Quality requirements for vaccines

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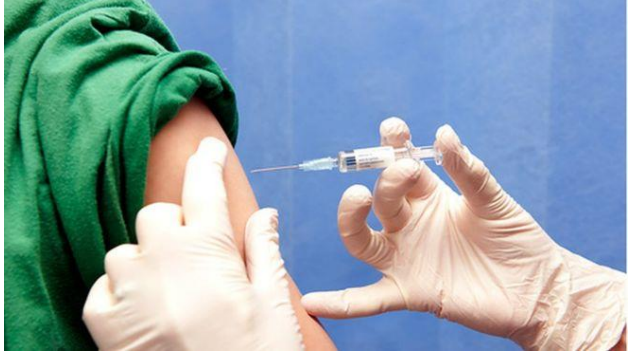
Sciensano

Disclaimer

- *Koen Brusselmans: Quality assessor for biological medicines*
- *performs quality assessment of scientific dossiers for:*
  - *Belgian Medicines Agency (FAMHP)*
  - *European Medicines Agency (EMA).*
- *Koen Brusselmans is alternate member (for Belgium) of the Biologics Working Party (BWP) at the EMA.*
- *This presentation represents a personal view and may not necessarily reflect in all aspects the official position of the FAMHP, BWP, CHMP, EMA, EDQM, WHO and/or any other regulatory bodies.*

Introduction



- Vaccination: one of most important factors for reduction of infectious diseases worldwide (2^{nd} after access to pure water).
- Vaccines: among the most widely used medicinal products.
  Belgium: + 1 million vaccine doses administered each year.
- Vaccines are used to prevent diseases (not for treatment)
  > mainly administered to healthy individuals/children.
- Quality of vaccines is of utmost importance: **Safe + Efficacious**
- Quality of vaccines is controlled at several levels.
- Vaccines: most stringent and extensive quality control system among all medicinal products.

Anti-vaccination movement

Anti-vaccination movement

"Vaccines induce autism in children"

- *No scientific proof for link between vaccines and autism.*
- *Only 1 publication (Wakefield, 1998), which was retracted by journal editorial board (data falsified, fraud committed).*
- *Hundreds of other studies > no link could be found between autism and vaccination.*

Anti-vaccination movement

- *"Vaccines contain toxic compounds (thiomersal, aluminium, squalene, formaldehyde,…)."*
- *"Vaccines contain elemental and metal impurities which are not mentioned on the label and are potentially toxic,"*
- *"MMR vaccine Priorix contains human fetal DNA: includes complete genome and is highly genetically modified and potentially carcinogenic."*
- *"Some vaccines contain foreign viruses."*

Sources: Gatti and Montanari, www.vaccinatieschade.be and www.corvelva.it

Anti-vaccination movement

- *"Presence of insoluble and indigestible macromolecules. Not possible to sequence/analyse. Is it a prion behavior?"*

- *"Vaccines do not contain the antigens that are claimed by the label"*
  - Infanrix hexa*: "none of the antigens (D, T, P, IPV, HepB, Hib) could be detected."*
  - Hexyon*: "only 3 antigens could be detected."*

Sources: www.vaccinatieschade.be and www.corvelva.it

Quality control of vaccines

Quality of vaccines is controlled at multiple levels:

1) Before approval > in depth review of the registration file (MA) by medicines agencies (BE: FAMHP; EU: EMA).
   MA contains all info on production process, materials and testing.

2) Quality control (QC) testing: every single vaccine batch has to be tested by the manufacturer according to a predefined set of specifications that has been approved by the authorities.

3) GMP inspections: before and after approval (every 2-3 years): verification of compliance with legislation and registration file.

4) Pharmacovigilance: post-approval clinical monitoring.

5) Additional QC testing by independent laboratory (OMCL).

1: Quality review of MA file

- All materials used for production need to be described in detail. Any biological materials used in the process need to be screened for possible contaminants (bacteria, viruses).
- Process, process controls, QC tests need to be described.
- Containers and process equipment has to be evaluated for possible leaching of elements into the vaccine.
  Impurity removal capacity of process needs to demonstrated.
  Assessment has to be made confirming the safety of the product.
  Any residuals need to be below acceptable safety limits.
- All this info has to be included in the MA file, which has to be reviewed and approved by the authorities (FAMHP / EMA).

afmps fagg
agence fédérale des médicaments et des produits de sa
federaal agentschap voor geneesmiddelen en gezondheidsproducten

EUROPEAN MEDICINES AGENCY
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2: QC testing by applicant

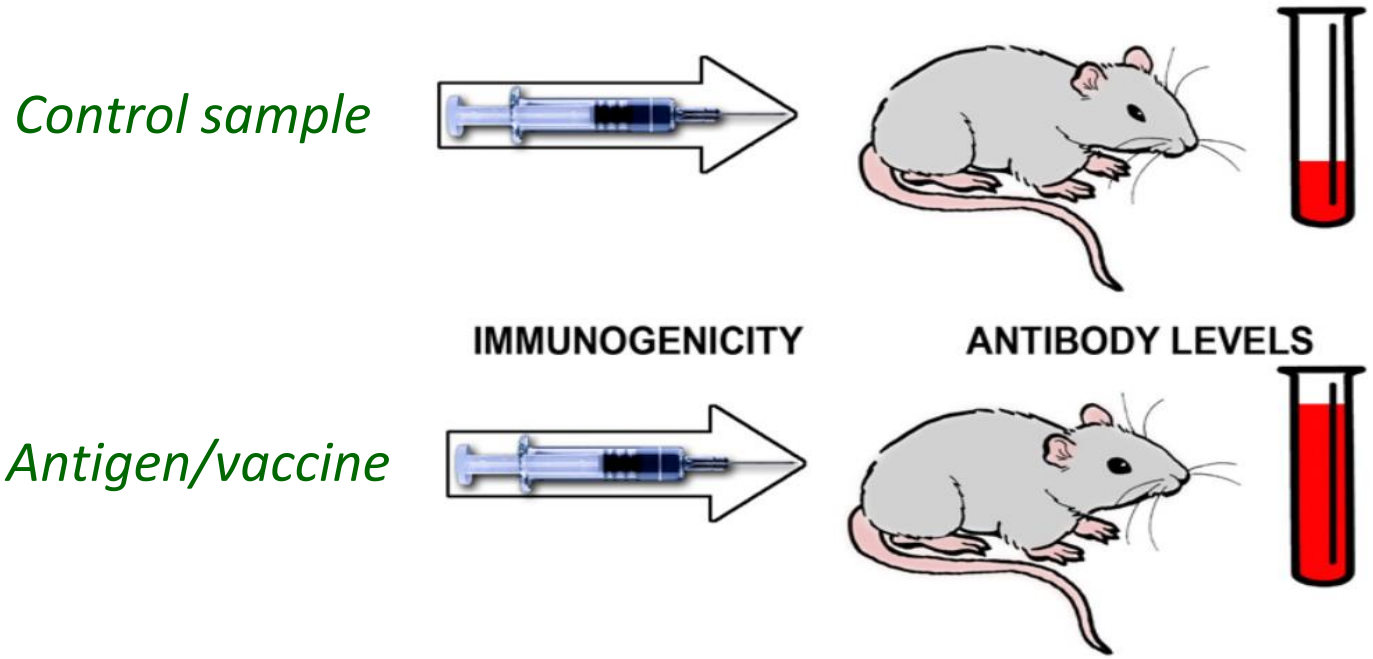
- Every single batch of active substance(s) and final product has to be tested by the manufacturer.
- Testing occurs using a fixed panel of methods and results need to comply with a predefined set of specifications (approved by the authorities; acceptance limits need to be safe and efficacious).
- Testing includes:
  - general parameters (pH, appearance,…)
  - identity (correct identity of antigen)
  - potency (verification of antigen content)
  - purity/integrity of antigen
  - safety (sterility, endotoxins,…)
- Specific tests for potency, purity, integrity: depend on the type of antigen or vaccine.

2: QC: in vivo testing

- In the past: vaccine potency was typically tested in animals (e.g. challenge assay in mice or Guinea pigs for tetanus and diphtheria).
  - Vaccine, positive control (reference) or negative control are administered to mice or Guinea pigs.
  - Animals are then challenged to tetanus/diphtheria toxin.
  - Protection induced by vaccine is compared to that of a reference standard.
- In vivo testing: > costly, high variability, not animal-friendly.
- Initiatives are ongoing to reduce in vivo testing: collaboration between companies and regulators/authorities (VAC2VAC).
- Try to limit use of animals > use a combination of in vitro assays to analyse potency (antigen content, integrity, size, purity).

2: 3Rs: replace, reduce, refine

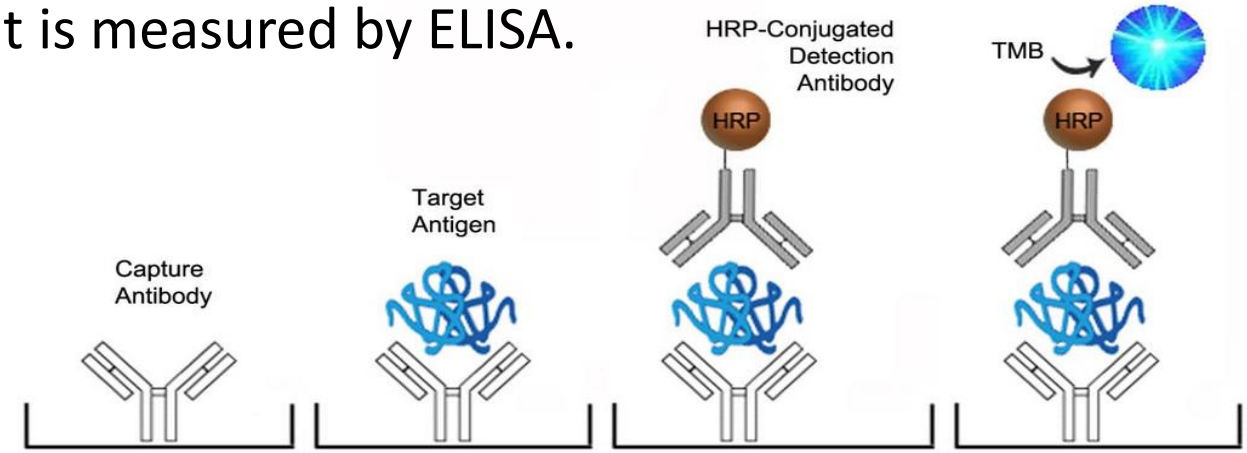
- Challenge tests may be replaced by immunogenicity tests.



- *In vivo* assays only for characterisation; replaced by *in vitro* assays for release testing, where possible.

2: Potency by immuno-assay

- *In vivo* assay can be replaced by an *in vitro* immuno-assay: Recombinant or sub-unit antigens: HepA and HepB vaccines, HPV vaccine, inactivated polio vaccine (IPV).
- Antigen content is measured by ELISA.

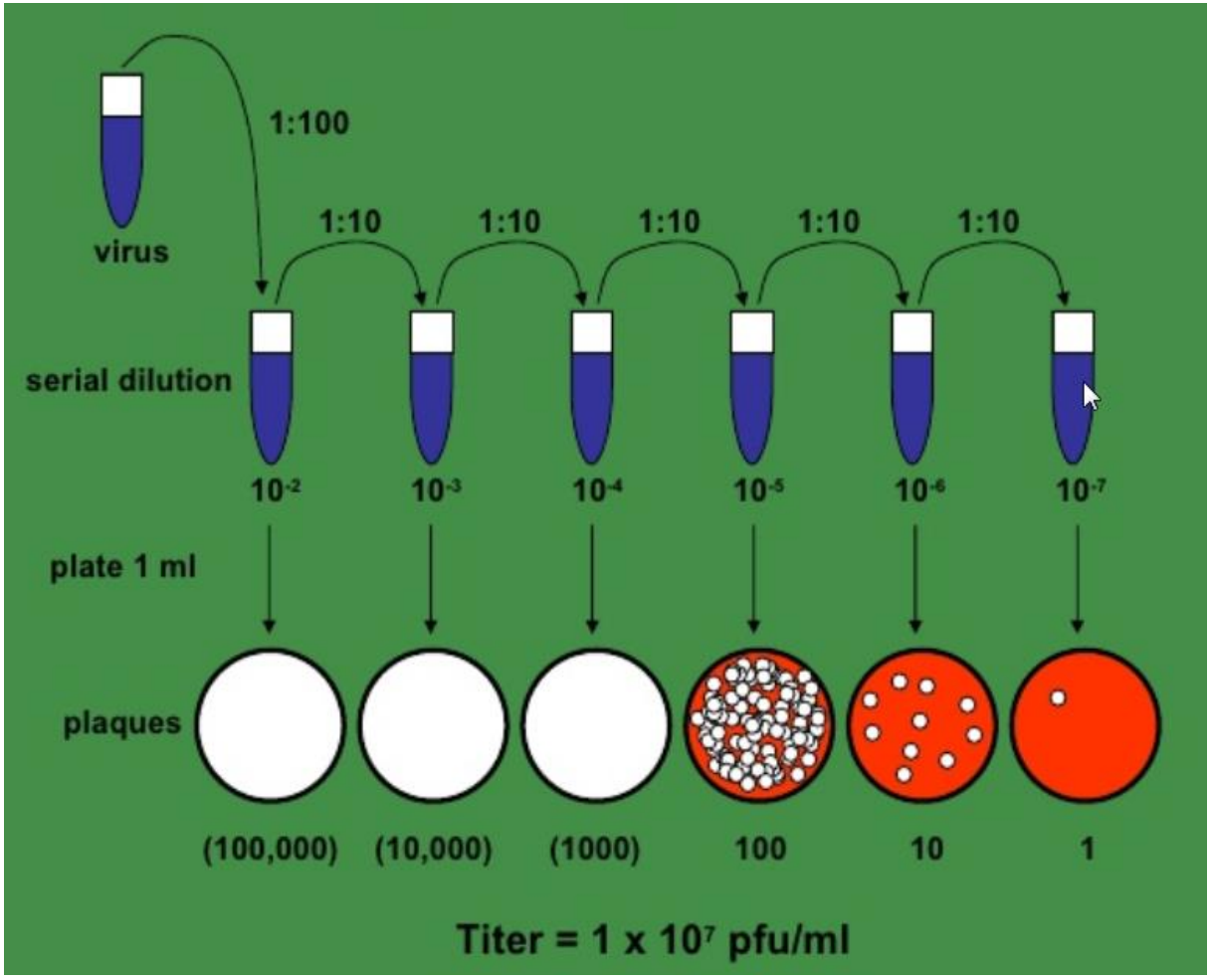


- In case of degraded antigen, an immuno-assays may still yield a positive test result > additional assays required to monitor size, purity and/or integrity of antigen (e.g. SDS-PAGE, SEC-HPLC,…).

2: Potency live viral vaccines

- Live viral vaccines (attenuated):
  measles, mumps, rubella, rotavirus, oral (live) poliovirus.

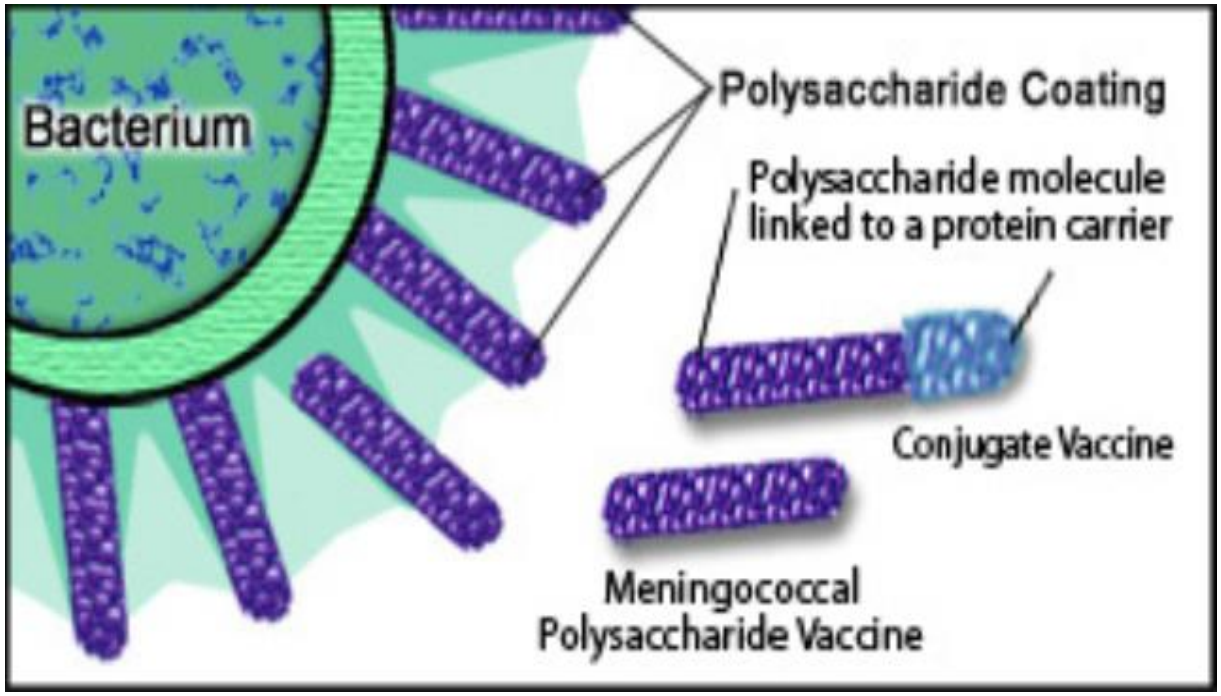
- Virus titer is measured using in vitro cell cultures.
- Cells are infected with various dilutions of vaccine
- Cell death is quantified.
- Potency expressed as PFU/ml or TCID50.



2: Potency polysaccharide vaccines

- Polysaccharide vaccines: pneumococcal, meningococcal, Haemophilus influenzae type B (Hib).

- Polysaccharide content
- Polysaccharide size
- Purity
- Degree of adsorption (for vaccines that are adsorbed on aluminium)



2: QC testing by applicant

- Regardless whether *in vivo* or *in vitro* methods are used, the approved specifications of a vaccine always contain a proper set of methods that allow to conclude on:
  - Correct identity of antigen
  - Antigen content
  - Antigen size, integrity and purity
  - Safety: sterility, endotoxin.

- Depending on the manufacturing process, vaccine specifications may also contain additional safety or purity tests:
  - If potentially toxic compounds are used in process
    > test for possible residuals (e.g. formaldehyde)
  - Inactivated vaccines:
    > test for residual live virus or bacteria.

3: GMP inspections

- GMP: Good manufacturing practices.
- All production sites have to be inspected (before vaccine approval, post-approval every 2-3 years).
- Verify whether manufacturing and QC testing by company is in compliance with the legislation:
  - Compliance with GMP guidelines and Ph.Eur ?
  - Full traceability ?
  - Manufacturing and testing done as described in MA file ?
  - Quality of raw materials: properly tested as described in MA ?
  - Have the analytical QC methods been properly validated ?
  - Are process controls performed? Results within acceptance limits?

5: OCABR testing by OMCL

- Vaccines and plasma-derived products: EU legislation (EU directive 2001/83/EC) allows member states to implement additional level of quality control (unique among medicinal products).
- In most EU member states (including BE), this testing is mandatory: Every vaccine batch that has been tested and released by the company, has to undergo a **second** testing and release (OCABR) by an independent laboratory (OMCL).
- OMCL: Official medicines control laboratory (accreditation from government and EDQM).
  OCABR: Official control authority batch release.
- OMCL network (17 MS): coordinated by EDQM (European directorate for quality of medicines).

edqm
European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

5: OCABR testing by OMCL

OMCL network: control laboratories in 17 EU countries

- Each OMCL has to operate under a quality system (ISO 17025).
- OMCL: audits/inspections performed by other OMCLs and EDQM.
- OMCL can only use validated methods for vaccine testing. Methods are standardized (based on Eur. Pharmacopoeia).
- Companies can choose OMCL (choice often depends on available expertise).
- If results of OCABR testing are compliant with specifications, then the OMCL can grant a batch release certificate. These certificates are valid in all EU member states (mutual recognition).
- Sciensano (BE OMCL): one of most important OMCLs for vaccines.

5: OCABR testing by OMCL

- EDQM and OMCL network define for each vaccine type a set of mandatory tests that need to be performed for OCABR.
  Discussed/approved by drafting group and advisory group (EDQM).
- OCABR testing represents a sub-set of the specification tests (typically selection of most important quality parameters):
  Appearance, identity, potency, antigen size/integrity, purity.
- Company sends samples for testing to an OMCL.
  > OMCL performs independent testing.
  > OMCL also verifies QC results obtained by company.
- If results of OCABR testing are compliant with specifications, the OMCL will grant a **batch release certificate**.
  If results are not compliant > a non-compliance certificate is issued and the batch must be destroyed.

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Quality of Vaccines and Blood Products

ISO 17025 Accreditation

EU OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE FOR IMMUNOLOGICAL PRODUCTS

EU/EEA OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE – Finished Product

Examined under Article 114 of Directive 2001/83/EC as amended by Directive 2004/27/EC (Immunological Medicinal Products) and in accordance with the Administrative Procedure for Official Control Authority Batch Release.

Trade name:	**INFANRIX HEXA**
International non-proprietary Name / Ph. Eur. name / common name:	**Diphteria, Tetanus, Pertussis (Acellular Component), Poliomyelitis (Inactivated), Hepatitis B (rDNA) with separate Haemophilus Type B Conjugate Vaccine (Adsorbed)**
Batch numbers appearing on the package and other identification numbers associated with this batch[1]:	**Final packaging lot: A21CD367** **DTPa-IPV-HepB lot: AC21B693D** **Hiberix component lot: AHIBD300B**
Type of container:	**Syringe + vial**
Total number of containers in this batch:	**AC21B693D : 324 748** **AHIBD300B : 124 099**
Number of doses per container:	**1 dose**
Date of start of period of validity:	**October 2017**
Date of expiry:	**September 2020**
Marketing Authorisation number (member state / EU) issued by:	**EU/1/00/152/001 to EU/1/00/152/008** **EU/1/00/152/019 to EU/1/00/152/021**
Name and address of manufacturer:	**GlaxoSmithKline Biologicals** **89, Rue de l'Institut** **B 1330 Rixensart** **Contact Person : E. Sarlet** **Phone: +32-10-85.62.82 Fax : +32-10-85.91.49**
Name and address of marketing authorisation holder if different:	**Same as above**

This batch has been examined using documented procedures which form part of a quality system which is in accordance with the ISO/IEC 17025 standard.
This examination is based on the relevant EU OCABR guideline for this product.

This batch is in compliance with the approved specifications laid down in the relevant European Pharmacopoeia monographs and the above marketing authorisation and is released.

Signed:	JULIEN AUQUIER VACCINE RESPONSIBLE NATIONAL CONTROL LABORATORY
Name and function of signatory:	p.o. **Geneviève Waeterloos, M.Sc., Head,** **Quality of Vaccines and Blood Products Service**
Date of issue:	27 JUIL. 2018

Certificate number: BE/18-1518

5: OCABR testing by OMCL

- Before vaccine registration/approval: company should contact OMCL for future OCABR testing.
- Discussion between OMCL and company as regards the vaccine characteristics and the methods to be used for OCABR testing.
  It has to be ensured that the OMCL has adequate and validated methods available in time (to start testing and release when the vaccine is approved by the authorities).
- Both manufacturing and testing are complex activities that need accurate organization and planning.
- Testing by OMCL and by company is done in parallel (more or less at the same time):
  > to save time and speed up release of vaccines to market.

5: OCABR testing by OMCL

Number of vaccine batches released (OCABR) by Sciensano (BE OMCL)

28 different vaccines from 4 different vaccine manufacturers.

- OCABR testing & critical protocol review: mainly for EU market, but also for some non-EU countries requesting a certificate.
- Critical protocol review: non-EU market (WHO)

Vaccines	2015	2016	2017	2018
EU Release Certificates	1394	1420	1306	1129
non-EU Release Certificates	1137	1333	1161	1356
Total release certificates	**2531**	**2753**	**2467**	**2484**

Quality of vaccines is controlled at multiple levels:

- Review and approval of the registration file (MA):
  > strict control of process and materials used.

- QC testing of each vaccine batch by the manufacturer:
  > results should fall within qualified limits.

- GMP inspections (on a regular base):
  > to verify if process is compliant with MA file,
  > to verify if QC testing is properly performed,…

- Additional level of control:
  Independent QC testing by OMCL (*unique for vaccines and plasma products*)
  > correct antigens present, quantity/potency correct,…

Vaccine safety

"Vaccines contain toxic compounds (thiomersal, aluminium, squalene, formaldehyde,…)."

- Thiomersal (Hg): Previously used as preservative, only at very low levels, not toxic. No longer used in vaccines.
- Aluminium: many vaccines contain aluminium (0,2-0,8 mg). These levels are not toxic. Essential function as adjuvant ! Daily aluminium intake via environment is about 10 mg !
- Squalene: present in some vaccines.
  Naturally occurring compound in human body: occurs in skin, precursor molecule for synthesis of sterols (cholesterol).
- Formaldehyde: often used to inactivate vaccines. Purification: formaldehyde removed. Residual levels: very low (< 0,1 mg). Daily environmental intake: air (1 mg), food (1-10 mg), plastics?

Vaccine safety

"Vaccines contain elemental impurities which are not mentioned on the label and are potentially toxic (Fe, Al, Cu, Cr, Zn, Ca, Mg, Si,…)."

- Researchers (*Gatti and Montanari*) used *Field emission gun electron scanning microscope using X-ray energy dispersive spectroscopy*.
- No details provided on the method. No info on sample preparation (elements may have been introduced at this step). No positive or negative controls included in analysis. Various vaccines were several years past expiry date at time of analysis.
- Scanning microscopy is excellent method to identify elements at very low quantities. But: not possible to accurately quantify !
- What is the relevance of these data ??? Trace elements are always present (unavoidable; everywhere in environment).
  But levels are extremely low > not toxic (toxicity ~ concentration).

Vaccine safety

"MMR vaccine Priorix contains human fetal DNA: includes complete genome, highly genetically modified and potentially carcinogenic."

- Rubella is produced using MRC-5 cells (fibroblast cell line derived from 14-week old aborted male fetus (1966).
- MMR vaccines: relatively 'old', lower purity than other vaccines. They contain small amounts of residual DNA (fragmented).
- Residual levels of fragmented DNA are low and not toxic.
  MRC-5 cells contain normal human DNA and are not tumorigenic.
  MRC-5 DNA is not genetically modified and not carcinogenic.
- MMR vaccines: used for more than 40 years > no evidence for any link with disease.

Vaccine safety

"Some vaccines contain foreign viruses."

- Researchers used next generation sequencing (NGS) to screen various vaccines.
  NGS: advanced technology that allows to detect low amounts of DNA/RNA. Data analysis (>software) is complex and difficult.
- In Gardasil-9 (HPV) they '*detected*' murine leukemia virus and human endogenous retrovirus K (HERV-K).
  (2 retroviruses that may induce cancer)
- Number of sequences is low: **results meaningless**.
  Only relevant if repeatable and if adjacent sequences positive.
  NGS need to be confirmed by more accurate test.
  > not done here.

Vaccine safety

"Presence of insoluble and indigestible macromolecules and aggregates in Infanrix hexa. Not possible to sequence/analyse."

"Is it a prion behavior?"

- SPC Infanrix hexa states: homogeneous turbid white suspension. Antigens are adsorbed onto aluminium,
- Vaccines are tested for appearance by manufacturer and OMCL. Only exceptionally deviating from specifications (> discarded).
  - Did research lab treat the samples correctly ???
- Many possible explanations why aggregation occurred in samples treated by research lab.
  But only hypothesis proposed is: "*these may be PRIONS*".
  > pure speculation, not science.

Vaccine safety

"Vaccines do not contain the antigens that are claimed by the label"
*(*Infanrix hexa*: "none of the antigens could be detected")*

- Infanrix hexa: 10 different antigens: Te, Di, Per (PT, FHA, PTN), IPV type 1, 2 and 3, Hepatitis B, Haemophilus influenzae type B.
- None of these could be detected by research lab ???
- To develop proper quantitative method: knowledge on antigens is needed (proprietary) > authorities and OMCLs have access.
- Lab used LC-MS (not suitable to quantify antigens). No details provided on method (impossible to assess if properly done).
- Antigens are adsorbed on aluminium. If lab only analysed supernatant > obvious that little or no antigen is found.
- Identity and potency are mandatory QC tests (company + OMCL) for each vaccine lot. Batches that do not comply (> rejected).

Vaccine safety

Findings and statements made by anti-vaccination movements:

When reviewed by independent experts: these findings and statements cannot be proven or confirmed.

- Often poor methodology is used, not suitable for intended use.
- Methodology used is not or poorly described; if no controls included > impossible to assess validity of results.
- Little or no information is given on sample collection / treatment (many mistakes may occur already at this stage, e.g. contamination during sample preparation).
- Results from high-tech analyses: wrong interpretations and wrong conclusions.
- Many statements are not proven or even absolutely incorrect.

Conclusion

Quality of vaccines is controlled at multiple levels:

- Review and approval of the registration file (MA).
- Quality control testing of each vaccine batch by the manufacturer.
- GMP inspections on a regular base (to verify if process is compliant with MA file, if QC testing is properly performed).
- Additional level of control: independent QC testing by OMCL (*unique for vaccines and plasma-derived products*).

➔ **This combination of control measures makes that vaccines are among the medicinal products with the most extensive and rigorous quality control.**

➔ **Safety and efficacy of vaccines is ensured !**