

Analytical and Manufacturing Challenges: Preparation of Bacterial Polysaccharide Conjugates

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Conjugate Vaccines: Manufacturing and quality control

Topics to be discussed:

- Characteristics of a conjugate vaccine
- Considerations in production of a conjugate
 - Polysaccharide activation
 - Conjugation
- Quality control and lot release
- Improving conjugation efficiency (yields)

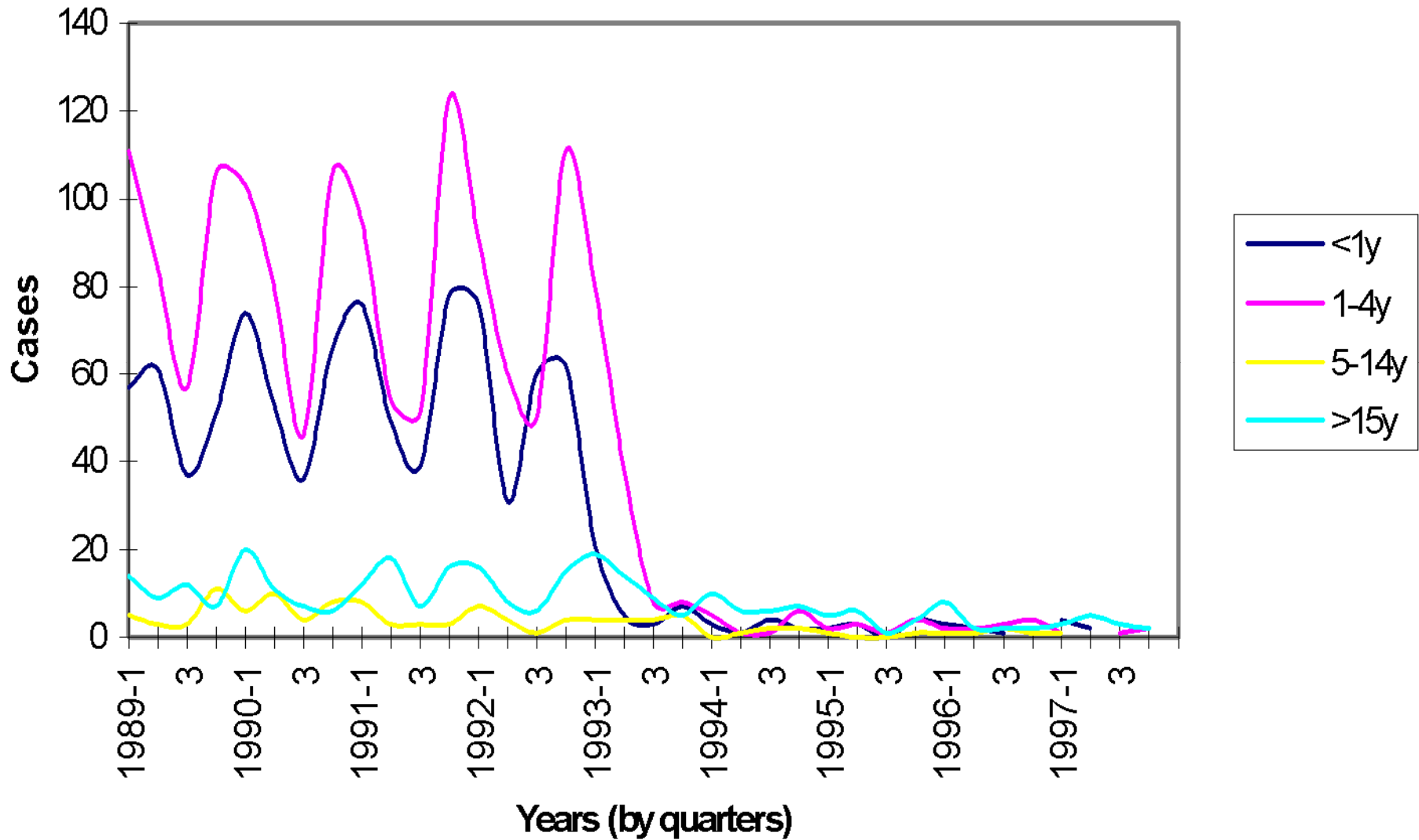
Properties of a good polysaccharide-protein conjugate vaccine

- Has defined chemical composition and structure
- Can be manufactured with consistent physical and chemical characteristics
- Is safe - has no inherent toxicity
- Induces high avidity bactericidal or opsonic antibodies
- Induces boostable IgG antibody in infants
- Primes for response upon natural exposure to the polysaccharide

Some currently licensed bacterial polysaccharide protein conjugate vaccines

Organism	Vaccine manufacturer	First Licensed	Saccharide	Protein carrier	Vaccine dose (μg)
<i>Haemophilus influenzae</i> b	Wyeth	1988	Hib oligo	CRM ₁₉₇	10
	Merck	1989	Hib Sz red	OMPC	7.5
	Sanofi	1993	Hib PS	Tet Tox	10
	GSK	1996	Hib PS	Tet Tox	10
Meningococcal	Wyeth	1999	C Sz red	CRM ₁₉₇	10
	Baxter	2000	C De OAc	Tet Tox	10
	Novartis	2000	C oligo	CRM ₁₉₇	10
	Sanofi	2005	A,C,Y,W Sz red	Diph Tox	4 each
Meningo/Hib	GSK	2005	Hib, MenC	Tet Tox	5 each
Pneumococcal	Wyeth	2000	4, 6B, 9V, 14, 18C, 19F 23F	CRM ₁₉₇	2 (4 μg 6B)

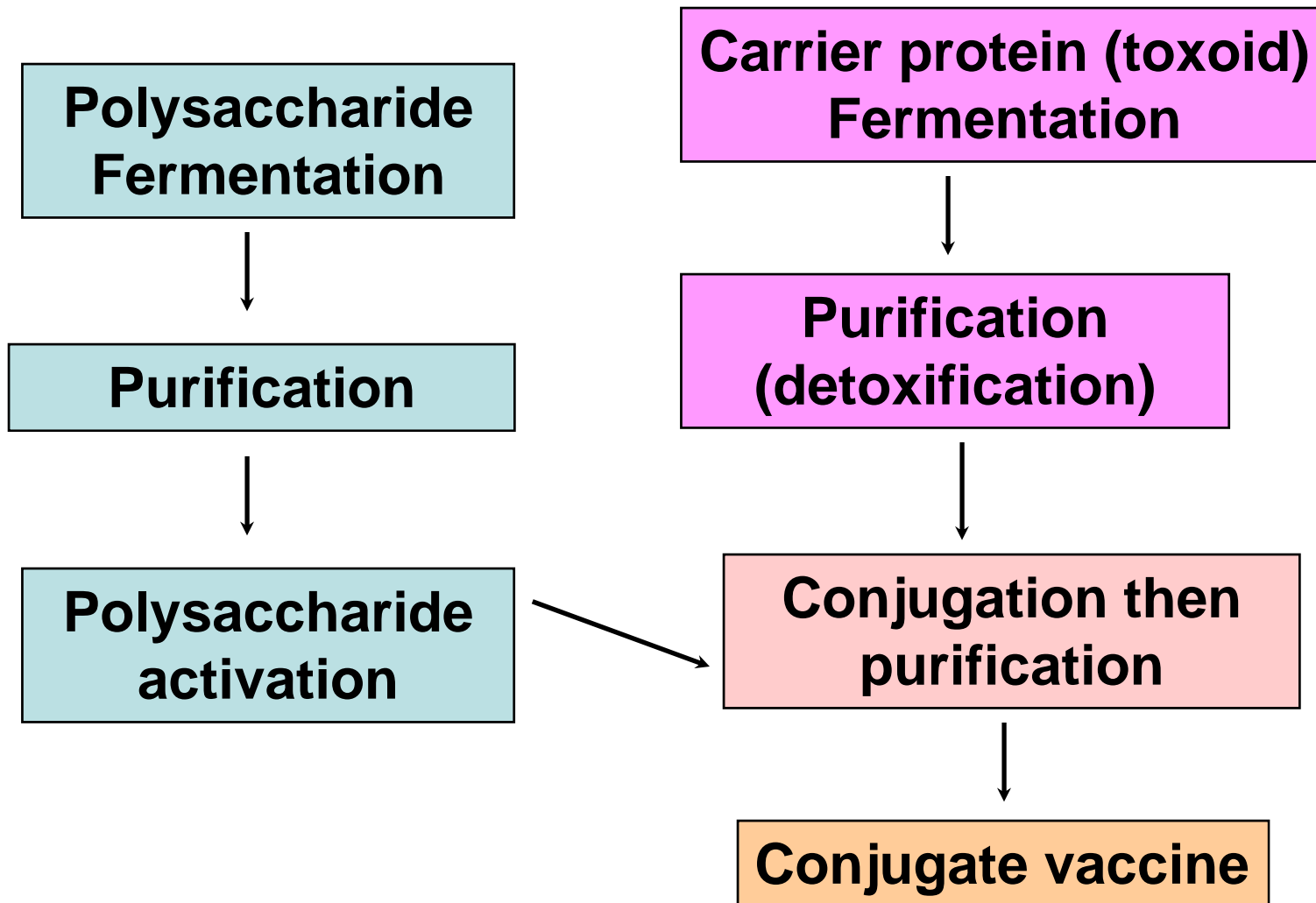
Laboratory reports of Hib to CDSC. England and Wales (bacteraemia + meningitis)



Major physical and chemical variables in production of glycoconjugate vaccines

1. Size of the polysaccharide or oligosaccharide
2. Chemistry for activation of the polysaccharide
3. Choice of carrier protein
4. Saccharide - protein conjugation chemistry
5. Saccharide loading onto protein carrier, ie, Saccharide to protein ratio

General process for manufacture of a conjugate vaccine



Making a conjugate

For a polysaccharide to be chemically linked to a protein, the polysaccharide must be activated, that is, chemically modified.

Methods include:

- Reductive amination
- Cyanylation
- Carbodiimide

Activation Chemistry

Important difference between methods used to activate the polysaccharide for conjugation

Periodate activation for reductive amination:

Activates by cutting carbon-carbon bonds between adjacent carbons having -OH groups to create active aldehyde groups

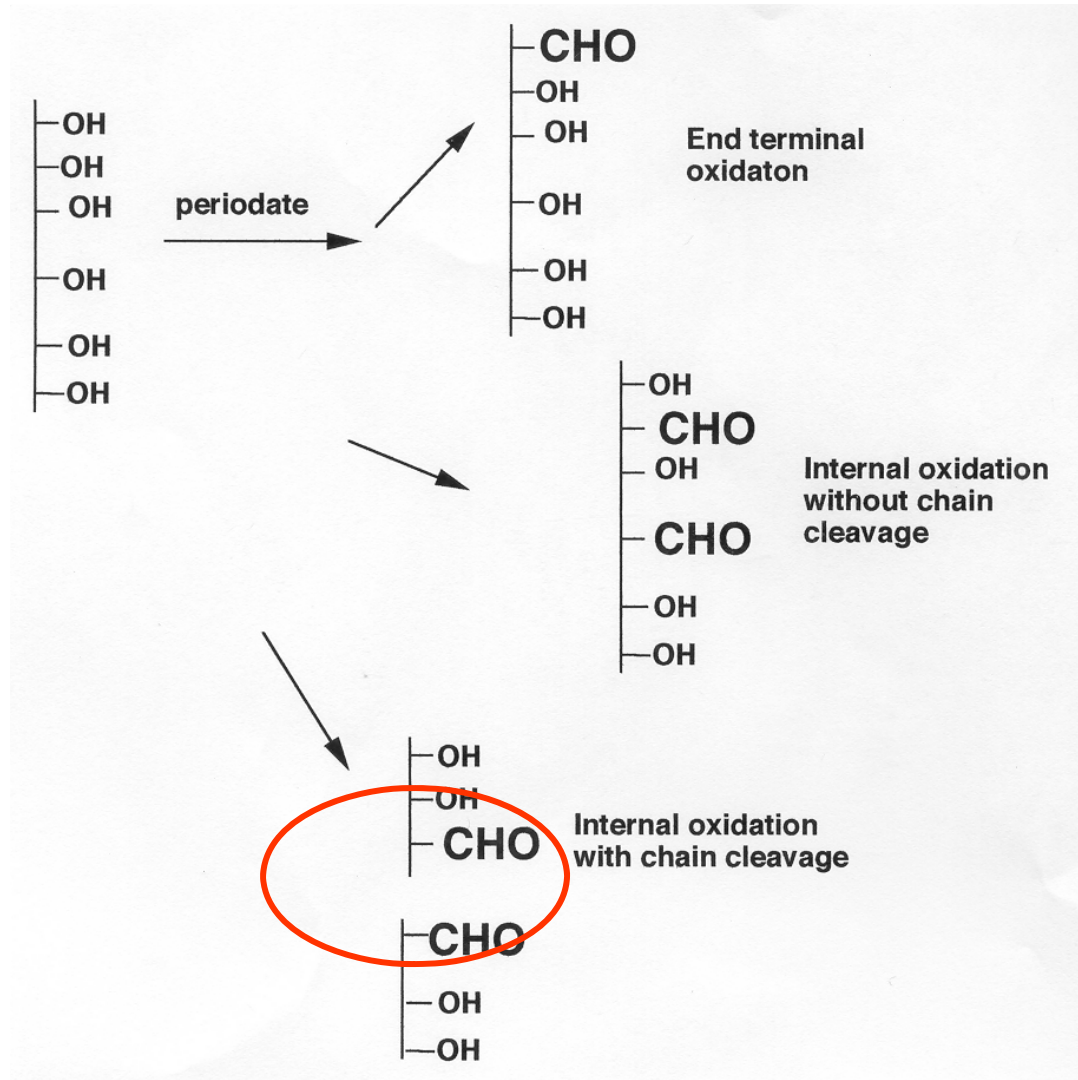
Cyanylation

Activates by randomly changing -OH groups to active -CN groups

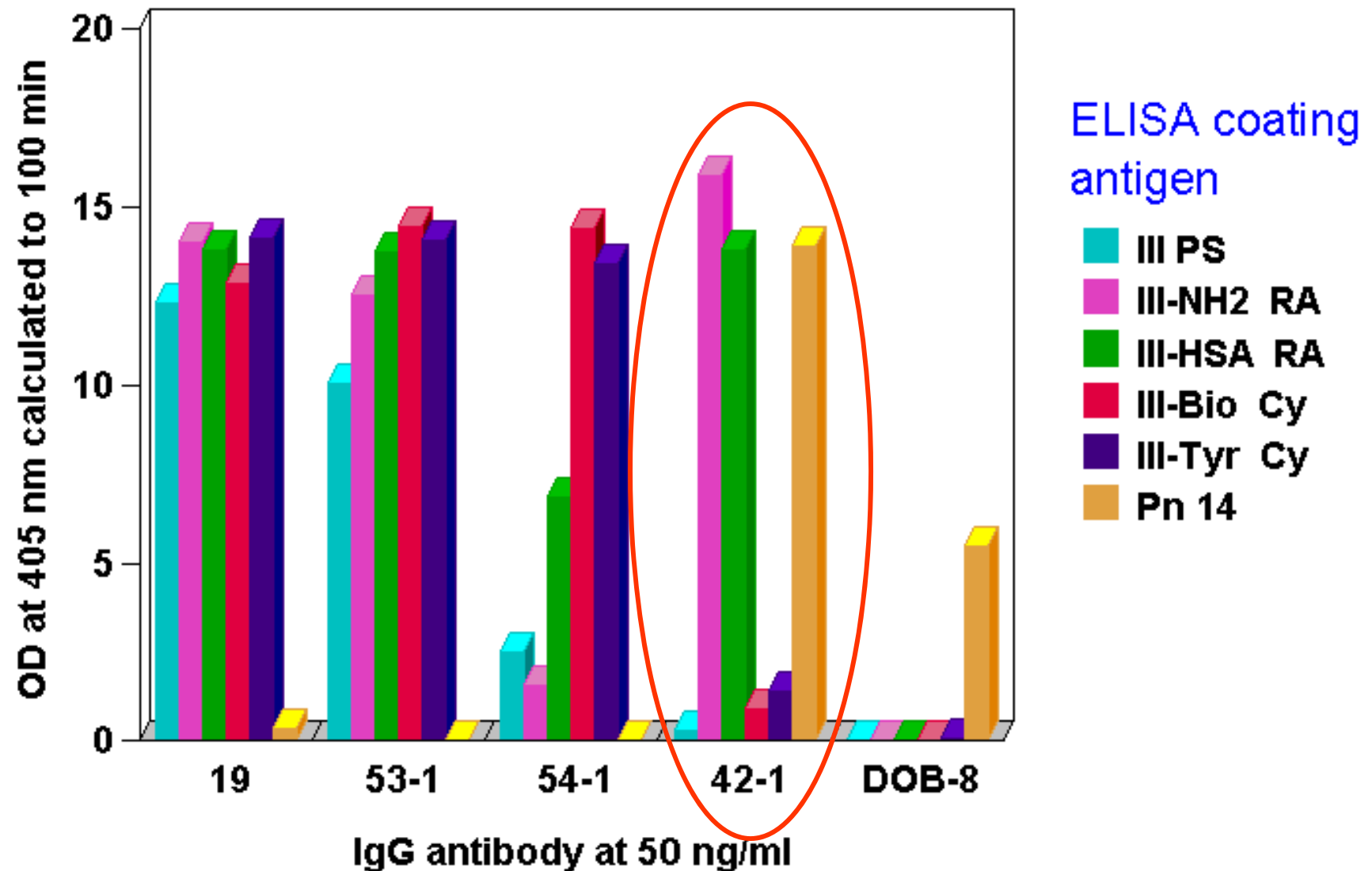
Carbodiimide

Activates at carboxyl groups by replacing -OH with carbodiimide

Periodate oxidation of polysaccharides



Comparative binding of antibodies at 50 ng/ml to different antigen preparations



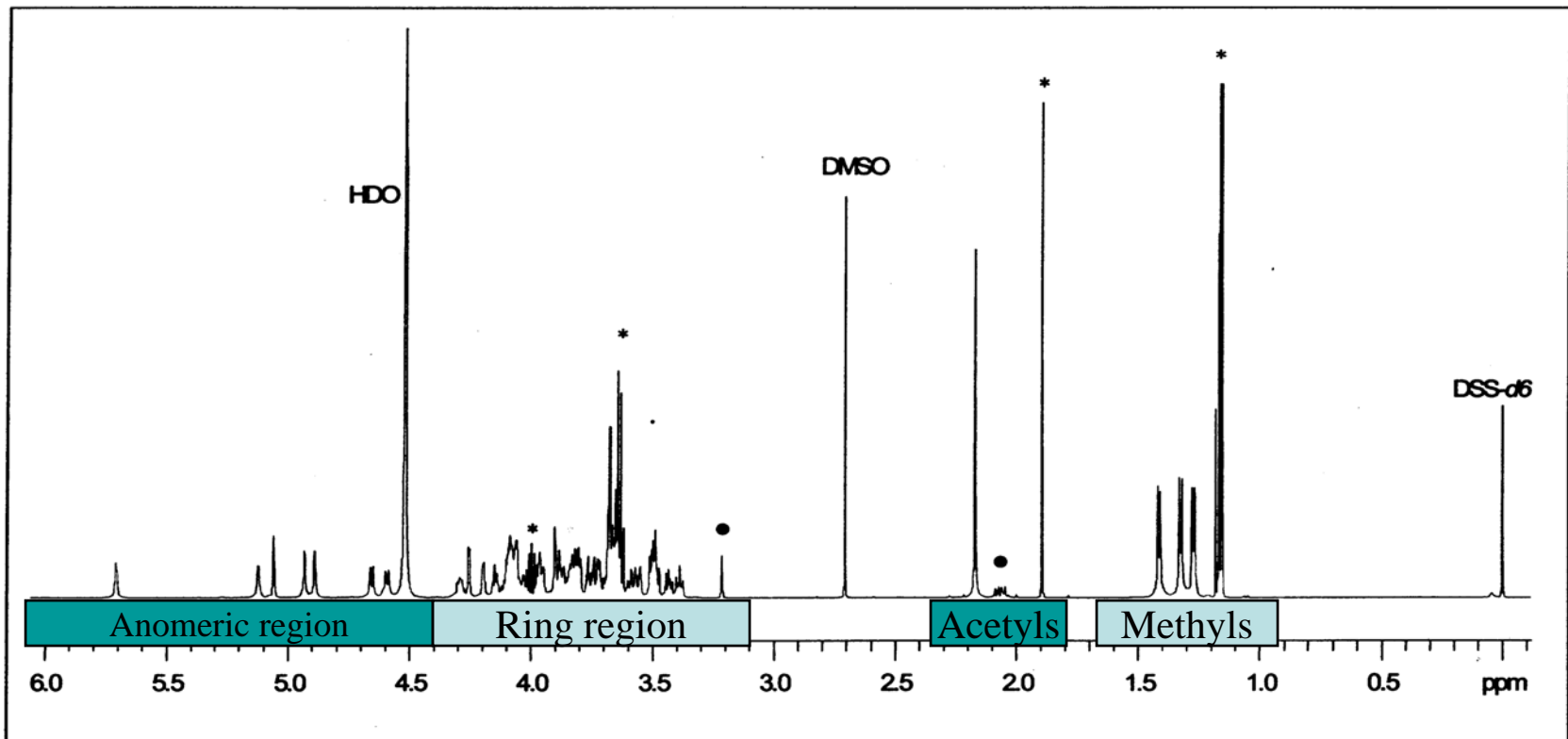
Important **lot release** tests for polysaccharide-protein conjugate vaccines

- Purity of polysaccharide and carrier protein
- Saccharide of known molecular size distribution
- Degree of saccharide activation
- Molecular size of conjugate (a stability measure)
- Vaccine Potency:
 - Polysaccharide to protein ratio in conjugate
 - Percent non-conjugated (free) saccharide present in monovalent conjugate bulks

Newer physical methods to analyze polysaccharide component of a conjugate

- Identity NMR
- Structure NMR
- Purity NMR
- Quantitation NMR
- Stability SEC-HPLC, NMR
- Size SEC-HPLC, MALLS

600-MHz proton spectrum of pneumococcal type 17F polysaccharide



Stability indicating quality control tests for monovalent bulks and final vaccine

- Change in the molecular size of conjugate during storage
- Increase in free (unbound) saccharide over time
- Change in conjugate solubility during storage
- Change in pH during storage
- Change in degree of adsorption, if vaccine adjuvant adsorbed

Conjugation efficiency (yields)

- It is very difficult to discern manufacturing yields
- Some manufacturers report high yields based upon recovery of the carrier protein (not relevant)
- Some do not account for losses during activation
- Yields can be much higher when both the polysaccharide and protein are activated before conjugation

Yields:

- Utilize native amino groups – about 10 to 30%
- Utilize activated protein -- about 50 %

New Aldehyde Conjugation Chemistry using **activated proteins** to improve **yields**

1. Hydrazone formation

Aldehyde/hydrazide/reduction

Benzaldehyde/hydrazide

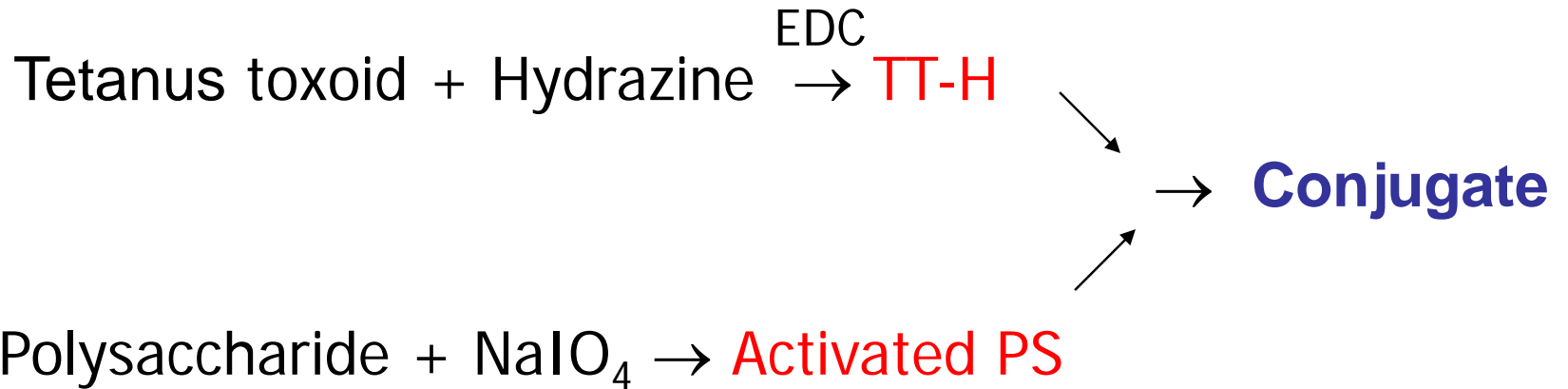
Activates through the carboxyl groups, not amino

Hydrazones are the condensation of hydrazide or hydrazine with aldehyde

2. Oxime formation

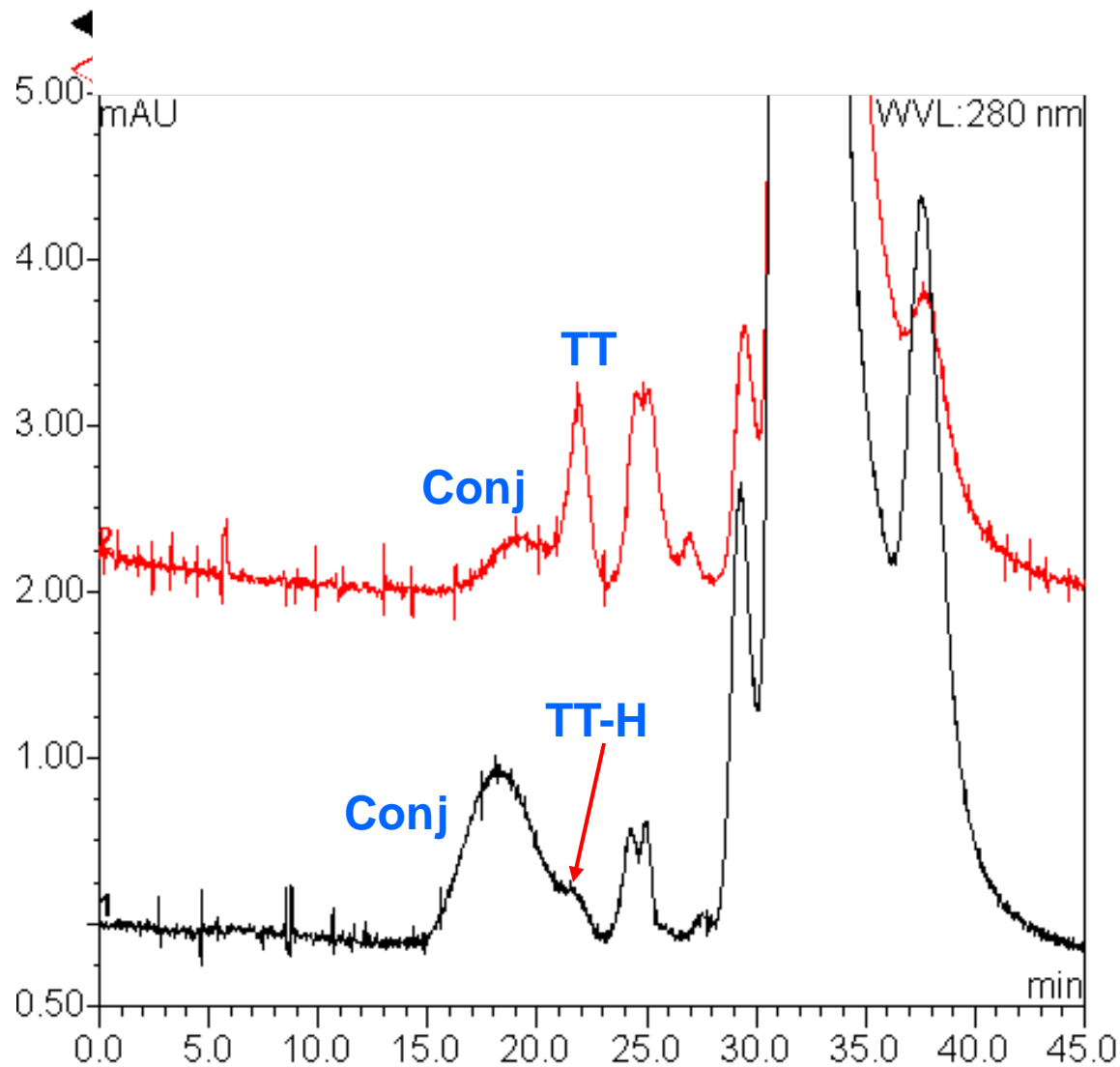
Creates a highly reactive aminooxy group

Schematics for Preparation of Polysaccharide-Tetanus Toxoid Conjugate using Hydrazide Chemistry



Conjugates made with hydrazide activated tetanus toxoid (TT-H) versus unmodified tetanus toxoid (TT)

SE-HPLC



Conclusions

- ✓ There are several methodologies for manufacture of saccharide-protein conjugate vaccines
- ✓ The polysaccharide or oligosaccharide must be chemically activated for conjugation to occur
- ✓ It is important to carefully consider the chemistry used for saccharide activation
- ✓ Activation of both the protein and the polysaccharide will improve conjugation yields
- ✓ Quality control and lot release testing are described in **WHO Technical Report** series publications for Hib, meningococcal and pneumococcal conjugate vaccines