Abstract: Introduction and objective: An important agent of nosocomial and community-acquired pneumonia is the bacterium Legionella pneumophila causing sporadic or epidemic disease worldwide. Flagellin is the major subunit of the flagella and Legionellae are flagellated. FLA is a virulence-associated factor. Flagellin not only promotes bacterial invasion but also induces immune responses by the interaction of its N-Terminus domain with host TLR-5. Hence eliciting immune response, flagellin is a candidate for vaccine design and here we report the expression of the protein in a recombinant form.

Method: The flaA gene was inserted into pJET1.2 and subcloned into pET28a(+) as cloning and expression vectors respectively. The recombinant plasmid (pET-flaA) was subjected to sequencing other than PCR and digestion analysis. Protein expression was induced by adding 1mM isopropy-β-D-thiogalactoside to cultures of E. coli strain BL21 transformed with pET-flaA. Protein expression assessed with SDS-PAGE and confirmed by western blot analysis.

Results: The restriction endonuclease digestion, PCR amplification analysis showed that the flaA gene of 1428 bp was amplified from legionella pneumophila DNA and Sequencing analysis of the pET-flaA confirmed the cloning accuracy and in frame insertion of flaA fragment. SDS-PAGE analysis showed the expression of an approximately 47000 Dalton protein consistent to our predicted molecular weight. Western blotting confirmed FlaA expression as it successfully recognized by specific polyclonal antibodies.

Conclusions: sequencing results along with SDS-PAGE and western blot analysis confirms the expression of recombinant FlaA in the heterologous E.coli BL21. Immuno-bloting results also imply that the protein retained its conformation at least partially in the E.coli host.