Application Note

MolYsis™ – Microbial DNA Isolation from Diverse Clinical Specimens for Sensitive Molecular Diagnosis

Keywords: pathogen enrichment, DNA isolation, bacteria, fungi, molecular testing, infectious diseases, sepsis, meningitis, joint infections, peritonitis, ascites, endocarditis

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The MolYsis™ Principle

Direct pathogen detection is challenged by extremely low loads of bacteria in clinical samples like blood [1]. Also, human DNA is a factor known to negatively influence sensitivity and specificity of pathogen-directed amplification assays [2]. MolYsis™ quantitatively removes human DNA by DNase treatment, followed by extraction and purification of highly enriched pathogen DNA (**Fig. 1**). Together with the human DNA, floating DNA from dead microorganisms is removed, enabling assaying of only DNA from live pathogens.

Tools

The effect of MolYsis™ bacterial DNA isolation is an improved performance of analytical assays. The main result of MolYsis™ treatment of samples is an increase in detection sensitivity and reduction of false positive rates due to unspecific amplification from human DNA [3]. The enhancement of detection sensitivity is an effect of human DNA removal and increased sample volume used for extraction [4].

MolYsis™ technology is available as a series of products for bacterial DNA isolation from a variety of specimens. The products comprise sample pre-treatment kits (*MolYsis™ Basic* series) which remove human DNA and enrich microorganisms from samples. They are meant to be combined with any other commercial kit or in-house procedure for DNA extraction, including manual and automated protocols. Pre-treatment and DNA extraction are combined in the *MolYsis™ Complete* kits. All products include solutions for small to large sample volumes (0.2 to 10 ml).

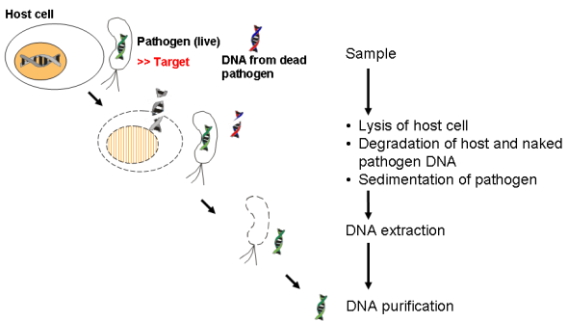

Fig. 1: The MolYsis™ procedure of human DNA removal and microbial DNA isolation.

Fig. 2: *SelectNA™ plus* instrument for full automation of MolYsis™ pathogen DNA isolation from blood and other clinical samples.

MolYsis™ microbial DNA isolation is further available as semi-automated (*SelectNA™*) and walk-away-automated versions (*SelectNA™ plus*, see **Fig. 2**).

Detection limits of PCR assays, such as those directed to the 16S rRNA gene of bacteria with MolYsis™-extracted DNA lie at <20-40 cfu/ml (*S. aureus*, *E. coli*) and thus match pathogen loads prevailing in the blood of septicaemic patients [1]. Processing of 10 ml blood decreases the detection limit even to <3-10 cfu/ml (*S. aureus*, *E. coli*, *M. tuberculosis*). This high sensitivity translates to acceptable diagnostic sensitivities (e.g., blood, 87.0%; orthopaedic samples, 88.5%) and specificities (85.8%; 83.5%) [5,6].

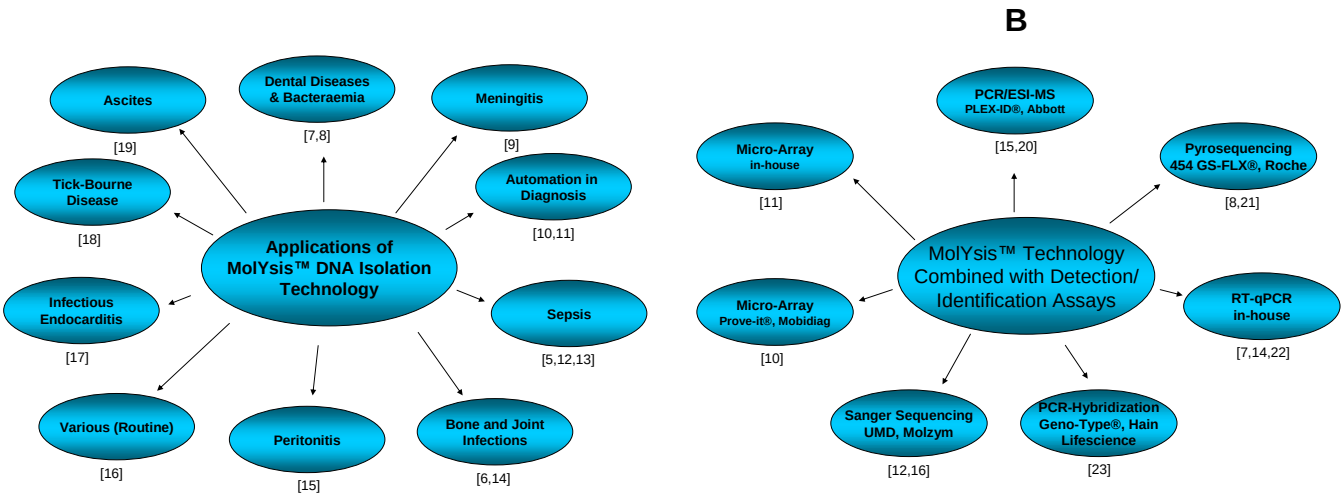

Fig. 3: Use of MolYsis™ for the enrichment and isolation of microbial DNA. A, clinical applications; B, combination of MolYsis™ with different analytical platforms.

Applications

The MolYsis™ procedure for the enrichment of microorganisms and DNA isolation has been employed with the analysis of a variety of clinical specimens (**Fig. 3, A**). In the studies performed up to now, more than 220 bacteria, 24 fungi and a protist have been identified. The direct analysis of samples has the advantage that non-growing pathogens are identified otherwise missed by culture diagnosis. For instance, among 40 patients under suspect of bacterial meningitis, PCR results from MolYsis™-extracted CSF samples matched culture/microscopy in 5 samples, whereas in another 8 PCR-positive samples culture/microscopy was negative [9]. The species identified represented strains known as aetiologies for meningitis (e.g., *Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae*). The authors conclude that the molecular analyis of CSF can add valuable information on the identity of etiologies and thereby aid culture diagnosis in the early management of patients. DNA isolation with MolYsis™ was also employed for the analysis of microorganisms by molecular assays based on hybridisation, pyrosequencing and DNA amplification followed by mass spectrometry (**Fig. 3, B**).

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