

Recommendations for the Use of Vaccines Manufactured with Bovine-Derived Materials

Bovine-derived materials have traditionally been used in the manufacture of many biological products, including vaccines. Bovine spongiform encephalopathy (BSE), so-called "mad-cow disease," was first recognized in the United Kingdom (UK) in the 1980s⁽¹⁾. The Center for Biologics Evaluation and Research (CBER) of the U.S. Food and Drug Administration (FDA) has been concerned about eliminating any potential for contamination of biological products with the BSE agent. This concern was heightened by the appearance of the human transmissible spongiform encephalopathy known as variant Creutzfeldt-Jakob Disease (vCJD, also referred to as new-variant CJD) in the UK in 1996; vCJD has been attributed, among other possibilities, to eating beef products from cattle infected with the agent of BSE ⁽²⁾. To date, there are no reports of BSE contamination of pharmaceutical or biological products. To minimize the possibility of contamination in such products, the FDA, in 1993 (published in the Federal Register on August 29, 1994, 59 FR 44591), and again in 1996, recommended that manufacturers not use materials derived from cattle that were born, raised, or slaughtered in countries where BSE is known to exist; the FDA referred manufacturers to the listing of such countries that is maintained by the U.S. Department of Agriculture (USDA)⁽³⁾.

In 1991 the USDA list included only countries and other regions in which BSE was known to exist, such as France, Great Britain, Northern Ireland, the Republic of Ireland, Oman, and Switzerland. In 1998, the USDA expanded the list to include countries and other regions in which BSE had not been documented but in which import requirements were less restrictive than requirements that would be acceptable for import into the United States or in which surveillance was inadequate. Thus, all European countries, even those that have had no reported BSE cases, are currently on the USDA list, which is published in the Code of Federal Regulations, title 9, part 94 (9 C.F.R. part 94).

In 2000, CBER learned that its recommendations regarding the sourcing of bovine materials for the manufacture of vaccines had not been followed in at least one instance. As a result of this finding, CBER requested all vaccine manufacturers to review the source for all bovine-derived materials used in the manufacture of their vaccines. This review identified additional vaccines manufactured with bovine-derived materials that had been obtained from European countries on the USDA list.

No evidence exists that any case of vCJD has resulted from the administration of a vaccine product⁽⁴⁾, and no cases of vCJD have been reported in the United States. To evaluate the risk of disease that might result from a vaccine manufactured with a process that utilizes bovine materials potentially contaminated with the BSE agent, CBER conducted risk assessments and convened a special joint meeting of the Transmissible Spongiform Encephalopathy Advisory Committee and the Vaccines and Related Biological Products Advisory Committee on July 27, 2000. In assessing the potential risk of vaccines, CBER and the joint Committees considered: (1) the likelihood that any cattle that were used might be infected (i.e., the time period and country of origin) and animal husbandry procedures; (2) the amount of bovine material that might be present in the final vaccine; and (3) the inherent infectivity of the various types of bovine materials that were used. The joint Committees concluded that the risk of vCJD posed by vaccines in the scenarios that were presented was theoretical and remote. They also noted that the benefits of vaccination far outweigh any remote risks of vCJD. The joint Committees made several recommendations.

- Bovine-derived materials used in the routine production of vaccines that are sourced from countries on the USDA list should be replaced with bovine-derived materials from countries not on the USDA list.
- Working bacterial and viral seed banks and working cell banks that were established using bovine-derived materials sourced from countries on the USDA list should be re-derived with bovine-derived materials from countries not on the USDA list. However, master bacterial and viral seed banks established in a similar manner do not need to be re-derived; the potential risk presented by the master seed banks is even more remote than that presented by the working seed banks and is outweighed by the risk of altering the bacterial or viral vaccine through re-derivation.
- These issues are of public interest and, therefore, the public should be informed about the safety of vaccines that used materials sourced from countries on the USDA list, and the assessment of the nature of any risk of vCJD from such vaccines.

As noted above, there is no evidence that any case of vCJD has been caused by or is related to vaccines manufactured with bovine-derived materials obtained from countries in which BSE or a significant risk of BSE exists (i.e., countries on the USDA list), and thus the risk of vCJD is theoretical. The joint Committees recommendation to replace such bovine-derived materials with bovine-derived materials from countries not on the USDA list is a precautionary measure intended to minimize even the remote risk of vCJD from vaccines.

The vaccines that use bovine-derived materials from countries on the USDA list include: Aventis Pasteur, S.A.'s *Haemophilus influenzae* type b conjugate vaccine, ActHIB[®] (ActHIB[®] is also marketed as OmniHIB[™] by SmithKline Beecham Pharmaceuticals); BioPort's anthrax vaccine; North American Vaccine Inc.'s diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine, Certiva[™] (the tetanus toxoid manufactured by Statens Seruminstitut for use in Certiva[™] is the only component of the vaccine manufactured with bovine-derived materials from a country on the USDA list); SmithKline Beecham Biological's DTaP vaccine, Infanrix[®] (the diphtheria toxoid manufactured by Chiron Behring GmbH & Co. for use in Infanrix[®] is the only component of the vaccine manufactured with bovine-derived materials from a country on the USDA list), hepatitis A vaccine, Havrix[®], and the hepatitis A inactivated and hepatitis B (recombinant) vaccine, TWINRIX[®].

In some other cases, the source of the bovine-derived materials is unknown, in part because manufacturers have not always maintained or had access to records of the source of such materials, particularly in the 1980s and early 1990s, before the connection between BSE and vCJD was first suggested. Vaccines that use bovine-derived material of unknown origin obtained in 1980 or thereafter (the current best estimate is that BSE first emerged in 1980) include: Aventis Pasteur, S.A.'s inactivated polio vaccine, IPOL[®] and Lederle Laboratories' pneumococcal polysaccharide vaccine, PNU-IMUNE[®] 23.

Vaccines using bovine-derived materials from a country on the USDA list or from an unknown source to manufacture only the master seed are not listed above; the joint Advisory Committees indicated that master seeds need not be re-derived. Additional information on such vaccines can be obtained upon request.

The FDA has requested that manufacturers of vaccines using bovine-derived materials obtained from countries on the USDA list or from an unknown source replace these materials with materials from countries not on the USDA list, consistent with the recommendations of the joint Advisory Committees. The manufacturers have agreed to fully implement these changes. Indeed, several manufacturers initiated a number of these changes before the July 27, 2000, joint Advisory Committee meeting. FDA anticipates that the majority of these changes will be completed within one year. The FDA will revise the list of vaccines using bovine-derived materials from countries on the USDA list or from an unknown source as the requested changes are implemented and the vaccines come to market (see [section VIII](#) for the current listing).

The Public Health Service (PHS) recommends that all children and adults continue to be immunized according to current immunization schedules⁽⁵⁾. At the present time, the PHS has no preference for using one licensed vaccine product over another based on the source of bovine-derived materials used in vaccine production. The recommendations of the FDA Advisory Committees and the actions of the FDA are, as described, precautionary and have been taken to reduce even the remote potential of a risk of vCJD and to maintain public confidence in the safety of vaccines. Failure to obtain the recommended vaccinations with licensed vaccines poses a real risk of serious disease.

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Bovine Spongiform Encephalopathy (BSE)

Estimating Risks for vCJD in Vaccines Using Bovine-Derived Materials

The risk of vCJD from bovine-derived materials

The risk of developing an illness such as vCJD from the use of bovine-derived material in the manufacture of vaccines is a function of a number of factors, including the nature and the amount of the bovine tissue that is used in manufacture, as well as the date and country of origin of the cows ⁽¹⁾. Other factors, such as how the cows were fed, are also important. In this regard, the CDC estimates that the risk, if any, for vCJD from eating a beef meal in Europe is less than approximately 1 in 10 billion [<http://www.cdc.gov/travel/madcow.htm>].

CBER's survey of vaccine manufacturers revealed a number of vaccines that utilized bovine materials that were obtained from countries where BSE or a significant risk for BSE exists. An estimate of the risk that the use of these materials might pose is presented in the following sections. Two examples have been chosen for presentation here, namely, the risk from the use of fetal calf serum sourced from the United Kingdom (UK) in the derivation of a viral working seed that is subsequently used in vaccine manufacture and the use of European-sourced (excluding the UK) beef broth in the production of a bacterial toxoid. Based on CBER's survey of the use of bovine-derived materials sourced from countries on the USDA BSE-list, the potential risk that would be associated with other uses of bovine-derived materials in vaccine production would be less than might be associated with these two situations.

The infectivity of most bovine-derived materials has not been determined experimentally. More is known about the infectivity of various ovine-derived (from sheep) materials. The knowledge of the infectivities of different ovine tissues relative to each other can be used to estimate the relative infectivities of bovine tissues. For example, if we know that, on a gram for gram basis, sheep brain is 100 million times more infective than sheep muscle, we can assume that bovine brain is also 100 million times more infective than bovine muscle. Thus, if the infectivity of bovine brain has been measured, and contains 10 million infective doses per gram, then we can estimate that bovine muscle is 100 million times less infective and contains 0.1 infective dose per gram.

Not surprisingly, since BSE and scrapie (the corresponding disease in sheep) are neural diseases, the greatest infectivity is found in neural tissue. Based on experimental studies, infected bovine brain contains approximately ten million infectious units per gram when administered to other cattle ^(2,3). In other tissues, such as serum or skeletal muscle, no infectivity has been detected. This does not mean that there is no infectivity associated with these materials; only that, if they are infectious, then the infectivity is at a level that is too low to be measured by current tests.

Table I presents the estimated infectivity of different bovine-derived tissues as determined by The Agency for the Evaluation of Medicinal Products (EMA) The actual infectivity of skeletal muscle or serum, for example, may be well below the values shown; we will, nevertheless, use these values in our risk estimates. It should be noted that these values are based on experiments in which animals were infected intra-cerebral injection with affected tissue; this is the most effective means of infecting experimental animals. When another route of administration, namely intramuscular injection, is used, infection rates are estimated to be approximately 200 fold lower ⁽⁴⁾.

The risk assessments follow.

Fetal calf serum used to derive viral seed and cell banks

Fetal calf serum from the United Kingdom was used in the production of certain viral seeds and cell banks. The calf serum that was used was produced in the mid-1980s, when the BSE epidemic was just getting underway in the UK ⁽⁵⁾. The U.S. Department of Agriculture estimated the incidence of BSE in adult cattle at about 1 in 200 at that time⁽⁶⁾. [Although many fewer cattle were observed to suffer from mad cow disease at that time, the long incubation time for the disease means that more cattle were infected than appeared diseased.] Since fetal calf serum was used in the production of the cell and viral seed banks, it is necessary to address the question of maternal-fetal transmission. Whether there is mother to fetus transmission of BSE is still unknown. One study may be interpreted as indicating that maternal-fetal transmission occurs at a rate of approximately 10%; i.e., that the calves of one of ten infected mothers may become infected with the BSE agent ⁽⁷⁾. However, other data indicate that maternal-fetal transmission does not occur or, if it does occur, it is below this 10% rate ⁽⁸⁾. As noted above, the U.S. Department of Agriculture estimates that, during the mid 1980s, approximately 1 in 200 cows in the United Kingdom was infected with BSE. Assuming that the rate of transmission from mother to fetus is 10% we would then estimate that 1 in 2000 fetal calves would have been infected.

When fetal calf serum is manufactured, the sera from approximately 1500 calves are pooled together. If 1 in 2000 calves is infected, it is likely that any given serum pool is infected. As mentioned above, although no infectivity has been observed with serum, there are limits to detectability. These experiments only rule out an infectivity that is greater than 1 infectious unit per milliliter (mL) of blood ^(3,9,10). Although serum is listed as category IV, we are using the highest estimate consistent with infectivity experiments. In the following risk estimate, we assume that the serum of an infected fetal calf can contain up to 1 infectious unit per mL.

In our risk calculation, we assume that the number of infectious BSE units that enters the vaccine production process is equal to the number of infectious units that remain in the vaccine at the end; that is, that the risk for vCJD is the input number of infectious units divided by the number of doses of vaccine that is in the batch. Thus, the risk estimate does not account for any purification step that might be present in the viral vaccine manufacturing process; although there are steps that probably remove infectivity, these are not considered in our risk estimate since none of the manufacturing steps have been demonstrated to remove BSE infectivity. We have also assumed that the BSE agent does not replicate during the manufacturing process; this is a reasonable assumption, bolstered by the many failed attempts to propagate the BSE agent in cell culture ⁽¹¹⁾. The BSE infectivities that are estimated in Table I are derived from data using direct intra-cerebral inoculation (direct injection of the material into the brain). Vaccines are given intramuscularly, a less efficient route of transmitting the disease. In our risk estimate, we have allowed a factor of 200 for reduced transmission by the intramuscular route.

In general, there is a species barrier for the transmissible spongiform encephalopathies; that is, it is easier to infect the same species of animal than another species (for example, bovine material is more infectious for cows than it is for other animals, such as mice) ^(3,4). The species barrier from cows to humans is not known; in our calculations, we will therefore assume that there is none.

Given these assumptions, we can estimate the risk for vCJD from fetal calf serum (FCS) being used to prepare a viral working seed as the product of four separate risk factors. The level of BSE agent in the serum of an infected calf is estimated at 1 infectious unit per mL. Approximately 1 infected calf is present in each pool, deriving from approximately 1500 calves, of fetal calf serum. The infectivity of the pooled FCS is thus diluted to 1/1500 infectious units per mL (ca. 6.7×10^{-4} infectious units/mL). The amount of FCS that was used to produce a vial of a working viral seed is approximately 4 mL, and the number of doses of vaccine coming from that batch is approximately 500,000. The risk for acquiring vCJD is therefore:

The number of infected calves in each pool	1/1500
Multiplied by	
The number of infectious units per mL of serum	1
Multiplied by	
The number of mLs of serum used	4

Divided by	
The number of doses of vaccine	500,000
Divided by	
The reduction in infectivity related to the route of	200

This yields a final risk estimate for vCJD of approximately 2.5 per 100 billion or 1 in 40 billion doses of vaccine $[(1/1500) \times 1 \times 4 \times (1/500,000) \times (1/200)]$. This level of risk would correspond to one case of vCJD arising every 5,000 years (assuming two doses per child) when vaccinating the entire birth cohort of the United States (four million children). Because of the assumptions that were used, this is an overestimate of the risk, and the true risk is likely to be significantly less. The risk that would be calculated for the use of a master seed that was prepared with fetal calf serum is again considerably less, due to an additional dilution that attends the preparation of the working seed from the master seed.

Beef broth used to manufacture a bacterial vaccine: a bacterial toxoid as an example

The potential risk of vCJD from a bacterial vaccine that used bovine-derived material in the nutrient broth to grow the bacterial strain during vaccine production is as follows. In the example that we are using, tissue derived from a single cow is used to prepare the fermentation broth. For this estimate, the incidence of BSE in European cows is taken to be 1 in 10,000. This value was derived by multiplying the average BSE rate in this region over the last five years by a factor of ten ⁽¹⁾ to account for any uncertainty in the actual rates. The nutrient medium that is used to grow the bacteria for the vaccine contains approximately 750 grams of skeletal muscle (a Category IV material) and 200 grams of a pancreatic extract (a Category III material); see [Table I](#). Because the broth is autoclaved (heated at high temperature), some of its potential infectivity is lost; a reduction factor of 20 is assigned to the autoclaving process⁽²⁾.

The risk, per dose of vaccine, for vCJD from a vaccine using a beef/pancreatic extract can be calculated as the product of the risk of using an infected cow (1 in 10,000) times the inherent risk of the bovine material after correction for the autoclaving process (approximately 1000 units; [200 grams of Category III material is estimated to contain no more than 20,000 infectious units and the 750 grams of Category IV material no more than 75 infectious units (20,075 units total); the autoclaving process reduces this infectivity to approximately 1000 units]), divided by the number of doses that are in a batch of vaccine (approximately 1 million), corrected for the route of administration (a reduction factor of 200).

Risk of an infected cow	1/10,000
Multiplied by	
Amount of infectious material	1000
Divided by	
The number of vaccine doses	1,000,000
Divided by	
The reduction in infectivity related to the route of	200

This yields a risk estimate for vCJD of 1 case in 2 billion doses of vaccine $[(1/10,000) \times 1,000 \times (1/1,000,000) \times (1/200)]$. A second scenario can also be considered, namely one in which a small amount of neural tissue inadvertently might contaminate the beef broth. We consider a 0.01% contamination with neural tissue. This would increase the amount of infectious material from 1000 units to 50,000 units, raising the total risk to 1 in 40 million. Because of the overestimates that were used in the risk calculation, the true risk is likely to be significantly less.

Potential sources of error

In estimating the risk of BSE contamination, it is important to note that each risk factor carries its own uncertainty. The overall risk, which is the product of these factors, compounds these uncertainties. For example, we have assumed no species barrier and no purification effect. The actual risk could be 10 to 1,000 fold lower, but probably no greater. On the other hand, we have assumed a 200-fold reduction due to an intramuscular route of administration. In fact, this risk could be 10-fold greater or 10-fold lower. Finally, in the case of viral vaccines, and based on experiments with analogous cell lines, we have assumed that BSE cannot replicate in cell cultures that were used. These uncertainties must be considered in order to

interpret the risk of BSE in viral vaccines. These calculations are not a formal risk assessment, but an attempt to estimate risk based on information currently available.

It should be noted that for both the viral and bacterial vaccine examples used, the exposure to this risk is temporary. Manufacturing changes have already been implemented which eliminate exposure during vaccine manufacture to bovine materials from countries at risk of BSE contamination. Vaccines made by these procedures are expected to be available in 2001.

Table 1

Estimated infectivity of bovine tissue by category

Category	Tissue	ID ₅₀ /gram*
I	Nervous tissue	10 ⁷
II	Spleen, lymph nodes, colon	<2.5 x 10 ⁴
III	Pancreas, liver, lung	<100
IV	Muscle, bone, heart	<0.1

Adapted from: Bader et. al, 1998 BioPharm *ID₅₀/gram = number of infectious units per gram of

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[Table of Contents](#)



Bovine Spongiform Encephalopathy (BSE)

Overview of Vaccine Manufacturing

The common childhood vaccines (www.cdc.gov/nip/recs/child-schedule.pdf) and many other vaccines, derive from bacteria, yeast, or viruses that are grown in culture. Bacteria are free-living organisms that are able to grow and reproduce when placed in a medium containing various nutrients (the culture medium) such as sugars, salts, or amino acids. Growing bacteria in culture simply refers to placing a small quantity of bacterial cells into a nutrient medium, and then allowing the cells to multiply.

Viruses, on the other hand, are not free-living organisms, and can reproduce (multiply) only in cells (they require many of the cellular enzymes and machinery for reproduction). Human- and animal-derived cell cultures are commonly used for growing vaccine viruses. The cells that are used as substrates for viral growth can, like bacterial cells, maintain themselves and grow only in a medium that contains their needed nutrients. Growing viruses in culture, therefore, involves adding a small quantity of virus to an existing culture of cells and letting the virus replicate in these cells. Commonly, the virus will kill the cell in which it is grown.

In order to manufacture vaccines consistently, it is essential to begin the manufacturing process (the growth of the virus or bacteria) with the exact same virus or bacterium; moreover, it is essential to start the process with a virus or bacterium that is pure. This is accomplished through the development and maintenance of "seed banks." A seed is the general term that is used to describe the small amount of bacteria or virus that is added to growth media or cell cultures to initiate further expansion of the bacterial cells or viruses.

Consider, for example, the manufacture of a bacterial vaccine; see [Figure 1](#). During the period when a new bacterial vaccine is being developed, a small amount of a pure culture of that vaccine bacterium is grown and frozen away; this material is termed the "master seed bank." A small portion of this master seed bank is then expanded (grown in culture) and frozen away in many small portions (for example, in a thousand different vials); these small portions are collectively referred to as the "working seed bank." The "master" and "working" cell banks are purified, characterized and shown to be free of known contaminants prior to using them in production. Each time a batch of the vaccine is manufactured, the process begins with one of the vials from the working seed bank. Through the use of "working seed banks" the manufacturing process always begins with the same material. If the vials of working seed are exhausted, another small portion of the master bacterial cell bank can be used to produce a new working seed bank, for example, another thousand vials. Since the number of batches of vaccine that are produced each year is limited, this banking system provides a sufficient amount of material to last, in a practical sense, indefinitely.

The viruses that are used in vaccine manufacturing also use a seed lot system. "Master" and "working" virus seed banks are produced and stored away in a similar fashion, the difference being that the viruses are propagated in cells as opposed to a simple nutrient medium. In order to ensure the consistency of the viral vaccines, "master" and "working" cell banks are also kept in those instances for which the virus is propagated in cell lines (as opposed to primary animal cell cultures). When a cell line is developed, a substantial amount is frozen away as a "master cell bank;" a small portion of the master cell bank is grown and stored away in many small vials, thus forming the "working cell bank."

The nutrient media that are used to support the growth of bacteria and the cell cultures in which viruses are grown often contain animal-derived components and, commonly, bovine-derived (from cows) components. As examples, the cells that are used to propagate viruses generally require calf serum for their

maintenance and growth and the nutrient broths that are commonly used to grow bacteria contain beef extracts (e.g., a beef broth).

A typical viral vaccine production scheme is outlined in [Figure 2](#). The seed banking system has been described above. At the start of vaccine production, the manufacturer thaws a vial of the working cell bank and grows it to large amounts, whereupon it is infected with a vial of the working seed virus, which will then grow to large numbers. One vial of the working cell bank and working seed virus may result in a half million or more doses of vaccine.

One of the major difficulties in the manufacture of vaccines arises from the possibility of introducing adventitious agents into the process. Obviously, if the seed virus or cells contain a viral agent other than the vaccine virus, it too may grow. Similarly, and the topic of current concern, if the BSE agent enters into the vaccine manufacturing process at any point, that agent may be carried through into the final vaccine formulation. Variant Creutzfeldt-Jakob disease, as mentioned in other sections of this Web Site, has been attributed to, among other possibilities, the consumption of products from BSE-infected cattle.

[Table of Contents](#)

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Figure 1. Manufacture of a bacterial vaccine

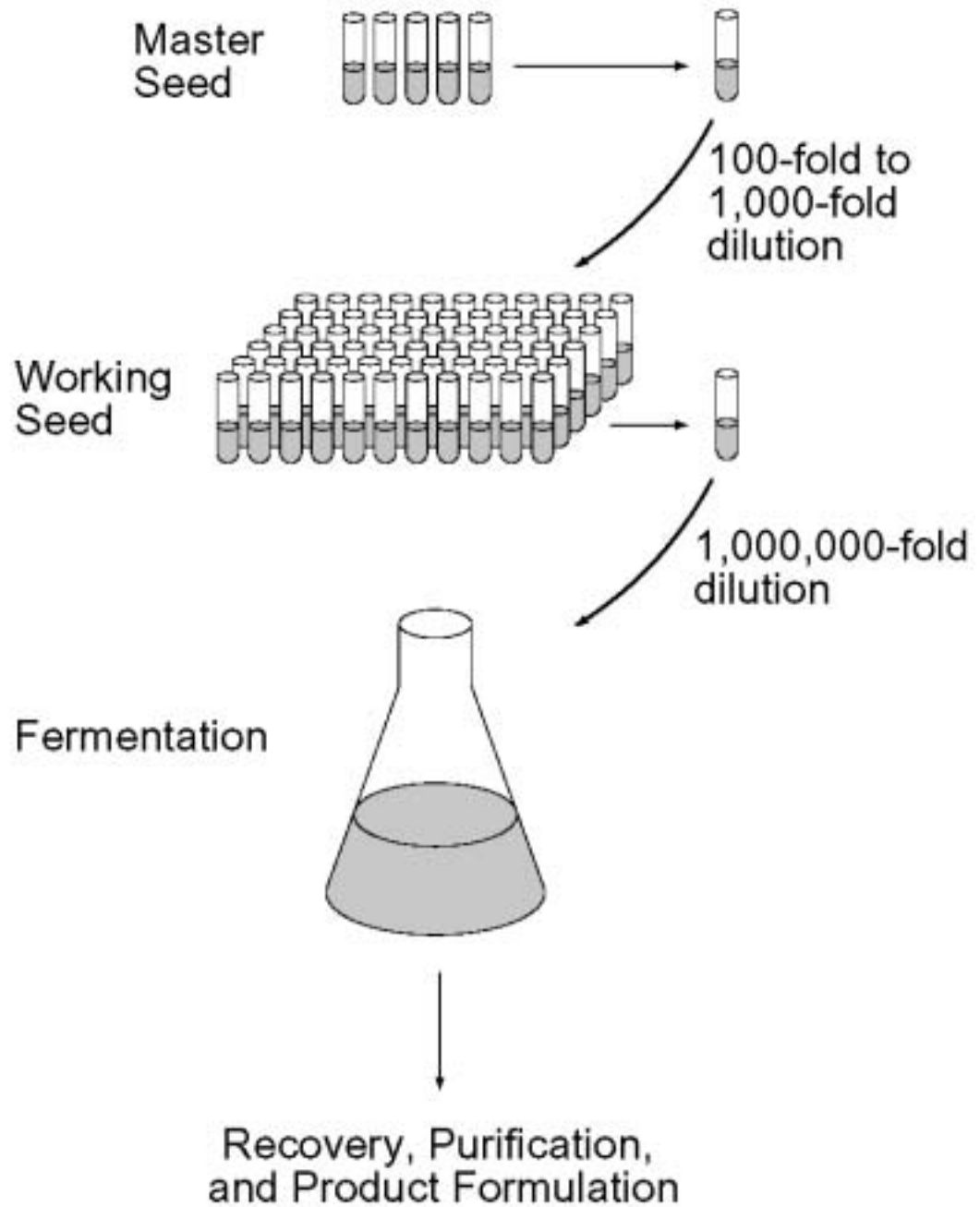


Figure 2: Viral Vaccine Production

