

## Zeta Plus™ Depth Filtration and Alternative Technologies for Cell Culture Clarification

### Introduction

Production of therapeutics and diagnostics by cell culture processes has become the cornerstone of the biotechnology industry. Cell culture systems can consist of bacterial, yeast, insect or mammalian cells, with mammalian cell culture Production becoming the most widely used method. Production of cell culture derived therapeutics begins with fermentation of the desired organism followed by purification of the cell-expressed therapeutic protein. The first step in purification involves separating cell mass from product. Separation of the cell culture fluid challenges the process engineer to select a separation method that results in maximum product yield, complies with FDA regulatory requirements, and offers acceptable economic performance.

This 3M Purification Inc. Application Brief presents issues associated with the cell separation technologies below and describes the advantages of a Zeta Plus™ depth filtration system.

- Zeta Plus depth filtration
- Tangential Flow Filtration (TFF)
- Centrifugation

### The Process

The most common method of producing therapeutic products such as monoclonal antibodies and other proteins is mammalian cell culture. Typically these products are secreted directly outside the mammalian cell into the culture fluid during fermentation. Fermentation cell culture processes range in volume from less than 5 liters to 10,000 liters and the first step in purification involves separating the cell mass from the product contained in the culture fluid. Figure 1 shows where in the process cell clarification is used. Alternatives for cell clarification include centrifugation and filtration.

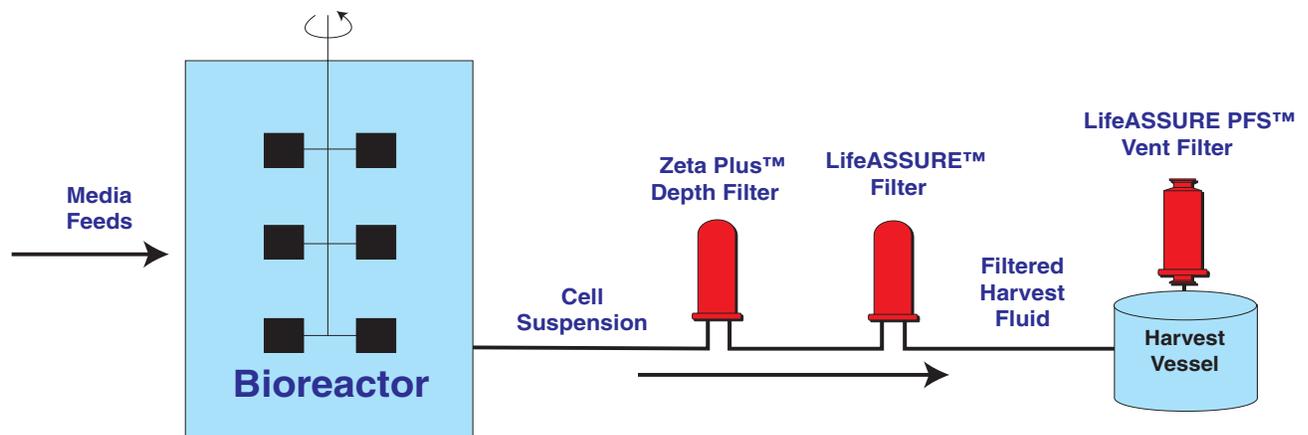


Figure 1 — Process Cell Clarification

## The Problem

Selection of a cell culture clarification system involves issues relating to process performance, regulatory compliance and economics. Process performance issues include scalability, production of consistent quality fluid for further downstream purification, product yield and flexibility of the separation system for process changes and future processes. Regulatory compliance includes issues relating to cleaning and cross batch contamination. Economic issues include maintenance costs, consumables cost and capital acquisition costs. Each of these issues is addressed in the 3M Purification Inc. Solution section following.

## The 3M Purification Inc. Solution

### *Process Performance Issues:*

Scalability — Zeta Plus™ filter media can be tested at small scale and the data collected can be used to specify production scale systems. A complete discussion of Zeta Plus scale-up testing entitled “Clarification of Animal Cell Culture Process Fluids Using Depth Microfiltration”, Singhvi et al. appeared in *BioPharm* Volume 9, April 1996. Using the apparatus shown in Figure 2, data were collected using 13.5 cm<sup>2</sup> filter area discs to size a 1200 liter cell separation system with 33.5 sq. ft of filter area. The scale up experiments were performed using constant flux (flow per unit area). When performed in this manner, results with Zeta Plus filters can be scaled up linearly. In order to obtain accurate scale up data, it is essential to use identical process fluid and process conditions as will be used at full scale.

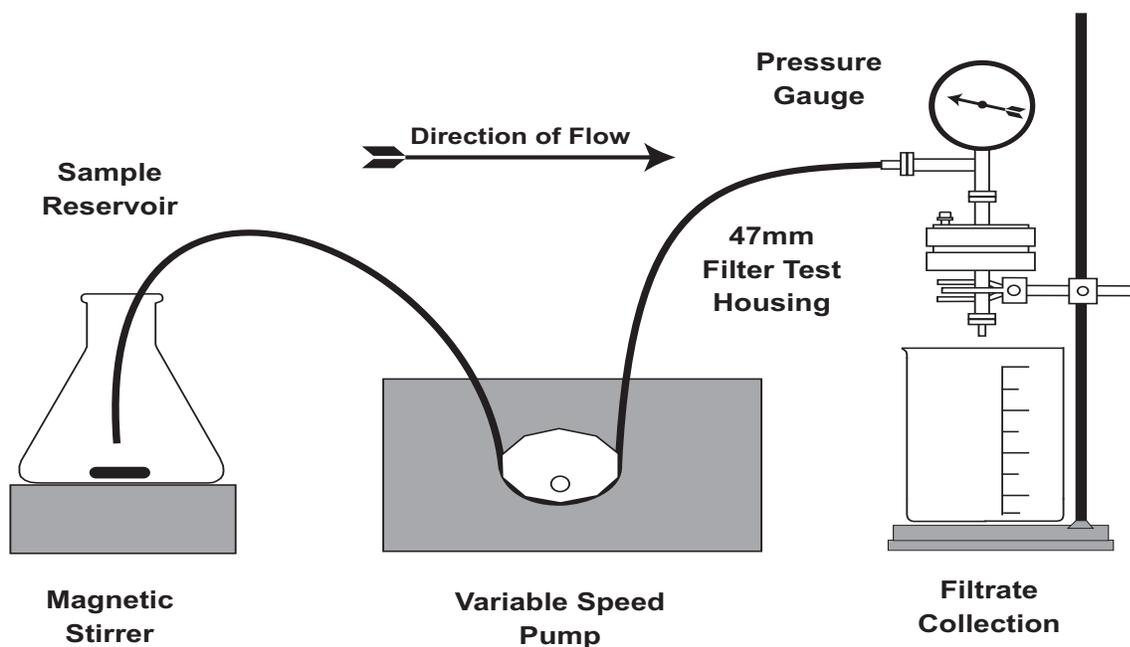


Figure 2 — Zeta Plus Filter Media Small-Scale Test Apparatus

Tangential flow filtration systems can also be evaluated at small scale and used to specify production size systems. In order to obtain accurate sizing data with TFF systems, it is essential to use test filter devices with the same flow path length as will be used in production system TFF filtration devices.

Centrifuges pose a more difficult scale up issue. In order to obtain accurate scale up information, the same g-force and flow path configuration device must be used. Due to mechanical constraints, it is not often possible to scale up centrifuge experiments.

Effluent quality — The efficiency of cell separation is dependent on the separation method selected and will greatly affect the downstream purification unit operations. Generally, Zeta Plus™ depth filters and TFF systems produce a high level of filtrate quality. Both Zeta Plus and TFF filter media consist of fixed pore filtration matrices that are reproducibly controlled during manufacture and thus produce filtrate of consistent quality.

Figure 3 shows results of particle counts vs. throughput for Zeta Plus filters. The objective of these tests is to demonstrate that the filter remains retentive over time and as filtrate volume increases. The fact that the particle count downstream of the test filter remains low provides evidence that filtrate quality will remain consistent over time as filtrate volume increases.

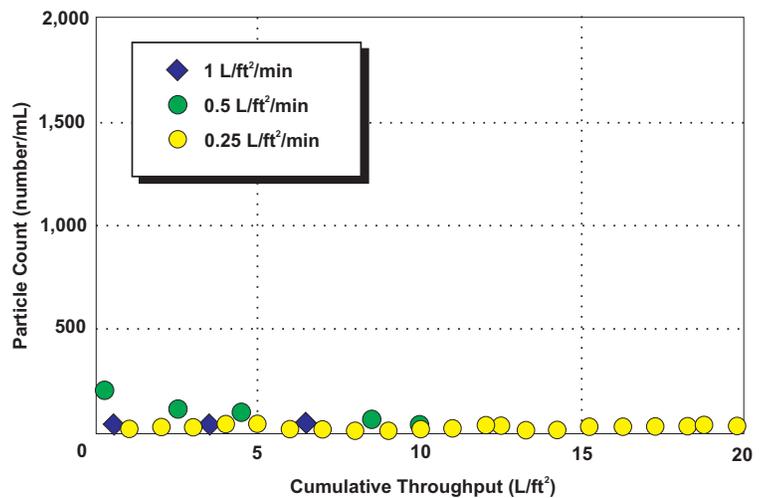


Figure 3 — Particle Count Vs. Throughput with Zeta Plus Filters

Centrifuges separate cell debris on the basis of density. As density differences among whole cells, cell debris and other colloidal matter may be small, the efficiency of separation is not as sharp as with filter media. For this reason, centrifuge overflow requires additional polishing by filtration in order to protect downstream systems.

Product yield — Effect of the cell separation system on product yield has a direct impact on process economics. Products produced by mammalian cell culture have high intrinsic value and any reduction in product yield is an economic loss.

Both TFF systems and centrifuges can limit product yield. Centrifuges used for cell separation are solids ejecting type. Limitation in yield is related to the level of dryness achievable in solids ejected. Solids dryness can vary from 50-70%, reducing product yield by as much as 30-50%.

TFF systems are limited by volume concentration factor (VCF) that can be achieved with the cell broth. In some cases the maximum VCF may be 10-fold, meaning that the total yield is 90%, or product yield loss is 10%. Product yield can be increased by diafiltration, however, this increases total process volume for downstream purification.

Zeta Plus depth filtration systems are direct flow meaning that all incident fluid passes directly through the filter. Product yield is essentially 100%. In addition to evaluating

yield based on volumetric throughput, product yield can also be reduced by adsorption or mechanical retention by the Zeta Plus filter medium. The results in Figure 4 compare total protein concentration and IgG concentration in filtrate of Zeta Plus filters. Total protein and IgG concentration in filtrate samples were taken at regular intervals in the various filtration experiments and compared with the same in the starting material. The results show essentially no loss in total protein or IgG concentration over the range of throughput. This indicates no loss in product yield due to filter adsorption or entrapment.

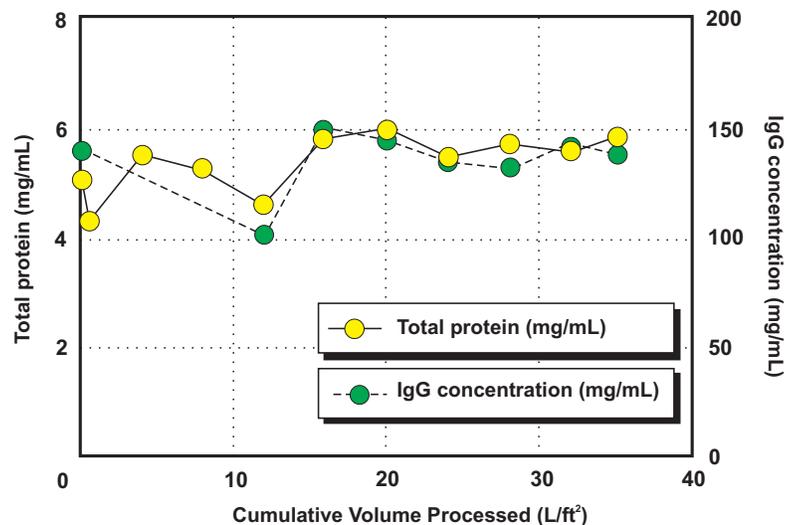


Figure 4 — Total Protein and IgG Concentration in Effluent from Zeta Plus Filters

System flexibility — Selection of a cell separation system may be dependent on variability in harvest volume or on future needs to increase batch volume. Of the alternatives for cell separation, only depth filtration offers flexibility of operation.

Zeta Plus™ depth filtration systems are direct flow as stated earlier. Increase in capacity requires only additional filter area, allowing users to adjust to variations in fermenter batch volumes. Where additional filtration area is required due to increase in batch volume, additional filter housings can be easily added. In most cases, the same pump package can be used. The only pressure required is that needed to force fluid through the filters. No re-circulation pumps are needed.

TFF systems for cell separation are sized according to specific process requirements. If the harvest volume decreases or increases, changes in crossflow re-circulation rate are necessary. If additional filtration area is required, it can be added in a modular fashion, however, re-circulation pump volumes must increase, often necessitating the need for a new pump. TFF systems are also typically automated to control performance and any process changes require reprogramming. In some cases, increase in batch volume will necessitate piping changes and flow control sensor changes.

Centrifuge systems are also sized for specific process parameters. If batch size increases, centrifuge capacity can not be added. The only recourse is to purchase a new centrifuge.

### *Regulatory Compliance Issues*

Cleaning — Validation of cleaning (CIP) processes is a considerable part of qualifying cell separation systems. Zeta Plus depth filters are single use, requiring change out following each use. For this reason, validation of cleaning is not required and there is no opportunity of cross batch contamination. TFF systems, however, are designed such that the membranes are used for multiple batches. Extensive CIP regimes are required following each filtration to remove contaminants that create resistance to flow. In addition to the membrane, all crevices such as re-circulation channels must also be freed of cellular debris. Cleaning efficiency is assessed by measuring return to baseline pressure drop or by measuring return to baseline TOC. Regardless of the measurement technique used, a possibility of cross batch contamination is always present. Similarly, centrifuges are used for multiple batches and require validation of CIP processes. In most instances the fluid contact surfaces associated with centrifuges are stainless steel and seal surface polymers. The ability to adequately clean these surfaces is dependent on CIP fluid access. Centrifuge ejection nozzles or other orifices may be difficult to clean *in-situ*, requiring labor intensive disassembly of equipment for thorough cleaning.

Scale up — All mammalian cell processes begin at small scale often with volumes of 5 liters or less. Even at these initial small scale stages, depth filters can be used and qualified. As batch volumes increase, the same filter media can be used, all the way from early clinical trial stages to full scale production. TFF filters can also be theoretically scaled up, however, with small volumes TFF systems are cumbersome to operate. In order to achieve seamless scale up, all flow control parameters must be maintained equivalent as will be used at full scale stages. Centrifuges also pose a problem for scale up qualification. Due to varying rotor design, as process volume increases it may not be possible to accurately model performance at small and large scale.

Contract facilities — Validation at contract facilities where multiple clients are served pose issues for re-use of cell harvest operations. As stated above, Zeta Plus depth filters are single use and thus, do not provide opportunity for cross-batch or even cross-client contamination. Due to the cleaning issues cited above, both TFF systems and centrifuges are vulnerable to cross use contamination.

### *Economic Issues:*

Product yield — Any reduction in product yield has a negative impact on overall process economics. In addition to lost product due to unrecoverable harvest fluid, shear forces can denature protein or rupture cells causing release of proteases which degrade product. Shear forces are associated with the high g-forces and air-liquid interface inherent in centrifugation. TFF systems require high re-circulation rates to prevent membrane fouling. Pumps responsible for re-circulation can cause cavitation resulting in protein denaturation and the high rate of re-circulation through membrane channels can also cause protein denaturation. Depth filters, however, are direct flow design ensuring maximum recovery of product and they operate at a relatively low pressure minimizing denaturation due to shear forces.

Validation — A major cost of system validation involves demonstrating that CIP processes are effective. Depth filters, which are replaced following each use, prevent cross-batch or cross-client contamination events. TFF membranes, in contrast, are generally used for repeated processing. Centrifuges offer obstacles to cleaning in terms of contact surfaces that may be difficult to access by CIP fluids.

Capital cost — Depth filters are a relatively simple solution to separating cell mass. Hardware components consist of filter housings, piping and pump packages. In contrast, TFF and centrifuge systems are more complex, incorporating sophisticated fluid control and automation which greatly increases initial capital cost, often by a factor of 5-10-fold compared to depth filtration systems.

Maintenance cost — Of depth filters, TFF systems and centrifuges, the highest costs are associated with centrifuges, with annual maintenance costs equaling 5% of purchase price. TFF systems are next due to the complexity of the control and fluid monitoring equipment. Depth filters require the least maintenance, as they are simple to operate and are not typically automated.

### Process related costs

CIP — all three cell separation technologies have associated CIP costs. TFF systems and centrifuges have the highest CIP costs as TFF membranes require extensive CIP following each use and centrifuges may require disassembly to provide assurance of complete cleaning. CIP costs associated with depth filters are minimal and relate mainly to wetted surfaces of the filter housing.

Power costs — centrifuges have the highest power requirements for operation followed by TFF systems and depth filters the lowest. TFF systems require relatively high fluid re-circulation rates to prevent membrane fouling which results in high pump horsepower requirements. Depth filter systems have minimal pump requirements as fluid flow direct path and requires little pressure.

Consumables — depth filter and TFF systems have the highest consumables costs related to membrane replacement. Depth filters require replacement following each filtration campaign. TFF membrane is reused, however, membrane life can vary depending on efficiency of cleaning. Although TFF membranes last significantly longer than depth filters, they are also significantly higher cost.

## Conclusion and Summary

This 3M Purification Inc. Application Brief has presented issues associated with alternative technologies that can be used for cell mass separation following fermentation. The focus of the technology comparison is based on mammalian cell separation applications. Table 1 below summarizes the comparisons made among Zeta Plus™ depth filters, TFF systems and centrifuge systems.

**Table 1 — Zeta Plus Depth Filters, Tangential Flow Filtration (TFF) Systems and Centrifuge Systems Comparison**

<b>Issue</b>	<b>Zeta Plus Depth Filtration</b>	<b>TFF</b>	<b>Centrifuge</b>
Scalability	Yes, linear	Yes, Linear	Difficult
Effluent Quality	Excellent	May require additional filtration	Requires additional filtration
Yield	Excellent, > 95%	Good, may require diafiltration	Dependent on solids dryness achievable
System Flexibility	Easy to size up or down	Difficult to scale up	None — Fixed process design
Fixed Process Design	No	Yes	Yes
Shear Forces	Low	Moderate	High
CIP Validation	Simple — Single use filters	Complex — Requires membrane re-use	Complex — May require equipment disassembly
Cross Batch Contamination	No — Single use filters	Yes — Membrane re-used	Yes — Difficult to CIP equipment
SIP Capability	Yes	No	Yes
Capital Cost	Low	High	High
Maintenance Cost	Low	Moderate	High
Consumables	Moderate — Filter replacement	Low/High — Dependent on membrane life	Moderate — Power consumption

## Additional 3M Purification Inc. Literature

Title	Old Literature Identification	New Literature Identification
3M Purification Inc. Filter Systems for Bioprocess and Biological Separations	LITCATBP	70-0201-8680-8
Zeta Plus™ EXT Series Filter Cartridges	LITZPMEXT	70-0201-8862-2
Zeta Plus Regulatory Support File	LITDRSFMAX	70-0201-8823-4
Zeta Plus S Series Filter Cartridges	LITZPS01	70-0201-8869-7
Zeta Plus SP Regulatory Support File	LITDRSFZP	70-0201-8831-7
ZPB Model Zeta Plus Filter Housings	LITHSZPBC	70-0201-8762-4
ZPB Operating Instructions	LITOPSZPB	—

## Scientific Applications Support Services

The corner stone of our philosophy is service to customers, not only in product quality and prompt service, but also in problem solving, application support and in the sharing of scientific information. The Scientific Applications Support Services (SASS) group is a market-oriented group of scientists and engineers who work closely with customers to solve difficult separation problems and aid in the selection of the most effective and economical filtration systems. 3M Purification Inc. offers specialized support to the pharmaceutical and biotechnology industry through our Validation Support Services Program.



SASS routinely provides end-users with:

- Validation and Regulatory Support
- Extractable and Compatibility Analysis
- Filter System Optimization Studies
- CUNOCheck™ 2 Integrity Tester Validation

For more information regarding our Validation Support Services, please contact 3M Purification Inc. Technical Services or your local Distributor.

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