Vaccine production: improved supply in the region through collaborations

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Workshop: Global Registration and Vaccine Shortage

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Vaccine types

√ Bacterial vaccines:

- Killed (chemical and/or heat), e.g. whole cell pertussis
- Toxoids, e.g. tetanus and diphtheria
- Atenuated: live modified micro-organisms in which the virulent properties have been modified. They are able to replicate and infect cells in the organism but they do not cause the disease, e.g. BCG
- Subunits: polysaccharide vaccines (meningococcal, pneumococcal), acellular pertussis vaccines

✓ Viral vaccines:

- Killed/ inactivated, e.g. Rabies, polio (Salk)
- Atenuated e.g. YF, JE, measles, rubella, mumps and polio (Sabin)
- Subunits: purified protection conferring antigens, e.g. influenza vaccine

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Recombinant DNA

- ✓ Identification of genes
- ✓ Transfer from one organism to another
- ✓ Expression vector for protein syntesis

Using genetic engineering techniques, a gene codifying for the relevant antigen is isolated and introduced into another organism or cell that will express the protein, which following the required purification steps will constitute the vaccine. Usually such technique will render virus like particles, e.g. hepatitis B and HPV

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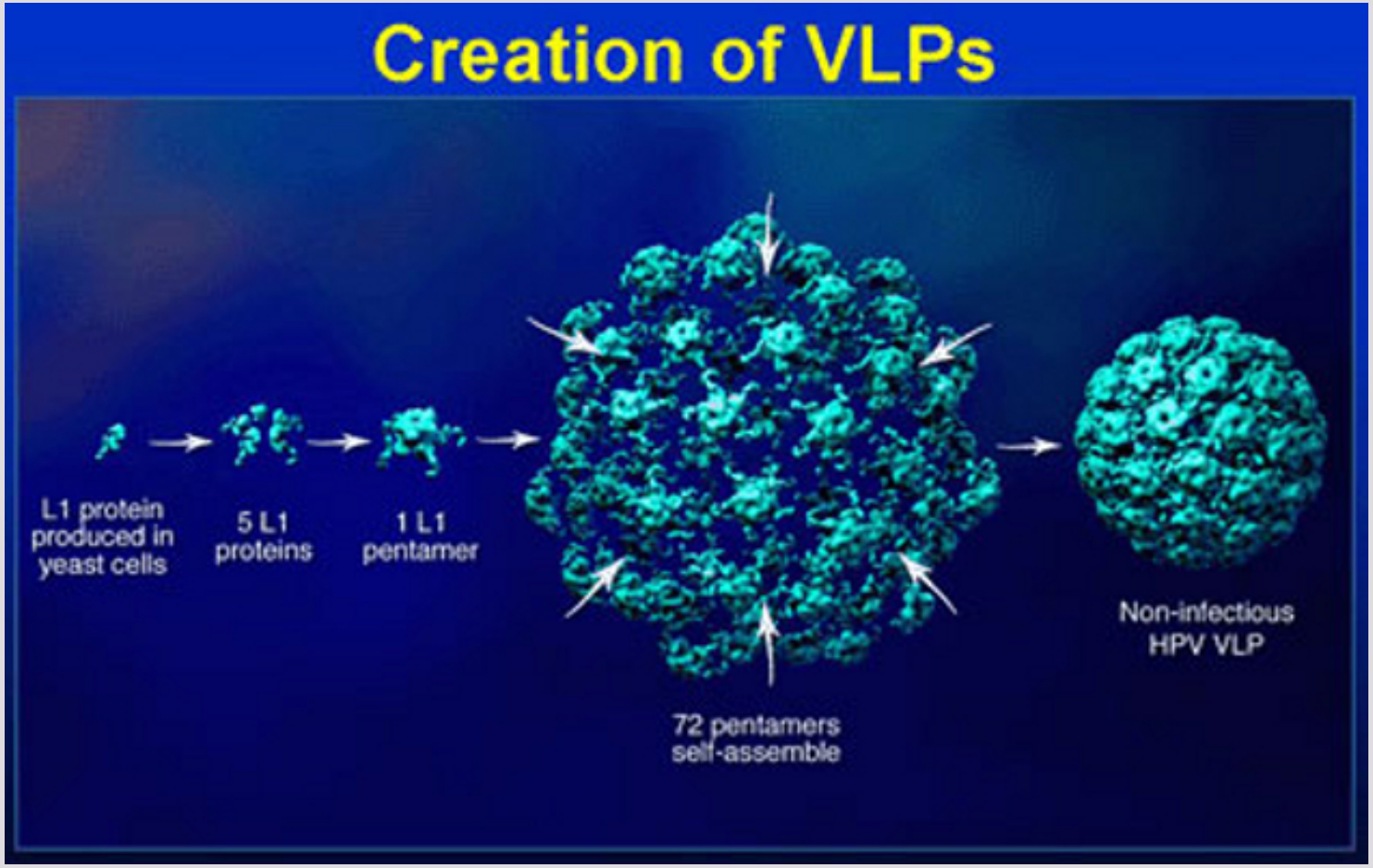
Recombinant HPV L1 VLP Vaccine



Courtesy of Dr. Umesh Shaligram, SIIPL

Live attenuated recombinant vaccine (dengue vaccine)

- ✓ The active substances contained in the CYD-TDV dengue vaccine are 4 live attenuated recombinant viruses representing serotypes 1, 2, 3, and 4.
- ✓ Each monovalent CYD recombinant is obtained separately by replacing the genes encoding the prM and E proteins of the attenuated yellow fever (YF) 17D virus genome with the corresponding genes of the 4 wild-type dengue viruses.
- ✓ The final formulation contains 4.5–6.0 log10 median cell-culture infectious doses (CCID50) of each of the live attenuated dengue serotype 1, 2, 3 and 4 vaccine viruses.

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Conjugate vaccines

Polysaccharide vaccines are not immunogenic in young infants, usually under the age of two. The method of conjugation has overcome this difficulty.

Immune response is improved by chemically linking the polysaccharide to a protein ‘ carrier’. The carrier is often either highly purified tetanus toxoid, or diphtheria toxoid (CRM)

Examples of conjugate vaccines are *haemophilus type b* vaccine, meningococcal A,C, W,Y and also pneumococcal vaccines (PCV 10 and 13)

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Vaccine combinations

Individual antigens can be combined in order to provide protection against several diseases, thus minimizing the number of injections and interventions. Examples of combos are

DTP-Hepatitis B

DTP-Hib

DTP-Hepatitis B – Hib

DTP-Hepatitis B-Hib-IPV

Measles, mumps and rubella

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Summary type of vaccines

- ✓ Bacterial vaccines: Killed, attenuated and subunits
- ✓ Toxoids: D and T
- ✓ Viral vaccines: Killed, attenuated and subunits
- ✓ Recombinant vaccines: Hepatitis B vaccine, HPV
- ✓ Live attenuated recombinant virus vaccine, dengue
- ✓ Conjugated vaccines: Hib, pneumococcal, meningo
- ✓ Combined vaccines

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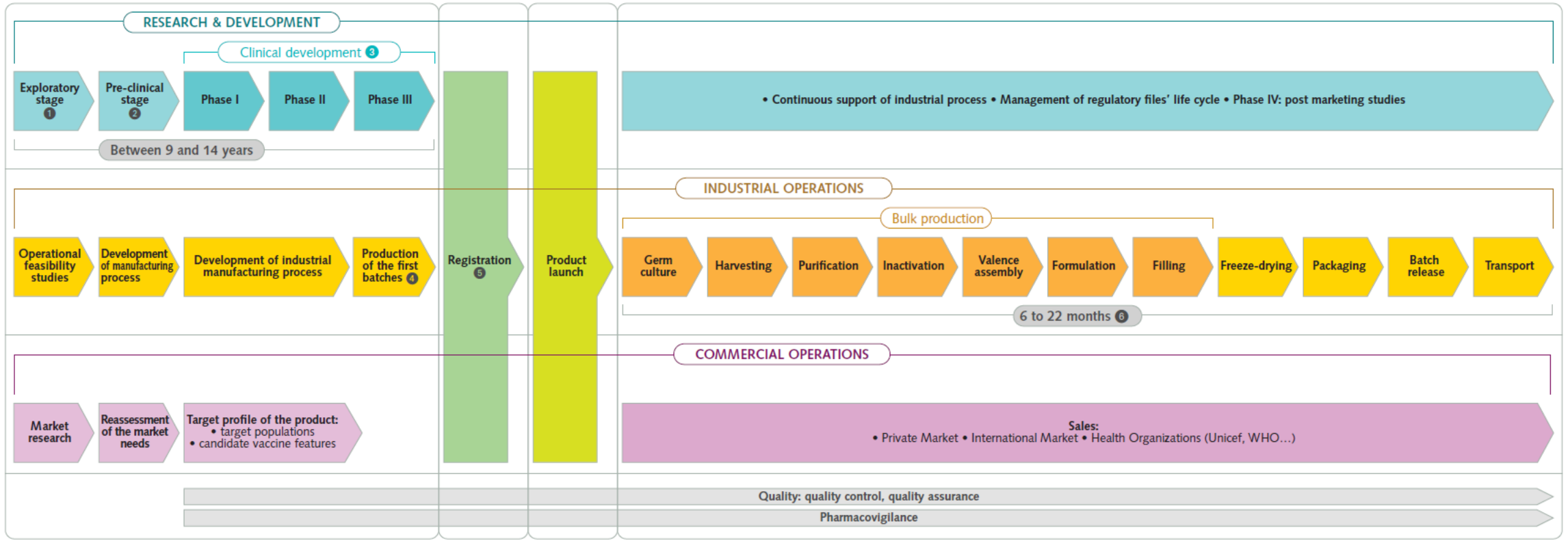
Source: SANOFI Pasteur website

The vaccine development cycle

Average development time for a vaccine: **12 years**

Overall cost to develop a vaccine investment: **More than half a billion US dollars**

70% of a vaccine's production time dedicated to quality control.



Comments

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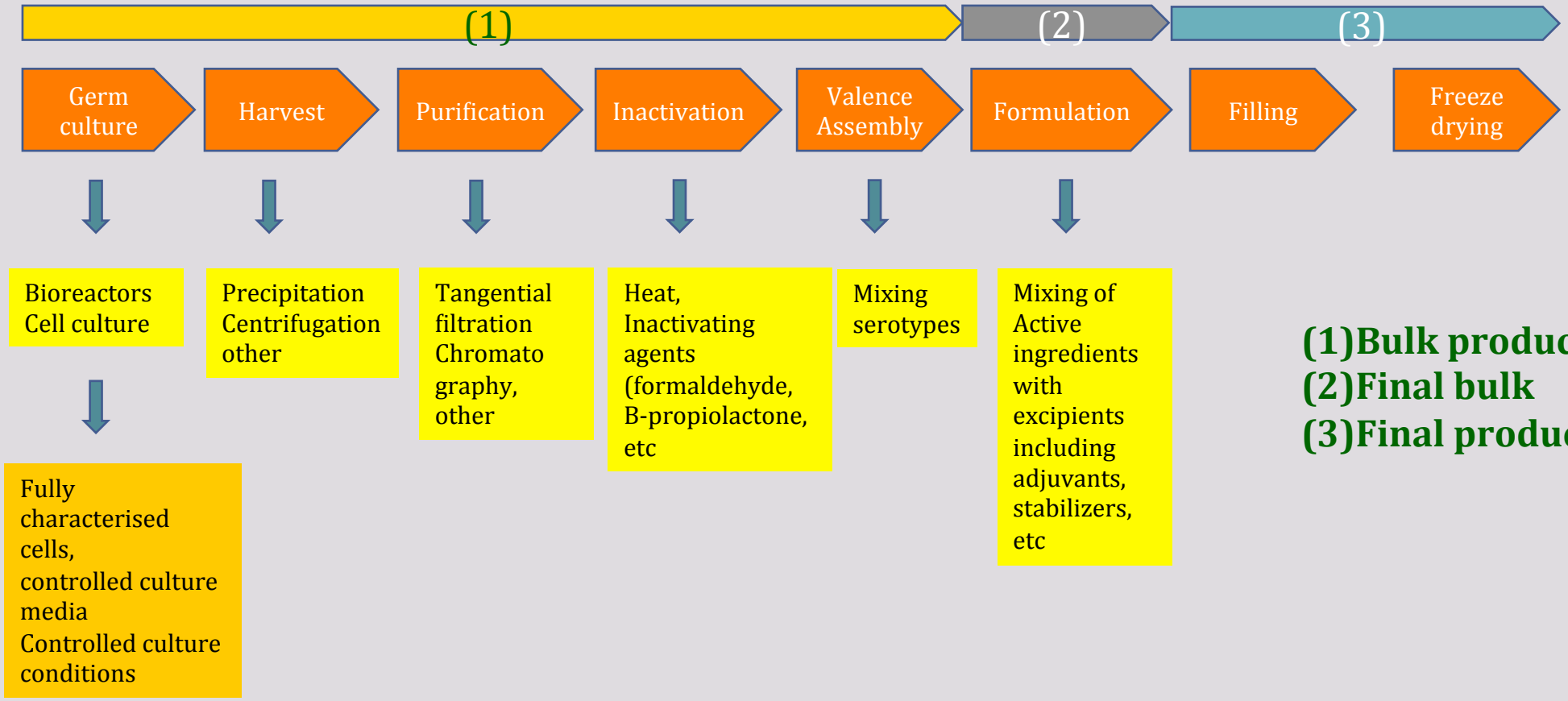
All of the data that have been collected during the preceding stages are gathered in a file and submitted to the health authorities in order to obtain a marketing authorization.

6 The infectious germs are cultured, harvested and purified. After formulation and freeze-drying (which stabilizes the more fragile vaccines), the vaccines are filled, primarily in vials and syringes and then packed. When the manufacturing process is complete, the cold chain must be constantly maintained during all stages, from distribution to vaccine administration to patients.

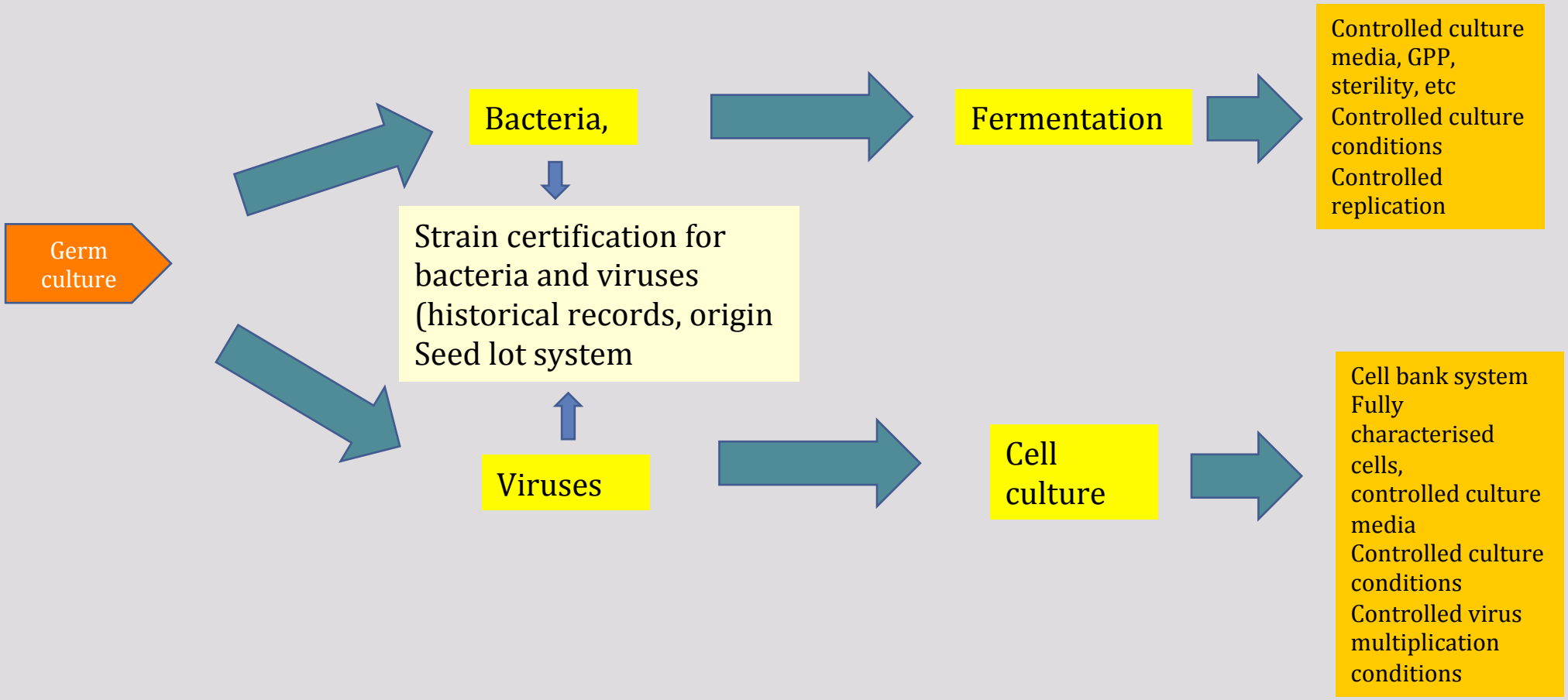
Steps involved in vaccine production



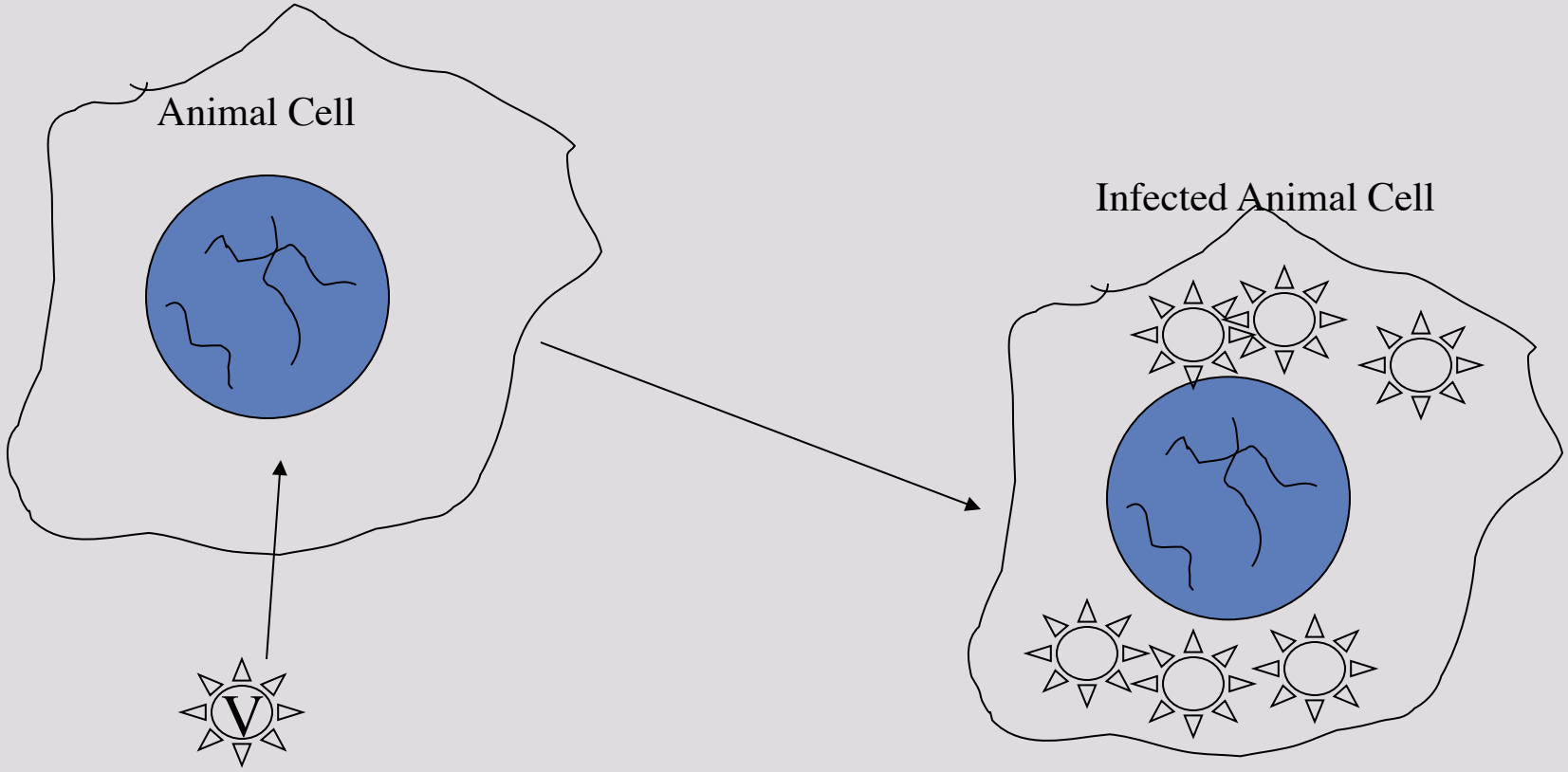
(1)Bulk production
(2)Final bulk
(3)Final product

GMP compliance: process validation for each step, cleaning validation, preventive maintenance, environmental monitoring, data trending and analysis, media fills, line clearance, etc
Quality Controls: IPC, control of intermediates, etc

Steps involved in vaccine production



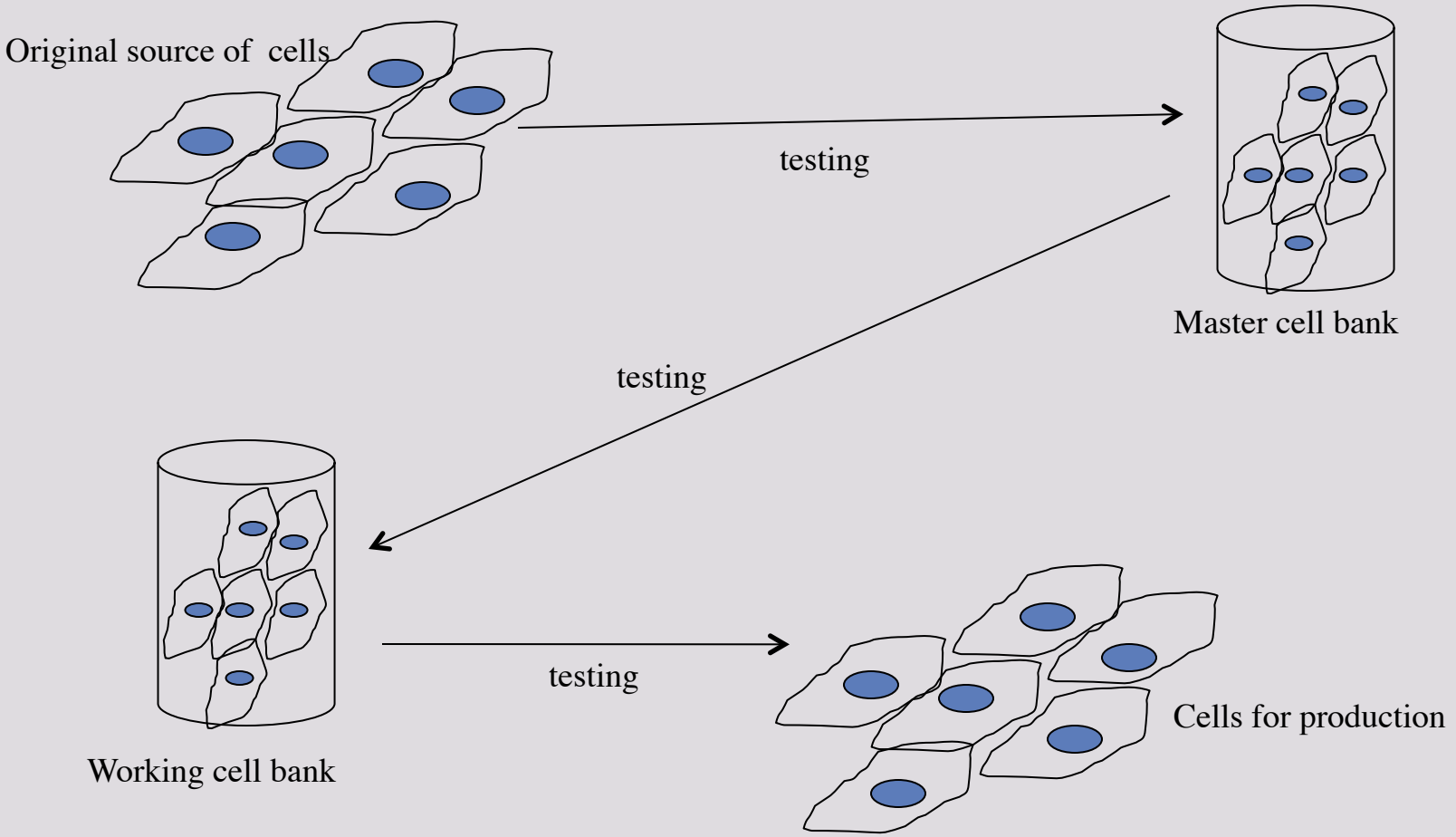
Virus Growth



Viruses cannot grow on their own, they require a host cell for multiplication

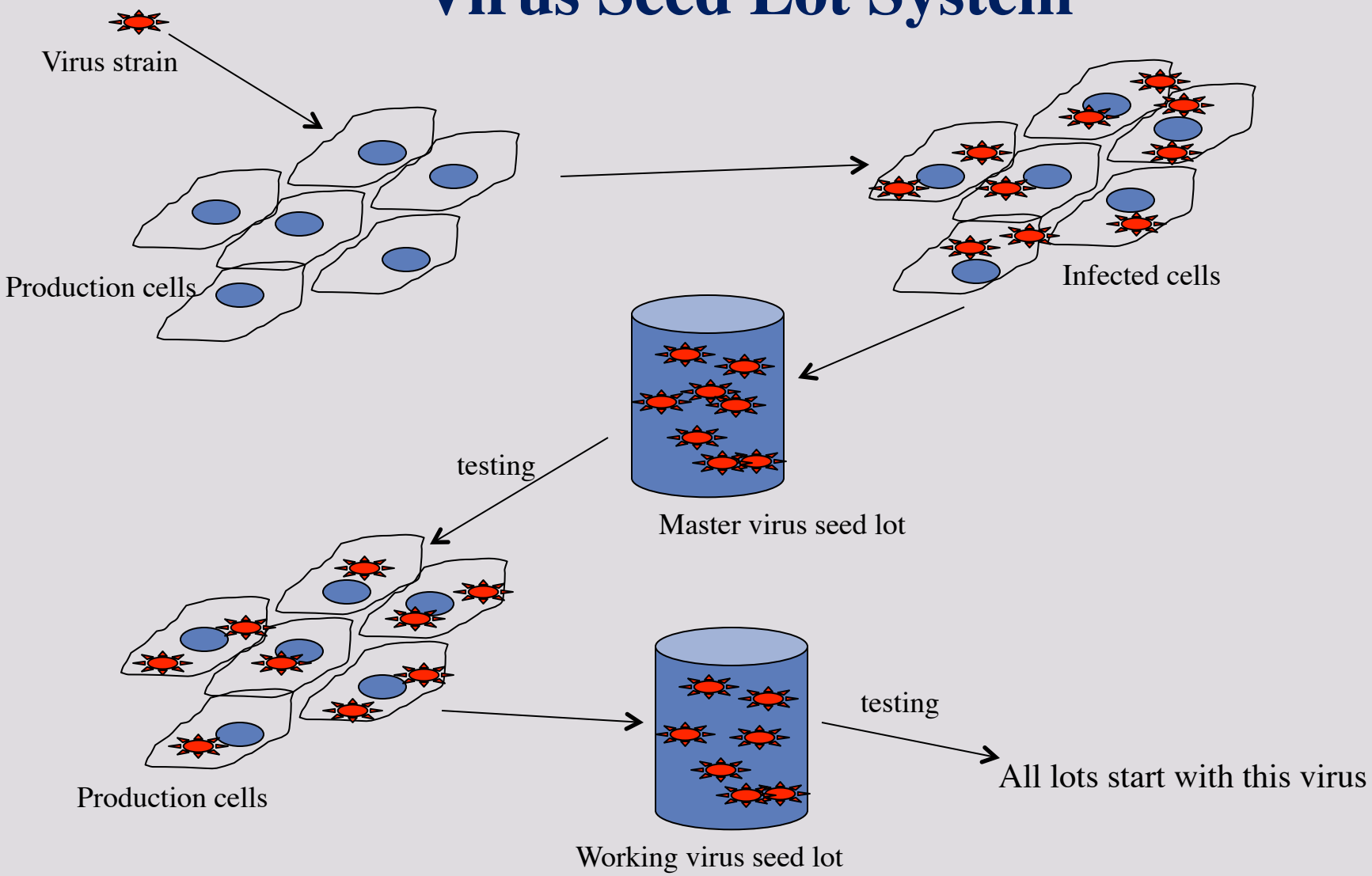
Courtesy: GTN Lot Release Course CDL India

Cell Bank System



Courtesy: GTN Lot Release Course CDL India

Virus Seed Lot System



Courtesy: GTN Lot Release Course CDL India

WHO references

- ✓ TRS No 978, Annex 3: 2013. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. Replacement of annex 1 of WHO TRS 878
- ✓ TRS 999, Annex 2: 2016 WHO good manufacturing practices for biological products Replacement of Annex 1 of WHO Technical Report Series, No. 822
- ✓ Vaccine specific requirements.

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Example of Hib polysaccharide conjugated vaccine

WHO Reference

WHO Technical Report Series, No. 897, 2000

Annex 1

Recommendations for the production and control of *Haemophilus influenzae* type b conjugate vaccines[1]

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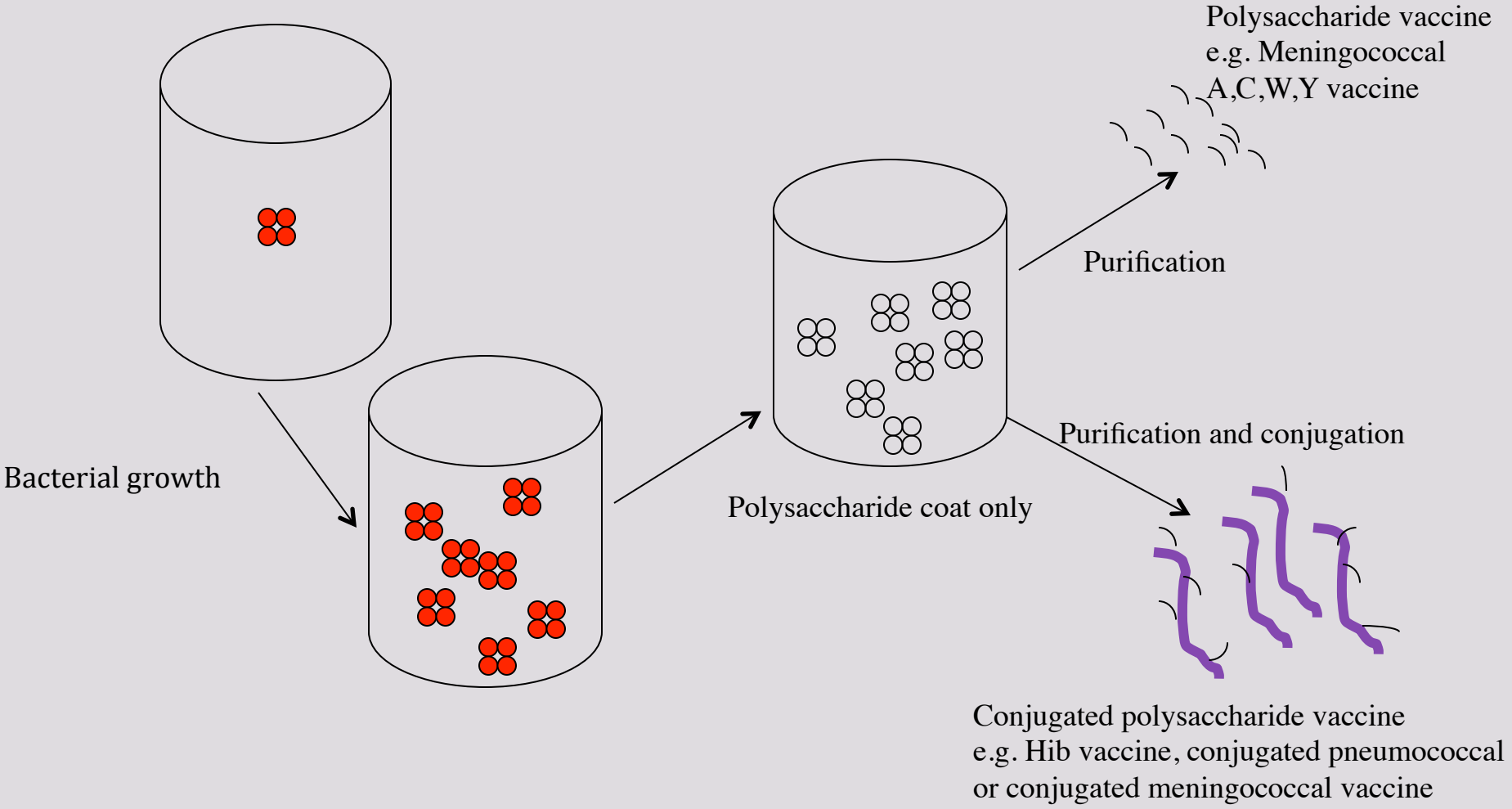
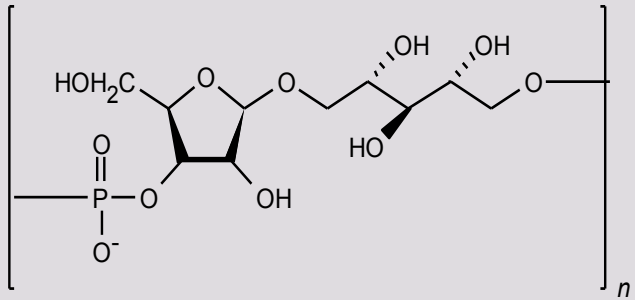
Example of a polysaccharide conjugate and non-conjugate vaccine



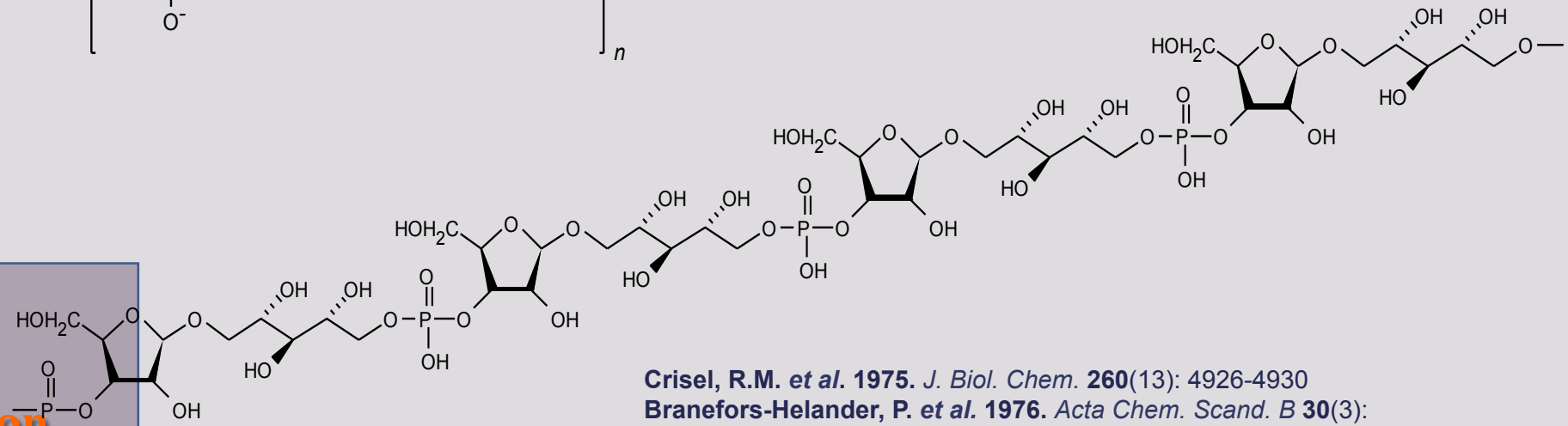
Haemophilus influenzae type b capsular polysaccharide (PRP)



→O)-P-(O→3)-β-D-Rib*f*-(1→1)-D-Ribol-(5→

$C_{10}H_{19}O_{11}P$ = 346.228

$C_{10}H_{18}NaO_{11}P$ = 368.210



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Crisel, R.M. *et al*. 1975. *J. Biol. Chem.* **260**(13): 4926-4930
Branefors-Helander, P. *et al.* 1976. *Acta Chem. Scand. B* **30**(3): 276-277

Formulation of different Hib vaccines

Table A1
Formulation of some currently available *H. influenzae* type b conjugate vaccines[a,b]

H. influenzae polysaccharide material	Polysaccharide per single human dose (µg)	Nature of carrier protein	Protein per single human dose (µg)
Polysaccharide (size-reduced)	25	Diphtheria toxoid	18
Polysaccharide (low relative molecular mass)	10	Diphtheria CRM 197 protein	25
Polysaccharide (size-reduced)	7.5	Outer membrane protein complex of *Neisseria meningitidis* group B	125
Polysaccharide	10	Tetanus toxoid	20

[a] For guidance only.
[b] *H. influenzae* type b conjugate vaccine is a preparation of capsular polysaccharide from *H. influenzae* type b covalently linked to carrier protein

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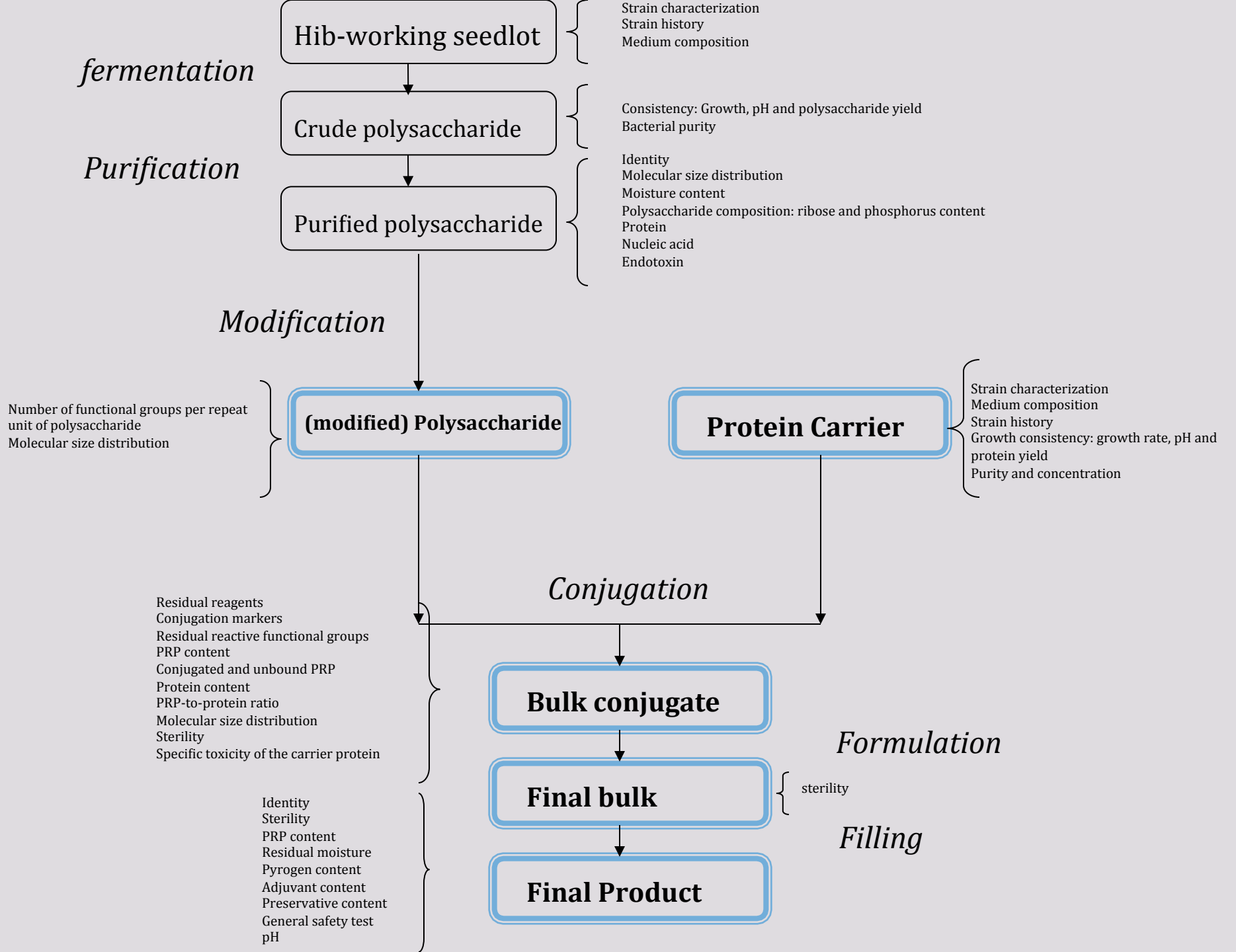
HIB TESTING


Hib-working seedlot
Strain characterization
Strain history
Medium composition
fermentation
Crude polysaccharide
Consistency: Growth, pH and polysaccharide yield
Bacterial purity
Purification
Purified polysaccharide
Identity
Molecular size distribution
Moisture content
Polysaccharide composition: ribose and phosphorus content
Protein
Nucleic acid
Endotoxin
Modification
Number of functional groups per repeat unit of polysaccharide
Molecular size distribution
(modified) Polysaccharide
Protein Carrier
Strain characterization
Medium composition
Strain history
Growth consistency: growth rate, pH and protein yield
Purity and concentration
Conjugation
Residual reagents
Conjugation markers
Residual reactive functional groups
PRP content
Conjugated and unbound PRP
Protein content
PRP-to-protein ratio
Molecular size distribution
Sterility
Specific toxicity of the carrier protein
Bulk conjugate
Formulation
Final bulk
sterility
Filling
Identity
Sterility
PRP content
Residual moisture
Pyrogen content
Adjuvant content
Preservative content
General safety test
pH
Final Product

Control of the Polysaccharide Specifications Summary

Process step	"Component"	Assay	Specification
Hib fermentation	Strain	NMR	Type b
	Seedlot system	x	Consistency
	Culture media	x	No human blood-group antigen-like material and no high-molecular-weight polysaccharide
	Harvest	pH, OD, polysaccharide	Consistency
	Contamination	Gram-smear	Pure
Polysaccharide purification	Identity test	NMR	PRP
	Molecular size distribution	HP-GPC	Consistency
	Moisture content	Karl Fisher	X
	Ribose	Orcinol	>32% dry weight
	Phosphorus	Ames	6.8%-9% dry weight
	Protein	Lowry	<1% dry weight
	Nucleic acid	UV260	<1% dry weight
	Endotoxin	LAL Rabbit test	<10 IU/ µg PRP 1 µg PRP / Kg
Polysaccharide modification	Degree of activation	TNBS	Consistency
	Molecular size distribution	HP-GPC	Consistency

Control of the carrier protein Specifications Summary

Process step	"Component"	Assay	Specification
Fermentation	Seedlot system	x	Consistency
	Culture media	x	Free from substances likely to cause toxic or allergic reactions in humans
	Harvest	pH, OD, Protein	Consistency
	Contamination	Gram-smear	Pure
Protein purification	Purity	LF test, HPLC or SDS-PAGE	D&T-toxoid: >1500 LF/mg protein N CRM197: >90% Outer-membrane complex of MengB: <8% lipopolysaccharide/weight + rabbit test
Protein modification	Extent of derivatization	x	Consistency

Table A2

Methods currently used for conjugation of *Haemophilus influenzae* type b polysaccharide and control of conjugates[a]

Method	Procedure	Assay for conjugation
Reductive amination	Combine carrier protein and aldehyde form of polysaccharide in presence of reducing agent	Formation of unique amino acid and gel filtration
Reductive amination and attachment of spacer linked to active ester	Selective reducing end group activation and coupling to carrier protein through spacer	Gel filtration or SDS-PAGE
Carbodiimide-mediated coupling	Combine reactants in presence of carbodiimide	Gel filtration
Cyanogen-bromide activation of polysaccharide	Addition of carrier protein to cyanogen-bromide-activated polysaccharide	Gel filtration and assay for bound polysaccharide
Thioether bonding	Combine haloacyl polysaccharide with protein thiol	Formation of unique amino acid and assay for bound polysaccharide

[a] For guidance only.

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Control of bulk conjugate Specifications Summary

Process step	"Component"	Assay	Specification
Polysaccharide-protein conjugation	Residual reagents	x	Removal to be confirmed
	Conjugation markers	PRP:Protein	consistency
	Residual reactive functional groups	x	No residual reactive group
	PRP content	Orcinol	X
	Conjugated and unbound PRP	Orcinol, sample pretreatment	<40% free PRP
	Protein content	BCA	X
	Polysaccharide-protein ratio	To be calculated	Diphteria & tetanus toxoid: 0.3-0.6 CRM197: 0.3-0.7 OMC: 0.05-0.1
	Molecular size distribution	HP-GPC	Consistency
	Sterility	Bacterial & mycotic	Pass
	Specific toxicity	guinea-pig test	Absence of specific toxicity

Control of final product Specifications Summary

Process step	"Component"	Assay	Specification
Polysaccharide-protein conjugation	Identity	Immunological test	PRP
	Sterility	Bacterial & mycotic	Pass
	PRP content	Orcinol and/or chromatographic	±20% of stated PRP content
	Residual moisture	Karl Fisher	<2.5%
	Pyrogen content	LAL or rabbit test	Acceptable
	Adjuvant content	Spectroscopy	<1.25 mg aluminium or 1.3 mg calcium per s.h.d.
	Preservative content	UV	Pass
	General safety	General safety test	Animals should survive for at least 7 days
	pH	pH test	Pass
	Inspection	visual	No clumping, lack of integrity and/ or particles

Source: SANOFI Pasteur website

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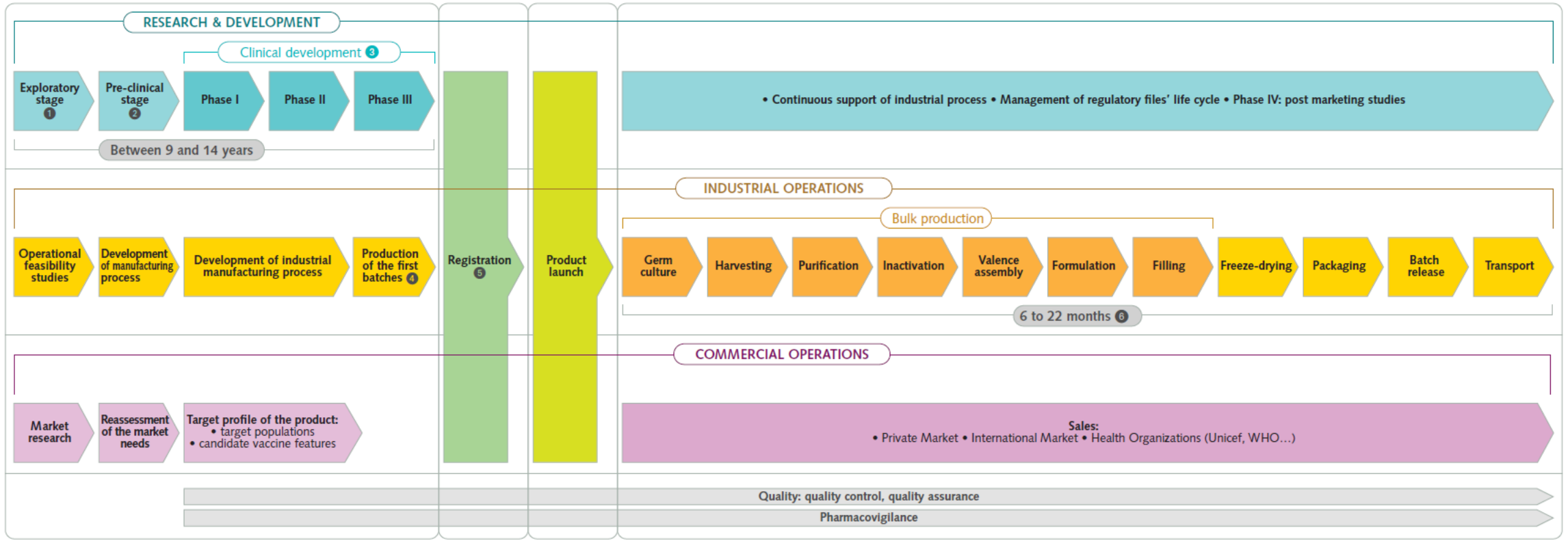

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Key factors to consider before launching vaccine production

- Cost of development
- Time for development
- Cost and difficulties of technological know how, commercial scale, consistency of production, GMP compliance
- Cost and difficulties for testing
- Technical difficulties to get appropriately characterized production strains
- IP related matters
- Cost and timeframe for non-clinical and clinical development
- Registration related issues and timelines
- Size of market for cost recovery and further profit

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Fostering collaborations between DCVMN members

- Information exchange
- Support to acquire specific technologies (freeze drying, cell culture, other)
- Support to acquire testing methodologies
- Sources of strains for vaccine production
- Sources of formulated bulk ready for filling, labelling and packaging
- Sources of concentrated bulk material for formulation, filling, labelling and packaging
- Full transfer of technology from seed

USE THE NETWORK FOR MUTUAL BENEFITS

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THANK YOU

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