

VAC.23 - Development of a Meningococcal W Conjugate Vaccine

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Introduction: *Neisseria meningitidis* is classified by their capsular polysaccharide composition. There are 12 different serogroups of this pathogen, however only six are responsible for the majority of cases. Meningococcal W produces a polysaccharide capsule composed by a disaccharide containing galactose and sialic acid. Several outbreaks caused by this serogroup were registered in Asia and South America and in the last years, it was observed an increase of these cases.

Objective: The aim of this study is to obtain bulks of meningococcal W conjugate vaccine using modified reductive amination methodology.

Methodology: Meningococcal W strain 2467, from Adolfo Lutz, was cultivated using Frantz medium in a 150L bioreactor under stirring, with pH and temperature under control for 4h. After that period, 10% of this culture was used as inoculum for the 150L culture for 16h. Cell growth, glucose consumption and polysaccharide production were evaluated during this step. After bacterial cells inactivation the supernatant was centrifuged and concentrated to 10% of volume using a 30KDa membrane. Concentrated supernatant (15L) was submitted to precipitation with 3% Cetavlon and Celite was used as a filtration assistant. Elution was done with different concentrations of ammonium chloride. Extractive solution was used to obtain the polysaccharide fraction that was mixed with calcium chloride and precipitated two times with ethanol to obtain a polysaccharide as required by WHO. Purified polysaccharide was dialyzed against 1% EDTA to improve solubility. Reductive amination assays started with evaluation of the polysaccharide oxidation. Modifications of sodium periodate (NaIO_4) concentrations and reaction times were studied. These modifications were evaluated using different exclusion chromatography with TSK G5000PWxl column. Using the best conditions found for polysaccharide oxidation, some conjugate bulks were obtained for evaluation of different reactant ratios (oxidized polysaccharide:activated protein; ratios 1:2, 1:1, 2:1).

Results: Bioreactor growth showed that cells reached stationary phase in 6h with continuous glucose consumption and polysaccharide production. Glucose consumption was about 95% after 16h of culture. Purified polysaccharide was obtained in accordance with WHO requirements and contained 2.38% nucleic acid, 0.69% protein, and 58.93% sialic acid. When reaction was done using 23.4mM NaIO_4 for 17h, all native polysaccharide was consumed as observed by homogenous chromatography peak with elution of 9.23mL. Conjugate bulks were obtained and exclusion chromatography assays demonstrated that lower elution times were observed in all batches as expected, suggesting that there is a polysaccharide:protein linkage. Increasing the reactant ratio revealed a tendency to observe chromatographic profiles with only one peak at ratios above 1:1, suggesting the presence of more homogenous products.

Conclusion: A method will be developed by capillary electrophoresis to determine the content of free components in conjugates batches. Produced conjugate bulks were formulated and inoculated intramuscularly in mice in order to obtain immunized serum for ELISA and bactericidal assays (CEUA: LW65/14).

Keywords: Meningococcal W vaccine; Conjugate vaccine; Reductive amination