

# Masters of Virology



Learn what the inventors of the most successful vaccines did to develop and produce some of the vaccines we use today.

We explain what they did in plain language and use simple examples from their own documents.

Featuring:  
Stanley Plotkin (Rubella),  
John Enders (Measles),  
Maurice Hilleman (Mumps)  
Jonas Salk (Polio)  
Leonard Hayflick (WI-38 cell line)

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# How are vaccines made?

The **National Immunisation Programme** of the **Dutch government** is a list of all the vaccines that are given to children in the Netherlands.

On the page entitled '**How does vaccination work?**' you can find the '**Vaccine factsheets**' for all the vaccines included in the Dutch government's vaccination schedule.

The first on the list is the **MMR (Measles, Mumps, Rubella) vaccine**, in the Netherlands this is the **M-M-RVAXPRO**.

## Vaccine factsheet

All the vaccines included in the National Immunisation Programme are listed below. There are also links to the vaccine factsheets, where you can read more about all the benefits and drawbacks of the vaccine. A 'vaccine factsheet' is an addendum to the official package leaflet. These Dutch factsheets were made by the Medicines Evaluation Board (CBG-MEB).

### MMR vaccine

Hide 

[Patient information leaflet for M-M-RVAXPRO \(go to page 37\)](#) 



[Product information on M-M-RVAXPRO](#) 



[Vaccine factsheet \(in Dutch\)](#) 



Website: **National Immunisation Program of the Dutch Government:**

<https://rijksvaccinatieprogramma.nl/en/how-does-vaccination-work/vaccine-information-leaflets>

(consulted 24 November 2024)

At first sight you might think that the Dutch government wants to inform its citizens well about the vaccines in its vaccine schedule.

**But is this so, how helpful are they really being?**

In the 'Product Information' about the **M-M-RVAXPRO vaccine** it says that the **rubella virus** is produced in **WI-38 human diploid lung fibroblasts**.

What are **WI-38 human diploid lung fibroblasts** exactly?

How can any parent make a **well informed decision** about giving this vaccine to their children without knowing what these are?

**Product Information about the MMR (Measles, Mumps, Rubella) M-M-RvaxPro vaccine from the Dutch National Immunisation Programme, see page 2**

**2. QUALITATIVE AND QUANTITATIVE COMPOSITION**

After reconstitution, one dose (0.5 ml) contains:

Measles virus<sup>1</sup> Enders' Edmonston strain (live, attenuated) .....not less than  $1 \times 10^3$  CCID<sub>50</sub>\*  
Mumps virus<sup>1</sup> Jeryl Lynn™ [Level B] strain (live, attenuated) .....not less than  $12.5 \times 10^3$  CCID<sub>50</sub>\*  
Rubella virus<sup>2</sup> Wistar RA 27/3 strain (live, attenuated) .....not less than  $1 \times 10^3$  CCID<sub>50</sub>\*

\*50% cell culture infectious dose

<sup>1</sup> produced in chick embryo cells.

<sup>2</sup> produced in WI-38 human diploid lung fibroblasts.

Website **National Immunisation Program of the Dutch Government**:

<https://rijksvaccinatieprogramma.nl/en/how-does-vaccination-work/vaccine-information-leaflets> also  
Website **European Medicines Agency**. [https://www.ema.europa.eu/en/documents/referral/m-m-rvaxpro-product-information-approved-chmp-13-december-2012-pending-endorsement-european-commission\\_en.pdf](https://www.ema.europa.eu/en/documents/referral/m-m-rvaxpro-product-information-approved-chmp-13-december-2012-pending-endorsement-european-commission_en.pdf) (consulted 24 November 2024)

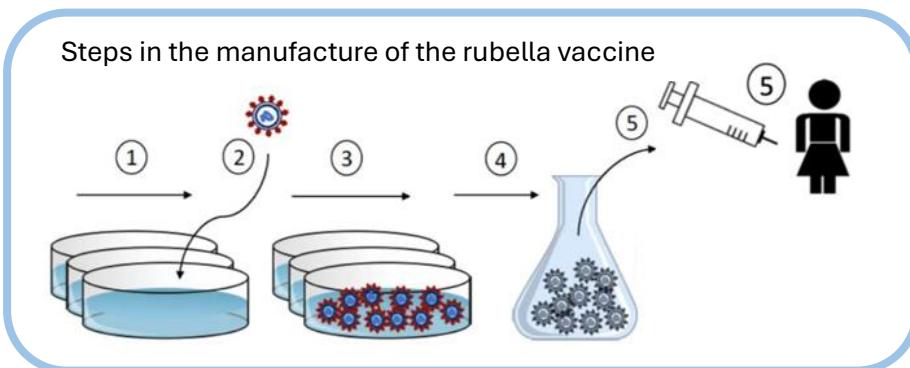
So according to the **M-M-RVAXPRO** Product Information the **rubella virus** is produced in **WI-38 human diploid lung fibroblasts**.

**WI-38 human diploid lung fibroblasts** are in fact the **lung cells of a human foetus** that was **aborted** when it was **16 weeks old**.

So in layman's terms, the so called **living** but **attenuated/weakened rubella virus**, Wistar RA 27/3, is added to these **lung cells** and allowed to multiply.

The rubella viruses that are produced are then collected and used in the in the **MMR (Measles, Mumps, Rubella) vaccine**.

Well that is the theory...



**1 Prepare growth medium for viruses e.g. (the lung cells of a human embryo) -> WI-38 human diploid lung fibroblasts.**

**2 Infect the human embryo cells with the rubella virus e.g. -> Wistar RA 27/3.**

**3 Allow the viruses to grow.**

**4 Collect the viruses and add adjuvants (to stimulate the immune system) and preservatives (to preserve the vaccine) etc.**

**5 Make doses of vaccine for injection.**

## The reality is a whole lot different.

But to understand how the **rubella vaccine** in the **MMR (Measles, Mumps, Rubella) - M-M-RvaxPro vaccine** is made we need to know a little about virology and how virologists make “things”.

As already mentioned, **WI-38 human diploid lung fibroblasts** are the **lung cells** of a **human foetus aborted** when it was 16 weeks.

In virology these **WI-38 human diploid cells** are called a **cell strain or cell line**. And believe it or not the **attenuated/weakened rubella virus**, the **Wistar RA 27/3**, is also a **cell strain/cell line**.

**(1)** So the **first thing** we need to understand in vaccine production is **how cell strains/cell lines are made** so that we can understand how the **WI-38 cell strain** and the so called **Wistar RA27/3 rubella cell strain** were made.

(It is also interesting to examine how the **Measles cell strain** was made as it was done in a very similar manner.)

**(2)** The **next step** is to understand **how these two cell strains/cell lines** are used in the **mass production** of the so called **rubella vaccine**.

**(3)** When virologists make cell strains such as the **Wistar RA27/3 rubella cell strain** and the **Measles cell strain** they never remove the alleged rubella or measles virus from the human samples that supposedly contain the virus.

Virologists only ever **culture/cultivate cell lines** that **allegedly contain viruses**.

**We examine why this is the case and what their motives are for doing this.**

**(4)** Some bacteriophages are even smaller than certain alleged viruses and have been isolated. So if virologists really wanted to isolate alleged viruses how could do this?

Here we look at **how bacteriophages** can be **isolated** from **sewage water** and **soil**.

**(5) Conclusion: What does all this tell us about the state of microbiology today?**

## Chapters In This Document

### **(1) How cell strains/cell lines are made**

- (a) How to make a cell strain/cell line in 4 steps
- (b) How cell strains/cell lines were made by Leonard Hayflick at the Wistar Institute in the 1960s
- (c) How Leonard Hayflick made the **WI-38 cell strain in 1962**
- (d) How Stanley Plotkin made the **Rubella virus Wistar RA 27/3 cell strain in 1964**
- (e) How John Enders made the **Measles virus Enders Edmonston cell strain in 1954**

### **(2) How the measles and rubella vaccines are mass produced**

### **(3) Why virologists have only ever cultured cells and never ‘isolated’ any microorganism called a virus?**

### **(4) If virologists really wanted to isolate alleged viruses how could they do this?**

### **(5) Conclusion: What does all of this tell us about the state of microbiology today?**

Appendix 1: Definition of Words Used in Microbiology and Virology

Appendix 2: References and Weblinks

## 1(a) How cell strains/cell lines are made in 4 steps

So let us look at the example of how to make a **cell strain/cell line** from the **hippocampal brain cells** of a **mouse embryo**.

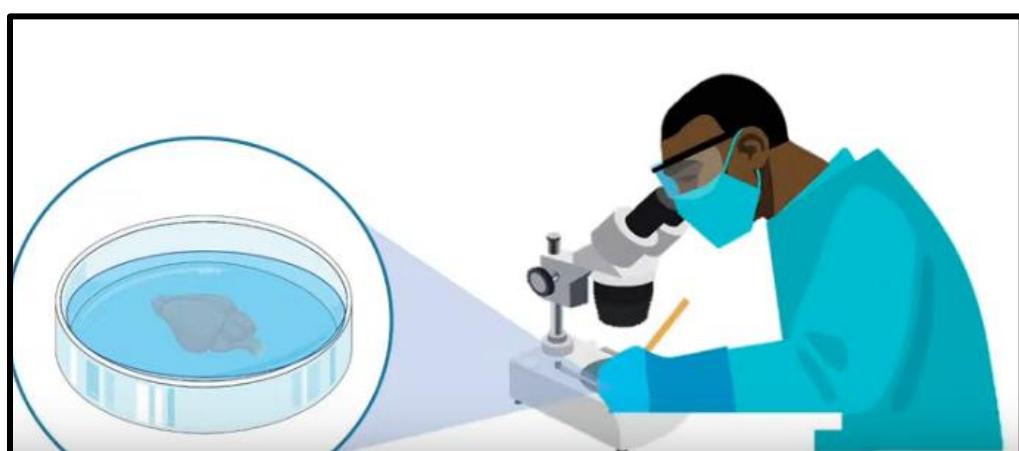
### Step 1 Obtain the required cells from a suitable embryo



- Select a **mouse embryo** of the required age and **remove** it from its **mother**.



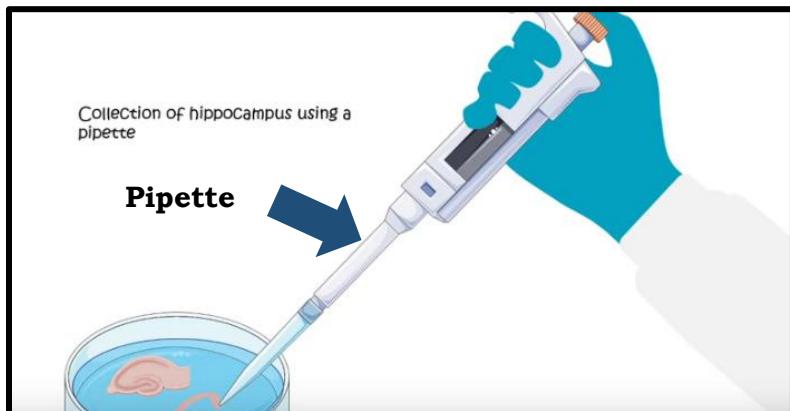
- Cut out the **brain** containing the desired “**explant organ or tissues**”.



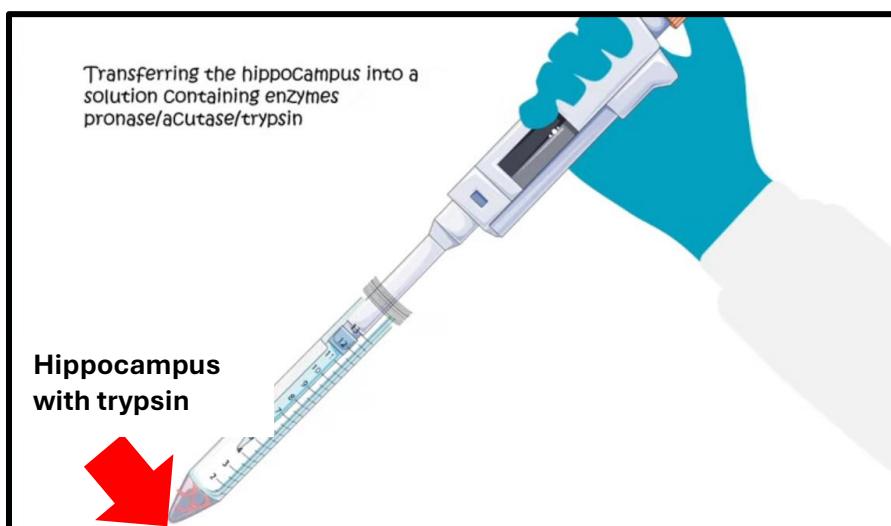
- Dissect the **hippocampus** from of the **brain** under the **microscope**.

(The **hippocampus** is part of the brain, human brains also have a **hippocampus**.)

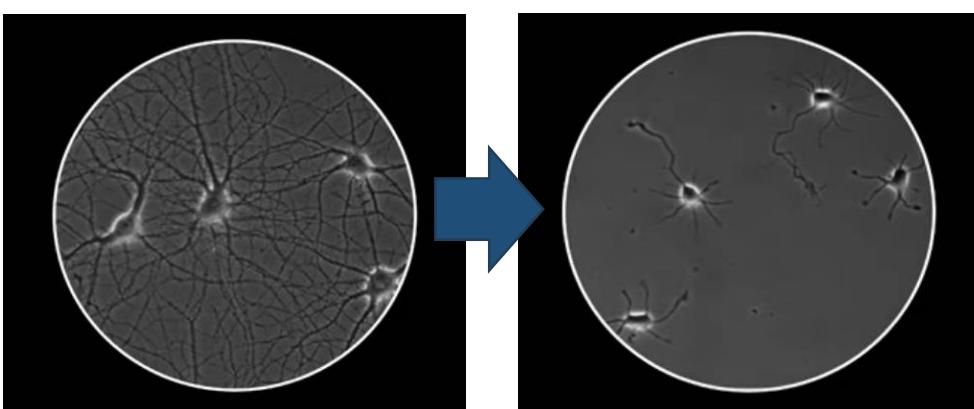
## Step 2 Prepare the cells for cultivation



- Suck the **hippocampus** up with a **pipette** and transfer it to a **test tube**.



- Add **trypsin** or similar enzymes. Trypsin breaks down the tissue connecting the cells in order to create a '**cell suspension**'. This is called '**cell disassociation**'.



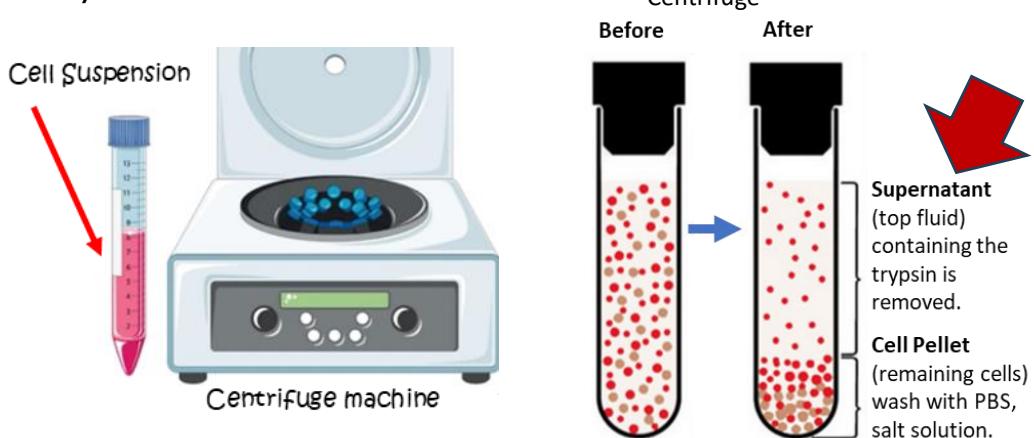
- Brain cells **before and after** the use of trypsin.

In the picture above you can see that the **cell tissue** connecting the cells has been **broken down**, the synapses between the brain cells have been removed. The cells are still alive however they are no longer growing as tissue but as single cells in a "**cell suspension**".

- Once the cells have been '**disassociated**' remove the **trypsin** as it is toxic and will harm cell growth. **This is done in 2 steps:**

(a) **Centrifuge** the cell suspension and remove the **supernatant** (top fluid) containing the trypsin.

(b) **Wash** (or rinse) the **cell pellet**, the remaining cells at the bottom of the test tube, with **PBS (salt solution)**.

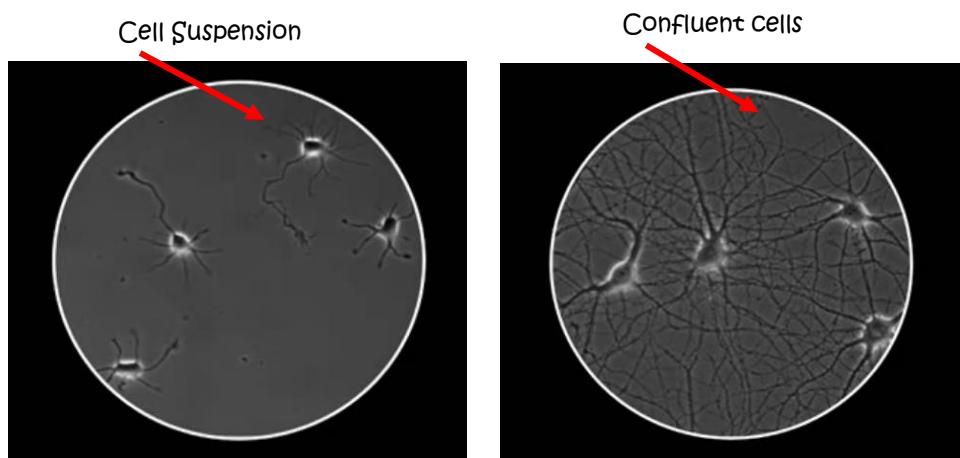


### Step 3. Let the cells grow

- Mix the cell pellet, the remaining cells in the test tube, with some **liquid food** called '**cell culture medium**' and place this in a **petri dish** or **other container** to grow. (This "food" is mainly made of serum (blood) of animal foetuses e.g. calves.)

After about **3 weeks (21 days)** the cells will reform connecting tissue. The cells start to grow as they would inside the body. (Because the dissected cells are brain cells they will reform synapses, as you can see below.)

In the beginning the cells in the culture will grow to form a monolayer. A **monolayer** is a **single sheet** of cells.



Brain cells before and after 3 weeks (21 days) of growth in a culture.

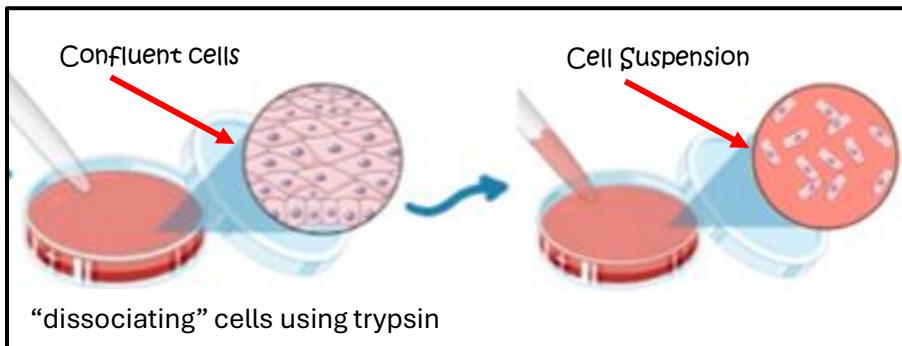
However, after some time the cells in a culture will stop growing as individual cells but will grow more as tissue, one on top of each other. Because of this, the growth of the cell culture slows or stops completely. Scientists call this **confluency**. They say that the cells have become **confluent**.

#### Step 4 Subcultivate / passage the cells

After about **3 weeks** (21 days) the cells are once again disassociated using **trypsin** and then **subcultured/passaged**, divided into **2 or more ‘subcultures’**.

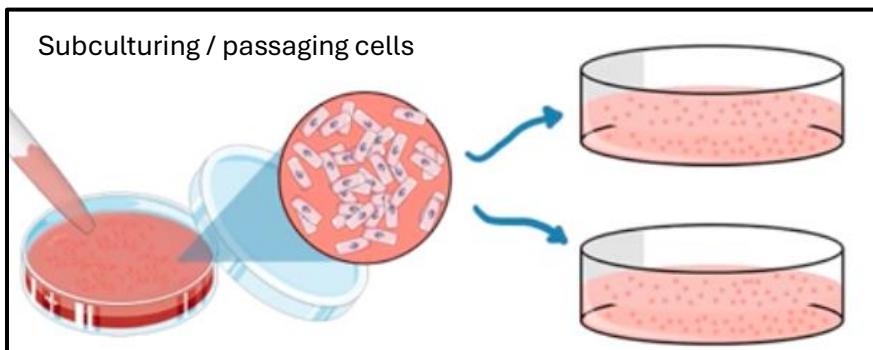
After about 3 weeks the cells in the culture have multiplied and cell growth has slowed or stopped. This happens because the cells have become **confluent**, they no longer grow as unique cells but more as cell tissue, their natural way of growing.

They therefore need to be ‘disassociated’ and ‘subcultured/passaged’.



- Add **trypsin** to ‘disassociate’ the cells and once again create a ‘**cell suspension**’.

As mentioned already **trypsin** breaks down the tissue connecting the cells but it is toxic **so it must be removed**. (See Step 2: Prepare the cells for cultivation)



- Divide the cell suspension into **two or more ‘subcultures’**.

Cells need to be regularly sub-cultured/passaged in order to maintain cell growth. (If not they will start to grow as tissue rather than suspended in liquid. This is called a “**cell suspension**”.)

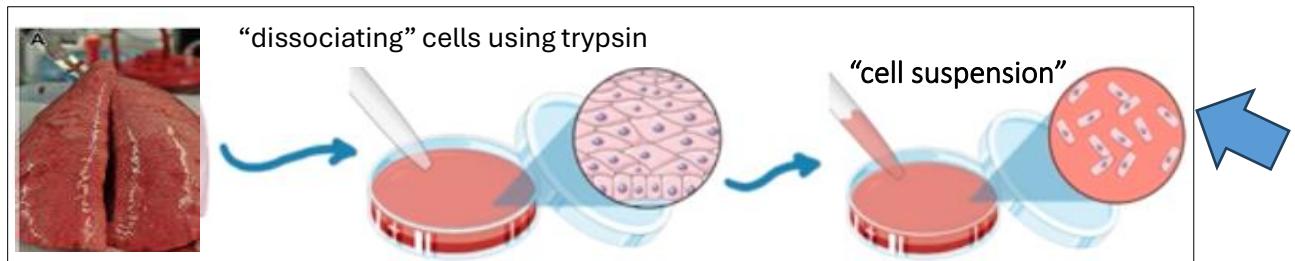
The number of times a cell strain or cell line has been subcultured is called the **population doubling level (PDL)**. A **PLD of 8**, for example, means that a cell strain or cell line has been **subcultured/passaged 8 times**.

**The explanation above is given in the following 3 videos:**

- Video: Primary Cell culture and cell line/Cell culture basics, <https://www.youtube.com/watch?v=9BvTFowr0rl&t=131s> (consulted 6 Nov 2024)
- Video: Sub-culturing cells/Cell culture basics, <https://www.youtube.com/watch?v=Oo36pvP0TrI> (consulted 9 Nov 2024)
- Video: Animal cell culture media, <https://www.youtube.com/watch?v=UV7T9JxsdXA> (consulted 9 Dec 2024)

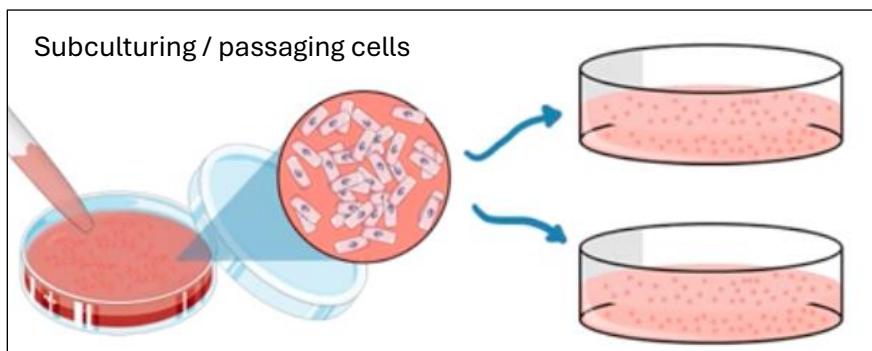
Let us now summarise this whole process by describing how you make a **cell line/cell strain** from the **lung tissue** of a **human embryo**.

1. Remove a baby from its mother and dissect. Cut out the **lungs which are the required “explant tissues” for your culture**.



2. Using **trypsin** -> ‘disassociate’ the lung cells and make a ‘**cell suspension**’.

Centrifuge the suspension and remove the supernatant (top fluid) with the trypsin. To further remove the trypsin, wash (rinse) the remaining cells with PBS (salt solution).



3. Leave the cells to grow in a ‘**cell medium culture**’, liquid food for cells.  
(This is mainly made of serum (blood) of animal foetuses e.g. calves.)

4. After about 10 days ‘disassociate’ the cells once again using **trypsin** and **subculture/passage** them, meaning **divide them into 2 or more ‘subcultures’**.

So this is how a **cell line / cell strain** is made using a **human fetus**,

It is by using this procedure that the **WI-38 cell line** was made. And as we have already mentioned the WI-38 cell line is an important part of the **Dutch vaccination schedule** because it is used in the production of the **MMR vaccine**.



Let us now see how **Leonard Hayflick** the maker of WI-38 made **cell lines** in the **1960s**. At the time he worked for the **Wistar Institute**. Indeed, in the 1960s the Wistar Institute was one of the most important laboratories in the world for the **development of vaccines**.

The processes and techniques that **Hayflick** used to make the **WI-38 cell-line** are the **basic cell-culture techniques** used in both the **development of vaccines** and in the **large scale production of vaccines**.

## 1(b) How cell strains/cell lines were made by Leonard Hayflick at the Wistar Institute in the 1960s

*Experimental Cell Research* 25, 585-621 (1961)

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### THE SERIAL CULTIVATION OF HUMAN DIPLOID CELL STRAINS<sup>1</sup>

L. HAYFLICK and P. S. MOORHEAD

*Wistar Institute of Anatomy and Biology, Philadelphia, Pa., U.S.A.*

Received May 15, 1961

L. Hayflick et al., "The Serial Cultivation of Human Diploid Cell Strains," *Experimental Cell Research*, Vol 25v(1961), pp 585-621. (consulted 6 Jan 2025) <https://irp.cdn-website.com/e4e1af55/files/uploaded/The+serial+cultivation+of+human+diploid+cell+strains.pdf>

#### Step 2. Preparation of cells for cultivation

As you can read below there were **two methods** used for preparing the **primary tissue** (e.g. the tissues and organs of **human embryos/fetuses** obtained from abortions) **for "cell cultivation"**.

Either **trypsin** was used to break down the cell tissue to create a 'cell suspension' or tissues were **fragmented**, in other words **minced up**. (Hayflick seems to prefer the use of **trypsin** as it yielded more cells from the collected tissues and organs.)



Blake Bottles

He gives an example of the **fragmented/minced up lungs of a three-month-old human fetus** being treated using **trypsin** and being put in **4 Blake bottles** (standard jars used in microbiology for cell cultivation).

*Isolation of primary cells.—* Two methods of cell cultivation from primary tissue were employed in this study with identical qualitative results. The use of trypsin yielded far more cells initially than cultures prepared from fragmented or minced tissue. Since high cell yields were not required from the starting tissue, most cultures were started from fragmented or minced tissue. Such preparations gave fewer cells initially than could have been obtained from tissue treated with the enzyme preparation. Minced preparations were obtained by cutting the tissue in a Petri dish containing GM with paired scalpels or a scissors until the size of each piece approximated 1-4 mm<sup>3</sup>. Fragmented preparations were obtained by tearing apart the tissue with two pairs of forceps in a Petri dish containing GM until the pieces could no longer conveniently be grasped and shredded. The entire contents of the dish were emptied into one or more Pyrex Blake bottles (surface area 100 cm<sup>2</sup>), depending on the size of the original starting tissue. The fragmented lungs, for example, from a three-month-old human fetus were usually placed in four Blake bottles. Treatment of tissue with trypsin was done, in general, according to the method of Fernandes [11].

### Step 3. Let the cells grow/multiply

Once the cells had been treated with trypsin or minced up/fragmented some **growth medium (GM)** was added and they were incubated. When Hayflick mentions “planting cells” all he means is that he has added some GM and thus started the **growth phase of the culture**.

He mentions that: If the foetal tissue that was collected was “viable” some cells could be found in the bottles after **3 days of incubation at 36C**.

It is interesting to note that according to Hayflick it is **not important to ‘process’** meaning prepare the fetus or fetal tissue **immediately** on receipt of it. If the fetus or fetal material (minced up body parts) are viable they can be kept **at least 5 days at room temperature, or 5C**, without any loss of viability.

*Initiation of cultures.*—If the fetal tissue was viable when received, cells could be found in bottles planted by any one of the methods described after about three days of incubation at 36°C. When growth was first observed the cultures were refed. The spent medium and any fragments present were discarded. If additional bottles were required these fragments could be replanted in a new bottle. Fresh GM was added and as soon as the cells formed a confluent sheet the cultures were subcultivated. This normally occurred in about 10 days. Periodic feeding of the cultures was done when a sharp drop in the pH of the medium made it necessary.

In the beginning of this study attempts were made to minimize the period of time elapsing between the receipt of the fetus or fetal tissue and its cultivation *in vitro*. It was subsequently found that if either was viable upon receipt it could be kept for at least 5 days at room temperature, or 5°C, without apparent loss of viability. Minced tissue, kept in a minimal amount of GM has been found to be viable for periods of time up to 3 weeks, either at room temperature or 5°C.

p 588 (GM stands for Growth Medium, *in vitro* – outside the body, *in vivo*- inside)

Eagle's Medium is a cell culture medium developed by Harry Eagle and first published in Science Magazine in 1959. It is based on six salts, glucose as well as thirteen essential amino acids, and eight vitamins.

The growth medium used contained **calf serum** which is the **blood of cow fetuses** which are killed and their blood drained to grow cell cultures. The blood of cow fetuses contains special growth hormones that are necessary to keep the cells multiplying in artificial settings.

The growth medium also contains two highly toxic **antibiotics** including penicillin and streptomycin. This is added to stop bacteria growing in the culture.

### MATERIALS AND METHODS

Media.—The growth medium (GM) used was Eagle's Medium in Earle's Balanced Salt Solution [8] supplemented with 10 per cent calf serum.<sup>1</sup> Twenty-five ml of 5.6 per cent NaHCO<sub>3</sub>, 10<sup>5</sup> units of penicillin and 10<sup>5</sup> µg of streptomycin, were added per liter. The final pH of the medium was 7.3, and before use it was brought to 37°C. Phosphate buffered saline (PBS) was prepared as described by Dulbecco and Vogt [7]. Difco trypsin (1:250) was prepared as a 0.25 per cent solution in PBS and supplemented after filtration with the antibiotics described above.

p 587

#### Step 4. Subcultivating/passaging the cells

As soon as the cell cultures were fully “sheeted” (had formed a monolayer of cells) they were subcultivated every three to four days.

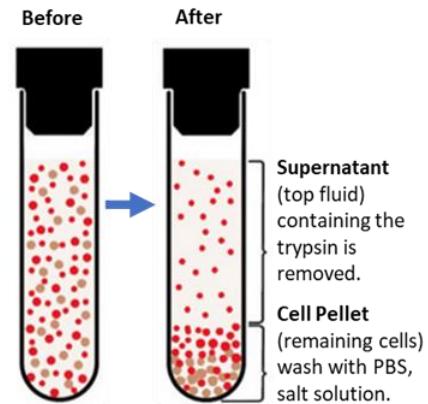
Hayflick describes how trypsin was used to “**dislodge**” cells meaning “**disassociate**” cells and create a cell suspension which was then centrifuged to remove the trypsin.

The “**trypsin solution**” was “**decanted**” meaning removed and some GM was added and then aspirated (sucked out with a pipet) to remove further the trypsin.

Interestingly he uses GM instead of the usual PBS (salt solution) to wash the cells.

**Finally**, the cells were **distributed** to two Blake bottles and GM was added.

**This was called a 2:1 split.**



Subcultivation of *confluent cultures*.—As soon as cell cultures were fully sheeted they were put on a strict schedule of subcultivations, which were done alternately every third and fourth day. The spent GM was discarded and trypsin solution was added to each bottle. After incubation at 37°C, or room temperature, for 15 min, the enzyme solution containing the dislodged cells was centrifuged for 10 min at 600 r.p.m. in an International Size 2 Model V Centrifuge. The trypsin solution was decanted after centrifugation and the cells were resuspended in a small amount of GM, aspirated with a 5 ml pipette, and evenly distributed to two Blake bottles. Sufficient fresh medium was added to each bottle to cover the surface adequately. This was called a 2:1 split. In the early part of this study split ratios of 3:1 were used with equal success. Incubation was carried out at 36°C.

p 588

#### Definitions according to Hayflick:

**primary cells** are cells obtained from the original tissue that have been cultivated in vitro (artificially outside the body) for the first time.

**a cell strain** is a population of cells derived from animal tissue, subcultivated more than once in vitro (artificially outside the body) and lacking the property of indefinite serial passage while preserving the chromosomal karyotype characterizing the tissue of origin.

**a cell line** is a population of cells derived from animal tissue and grown in vitro (artificially outside the body) by serial subcultivations for indefinite periods of time with a departure from the chromosome number characterizing its source.

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Related Transcript Biography [In](#)

Title: How to isolate your own cells

Listeners: Christopher Sykes

Tags: human cells, trypsin, skin, bovine serum, growth medium

Duration: 2 minutes, 28 seconds

Date story recorded: July 2011

Date story went live: 08 August 2012

How to isolate your own cells

Leonard Hayflick Scientist

Play all

## Interview with Leonard Hayflick in July 2011 (part 50) : How to isolate your own cells (Interviewer Christopher Sykes)

<https://www.webofstories.com/play/leonard.hayflick/50> (consulted 7 Jan 2025)

Hayflick explains in his own words the use of trypsin to disassociate the cells in a cell culture. He also describes how they use **serum** meaning the **blood** from **calves and other animals** to “feed” the cells in the cell cultures and keep them alive.

1. Obtain the required cells from part of your own tissue. 2. Preparation of cells for cultivation,

### Transcript:

You can isolate cells very easily from your own person or from volunteers, usually without too much pain. And, for example, I've done it to myself and I'll illustrate it. **You can pinch, for example, part of your tissue like your wrist until it's a bit numb. Then you pull up one hair with a forceps, so you have a pyramid of skin, then you knock the top of that pyramid off with a sterile scalpel, with a bit of pain obviously, but easily endured, and you take that hair with its attached skin, put it into a vessel that contains an enzyme called trypsin. The trypsin dissolves the substances that keep cells together in a tissue. Very much like a brick wall and what you're doing is dissolving the mortar in a brick wall and releasing the millions of cells, and there are millions of cells, in that tiny scrap of tissue, and now you take those cells, put them in a growth media.**

The media consists of chemicals that are well known, that you might expect, like amino acids, certain salts, sodium, potassium, calcium, phosphorous, etc. And because we do not know, for most cells, certainly normal cells, what their growth requirements are in chemical terms, we have to cover our ignorance by including in the media serum from an animal. **Usually about 10% of the volume of the growth media consists of serum. It might be humbling to realise that your own cells, or human cells, grow very well in serum from a cow, a horse, or several other species.** You do not need human serum to grow human cells, although they will grow in human cells, but that obviously is much more difficult to obtain, more costly. **So generally people use bovine serum as the cover-up for ignorance and that continues to this day.**

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Related Transcript Biography Info

**Title:** Cell division

**Listeners:** Christopher Sykes

**Tags:** normal cells, growth medium, glass flask, incubator, cell division, cancer cells, trypsin, subcultivation

**Duration:** 3 minutes

**Date story recorded:** July 2011

**Date story went live:** 08 August 2012

**Cell division**  
Leonard Hayflick Scientist

### Interview with Leonard Hayflick in July 2011 (part 51) : Cell division

(Interviewer Christopher Sykes)

<https://www.webofstories.com/play/leonard.hayflick/51> (consulted 29 Jan 2025)

[https://www.youtube.com/watch?v=NC65\\_b1sGiY&list=PLVV0r6CmEsFyL\\_YyHe6RzAARP6eJ\\_8wC&index=51](https://www.youtube.com/watch?v=NC65_b1sGiY&list=PLVV0r6CmEsFyL_YyHe6RzAARP6eJ_8wC&index=51) (consulted 29 Jan 2025)

3. Let the cells grow/multiply 4. Subcultivating/passaging the

#### Transcript:

So the cells are... **now you have 1 million cells or more, you put them into a rectangular vessel, for example, and then you add the media that I've just described.** The culture vessel is then stoppered with usually a rubber stopper and there are more sophisticated ways of doing this today, but this is in general how it's done. **And you lay this flask on its side so that the long side of the rectangle is flat to the surface of the incubator in which you put the culture.**

So now you have the cells essentially settling down on to the floor of this long side of the flask that you laid flat on the surface of the incubator. **In a couple of hours, the cells, because of their weight, although they weigh very little, will settle down to the floor of the flask, stick to the glass surface and begin to spread out.** They are in a spherical form at this time. **In a couple of hours, they spread out and in a day or two, they begin to divide, so that within a week or so – these are rough numbers – the cells have divided to the point where they have covered the floor of this single flask** and then they do something very important. **They stop dividing because the signals that they receive, when they touch neighbour cells, tells them to stop dividing.** Prior to that, of course, there is much denuded glass so they have plenty of room to divide. Unlike cancer cells that will continue to grow to some extent when they receive that signal, and they'll grow on top of each other to some small extent, normal cells won't do that unless you use some very sophisticated conditions that are not relevant to this story.

**Now, if you want more cells – they have stopped dividing because they have covered the floor of the flask – it is then necessary, if you want more cells and that's a key part of the story, you pour off the spent media.** The cells remain behind because they're stuck to the glass floor. **You then introduce this enzyme called trypsin, which does the same thing it did in the initial scrap of tissue and that then releases the cells from each other and from the glass surface.** And now, for example, you can divide them into two equal parts, introduce them to two equivalent size rectangular bottles and start that process all over again. **That in laboratory jargon is called a split, or a subcultivation,** and I'll be using those terms subsequently.

## 1(c) How Leonard Hayflick made the WI-38 cell strain in 1962

# Cell division

In 1962, Leonard Hayflick created a cell strain from an aborted fetus.



**T**he woman was four months pregnant, but she didn't want another child. In 1962, at a hospital in Sweden, she had a legal abortion.

The fetus — female, 20 centimetres long and wrapped in a sterile green cloth — was delivered to the Karolinska Institute in northwest Stockholm. There, the lungs were dissected, packed on ice and dispatched to the airport, where they were loaded onto a transatlantic flight. A few days later, Leonard Hayflick, an ambitious young microbiologist at the Wistar Institute for Anatomy and Biology in Philadelphia, Pennsylvania, unpacked that box.

Working with a pair of surgical scalpels, Hayflick minced the lungs — each about the size of an adult fingertip — then placed them in a flask with a mix of enzymes that fragmented them into individual cells. These he transferred into several flat-sided glass bottles, to which he added a nutrient broth. He laid the bottles on their sides in a 37°C incubation room. The cells began to divide.

So began WI-38, a strain of cells that has arguably helped to save more lives than any other created by researchers. Many of the experi-

*M. Wadman., "Medical research: Cell Division," Nature, Vol 498 (27 June 2013), pp. 422-426.  
<https://www.nature.com/articles/498422a> (not consulted, paid link)  
<https://www.researchgate.net/publication/242333147> Medical research Cell division (consulted 12 Jan 2025)*

In **1962**, Leonard Hayflick created the WI-38 cell strain from an **aborted fetus**.

Here he describes how he did it.

Using **surgical scalpels**, Hayflick **minced the lungs** and then placed them in a flask with a **mix of enzymes** (probably trypsin) that fragmented (disassociated) them into individual cells.



These he transferred into several flat sided glass bottles, to which he added a **nutrient broth** meaning **cell culture medium/growth medium**.

He laid the bottles on their sides in a **37 °C incubation room**. The cells began to divide. **So began the WI-38 cell strain/cell line.**

## 1(d) How Stanley Plotkin made the Rubella virus Wistar RA 27/3 strain in 1964

Hayflick also supplied WI-38 liberally to aspiring vaccine-makers. One was **Stanley Plotkin**, a Wistar scientist and a physician who had seen at first hand the effects of the huge rubella epidemic that swept the United Kingdom and the United States in the early 1960s. Rubella can be



M. Wadman., "Medical research: Cell Division," *Nature*, Vol 498 (27 June 2013), pp. 422-426.  
<https://www.nature.com/articles/498422a> (not consulted, paid link)  
[https://www.researchgate.net/publication/242333147\\_Medical\\_research\\_Cell\\_division](https://www.researchgate.net/publication/242333147_Medical_research_Cell_division) (consulted 12 Jan 2025)

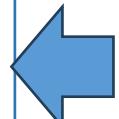
In 1964 Leonard Hayflick supplied Stanley Plotkin who was also working at the Wistar Institute with the WI-38 cell strain.

Plotkin used it to make the **Rubella virus Wistar RA 27/3 strain** and his **rubella vaccine** containing the **alleged rubella virus**.

Let us examine how the **Rubella virus Wistar RA 27/3 strain** was made.

Plotkin describes how he and others made this cell strain in a document entitled:

**"Studies of Immunization With Living Rubella Virus, Trials in Children With a Strain Cultured From an Aborted Fetus",**  
S. Plotkin et al., *American Journal of Diseases of Children*, Vol 110 (1 Oct 1965), pp 381-382, 389.



**Published online by:**

- Semanticscholar: <https://www.semanticscholar.org/paper/Studies-of-immunization-with-living-rubella-virus.-Plotkin-Cornfeld/0c65acb5c182860c0c80263e37fdf1c6e40d48b1> (consulted 30 Sep 2025, only the diagram by Stanley Plotkin can you consult for free, the rest is a paid link) or
- <https://irp.cdn-website.com/e4e1af55/files/uploaded/AmJDisChildPlotkinRubellaVirus.pdf> (consulted 27 Nov 2024)

# Studies of Immunization With Living Rubella Virus

*Trials in Children With a Strain Cultured From an Aborted Fetus*

STANLEY A. PLOTKIN, MD; DAVID CORNFELD, MD; AND THEODORE H. INGALLS, MD, MPH, PHILADELPHIA

The fetus was surgically aborted 17 days after the maternal illness and dissected immediately. Explants from several organs were cultured and successful cell growth was achieved from lung, skin, and kidney. All cell strains were found to be carrying rubella virus. As illustrated in Fig 1, fibroblastic cells from the kidney explant were subcultivated four times by trypsinization and 1:2 split, after which the supernatant fluid was harvested. This harvest was inoculated on stationary WI-38 diploid lung fibroblasts, to initiate infection in these cells. Three further passages were subsequently performed by serially inoculating supernatant tissue culture fluids on fresh WI-38 cultures. Two weeks after inoculation of the fourth set of WI-38 cell cultures, the supernatant fluid was harvested, pooled, and divided into aliquots.

Safety Tests: Before use in children, the following safety tests were performed: The material

(See pp 382)

## Materials and Methods

*Derivation of Rubella Virus for Administration to Children.—Virological Techniques:* The nutrient medium used for tissue culture was Eagle's Basal Medium with 10% inactivated calf serum added when cell growth was desired. Double strength of amino acids and vitamins was incorporated into the medium for culture of explants. The concentrations of antibiotics in each milliliter of medium were 100 $\mu$ g of penicillin, 40 $\mu$ g of streptomycin, 50 $\mu$ g of chlortetracycline or 50 $\mu$ g of neomycin, and 20 $\mu$ g of nystatin. Subcultivation of cells was performed with the aid (See pp 381)

So all Plotkin did was injected WI-38 cells with a toxic mix of antibiotics and trypsin.

(This is how he made the Rubella virus Wistar RA 27/3 strain. He never "isolated" (removed) the alleged rubella virus from the aborted foetus to study it ever.)

**Step 3** was repeated 3 more times. (In other words, the **kidney cells** were **passaged/subcultured 3 more times** and the **WI-38 cells** injected **3 more times**.)

Finally, **two weeks** after this so called "**inoculation of the WI-38 cells**", the **supernatant fluid** from the **WI-38 cells** was '**harvested**' meaning it was collected and then '**safety tested**' before being **injected into children** as a **so called "vaccine"**.

1. First he describes how a baby was aborted and dissected immediately.

2. He then says that "explants" from several organs were successfully "cultured". However **only the cells of the kidney explant were used for further experimentation.**

He goes on to explain that the "**fibroblastic cells from the kidney explant**" were **subcultivated 4 times using trypsin.**

3. The **supernatant fluid** from this kidney culture was then inoculated meaning injected onto **WI-38 cells**.

(He claims that by doing this he is "initiating the infection of the cells".

In other words he is "infecting" the healthy WI-38 cells with Rubella virus.)

This is a ridiculous claim to make given as he made no analysis of the content of the **supernatant fluid**. This fluid could have contained more than one type of virus or even bacteria resistant to antibiotics.

What we do know and what Plotkin knew was that the **supernatant fluid** contained at least **4 types of antibiotics**, present in the GM, and also some **trypsin** as it was used to **subcultivate** the cell cultures.

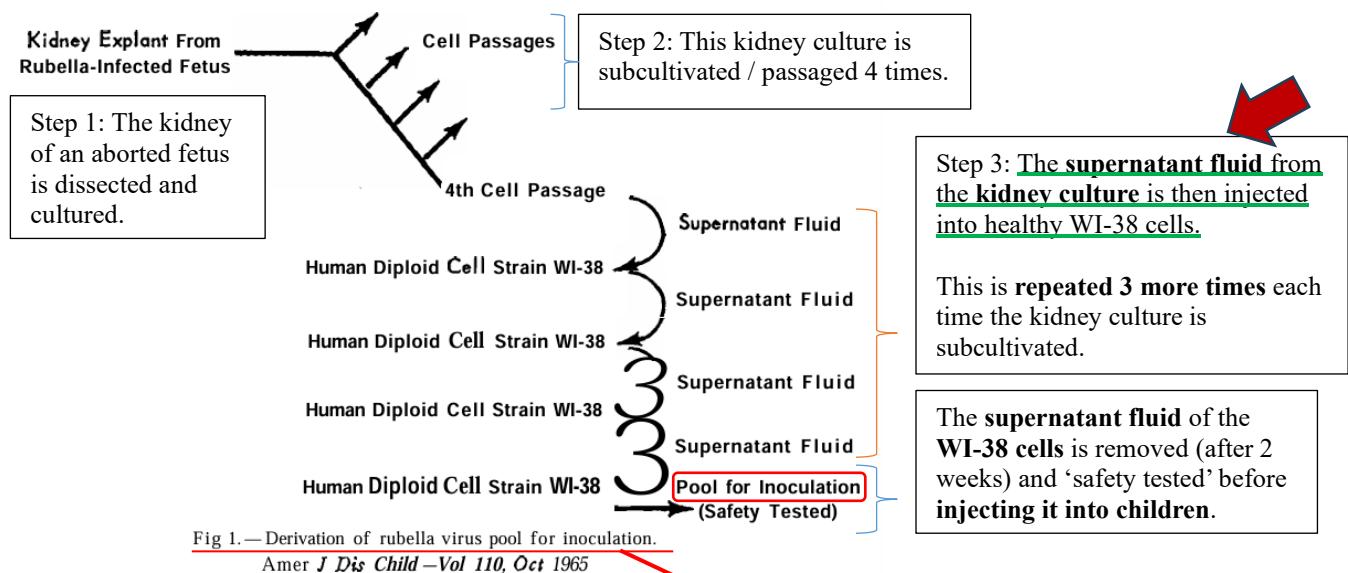
(See pp 381).

So in the words of Stanly Plotkin himself his **Rubella virus Wistar RA 27/3 strain** is nothing more than a **WI-38 cell culture** that he **injected** with some **supernatant fluids** full of antibiotics and trypsin from the **kidney culture of an aborted baby**.

The title of his 'scientific paper' explains perfectly what he made:

"Studies of Immunization With Living Rubella Virus, Trials in Children With **a Strain Cultured From an Aborted Fetus**", American Journal of Diseases of Children, Vol 110 (Oct, 1965), pp 381-382, 389.

The following diagram from **page 382** of Plotkin's paper summarises how he made his so called **Rubella virus Wistar RA 27/3 strain**.



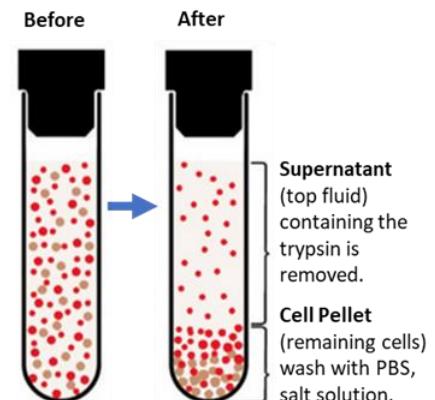
Plotkin called the **supernatant fluids** he collected a **virus pool for inoculation** in other words a "vaccine", a substance that contained the supposed rubella virus.

As you can read for yourself the 'nutrient medium' or **GM (growth medium)** that was used to culture the cells in Plotkin's experiments contained at least **four types of antibiotics**.

The **supernatant fluids** taken from the cultures would have therefore contained these toxins as well as traces of trypsin used to subculture the cells.

There may have been several different **microorganisms** in the supernatant fluids.

However, Plotkin never "isolated" these from the supernatant to study them. **At least, there is no description of this anywhere in his document.**



In 1969 Plotkin published a paper where once again he describes the procedure he used to make the RA 27/3 rubella virus strain. He writes that after **subcultivating the kidney fibroblasts** of an aborted foetus **four times** he **inoculated**, meaning injected, the **supernatant fluid** from the culture “**directly into a WI-38 culture**”. He then states that the cells were subcultured further in the same cell strain namely the **WI-38 cell culture**.

This is more proof that Plotkin never “isolated” the alleged rubella virus. All he did was cultivate the cells of an aborted baby and then inject the supernatant fluids from this cell culture into another cell culture.

## Attenuation of RA 27/3 Rubella Virus in WI-38 Human Diploid Cells

Stanley A. Plotkin, MD; John D. Farquhar, MD; and Michael Katz, MD, Philadelphia; and Fritz Buser, MD, Bern, Switzerland

THE PROVENANCE of the RA 27/3 attenuated rubella strain has already been described in print.<sup>1,2</sup> Detailed information was given at the London Conference just three months ago.<sup>3</sup> Therefore we shall only outline the history of this strain, before proceeding to examine its in vitro characteristics and its behavior when inoculated in man.

In order to avoid the problem of passenger viruses, the RA 27/3 strain was isolated directly from naturally infected material in WI-38 human diploid fibroblast~.~

Explant cultures were made of the dissected organs of a particular fetus aborted because of rubella, the 27th in our series of fetuses aborted during the 1964 epidemic. The third explant, which happened to be from kidney, was selected arbitrarily for further study. Fibroblast cells that grew out from this explant

Received for publication March 10, 1969.

From the Wistar Institute of Anatomy and Biology (Drs. Plotkin and Katz), and the Department of Pediatrics, University of Pennsylvania (Drs. Plotkin, Farquhar, and Katz), Philadelphia. Dr. Buser is in private practice in pediatrics, Bern, Switzerland.

Read before the International Conference of Rubella Immunization, Bethesda, Md, Feb 19, 1969. It was also read before the 23rd Symposium on Microbiological Standardization: Rubella Vaccines, London, Nov 19, 1968.

Reprint requests to the Wistar Institute, Philadelphia 19104 (Dr. Plotkin).

could be subcultivated after several weeks. The presence of rubella virus in the supernatant fluids was confirmed. After four subcultivations of the infected kidney fibroblasts, the supernatant fluid was inoculated directly into a WI-38 culture. Once transferred to WI-38, the RA 27/3 rubella strain was passaged further in the same cell strain.

The RA 27/3 strain was tested again after four and eight passages in WI-38 incubated at 35°C. Subcutaneous inoculation of virus provoked much virus excretion, rash, and spread to contacts.

At this point, two sublines were developed, as illustrated in Table 1, the first by passage in WI-38 cells incubated at 35°C, and the second in the same cultures incubated at 33°C. After reaching the 13th WI-38 passage, the second subline was passaged in cultures incubated at 30°C.

Virus pools at four medium-passage levels were tested in man: the 11th and 14th passage levels of the first subline, and the 15th and 17th passage levels of the second. Although only two subjects were tested for each pool, the results were nevertheless striking (Table 2). The passages of the 35°C subline produced more virus excretion and more clinical reaction than the passages of the 30°C subline. In view of these results, the 35°C subline was

Plotkin said that he confirmed the presence of the virus in the supernatant fluids by injecting these into human subjects “subcutaneously” meaning under the skin.

Amer J Dis Child—Vol 118, Aug 1969

S. Plotkin et al., “Attenuation of RA 27/3 Rubella Virus in WI-38 Human Diploid Cells,” American Journal of Diseases of Children, Vol 118 (1969), pp 178-179.

<https://irp.cdn-website.com/e4e1af55/files/uploaded/AmJDisChildRA273inWI-38.pdf> (consulted 27 Nov 2024)

Plotkin writes in his article that he **confirmed the presence of the virus** in the **supernatant fluids** by injecting these into human subjects “subcutaneously” meaning under the skin. Because some got a **rash** and **excretion**, he concluded that this was due to the alleged virus. He also claimed the alleged virus “spread to contacts” though he gives no explanations as to how he verified this scientifically.

As already mentioned the **supernatant fluids** would have contained at least **four types of antibiotics** including Penicillin. Penicillin is known to cause skin rash in many patients and is a common side effect and allergic reaction to the antibiotic.

Allergy to a medication in the Penicillin (PCN) class is reported to occur in approximately 10% of patients internationally.<sup>1,2,3</sup> The majority of patients report low-risk symptoms of allergy, such as **delayed rash**, at a very young age which is unlikely to recur with subsequent exposures.<sup>4,5,6</sup> When a PCN allergy is reported in a child there are increased

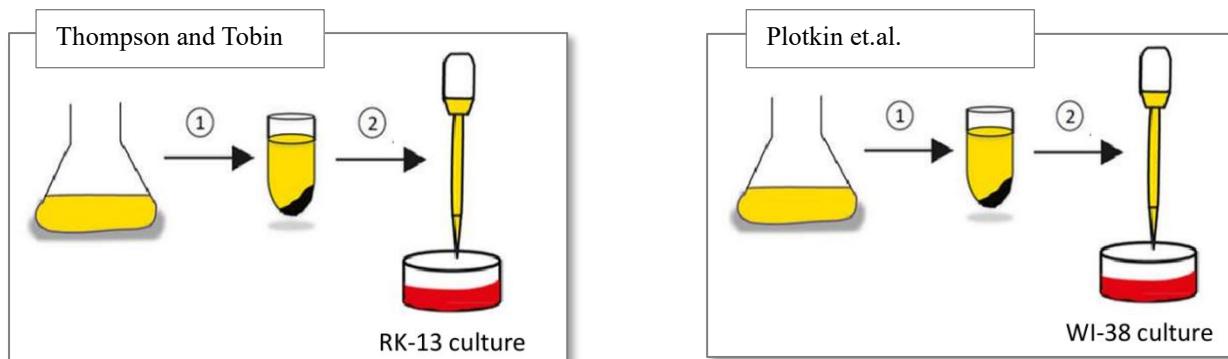
*D. Vyles et.al., Children with Reported Penicillin Allergy: Public Health Impact and Safety of De-labeling, Ann Allergy Asthma Immunol. 26 Mar 2020  
<https://pmc.ncbi.nlm.nih.gov/articles/PMC7255916/> (consulted 17 Jan 2025)*

**The whole thing is ridiculous “pseudo-science”.**

However, Plotkin was not the only virologist in the **1960s** and **1970s** to claim he had “**isolated**” the Rubella virus from **aborted foetal material**.

In 1970 Thompson and Tobin published a paper in which they claimed to have isolated the rubella virus from **abortion material** in other words **human foetuses**.

They **minced up the tissues of human foetuses**, added some basic cell culture medium (basal medium) and injected the **supernatant fluids** into **RK13 rabbit kidney cells**.



- 1 The **minced up tissues from the aborted foetuses** are **centrifuged**.
- 2 The **supernatant** is injected on to a culture of **RK-13 cells**.

Plotkin's procedure was slightly different in the sense that he cultured the cells of an aborted foetus rather than simply mincing them up before injecting the supernatant fluids onto a culture of WI-38 cells.

- 1 The **kidney culture from the aborted foetus** is **centrifuged**.
- 2 The **supernatant** is injected on to a culture of **WI-38 cells**.

## Isolation of Rubella Virus from Abortion Material

K. M. THOMPSON,\* F.I.M.L.T. ; J. O'H. TOBIN,† B.M., DIP.BACT., M.R.C.PATH.

*British Medical Journal, 1970, 2, 264-266*

K. M. Thompson et al., "Isolation of Rubella Virus from Abortion Material," *British Medical Journal*, Vol 2 (1970), pp. 264-266. <https://www.bmjjournals.org/lookup/doi/2/5704/264> (consulted 27 Nov 2024)

### Materials and Methods

The RK13 line of rabbit kidney cells (Beale *et al.*, 1963) was used for virus isolation, and cultures were treated as previously described (Hutchinson and Thompson, 1965), except that the

Specimens were sent in bottles or plastic bags (which are not recommended unless being delivered by hand) by either road or post from women aborted from a few hours to a few days previously because of rubella infection. If a fetus was received in its amniotic sac this fluid was removed before selected organs, usually the lungs, liver, kidney, eye, and brain were dissected out. If the embryo had already been damaged by the operative procedure selected material was washed well in basal

Tissues were finely minced with scissors and suspended in about four times their volume in basal medium in 1-oz. (28-ml.) universal containers and shaken vigorously by hand before being spun in a refrigerated M.S.E. medium centrifuge at about 800 r.p.m. for five minutes. Three or four fivefold to tenfold dilutions of supernatant fluid from each sample or of amniotic fluid were then made and inoculated into RK13 cultures, usually using only one tube culture per dilution. The

Here they say that they **minced up the tissues** of the aborted foetuses with scissors and added some **basal medium** (basic cell culture medium) thus creating a suspension. This **mixture** was then shaken vigorously and **centrifuged**.

They then **injected the supernatant fluid** into **RK13 cultures**, rabbit kidney cells.

virus isolation from fetal material. Rawls *et al.* found that growing out cell cultures from the embryos produced higher yields than that by the more usual method of grinding up material and inoculating supernatant fluids. Our methods of preparing cell suspensions are perhaps more gentle than the usual methods and explain the high isolation rate we obtained without growing out cell cultures from the embryo. We also used RK13 cells for isolation instead of vervet monkey kidney cells, a cell system apparently preferred by most American workers. None of our material was frozen at any time before processing and inoculation into cell cultures.

We read that according to Rawls et al. **grinding up material and inoculating supernatant fluids** as described above was a "**more usual method**" of isolating the rubella virus than "**growing out cell cultures from embryos**" as Plotkin described.

**In any case none of these scientists ever "isolated" the supposed rubella virus from any of the human tissue they collected not in 1970, not ever.**

## 1(e) How Thomas Peebles and John Enders made the Measles virus Enders Edmonston strain in 1954

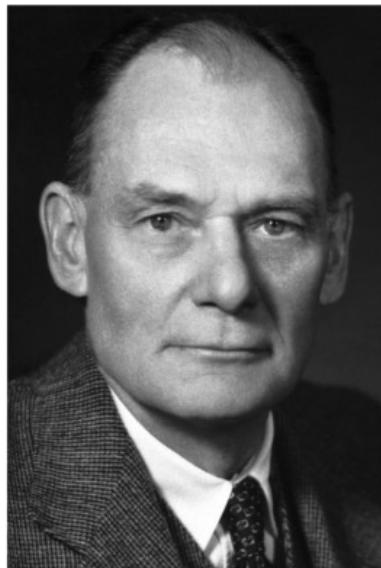
Let us now look at how Thomas Peebles and John Enders used a 'human kidney cell culture' (meaning human kidney cell strain) to make the **measles vaccine** that is part of the **MMR (Measles, Mumps, Rubella) M-M-RvaxPro vaccine**.

As you can read below **Thomas Peebles** working with John Enders collected **samples of mucus** (nasopharyngeal) and **blood** from an 11 year old boy with measles called **David Edmonston** and 'cultured' them on a 'human kidney cell culture'.

He and Enders then used **one** of these **cultures** to make what they called the **Edmonston strain of measles**.

### 5. The discovery of the measles virus

The viral nature of the disease was demonstrated in 1911 by John Anderson and Joseph Goldberger who successfully transmitted measles to rhesus monkeys from blood samples of measles patients, resulting in a discrete rash with a febrile peak [19]. The virus was then cultured in the 1940's on chicken embryos [20, 21]. In 1954, Thomas Chalmers Peebles (1921–2010), working in Boston with John Franklin Enders (1897–1985), future winner of the 1954 Nobel Prize in Medicine (Fig. 4), was sent to a nearby elementary school during a measles epidemic. He cultured nasopharyngeal and blood samples from an 11-year-old child named David Edmonston on a human kidney cell culture. After a few days, he observed the appearance of syncytia scattered in foci with multinucleated giant cells. Peebles injected the culture supernatant into monkeys, which developed a mild form of measles with a discrete rash [22, 23]. It was from this Edmonston strain that a live attenuated vaccine was developed



John Enders (1897-1985)



Maurice Hilleman (1919-2005)

P. Berche, "History of measles", *Quarterly Medical Review – History of Modern Pandemics*, Volume 51, Issue 3, September 2022, (consulted 27 Nov 2024)  
<https://www.sciencedirect.com/science/article/pii/S0755498222000422?via%3Dihub>

In 1954 John Enders wrote a scientific paper in which he describes the collection of 'throat washings' and 'blood' from patients with measles. And, how these were **propagated (cultured) in human and monkey cells.**

**10 ml of blood** was taken from each patient and **heparin** was added to stop it from coagulating. **0.5 ml to 2 ml** of whole blood was then used as '**inocula for tissue cultures**'. (An 'inocula' or 'inoculum' is a material used to 'inoculate' (inject) a culture, person or animal.)

So all Enders did was **inoculate (inject)** between **0.5ml to 2ml of whole blood**, with a bit of **heparin**, into '**tissue cultures**' in order to '**propagate**' (**culture**) them.

The alleged virus was **never isolated** (i.e. removed) from the **blood samples** ever.

*"Materials and methods. Collection of Specimens.*

**Throat washings, venous blood** and **feces** were obtained from **7 patients** as early as possible after a clinical diagnosis of measles was established. In 5 instances the time at which specimens were collected in relation to the onset of exanthem<sup>3</sup> is given in the case histories described below or in Table I. When capable, patients were asked to gargle with 10-15 ml of sterile neutralized fat-free milk. Certain specimens from the throats of younger children were obtained by cotton swab previously moistened in milk. After swabbing the throat the swab was immersed in 2 ml of milk. Penicillin, 100 u/ml, and streptomycin, 50 mg/ml, were added to all throat specimens which were then centrifuged at 5450 rpm for about one hour. Supernatant fluid and sediment resuspended in a small volume of milk were used as separate inocula in different experiments in amounts varying from 0.5 ml to 3.0 ml. **About 10 ml of blood** immediately after withdrawal were placed in tubes containing 2 ml of 0.05% solution of **heparin**. **As inocula for tissue cultures** **amounts varying from 0.5 ml to 2.0 ml of the whole blood were employed**. After addition of antibiotics as described above 10% fecal suspensions were prepared by grinding the material in bovine amniotic fluid medium. The suspensions were then centrifuged at 5450 rpm for about one hour and the supernatant fluids used as inocula., in amounts varying from 0.1 ml to 3 ml.

**All specimens were refrigerated** in water and ice or maintained in the cold at about 5°C from the time of collection **until they were added to the cultures**. The maximum time that lapsed between collection of specimens and inoculation was 3 and a quart hours.

*Tissue culture technics.*

**In the initial isolation attempts** roller tube **cultures** (11,12) **of human kidney, human embryonic lung, human embryonic intestine, human uterus and rhesus monkey testis were employed**. Subsequent passages of the agents isolated were later attempted in human kidney, human embryonic skin and muscle, human foreskin, human uterus, rhesus monkey kidney and embryonic chick tissue." (Enders J. et al, 1954, page 3)

**"Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles". John F. Enders et al, Proceedings of the Society for Experimental Biology and Medicine 1 June 1954**  
<https://www.semanticscholar.org/paper/Propagation-in-Tissue-Cultures-of-Cytopathogenic-Enders-Peebles/a8d3a62cbed04eca654ec5888684c97adb376143> (consulted 4 Dec 2024)

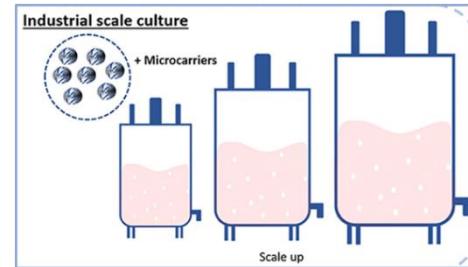
## 2 How the measles and rubella vaccines are mass produced

Let us now look at how the **Edmonston strain of measles** is massproduced.

As you can read from the **Product Information** about the M-M-Rvaxpro vaccine this is done in **“chicken embryo cells”**. The following document from the European Medicines Agency gives us further details about how this is done.

We read that the **chicken embryos** are removed from the eggs, **dissociated** using trypsin, **clarified** meaning washed and then **centrifuged** before the alleged ‘measles virus’, is allowed to so called ‘infect’ the cells.

A **stainless steel tank** is filled with this **Chicken Embryo Cell suspension (CEC)** and the **measles stock seed**, **meaning the Edmonston strain**, is added and the whole mixture is allowed to “grow” incubated at 37C (body temperature).



“The **cell sheets** are rinsed and **refed several times**, and the **virus propagators harvested**”.

In other words, the **cells are subcultivated** and **refed several times** (with culture medium) before the **supernatant fluids** are harvested meaning collected.

The measles **harvested virus fluids (HVF)** just refers to the **supernatant fluids** from the **cell cultures of human and chicken embryos**.

**It's absolutely disgusting.**

Active substance - measles

- Manufacture

Seed lot system

The Enders' Edmonston strain of measles virus was isolated in primary human kidney cell tissue culture from the blood of a child (Edmonston) in the early acute phase of measles. The virus (10 ml) was received by Merck from Dr. John Enders at the Children's Hospital of Harvard Medical School in 1960. Further passages were performed at Merck to develop the Moraten (more attenuated Enders) strain that served as a pre-master seed from which the Master Seed was derived. The preparation of the Master Seed and the Stock Seed is appropriately described in the dossier.

What Merck received from Enders was **not 10 ml of pure virus** but a **cell culture with the alleged virus in it**.

**Chicken embryo cells (CEC) as cell substrate**

Chick embryo cells, the cell substrate for measles and mumps virus propagation, are sourced from eggs from a specific-pathogen-free (SPF) chicken flock. Embryos are removed from the eggs, dissociated with trypsin, clarified and centrifuged prior to virus infection.

Manufacture of measles harvested virus fluids (HVF)

A virus propagator, a stainless steel tank, is planted with CEC suspension. The cells are infected with an appropriate volume of thawed measles stock seed, added to the seeding medium, stirred and incubated. The cell sheets are rinsed and refed several times, and the virus propagators are harvested. HVF is sampled for virus potency and sterility.

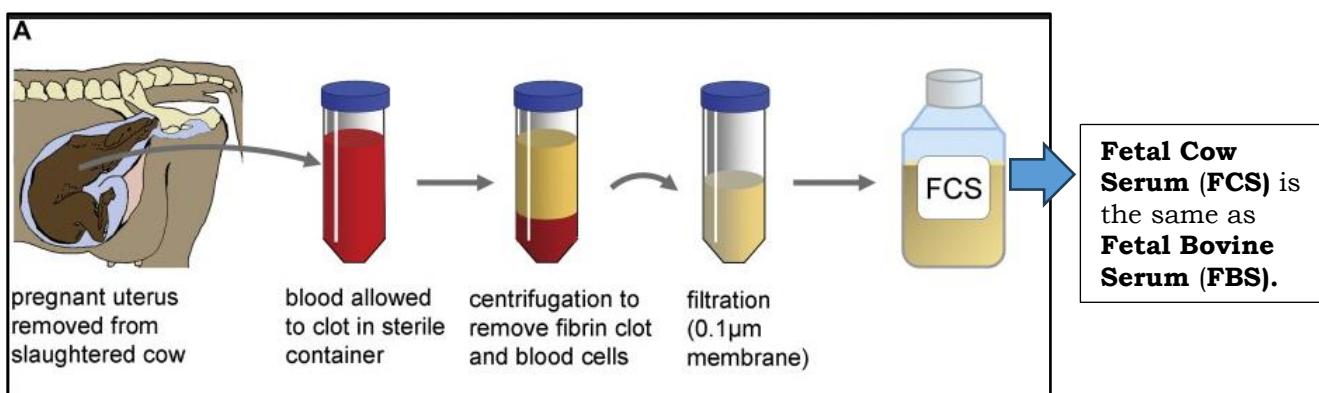
Website of the **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, published 2006, page 2, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf)** (consulted 27 Nov 2024)

The European Medicines Agency goes on to admit that there are so called “**process-related impurities**” related to the use of **cell substrates** and **cell cultures** in the manufacturing process of the so called measles vaccine.

The **cell culture related impurities** include antibiotics (e.g. neomycin) and serum which is blood. This is of course because the ‘cell culture medium’ used is a mixture of **antibiotics** and **Fetal Bovine Serum (FBS) the blood of cow foetuses**.

Approximately 500,000 L of FBS are produced annually for use in cell culture, requiring the killing of about one million bovine (cow) fetuses. <https://novapublishers.com/wp-content/uploads/2019/01/The-Impact-of-BVDV-Presence-on-Fetal-Bovine-Serum-used-in-the-Biotechnology-Industry.pdf> see page 77 (consulted 17 Jan 2025)

In the mass production of the so called measles virus hundreds of litres of fetal cow blood is used to cultivate the **cells of aborted babies** and the **cells of chicken embryos**.



[https://www.isct-cyotherapy.org/article/S1465-3249\(13\)00777-9/fulltext](https://www.isct-cyotherapy.org/article/S1465-3249(13)00777-9/fulltext) (consulted 17 Jan 2025)

The so called **harvested virus fluids (HVF)** meaning the **supernatant fluids** from the **cell cultures** contain a toxic cocktail of **antibiotics** and the **residues** from the cells of **human foetuses, chicken foetuses and cow foetuses**.

As already mentioned **human embryos** and **animal embryos** are at the heart of vaccine production world-wide.

The scientists who do this are absolutely sick.

Process-related impurities arising from the measles vaccine bulk manufacturing processes are classified as cell substrate- or cell culture-derived. Cell substrate-derived impurities may include proteins derived from the host organism, such as CECs used as substrate for measles vaccine bulk production. Cell culture-derived impurities may include antibiotics (e.g., neomycin), serum, or other media components. Also low levels of particle-associated reverse transcriptase activity are found; however, no signal of infectious retrovirus could be detected.

Since the measles process uses cell growth medium containing fetal bovine serum (FBS), measures have been taken to minimize the concentration of bovine serum proteins in the vaccine bulk. The concentration of bovine serum albumin (BSA) is used as a surrogate marker for other bovine serum proteins. Each measles final bulk is tested for BSA.

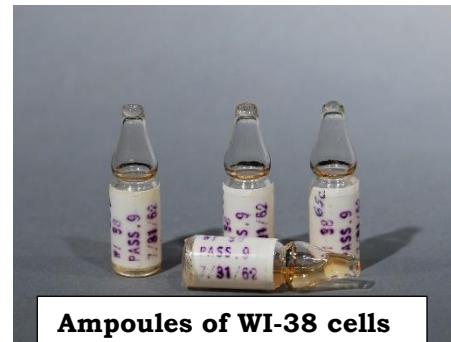
Website of the European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, published 2006, page 3, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf) (consulted 27 Nov 2024)

In the same document published in 2006 by the **European Medicines Agency** we read how the rubella vaccine is mass produced using a similar process to that of the measles vaccine.

But instead of preparing a **suspension of chicken embryo cells**, **WI-38 Cell Bank Ampoules** (mini capsules that contain the WI-38 cells) from the ATCC (American Type Culture Collection) are used.

Like with the measles vaccine a tank is filled with these WI-38 cells and some culture medium, and then the so called "**rubella stock seed**" is added.

As already described this is nothing more than a **WI-38 cell culture** that **Stanley Plotkin injected with supernatant fluids from the kidney culture of an aborted baby** in 1964.



They then "feed" the cells with **cell culture medium** and **incubate** them at 37C so that they will grow.

The sheets of cells are rinsed and refed before the so called "**harvested virus fluids (HVF**s)" are collected, in other words, before they centrifuge the cell suspension and remove the supernatant.

As with the measles vaccine all the "**harvested virus fluids (HVF**s)" are is a toxic cocktail of **antibiotics** and the **residues** from the cells used in the cultures in this case **human foetus cells**, and **cow foetus cells**.

(Chicken foetus cells were not used.) It is just disgusting.

**WI-38 working cell banks (WCBs)** are prepared using appropriate cells from the ATCC. WCB lots have been used in clinical trials; in the meantime, the stock for these two WCBs has been depleted and a new WCB lot was manufactured by the method described in the dossier and has passed all release testing.

#### Manufacture of rubella harvested virus fluids (HVFs)

An appropriate number of **WCB** ampoules are expanded to create a sufficient amount of cell substrate. Post-plant, the spent medium is removed and discarded. A sufficient quantity of rubella stock seed is added. Following virus adsorption, the infected cells are refed and incubated.

Post-infection, the spent medium is removed and discarded; the cell sheets are rinsed, refed and incubated.

The first HVF are collected, pooled and mixed with a stabilizer. The HVF is stored frozen and sampled for virus potency and sterility.

Website **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, page 6**, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf), published 2006, (consulted 27 November 2024)

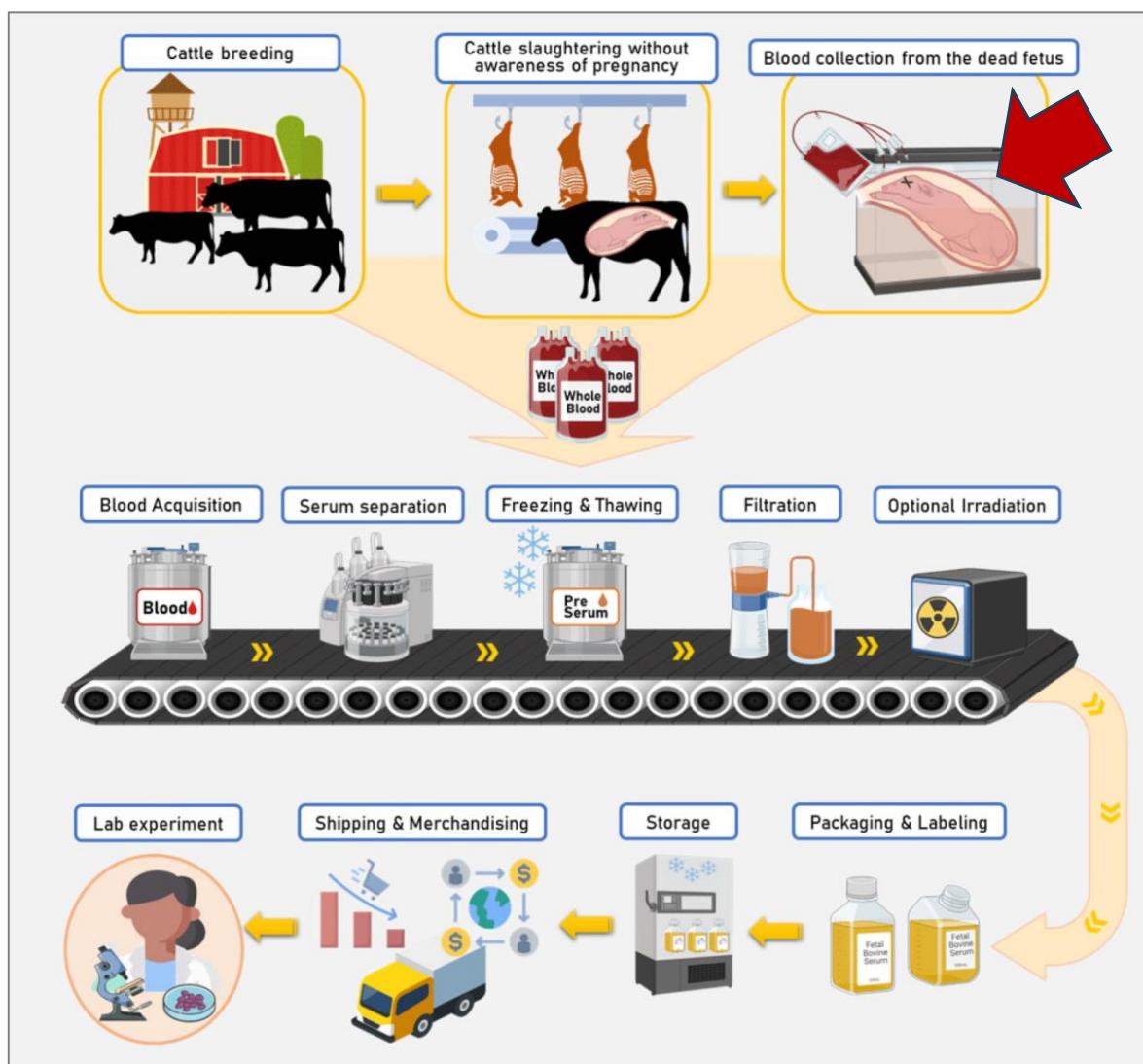
The European Medicines Agency goes on to admit once again that there are so called “**process-related impurities**” related to the use of **cell substrates** and **cell cultures** in the manufacturing process of the so called rubella vaccine.

The **cell culture related impurities** include **fetal bovine serum (FBS)**. This is of course because the ‘cell culture medium’ used to culture the WI-38 cells is made of **the blood of cow foetuses**. **(See the manufacturing process below.)**

Interestingly they do not mention that the “**harvested virus fluids (HVF**s)” also contain antibiotics as they are also used in the cell culture medium.

Process-related impurities arising from the rubella vaccine bulk manufacturing processes are classified as cell substrate- or cell culture-derived. Since the rubella process uses cell growth medium containing fetal bovine serum (FBS), rubella bulk lots were tested for BSA and the results for all of these lots were within the specification.

Website of the **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, published 2006, page 7, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-r-vaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-r-vaxpro-epar-scientific-discussion_en.pdf)** (consulted 27 Nov 2024)



[https://www.kosfaj.org/archive/view\\_article?pid=kosfa-42-5-775](https://www.kosfaj.org/archive/view_article?pid=kosfa-42-5-775) (consulted 17 Jan 2025)

### 3 Why virologists have only ever cultured cells and never ‘isolated’ any microorganism called a virus?

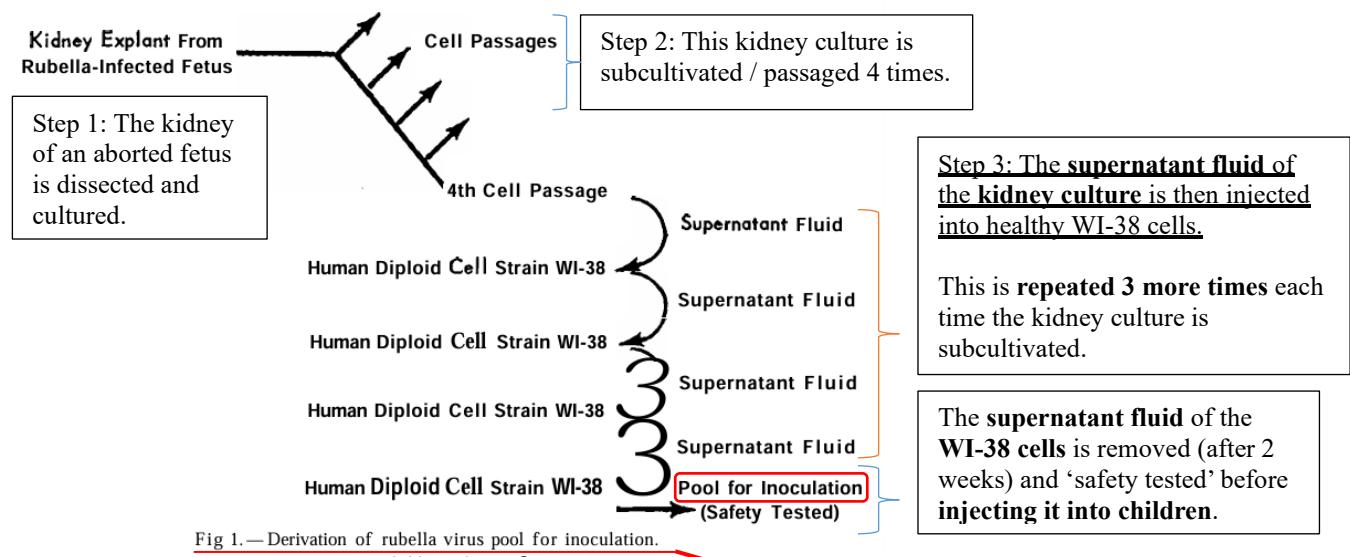
As explained in this document the alleged rubella and measles viruses have never been “isolated” from any tissue ever.

Stanley Plotkin describes very clearly how he made his so called “rubella virus strain” called the **RA 27/3 strain**.

All he did was **inject** some **supernatant fluids** from the **kidney culture of an aborted fetus** into a **WI-38 cell strain** which he then further “subcultured”.

The following diagram from **page 382** of Plotkin’s paper summarises how he made his so called **Rubella virus Wistar RA 27/3 strain**.

*“Studies of Immunization With Living Rubella Virus, Trials in Children With a Strain Cultured From an Aborted Fetus”, S. Plotkin et al., American Journal of Diseases of Children, Vol 110 (1 Oct 1965)*  
<https://irp.cdn-website.com/e4e1af55/files/uploaded/AmJDisChildPlotkinRubellaVirus.pdf> (consulted 27 Nov 2024)



Plotkin deceptively called the **supernatant fluids** he collected a **rubella virus pool for inoculation**, in other words a **vaccine**.

Plotkin never found the alleged rubella virus in the kidney cells of the baby he used in his experiments.

You cannot **isolate** a microorganism and study it by injecting it onto a **cell culture**.

Thomas Peebles working with John Enders didn’t isolate the **measles virus** either.

Enders describes inoculating whole blood mixed with an anticoagulant called heparin onto human kidney cells and propagating (culturing) them.

This is written clearly in his own words in a document published in 1954.

*“Materials and methods. Collection of Specimens.*

**Throat washings, venous blood and feces** were obtained from 7 patients as early as possible after a clinical diagnosis of measles was established. In 5 instances the time at which specimens were collected in relation to the onset of exanthem<sup>3</sup> is given in the case histories described below or in Table I. When capable, patients were asked to gargle with 10-15 ml of sterile neutralized fat-free milk. Certain specimens from the throats of younger children were obtained by cotton swab previously moistened in milk. After swabbing the throat the swab was immersed in 2 ml of milk. Penicillin, 100 u/ml, and streptomycin, 50 mg/ml, were added to all throat specimens which were then centrifuged at 5450 rpm for about one hour. Supernatant fluid and sediment resuspended in a small volume of milk were used as separate inocula in different experiments in amounts varying from 0.5 ml to 3.0 ml. About **10 ml of blood** immediately after withdrawal were placed in tubes containing 2 ml of 0.05% solution of heparin. **As inocula for tissue cultures** amounts varying from **0.5 ml to 2.0 ml of the whole blood were employed**. After addition of antibiotics as described above 10% fecal suspensions were prepared by grinding the material in bovine amniotic fluid medium. The suspensions were then centrifuged at 5450 rpm for about one hour and the supernatant fluids used as inocula., in amounts varying from 0.1 ml to 3 ml.

**All specimens were refrigerated** in water and ice or maintained in the cold at about 5°C from the time of collection **until they were added to the cultures**. The maximum time that lapsed between collection of specimens and inoculation was 3 and a quart hours.

*Tissue culture technics.*

**In the initial isolation attempts** roller tube **cultures** (11,12) of **human kidney, human embryonic lung, human embryonic intestine, human uterus and rhesus monkey testis were employed**. Subsequent passages of the agents isolated were later attempted in human kidney, human embryonic skin and muscle, human foreskin, human uterus, rhesus monkey kidney and embryonic chick tissue.” (Enders J. et al, 1954, page 3)

**“Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles”**. John F. Enders et al, *Proceedings of the Society for Experimental Biology and Medicine* 1 June 1954  
<https://www.semanticscholar.org/paper/Propagation-in-Tissue-Cultures-of-Cytopathogenic-Enders-Peebles/a8d3a62cbed04eca654ec5888684c97adb376143> (consulted 4 Dec 2024)

**Why did they do this? It's big business, no it's huge business.**

The **WI-38 cell strain** was not only used to make the **rubella vaccine**, it was used by Koprowski to make a **rabies vaccine**, Wyeth (now part of Pfizer) an **oral adenovirus vaccine**, Pfizer a **polio vaccine** and Merck **chickenpox** and **shingles vaccines**.

The **rubella vaccine** was only one of many made using **WI-38**. In the 1960s, a WI-38-based measles vaccine was licensed in the former Soviet Union and **Koprowski developed a rabies vaccine** using the cells. In the early 1970s, the pharmaceutical company **Wyeth** (now part of Pfizer) launched an **oral adenovirus vaccine** developed using **WI-38** and **Pfizer**, based in New York, used **WI-38** to make a vaccine against **polio**. Today, the cells are also used by **Merck** to make vaccines against **chickenpox** and the painful nerve infection **shingles**.

M. Wadman., “Medical research: Cell Division,” *Nature*, Vol 498 (27 June 2013), pp. 422-426.  
[https://www.researchgate.net/publication/242333147\\_Medical\\_research\\_Cell\\_division](https://www.researchgate.net/publication/242333147_Medical_research_Cell_division) (consulted 12 Jan 2025)

Hilary Koprowski was the director of the Wistar Institute between 1957 and 1991 and the boss of **Hayflick** and **Plotkin**. He is best known for his **so called vaccine against polio** that was administered orally.

Just like his rival **Albert Sabin** he so called attenuated/weakened the **polio virus** by culturing it in monkey kidney cells. Sabin apparently cultured several “strains”.

Just like **Plotkin, Koprowski** and **Sabine** never “isolated” any virus ever.

Sabin’s version of the polio vaccine was licensed in the United States and **adopted by the WHO for use throughout the world**. **The polio vaccine alone makes the pharmaceutical industry billions and billions every year**.

In the mid-1950s, cell culture became available, and Koprowski and Albert Sabin separately began to attenuate polioviruses by passage in monkey kidney cells. Both succeeded, and the Koprowski strains were tested extensively in the former Belgian Congo, his native Poland, and elsewhere (2). Nevertheless, because the Sabin strains were less neurovirulent in monkeys and were given successfully to millions of children in the former Soviet Union, **they achieved licensure in the United States and adoption by the WHO for use throughout the world**. During the battle between the oral polio vaccines, the atmosphere between Sabin and Koprowski became quite heated, with many colorful exchanges of insults, but afterwards they reestablished a friendship.

S. Plotkin, “In Memoriam: Hilary Koprowski, 1916–2013” *Journal of Virology*, Vol. 87 Number 15, Aug 2013 <https://pmc.ncbi.nlm.nih.gov/articles/PMC3719820/> (consulted 19 Jan 2025)

## **SALK POLIO VACCINE PROVES SUCCESS; MILLIONS WILL BE IMMUNIZED SOON; CITY SCHOOLS BEGIN SHOTS APRIL 25**



### **TRIAL DATA GIVEN**

Efficacy of 80 to 90%  
Shown—Salk Sees  
Further Advance

*Abstract of report, summary  
of data on tests, Page 22.*

By WILLIAM L. LAURENCE  
Special to The New York Times.  
ANN ARBOR, Mich., April 12  
—The world learned today that  
its hopes for finding an effective  
weapon against paralytic polio  
had been realized.

*The New York Times*, 16 Mar 2020, Corona Vaccine Dreams,  
<https://www.nytimes.com/2020/03/16/well/family/coronavirus-vaccine.html> (29 Jan 2025)

**Maurice Hilleman** who lead the virus and vaccination research programs at the **pharmaceutical giant Merck** for a total of **47 years** also helped make his company millions from selling vaccines. **And to this day Big Pharma make billions from the so called vaccines he and his team made.**

Hilleman and his team made more than 40 experimental and licensed human and animal vaccines.

The **two most notable vaccines** he made were the so called **measles** and **mumps vaccines**.

In 1957, at age 38, Hilleman was recruited by the pharmaceutical company Merck & Company at West Point, Pennsylvania, to lead its virus and vaccination research programs for the next 47 years, continuing to direct the Merck Institute for Vaccinology for another 20 years—after compulsory retirement from Merck Research Labs in 1984 at age 65—until his death at age 85. From the 1950s to the 1990s, Hilleman and his team created more than **40 experimental and licensed human and animal vaccines**, including those in use currently offering protection against **measles, mumps, chickenpox, rubella, hepatitis A, hepatitis B, pneumococcal pneumonia, meningitis, pandemic influenza, and chlamydia**.

*T. Tulchinsky. "Maurice Hilleman: Creator of Vaccines That Changed the World". Case Studies in Public Health: 443–4702, Mar 2018 <https://pmc.ncbi.nlm.nih.gov/articles/PMC7150172/> (consulted 19 Jan 2025)*

In **1963 Maurice Hilleman** made a so called vaccine for mumps. But just like Plotkin and Enders before him, Hilleman never isolated the alleged **mumps virus**.

When his daughter got sick supposedly with **mumps** he **swabbed** the inside of her **mouth** and placed the **swab** in some **broth** (probably cell culture medium).

He then **subcultured** this mixture multiple times in **chick embryo cells**.

**That's right he never isolated the mumps virus from any human tissues.**

But his company **Merck** made millions from selling **the supernatant fluids** from **cell cultures** containing **his daughter's mouth swab**.

On March 23, 1963, at 1:00 a.m. Jeryl Lynn, Maurice's five-year-old daughter, walked into her father's room and stood at the edge of his bed. "Daddy," she whispered, "my neck hurts." Maurice woke up and gently touched the side of his daughter's face. There, at the angle of her jaw, he felt a lump. Jeryl winced in pain. She had swollen parotid glands, a sure sign of mumps. Maurice then did something that only a scientist would do. He walked down the hall and told the housekeeper that he would be going back to the lab but would be back soon. (Maurice's wife, Thelma, had passed away years earlier.) Maurice picked up swabs and broth from the lab, drove back home, gently woke up his daughter, swabbed the inside of her mouth, placed the swab in broth, and drove back to the lab. Between 1963 and 1967, Maurice attenuated his daughter's strain of mumps virus by serially passaging it chick embryo cells. The final vaccine, termed the "Jeryl Lynn" strain, has dramatically reduced the incidence of mumps and consequent deafness in the United States and the world.

*P. Offit, A Biographical Memoir, National Academy of Sciences, 2021: <https://www.nasonline.org/wp-content/uploads/2024/06/hilleman-maurice.pdf> (consulted 19 Jan 2025)*

In 1968 Hilleman made a so called “**improved version**” of the **Ender’s measles vaccine**. And then in 1971 he made the so called “**combined MMR (Measles, Mumps Rubella) vaccine**”.

To this day, this vaccine is sold worldwide with the help of the **World Health Organisation** and their so called “**program of immunization**”.

vaccinate from the 9th month of life when maternal antibodies had disappeared [37]. In 1974, the WHO introduced measles vaccination into its expanded program of immunization. Maurice Hilleman (1919–2005) (Fig. 4), a pioneer in vaccine development working at Merck & Co. [38], developed an improved version of the measles vaccine in 1968, and then the combined vaccine with mumps and rubella (MMR) in 1971, with a single dose at 9–12 months and then a booster before 18 months.

*P. Berche, “History of measles”, Quarterly Medical Review – History of Modern Pandemics, Volume 51, Issue 3, September 2022, (consulted 27 Nov 2024)*  
<https://www.sciencedirect.com/science/article/pii/S0755498222000422?via%3Dihub>



The **European Medicines Agency** document about the MMR vaccine summaries how Plotkin, Enders and Hilleman so called “isolated” the alleged rubella, measles and mumps viruses. However as has been shown in this document none of these men ever isolated any microorganism from any human tissue ever.

The European Medicines Agency has indeed worked tirelessly **to deceive and outright mislead people into** believing pseudo-science.

#### Active substance - rubella

- *Manufacture*

##### Seed lot system

The Wistar RA 27/3 strain of rubella virus was isolated in 1964 by Dr. Stanley Plotkin, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania, U.S., from a kidney explant obtained from a surgically aborted foetus. It was directly inoculated into WI-38 cells, and then attenuated.

Website **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, page 5**, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf), published 2006, (consulted 27 November 2024)

Here we read that Plotkin “isolated” the rubella virus by injecting it onto WI-38 cells and then attenuating it meaning subculturing it. You cannot isolate a microorganism by injecting it onto a cell line and cultivating it.

This is not “isolation” and cannot be called “isolation”.

#### Active substance - measles

- *Manufacture*

##### Seed lot system

The Enders' Edmonston strain of measles virus was isolated in primary human kidney cell tissue culture from the blood of a child (Edmonston) in the early acute phase of measles. The virus (10 ml) was received by Merck from Dr. John Enders at the Children's Hospital of Harvard Medical School in 1960. Further passages were performed at Merck to develop the Moraten (more attenuated Enders) strain that served as a pre-master seed from which the Master Seed was derived. The preparation of the Master Seed and the Stock Seed is appropriately described in the dossier.

Website **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, page 2**, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf), published 2006, (consulted 27 November 2024)

Here we are told that the measles virus was isolated in primary human kidney cell tissue culture from the blood of a child diagnosed with measles. John Enders collected 10 ml of blood from a boy called David Edmonston added some anticoagulant called heparin and cultured this mixture in human and monkey kidney cells. That is what he did. He did not “isolate” the alleged measles virus from Edmonston’s blood ever.

The European Agency’s description is **outright misleading**.

Maurice Hilleman, at the time the director of the **pharmaceutical giant Merck**, then allegedly made a more attenuated (weaker) version of Ender's cell strain called **Moraten** by passaging/subculturing it further. Why passaging/subculturing a cell culture would weaken a microorganism such as a virus is not explained anywhere.

**Active substance - mumps**

- **Manufacture**

Seed lot system

The Jeryl Lynn strain of mumps virus was isolated from a throat washing specimen collected in 1963 from a clinical case of mumps (Jeryl Lynn) by Dr. M. R. Hilleman, Merck Research Laboratories, Merck & Co., Inc. Virus strain isolation was performed at the Merck West Point, Pennsylvania facility. The preparation of the master seed and the stock seed is described in detail in the dossier.

Website **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, page 4**, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf), published 2006, (consulted 27 November 2024)

Here we read that Hilleman isolated the mumps virus from a “throat washing specimen” from a clinical case of mumps (Jeryl Lynn).

Hilleman collected a **mouth swab** from his daughter Jeryl Lynn and not a “**throat washing**”. He then cultured this in chicken embryo cells to so called attenuate/ weaken the alleged virus in the mouth swab.

Here the European Medicines Agency doesn't even bother to mention that Hilleman cultivated the so called mumps virus in chicken embryo cells for some 4 years.

Just like the rubella and measles virus they deceptively claim that the mumps virus was actually isolated. Though the only thing **Maurice Hilleman** ever did according to the **Academy of Sciences** was swab his daughter's mouth and cultivate the swab in chicken embryo cells.

That procedure does not constitute the isolation of a microorganism.

Neither Stanley Plotkin, John Enders or Maurice Hilleman ever isolated meaning removed the alleged rubella, measles or mumps virus from any human tissue.

And together with Hilary Koprowski and Albert Sabin who before them never isolated the alleged polio virus committed the “biggest medical fraud” of the “twentieth century”. That is a fact.

**By working their lies these scientists gained a power and influence over people's lives that they would never have had otherwise. They also made billions for Big Pharma.**

**Their lies enabled them to create a false image of themselves as “creators of medicines” that “saved lives” when nothing could be further from the truth.**

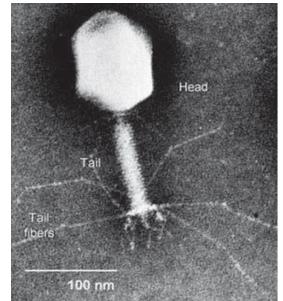
## 4 If virologists really wanted to isolate alleged viruses how could they do this?

There is a group of microorganisms that are as small as alleged viruses that have been isolated and studied by microbiologists.

These are called bacteriophages and they eat bacteria.

Let us look at how bacteriophages are isolated.

(Here is one textbook example.)



### Isolation of Bacteriophage from Sewage and Determination of Phage Titer EXERCISE 37 281

#### Second Session: Bacteriophage Isolation and Plating:

Prior to today's lab, 2 ml of 1× nutrient broth was inoculated with *E. coli* B for overnight growth at 37°C with shaking. Earlier today, 100 ml of 1× nutrient broth was inoculated with a small volume of the overnight. This was done to obtain a culture in log growth by class time. *Note:* The instructor may choose to inoculate today's culture with the day-old "overnight" stored in the refrigerator.

1. Transfer 10 ml of the sewage-bacteria-bacteriophage culture into a centrifuge tube, and centrifuge the sample at 2,000 RPM for 5 minutes. Most of the remaining cells will be pelleted. The supernatant contains bacteriophage.
2. Prepare a 10 ml storage tube for the collection of bacteriophage supernatant as it is filtered. Then pipette the supernatant into a 10 ml syringe barrel fitted with a 0.45 micron filter. Gently slide the plunger, allowing the flow-through to drip into

the storage tube. This step removes any remaining bacteria from the phage sample. The storage tube contains bacteriophage. It can be stored at 4°C and is stable for several months.

3. Prepare a series of microfuge tubes for making serial 10-fold dilutions of the bacteriophage suspension (performing the same dilution repeatedly in series is called serial dilution; see figure 37.4). Label six tubes 1–6. Into each tube, pipette 0.9 ml of sterile PBS.
4. **Perform serial dilutions:** Transfer 0.1 ml of phage suspension (that has been mixed well) into tube 1, and mix. Using the same pipette, transfer 0.1 ml of the sample from tube 1 into tube 2, and mix. Repeat this process, transferring 0.1 ml from tube 2 to tube 3, and so on, mixing each time, as shown in figure 37.4. Store the remaining phage suspension in the refrigerator.
5. Distribute 0.5 ml of log-phase *E. coli* into each of six microfuge tubes, labeled 1–6.

*Bacteriophage isolation technique a document of North West University, published by studeersnel.nl*  
<https://www.studeersnel.nl/nl/document/north-west-university/industrial-microbiology-and-biotechnology/bacteriophage-isolation-technique-2022/52866265?sid=01734251982&shared=u>  
(consulted 21 Jan 2025)

- Step 1: **Collect 40ml of raw sewage.** Then add some nutrient broth and *E. coli* bacteria and incubate overnight at 37°C.
- Step 2: **Centrifuge 10 ml of sewage bacteriophage culture at 2000 RPM for 5 mins.** The supernatant contains the bacteriophage.
- Step 3: **Filter the supernatant.** Using a pipette put the supernatant in to a 10ml syringe barrel fitted with a 0.45 micron filter. (This stage removes any remaining bacteria from the tube.) The storage tube contains bacteriophage. It can be stored at 4C and is stable for several months.

Let's now look at how a microbiologist would do this in practice.





Finding and Isolating Phages

Step 1: Collect the poo of some geese and mix with some buffer (PBS salt solution, distilled water and some salt). About 50% poo and 50% buffer (PBS) then shake well.



Finding and Isolating Phages

Step 2: Centrifuge



Finding and Isolating Phages

Step 3: Filter

Step 2: Then you **centrifuge** the mixture and remove the supernatant.

Step 3: Then take the supernatant and **filter** it to remove any bacteria. (Use a 0.22 micron filter.)

(Sometimes you may need to centrifuge a second time to get the supernatant through the filter.)



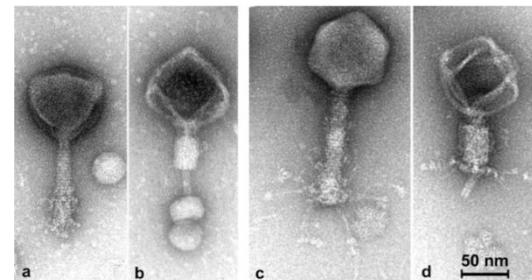
Finding and Isolating Phages

Use a **glass vial** (small container) to collect the liquid containing the bacteriophage. (A lysate – in this case, is the product of filtering the supernatant.)

**Finding and Isolating Phages by Dr Sabrina Green, 6 Jan 2021:**  
<https://www.youtube.com/watch?v=Kt0miFrXMaY> (consulted 12 Dec 2024)

Dr. Sabrina Green goes on to cultivate the bacteriophages she collected and then isolate them once again. Though microbiologists seem to use slightly different methods for doing this it is not the case.

All microbiologists use a combination of **centrifuge** and **filtration** to isolate bacteriophages and other microorganisms.

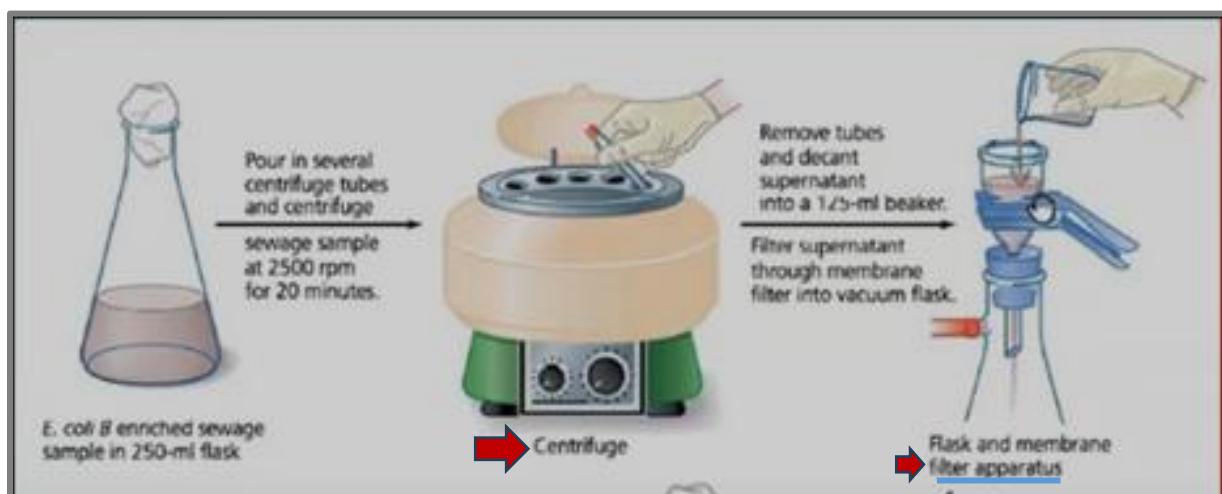


See p. 40 for a bigger picture of a bacteriophage.

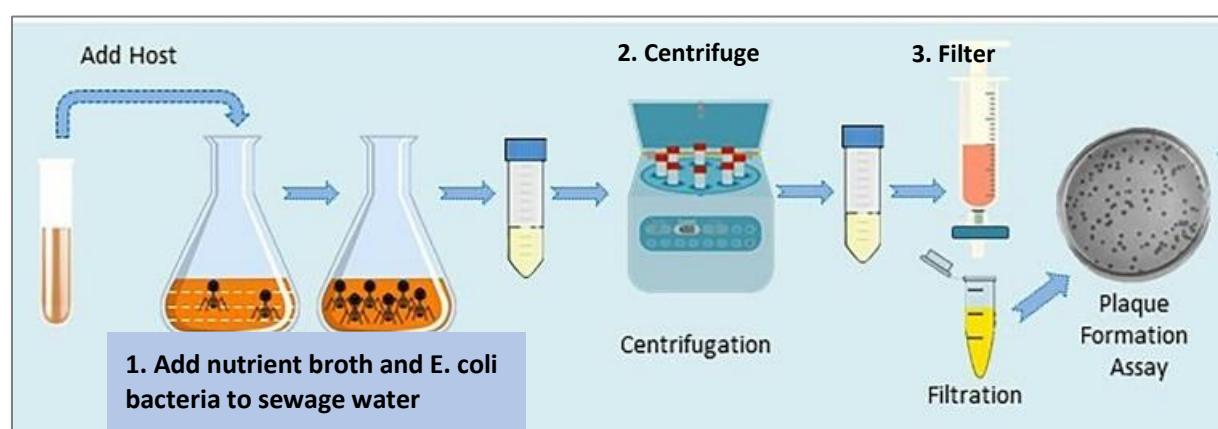
Sometimes microbiologists talk of **purification**. This usually refers to isolating a microorganism after it has been cultivated artificially in a lab. Purification is therefore essentially the same as isolation.

Bacteriophages are abundant in nature. You can find them in the excrement (poo) of animals, in sewage water, in soil, and even in the sea. They have a head, body and legs and are approximately 100 nm (nanometres).

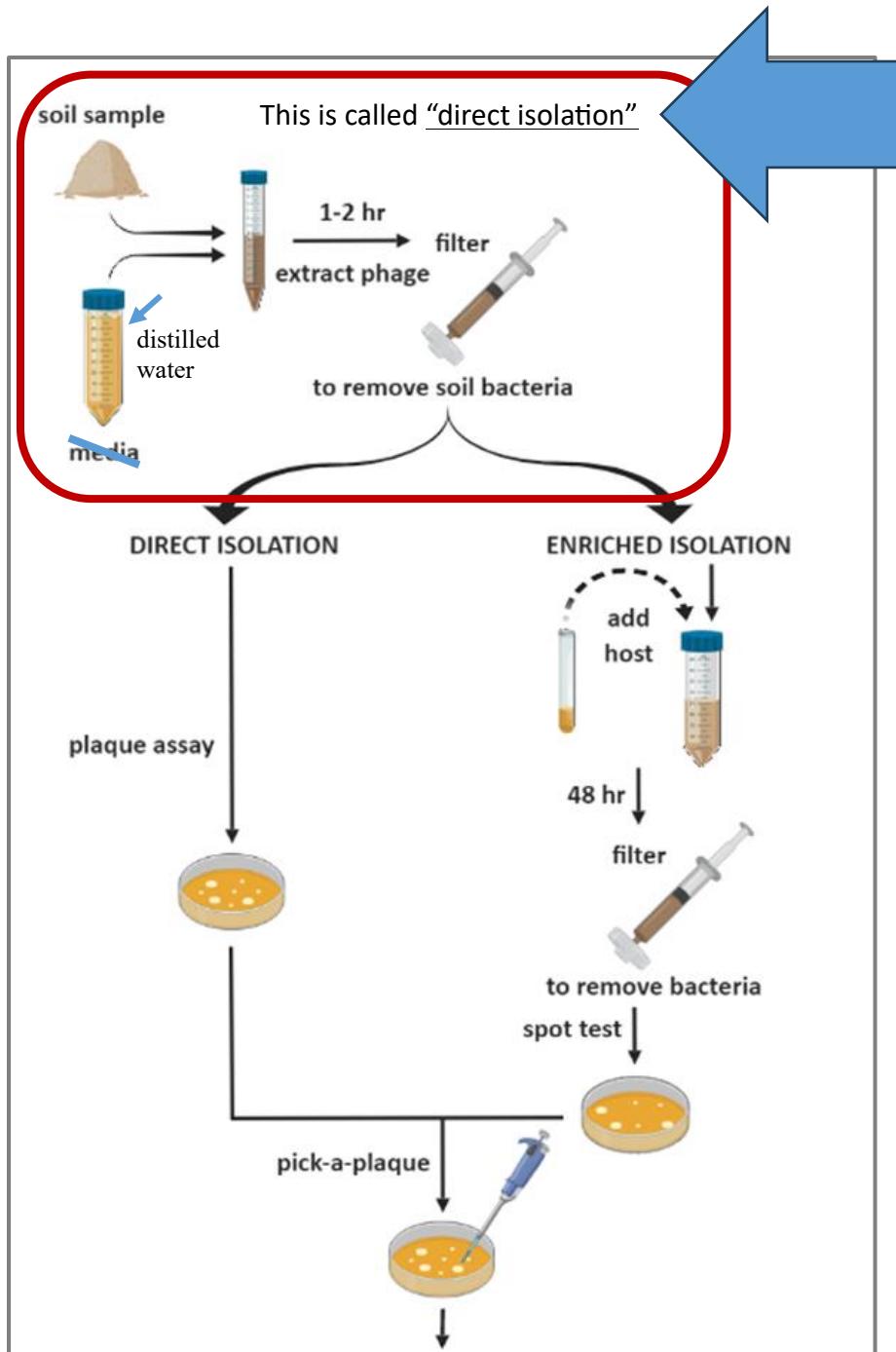
**How To Purify A Phage by Dr Sabrina Green, 23 Feb 2021:**  
<https://www.youtube.com/watch?v=-t85C04Ueio> (consulted 12 Dec 2024)



*Isolating bacteriophages by centrifuge and filtration*  
<https://i.ytimg.com/vi/nvJGOjYVJQI/maxresdefault.jpg> (consulted 21 Jan 2025)



*Isolating bacteriophages by centrifuge and filtration*  
<https://www.frontiersin.org/articles/10.3389/fmcb.2022.993990/full> (consulted 21 Jan 2025)



The above diagram shows how it is possible to isolate a bacteriophage from a soil sample. You will notice that it is possible to isolate the bacteriophage directly from the sample. In other words, it is possible to isolate a bacteriophage without culturing /cultivating it.

All you need to do is add some distilled water to your soil sample in a test tube and shake well. Leave the mixture to settle for 1 to 2 hours or centrifuge (5 minutes).

Finally, remove the **supernatant** (top fluid) with a syringe **and filter**. (Push the supernatant through a 0.22 micron filter.)

**Virus Isolation Course see -> Direct Isolation.**

<https://dustinedwards.info/virus-isolation/> (3 Feb 2025)

**Virus Isolation Course by Dustin Edwards see -> Direct Isolation.**

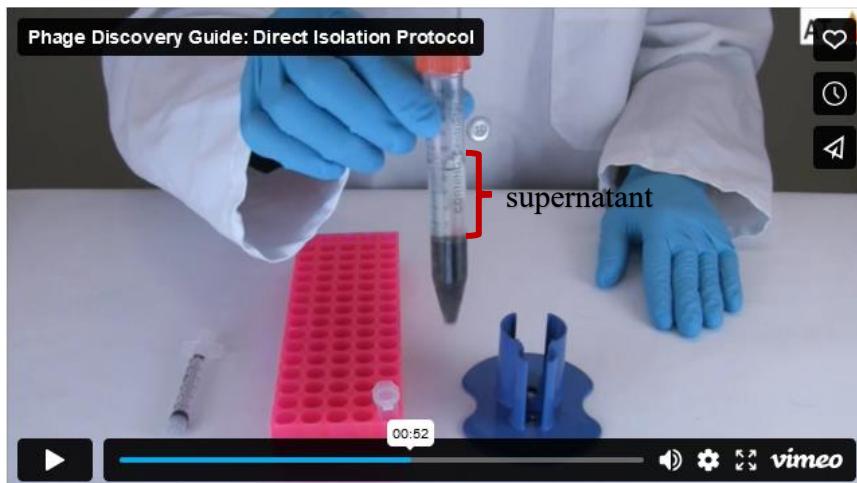
<https://dustinedwards.info/virus-isolation/> (3 Feb 2025)

Bacteriophages are microorganisms that can be isolated directly from excrement (poo), sewage water or soil. Here is how you can do this.

**-Direct Isolation Procedure of Bacteriophages**



1. Collect a soil sample, add some distilled water and shake well to mix.



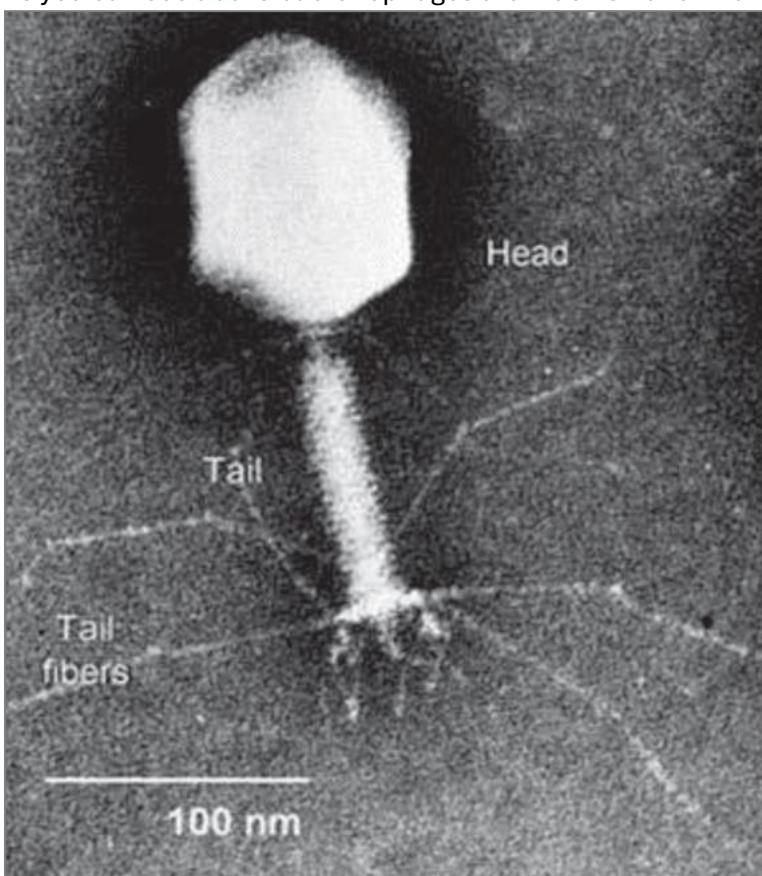
2. Leave the test tube untouched until all the large particles have sunk to the bottom (see above) or **centrifuge**.



3. Remove/draw up some of the supernatant with a syringe and **filter the supernatant**. Push the fluid through the filter into a small test tube. (Use a 0.22 micron filter.)



As you can see above bacteriophages are much smaller than bacteria.



Bacteriophages have a head, body, and legs. They call the body a tail but it is not a tail. And they call the legs tail fibres but these are not really tail fibres but legs.

## (5) Conclusion: What does all of this tell us about the state of microbiology today?

As Dr. Dustin Edwards and Dr. Sabrina Green demonstrate, it is very, very easy to directly isolate a microorganism.

Step 3. Draw up some of the **supernatant** with a syringe and **filter**.



But Plotkin, Enders and Hilleman never “isolated” the alleged rubella, measles or mumps viruses from any human tissue ever. You simply cannot isolate a virus/ microorganism **“in a human tissue cell culture”** (a human kidney cell culture, a human lung cell culture, a human intestine cell culture) **or any other culture**.

This is not “isolation” and cannot be called “isolation”.

### Active substance - measles

- *Manufacture*

#### Seed lot system

The Enders' Edmonston strain of measles virus was isolated in primary human kidney cell tissue culture from the blood of a child (Edmonston) in the early acute phase of measles. The virus (10 ml) was received by Merck from Dr. John Enders at the Children's Hospital of Harvard Medical School in 1960. Further passages were performed at Merck to develop the Moraten (more attenuated Enders) strain that served as a pre-master seed from which the Master Seed was derived. The preparation of the Master Seed and the Stock Seed is appropriately described in the dossier.

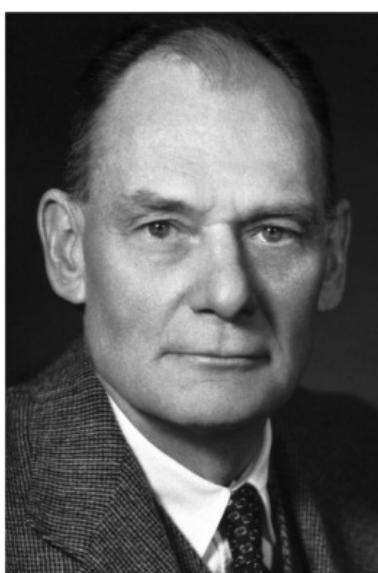
Website **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, page 2**, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf), published 2006, (consulted 27 November 2024)

John Enders in his 1954 scientific paper describes his procedure very clearly. He collected 10 ml of blood from a boy who was sick, added an anticoagulant called heparin and cultured this blood sample in **human kidney cells**, as well as, several other kinds of human and monkey cells.

He never once “removed” the alleged measles virus from the blood sample.

## 5. The discovery of the measles virus

The viral nature of the disease was demonstrated in 1911 by John Anderson and Joseph Goldberger who successfully transmitted measles to rhesus monkeys from blood samples of measles patients, resulting in a discrete rash with a febrile peak [19]. The virus was then cultured in the 1940's on chicken embryos [20, 21]. In 1954, Thomas Chalmers Peebles (1921–2010), working in Boston with John Franklin Enders (1897–1985), future winner of the 1954 Nobel Prize in Medicine (Fig. 4), was sent to a nearby elementary school during a measles epidemic. He cultured nasopharyngeal and blood samples from an 11-year-old child named David Edmonston on a human kidney cell culture. After a few days, he observed the appearance of syncytia scattered in foci with multinucleated giant cells. Peebles injected the culture supernatant into monkeys, which developed a mild form of measles with a discrete rash [22, 23]. It was from this Edmonston strain that a live attenuated vaccine was developed



John Enders (1897-1985)



Maurice Hilleman (1919-2005)

P. Berche, "History of measles", *Quarterly Medical Review – History of Modern Pandemics*, Volume 51, Issue 3, September 2022, (consulted 27 Nov 2024)  
<https://www.sciencedirect.com/science/article/pii/S0755498222000422?via%3Dihub>

Nevertheless, even the prestigious Medical Journal - **Quarterly Medical Review** - tries to tell us that you can "isolate" a virus "on a human kidney cell culture". This is totally ridiculous. But why do they do this? You ask.

**They do this for two very simple reasons. Power and Money!!**

Albert Sabin for example, attenuated/weakened the so called **polio virus** by culturing it in monkey kidney cells.

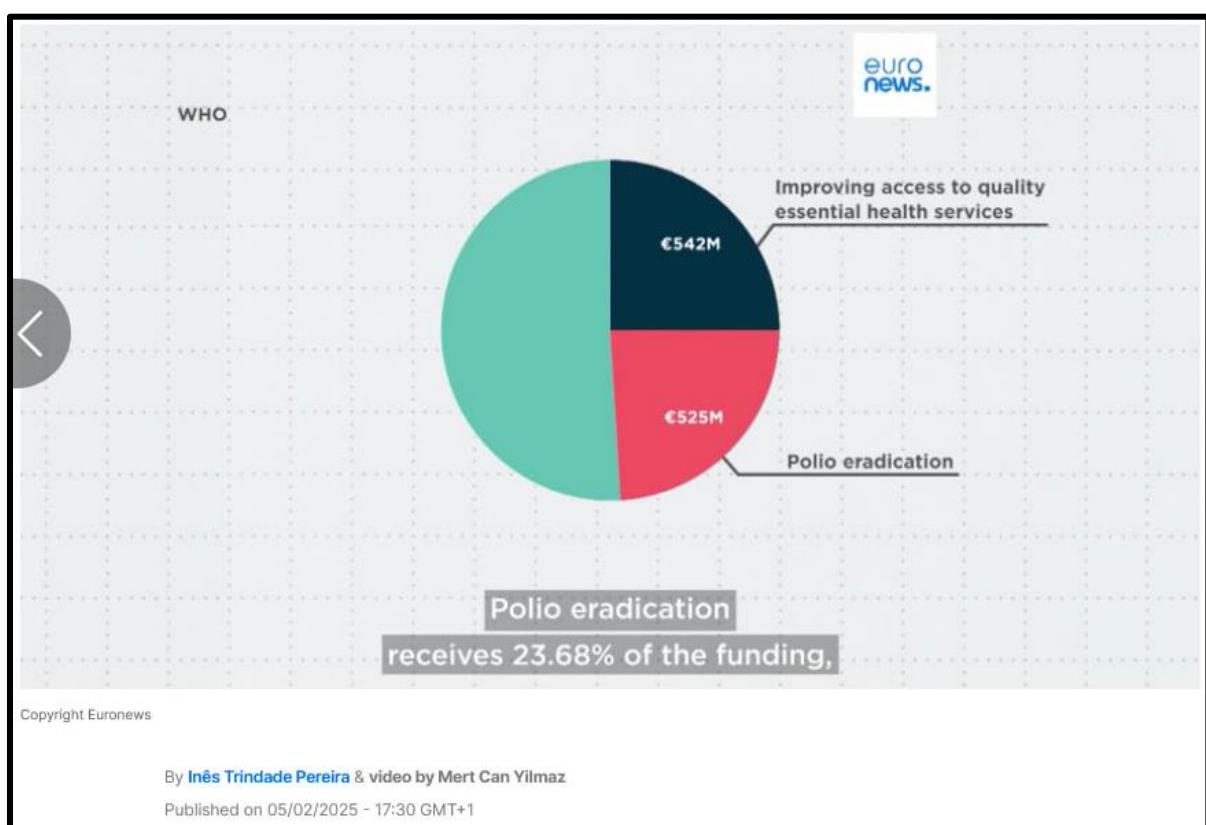
After his version of the polio vaccine was licensed in the United States it was **adopted by the WHO for use throughout the world**.

Albert Sabin's "culturing" of "monkey kidney cells" made him a very rich and powerful man.

In the mid-1950s, cell culture became available, and Koprowski and Albert Sabin separately began to attenuate polioviruses by passage in monkey kidney cells. Both succeeded, and the Koprowski strains were tested extensively in the former Belgian Congo, his native Poland, and elsewhere (2). Nevertheless, because the Sabin strains were less neurovirulent in monkeys and were given successfully to millions of children in the former Soviet Union, they achieved licensure in the United States and adoption by the WHO for use throughout the world. During the battle between the oral polio vaccines, the atmosphere between Sabin and Koprowski became quite heated, with many colorful exchanges of insults, but afterwards they reestablished a friendship.

S. Plotkin, "In Memoriam: Hilary Koprowski, 1916–2013" *Journal of Virology*, Vol. 87 Number 15, Aug 2013 <https://PMC3719820/> (consulted 19 Jan 2025)

Since the 1950s the selling of Polio vaccines has become one of the biggest money makers, in human history, for the pharmaceutical industry.



Euronews, 5 Feb 2025, Before Trump's WHO cutoff, who was funding the United Nations' health organisation? <https://www.euronews.com/my-europe/2025/02/05/before-trumps-who-cutoff-who-was-funding-the-united-nations-health-organisation> (consulted 6 Feb 2025)

In February 2025, Euronews reported that approximately 24% of WHO funding goes to pay for Polio vaccines and Polio vaccination. So according to Euronews almost a **quarter** of the **WHO budget** (525M Euros) goes to paying **Polio vaccinations alone**.

In 1964 Stanley Plotkin simply followed in the footsteps of his boss Hilary Koprowski who was famous for his polio vaccine just like his rival Albert Sabin.

Stanley Plotkin describes very clearly how he made his so called “rubella virus strain” by culturing the “human kidney cells” of an aborted fetus.

All he did was **inject** some **supernatant fluids** from the **kidney culture of an aborted fetus** into a **WI-38 cell strain** which he then further “subcultured”.

The following diagram from **page 382** of Plotkin’s paper summarises how he made his so called **Rubella virus Wistar RA 27/3** strain.

*“Studies of Immunization With Living Rubella Virus, Trials in Children With a Strain Cultured From an Aborted Fetus”, S. Plotkin et al., American Journal of Diseases of Children, Vol 110 (1 Oct 1965) <https://irp.cdn-website.com/e4e1af55/files/uploaded/AmJDisChildPlotkinRubellaVirus.pdf> (consulted 27 Nov 2024)*

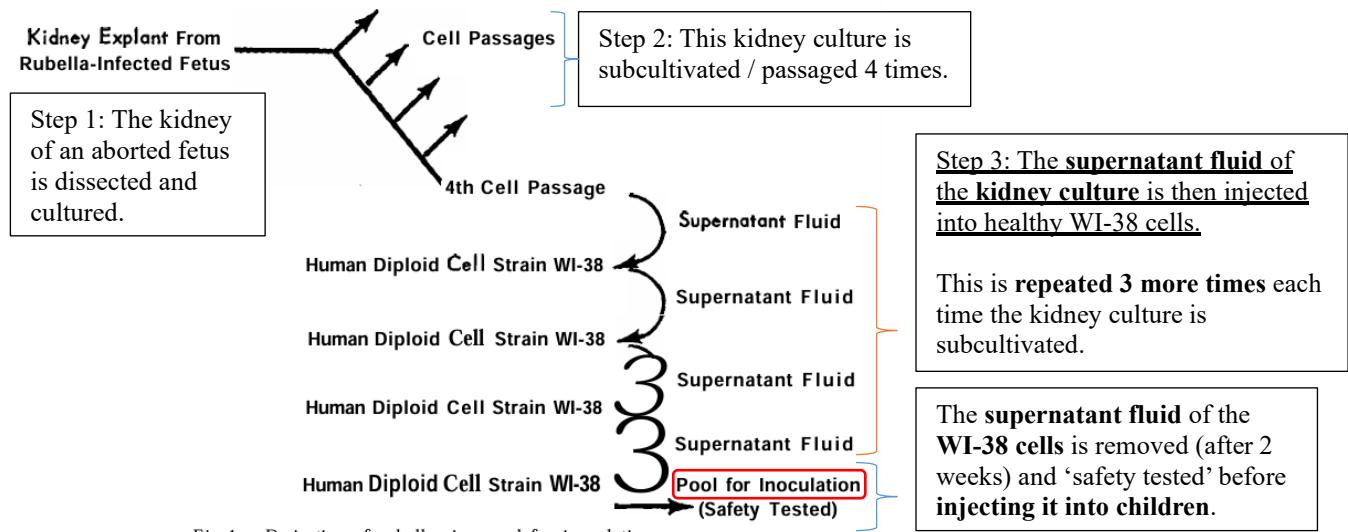


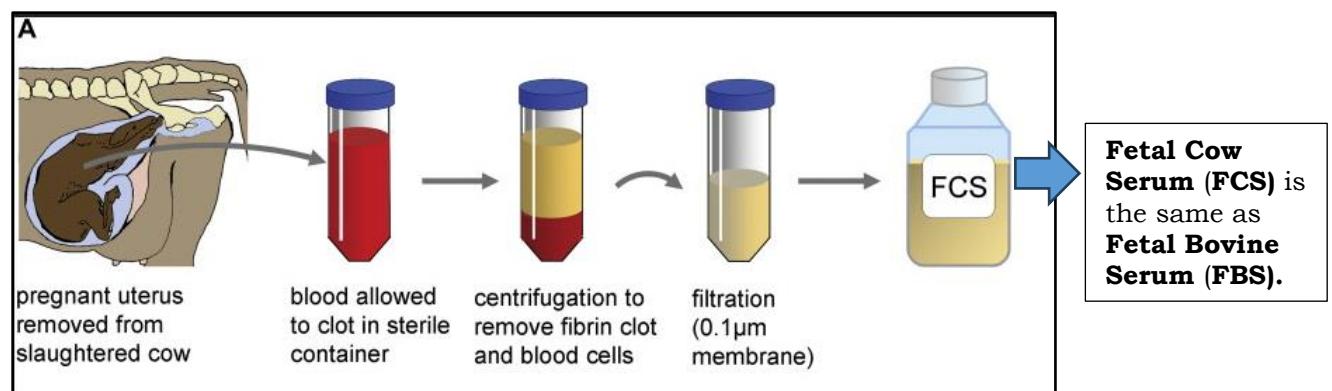
Fig 1.—Derivation of rubella virus pool for inoculation.  
Amer J Dis Child—Vol 110, Oct 1965

Plotkin deceptively called the **supernatant fluids** he collected a **rubella virus pool for inoculation**, in other words a **vaccine**.

You cannot **isolate** a microorganism and study it by injecting it onto a **cell culture**.

Plotkin never found or studied the alleged rubella virus in the kidney cells of the baby he used in his experiments. Not in 1965, not ever.

In the mass production of Plotkin’s the so called rubella vaccine and Ender’s measles vaccine, hundreds of litres of fetal cow blood (the blood of cow fetuses) is used to cultivate the **cells of aborted babies** and **chicken embryos**.



[https://www.isct-cytotherapy.org/article/S1465-3249\(13\)00777-9/fulltext](https://www.isct-cytotherapy.org/article/S1465-3249(13)00777-9/fulltext) (consulted 17 Jan 2025)

The so called **harvested virus fluids (HVF)**s meaning the **supernatant fluids** from the **cell cultures** contain a toxic cocktail of **antibiotics** and the **residues** from the **cells of human and chicken foetuses**.

As already mentioned **human embryos** and **animal embryos** are at the heart of measles and rubella vaccine production world-wide.

Process-related impurities arising from the rubella vaccine bulk manufacturing processes are classified as cell substrate- or cell culture-derived. Since the rubella process uses cell growth medium containing fetal bovine serum (FBS), rubella bulk lots were tested for BSA and the results for all of these lots were within the specification.

Website of the **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, published 2006, page 7**, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf) (consulted 27 Nov 2024)

Process-related impurities arising from the measles vaccine bulk manufacturing processes are classified as cell substrate- or cell culture-derived. Cell substrate-derived impurities may include proteins derived from the host organism, such as CECs used as substrate for measles vaccine bulk production. Cell culture-derived impurities may include antibiotics (e.g., neomycin), serum, or other media components. Also low levels of particle-associated reverse transcriptase activity are found; however, no signal of infectious retrovirus could be detected.

Since the measles process uses cell growth medium containing fetal bovine serum (FBS), measures have been taken to minimize the concentration of bovine serum proteins in the vaccine bulk. The concentration of bovine serum albumin (BSA) is used as a surrogate marker for other bovine serum proteins. Each measles final bulk is tested for BSA.

Website of the **European Medicines Agency EMA, M-M-R Vaxpro epar scientific discussion, published 2006, page 3**, [https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-discussion/m-m-rvaxpro-epar-scientific-discussion_en.pdf) (consulted 27 Nov 2024)



Thermo Fisher Scientific

<https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-cult...>

\*\*\*

### Fetal Bovine Serum Collection and Manufacturing | Thermo Fisher ...

Approximately 500,000 L of FBS are produced annually, requiring the harvest of about one million bovine fetuses [1]. Examples of quality tests performed during FBS production Processing and quality testing are vital to ensuring that FBS is suitable for cell culture.

<https://www.thermofisher.com/nl/en/home/references/gibco-cell-culture-basics/cell-culture-environment/culture-media/fbs-basics/steps-taken-manufacture-fbs.html> (consulted 17 Jan 2025)

Thermo Fisher Scientific estimates that 1 million bovine fetuses are “harvested” every year to supply “scientists” with the blood they require for “cell culture”.

So about **one million cow fetuses** are killed and their blood drained every year to supply the **pharmaceutical industry** with the blood they require to “culture cells” in their **labs** and **factories**.

Today most of the cells they culture are taken from the tissues and organs of aborted human babies including the **kidneys** and **lungs** and even the **eyes of aborted fetuses**.

*“Materials and methods. Collection of Specimens.*

**Throat washings, venous blood and feces** were obtained from 7 patients as early as possible after a clinical diagnosis of measles was established. In 5 instances the time at which specimens were collected in relation to the onset of exanthem<sup>3</sup> is given in the case histories described below or in Table I. When capable, patients were asked to gargle with 10-15 ml of sterile neutralized fat-free milk. Certain specimens from the throats of younger children were obtained by cotton swab previously moistened in milk. After swabbing the throat the swab was immersed in 2 ml of milk. Penicillin, 100 u/ml, and streptomycin, 50 mg/ml, were added to all throat specimens which were then centrifuged at 5450 rpm for about one hour. Supernatant fluid and sediment resuspended in a small volume of milk were used as separate inocula in different experiments in amounts varying from 0.5 ml to 3.0 ml. **About 10 ml of blood** immediately after withdrawal were placed in tubes containing 2 ml of 0.05% solution of heparin. **As inocula for tissue cultures** amounts varying from **0.5 ml to 2.0 ml of the whole blood were employed**. After addition of antibiotics as described above 10% fecal suspensions were prepared by grinding the material in bovine amniotic fluid medium. The suspensions were then centrifuged at 5450 rpm for about one hour and the supernatant fluids used as inocula, in amounts varying from 0.1 ml to 3 ml.

**All specimens were refrigerated** in water and ice or maintained in the cold at about 5°C from the time of collection **until they were added to the cultures**. The maximum time that lapsed between collection of specimens and inoculation was 3 and a quart hours.

*Tissue culture technics.*

**In the initial isolation attempts roller tube cultures (11,12) of human kidney, human embryonic lung, human embryonic intestine, human uterus and rhesus monkey testis were employed.** Subsequent passages of the agents isolated were later attempted in human kidney, human embryonic skin and muscle, human foreskin, human uterus, rhesus monkey kidney and embryonic chick tissue.” (Enders J. et al, 1954, page 3)

**“Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles”.** John F. Enders et al, *Proceedings of the Society for Experimental Biology and Medicine* 1 June 1954  
<https://www.semanticscholar.org/paper/Propagation-in-Tissue-Cultures-of-Cytopathogenic-Enders-Peebles/a8d3a62cbed04eca654ec5888684c97adb376143> (consulted 4 Dec 2024)

In 1954 John Enders cultivated the blood sample from a sick boy called David Edmonston in no less than **five different kinds of cells** including:

->the **kidneys and lungs**, as well as, the **intestines of aborted human embryos**.

He then passaged/subcultured the blood sample in **human kidney, human embryonic skin and muscle, human foreskin, human uterus, rhesus monkey kidney and embryonic chick tissue**.

Then he took the supernatant fluids from these cultures and told the world that he had made a **vaccine against measles**.

Not once did John Enders “**isolate**” the alleged measles virus from **any human or animal tissue** not in **1954 not ever**.

**It is a scientific fact that John Enders never published a single scientific paper in which he actually describes isolating any microorganism.**

In 1954 **Jonas Salk** started mass producing the alleged Polio virus using “monkey kidney cells”. And in 1955 Natan Goldblum went to the United States on behalf of the Israeli Ministry of Health to learn from Salk how to produce his Polio vaccine.

He wrote a report about this which was published by the **World Health Organisation** in **1957**.

*N. Goldblum et al., “Production of formalinized poliomyelitis vaccine (Salk-type) on a semi-industrial scale”, Bulletin World Health Organisation, Vol 17(1957), pp 1001–1023.  
<https://PMC2537626/?page=14> (consulted 18 May 2025)*

**This article describes a semi-industrial method for the production of formalinized poliomyelitis vaccine developed during 1956. The general technique followed that originally devised by Salk with modifications developed locally. The vaccine was tested for safety**

p.1001

### **Methods of Production and Testing**

#### *Preparation of tissue cultures and virus suspensions*

Monkey kidneys were used exclusively in the production of tissue cultures for the preparation of virus suspensions. The monkeys were *Macaca mulatta* imported from India, and weighed on the average 2-4 kg. On

The kidneys were weighed and decapsulated, and the pelvis was removed. Inside a 50-ml thick-wall centrifuge tube, they were cut into small 3- to 5-mm pieces, which were washed in phosphate-buffered saline (PBS) and placed in a trypsinization flask. The minced tissue in the flask was covered with a 0.25% trypsin solution in PBS and incubated at 37°C for 30 minutes. The pH of the trypsin solution was 7.2.

p.1002

were resuspended in 30-40 ml of medium Mixture No. 199 <sup>7, 12</sup> containing 2% calf serum, 200 units/ml penicillin, 200 µg/ml dihydrostreptomycin and 50 units/ml Mycostatin (nystatin Squibb); the pH of the medium was brought to 7.0 by the addition of a NaHCO<sub>3</sub> solution. This medium will be referred to as medium 199-T. The cell clumps were dispersed in the

p.1004

Goldblum describes how they used monkey kidneys to make “tissue cultures” in order to prepare so called “virus suspensions”. They did this by mincing up the monkey tissue, washing it with PBS and adding trypsin. Four monkeys kidneys resulted in about 12 liters of cell suspension (see p. 1004).

The growth medium they then used to cultivate the trypsinized monkey kidney cells contained 2% calf serum and the antibiotics penicillin and dihydrostreptomycin, as well as, the antifungal Mycostatin (nystatin).



*Infantile Paralysis Polio Development of Poliomyelitis Vaccine 50664, published 29 Dec 2016 by Periscope Film LLC archive. <https://www.youtube.com/watch?v=vghbjh7105g&t=289s> (consulted 18 May 2025) (A Film from the mid-1950s made to promote the newly-developed poliomyelitis vaccine.)*

**On the average, 12 litres of cell suspension containing 300 000 cells/ml were obtained from the kidneys of four monkeys. The cultures were incubated at 37°C for 6-8 days—bottles in a horizontal, and tubes in a slanted, position. All cultures were tightly closed with rubber stoppers. At the end of 6-8 days the surface of the bottles and tubes was completely covered with a confluent sheet of cell growth.**

p.1004

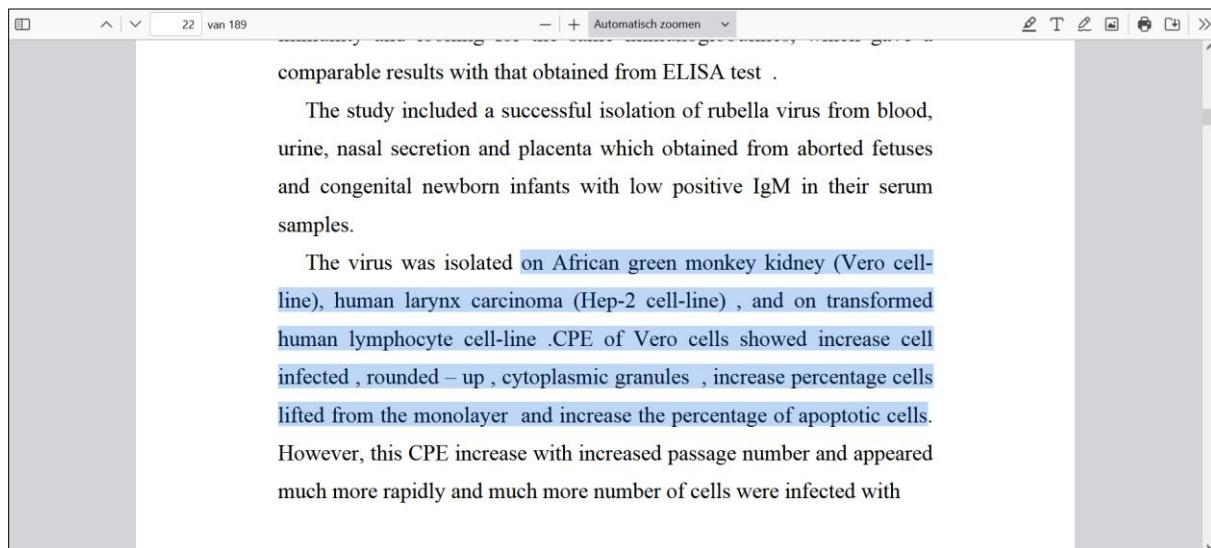
They then added formalin to so call “kill” the virus. Inactivate as they called it. The end result was a toxic soup of monkey kidney cells, calf serum, the antibiotics penicillin and dihydrostreptomycin, the antifungal Mycostatin (nystatin), as well as formalin (liquid formaldihyde). From this soup they collected the supernatant fluids to produce the vaccine which they first filtered before starting animal testing.

They killed and removed the kidneys of about **1500 monkeys** to make **1 million** doses of Salk’s so called **Inactivated Polio Vaccine**.

000 in 1954 to 0.8 cases per 100 000 in 1961[14]. Some disadvantages of the Salk vaccine in that time were the decrease of the titres of the circulating antibody within a few years of vaccination, the further circulation of wild PV and its implications in outbreaks, and **the large number of monkeys (about 1500) needed to be sacrificed to produce every 1 million inactivated doses**. The strains of virus used in the vaccine were Mahoney (type I), MEF-I (type

A. Baicus, “History of polio vaccination”, *World Journal of Virology*, Vol 1(4) (2012), pp 108-114, published online: 12 Aug 2012, <https://pmc.ncbi.nlm.nih.gov/articles/PMC3782271/> (consulted 16 May 2025)

**To this day scientists claim they can “isolate” viruses “on cell-lines” including “monkey kidney” cell-lines just like John Enders did in the 1954.**



S. Jabbar Yasi, “Isolation of Rubella Virus from Aborted Fetuses and Congenitally Malformed Infants and Evaluation of Immunological Response among Aborative Pregnant Women”, September 2023  
[https://www.researchgate.net/publication/373597868\\_Isolation\\_of\\_Rubella\\_Virus\\_from\\_Aborted\\_Fetuses\\_and\\_Congenitally\\_Malformed\\_Infants\\_and\\_Evaluation\\_of\\_Immunological\\_Response\\_among\\_Abo\\_rative\\_Pregnant\\_Women](https://www.researchgate.net/publication/373597868_Isolation_of_Rubella_Virus_from_Aborted_Fetuses_and_Congenitally_Malformed_Infants_and_Evaluation_of_Immunological_Response_among_Abo_rative_Pregnant_Women) (consulted 17 May 2025)

Saif Jabbar Yasi at the University of Kufa in Iraq writes that he isolated the so called **rubella virus** from the blood, urine and nasal secretions (snot) of aborted fetuses and new born babies on **African green monkey kidney cells**.

The date of his publication, September 2023.

The **growth medium** used to grow cells has not changed in 60 years. It is almost always **supplemented** with **calf serum** which is the **blood of cow fetuses**. Why? Because, the blood of cow fetuses contains special growth hormones that are necessary to keep the cells multiplying in artificial settings.

The **growth medium** also contains highly toxic **antibiotics** e.g. penicillin and streptomycin. Sometimes neomycin is used.

Thus any supernatant fluids also called Harvested Virus Fluids (HVF) will also contain these toxic substances.

## MATERIALS AND METHODS

Media.—The growth medium (GM) used was Eagle's Medium in Earle's Balanced Salt Solution [8] supplemented with 10 per cent calf serum.<sup>1</sup> Twenty-five ml of 5.6 per cent NaHCO<sub>3</sub>, 10<sup>5</sup> units of penicillin and 10<sup>5</sup> µg of streptomycin, were added per liter. The final pH of the medium was 7.3, and before use it was brought to 37°C. Phosphate buffered saline (PBS) was prepared as described by Dulbecco and Vogt [7]. Difco trypsin (1:250) was prepared as a 0.25 per cent solution in PBS and supplemented after filtration with the antibiotics described above.

L. Hayflick et al., “The Serial Cultivation of Human Diploid Cell Strains,” Experimental Cell Research, Vol 25v(1961), pp 585-621. <https://cogforlife.org/wp-content/uploads/Hayflick1961ExpCell.pdf>

THE CHECKUP

## Coronavirus Vaccine Dreams

If we get a vaccine for the coronavirus, it will immediately make our world a safer, easier, more reassuring place once again. That's what vaccines do.

Corona Vaccine Dreams, The New York Times, 16 Mar 2020,

<https://www.nytimes.com/2020/03/16/well/family/coronavirus-vaccine.html> (consulted 29 Jan 2025)

The Big Pharma corporations tell us that vaccines “make our world a safer place”. However it has been demonstrated conclusively in this document that the measles, mumps and rubella vaccines are nothing more than a toxic cocktail of antibiotics, trypsin, and the residues of the cells of human embryos and animal embryos that have been grown in fetal bovine serum, the blood of cow embryos. (Vaccines also contain highly toxic stabilisers, adjuvants and preservatives.)

Vaccines have never and will never promote the health of any child ever. This is an undeniable and irrefutable scientific fact. There is nothing more to say about this.

### COMMON COMPONENTS OF VACCINES

As well as the active ingredients, vaccines contain a number of other substances. This graphic examines these and the reasons for their inclusion.

**ACTIVE COMPONENTS**

From the virus or bacteria that causes the disease is used as the antigen. This antigen is modified from the original form so it no longer causes disease, but still elicits an immune response from the body. To modify the disease-causing agent, it can be treated with specific chemicals, so it cannot replicate. It can also be treated so it does not cause serious disease, or only parts of the disease-causing agent that do not cause serious symptoms can be used.

**STABILISERS**

Vaccines need to be stable, so stabilisers are added to ensure the various components remain stable and effective. A variety of different stabilisers are used; either inorganic magnesium salts such as magnesium sulfate or magnesium chloride, or mixtures of lactose, sorbitol and gelatin. Monosodium glutamate and glycine are also used in some cases.

**ADJUVANTS**

Used to enhance the body's immune response to the vaccine. How they work isn't entirely understood, but it's thought they help keep antigens near the site of injection. This means they can be easily accessed by the immune system cells. There is no evidence of any serious adverse effects from adjuvants, though they can cause some minor reaction near the injection site.

**PRESERVATIVES**

Preservatives help prevent contamination of vaccines. They are used particularly in multi-dose vaccines. Thiomersal is a common preservative, though its use declined in the late 1990s when vaccines were falsely linked to child autism. This link was later shown to be an elaborate medical hoax, and there is no link between thiomersal and autism.

**ANTIBIOTICS**

Antibiotics are used in the manufacturing process of the vaccine to prevent bacterial contamination. They are later removed, and only residual quantities remain in the vaccine after the production process.

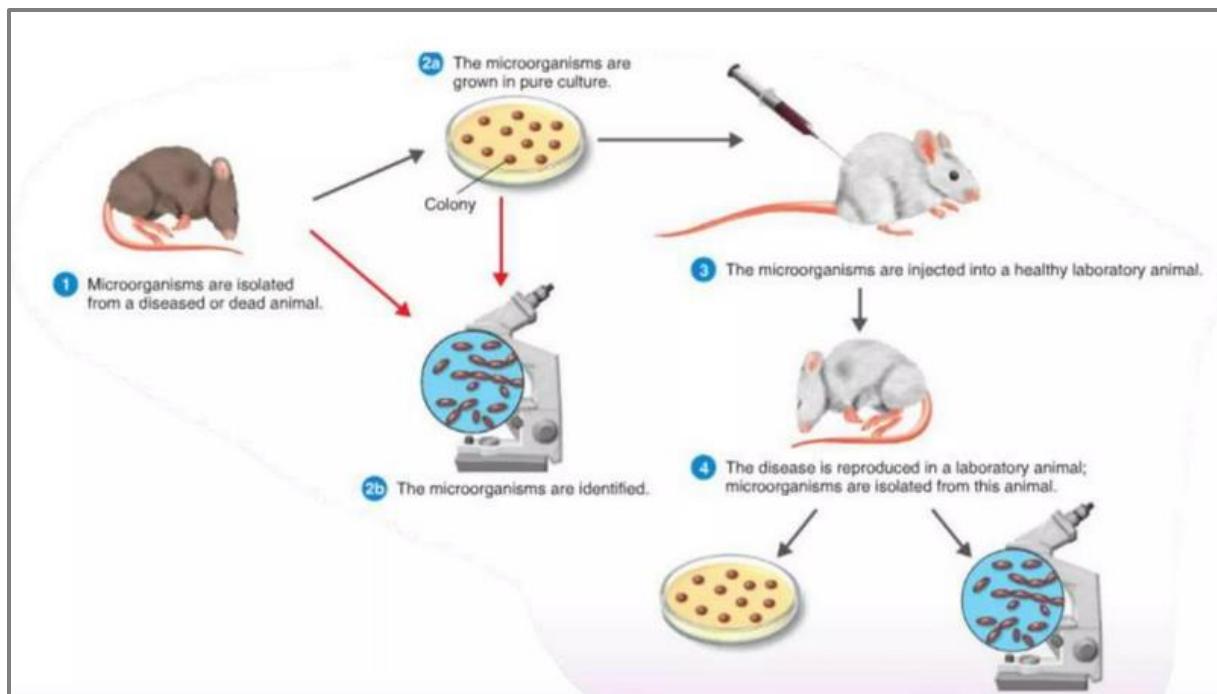
**TRACE COMPONENTS**

These are left over from the vaccine production process, though they are purposefully removed, residual amounts remain. Formaldehyde is one such agent, used to deactivate viruses and detoxify bacteria, but amount remaining is several hundred times lower than the smallest amount known to cause harm in humans.

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A Summary of Common Vaccine Components, [compoundchem.com](https://www.compoundchem.com/2015/02/10/vaccines/)  
<https://www.compoundchem.com/2015/02/10/vaccines/> )



<https://www.slideshare.net/slideshow/infection-and-disease/33336417#13> (see slide 13) (consulted 3 Feb 2025)

### Robert Koch's Postulates

*“Based in part on the earlier perceptions of Jakob Henle, and in consultation with Friedrich Loeffler, Robert Koch devised guidelines to demonstrate that certain human diseases were caused by specific micro-organisms (Table 1).*

*As applied to viral agents (viruses), “Koch's Postulates” for establishing causation require virus isolation from a diseased organism, growth of the agent in pure culture, and the development of disease when the virus is re-introduced into a healthy organism (Koch, 1884; Rivers, 1937)”*

#### Table 1: Koch's postulates to identify the causative agent of an infectious disease.

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms
2. The microorganism must be isolated from a diseased organism and grown in pure culture (**Pure culture means without trypsin, antibiotics or other toxic chemicals.**)
3. The microorganism (from the pure culture) should cause disease when inoculated into a healthy organism
4. The microorganism must be re-isolated from the inoculated, diseased experimental host (animal) and identified as being identical to the original specific causative agent (the original microorganism).

*J. Prescott et al., “Amending Koch's postulates for viral disease: When “growth in pure culture” leads to a loss of virulence”, Antiviral Research, Vol 137, 5 Nov 2016, Pages 1-5, see p2 Table 1, <https://www.sciencedirect.com/science/article/pii/S0166354216302595> (consulted 3 Feb 2025)*

This is the procedure Enders, Plotkin, and Hilleman should have carried out in order to determine if the samples of human tissue (e.g. blood, lung tissue etc.) they collected contained any microorganisms that were the cause of the symptoms they observed in sick patients.

They never did perform this procedure because it would prove conclusively that viruses are pure theory and not entities that exist in reality.

**We do not ask you to believe us, but to read for yourselves what is in the documents presented and understand what is written in them.**

Cell lines such as WI-38 have not only been used in the production of Rubella and Measles but they have also been used in the production of Covid 19 vaccines by Astra Zeneca and Johnson & Johnson. They were also used in the test phase of every single major Covid-19 vaccine on the market.

Read our detailed study on the use of human embryonic cell lines in the production and development of Covid-19 vaccines.

[https://irp.cdn-website.com/e4e1af55/files/uploaded/Babycellen\\_in\\_covid-19\\_vaccins-8e080e7f.pdf](https://irp.cdn-website.com/e4e1af55/files/uploaded/Babycellen_in_covid-19_vaccins-8e080e7f.pdf) (consulted 9 Oct 2025)

And for more information about the use of fetal cell lines in other vaccines click here.

<https://www.diederikengelen.nl/vaccines-and-fetal-cells>

(consulted 9 Oct 2025)

### **The Germ Hypothesis Part 2: Koch's Crisis**

On the pages of [viroliegy.com](https://viroliegy.com) you can find more information about Koch's postulates and how Robert Koch one of the fathers of the "Germ Theory of Disease" was never able to show that any bacteria or other entities he called viruses ever caused any disease.

<https://viroliegy.com/2024/06/07/the-germ-hypothesis-part-2-kochs-crisis> (consulted 9 Oct 2025)

### **Monkey Business: Polio, Measles And How It All Began**

This is a documentary about the discovery of the so called Polio and Measles viruses.

It features among others Dr. Tom Cowan, MD, author of 'The Contagion Myth', Claus Kohlein, MD, Torsten Engelbrech, co-authors of 'Virus Mania' and Jim West, author of 'Virology vs. Toxicology'.<https://thenviraldelusion.substack.com/p/the-viral-delusion-episode-three>  
(consulted 9 Oct 2025)



### **The Viral Delusion Episode Three: Monkey Business: Polio, Measles And How It All Began**

The magic formula for how to discover a "virus" is discovered and modern virology is born.

 MICHAEL WALLACH  
AUG 30, 2025



Mike Wallach: The Viral Delusion

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## Appendix 1: Definition of Words Used in Microbiology and Virology

### 1. Obtaining the required cells

**to isolate:** another word for **dissect** or **cut out** cells from of a living animal or human.

**an explant :** tissue or organ from an organism. (e.g. fresh blood, kidney cells, snot)

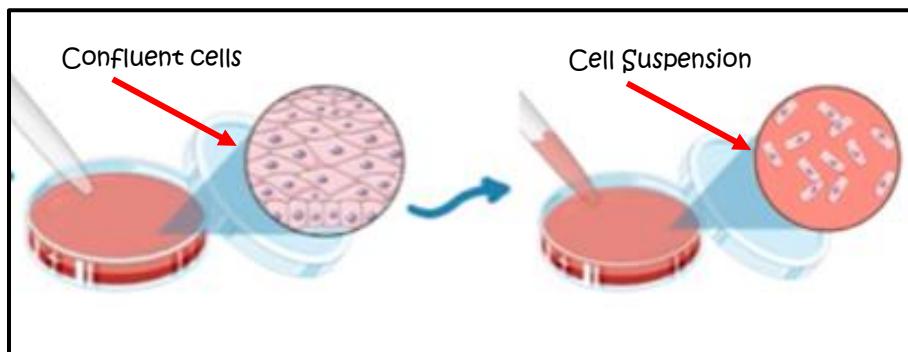
**primary cells:** cells directly obtained from a living organism.

### 2. Preparation of cells for cultivation

**to mince/fragment:** sometimes tissues are minced up or fragmented into small pieces in order to create a **cell suspension**.

**trypsin:** is an enzyme used to break down the natural tissue connecting cells in order to create a **cell suspension**. Once the connecting tissue has been broken down trypsin must be removed because it is toxic and will stop or slow cell growth. Trypsin is usually removed by centrifuging the cell suspension, removing the supernatant fluid (top fluid) and washing (rinsing) the remaining cells with PBS (salt solution).

**cell disassociation:** the process of breaking down the natural tissue connecting cells. This is usually done with the use of trypsin. Sometimes this is done by mincing the organs or the collected tissues and adding PBS (salt solution).



Cells before and after the use of **trypsin**.

**supernatant:** the **top fluid** after the cell suspension has been centrifuged.

**cell pellet:** the **cells at the bottom of the test tube** after the cell suspension has been centrifuged. (This is washed or rinsed with PBS salt solution to remove the trypsin.)

**Phosphate Buffer Saline (PBS) / Buffer (salt solution):** is used to wash (rinse) the cell pellet after the cells have been disassociated with trypsin. It is also sometimes used to maintain the PH of the cell culture at 7 (neutral).

Purpose and Products of Centrifugation

Small diameter, low density.  
Small diameter, high density.  
Large diameter, high density.

Instruction

A centrifuge is used to separate particles from a liquid or to separate a number of different particles based on their size or density. After centrifugation, a suspension is separated into a pellet and some supernatant. The largest and densest particles will appear near the bottom of the tube. Roll over the orange icons for more information about this process.

NEXT →

Purpose and Products of Centrifugation: <https://www.learnsci.com/resources/purpose-and-products-of-centrifugation> (consulted 11 Jan 2025)

### 3. Let the cells grow/multiply

'cell culture medium' also know a growth medium (GM) : liquid food for cells.

Most microbiologists and virologists use what is called **Eagle's Medium** as the cell culture medium to grow/cultivate cell cultures.

**Eagle's Medium** is a cell culture medium developed by Harry Eagle and first published in Science Magazine in 1959. It is based on six salts, glucose as well as thirteen essential amino acids, and eight vitamins.

The **growth medium** used is **supplemented** with **calf serum** which is the **blood of cow fetuses** which are killed and their blood drained to grow cell cultures. The blood of cow fetuses contains special **growth hormones** that are necessary to keep the cells multiplying in artificial settings.

Calf serum is also called **Fetal Cow Serum (FCS)** or **Fetal Bovine Serum FBS**.



Thermo Fisher Scientific

<https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-cult...> \*\*\*

#### Fetal Bovine Serum Collection and Manufacturing | Thermo Fisher ...

Approximately 500,000 L of **FBS** are produced annually, requiring the harvest of about **one million bovine fetuses** [1]. Examples of quality tests performed during **FBS** production Processing and quality testing are vital to ensuring that **FBS** is suitable for cell culture.

<https://www.thermofisher.com/nl/en/home/references/gibco-cell-culture-basics/cell-culture-environment/culture-media/fbs-basics/steps-taken-manufacture-fbs.html> (consulted 17 Jan 2025)

The **growth medium** also contains highly toxic **antibiotics** e.g. penicillin and streptomycin. This is added to stop bacteria growing in the culture.

## MATERIALS AND METHODS

Media.—The growth medium (GM) used was Eagle's Medium in Earle's Balanced Salt Solution [8] supplemented with 10 per cent calf serum.<sup>1</sup> Twenty-five ml of 5.6 per cent NaHCO<sub>3</sub>, 10<sup>5</sup> units of penicillin and 10<sup>5</sup> µg of streptomycin, were added per liter. The final pH of the medium was 7.3, and before use it was brought to 37°C. Phosphate buffered saline (PBS) was prepared as described by Dulbecco and Vogt [7]. Difco trypsin (1: 250) was prepared as a 0.25 per cent solution in PBS and supplemented after filtration with the antibiotics described above.

L. Hayflick et al., "The Serial Cultivation of Human Diploid Cell Strains," *Experimental Cell Research*, Vol 25v(1961), pp 585-621. <https://cogforlife.org/wp-content/uploads/Hayflick1961ExpCell.pdf>

**roller tube culturing technique** (used by John Enders)

Roller drum used to hold cell culture tubes during incubation. Slow rotation continually bathes the cells in the medium.



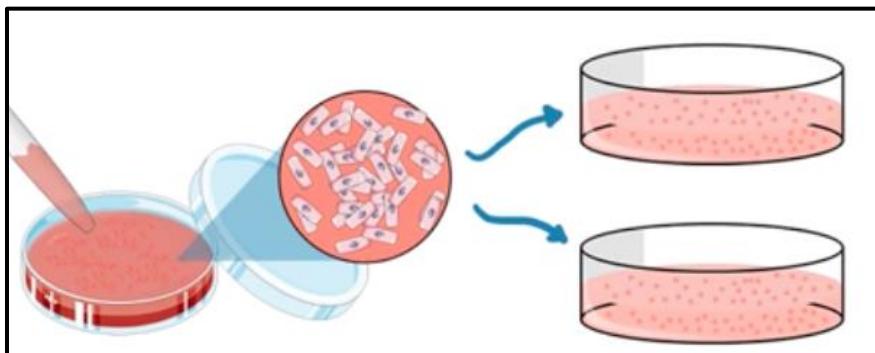
**Overview of the Methods and Strategies in Virology Chapter 65**

<https://basicmedicalkey.com/overview-of-the-methods-and-strategies-in-virology/> (consulted 30 Jan 2025)

#### 4. Subcultivating/passaging the cells

**subcultivate/passage:** dividing a cell suspension into **two or more ‘subcultures’**.

Subcultivation is usually done after cells in a cell culture have become “confluent” and are no longer growing. Trypsin is usually used to break the natural tissue connecting the cells so that they can be divided into **two or more ‘subcultures’**.



**Cytopathogenic Agents:** another word for virus

**Propagate:** another word for culturing or growing.

#### Basal Media vs. Complete Media

What's the Difference?

Basal media and complete media are both types of culture media used in laboratory settings for the growth and maintenance of cells. Basal media, also known as minimal media, provide the essential nutrients required for cell survival and growth, such as salts, amino acids, and vitamins. However, they lack certain components like growth factors and serum, which are present in complete media.

Complete media, on the other hand, contain all the necessary nutrients, growth factors, and serum to support optimal cell growth and proliferation. While basal media are often used for specific research purposes where the absence of certain components is desired, complete media are more commonly used for routine cell culture and are ideal for supporting a wide range of cell types.

<https://thisvsthat.io/basal-media-vs-complete-media> (consulted 11 Jan 2025)

Growth factors = growth hormones and nutrients

**Formalin** = formaldehyde and water used to so call “inactivate” alleged viruses. It is used in mortuaries to preserve dead bodies.



Pediaa.Com

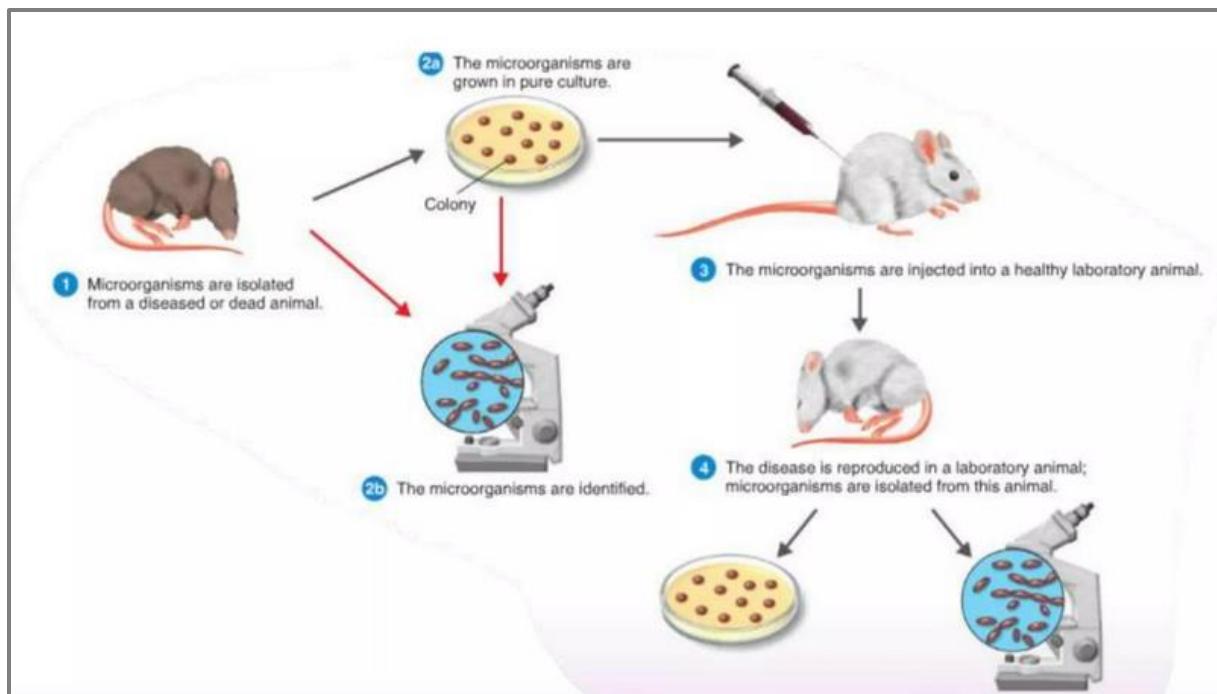
<https://pediaa.com/difference-between-formalin-and-formaldehyde>

...

#### Difference Between Formalin and Formaldehyde - Pediaa.Com

Basically, **formaldehyde** is a colorless, water-soluble, flammable gas at room temperature with a sharp, irritating smell. However, **formalin** is a liquid, which is prepared by mixing **formaldehyde** gas and water. This is the main difference between **formalin** and **formaldehyde**.

<https://pediaa.com/difference-between-formalin-and-formaldehyde/> (consulted 18 May 2025)



<https://www.slideshare.net/slideshow/infection-and-disease/33336417#13> (see slide 13) (consulted 3 Feb 2025)

### Robert Koch's Postulates

*“Based in part on the earlier perceptions of Jakob Henle, and in consultation with Friedrich Loeffler, Robert Koch devised guidelines to demonstrate that certain human diseases were caused by specific micro-organisms (Table 1).*

*As applied to viral agents (viruses), “Koch's Postulates” for establishing causation require virus isolation from a diseased organism, growth of the agent in pure culture, and the development of disease when the virus is re-introduced into a healthy organism (Koch, 1884; Rivers, 1937)”*

Table 1: Koch's postulates to identify the causative agent of an infectious disease.

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms
2. The microorganism must be isolated from a diseased organism and grown in pure culture (**Pure culture means without trypsin, antibiotics or other toxic chemicals.**)
3. The microorganism (from the pure culture) should cause disease when inoculated into a healthy organism
4. The microorganism must be re-isolated from the inoculated, diseased experimental host (animal) and identified as being identical to the original specific causative agent (microorganism).

*J. Prescott et al., “Amending Koch's postulates for viral disease: When “growth in pure culture” leads to a loss of virulence”, Antiviral Research, Vol 137, 5 Nov 2016, Pages 1-5, see p2 Table 1, <https://www.sciencedirect.com/science/article/pii/S0166354216302595> (consulted 3 Feb 2025)*

Not one virologist has ever performed this procedure formulated by Robert Koch in 1884 not even Robert Koch himself. Why?

It would prove conclusively that viruses are pure theory and not entities that exist in reality.

If you do experiments correctly, they do not lie.

## Appendix 2: References and Weblinks

For more information about the use of foetal cells in vaccines see my website:  
<https://diederikengelen.nl/vaccines-and-fetal-cells> (English)  
<https://diederikengelen.nl/vaccins-en-foetuscellen> (Dutch)

About the **WI-38 cell line** from the lungs of an aborted baby girl when she was 16 weeks old.  
[https://www.cellosaurus.org/CVCL\\_0579](https://www.cellosaurus.org/CVCL_0579)

A detailed study about the use of **the cells of aborted babies (human embryonic cell lines)** in the development and manufacture of **COVID-19 vaccines**: [https://irp.cdn-website.com/e4e1af55/files/uploaded/Babycells\\_in\\_covid-19\\_vaccins.pdf](https://irp.cdn-website.com/e4e1af55/files/uploaded/Babycells_in_covid-19_vaccins.pdf)

Copies of all these documents have been downloaded and printed on paper as well as stored in digital format. They are the property of mankind as evidence of the greatest mass murder that was ever carried out in the history of man.

They are evidence that can be used in a court of law anywhere in the world against any government or corporation who claims that viruses have ever been isolated and that vaccines are of any effect in preventing disease.

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[https://www.youtube.com/watch?v=NC65\\_b1sGiY&list=PLVV0r6CmEsFyL\\_YYxHe6RzAARP6eJ\\_8wC&index=51](https://www.youtube.com/watch?v=NC65_b1sGiY&list=PLVV0r6CmEsFyL_YYxHe6RzAARP6eJ_8wC&index=51) (consulted 29 Jan 2025)

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[https://www.researchgate.net/publication/242333147\\_Medical\\_research\\_Cell\\_division](https://www.researchgate.net/publication/242333147_Medical_research_Cell_division) (consulted 12 Jan 2025)

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