the PCR amplification are free of contaminating exogenous DNA. Due to the universal nature of amplification, essentially all microorganisms can be identified. In fact, more than 200 species from a great variety of taxonomic groups have been identified in clinical studies so far (the list is available upon request). Table 2 gives an overview of published studies performed until now using SepsiTest™/UMD. The references address the diagnosis of a variety of diseases, including joint and bone infections, infective endocarditis, sepsis and meningitis.

Table 1: Broad-range PCR in the diagnosis of infectious agents.

Disease	Material	Target gene	Target organisms	Identification method	Patients	Sensitivity (%)	Ref.
Empyema	pleural fluid	16S	bacteria	sequencing	32	100	[14]
Endophthalmitis (case studies)	vitreous fluid	16S ITS1	bacteria fungi	microarray hybridization	3	n.a.	[15]
Infective endocarditis	heart valve tissue	16S	bacteria	sequencing	147	n.d.	[12]
Meningitis, pneumonia	CSF, pleural fluid, bone marrow	16S	bacteria	sequencing	46	n.d.	[7]
Sepsis	blood culture	23S	bacteria	sequencing	295	88.0	[13]
Sepsis	blood culture	16S	bacteria	microarray hybridization	172	100	[17]
Sepsis	blood	16S	bacteria	none	53	96.2	[4]
Sepsis	blood	18S	fungi	probe Real-Time PCR	384	87.5	[20]

Application: Diagnosis of Infections of Bones and Joints

SepsiTest™/UMD were evaluated recently by the molecular diagnosis of clinical material coming to the routine laboratory [2]. In particular, 83 orthopedic and 21 specimens from other body sites from 84 patients were diagnosed for the presence of bacteria and fungi by culture and PCR. Compared to culture, the diagnostic sensitivity and specificity of PCR were 88.5% and 83.5%, respectively. The benefit of PCR was seen by the authors in the identification of a considerable rate (15.5%, see Table 2, line 1) of clinically relevant microorganisms that did not grow in culture. The authors concluded that SepsiTest™/UMD constitutes a valuable supplemental tool for the rapid detection of pathogens, in particular culture-negative infections, allowing earlier initiation of adequate therapy in patients with bone and joint infections.

Laboratory Service

Molzym offers an identification service for the culture-independent molecular identification of pathogens from many kinds of specimens using SepsiTest™/UMD. After receipt of samples, reports are usually supplied within 1-3 weeks, including the identity of organisms in positive samples. Please call for further information.

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Table 2: Performance of SepsiTest™/UMD using diverse clinical material

Disease	Material	Patients	Sensitivity (%)	Culture-negative infections (%) [a]	Ref.
Diverse - bone, joint and other infections	synovial fluid, tissues, CSF, peritoneal fluid	84	88.5	15.5	[2]
Diverse - infective endocarditis, joint infections, meningitis, sepsis	heart valves, synovial fluid, CSF, blood, blood culture	66	n.d.	21.2	[8]
Diverse - sepsis, respiratory, urinary, skin, bone and joint infections	blood	120	80.0 (sepsis)	16.7	[3]
Infective endocarditis	blood and heart valves	30	85.0	53.8	[5]
Corynebacterium striatum endocarditis (case study)	lead tips	1	n.a.	n.a.	[9]
Meningitis	CSF	12	culture-negative	50.0	[6]
Sepsis	blood	187	87.0	13.4	[19]
Sepsis	blood	1	n.a.	n.a.	[16]
Tick-borne - *Neoehrlichia* systemic infection (case studies)	blood	2	n.a.	n.a.	[18]

[a] PCR-positive, culture-negative results with clinically relevant species identified among all patients

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