Platform approach to purification of bacterial capsular polysaccharides for vaccine production

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Capsular polysaccharides (CPS) of encapsulated bacterial pathogens can give rise to an effective immune response in humans, and are commonly used in vaccine production. This whitepaper offers an overview of modern tools and technologies that can facilitate CPS-based vaccine production. An alternative purification approach based on chromatography, replacing many of the ethanol and phenol extraction steps of the traditional process, is also presented. Using the proposed purification platform, 28 different CPS of three different species could be processed to high purity and yield in a secure and environmentally friendly way.

Introduction

The growth rate in vaccines looks promising, with a total market worth of 30 billion USD in 2016 and estimated to reach 45 billion USD in 2022 (1). As of 2016, there were more than 250 vaccines in development of US based companies or foreign companies conducting clinical trials in the USA (2). Of the vaccines in development against infectious diseases, about 25 could be attributed to diseases caused by bacteria. In the vaccine pipeline for bacterial diseases, vaccines against *Neisseria meningitidis, Streptococcus pneumoniae*, and *Haemophilus influenza* could be found. Capsular polysaccharides (CPS) are the primary cause of the virulence of these bacteria, and are often used in production of vaccines against these pathogens.

As vaccines are often distributed by national ministries of public health, development and production costs need to be low to enable a global reach of the vaccines, especially in emerging markets. Traditionally, CPS purification is performed by extractions, using solvents such as ethanol and phenol to remove nucleic acids and protein, followed by ethanol precipitation and centrifugation to remove endotoxins. For the Gram-negative bacteria *S. pneumoniae* and *H. influenza,* an ultracentrifugation step is commonly included to remove lipopolysaccharides. This purification strategy is both time- and resource-consuming. The extractions are often carried out in explosion-proof rooms as the flammability of the required solvents is high, and ultracentrifugation is costly and not all manufacturing facilities have access to such equipment for manufacturing (3). In addition, handling and recycling of the toxic solvents and other components constitute safety and environmental risks. Such a process is also difficult to scale to meet market demands.

Replacement of these traditional purification strategies can help enhance productivity and result in more cost-efficient and more easily scaled processes. A simple platform approach to purification of CPS can contribute to more rapid process development to reduce time to market.

CPS production for vaccine manufacturing

CPS, composed of monosaccharide units joined together through glycosidic and phosphodiester linkages, constitute a highly diverse group of polymers that surround both Gram-positive (e.g., *N. meningitidis*) and Gram-negative (e.g., *S. pneumoniae* and *H. influenza*) bacteria. Of the 13 clinically significant *N. meningitidis* serotypes, classified according to the structure of their CPS, the serotypes A, C, Y, and W-135 are responsible for 90% of all global cases. For *S. pneumoniae*, only a few of the 90 different serotypes leads to diseases in humans. *H. influenza* can occur in both unencapsulated and encapsulated form. There are six types of encapsulated strains (a, b, c, d, e, and f), of which type b is the most familiar. For encapsulated pathogenic bacteria, the concealment

Table 1. Repeated units of selected bacterial CPS involved in vaccine production (4)

Organism	Structure
H. influenzae type B	→3)-β-D-Rib*f*-(1→1)-D-Ribitol-(5→OPO3→
N. meningitidis group A	→6)-α-D-ManpNAc(3/4OAc)-(1→OPO3→
N. meningitidis group C	→9)-α-D-Neup5Ac(7/8OAc)-(2→
N. meningitidis group Y	→6)-α-D-Galp-(1→4)-α-D-Neup5Ac(9OAc)-(2→
N. meningitidis group W-135	→6)-α-D-Glcp-(1→4)-α-D-Neup5Ac(9OA)-(2→
S. pneumoniae type 1	→3)-D-AAT-α-Galp-(1→4)-α-D-GalpA(2/3OAc)-(1→3)-α-DGalpA-(1→
S. pneumoniae type 2	→4)-β-D-Glcp-(1→3)-[α-D-GlcpA-(1→6)-α-D-Glcp-(1→2)]-α-L-Rhap-(1→3)-α-L-Rhap-(1→3)β-L-Rhap-(1→
S. pneumoniae type 3	→3)-β-D-GlcA-(1→4)-β-D-Glcp-(1→
S. pneumoniae type 4	→3)-β-D-ManpNAc-(1→3)-α-L-FucpNAc-(1→3)-α-D-GalpNAc-(1→4)-α-D-Galp2,3(S)Py-(1→
S. pneumoniae type 5	→4)-β-D-Glcp-(1→4)-[α-L-PnepNAc-(1→2)-β-D-GlcpA-(1→3)] -α-L-FucpNAc-(1→3)-β-D-Sugp-(1→
S. pneumoniae type 6B	→2)-α-D-Galp-(1→3)-α-D-Glcp-(1→3)-α-L-Rhap-(1→4)-D-Rib-ol-(5→P
S. pneumoniae type 9N	→4)-α-D-GlcpA-(1→3)-α-D-Glcp-(1→3)-β-D-ManpNAc-(1→4)-β-D-Glcp-(1→4)-α-D-GlcpNAc-(1→
S. pneumoniae type 9V	→4)-α-D-GlcpA(2/3OAc)-(1→3)-α-D-Galp-(1→3)-β-DManpNAc(4/6OAc)-(1→4)-β-D-Glcp-(1→4)-α-D-Glcp-(1→
S. pneumoniae type 12F	→4)-[α-D-Galp-(1→3)]α-L-FucpNAc-(1→3)-β-D-GlcpNAc-(1→4)-[α-D-Glc-(1→2-α-D-Glc-(1→3)]-β-D-ManNAcA-(→
S. pneumoniae type 14	→4)-β-D-Glcp-(1→6)-[β-D-Galp-(1→4)]-β-D-GlcpNAc-(1→3)-β-D-Galp-(1→
S. pneumoniae type 18C	→4)-β-D-Glcp-(1→4)-[α-D-Glcp(6OAc)(1→2)][Gro-(1→P→3)]-β-D-Galp-(1→4)-α-D-Glcp-(1→3)-β-L-Rhap-(1→
S. pneumoniae type 19F	→4)-β-D-ManpNAc-(1→4)-α-D-Glcp(1→2)-α-L-Rhap-(1→P→
S. pneumoniae type 23F	→4)-β-D-Glcp-(1→4)-[α-L-Rhap (1→2)]-[Gro-(2→P→3)]-β-D-Galp-(1→4)-β-D-Galp-(1→4)-β-L-Rhap-(1→

of extracellular proteins has prevented the development of protein-based vaccines. However, CPS has shown to give rise to an effective immune response in humans and thus are commonly used for vaccine production (Table 1).

CPS vaccines can be based on purified CPS alone, so called, naked vaccine. More commonly, the CPS is covalently coupled to a carrier antigen in, what is called, a conjugate vaccine. CPS-based vaccines can also be multivalent, where multiple strain-specific components are included to protect against the most prevalent pathogenic serotypes. When looking at the breadth of disease causing strains across the world, it is forecasted that multivalent conjugate vaccines will constitute more than 50% of the world market in the near future (5).

Upstream production

In the industry today, production of CPS by bacterial fermentation is commonly conducted in stainless steel systems. The adoption of single-use technology by biomanufacturers using microbial systems has been limited by the lack of single-use bioreactor systems capable of accommodating the unique requirements of microbial cultures. Bioreactors designed for mammalian culture fall short of meeting bacterial cultivation requirements, for example, high oxygen transfer capacity and increased cooling capacity for efficient temperature control.

With the advances in single-use technologies, today's microbial systems are purpose-designed to overcome these limitations, and can fulfill the demands of bacterial fermentation (Fig 1).

Fig 1. Single-use Xcellerex™ XDR-50 MO and XDR-500 MO fermentor systems together handle working volumes from 25 to 500 L. The systems deliver performance comparable with hard-piped, stainless steel fermentor systems, while eliminating the time-consuming and costly clean-in-place (CIP), steam-in-place (SIP), and cleaning validation procedures. Single-use technology also increases operator safety, as the system is closed and process components that have been in contact with the process material are disposed after use without the need for open handling of the product.

Downstream purification

The upstream process determines the type and concentration of impurities that the downstream process will need to be capable of removing. Typical impurities in purification of CPS include host cell protein (HCP), host cell DNA (hcDNA), pigments, and other polysaccharides such as cell wall polysaccharides and lipopolysaccharides.

The traditional purification process commonly comprises a sample preparation step to separate CPS from cell debris, followed by removal of remaining impurities in several selective precipitation steps involving solvents like ethanol and phenol as well as cationic detergents. Separation of solid from liquid is performed by centrifugation.

To limit the use of flammable and health hazardous solvents, the extraction steps can be greatly reduced by using modern chromatography resins. Multimodal resins, for example, provide multiple modes of actions such as ion exchange, hydrophobic interaction, and hydrogen bonding, enabling efficient removal of impurities such as HCP and nucleotides, often in one single step (Fig 2).

The ultracentrifugation step for lipopolysaccharide removal can be replaced by tangential flow filtration (TFF) (6). In TFF, the feed stream moves tangential to the membrane. Particles larger than the pore size are kept in constant movement, avoiding the formation of a filtration cake, which can clog the filter. Hollow fiber filters are commonly used for the TFF step (Fig 3). Because of their open channel structure, hollow fiber filters are well-suited for microfiltration applications like recovery of proteins expressed in bacteria.

Purification techniques such as chromatography and filtration allow fast and easy process development. Design of experiment (DoE) methodologies can be used to perform experiments to identify conditions for maximized productivity (Fig 4). This approach produces maximum amount of data with minimum number of experiments, and meets the demands from regulatory authorities for better process understanding, one of the cornerstones of the quality by design (QbD) initiative.

In addition, chromatography and filtration operations are easy to scale and compatible with both single-use and conventional technologies.

Process integration and automation

For automated operations in a closed or functionally closed fashion, the different unit operations, from upstream fermentation to downstream filtration and chromatography purification steps, can be integrated. Process integration can help reduce the number of process steps, which in turn, can help reduce floor space (facility overhead) requirements and shorten overall process time. Automated workflows in closed system operation minimizes cross-contamination risk and contributes to increased operator safety by eliminating the need for open handling of potentially infectious process materials.

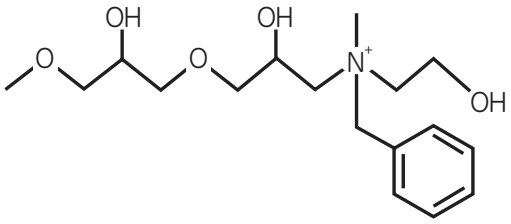

Fig 2. The ligand, N-benzyl-N-methylethanolamine, of Capto™ adhere multimodal anion exchange resin interacts with the target molecule through multiple modes of action, of which the most pronounced are ionic interaction, hydrogen bonding, and hydrophobic interaction.

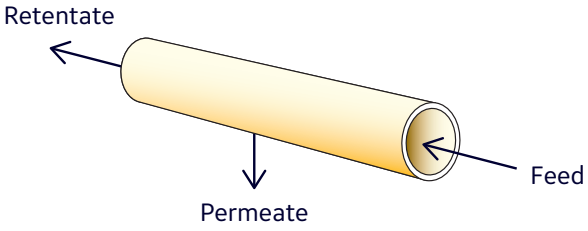

Fig 3. Configuration of hollow fiber filters. The membrane forms a set of parallel hollow fibers. The feed stream passes through the lumen of the fibers and the permeate is collected from outside the fibers. Cartridges are characterized in terms of fiber length, lumen diameter and number of fibers, as well as filter pore size.

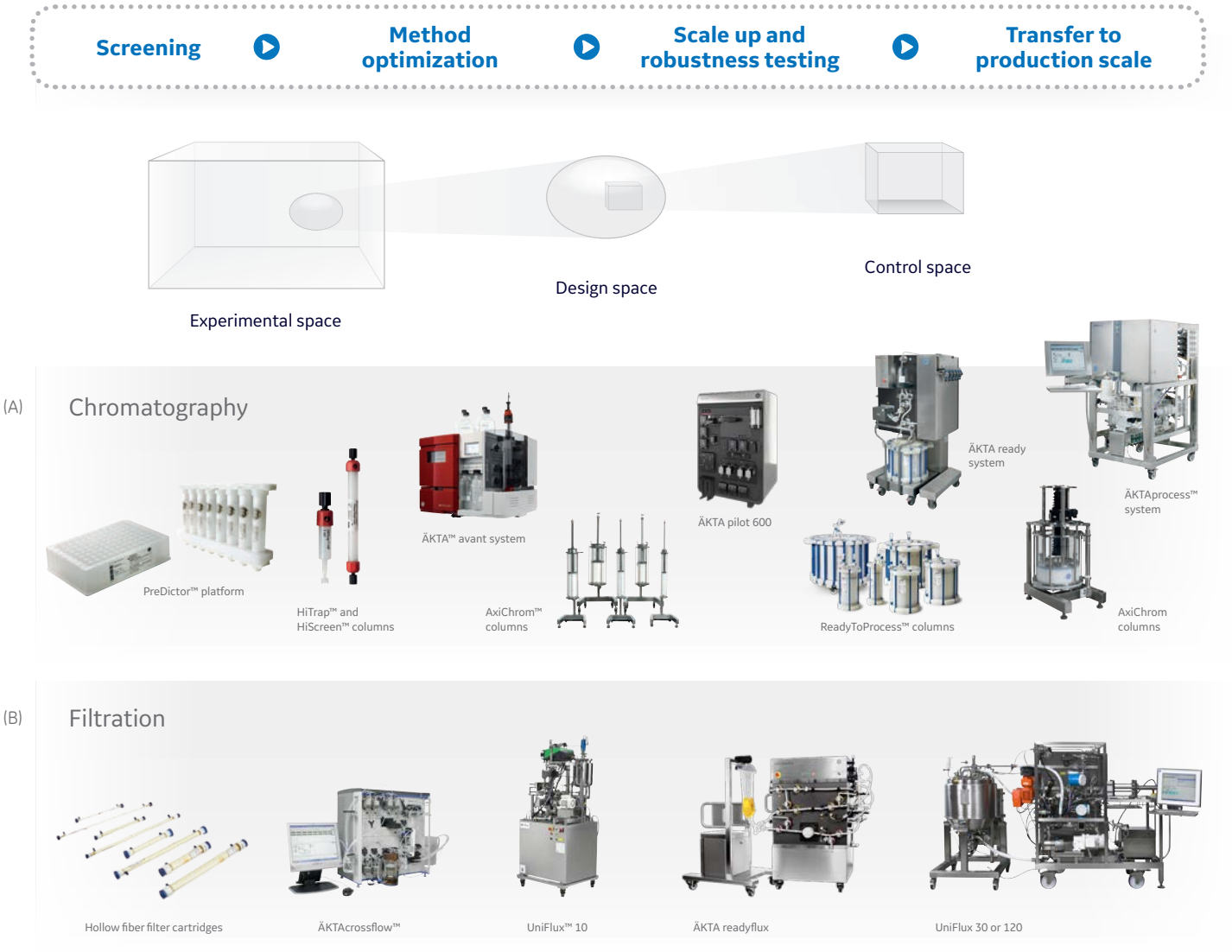

Fig 4. Process development workflow, including initial screening, verification, and further optimization of (A) chromatography and (B) filtration conditions in the purification of a target biomolecule.

Development of a CPS purification platform based on chromatography

A purification strategy for CPS of *N. meningitides*, *S. pneumoniae*, and *H. influenza* based on chromatography was developed by GE Healthcare's Fast Trak services team in Shanghai, CN. For most serotypes, the purification procedure constitutes a one-step chromatography process using Capto adhere resin. Capto adhere is a multimodal anion exchange resin designed to remove key contaminants such as hcDNA, HCP, larger aggregates, and viruses in a single step. For some serotypes, remaining impurities were removed by including a second chromatography step using Capto DEAE resin. Capto DEAE is a weak anion exchange resin designed for capture of protein from large feed volumes to meet industry demands for fast, efficient, and cost-effective processes.

A general overview of the CPS production process is given in Figure 5. More detailed information is given in application notes 29216880, 29216881, and 29258288 (7–9). Chromatography conditions used for purification of the various CPS serotypes are summarized in Table 2. The chromatographic processes offered good separation of CPS from impurities. Compared with the recoveries that can be expected from the more time-consuming traditional purification processes, the described process designs significantly improved recovery and only required one week from sample preparation to desalted product.

As shown from the results listed in Table 3, impurity levels met the requirements of European Pharmacopoeia (EP) for protein and DNA. Eliminating the need for time-consuming, multi-step phenol and ethanol extractions, the suggested processes allowed CPS to be successfully purified with high recovery and purity in a way that benefits the environment and operator health.

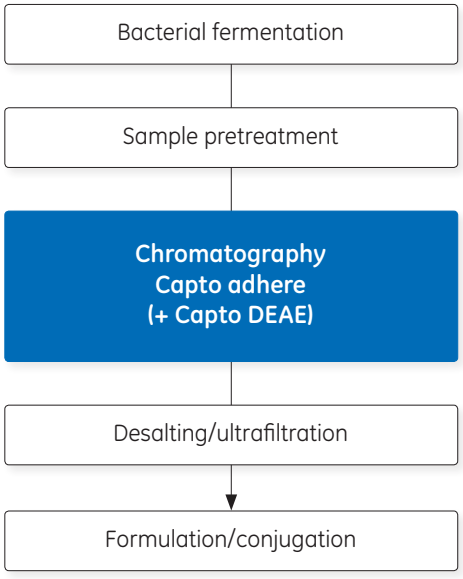

Fig 5. CAP production.

Table 2. Chromatography conditions for purification of CPS serotypes

N. meningitidis	Sample pH	Sample buffer	Sample load	Resin	Buffers
A, C, Y, W-135	8.0	20 mM Tris-HCl + 1% SDC	0.65 column volumes (CV)	Capto adhere (FT) + Capto DEAE (FT)	Loading: 20 mM Tris-HCl, pH 8.0 + 1% SDC Equilibration: 20 mM Tris-HCl, pH 8.0 + 1% SDC
S. pneumoniae	**Sample pH**	**Sample conductivity**	**Sample load**	**Resin**	**Buffers***
2, 9N, 9V, 10A, 12F, 15B, 20, 22F, 23F, and 33F	7.6	23 mS/cm	1 CV	Capto adhere (FT)	Loading: Buffer A, pH 7.6 Equilibration: 80% Buffer A, pH 7.6 + 20% Buffer B, pH 7.6
1, 5, 7F, 14, 17F, and 18C	7.6	23 mS/cm	0.5 CV	Capto adhere (FT) + Capto DEAE (FT)	Loading: Buffer A, pH 7.6 Equilibration: 80% Buffer A, pH 7.6 + 20% Buffer B, pH 7.6
3, 4, 6B, 8, 11A, and 19F	7.6	54 mS/cm (type 4: 45 mS/cm) (type 8: 35 mS/cm)	1 CV (type 8: 0.8 CV)	Capto adhere (FT)	Loading: Buffer A, pH 7.6 Equilibration: 40% Buffer A, pH 7.6 (type 4: 60%) (type 8: 65%) + 60% Buffer B, pH 7.6 (type 4: 40%) (type 8: 35%)
Serotype 19A	6.0	54 mS/cm	0.2 CV	Capto adhere (FT)	Loading: Buffer A, pH 6.0 Equilibration: 40% Buffer A, pH 6.0 + 60% Buffer B, pH 6.0
H. influenzae	**Sample pH**	**Sample buffer**	**Sample load**	**Resin**	**Buffers***
b	7.6	50 mM phosphate	2 CV	Capto adhere (B/E)	Loading: Buffer A, pH 7.6 Equilibration: 14% Buffer B, pH 7.6 Elution: 15% Buffer B in Buffer A + 40% Buffer B in Buffer A + 70% Buffer B in Buffer A (target eluent)

* Buffer A: 50 mM sodium phosphate, pH 7.6 or 6.0, Buffer B: 50 mM sodium phosphate + 1.0 M NaCl, pH 7.6 or 6.0
SDC = sodium deoxycholate, FT = flow-through mode, B/E = bind-elute mode

Table 3. Process results

Polysaccharide (PS) serotype	CPS (µg/mL)	Recovery (%)	Protein/CPS (%)	Protein required by EP (%)	DNA/CPS (%)	DNA required by EP (%)
N. meningitidis						
A	789	77	BDL	≤ 1%	0.5	≤ 1%
C	613	73	BDL	≤ 1%	0.7	≤ 1%
W135	716	69	BDL	≤ 1%	0.1	≤ 1%
Y	523	58	BDL	≤ 1%	0.4	≤ 1%
S. pneumoniae						
1	329	40	1.33	2	1.3	≤ 2
2	285.4	84	1.3	2	0.75	2
3	841.0	56	0.46		0.19	2
4	498	60	1.06		0.78	2
5	381	54	4.85	7.5	1.4	2
6B	867.3	55	0.50		0.16	2
7F	716.8	70	4.0	5	0.4	2
8	318.4	50	1.37		1.22	2
9N	281.5	86	1.8	2	0.11	1
9V	290.8	70	1.9	2	0.12	2
10A	163.8	72	5.6	7	0.21	2
11A	2063.4	70	0.62	3	0.10	2
12F	107.8	39	2.6	3	0.19	2
14	320.1	50	2.67	5	0.78	2
15B	275	88	2.5	3	0.16	2
17F	933.7	66	1.27		0.18	2
18C	650.4	58	2.11	3	0.9	2
19F	1922.5	75	0.64	3	0.1	2
19A	130.4	32	1.93		0.84	2
20	731	90	1.2	2	0.06	
22F	292	90.9	1.4	2	0.10	
23F	256	40	1.7	2	0.55	
33F	299	76	0.5	2.5	0.10	
H. influenzae						
b	868	31.2	0.45	≤ 1%	0.30	≤ 1%

Note! Recovery is for the total process including pretreatment and chromatography.
BDL = below detection limit.

Increase speed to market with collaborative services

GE's Fast Trak Services are specifically designed to help biomanufacturers increase process productivity, reduce cost, and enable bringing product to market faster. The Fast Trak Services centers are equipped with the latest technologies for bioprocessing in an environment and at a scale that closely replicates the real-life industrial setting. For over 30 years, thousands of customers have had access to industry expertise encompassing process and analytical development, process scale-up, as well as manufacture of drug substances for use in toxicology studies or phase I and II clinical testing. The Fast Trak Services centers are located in South Korea, the USA, Sweden, India, and China, with satellite centers in Turkey, Japan, and Singapore.

GE's Fast Trak team of scientists has a wide experience in vaccine process development and production.

Conclusion

This white paper gives an overview of modern products and technologies that can help solve many challenges in production of bacterial CPS vaccines. Fermentor systems based on single-use technology support significant time-savings, while increasing process- and operator-safety. Process steps involving flammable or health hazardous solvents can be replaced by modern chromatography resins. Cross-flow filtration offers an alternative to costly ultracentrifugation operations. With tools and formats for process development workflows, the time for such activities can be significantly reduced to contribute to an overall shorter time to market. Integrating the different process units for automated operations in a closed system will reduce the number of process steps, thereby minimizing facility footprint. The described purification platform allowed multiple CPS serotypes to be successfully purified with high recovery and purity in a health and environmentally friendly way.

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

gelifesciences.com/bioprocess

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