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# Vaccinegate:

## Metagenomic analysis report on vaccine samples

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# Brief presentation of the results

The Corvelva Association, an historic Italian association that support vaccine freedom, has commissioned the analysis of biological contamination in some batches of vaccines currently commercialized in Italy, to a highly qualified scientific institute specialized in sequencing the genetic material.

The results are alarming, on 7 types of vaccines, as many as 5 do not conform to the guidelines for the quantity of biological material, DNA or foreign RNA of human or animal origin, or for the presence of genetic mutations of the antigens!!!

1. *Priorix Tetra, GlaxoSmithKline - NOT CONFORMING*
2. *Infanrix hexa, GlaxoSmithKline - NOT CONFORMING*
3. *Measles live vaccine B.P., Poonawalla Group - NOT CONFORMING*
4. *PolioInfanrix, GlaxoSmithKline - NOT CONFORMING*
5. *Vivotif, PaxVax - NOT CONFORMING*

**These results throw a shadow on the quality of the checks carried out by the controllers bodies.**

We can not yet release the original documentation and communicate the names of the laboratories, that are internationally certified, because we are completing further investigations to understand other aspects that are decisive for safety and effectiveness. Vaccines such as Infanrix hexa and PolioInfanrix have the viral DNA of the poliovirus in quantities below the limits of detection of both standard instruments and the sensitivity of deep sequencing, this raises the following questions: is the antigen really present? those vaccines immunize? These questions and many others will be answered in the second line of our research.

In Italy already in the coming days will be presented complaints to the competent authorities and we will keep you updated.

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# Metagenomic analysis report on vaccine samples

## Introduction

As is known, vaccines are biological drugs used for the prevention of certain infectious diseases and are made up of several components: antigens (viruses, inactivated or attenuated bacteria, inactivated toxins, proteins or complex molecules derived from viruses and bacteria, capable of stimulating the immune response), adjuvants (substances that increase the ability of vaccine antigens to induce antibody immune response), excipients (substances necessary to formulate the vaccine, or to preserve it from bacterial contamination) and contaminations (substances present in traces from raw materials, eg cell lines for bacteria and virus growth, or from the production process, eg formaldehyde, antibiotics).
During the registration phase of a biological drug, the vaccine is subjected to the controls provided by the EMA guidelines and agreed with the regulatory body according to the specific type of vaccine. These checks are then carried out on a representative number of samples on each lot before marketing.

**The responsibility for the conformity of the product sold is therefore of the producer and of the regulatory bodies in charge of the control.**

Since the safety of a vaccine depends on its compliance with the quality criteria, especially regarding the control of the absence of toxic or potentially toxic contamination (ie for which no effects in humans are known) it is of great importance that such compliance is respected in a very strict manner.
Various studies in the literature have put the issue of the presence of various types of contamination, both chemical and microbiological, thus opening the question if the vaccines actually comply with the directives imposed by the regulatory bodies, if in turn the regulatory bodies apply the control for the respect of these directives and if the regulatory bodies have defined the criteria for the control and containment of such contamination with effective guidelines.
In order to answer these questions, Corvelva commissioned the analysis of biological contamination, which should never be present in vaccines, at a highly qualified centre of services specialized in genomics DNA and RNA sequencing.

The study commissioned by Corvelva is based on two types of analysis:

1. **Testing the presence of nucleic acids** (DNA / RNA) of human and animal origin and by microorganisms (viruses, bacteria) using the Next Generation Sequencing method, which allowed to quantify in a highly specific and accurate manner the sequence of the genetic material contained in the vaccines examined

2. **Verification of the correspondence of the genome sequences** of live attenuated or inactivated bacteria and viruses present in the vaccines (presence of genetic variants)

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## Description of the method used for the analysis

Next Generation Sequencing, also known as deep sequencing, generates a single sequence from each DNA fragment, or cDNA, present in a sample. The downstream bioinformatics analysis then allows the differentiation between the origin of the sequence fragments, for example human, bacterial species or a particular virus. This means that mixed biological samples can be easily resolved with this technology, which has now entered the routine of genomic research and diagnostics. Moreover, from NGS data it is possible to reconstruct the entire sequence of viral DNA and RNA genomes and bacterial genomes present in the sample and compare it with the reference genomes present in public databases.
The samples examined are shown below together with the results obtained, grouping them by classes of similar vaccines:

* ssRNA: single strand RNA; dsDNA: double strand DNA.

## Examined samples

### Sample 1.

**Product Name:** **Priorix Tetra**
**Tipo di prodotto:** Tetravalent Vaccine Measles, Mumps, Rubella, Varicella
**Manufacturer:** GlaxoSmithKline, Belgium
**Composition[1]:** Live attenuated viruses: 1) Measles (ssRNA *) Swartz strain, grown in embryo chicken cell cultures ; Mumps (ssRNA) strain RIT 4385, derived from the Jeryl Linn strain, grown in embryo chicken cell cultures; Rubella (ssRNA) Wistar RA 27/3 strain, grown in human diploid cells (MRC-5); Varicella (dsDNA *) OKA strain cultivated in human diploid cells (MRC-5)

### Sample 2.

**Product Name:** **Measles vaccine live B.P.**
**Product Type:** Measles monovalent vaccine
**Manufacturer:** Poonawalla Group (Profarma AG, Baar)
**Composition[2]:** Live attenuated Measles virus (ssRNA): Edmonson-Zagreb strain propagated in human diploid cells MRC-3.

### Sample 3.

**Product Name:** **MMR vax Pro**
**Product Type:** Trivalent Vaccine measles, mumps, rubella
**Manufacturer:** MSD Vaccins, France
**Composition[3]:** Live attenuated viruses: 1) Measles (ssRNA) Enders Edmonston strain grown in embryo chicken cell cultures; Mumps (ssRNA) Jeryl Linn strain (Level B), grown in embryo chicken cell cultures; Rubella (ssRNA) Wistar RA 27/3 strain, grown on fibroblasts of human diploid cells WI-38.

### Sample 4.

**Product Name:** **Infanrix hexa**
**Product Type:** Pediatric hexavalent vaccine: diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis, Haemophilus influenzae type b
**Manufacturer:** GlaxoSmithKline, Belgium
**Composition[4]:** Diphtheria and tetanus toxoids; Bordetella pertussis antigens; recombinant antigens (produced in Saccaromyces cells) of sup. for hepatitis B; polysaccharide from H. influenzae; 3 types of inactivated polio virus (ssRNA): type 1 (Mahoney strain) -type 2 (MEF- strain) -type 3 (Saukkett strain), propagated in VERO (monkey) cells. Toxoids + Bordetella antigens adsorbed on aluminum hydroxide hydrate; H. influenzaea polysaccharide adsorbed on aluminum phosphate

[1] https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_000200_038200_RCP.pdf&retry=0&sys=m0b1l3
[2] http://compendium.ch/mpro/mnr/28396/html/de
[3] http://ec.europa.eu/health/documents/community-register/2017/20171215139691/anx_139691_it.pdf
[4] http://www.ema.europa.eu/docs/it_IT/document_library/EPAR_-_Product_Information/human/000296/WC500032505.pdf

## Sample 5.

**Product Name:** **PolioInfanrix**
**Product Type:** Pediatric pentavalent vaccine: diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis
**Manufacturer:** GlaxoSmithKline, Belgium
**Composition[5]:** Diphtheria and tetanus toxoids; Bordetella pertussis antigens; surface recombinant antigens (produced in Saccaromyces cells) of hepatitis B ; 3 types of inactivated polio virus (ssRNA): type 1 (Mahoney strain) -type 2 (MEF- strain) -type 3 (Saukkett strain), propagated in VERO (monkey) cells. Toxoids and Bordetella antigens adsorbed on aluminum hydroxide hydrate.

## Sample 6.

**Product Name:** **Fluad**
**Product Type:** Vaccine influenza season 2017/2018
**Manufacturer:** Seqirus Srl, Siena
**Composition[6]:** Surface antigens of influenza virus (haemagglutinin = surface glycoprotein) grown in eggs and adjuvanted with MF59C. 1, of the strains: A / Michigan / 45/205 (H1N1) pdm09-A / Hong Kong / 4801/2014 (H3N2) -B / Brisbane / 60/ 2008 . Adjuvant MF59C.1 = squalene, polysorbate 80, sorbitan trioleate, sodium citrate, citric acid, water for injectable suspension

## Sample 7.

**Product Name:** **Vivotif**
**Product Type:** Typhoid fever vaccine
**Manufacturer:** PaxVax, United Kingdom
**Composition[7]:** Salmonella typhi Ty21a, attenuated live strain

[5] https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_000200_037157_RCP.pdf&retry=0&sys=m0b1l3
[6] https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_004166_031840_RCP.pdf&retry=0&sys=m0b1l3
[7] https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_004175_025219_RCP.pdf&retry=0&sys=m0b1l3

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# Results of the analysis of vaccine samples 1, 2, 3

| Priorix Tetra | MMR vax Pro | Measles vaccine live B.P. |
|---|---|---|
| **DNA analysis**<br><br>**Total DNA extracted: 1729.8 ng**<br><br>Quantity (about 2 micrograms) in line with that found for a sample of Priorix Tetra previously analyzed with the same method.<br><br>DNA sequencing analysis performed using a metagenomic approach, out of a total of 13.11 million sequences produced<br><br>**Presence of genomic DNA of:**<br><br>Varicella **14%**<br>Chicken **4%**<br>Human (MRC-5) **78%** | **DNA analysis**<br><br>**Total DNA extracted**:<br>not quantifiable through standard fluorimetric methods (limit of detection 0.1 ng/µl).<br><br>DNA sequencing analysis performed using a metagenomic approach, out of a total of 20.89 million sequences produced.<br><br>**Presence of genomic DNA of:**<br><br>Chicken **28%**<br>Human **14%** | **DNA analysis**<br><br>**Total DNA extracted: 13.6 ng**<br><br>DNA sequencing analysis performed using a metagenomic approach, out of a total of 10.53 million sequences produced.<br><br>**Presence of genomic DNA of:**<br><br>Human (MRC-3) **56%** |
| **RNA analysis**<br><br>Total RNA extracted:<br>not quantifiable through standard fluorimetric methods (limit of detection 0.1 ng/µl).<br><br>**Presence of RNA of:**<br><br>Measles **0.02%**<br>Mumps **0.22%**<br>Rubella **0%**<br>Varicella **5.15%**<br>Chicken **0.20%**<br>Human (MRC-5) **89.65%** | **RNA analysis**<br><br>**Total RNA extracted**:<br>not quantifiable through standard fluorimetric methods (limit of detection 0.1 ng/µl).<br><br>RNA sequencing analysis performed using reverse transcription approach, out of a total of 29.57 million sequences produced.<br>.<br>**Presence of RNA of:**<br><br>Measles **8%**<br>Mumps **17.70%**<br>Rubella **0.2%**<br>Chicken **23%**<br>Human **12.75%** | **RNA analysis**<br><br>**Total RNA extracted:**<br>not quantifiable through standard fluorimetric methods (limit of detection 0.1 ng/µl).<br><br>RNA sequencing analysis performed using reverse transcription approach, out of a total of 21.56 million sequences produced.<br><br>**Presence of RNA of:**<br><br>Measles **15.52%**<br>Human (MRC-3) **35.82%** |

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## From the comparison of these three vaccines it is possible to highlight the following criticalities:

Priorix Tetra is the vaccine with the highest amount of contaminant extraneous DNA (total DNA extracted = 1729.8 ng, of which 78 % is human, thus coming from MRC-5 cells , and 4% from chicken embryonic cells); follows the Measles vaccine with 13.6 ng of which 56% is human, coming from the MRC-3 cells, and finally the MMR vax Pro for which the extracted DNA is in amounts lower than 0.1 ng / µl of which 28% coming from chicken cells and 14% from WI-38 cells.
In the Priorix Tetra vaccine, human genomic DNA is high molecular weight (> 10,000bp) and the total sequential coverage of the entire human reference genome (HG-19) **shows that the entire genome of fetal cells used for the culture of vaccinia viruses is present and not just portions of it.**
The amount of this DNA is so high as to prevent the fluorimetric quantification of the RNA of the vaccinia viruses with lower number of nucleotide bases (Rubella, Measles); in MMR vax Pro, in which the genomic contaminant DNA is below the limits of detection of the instrument, it is in fact possible to quantify the RNA of the vaccine viruses with more accuracy.

From the EMA's answer to our question(8) about the limits imposed on residues of foreign genetic material in vaccines, it appears that in fact there are no limits for each vaccine but only for some, reported in the monographs of the product; **the maximum limit envisaged ranges from 10 pg to 10 ng**, based on the theoretical calculation of the possibility of foreign genomic DNA to cause oncogenic mutations.
It is noteworthy that regulatory authorities do not require that these contaminations be tested in the final product, but only in the initial preparation phase, and that for the attenuated virus vaccines the purification of these contaminations is a critical step. The EMA has not provided specific studies on the dangers of fetal residual DNA, which allow assessing the risk to human health of these contaminations, so this limit remains arbitrary today.

It follows that of these three samples only the MMR vax Pro complies with the limit of 10 ng, while the Priorix Tetra is about 140 times higher than the maximum limit of 10 ng and 140,000 times higher than the minimum limit of 10 pg.

On the question of contaminating human DNA, the World Health Institute in an official 2011 document entitled 'Recommendations for the evaluation of animal cell cultures as subsitrates for the manifacture of biological medicine products and for the characterization of cell banks' argues that what is necessary to take into consideration with respect to rcDNA (residual cellular DNA) in vaccines is:

**A.** a reduction in the amount of contaminating DNA during the manufacturing process;
**B.** a reduction in the size of the contaminating DNA during the manufacturing process;
**C.** a chemical inactivation of the biological activity of DNA occurred during the manufacturing process.

Taking into account the three requests described above, the product is considered by their regulatory organs (NRA) and control laboratories (NLC) to be at an acceptable level of risk regarding the presence of DNA from the cell substrate, based on (a) and / or (b) and / or (c), when the data show that adequate levels of safety have been achieved.

In particular, in the 2 batches of Priorix Tetra vaccine tested to date, point A. does not occur because the quantity is about 140 times higher than that recommended by the FDA (in Briefing Document September 19, 2012: Vaccines and Related Biological Products Advisory Committee Meeting) and the EMA, ie ≤ 10ng per dose; point B) does not occur because the DNA is high molecular weight (most> 10,000 bp, as can easily be verified using a simple agarose gel to control the quality of the DNA extracted from the vaccine), ie 50 times greater than the size recommended by the FDA (200bp or less). Finally, in the same vaccine, point C) does not occur because, containing attenuated viruses, a possible chemical inactivation of DNA would also inactivate viruses.

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# Results of the analysis of vaccine samples 4, 5, 6, 7

## Infanrix hexa

The assay was performed on the DNA extracted from a solution prepared by resuspending the powder of the vial of the vaccine with the sterile physiological solution supplied together with it.

**DNA analysis**

**Total DNA extracted**:
not quantifiable through standard fluorimetric methods
(limit of detection 0.1 ng/µl).

DNA sequencing analysis performed using a metagenomic approach, out of a total of 27.28 million sequences produced.

**Presence of genomic DNA of:**

Monkey **4.69%**

**RNA analysis**

**Total RNA extracted:**
not quantifiable through standard fluorimetric methods
(limit of detection 0.1 ng/µl).
Failed RNA sequencing analysis performed using reverse transcription approach.

## PolioInfanrix

The assay was performed on the DNA extracted from a solution prepared by resuspending the powder of the vial of the vaccine with the sterile physiological solution supplied together with it.

**DNA analysis**

**Total DNA extracted**:
not quantifiable through standard fluorimetric methods
(limit of detection 0.1 ng/µl).

DNA sequencing analysis performed using a metagenomic approach, out of a total of 23.03 million sequences produced.

**Presence of genomic DNA of:**

Monkey **5.14%**

**RNA analysis**

**Total RNA extracted:**
not quantifiable through standard fluorimetric methods
(limit of detection 0.1 ng/µl).
Failed RNA sequencing analysis performed using reverse transcription approach.

## Fluad

The assay was performed on the DNA extracted from a solution prepared by resuspending the powder of the vial of the vaccine with the sterile physiological solution supplied together with it.

**DNA analysis**

**Total DNA extracted**:
not quantifiable through standard fluorimetric methods
(limit of detection 0.1 ng/µl).

DNA sequencing analysis performed using a metagenomic approach, out of a total of 22.48 million sequences produced.

**Presence of genomic DNA of:**

Chicken **8%**

**RNA analysis**

**Total RNA extracted:**
not quantifiable through standard fluorimetric methods
(limit of detection 0.1 ng/µl).
Failed RNA sequencing analysis performed using reverse transcription approach.

## Vivotif

| | |
|---|---|
| | The assay was performed on the DNA extracted from powdered gastrointestinal hard capsule. |
| **DNA analysis** | Total DNA extracted: 2500 ng per tablet. DNA sequencing analysis performed using a metagenomic approach, out of a total of 1.2 million sequences produced. |
| **Presence of genomic DNA of:** | Salmonella typhi Ty21a **97%** |
| **RNA analysis** | **Total RNA extracted**: 2050 ng per tablet. RNA sequencing analysis performed using reverse transcription approach, out of a total of 0.34 million sequences produced. |
| **Presence of RNA of:** | Salmonella typhi Ty21a **90 %**<br>Human **8%** |

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## From the examination of the findings, the following can be highlighted:

**Infanrix hexa and polio infanrix vaccines**: viral DNA of the poliovirus (virus inactivated during the vaccine production) is in quantities below the limits of detection of both standard instruments (eg. fluorimeter to detect the concentration of DNA) and also below the sensitivity of deep sequencing, which is the most sensitive state-of-the-art method in detecting DNA traces.
Traces of monkey DNA from Vero cell line are present in both vaccines.

**Fluad vaccine**: traces of DNA from chicken cell line are present.

**Vivotif vaccine**: 8% of human DNA is present for unexplained reasons.

## Analysis of genetic variants

With Next Generation Sequencing technology it is possible to reconstruct the entire sequence of viral DNA and RNA genomes and bacterial genomes which are present in the sample and compare it with the reference genomes from public databases. Therefore the technology can also enable to monitor over time if and how the viral or bacterial genome sequence changes during the process of vaccine production.

The result of the call of the variants (single nucleotide and small insertions/deletions) from the reference strains available in the public databases (NCBI, National Center for Biotechnology information, https://www.ncbi.nlm.nih.gov/) performed in vaccine samples containing live attenuated virus or bacteria proved the following :

### Campione 1. - Priorix Tetra

**1)** The measles virus genome contained in the vaccine is identical to the Edmonston Swartz strain sequence filed into the databases with accession number AF266291.1. The number of variants detected was in fact equal to 0;
**2)** The mumps virus genome contained in the vaccine showed a single mutation from the Jeryl-Lynn viral strain filed in the public databases with accession number AF338106.1;
**3)** The rubella virus genome was not detected;
**4)** The chicken pox virus contained in the vaccine showed 4 mutations compared to the Human herpesvirus 3 filed in public databases with accession number AB097932.1;

### Sample 2. - Measles vaccine live B.P.

The measles virus genome contained in the vaccine showed 6 mutations compared to the Edmonston Zagreb viral strain filed in public databases with accession number AF266290.1.

### Sample 3. - MMR vax Pro

**1)** The measles virus genome contained in the vaccine is identical to the Edmonston Swartz strain sequence filed in the databases with accession number AF266291.1. The number of variants detected was in fact equal to 0.
**2)** The mumps virus genome contained in the vaccine was identical to the Jeryl-Lynn virla strain sequence filed in public databases with accession number AF338106.1. the number of variants detected was in fact equal to 0;
**3)** The rubella virus genome contained in the vaccine is identical to the Wistar RA 27/3 strain sequence filed in the public databases with accession number FJ211587. The number of variants detected was in fact equal to 0;

### Sample 7. - Vivotif

The bacterial genome contained in the vaccine showed 154 mutations compared to the Salmonella typhi Ty21a (NCBI accession number NC_021176.1) sequence, reported in public databases as a vaccine strain sequence.

The viral antigens/genomes sequence is a strictly confidential data which is not provided by the EMA. There are no guidelines to regulate the analysis of genetic mutations and the research of the effects on human health.

The high rate of genetic mutations in viruses and bacteria, as well as in the cell lines DNA culture, is a major issue regarding safety, as it is not known how any found variants can modify the infectious capacity and the stimulation of the immune system leading to autoimmune reactions.

As an example, Efsa now requires the genomic characterization of probiotic strains for human/animal use and subsequently the evidence, over time, of matching the microorganism sequence with the one declared, while in the case of vaccines, like Vivotif, are tolerated as many as 154 genetic variants compared to that reported in the data sheet and public databases as a reference vaccine strain.

***We consider the presence of genetic variants in vaccine samples compared to the declared strains as a non-compliance of the drugs.***

**NOTE**: the original documents are protected subject-matter by a nondisclosure agreement with the analysis laboratory and the researchers who performed the tests. All subject-matter will be presented to the investigation bodies as an attachment to a Complaint to the Public Prosecutor's Office.

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**Attached 1.**

| TABLE OF NUMBER OF VARIANTS DETECTED | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Genome of reference (NCBI Accession Number) | 1. Priorix Tetra lot. A71CB205A | 2. Measles vaccine live B.P. lot. B.N. 001M7001A | 3. MMR vax Pro lot. N023345 | 4. Infanrix hexa lot. A21CD072D | 5. Polio Infanrix lot. AC20B351B C | 6. Fluad lot. 170901 (Influence period 2017/2018) | Vivitof strain Ty21a lot 3003187§ | 11. H20 CTRL NEG |
| Measles Edmonston Swartz (AF266291.1) | 0 | 43 (13) | 0 (18) | not done | not done | not done | not done | 0* |
| Measles Edmonston Zagreb (AF266290.1) | 39 (2) | 6 (14) | 42 (10) | not done | not done | not done | not done | 0* |
| Measles Edmonston Enders (Morten) (FJ211583.1) | not done | not done | 0 (18) | not done | not done | not done | not done | 0* |
| Mumps_JERYL-LYNN_mayor_component JL2 (AF338106.1) | 1 (28) | 0* | 0 (322) | not done | not done | not done | not done | 0* |
| Mumps_JERYL-LYNN_minr_component JL2 (AF345290.1) | 411 (26) | 0* | 76 (356) | not done | not done | not done | not done | 0* |
| Rubella Wistar RA 27/3 (FJ211587) | 0* | 0* | 0 (22) | not done | not done | not done | not done | 0* |
| Varicella Human herpesvirus 3 (AB097932.1) | 4 (28) | not done | not done | not done | not done | not done | not done | not done |
| Salmonella typhi Ty21a (NC_021176.1) | not done | not done | not done | not done | not done | not done | 154 (16) | not done |

§ Vivitof, lot. 3003187 (Composition Salmonella typhi Ty21a, strain live attenuated, Manufacturer PaxVax, United Kingdom), call of the variants done with data of metagenome DNA-seq producted in the 2017 for the same customer

* the organism is not present

further putative variants to be confirmed

11. Negative check (bidistilled sterile water)