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Step-by-Step Transition to In Vitro Potency Testing

Webinar 1: Use of Reference Standards in the Transition from In Vivo to In Vitro Methods for Vaccine Release Testing

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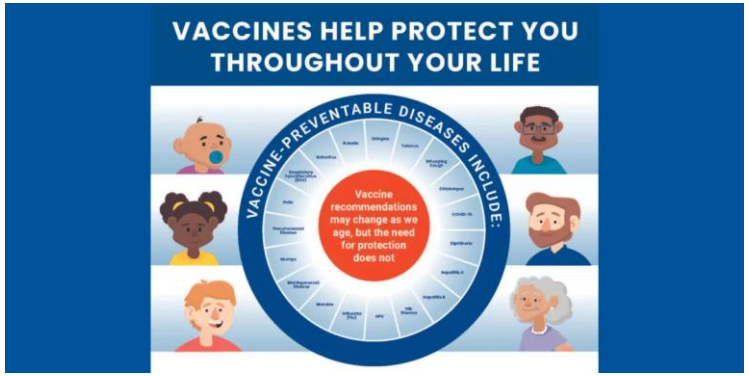
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Vaccine potency

Some definitions

- Vaccine potency assay is a critical component in the development and quality control of vaccines.
- It is a **quantitative measure** of the vaccine's ability to induce a specific **immune response**, which is essential for ensuring vaccine efficacy and safety **in humans**
  - ! not potent enough may not provide adequate protection against a disease,
  - ! too potent may cause adverse reactions.
- Vaccine potency assay is a **quality control laboratory test** used to **routinely** measure the **strength** or **potency** of a vaccine.
- Typically measured by its **ability to protect** against a specific disease or infection.

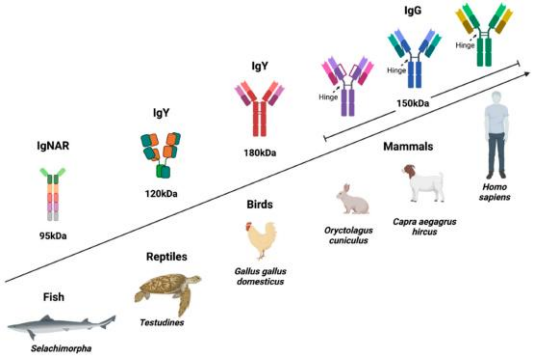


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In vivo bioassay

A model to measure strength



- Vaccine dose setting and efficacy are determined **during clinical study**
- Assumption behind a model is that the measured effect is a relevant property somehow related to clinical outcome
- Animal model used **as proxy** to humane response in **pre-clinical** development …

  …and for some (old / legacy) vaccines, is/was used as **QC release test.**
- Model does not recapitulate human response, but animal model offers **a functional** reaction
- In relative potency, as one model, test sample and standard are compared by an appropriate randomised experimental design and evaluated with a relevant statistical approach
- In a quality control setting, the animal model often displays a high variety of interindividual responses that scientist **struggle to offset** (increase N, introduce reference standard, randomization, training, …)
- **None** of the in vivo QC methods are validated as per **ICHQ2 (R2) principle**

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Legacy

2005-2008 ICHQ8 to 10

1930 1940 1950 1960 1963 1967 1980 1986 1987 1990 ...2024



ELISA...cell based assay...

Disease / Vaccine	First market approval (brand – date – market)	Vaccine technology	Legacy Animal use in routine QC
Diphtheria (component in D/T/dTap combos)	Early toxoid use pre-1930s; modern adsorbed toxoid e.g., "Diphtheria Toxoid Adsorbed" widespread by 1940s US; EU national licenses 1950s–60s	Toxoid (inactivated diphtheria toxin), alum-adsorbed	Historically guinea pig/mouse challenge neutralization; to reduce animal number some use single-dilution assay, and to reduce pain and avoid challange in vivo immunogenicity test (vero-cell based)
Tetanus (component in D/T/dTap combos)	Toxoid use widespread by 1940s US; EU national licenses 1950s–60s	Toxoid (formalin-inactivated), alum-adsorbed	Historically mouse/guinea pig toxin challenge neutralization assays; to reduce animal number some use single-dilution assay.
Whole-cell Pertussis (wP)	First licensed 1940s US (DTwP combinations); varied national dates	Killed whole Bordetella pertussis (inactivated)	Mouse Kendrick protection test; animal use persists in many jurisdictions
Oral Poliovirus Vaccine (OPV)	US licensure 1960s; WHO-prequalified globally in 1960s; many markets	Live attenuated (Sabin strains)	No routine animal test: efficacy; Infectivity/identity and potency by CCID50/tissue culture infectious dose for each serotype. Note that Neurovirulence (safety test) still use animal in many juridiction.
Rabies	HDCV / Imovax Rabies – 1980 – United States	Cell-culture inactivated vaccine; modern rabies vaccines moved from nervous-tissue substrates to tissue-culture production	Historical mouse challenge test is still used in many juridiction as part of rabies vaccine testing.
Tick-Borne Encephalitis (TBE)	FSME-IMMUN – early 1980s – Austria / European market	Inactivated whole-virus vaccine grown in SPF chicken embryo cells	Historical animal challenge test is still used in many juridiction as part of rabies vaccine testing
Hepatitis B	Recombivax HB US 1986; Engerix-B EU 1988, US 1989	Recombinant HBsAg (yeast-derived), alum-adsorbed	In vivo immunogenicity test, replaced by In vitro HBsAg antigenicity ELISA; sometimes competitive immunoassay

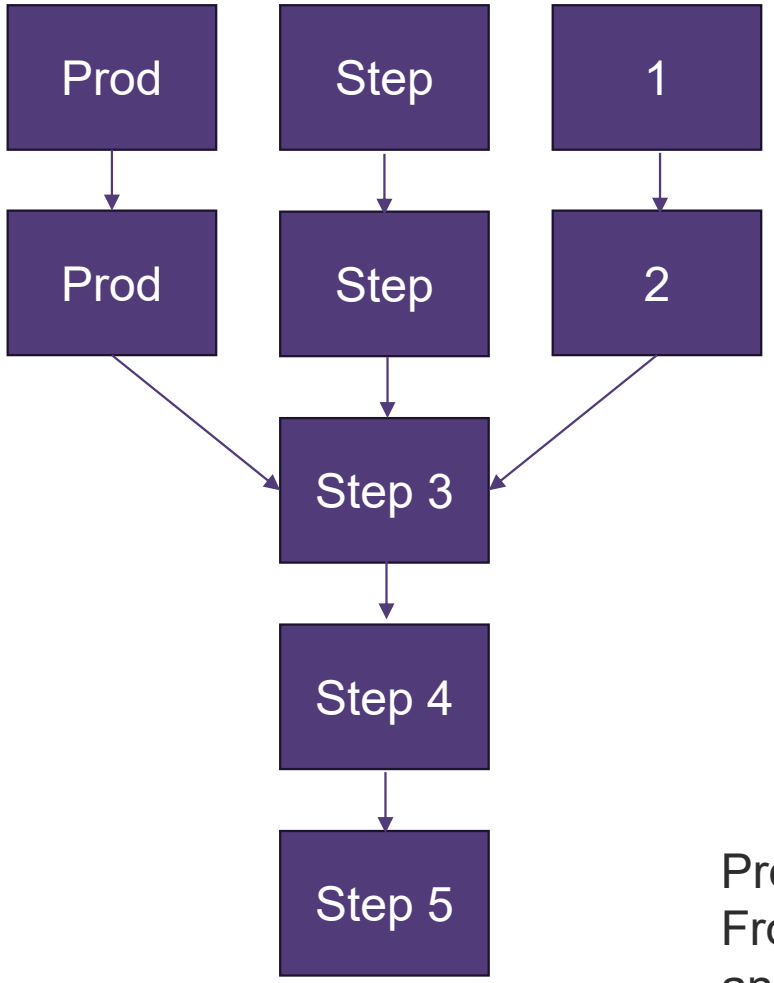
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Disease / Vaccine	First market approval (brand – date – market)	Vaccine technology	Legacy Animal use in routine QC
Inactivated Poliovirus Vaccine (IPV)	IPOL licensed US Dec 21, 1990 (enhanced potency IPV) – US	Inactivated (formalin-killed) polio types	Historically rat serology ELISA test to calibrated reference, replaced by In vitro D-antigen ELISA for each serotype against reference
Hepatitis A	Havrix first licensed EU 1991; US licensure 1995; Vaqta US 1996	Inactivated whole virus, alum-adsorbed	In vitro antigen content/antigenicity ELISA against reference
Acellular Pertussis (aP)	DTaP combos licensed US early 1990s; e.g., Infanrix US 1997	Subunit (purified pertussis antigens: PT, FHA, pertactin, fimbriae), adsorbed	mouse immunogenicity test to be replaced by in vitro antigen content
Human Papillomavirus (HPV)	Gardasil (quadrivalent) US 2006; Cervarix (bivalent) EU 2007	Virus-like particles (recombinant L1 VLPs), alum-based adjuvant	No routine animal test; Antigen-specific in vitro ELISAs per type; often singlex release per antigen; mAb specificity critical
Rotavirus	Rotarix EU 2006; RotaTeq US 2006	Live attenuated (Rotarix monovalent human strain; RotaTeq pentavalent human–bovine reassortants)	No routine animal test; Potency by infectious titer per strain (CCID50) vs. reference
Pneumococcal (conjugate)	Prevnar (PCV7) US 2000; Prevenar/Prevnar13 expansions later; EU 2001	Polysaccharide-protein conjugate (CRM197 carrier)	No routine animal test; historical mouse passive protection used in development; In vitro antigenicity by serotype-specific ELISAs or inhibition immunoassays; free saccharide content; conjugation ratio
Zoster (Shingles)	Zostavax (live) US 2006; Shingrix (recombinant) EU 2017, US 2017	Live attenuated (Zostavax); Recombinant glycoprotein E + AS01B (Shingrix)	Zostavax: no animal test in routine; Shingrix: none; Zostavax: CCID50 potency vs. reference. Shingrix: in vitro antigenicity of gE by ELISA; adjuvant QC
COVID-19	Comirnaty EU conditional Dec 21, 2020; US EUA Dec 11, 2020; Spikevax US EUA Dec 18, 2020; Vaxzevria UK Dec 30, 2020; Jcovden US EUA Feb 27, 2021	mRNA (Comirnaty/Spikevax); Adenoviral vector (AZ, J&J)	No routine animal test; Potency by in vitro cell-based expression assays (reporter/ELISA for spike), RNA content, integrity, capsid infectivity (for vectors)
Malaria	RTS,S/AS01 (Mosquirix) first WHO recommendation 2021; first authorizations 2022–2023 (e.g., Ghana); R21/Matrix-M approvals 2023–2024 in several African countries and India	Recombinant protein (RTS,S VLP with HBsAg) with AS01 adjuvant; R21 also recombinant with Matrix-M	No routine animal test, In vitro antigen content/antigenicity ELISAs vs. reference; adjuvant content checks

Paradigm shift in Potency Testing: From Animal-Based-End-Product Testing to Quality by Design and Consistency Approach

- Traditional potency testing relies on **end-product testing** to demonstrate compliance.
- In contrast, the modern paradigm integrates **Quality By Design** (QbD), deep process understanding, validated analytical methods, and **Consistency approach** to build quality into the product rather than merely testing it at the end.
- Potency assays become one component of an **integrated control strategy** rather than sole determinant of product quality.



CQA 1 to 4

CQA 2 to 5

CQA 2 to 5

CQA 4 & 5

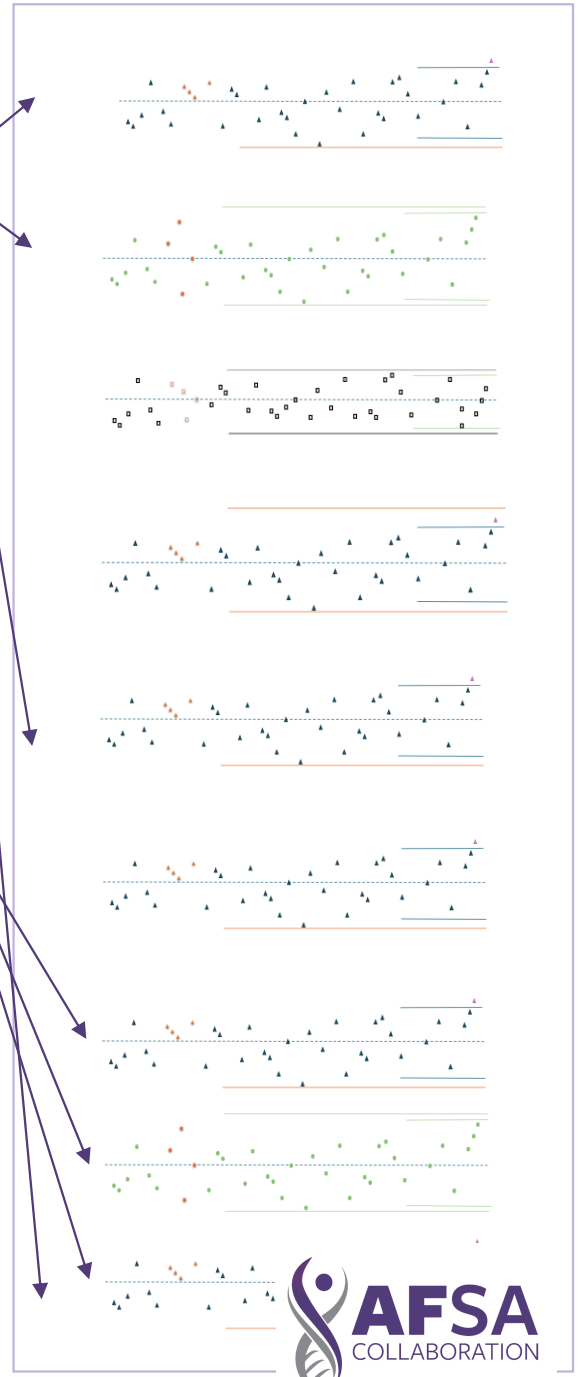
CPP 1 to 4

CPP 2 to 5

CPP 2 to 5

CPP 4 & 5

PCS



Production step from seed to vaccine. From drug substances to drug product and finished product.

Critical Process Parameters have impact on **Critical Quality Attributes** that are controlled by **Product Control Strategy**.

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"The consistency approach implies the use of a (set of) parameter(s) to constitute an integrated ***control strategy*** *preferably relying on non-animal testing (e.g. antigen content, antigen integrity, etc.) and taking into account production controls (e.g. defined ranges, harvest criteria etc.). Taken together it can be seen as an approach which can replace the current approach for many established vaccines which involves in vivo testing. The new integrated control strategy has to be established to the satisfaction of the regulators and is monitored throughout production under a strict quality system. The integrated control strategy* ***ensures that each batch or lot released is similar to a manufacturer-specific vaccine of proven clinical efficacy and safety****, with respect to all characteristics agreed upon at licensing between manufacturer and regulator.*

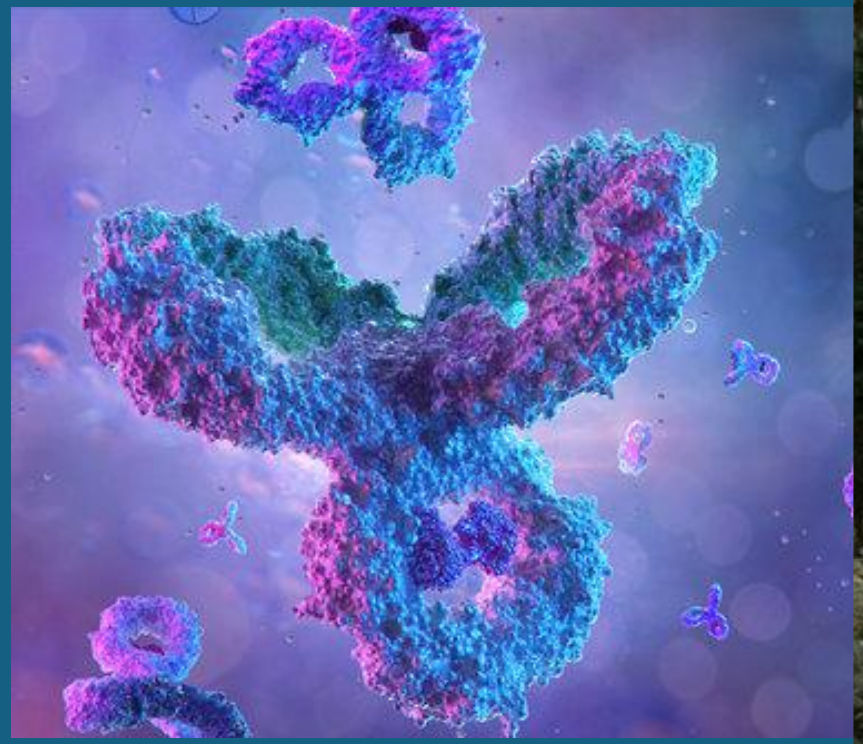
If available, comparison to the batches used in the clinical studies / challenge studies / field trials to support the marketing authorization application may be an option. For older vaccines, parallel testing of batches may be the better way. The number of batches to be tested in parallel should be reasonable, depending on the number of batches normally manufactured in one year (blockbuster versus orphan products / MUMS)."

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The VAC2VAC consortium proposes the following update of definition from de Mattia et al. (2011) to reflect current developments, Dierick et al. open letter; The consistency approach for the substitution of in vivo testing for the quality control of established vaccines: practical considerations and progressive vision

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From development to effective alternative







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Steps to cross the bridge

1. Engage Management
2. Assess technology
3. ICHQ2 R2 Validation
4. Set up specification
5. Submit & approve file
6. Method Lifecycle

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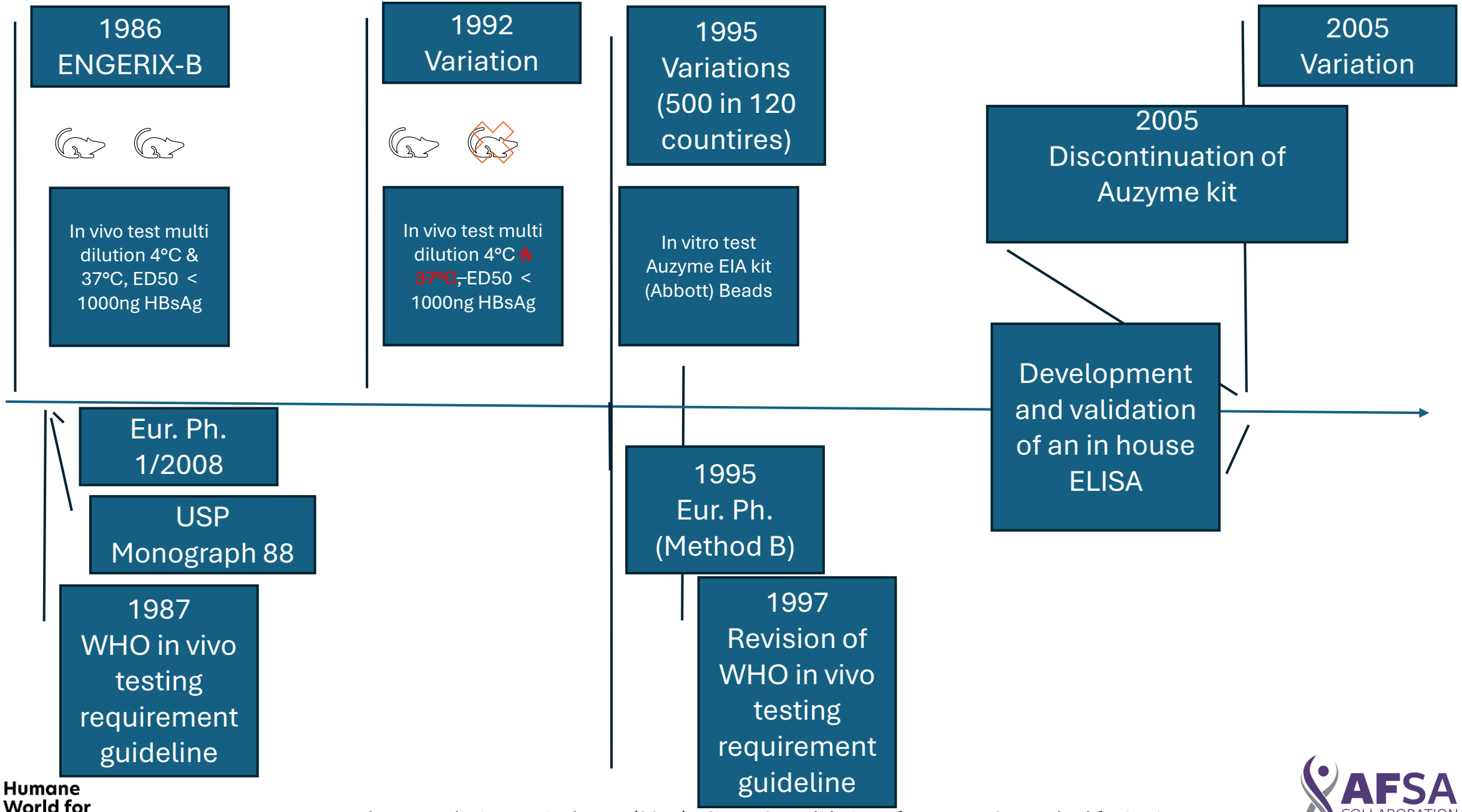
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Hepatitis B vaccine case study

J. Descamps et al. / Procedia in Vaccinology 5 (2011) 184 – 191 Validation of a new ELISA method for in vitro potency assay of hepatitis B-containing vaccines

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J. Descamps et al. / Procedia in Vaccinology 5 (2011) 184 – 191 Validation of a new ELISA method for in vitro potency assay of hepatitis B-containing vaccines

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The WHO potency requirement :

FROM

« an appropriate quantitative test for antigen content **and** an immunogenicity assay be performed on samples representative of the final filling lot. The vaccine potency should be compared with that of a reference preparation and the NRA shall determine the lower limit of potency »

TO

« The vaccine shall be identified as envelope antigens of hepatitis B virus by appropriate methods. An appropriate quantitative test for potency by an in vitro **or** in vivo method shall be performed on samples representative of the final filling lots. Any in vitro method shall be appropriately validated and the test method approved by the NRA. The vaccine shall be compared with a reference vaccine and the NRA shall establish limits of potency »

Hepatitis B Potency Testing Standardization Report of a Meeting, WHO/SEARO, New Delhi, 9-11 April 2001

Legacy in vivo potency for human vaccine, what's next as alternatives

- In vivo HBsAg bioassay replaced by ELISA antigenicity test;
- In vivo IPV in rat bioassay replaced by D-antigen ELISA antigenicity analytical method
- Rabies is on its way

- And DTaP…

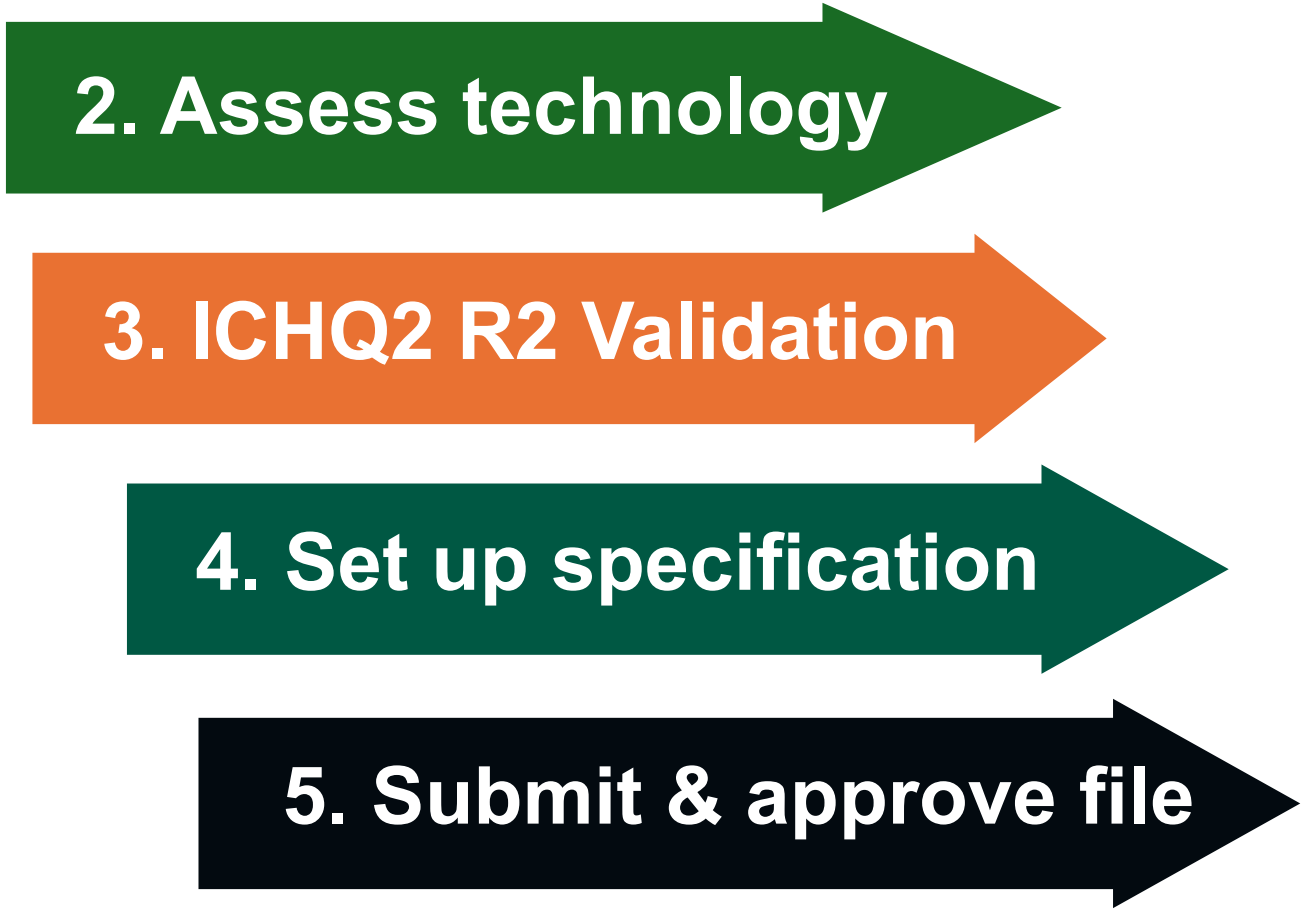
- … VAC2VAC is a wide-ranging collaborative research project funded by IMI2 (2016–2022), which aims to develop and validate quality testing approaches (physicochemical, immunochemical methods, cell-based and multi-parametric) for routine batch quality, safety and efficacy testing of human and veterinary vaccines using non-animal methods.

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Immunotools are there, method is there, what is missing?...

- Assess technology in your facility set up, with your equipment and your technician,…fine tune, assure critical reagent continuity, improve, standardize, gear up for next step…validation
- Product matrix per product matrix validation, combined product, adsorbed or non adsorbed, assess paralalism do you need homologous reference material or common reference works?...
- Define PASS / FAIL criteria, based on what?
- Assess stability do you need specific limit adapted to product shelf live?
- Write and submit file
- Manage delayed approval with stock or parallel testing



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Produit | Info prof. | Risques | Similaire | Content

INFANRIX DTPa-IPV susp inj (hc 03/25) QAP+
GlaxoSmithKline AG

Questions concernant le produit?

- Composition
- Forme pharmaceutique et quantité de principe actif par unité
- Indications/Possibilités d'emploi
- Posologie/Mode d'emploi
- Contre-indications
- Mises en garde et précautions
- Interactions
- Grossesse/Allaitement
- Effet sur l'aptitude à la conduite et l'utilisation de machines
- Effets indésirables
- Surdosage

Forme pharmaceutique et quantité de principe actif par unité

Suspension injectable (i.m.).

Une dose vaccinale (0,5 mL) contient:

Toxoïde diphtérique[1]: au moins 30 UI

Toxoïde tétanique[1]: au moins 40 UI

Antigènes de *Bordetella pertussis:*
- Toxoïde de la coqueluche (PT)[1] 25 µg
- Hémagglutinine filamenteuse (HAF)[1] 25 µg
- Pertactine (PRN)[1] 8 µg

Poliovirus inactivé de type 1 (souche Mahoney)[2] 40 unités antigène D

Poliovirus inactivé de type 2 (souche MEF-1)[2] 8 unités antigène D

Poliovirus inactivé de type 3 (souche Saukett)[2] 32 unités antigène D

[1] adsorbé sur hydroxyde d'aluminium hydraté ($Al(OH)_3$) 0,5 mg Al^{3+}

[2] produit sur des cellules VERO

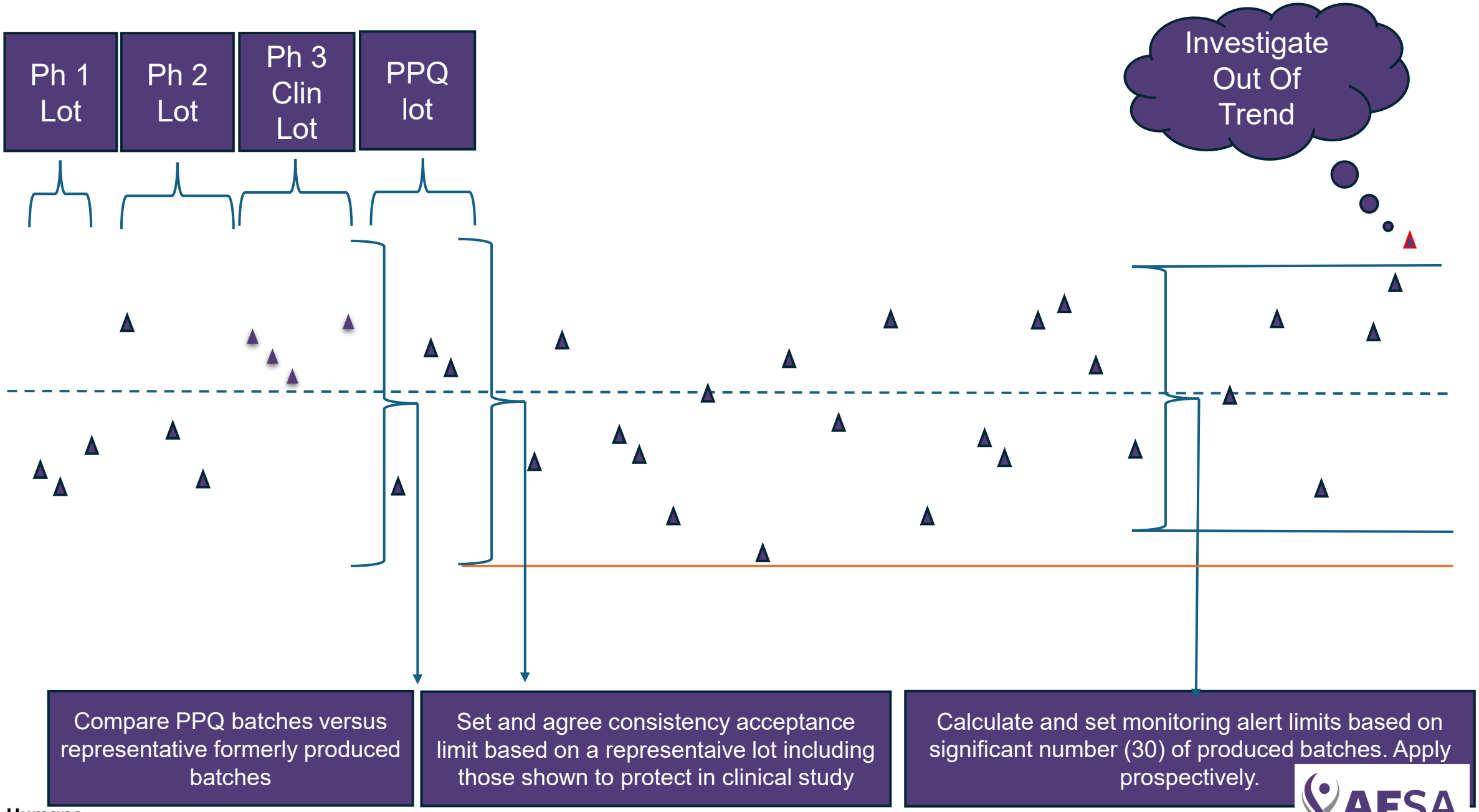
Infanrix DTPa-IPV est une suspension trouble blanchâtre.

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How to set the acceptance (PASS / FAIL) limit? (e.g. INFANRIX not less than 30 IU toxoid D.)

- Specification is derived from decision taken in the test model: **Relative Potency**, using an **int'l reference**, or a **in-house primary standard**, or a **in-house calibrated standard**…result can be expressed **as IU**, as **Ratio**, or as **quantity (weight) per volume**, but also as **mean** probability, **or lower confidence interval** or both, or even semi-quantitative (**above a defined threshold** e.g. single dilution assay).
- A **compendial** specification or **Company** specific specifications ?
- Based on titer of known batches that has **not met clinical efficacy** criteria
- Based on known affects QTPP Quality Target Product Profile obtained by (laboratory) **degraded** vaccine
- Based on known vaccine with substanciated record of having **failed** to protect patient (reported by pharmacovigilence system)
- Alternatively, in absence of such information, consistency limits are set **statistically based** on a significant number of results of produced lot that are comparable to results obtained in clinical lot.

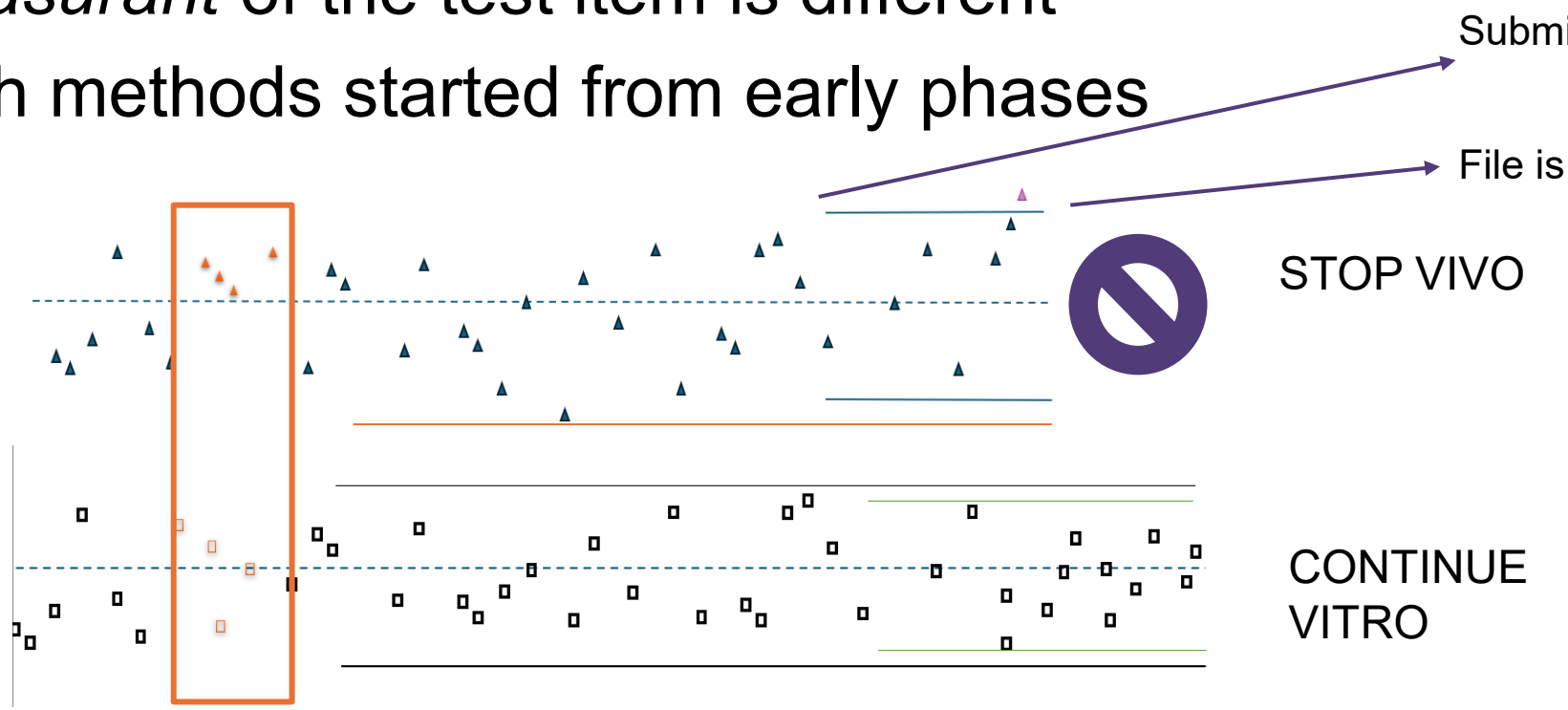


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Transitioning from in vivo assay to in vitro alternative assay (new product)

- Method is different
- Read out is different
- *Measurant* of the test item is different
- Both methods started from early phases



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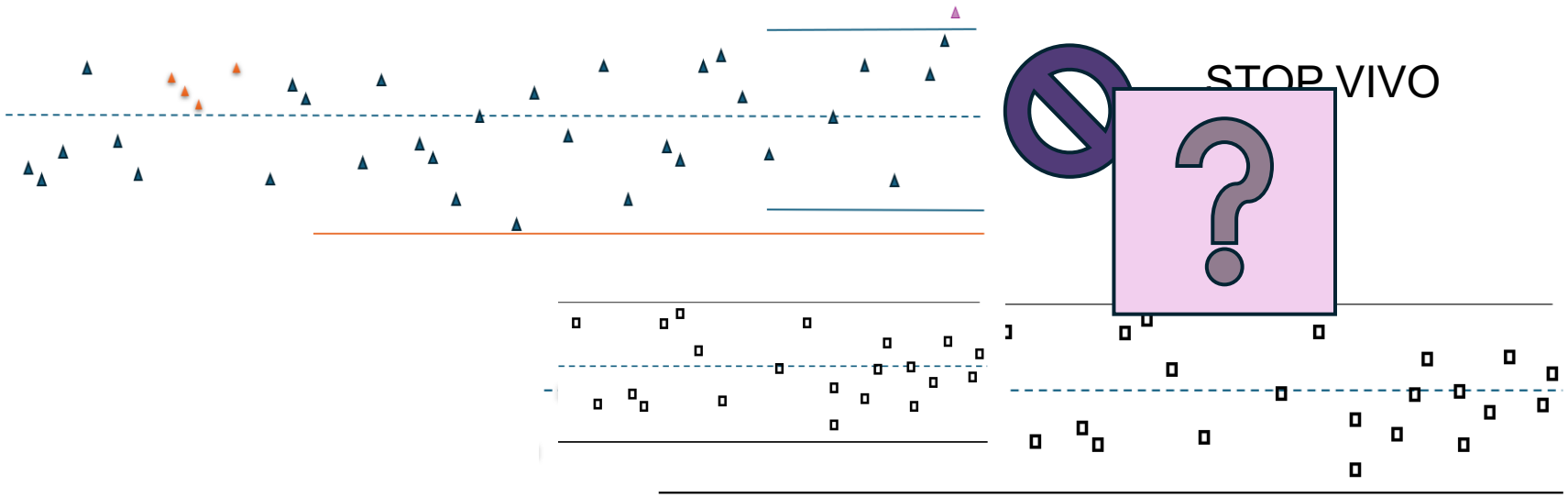
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Transitioning from established assay to new alternative assay (legacy product)

- No more access to initial old clinical lot material (nor it is possible to run new efficacy clinical study)
- **QbD** consider batches produced recently conforming all aspects of Product Control Strategy **as consistant with clinical batches** an therefore select one representative as candidate in house reference?

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Development & validation of an ELISA

you are halfway there !



- It is **manufacturer's responsibility** to develop and validate in vitro QC analytical test and show consistency of the production and stability
- Product per product validation of dilution, verification of interactions, stability indicating
- Establishment of a internal **reference material** used as a standard (homologous/ common)
- Definition of **acceptance limit** based on method variability, CQA, discussed and approved by NRA
- **Submit variations** on all marketed countries
- **Transfer** method and reagent to NCL
- **Maintain** reagents (incl. Standard) material stock and periodically assess method performance
- Include embedded **controls** of method performance to assure early detection of drift; orthogonal method.

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Thank you

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