Claims:

1) A method for isolation of polysaccharide from Neisseria meningitidis selected from serotype C, serotype W, and serotype y, said method, comprising of following steps:

a) addition of Sodium dodecyl sulfate (SDS) to crude polysaccharide solution;

b) addition of sodium hydroxide (NaOH) to solution;

c) pH neutralization of solution;

d) subjecting solution to alcohol based precipitation, followed by centrifugation and retention of supernatant;

e) removal of detergent present in the supernatant;

f) concentration and diafiltration of polysaccharide;

wherein, purified polysaccharide obtained has recovery of 60-80%.

2) The method of claim 1, wherein the method further comprises of following steps:

g) mixing of polysaccharide solution with cationic detergent;

h) subjecting solution to centrifugation and collection of pellet; and

i) dissolution of pellet in alcohol, followed by salt based precipitation of polysaccharides.

3) The method of claim 1, wherein

(i) purified polysaccharide from Neisseria meningitidis serotype C has O-acetylation levels >1.5 mMol/ gm, protein impurity <1 %, nucleic acid impurity <1 %, and endotoxin impurity <50 EU/µg;

(ii) purified polysaccharide from Neisseria meningitidis serotype Y has O-acetylation levels >0.3 mMol/ gm, protein impurity <5 %, nucleic acid impurity <2 %, and endotoxin impurity <60 EU/µg; and

(iii) purified polysaccharide from Neisseria meningitidis serotype W has O-acetylation levels >0.3 mMol/ gm, protein impurity <5 %, nucleic acid impurity <2 %, and endotoxin impurity <50 EU/µg.

4) The method of claim 1, wherein final concentration of anionic detergent (SDS) in solution is in the range of 0.1% to 4.0%, preferably less than 1 %.

5) The method of claim 1, wherein final concentration of sodium hydroxide (NaOH) in solution is in the range of 5-50 mM,

preferably in the range of 5-20 mM, and most preferably in the range of 8-15 mM.

6) The method of claim 1, wherein the pH of the solution after alkali addition is in the range of pH 9 - 11, preferably pH 10.5.

7) The method of claim 1, wherein solution is neutralized (pH 7.0) by addition of a mild organic acid.

8) The method of claim 7, wherein the mild organic acid is one of Formic acid, Acetic acid, Propionic acid, Oxalic acid; preferably Acetic acid.

9) The method of claim 1, wherein the removal of detergent is carried out using at least one or more of Potassium salt treatment, Gel filtration, Ethanol treatment, and Ion exchange resins / Amberlite columns.

10) The method of claim 1, wherein the removal of detergent is carried out by precipitation of detergent using Potassium salt.

11) The method of claim 10, wherein potassium salt is one of potassium chloride, potassium acetate, potassium sulfate, potassium carbonate, potassium bicarbonate, potassium phosphate, potassium hydrogen phosphate, potassium dihydrogen phosphate, potassium nitrate, and other potassium salts, or a combination of two or more thereof.

12) The method of claim 11, wherein potassium salt is potassium chloride.

13) The method of claim 10, wherein final concentration of the potassium salt in the solution is higher than or equal to the concentration at which the anionic surfactant is sufficiently precipitated.

14) The method of claim 10, wherein final concentration of the potassium salt in the solution is less than 300 mM, preferably 100 mM.

15) The method of claim 1, wherein the polysaccharide is a polymer of sialic acid residues.

16) The method of claim 2, wherein the cationic detergent(s) is one of cetyltrimethylammonium salt, tetrabutylammonium salt, myristyltrimethylammonium salt and hexadimethrine bromide; or a combination of two or more thereof.

17) The method of claim 2, wherein the cationic detergent(s) is cetyltrimethylammonium bromide (CTAB).

18) The method of claim 2, wherein the concentration of cationic detergent(s) is less than 3%, preferably 1%.

19) The method of claims 1 & 2, wherein the alcohol is a hydrophilic alcohol.

20) The method of claim 19, wherein hydrophilic alcohol is one of methanol, ethanol, n-propyl alcohol, isopropyl alcohol, acetone, and t-butyl alcohol; or a combination of two or more thereof; and its concentration is <65% or >95%.

21) The method of claims 1 & 2, wherein alcohol is ethanol.

22) The method of claim 1, wherein the concentration and diafilration of polysaccharide is done using tangential flow filtration, with 100 kDa MWCO membrane.

23) The method of claim 2, wherein salt used for precipitation is one of Sodium chloride, Ammonium Chloride, Potassium chloride, or combination of two or more thereof.

24) The method of claim 2, wherein salt used for precipitation is Sodium chloride.

25) The method of claim 2, concentration of salt is in the range of 0.25M to 2M.

26) A method for isolation of polysaccharide from Neisseria meningitidis selected from serotype A, and serotype X, said method comprising of following steps:

a) addition of Sodium dodecyl sulfate (SDS) to crude polysaccharide solution;

b) addition of EDTA and Sodium Acetate to solution;

c) subjecting solution to alcohol precipitation, followed by centrifugation and retention of supernatant;

d) removal of detergent present in the supernatant;

e) concentration and diafiltration of polysaccharide;

wherein, purified polysaccharide obtained has recovery of 60-80%.

27) The method of claim 26, wherein the method further comprises of following steps:

a) mixing of bacterial polysaccharide solution with cationic detergent;

b) subjecting solution to centrifugation and collection of pellet; and

c) dissolution of pellet in alcohol, followed by salt based precipitation.

28) The method of claim 26, wherein

(i) purified polysaccharide from Neisseria meningitidis serotype A has O-acetylation levels >2 mMol/ gm, protein impurity <3 %, nucleic acid impurity <1 %, and endotoxin impurity <50 EU/µg; and

(ii) purified polysaccharide from Neisseria meningitidis serotype X has protein impurity <1 %, nucleic acid impurity <1 %, and endotoxin impurity <50 EU/µg.

29) The method of claim 26, wherein the final concentration of sodium dodecyl sulphate (SDS) is in the range of 0.1% to4.0%, preferably less than 1 %.

30) The method of claim 26, wherein the removal of Sodium dodecyl sulfate (SDS) is carried

out using atleast one or more of Potassium salt treatment, Gel filtration, Ethanol treatment, and Ion exchange resins / Amberlite columns.

31) The method of claim 26, wherein the removal of detergent is carried out by precipitation of detergent using Potassium salt.

32) The method of claim 26, wherein final concentration of potassium salt in solution is higher than or equal to concentration at which Sodium dodecyl sulfate (SDS) is sufficiently precipitated.

33) The method of claim 31, wherein potassium salt is one of potassium chloride, potassium acetate, potassium sulfate, potassium carbonate, potassium bicarbonate, potassium phosphate, potassium hydrogen phosphate, potassium dihydrogen phosphate, potassium nitrate, and other potassium salts, or a combination of two or more thereof.

34) The method of claim 27, wherein the cationic detergent(s) is one of cetyltrimethylammonium salt, tetrabutylammonium salt, myristyltrimethylammonium salt and hexadimethrine bromide; or a combination of two or more thereof.

35) The method of claim 27, wherein the cationic detergent(s) is cetyltrimethylammonium bromide (CTAB).

36) The method of claim 35, wherein the final concentration of CTAB is in the range of 0.5 -3.0%, preferably less than 1%.

37) The polysaccharide obtained by method as claimed in claim 1 or 26.

38) The polysaccharide as claimed in claim 37, is a capsular polysaccharide, sub-capsular polysaccharide, or exopolysaccharide.

39) The polysaccharide as claimed in claim 37is subjected to sizing by chemical or mechanical means.

40) Vaccine comprising polysaccharide antigen obtained by method claimed in claim 1 or 26.

41) The polysaccharide as claimed in claim 37, is conjugated to a carrier protein, wherein carrier protein is one of CRM197, Pneumococcal surface adhesin A (PsaA), diphtheria toxoid (DT), tetanus toxoid (TT), fragment C of tetanus toxoid, pertussis toxoid, protein D of H. influenzae, E. coli LT, E. coli ST, exotoxin A from Pseudomonas aeruginosa, outer membrane complex c (OMPC), porins, transferrin binding proteins, pneumolysin, pneumococcal surface protein A (PspA), pneumococcal surface adhesin A (PsaA), pneumococcal PhtD, pneumococcal surface proteins BVH-3, BVH-11, protective antigen (PA) of Bacillus anthracis, detoxified edema factor (EF), lethal factor (LF) of Bacillus anthracis, ovalbumin, keyhole limpet hemocyanin (KLH), human serum albumin, bovine serum albumin (BSA), purified protein derivative of tuberculin (PPD); particularly CRM197,tetanus toxoid, diphtheria toxoid and/or PsaA.

42) The polysaccharide as claimed in claim 37, is conjugated to a carrier protein using reductive amination, cyanylation, or carbodiimide chemistry.

43) An immunogenic composition comprising atleast one of:

a) a conjugate of (i) the capsular polysaccharide of Neisseria meningitidis serogroup A and (ii) tetanus toxoid;

b) a conjugate of (i) capsular polysaccharide of Neisseria meningitidis serogroup C and (ii)CRM197;

c) a conjugate of (i) capsular polysaccharide of Neisseria meningitidis serogroup Y and (ii) CRM 197;

d) a conjugate of (i) capsular polysaccharide of Neisseria meningitidis serogroup W135 and (ii)CRM197; and

e) a conjugate of (i) capsular polysaccharide of Neisseria meningitidis serogroup X and (ii)tetanus toxoid.

wherein the said composition comprises of:

a) at least one polysaccharide-protein conjugate;

b) sucrose at a concentration of approximately 3% w/v; and

c) sodium citrate at a concentration of approximately 0.5% w/v, wherein the polysaccharide is purified by method according to any of the preceding claims.