# How promising is the development of a semi-synthetic meningococcal conjugate vaccine ?

## - A CASE STUDY

**5th Asia Pacific Global Summit and Expo on Vaccines & Vaccination**
**29th July 2015**

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- Need for an affordable multivalent meningococcal conjugate vaccine
- Challenges and avenues in conjugate vaccine manufacture
- Promises from synthetic vaccine technologies for conjugate vaccines
- Semi-synthetic conjugate vaccine research at Hilleman Labs
- Conclusions

# Need for an affordable multivalent meningococcal conjugate vaccine

# Meningitis

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- Inflammation of meninges
- Can be bacterial, viral, sometimes fungal
- Bacterial meningitis: Meningitis and /or Septicaemia
- Causes: ***N. meningitidis***,
  *Str. pneumoniae,*
  *H. influenzae,*
  *E. coli,*
  *GBS, GAS*
  *L. monocytogenes and several others*
- Symptoms: Fever, vomiting, rashes, headache, delirium, stiffness, drowsiness, muscle and/or joint pain

# Meningococcal Epidemics

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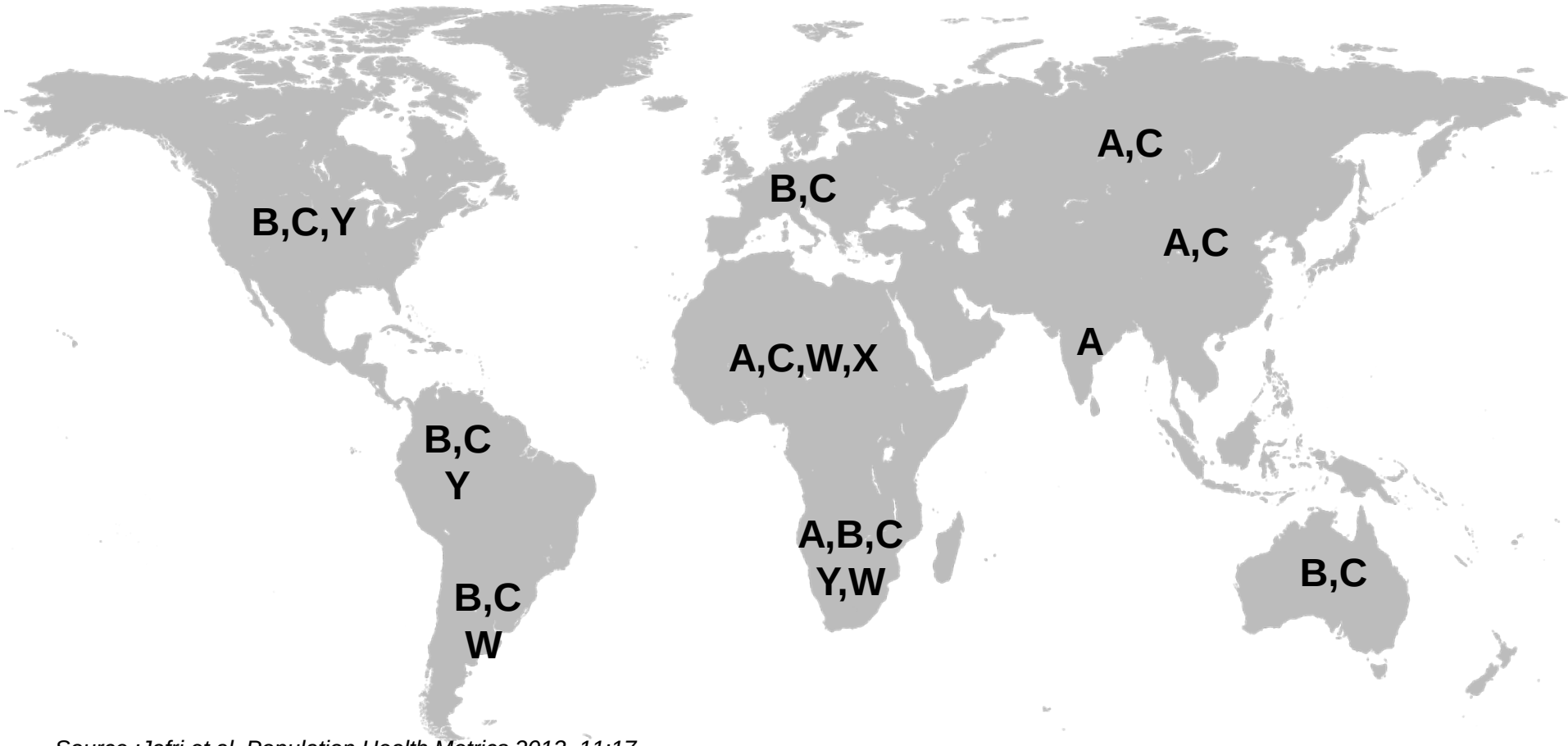
***N. meningitidis epidemics:***

- 1996 epidemic: Africa; 20,000 reported deaths
  https://www.nathnac.org/pro/factsheets/documents/meningitis.pdf
- In 2000 and 2001 several hundred pilgrims attending the Hajj in Saudi Arabia were infected with *N. meningitidis* W. Then in 2002, W emerged in Burkina Faso, striking 13,000 people and killing 1,500.
  http://www.cdc.gov/meningococcal/global.html
- 2007 epidemic: Burkina Faso; 22,255 suspected cases, 1490 deaths
  https://www.nathnac.org/pro/factsheets/documents/meningitis.pdf
- 2009 epidemic: 14 countries from African meningitis belt 88,199 suspected meningitis cases, 5352 deaths. (http://www.who.int/mediacentre/factsheets)
- 2014 epidemic season: 19 African countries; 11 908 suspected cases including 1146 deaths, lowest numbers since 2004
  http://www.who.int/mediacentre/factsheets/fs141/en/
- Up to 20% mortality in epidemics and serious disability after survival in several cases

# Major Meningococcal serogroups by geography: A, B and C account for 90% of IMD worldwide




*Source :Jafri et al. Population Health Metrics 2013, 11:17*

Public Health England

# Health Protection Report

weekly report

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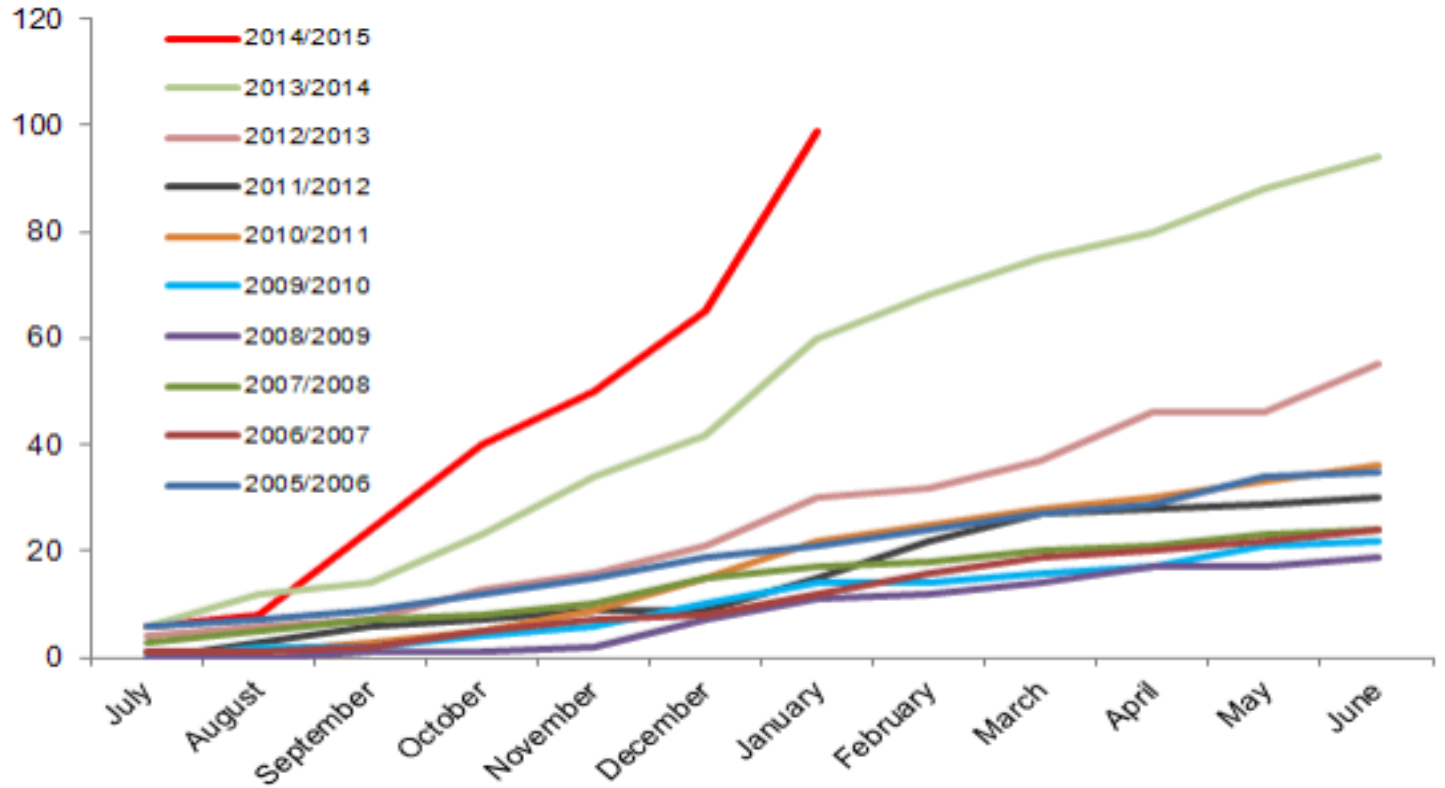
## Infection (news) report

**Volume 9 Number 7 Published on: 27 February 2015**

## Continuing increase in meningococcal group W (MenW) disease in England

**Cumulative cases of laboratory-confirmed invasive meningococcal group W disease by epidemiological year in England, to end-January 2015**




https://www.gov.uk/government/publications/meningococcal-disease-laboratory-confirmed-cases-in-england-and-wales

Public Health England

NHS England

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Protecting and improving the nation's health

22 June 2015

NHS England Gateway Number: 03516

**Meningococcal ACWY conjugate vaccination (MenACWY)**

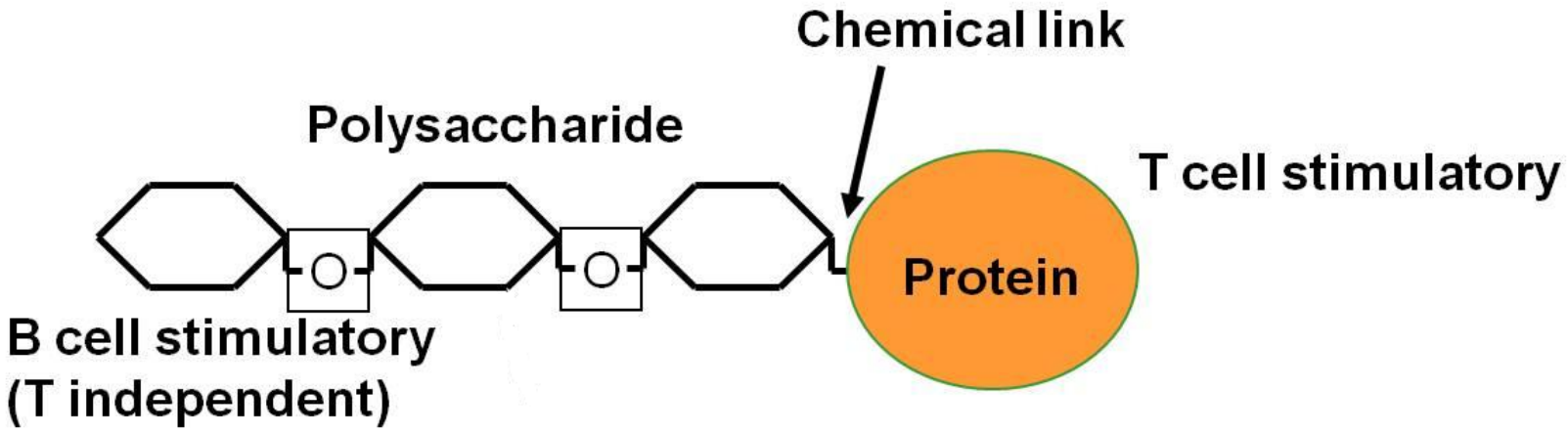
This vaccination is being introduced into the national immunisation programme for England this year to respond to a rapid and accelerating increase in cases of invasive meningococcal group W (MenW) disease, which has been declared a national incident. The MenACWY conjugate vaccine will provide direct protection to the vaccinated cohort and, by reducing MenW carriage, will also provide indirect protection to unvaccinated children and adults. This follows advice from the Joint Committee on Vaccination and Immunisation (JCVI).

The overall programme is comprised of:

- an urgent catch-up* campaign for current school year 13 age adolescents through general practice using a call and recall system
- a catch-up* campaign for current school year 10 students through schools from January 2016
- adding MenACWY vaccine to the routine adolescent schools programme (school year 9 or 10) from Autumn 2015, as a direct replacement for the MenC vaccination
- adding MenACWY vaccine to the existing time-limited 'freshers' programme (ie for older first time university entrants who have not already received

https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/437901/150622_ACWY_bipartite_letter.pdf

# The solution is:
# multivalent meningococcal conjugate vaccine



# Opportunities in development of cost effective conjugate vaccines

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- Existing poly-valent meningitis vaccines are effective but are too expensive to be marketed in developing countries.
- Need to provide the right serogroup combination to provide protection for the specific needs of the countries/regions
- Need to reduce dependency on one or few low cost vaccine suppliers
- The existing cost of conjugate vaccines can be reduced by adapting to novel technologies and thermo-stabilization.

# GAVI Alliance: Strategic Demand Forecast as of Second Quarter 2014

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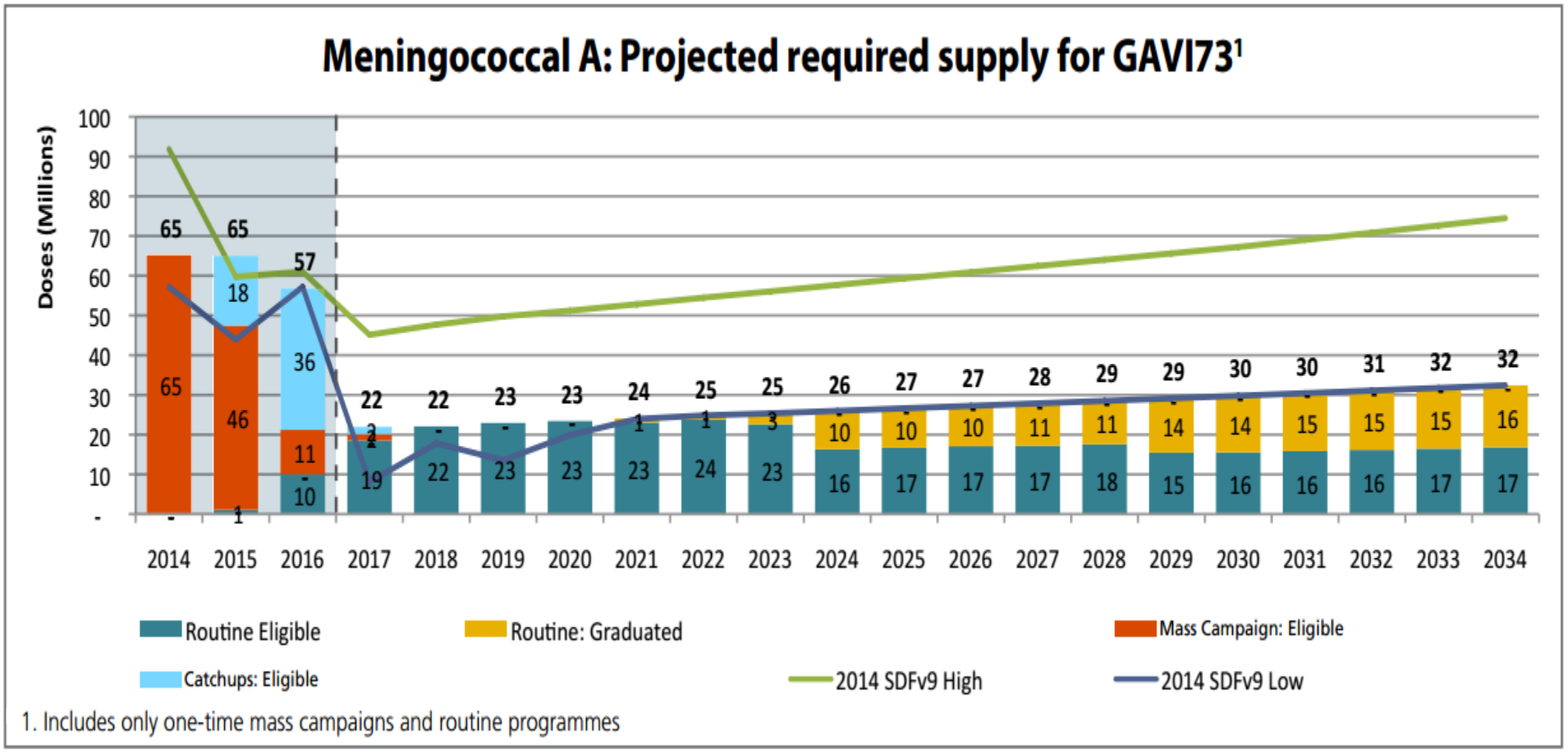


1. Includes only one-time mass campaigns and routine programmes

**Based on increasing prevalence of other serogroups the need for cost effective multivalent meningococcal conjugate vaccine is warranted**

# Challenges & avenues in multivalent conjugate vaccine manufacture

# 13 serogroups, regional variations

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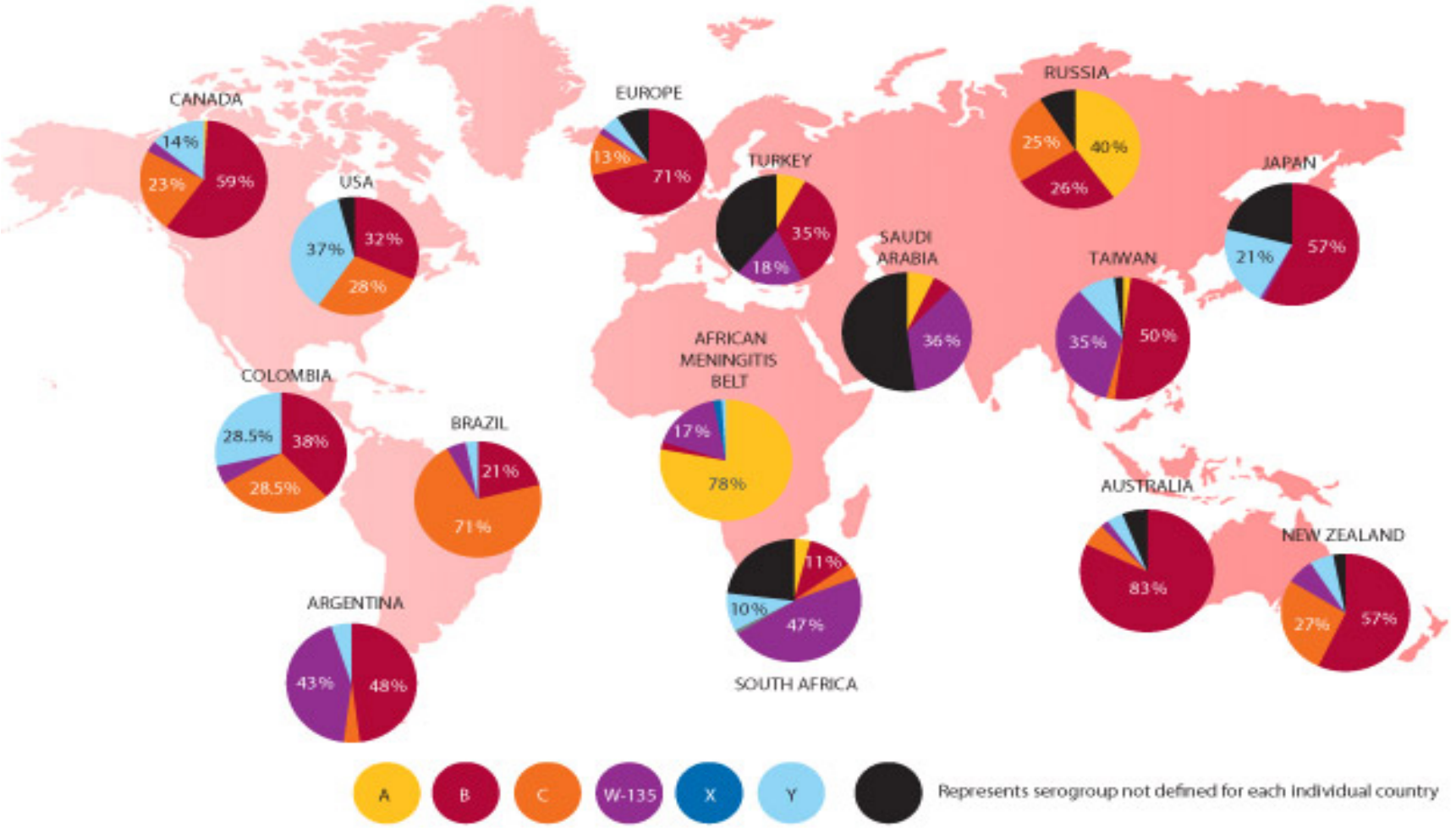


. Global Distribution of Invasive Meningococcal Disease by Serogroup[5-17] **in year 2011**

http://www.meningitisinfo.com/Epidemiology_ssi.aspx

# Seroprevalence varying by age groups and time

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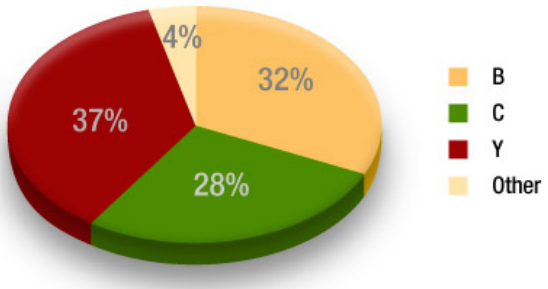
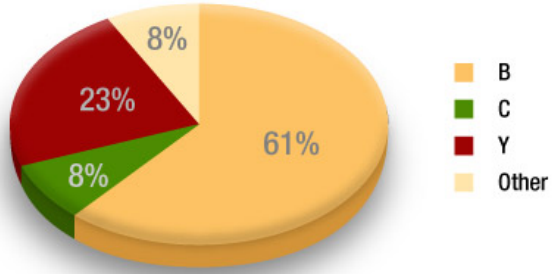
## All ages




2009

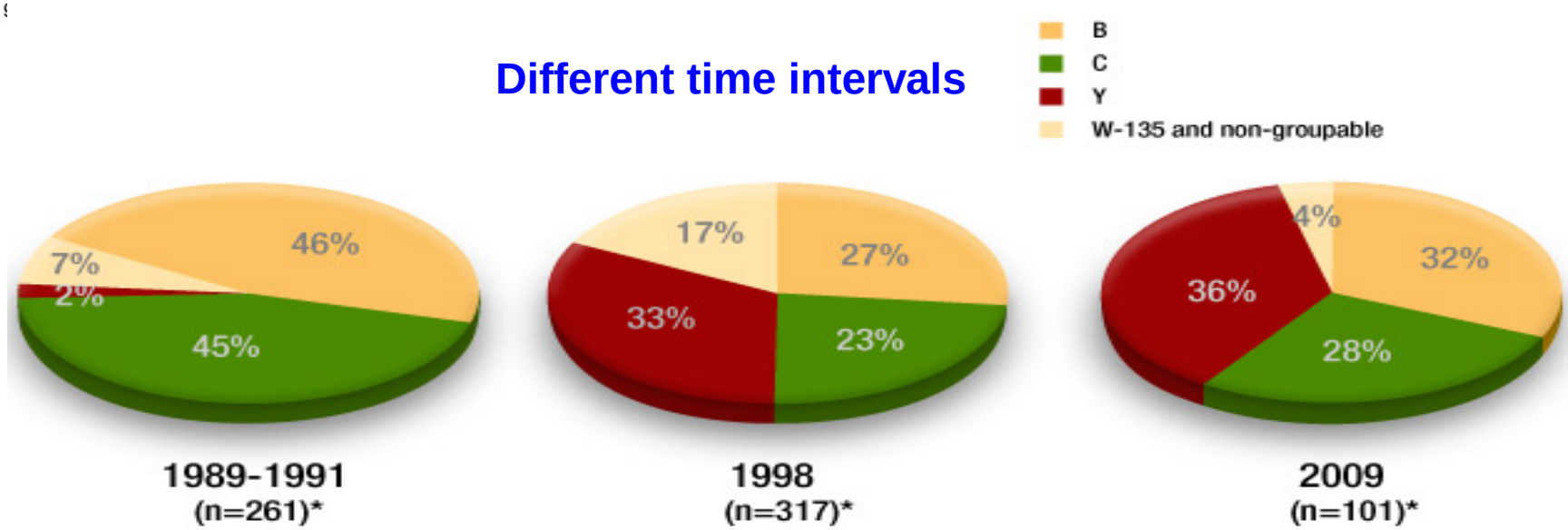
## Infants <1yr




2009

Figure 9

## Different time intervals




n = number of cases from specific surveillance sites (not total incidence per year).

Figure 11. Serogroup Distribution in the United States: 1989-2002[5,18]

http://www.meningitisinfo.com/Epidemiology_ssi.aspx

# Complex processes & analytics

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1. Identity
2. Molecular Size
3. Composition
4. Protein impurity
5. Nucleic acid impurity
6. Endotoxin
7. Moisture content

Polysaccharide

Activation

1. Number of functional groups
2. Mol. Size distribution

Activated saccharide

Carrier protein

1. Identity
2. Purity
3. Extent of derivatization
4. Endotoxin
5. Specific toxicity

Conjugation

Bulk Conjugate

1. Identity
2. Mol. Size distribution
3. Saccharide content
4. Protein content
5. Saccharide:protein ratio
6. Endotoxin
7. Conjugation markers
8. Residual reagents
9. Sterility

Formulation


1. Identity
2. Saccharide content
3. Residual moisture (if lyophilized)
4. Adjuvant content (if used)
5. Preservative content (if used)
6. Pyrogen content
7. General safety test
8. pH
9. Sterility
10. Physical inspection

# Novel Technology solutions to Conjugate vaccine development

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1. Novel/improved polysaccharide production processes
2. Antigen Synthesis
    a. Organic synthesis
    b. Enzymatic synthesis
3. Reverse engineering (Broader coverage)
4. Novel/improved conjugation processes
    a. Reductive amination
    b. Cyanylation
    c. Oxime chemistry
    d. Thio-ether conjugation etc.
5. Novel formulations
5. Novel Analytics
    a. Sophisticated physico-chemical analyses
    b. Multiplexed immunoassays (xMAP, MSD etc.)

# Developments in semi-synthetic conjugate vaccine R&D

# How interest in semi-synthetic conjugate vaccines started ?

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J Clin Invest. 1992 Jan;89(1):203-9.

**Effects of chain length on the immunogenicity in rabbits of group B Streptococcus type III oligosaccharide-tetanus toxoid conjugates.**

Paoletti LC[1], Kasper DL, Michon F, DiFabio J, Jennings HJ, Tosteson TD, Wessels MR.

# The initial success…with Hib

*Science* 23 July 2004:
Vol. 305 no. 5683 pp. 522-525

**A Synthetic Conjugate Polysaccharide Vaccine Against *Haemophilus influenzae* Type b**

V. Verez-Bencomo[1,*], V. Fernández-Santana[1,*], Eugenio Hardy[2,*], Maria E. Toledo[3,*], Maria C. Rodríguez[1], Lazaro Heynngnezz[2], Arlene Rodriguez[2], Alberto Baly[3], Luis Herrera[2], Mabel Izquierdo[2], Annette Villar[1], Yury Valdés[1], Karelia Cosme[2], Mercedes L. Deler[1], Manuel Montane[2], Ernesto Garcia[1], Alexis Ramos[1], Aristides Aguilar[2], Ernesto Medina[2], Gilda Toraño[3], Iván Sosa[2], Ibis Hernandez[3], Raydel Martínez[3], Alexis Muzachio[2], Ania Carmenates[4], Lourdes Costa[2], Félix Cardoso[1], Concepción Campa[5], Manuel Diaz[3], René Roy[6,*]

# Research in developing semi-synthetic conjugate vaccines

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| | |
|---|---|
| ❖ *Cryptococcus neoformans:* | Vaccine (2005), 3961 |
| ❖ *Clostridium difficile*: | Chem. Biol. (2011); J. Am. Chem. Soc. (2013) |
| ❖ Group A Streptococcus: | Bioorg. Med. Chem. Lett. (2013) |
| ❖ *Vibrio cholerae*: | Glycoconj. J. (2013) |
| ❖ *Neisseria meningitidis* group W: | Ange. Chemie. Int. Ed. (2013) |
| ❖ *Streptococcus pneumoniae* type 14: | Russian Chem. Bull. (2014) |
| ❖ *Bacillus anthracis*: | Curr. Org. Chem. (2014) |
| ❖ *Shigella*: | Chemi. Commn. (2015) |
| ❖ *Neisseria meningitidis* group X: | Archivoc (2013); Baielstein J.Org. Chem. (2014); RSC Adv. (2015) |

Why there is no second semi-synthetic vaccine yet?

# Questions in front of a manufacturer before adapting a novel technology

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| Parameter | Conventional technology | Novel technology |
|---|---|---|
| GMP requirements | ? | ? |
| Duration for production | ? | ? |
| Cost of production | ? | ? |
| Batch to batch consistency | ? | ? |
| Host cell impurities (e.g. Protein/nucleic acid) | ? | ? |
| Endotoxin content | ? | ? |
| Residuals | ? | ? |
| Analytics | ? | ? |
| Stability | ? | ? |
| Immunogenicity | ? | ? |
| Ease of modifying structure | ? | ? |
| Loss of epitopes during conjugation | ? | ? |
| Conjugation yield | ? | ? |

# Mening Conjugate Vaccine Program at Hilleman Labs

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## Mening ACWXY

### Technology

- Conventional conjugates (Bacterial PS and carrier protein)
- Semi-synthetic conjugates (Synthetic PS and bacterial carrier protein)

## Options for development

### Valency

- Regional vaccine(s)
- Pentavalent ACYWX vaccine

# R&D AT HILLEMAN LABS

## Polysaccharide production VERSUS Oligosaccharide synthesis

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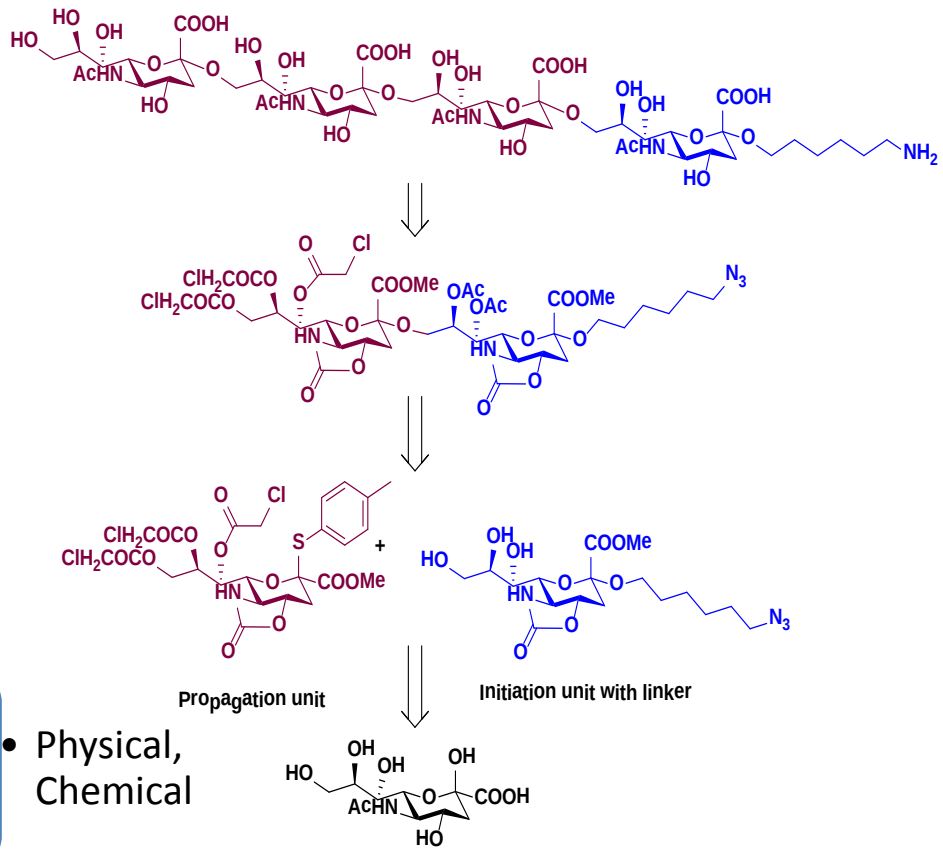
### Bacterial Polysaccharide production

- **Cell Banking** — Extensive characterization
- **Fermentation** — Handling pathogenic organisms
- **PS purification** — Several steps
- **Size reduction** — Physical, Chemical

### Retro-synthesis of Mening C Tetramer




**Significant difference in GMP requirements and raw material required for the two options**

# Oligomers synthesized at Hilleman Labs

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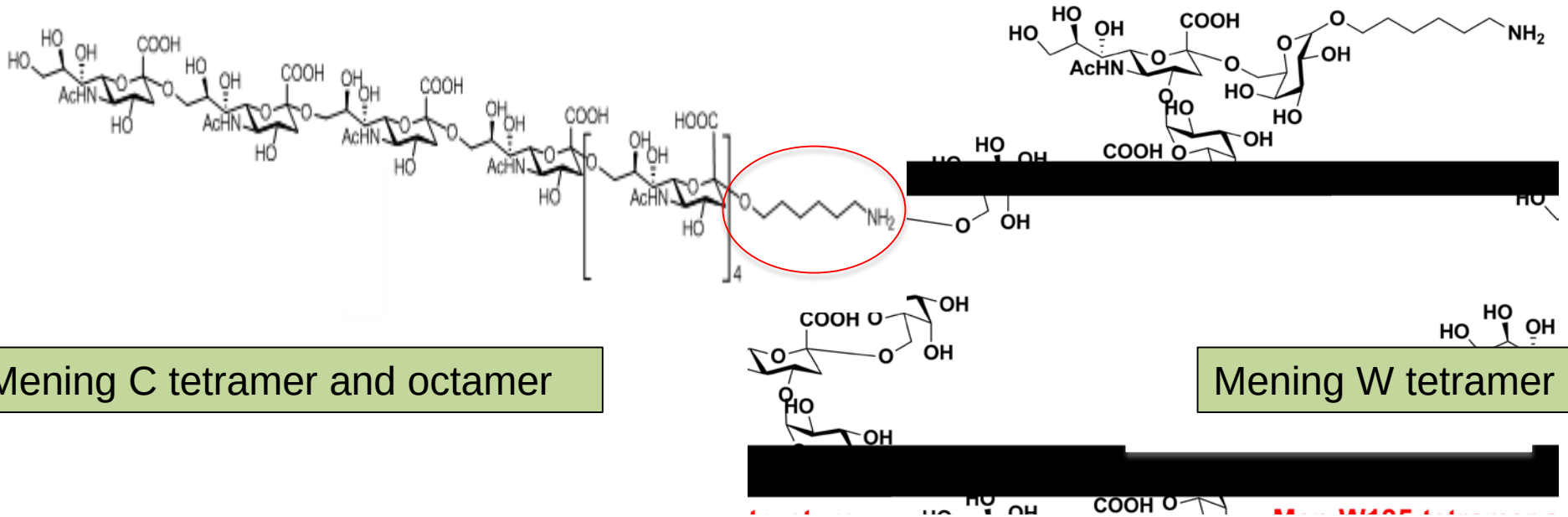
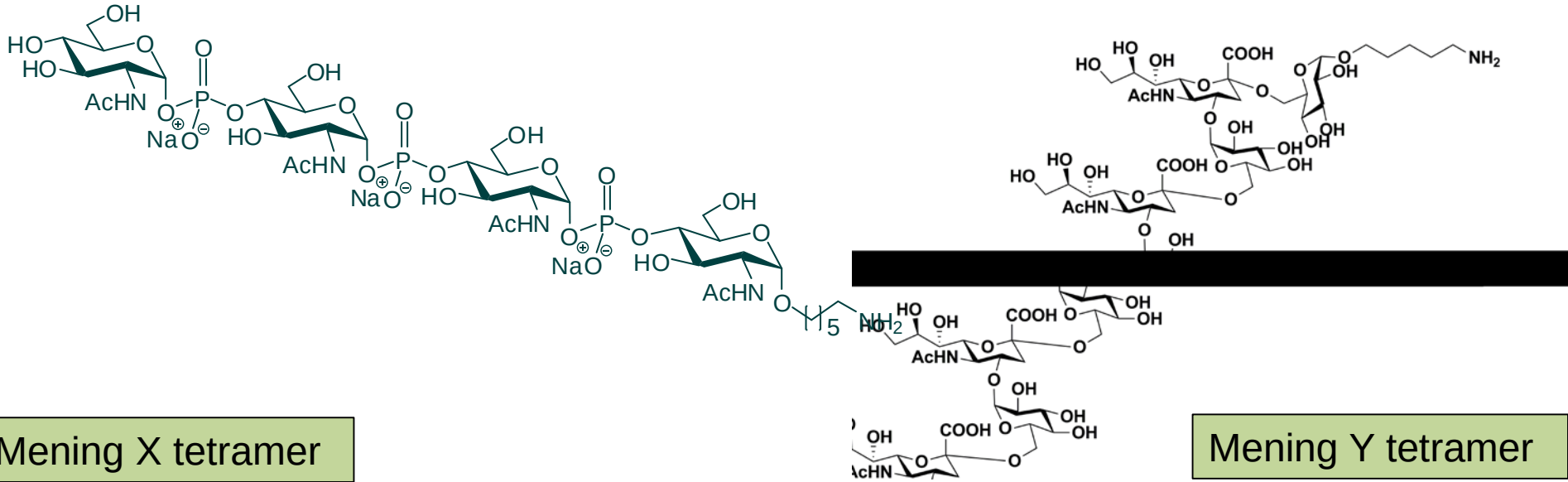


Mening C tetramer and octamer

Mening W tetramer



Mening X tetramer

Mening Y tetramer

# Highly defined oligomers

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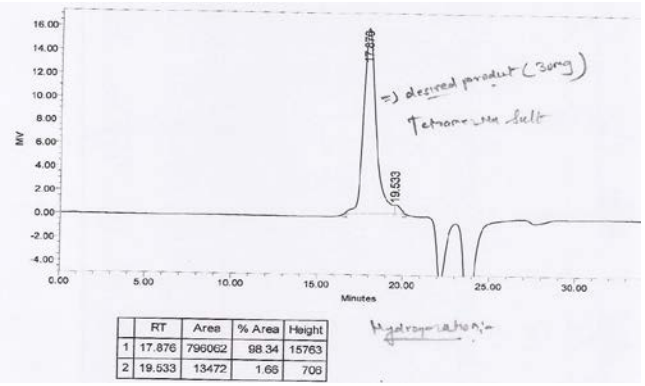
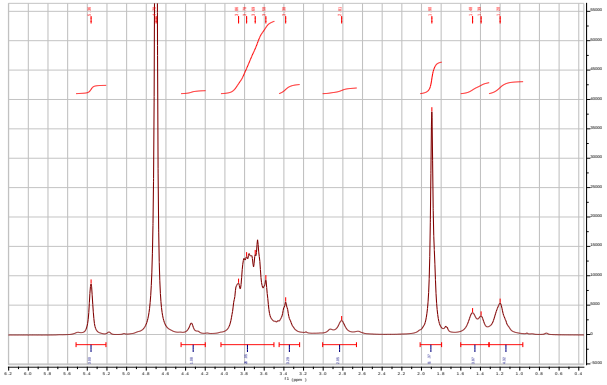
## HPSEC

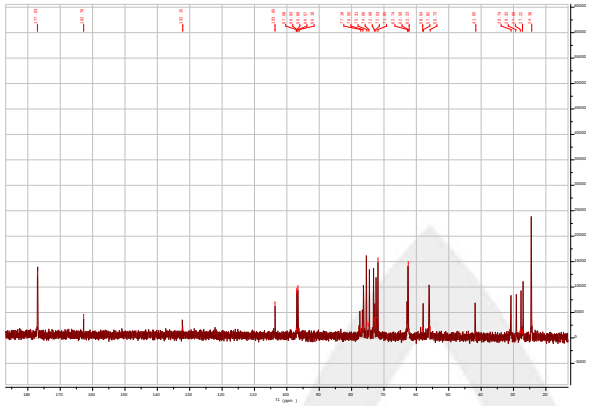



| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 17.876 | 796062 | 98.34 | 15763 |
| 2 | 19.533 | 13472 | 1.66 | 708 |

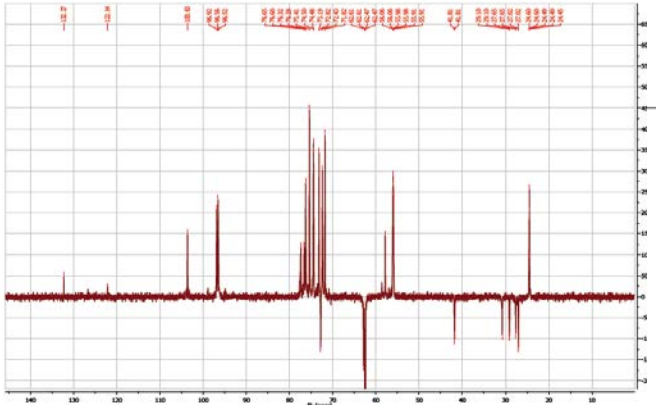
## $^{1}$H-NMR



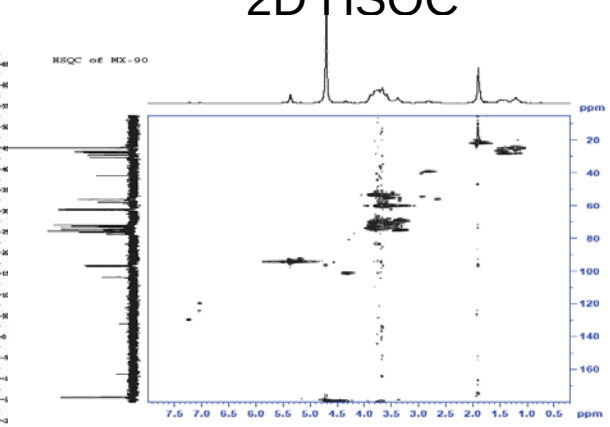
## $^{13}$C-NMR



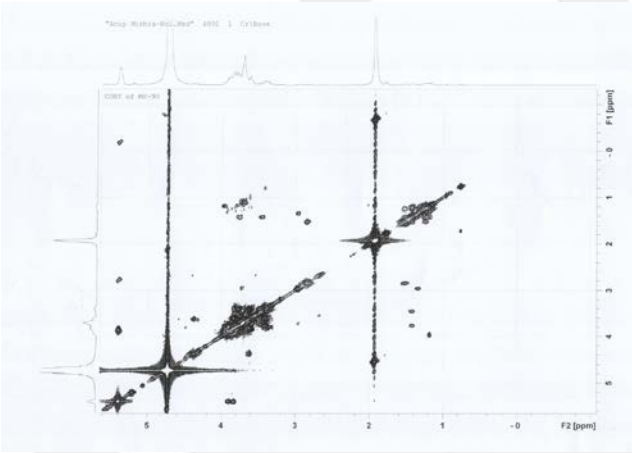
## DEPT



## 2D HSOC

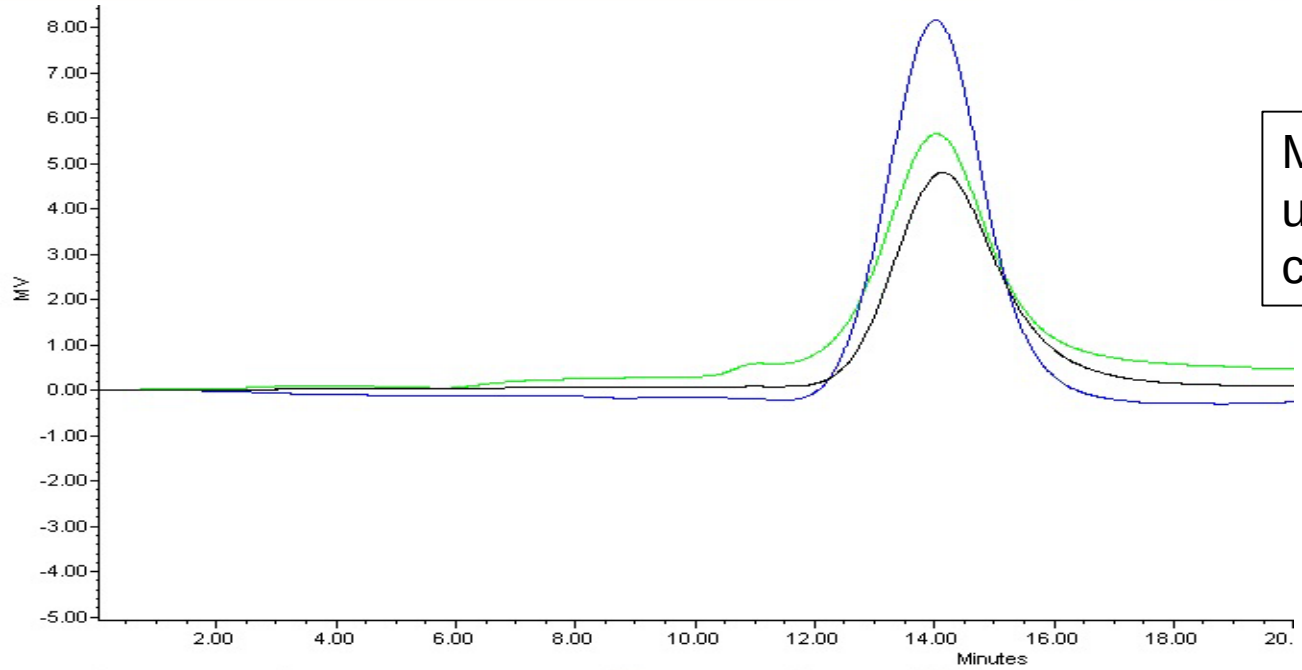



## 2D COSY

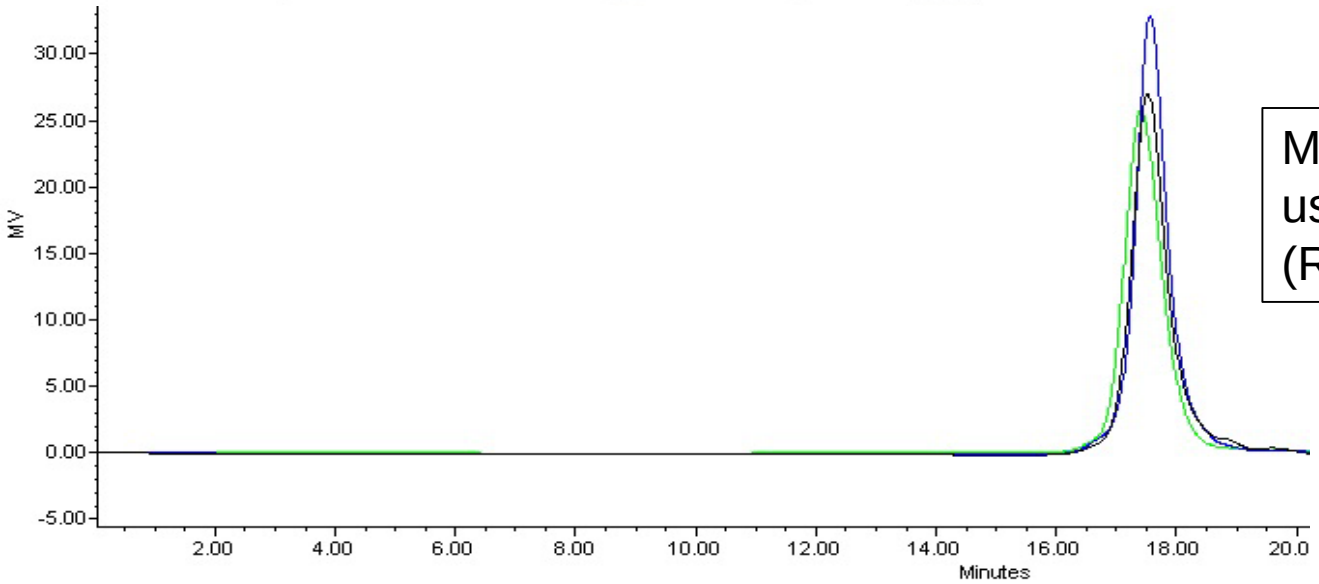



**Additional testing:** MS; IR; TLC

# Advantages in size distribution




MenC PS lots analyzed using PWXL 4000-5000 columns in series (RI signal)




MenC tetramer lots analyzed using PWXL 3000 column (RI signal)

# Different Conjugation Methods affect the polysaccharide epitopes

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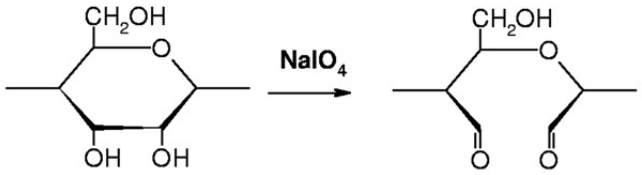
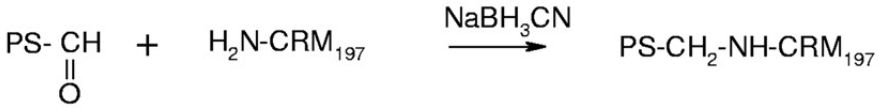
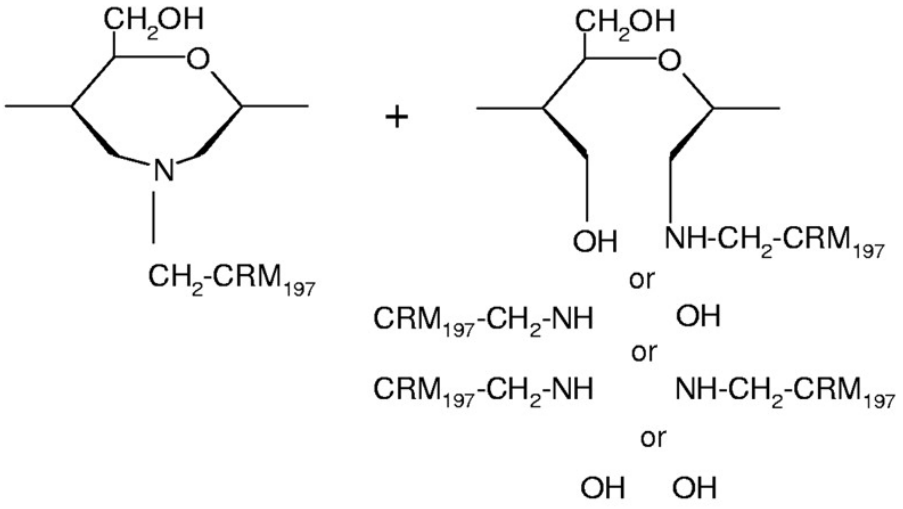
**1. Oxidation (activation at vicinal OH)**



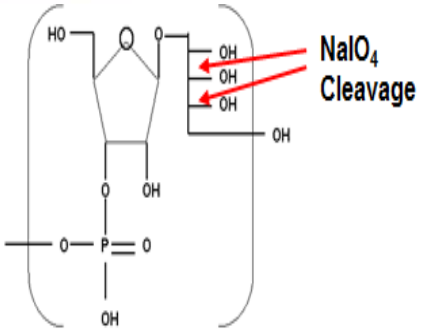

**2. Reductive amination**



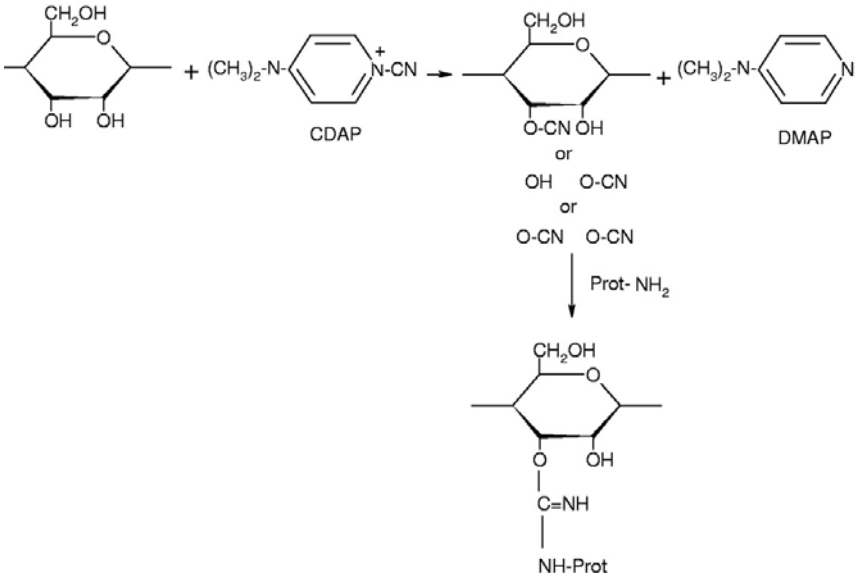

**3. After conjugation, a new epitope can be produced**




## Reductive amination of *Str. pneumoniae* Serotype 19F Polysaccharide




## Periodate oxidation of Hib-PRP

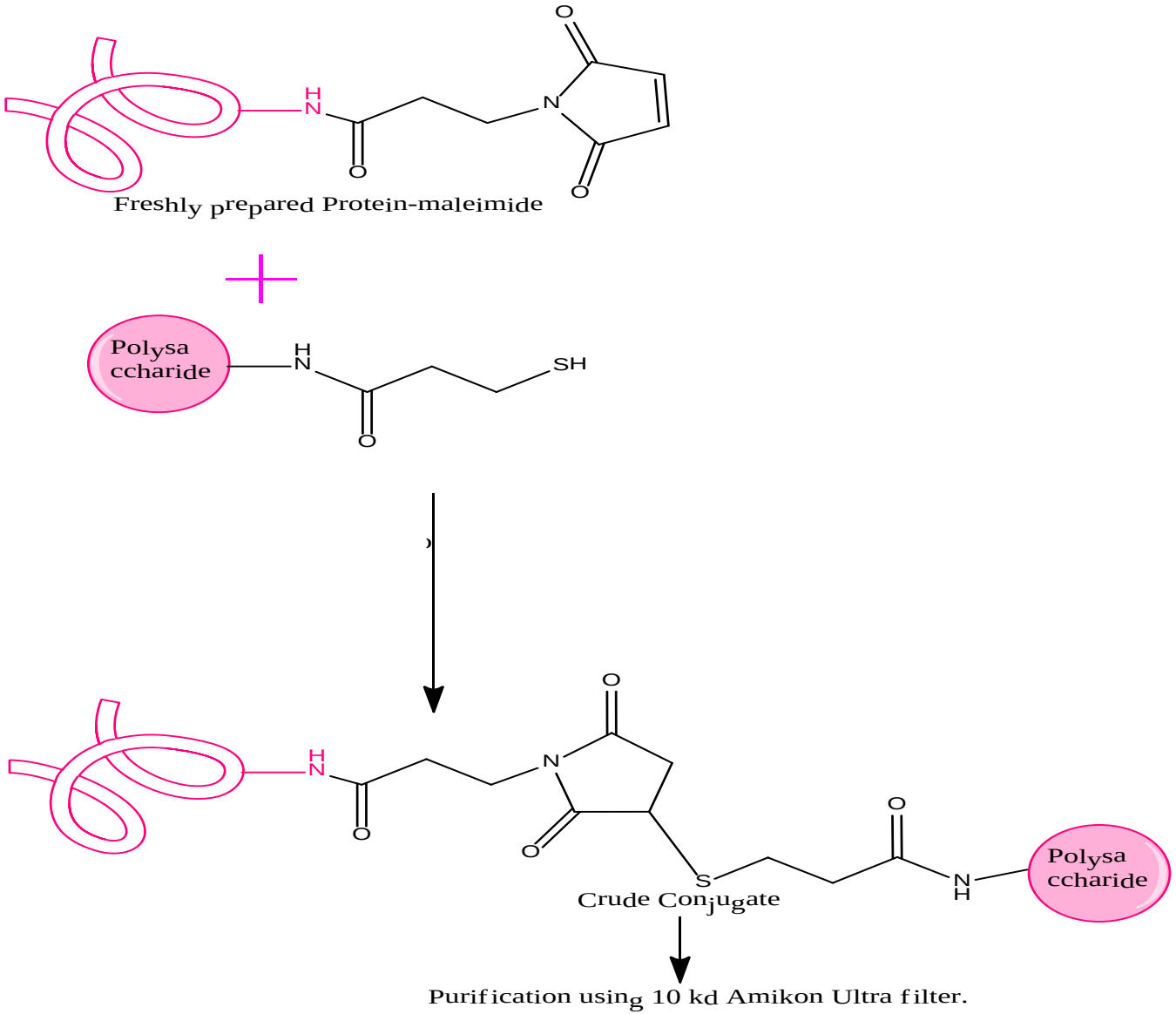



## Cyanylation of 19F-PS using CDAP

Clin Vaccine Immunol **2011** 18(2): **327-336**

Clin Vaccine Immunol **2011** 18(2): **327-336**

# Synthetic oligosaccharides can be conjugated without impacting OS epitopes with high yields




**Same chemistry for all oligosaccharides**

# Consistent, Repeatable process with high yields

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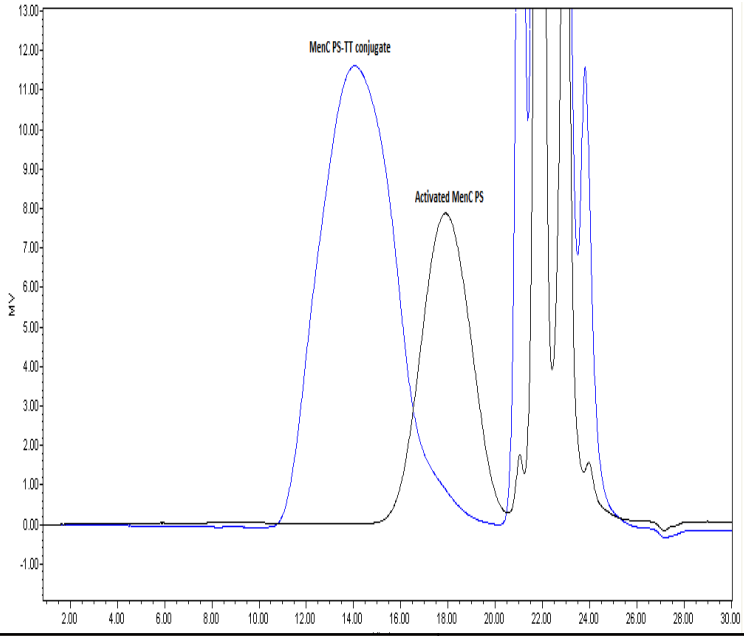
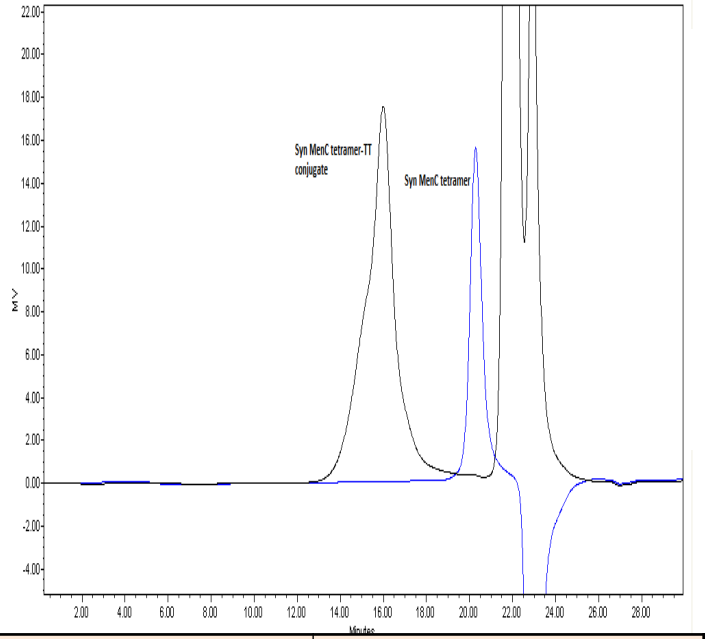
**MenC-PS & Conjugate**




**MenC-Tetramer & Conjugate**




**Sharp contrast in OS and conjugate profile and minimum heterogeneity**

| Conjugate Lot No. | OS:Pr Ratio | Free OS % | Conjugation yield |
|---|---|---|---|
| MenCTM-TT-1 | 0.25 | 2.5 | 21% |
| MenCTM-TT-2 | 0.25 | 1.2 | 32% |
| MenCTM-TT-3 | 0.24 | 3.0 | 38% |
| MenCTM-TT-4 | 0.28 | <1 | 47% |
| MenCTM-TT-5 | 0.23 | 1.5 | 38% |
| MenCTM-TT-6 | 0.28 | 5.9 | 39% |
| MenCTM-TT-7 | 0.25 | 9.9 | 35% |
| MenCTM-TT-8 | 0.26 | <1 | 27% |

# MenC Octamer-TT conjugates are immunogenic and non-inferior to licensed vaccine

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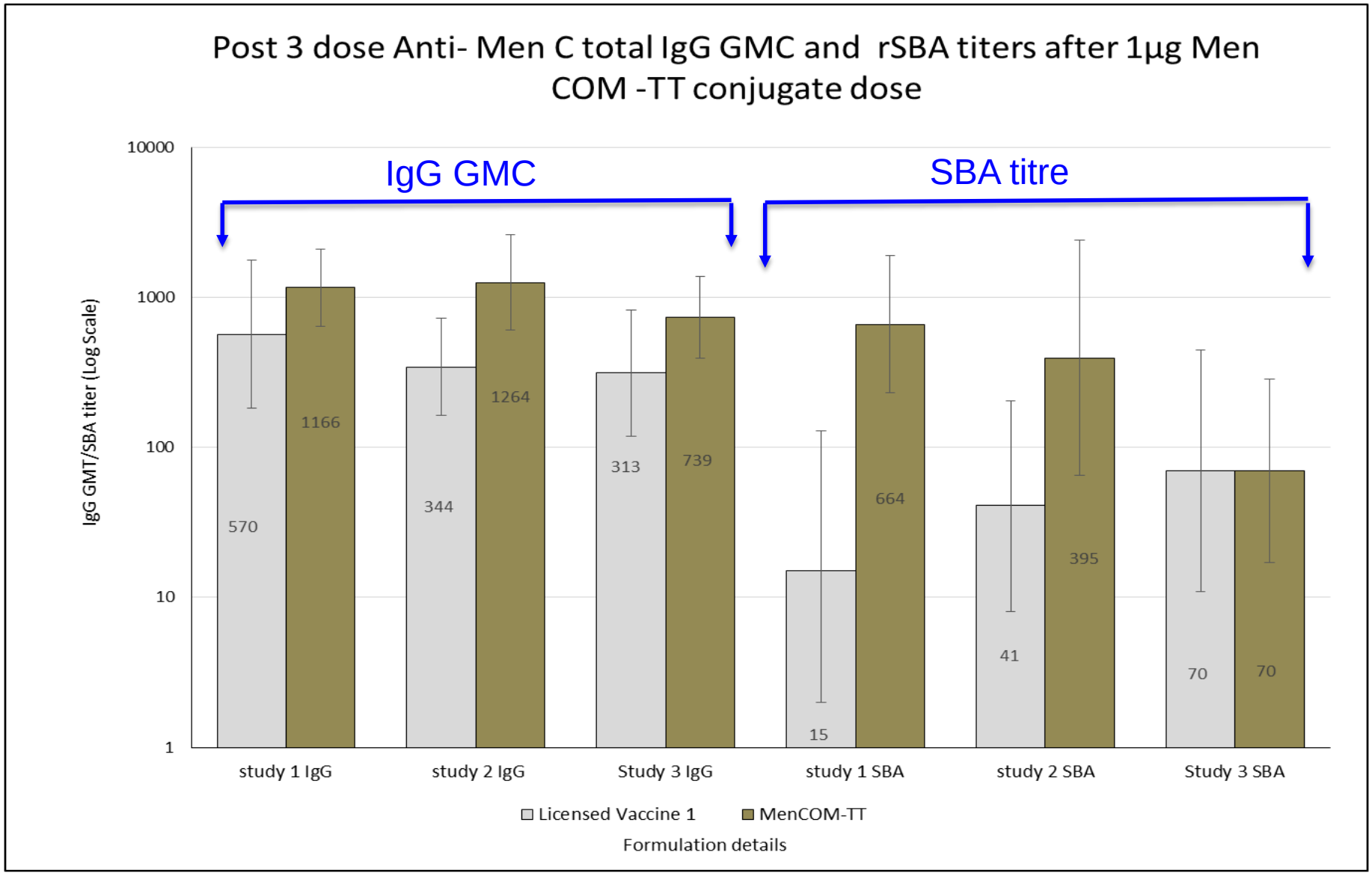

# MenC Tetramer-TT & Octamer –TT conjugates show comparable immunogencity

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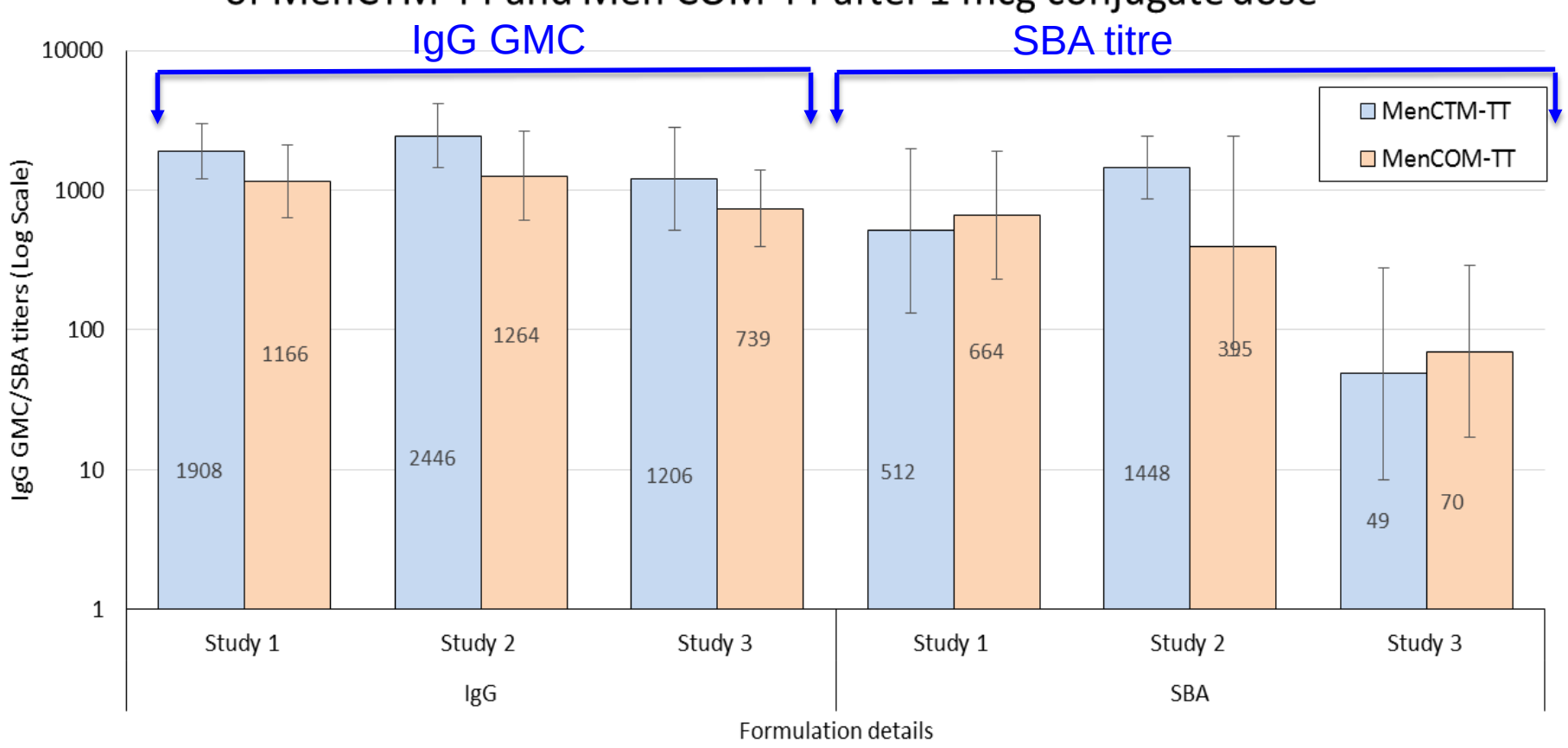
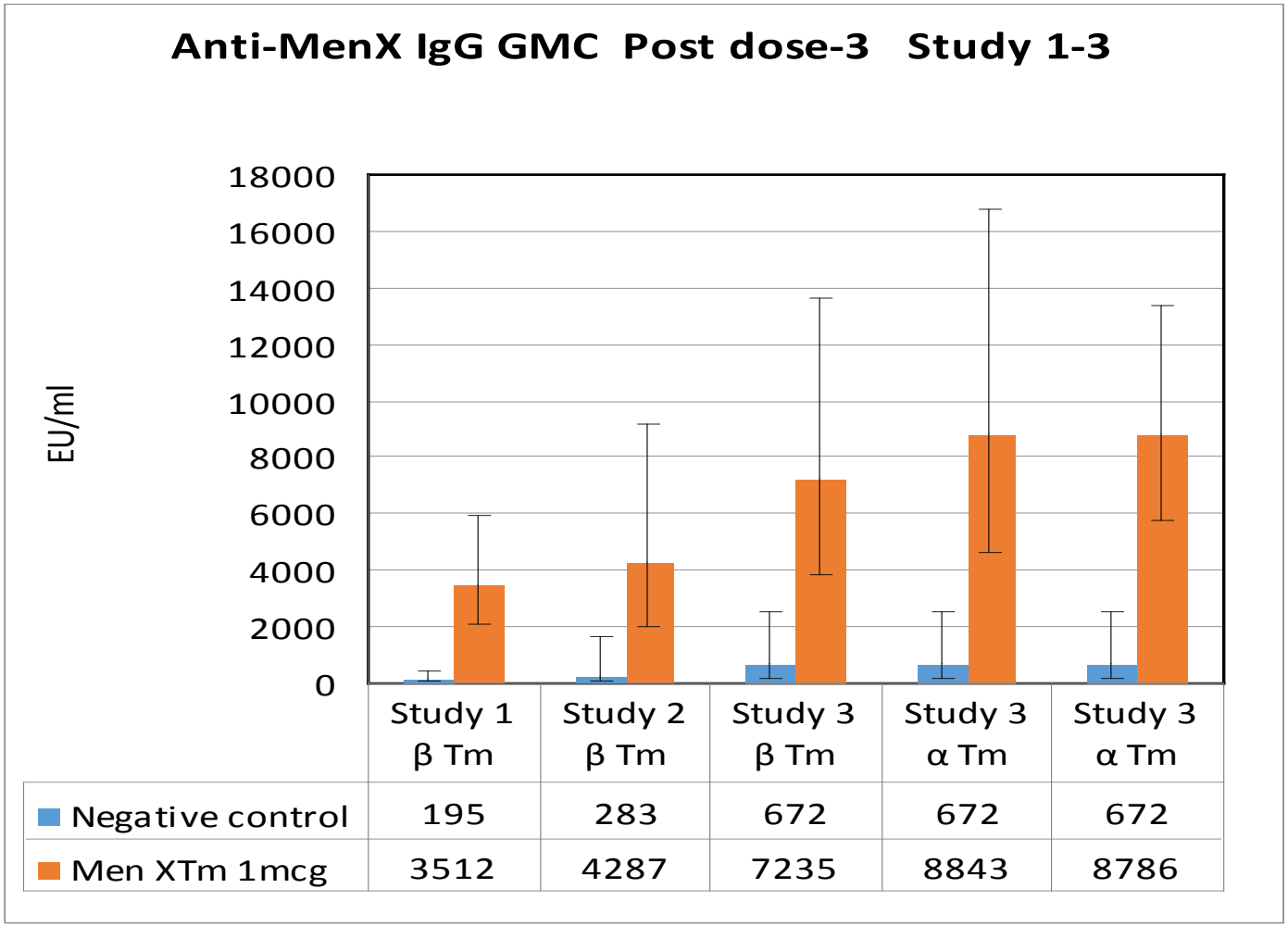


# MenX-TT Conjugate give rise to high IgG concentration in mice




**INFERENCE:** 1µg of conjugated MenX-tetramer-TT gives 10-15 fold higher response than vehicle control



SBA data awaited

# Efforts towards automation…

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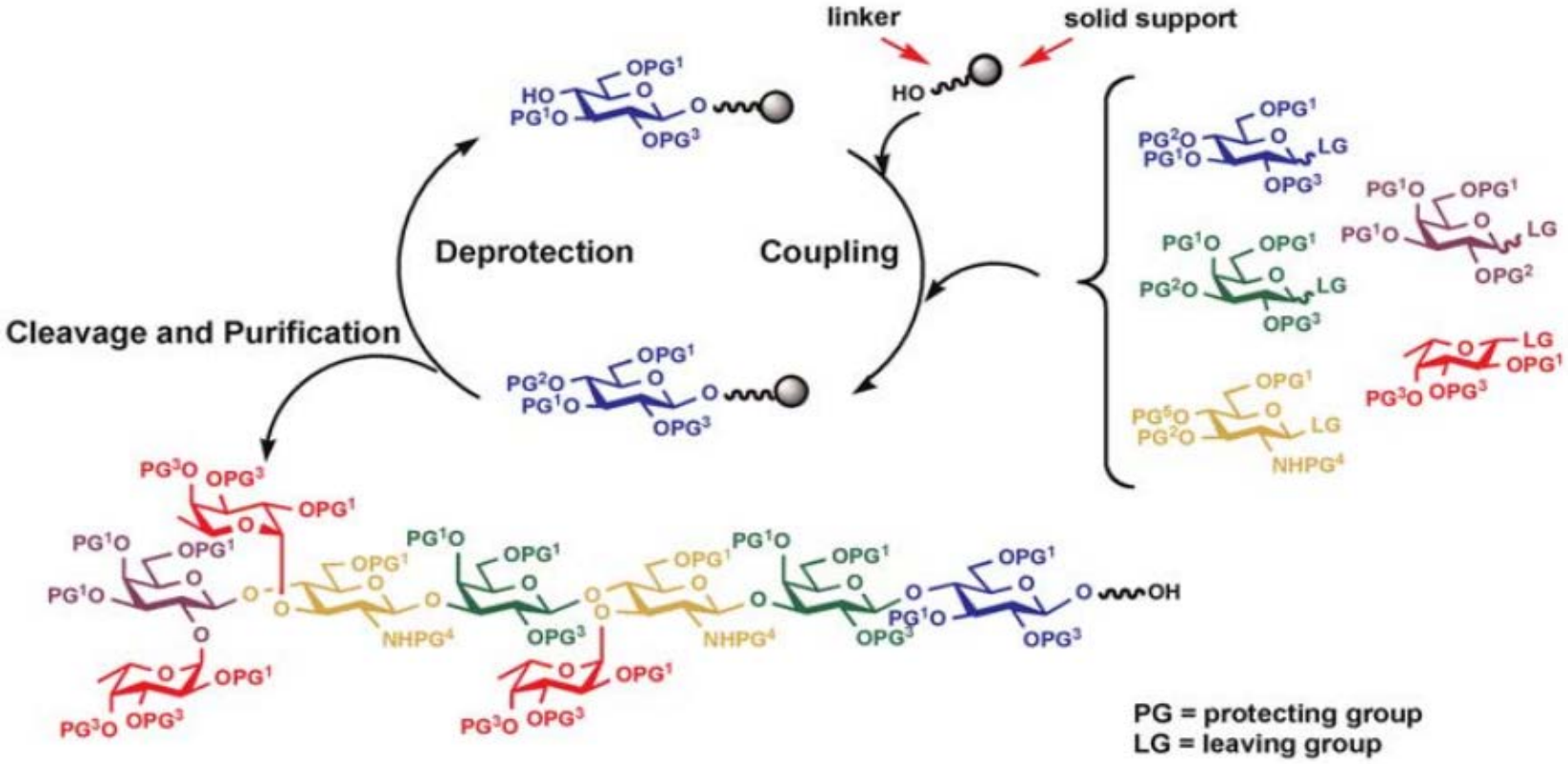
Automated synthesis of oligosaccharides as a basis for drug discovery
**Peter H. Seeberger & Daniel B. Werz**
*Nature Reviews Drug Discovery* **4**, 751-763 (September 2005)




**Scheme 1.** Automated solid-phase synthesis of a $Le^y$ -$Le^x$ nonasaccharide.

**Automation reduces significant time for organic synthesis**

# Comparative analysis

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| Parameter | Bacterial PS | Synthetic OS | Remarks |
|---|---|---|---|
| GMP requirements | - | + | |
| Duration for production | +++ | - | Significantly longer time for synthesis, (Scope for Automation) |
| Cost of production | + | ± | Still under evaluation |
| Batch to batch consistency | - | ++ | PS from live organisms vs chemical synthesis |
| Protein/nucleic acid impurities | - | ++ | |
| Endotoxin content | - | ++ | |
| Residuals | + | ± | Still under evaluation |
| Ease of modifying structure | - | ++ | |
| Loss of epitopes during conjugation | - | ++ | |
| Conjugation yield | - | + | |
| Analytics | - | + | Highly defined oligomers |
| Immunogenicity | + | + | |
| Stability | - | ± | Multiple vs Single link and Long vs small repeats |

**+ : a positive attribute of the technology** **- : a negative attribute**

# Conclusions

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1. Among all the other novel technologies, synthetic approaches to develop new conjugate vaccines is an emerging subject

2. Initial successes have paved the way for exploration of synthetic platform technology for other vaccine candidates

3. The advantages of synthetic approach over the conventional approaches have upper hand as compared to the possible short falls

4. The approach may lead to successful candidates which are difficult to be produced by conventional approaches

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