Methods for vaccine production

Development of Drugs and Heath Products Master
TU08 Biotechnology

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Vaccines are biopharmaceutical products unlike any others

Vaccines:

Suspensions of killed or attenuated microorganisms (bacteria, viruses, fungi, protozoa), antigenic proteins, synthetic constructs, or other bio-molecular derivatives, administrated for the prevention, amelioration, or treatment of infectious and other diseases (WHO)

Goal: Safe reproduction of the immune response elicited by a pathogen

➔Induction of a memory immune response that is activated upon contact with the virulent wild-type pathogen

Biodrug:

Drug substance produced by or extratcted from a **biological source**
Well characterized & described **manufacturing and purification process**
Characterization and **quality** assessment need a **combination** of physical, chemical and biological assays

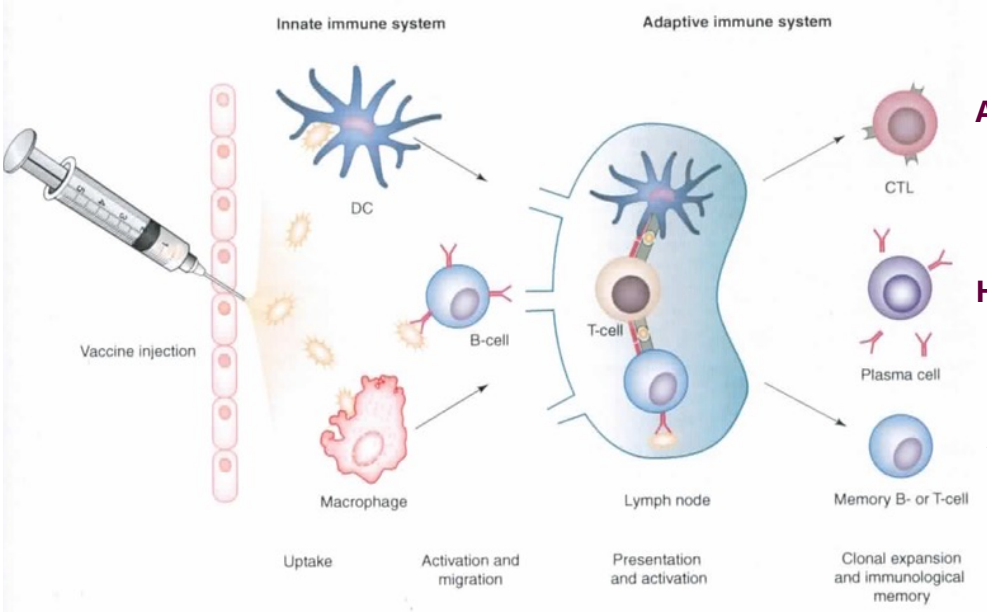
EU definition of Biologic (Directive 2001/83/EC as amended, Annex 1 Active substance 3.2.1.1.b)

- Long development
  - Long clinical experimentation
  - Complex manufacturing process
  - Highly regulated quality and control environments for maximum safety

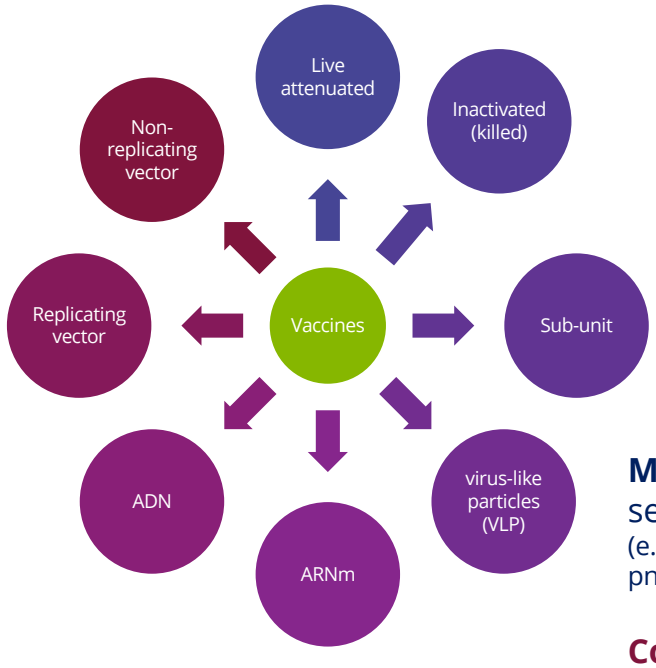
- Preventive approach
  - Healthy individuals (including paediatric population)➔Benefit/risk ratio
  - Perception of benefit is not visible (not immediate)
  - Large treated populations + paediatric populations
  - Side effects not accepted

General vaccine mechanism of action

Goal: to induce specific and adaptive immune responses to protect from a micro-organism infection: bacteria (extra- cell) or virus (intra-cell)
= Active immunization



Different vaccine strategies



Purified antigen, recombinant antigen, polysaccharidic, conjugated

Multivalent vaccines = targeting several strains of the same pathogen
(e.g. 23-valent vaccine prevents pneumococcal infection)

Combined vaccines = targeting several pathogens
(e.g. DT-Polio prevents against 3 diseases: diphtheria, tetanus and polio)

Not all strategies are commercially available

Commercialized vaccines

Killed vaccines(inactivated, inert)

Typhoid
Cholera
Plague
Pertussis
InfluenzaPolio (injectable)
Japanese encephalitis
A Hepatitis

Live attenuated vaccines

Smallpox
BCG
Rage
Yellow Fever
Polio (oral Sabin)
Measles
Mumps
Rubeole
Adenovirus
Chickenpox
Rotavirus
Japanese encephalitis (inactivated)

Sub unit vaccines

Recombinant:
- B Hepatitis
- Papillomavirus
- Pertussis (acellular)

Purified:
Polysaccharidic:
- Meningococcus
- Pneumococcus
- Typhoid

Conjuguated:
- Hib
- Meningococcus
- Pneumococcus

Anatoxin:
- Tetanus
- Diphteria

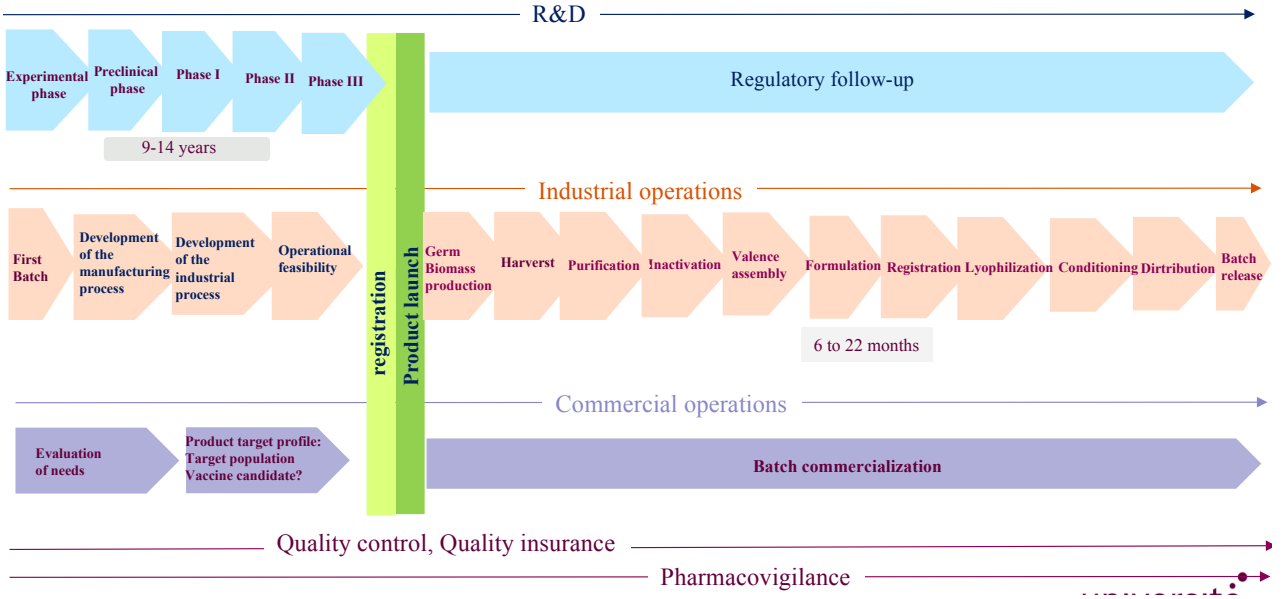
ARNm
Non-replicable vector COVID-19

General scheme for a vaccine development

Average development time : 12 years

Average of global investment: >0.5 Billions €

70% time line is for Quality control



General scheme for vaccine production
(industrial operations)

USP
Germ Biomass Production

DSP

Clarification/Concentration:
Filtration
Chromatography
Purification
Chromatography
Précipitation

No inactivation step for live attenuated vaccines

Harvest → purification → inactivation → Filtration

Centrifugation
Filtration
Keep supernatant OR cells

- Solvent/detergent:
Hydrogen peroxide
Formaldehyde,
Glutaraldehyde
- Pepsin pH4
- Damp heat

Formulation

- **Purpose :**
  - **Maintenance of the structure and stability of the active substance**
  - **Vaccine increased and sustained stability**
  - **Increasing the potency of the vaccine with added adjuvant Minimising adverse effects**

- **Stabilisers: lactose, sorbitol**

- **Adjuvants: The European Pharmacopoeia recommends a maximum amount of 1.25 mg of aluminium (Al^{3+}) per dose (between 0.125 and 0.82 mg for children <2 years).**

Adjuvant category	Adjuvant
Salts	Alum
Oil-in-water emulsions	MF59 (squalene, Span 85, polysorbate 80)
	AS03 (squalene, DL-α-tocopherol, polysorbate 80)
	AF03 (squalene, Brij 76)

Filling, lyophilisation, packaging

VIAL

Cleaning → Sterilisation → Filling → Lyophylisation → Capping → Visual inspection /automatic candling → Labelleing

SYRINGE

Sterilisation → Filling → Visual inspection /automatic candling → Labelling

Some vaccines production examples

Example 1: Infanrix Hexa, GlaxoSmithKline Biologicals s.a. , a combined vaccine

1. Diphteric Anatoxin: purified from the bacterial culture ➔Diphteria

2. Tetanic Anatoxin: purified from the bacterial culture ➔ tetanus

3. *Bordetella pertussis* Antigens: purified from the bacterial culture ➔ Whooping cough
   - Pertusic anatoxin (PT)
   - Filamentous Haemagglutinin (HA)
   - Pertactin (PRN)

4. B Hepatitis Surface antigen (HBs Antigen): recombinant protein ➔ HBV

5. Inactivated Poliomyelitis virus : Purified from infected cells and inactivated
   - Type1 strain
   - Type 2 strain
   - Type 3 strain

6. Haemophilus influenzae type b (conjugated to tetanic toxin as a vector protein): purified polyoside from the bacterial strain AND combined with the purified toxin from *Clostridium tetani* culture.

List of excipients
Anhydrous lactose Sodium chloride (NaCl),
Medium 199 containing mainly amino acids, mineral salts, vitamins, etc.
Water for injectable preparations.

May contain traces of formaldehyde, neomycin and polymyxin that have been used during the manufacturing process

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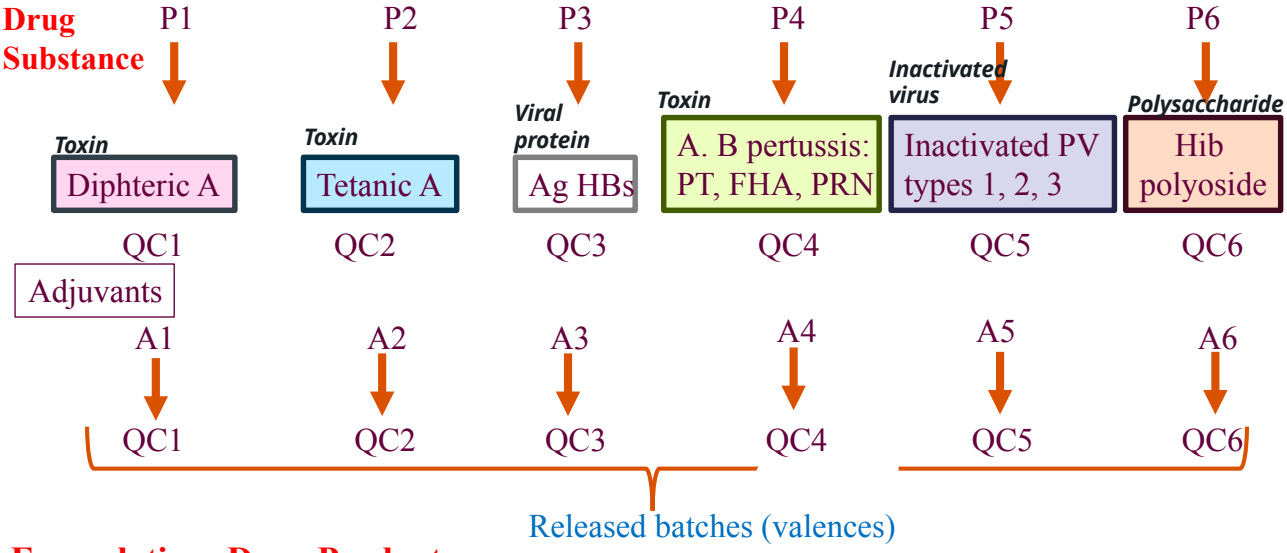
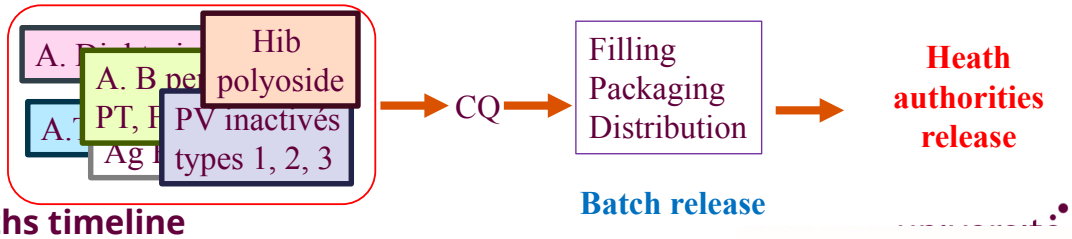
6 Production process

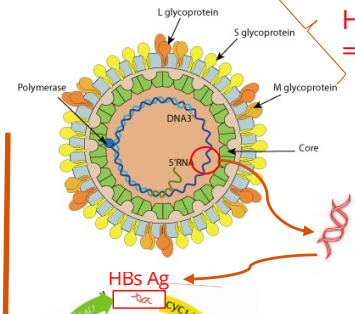


Formulation: Drug Product



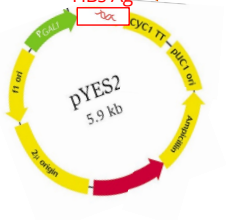
24-36 months timeline

P3: Recombinant HBs Antigen production (HBV)



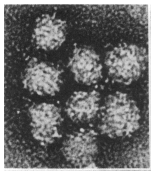
HBS antigen Protein
=L+S+M

Isolated surface antigen
mRNA coding for HBS
Ag Prot
Transformed into cDNA

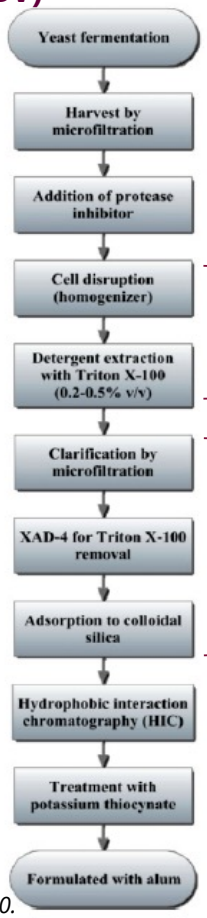


Expression vector
= plasmid bearing the coding sequence for protein HBs Antigen

Yeast transfection



Virus Like Particles
(VLP) production



Production diagram

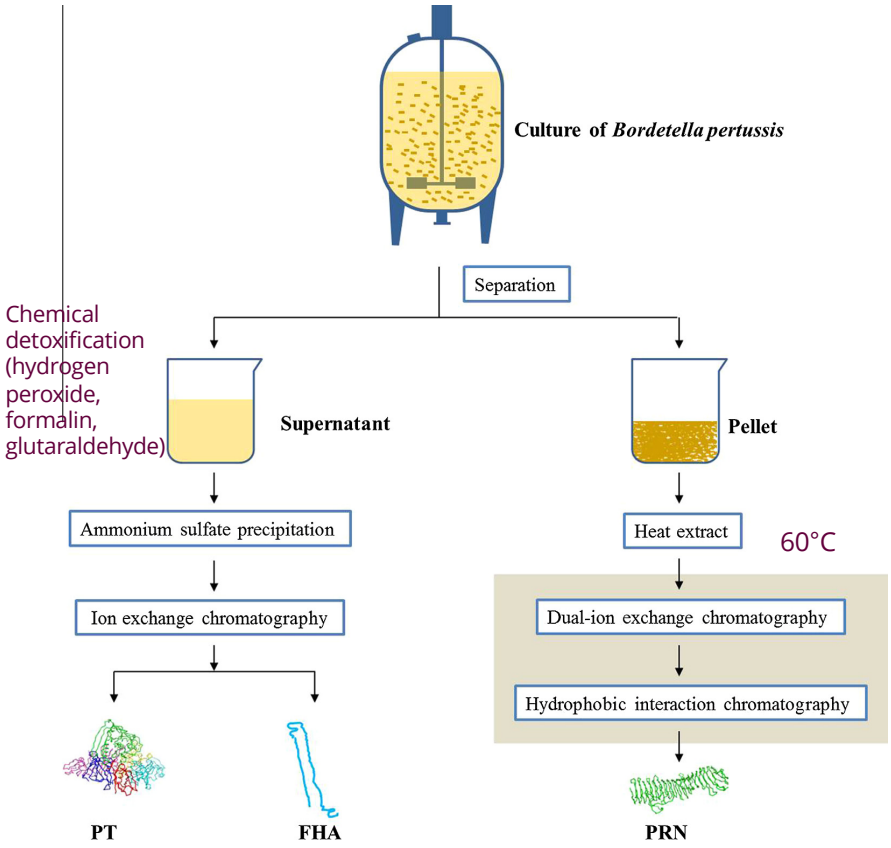
Extraction

Enrichment

Impurities removal

Biotechnol. Bioeng. 2012;109: 1443–1460.

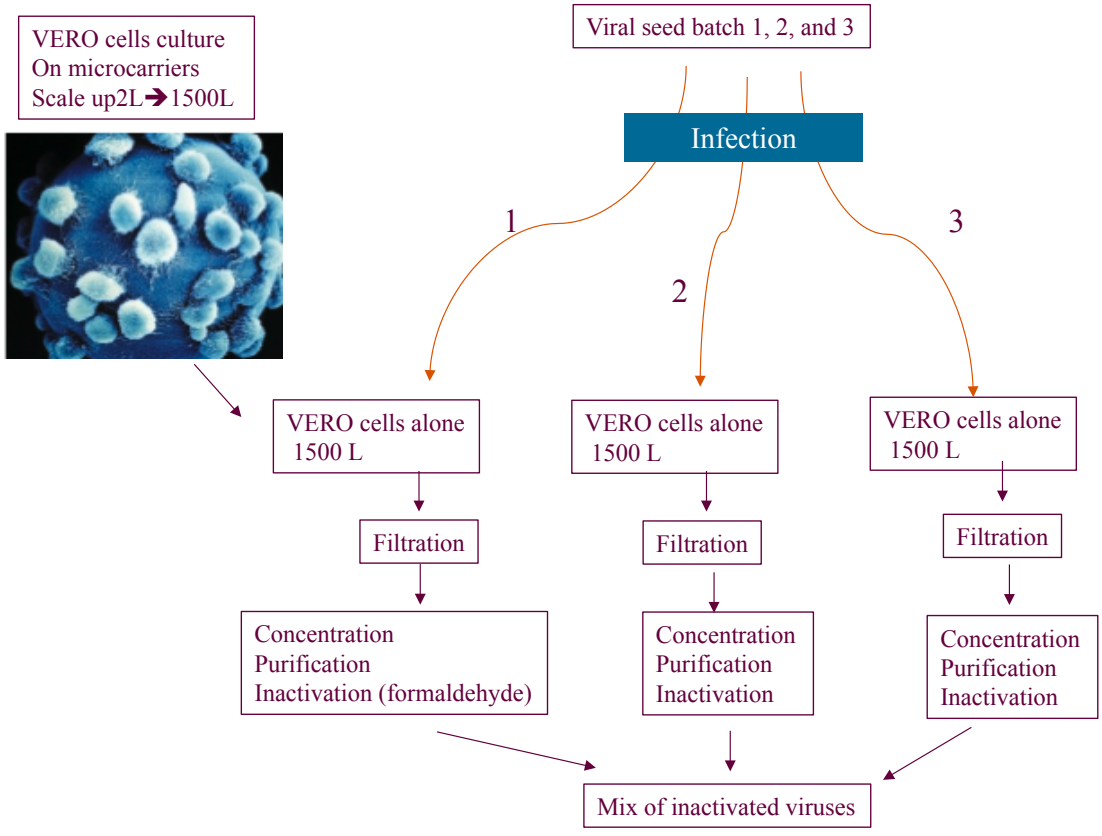
P4: Bordetella pertussis valences production: pertussic anatoxin (PT), Filamentous haemagglutinin (FHA) and pertactin (PRN) = purified proteins



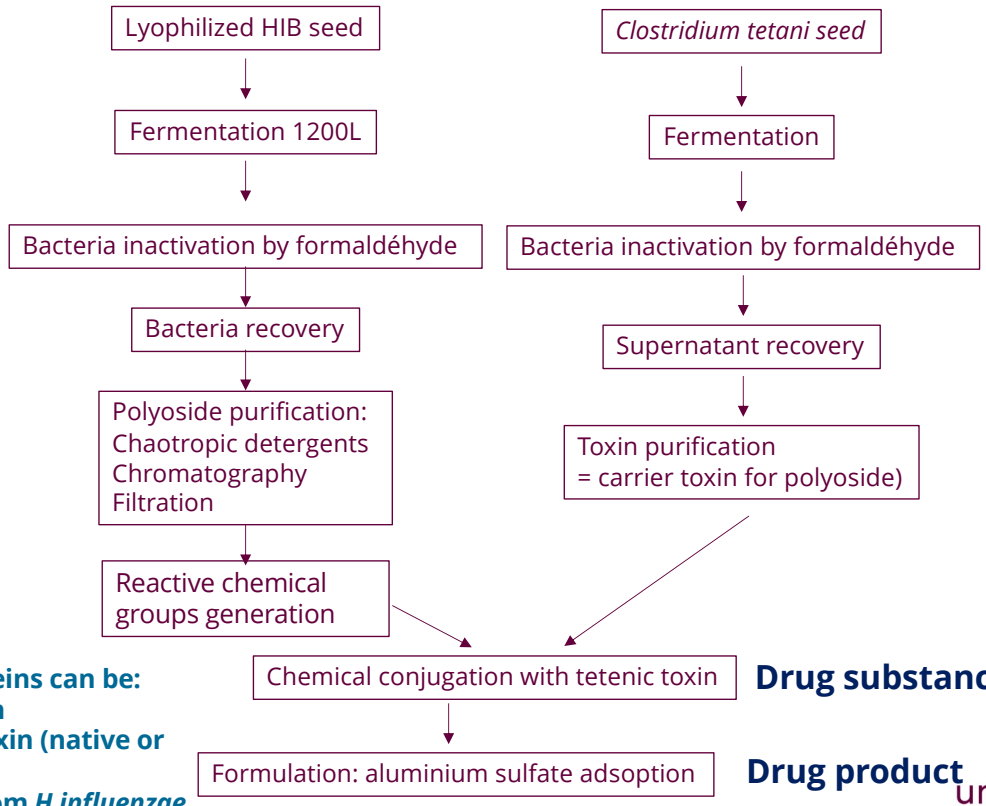
« The initial anionic exchange chromatography concentrated the product from 1.7% to 14.6%, with recovery of 80%. The second cationic exchange chromatography increased the purity to 33%, with recovery of 83%. The final purification was accomplished by hydrophobic interaction chromatography, yielding a purity of 96%. The total recovery of the three columns was 61%. «

Li et al. Vaccine 34 (2016) 4032–4039

P5: Poliomyelitis valences production (3 virus types)



P6: Production/Purification of Haemophilus influenza type b polyoside (Combination of purified polysaccharide with a carrier)



Carrier proteins can be:
Tetanic toxin
Diphteria toxin (native or mutated)
D protein from *H influenzae*

The manufacturing operations for vaccines multivalent vaccines are often carried out in geographically distant sites, or even in different countries (as a result of the evolution of pharmaceutical companies, but also of market access, technical or intellectual property considerations).

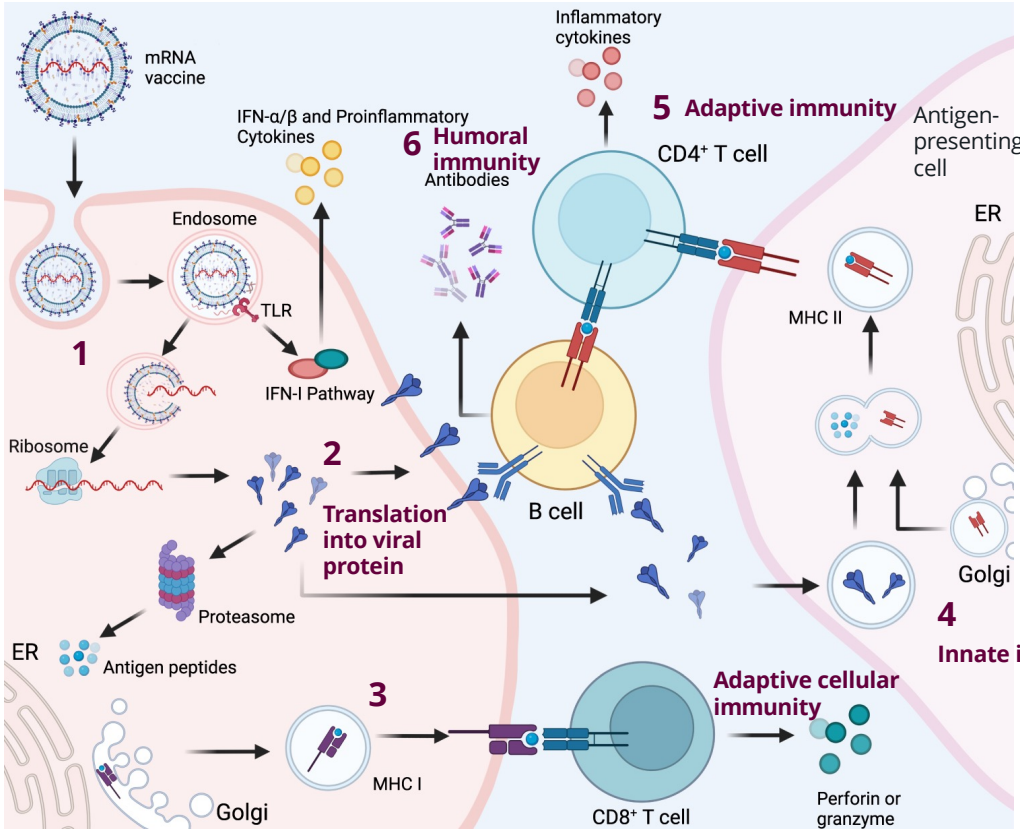
In this context, the management of material flows, product batches, reagents for QC, as well as production areas, becomes fundamental for the success of vaccine distribution.

All the drug substances + adjuvants must be brought together to produce (formulate) the drug product (the vaccine)

Formulation constraints:
Avoiding incompatibility between the different antigens, adjuvants, preservatives
Avoid degradation of epitopes, destabilisation of proteins, aggregation
Avoid induction of "opposite" immune responses (i.e.Th1 vs Th2)

Example 2: mRNA vaccines production

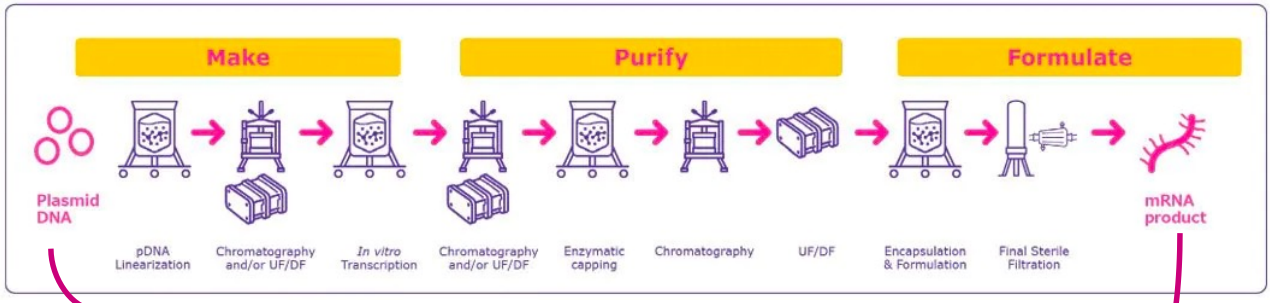
How mRNA vaccines work??



Fang et al. Signal Transduction and Targeted Therapy ***volume 7****, : 94 (2022)*

Example 2: mRNA vaccines production

Overall process



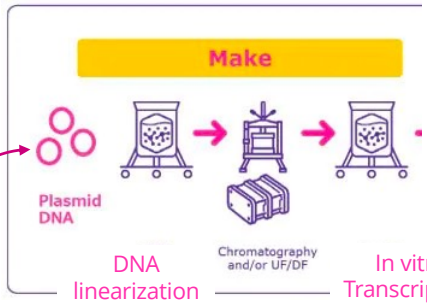
Double-stranded DNA coding for the Drug substance

Drug substance

UTR: untranslated region, for translation efficacy
RDRP: RNA-dependent RNA polymerase: enable self-replication
Immunogen: i.e. viral protein coding sequence

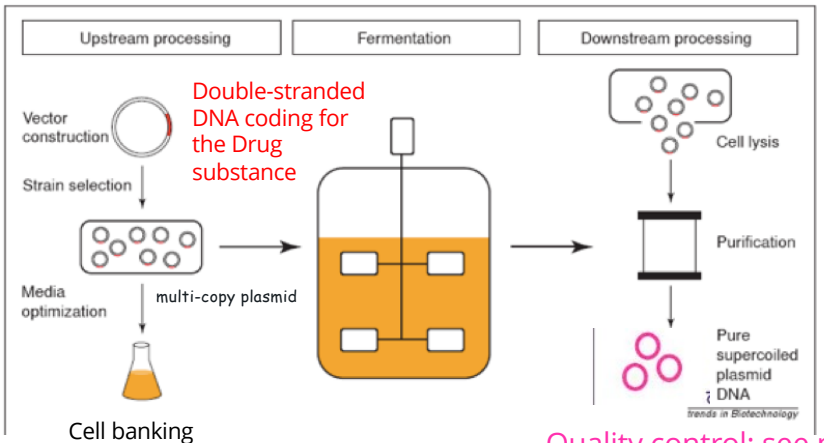
https://www.sigmaaldrich.com/FR/en/technical-documents/technical-article/pharmaceutical-and-biopharmaceutical-manufacturing/vaccine-manufacturing/manufacturing-strategies-for-mrna-vaccines

Step 1: Producing the mRNA



Plasmid DNA production and purification

- putting the cDNA into a plasmid vector
- Bacterial amplification
- plasmid purification



Endotoxins!

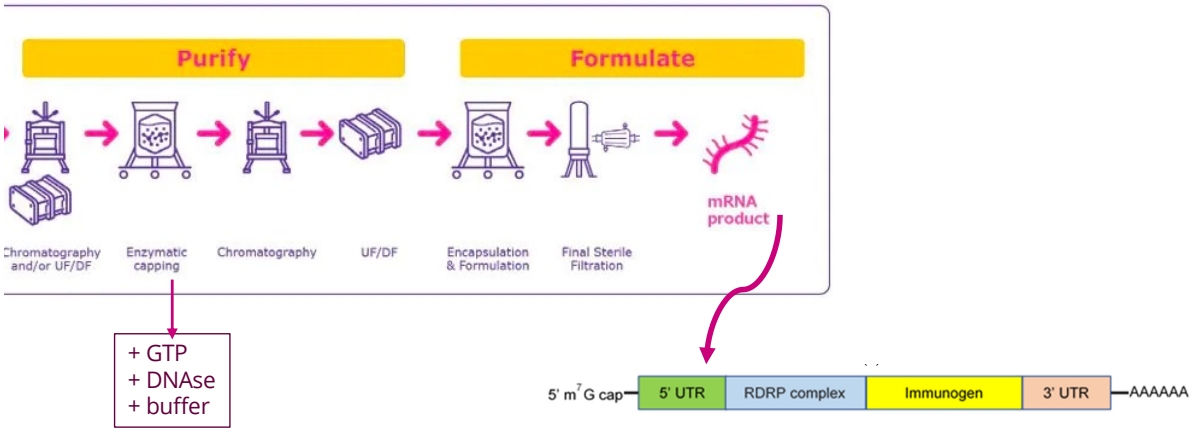
≈ 200-300 mg plasmide /L medium

Quality control: see next slide

Quality control of purified DNA

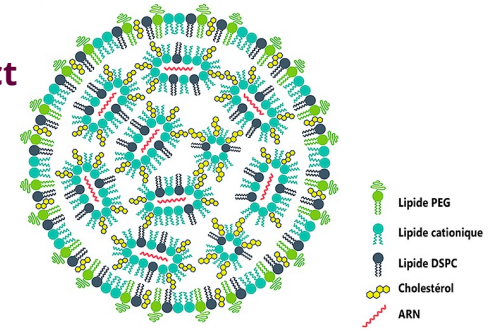
Impurities result	Recomended assay	Expected
Bacterial DNA	Agarose gel Southern Blot PCR	Undetectable <0.01µg/ µg plasmid
RNA	Agarose electrophoresis	Undetectable
Plasmid isoforms	Agarose electrophoresis	<5%
Proteins	Protein dosage SDS-PAGE+ Ag staining	Undetectable
Endotoxins	LAL test	<0.1 U/ µg plasmid
Sterility		No micro-organisms
DNA identity	Restriction map, sequencing	Comaprison with the reference molecule

Next steps: chemical modification, further purification and formulation



Cap: confers mRNA protection and enables maturation and efficient translation

Formulation
➔ Drug product



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Recommandations for vaccine manufacture and control

✓ World Health Organization. « Guidelines for Independent Lot Release of Vaccines by Regulatory Authorities ». In: WHO Experts Committee on Biological Standardization. Sixty-first report. Geneva, WHO, 2012, Annex 2, WHO Technical Report Series, no. 978.

« Recommendations to assure the quality, safety and efficacy of ... »
- poliomyelitis vaccines (inactivated)
- tetanus vaccines (adsorbed)
- diphtheria vaccines (adsorbed)
- haemophilus influenzae type b conjugate vaccines
- recombinant hepatitis B vaccines
- acellular pertussis vaccines

✓ Quality control are developped in the European Pharmacopeia

✓ Standards controls are provided by EDQM

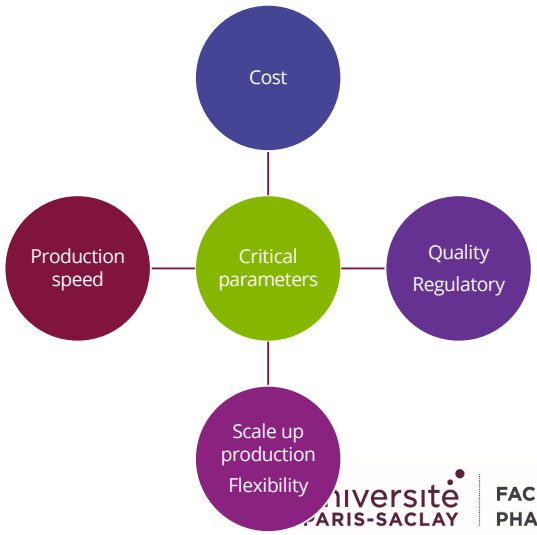
edqm European Directorate for the Quality of Medicines & HealthCare

Conclusion

Vaccines are sensitive bioproducts: their manufacture involves raw materials of biological origin and a complex and variable process. Their marketing conditions are reinforced through a release process by a national authority in addition to the control generally carried out by the manufacturer.

The release control of each batch by medical authorities, in parallel with the control carried out by the manufacturer, is an additional guarantee of the safety of the vaccines.

« For every US$ 1 spent on vaccination against diseases associated with 10 antigens in low-income and middle-income countries, the estimated return on investment for society is US$16 due to direct savings on healthcare and increased productivity »



To go further, a reference to read....

Vaccine Process Technology

Jessica O. Josefsberg,[1] Barry Buckland[2]

Biotechnol. Bioeng. 2012;109: 1443–1460.