Poster Session Details

P551 - CIV01 - Clinical Studies of Adult Infectious Diseases: New Diagnostics

Sunday, Jun 23
10:30am - 4:00pm

SUNDAY - CIV-169 - **Depletion of Human DNA and Reduction of Bacterial Contamination towards a Standardized Method for Infection Diagnosis with Next Generation Sequencing**

Author Block:

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Background: Next-generation sequencing (NGS) is a powerful tool for analysing (poly-)microbial infections. A major challenge in the analysis of clinical samples is the non-standardised workflow. On one side, human DNA is in vast excess to the bacterial DNA of interest which leads to a substantial unspecific amplification of non-target material resulting in many irrelevant sequencing reads. On the other side, the contamination of extraction and PCR reagents with bacterial DNA leads to spurious signals. In this study we assess the removal of human DNA before library preparation and the reduction of bacterial contaminants in 16S-PCR reagent kits to obtain specific and informative NGS results.

Methods: EDTA-blood samples of a healthy donor were spiked with serial dilutions (1:2, 1:200, 1:2000, 1:10000) of *S. aureus* or *B. cereus*. DNA extraction was performed with the MolYsis™ technology for the depletion of human DNA integrated in the Molzym SelectNA™*plus* system (UMD), Qiagen QIAamp® UCP Pathogen Mini Kit (QiaU), QiaU in combination with Looxster® Enrichment Kit (QiaL) and QIAamp® DNA Microbiome Kit (QiaM). DNA was analysed either genome-wide with Nextera® XT DNA Library Preparation Kit (NeXT) or targeted to V3/V4 in 16S-rRNA genes with the contaminant-purified Molzym NGS Mastermix (MolzM) or the Roche KAPA HiFi PCR Kit (KapaM). Libraries were normalized, pooled and sequenced with the MiSeq™ system using v3 reagents.

Results: At first, we analysed four DNA extraction kits (UMD, QiaU, QiaL, QiaM) by means of depletion of human DNA. The relative content of human DNA in NeXT samples was reduced most efficiently with UMD (~16-fold). Then, four DNA extraction kits were analysed with two master mixes with 16S-V3/V4 primers (MolzM, KapaM) for bacterial contamination by quantification of relative contents of bacterial DNA in pure blood. For MolzM, ≤5% sequencing reads corresponded to bacterial DNA whereas, for KapaM, ≥90% reads matched to bacterial DNA.

Conclusion: The combination of UMD DNA extraction and MolzM 16S-rRNA amplification substantially depletes human DNA and reduces bacterial contamination, respectively, aiding a resource-efficient and reliable NGS-based workflow towards a standardized method in non-targeted infection diagnosis.