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Paper Poster Session
Non-culture techniques for challenging situations in diagnostics

Increased detection of bacterial and fungal pathogens in culture negative specimens using Universal Microbe Detection assay compared to a routine 16S and 28S analyses in a diagnostic laboratory

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Background: Infections remain a considerable cause of morbidity and mortality in the western world. Culturing is the gold standard for identifying bacterial and fungal pathogens. However, some pathogens are difficult to culture due to low numbers, slow growth, very strict growth requirements or previous antibiotic treatment. Molecular methods have been developed to aid the detection of pathogens in infections. At our Department of Clinical Microbiology in a tertiary referral hospital, 16S and 28S analyses were implemented 6 years ago improving diagnostics, however, the majority of the samples analysed are negative. The broad-range Universal Microbe Detection (UMD) assay (Molzym, Germany) has previously been demonstrated to have an increased detection rate compared to culture. We, therefore, wanted to evaluate the UMD assay for identification of bacteria and fungi in culture negative specimens and compare to the routine molecular analyses in the Department.

Material/methods: 96 culture negative samples from patients suspected of having an infection were processed with the UMD SelectNA™ kit according to the manufacturer's recommendation. In brief, human cells in tissues, fluids and E-swaps were lysed by addition of chaotropic buffer and human DNA (and free-floating microbial DNA) was degraded by a DNase. DNA from intact microbial cells was extracted in the Arrow instrument. Real-Time PCRs amplifying part of the 16S and 18S gene were performed. Positive samples were sequenced by Sanger sequencing and the resulting sequences were BLASTed to the SepsiTest-BLAST database and the NCBI BLAST database. Results were compared to the department's routine 16S and 28S analyses (DNA extraction with DNeasy kit, Qiagen followed by idenitifcation with the MicroSeq ID kit, Applied Biosystems) performed on the same sample and to other microbiological findings from the patient.

Results: 31 samples (32%) were positive with the UMD assay and 15 samples (16%) were positive with the routine 16S and 28S analyses. The results were concordant in 10 samples (see figure).The UMD assay identified 11 additional true positives verified by other microbiological findings, 8 possible pathogens based on literature and 2 contaminants. The routine analyses identified 2 possible pathogens and 3 true positives of which 2 were the same genus level as the organisms identified with the UMD assay and 1 were not identified with the UMD assay. The intracellular parasite *Toxoplasma gondii* was found in this sample; it is possible that the cell wall of the parasite is disrupted by the chaotrop resulting in degradation of the DNA during the UMD DNA extraction.

Conclusions: The UMD assay had a higher positivity rate compared to the department's 16S and 28S analyses and was able to identify more clinical relevant pathogens. It also identified some

contaminants and possible pathogens, necessitating that the findings are evaluated for clinical relevance.