



# **Vaccine and Virus Purification using Toyopearl**

Tosoh corporation

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# Characteristics of Vaccine and Virus

## ■ Vaccine

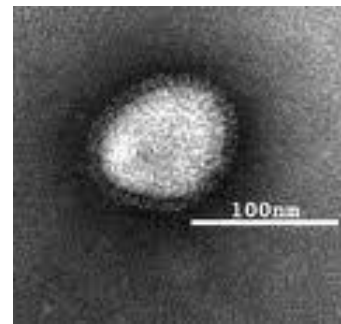
- Protein as surface antigen, toxin, and polysaccharide
- Native protein, polysaccharide or recombinant protein
- Similar purification strategy as protein separation
- SEC, IEC, HIC and AFC

## ■ Virus

- Huge biomolecule complex particles
- Influenza; 100-120 nm, Adenovirus; 80 nm, Smallpox; 30 nm
- Slightly different purification strategy against protein separation
  - Large pore chromatographic resin
  - Polymethacrylate-grafting type IEC may be effective.
  - Flow through mode is effective as mild separation (SEC, IEC)
  - Ultrafiltration

## ■ Plasmid (DNA)

- Huge DNA molecule
- Purification by SEC, AIEC and HIC resins with large pore
- Ultra-high Purity is required for gene therapy



Influenza virus



# Chromatographic Resins for Vaccine

## ■ Size-Exclusion chromatography(SEC)

- Large pore resin is required for virus separation
  - Virus size -100 nm; TOYOPEARL HW-65, Sepharose 6 FF
  - Virus size - 200 nm, HW-75, Sepharose 4 FF
- **Rigidity of resin is more important for process column.**
  - TOYOPEARL has better rigidity, flow stability than Sepharose matrix.

## ■ Ion-exchange chromatography(IEC)

- Large pore resin; TOYOPEARL 650 series
- **Polymethacrylate, grafting type may be effective**
  - Grafting type; TOYOPEARL GigaCap Q-650M, GigaCap S-650M, SuperQ-650M

## ■ Hydrophobic interaction chromatography(HIC)

- Various hydrophobicity on resin
- **Less hydrophobic resin may be effective for virus separation**
- DNA is less retained to HIC resin

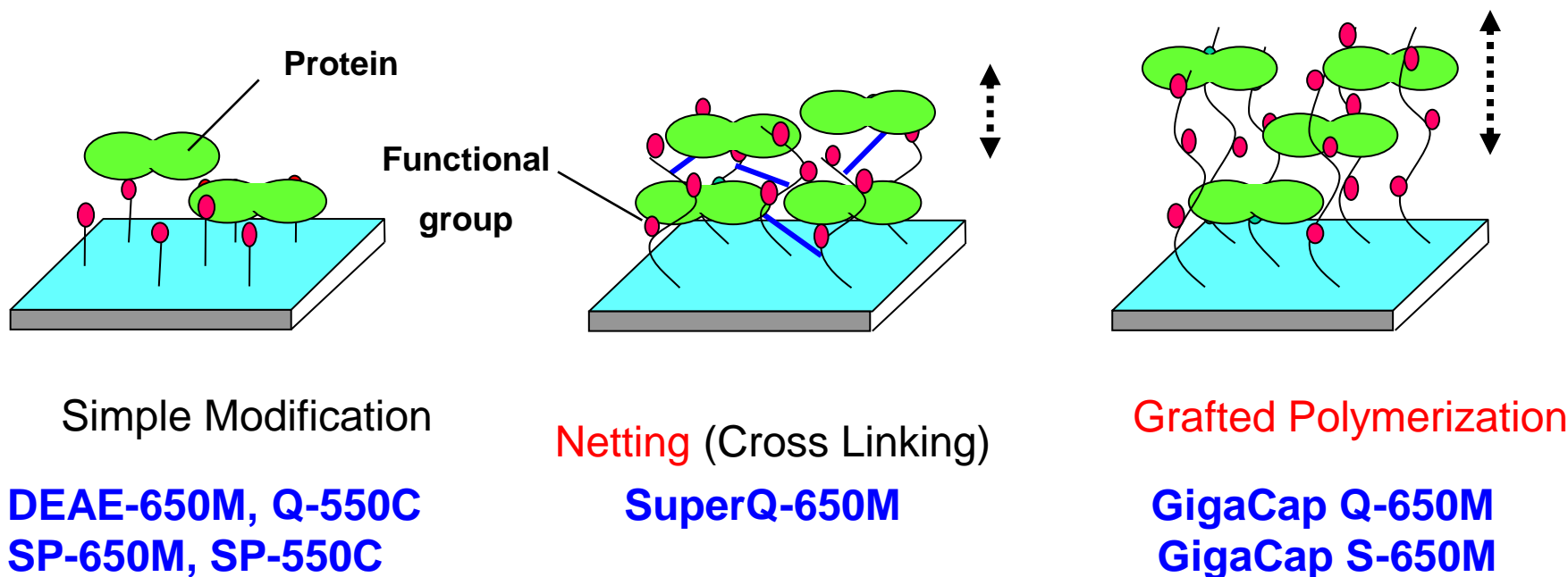
## ■ Affinity chromatography(AFC)

- Dye affinity; TOYOPEARL AF-Blue-650M
- Heparin; TOYOPEARL AF-Heparin-HC-650M
- Other protein immobilized AFC resin



# Chemistry for Functional Group on IEC Resins

## Resins



Functional Group:

Adsorption Capacity:

Operational Pressure Drop:

Flat Surface

Not So High

Low

Loose Cross Linking

High

Medium

Graft Polymer

Very High

High



# Application of TOYOPEARLS for Vaccines, Viruses and Viral Vectors/Plasmid

## ■ Vaccine (including toxin, rec-antigen and polysaccharide)

- SEC; HW-40S, HW-55S, HW-55F, HW-65S, HW-65F
- IEC; DEAE-650M, SuperQ-650M, QAE-550C, SP-650M, SP-550C (predicted; GigaCap Q-650M, GigaCap S-650M, Q-600C AR)
- HIC; Butyl-650M, Phenyl-600M, Phenyl-650M, Ether-650M
- AFC; AF-Heparin-(HC)-650M, Fetuin-immobilized-650M

## ■ Virus

- SEC; HW-65F, HW-75F
- IEC; SuperQ-650M
- HIC; Butyl-650M

## ■ Viral vector (plasmid)

- SEC; HW-65F
- IEC; DEAE-650M
- HIC; Hexyl-650C

# Vaccine Purification (1)

Class	Year	Title	Authors	Journal	Summary
Adjuvant	2009	New adjuvant component: recombinant allergen Derf2 vaccine	T. Tsukui et al	Patent WO/2009/057763	Recombinant allergen Derf2 purified by <b>EC</b> on <b>Toyopearl SP-550C</b> and Q-Sepharose Fast Flow
Allergen vaccine	2010	Production-scale purification of the recombinant major house dust mite allergen Der f2 mutant	S. Kayanagi et al	J. Bioscience and Bioengineering, 110 (2010) 597-601	C8/119S was purified by three chromatography including <b>TOYOPEARL QAE-550C</b> .
Bordetella	2000	Process for purifying dornecrotic toxin produced by Bordetella and toxoid	T. Kawai et al	US patent No. 6124432	Dornecrotic toxin purification including <b>Toyopearl SP-650M</b> .
Botlinus	2003	Purification of Fully Activated Clostridium botulinum Serotype B Toxin for Treatment of Patients with Dystonia	H. Arimitsu et al	Infection and Immunity, 71 (2003) 1599-1603	(1) Ammonium sulfate precipitation, and <b>Toyopearl SP-650M</b> , NaCl gradient from 0 to 0.5 mol/L in 0.05 mol/L sodium acetate buffer (pH 4.2), (2) aminophenylbeta lactose gel column
Botlinus	2003	Scale-up of the fermentation and purification of the recombinant heavy chain fragment C of botulinum neurotoxin serotype F, expressed in Pichia pastoris	S. K. Johnson et al	Protein Expression and Purification, 32 (2003) 1	Pilot scale purification of BoNTF (Hc). Final step on <b>Toyopearl SP-650M</b> and HIC
Botlinus	2009	Bivalent recombinant vaccine for Botulinum neurotoxin types A and B based on a polypeptide comprising their effector and translocation domains that is protective against predominant A and B subtypes	C. Shone et al	Infection and Immunity, 77 (2009) 2795-2801	LH-N A and B fragments derived from BoNT serotypes A and B. Purification by <b>Toyopearl Phenyl-650M</b> , Q-Sepharose and hydroxyapatite.
Botlinus	2002	Mucosal immunization with Clostridium botulinum type C 16S toxoid and its non-toxic component	N. Mahmut et al	J. Med. Microbiol 51 (2002) 813-820	Type D-16S toxin and non Tox were purified by <b>EC</b> on <b>Toyopearl SP-650M</b> and <b>Toyopearl HW-65S</b> .
Erisperas	2002	Purification and recovery of recombinant Erysipelothrix rhusiopathiae vaccine: 46.5 K PA : 46.5 KDa protective	N/A	Patent 2002-34568 (P2002-34568A)	Purification of recombinant antigen by <b>EC</b> on <b>Toyopearl DEAE-650M</b>
HBV	1989	Process for the extraction and purification of proteins from culture media producing them	F. V. Wijendaele	United States Patent 4857317	Hepatitis B surface antigen vaccine purified by SEC on Fractogel TSK HW-55F and HW-65F, and <b>EC</b> on Fractogel TSK DEAE-650M
HTLV-1	2009	Efficient induction of human T-cell leukemia virus-1-specific CTL by chimeric particle without adjuvant as a prophylactic for adult T-cell leukemia	T. Kozako et al	Molecular Immunology, 47 (2009) 606-613	Purification including <b>Toyopearl DEAE-650M</b>
HVB	2006	Expression and purification of Hepatitis B surface antigen (S-protein) from methylobrophic yeast Pichia	N. Bardiya	Anaerob, 12 (2006) 194	Recombinant HBsAg vaccine, <b>Toyopearl DEAE-650M</b>
HVB	2004	Purification of recombinant HBsAg expressed in methylobrophic yeast Pichia pastoris	N. Bardiya et al	Theories and Applications of Chem. Eng. 10 (2004) 1604	Purification of HBsAg by <b>EC</b> on <b>Toyopearl DEAE-650M</b> .

# Vaccine Purification (2)

Class	Year	Title	Authors	Journal	Summary
Leishmania	1987	Lipophosphoglycan of Leishmania major that vaccinates against cutaneous leishmaniasis contains an alkylglycerophosphoinositol	M. J. McConville et al	Proc. Natl Acad. Sci USA, 84 (1987) 8941-8945	Dephosphorylated lipophosphoglycan was separated by Fractogel TSK HW-40S
Malaria	2008	Purification, quality control, stability and pharmacotoxicity of cGMP-produced Plasmodium falciparum AMA1 FVO strain ectodomain expressed in Pichia pastoris	B. W. Faber et al	Vaccine, 26 (2008) 6143-6150	PfAMA1 ectodomain purification, <b>Toyopearl Butyl-650M</b>
Malaria	2006	Production and characterization of clinical grade Escherichia coli derived Plasmodium falciparum 42kDa merozoite surface protein 1 (MSP1-42) in the absence of	R. L. Shimp Jr. et al	Protein Expression and Purification, 50 (2006) 58	Malaria vaccine target A pilot scale production of EcMSP1-42-FUP in a cGMP environment. <b>Toyopearl Butyl-650M</b> , <b>SuperQ-650M</b> (?) and SEC were applied.
Malaria	2003	Development and pre-clinical analysis of a Plasmodium falciparum Merozoite Surface Protein-1-42 malaria vaccine	E. Angov et al	Molecular and Biochemical Parasitology, 128 (2003) 195-204	Merozoite Surface Protein-1-42 (MSP-1-42) was purified by MAC, IEC on <b>Toyopearl SuperQ-650M</b> and IEC on CM-resin.
Malaria	2007	Single-chain antibody fragment specific for Plasmodium vivax Duffy binding protein	S.-H. Kim et al	Clinical and Vaccine Immunology, 14 (2007) 726-731	Recombinant PvRII protein purified by IEC on <b>Toyopearl SP-650</b> and SEC on Superdex-75
Parvovirus	2009	Parvovirus B19 VLP vaccine manufacturing	D. Shelly et al	GEN, 29 (2009) Sept. 15	HPVB19 vaccine was purified by EBA and IEC on Fractogel DEAE and Fractogel TMAE.
Pertussis	2001	Efficacy of chemically cross-linked antigens for acellular pertussis vaccine	M. Watanabe et al	Vaccine, 19 (2001) 1199-1203	<b>Fetuin-immobilized Toyopearl PT</b> eluted with 0.05 mol/L diethanolamine containing 0.5 mol/L NaCl
Pertussis	2002	Efficacy of pertussis components in an acellular vaccine, as assessed in a murine model of respiratory infection and a murine intracerebral challenge model	M. Watanabe et al	Vaccine, 20 (2002) 1429	Purification including <b>Toyopearl AF-Heparin 650M</b>
PRV	1994	Vaccine for Aujeszky's disease	N/A	Patent JPH05-246888	Porcine herpes 1 virus membrane glycoprotein gIII Purification using <b>Toyopearl DEAE-650M</b> and <b>AF-Heparin 650M</b>
Staphylococcal	2003	Methods and compositions for production and purification of recombinant staphylococcal enterotoxin B (SEB)	J. D. Coffman et al	Patent W/O/2003/031471	Purification of rSAg including HIC on <b>Toyopearl Butyl-650M</b> and <b>Phenyl-650M</b> and IEC on <b>Toyopearl SP-650M</b>

# Virus and Viral Vector Purification

Class	Year	Title	Authors	Journal	Summary
Adenovirus	2001	Method for the production and purification of adenoviral vectors	S. Zhang et al	US Patent 6194191	Purification of Ad5 by EC on <b>Toyopearl SuperQ-650M</b> , where SuperQ-650 showed best capacity and recovery
Adenovirus	2007	Sorption process in ion-exchange chromatography of viruses	E.I Trilisky et al	J. Chromatography, 1142 (2007) 2-12	Adenovirus separation by EC. Referred to <b>Toyopearl SuperQ-650M</b> and Fractogel DEAE-650M
Adenovirus	2008	Challenges in producing viral vectors for gene therapy or vector vaccine	P. S. Chahal et al	not applicable	Purification of adeno-associated virus including EC and HC on <b>Toyopearl Butyl-650M</b>
HVA	1996	Vaccine for hepatitis A virus	N/A	Patent JPH07-48277	Purification using <b>Toyopearl DEAE-650M</b> and <b>Toyopearl HW-55S</b> and <b>HW-65S</b>
Influenza	2008	Method for influenza virus protection	P. Matjaz et al	Patent No. WO 08006780	<b>Toyopearl HW-75</b> and other SEC resins were packed in a HR 16/50 column to 100 ml bed volume and compared for their separation profile and recovery. For all experiments SPG buffer (0.218 M sucrose, 0.0038 M KH <sub>2</sub> PO <sub>4</sub> , 0.0072 M K <sub>2</sub> HPO <sub>4</sub> , 0.0049 M L-glutamate pH 8.0 ± 0.2) was used
Rabies	2010	Methods for purifying the rabies virus	V. Fabre et al	US patent 20100260798	Inventor suggested grafted polymethacrylate cation-exchanger would be better like Fractogel EMD S03-, which suggests <b>TOYOPEARL GigaCap S-650M</b> may be applicable as well
Smallpox	2009	Method using ion-exchange and gel filtration chromatography for poxvirus purification	Y. Xiong et al	Patent No. WO 09100521	For the purification of poxviruses, EC and SEC on <b>Toyopearl HW series (HW-65, HW-75)</b> with a Tris-HCl buffer (e.g., 5 mM, 10 mM, 15 mM, 20 mM) at a pH of between approximately 7.0-9.0 may be suitable.
Smallpox	2010	Vaccinia virus particles	N/A	P2010-507827	Purification of viruses by AFC on <b>Toyopearl IAF-Heparin-650M</b>
West Nile Virus	2004	Chromatographic method to obtain highly enriched supercoiled DNA	J. Balantyne et al	Alderon LLC presentation	Purification of West Nile Virus DNA plasmid vaccine using Fractogel DEAE and RPC
HVJ	2009	Method for purifying virus envelope	S. Ioka	US patent 20090042274	Purification of HVJ by EC, HC on <b>Toyopearl Ether-650M</b> and SEC





# Vaccine for Aujeszkie's Disease

JPH05-246888

Component vaccine

Porcine Herpes 1 virus membrane glycoprotein gIII

(Procedure)

- 1) Solubilization of porcine Herpes 1 virus infected cell
- 2) AIEC to remove membrane glycoprotein gX
  - **Toyopearl DEAE-650** or DEAE-Sepharose CL-6B
- 3) Heparin AFC to purify membrane glycoprotein gIII
  - **Toyopearl AF-Heparin 650** or Heparin Sepharose CL-6B



# Hepatitis B Vaccine (Surface antigen)

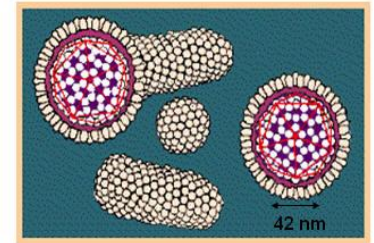
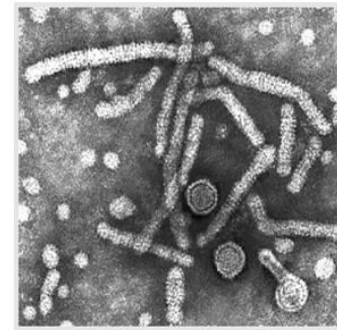
United States Patent 4857317

## HBsAg Structure

HBsAg expressed by yeast

(Procedure)

- 1) PEG400 treatment
- 2) Ultrafiltration
- 3) SEC on Fractogel **TSK HW-55F**
- 4) IEC on Fractogel **TSK DEAE-650M**
- 5) SEC on Fractogel **TSK HW-65F**



~10<sup>4</sup> excess of HBsAg vs. viron

8

CDC



# **Bordetella bronchiseptica(Bb) Toxoid Pasteurella multocida(Pm) Toxoid Mixed Vaccine**

P2006-342175A

Animal vaccine

(Procedure)

1) Cellulose sulfide

or Blue-dye AFC on **Toyopearl AF-Blue 650M**,

- Cellulofine (GC-15) sulfide or Heparin-AFC
  - Heparin-Sepharose CL-6B, Affi-Gel Heparin, Heparin-Cellulofine or Blue Cellulofine

2) Sulfo-CIEC on **Toyopearl SP-650M**,  
SP-Sephadex

3) SEC on **TSKgel G3000SW**, **Toyopearl HW-75/HW-65**





# New Adjuvant Composition; Allergen Derf 2 Vaccine

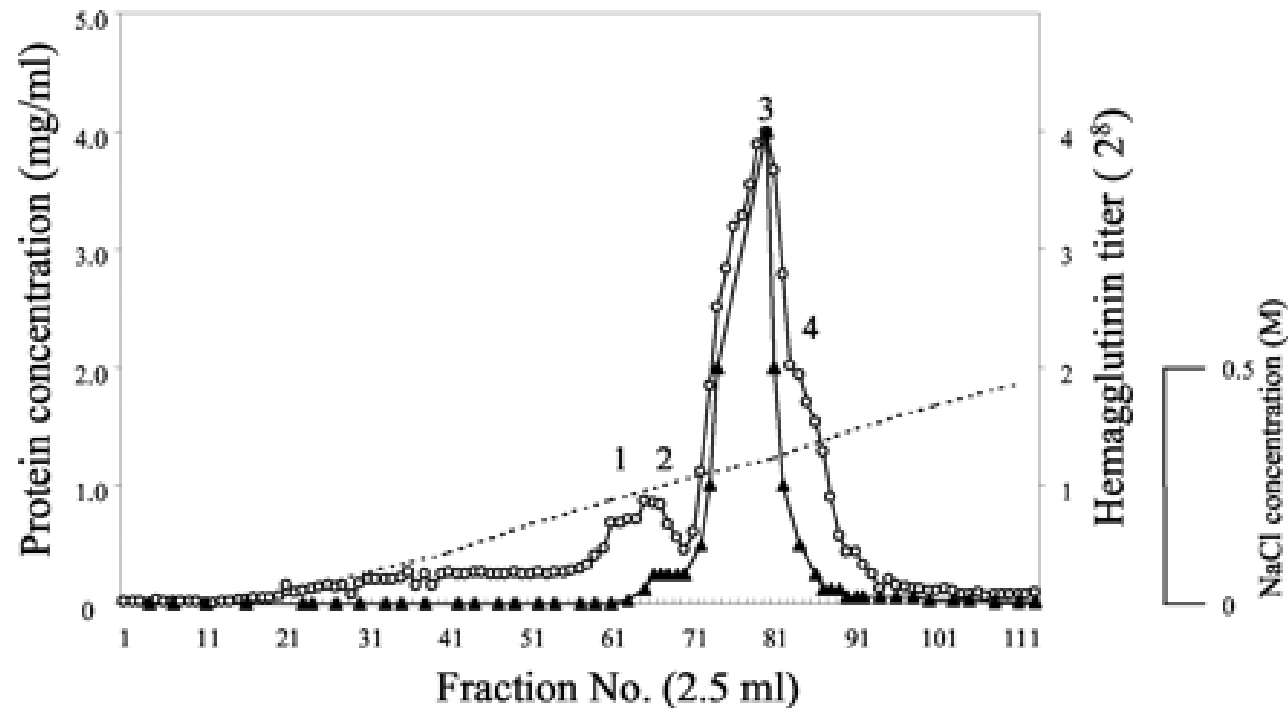
WO2009/057763



To provide a vaccine adjuvant composition which is to be used in combination with an allergen vaccine, an infection vaccine, a mucosal vaccine or a tumor vaccine. An adjuvant composition which is to be used in combination with an allergen vaccine, an infection vaccine, a mucosal vaccine or a tumor vaccine containing hemozoin or  $\beta$ -hematin; and a vaccine composition which contains the above-described adjuvant composition together with an allergen vaccine, an infection vaccine, a mucosal vaccine or a tumor vaccine.

- 1) Silkworm body fluids Supernatant
- 2) CIEC on **Toyopearl SP-550C**
- 3) AIEC on Q Sepharose Fast Flow

# Purification of Fully Activated *Clostridium botulinum*

## Serotype B Toxin for Treatment of Patients with Dystonia



Separation of progenitor toxins by **SP-Toyopearl 650 M** cation-exchange column chromatography. An ammonium sulfate-precipitated preparation treated with protamine was applied to the column and eluted with an NaCl gradient. The HA activities of some fractions were determined. , protein; , HA titer.



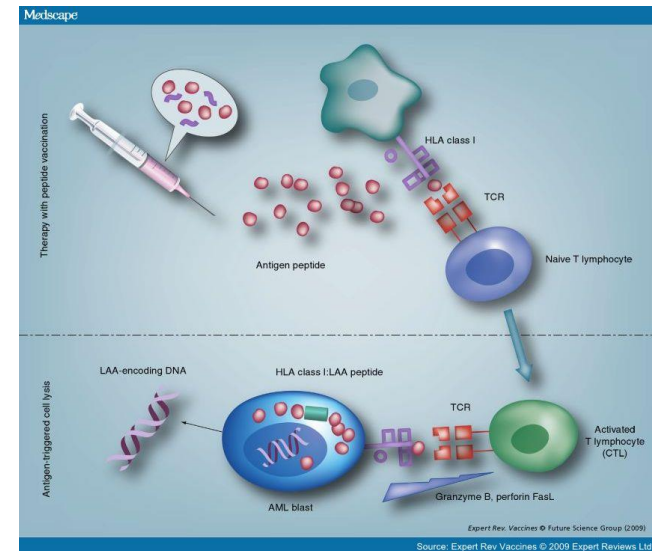
# Peptide Vaccine; Recombinant Protective Polypeptide Antigen(rPPA)

P2000-279179A

Porcine vaccine

(Procedure)

- 1) Butilus vrevisCulture media
- 2) AIEC on **Toyopearl DEAE-650M**
- 3) Ultracentrifugation
  - Millipore membrane (MW 10,000 cut)
- 4) Purity; 80%, Recovery; 45%





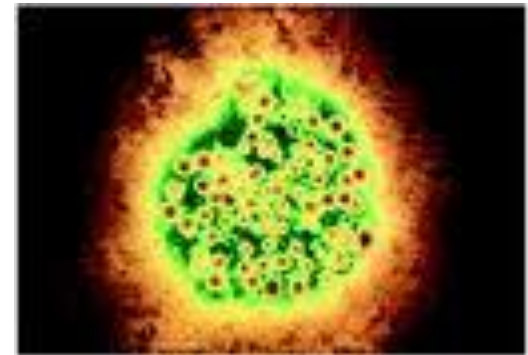
# Vaccine for Hepatitis A Virus (HAV)

JPH07-48277

Production of HAV over 95% purity

(Procedure)

- 1) Concentration by AIEC on **Toyopearl DEAE-650M**
  - Removal of non-HAV specific proteins and host DNA
- 2) Purification by SEC on **Toyopearl HW-65S + HW-55S**
- 3) Inactivation of HAV





# Rabies Virus Vaccine

US patent 20100260798

(Procedure)

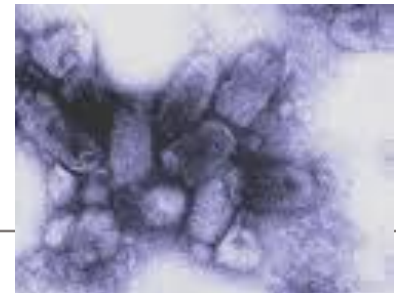
- Cation-Exchanger Fractogel EMD SO3-

- Except for the chromatography carried out on a Fractogel® EMD SO3- support where the amount of virus found in the filtrate was about 5% of the total amount of virus injected in the chromatography column, the amounts of virus found in the filtrates of the other chromatography carried out on the other 3 supports (Capto™ S, SP Sepharose XL and Toyopearl® SP 650) were between 80 and 99% of the total amount injected. Accordingly, the harvested virus yields were very low (<10%).
- Therefore, the structure of the ligands as well as the matrix play an important role since among all the **strong cation exchanger chromatographic supports tested only the chromatographic support with sulfoisobutyl ligands grafted onto a polymethacrylate matrix gave good results.**

- Ultrafiltration (UF)

- Benzonase treatment

•**Toyopearl GigaCap S-650M** may be applicable as Fractogel EMD SO3- as grafted ion-exchanger.





# Vaccinia Virus (Small Pox)

P2010-507827

Vaccinia virus particles

(Procedure)

- Prepurified and concentrated viruses
- AFC on **Toyopearl AF-Heparin-650M**  
(via surface protein A27 of virus)
  - Washing with PBS, 0.1 and 0.15 mol/L NaCl
  - Gradient elution of NaCl from 0.15 to 2.0 mol/L in PB (pH 7.2)
- Recovery of virus; 70-90%
- Purity; DNA 60 % removed





# Adenovirus (Ad5)

US 2001-6194191

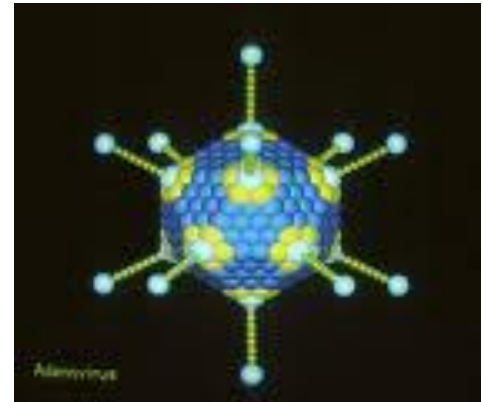
Method of the production and purification of adenoviral vectors

(Procedure)

1) Single step purification by IEC on

**Toyopearl SuperQ-650M**

- In addition to the strong anionic ion exchange chromatography, other modes of chromatographic methods, were also evaluated for the purification of AdCMVp53 virus (e.g. size exclusion chromatography, hydrophobic interaction chromatography, cation exchange chromatography, or metal ion affinity chromatography). Compared to the Toyopearl Super Q, all those modes of purification offered much less efficient purification with low product recovery. Therefore, Toyopearl Super Q resin is recommended for the purification of AdCMVp53.





# Conditions on Preparative IEC for Adenovirus Ad5

Resin	Buffer	pH	Concentration of NaCl (mol/L)	
			Loading	Elution
TOYOPEARL SuperQ-650M	0.02 mol/L Tris-HCl	9.0	0.3	2.0
Fractogel DEAE-650M	0.05 mol/L HEPES	7.5	0.3	0.6
Fractogel DEAE-650M	0.05 mol/L PBS	7.5	0.3	0.6
SOURCE 1Q	0.05 mol/L Tris-HCl	8.0	0.0	0.75
Q Sepharose XL	0.05 mol/L Tris-HCl	8.0	0.0	1.0

Data was modified from J. Chromatogr., 1142 (2007) 2



# Hemagglutinating Virus of Japan (HVJ)

US 20090042274

Production of HVJ at higher recovery

(Procedure)

1) AIEC

2) HIC on **Toyopearl Ether-650M**

- Elution on Phenyl or Butyl HIC resin is too hydrophobic for adsorption and elution. (Mild HIC resin is required.)

3) SEC

