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# Using In-Line Disposable Pressure Sensors to Evaluate Depth Filter Performance

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Development of a recovery process for a fed-batch mammalian cell culture product involves several objectives: process scalability, robustness, maximizing product yield, elimination of subsequent purification steps, and low cost of goods. In an effort to achieve those objectives, we developed a three-stage primary recovery process to remove biomass and clarify the feed stream for downstream column chromatography (Figure 1). The initial stage involves removal of whole cells and larger cellular debris using a continuous disc-stack centrifuge. Depth filtration is the second stage, removing smaller particulates based on size exclusion and adsorption. The third stage consists of 0.2-µm filtration, which removes potential bioburden. To assist in process development, we are investigating innovative approaches to achieve better process control and maximize processing efficiency. Monitoring pressure in the depth filtration process step is one potential area for improving throughput and efficiency.

**Product Focus:** All biologics

**Process Focus:** Downstream processing

**Who Should Read:** Process development and manufacturing

**Keywords:** Harvest, filtration, pressure differential, process control, data management

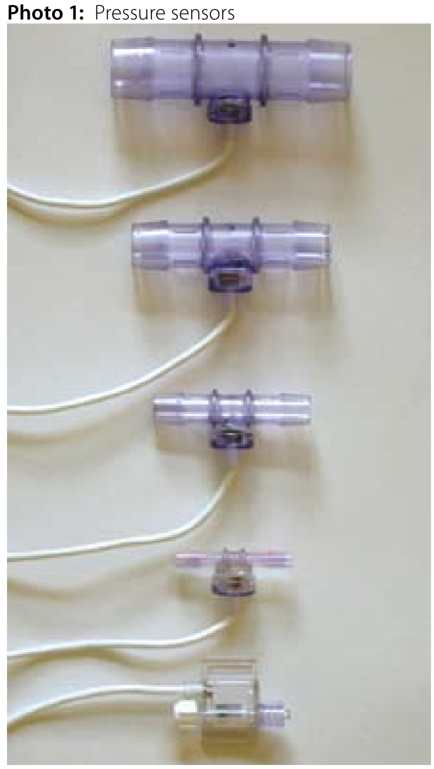
**Level:** Intermediate

Depth filtration coupled with 0.2-µm filtration adequately removes cellular debris and contaminants from a feed stream. It offers two different methods for removing biomass and other contaminants. The depth filter medium is a porous mix of diatomaceous earth and cellulosic fibers that removes small particles (<1 µm) by size exclusion. That medium may also contain positively charged adsorptive binding sites. They can also effectively remove smaller charged particles that are too small to be removed by size exclusion but can impair subsequent column chromatography operations.

Because of inherent differences in cell lines, a purification process needs to be developed individually for each mammalian cell culture. There is a shortage of relevant data on process feed streams that can accurately predict the reliability of depth filtration. But pressure differential (pressure drop across a depth filter) is an important means of monitoring the overall performance of a depth filter during use. After initial sizing for a particular clarification process, depth filter performance still needs to be closely monitored to prevent premature fouling, which can be caused by unforeseen impurities or less-than-optimal process parameters, such as a high flow rate. This would require additional depth filter area to clarify the remaining cell culture harvest.

No correlation currently exists between the characteristics of a cell culture (e.g., cell viability, cell density, viscosity) and the ability of a depth filter to successfully clarify it.

**Photo 1:** Pressure sensors



Changes in media feeding rates and other growth parameters play a role in the characteristics of a cell culture to be clarified. So even with proper filter sizing for a given volume of a particular culture, depth filter performance can still suffer if key variables are not monitored closely. Monitoring the pressure differential is a way to ensure that a cell culture harvest is not prematurely plugging the porous medium of a depth filter.

Traditional analog, stainless steel pressure gauges have been used to record both pre- and post-depth filter pressure. A major drawback of such gauges is their need for frequent calibration and cleaning verification and validation. Another drawback is

**Figure 1:** Schematic presentation of a cell culture harvest clarification train

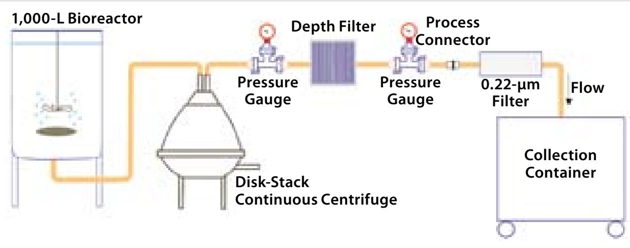



**Figure 2:** Inlet pressure profiles of depth filter and 0.2-µm filter with the pressure differential using traditional stainless steel pressure gauges (manually collected data)

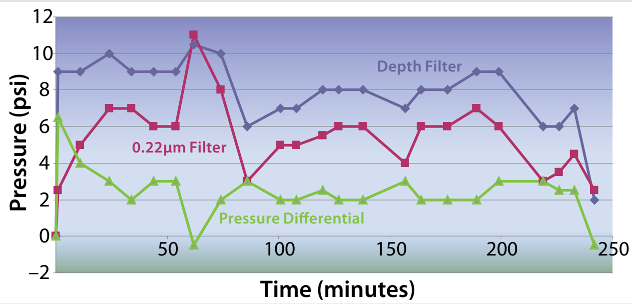



**Figure 3:** Schematic presentation of a cell culture harvest clarification train with PendoTECH pressure sensors and PressureMAT system

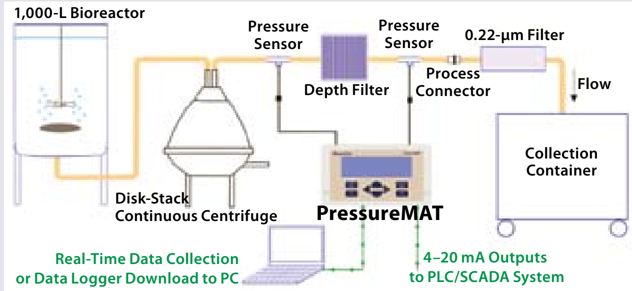
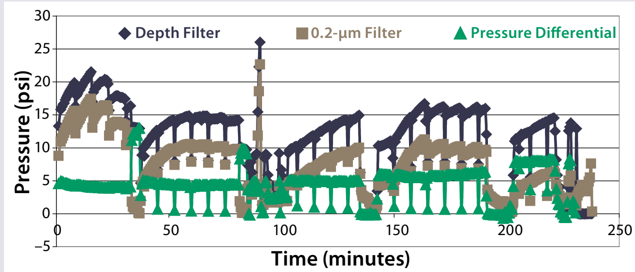



**Figure 4:** Filter pressure profiles and pressure differential using PendoTECH pressure sensors and PressureMAT system; data points were transmitted continuously, then collected on a laptop computer.




the need for an in-line stainless steel sanitary "tee" to install each pressure gauge into a flow path. That causes a short deadleg with a hold-up volume, which can lead to inaccurate readings. For mammalian cell culture operations, pressure readings have to be taken by operators and recorded manually. Frequent data collection can put a strain on limited manpower and may also lead to transcription errors.

## Single-Use Pressure Sensors and Data Collection

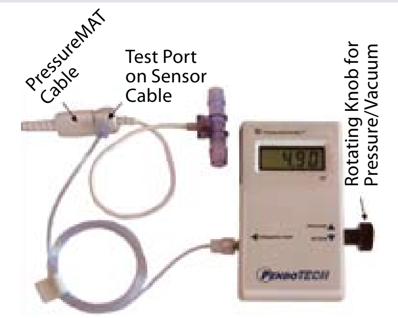
At Centocor, we were interested in PendoTECH single-use pressure sensors as an alternative to stainless steel pressure gauges to be used in conjunction with the company's PressureMAT monitor, alarm, and transmitter system. The combination can be used to record and transmit pressure information to a data collection system. Each pressure sensor has an in-line, flow-through design, and sizes are available from Luer to 1-in. hose barb fittings (Photo 1), eliminating the need for sanitary tees and their associated hold-up volumes. This reduces the number of process components (e.g., gaskets, clamps, tubing adapters), and the disposability of the pressure sensors reduces demand on cleaning verification/validation.

The pressure sensors use an innovative microelectromechanical (MEM) chips. MEM technology integrates mechanical elements, sensors, actuators, and electronics on a common silicon substrate through microfabrication technology (**1**). These chips are manufactured using a silicon piezoresistive sensing element in a Wheatstone bridge circuit, through which an applied pressure gives a proportional output voltage. Before installation into finished devices, the chips undergo an accuracy test and integrity testing that includes a pressure stress test to >100 psi.

The plastic material used to mold the sensor body is either polycarbonate or polysulphone that, along with the other fluid contact materials used in the sensor, meets USP Class VI requirements. The sensors are manufactured in a clean room at an ISO13485-certified, FDA-registered facility. Each individual device undergoes several tests to determine electrical integrity, to confirm the absence of leaks, and to ensure proper calibration within a tight specification range. These sensors are qualified for use ≤75 psi, with burst testing conducted up to 150 psi. Gamma irradiation has been qualified for ≤50 kGy, so the sensors can be preassembled with ready-to-use tubing, filter, and bag assemblies. The accuracy specification of 30 psi (2 bar), +/-3% is sufficient for most clarification process operations (**2**). All these factors ensure that PendoTECH pressure sensors provide highly accurate pressure monitoring.

Each pressure sensor chip circuit requires a narrow range of applied voltage. The circuit voltage output directly proportional to pressure is not a traditional field output signal such as 4–20 mA or 0–10 V, which gives a higher resolution for analog-to-digital conversions. The PressureMAT system serves as a voltage source and processes the output signal from the sensor into a pressure reading. It is therefore required as an intermediate device to integrate the sensors into a control system for building a feedback control loop. Once the sensors are inserted into a flow path, data will be transmitted to the PressureMAT system at intervals as frequent as 1 data point per second. Pressure data can be viewed on the PressureMAT monitor display. Outputs (4–20 mA) from the PressureMAT transmitter can be brought into a data handling system, which facilitates data recording and processing.

**Figure 5:** PressureChecker assembly




### A Case Study

We clarified cell culture harvest from a 1,000-L bioreactor using an LAPX404 disc-stack centrifuge from Alfa Laval (www.alfalaval.com), followed by depth filtration using Pod A1HC filters and 0.2-µm filtration with Express SHC filters, both from Millipore Corporation (www.millipore.com). The cell culture in defined media was harvested after 14 days, showing a viable cell density of 2.96 cells/mL and cell viability of 68%. This process was continuous, with no break tanks in between the steps (Figure 1). Flow rate throughout the clarification train remained constant at 5 L/min. We collected filtrate in 200-L collection containers, each with a 0.2-µm filter attached. As each collection bag was replaced during culture clarification, the 0.2-µm filter was also replaced to reduce the number of interruptions in the overall process and reduce potential bioburden or endotoxin contamination of the final filtrate.

Before obtaining the sensors, we set up stainless steel pressure gauges in front of both filters (Figure 2). We recorded pressure readings manually and calculated the pressure differential as the difference between readings from those two gauges. The stability of the pressure differential at a constant flow rate indicates almost no change on the depth filter's performance throughout the process.

To test the pressure sensors and PressureMAT system, we installed half-inch sensors into the flexible tubing directly upstream of both the depth filter and the 0.2-µm filter without tee connectors. Cell culture was clarified from a 1,000-L bioreactor using the same process train described above. A similar (but not identical) feed stream was used in this particular case study.

Cell culture in defined media was harvested after 16 days, showing a viable cell density of 3.56 cells/mL and viability of 63%. Pressure data were collected using the PendoTECH single-use pressure sensors and PressureMAT system (Figure 3). We took pressure readings (Figure 4), every 30 seconds during processing. The "valleys" in those pressure readings (about every 50 minutes) represent replacement of a 0.2-µm filter and a 200-L filtrate collection bag. Small pressure decreases seen at ~7-minute intervals are due to the discharge of solids from the centrifuge bowl. Each time the centrifuge enters a discharge period, the feed flow is stopped until that discharge period ends, so the pressure differential dropped to zero at those times.

Because the pressure differential remained unchanged, we attributed the increase in pressure readings from both filters during the filling of each individual bag to increased fouling of the 0.2-µm filter. The pressure differential remained constant at ~5 psi for the first 120 minutes of processing, at which point 600 L of cell culture harvest were filtered. At the end, the pressure differential reached 8 psi. Regardless of that minor increase, the pressure differential did not approach the maximum allowable value for this depth filter (20 psi).

A spike in pressure at ~85 minutes was due to a piece of kinked tubing. The PressureMAT monitor's audible alarm, which can be triggered by a predetermined high or low pressure value, alerted the operator to the flow-path obstruction. Without that alarm, it could have gone unnoticed. The alarm set point can also trigger an internal relay that can be easily wired to automate process control by shutting off a pump or opening a valve.

Comparing Figures 3 and 5, it's clear that the combined PendoTECH pressure sensor and PressureMAT system are superior to the analog stainless steel pressure gauges alone. This new approach offers frequent, accurate, and automatic pressure readings that provide a complete picture of depth filter performance. The information assists in process monitoring, process improvement, and trouble-shooting. In addition, the automated pressure monitoring and recording function can free up operators for other tasks, reducing the labor demand of these operations.

### Implementation, Scale-Up

PendoTECH single-use pressure sensors are available in a wide range of sizes. They are easily adaptable to filter screening experiments with small disc filters or scale-up to high flow rates with sensors for 1-inch tubing size. Each sensor is tested during manufacturing to be in calibration, but there is no ability to directly calibrate the sensors or PressureMAT monitor. If a demand for verifying proper functioning of the monitor and the output of a sensor exists, particularly for a GMP process, it is feasible to test the sensors and the system without interfering with the flow path. Each pressure sensor has a test port on its connector cable that can be used to access the atmospheric reference side of the pressure-sensing chip. The test port is a female Luer port, and by applying a calibrated vacuum source to this port, it will give a pressure reading with the same absolute value on the monitor.

For demands on validating the pressure readings, PendoTECH has developed the National Institute of Standards and Technologies (NIST) traceable PressureChecker device to perform this operation (Figure 5). It has an internal cylinder with an external adjustment knob that can create vacuum and pressure to the sensor test port. This testing can be done without flow-path interferance and serves to verify proper functioning of a newly installed sensor. It has a pressure sensing chip simulator embedded that references the internal cylinder. In addition, the PressureMAT monitor cable can be connected to the simulator port and the PressureMAT can be tested to verify it is functioning properly.

### Advantage: Process Control

At Centocor, we found PendoTECH single-use pressure sensors and the PressureMAT monitoring system to provide many advantages. Disposables eliminate the demand for cleaning verification or validation, saving time and cost. Set-up is easy, and the configuration does not create deadlegs in process streams. This system could also provide for automation and feedback control functions.

The PressureMAT system offers 4–20 mA outputs that can be interfaced with a distributed control system (DCS).

This feature would allow end users to connect it with other instruments (e.g., pumps and scales) that might not be directly connected to the system, but that would offer additional control over a particular process. Analog inputs configured in DCS can also be sent to data historian programs for data collection purposes.

These pressure sensors provide accurate and reliable data that can be automatically recorded into data historians and control systems, allowing operators to focus on other tasks. This is ideal for pilot plants and multiproduct facilities, where product turnover and equipment changeover are frequent. This new technology is being tested and evaluated in development operations, and its application in routine manufacturing operations will be further evaluated later. The system also can be used on other applications in which pressure monitoring is needed, such as for filter capacity testing, determining chromatography column pressure flow curves, and controlling tangential flow filtration operations.

## References

**1** What Is MEMs Technology? *MEMs and Nanotechnology Clearinghouse.* 11 May 2009; www.memsnet.org/mems/what-is.html.

**2** PendoTECH. 11 May 2009; www.pendotech.com/pressure.

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