[O44] APPLICATION OF NEXT GENERATION SEQUENCING FOR THE DIAGNOSIS OF ORTHOPAEDIC INFECTION; AN EVALUATION OF FOUR DNA EXTRACTION TECHNIQUES

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Aim: Diagnosing Orthopaedic infection is limited by the sensitivity of culture methods. Next generation sequencing (NGS) offers an alternative approach for detection of microorganisms from clinical specimens. However, the low ratio of pathogen DNA to human DNA often inhibits detection of microorganisms from specimens. Depletion of human DNA may enhance the detection of microbial DNA[1]. Our aim was to compare four DNA extraction methods for the recovery of microbial DNA from orthopaedic samples for NGS.

Method: Simulated samples; pooled culture negative sample matrix was spiked with known concentrations of microorganisms, each panel consisting of 7 samples. Broth culture was performed on simulated samples for comparison with NGS*.
DNA Extraction; total nucleic acid extraction was performed on a automated extraction platform** using the viral NA assay. Modifications included:

1. mechanical lysis (glass beads)
2. lysis of human cells (saponin 0.025%), turbo DNase treatment and mechanical lysis
3. addition of MspJI enzyme post-extraction for methylated DNA digestion

Detection of human and microbial DNA; human endogenous (HE) gene rtPCR*** was utilised following manufacturer's recommendations. Microbial DNA was detected using SYBR green 16s ribosomal RNA rtPCR with high resolution melt-curve analysis****.

Results: Broth culture recovered 64% (9/14) of the microorganisms from simulated samples.
A significant increase ($p<0.01$) in the cycle threshold (C_T) (median C_T ***25.9 IQR 25.5, 26.1***) of the HE gene rtPCR was observed using extraction method b, indicating a significant reduction in human DNA. No significant change ($p=0.38$) in the C_T of the HE gene rtPCR was observed between the baseline method (median C_T 19.2 IQR 18.5, 19.7) and modifications a (median C_T 18.4 ***IQR 18.2, 19.4***) and c (median C_T 19.3 IQR 18.6, 19.4).
Detection of microbial DNA was successful using the base line extraction method and modification a. Microbial DNA was not detected using the 16s ribosomal RNA rtPCR for modifications b and c.

Conclusions: This study has demonstrated that modification of DNA extraction methods using selective enzymatic digestion of human DNA negatively impacts on the recovery of microbial DNA from simulated specimens. Total DNA extraction allows the successful recovery of microbial DNA alongside a significant amount of human DNA. The effect of the presence of human DNA will be subsequently assessed through NGS CosmosID analysis to establish if NGS is more sensitive than broth based culture.

References

S. Oyola ***et al***: Journal of Clinical Microbiology

*BD Bactec; Becton Dickinson, USA, **iXT (DiaSorin, Italy), ***Fast-Track Diagnostics, Malta, ****RotorGene 600