

VIROLOGIA

Viral vaccines

VACCINATION:
OUR PROVEN BEST DEFENSE AGAINST
VIRUSES

the vaccine concept

- Protects a recipient from a pathogenic agent
- Establishes an immunological resistance to an infection (immune memory)
- Breaks the chain of transmission

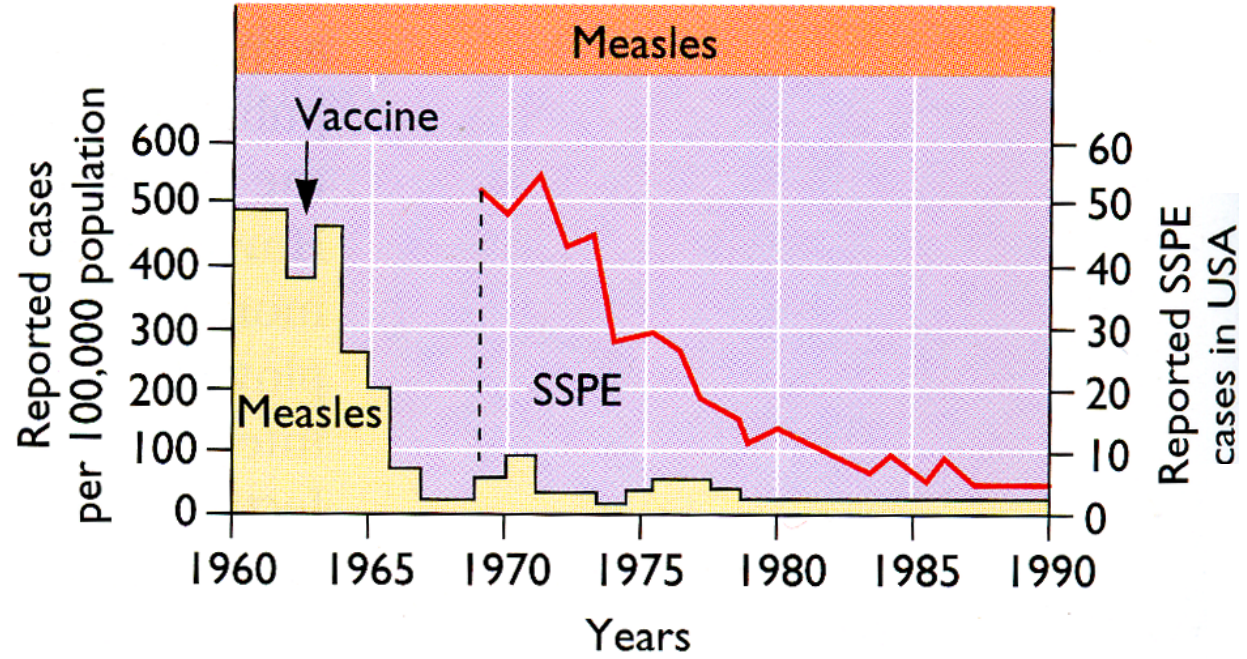
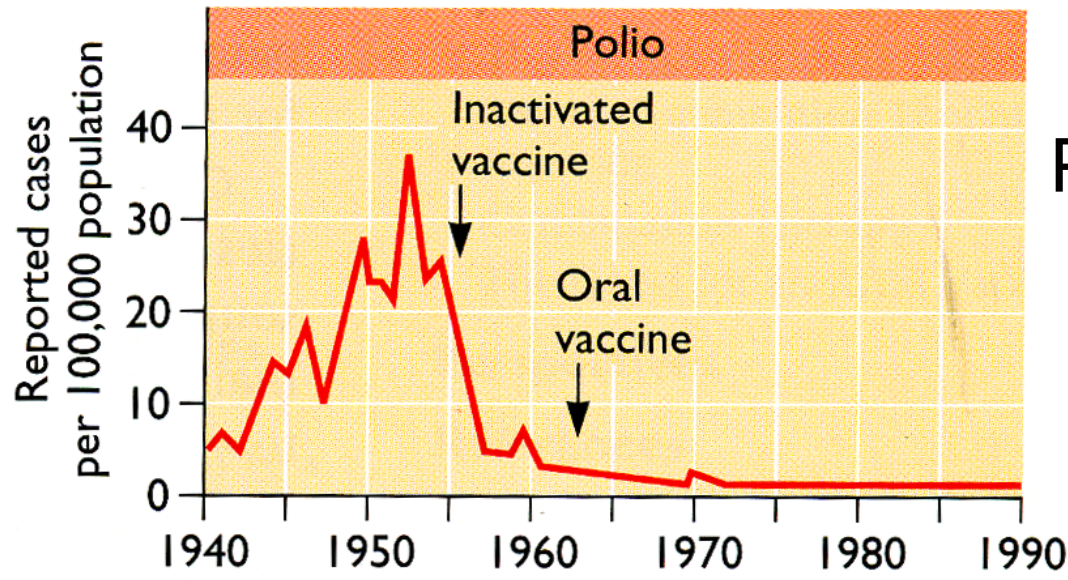
VACCINES:

the proven best defense against viruses

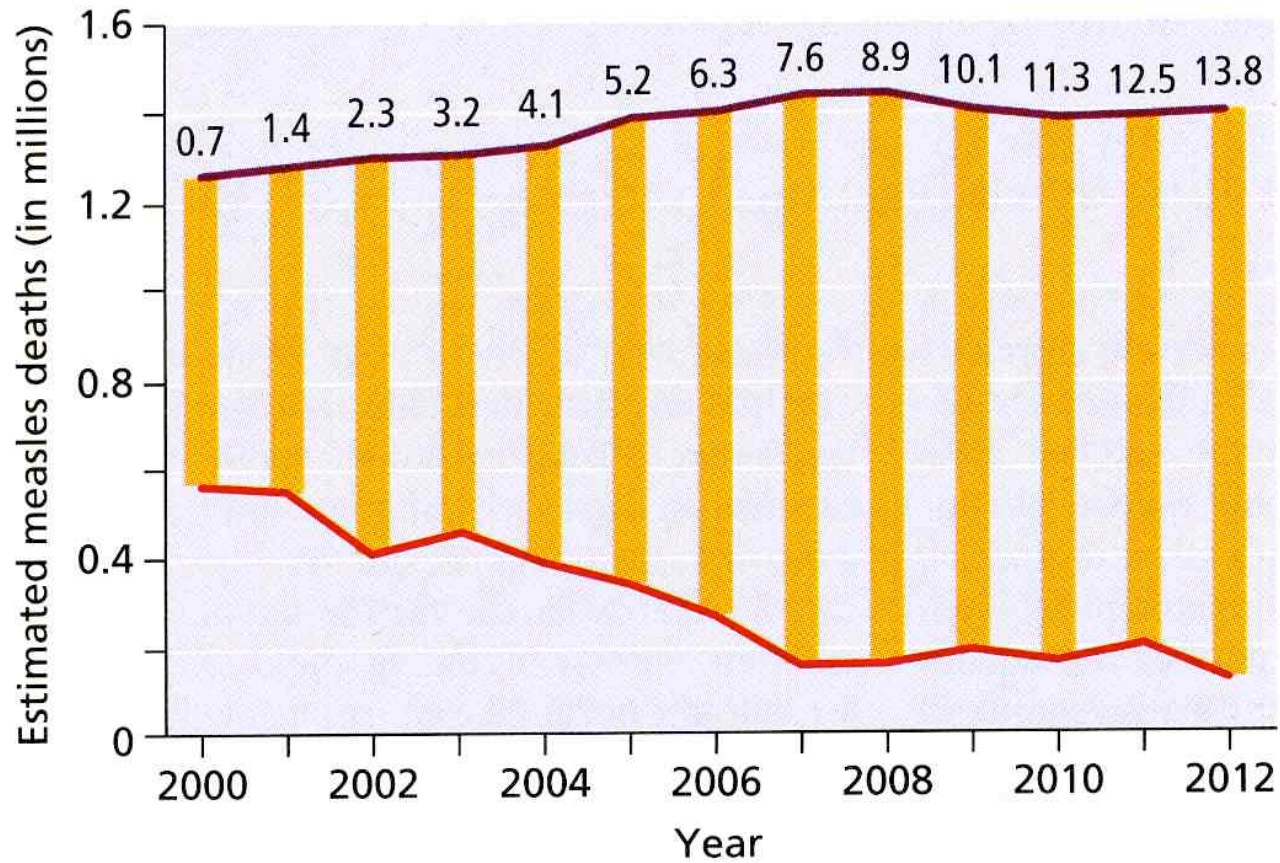
Impact of antiviral vaccines on diseases frequency:
pre-vaccination era vs. 1998 (USA)

Disease	Dates	Annual reported cases:		Decrease (%)
		Pre-vaccination	Post-vaccination (1998)	
Smallpox	1900–04	48,164	0	100
Poliomyelitis (paralysis)	1951–54	1,314	1*	100
Measles	1958–62	503,282	89	100
Mumps	1968	152,209	606	99.6
Rubella	1966–68	47,745	345	99.3
Congenital rubella syndrome	1958–62	823	5	99.4

Profiles of successful vaccination campaigns



Decline in worldwide measles deaths due to vaccination



— Estimated measles deaths in absence of vaccination

■ Estimated deaths prevented by measles vaccination

— Estimated measles deaths with vaccination

Milestones in antiviral vaccine development

Pre-1700s	Chinese doctors use powdered smallpox scabs to “immunize” intranasally. Mediterranean-area doctors use directed leishmania-infected sandfly bites to induce long-term protection from reinfection.
1721	Lady Montagu brings concept of variolation (inoculation with pus from recovering smallpox victim) from Turkey to England.
1798	Jenner publishes <i>Variolae Vaccinae</i> , the use of cowpox inoculation to protect against smallpox.
1885	Pasteur and collaborators introduce air-dried rabbit spinal cord as rabies vaccine.
1900	Walter Reed demonstrates that yellow fever is caused by a filterable virus.
1930–45	Introduction of vaccines for Japanese B encephalitis (1930), yellow fever (1935), and influenza (1936).
1946–75	Introduction of vaccines for polioviruses types 1–3 (Sabin attenuated strains and Salk inactivated virus); measles, mumps, and rubella viruses; tick-borne encephalitis virus; mouse brain, duck embryo, and tissue culture vaccines for rabies virus; inactivated influenza A and B viruses; and adenoviruses.
1975–present	Introduction of vaccines for hepatitis B virus, hepatitis A virus, varicella zoster virus (chickenpox), live, cold-adapted influenza virus, rotavirus, and human papillomavirus.

VACCINES:

the proven best defense against viruses

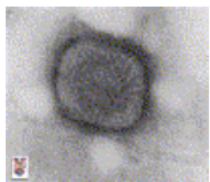
- Development of vaccines
- Eradicating a viral disease: is it possible?
- The eradication of smallpox (1978)
- The eradication of rinderpest virus (2011)
- What make eradication conceivable?
- The **poliovirus** case: should be vaccine eradication be next?

Viral vaccines

Smallpox: a Historical Perspective

Smallpox: an historical perspective

Figure 2



A. Smallpox virus
Copyright
1994 Veterinary Sciences
Division Queen's University Belfast



B. The mummified head of Ramses V (died 1157 BCE) with rash that is probably the result of smallpox



C. Infant with smallpox



D. Smallpox lesions on skin of trunk. Photo taken in Bangladesh.
CDC/James Hicks



E. Powdered smallpox scabs were inhaled to protect against smallpox in Chinese medicine

Figure 3



A. Edward Jenner



B. Dr Jenner about to vaccinate a child



C. Blossom the cow



F. The last known person in the world to have a natural case of smallpox. Variola minor in 23-year-old Ali Maow Maalin, Merka, Somalia
CDC

Table 19.1 Features of smallpox that enabled its eradication

Virology and disease aspects

No secondary hosts; it is a human-only virus

Long incubation period

Infectious only after incubation period

Low communicability

No persistent infection

Subclinical infections are not a source of spread

Easily diagnosed

Immunology

Infection confers long-term immunity

One stable serotype

Effective vaccine available

Vaccine is stable and cheap

Social political aspects

Severe disease with high morbidity and mortality

Considerable savings to developed, nonendemic countries

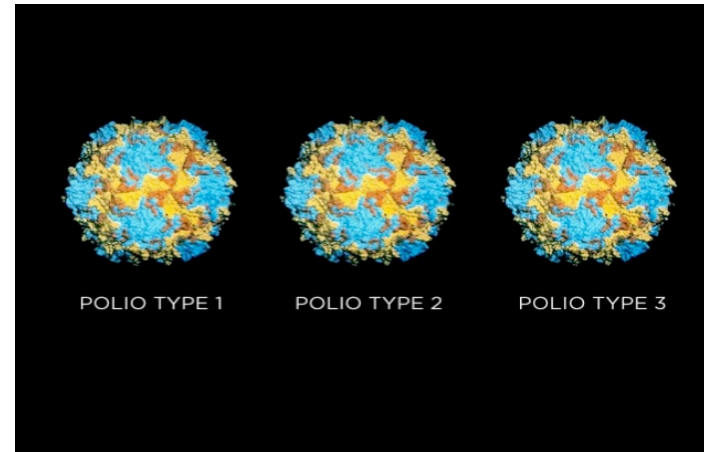
Eradication from developed countries demonstrated its feasibility

Few cultural or social barriers to case tracing and control

Viral vaccines

The poliomyelitis eradication: should vaccine eradication be next?

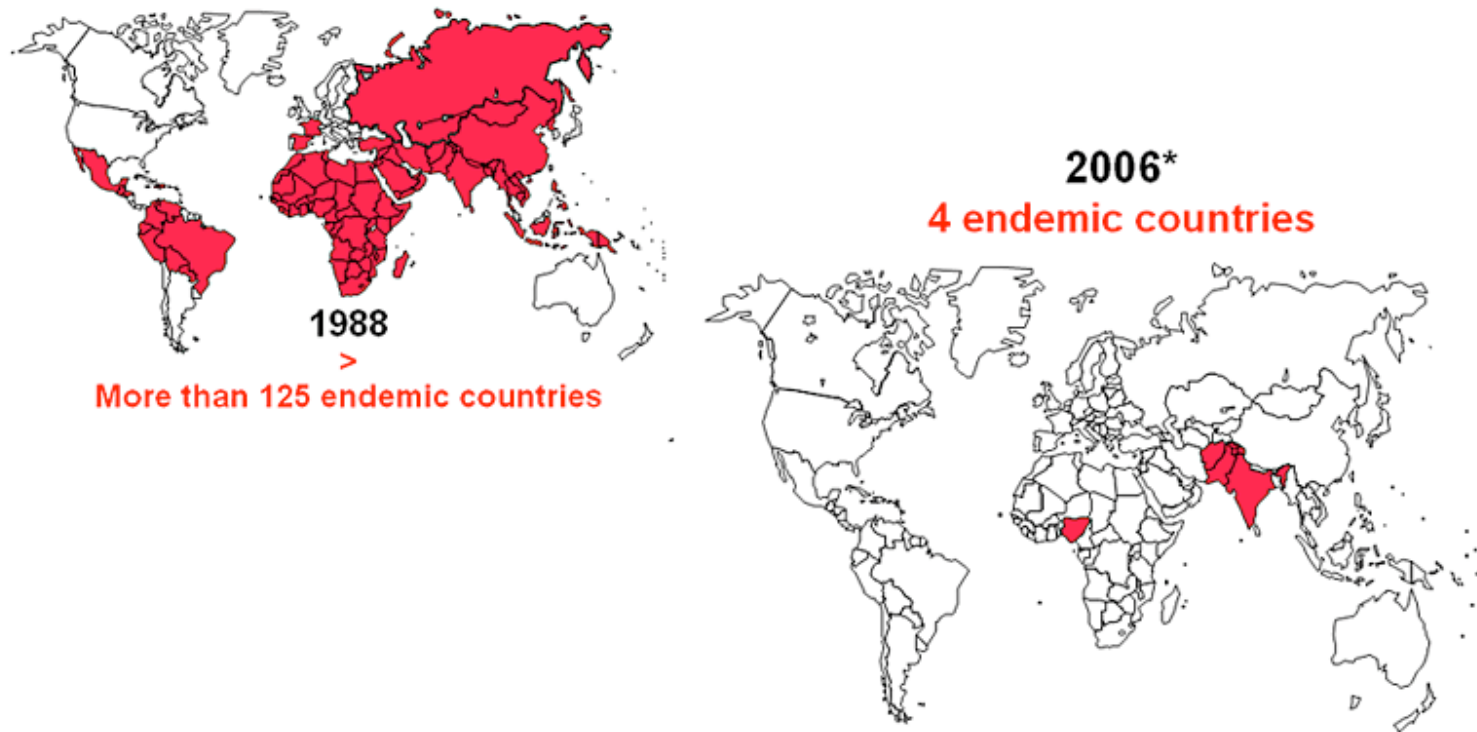
Polio Across the Centuries



THE ROLE OF INACTIVATED POLIO VIRUS (IPV)

“The next step towards a sustainable polio-free world”



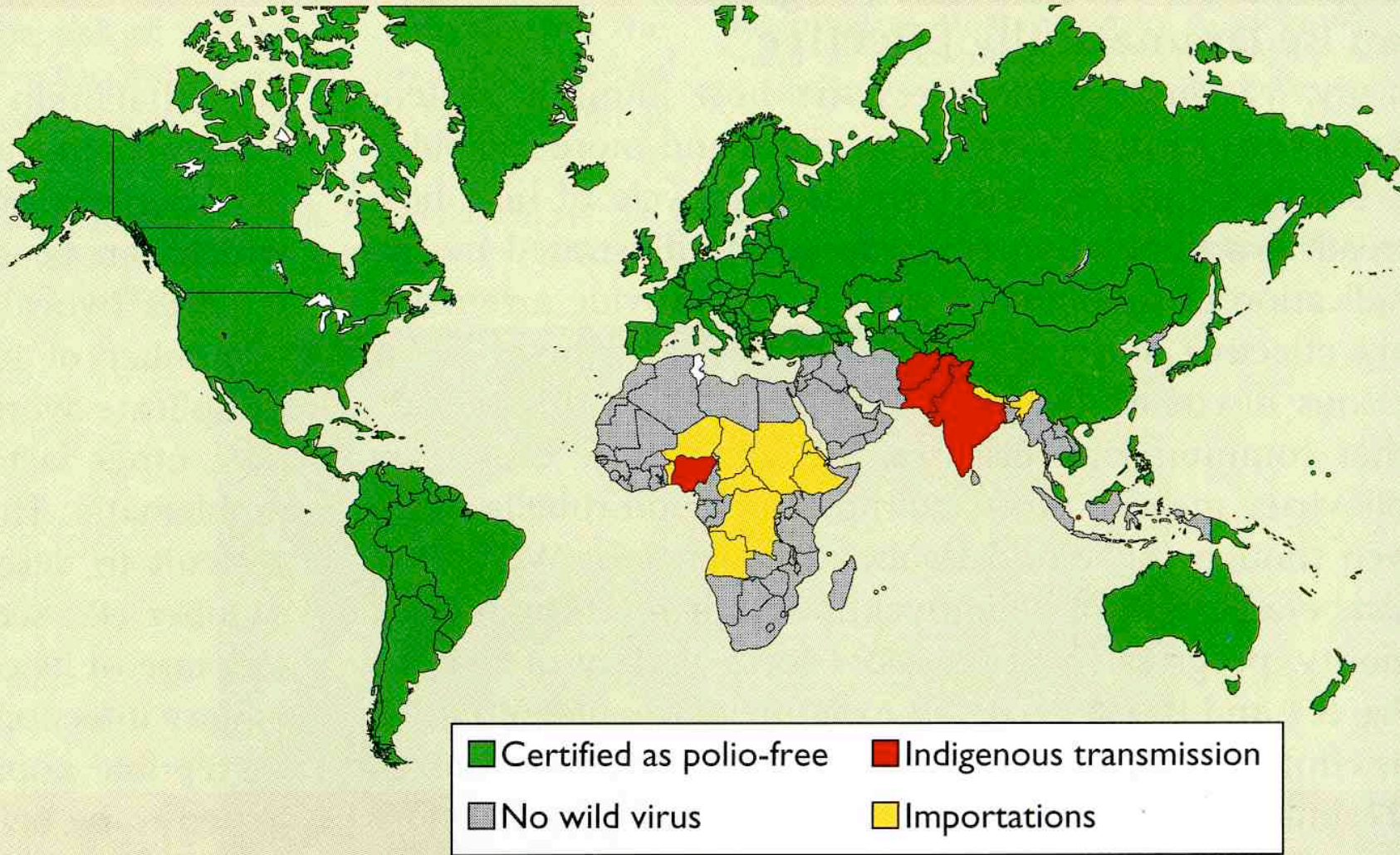


* as of 8 July 2006

Since the momentous launch of the Global Polio Eradication Initiative in 1988 during the World Health Assembly in Geneva, nearly five million children, who otherwise would have been paralyzed and incapacitated by polio, are walking, able and symptoms-free. More than 1.5 million deaths have been prevented.

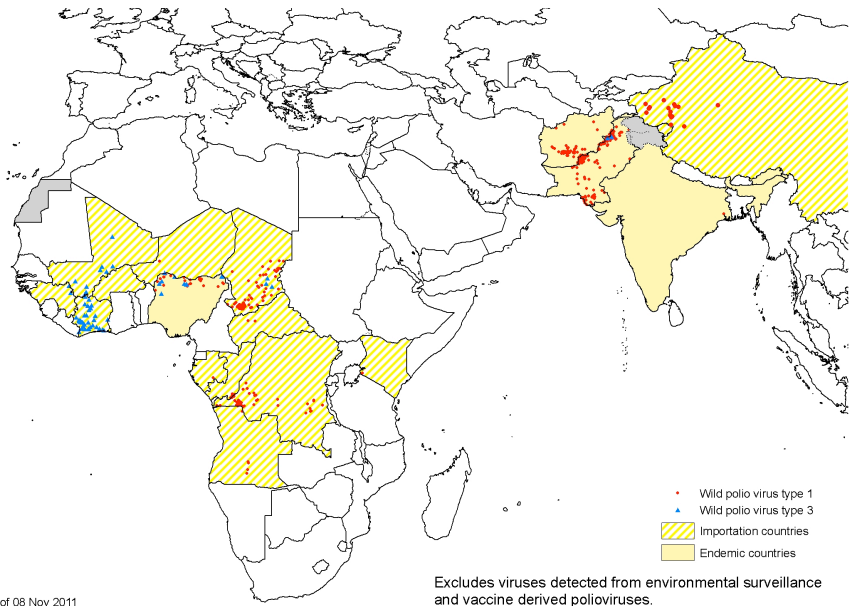
The number of polio cases reported annually has decreased by 99% from 350,000 in 1988 to 2,000 cases in 2006.

Globally reported incidence of poliomyelitis in 2008. The Americas, Western Pacific, and European regions have been declared poliomyelitis free by the WHO. The number of cases has declined from an estimated 350,000 in 1988 to ca. 1,300 in 2008. At the same time, the number of countries in which poliovirus is endemic has decreased from >125 to 4.



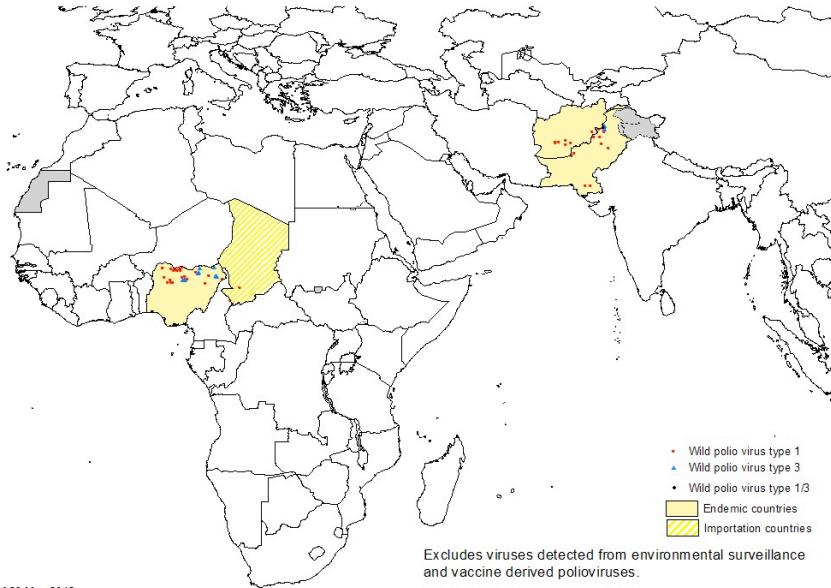


Wild Poliovirus - 2011



Data at HQ as of 08 Nov 2011

Wild Poliovirus - 2012

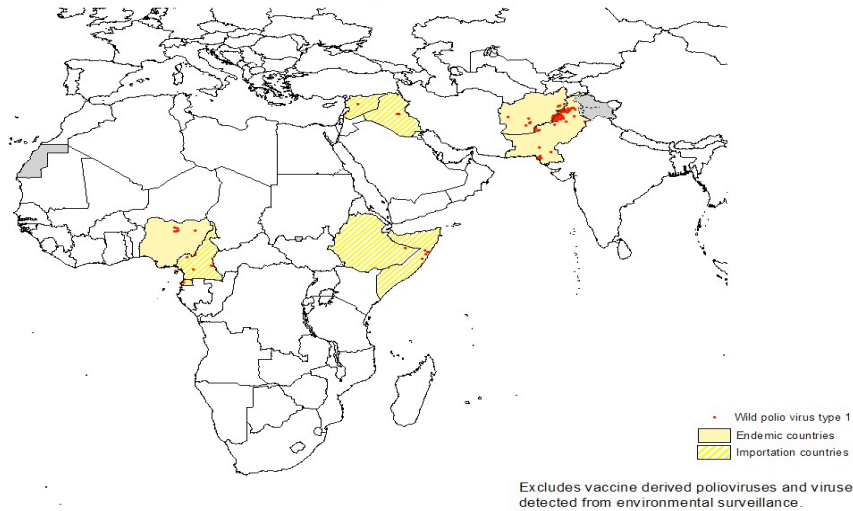


Data at HQ as of 29 May 2012

As 2012, Polio remained endemic in four countries – **Afghanistan, India, Nigeria and Pakistan** – with a further four countries known to have (**Angola, Chad and Democratic Republic of the Congo**) or suspected of having (**Sudan**) re-established transmission of poliovirus. Several more countries had outbreaks in 2010 due to importations of poliovirus.

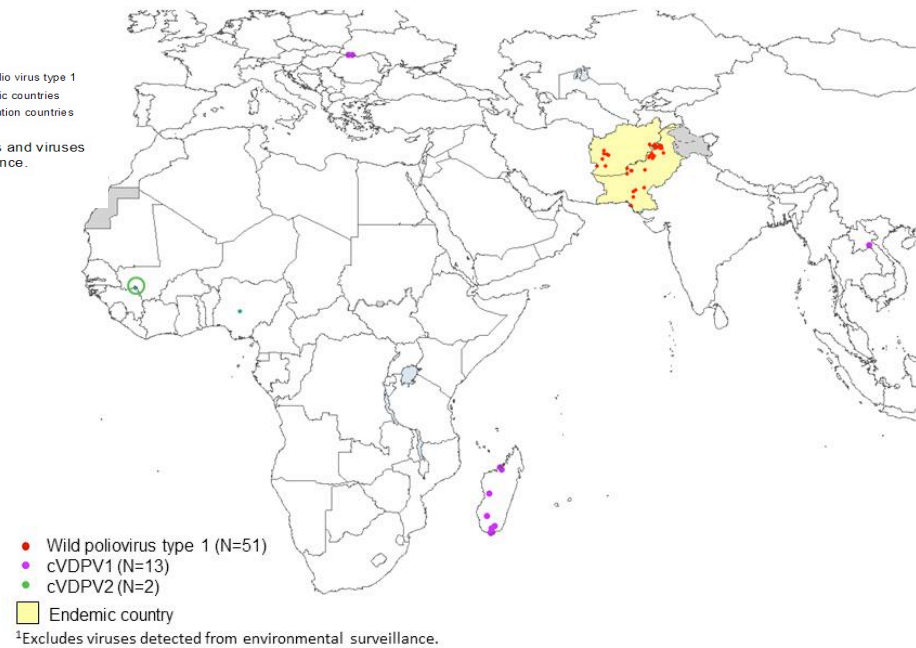


Wild Poliovirus - 2014
01 January - 28 October



Data in HQ as of 28 October 2014

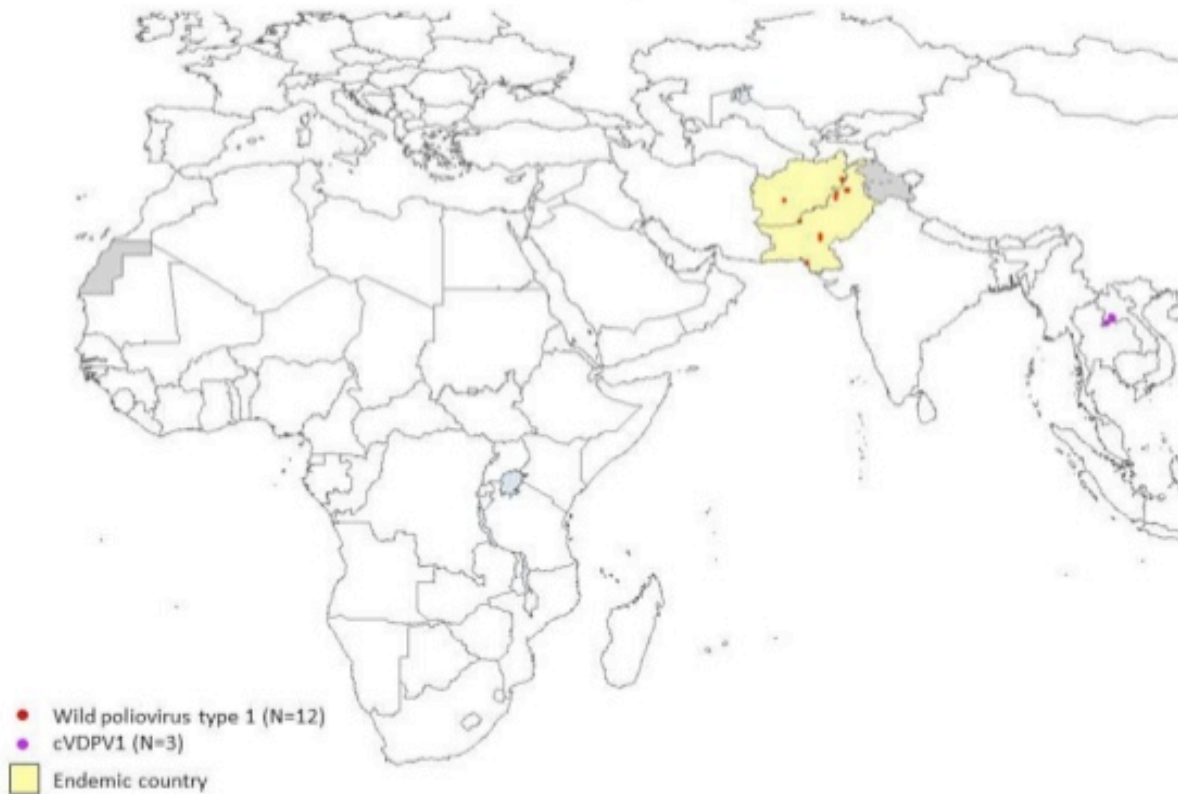
Wild Poliovirus & cVDPV Cases¹, 2015
01 January – 27 October



Data in WHO HQ as of 27 October 2015



Wild Poliovirus & cVDPV Cases¹, 2016
01 January – 26 April




¹Excludes viruses detected from environmental surveillance.

Viral vaccines

Vaccination in Italy

Tabella 3 - Calendario nazionale delle vaccinazioni offerte attivamente a tutta la popolazione

Vaccino	Nascita	3° mese	5° mese	6° mese	11° mese	13° mese	15° mese	5-6 anni	11-18 anni	>65 anni	ogni 10 anni
DTPa		DTPa	DTPa		DTPa			DTPa ¹	dTpa		dT ²
IPV		IPV	IPV		IPV			IPV			
HBV	HBV ³	HBV	HBV		HBV						
Hib		Hib	Hib		Hib						
MPR						MPR		MPR	MPR ⁴		
PCV		PCV	PCV		PCV						
Men C						Men C ⁵			Men C ⁵		
HPV	 <p>Planner Vaccinazioni Nuova App del Ministero Vaccina il tuo bambino e schiaccia il virus!</p>								HPV ⁶ (3 dosi)		
Influenza										Influenza	
Varicella									Var ⁷ (2 dosi)		

In Italia sono obbligatorie per tutti i nuovi nati le vaccinazioni contro difterite, tetano, **poliomielite**, **epatite B**.

Oltre alle quattro vaccinazioni obbligatorie, il Ministero della salute raccomanda le vaccinazioni per prevenire: **pertosse**, **meningite (haemophilus influenzae tipo B, pneumococco, meningococco C)**, **morbillo**, **parotite**, **rosolia**, **varicella**, **infezione da papillomavirus**.

DTPa: vaccino antidifterite-tetano-pertosse acellulare

Hib: vaccino contro le infezioni invasive da *Haemophilus influenzae* tipo b

HPV: vaccino antipapilloma virus umano

dTpa: vaccino antidifterite-tetano-pertosse per adulti

PCV: vaccino antipneumococcico coniugato eptavalente

Varicella: vaccino antivariella

IPV: vaccino antipolio inattivato

HBV: vaccino antiepatite B

MPR: vaccino anti morbillo-parotite-rosolia

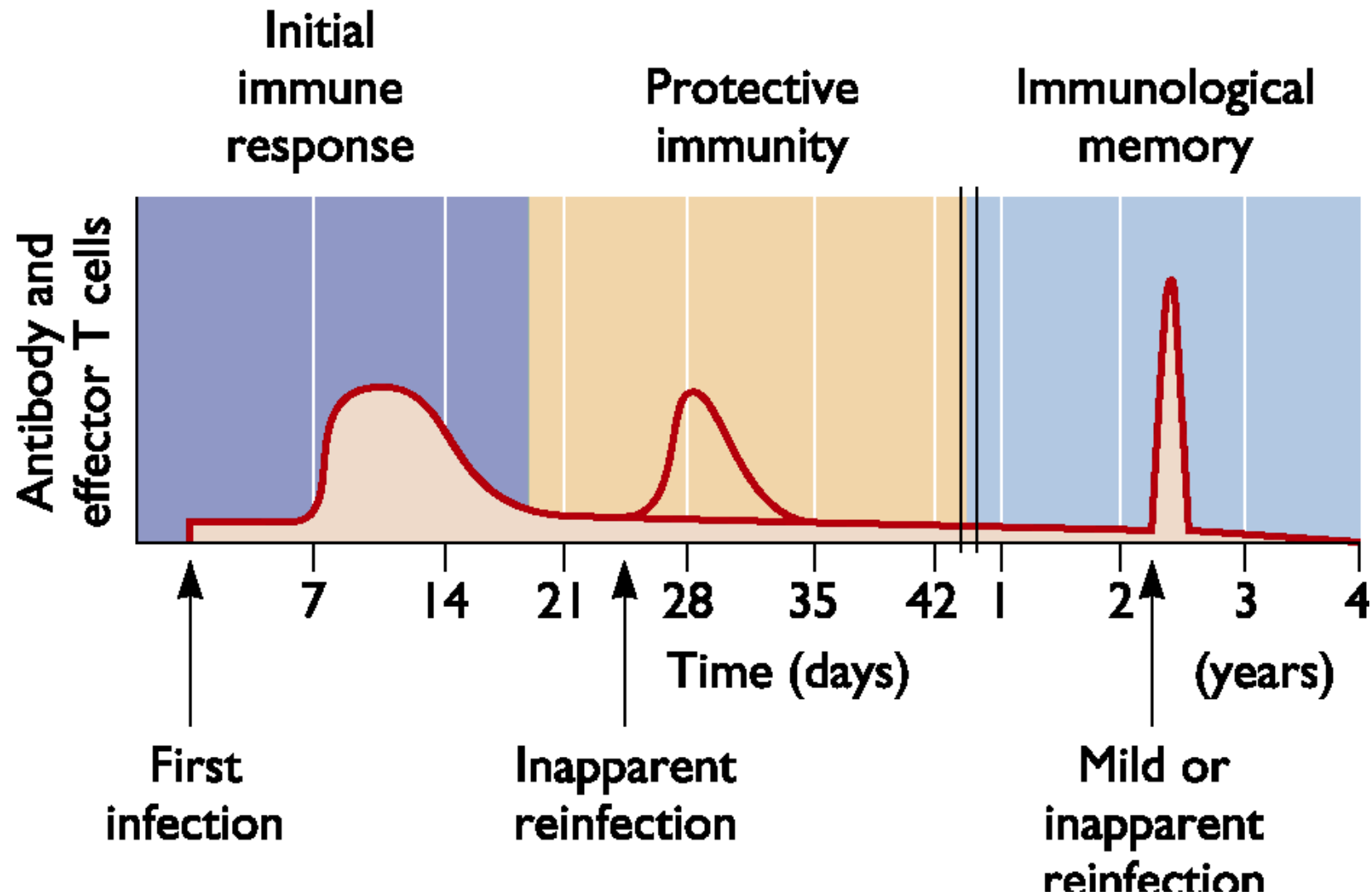
Influenza: vaccino antiinfluenza

MenC: vaccino antimeningococco C coniugato

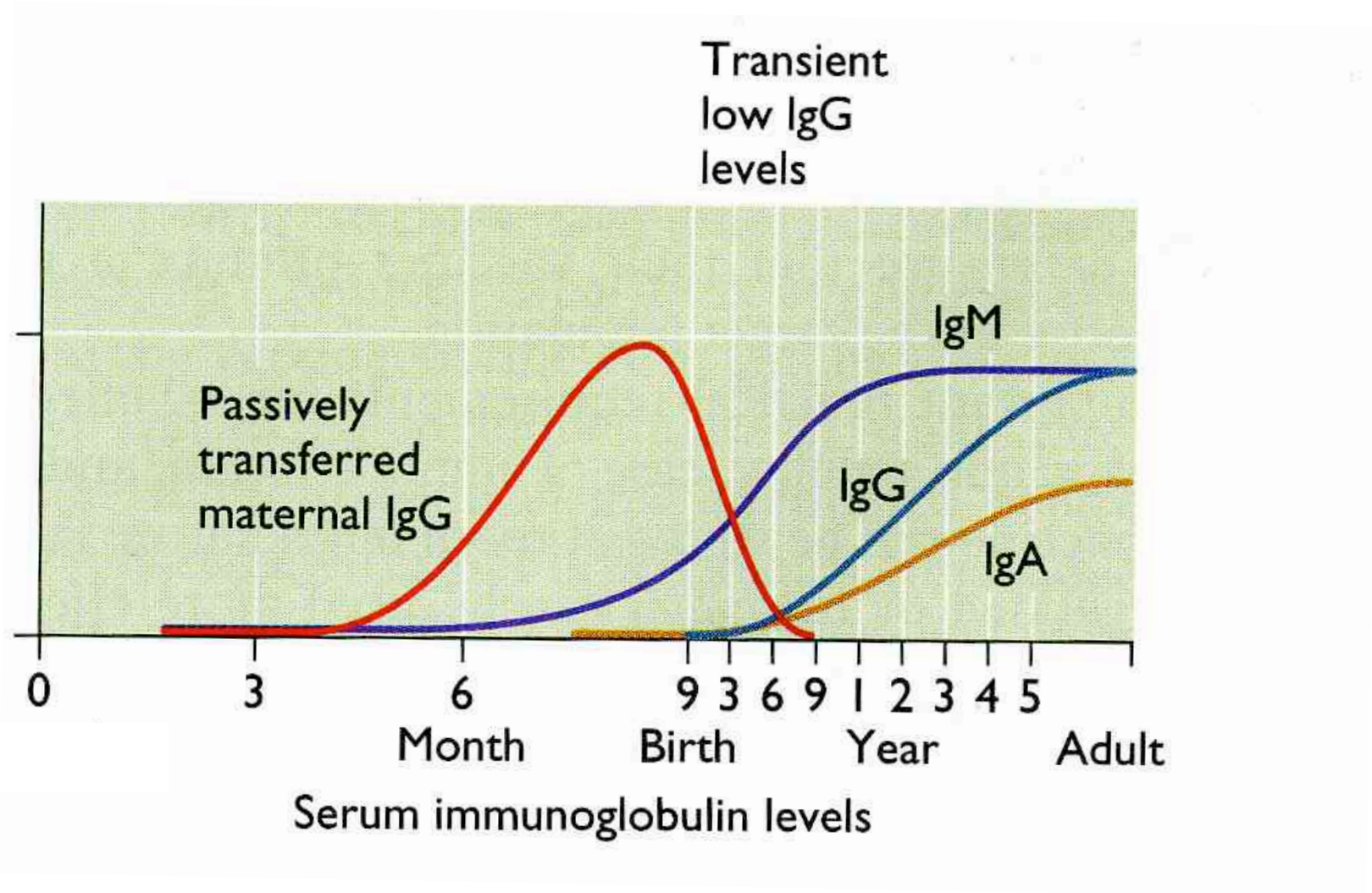
Viral vaccines

Vaccine Basics

VACCINATION: basis for protective immunity



NATURAL PASSIVE IMMUNIZATION: transfer of antibody from mother to infant

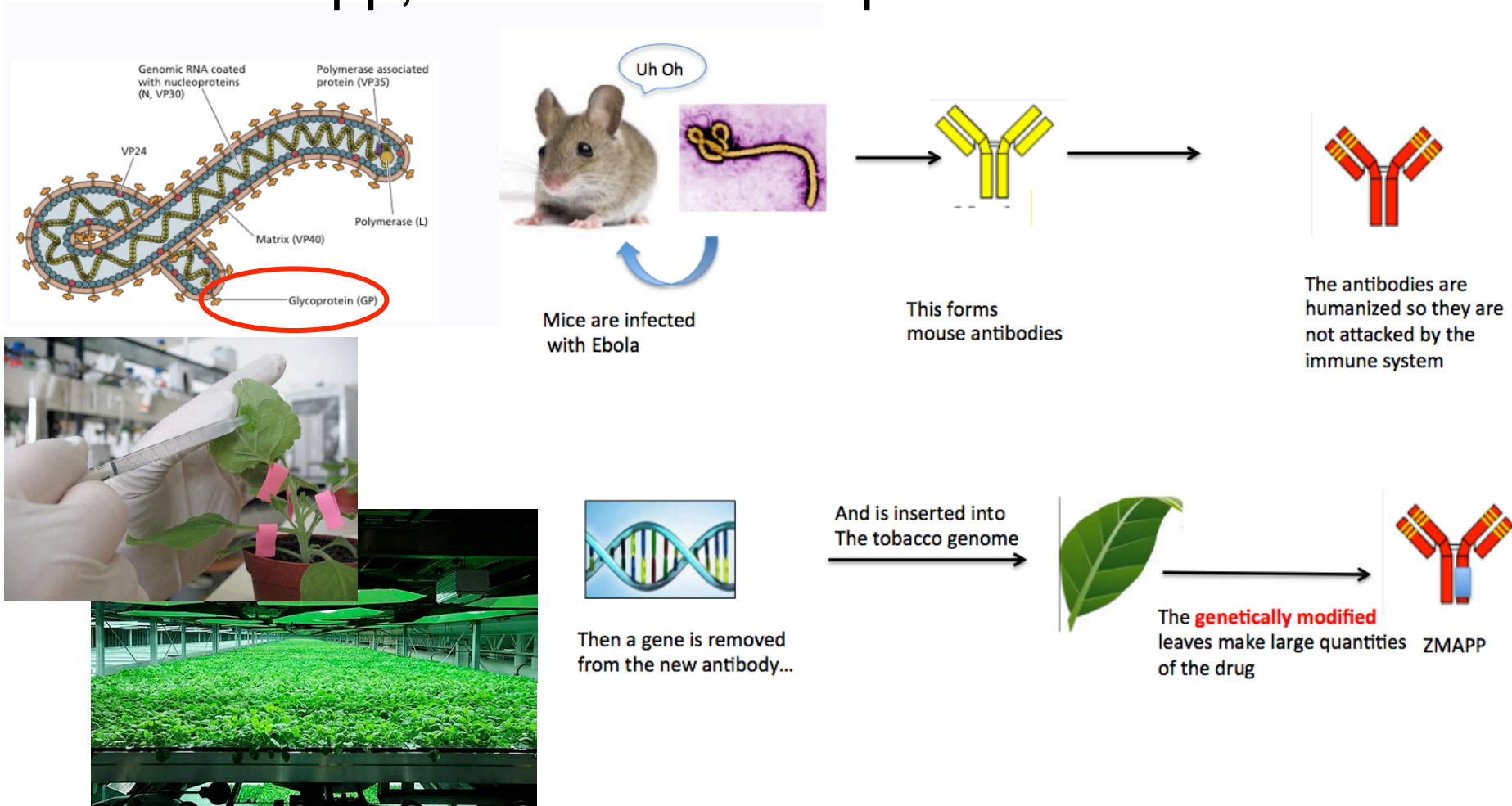


VACCINATION:

vaccines can be *active* or *passive*

- **Active** – instilling into the recipient a modified form of the pathogen or material derived from it that induces immunity to disease (long term protection).
- **Passive** – instilling the products of the immune response (antibodies or immune cells) into the recipient (short term protection)

Zmapp, the best known passive vaccine



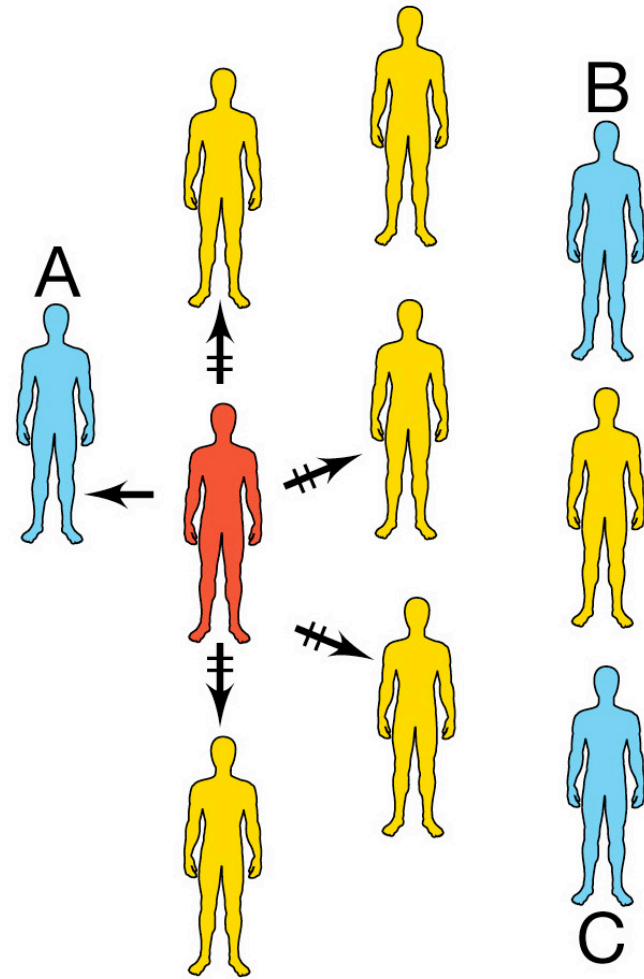
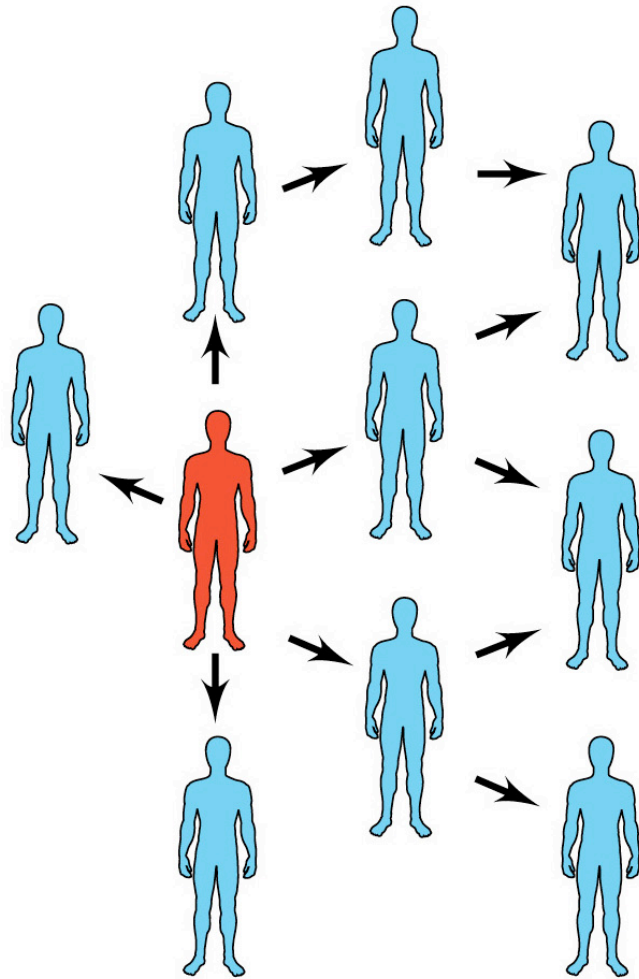
- Raised in mice immunized with virus-like particles
- Chimerized into human IgG1 scaffold
- Produced in tobacco plants

VACCINATION:

requirements for an effective vaccine

Safety	The vaccine must not cause disease
Side effects must be minimal	
Induction of protective immune response	Vaccinated individuals must be protected from illness due to pathogen Proper innate, cellular, and humoral responses must be evoked by vaccine
Practical issues	Cost per dose must not be prohibitive The vaccine should be biologically stable (no genetic reversion to virulence; able to survive use and storage in different surroundings) The vaccine should be easy to administer (oral delivery preferred to needles) The public must see more benefit than risk

THE HERD IMMUNITY CONCEPT



Herd immunity threshold (HT): $1 - 1/R_0$

R_0 reproduction number

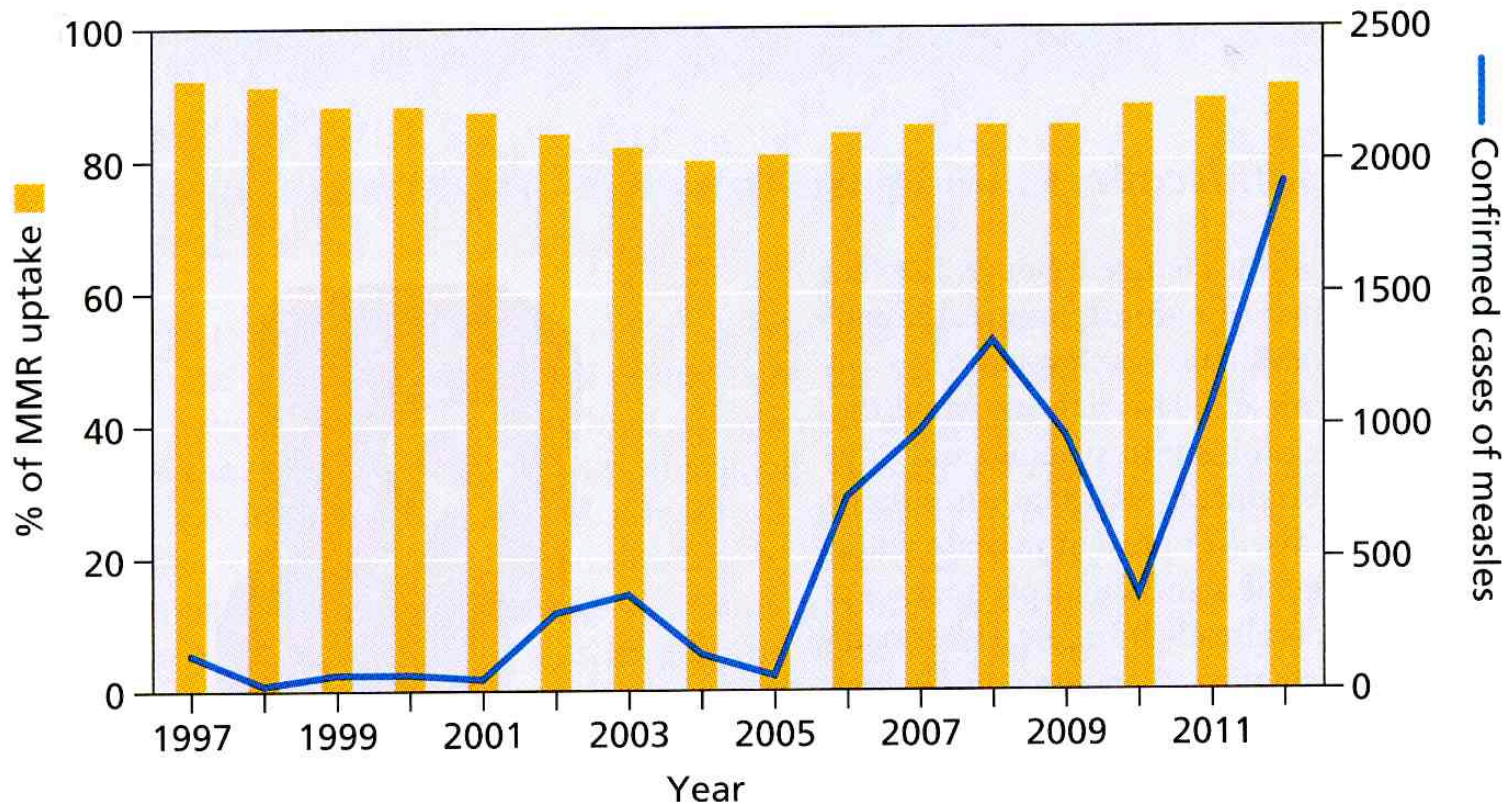
R_0 smallpox: 5-7 HT: 80-85%

R_0 measles: 12-18 HT: 93-95%

THE HERD IMMUNITY CONCEPT

The correlation between herd immunity and the potential for outbreaks.

As the % of vaccinated individuals dip below 90%, a corresponding rise in the number of acute cases is observed



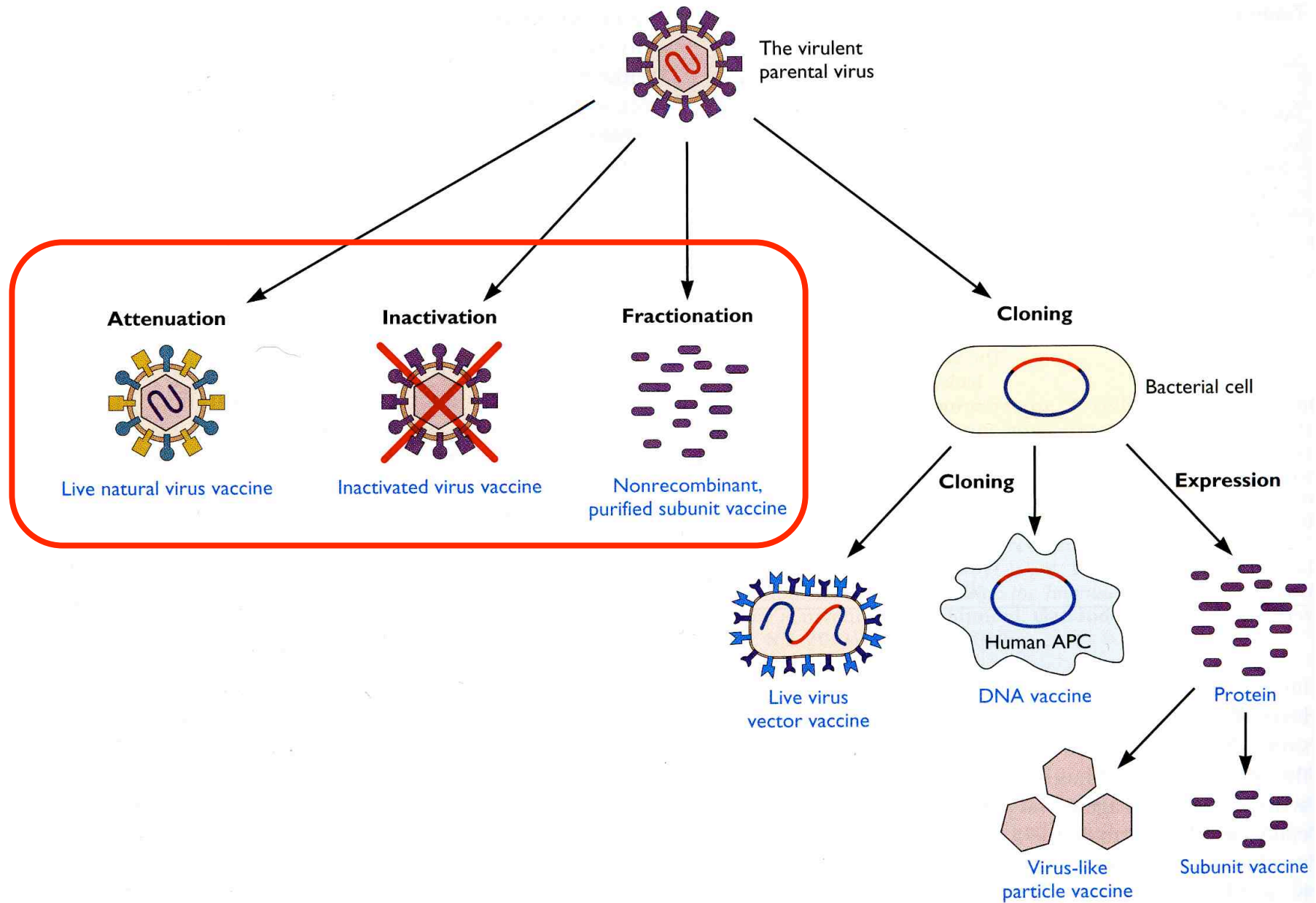
When 80% of population is immunized with measles, 76% of population is immune

VACCINATION:

viral vaccines in the USA

Disease or virus	Type of vaccine	Indications for use	Schedule
Adenovirus	Live attenuated, oral	Military recruits	One dose
Hepatitis A	Inactivated whole virus	Travellers, other high-risk groups	0, 1, and 6 mo
Hepatitis B	Yeast-produced recombinant surface protein	Universal in children, exposure to blood, sexual promiscuity	0, 1, 6, and 12 mo
Influenza	Inactivated viral subunits	Elderly and other high-risk groups	One dose seasonally
Influenza	Live attenuated	Children 2–8 yr old, not previously vaccinated with influenza vaccine	Two doses at least 1 mo apart
		Children 2–8 yr old, previously vaccinated with influenza vaccine	One dose
		Children, adolescents, and adults 9–49 yr old	One dose
Japanese encephalitis	Inactivated whole virus	Travelers to or inhabitants of high-risk areas in Asia	0, 7, and 30 days
Measles	Live attenuated	Universal vaccination of infants	12 mo of age; 2nd dose, 6 to 12 yr of age
Mumps	Live attenuated	Universal vaccination of infants	Same as measles, given as MMR
Papilloma (human)	Yeast- or SF9-produced virus-like particles	Females 9–26 yr old	Three doses
Rotavirus	Live reassortant	Healthy infants	2, 3, and 6 mo or 2 and 4 mo of age depending on vaccine
Rubella	Live attenuated	Universal vaccination of infants	Same as measles, given as MMR
Polio (inactivated)	Inactivated whole viruses of types 1, 2, and 3	Changing: commonly used for immunosuppressed where live vaccine cannot be used	2, 4, and 12–18 mo of age, then 4 to 6 yr of age
Polio (live)	Live, attenuated, oral mixture of types 1, 2, and 3	Universal vaccination; no longer used in United States	2, 4, and 6–18 mo of age
Rabies	Inactivated whole virus	Exposure to rabies, actual or prospective	0, 3, 7, 14, and 28 days postexposure
Smallpox	Live vaccinia virus	Certain laboratory workers	One dose
Varicella	Live attenuated	Universal vaccination of infants	12 to 18 mo of age
Varicella-zoster	Live attenuated	Adults 60 yr old and older	One dose
Yellow fever	Live attenuated	Travel to areas where infection is common	One dose every 10 yr

VACCINATION: **how to make a vaccine?**



VACCINATION: **traditional vaccines**

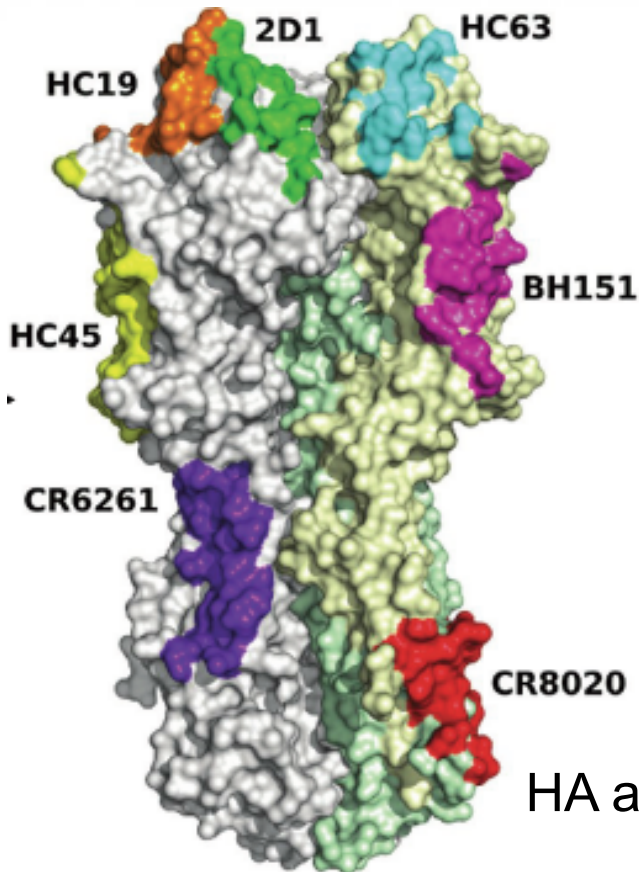
Traditional vaccines are of two types:

- **Inactivated** (killed) - (whole cells, viruses, inactivated toxin proteins (toxoids))
- **Attenuated** (live) –viral or bacterial strain

VACCINATION:

commonly used traditional antiviral vaccines

- **Live viral vaccines**
 - Polio
 - Mumps
 - Measles
 - Rubella
 - Yellow fever
 - Varicella
- **Inactivated viral vaccines**
 - Influenza
 - Polio
 - Rabies

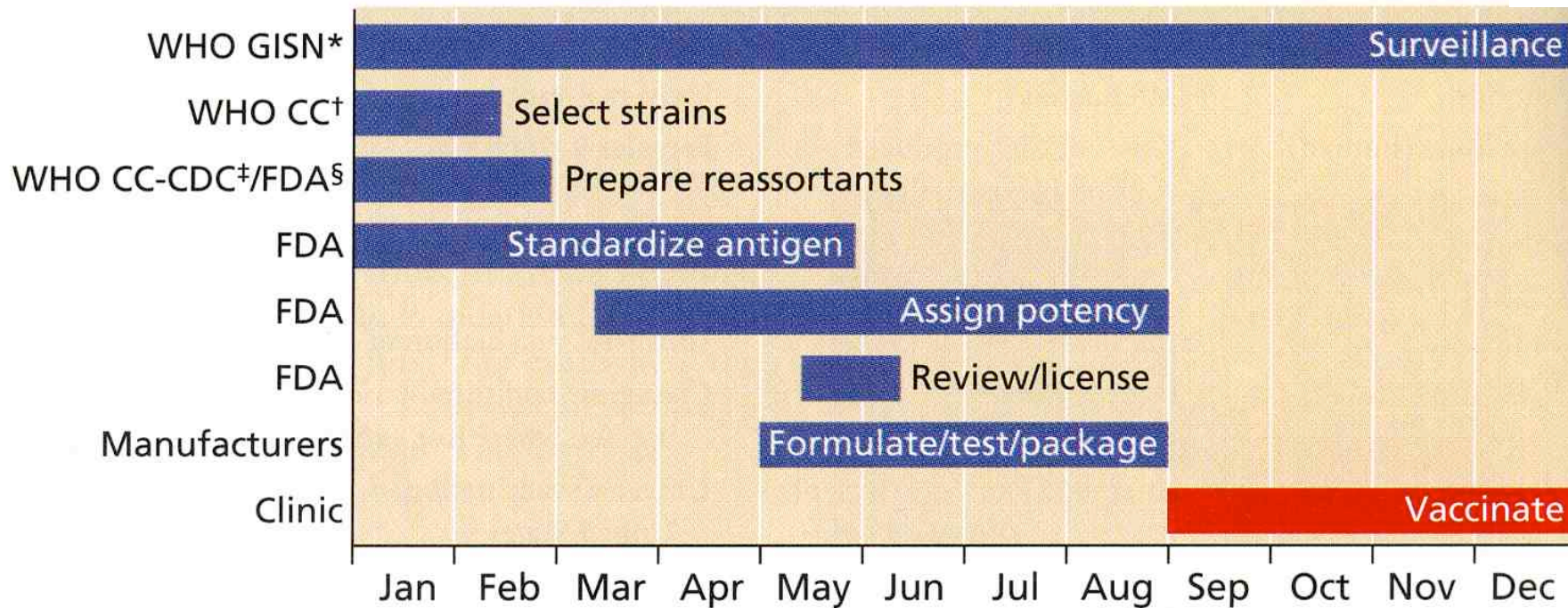


HA antigenic drift: influenza virus

Inactivated influenza vaccine

- >7000 deaths/yr in Italy due to influenza virus
- Vaccine: virus grown in embryonated chicken eggs, formalin-inactivated or detergent or chemically disrupted virions.
- Hundreds million of doses manufactured each year.
- 60% effective in healthy children and adults <65 yr.
- Protection correlates with antibodies to HA and NA.
- Envelope proteins change each year; new strains must be selected in the first months for manufacture.
- Use reassortants with most RNA segments from high-yielding strain, HA, NA from selected strain.

Annual timeline for creating an influenza virus vaccine



*World Health Organization Global Influenza Surveillance Network

†WHO Collaborating Centres

‡US Centers for Disease Control and Prevention

§US Food and Drug Administration





NIC - MIPI

Istituto Superiore di Sanità

SORVEGLIANZA VIROLOGICA

dell'INFLUENZA



26 febbraio 2016

Raccomandazioni dell'OMS per la composizione del vaccino antinfluenzale per la stagione 2016-2017 (Emisfero Nord)

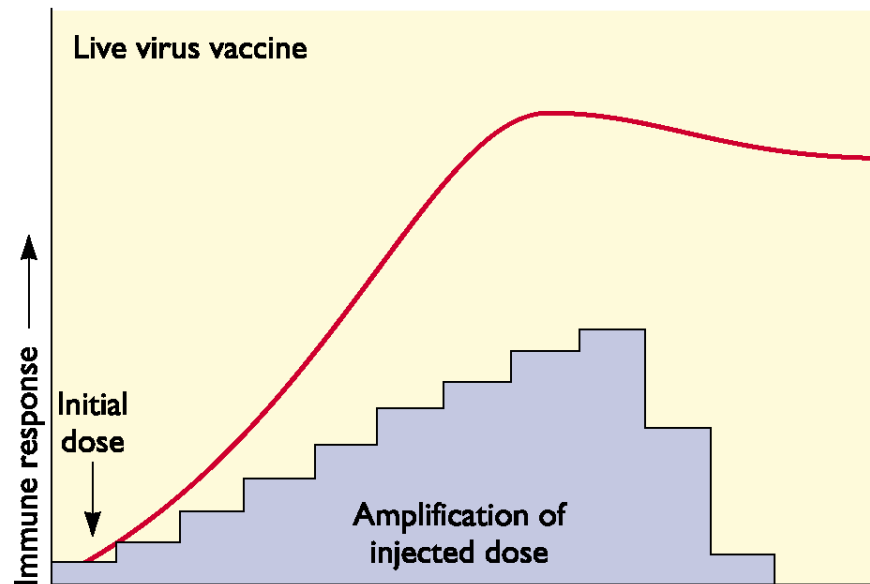
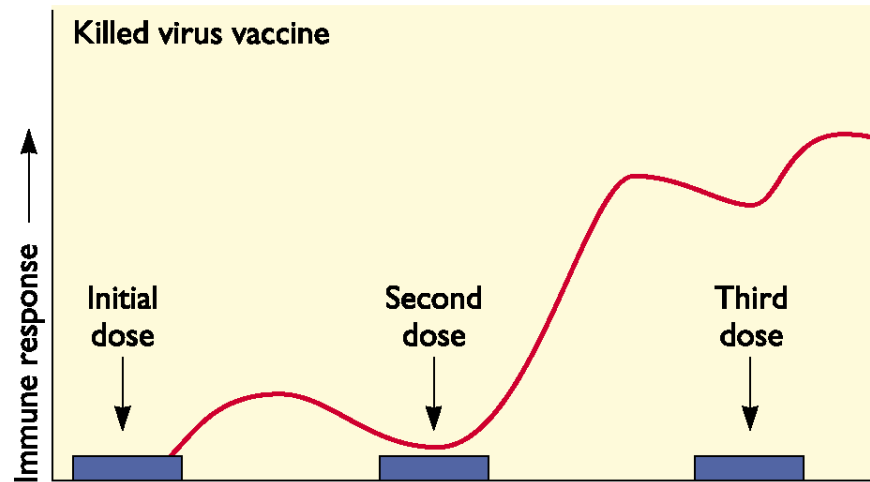
Dal 22 al 24 febbraio 2016 si è svolto a Ginevra il *meeting* annuale dell'OMS per l'aggiornamento della composizione del vaccino antinfluenzale per la stagione 2016/2017.

Le raccomandazioni emanate sono il risultato dei dati di sorveglianza virologica forniti da tutti i Centri Nazionali di riferimento (NIC), afferenti alla rete internazionale dell'OMS, attualmente composta da oltre 140 laboratori.

Qui di seguito viene riportata la nuova composizione vaccinale 2016/2017:

A/California/7/2009 (H1N1)	Presente anche nel vaccino 2015/2016
A/Hong Kong/4801/2014 (H3N2)	Nuova variante
B/Brisbane/60/2008 (lineaggio B/Victoria)	Nuova variante

VACCINATION: comparison of the predicted immune response to live and killed viruses used as vaccines



Comparison of immune responses
to live and killed viruses

VACCINATION:

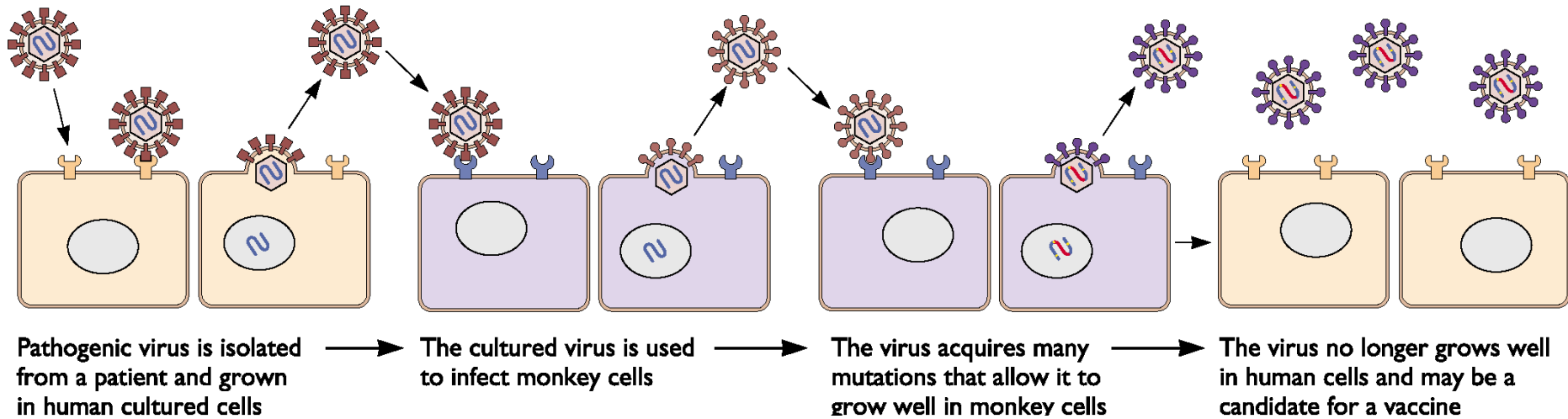
problems with traditional vaccines

- Disadvantages of **inactivated** vaccines:
 - they themselves can cause severe reactions.
 - the organism or the toxin may not be completely killed or inactivated.
 - in some cases producing a sufficient quantity of infectious agent is extremely costly or even impossible (HBV, HPV)

Severe adverse events associated with antiviral vaccine administration

Vaccine implicated	Event
Smallpox (“lymph” vaccines)	Sepsis due to bacterial contamination
Yellow fever (1942)	Vaccine lot contaminated with hepatitis B virus, leading to 28,000 cases of hepatitis B
Inactivated polio (1955)	Incomplete inactivation of virus, leading to 204 cases of paralytic disease (Cutter incident)
Inactivated measles (1960s)	Atypical (severe) disease upon exposure to natural measles infection
Attenuated polio (Sabin)	Vaccine-associated paralysis (~1 per 2.4 million persons vaccinated) due to reversion to more pathogenic strain
Live vaccines (vaccinia, polio, measles)	Dissemination and death in immunocompromised individuals
Attenuated live measles	Unexplained mortality in girls who received high titer formulations (relative risk of death doubled to age 5); more than 20 million doses of vaccine distributed
Inactivated influenza A	Apparent risk of Guillain Barré Syndrome (~1 per 1 million persons vaccinated), only in some years
Rotavirus (1999)	Intussusception—bowel folding and obstruction (vaccine withdrawn)

VACCINATION: producing attenuated human viruses



Passage history of the Oka-strain VZV vaccine

Isolated by Takahashi et al. in 1974 from three-year-old Japanese boy (family name Oka)

Initial growth in human lung fibroblast cells

Twelve passages in primary guinea pig fibroblasts

Two passages in WI-38 cells (human diploid fibroblasts)

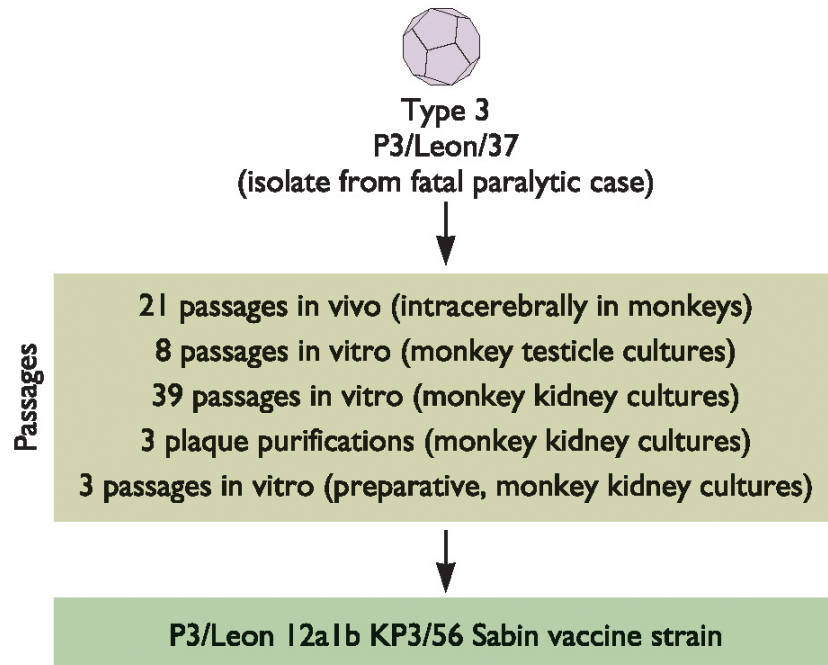
Three to six passages in MRC-5 cells (human diploid lung cells)

Licensed for use in Japan and Korea in late 1980s, and in USA in 1995

VACCINATION:

live attenuated oral poliovirus vaccines

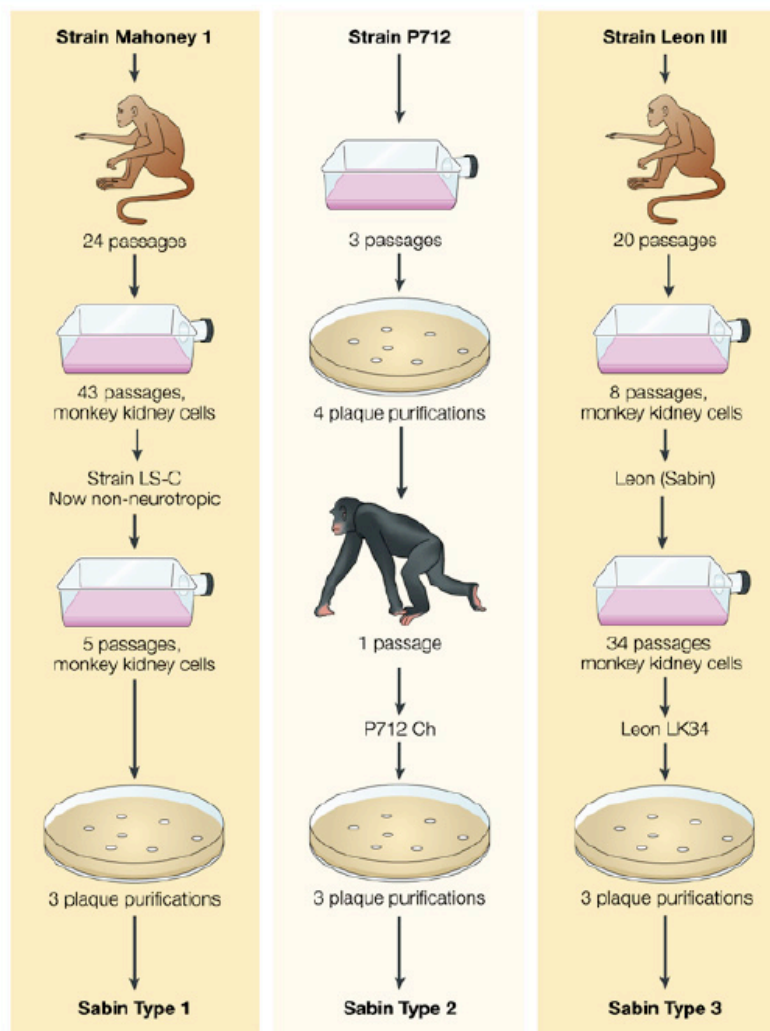
A Derivation of Sabin type 3 attenuated poliovirus



B Determinants of attenuation in the Sabin vaccine strains

Virus	Mutation (location/nucleotide position)
P1/Sabin	5'-UTR (480) VP1 (1106) VP1 (1134) VP3 (3225) VP4 (4065)
P2/Sabin	5'-UTR (481) VP1 (1143)
P3/Sabin	5'-UTR (472) VP3 (3091)

VACCINATION: live attenuated oral poliovirus vaccines



Attenuation of poliovirus neurovirulence: the Sabins OPV licensed in 1961

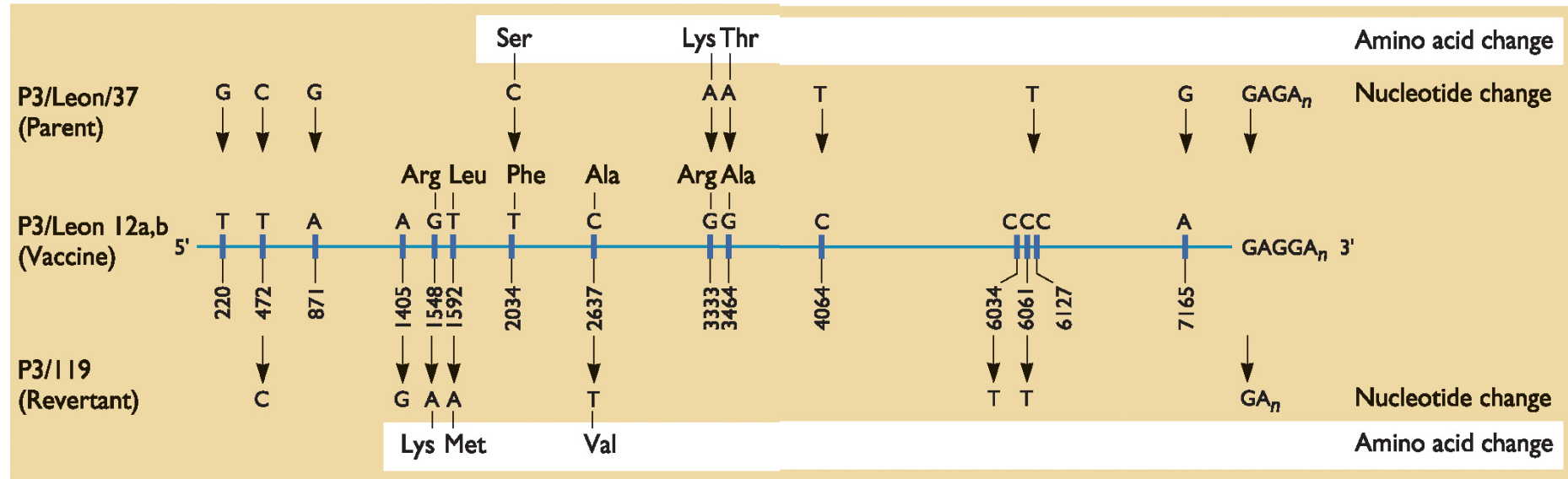
VACCINATION:

problems with traditional vaccines

- Disadvantages of **attenuated** vaccines:
 - reversion to the virulent state (polio).
 - growth in tissue culture cells or in animals poses the risk of introducing hidden viruses from host cells (early lots of polio vaccines-SV40).
 - even attenuated pathogens can produce severe disease in people with immune system deficiencies.

VACCINATION: reversion of P3/sabin (1/790.000)

C Reversion of P3/Sabin



Virus strain	Number of mutations	Number of amino acid changes	Number of mutations required for return to neurovirulence
Sabin strain 1	57	23	>10
Sabin strain 2	23	5	5-6
Sabin strain 3	~6	3	1-2

Limitations to Current Vaccine Production Methods

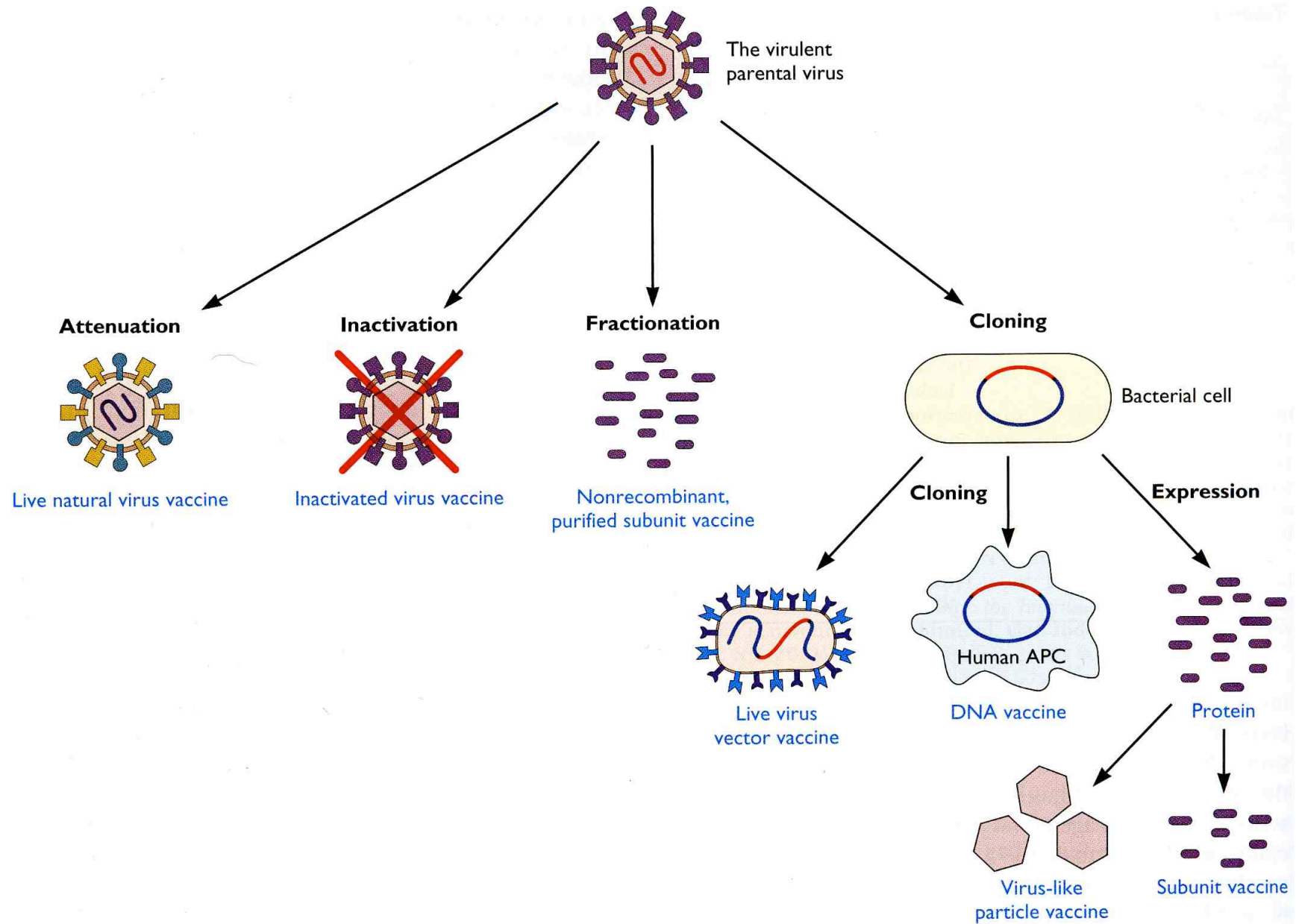
- Not all pathogens can be grown in culture
- Cell culture is expensive
- Yield and rate of production may be low
- Extensive safety precautions needed
- Inactivation/attenuation must be 100%
- Attenuated strains might revert
- Limited shelf-life and refrigeration requirements
- Not all diseases preventable by vaccines

NEW VACCINE TECHNOLOGY:

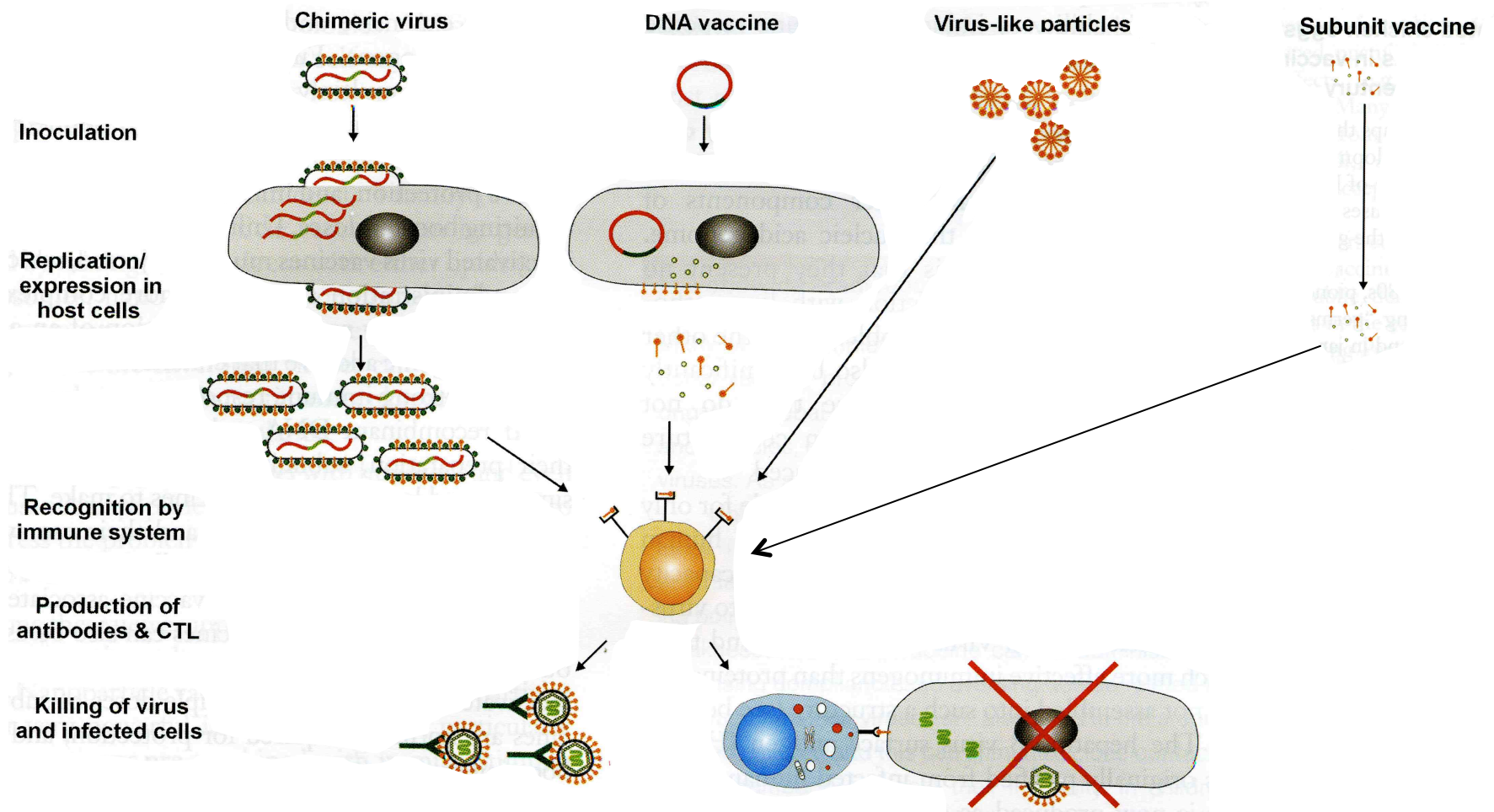
Impact of rDNA technology approach to vaccine production

1. Attenuate organism by specific deletion of virulence gene (bacmid technology)
2. Engineer live, non pathogenic carrier (vector) to contain antigenic determinant for disease organism (viral vector technology)
3. If non-culturable, clone pathogen's genes, and overexpress a subset of viral proteins to use as vaccines (subunit vaccines, VLPs)

VACCINATION: how to make a vaccine?



VACCINATION: **how to make a vaccine?**



Recombinant antiviral vaccines

MODERNS VACCINES

- Subunit Vaccines
- Peptide Vaccines
- Genetic Immunization
- Attenuated Vaccines
- Vector Vaccines

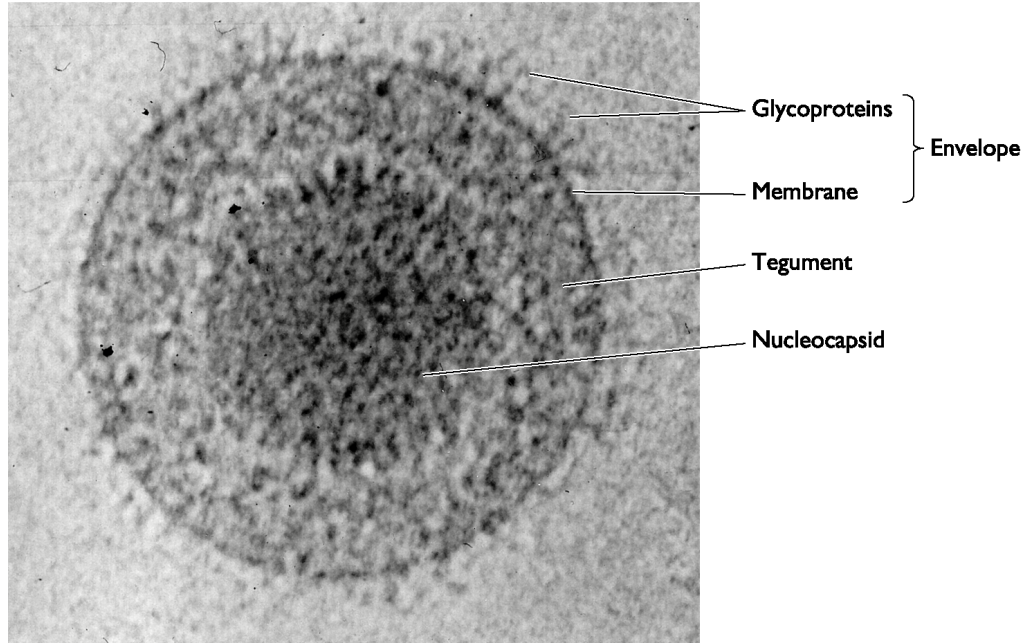
MODERNS VACCINES

- **Subunit Vaccines**
- Peptide Vaccines
- Genetic Immunization
- Attenuated Vaccines
- Vector Vaccines

Subunit Vaccines

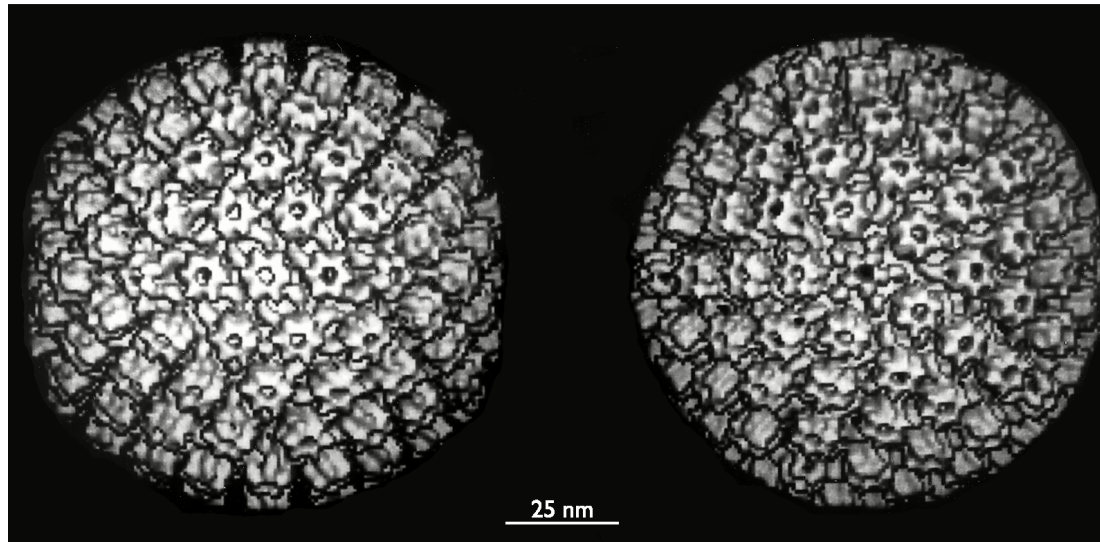
- Generally whole pathogenic agent used to construct attenuated or inactivated vaccine
- Immune response generally elicited by interaction with proteins on outer surface of pathogen

A



Subunit vaccines for viruses

B



Subunit Vaccine

- So, is the entire pathogen required?
- No, only outer surface proteins are needed to elicit an immune response
- Vaccines that use components of a pathogen rather than the whole organism are “subunit” vaccines

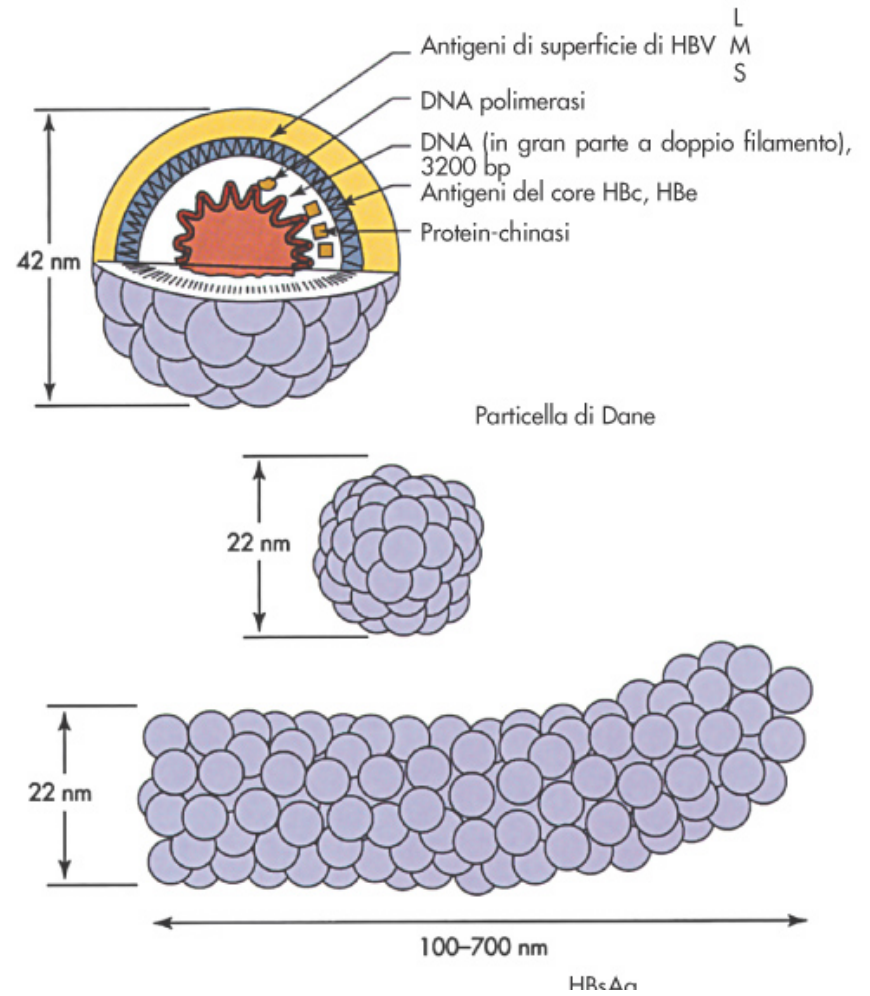
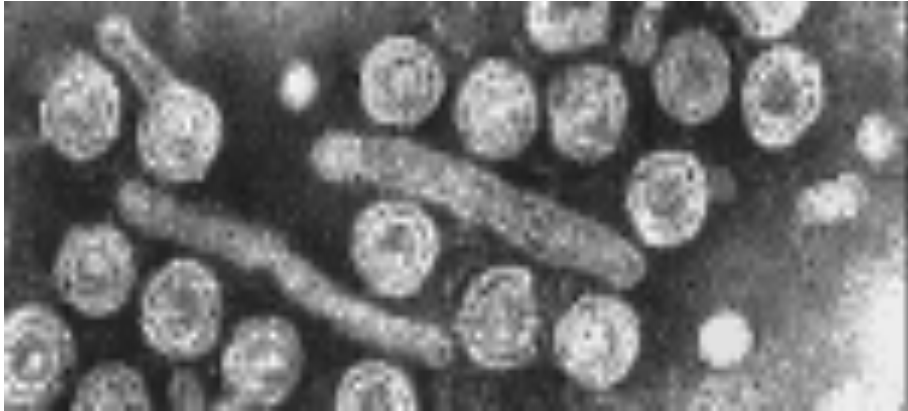
Subunit Vaccine

- **Advantages**

- Easily to produce
- Using a purified protein ensures that the vaccine is safe and stable
- Inexpensively

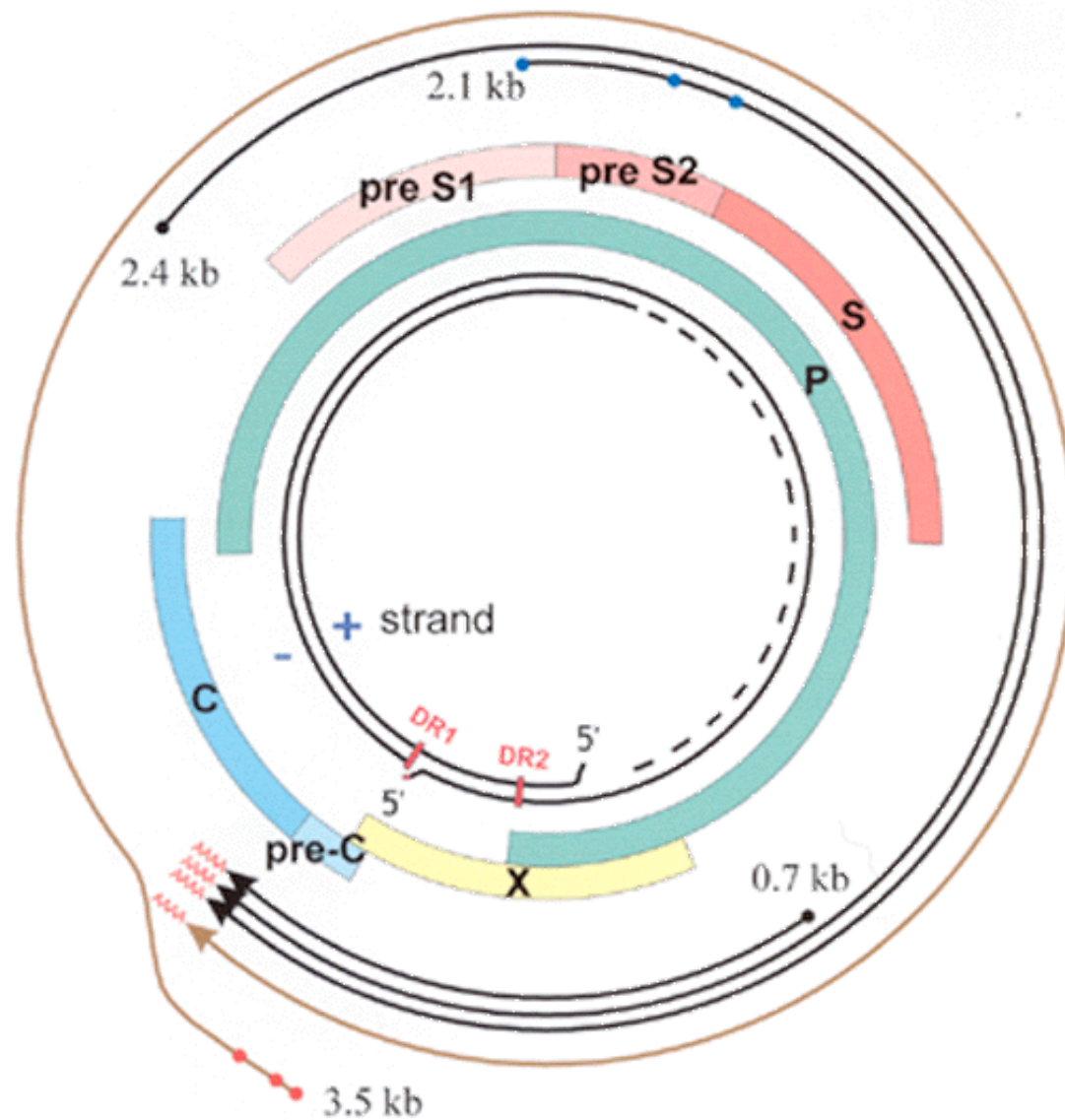
- **Disadvantages**

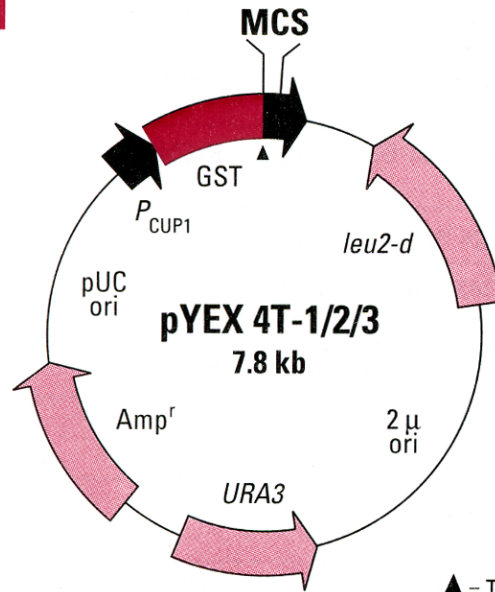
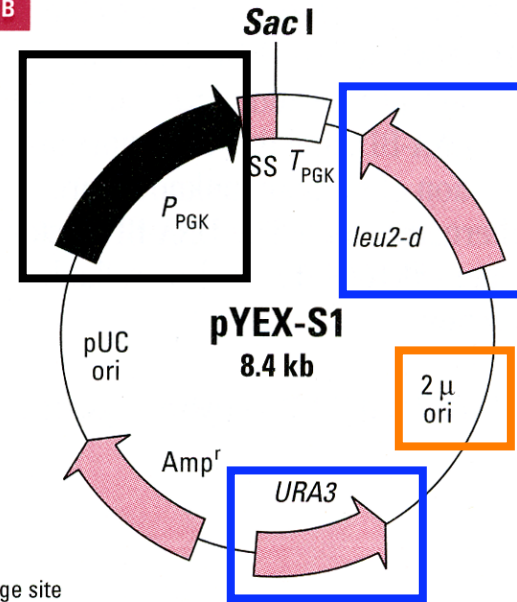
- Isolated protein may not have the same conformation as in the pathogen, so may not have the same antigenicity (weakness and short duration of immunity)
- It may also be necessary to administer the antigen in a specific manner (in a concentrated form, with adjuvants)



Subunit Vaccine - Example: HBV

Subunit Vaccine - Example: HBV



A**B****C****pYex 4T-1 MCS**

Thrombin recognition site
 CTG GTT CCG CGT GGA TCC CCG GAA TTC CCG GGT CGA CTC GAG CGG CCG CAT CGT GAC TGA CTG A
 BamHI EcoRI SmaI SalI XhoI NotI

STOPs

pYex 4T-2 MCS

Thrombin recognition site
 CTG GTT CCG CGT GGA TCC CCA GGA ATT CCC GGG TCG ACT CGA GCG GCC GCA TCG TGA CTG ACT GA
 BamHI EcoRI SmaI SalI XhoI NotI

STOPs

pYex 4T-3 MCS

Thrombin recognition site
 CTG GTT CCG CGT GGA TCC CCG AAT TCC CGG GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA
 BamHI EcoRI SmaI SalI XhoI NotI

STOPs

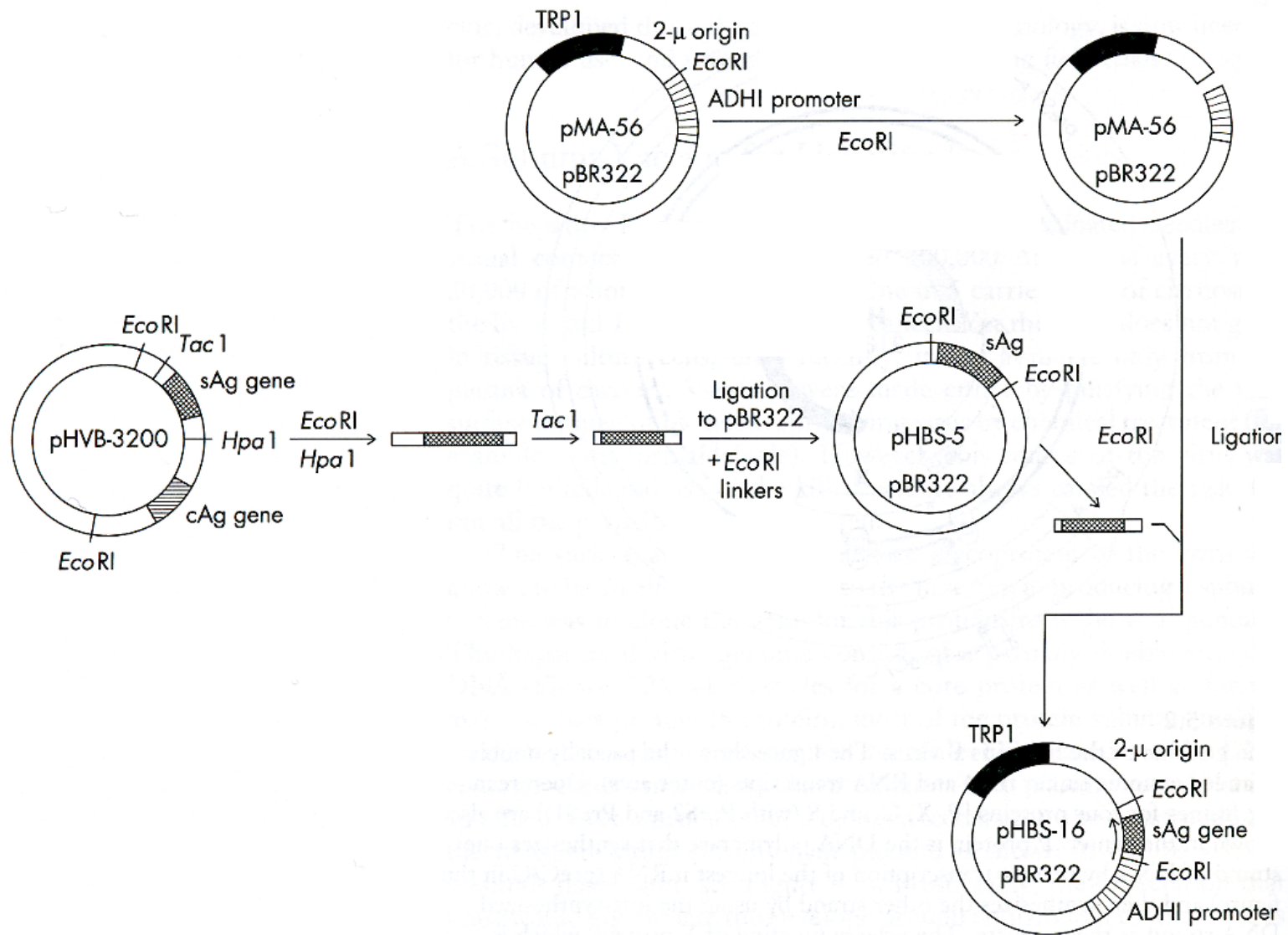
pYex-Bx MCS

GGA TCC GCA GCT GTC GAC TGC AGA ATT C
 BamHI PvuII SalI PstI EcoRI

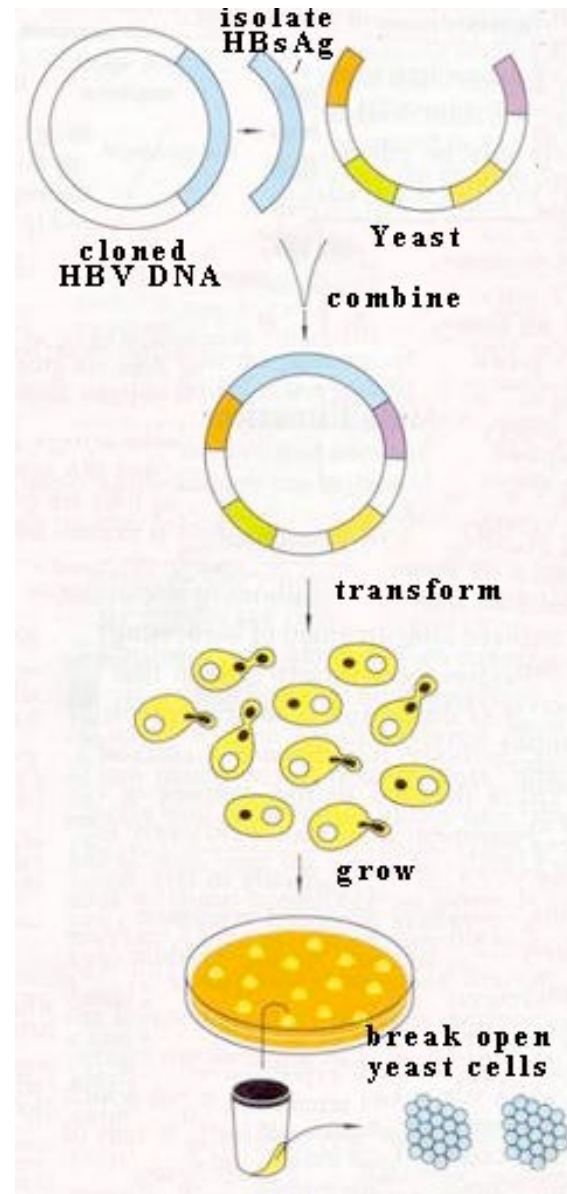
pYex-S1 MCS

KEX2
 AAA AGG AGC TCG AAT TC
 SacI EcoRI

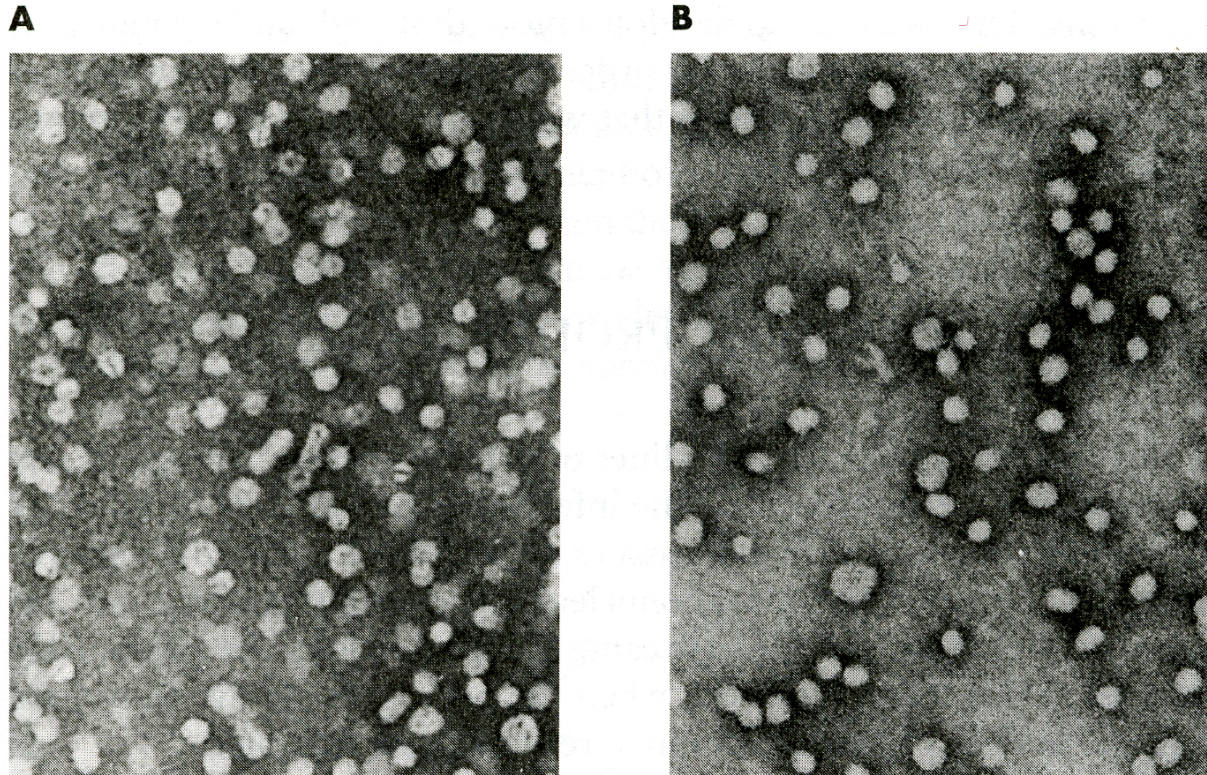
Subunit Vaccine - Example: HBV



Subunit Vaccine - Example: HBV



Subunit Vaccine - Example: HBV



Negative stained electron micrographs of (A) plasma-derived and (B) yeast-derived hepatitis B surface antigen vaccines

RECOMBIVAX HB® **HEPATITIS B VACCINE (RECOMBINANT)**

DESCRIPTION

RECOMBIVAX HB® Hepatitis B Vaccine (Recombinant) is a non-infectious subunit viral vaccine derived from hepatitis B surface antigen (HBsAg) produced in yeast cells. A portion of the hepatitis B virus gene, coding for HBsAg, is cloned into yeast, and the vaccine for hepatitis B is produced from cultures of this recombinant yeast strain according to methods developed in the Merck Research Laboratories.

The antigen is harvested and purified from fermentation cultures of a recombinant strain of the yeast *Saccharomyces cerevisiae* containing the gene for the *adv* subtype of HBsAg. The fermentation process involves growth of *Saccharomyces cerevisiae* on a complex fermentation medium which consists of an extract of yeast, soy peptone, dextrose, amino acids and mineral salts. The HBsAg protein is released from the yeast cells by cell disruption and purified by a series of physical and chemical methods. The purified protein is treated in phosphate buffer with formaldehyde and then coprecipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvanted with amorphous aluminum hydroxyphosphate sulfate. The vaccine contains no detectable yeast DNA but may contain not more than 1% yeast protein. The vaccine produced by the Merck method has been shown to be comparable to the plasma-derived vaccine in terms of animal potency (mouse, monkey, and chimpanzee) and protective efficacy (chimpanzee and human).

The vaccine against hepatitis B, prepared from recombinant yeast cultures, is free of association with human blood or blood products.



PRESCRIBING INFORMATION

ENGERIX-B® **[Hepatitis B Vaccine (Recombinant)]**

DESCRIPTION

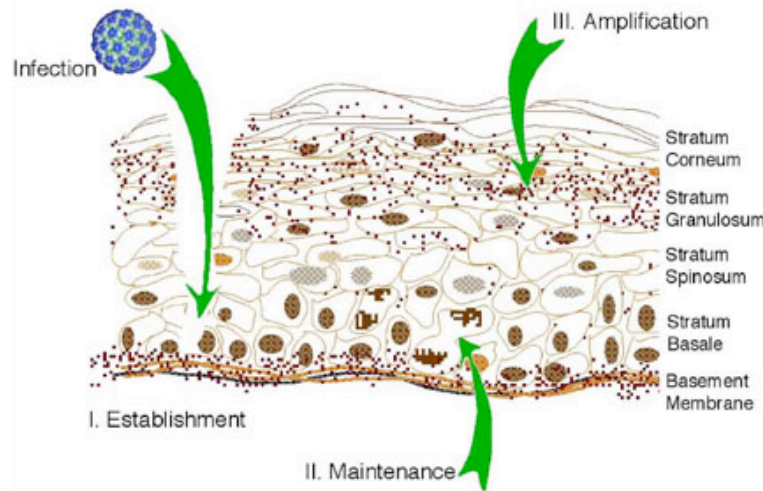
ENGERIX-B [Hepatitis B Vaccine (Recombinant)] is a noninfectious recombinant DNA hepatitis B vaccine developed and manufactured by GlaxoSmithKline Biologicals. It contains purified surface antigen of the virus obtained by culturing genetically engineered *Saccharomyces cerevisiae* cells, which carry the surface antigen gene of the hepatitis B virus. The surface antigen expressed in *Saccharomyces cerevisiae* cells is purified by several physicochemical steps and formulated as a suspension of the antigen adsorbed on aluminum hydroxide. The procedures used to manufacture ENGERIX-B result in a product that contains no more than 5% yeast protein. No substances of human origin are used in its manufacture.

ENGERIX-B is supplied as a sterile suspension for intramuscular administration. The vaccine is ready for use without reconstitution; it must be shaken before administration since a fine white deposit with a clear colorless supernatant may form on storage.



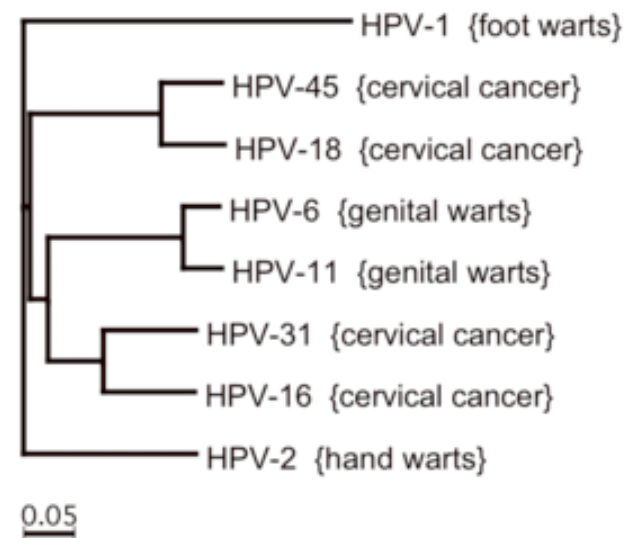
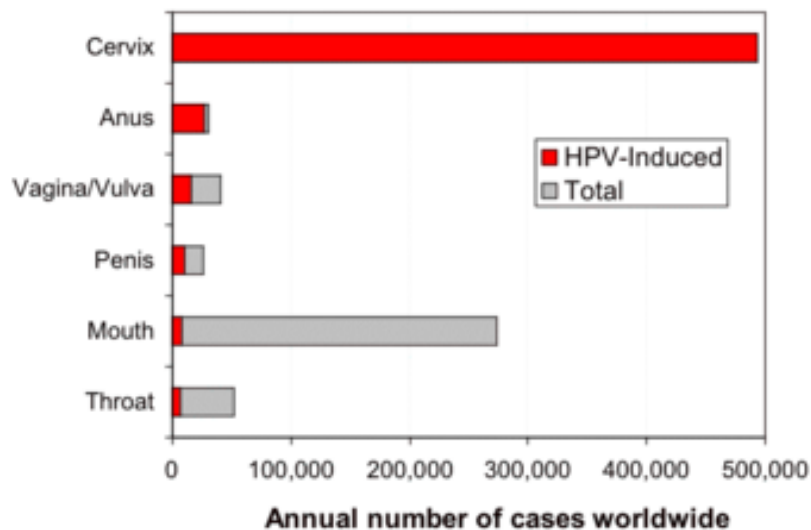
Subunit Vaccine - Example: HPV

The Differentiation-Dependent Life Cycle of Papillomaviruses

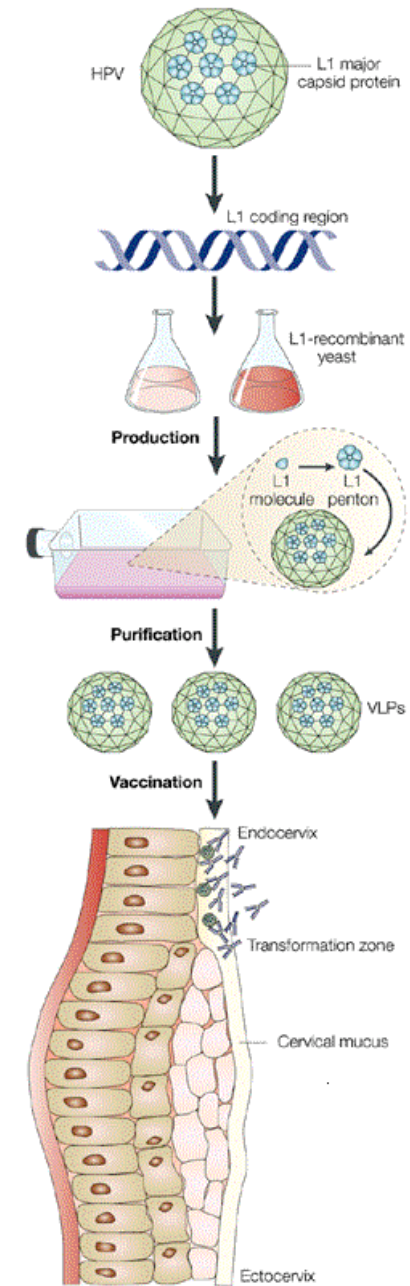
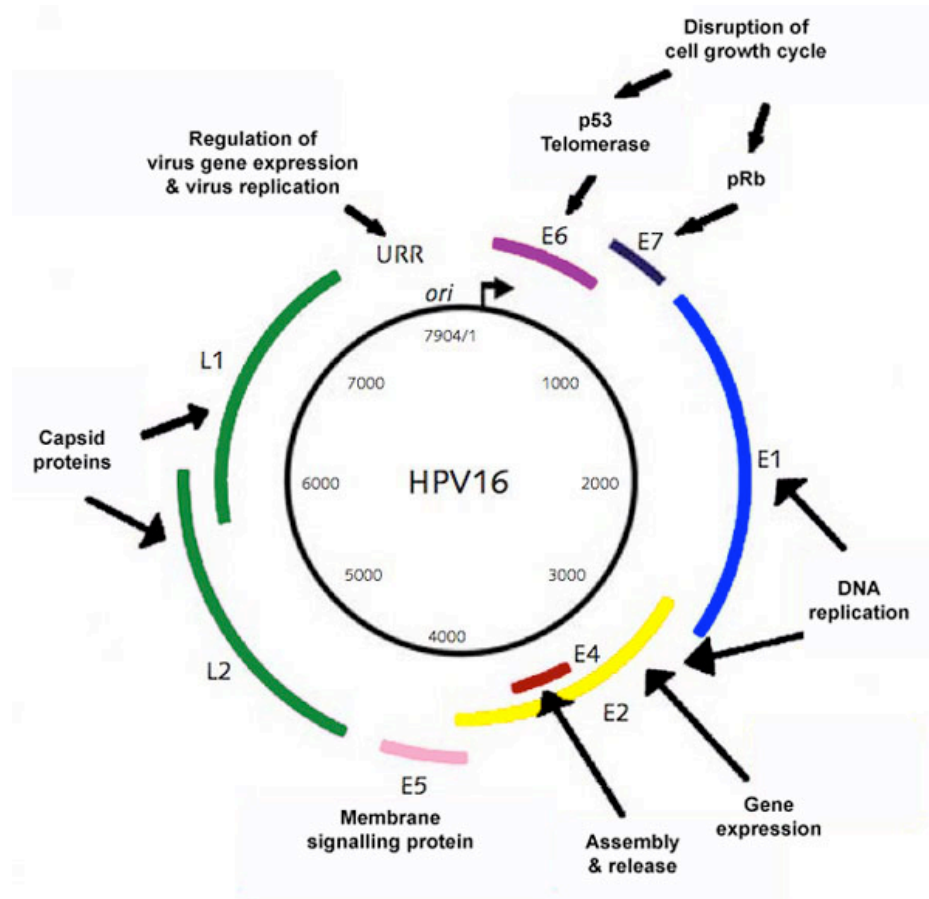


VIRAL FUNCTIONS			CELL ATTACHMENT PROTEINS			
Early Proteins	DNA Synthesis	Capsid Proteins	HSPG	Synde-can-1	α -6 Integrin	Laminin 5
-	-	+	++	++	-	-*
++	+++	++	++	++	-	-*
++	++	-	++	++	-	-*
++	++	-	+	+	++	-*
-	-	-	+++	+++	+++	++

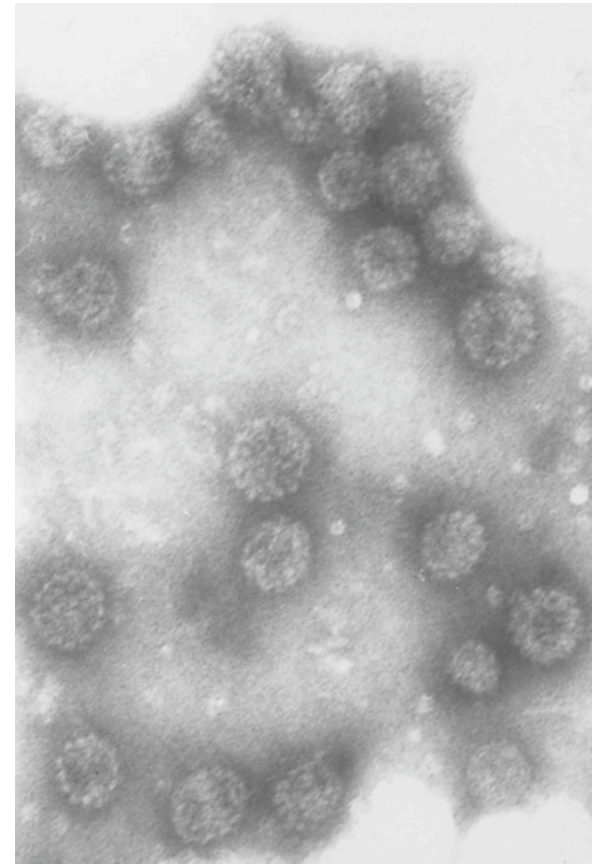
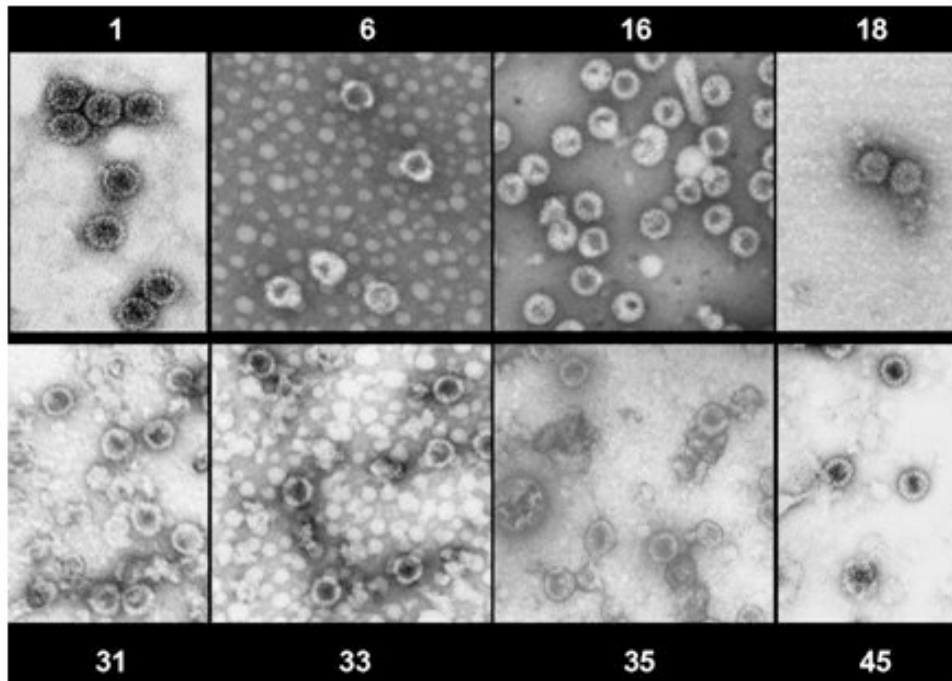
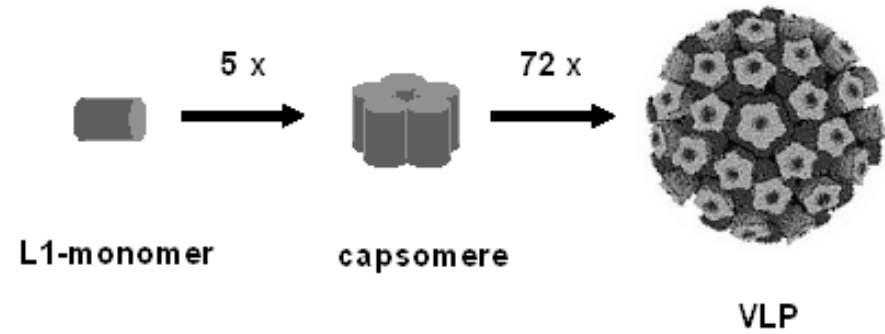
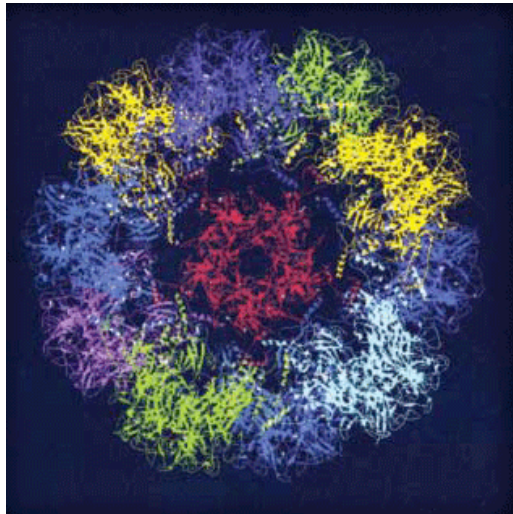
M. Ozbun © 2006



Subunit Vaccine Example: HPV

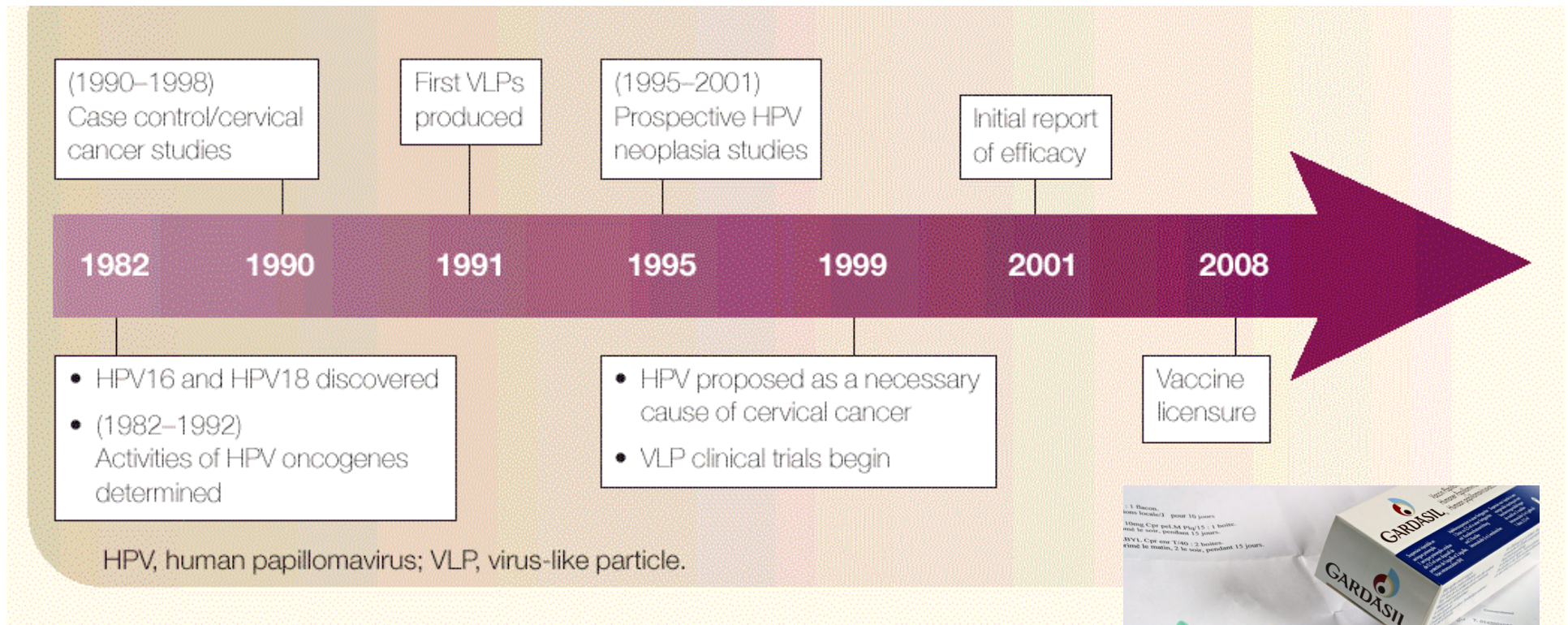


Subunit Vaccine - Example: HPV



Subunit Vaccine - Example: HPV

HPV VLP vaccine development: timeline



Subunit Vaccine - Example: HSV

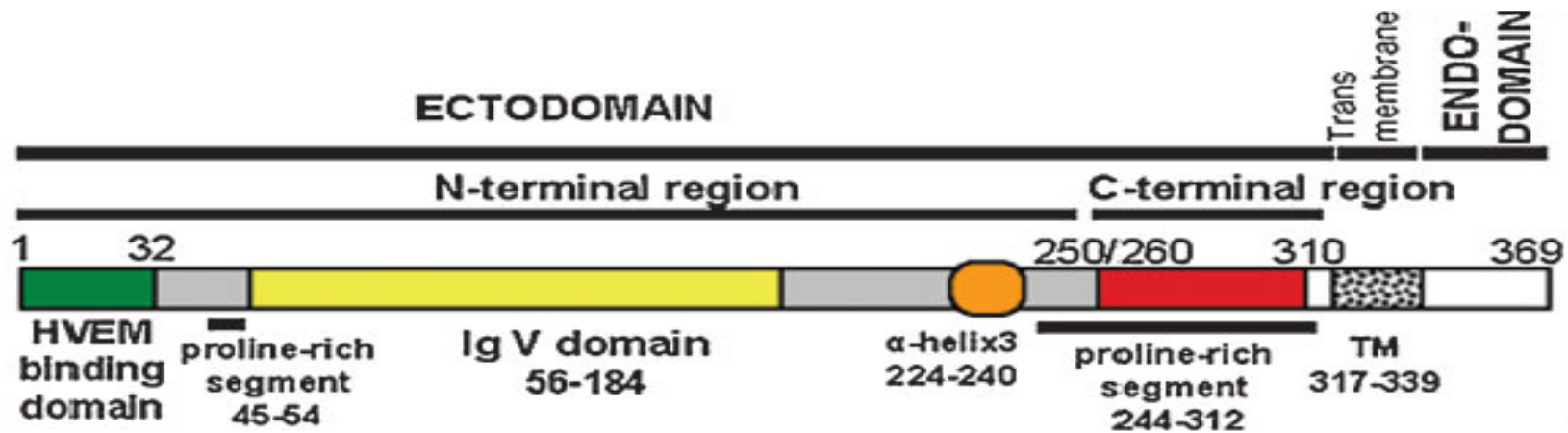
- Herpes Simplex Viruses (HSV-1,2)
 - sexually transmitted disease (HSV-2)
 - cancer associated agent (HSV-2)
 - encephalitis and severe eye infections
- Subunit vaccine would be best
 - inactivated or attenuated virus would have to be 100% or risk infecting patient

Subunit Vaccine - Example: HSV

Target Antigenic Protein

- Herpes Simplex Virus
 - What is the antigenic target?
 - HSV viral envelope glycoprotein D
 - gD interacts with entry receptors (HVEM, nectin-1, 3-O-sulphated HS)

Structure and function of HSV gD glycoprotein



Campadelli *et al.*, 2007

• **369 aa**

• **organized in three domains:**

1) **ecto-domain: aa 1-317**

N-terminal: aa 1-260

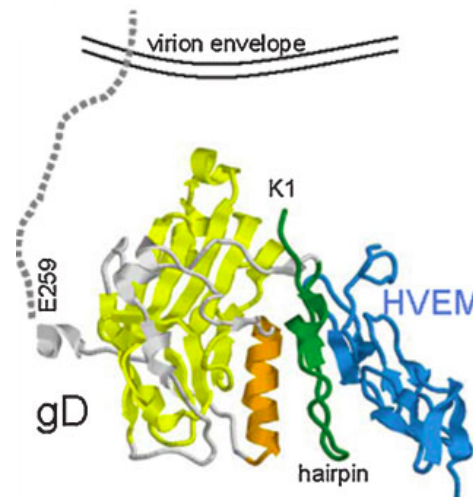
core: aa 56-184;

α -helix, aa 224-240

C-terminal: aa 261-310

2) **trans-membrane domain: aa 318-339**

3) **endo-domain: aa 340-369**



gD functions:

1) **receptor recognition**

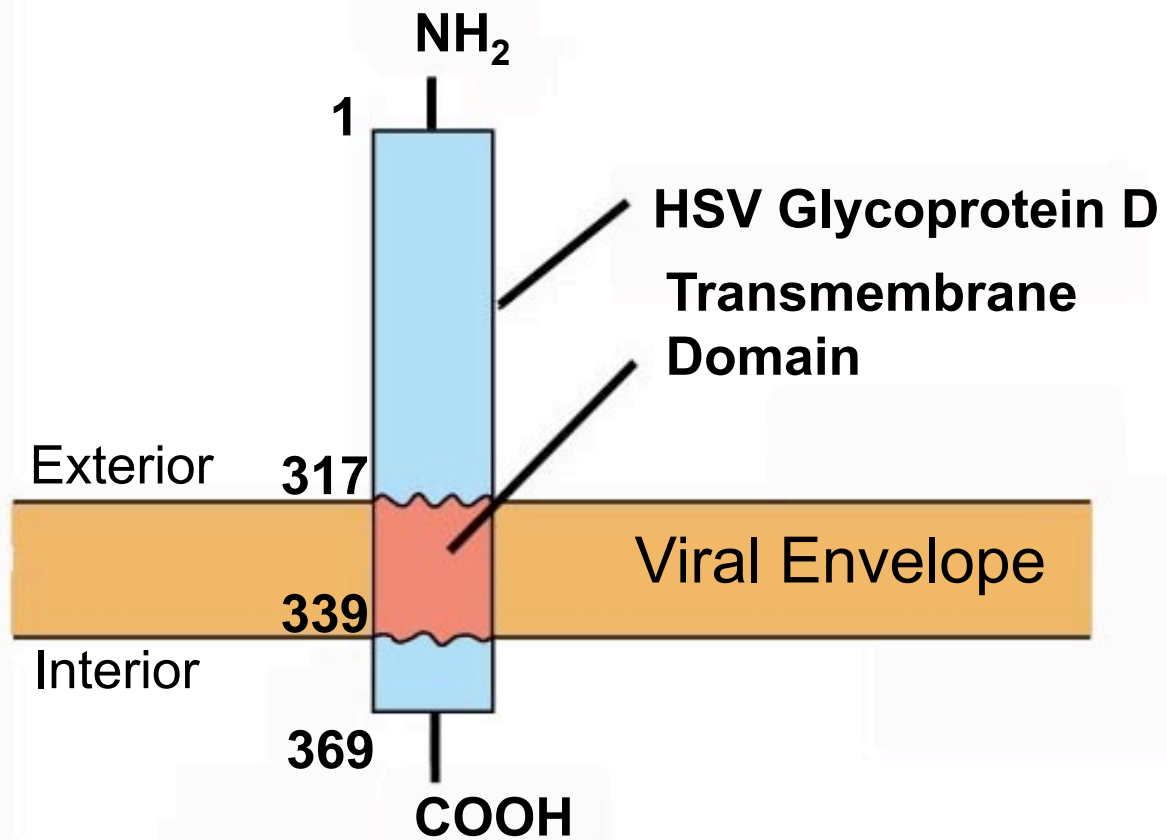
2) **triggering of fusion**

Functions of HSV **gD**: receptor recognition

The three natural **gD** receptors:

1. **herpesvirus entry mediator (HVEM):**
tumor necrosis factor receptor family;
in T-lymphocytes or lymphoid organ.
HVEM binding-site: aa 1-32 (contact residues between aa 7-15 and 24-32).
2. **nectin 1:**
intercellular adhesion molecules family;
in sensory neurons, muco-epithelia or epithelia cells.
nectin-1 binding-site: critical aa residues (aa 34, 38, 215 and aa 222-223).
3. **O-sulphated HS (heparan sulfate):**
modified heparan sulfate by enzymes in neuronal and endothelial cells, corneal fibroblasts.

HSV Glycoprotein D



HSV glycoprotein D vaccine

- Isolate gene
- Clone into mammalian exp. system
- Purify expressed protein

- Membrane proteins typically difficult to purify - aggregate
 - Mutate so not membrane bound

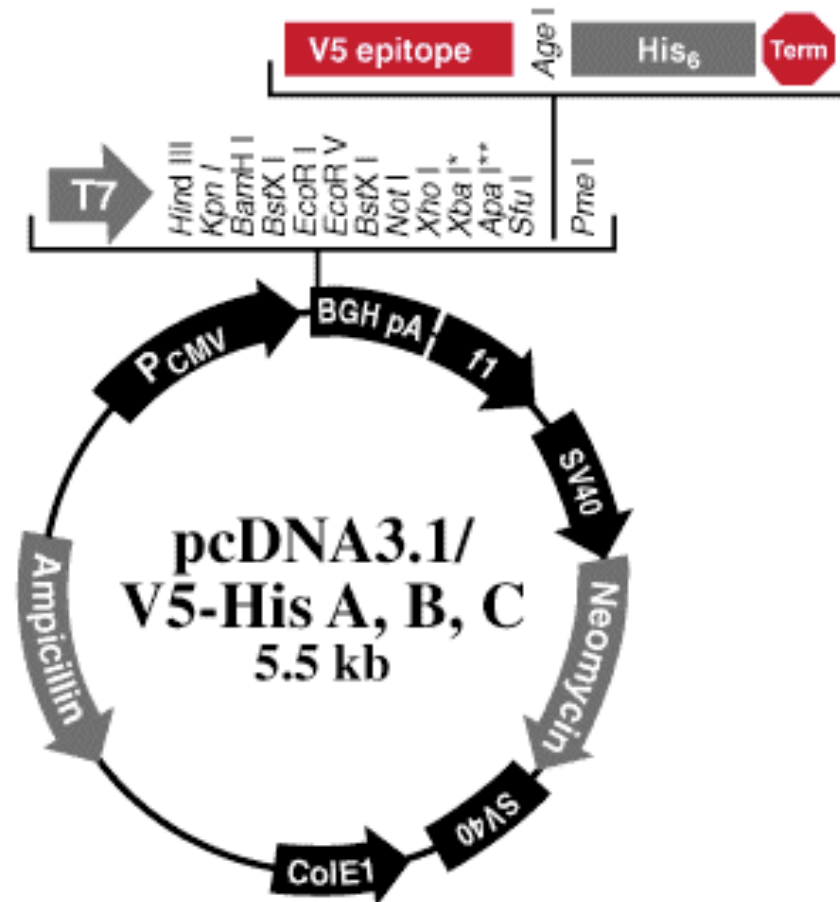
A Mammalian expression vector

Comments for pcDNA3.1/V5-His A
5503 nucleotides

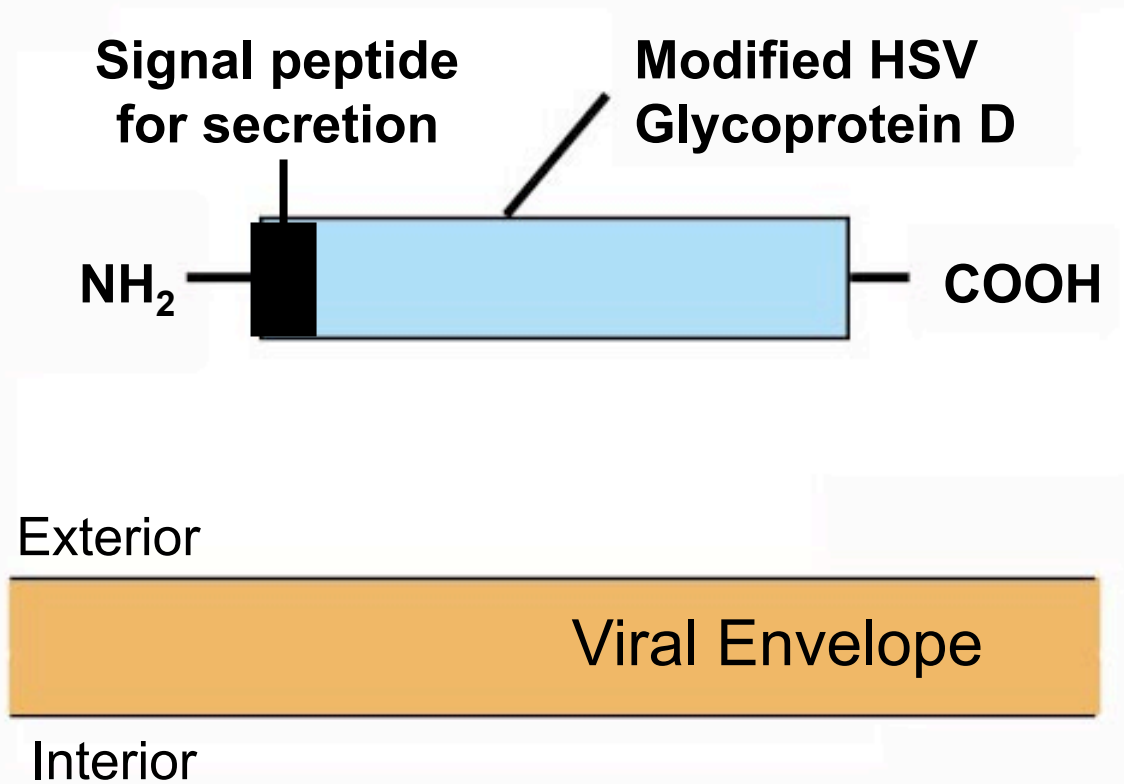
CMV promoter: bases 209-863
 T7 promoter/priming site: bases 863-882
 Multiple cloning site: bases 902-999
 V5 epitope: bases 1000-1041
 Polyhistidine tag: bases 1051-1068
 pcDNA3.1/BGH reverse priming site: bases 1091-1108
 BGH polyadenylation signal: bases 1090-1304
 f1 origin of replication: bases 1357-1780
 SV40 promoter and origin: bases 1845-2170
 Neomycin resistance gene: bases 2206-3000
 SV40 polyadenylation signal: bases 3019-3257
 ColE1 origin: bases 3689-4362
 Ampicillin resistance gene: bases 4507-5367

* After the *Xho* I site, there is a unique *Bst*E II site, but no *Xba* I or *Apa* I sites in version C.

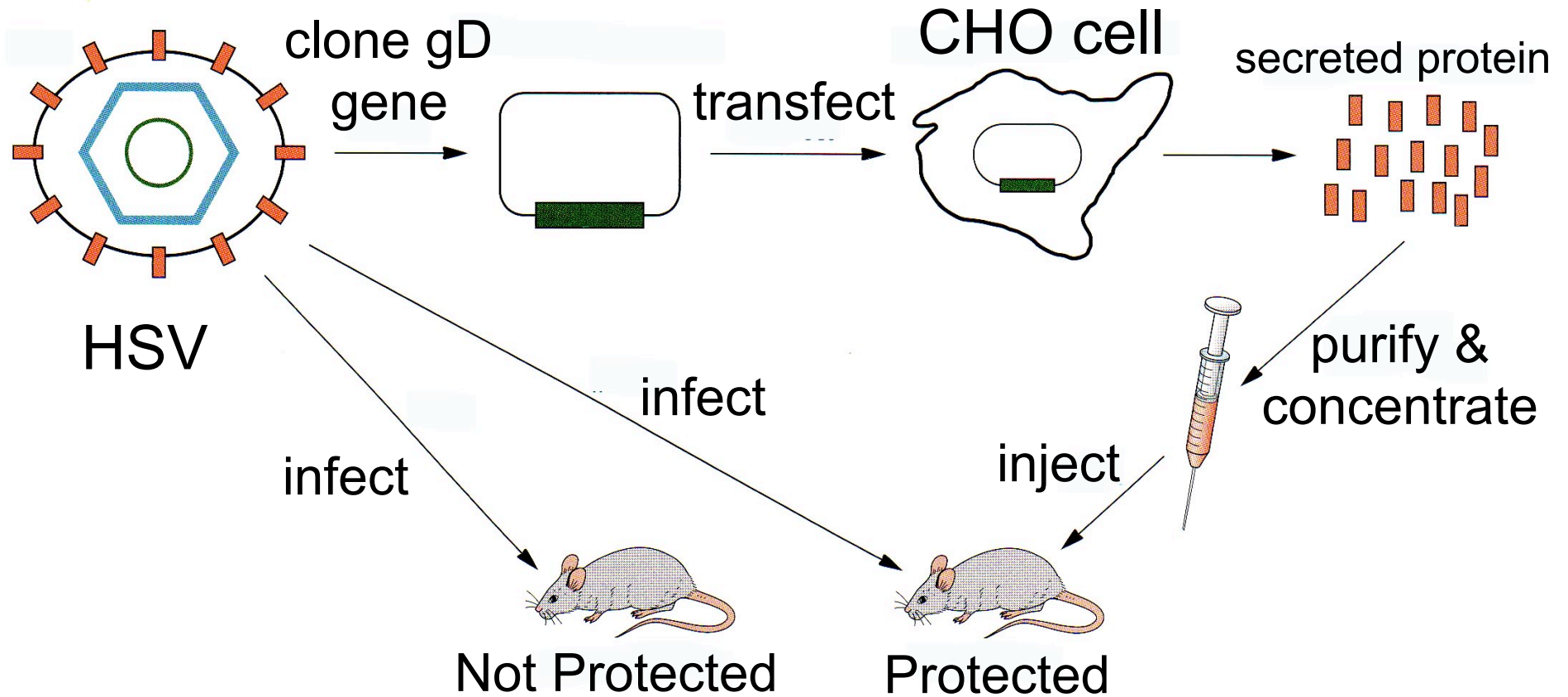
** There is a unique *Sac* II site between the *Apa* I site and the *Sfu* I site in version B only.



Modified HSV glycoprotein D



Development of Subunit Vaccine against HSV



HSV glycoprotein D vaccine

- HSV Subunit vaccine production
 - Transfect modified glycoprotein gene into mammalian cells
 - Overexpress
 - Purify
- Modified HSV glycoprotein D was effective against both HSV-1 and HSV-2 in animal lab tests

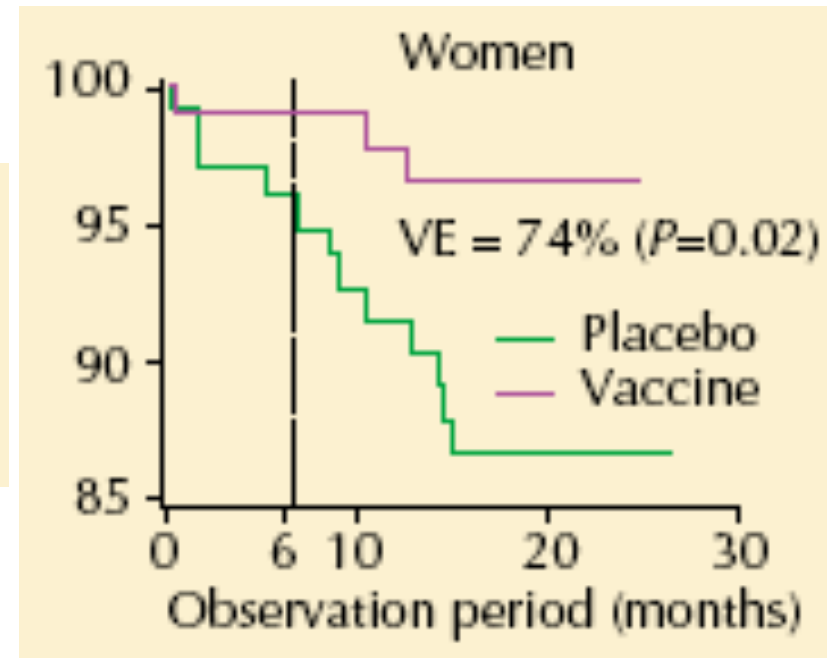
HSV glycoprotein D vaccine

Table 4: Summary of human trials of prophylactic herpes simplex virus (HSV) vaccines

Vaccine	Manufacturer
Live-attenuated/replication disabled	
R7020	Pasteur Merieux ^{2*}
ICP10DPK	AuRx Inc. ³²
Killed/viral component	
Skinner ⁷⁻⁹	
Cappel et al. ¹⁴	
HSV-2 GS	Merck, Sharpe & Dohme ¹⁶
Biocine	Chiron ^{60*}
gD2/gB2/ MF59	Chiron ³⁵
Simplirix (gD2/MPL-alum)	GlaxoSmithKline³

*Trial referred to within this reference.

Percentage without GHD



ORIGINAL ARTICLE

Efficacy Results of a Trial of a Herpes Simplex Vaccine

Robert B. Belshe, M.D., Peter A. Leone, M.D., David I. Bernstein, M.D., Anna Wald, M.D., Myron J. Levin, M.D., Jack T. Stapleton, M.D., Iris Gorfinkel, M.D., Rhoda L. Ashley Morrow, Ph.D., Marian G. Ewell, Sc.D., Abbie Stokes-Riner, Ph.D., Gary Dubin, M.D., Thomas C. Heineman, M.D., Ph.D., Joann M. Schulte, D.O., and Carolyn D. Deal, Ph.D., for the Herpevac Trial for Women

ABSTRACT

BACKGROUND

Two previous studies of a herpes simplex virus type 2 (HSV-2) subunit vaccine containing glycoprotein D in HSV-discordant couples revealed 73% and 74% efficacy against genital disease in women who were negative for both HSV type 1 (HSV-1) and HSV-2 antibodies. Efficacy was not observed in men or HSV-1 seropositive women.

METHODS

We conducted a randomized, double-blind efficacy field trial involving 8323 women 18 to 30 years of age who were negative for antibodies to HSV-1 and HSV-2. At months 0, 1, and 6, some subjects received the investigational vaccine, consisting of 20 µg of glycoprotein D from HSV-2 with alum and 3-O-deacylated monophosphoryl lipid A as an adjuvant; control subjects received the hepatitis A vaccine, at a dose of 720 enzyme-linked immunosorbent assay (ELISA) units. The primary end point was occurrence of genital herpes disease due to either HSV-1 or HSV-2 from month 2 (1 month after dose 2) through month 20.

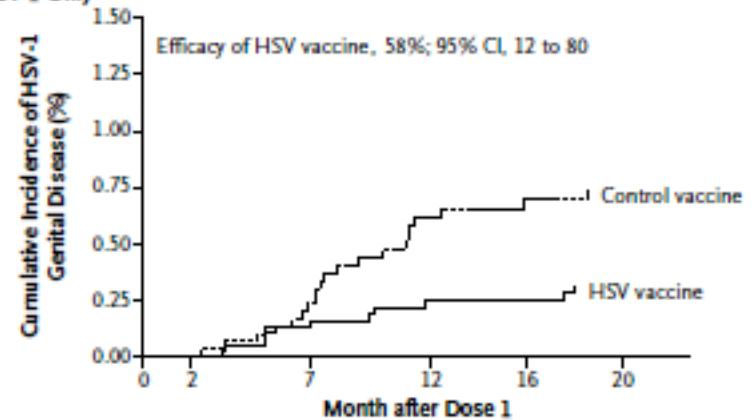
RESULTS

The HSV vaccine was associated with an increased risk of local reactions as compared with the control vaccine, and it elicited ELISA and neutralizing antibodies to HSV-2. Overall, the vaccine was not efficacious; vaccine efficacy was 20% (95% confidence interval [CI], -29 to 50) against genital herpes disease. However, efficacy against HSV-1 genital disease was 58% (95% CI, 12 to 80). Vaccine efficacy against HSV-1 infection (with or without disease) was 35% (95% CI, 13 to 52), but efficacy against HSV-2 infection was not observed (-8%; 95% CI, -59 to 26).

CONCLUSIONS

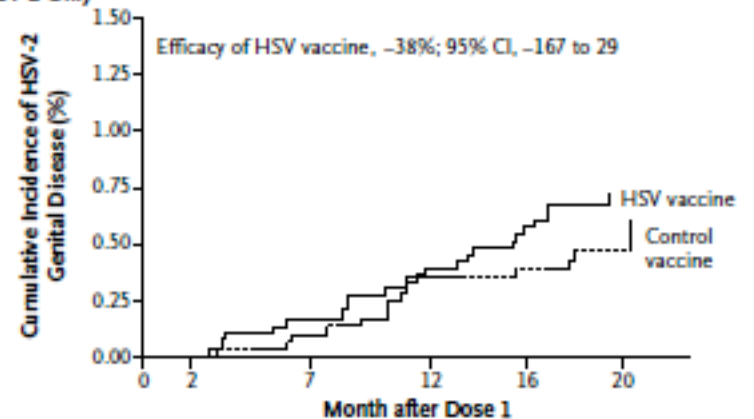
In a study population that was representative of the general population of HSV-1- and HSV-2-seronegative women, the investigational vaccine was effective in preventing HSV-1 genital disease and infection but not in preventing HSV-2 disease or infection. (Funded by the National Institute of Allergy and Infectious Diseases and GlaxoSmithKline; ClinicalTrials.gov number, NCT00057330.)

B HSV-1 Only



No. at Risk						
HSV vaccine	3798	3637	3345	3229	1192	
Control vaccine	3076	2950	2675	2570	969	
Cumulative No. of Events						Total
HSV vaccine	0	6	9	9	11	11
Control vaccine	0	7	18	20	21	21

C HSV-2 Only



No. at Risk						
HSV vaccine	3798	3638	3340	3219	1190	
Control vaccine	3076	2955	2684	2580	971	
Cumulative No. of Events						Total
HSV vaccine	0	6	14	20	24	24
Control vaccine	0	3	10	11	13	14

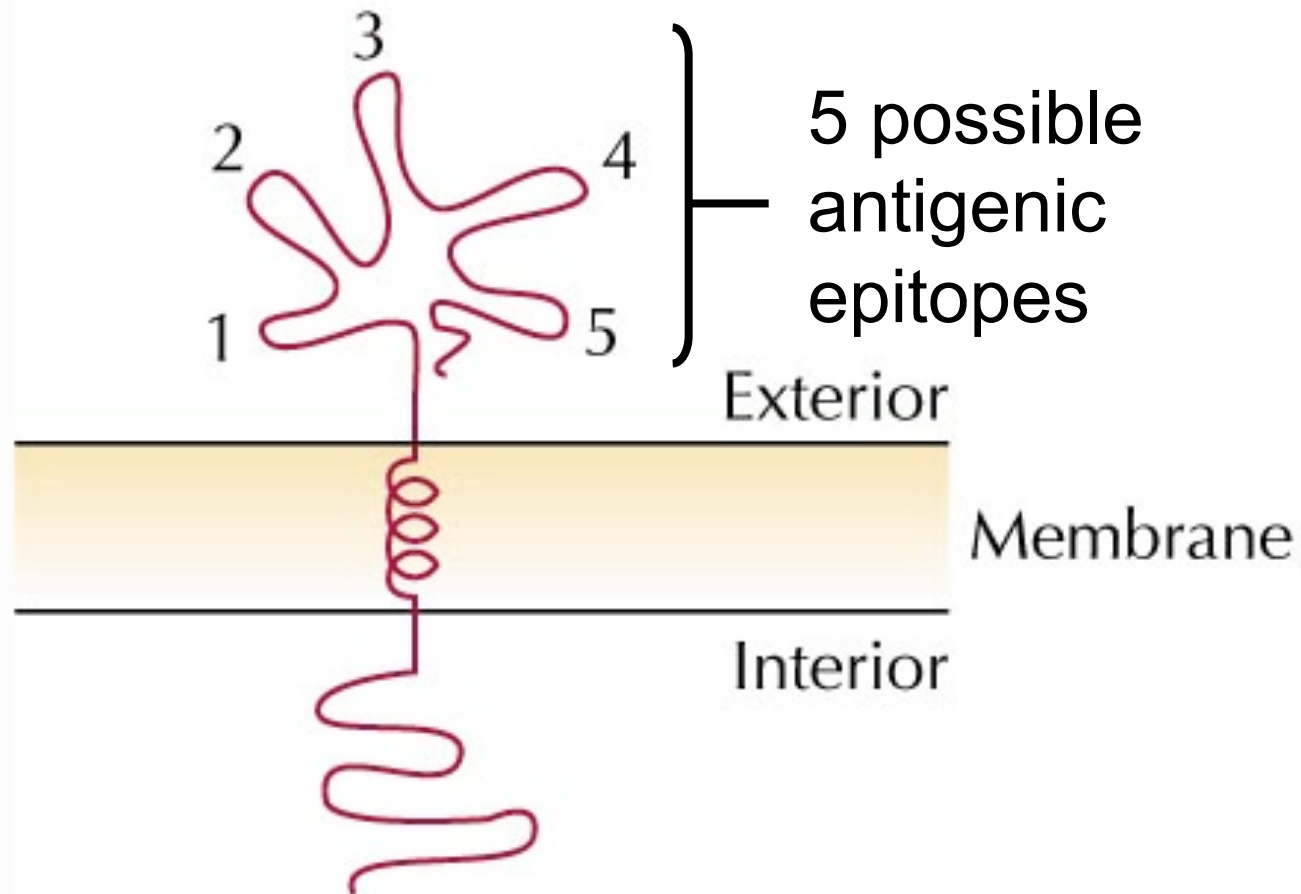
MODERNS VACCINES

- Subunit Vaccines
- **Peptide Vaccines**
- Genetic Immunization
- Attenuated Vaccines
- Vector Vaccines

Peptide Vaccines

- Subunit vaccine uses entire protein
 - Contains several antigenic determinants (B and T cells epitopes)
- **Peptide Vaccine**
 - vaccine from a specific domain of an antigenic protein
 - single epitope or antigenic determinant (immunogenic epitope)

Generalized membrane-bound protein with external epitopes



Peptide Vaccines

- **Advantages of peptide vaccines:**
 - can be made by chemical synthesis and eliminate the need of expensive process of protein purification (remove host DNA, LPS).
 - they are less expensive, purer and more stable than protein-containing subunit vaccines.
 - in addition, using only a part of the antigenic protein there will be no unwanted immunological reactions.

Peptide Vaccines

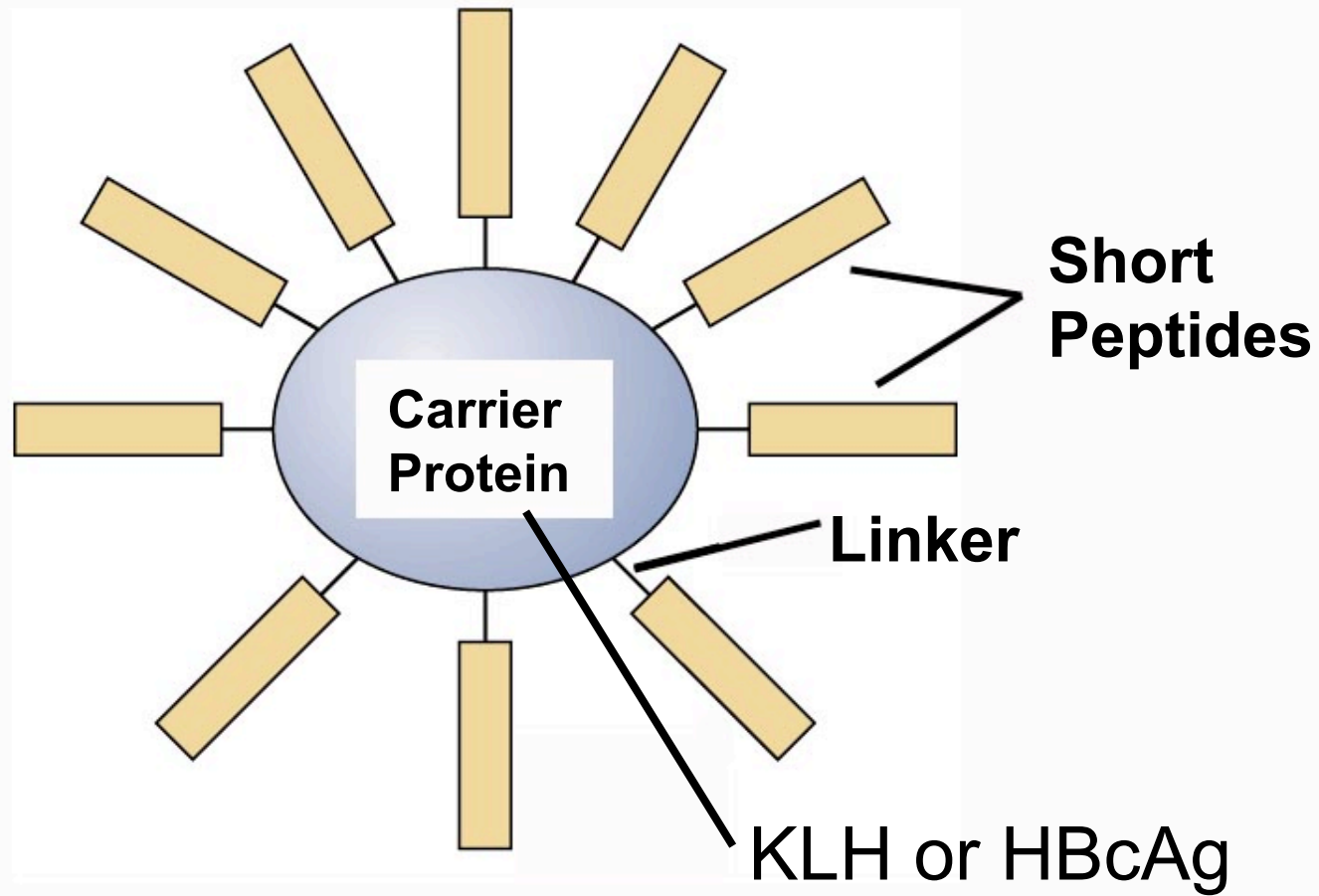
Peptides need to be linked to another molecule to prevent rapid degradation

- Keyhole limpet hemocyanin
 - an inert carrier protein
 - from a marine gastropod mollusk
- Hepatitis B core protein (HBcAg)
 - highly immunogenic carrier protein
 - self assembles into small particles

Keyhole Limpet (*Diodora aspera*)



Structure of a peptide vaccine

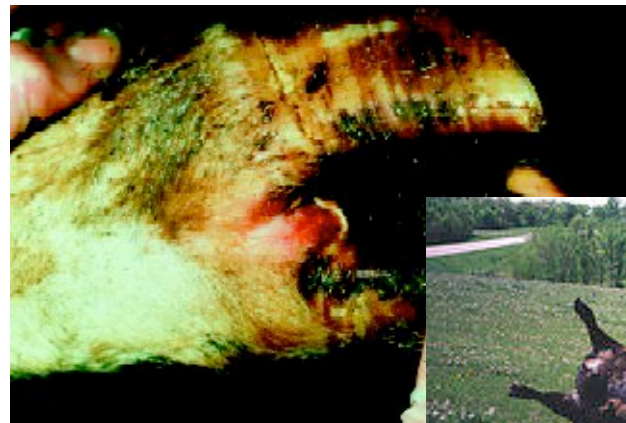
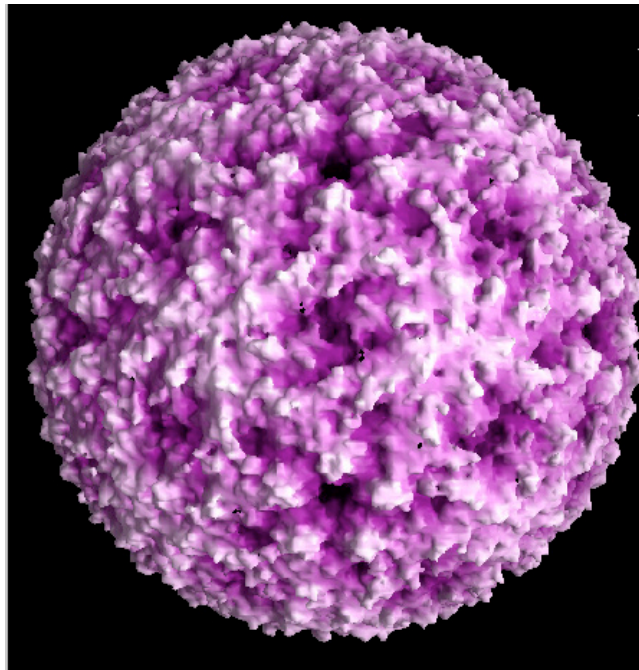


Limitations of Peptide Vaccines

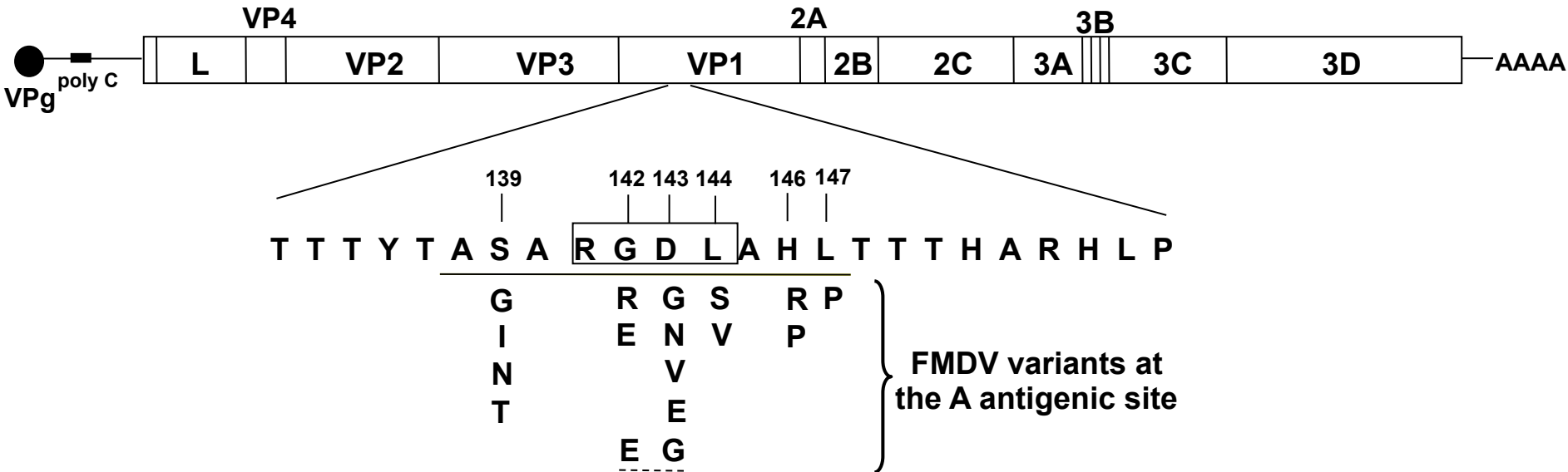
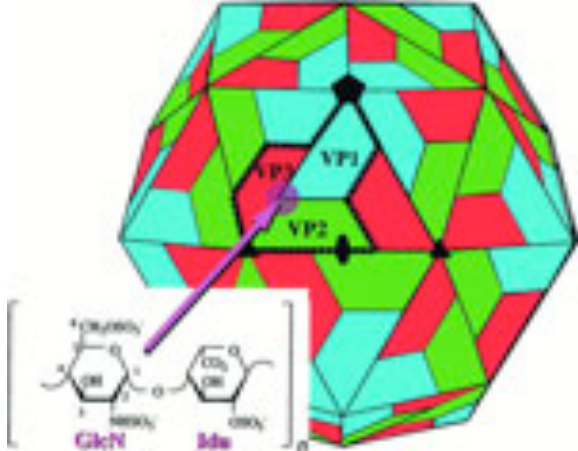
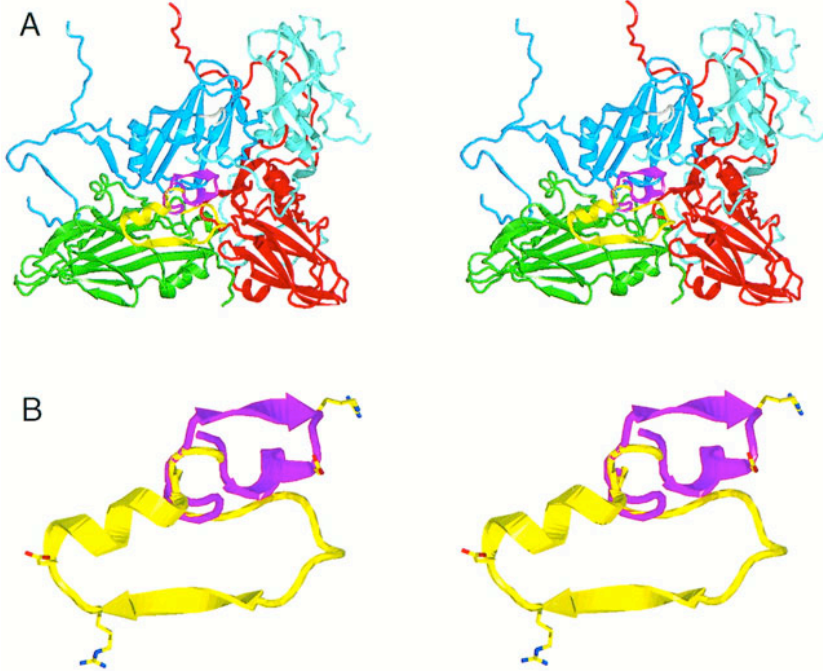
- Epitope must consist of a **contiguous stretch** of amino acids
- Not all peptides are effective in eliciting an immune response (may need 2 or more)
- Peptide must have the same conformation as in pathogen
- Selection of immune escape mutants is highly probable (may need 2 or more)
- Amount of peptide required to elicit an immune response may be 1000X more than for inactivated pathogen

Peptide Vaccine Effectiveness

Foot and Mouth Disease Virus (FMDV)



FMDV peptide vaccines



Peptide Vaccine Effectiveness - Example FMVD

Foot and Mouth Disease peptide vaccines

FMDV particles	1
VP1-HBcAg particles	1/10
VP1=142-160 aa	
VP1- β -gal fusion	1/350
VP1=137-162 aa	
VP1 142-160 aa	1/5000

MODERNS VACCINES

- Subunit Vaccines
- Peptide Vaccines
- **Genetic Immunization**
- Attenuated Vaccines
- Vector Vaccines

GENETIC IMMUNIZATION

- Novel variation of recombinant vaccine strategy
 - First reported in 1992
 - Instead of injecting protein antigen to elicit immune response, inject gene encoding antigen
- Also called **“DNA Vaccine”**

Genetic Immunization / DNA Vaccine

- Gene directs production of encoded protein antigen directly into tissues of the vaccinee
- A relative low dose of immunizing protein (ng range) seems to be sufficient to produce long-lasting immune response
- Antigen activates an immune response
 - Antibody production
 - Cell mediated response

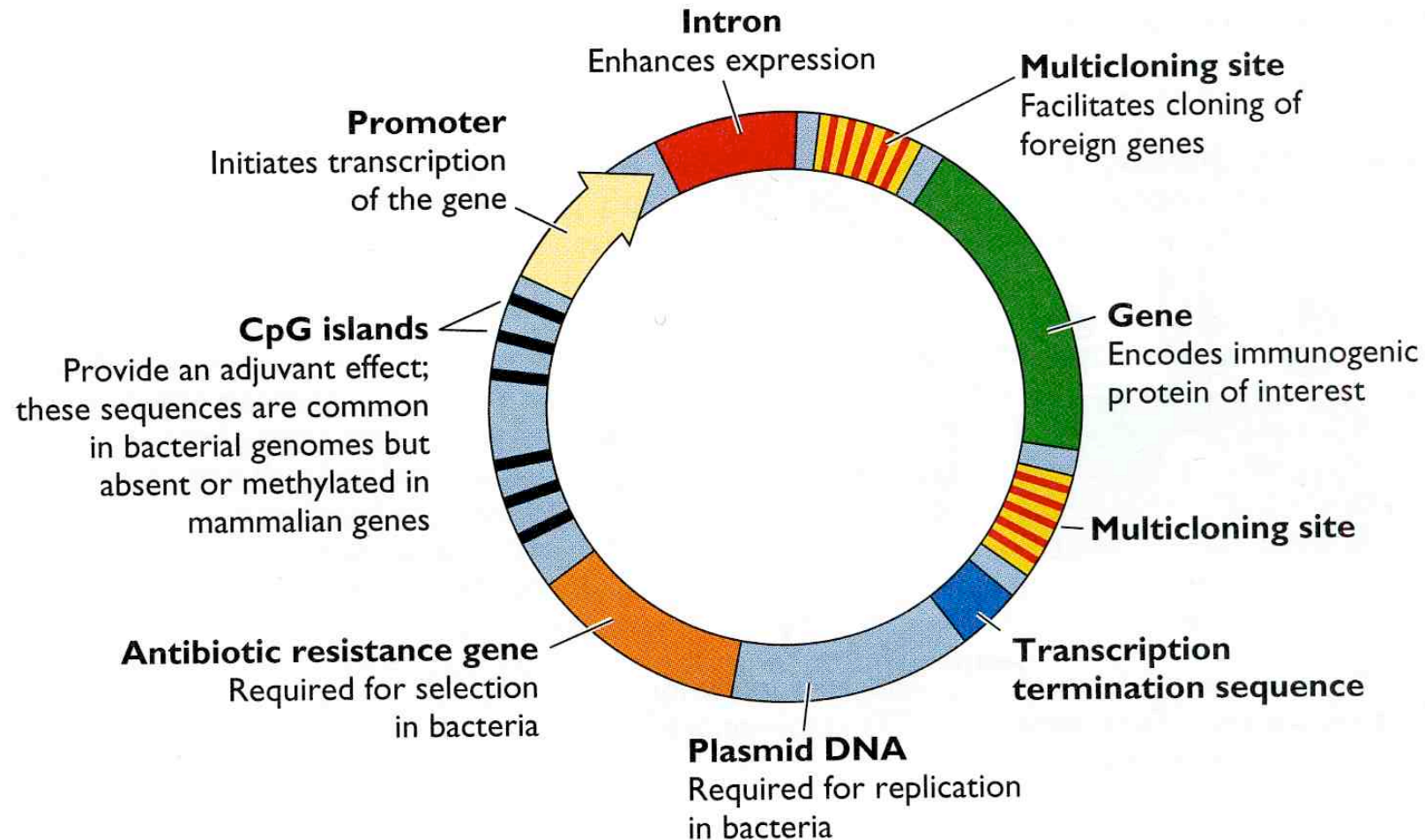
Genetic Immunization / DNA Vaccine

- The method of inoculation appears to dictate the type of immune response:
 - Direct Injection into muscle of an aqueous DNA solution (DNA travels to spleen - Th1 response)
 - Biolistic method - DNA coated metal beads (keratinocytes express ag, Langerhans cells move to the draining lymph node – Th2 response)

Genetic Immunization / DNA Vaccine

- Advantages of **genetic immunization**
 - Cultivation of pathogen not required
 - No chance of reversion
 - No adjuvants are necessary to stimulate an immune response
 - Inexpensive - no need to purify protein
 - Inexpensive – purified DNA is stable
 - One plasmid could encode several antigens

Genetic Immunization / DNA Vaccine



Genetic Immunization / DNA Vaccine

- **Disadvantage**
 - Don't know fate of DNA
 - Transient - will repeated treatments be needed?
 - Integration into genome - mutations?

Genetic Immunization / DNA Vaccine

Representative results of DNA vaccine trials

Virus	Proteins	Induction of antibody	Induction of CTL response	Protection against challenge
Bovine herpesvirus	gD	+	ND	+ (cattle)
Hepatitis B virus	Surface and core antigens	+ (chimpanzees); ND (humans)	+ (chimpanzees)	+ (chimpanzees)
Hepatitis C virus	Nucleocapsid	+	+	+ (mice)
Herpes simplex virus type 1	gD, gB	+	+	+ (mice)
HIV type 1	Env, Gag, Rev	+	+	+ (rhesus macaques)
Influenza virus	HA, M1, Np	+	+	+ (chickens, mice)
Lymphocytic choriomeningitis virus	NP	+	+	+ (mice)
Rabies virus	Glycoprotein, NP	+	+	+ (cynomolgus monkeys)
Respiratory syncytial virus	Glycoprotein	+	+	+ (mice)

^aData from A. Reyes-Sandoval and H. C. Ertl, *Curr. Mol. Med.* 1:217–243, 2001, with permission. ND, not detected.

Genetic Immunization/ DNA Vaccine

Example Influenza Virus

Inject mice with plasmid

Gene

influenza virus nucleocapsid NP coding sequences

Promoter

Cytomegalovirus (CMV) promoter

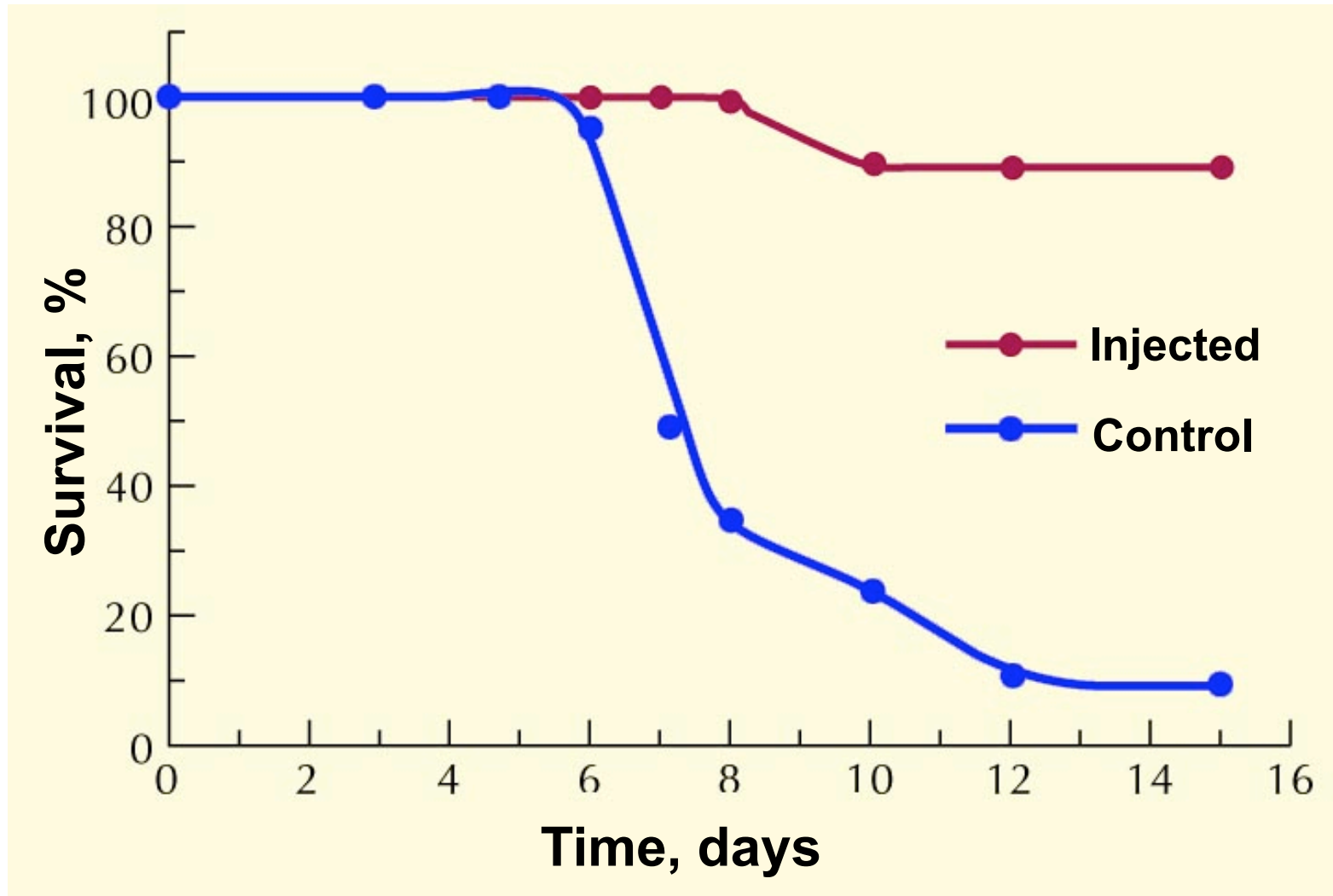
Injection site

quadriceps muscles of both rear legs

Genetic Immunization of Mice Against Influenza Virus

- Couldn't detect expression of protein
- Could detect Abs to flu virus nucleoprotein
- Injected mice were protected from Influenza virus (lv)

Survival of DNA-immunized Mice



Genetic Immunization / DNA Vaccine

The immunostimulatory sequences of plasmid vector DNA have adjuvant effects:

- Bacterial DNA has intrinsic immunostimulatory activity in mammalian cells through TLR9 stimulation.
- DNA motifs containing unmethylated CpG dinucleotides flanked by two 5' purines and by two 3' pyrimidines (5'-GACGTC-3') induce synthesis of IL6, IFN- γ , IL12, TNF.
- CpG DNA as a PAMP such as LPS, PGN, LTA dsRNA

MODERNS VACCINES

- Subunit Vaccines
- Peptide Vaccines
- Genetic Immunization
- **Attenuated Vaccines**
- Vector Vaccines

ATTENUATED VACCINES: a modern approach

- Live (attenuated) vaccines
 - generally more effective vaccines
 - BUT - must not have virulent forms present
- rDNA techniques permit genetic manipulation for generating attenuated vaccines

rDNA Technology Based Approach for Modifying Organisms to Generate Attenuated Vaccines

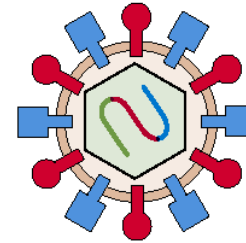
- A non-pathogenic organism engineered to carry and express antigenic determinants from a target pathogenic agent (vector vaccines)
- A pathogenic organism engineered such that the virulence genes have been modified or deleted (Bacmid technology)

Benefits of Attenuated Vaccines generated by rDNA technology

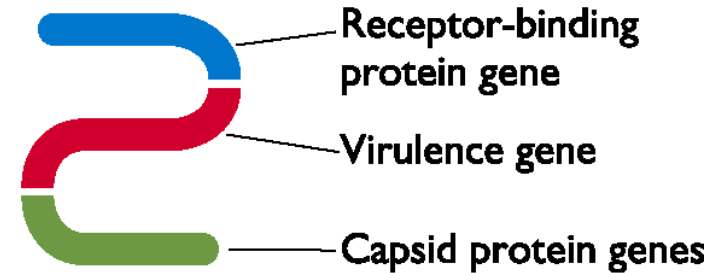
- Only a **selected antigenic determinant** from the pathogen is put into the non-pathogen, so there is no chance of vaccine causing disease
- **Deletion of virulence genes** greatly decreases the likelihood of reversion back to the virulent form
- Whole pathogen much more immunogenic than subunit or peptide vaccines (concentrated forms, in the presence of PAMPs)

Construction of attenuated Viruses by using recombinant DNA technology

Isolate pathogenic virus

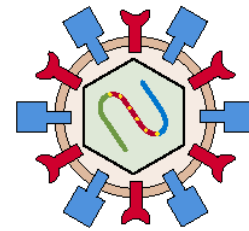


Clone genome

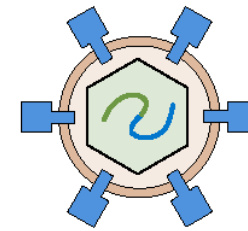


Isolate virulence gene

Mutate
virulence
gene

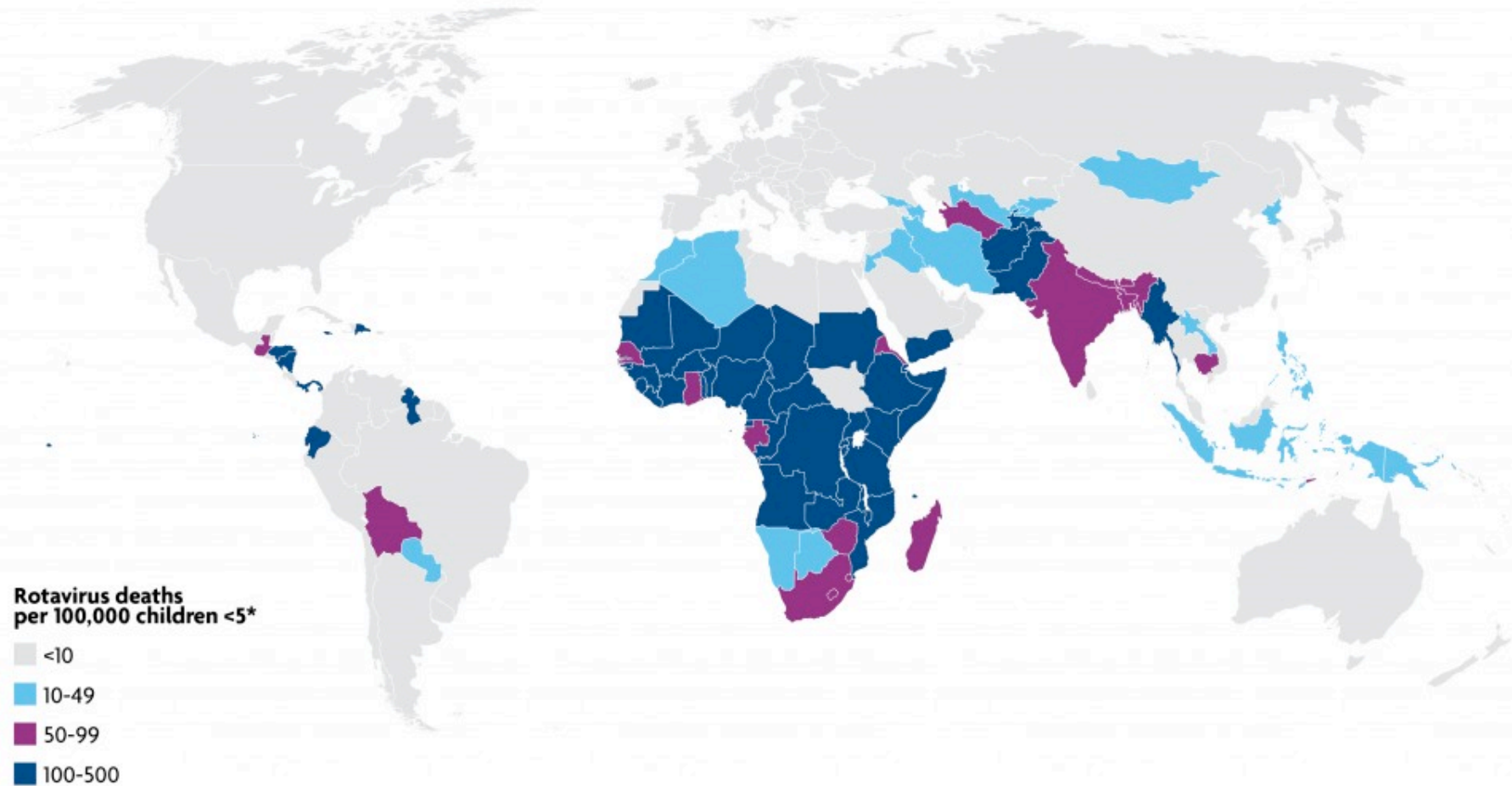


Delete
virulence
gene



The resulting virus is viable and immunogenic
but not virulent. It may be used as a vaccine.

Estimated Global Rotavirus Deaths in 2008



*Tate JE, Burton AH, Boschi-Pinto C, Duncan Steele D, et. al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *The Lancet*. Published online October 25, 2011.

Rotaviruses are the leading cause of severe diarrhea among infants and young children. Each year an estimated 453,000 children die from diarrhoeal disease caused by Rotavirus, most of whom live in developing countries, and another two million are hospitalised.

Rotavirus pathogenesis

Reoviruses

Rotavirus

Virus	Disease
Orthoreovirus	Mild upper respiratory tract disease, gastroenteritis, biliary atresia
Orbivirus/coltivirus	Colorado tick fever: febrile disease, headache, myalgia (zoonosis)
Rotavirus	Gastroenteritis

Epidemiology

Transmission

- Fecal-oral route

At risk

- Rotavirus type A
 - Infants <24 months of age (gastroenteritis, dehydration)
 - Older children (mild diarrhea)
 - Undernourished persons in underdeveloped countries (diarrhea, dehydration, death)
- Rotavirus type B
 - Infants, older children, adults in China (severe gastroenteritis)

Distribution of virus

- Ubiquitous (type A)
- Less common in summer

Vaccines or antiviral drugs

- None

Disease mechanisms

Transmitted by fecal-oral route

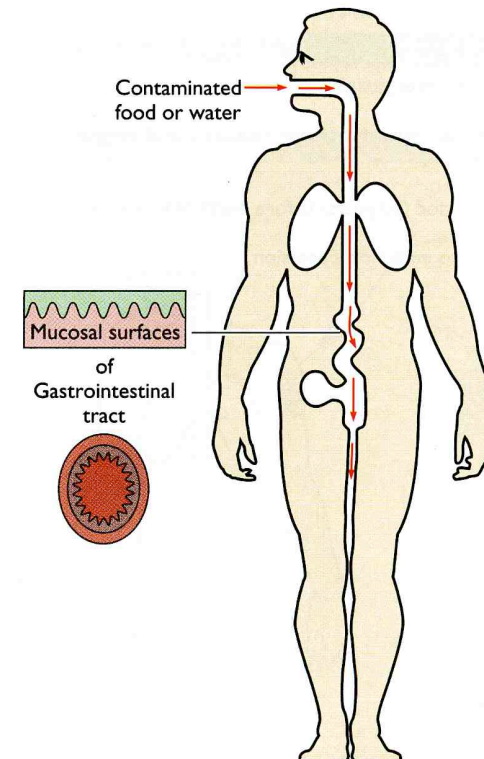
nsP4 is a viral enterotoxin that causes diarrhea

Disease is serious in infants <24 months old, asymptomatic in adults

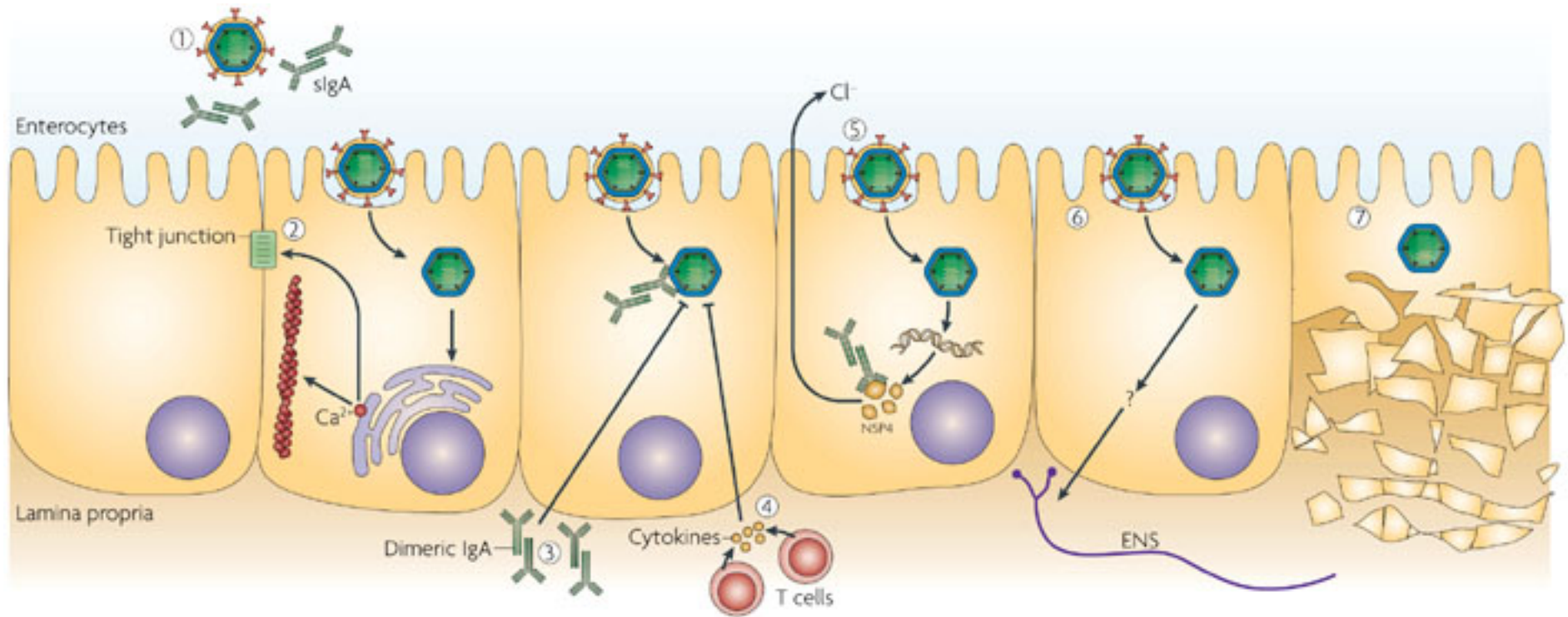
Large quantities of virions released in diarrhea

Immunity to infection depends on IgA in gut lumen

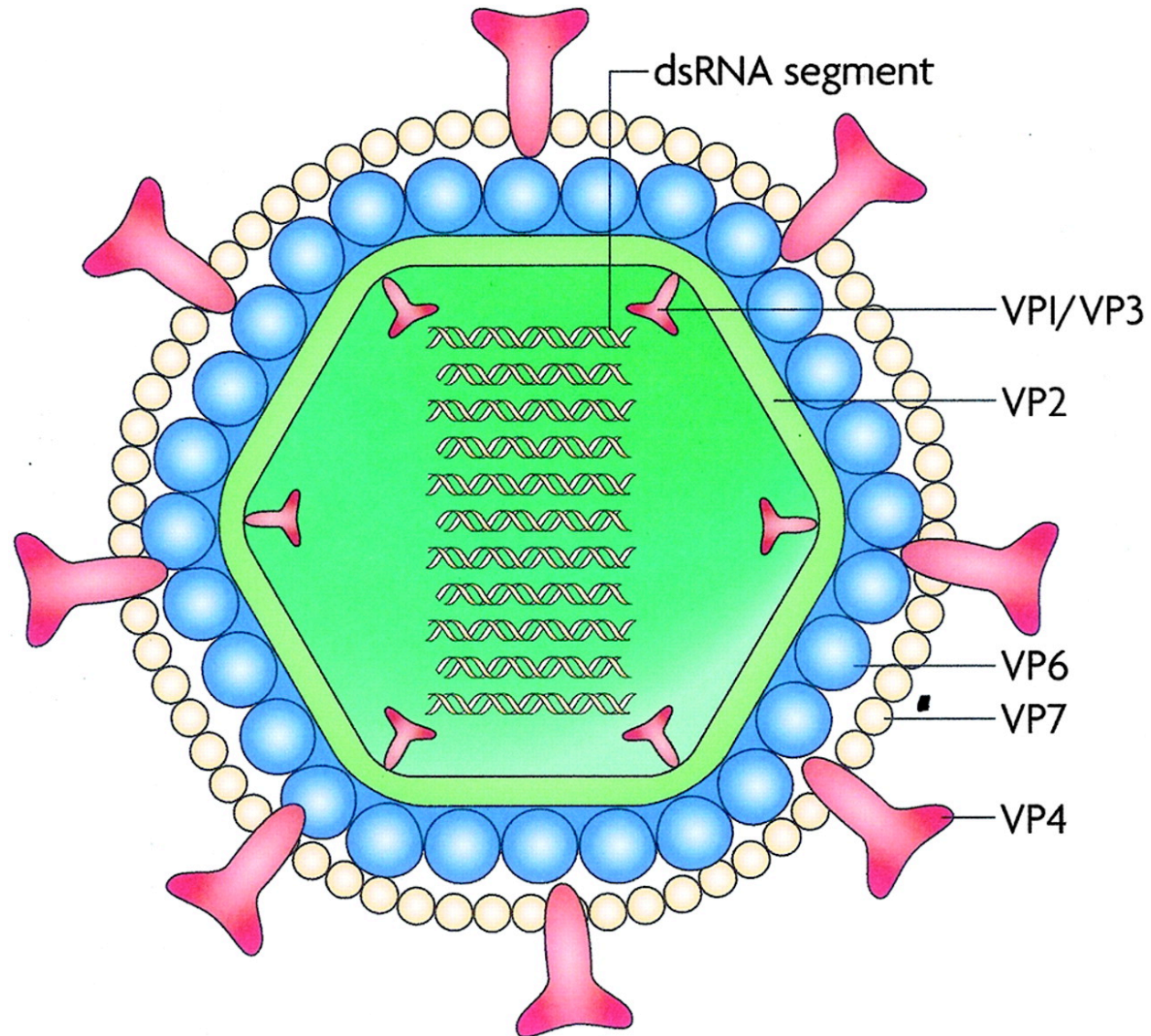
Rotavirus is highly contagious and resistant and, regardless of water quality and available sanitation nearly every child in the world is at risk of infection.



The mechanisms of Rotavirus pathogenesis and immunity



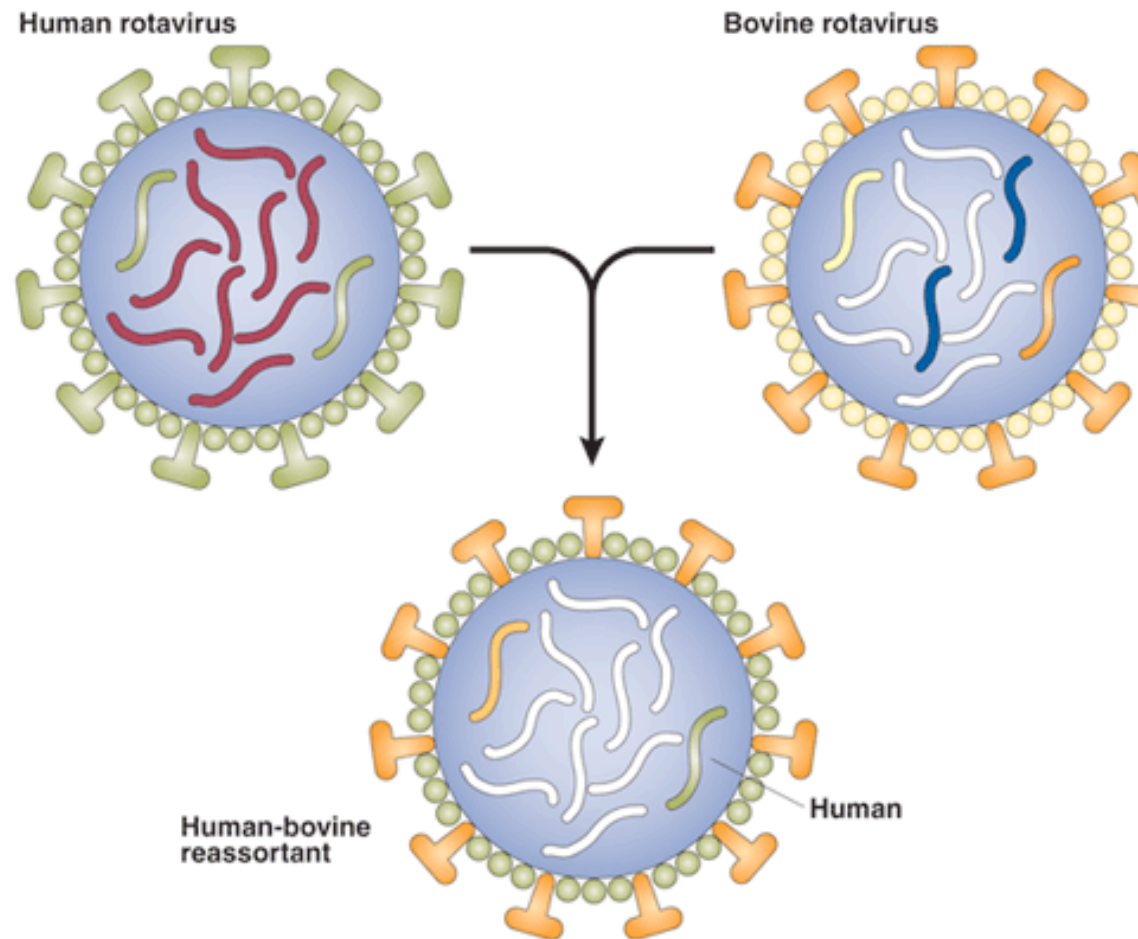
Schematic representation of a rotavirus virion.

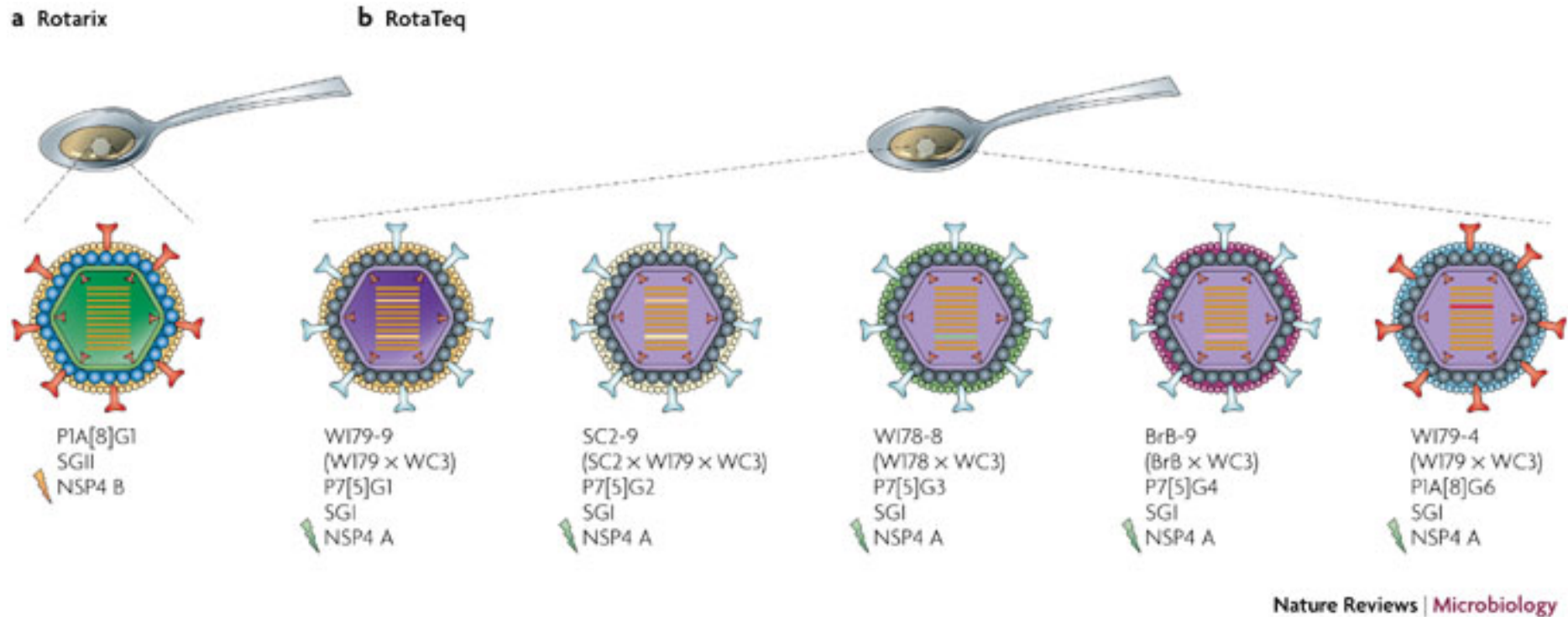


Rotavirus vaccine

- Rotaviruses contain 11 segments of double-stranded RNA.
- Rotaviruses are classified into seven groups (A to G) on the basis of their distinct antigenic and genetic properties. Human infection has been reported with group A, B, and C rotaviruses. Of these, group A rotavirus is the most important, being a significant cause of severe gastroenteritis in children worldwide.
- The two outer capsid proteins, **VP4** and **VP7**, allow classification of rotavirus into **P** and **G** genotypes, respectively. In rotavirus, at least **15 G** genotypes have been recognized by neutralization assay and **27 P** genotypes have been identified by hybridization or sequence analysis. Of these, four rotavirus **G-P** combinations, i.e., **G1P[8]**, **G2P[4]**, **G3P[8]**, **G4P[8]**, and **G9P[8]**, are the most common globally and are therefore the targets for current vaccine development strategies.
- Since effective antirotavirus drugs have not been developed, a rotavirus vaccine would be very useful.

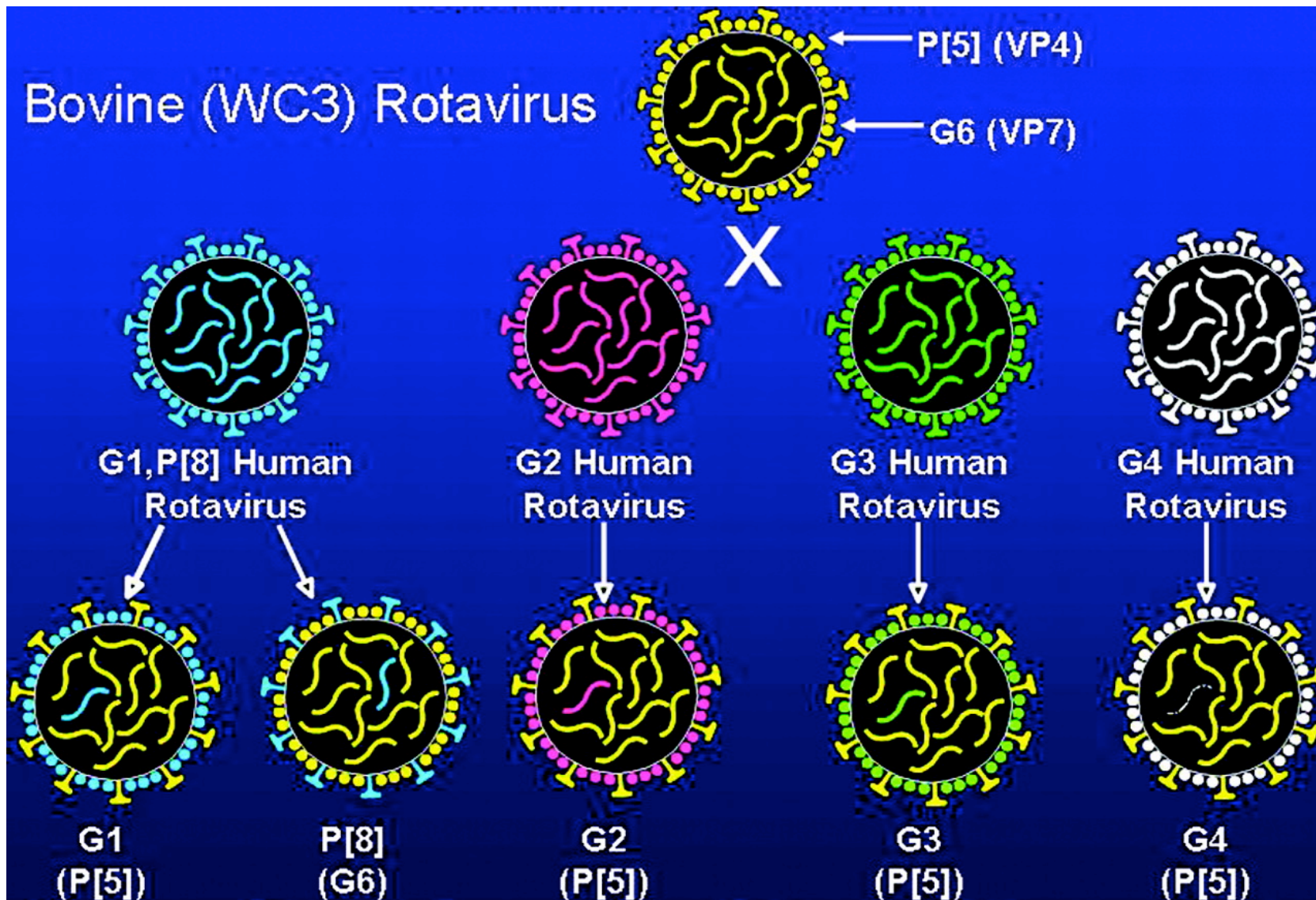
Rotavirus reassortant to generate oral live virus vaccine





a) **Rotarix** is an attenuated human rotavirus vaccine made of a tissue-culture-adapted human P1A[8]G1, VP6 subgroup II and NSP4 geno-group B strain.

b) **RotaTeq** is a bovine (WC3)–human reassortant vaccine. RotaTeq is a live, oral pentavalent vaccine that contains five rotaviruses produced by reassortment. The rotavirus A parent strains of the reassortants were isolated from human and bovine hosts. Four reassortant rotaviruses express one of the outer capsid, **VP7**, proteins (serotypes G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein VP4 (type P7[5]) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein **VP4**, (type P1[8]), from the human rotavirus parent strain and the outer capsid protein VP7 (serotype G6) from the bovine rotavirus parent strain.



RotaTeq is a bovine (WC3)–human reassortant vaccine. RotaTeq is a live, oral pentavalent vaccine that contains five rotaviruses produced by reassortment. Four reassortant rotaviruses express one of the outer capsid, VP7, proteins (serotypes G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein VP4 (type P7[5]) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein VP4, (type P1[8]), from the human rotavirus parent strain and the outer capsid protein VP7 (serotype G6) from the bovine rotavirus parent strain.

RotaTeq®

[Rotavirus Vaccine, Live, Oral, Pentavalent]

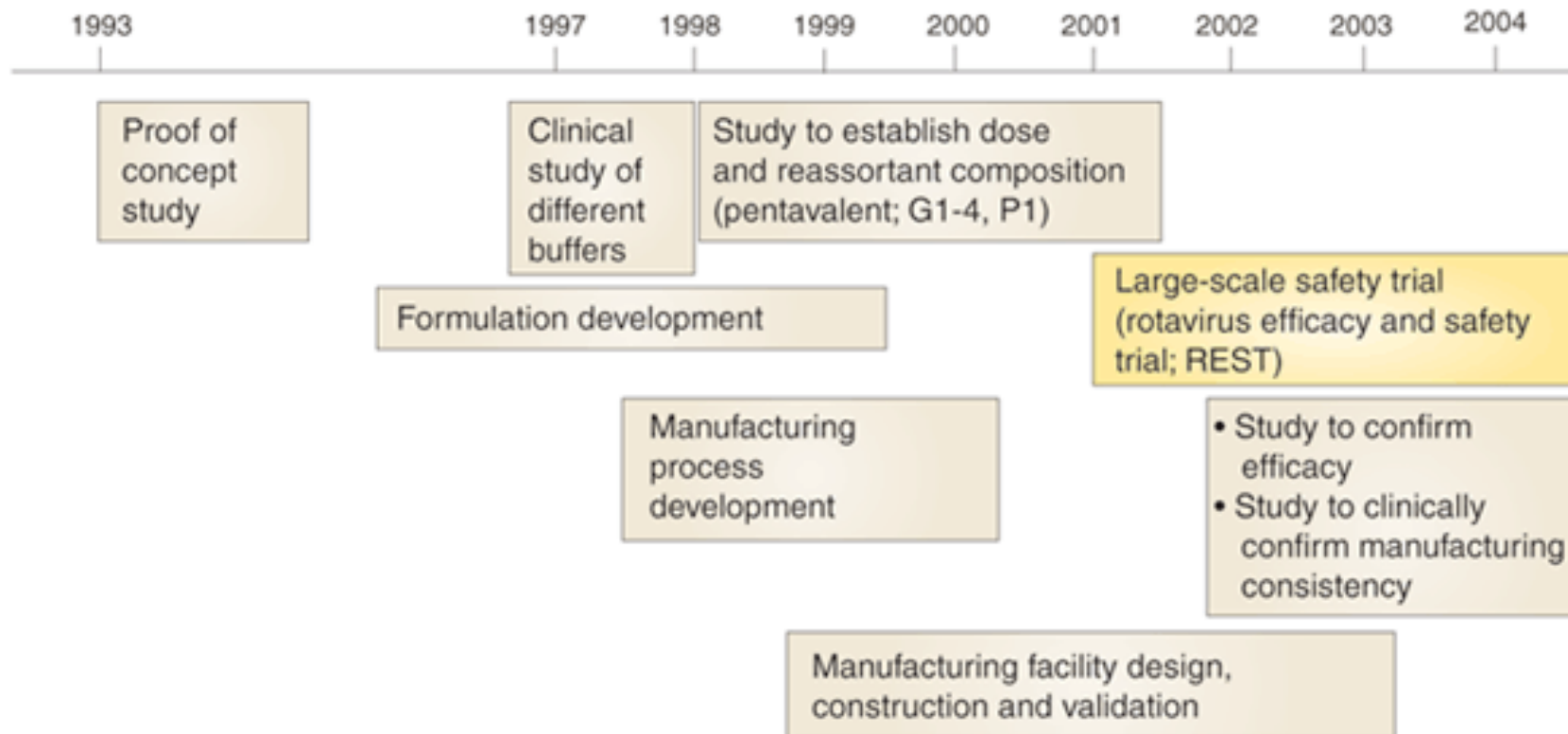
Name of Reassortant	Human Rotavirus Parent Strains and Outer Surface Protein Compositions	Bovine Rotavirus Parent Strain and Outer Surface Protein Composition	Reassortant Outer Surface Protein Composition (Human Rotavirus Component in Bold)	Minimum Dose Levels (10 ⁶ infectious units)
G1	WI79 – G1, P1[8]	WC3 - G6, P7[5]	G1 , P7[5]	2.2
G2	SC2 – G2, P2[6]		G2 , P7[5]	2.8
G3	WI78 – G3, P1[8]		G3 , P7[5]	2.2
G4	BrB – G4, P2[6]		G4 , P7[5]	2.0
P1[8]	WI79 – G1, P1[8]		G6, P1[8]	2.3

DESCRIPTION

RotaTeq* is a live, oral pentavalent vaccine that contains 5 live reassortant rotaviruses. The rotavirus parent strains of the reassortants were isolated from human and bovine hosts. Four reassortant rotaviruses express one of the outer capsid proteins (G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein (P7) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein, P1A (genotype P[8]), hereafter referred to as P1[8], from the human rotavirus parent strain and the outer capsid protein G6 from the bovine rotavirus parent strain (see Table 1).

Overview of the development of a vaccine against Rotavirus (Rotateq)

Overview of development of RotaTeq



MODERNS VACCINES

- Subunit Vaccines
- Peptide Vaccines
- Genetic Immunization
- Attenuated Vaccines
- **Vector Vaccines, see. Viral vectors**

Some antiviral vaccines currently under development

Virus family	Virus species	Most promising strategies
Arenavirus	Junin	Live attenuated
	Lassa fever	Recombinant vaccinia virus
Calicivirus	Norwalk	Recombinant capsid “ghosts”
Filovirus	Ebola/Marburg	DNA
		Recombinant vesicular stomatitis virus
Flavivirus	Hepatitis C	Recombinant envelope glycoprotein, peptide, polypeptide
	Dengue	Tetravalent attenuated, DNA
Hepadnavirus	Hepatitis B	DNA, recombinant surface antigen including pre-S1/S2 regions; curative hepatitis B vaccine: DNA with CpG motifs
Herpesvirus	Epstein–Barr	Whole inactivated, subunit (gp350)
	Herpes simplex 1, 2	Recombinant polypeptide, vectored
Orthomyxovirus	Influenza A	Vectored, DNA, peptide
Paramyxovirus	Measles	Vectored, DNA
	Parainfluenza	Live attenuated
	Respiratory syncytial	Subunit G and fusion protein, DNA, vectored, peptide
Parvovirus	Parvovirus B19	Empty capsid with VP1 protein
Picornavirus	Enterovirus 71	Whole inactivated
Reovirus	Rotavirus	Live reassortants of simian or human origin
Retrovirus	HIV-1, 2	Recombinant polypeptide, DNA, vectored, chimeric
Unclassified	Hepatitis E	Recombinant protein ORF2