

COMPARISON OF THE MOLYSIS SELECTNA PLUS, QIAAMP DNA MICROBIOME AND PURELINK GENOMIC DNA MINI KITS USING 16S-23S RDNA NEXT GENERATION SEQUENCING

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INTRODUCTION

The implementation of Next Generation Sequencing (NGS) for the detection of pathogens in clinical samples is an emerging area in clinical diagnostics. Certe and the UMCG have developed a novel, culture independent, method that detects and identifies bacterial species directly from clinical samples by targeted NGS of the 16S-23S rDNA region (1). A barrier is the ratio of eukaryote to pathogen DNA isolated from samples with a low pathogen load. DNA extraction methods that feature selective lysis of eukaryote cells and subsequent degradation of the released eukaryote DNA followed by isolation of purified microbial DNA could improve the sensitivity of 16S-23S rDNA NGS. We compared the MolYsis SelectNA plus DNA extraction kit (Molzym) and QIAamp DNA Microbiome DNA extraction kit (Qiagen), both featuring eukaryote DNA depletion, with the Purelink Genomic DNA mini kit (Invitrogen) which extracts total DNA, for sensitivity and depletion of eukaryote DNA using 16S-23S rDNA NGS.

METHODS

From a urine and EDTA blood sample a series of mock samples were prepared containing a 10-log dilution of *Staphylococcus aureus* and *Acinetobacter baumannii*. CFU count was established by overnight culture of 10 µl of the prepared dilution series on 5% sheep blood agar. Input for each extraction method was 500 µl of mock sample. DNA extractions were performed according to the manufacturer's instructions. DNA elution volume for the MolYsis SelectNA plus was 200 µl and 50 µl for both QIAamp DNA Microbiome and Purelink Genomic DNA mini kit. Targeted 16S-23S rDNA NGS was performed on the DNA eluates as described by Sabat *et al.* (1) Briefly, the 16S-23S rDNA region was amplified by conventional PCR followed by DNA purification and quantification. Illumina Nextera XT library preparation was performed followed by Illumina MiSeq sequence detection using the Nextera XT v3-600 kit according to the manufacturer's instructions. Data was analysed using CLC bio (Qiagen). Contigs were identified using the NCBI Blast nt database.

RESULTS

Tables 1, 2 and 3 show the results for the detection of eukaryote, bacterial and target bacterial DNA obtained using the MolYsis SelectNA plus, QIAamp DNA Microbiome and Purelink Genomic DNA mini DNA extraction kits respectively. Table 4 shows the average percentage of detected eukaryote DNA in blood and urine samples for the three extraction methods.

Table 5 shows the estimated sensitivity of 16S-23S rDNA for the MolYsis SelectNA plus-, QIAamp DNA Microbiome- and Purelink Genomic DNA mini-DNA extraction kits.

Table 4: Detected eukaryote DNA for the extraction methods.

	MolYsis SelectNA	QIAamp Microbiome	Invitrogen Purelink
Average % eukaryote DNA in EDTA Blood	1 %	25 %	27 %
Average % eukaryote DNA in Urine	0 %	0 %	22 %

Table 5: Detection limits of the extraction methods using 16S-23S rDNA NGS.

	MolYsis SelectNA (CFU/PCR)	QIAamp DNA Microbiome (CFU/PCR)	Purelink DNA mini (CFU/PCR)
Blood <i>S. aureus</i>	~40	~2	~160
Blood <i>A. baumannii</i>	~16	~1	~7
Urine <i>S. aureus</i>	~4	~16	~160
Urine <i>A. baumannii</i>	~16	~7	~1

CONCLUSIONS

- The MolYsis SelectNA plus DNA extraction kit achieved excellent eukaryote DNA depletion in both urine and blood samples.
- The QIAamp DNA Microbiome DNA extraction kit achieved excellent eukaryote DNA depletion in urine but no significant difference was observed in comparison to the Purelink Genomic DNA mini DNA extraction kit for EDTA blood.
- The MolYsis SelectNA plus, QIAamp Microbiome and Purelink Genomic DNA mini DNA extraction kits show comparable sensitivity for *Acinetobacter baumannii*.
- The MolYsis SelectNA plus and QIAamp Microbiome DNA extraction kits show a comparable sensitivity for *Staphylococcus aureus*. The Purelink Genomic DNA mini extraction kit appears a factor 10-100 less sensitive detecting *Staphylococcus aureus*.

RESULTS

Table 1: Results for the MolYsis SelectNA DNA plus extraction kit.

MolYsis SelectNA plus	Illumina Nextera XT NGS			
	Calculated CFU/PCR	Eukaryoot DNA (%)	Bacterial DNA (%)	Bacterial target DNA (%)
Blood <i>S.aur</i> 10 ⁻²	400	0	100	100
Blood <i>S.aur</i> 10 ⁻³	40	2	97	97
Blood <i>S.aur</i> 10 ⁻⁴	4	0	100	0
Blood <i>S.aur</i> 10 ⁻⁵	0.4	4	96	0
Blood <i>A.baum</i> 10 ⁻²	162	0	100	99
Blood <i>A.baum</i> 10 ⁻³	16	0	100	39
Blood <i>A.baum</i> 10 ⁻⁴	1.6	0	99	0
Blood <i>A.baum</i> 10 ⁻⁵	0.16	0	100	0
Urine <i>S.aur</i> 10 ⁻²	400	0	100	100
Urine <i>S.aur</i> 10 ⁻³	40	0	100	100
Urine <i>S.aur</i> 10 ⁻⁴	4	0	100	67
Urine <i>S.aur</i> 10 ⁻⁵	0.4	0	100	0
Urine <i>A.baum</i> 10 ⁻²	162	0	100	100
Urine <i>A.baum</i> 10 ⁻³	16	0	100	100
Urine <i>A.baum</i> 10 ⁻⁴	1.6	0	100	0
Urine <i>A.baum</i> 10 ⁻⁵	0.16	0	100	0

Table 2: Results for the QIAamp DNA Microbiome extraction kit.

QIAamp DNA Microbiome	NGS			
	Calculated CFU/PCR	Eukaryoot DNA (%)	Bacterial DNA (%)	Bacterial target DNA (%)
Blood <i>S.aur</i> 10 ⁻²	1600	18	82	82
Blood <i>S.aur</i> 10 ⁻³	160	94	6	6
Blood <i>S.aur</i> 10 ⁻⁴	16	17	83	75
Blood <i>S.aur</i> 10 ⁻⁵	1.6	0	100	100
Blood <i>A.baum</i> 10 ⁻²	650	4	96	96
Blood <i>A.baum</i> 10 ⁻³	65	3	97	97
Blood <i>A.baum</i> 10 ⁻⁴	6.5	6	94	64
Blood <i>A.baum</i> 10 ⁻⁵	0.6	60	40	14
Urine <i>S.aur</i> 10 ⁻²	1600	0	100	100
Urine <i>S.aur</i> 10 ⁻³	160	0	100	100
Urine <i>S.aur</i> 10 ⁻⁴	16	0	100	0,21
Urine <i>S.aur</i> 10 ⁻⁵	1.6	0	100	0
Urine <i>A.baum</i> 10 ⁻²	650	0	100	100
Urine <i>A.baum</i> 10 ⁻³	65	0	100	92
Urine <i>A.baum</i> 10 ⁻⁴	6.5	0	100	83
Urine <i>A.baum</i> 10 ⁻⁵	0.6	0	100	0

Table 3: Results for the Purelink Genomic DNA mini kit.

Purelink Genomic DNA mini kit	NGS			
	Calculated CFU/PCR	Eukaryoot DNA (%)	Bacterial DNA (%)	Bacterial target DNA (%)
Blood <i>S.aur</i> 10 ⁻²	1600	48	52	51
Blood <i>S.aur</i> 10 ⁻³	160	35	65	35
Blood <i>S.aur</i> 10 ⁻⁴	16	25	75	0
Blood <i>S.aur</i> 10 ⁻⁵	1.6	44	56	0
Blood <i>A.baum</i> 10 ⁻²	650	4	96	96
Blood <i>A.baum</i> 10 ⁻³	65	9	91	90
Blood <i>A.baum</i> 10 ⁻⁴	6.5	26	74	39
Blood <i>A.baum</i> 10 ⁻⁵	0.6		no id	
Urine <i>S.aur</i> 10 ⁻²	1600	9	91	78
Urine <i>S.aur</i> 10 ⁻³	160	21	79	1
Urine <i>S.aur</i> 10 ⁻⁴	16	95	5	0
Urine <i>S.aur</i> 10 ⁻⁵	1.6	17	83	0
Urine <i>A.baum</i> 10 ⁻²	650	0	100	100
Urine <i>A.baum</i> 10 ⁻³	65	4	96	93
Urine <i>A.baum</i> 10 ⁻⁴	6.5	12	88	47
Urine <i>A.baum</i> 10 ⁻⁵	0.6	20	80	5

1. Targeted next-generation sequencing of the 16S-23S rRNA region for culture-independent bacterial identification - increased discrimination of closely related species. Sabat *et al.* Nature Scientific Reports. 2017 Jun.