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Research paper

Inhibition ELISA as a putative tool for the identification and quantification of meningococcal A and X polysaccharides at various stages of vaccine development

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ABSTRACT

The multivalent glycoconjugate vaccines against *Neisseria meningitidis* are extremely high-priced for the developing world. The high cost is due to the manufacturing set-up required to produce an effective vaccine and other inflators like complex production steps including the production and purification of the polysaccharide and consequently its conjugation with a protein and finally formulating the finished multivalent product. There is an urgent need for assays which are simple, precise, can be applicable at multiple steps and contribute in reducing the overall manufacturing cost, thereby making the vaccines more equitable to the developing world. WHO recommends serological tests for polysaccharide identification and quantitation at different stages of conjugate vaccine production. We report development of inhibition ELISAs for the identification and quantification of *N. meningitidis* serogroup A (MenA) and *N. meningitidis* serogroup X (MenX) polysaccharides (PSs) in samples from stage of cell banking till production of finished product. The method was qualified on various parameters such as specificity, intermediate precision, sensitivity and accuracy. Our results provide a proof of concept for the use of an inhibition ELISA as a common tool for the identification and quantification of PS at various stages of vaccine development and manufacture.

1. Introduction

Meningitis is a bacterial disease caused by the encapsulated bacteria, *Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae* (Pollard et al., 2009). Although, there is a decrease in global meningitis deaths by 21.0% from 1990–2016, the global cases have increased from 2.50 million in 1990–2.82 million in 2016 (GBD Meningitis Collaborators, and Global, regional, and national burden of meningitis, 1990–2016, 2018). On the basis of the structure of polysaccharide (PS) capsules, *N. meningitidis* is classified into 13 distinct serogroups, while, majority of invasive meningococcal disease is caused by serogroups A, B, C, W, Y and X. Historically, *N. meningitidis* serogroup A (MenA) has been the major cause of meningococcal meningitis in Africa. However, increasing incidences of *N. meningitidis* serogroup X (MenX) have also been observed in Africa (Berti et al., 2012).

The PS vaccines against meningitis have shown poor immune response in infants however with the introduction of glycoconjugate vaccines, a new wave of success was achieved (Eskola et al., 1987; Peltola et al., 1992; Costantino et al., 2011). Glycoconjugate vaccines basically based on the research of Avery and Goebel (1929) overcame the disadvantages of the plain PS vaccines (Maiden, 2013; Avery and Goebel, 1929). The linkages of (poly) saccharides with a (suitable) T-cell dependent protein carrier made the former more immunogenic and induce long lasting immune response. Consequently, glycoconjugate vaccines can be perceived as a potent tool in the reduction of many infectious diseases.

However, for the construction of meningococcal conjugate vaccines, there are series of complex steps such as the production and purification of PS, conjugation of the purified PS to a suitable protein carrier and consequently its formulation, mostly in form of multivalent vaccine which is potent and stable. All these complex stages of vaccine development involve several important analytical tests including those for identification and quantitation of PS at various steps. Nuclear Magnetic Resonance (NMR) is used to verify the purity, structure and identity of PS, while, various serogroup specific physico-chemical assays and high-performance anion exchange chromatography coupled with pulsed amperometry detector (HPAEC-PAD) have been employed for

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quantitation of PS (WHO, 2011). However, all the above options are not feasible as common tool at different stages of vaccine development and manufacture due to interfering metrices. World Health Organization (WHO) also suggests the use of immunoassays such as enzyme linked immunosorbent assay (ELISA) for the identification and quantitation of PS. Furthermore, there are reports where authors have used inhibition ELISA for serotype identification of group B streptococcus PS (Arakere et al., 1999). Our laboratory has developed and qualified an inhibition ELISA as a common tool for the identification and quantification of PSs of MenA and MenX in samples from stage of cell banking, shake flask experiments for media optimization, upstream PS production process (USP), downstream PS purification process (DSP), conjugation and at vaccine formulation.

2. Materials and methods

2.1. Bacterial strains

N. meningitidis serogroup A (Albrecht and Ghon) Murray (ATCC® 13,077™), *N. meningitidis* Serogroup C (Albrecht and Ghon) Murray (ATCC® 13,102™) and *N. meningitidis* Serogroup X (Albrecht and Ghon) Murray (ATCC® 35,560™) strains were procured from ATCC (American Type Culture Collection), USA for the production and purification of PS.

2.2. Polysaccharides, conjugates and primary antibodies

The MenA PS, MenX PS, MenA-Tetanus Toxoid (TT) and MenX-TT conjugates were prepared in-house at MSD Wellcome Trust Hilleman Laboratories Pvt. Ltd. using standardized processes. All the 4 antigens met the desired specifications as per World Health Organization Technical Report Series (WHO-TRS) 962 Annex 2 or in-house specifications. In-house primary antibodies were prepared by pooling of rabbit hyper-immune sera obtained after immunizations with monovalent MenA-TT or MenX-TT conjugates at a reputed Contract Research Organization with protocols approved by the Institutional Animal Ethics Committee. The hyper-immunization study was conducted following guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Indian Ministry of Environment and Forest.

2.3. Inhibition ELISA protocol

Inhibition ELISA involves the incubation of an antigen (sample) with primary antibody for specific binding. After the incubation, this antibody-antigen mixture is added to the plate coated with the respective standard antigen in order for the free primary antibody to bind with it. As a result of prior binding of sample antigen to primary antibody, the reaction in the ELISA plate wells is reduced and the reduction of absorbance in the wells is inversely proportional to the concentration of the analyte in the test sample. Following protocol was used to develop the Inhibition ELISAs in our study.

The identity and the quantification experiments were performed using inhibition ELISA (Fig. S1). The antigen concentrations and primary and secondary antibody dilutions for eeach assay were optimized by checker-board analysis in order to ascertain suitable assay working range while keeping antigen-antibody reaction in zone of equivalence and also minimize amount of precious antigen and antibodies used in each assay. In this assay, the in-house rabbit primary antibodies raised against the respective serogroups of *N. meningitidis* (MenA, MenC and MenX) were incubated for 1 h (hr) at 37 °C with threefold dilutions of the respective internal quality control (IQC) (of either PS or the conjugate) and samples. Each well in 96 well microtiter plate (Plate B), finally contained 100 µl of primary antibody (diluted 1:250 for PS experiments, and 1:500 for conjugate and formulation experiments) and 100 µl of the IQC or test samples diluted to different concentrations as below. All the experiments had their respective IQC (of either PS or the conjugate) and the corresponding test samples in duplicate at various concentrations as 0.03, 0.11, 0.33, 1, 3, 9 and 27 µg/ml (unless otherwise stated) diluted in assay buffer (phosphate-buffered saline (PBS) (pH 7.3 ± 0.1) containing 0.1% *v*/v Brij35 (Sigma) and 5% fetal bovine serum (FBS, Gibco)).

A separate plate (plate A) was coated with a 100 µl mixture of *methylated*-Human Serum Albumin (m-HSA) (NIBSC) and the respective in-house purified PS (MenA, MenC and MenX) each at 5 µg/ml (i.e. 500 ng/well) and subsequently blocked with assay buffer after overnight incubation at 2–8 °C. The plate A was washed and, 100 µl pre-incubated antibody-antigen mix from plate B was added to each well and incubated at 37 °C for 1.5 h followed by 30 min at room temperature (RT, 25 ± 2 °C). The plate was washed and incubated for 1 h at RT with 100 µl/well (1: 1000 dilution) of peroxidase labelled anti-rabbit IgG antibodies (Sigma). Plate was washed again and incubated for 10 ± 1 min at RT with the 100 µl/well of 3,3′,5,5′-tetramethylbenzidine (TMB, Sigma). The reaction was stopped by adding 50 µl of 2 M H_2SO_4/well. The plate absorbance was recorded at 450 nm (A_{450nm}) on an ELISA reader (Tecan microplate reader) with a reference to A_{630nm}. ($A_{450–630}$ or OD). The "No Antigen Control" (NAC) consisted of coated wells incubated with sample from Plate B without any antigen (assay buffer with primary antibody only) and had the maximum OD. Percentage inhibition of OD of the different wells containing a particular sample or IQC dilution was calculated against the OD of the NAC with the following formula:

$$\text{Percent inhibition} = (\text{NAC OD} - \text{Test sample OD})/\text{NAC OD} * 100.$$

For quantitation assays, the A_{450nm} of IQC dilutions was used to prepare standard curve using CombiStats software developed by European Directorate for the Quality of Medicines and HealthCare (EDQM) which utilizes a four-parameter logistic sigmoid curve model. The PS or conjugate standard curves were used to extrapolate the respective test samples antigen concentrations.

2.4. Qualification of inhibition ELISA

The MenA and MenX PS and conjugate inhibition ELISAs were qualified for the following parameters namely, specificity, intra and inter-assay repeatability, sensitivity and accuracy referring to the guidelines mentioned in international conference of harmonization (ICH) (ICH, 2005).

Specificity of the assay was analysed by examining how specific the assay is for homologous serogroup while being non-specific for the heterologous serogroup (s). For the specificity experiments, the percent inhibition was calculated with standardized assays as above for representative samples belonging to three serogroups (MenA, C and X) at different concentrations for PS (27 µg/ml till 0.03 µg/ml) and conjugates (27 µg/ml till 1 µg/ml) of MenA and MenX. The precision of the assay was checked by comparing the intra- and inter-assay repeatability with the IQC for both MenA and MenX PS and conjugate experiments. For intra-assay repeatability, 6 replicates of each IQC at each antigen concentration (27 µg/ml till 0.03 µg/ml) were analysed in the same assay plate, while for inter-assay repeatability each assay was run 5 times on different days. The percentage coefficient of variation (% CV) was calculated for the OD and % inhibition at each antigen concentration.

Sensitivity was analysed by conducting inhibition ELISA of the IQC of both serogroups starting with concentration of 600 µg/ml with threefold serial dilutions up to 0.01 µg/ml. Twenty blank controls which comprised of assay buffer in place of the analyte in the assay were used for calculation of Mean and Standard deviation (SD) of the % inhibition for the Blank control. Referring to Armbruster and Pry (2008), the limit of detection (LOD) and lower limit of quantitation (LLOQ) of an assay were calculated as per the following formulae based on signal and noise approach and analysis of overall results from intermediate precision in the assay:

LOD = Concentration of antigen in well with%inhibition > Mean + 2 ∗ SD of Blank controls.

LLOQ = Lowest concentration of antigen in assay range with% inhibition inter − assay CV of ≤ 20% (minimum analyte concentration in assay range with desired precision).

Accuracy of the assay was assessed by calculating percent recovery of the antigen quantity in spiking experiments. The 75 µl of test sample (USP, DSP and purified PS samples for PS assay and vaccine formulation containing both MenA and MenX for conjugate assays) was added with 75 µl of PS or conjugate IQC of defined concentration to find out how close is the observed value to the expected value of the spiked sample. The following formula was used to calculate % recovery:

%recovery = observed value/expected value ∗ 100

2.5. Applications of inhibition ELISA

2.5.1. Serogroup identification of MenA and MenX master cell banks (MCB), working cell banks (WCB) and purified PS samples after fermentation

Inhibition ELISAs as described in Section 2.3 was utilized for the identification of MenA and MenX PSs in MCB, WCB. The thawed vials of MCB or WCB of either MenA or MenX were added with 1 ml sterile PBS (pH 7.3 ± 0.1). Afterwards, the vial was centrifuged at 2000 x*g* at RT for 10 min, the collected pellet was again resuspended in sterile PBS followed by the inactivation of bacterial cells at 60 ± 5 °C for 30 ± 5 mins. Sonication of the inactivated cells was done in the water bath sonicator for 60 ± 5 mins at 30 ± 5 °C. Post sonication, centrifugation was performed at 2000 x*g* at RT for 10 min and supernatant having the respective PS was collected for analysis. The MCB or WCB supernatant samples were then diluted in PBS to 1:3 and 1:9, whereas for the respective PS IQC, three concentrations of 27, 9 and 3 µg/ml were taken for confirming appropriate conduct of the assay and the percent inhibition was calculated.

The inhibition ELISA protocol for the identification of purified PS samples after fermentation of either MenA or MenX was followed as mentioned in Section 2.3 and the percent Inhibition was calculated.

2.5.2. PS estimation at shake flask level

Six shake flasks, each containing different compositions of fastidious media for the growth of meningococci were used to evaluate the optimal media composition for growth (OD_{550nm}) and PS production by the bacteria. The PS was estimated using inhibition ELISA as mentioned in Section 2.3. As a representative, MenA was utilized for shake flask experiments and the culture supernatant samples were tested at the 8th, 10th and 12th hour for OD_{550nm} and PS concentrations.

2.5.3. Estimation of PS at USP and DSP of MenA and MenX

PS estimation for both MenA and MenX was done at the USP level for culture supernatant samples collected at different hours of fermentation and at the DSP level for samples collected after important purification steps for respective serogroups, using qualified inhibition ELISA as mentioned in Section 2.3.

2.5.4. Identification and quantification MenA and MenX PS in monovalent conjugates

MenA and MenX PS was identified and quantified in respective monovalent conjugate samples (A1, A2 and X1, X2, respectively). Inhibition ELISA as described in Section 2.3 was performed for the identification and quantification of PS in the monovalent MenA and MenX conjugates. Percent inhibition was calculated for the identification of MenA and MenX PS in the samples whereas the PS concentration was estimated using IQC standard curve as described in Section 2.3.

2.5.5. Identification and quantification of MenA and MenX PS in vaccine formulations

MenA and MenX PS were identified and quantified in the vaccine formulation samples designated as S1 and S2, containing both MenA and MenX conjugates each at concentration of 10 µg/ml. S1 was adjuvanted (with aluminium phosphate) and S2 was non-adjuvanted formulation. The S1 was centrifuged at 2000 x*g* for 10 min at RT and the supernatant was used as sample for analysis, while S2 was used as such in the assay. Inhibition ELISA as described in Section 2.3 was performed for the identification and quantification of MenA and Men X PS in the multivalent formulations using respective monovalent conjugates as IQC from 27 to 0.03 µg/ml. Percent inhibition was calculated for the identification of MenA and MenX PS whereas the PS concentrations for both were estimated using IQC standard curve as described in Section 2.3.

3. Results and discussion

The development of glycoconjugate vaccines have shown tremendous success in reducing the scourge of infectious diseases (Sharma et al., 2015). However, to produce an effective glycoconjugate, it must undertake several manufacturing and analytical challenges to finally achieve the desired characteristics. One of the quality control measures is to determine the purity, structure and identity of the PS by NMR (Frasch, 2009). However, the set-up required for the establishment of NMR lab including the equipment as well as the skilled manpower requires significant investments which inflates the vaccine cost. Further, an estimation of PS content is required at different stages of vaccine production. WHO recommends the use of ELISA as an alternative test for the identity of PS (WHO, 2011).

The development of the assay in our laboratory used IQC (comprising either purified PS or conjugate of the respective serogroup). Seven-point calibration curve for the IQC was generated for the respective serogroups (MenA or MenX) after three-fold serial dilution with a range from 27 µg/ml to 0.03 µg/ml in association with optimized dilutions of in-house primary antibodies. There are reports which suggest the use of monoclonal antibodies (MAbs) because polyclonal antibodies (PAbs) are likely to cross react with other antigens (or PS) having similar structures. However, authors also suggest the use of PAbs, if the antigen is highly purified (Inzana and Champion, 2007). Moreover, the cost for developing specific MAbs, its production and clone maintenance would be exorbitantly high as compared to raising PAbs especially for low- and middle-income country vaccine manufacturers when MAbs will be utilized at several stages of vaccine development. Keeping into consideration the above points, the assay was developed using PAbs. Also, as described earlier (Sharma et al., 2015), highly purified PSs were used for the establishment of the assay therefore the use of in-house PAbs worked well with the identification and quantification experiments.

This immunoassay was qualified as per the guidelines stated in international conference of harmonization (ICH) (ICH, 2005), as its validity was of great significance. The identification of optimal dilutions of the IQC, primary antibodies and the secondary antibodies marks the first step for the successful establishment of an assay. Consequently, checker board analysis was performed with several dilutions of IQC, primary antibody and secondary antibody. Based on checker board analyses, the range of IQC for both PS and conjugate was selected as 27 µg/ml till 0.03 µg/ml and an optimal dilution of 1:250 and 1:500 of primary antibodies were selected for PS and conjugates ELISAs, respectively. Furthermore, 1:1000 was found to be an optimal dilution of secondary antibody for both the PS and conjugates ELISAs. The OD of NAC for the assays was selected preferably in the range of 2.5 ± 1.0 without reaching saturation. Furthermore, the percent inhibition achieved with the IQC antigen was also considered so as to have at least

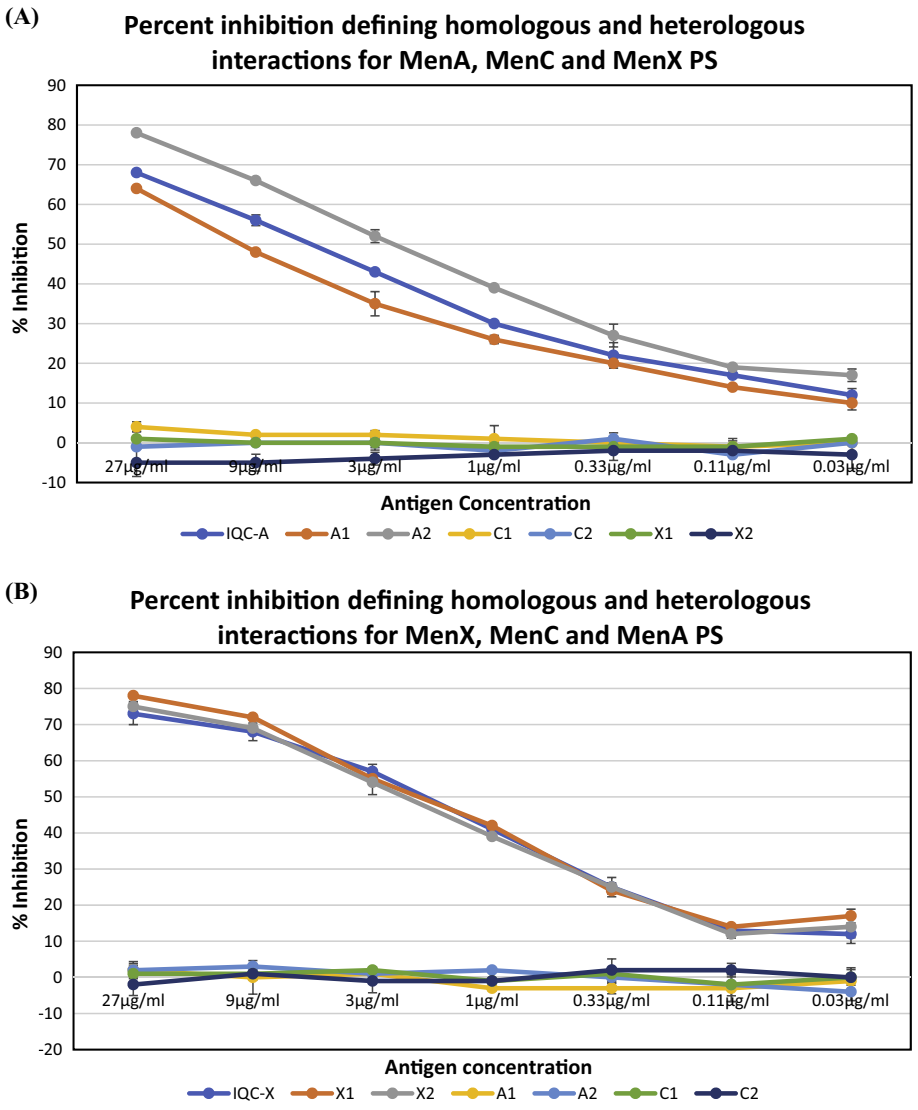

Fig. 1. Inhibition ELISA curves showing percent inhibition for homologous and heterologous interactions in (A) MenA PS and (B) MenX PS assays for MenA (A1, A2), MenX (X1, X2) and MenC (C1, C2) PS samples compared to the respective internal quality control (IQC). Error bars represent standard deviations of the per cent inhibition of the replicates.

60% inhibition for the highest antigen concentration used. As several experiments were conducted for checker board analyses, the results of a representative experiment have been presented as Table S1.

A suitable identification tests should be able to discriminate between closely related compounds and other components in the sample. The specificity of the assays was determined by percent inhibition observed with different concentrations of homologous (e.g. MenA) and 2 other heterologous antigens (e.g. MenC and MenX for MenA ELISA). A percent inhibition of above 10% was observed at all the PS concentrations for homologous serogroups for both MenA and MenX PSs (Fig. 1A, B) and conjugates (data not shown). Whereas for the heterologous interactions, at all the different concentrations tested, the percent inhibition was observed to be below 10% for both heterologous PSs and conjugates. Based on overall specificity results for both the serogroup assays, percent inhibition of above 30% in a sample was set as the identification criteria for the homologous serogroup. A higher cut-off than 10% was set to ascertain assay robustness.

The intra assay percent CV of the percent inhibition values was below 20% at various concentrations of the IQC for MenA PS (3–17%) from 0.33–27 µg/ml and MenA conjugate (2–19%) from 0.03–27 µg/ml, while for MenX PS (3–17%) from 0.11–27 µg/ml and MenX conjugate (2–12%) from 0.03–27 µg/ml, respectively. The inter assay percent CV of the percent inhibition values was below 20% at various concentrations of the IQC for MenA PS (9–19%) from 0.33–27 µg/ml and MenA conjugate (2–13%) from 0.03–27 µg/ml while for MenX PS (4–20%) from 0.33–27 µg/ml and MenX conjugate (2–10%) from 0.03–27 µg/ml, respectively. The results indicate an appreciable intra- and inter-assay CVs of ≤ 20% for the full range of the 4 assays except for the variations in % inhibition values for MenA and MenX PS ELISAs where the %CV values were observed to be higher than 20% at concentrations of 0.03 and/or 0.11 µg/ml. Further, the intra- and inter-assay percent CV of the OD values at all concentrations in all assays was also found to be below 20%.

Several approaches are applied for determining the sensitivity of analytical assays (ICH, 2005; Armbruster and Pry, 2008). Determining LOD and LOQ of the analytical assay are required for setting limit for identity assay and the working range for quantitation assay. Based on the percent inhibition results from the assay sensitivity experiments, the LOD for both PS ELISAs was calculated to be 0.09 µg/ml, and that for MenA and MenX conjugate ELISAs were calculated to be 0.01 and 0.03 µg/ml, respectively as per the formula in section 2.4. Generally, mean of Blank OD plus 2–10*SD of Blank OD values are used for

determination of LOD of various quantitative assays including indirect ELISAs (Armbruster and Pry, 2008). We used Mean + 2*SD of % inhibition of the Blank control for LOD estimation. A representative data for MenA PS Inhibition ELISA sensitivity assay results are presented as Table S2. Further, LLOQ of each of the four assays was calculated and found to be 0.33 μg/ml for both PS ELISAs and 0.03 μg/ml for both conjugate ELISAs based on the formula in Section 2.4. For analysing the assay accuracy, spiking recovery experiments were conducted for both the purified MenA, MenX PS and vaccine formulation samples. The spiking recovery was found to be within the acceptable range of 70% and 130% (90–117% and 85–110% for MenA and MenX PS assay, respectively; 70–80% and 100–110% for MenA and MenX conjugate assay, respectively).

Identification of serogroup of a meningococcal strain and its products including purified PS, bulk conjugate and in finished product are required as part of regulatory requirement (WHO, 2004; WHO, 2011). The inhibition ELISAs, developed in our laboratory were utilized for the identification of PS for both MenA and MenX MCBs and WCBs as well as for the purified PS samples after fermentation. The percent inhibition for both the MCBs and WCBs samples of MenA and MenX, was found to be above the set point of 30% at both the dilutions (1:3 and 1:9) used indicating the presence of the respective serogroup. The percent inhibition for the purified MenA PS (Fig. 1A) or MenX PS (Fig. 1B) at a concentration of 27 μg/ml, was above 30%, demonstrating the presence of respective PSs.

We could successfully quantify the PS concentrations at the shake flasks, USP and DSP levels using qualified inhibition ELISAs. Fig. 2 explains the estimation of MenA PS at shake flasks level. Six shake flasks, each having different compositions of fastidious media for the growth of MenA were used. Media for each of the six shake flasks was adapted from published literature (Reddy, 2009; Sharma et al., 2015) and comprised of different concentrations of glucose (carbon source), yeast extract (nitrogen source), salts and amino acids. The samples for PS estimation consisted of growth phase (8th and 10th hour) and decline phase (12th hour) of the culture, respectively. The results could indicate the optimal media for bacterial fermentation experiments. After the estimation of PS at the shake flask levels, the assay was utilized to estimate the PS concentrations at the USP and DSP levels. The PS estimation of both MenA (Fig. 3A) and MenX (Fig. 3B) was done for the USP samples at the 8th, 10th hr and harvest of fermentation culture, and at the consecutive DSP steps and represented as total PS per batch at different stages. Hence, the immunoassays may be used for the selection of high yielding processes as well as for the process improvements.

The proposed MenA and MenX conjugate assays were also able to identify and quantify PS in the MenA (A1, A2) and MenX (X1, X2) monovalent conjugate samples. The percent inhibition at a concentration of 27 μg/ml was above the set point of 30% for the MenA (Table S3) or MenX conjugate. Further, the estimated PS concentration in the quantitative assay lied within the acceptable range of 70% and 130% (24.60, 29.40, 25.02 and 32.09 μg/ml for samples A1, A2, X1 and X2, respectively as compared to expected concentration of 27 μg/ml for each of them based on biochemical assay).

Furthermore, the inhibition ELISA could identify and quantify MenA and MenX PS in the multivalent vaccine formulations containing both MenA and MenX conjugates. Both the adjuvanted (S1) and non-adjuvanted (S2) formulations gave percent inhibition above 30% at a concentration of 10 μg/ml of both MenA (Table S3) and MenX. The estimated concentrations for MenA were 7.1 μg/ml and 7.3 μg/ml for S1 and S2, respectively, whereas those for MenX, were 11 μg/ml and 9.5 μg/ml, respectively, which is within the acceptable range of 70% and 130% of the expected concentration. Although we did not perform extensive percent adsorption studies for both the formulations of MenA and MenX, however the assay results suggest a low percent adsorption of PSs (via the TT linkage) of both MenA and MenX to the adjuvant. There are also reports which show low adsorption of *Neisseria meningitidis* serogroup C TT conjugate to aluminium phosphate (Otto et al., 2015). Based on our results it can be conveniently construed that the developed immunoassay can be utilized to identify and quantitate meningococcal PS antigen at conjugation as well as at vaccine formulation level also. In addition, it is worth mentioning that application of the developed Inhibition ELISAs in our laboratory has also been demonstrated in evaluation of the stability of MenA and MenX serogroups PS and conjugates (Sharma et al., 2019).

There are several published methods for the identification and quantification of meningococcal PSs, bulk conjugates individually or in multivalent formulation e.g. NMRs, various physico-chemical assays and HPAEC-PAD etc. (WHO, 2011; Reyes et al., 2014). However, the immunoassays confer higher specificity at all different stages in contrast to the interference observed with various physico-chemical assays and NMRs especially at the crude level of USP and DSP due to various impurities and during analyses of a multivalent formulation due to structural similarities. The use of immunoassays such as rate nephelometry and sandwich ELISA are also reported for PS quantification (Aznar et al., 2016). Studies by Inzana and Champion (2007) describe the use of inhibition ELISA for the quantification of capsular PS or

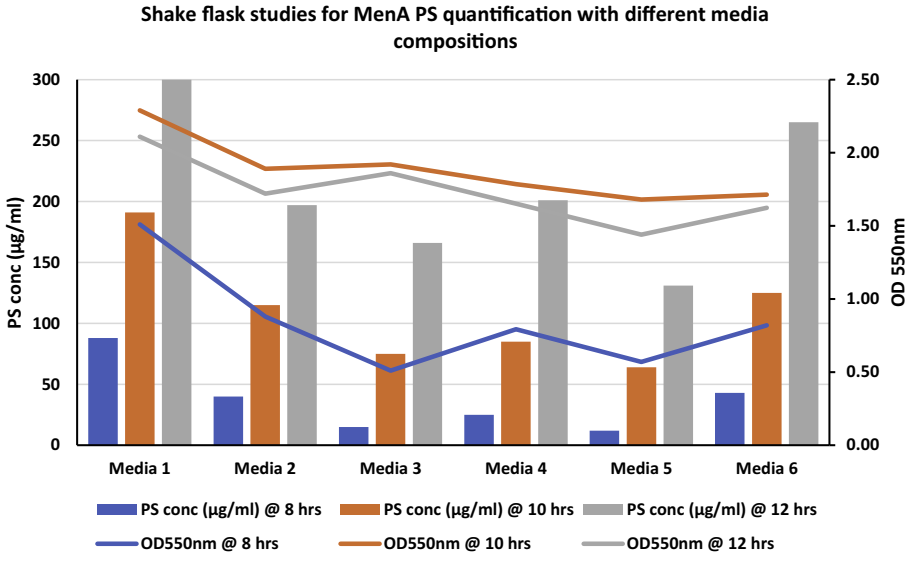

Fig. 2. MenA PS quantification in shake flask experiment for media optimization.

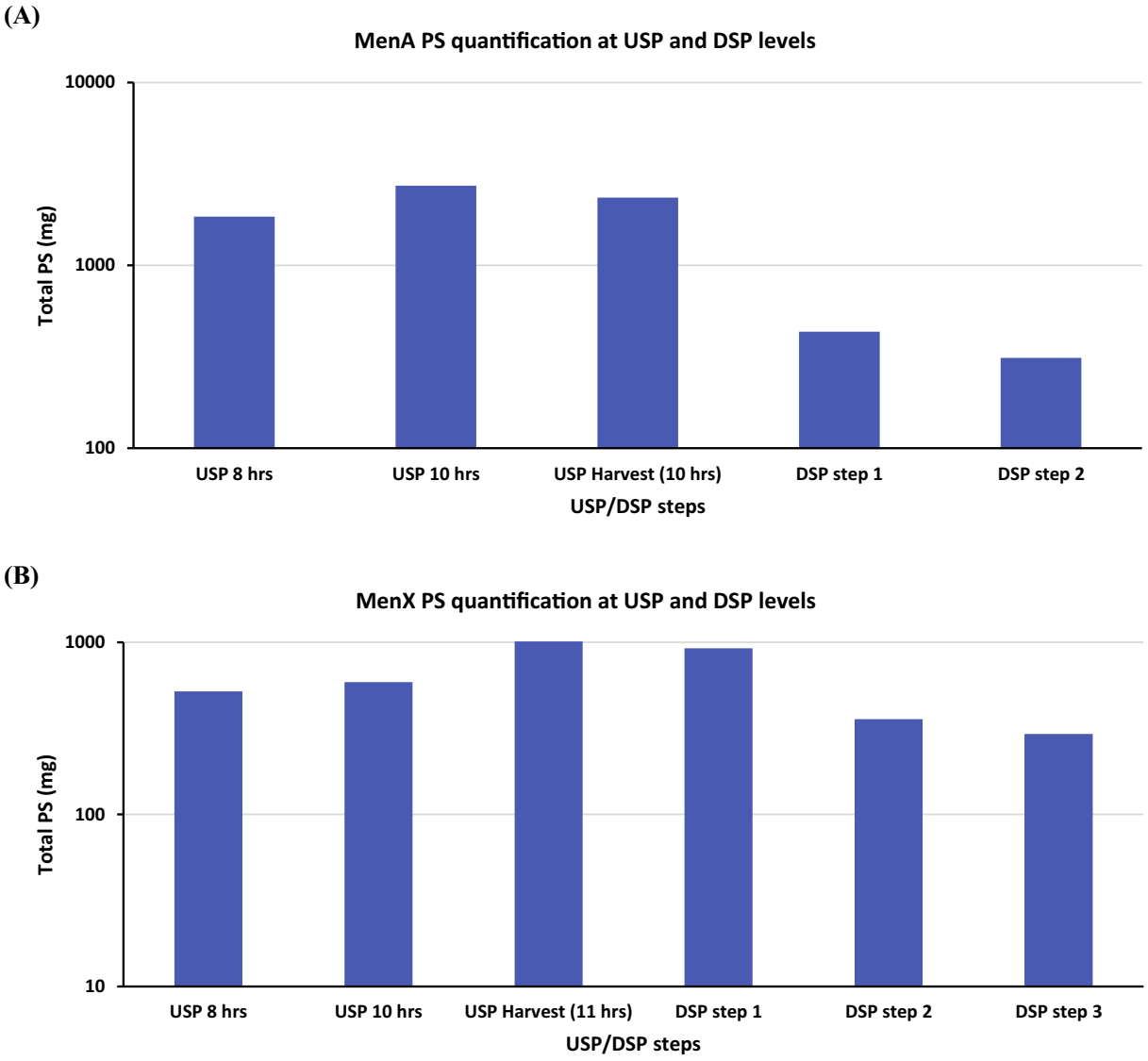

Fig. 3. PS quantification at USP and DSP stages for (A) MenA and (B) MenX.

proteins in the whole cells or extracts of *Actinobacillus pleuropneumoniae*. Furthermore, the use of inhibition ELISA for serotyping of Group B Streptococcal isolates has also been reported (Arakere et al., 1999).

Indeed, these reports validate the potential use of inhibition ELISA in vaccine development. However, it is important to mention that till date there are no reports which describe the identification and quantification of *N. meningitidis* PS using inhibition ELISA. As a step in this direction, our laboratory is the first to report development, qualification and application of Inhibition ELISAs at all different stages of conjugate vaccine development using MenA and MenX as representative serogroups and the assays are equally applicable to other meningococcal serogroups as well.

Contributors

MKC conceptualized and designed the experiments. NS conducted the experiments. NS and SH drafted the manuscript. MKC and DU reviewed the manuscript.

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Declaration of Competing Interest

None of the authors has conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2019.112634.

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