

Application Note

Applications of MoLYsis™ Host DNA Removal in Culture-Independent Molecular Analysis of Bacteria and Fungi

Keywords: *direct testing, DNA isolation, DNA-free reagents, next generation sequencing, NGS, microbiome, metagenome, Real-Time PCR, DNA array, liquid and tissue biopsies, automation*

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Abstract: In samples from infected humans and animals host DNA is present in vast excess to microbial DNA. Un-specific primer binding to host DNA decreases the sensitivity and specificity of molecular analysis of the microbial DNA. The MoLYsis™ technology provides a solution to this problem by removing the host DNA before extraction of microbial DNA. The MoLYsis™ Basic kits can be prefixed to any commercial or in-house DNA purification procedure. The MoLYsis™ Complete and Ultra-Deep Microbiome Prep kits comprise the entire process of human DNA removal and microbial DNA purification. With the MoLYsis-SelectNA™plus a fully automated version of the technology is available. All kit components are guaranteed free of contaminating microbial DNA. This application note summarizes clinical and other applications of MoLYsis™ sample pre-treatment in conjunction with a variety of analytical methods, including next generation sequencing, amplicon cloning, real-time PCR, Sanger sequencing and DNA array.

Introduction

Culture-independent molecular analysis of microorganisms in clinical samples is often challenged by low loads of bacteria and fungi [1]. A major problem is that host DNA can exceed microbial target sequences several thousand fold which negatively influences sensitivity and specificity of amplification assays targeting 16S rRNA and other genes [2]. MoLYsis™ is a technology that lyses human cells and quantitatively removes human DNA by DNase treatment. The following extraction and purification of DNA from the intact microorganisms provide highly enriched microbial DNA. Notably, free floating DNA is removed.

The MoLYsis™ technology is available for bacterial and fungal DNA isolation from a variety of specimens. Using MoLYsis™-extracted DNA, detection limits of PCR assays, such as those directed to 16S and 18S rRNA genes of bacteria and fungi, respectively, lie at 10-40 cfu/ml (*Candida albicans*, *S. aureus*, *E. coli*) and thus match pathogen loads prevailing in the blood of septicemic patients [1]. Processing of 5-10ml blood decreases the detection limit even more.

So far, MoLYsis™ extraction and real-time PCR with sequencing analysis identified species from 86 Gram-positive and 120 Gram-negative bacterial and 65 fungal genera.

Fig. 1: Kits available for the enrichment of pathogens from complex fluid and tissue samples and DNA isolation (coloured arrows). For further details, please refer to the text.

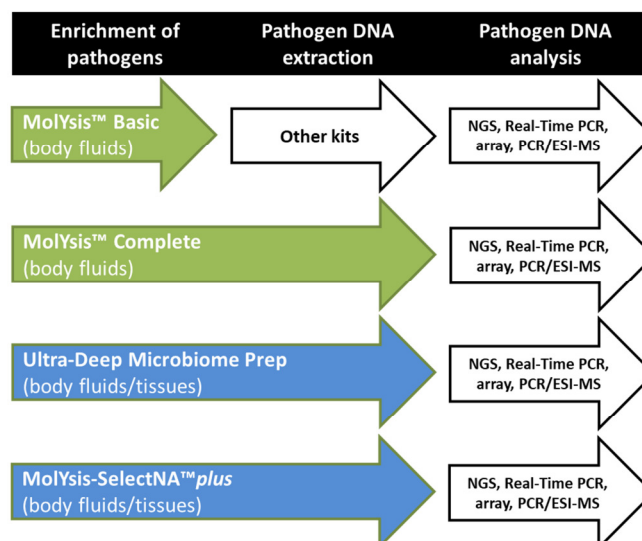


Table 1: Applications of MolYsis™ host DNA removal for molecular analysis of microorganisms.

Topic	Specimen(s)	Host DNA removal	DNA isolation	Assay	Target gene(s)	Organism(s)	Reference
Human diseases							
Ascites	ascites aspirate	MolYsis™ Complete 5		quantitative real-time PCR	16S rRNA gene	bacteria	[3]
Infant nasal microbiome	nasal swabs	MolYsis™ Basic	Agencourt Genfind™ (Beckman Coulter)	NGS (454® GS-FLX, Roche)	<i>cpn60</i>	microbiota	[4]
Oral infection	subgingival plaque	MolYsis™ Basic	mirVana® (Life Technologies)	NGS (MSeq®, Illumina)	metagenome (amplification)	microbiota	[5]
	saliva, subgingival plaque	MolYsis™ Basic	QIAamp® DNA Mini Kit (Qiagen)	quantitative real-time PCR	16S rRNA gene, <i>glyF/R</i>	<i>S. mutans</i> , <i>P. gingivalis</i>	[6]
Pneumonia	broncho-alveolar lavage	Ultra-Deep Microbiome Prep		NGS (HiSeq® 2500, Illumina)	metagenome (amplification)	microbiota	[7]
Bone and joint infection	sonicate fluid from resected samples	MolYsis™ Basic5	Mobio BiOstic® (Qiagen)	real-time PCR/NGS (HiSeq®, Illumina)	16S rRNA gene, metagenome (amplification)	<i>S. aureus</i> , microbiota	[8]
	orthopedic implants	MolYsis™ Complete5		PCR and array	16S rRNA gene	bacteria	[9]
	soft tissue, spongius bone	MolYsis™ Basic	DNeasy® Blood & Tissue kit (Qiagen)	PCR, cloning, sequencing	16S rRNA gene	bacteria	[10]
	tissue biopsies	Ultra-Deep Microbiome Prep		NGS (HiSeq®, Illumina)	metagenome	bacteria	[11]
Urinary tract infection	Urine	MolYsis Basic5	MagNa Pure (Roche)	NGS (MiniON, Oxford Nanopore)	metagenome, antimicrobial resistance	bacteria	[12]
Sinus infection	intramucosal abscess	MolYsis™ Basic	FastDNA SPIN Kit for Soil (MP Biomedicals)	quantitative real-time PCR	16S rRNA gene, <i>gyrB</i> , <i>femA</i>	<i>P. aeruginosa</i> , <i>P. acnes</i> , <i>S. aureus</i>	[13]
Systemic infection	EDTA blood	MolYsis™ Basic	QIAamp® (Qiagen)/ MagNA Pure® (Roche)/ MagSi® (Magna-Medics)	real-time PCR	<i>mecA</i> , <i>femA</i> , <i>sa442</i>	<i>S. aureus</i> (MRSA)	[14]
	EDTA blood	MolYsis™ Basic5	MasterPure yeastDNA (Epicentre)	real-time PCR	ITS	fungi	[15]
	EDTA blood	MolYsis™ Complete 5		NGS (454® GS-FLX, Roche)	16S rRNA gene	bacteria	[16]
	EDTA blood	MolYsis™ Complete 5		NGS (MSeq®, Illumina)	metagenome (amplification)	microbiota	[17]
	EDTA blood	MolYsis™ Complete 5		NGS (HiSeq® 2500, Illumina)	metagenome (amplification)	microbiota	[18]
	EDTA blood	MolYsis™ Complete 5		quantitative real-time PCR	16S rRNA gene	<i>P. gingivalis</i> , <i>A. actinomycetem-comitans</i>	[19]
	EDTA blood	MolYsis™ Complete 5		PCR and array	16S/28S rRNA genes	bacteria and fungi	[20]
Other applications							
Insecticide resistance	whole mosquitos	MolYsis™ Complete 5		NGS (HiSeq®, Illumina)	metagenome	microbiota	[21]
Intestinal <i>M. avium</i> infection (rabbit)	sacculus rotundus tissue	Ultra-Deep Microbiome Prep		NGS (MSeq®, Illumina)	16S rRNA gene	bacteria	[22]
Quality Control	mammalian cell culture	MolYsis™ Basic 5	MagNA Pure (Roche)/ UltraClean® (Qiagen)/ ZR DNA MiniPrep™ (Zymo Research)	quantitative real-time PCR	16S/18S rRNA genes	bacteria, fungi	[23]
Systemic infection model (canine)	EDTA blood	MolYsis™ Complete 5		NGS (PyroMark®, Qiagen)	16S/23S rRNA genes	<i>S. aureus</i> , enteric Gram-negative rods	[24]

Pre-Analytical Processing

The MolYsis™ pre-analytical kit series (see Fig. 1, previous page) encompasses three optional manual usage categories as regards sample processing steps, fluid volume (0.2-10ml) and sample type (fluid, tissue) as well as one automated solution.

MolYsis™ Basic kits. The kits provide protocols for the removal of host DNA and enrichment of microorganisms from liquid specimens, including whole blood, aspirates, cell culture and swabs (Table 1). The kits are ideal to be combined with other commercial kits or in-house

procedures for DNA extraction, including manual and automated protocols (Table 1, column 4, previous page). Small volume liquid samples (0.2ml) are processed by *MolYsis™ Basic*. Higher volumes are handled by the *MolYsis™ Basic 5* (0.2-1ml; 5ml) and *MolYsis™ Basic 10* kits (5-10ml).

Reported applications address NGS analysis of microbiota in nasal communities, oral, PJI and systemic infections as well as QC of mammalian cell culture production (Table 1).

MolYsis™ Complete kits. Molzym supplies kits that comprise of all pre-analytical steps for processing of body fluids, i.e., host DNA removal, enrichment of microorganisms and purification of microbial DNA. An important advantage of this solution is that buffers, reagents and consumables supplied are guaranteed free of bacterial and fungal DNA. This is the best way to exclude contamination by reagent-borne microbial DNA from eluates that are used for highly sensitive analytical protocols like next generation sequencing and real-time PCR analysis (Table 1). Kits are available for the extraction of host DNA-depleted microbial DNA from 0.2-1ml and 5ml (*MolYsis™ Complete5*) and 5-10ml liquid samples (*MolYsis™ Complete10*).

MolYsis™ Complete kits were used for the detection of bacterial DNA in ascites, PJI and systemic infections. Further applications include the metagenomic analysis of mosquito insecticide resistance and canine sepsis (Table 1).

Ultra-Deep Microbiome Prep kit. This kit extracts host DNA-depleted microbial DNA from fluid and tissue specimens for use with Next Generation Sequencing systems (Table 1). Besides NGS, the kit can be used also with real-time PCR, cloning and DNA array assays targeting microbial sequences.

Published studies employing *Ultra-Deep Microbiome Prep* kit are available on the microbial community structure in BAL of a patient suffering from pneumonia and on the influence of pathogenic *Mycobacterium avium* on the microbiota in the intestine of rabbits (Table 1).

MolYsis-SelectNA™plus automated host DNA removal and microbial DNA extraction. The MolYsis™ procedure of human DNA removal, microbe enrichment and microbial DNA isolation from fluid and tissue specimens is also available as an automated system (Fig. 2). The system consists of the *SelectNA™plus* instrument and the *MolYsis-SelectNA™plus* kit. Pre-filled buffer cartridges, reagents and consumables are guaranteed free of contaminating microbial DNA. Handling is limited to the loading of the instrument with cartridges, other consumables

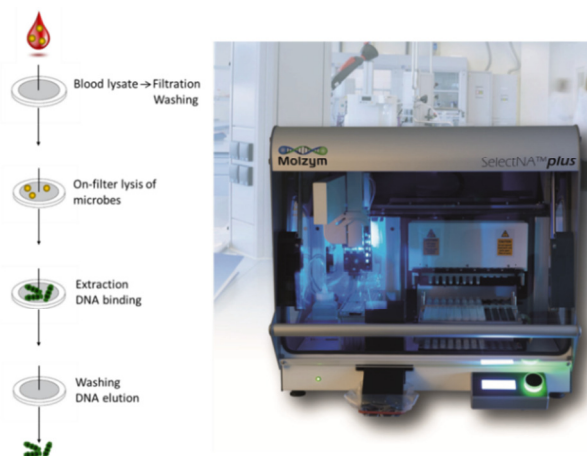


Fig. 2: *MolYsis-SelectNA™plus* automated procedure for the removal of host DNA, enrichment of microorganisms and isolation of microbial DNA.

and samples. 1 to 12 samples can be processed by the instrument at a time. *MolYsis-SelectNA™plus* has been validated using a variety of body fluids and tissue biopsies, including whole blood, BAL, aspirates of ascites, CSF and others, abscess material, tissue biopsies and swabs.

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