Abstract No: 96 ***Life Sciences***

DEVELOPMENT OF A COST-EFFECTIVE IN-HOUSE REAL TIME-PCR ASSAY TO DETECT *Clamydia trachomatis*

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Chlamydia trachomatis (CT) is a bacterium causing various infections primarily transmitted through sexual contact or from mother to child during childbirth. It encompasses 15 serovars categorized by antigenic variation, affecting different anatomical sites causing conditions ranging from ocular disease to sexually transmitted infections. CT's ability to evade immune responses and establish chronic infections, particularly asymptomatic in females, underscores its public health challenge. Objectives of this research include optimizing PCR for CT, analysing costs versus commercial kits, and standardizing protocol for reproducibility. The research details both conventional and real-time PCR techniques for CT detection. Three samples were analysed using a 25 μL reaction volume with master mix components, including 1X PCR buffer, 25 mM $MgCl_2$, 10 mM dNTP, primers, and Taq DNA polymerase. Negative controls (tubes 1 and 2) contained distilled water, and tube 3 with CT DNA. Thermal cycling conditions were initial denaturation at 94 °C for 5 mins (1 cycle), denaturation at 94 °C for 30 secs, annealing at 59 °C for 30 secs, extension at 72 °C for 1 min (30 cycles), and final extension at 72 °C lasted for 7 mins (1 cycle). Gel electrophoresis was performed on a 2% agarose gel stained with ethidium bromide alongside a 100 bp DNA ladder. Lane 1 showed no bands, indicating the presence of a negative control; lane 2 displayed faint bands below 100 bp, representing primer dimers; lane 3 exhibited a band confirming successful amplification of the DNA template. Real-time PCR validation included SYBR green dye, primers, and a CT DNA template in a Rotar-gene Q machine. Melting curve analysis identified ideal detection at 1.0 ng/μL and 0.1 ng/μL, with the lowest detection point determined as 0.005 ng/μL. The samples were run on a 2% agarose gel. The gel image was taken after 35 mins, and bands corresponding to the peaks obtained from the melting curve were observed. Cost analysis compared reagents for conventional PCR and real-time PCR. It confirmed real-time PCR's cost-effectiveness due to lower reagent requirements. Successful amplification and detection of CT DNA using real-time PCR have reduced end-point detection time and the need for post-PCR analysis. Real-time PCR demonstrated superior sensitivity and cost-effectiveness compared to conventional PCR for CT detection. Melting curve and gel electrophoresis confirmed its reliability. Future studies should focus on optimizing detection limits and incorporating internal controls for clinical applicability.

Financial assistance from the International College of Business and Technology, Sri Lanka and Cardiff Metropolitan University, United Kingdom are acknowledged

Keywords: *Chlamydia trachomatis*, Cost-effectiveness, Real-time PCR, Sensitivity