

## ▶ Cell Banking Application

### Cell Banking Introduction

The cell bank is a critical element of the manufacturing process. Procedures for creating and subsequent use of cell bank repositories are well established and have not changed significantly in years. These procedures often involve significant manual handling with an associated potential for contamination and variation. Furthermore, the time required from cell bank to inoculation of the first bioreactor is significant. It is a period which exposes the inoculum to various potential hazards.

Through the novel use of the ATF™ System, it is possible to achieve a more robust cell bank with significant manufacturing advantages. Cells are rapidly grown to high cell concentrations, then prepared for freezing within the same culture vessel without removal. Cells are dispensed into single-use bags pre-attached to the culture vessel, speeding the creation of a more homogenous cell bank. The need for centrifugation and dilution/concentration is eliminated.

This high density cell bank stores cells at sufficiently high cell numbers, eliminating the need for inoculum scale up and allowing the direct inoculation of a bioreactor.

### ATF-cellbanking Overview

High density cell banking is not new – it is just difficult to achieve and validate. However ATF-cellbanking solves most if not all the problems encountered in this operation. It has the following advantages over other processes and equipment:

- All steps are performed within the sterile reactor environment, including the final step of filling
- Single use large volume bags are used for the bank, no vials
- No centrifuge required
- No individual batches, one reactor volume is identical
- No individual dilution nor concentration steps
- Better reproducibility: identical cell concentration & viability per batch
- Significantly reduced manual handling of cells
- No open handling of cells
- Significantly faster creation of bank
- Thaws directly into a seed reactor

Due to the high cell concentrations achieved and subsequent large cell banks produced, the ATF-cellbanking process becomes more advantageous for cells used in clinical manufacturing and beyond.

### Set-up

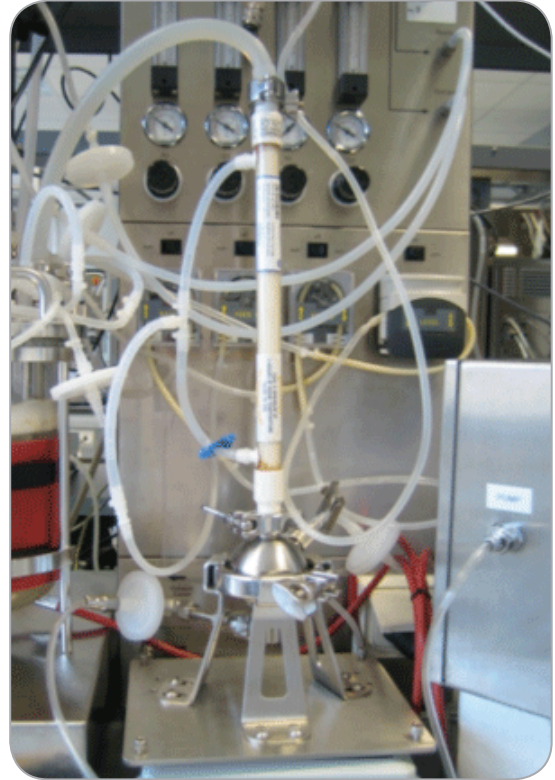
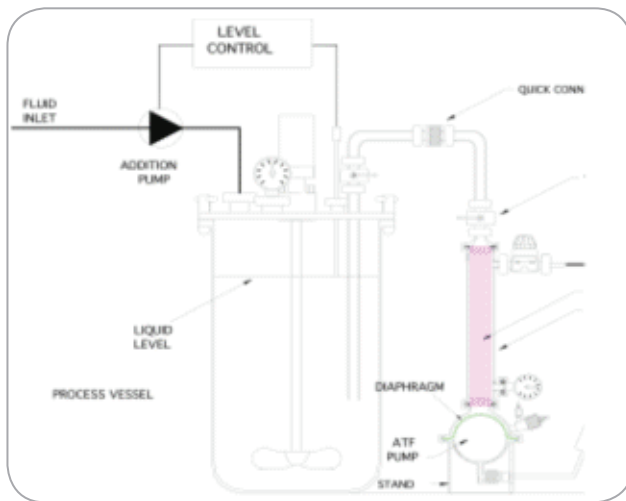
The ATF™ System is prepared and connected to a spinner or small bioreactor (glass or single use) according to the manufacturer's procedures. Often this would be 5-10L in working volume, but can be as high as 200L depending on the size of cell bank required.

## ▶ Cell Banking Application

### Set-up

#### Autoclavable glass reactors

The ATF is attached with special molded tubing via a diptube, and both are autoclaved together.



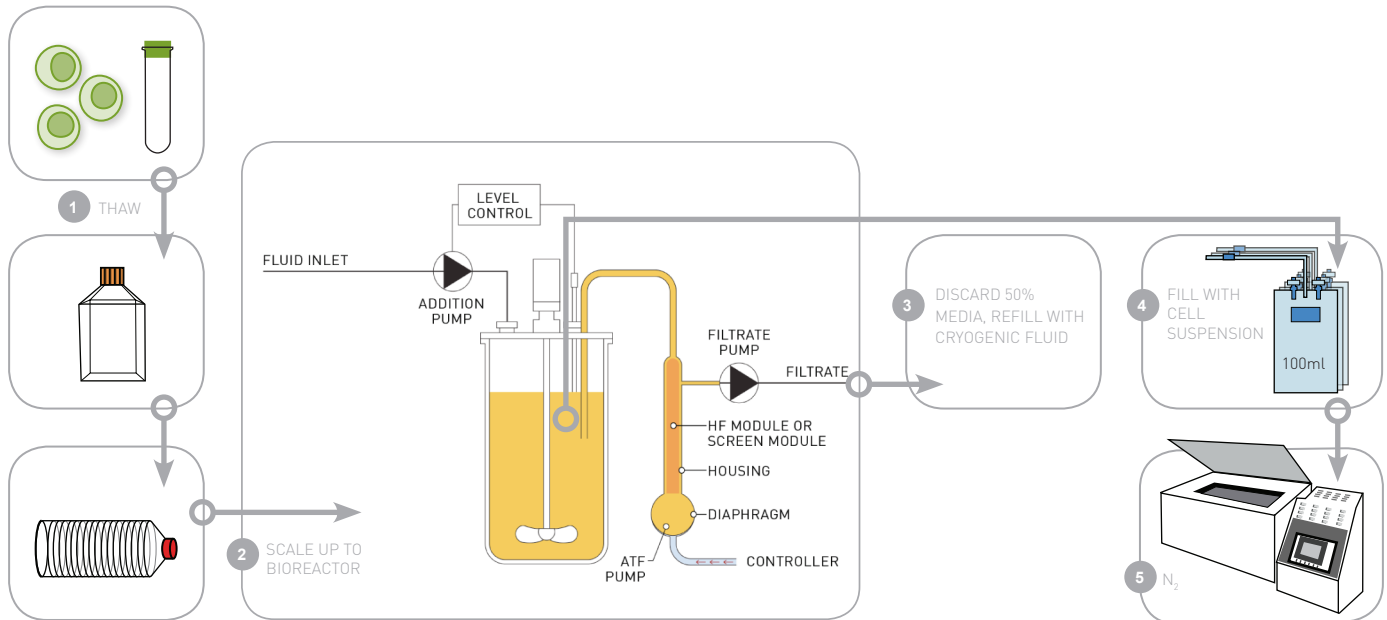
#### Single-use reactors

The ATF is autoclaved with tubing and a single-use aseptic connector, such as Pall Kleenpack or GE ReadyMate, and then a sterile connection is made to the reactor.



## ▶ Cell Banking Application

### Process Overview



From an existing cell bank, cells are thawed and grown normally to inoculate the reactor or spinner where the ATF System is already attached. A concentrated fed-batch culture is then run until the desired cell concentration has been reached, for example  $50 \times 10^6$  cells / ml.

The reactor is then cooled down and a rapid media exchange is performed, for example 50% removal and replacement by the preferred cryogenic fluid for the cell line, generally in 20-40 minutes depending on the culture conditions. Uniform mixing in the bioreactor results in a homogenous suspension.

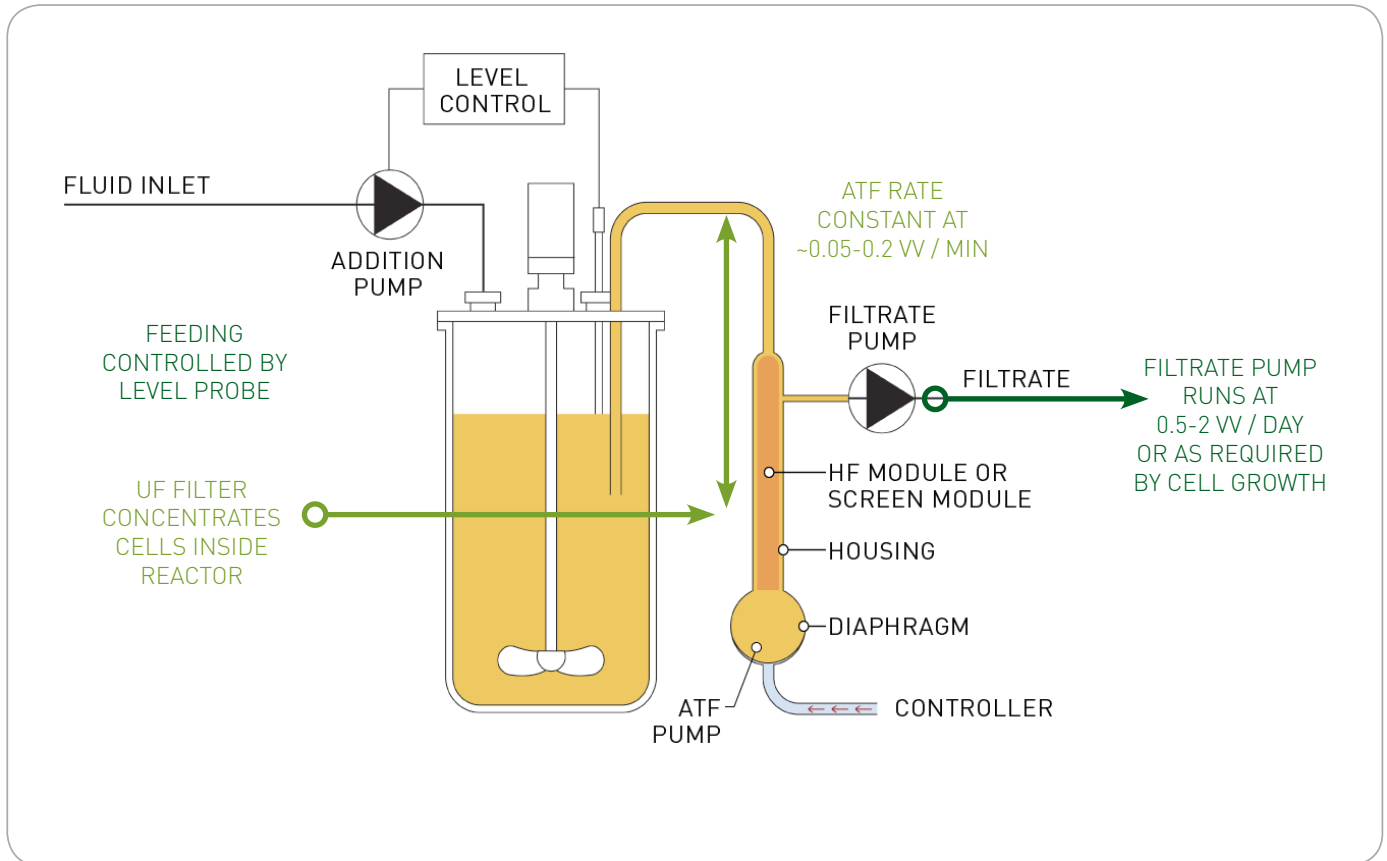
A manifold of single use bags is then attached to the reactor, for example 10 at a time, and filled simultaneously with the use of a peristaltic pump and either manual or weight controlled flow. Further bags or manifolds are welded on and off as required. Depending on the reactor size and number of bags used in the cell bank, the total filling time could often be 30-60 minutes.

The bags are then placed into a suitable cryogenic freezer. Freezers of this type are common in hospitals (for example for blood banks) and commercially available from many suppliers.

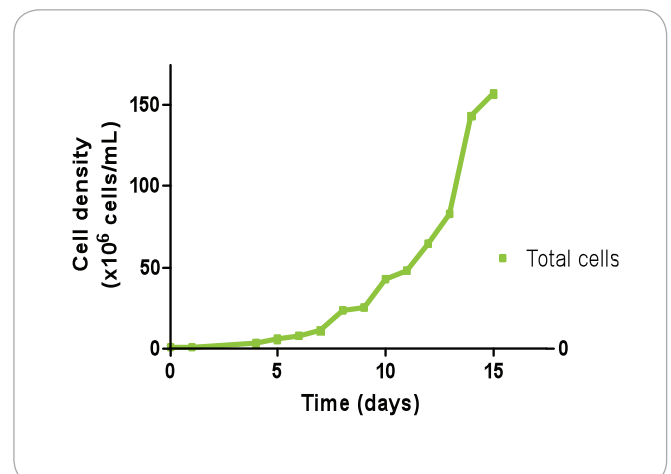
Each bag will contain the same culture volume at identical cell concentration and therefore will therefore have almost identical characteristics upon thawing. Consequently, the cell bank can be validated for performance with good confidence.

## ▶ Cell Banking Application

### Cell Growth in Concentrated Fed-Batch Culture (CFB)



A typical fed-batch culture may reach  $10\text{-}20 \times 10^6$  cells/ml over approximately a two week period (with viability dropping at the end), while the harvest point for cell banking may be around half way through the culture at a suitable exponential growth point and at high viability. In a comparable concentrated fed-batch culture, in the same two week period, one would generally expect to reach  $80\text{-}200 \times 10^6$  cells/ml. In a CFB process the viability remains high to the end of the process. The culture environment is more constant, minimizing the build-up of potentially inhibitory by-products therefore, providing the user the greater flexibility of when to harvest the cells and at what concentrations and how to use the banked cells subsequently in your manufacturing facility. For example, at approximately  $40\text{-}60 \times 10^6$  cells / ml, a 80-100ml bag could be used to inoculate a 10L reactor, or a 0.5-1L bag could be used to inoculate a 100L reactor.

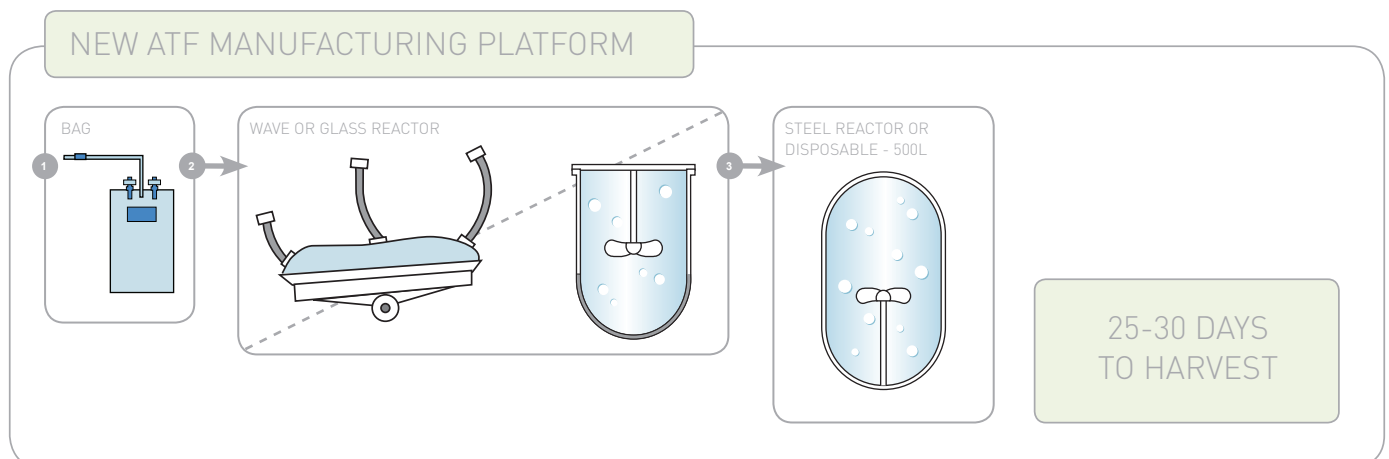
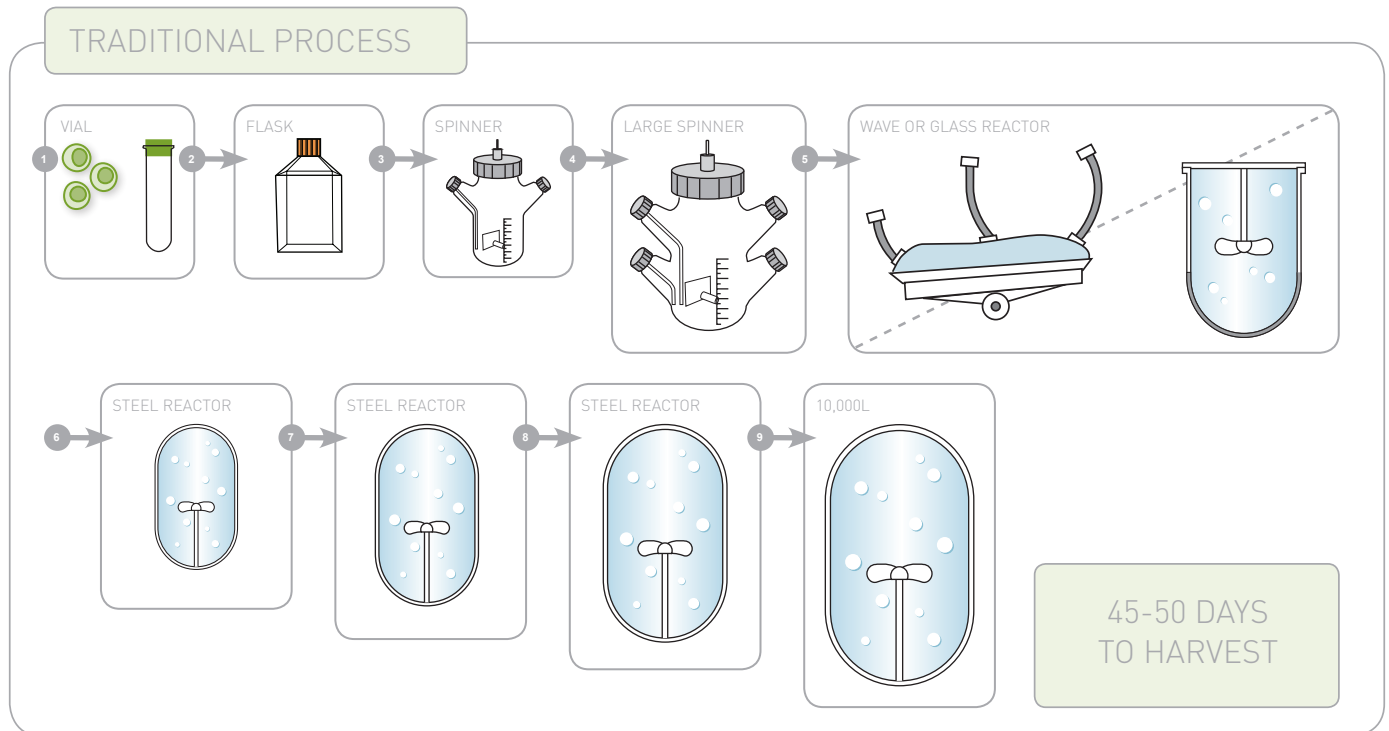


## ▶ Cell Banking Application

### ATF-seedexpansion and RAPIDstart™ Manufacturing

A key benefit of the ATF-cellbanking process is the ability to inoculate a seed reactor directly from the freezer. An obvious gain from this is a reduced process time by the removal of all passaging steps prior to the reactor suite. Equally or more important is the significant reduction in risk of the seed expansion, since cell growth, viability and productivity will be the same, while manual handling and possible contamination risk are reduced to nearly zero.

Some typical timescales for traditional cell banks and fed-batch operation are: 1ml to 2000L in 45-50 days (Wolfgang Noe, Biogen Idec, IBC BPI Oct-09) and 1ml to 10,000L in 40 days (Marcel de Vocht, Crucell, 5th Optimising Biomanufacturing Processes, Dec-08). A typical time saving when moving to a direct inoculation methodology is 2-3 weeks, reducing upstream production times from 40-50 days to 20-30 days, depending upon cell line and final process scale.



## ▶ Cell Banking Application

### Discussion Points

There are now many companies performing ATF-cellbanking. Their results show that the optimum conditions for this process are varied. The following items should be taken into account when moving from the older traditional process of 1-10ml vials to a large volume high density ATF-cellbanking process.

- Harvest decision: growth rate, viability, cell concentration
- Cryogenic fluid composition and concentration for high cell density
- Material of bag for freezing, working and total volumes
- Freezer type, plate size vs bag size, surface area
- DMSO removal post thaw: ATF-mediaexchange or ATF-perfusion?
- Validation of cell bank: old vs new bank, reproducibility of bank

### Conclusions

ATF-cellbanking offers significant advantages to forming a cell bank and to using the cell bank in a manufacturing facility. Its a robust procedure that may be readily implemented into a clinical or commercial manufacturing process with the potential of significant reduction in risk, process variation and cost; however, as with any new process, some initial optimization work may be required.