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Application Note

Culture-Independent Broad-Range 16S rDNA PCR and Sequencing Diagnosis of Bone and Joint Infections

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Abstract: DNA-based identification of pathogens without cultivation is an integral part of routine diagnosis. PCR and sequencing analysis directly on samples rapidly uncovers microorganisms that do not grow in culture because of inhibition by antibiotics or fastidious growth requirements and thereby significantly contributes to the management of patients with bone and joint infections.

Introduction

Infections of joints, prostheses, bones and stabilized fractures with internal fixation cause severe morbidity associated with high costs. Diagnosis by microbiological and other methods is difficult, because microorganisms may be inhibited by applied antibiotics, exhibit slow growth or are uncultivable [1, 2].

Molecular methods have been accepted in routine as a means of detecting microorganisms by the analysis of their DNA in samples – without the need for cultivation. Molecular tools are regarded as valuable support to culture due to their independence on growth, rapidity, sensitivity and reproducibility [2]. In this contribution Molzym's CE IVD molecular diagnostic systems are reviewed in respect to their performance and benefits in the diagnosis of bone and joint infections.

Routine Diagnosis of Infected Bones and Joints

In a large study, various specimens from Motol University Hospital, Prague, Czech Republic, were diagnosed for pathogens. Among the 1,370 samples from 973 hospitalized patients, 381 samples (27.8%) were joint tissues and aspirates [4]. Similar rates of material from joint infections (27.1%) were analyzed among 96 samples from tertiary orthopedics, tertiary cardiac clinic and general hospitals in Germany [5]. At Austrian laboratories the incidence of bone and joint specimens even reached 80% [2]. Thus, the diagnosis of infections of joints and bones constitute an important part of daily microbiological routine.

Culture-Independent Molecular Diagnosis

Molzym provides kits and automated systems for the *in-vitro* diagnosis following 98/79 EC regulations (see Table 2). The method follows a single

Fig. 1 The SelectNA™*plus* robot for the automated extraction of microbial DNA from clinical samples.

protocol of microbial DNA enrichment and extraction (MolYsis™) from various specimens, including bone and joint samples, followed by PCR or Real-Time PCR and sequencing analysis. The principle behind the kits is the sensitive detection of microbial DNA by PCR or Real-Time PCR amplification of hypervariable parts of the 16S (V3/V4) and 18S (V8/V9) rRNA genes of bacteria and fungi, respectively. If positive, the amplicon is subjected to a sequencing analysis which provides the best phylogenetic hit by comparison of the sequence with a high quality 16S and 18S rRNA gene library of cultured microorganisms. This broad-range, also called universal approach has identified more than 1,300 microorganisms in clinical and other studies so far [3]. An automated, walk-away solution for DNA extraction is available on the SelectNA™*plus* robot (Fig. 1).

Diagnostic Performance of Molecular Tests

Validated materials included infected synovial fluids, synovial tissues, spinal disks, epidural tissues, vertebral body biopsies, hip tissues and prosthe-

Table 1: Diagnostic performance of Molzym's CE IVD tests for analysis of orthopaedic samples.

Kit	N	Relation	Sensitivity	PCR-positive, culture-negative	Added value of PCR results	Reference
UMD-SelectNA™	381	samples	92%	26%	20%	[4]
SepsiTest™-UMD	17	patients	100%	29%	not determined	[5]
SepsiTest™-UMD	84	patients	89%	13%	not determined	[2]

ses, spine tissues, spinal and other implant materials (spondylodiscitis), and knee prostheses [2, 4-6]. Three independent studies used diverse materials coming in for routine diagnosis, including samples from bone and joint infections. A high degree of agreement of positive PCR and sequencing results with culture was observed. Sensitivities compared to culture accounted for 89 to 100% (Table 1). Obviously, Molzym's tests have a high accuracy of detecting pathogens in orthopedic samples.

Added Value of Culture-Independent Diagnosis

Importantly, all of the studies uncovered positive PCR results at considerable incidences (13 to 29%) with samples that were negative by culture (Table 1). Tkadlec et al. [4] judged PCR positive results in culture-negative samples of 'added value' if (i) a particular organism was identified in another sample from the same site of infection, (ii) the found organism was a pathogen recorded by literature to be the causative agent of the infection or (iii) clinical and/or laboratory signs of infection were apparent, and the result reassured clinicians to continue with or change a certain antibiotic therapy. The majority (20%) of PCR-positive, culture-negative samples (26%) (Table 1) provided clinically relevant information to the treating physician.

Culture-Negative Infections

Culture typically grows coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, streptococci, enterococci, Enterobacteriacae, non-fermenting gram negative bacilli and others from infected orthopedic samples [2, 4, 5]. Similar organisms were found by direct PCR and sequencing analysis in culture-negative samples [2, 4-6]. In addition, rare pathogens some of which are hard to grow if at all were found in single cases, including, for instance, *Granulicatella adiacens*, *Finegoldia magna*, *Tropheryma whipplei* [2] and *Parvimonas micra* [5].

Conclusion

Molzym's PCR and sequencing tests have proven of clinical benefit in the routine diagnosis of bone and joint infections by the identification of significant numbers of common, fastidious and rare pathogens that do not grow in culture. Solutions are provided that can be integrated easily into the workflow of routine practice.

References

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Table 2: Molzym's diagnostic tests provided for the direct analysis of pathogens of bone and joint infections.

Kit	Sample	DNA extraction	Automated	Robot	PCR/Real-Time PCR	Sequencing	Tests	Order no.
SepsiTest™-UMD	1-10* ml fluids, swabs & tissues	yes	no	none	yes	yes	24 48	U-010-024 U-010-048
UMD-SelectNA™	1-10* ml fluids, swabs & tissues	yes	yes (semi)	Liaison® Ixt (Diasorin), Arrow® (Nordiag), Seeprep12™ (Seegene), GenoXtract® (Hain Lifescience/Bruker)	yes	yes	24 48	U-050-024 U-050-048
Micro-Dx™	1 ml fluids, swabs & tissues	yes	yes	SelectNA™*plus* (Molzym)	yes	yes	24 48	U-200-024 U-200-048

* For large volume processing (>1-10 ml), the kits are supplemented with another kit, Add-On 10, for the enrichment of microorganisms from fluid samples

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