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(54) Title: OPTIMIZED CELL-FREE SYNTHESIS OF INVASION PLASMID ANTIGEN B AND RELATED COMPOSITIONS AND METHODS OF USE

(57) Abstract: The present disclosure provides a cell-free method for synthesizing an Invasion Plasmid Antigen B (IpaB) antigen associated with a *Shigella* bacterium comprising exogenous addition of the purified chaperone protein IpgC to the cell-free synthesis mixture. The disclosure further provides IpaB antigen mutants comprising non-natural amino acids incorporated during cell-free synthesis, enabling covalent conjugation to a *Shigella* O-antigen polysaccharide. Further provided are Ipa B antigens and conjugates thereof, as well as immunogenic compositions prepared with the synthesized IpaB antigens and conjugates thereof and methods of use.



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