Application Note

Micro-Dx™ – Automation of Microbial DNA Extraction in Direct Molecular Pathogen Diagnosis

Keywords: *molecular testing, culture-independent diagnosis, automated microbial DNA extraction, bacteria, fungi, sepsis, meningitis, endocarditis, Real-Time PCR, liquid and tissue biopsies, sequencing*

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Abstract: Enrichment of microbial DNA from samples by selective degradation of human DNA is Molzym's technology, MolYsis™ that greatly enhances the sensitivity and specificity of detection of pathogens by PCR assays. The Micro-Dx™ kit uses MolYsis™, integrated in a solution for the automated isolation of even traces of microbial DNA from liquid and tissue biopsies on the SelectNA™*plus* robotic platform. Micro-Dx™ further supplies reagents for the PCR and Real-Time PCR amplification of the 16S and 18S rRNA genes of eubacteria and fungi, respectively, and primers for the sequencing analysis to identify the species. Micro-Dx™ automated DNA extraction on the SelectNA™*plus* robot greatly reduces hands-on times and the risk of sample contamination. The system has been evaluated against in-house methods using a variety of specimens, including pleural and joint aspirates, EDTA blood, cerebrospinal fluid, swabs, abscesses, heart valves, hematoma, muscle and other tissues. Micro-Dx™ proved to detect more pathogens than standard culture tests.

Introduction

Bacteria and fungi are present at low loads in clinical samples [1]. Culture independent molecular analysis of microorganisms at the limit of detection is challenging because of a variety of parameters negatively influencing the sensitivity and specificity of the assay. First of all, host DNA mostly which exceeds microbial DNA by several orders of magnitude in mass tends to bind microbial sequence-specific primers and thereby reduce the sensitivity and specificity of amplification [2]. Further, contamination of extraction and PCR reagents and consumables as well as handling-borne contamination may produce false positive results [3]. Molzym's MolYsis™ technology selectively removes human DNA and enriches microbes for the extraction of pure DNA. Molzym's kits for MolYsis™ extraction and Real-Time PCR assaying are supplied with reagents and consumables that are guaranteed free of microbial DNA contaminations. Another problem in routine molecular analysis of microbial infections is the high hands-on time that has to be spent, in particular for DNA extraction. Automation therefore is highly desired. Molzym now has released the SelectNA™*plus* robotic system which operates on the grounds of vacuum driven filtration of microorganisms and DNA extraction (Fig. 1).

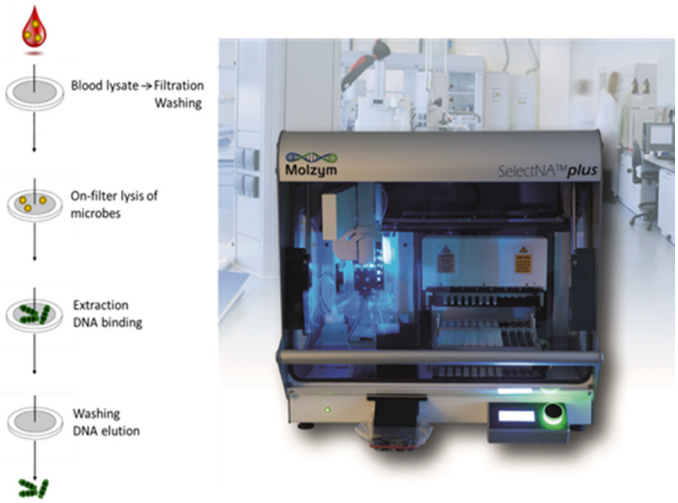

Fig. 1: SelectNA™*plus* automated procedure for the removal of host DNA, enrichment of pathogens and isolation

The Micro-Dx™ Kit

SelectNA™*plus* automated microbial DNA extraction. The SelectNA™*plus* robot (Fig. 1) is a robust, contained desk top instrument that can run single and multiple samples (up to 12). This high flexibility allows for the instant processing of samples as they come to the laboratory. The SelectNA™*plus* robot is equipped with a strong UV lamp for decontamination of the instrument's interior after each run.

For processing of samples, buffer and vacuum cartridges, enzyme vials, elution and sample tubes are supplied with Micro-Dx™. Handling is limited to the loading of the SelectNA™*plus* robot which extracts samples fully automatically. A single run protocol processes a wide variety of liquid and tissue biopsies. A non-microbial DNA which is present in the buffer cartridge is co-extracted with the sample to control the process. At the end of extraction, the resultant eluate is used for PCR based analysis of bacterial and fungal DNA in two Real-Time PCR or PCR assays.

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Specifications	
Robot:	SelectNA™*plus*
Samples:	1 to12
Decontamination:	UV irradiatiion
Kit:	Micro-Dx™
Time to detection:	3.5 to 4 hours
Time to sequence identification:	3 to 4 hours
Assay:	Real-Time PCR
Targets:	16S rRNA gene (V3, V4)
	18S rRNA gene (V8, V9)
Typical LODs (blood):	*E. coli* 100 cfu/ml
	S. aureus 50 cfu/ml
	C. albicans 10 cfu/ml
Specimens:	liquid and tissue biopsies

Broad-range detection and identification. All reagents needed for performing the broad-range 16S and 18S rRNA gene Real-Time PCR and PCR assays together with extraction and run controls are supplied with Micro-Dx™. The assays target the amplification of the hypervariable V3/V4 and V8/V9 regions of bacteria and fungi, respectively. A first result of the presence or absence of bacteria and/or fungi is obtained in less than 4 hours.

Amplicons from positive tests are sequenced and BLAST analysed against a validated gene library using Molzym's open access online tool, SepsiTest™-BLAST, which provides the identification of the pathogen at the species level. Species of more than 200 bacterial and 65 fungal genera have been found so far by clinical evaluations using the Real-Time PCR assay provided in all diagnostic products. Differentiation to the species level is possible for yeasts and *Aspergillus* spp. which are the most common among fungal infections. The use of alternative gene libraries to double check the results is recommended.

Evaluation of Micro-Dx™

Micro-Dx™ has been evaluated in routine laboratories against in-house methods for the identification of pathogens. The list of liquid samples having been evaluated comprises aspirates from joint, pleural, peritoneal and cerebrospinal fluids, wound and other swabs, EDTA- and citrate blood, bone marrow, broncho-alveolar lavage and other fluids. Tissue biopsies are subjected to a short pre-treatment with proteinase K to partially digest the specimen and release microorganisms from biofilms. The liquid supernatant is then fed into the automatic processing for DNA extraction. Examples of tissues analysed using Micro-Dx™ include biopsies from pericardium, different heart valves, meninges, vertebra, aorta, pleura, muscle, necrotized tissue, joints, bones, hematoma and others. For instance, in an evaluation of a routine laboratory, 64 clinical samples were analysed by Micro-Dx™ and the in-house universal PCR test. Among the samples 36 were liquid and 28 tissues biopsies. In total, 26 and 16 samples were positive with Micro-Dx™ and the in-house test, respectively (Table 1 A). The interesting result was that 12 samples were positive with Micro-Dx™, but negative with the in-house test and all except one also culture negative. When organisms were sequence identified in 11 of the 12 Micro-Dx™-positives, all species were judged relevant on the grounds of patient disease and history, including the only culture positive sample (heart valve; *S. epidermidis*), which Micro-Dx™ identified as *Streptococcus* spp.

Table 1: Comparison of Micro-Dx™ with an in-house method. **A:** numbers of concordant and discordant samples; **B:** species identified in 11/12 samples that were Micro-Dx™- positive and all but one in-house test-negative.

A

	In-house PCR positive	In-house PCR negative	Sum
Micro-Dx™ positive	14	12	26
Micro-Dx™ negative	2	36	38
Sum	16	48	64

B

Sample	Micro-Dx™	Culture
Aspirate	*Staphylococcus aureus*	neg
Heart valve	*Streptococcus mutans*	neg
CSF	*Staphylococcus aureus*	neg
Heart valce	*Streptococcus* spp.	Staphylococcus epidermidis *
Abscess tissue	*Streptococcus pyogenes*	neg
Aortic valve	*Streptococcus dysgalactiae*	neg
Pleura aspirate	*Streptococcus pyogenes*	neg
Tissue proximal	*Streptococcus sanguinis*	neg
Heart valve	*Streptococcus mitis* group	neg
Aortal swab	*Streptococcus mitis* group	neg
CSF	*Streptococcus* spp. (mixed)	neg

* regarded as contaminant

In another evaluation Micro-Dx™ was compared with a commercial sequencing-based test system for bacteria [4]. Total DNA extracts were used to run a broad-range 16S rRNA gene PCR followed by amplicon sequencing. Among the 41 liquid, swab and tissue samples, which all were culture-negative, the in-house test found 3 and Micro-Dx™ 16 positives. Micro-Dx™ matched the in-house test-positives, and another 3 were regarded true positives as judged from other microbiological findings. Among the remaining 10 Micro-Dx™-positives, a part could have contained species relevant by literature record and another part contaminations.

Conclusions

The Micro-Dx™ diagnostic system of automated extraction on the SelectNA™*plus* robot and broad-range Real-Time PCR with sequence identification of bacteria and fungi greatly reduces hands-on time and the risk of contamination in culture independent identification of bacteria and fungi. The system processes a great variety of liquid and tissue biopsies and thus constitutes a valuable tool for the precise diagnosis of pathogens.

References

[1] Kellogg JA et al. (2000) J Clin Microbiol 38, 2181-2185.
[2] Disqué C (2007) BIOspektrum 06, 627-629.
[3] Lorenz MG (2016) Res Mol Microbiol (newsletter Molzym) 2/16, 1-8.

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