

Filtration Processes Applied in Vaccine Production

Introduction

The subject of this 3M Purification Inc.TM Application Brief is filtration applied in vaccine production. Vaccines are derived from a variety of sources including tissue extracts, bacterial cells, virus particles, recombinant mammalian, yeast and insect cell produced proteins and nucleic acids. Typically, vaccines are produced to treat the general population, however, vaccines can be tailor made to treat specific individuals. Veterinary vaccines also have important economic significance in the protection of animal stocks.

All vaccines have at least two common elements: they must be immunogenic and they must be produced in a highly purified formable to be safely administered to recipients. Vaccines typically employ an immunogen, a molecule which when injected into the body causes the body to recognize it as foreign and to raise an immune response against the intruding molecule. The vaccine substance can be a whole particle such as a virus or bacterial cell, a membrane fragment from a bacterial cell wall, a portion of the capsule layer surrounding a bacteria, nucleic acid (RNA, DNA) coding for a disease causing protein, certain proteins associated with disease or receptors or other cell surface markers. When whole cell or virus vaccine therapies are used, the injected material can be live or attenuated such that the injected form does not cause a severe case of the disease the vaccine is designed to prevent. Attenuation can be done by chemical treatment or genetic modification of the vaccine agent.



The key to vaccine formulation is large scale production of the immunogenic agent followed by purification resulting in a homogenous species of the immunogen able to be safely administered to the patient. Quite often, the vaccine is produced by large scale fermentation and the cells, their internal products or extracellular secretions are purified following fermentation. Where whole cell or virus vaccines are the end product, the purification process begins by separating the cells or viral particles from the bulk of the fermentation liquor. In some cases, the vaccine may derive from the cell membrane or outer capsule and following cell separation, an extensive extraction process of the specific cell or virus associated molecule takes place. In other processes, the vaccine product may be contained within the cell envelope and again separation of the cellular material is required, followed by cell lysis and purification of the internal components. Finally, vaccine products may be secreted by cells grown in culture and the purification process involves an initial separation step resulting in discarding the cells and collection of clarified extracellular fluid for down stream purification. This 3M Purification Inc. Application Brief discusses the different adsorptive and exclusion based filtration separation technologies used in vaccine purification.

The Process

Vaccines can be produced for specific individuals or for the general population based on purification of the vaccine product (immunogen) from tissue extracts or from a fermentation process. The most common method of vaccine production is based on an initial fermentation process followed by purification. The schematic and outline (Figure 1) describe the variety of processes that can be used to produce vaccines.

- **Tissue extraction** — the starting material is collected from an individual or pooled tissue source and the immunogen is obtained by a series of extraction and purification procedures.
- **Fermentation** — the vaccine is produced by growing bacterial, yeast, insect or mammalian cells in culture. The vaccine can be the cells themselves or other cell produced material such as nucleic acid, proteins, portions of the cell membrane or capsule material or virus or viral particles grown inside the host cells.

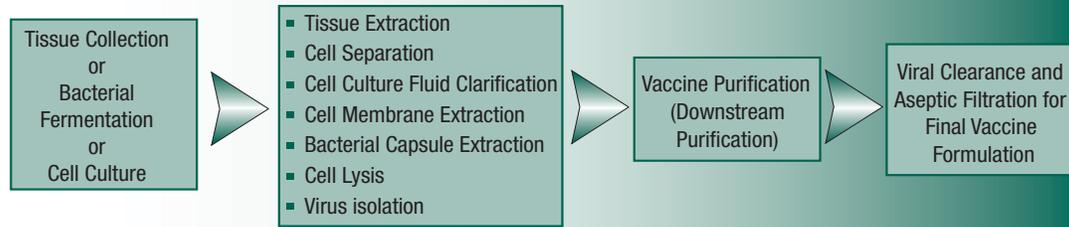


Figure 1 — Process Flow of Vaccine Production

- **Cell harvest** — following fermentation, the cells are collected if the vaccine product is contained inside the cells or if the cell components are to be extracted. If the vaccine product is produced inside the cell and secreted into the fermentation fluid, the cells are separated from the fluid and discarded. The extracellular fluid is clarified and sterile filtered for further downstream purification.
- **Collected cells** — collected cells may be lysed to release viral particles, nucleic acid, inclusion bodies, or to extract membrane or capsule components. Cells are usually collected by centrifugation. The collected cells may be washed several times and lysed to release internal, membrane or capsule components. The lysed cell suspension must be filtered to remove unwanted cell debris and to produce a clarified and sterile material for further downstream purification.
- **Virus harvest** — if the vaccine product is a virus, the viral particles must be separated from the host cell lysate. This requires filtration of the lysate to remove the host cell debris and allow the viral particles to pass through the clarification filter. Good yield of clarified viral particles is required. The viral particles are either purified further and used directly, or the components of the viral particles are extracted, purified and used to formulate the vaccine.
- **Nucleic acid** — nucleic acid is obtained from collected and lysed cells. The nucleic acid is purified by solvent extraction and chromatographic or other techniques and formulated for the final vaccine product. Nucleic acid vaccines can be regions of RNA or DNA that code for disease associated proteins.
- **Inclusion bodies** — bacterial cells often are used to produce proteins that can function as vaccines. Bacteria produce proteins intracellularly and store the produced proteins in internal structures called inclusion bodies. Following bacterial cell collection and lysis, the inclusion bodies are collected and disrupted. This often involves a series of steps involving protein denaturation followed by protein renaturation or folding. Filtration is employed to achieve clarification of the protein solution during this process.
- **Membrane extraction** — vaccine products can be portions of bacterial or mammalian cell membrane structures. These membrane structures are typically protein, but, can be lipid or carbohydrate molecules. The membrane components are usually associated with a disease state. The vaccine product is formulated from the extracted and purified membrane structure.
- **Capsule extraction** — some bacteria grow and secrete a complex carbohydrate material forming an external capsule. This capsular material can be isolated and purified to formulate a vaccine. The capsule extraction process usually requires multiple steps of solvent extraction, followed by chromatographic separation or other standard purification techniques.
- **Aseptic filling** — all vaccine products must be extensively purified to produce a homogeneous solution of the vaccine final product. The purified vaccine must be treated to achieve satisfactory viral clearance (other than the intended virus if this is a viral vaccine) and sterile filtered to remove any harmful bacteria.

The Problem

As described above, production of vaccines is a complex process involving many different steps and processes. Selection of the appropriate filtration technique can be difficult from the various options available. Once the appropriate filtration or separation technique is selected, further optimization of the process is required. The 3M Purification Inc. Solution section provides manufacturers with assistance in selecting the most efficient 3M Purification Inc. separation technology for vaccine production.

The 3M Purification Inc. Solution

3M Purification Inc. produces a wide variety of depth and surface filters and adsorption based separation technologies for vaccine production. Depth filters (Zeta Plus™, Zeta Plus EXT Series and Betapure NT-P™ filters) are usually used upstream in vaccine production for cell separation or clarification and removal of colloidal protein, haze, nucleic acid or other biological material. Zeta Plus depth filters are manufactured in a variety of formulations to address specific separation needs. Zeta Plus depth filters should be considered for clarification of any fluid that is slightly turbid or hazy.

Zeta Plus media is also formulated for adsorptive based separations. Adsorptive Zeta Plus media products contain adsorbents such as carbon, precipitated silica or cationic resins which have specific functional properties. Zeta Plus Activated Carbon cartridges are used to remove color bodies, phenolic compounds or nucleic acids. Zeta Plus DELI and DELP filters contain precipitated silica and are effective in removing detergents, hydrophobic compounds and lipids. Zeta Plus ZA Series and Zeta Plus EXT Series with Z media contain strong cationic functional groups to remove an ionic molecules such as endotoxins, virus and general cellular debris. Zeta Plus VR (virus retentive) media is available in a variety of formulations for effective viral clearance.

3M Purification Inc. surface filters include unique membrane filters to remove colloidal material and bacteria. 3M Purification Inc. LifeASSURE™ membrane filters have exceptionally high contaminant capacity based on their proprietary multi-zone microporous membrane pore structure. LifeASSURE filters are used for high efficiency bacteria removal, chromatography column protection or general prefiltration to sterilizing grade membrane filters. LifeASSURE PSA sterilizing grade 0.1 µm or 0.2 µm absolute rated filters provide quantitative bacteria and mycoplasma removal for sterilizing grade final filtration. The table below provides guide lines for selecting the best 3M Purification Inc. solution.

Table 1 — Guidelines For Selecting the Best 3M Purification Inc. Solution.

Separation Application	Recommended 3M Purification Inc. Solution	Points to Consider
Cell culture media feeds	Zeta Plus and LifeASSURE™ Filters	Zeta Plus for clarification. LifeASSURE for bacteria and mycoplasma removal.
Fermentation air, CO ₂ or exhaust gas sterilization	LifeASSURE PFS Filters	Reliable bacteria and bacteriophage control
Tissue extraction	Zeta Plus and Zeta Plus EXT Series Filters	Select among open to fine removal grades and moderate to strong positive charge
Cell harvest	Zeta Plus and Zeta Plus EXT Series Filters	Choose Zeta Plus for primary stage and Zeta Plus EXT Series (Z, L, M) for extended life and membrane protection
Cell lysate clarification	Zeta Plus and Zeta Plus EXT Series Filters	Select open grades for initial stage and fine grades with moderate to strong positive charge for final stage clarification
Virus harvest	Zeta Plus and Betapure NT-P™ Filters	Choose Zeta Plus C grade or Betapure NT-P if virus adsorption with Zeta Plus S grades is a problem.
Nucleic acid removal or purification	Zeta Plus, and Zeta Plus EXT Series Filters	Choose open Zeta Plus grades for nucleic acid passage. Choose 60 or 90 grades of Zeta Plus ZA or S series or Zeta Plus Activated Carbon for nucleic acid removal.
Inclusion body processing	Zeta Plus Filters	Choose open grades for unfolded protein passage and fine grades for folded protein passage and colloidal material removal
Membrane extraction	Zeta Plus and Zeta Plus EXT Series Filters	Select open grades for initial stage and fine grades with moderate to strong positive charge for final stage clarification
Bacterial capsule extraction	Zeta Plus and Zeta Plus EXT Series Filters	Select open grades for initial stage and fine grades with moderate to strong positive charge for final stage clarification
Solvent and detergent removal	Zeta Plus DELI/DELP Filters	Choose Zeta Plus DELP for maximum solvent or detergent contaminant removal
Lipid, hydrophobic material removal	Zeta Plus DELI/DELP Filters	Choose Zeta Plus DELP for maximum lipid or hydrophobic contaminant removal
Decolorization	Zeta Plus Activated Carbon Cartridges	Choose among 5 distinct carbon formulations
Buffer filtration	LifeASSURE and LifeASSURE SP Filters	Choose LifeASSURE SP if endotoxin contamination is a problem
Endotoxin removal	Zeta Plus ZA, LifeASSURE SP Filters	Use Zeta Plus ZA upstream and LifeASSURE SP downstream for clarified solutions
TFF protection	Zeta Plus Filters	Choose Zeta Plus 60S or 90S grades
Chromatography column protection	LifeASSURE Filters	Choose LifeASSURE for bacteria control and LifeASSURE PSA for sterile filtration
Air/ vent filtration	LifeASSURE PFS Filters	Choose cartridge or capsule filter configurations
Viral clearance	Zeta Plus VR Filters	Choose among 7 formulations
Aseptic filtration	LifeASSURE Filters	Choose LifeASSURE for prefiltration and LifeASSURE PSA 0.2 or 0.1 µm rated cartridge and capsule configurations for sterilizing filtration

Conclusion and Summary

Vaccine production involves a variety of production methods and separation challenges. This 3M Purification Inc. Application Brief has described different methods of vaccine preparation and provided suggestions and guide lines for selection of the appropriate 3M Purification Inc. filtration or adsorptive separation solution. For more information, please consult the additional 3M Purification Inc. Application Briefs referenced or consult 3M Purification Inc.

Additional 3M Purification Inc. Literature

Title	Old Literature Identification	New Literature Identification
3M Purification Inc. Application Brief - Filtration of Cell Culture Growth Media and Process Buffers	LITCABLA3	70-0201-8633-7
3M Purification Inc. Application Brief - Zeta Plus Depth Filtration and Alternative Technologies for Cell Culture Clarification	LITCABZPS1	70-0201-8667-5
3M Purification Inc. Application Brief - Filtration Processes Applied in Viral Vector Based Gene Therapy Production	LITCABZPS3	70-0201-8669-1
3M Purification Inc. Application Brief - DNA Removal from Bioprocess Purification Processes	LITCABZPLA2	70-0201-8665-9
3M Purification Inc. Application Brief - A Review of the Practices of Using Carbon in the Production of Fine Chemicals	LITCABZC1	70-0201-8662-6
3M Purification Inc. Application Brief - Chromatography Column Protection with 3M Purification Inc. LifeASSURE PLA Membrane Filters	LITCABLA2	70-0201-8632-9
3M Purification Inc. Application Brief - Zeta Plus™ VR Series Filters for Viral Reduction in Biopharmaceutical Processes	LITCABZPVR2	70-0201-8676-6

Important Notice

3M Purification Inc. MAKES NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Since a variety of factors can affect the use and performance of a 3M Purification Inc. product in a particular application, some of which are uniquely within the user's knowledge and control, the user is responsible for determining whether or not the 3M Purification Inc. product is fit for a particular purpose and suitable for user's method of application.

Limitation of Remedies and Liability

If the 3M Purification Inc. product is proved to be defective, THE EXCLUSIVE REMEDY, AT 3M Purification Inc.'s OPTION, SHALL BE TO REFUND THE PURCHASE PRICE OR TO REPAIR OR REPLACE THE DEFECTIVE PRODUCT. 3M Purification Inc. shall not otherwise be liable for loss or damages, whether direct, indirect, special, incidental or consequential, regardless of the legal theory asserted, including, but not limited to, contract, negligence, warranty or strict liability.



3M Purification Inc.
400 Research Parkway
Meriden, CT 06450, U.S.A.
Tel (800) 243-6894
(203) 237-5541
Fax (203) 630-4530
www.3Mpurification.com

Please recycle. Printed in U.S.A.
Betapure, LifeASSURE and Zeta Plus are
trademarks of 3M Company used under license.
3M is a trademark of 3M Company.
© 2011 3M Company. All rights reserved.
70-0201-8674-1
REV 0411b