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P2193 Evaluation of DNA extraction methods for *Pneumocystis jirovecii* from respiratory samples

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Background: *Pneumocystis jirovecii* (PJP) is the causative agent of Pneumocystis pneumonia, a potentially life threatening respiratory infection in immunocompromised patients. There are no viable culture techniques for this mono-cellular fungus, so detection is reliant on either microscopic immunofluorescence observation of cysts or nucleic acid amplification methods. Bronchoalveolar lavage (BAL), sputum and lung biopsy samples have the greatest sensitivity. Automated DNA extraction is the most appropriate option for routine diagnostic laboratories. Current published literature focuses on non-automated methods, resulting in limited data regarding the most efficient automated method of extracting PJP nucleic acids from respiratory samples.

Materials/methods: This study compared four automated nucleic extraction protocols:

A. Qiagen EZ1® Virus Minikit

B. Qiagen EZ1® DNA Tissue Kit

C. Promega Maxewell® 16 FFPE Plus LEV DNA Purification Kit

D. Promega Maxewell® 16 Blood DNA Purification Kit

Dilutions of PJP cells (©Qnostics) in a ten-fold serial dilution series in water, starting from 10,000 organisms/ml, were extracted by all four methods in triplicate. Two methods were then selected for testing with spiked PJP negative BAL in triplicate. These were subsequently tested in spiked PJP negative sputum in duplicate. Target nucleic acids were detected using a commercial PCR kit from Fast Track Diagnostics™. The evaluation compared cycle threshold (Ct) values for both the PCR target and internal control. We also assessed the agreement of internal control, the amount of total DNA extracted, quantified using the Qubit™ 2.0 (Thermofisher) and overall ease of use.

Results: Methods B, C and D successfully extracted PJP from water solutions down to 100 organisms/ml. Methods B and C were selected for testing in BAL and sputum and successfully extracted PJP. Only method C successfully extracted detectable quantities of PJP down to 100 organisms/ml from sputum.

Conclusions: Overall method C, the Promega® FFPE Kit, was found to yield consistently lower Ct values for target and internal control from BAL and sputum. Additionally the methodology was conducive to routine implementation.