

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?

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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 virus¹ Enders' Edmonston strain (live, attenuated)not less than 1×10^3 CCID₅₀*
Mumps virus¹ Jeryl Lynn™ [Level B] strain (live, attenuated).....not less than 12.5×10^3 CCID₅₀*
Rubella virus² Wistar RA 27/3 strain (live, attenuated)not less than 1×10^3 CCID₅₀*

*50% cell culture infectious dose

¹ produced in chick embryo cells.

² 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 (consulted 24 November 2024)

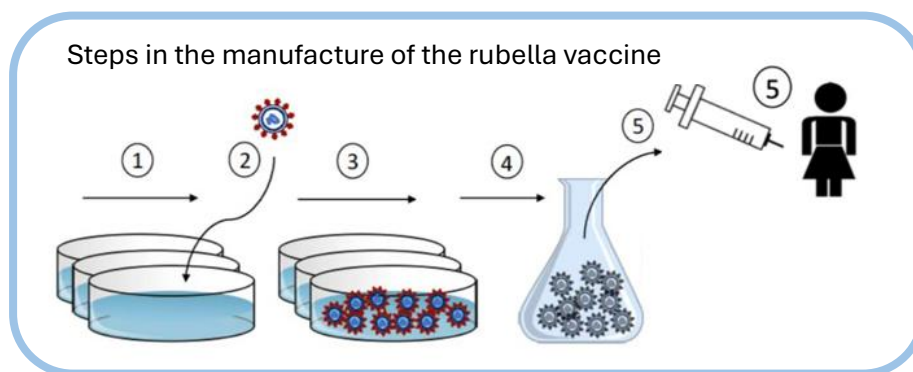
So according to the **M-M-RVAXPRO** Product Information the **rubella virus** is produced in **WI-38 human diploid lung fibroblasts**.

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.

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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?**

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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

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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**.

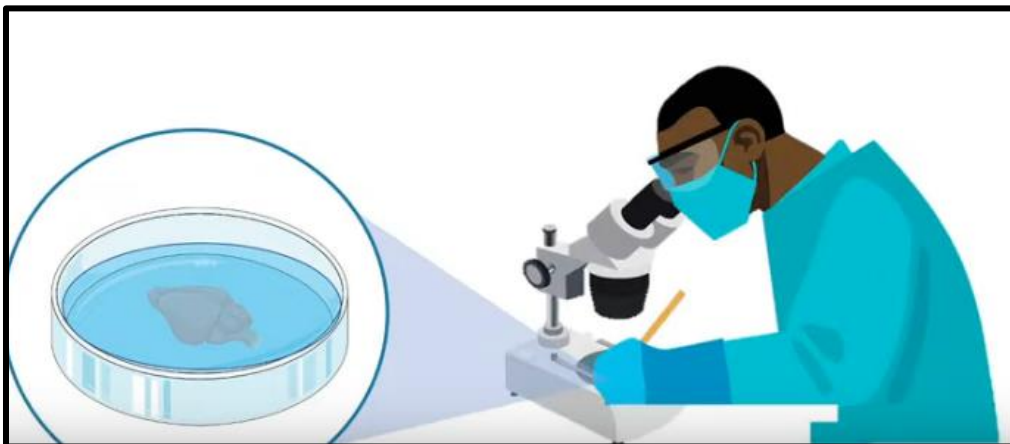
Step 1 Obtain the required cells from a suitable embryo



- 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**”.

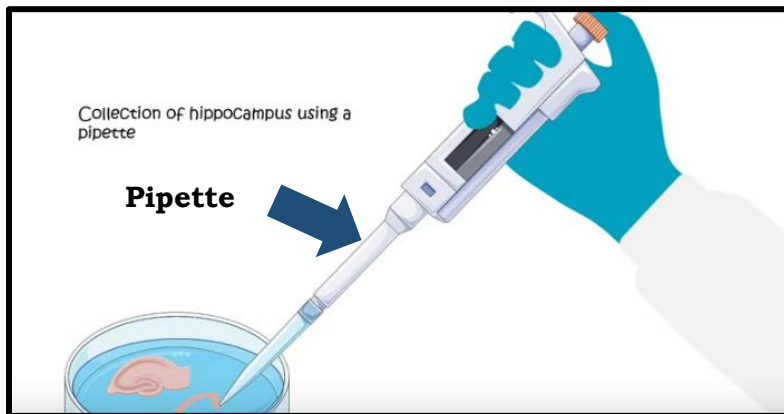


- Dissect the **hippocampus** from of the **brain** under the **microscope**.

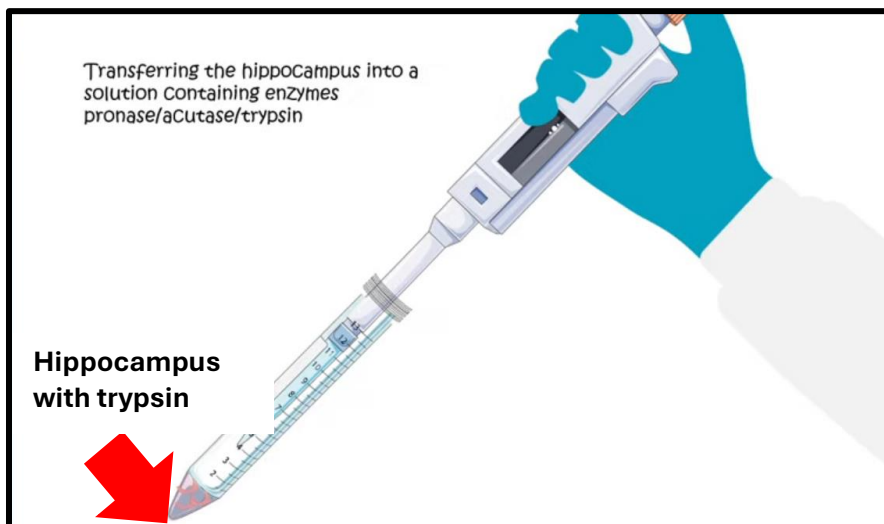
(The **hippocampus** is part of the brain, human brains also have a **hippocampus**.)

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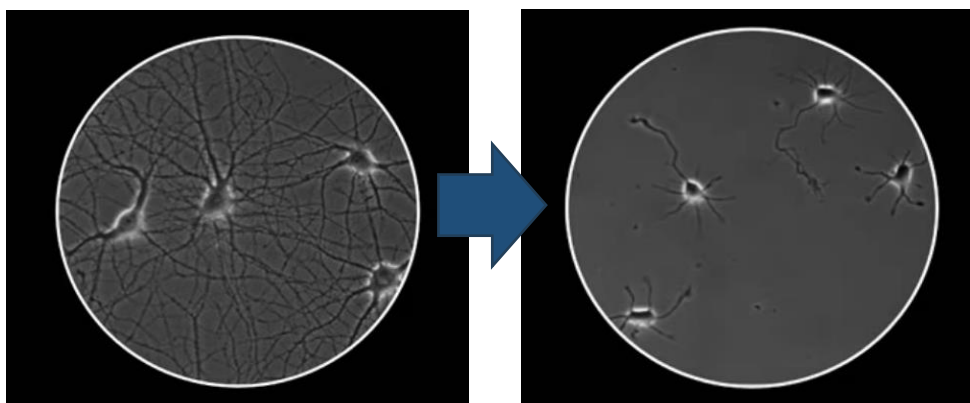
Step 2 Prepare the cells for cultivation



- 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.

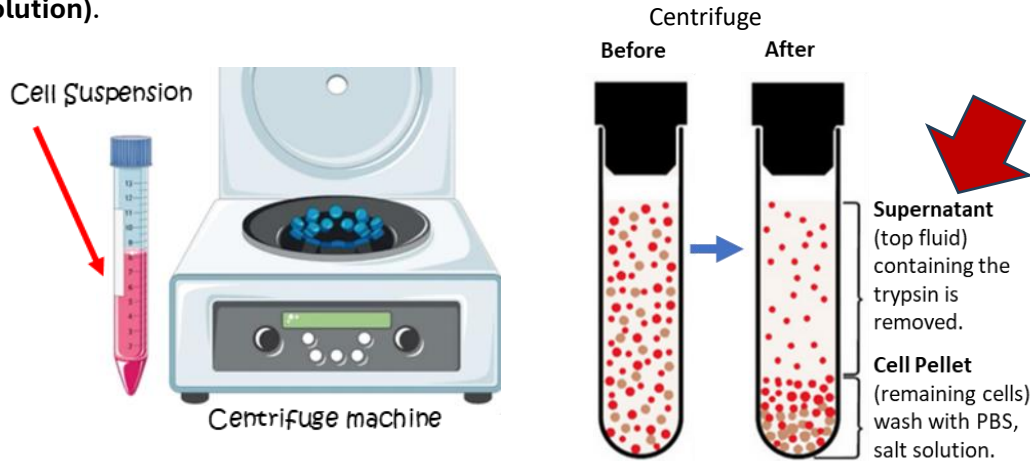
In the picture above you can see that the **cell tissue** connecting the cells has been **broken down**, the synapses between the brain cells have been removed. The cells are still alive however they are no longer growing as tissue but as single cells in a "**cell suspension**".

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- 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)**.

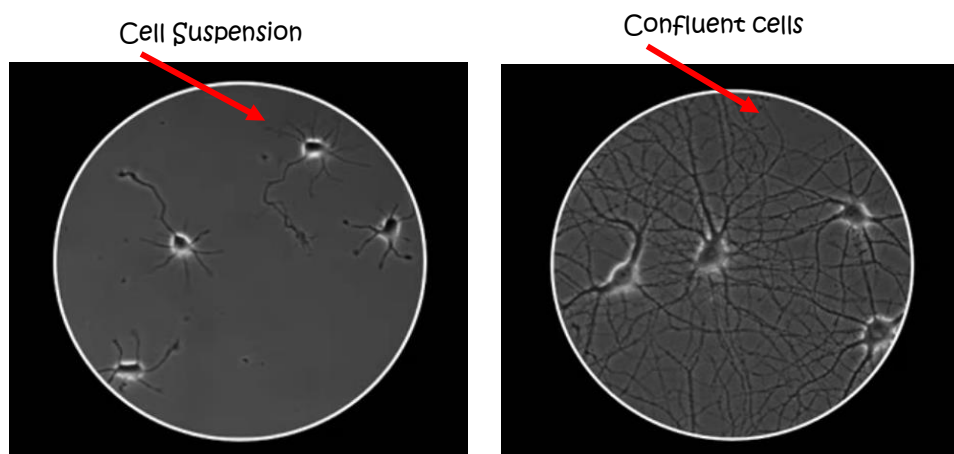


Step 3. Let the cells grow

- 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.



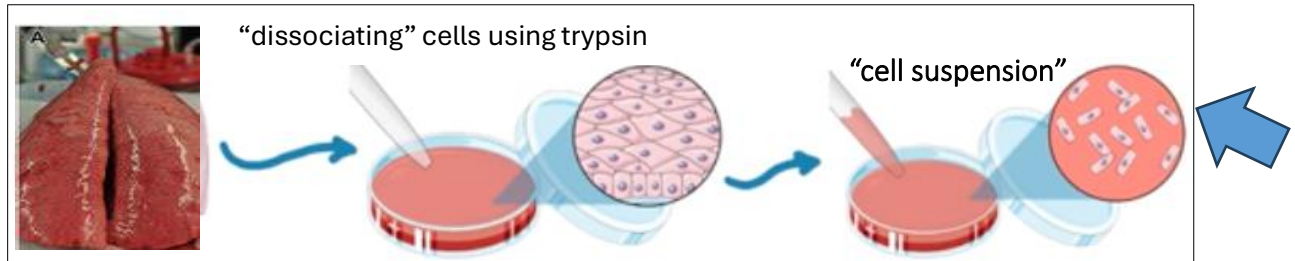
Brain cells before and after 3 weeks (21 days) of growth in a culture.

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**.

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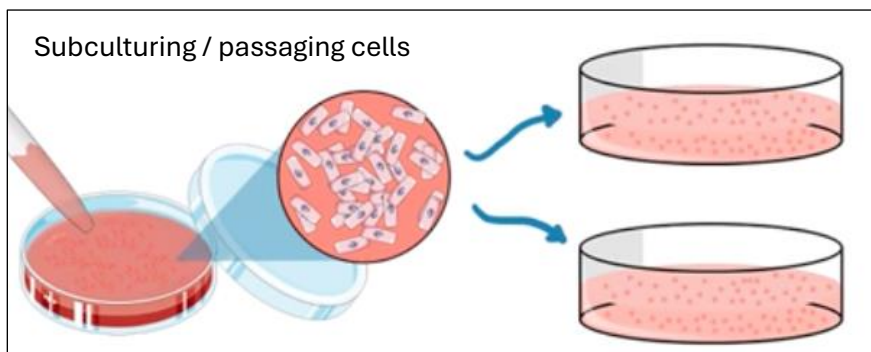
Let us now summarise this whole process by describing how you make a **cell line/cell strain** from the **lung tissue** of a **human embryo**.

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’**.

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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 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**.

