Background – Laboratory Diagnostics

Direct Eubacterial PCR Diagnosis of Primary Sterile Body Fluids and Tissues

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Culture-Independent, Broad-Range PCR Diagnosis

Culturing is the standard method for the detection and identification of pathogens. However, the rate of culture-negative infections can be considerably high [17]. Well studied is infectious endocarditis for which the rate of culture-negative infections can account for 12% and thus be nearly double as high as culture [11]. The high rate of false-negative results is primarily due to fastidious organisms, including species of *Coxiella*, *Bartonella*, *Chlamydia*, *Legionella* and *Tropheryma* some of which can be detected only by molecular means [3, 5]. In the case of sepsis and its progressive forms the cultures remain negative primarily because of the administration of antibiotics before blood collection [4]. Examples of culture-negative infections are summarized in Table 1. Interestingly, the incidence of culture-negative infections, e. g., infectious ascites as judged by clinical parameters, can be two to three times higher than culture proven infections.

Broad-range 16S rRNA gene PCR and sequencing are acknowledged methods for the precise diagnosis of pathogenic organisms. Frequently used as an *ultima ratio* method, 16S rRNA gene PCR allows for the most comprehensive detection and identification of pathogens. It is especially useful for the etiological diagnosis of diseases which can be caused by a variety of organisms from very diverse taxons. PCR methods rely on the detection of nucleic acids and, thus, do not depend on the growth of organisms. For this reason, the major strength of PCR is the proof of pathogens that do not grow in culture because of being fastidious or inhibited by antibiotics (Table 1). For molecular diagnosis, the material is extracted and the purified DNA from potentially present pathogens is analyzed using PCR or Real-Time PCR assays. Detection of DNA from pathogens by broad-range PCR or Real-Time PCR is followed by sequence analysis of amplicons which results in the identification of the species.

Direct Diagnosis of pathogens by SepsiTest™

SepsiTest™ is a CE-marked test for the *in-vitro* diagnosis by broad-range rRNA gene PCR of bacteria and fungi. The diagnosis is performed using clinical material without cultivation. The specimens comprise EDTA, citrate or heparin-stabilized whole blood, aspirates from joints or the peritoneum, abscesses, diverse tissues

Table 1: Culture-negative infections

Disease	Study patients	Specimens	Issue	Culture-positive infections (%) [a]	Culture-negative infections (%) [b]	Method	Ref.
Acute osteoarticular infections	390	blood, aspirates, tissues	MRSA incidence	50.1	49.9	clinical parameters	[22]
Brain abscess	1 (case study)	tissue	*Fusobacterium nucleatum*, *Porphyromonas endodontalis* mixed infection	n.a.	n.a.	broad-range 16S rRNA gene PCR plus sequencing	[8]
	1 (case study)	tissue	*Streptococcus intermedius*	n.a.	n.a.	broad-range 16S rRNA gene PCR plus sequencing	[18]
Bloodstream infection	218	EDTA-blood	detection of bacteremia	19.3	2.3	Gram-specific Real-Time PCR	[1]
	233	blood culture	rate of culture-negative infections	54.9	4.7	broad-range 16S rRNA gene PCR plus array hybridization	[12]
	384	EDTA-blood	MRSA incidence	1.6	2.6	target-specific Real-Time PCR	[21]
Ascitic fluid infection	130	aspirates	bacterial infection in hepatitis B virus cirrhotic patients	28.5	71.5	clinical parameters	[9]
	187	aspirates	viral causes of cirrhosis along with ascites	23.5	76.4	clinical parameters	[7]
	100	tissues, aspirates		9.9	2.0	broad-range 16S rRNA gene PCR plus pyro-sequencing	[2]
Infectious endocarditis	516	blood	incidence of culture-negative infections	6.6	12.2	pathologically, other cultures, serology	[11]

[a] results among all patients without assessment of clinical relevance

[b] clinically relevant results among all patients

(e.g., skin, organ, bone biopsies), and swabs from wounds, catheters and other material. Samples are processed following manual or automated protocols for the enrichment and purification of microbial DNA exclusively from live or intact dead cells. The DNA is analyzed by Real-Time PCR assays for bacterial and fungal sequences. In case of positive results, the amplicon is sequence-analyzed. For this purpose, a BLAST algorithm (sepsitest-blast.de) is available based on more than 7,000 quality-controlled sequence entries of 16S and 18S rRNA genes. Mixed infections of up to 4 strains can be identified by the Gram-specific primer sequencing approach combined with Ripseq® software algorithm (Isentio, Bergen, Norway).

The diagnostic performance of SepsiTest™ has been evaluated employing a variety of samples from more than 500 patients. An international multicenter study on sepsis diagnostics by SepsiTest™ has now been completed. The diagnostic values with blood culture as standard were comparable (e.g., 80-88.5% sensitivity [4, 6, 10, 20]). Besides the rapidity of diagnosis, SepsiTest™ uncovered its benefit in the identification of aetiologies in substantially less time fraction of suspected culture-negative infections (Table 2). In fact, SepsiTest™-approved infections accounted for rates of detection comparable to or even higher than those of culture. Hence, SepsiTest™ constitutes a valuable tool complementing culture diagnosis.

Laboratory Service

Molzym offers an identification service for the culture-independent molecular identification of pathogens from a variety of specimens. After receipt of samples, reports are usually provided within 1-3 weeks, including the identity of organisms in positive samples. Please call for further information.

Table 2: Culture-negative infections determined by SepsiTest™

Primary Diagnosis	Material	Patients	Culture-positive infections (%) [a]	Culture-negative infections (%) [b]	Ref.
Diverse	synovial and peritoneal aspirates, tissues, CSF	84	25.0	15.5	[4]
	heart valves, synovial aspirates, CSF, blood, blood culture	66	18.2	21.2	[14]
	blood	120	13.3	18.3	[6]
Infectious endocarditis	blood and heart valves	29	31.0	48.3	[10]
	catheter tip, *Coryne-bacterium striatum*	1	- [c]	+	[16]
Meningitis	CSF	12	- [c]	50.0	[13]
Sepsis	blood	187	18.2	13.4	[20]
	blood	26	30.8	7.7	[15]
Tick-borne disease	blood, *Neoehrlichia mikurensis*	2	- [c]	+	[19]

[a] cultivation of samples in blood culture bottles; results among all patients clinically not assessed

[b] PCR-positive, culture-negative, clinically relevant results among all patients

[c] culture-negative patients

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