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Application Note

SepsiTest™-UMD – Single Protocol Direct Molecular Diagnosis of Pathogens in Diverse Samples

Keywords: *in-vitro* routine diagnosis, *culture-independent, bacteria, fungi, yeasts, molds, Real-Time PCR, 16S, 18S rRNA genes, broad-range bacterial, pan-fungal assays*

Michael Lustig - Molzym GmbH & Co. KG, Bremen, Germany

Abstract: SepsiTest™-UMD is the new CE-IVD approved brand that marks the fusion of two former tools for the culture-independent molecular diagnosis of pathogens, SepsiTest™ and UMD-Universal. SepsiTest™-UMD uses only a single protocol for the DNA extraction and Real-Time PCR analysis of a variety of routine fluid and tissue specimens. The tool is characterized by reported clinical evaluations. Like culture diagnosis, SepsiTest™-UMD follows an unbiased approach of analyzing microorganisms. As yet, >750 bacteria and fungi have been identified in clinical material. Unlike culture which needs days to results, SepsiTest™-UMD can identify pathogens within hours, including those prevailing but not proliferating in the body due to antibiotic inhibition or not growing because of fastidious growth demands.

Introduction

At suspect of an infection, classical microbiological diagnosis is performed by culturing of specimens (Fig. 1). The supply of nutrients promotes the growth of potentially present microorganisms which is detected by automated signalling systems in response, for instance, to certain CO_2 levels produced during growth. Gram staining gives a first direction to the selection of more specific antibiotics. Ultimately, biochemical and microbiological fingerprinting analysis leads to the identification of species followed by antibiotic susceptibility testing. The whole process is time consuming, lasting 2 to 7 days, depending on the organism. Furthermore, culture diagnosis produces false negative results in cases of antibiotic treatment before sampling or because media do not meet conditions for growth of fastidious microorganisms.

SepsiTest™-UMD follows, on a molecular level, the line of the microbiological pathway from sample to result (Fig. 1). Instead of culturing and cell proliferation, SepsiTest™-UMD selectively isolates microbial DNA and PCR or Real-Time PCR amplifies DNA targets, the 16S and 18S rRNA genes which are present on all genomes of bacteria and fungi, respectively. Detection of the amplicon occurs by gel electrophoresis or melting curve analysis. At this point, after 4 hours, a first differentiation takes place: test results indicate whether bacteria or fungi or both are present in a sample. Precise identification at the species level is achieved by Sanger sequencing of the amplicon and analysis using Molzym's SepsiTest™-BLAST. The freely available online tool (sepsitest-blast.net) contains quality-edited entries of complete 16S (>7,000 eubacteria) and 18S (>300 *Candida, Cryptococcus, Aspergillus*) rRNA genes of cultivated microorganisms. The analysis is completed within approximately 7 hours or, latest, the next morning if sequencing is run over night.

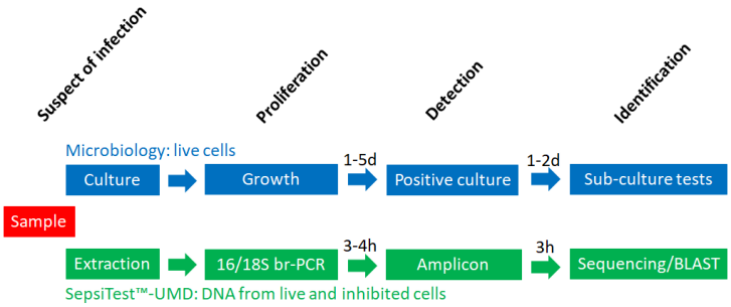

Fig 1: *Analogy of the pathway of in-vitro diagnostics by culture and SepsiTest™-UMD (br-PCR: broad-range 16S and 18S rRNA genes PCR or Real-Time PCR).*

Diverse Sample Analysis by One Protocol

SepsiTest™-UMD provides only one protocol for the extraction of microbial DNA from diverse clinical materials. Tissue biopsies are liquefied by a short (10min) proteinase digestion step. The kit has been evaluated using typical samples coming in routine laboratories, including, for instance, various valves, valve prostheses, pleura biopsies, necrotic tissues, abscesses, cerebral biopsies, bronchoalveolar lavage, diverse muscle biopsies, haematomas, aspirates from pleura, joints, bone marrow, cerebrospinal fluid, EDTA blood, blood culture and various swabs. Sample pre-treatment comprises of the lysis of human cells and the digestion of released human DNA and free floating DNA. Microorgan-

Table 1: *Diagnostic characteristics of SepsiTest™-UMD (UMD) from a selection of published evaluations. PPV: positive predictive value; NPV: negative predictive value.*

Clinical material	No. samples or episodes	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	UMD-positive Culture-negative (%) *	Reference
Reference to culture							
Whole blood	342	87.0	85.8	53.4	97.2	12	[1]
	23	100	70	33	100	26	[2]
	160	78.6	88.4	39.3	97.7	10	[3]
	236	66.7	94.4	53.8	96.7	6.4	[4]
CSF	38	100	nd	nd	nd	24	[5]
	40	86	75	nd	nd	25	[6]
Orthopaedic samples	104	88.5	83.3	63.9	95.6	12.5	[7]
	54	86	98	nd	nd	2	[8]
Various (routine)	66	nd	nd	nd	nd	31.8	[9]
Reference to Duke criteria							
Tissue, heart valve							
UMD	30	85	40	73.9	57.1	30	[10]
Culture		45	100	100	45	na	
UMD	40	88	83	nd	nd	12.5	[11]
Culture		18	100	nd	nd	na	
UMD	46	60.9	nd	nd	nd	41.3	[12]
Culture		19.6	nd	nd	nd	na	
Sum	1179						

isms stay intact and are concentrated from the lysate. In the following, microorganisms are lysed by an enzymatic treatment and the enriched DNA is extracted and purified by a bind-wash-elute process. The eluate is analysed for bacterial and fungal DNA in broad-range PCR assays. Finally, in case of positive results, amplicons are Sanger sequenced (see 'Introduction').

Clinical Evaluation

Table 1 compiles the results from a selection of 12 studies using a total of 1,179 samples. Eight studies including 997 samples used culture results as a reference and provided data on the clinical performance of SepsiTest™-UMD. With a median diagnostic sensitivity and specificity of 86% (range: 66.7 to 100%) and 85.8% (range: 70 to 98%), respectively, SepsiTest™-UMD matched culture results well. The high negative predictive values (median: 97.2; range: 92.3 to 100%) indicate accuracy of negative test results.

Low positive predictive values (median: 53.4%, range: 33 to 63.9%) reflect the identification of pathogens by SepsiTest™-UMD in culture-negative samples. Rates of clinically relevant species can be as high as observed in a routine diagnostic study [9] (31.8%, Table 1).

Three studies employing in total 116 resected valve samples from endocarditis patients according to Duke criteria compared the diagnostic performances of SepsiTest™-UMD and culture (Table 1). The diagnostic sensitivities of SepsiTest™-UMD (median: 85%; range 61 to 88%) were 1.9 to 4.9 times higher than culture (median: 19.6%; range: 18 to 45%). SepsiTest™-UMD detected non-growing pathogens (culture-negative) at considerable rates (12.5 to 41.3%).

Summary

SepsiTest™-UMD is a versatile tool for the *in-vitro* diagnosis of bacterial and fungal pathogens without culturing. Diverse specimens are processed by only a single protocol where tissues are shortly digested before extraction. Broad-range PCR and sequencing identification analysis hit >750 species of eubacteria, yeasts and molds. The tool proved to be reliable by high sensitivities and NPVs in clinical evaluations. Further, non-growing, but persisting pathogens were detected at considerable rates which may lead to a change of the antibiotic regime [12].

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Contact: Dr. Michael Lustig, Molzym GmbH & Co. KG, D-28359 Bremen, Germany
Tel.:+49(0)421 696 162 17; e-mail: lustig@molzym.com