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Improving Orthopedic Diagnostics: The Impact of Human DNA Depletion Combined with 16S /18S PCR & Sequencing

Keywords: culture-independent, bacteria, fungi, orthopedic samples, culture-negative infections, 16S rDNA PCR, 18S rDNA PCR, antibiotic treatment, therapy change, Micro-Dx™, SelectNA™*plus*, SepsiTest™-UMD, UMD-SelectNA™, automated DNA extraction, DNA-free reagents, Sanger sequencing

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Abstract

Identification of the causative agent(s) in bone and joint infections (BJIs) is a crucial but important challenge. Recent studies have highlighted the limitations of traditional culture methods leading to what is known as "culture-negative BJI", reportedly occurring in up to 50% of cases [1]. Molzym, a company specializing in broad-range molecular diagnostic solutions, has developed a workflow for the culture-independent detection and identification of bacteria and fungi directly from samples. This workflow offers significant value for the analysis of culture-negative orthopedic samples as demonstrated in this application note.

Introduction

The diagnosis of bone and joint infections is complex and often requires a multidisciplinary approach. Rapid pathogen identification and targeted treatment are critical to prevent serious complications such as bone destruction, joint damage, and sepsis. A considerable number of BJIs are culture-negative or mistakenly identified as aseptic failures, even when diagnostic culture techniques are correctly performed. Common causes include age, smoking status, fastidious growth requirements of the organism(s), with perhaps the most common being preoperative antimicrobial treatment [1, 2]. Several studies also propose the ability of a BJI-causing organism to form a biofilm as a contributing factor [2]. Modern molecular biology techniques are now routinely employed to detect microorganisms by analysing their DNA, without the need for cultivation. These methods are highly valued as complementary tools to traditional culture techniques due to their independence from growth conditions, as well as their speed, sensitivity, and reproducibility. However, their scope is often limited to panels that target only a limited number of microorganisms, potentially missing rare or untypical pathogens, or they are only applicable for a specific sample type. The molecular diagnostic solutions from Molzym, SepsiTest™-UMD, UMD-SelectNA™ and Micro-Dx™ (hereafter referred to as MMDx™), address such limitations with a culture-independent, molecular broad-range approach directly from sample. This application note outlines the clinical applications of MMDx™ and its performance in pathogen diagnostics, with a particular focus on BJIs.

Molecular Diagnostic Workflow

MMDx™ are based on a three-step streamlined workflow that includes (1) manual or automated human DNA depletion and microbial DNA extraction from body fluids, swabs and tissue samples, followed by (2) broad-range 16S and 18S rDNA PCR and (3) amplicon sequencing analysis.

The combination of DNA-free reagents and plastics, in addition to host DNA depletion, ensures high sensitivity for the detection of bacterial and fungal DNA, even in low-load samples. To date, more than 1,300 bacteria and fungi have been identified down to genus or species level using MMDx™ in clinical studies, routine analyses, and other applications. The full list of identified microorganisms is available here. The diagnostic workflow of MMDx™ is illustrated in Figure 1.

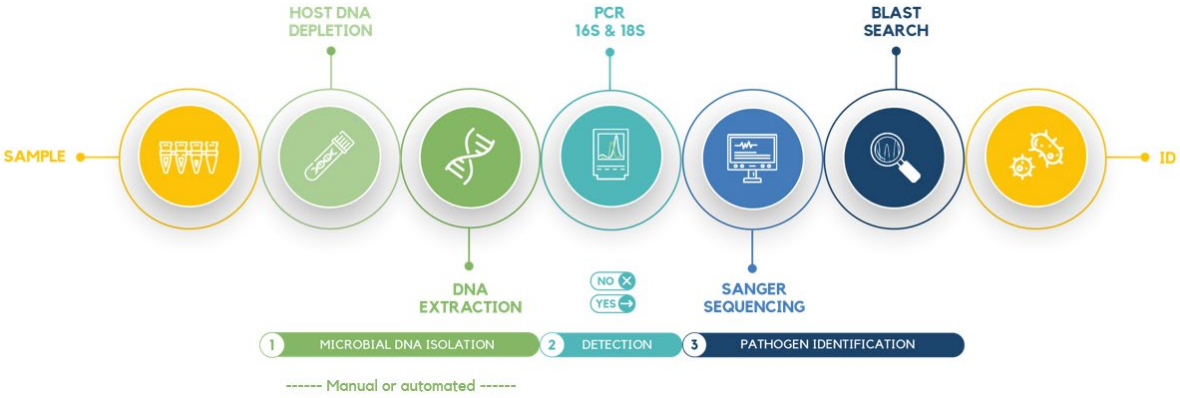

Figure 1: MMDx™ broad-range diagnostic workflow.

Clinical Evaluations

Micro-Dx™ provides significant diagnostic value in culture-negative samples with positive impact on antimicrobial treatment

The diagnostic value of Micro-Dx™ on culture-negative specimens from normally sterile body sites was investigated by the Department of Clinical Microbiology, Region Zealand, Denmark [3]. The retrospective study encompassed microbiological and clinical data from a 2.5 year period, including a total of 529 specimens, predominantly (71.5%) orthopedic specimens, from 223 patients, representing 251 episodes. The findings indicate that in 191 samples (36.1%), bacterial/fungal DNA was detected (see Figure 2) and positive results were judged clinically relevant in 79 (31.5%) episodes. A dominance of Gram-positive bacteria was anticipated reflecting the distribution of sample types which was indeed the case, demonstrated by the dominance of S. aureus, CoNS, and various streptococci in the results.

However, yet more unusual microorganisms were identified that can be difficult to culture, such as *F. necrophorum, Capnocytophaga canimorsus*, Legionella species, Borrelia species, and *Mycobacterium tuberculosis*. Pathogens not normally covered by empiric treatment were also diagnosed, e.g., *Candida albicans* in joint infection. Moreover, results generated by Micro-Dx™ prompted changes in antimicrobial treatment in 42 (16.7%) episodes, demonstrating the clinical

relevance of the results. The study concludes that the results are of significant clinical importance, offering support for the use of Micro-Dx™ in routine clinical practice.

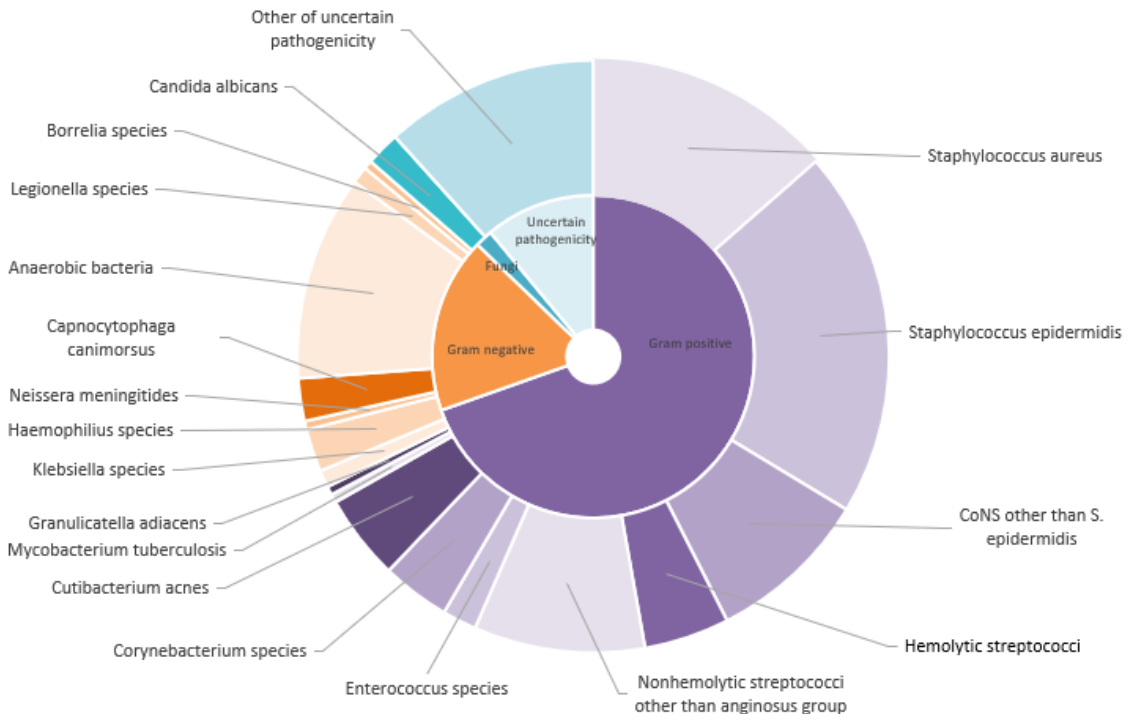

Figure 2: Taxons (genera/species) identified by Micro-Dx™ from 191 positive samples. Original data extracted from [3].

SepsiTest™-UMD demonstrates higher detection rate in comparison to bacterial culture

A team at the Medical University of Innsbruck, Austria, investigated the performance of SepsiTest™-UMD (previously named UMD-Universal) for rapid identification of pathogens in the diagnosis of bone and joint infections [4]. Eighty-three orthopedic specimens and 21 specimens from other normally sterile body sites were tested in parallel by culture and broad-spectrum PCR for the detection of bacterial and fungal DNA. Compared with culture, the diagnostic sensitivity and specificity of MMDx™ were 88.5% and 83.5%, respectively. The detection rate was higher (34.6%) relative to bacterial culture (25.0%). In 13 culture-negative samples, pathogens were identified by MMDX™, including verified polymicrobial infections. In particular, anaerobic, fastidious, and non-cultivable organisms were identified, e.g., Clostridia bacterium, *Finegoldia magna*, *Granulicatella adiacens*, and *Tropheryma whipplei*.

Focussing only on the orthopedic samples from the study, MMDx™ demonstrated a significant added diagnostic value as evidenced by 40% of the positive results being obtained by MMDx™ alone while culture remained negative (see Figure 3).

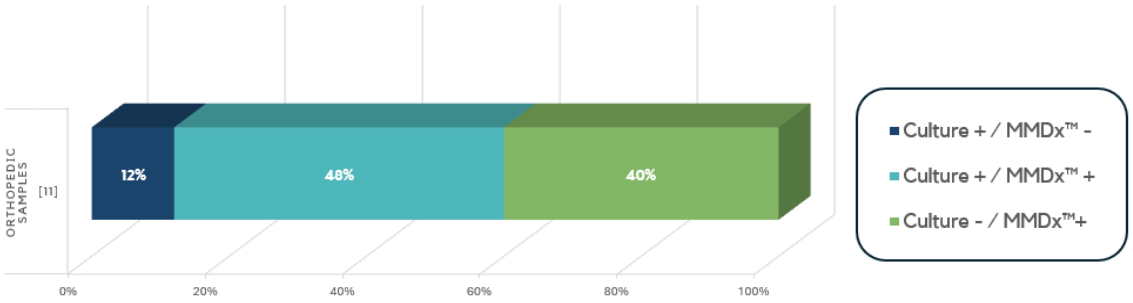

Figure 3: Ratios of positive results by culture, MMDx™ or both methods from orthopedic samples. Original data extracted from [4].

The study concludes that MMDx™ performed as a valuable complementary tool for rapidly identifying pathogens, especially for difficult-to-culture and fastidious organisms, allowing for earlier start of treatment tailored to the specific pathogen in patients with bone and joint infections.

Sensitivity of UMD-SelectNA™ much higher compared to culture

A large prospective study was conducted at Motol University Hospital, Prague, Czech Republic analysing a wide range of specimens, including 151 joint tissue samples and 230 joint aspirate samples with MMDx™ (UMD-SelectNA™ kit) [5]. MMDx™ results were compared with culture, and the additional diagnostic value of the MMDX™ approach evaluated in the cases of negative culture results. Focussing only on joint infection samples from the study, a total of 232 out of 381 (61%) samples were found to be positive by culture, MMDx™ or both. MMDx™ concordance with culture positive was 96% and 86% for joint aspirate and joint tissue respectively. As a result of the greater number of unique PCR-positive samples, the overall sensitivity of MMDx™ was much higher than that of culture, 91% and 66% respectively for joint aspirate samples and 97% and 65% respectively for joint tissue samples (calculated from the data in Table 1).

	Joint Tissue	Joint Aspirate
No. of samples tested	151	230
Positive by culture and MMDx™	49	73
Positive only by MMDx™ (added value)	30	41
Positive only by MMDx™ (not clinically relevant)	10	18
Positive only by culture	8	3
Negative by culture and MMDx™	54	95

Table 1: Results obtained by culture and MMDx™ for joint tissue and joint aspirate fluid samples. Original data extracted from [5].

Pure diagnostic added value, i.e. clinically relevant results obtained by MMDx™ in culture-negative samples, was shown in 30 joint tissue samples and 41 joint aspirate samples. This corresponds to 34% and 35% of the samples, respectively, as shown in Figure 4. The authors of the study conclude that this broad-spectrum PCR approach should be used as a standard method for the analysis of such samples as it allows analysis of samples that were negative with other microbiological methods.

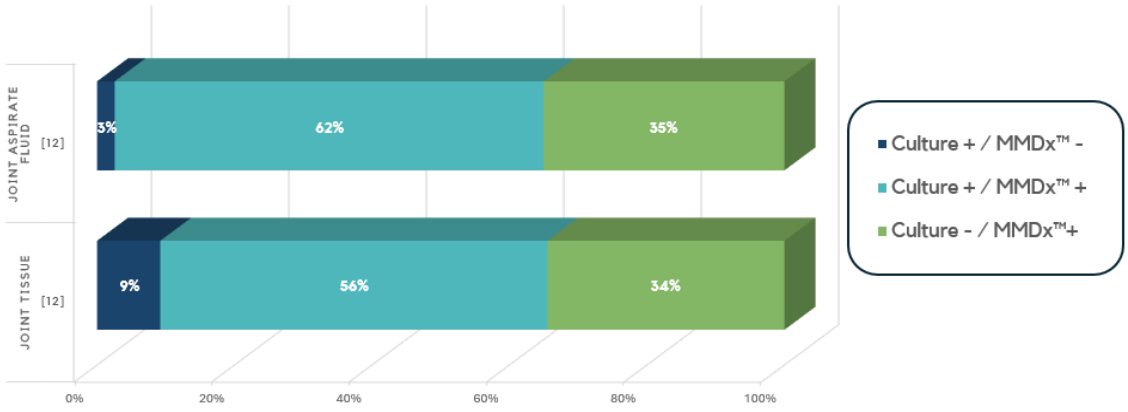

Figure 4: Ratios of positive results by culture, MMDx™ or both methods from orthopedic samples (joint tissue and joint aspirate fluid).

Conclusion

These independent studies clearly show that conventional culture methods have their limitations in the diagnostic examination of difficult, anaerobic or uncultivable microorganisms. MMDx™ has proven to be a reliable and complementary method for the identification of such rare and difficult to culture bacterial and fungal pathogens in standard clinical practice. Contamination-free kits and reagents in combination with human DNA depletion allow for amplification of even small amounts of pathogen DNA for up to 40 PCR cycles without background. Thus, Molzym significantly addresses the obstacles currently encountered in molecular detection methods.

With MMDx™ in combination with Sanger Sequencing, pathogens can be identified within one day, significantly improving the treatment of patients with orthopedic infections through faster pathogen-specific antimicrobial therapy.

Bone and Joint Infections: morbidity, mortality and further statistics

BJIs encompassing conditions such as osteomyelitis, septic arthritis, and prosthetic joint infections (PJI), can lead to severe complications, including chronic pain, functional impairment, and in some cases, permanent disability [6]. The incidence of e.g. osteomyelitis in Europe varies but is estimated at 16.7 cases per 100,000 people in Germany [7]. Prosthetic joint infections are a major concern in Europe, with an incidence rate of 1-2.5% following primary joint replacement surgeries and up to 5.8% following revision surgeries [8].The prevalence of most BJIs is steadily increasing, influenced by increased life expectancy of the population with a corresponding increased use of prosthetic joints and bone fixation devices and changing demographic characteristics, including rates of obesity [9]. Morbidity and mortality rates associated with BJI can be significant, particularly in immunocompromised or elderly patients, with >10% mortality for patients aged over 80 [10]. Septic arthritis can have a mortality rate as high as 16.3% in severe cases [11], while PJI patients have a mortality rate of 45% compared to a rate of 29% in non-infection patients [6].

The economic burden of BJIs on healthcare systems in Europe and globally, is significant. For example: the cost of treating an osteomyelitis patient with a severe open fracture can be increased by 63% and the length of hospital stay increased by 80% when there is an onset of infection [12], and the cost of treating prosthetic joint infection can be more than four times that of the initial joint replacement surgery, due to the need for prolonged antibiotic therapy, additional surgeries, and extended hospital stays [10].

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