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Application Note

Clinical Evaluation of Micro-Dx™ CE IVD – Eubacterial and Panfungal PCR Diagnosis Using Automated DNA Extraction

Keywords: *routine molecular testing, in-vitro diagnosis, culture-independent, eubacteria, fungi, pathogens, sepsis, meningitis, endocarditis, joint infections, broad-range Real-Time PCR, sequencing, fluid and tissue biopsies*

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

Abstract: Micro-Dx™ is the new kit for the *in-vitro* diagnosis of microbial pathogens without the need for cultivation. The kit is used with the SelectNA™*plus* instrument which automatically processes a variety of routine specimens ending up with human DNA depleted, pure DNA from eubacterial and fungal pathogens. Assaying is performed by broad-range 16S and 18S rRNA genes PCR or Real-Time PCR analysis which provides a positive-negative result after 3.5 to 4 hours. Identification of species by Sanger sequencing analysis is completed within another 3 to 4 hours. Here, the results of multi-sites evaluations by routine laboratories are presented which either used results from other molecular tests or culture as reference systems. In total, 409 samples were analyzed using Micro-Dx™ in 3 laboratories from Germany and in one laboratory each from Denmark, France, Switzerland and UK. Samples comprised of a variety of tissues, fluid biopsies and swabs. The evaluations indicated acceptable diagnostic performance of Micro-Dx™ with mean values of sensitivity of 89%, specificity of 85%, PPV of 76%, NPV of 88%, and concordance of 85%. In 7 of the 9 evaluations, Micro-Dx™ identified pathogens in reference-negative samples. By its automated sample extraction, Micro-Dx™ contributes greatly to the demand for a reduced hands-on workflow in routine molecular pathogen testing.

Introduction

Automation in routine molecular pathogen testing is crucial for the provision of timely and accurate results. Handling errors with resulting false findings can be greatly reduced by robotic solutions. Reduction of exogenous contaminations becomes even more important when pathogens are to be diagnosed at low loads such as in systemic, joint and nervous system infections. Another demand in routine testing is the limitation of handling time to a minimum, regarding DNA extraction in particular.

Molzym developed an automated, contained system suitable for the safe and reliable enrichment and extraction of microbial DNA from clinical samples (Fig. 1, next page). The SelectNA™*plus* instrument is run with the Micro-Dx™ kit containing DNA-free reagents and consumables for the simultaneous extraction of microbial DNA from up to 12 samples (see box, 'Specifications of Micro-Dx™'). The kit further contains DNA-free reagents for the broad-range amplification with 40 cycles of parts of the 16S and 18S rRNA genes of eubacteria and fungi, respectively. Amplicon sequencing results in the precise identification of microbial species.

Specifications of Micro-Dx™	
Application:	*In-vitro* diagnosis of bacterial and fungal pathogens
Certification:	CE-IVD, 98/79/EC
Specimens:	Liquid and tissue biopsies
DNA extraction robot:	SelectNA™*plus*
Extracted DNA:	Bacterial, fungal; human DNA-depleted
Sample processing:	1 to12
Decontamination:	UV irradiation
Internal process controls:	Extraction control; PCR run control
Hands-on time, extraction (6 samples):	15 min
Assay (detection):	40 cycles PCR or Real-Time PCR with melting curve analysis
Identification:	Amplicon Sanger sequencing
Targets:	16S rRNA gene (V3, V4) 18S rRNA gene (V8, V9)
Time to detection (positive, negative):	3.5 to 4 hours
Time to sequence identification:	3 to 4 hours
Typical LODs:	*K. pneumoniae* 200 cfu/ml *S. aureus* 175 cfu/ml *C. albicans* 2 cfu/ml

In the following the results of multi-site evaluations of Micro-Dx™ are shown and discussed with respect to performance and benefit over reference methods.

Evaluation of Micro-Dx™

In total, 9 evaluations were conducted in 7 routine laboratories in Germany (3 sites), Denmark, Switzerland, France and UK in the time from February 2016 until June 2017. Daily incoming samples included various aspirates from primary sterile body sites, wound and other swabs, and tissue and prosthesis biopsies (Table 1, 4th column; next page). Tissue samples were subjected to a short pre-treatment with proteinase K (10min) to partially digest the specimen and release microorganisms from biofilms for DNA extraction and PCR analysis. In-house used methods served as references for Micro-Dx™, including various self-developed and commercial PCR-based assays, Molzym's SepsiTest™-UMD and culture.

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

In total, 409 samples were analyzed by Micro-Dx™ and reference methods. The median number of samples per evaluation was 37 ± 28 (range: 18 to 110). The diagnostic values observed against the reference systems are shown in Table 1. The mean values were as follows (95% CI): sensitivity 89% (83.8 to 94.2%), specificity 85% (76.5 to 93.5%), PPV 76% (59 to 93%), NPV 88% (81.5 to 94.5%), and concordance of positive and negative results 85% (78.5 to 91.5%). The sensitivity and specificity data is comparable with those available from sepsis studies employing other direct molecular testing systems and culture as reference. With Roche SeptiFast®, SIRS Lab VYOO® and Molzym SepsiTest™ the estimates of sensitivity were in the range 60 to 90%, 30 to 51% and 61 to 88.5%, respectively [1]. Specificity data were available for SeptiFast® and SepsiTest™ which accounted for 74 to 99% and 83.5 to 85.8%, respectively.

Culture-Negative Infections

In 7 of the 9 evaluations Micro-Dx™ found microorganisms that were not detected by the reference methods. The species identified by sequencing were judged possible by record or clinical considerations. The observed rates lay between 5 and 32%. The reason may be that pathogens identified only by Micro-Dx™ were fastidious or inhibited by antibiotics. Thus, Micro-Dx™ contributed to the diagnosis by detecting non-growing clinically relevant microorganisms.

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 showed acceptable diagnostic performance and detected pathogens in samples that were negative in the reference systems. Also, rare pathogens were identified with accuracy in a timely manner (Table 1), some of which grow slowly (e.g., fungi) or not at all in culture (e.g., Candidatus *Neoehrlichia mikurensis*). With its automated, walk-away DNA extraction, Micro-Dx™ greatly reduces hands-on time and the risk of handling- and environment-related contamination. The system processes a great variety of liquid and tissue biopsies and thus constitutes a tool suitable for the precise identification of pathogens in clinical routine without the need for cultivation.

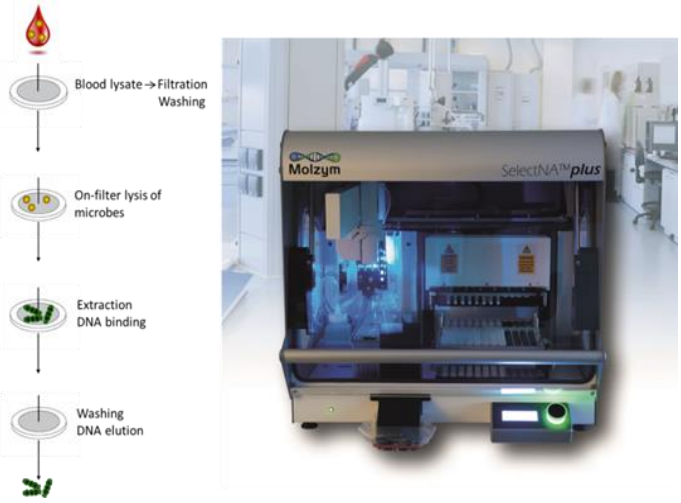

Fig. 1: SelectNA™*plus* instrument for the removal of host DNA from fluid and tissue samples (blood shown as an example), enrichment of pathogens and isolation of microbial DNA. The robot is operated with the Micro-Dx™ kit.

Reference

[1] Zhang et al. (2013) Exp Rev Mol Diagn DOI: 10.1080/14737159.2017.1275964.

Table 1: Comparison of Micro-Dx™ with in-house methods.

Evaluation laboratory	Samples n	Reference method	Specimens	Sensitivity %	Specificity %	PPV %	NPV %	Concordance %	Micro-Dx™ pos / reference neg (%)	Rare etiological species found by Micro-Dx™
Germany	66	SepsiTest™-UMD	AV, BM, CS, EB, ES, HV, HT, JA; MT, NT, SC	82	92	88	88	88	6	*A. defectiva, Cladosporium spp., M. osloensis, N. meningitidis*
Germany	33	SepsiTest™-UMD	AB, AS, BA, BB, CS, EB, EF, ES, HV, MT, PM, WB	93	100	100	95	97	0	*C. parapsilosis, F. nucleatum, H. influenzae, P. agglomerans, P. micra*
Germany	110	SepsiTest™-UMD	AB, AV, BA, EB, HT, HV, JA, PT, PM, SC, TE	82	92	89	86	87	5	*A. fumigatus, A. defectiva, B. quintana, Cand. Neoehrlichia mikurensis, C. diphteriae, F. necrophorum, M. morganii, P. boydii, Rhodococcus* spp.
Germany	64	In-house PCR / culture	AS, CS, EA, HV, JA, SD, WB	88	75	54	95	78	19	none
Germany	21	SepsiTest™-UMD	EF, ES, HV, JA, SC, WB	80	91	89	83	86	5	*F. nucleatum, G. morbillorum*
Denmark	41	In-house PCR / culture	tissues, swabs, body fluids not further specified	100	66	19	100	68	32	none
Switzerland	19	SepsiTest™-UMD / culture	MT, SC, WB	94	100	100	67	95	0	*C. mucifaciens, M. morganii, S. marcescens*
France	37	culture	AS, AV, HV, JA, MT, PS, SC, SD,	79	61	55	82	68	24	*C. burnetii, G. taiwansensis, H. parainfluenzae, Salmonella* spp., *S. marcescens*
UK	18	In-house PCR / culture	AS, HV, MT, PS, SD	100	88	91	100	94	6	*C. parapsilosis, F. nucleatum*

AB: Abscess; **AS:** ascites/peritoneal/pleural aspirate; **AV:** aortic valve; **BA:** broncho-alveolar lavage; **BB:** brain biopsy; **BM:** bone marrow; **CS:** cerebrospinal fluid; **EA:** endotracheal aspirate; **EB:** EDTA blood; **EF:** effusion; **ES:** eSwabs; **HT:** haematoma; **HV:** heart valve; **JA:** joint aspirates; **MT:** tissue/bone biopsy, **NT:** necrotic tissue; **PM:** pace maker; **SC:** sample culture; **TE:** tissue, parafin embedded; **PS:** pus; **PT:** prothesis; **SD:** spinal disc; **WB:** wound biopsy/swab

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