Purification: Virology's Black Sheep

> PURITY: ***Relative* freedom from extraneous matter in the finished product,** whether or not harmful to the recipient or deleterious to the product. (21 CFR 600.3(r))
>
> *FDA Guidance Definition for vaccine purity*

The above definition for purity is taken from the FDA's *Guidance for Industry*. According to the FDA, purity is considered *relative* freedom from extraneous matter. What is "*relative* freedom?" Relative implies that something is true to a certain degree. In other words, there is freedom from extraneous matter to a certain degree. However, in relation to purity, there can be no degrees of freedom from extraneous matter. A substance is either pure, meaning that it is free of all contaminants and foreign matter, or it is impure, meaning it is mixed with contamination and foreign matter. There is no such thing as purity to a certain extent yet you will commonly find discussions of purity within the "scientific" papers as if this can be the case. Terminology such as *low* purity, *partially* purified, *somewhat* purified, *relatively* purified, *highly* purified, etc. are thrown about as vague descriptors used to wiggle around a concept that is so simple and straight forward that most children can understand it. Something is either pure or impure. There is no in-between.

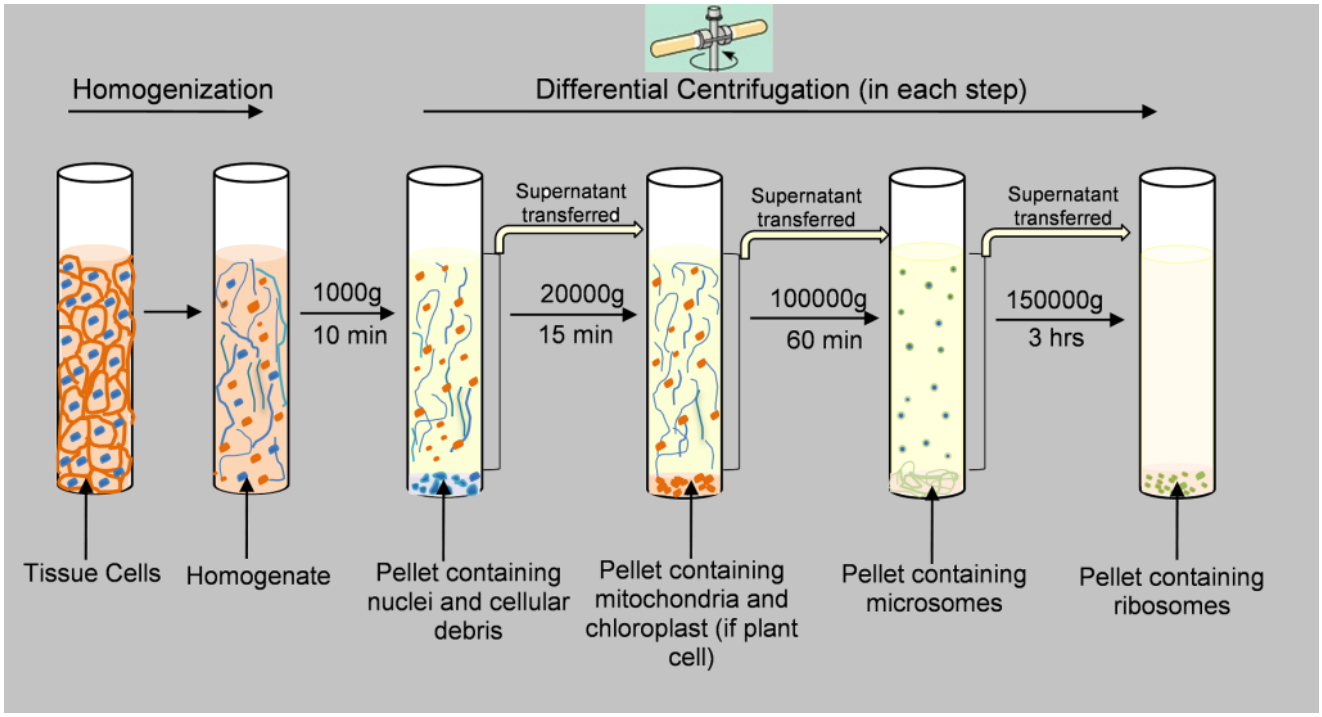
The issue of purification is absolutely vital to the foundational problems regarding virology. In order to prove the very existence of the fictional creations we are supposed to fear, it must be shown that "viruses" exist in a purified and isolated state. However, purification is mostly ignored in any original "virus" study when it should be a prominent focus. If we are to believe that these invisible intracellular parasitic entities exist, we need to be able to observe them alone. As we can not observe "viruses" in nature nor with our own eyes, this means that it is essential that the particles assumed to be "viruses" are taken directly from the fluids of a sick human and then purified free of any contaminants, pollutants, foreign materials, etc. contained within so that nothing else remains other than the assumed "viral" particles. Only then could pathogenicity be proven by having a valid independent variable separated from everything else in order to establish a cause-and-effect relationship for the particles claimed as the causative agent. Only then would electron microscope images of just those particles with nothing else contained within the sample be valid as evidence.

However, as you will see, virologists have admitted numerous times that "viruses" can not be purified and isolated directly from the fluids of a sick human. They state that "viruses" must be cultured with a host cell first before purification and isolation can occur. This creates many problems as:

1. The methods used to purify and isolate "viruses" are ineffective
2. "Viruses" can not be separated from exosomes and other MVB's within the sample
3. The cell culture method is impure and the media and host cell DNA can not be purified away from the invisible "viruses"

In this article, I will break these various problems down and show that, from their own sources, virology admits that complete purification and isolation of the assumed "viral" particles is an impossibilty.

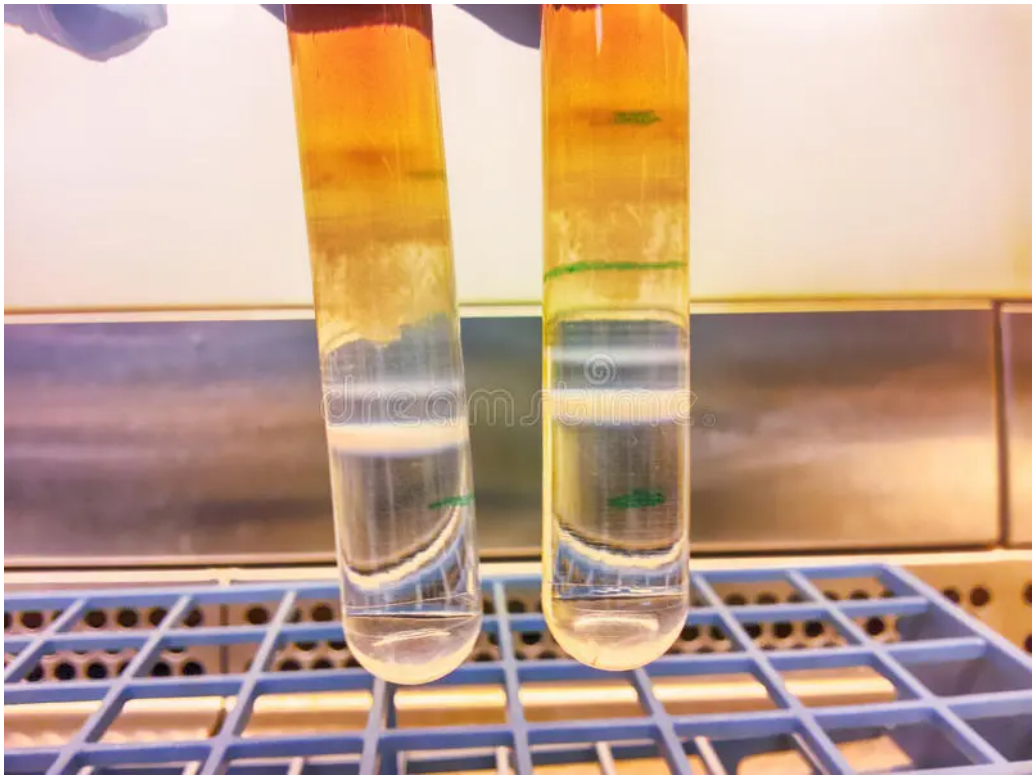
Ineffective Methods



A big hindrance to obtaining evidence of purification of the particles assumed to be "viruses" is that there is no standardized universal purification method. The techniques used vary by "virus" and the researchers performing the study. What "works" for one "virus" may not work for another as noted in this source on purifying plant "viruses:"

"Purification refers to the separation of virus particles from host components in a biologically active state. Purified virus is required for the production of antibodies, physical, biochemical and molecular characterization of virus isolates. Purification of virus involves several steps such as propagation of the virus in the host, extraction of sap, clarification, concentration and further purification. Purity of purified preparation can be checked through UV absorption spectra and its infectivity by inoculating to a susceptible host under optimal environmental conditions in an insect-proof glasshouse. **Purification methods vary with different viruses, and there are no universal methods of virus purification. Procedures that are effective for one virus may not work with the other."**

https://experiments.springernature.com/articles/10.1007/978-1-0716-0334-5_21



Complicating the issues related to the lack of any standardized methods or procedure to achieve purification is the existence of various methods virologists can choose from in order to attempt to obtain purification. These include but are not limited to:

1. Centrifugation

2. Filtration
3. Precipitation
4. Chromatography
5. Adsorption

However, you will be hard pressed to find much mention of any of these methods being used in "virus" studies at all. If you do happen to find purification methods being performed, it is typically just a spin on the ol' centrifugation for a bit. While this may separate some of the larger substances from the smaller ones, it does not offer complete purification of all particles.

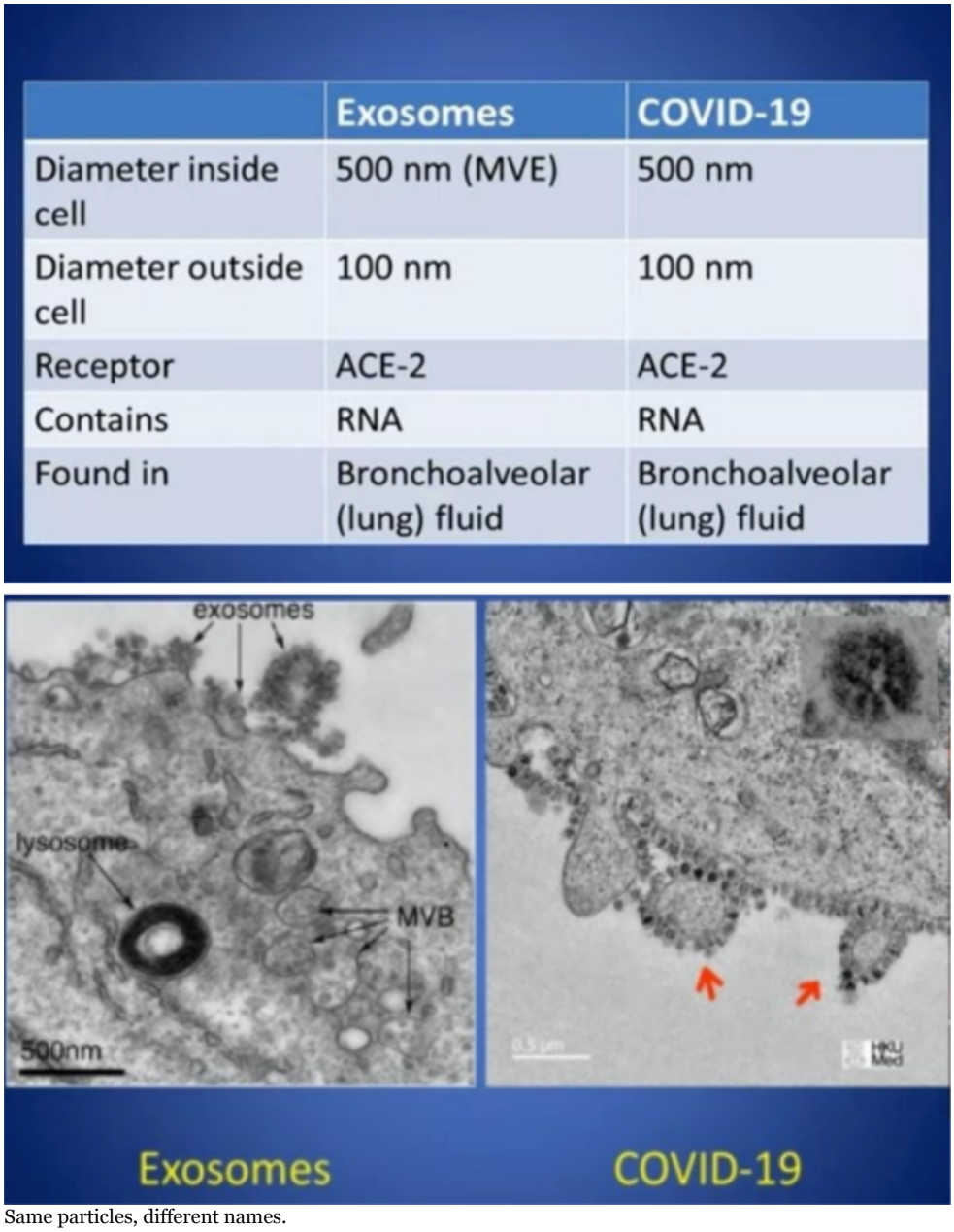
"Viruses"/Exosomes: Inseparable "Cousins"

> How can we be sure that we are isolating and quantifying extracellular vesicles **rather than enveloped viruses present in the sample?** Equally, **how can viral researchers know that they are not detecting similarly sized non-viral vesicles or empty vectors** during vaccine production?"
>
> *https://www.google.com/amp/s/www.nanoviewbio.com/exosome-blog/2020/5/5/extracellular-vesicles-and-viruses-two-sides-of-the-same-coin%3fformat=amp*

We can easily find this lack of complete purification to be the case for all of the methods used for "viruses" if we look to exosome research as it shares the exact same procedures as those used in virology. For those who may not know, exosomes are "viruses" in every sense of the word. They share the same biochemical and morphological characteristics. The main difference (beyond the name) is that exosomes are considered "non-infectious" and they are assumed to play a role in intracellular communication. In other words, they are the exact same particles as "viruses" but they were given different names and different functions than their "viral" cousins. Fortunately, as I noted earlier, the same methods of purification are used for these identical particles. If the purification methods are unable to completely purify exosomes, the same can be said of "viruses." For instance, in this 2020 study on the attempts to separate "viruses" from exosomes, we can see that ultracentrifugation, the "gold standard" method of purification for both entities, is considered inadequate:

"The method is **not ideally suited for the separation of EV and viruses, particularly from infected cultures and/or body fluids** and it is difficult to adopt ultracentrifugation to large-scale production environments or to environments that present biosafety concerns."

https://link.springer.com/article/10.1007/s11481-019-09874-x



Same particles, different names.

This inability to purify exosomes from "viruses" does not stop with ultracentrifugation. No matter what method is utilized, each one has its own drawbacks and limitations which lead to a lack of complete purification and the inability to separate "viruses" from exosomes. These next two sources show that the same methods shared between "viruses" and exosomes are all equally unable to successfully purify and isolate these particles from each other:

Exosomes and viruses

"Because of their similar physical properties, **the techniques used by researchers for purification of exosomes and virions are also similar.**

Ultracentrifugation at greater than 100,000 x g is used to concentrate both exosomes and viruses. This technique retains intact, functional viruses and exosomes. **Because exosomes and viruses are similar in size and density, separation of the two is not possible using this technique.**

Polyethylene glycol (PEG) can be used to precipitate both exosomes and viral particles. PEG precipitation is commonly used in commercially available exosome isolation kits and has been harnessed for isolation of many different viruses for many years. **Again, you cannot separate viruses and exosomes with this protocol.**

Exosomes and viruses can also be purified using chromatography techniques based on size, affinity, hydrophobicity, or other characteristics. Size exclusion chromatography (SEC) purification such as Exo-spin can be used to isolate both exosomes and viral particles. **Again, exosomes and virions cannot be distinguished from one another using this method."**

https://www.cellgs.com/blog/exosomes-and-viruses.html

Review on Strategies and Technologies for Exosome Isolation and Purification

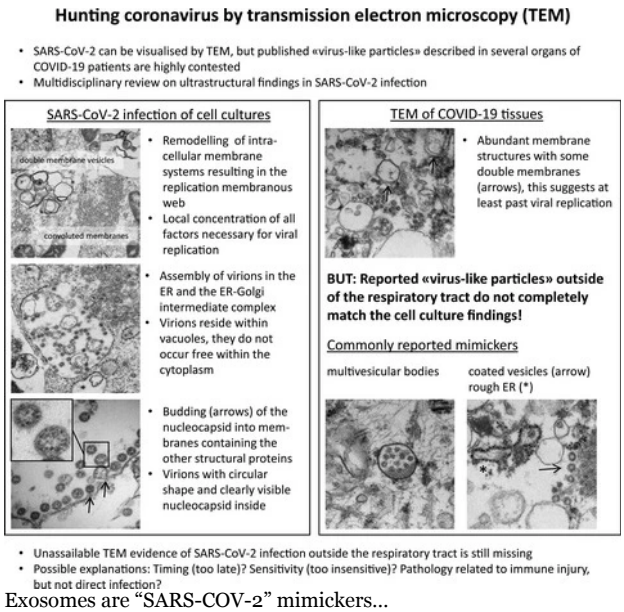
"A method that can efficiently provide intact and pure exosomes samples is the first step to both exosome-based liquid biopsies and therapeutics. Unfortunately, **common exosomal separation techniques suffer from operation complexity, time consumption, large sample volumes and low purity,** posing significant challenges for exosomal downstream analysis."

“At present, common exosome isolation technologies, such as ultrafiltration, immunoaffinity, ultracentrifugation (“gold standard” for the isolation of exosomes) are expensive instruments, large volumes of sample, **possible protein contamination,** complete isolation steps, **but they result in low isolation efficiency, sample loss, low exosome recovery and purity** (LeBleu and Kalluri, 2020).”

“Although exosomes play an irreplaceable role in early detection and treatment, **they are small in size (30–150 nm), low in density (1.13–1.19 g/ml), and mixed with similar components (e.g., cell fragments, proteins) in the body fluids, which pose tremendous challenges for their separation (Cui et al., 2018; Lin et al., 2020).** In addition, the biological activity of exosomes will be affected by different separation techniques (Paolini et al., 2016).”

“Although the above-mentioned traditional separation methods are the most widely used, there are also many disadvantages, such as large sample consumption, risk of damage to exosomes, **low purity,** and long time consuming, which are hard to meet the current increasing scientific research needs.”

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8766409/



Exosomes are “SARS-COV-2” mimickers…

As can be seen, the methods listed above do not offer the ability to actually separate the particles believed to be exosomes from those believed to be “viruses.” This is a major problem as purification is absolutely necessary not only in order to prove cause and effect but also to characterize the “virus” physically, molecularly, and biochemically. Without having just the assumed “viral” particles alone, the “virus” can not be studied independently nor differentiated from the millions of similar and/or identical particles within the sample. There can be no direct proof for the existence of any “virus” as there are numerous other microorganisms, substances, and contaminants within a sample that could also be the potential cause of the disease rather than the assumed-yet-never-seen “virus.” Exosome literature is rife with papers explaining the impossibility of separating these particles from their “viral” cousins. As exosomes and “viruses” are the same size and shape, this means that complete purification and isolation of any “virus” is impossible. There will always be exosomes and/or any other particle that is either the same size or smaller than the assumed “virus” particles left remaining. Highlights from the below article from 2019 presents further evidence to this effect:

How are Exosomes Isolated?

“Exosomes are extracellular vesicles that **are believed** to play a role in communication between cells by transporting materials inside the vesicle.

Exosomes can be isolated from cells using typical methods for purifying cell fractions. **The challenge in isolating vesicles is differentiating them from other types of membrane material in the cell culture supernatant.**

This is done through successive steps of centrifugation at increasing speeds. The final supernatant is ultracentrifuged at 100,000g to pellet the exosomes. This is then washed to remove contaminating proteins and centrifuged at high speed one more time.

However, that type of preparation is more an enrichment of the sample than a purification. Further analysis of the sample through biochemistry or microscopy is still required to characterize the vesicles.

Characterization

In addition to exosomes, the extracellular milieu contains extracellular RNA, other types of vesicles, protein complexes, and lipoproteins. These are not fully separated from exosomes through the centrifugation protocol. There are also commercial kits available to isolate exosomes that make use of polymers, but these kits tend to co-isolate other molecules, particularly RNA-protein complexes.

The Executive Committee of the International Society for Extracellular Vesicles (ISEV) proposed criteria for characterizing exosomes to aid in consisting reporting of experimental results.

The criteria are that all exosome samples must adhere to are given below:

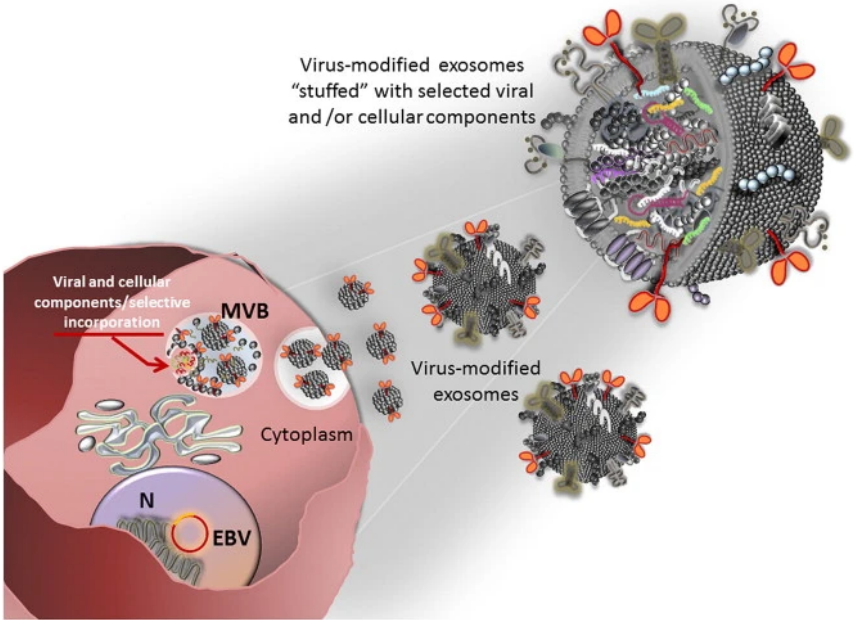
- ***Be isolated from extracellular fluids like cell culture medium or body fluids*. The collection should be gentle to minimize cell wall disruption that could contaminate the sample with intracellular compartments.**
- *Have an overview of protein composition included in its description*. The amount of 3 or more relevant proteins should be reported in a semi-quantitative manner in any exosome preparation.
- ***Have levels of proteins not expected to be enriched* to determine the extent of co-isolation of intracellular vesicles.**
- *Have single vesicles characterized as an indication of heterogeneity*.
- *Have a quantitative analysis of dose-function relationships* in the case that in vitro functional studies are carried out.

- *Have demonstration of association of molecules with the exosome*, in the case that functional changes are ascribed to single molecules or clusters of molecules.

Study of exosomes is a rich area of research due to the large number of **unanswered questions,** including exactly how they are formed, **what their functions are,** how they communicate with acceptor cells, and their role in various diseases.

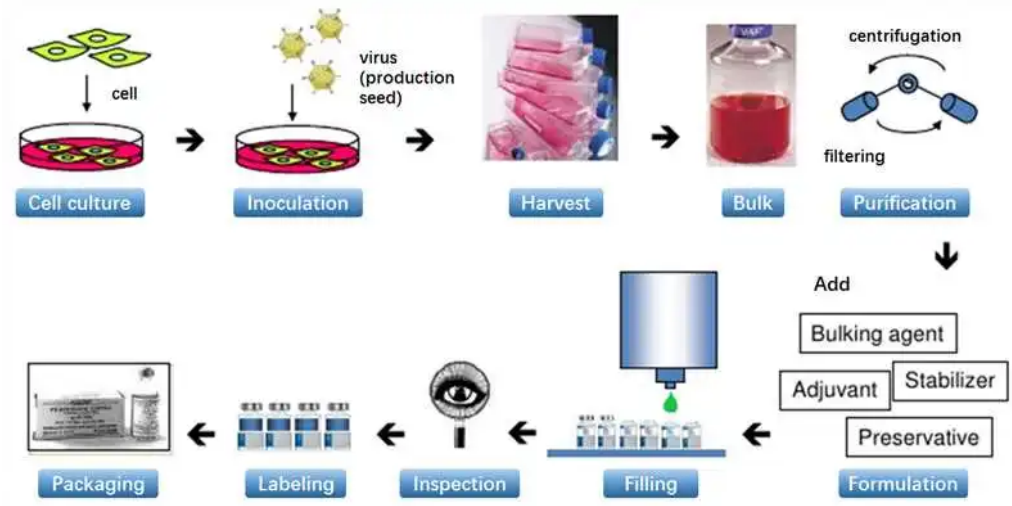
Consistent methods for isolating and characterizing exosomes and distinguishing them from other types of extracellular and intracellular vesicles are needed to enable these advances."

https://www.news-medical.net/amp/life-sciences/How-are-Exosomes-Isolated.aspx



There are no consistent methods used for purifying (i.e. "isolating") exosomes nor ways for distinguishing them from other extracellular and intracellular organisms. In other words, exosomes (the "non-infectious virus") can not be completely separated from everything else nor can they be differentiated from everything else within the cell culture supernatant. This should tell you everything you need to know regarding the impossibility of complete purification and isolation of these entities.

Purifying "Viruses" from Culture for Vaccines?



Beyond being unable to completely purify and isolate exosomes (cough "viruses" cough) from everything contained within a sample, it is also admitted that "viruses" can not be completely purified from the cell culture materials that they are supposedly grown in. Take, for instance, the Jynneos vaccine for monkeypox. It is claimed that the vaccine, which utilized chicken embryo fibroblast cells for culturing the assumed "virus," was purified and that it contained no animal material. However, it is later revealed within the same paragraph that not only does the vaccine contain host-DNA (i.e. chicken DNA from the CEF cell), it contains other proteins (source unknown) as well as antibiotics used for culturing and an enzyme used for purification:

- Benzonase – a genetically engineered endonuclease from Serratia marcescens produced and "purified" from E. coli strain
- Gentamicin – an antibiotic that can cause severe damage to the kidneys
- Ciprofloxacin – another antibiotic used to treat urinary bacterial infections

"JYNNEOS is a live vaccine produced from the strain Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN), an attenuated, non-replicating orthopoxvirus. **MVA-BN is grown in primary Chicken Embryo Fibroblast (CEF) cells suspended in a serum-free medium containing no material of direct animal origin, harvested from the CEF cells, purified and concentrated by several Tangential Flow Filtration (TFF) steps**

including benzonase digestion. Each 0.5 mL dose is formulated to contain 0.5 x 108 to 3.95 x 108 infectious units of MVA-BN live virus in 10 mM Tris (tromethamine), 140 mM sodium chloride at pH 7.7. **Each 0.5 mL dose may contain residual amounts of host-cell DNA (≤ 20 mcg), protein (≤ 500 mcg), benzonase (≤ 0.0025 mcg), gentamicin (≤ 0.163 mcg) and ciprofloxacin (≤ 0.005 mcg).”**

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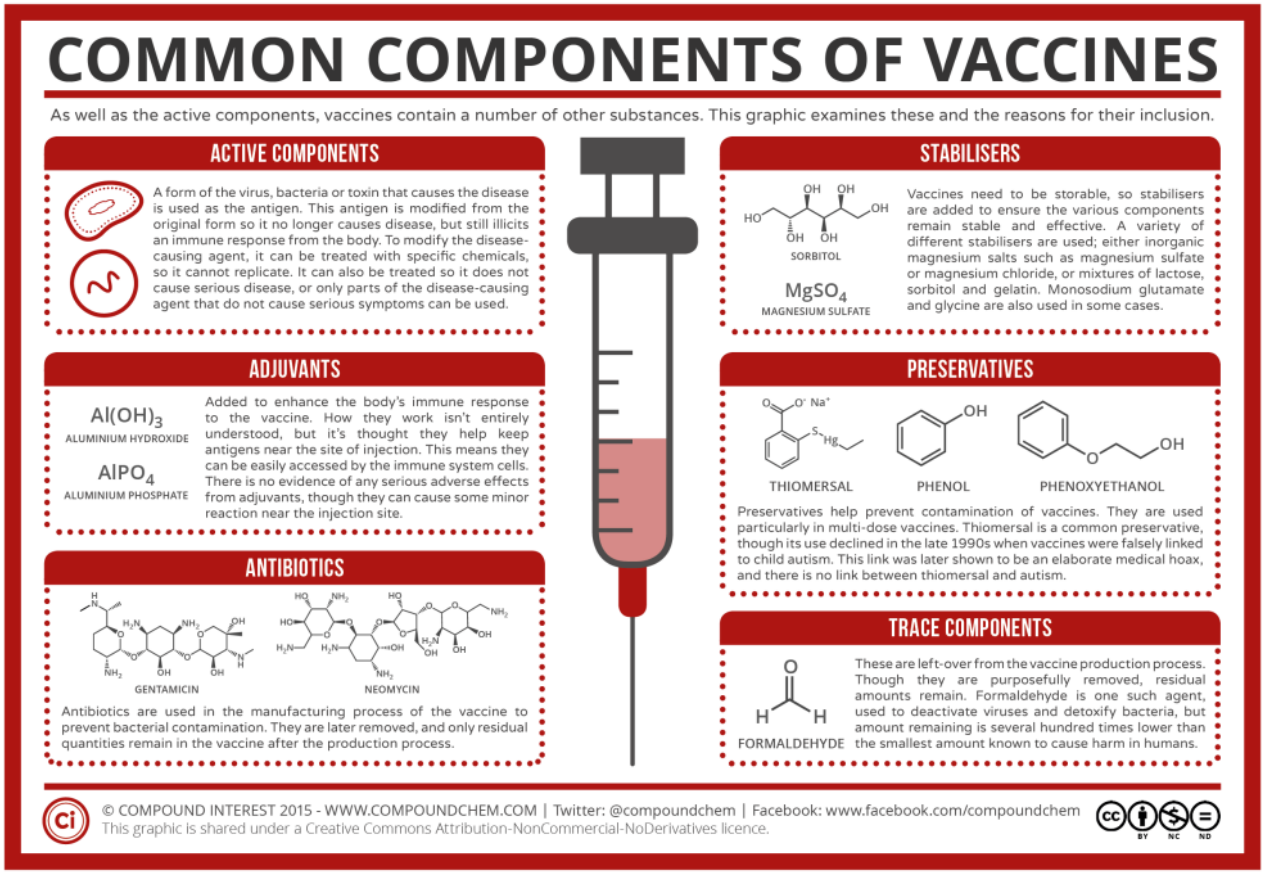
This is also seen in the Johnson & Johnson “Covid” vaccine which contains trace amounts of host DNA belonging to the PER.C6 TetR cell line derived from human embryonic retinal cells from an aborted fetus supposedly genetically modified with “adenovirus.” This cell line is known to cause tumors in mice:

“The Ad26 vector expressing the SARS-CoV-2 S protein is **grown in PER.C6 TetR cells,** in media containing amino acids and no animal-derived proteins. After propagation, the vaccine is processed through several purification steps, formulated with inactive ingredients and filled into vials.

Each 0.5 mL dose of Janssen COVID-19 Vaccine is formulated to contain 5×10^10 virus particles (VP) and the following inactive ingredients: citric acid monohydrate (0.14 mg), trisodium citrate dihydrate (2.02 mg), ethanol (2.04 mg), 2-hydroxypropyl-β-cyclodextrin (HBCD) (25.50 mg), polysorbate-80 (0.16 mg), sodium chloride (2.19 mg). **Each dose may also contain residual amounts of host cell proteins (≤0.15 mcg) and/or host cell DNA (≤3 ng).”**

Click to access Janssen+COVID-19+Vaccine-HCP-fact-sheet.pdf



Antibiotics and trace components remain in the “purified” vaccines.

Remember, purification means to free from contaminants, pollutants, foreign materials, etc. Do these vaccines sound like purified concoctions to you? If they can not separate out the cell culture additives as well as the benzonase substance used for “purification” as in the Jynneos vaccine, how can they purify a “virus” to study for cause and effect, let alone for use in a vaccine? If they can not separate the host cell DNA as noted in both vaccines, whatever cell line they use, from cancer cells to aborted fetuses to monkey kidneys, will remain within the vaccine and injected into the body. This is why it is imperative to be able to show that these assumed “virus” particles exist directly in the fluids of a sample from a sick human first before it is subjected to the cell culture process and mixed with all sorts of outside contaminants and foreign materials that are subsequently unable to be purified away from the assumed “viral” particles.

We can gain further insight into this inability to separate the “virus” from the culture materials in these highlights from a 270-page “virus” purification manual:

VIRUS PURIFICATION, DETECTION AND REMOVAL

“In vaccine manufacturing, the cell culture is commonly accompanied with cellular debris, unwanted media proteins, adventitious agents, residual DNA, nucleic acid and many process related leachable contaminants. As per the FDA vaccine approval requires the freedom from extraneous material whether or not harmful to the recipient [12]. Viruses have a unique size, shape and surface chemistry, i.e. hydrophobicity and charge. Most often a series of unit operations are required to increase the yield of virus particles. The currently used operations involve a combination of precipitation, centrifugation, filtration and chromatography [13, 14].”

“Vaccine production in therapeutic industry is currently performed on a case-by-case basis for each virus and **a single platform system to purify viruses is still lacking.** A traditional ATPS system was used for virus precipitation but **lack of selectivity for virus against protein contaminants and difficulty in separation from polymer phase** inhibited ATPS progress towards vaccine development systems.”

“Traditionally, virus is purified by ultracentrifugation on cesium chloride (CsCl) or sucrose gradients [10, 11]. However, the shear force has been known to reduce virus infectivity and moreover, it is time consuming, labor intensive and difficult to scale up [12]. Virus precipitation with modest results have been obtained using salts ammonium sulfate [13] or calcium phosphate [14]. **Polyethylene glycol (PEG)/aqueous two-phase system has also been studied for virus purification [15], but the technique lacks the ability to purify virus from many cell culture contaminants. Chromatography a popular technique for biomolecular purification has been seldom used for virus recovery due to diffusional limitations and large virus size, restricting access to internal surface area of beads.** The technique has not provided high virus yield but it is known to work well for protein biomolecules < 5 nm in size [16]. Chromatography has gained wide spread attention due to its ability to create specificity for a biomolecule based on several variables as charge, size and hydrophobicity [17-19]. **The virus produced in the lab is heavily contaminated with cell media proteins** and our goal is to consider each variable individually (charge, size and hydrophobic) for maximum protein removal with best possible recoverable virus. The purified virus can be very useful to analyze cell protein and contaminant interference during lab scale experimentation.”

“Purification of virus is usually performed from density gradient centrifugation or salt or polymer precipitation techniques. **The methods are difficult to scale up or lack specificity from co-precipitating impurities.** Filtration is another technique for virus purification but this may cause virus degradation from shear stress, especially in tangential flow filtration conditions. **An efficient and fast paced system to achieve high purity virus is required.”**

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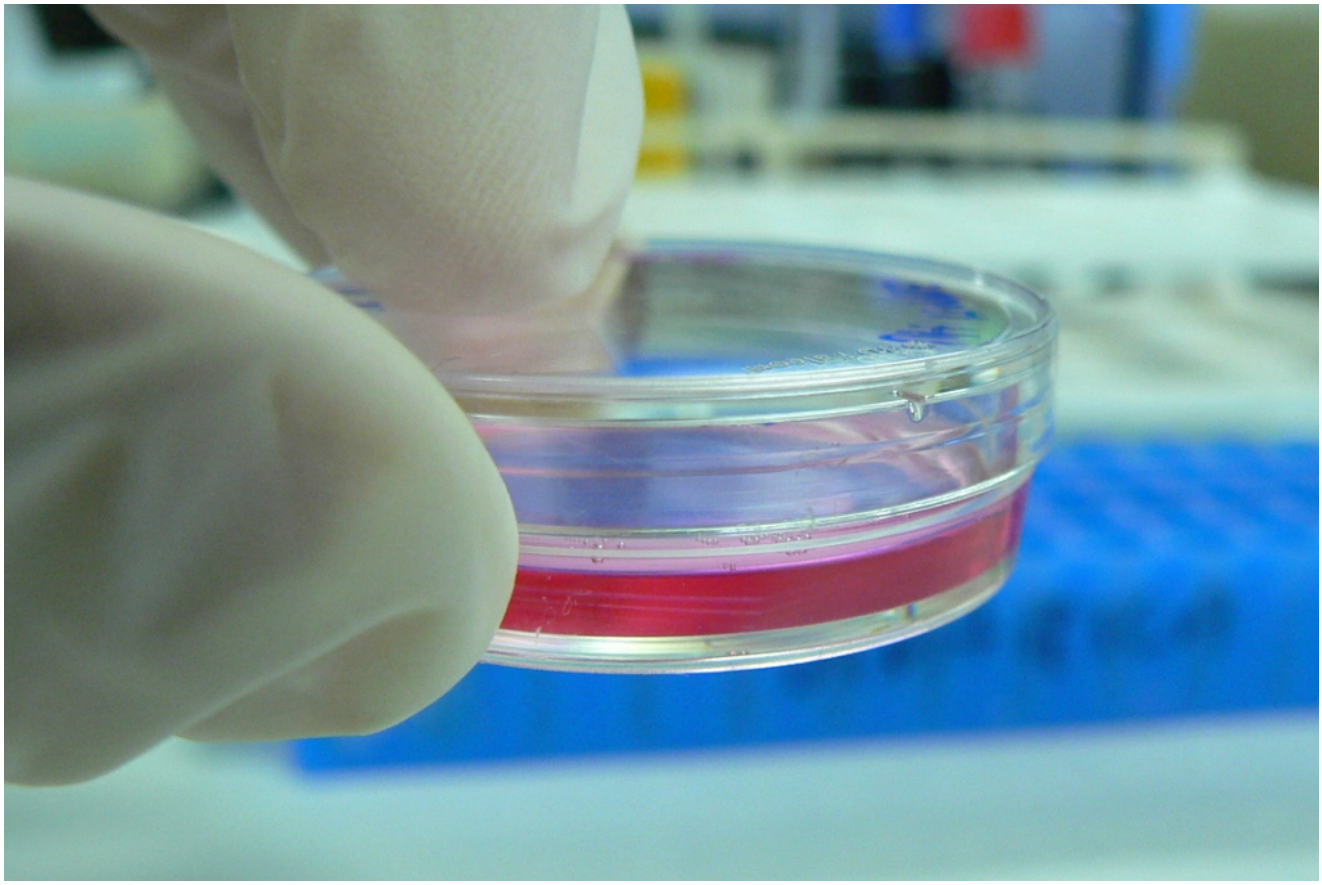


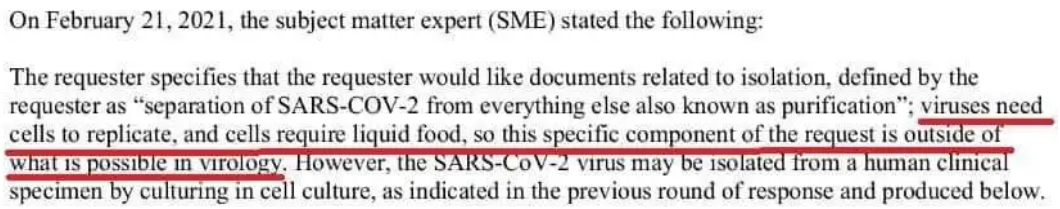
It should be clear from the above passages that it is an impossibility to purify “viruses” from the cell culture supernatant and the material used for propagation. This should be the final nail in the coffin for both cell culture and virology as it has been admitted numerous times that the only way virologists can “isolate” a “virus” is by means of cell culture.

From University of Auckland associate professor and microbiologist Siouxsie Wiles in an AAP “Fact” check:

“Viruses are basically inanimate objects which need a culture to activate in. **But the way they are phrasing the requests is that the sample must be completely unadulterated and not be grown in any culture – and you can’t do that,”** she told AAP FactCheck in a phone interview.

“You can’t isolate a virus without using a cell culture, so by using their definition it hasn’t been isolated. But it has been isolated and cultivated using a cell culture multiple times all around the world.”

From the CDC:



On February 21, 2021, the subject matter expert (SME) stated the following:

The requester specifies that the requester would like documents related to isolation, defined by the requester as "separation of SARS-COV-2 from everything else also known as purification"; viruses need cells to replicate, and cells require liquid food, so this specific component of the request is outside of what is possible in virology. However, the SARS-CoV-2 virus may be isolated from a human clinical specimen by culturing in cell culture, as indicated in the previous round of response and produced below.

https://www.fluoridefreepeel.ca/fois-reveal-that-health-science-institutions-around-the-world-have-no-record-of-sars-cov-2-isolation-purification/

For more on this impossibility of purifying the "virus" from the cell culture supernatant, let's look at the FDA's *Guidance for Industry* for vaccines which you will find highlights for below. The FDA states that live attenuated "viruses" can not be purified as rigorously as subunit vaccines (a vaccine that contains "purified" *parts of the pathogen* that are used to elicit an immune response) which leads to greater contamination. Due to this, it is not possible to validate the clearance of adventitious agents in the live "virus" vaccines which means that purification is impossible. For inactivated "virus" vaccines, there is the possibility that not all of the adventitious agents are inactivated as was seen in the case of the polio vaccine.

The FDA admits to numerous sources of contamination just from the cell culturing process itself such as:

1. Exposure to the person or animal from which the "virus" was "isolated"
2. The cells and raw materials (e.g., serum or trypsin) used
3. Materials used in banking and propagation of cells for "viral" seed growth
4. Other materials used during production and filling of the seed
5. Any infectious "viruses" (including those that infect nonhuman species)
6. Retroviruses that may either be endogeneous (coming from within the host) or exogenously acquired

The FDA's laundry list of sources of contamination establishes that culturing the sample immediately invalidates any claims of purity. Even when checking for contamination, the FDA allows for the use of "non-infected control" cultures to establish whether or not the culture is free of contamination rather than checking the actual "infected" culture to be used for the vaccine. While it should be obvious that a stand-in culture should not be used to judge whether or not a separate culture is free of contamination, the conditions used for the control culture only need to be *similar* to the "infected" culture rather than identical which immediately invalidates this process as a control.

It is also admitted by the FDA that cell lines such as the Vero cells used in culturing many "viruses" (including "SARS-COV-2") have biochemical, biological, and genetic characteristics that are different from primary cell lines which may be a result of transformation caused by an adventitious agent. There also may be potential risks from residual DNA within the vaccine as well. As the mechanism by which most cell lines become immortal is unknown, and due to the fact some cell lines cause tumors in inoculated mice, there are many concerns with using these cell lines in vaccines. This is yet another example which shows that complete purification of "viruses" coming from these cell lines is an impossibilty.

Finally, it is admitted by the FDA that the cytopathogenic effect, which is used to determine whether or not a "virus" is present in the culture, can in fact be caused by substances other than a "virus:" Obviously, if other factors can cause the same effect ascribed to a "virus," there is no need for the vitus" to enter into the equation as a possible explanation as the cause of the effect, especially when the particles assumed to be "viruses" are unable to be seen in a completely purified and isolated state without culturing first:

Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious DiseaseIndications

"Live attenuated viruses, whole inactivated virions, or virus-like particles **often cannot be purified** as rigorously as viral subunit vaccines; as a consequence, **the potential for contamination is greater than that of subunit vaccines.** Generation of live viral vaccines often involves cell disruption, **which may add cellular components to the vaccine bulk.** In addition, such vaccines often are **minimally purified** and are not subjected to inactivation steps. Comprehensive testing and qualification of the biological starting materials and lot-by-lot testing for adventitious agents are particularly important **because it is not possible for a manufacturer of live viral vaccines to validate the process for clearance of adventitious agents.**

In contrast, for more highly purified products where viral clearance can be demonstrated during downstream processing, you can place more reliance on process validation (Ref. 2). **For inactivated vaccines, the concern is that the process used to inactivate the vaccine virus may not inactivate all adventitious agents potentially present (as occurred with early inactivated poliovirus vaccines [Ref. 4]).** Therefore, you should validate your process for inactivation of adventitious agents using different model viruses (Ref. 2). The choice of tests and the stages at which the tests are applied will depend on your inactivation process. **The degree of viral clearance that is feasible may influence the sensitivity of the testing you should perform to demonstrate the absence of contaminants in your product."**

2. Potential Sources of Contamination

"It is important that you identify and examine all potential sources of contamination of your product. For example, a viral seed could be exposed to the following potential sources of contamination: the person or animal from which it was isolated; the cells and raw materials (e.g., serum or trypsin) used in its isolation and attenuation; materials used in banking and propagation of cells for viral seed growth; and other materials used during production and filling of the seed. You should also consider the species of origin of your cell substrates, viral seeds, and other biological starting materials in selecting your tests to ensure the absence of contaminants. **Furthermore, you should consider any infectious viruses (including those that infect nonhuman species) as potential contaminants if there is the possibility of contact with your product or cell substrate at any time during development or production. Retroviruses may be either endogeneous (i.e., encoded by the cell substrate genome) or exogenously acquired.** Retrovirus testing should address the possibility that either type of retrovirus could contaminate a product. Finally, you should consider the possibility of contamination from unusual sources, as exemplified by the reported presence of minute virus of mice (MVM) in some lots of recombinant proteins (Ref. 5). **The susceptibility of the cell substrate to infection by agents of potential concern can influence the tests needed to assure absence of contamination."**

4. Use of Control-Cell Cultures

"We recommend the use of control cells when it is not feasible to directly test cells or product at various stages of manufacture. For example, if you are using primary cell cultures to propagate your vaccine virus, **complete testing of the primary culture might not be feasible prior to inoculation of**

virus. In this situation, when possible, you should **produce and test uninfected control-cell cultures** that are derived in parallel with, and handled in the same manner as, the production culture."

"Control cell cultures should be propagated under conditions similar to conditions of manufacture for a time appropriate to the reason for performing the cultures. This time period may be 14 days or more, when needed to allow for detection of potential adventitious agents that may be latent, endogenous, or poorly replicating. **Control-cell cultures should be processed simultaneously with your production culture, but left uninfected.** Control cells should be evaluated for the presence of adventitious agents using the same tests that would have been performed on the production cultures, if it were feasible. Tests for adventitious agents should be performed on control cells at times at which you would perform similar tests on your manufactured product."

5. Special Considerations for Continuous Cell Lines

"Some continuous cell lines, **including Vero cells and CHO cells,** have been used as substrates for licensed biologicals. **Cell lines might have biochemical, biological, and genetic characteristics that differ from primary or diploid cells** (e.g., they are typically aneuploid and have accumulated genetic changes). **Because the mechanism by which most cell lines become immortal is generally unknown,** and because some cell lines form tumors when inoculated into immunodeficient rodents, **there are additional concerns for continuous cell lines compared with diploid cells, including the potential that transformation was caused by an adventitious agent and potential risks from residual DNA.**

Products prepared in cell lines (including viral vaccines) **should be purified to be free** (see Section V. Glossary) **of adventitious agents and residual cells and should have low levels of cell-substrate DNA.** When potential biological activity of residual cell substrate DNA is a concern, **you should introduce procedures that extensively remove or degrade DNA.** If you are considering the use of cell lines, **we encourage you to develop and evaluate efficient methods for the purification of your product as an essential element of any product development program."**

"Your biological starting materials should be characterized sufficiently **to ensure that they do not contaminate the final product with extraneous infectious organisms,** such as bacteria, fungi, cultivatable and non-cultivatable mycoplasmas and spiroplasma, mycobacteria, viruses, and the agent(s) responsible for transmissible spongiform encephalopathies (TSEs). For a substance to be considered free of a contaminant, your assay should demonstrate, at a predefined level of sensitivity, **that a certain quantity of the substance is free of that contaminant.** Alternatively, a validated process that is known to remove a contaminant to a defined level **may be used to demonstrate the absence of that contaminant."**

"An appropriate volume should be inoculated onto monolayer cultures of at least 3 cell types. The sample to be tested should be diluted as little as possible. **The cell cultures should be observed for at least 2 weeks. After 2 weeks of observation, the original cultures of supernatants or lysates from cell banks are subcultured onto fresh cells and observed for at least an additional 2 weeks. This subculture onto fresh cells might help to distinguish between non-specific CPE and virus-induced CPE, as toxic effects of the initial specimen or length in culture will be diluted, whereas virus-induced CPE should be amplified."**

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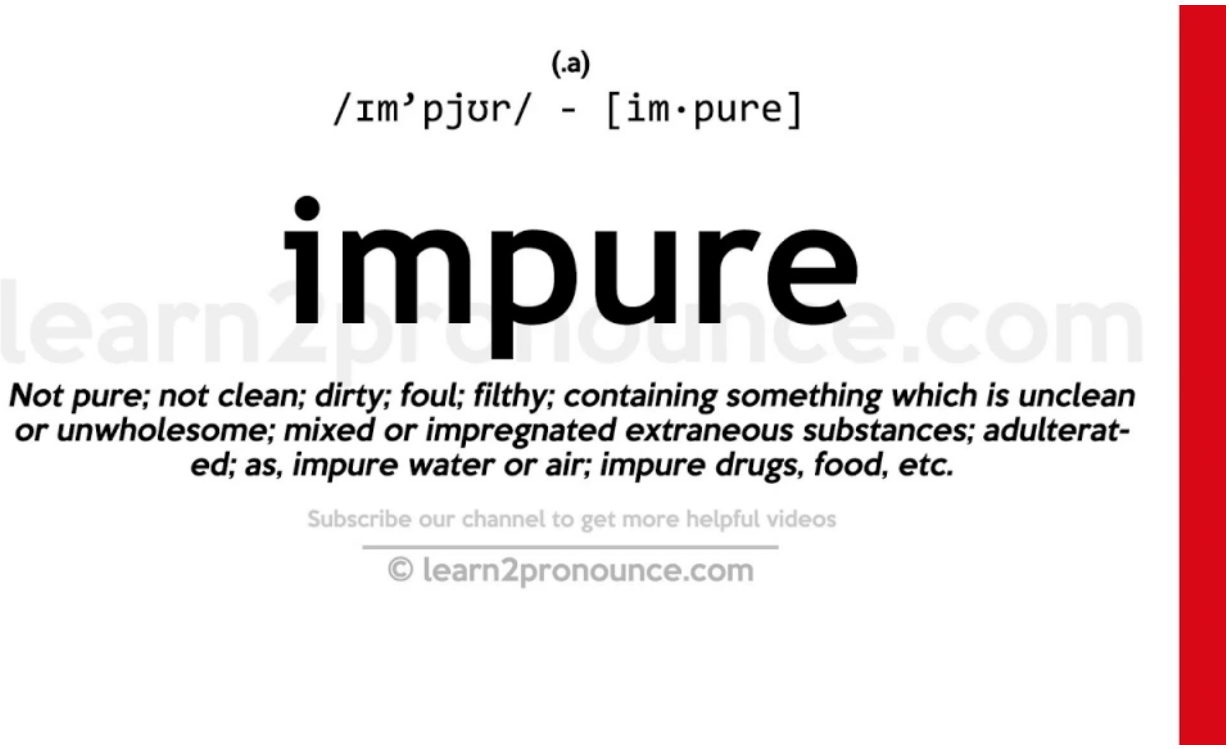
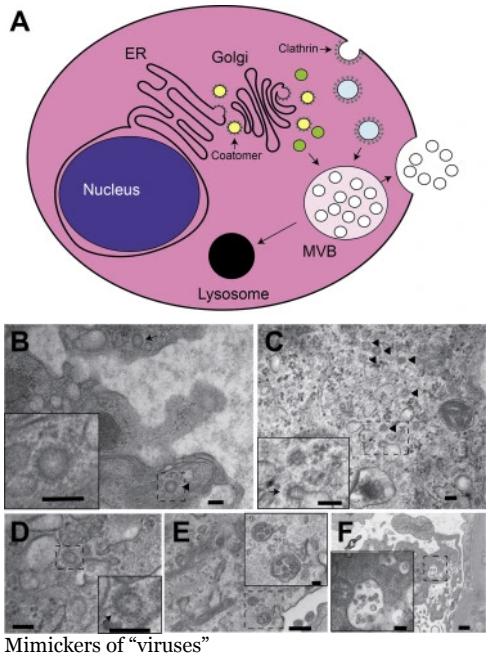


In Summary:

- Purification refers to the **separation of "virus" particles from host components** in a biologically active state
- Purified "virus" is **required** for the production of antibodies, **physical, biochemical and molecular characterization** of "virus" isolates
- Purification methods vary with different "viruses," **and there are no universal methods of "virus" purification**
- Procedures that are effective for one "virus" **may not work with the other**
- As the most common purification method, ultracentrifugation **is not ideally suited for the separation of exosomes (i.e. non-infectious**

"viruses") and "viruses," particularly from infected cultures and/or body fluids
- The **techniques used** by researchers for purification of exosomes and "virions" **are similar**
- **None of the methods,** including ultracentrifugation, precipitation, and chromatography, **can separate exosomes from "viruses"**
- Common exosomal separation techniques suffer from operation complexity, time consumption, large sample volumes **and low purity**
- At present, common exosome isolation technologies, such as ultrafiltration, immunoaffinity, ultracentrifugation ("gold standard" for the isolation of exosomes) are expensive instruments, large volumes of sample, **possible protein contamination,** complete isolation steps, **but they result in low isolation efficiency, sample loss, low exosome recovery and purity**
- Like "viruses," exosomes are small in size (30–150 nm), low in density (1.13–1.19 g/ml), **and mixed with similar components (e.g., cell fragments, proteins) in the body fluids, which pose tremendous challenges for their separation**
- Exosomes are extracellular vesicles that **are believed to play a role** in communication between cells by transporting materials inside the vesicle
- The challenge in isolating vesicles is **differentiating them from other types of membrane material in the cell culture supernatant**
- This is done through **successive steps of centrifugation** at increasing speeds
- However, that type of preparation is more an enrichment of the sample **than a purification**
- In addition to exosomes, the extracellular milieu **contains extracellular RNA, other types of vesicles, protein complexes, and lipoproteins**
- These are **not fully separated** from exosomes through the centrifugation protocol
- The Executive Committee of the International Society for Extracellular Vesicles (ISEV) **proposed criteria for characterizing exosomes** to aid in consisting reporting of experimental results and within these criteria, they state:
    1. *Be isolated from extracellular fluids like cell culture medium or body fluids*. The collection should be gentle to minimize cell wall disruption **that could contaminate the sample with intracellular compartments**
    2. *Have levels of proteins not expected to be enriched* **to determine the extent of co-isolation of intracellular vesicles**
- *In other words, it is well-known that other intracellular components will be "isolated" along with the exosomes*
- Study of exosomes is a rich area of research due to the **large number of unanswered questions,** including:
    1. Exactly how they are formed
    2. What their functions are
    3. How they communicate with acceptor cells
    4. Their role in various diseases
- Consistent methods for isolating and characterizing exosomes **and distinguishing them from other types of extracellular and intracellular vesicles** are needed to enable these advances
- In vaccine manufacturing, the cell culture is **commonly accompanied with:**
    1. Cellular debris
    2. Unwanted media proteins
    3. Adventitious agents
    4. Residual DNA
    5. Nucleic acid
    6. Many process related leachable contaminants
- Vaccine production in therapeutic industry is currently performed on a case-by-case basis for each "virus" and **a single platform system to purify "viruses" is still lacking**
- A traditional ATPS system was used for "virus" precipitation but **lack of selectivity for "virus" against protein contaminants and difficulty in separation from polymer phase** inhibited ATPS progress towards vaccine development systems
- Polyethylene glycol (PEG)/aqueous two-phase system has also been studied for "virus" purification, but the technique **lacks the ability to purify "virus" from many cell culture contaminants**
- Chromatography a popular technique for biomolecular purification has been seldom used for "virus" recovery **due to diffusional limitations and large "virus" size,** restricting access to internal surface area of beads
- The "virus" produced in the lab **is heavily contaminated with cell media proteins**
- Purification of "virus" is **usually performed from density gradient centrifugation** or salt or polymer precipitation techniques
- The methods are difficult to scale up or **lack specificity from co-precipitating impurities**
- An efficient and fast paced system **to achieve high purity "virus" is required**
- According to University of Auckland associate professor and microbiologist Siouxsie Wiles, "Viruses are basically inanimate objects which need a culture to activate in. **But the way they are phrasing the requests is that the sample must be completely unadulterated and not be grown in any culture – and you can't do that**. **You can't isolate a virus without using a cell culture, so by using their definition it hasn't been isolated.** But it has been isolated and cultivated using a cell culture multiple times all around the world."
- According to the CDC, purifying "viruses" **without cell culturing** is outside of what is possible in virology
- *In other words, it is impossible to purify "viruses" without culturing and it is also impossible to purify and isolate them with culturing*
- From the FDA's *Guidance for Industry,* we learn that live attenuated "viruses," whole inactivated "virions," or "virus-like" particles **often cannot be purified as rigorously** as "viral" subunit vaccines; as a consequence, **the potential for contamination is greater than that of subunit vaccines**
- Generation of live "viral" vaccines often involves cell disruption, **which may add cellular components to the vaccine bulk**
- In addition, such vaccines often are **minimally purified** and are not subjected to inactivation steps
- Comprehensive testing and qualification of the biological starting materials and lot-by-lot testing for adventitious agents are particularly important because **it is not possible for a manufacturer of live "viral" vaccines to validate the process for clearance of adventitious agents**
- For inactivated vaccines, the concern is that the process used to inactivate the vaccine "virus" **may not inactivate all adventitious agents potentially present** (as occurred with early inactivated poliovirus vaccines)
- The **degree of "viral" clearance that is feasible may influence the sensitivity of the testing** that should be performed to demonstrate the absence of contaminants in the product
- It is important to identify and examine **all potential sources of contamination** of the product such as:
    1. The person or animal from which it was isolated
    2. Tthe cells and raw materials (e.g., serum or trypsin) used in its isolation and attenuation
    3. Materials used in banking and propagation of cells for "viral" seed growth
    4. Other materials used during production and filling of the seed
- Furthermore, any infectious "viruses" (including those that infect nonhuman species) **should be considered as potential contaminants** if there is the possibility of contact with the product or cell substrate at any time during development or production
- "Retroviruses" **may be either endogeneous** *(from the host genome)* or exogenously acquired
- The susceptibility of the cell substrate to infection by agents of potential concern **can influence the tests needed to assure absence of contamination**
- If using primary cell cultures to propagate vaccine "virus," **complete testing of the primary culture might not be feasible prior to inoculation of "virus"**
- In this situation, when possible, they are to produce and test **uninfected control-cell cultures that are derived in parallel with, and handled in the same manner as, the production culture**
- *In other words, if they decide that they can not check the uninfected culture used for the vaccine for contamination, using another uninfected culture as a stand-in suffices*

- Control cell cultures should be propagated under conditions **similar** *(i.e. not the same)* to conditions of manufacture for a time appropriate to the reason for performing the cultures
- Control-cell cultures should be processed simultaneously with the production culture, **but left uninfected**
- Cell lines, such as Vero and CHO, **might have biochemical, biological, and genetic characteristics that differ from primary or diploid cells** (e.g., they are typically aneuploid and have accumulated genetic changes)
- Because the mechanism by which most cell lines become immortal **is generally unknown,** and because some cell lines form tumors when inoculated into immunodeficient rodents, **there are additional concerns for continuous cell lines** compared with diploid cells, **including the potential that transformation was caused by an adventitious agent and potential risks from residual DNA**
- Products prepared in cell lines (including "viral" vaccines) **should be purified to be free of adventitious agents and residual cells and should have low levels of cell-substrate DNA**
- When potential biological activity of residual cell substrate DNA is a concern, **procedures that extensively remove or degrade DNA should be introduced**
- If considering the use of cell lines, **it is *encouraged*** *(not required)* **to develop and evaluate efficient methods for the purification of the product as an essential element of any product development program**
- Biological starting materials should be characterized sufficiently **to ensure that they do not contaminate the final product with extraneous infectious organisms** such as:
    1. Bacteria
    2. Fungi
    3. Cultivatable and non-cultivatable mycoplasmas and spiroplasma
    4. Mycobacteria
    5. "Viruses"
    6. Agent(s) responsible for transmissible spongiform encephalopathies (TSEs)
- For a substance to be considered free of a contaminant, the assay should demonstrate, at a predefined level of sensitivity, **that a certain quantity of the substance** is free of that contaminant *(i.e. not all of it has to be shown free of contamination)*
- Alternatively, a validated process that is known to remove a contaminant to a defined level **may be used to demonstrate** *(i.e. to infer)* **the absence of that contaminant**
- The cell cultures should be observed for at least 2 weeks and after 2 weeks of observation, the original cultures of supernatants or lysates from cell banks **are subcultured onto fresh cells** and observed for at least an additional 2 weeks
- This subculture onto fresh cells **might help to distinguish between non-specific CPE and "virus-induced" CPE,** as toxic effects of the initial specimen or length in culture will be diluted, whereas "virus-induced" CPE should be amplified
- *In other words, it is admitted that the CPE used to identify "viruses" can be caused by factors other than "viruses" thus destroying this effect as an indirect indicator of the presence of any "virus"*



Mimickers of "viruses"
https://ajp.amjpathol.org/article/S0002-9440%2820%2930503-4/fulltext

Obviously the **main objective** in the purification and isolation of viruses is **the separation of the virus from the host tissues and cell organelles."**

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It should be clear by now that virology can not completely purify and therefore isolate the particles assumed to be "viruses" from everything else that is sure to be within the sample. Virologists openly admit that they:

1. Can not purify directly from the fluids of humans
2. Can not purify the particles from cell culture supernatant
3. Can not separate "viruses" from exosomes and other similar MVB's
4. Can not separate the "viruses" from the cell culture host material and DNA
5. Can not separate the cell culture media from the sample

As purification can not be achieved, virology lacks the physical particles necessary for use as an independent variable to vary and manipulate in order to determine cause and effect. There is no direct evidence as to the existence of the particles assumed to be "virus" ever being found within humans. The only evidence is from indirect cell culture experimentation citing patterns of cell death known as the cytopathogenic effect (CPE) seen in the cells after being poisoned and starved for days which is then blamed on an invisible "virus." As other contaminants, chemicals, and impurities remain within the cultured sample, there can be no certainty that the other substances within may be the potential cause of the CPE seen within the culture over the assumed "virus." It

is a fact that other substances will be there within the fluids, especially in any sample processed through cell culture, as can be seen from the exosome studies as well as from attempts to purify “viruses” from cell culture supernatant for vaccine use. The FDA admitted that there are other factors besides a “virus” which can cause the CPE seen which is instead blamed on the invisible “virus.” The cell culture method is by definition an impure process as many foreign elements, chemicals, and contaminants are added to the culture which can not be separated out. As virology admits that “viruses” can not be purified and isolated directly from the fluids of a sick human and must be cultured, there is no purification that can ever be achieved by subjection to an impure process.

Without purification, virology can not adhere to the scientific method and is thus a pseudoscience. We know this and virologists know this which is why purification methods are rarely performed and why this criteria is rarely discussed in virology papers. Purification is the black sheep of the virology family and it will remain as such until virology can find a way to do what it has been unable to do for well over 100 years.