

Application Note:

# Performance Characterization of Batch Fed Non-Adherent CHO Cell Culture in Rocker Bags

## Application

Disposable cell culture bags are used in conjunction with rocker platforms in an array of cultivation applications (insect and mammalian cell lines, virus, protein expression). Rocking platforms provide the dynamic motion and oxygen transfer for cell culture environments. Disposable culture bags are designed to allow for a large air-liquid surface for oxygen transfer, easy access for sampling/filling, and a sterile environment. Venting issues have been reported in the field during rocking of disposable cell culture bags. Therefore, venting and cell growth testing were performed for optimized Charter Medical Clear-Pak® Cell Culture bags.

## Product Optimization

An optimal cell culture design was prototyped with modifications defined in Table 1. These changes were chosen to address venting in the cell culture bag, and incorporated into standard Charter Clear-Pak® 10L cell culture bags (5 L working volume). Bags were gamma irradiated.

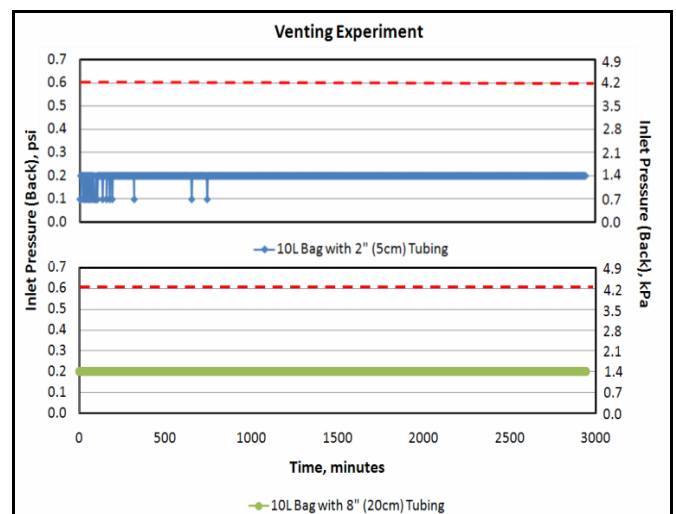
**TABLE 1**

Attribute	Reasons for Modification
Tubing from ports (inlet/exhaust) to filters	Tubing walls being increased in thickness to better resist kinking and/or collapsing.
Check Valve	New check valve design to eliminate potential clogging of filters.
Distance between inlet/exhaust filter ports	Closer inlet/exhaust ports will reduce propensity for filled bag to get media in tubing when on rocker.

## Venting Experiment

A venting study was first executed to gage suitability of the modifications prior to cell cultivation. A BIOSTAT® CultiBag RM 20L rocking platform and control unit were used to continually apply air pressure and rock the culture bag. Rocker settings are located in Table 2. A typical media dissolved in high purity water was used to fill the bags (Sodium bicarbonate=2.22g/L, NaCl=7.9 g/L, Pluronic F-68=1.0 g/L, anti-foam C reagent=0.8 g/L). The inlet (back) pressure was monitored to ensure suitable venting of the bag through the filters and check valve. Two 10L bags with the proposed modifications were tested: 1 bag with 2" (5cm) tubing from chamber to filter and 1 bag with 8" (20cm) tubing from chamber to filter.

**FIGURE 1**



**TABLE 2**

Study	Rocker Settings	Fill Volume	Duration	Temperature	Filter Heater
Venting	8°, 25 rpm. 0.5 L/min airflow	5 L	48 hrs	37°C	Yes (40°C)
Cell Growth	8°, 25 rpm. 0.5 L/min airflow	5 L	7 days	37°C	Yes (40°C)

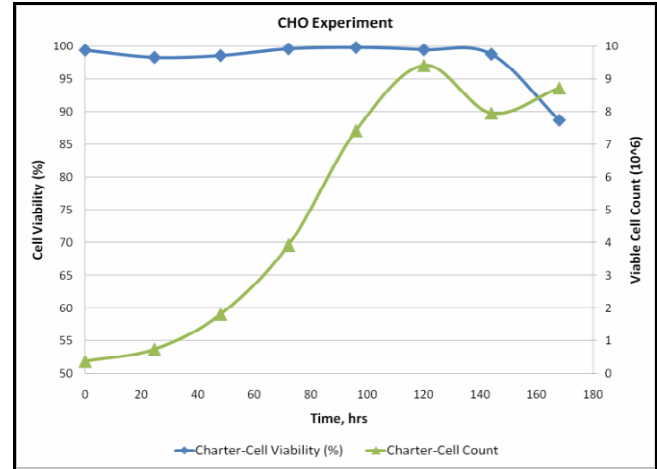
As shown in Figure 1, the venting study showed acceptable results for the Charter cell culture bags. Acceptable results were defined as no clogging of filter or no back pressure spikes (>0.6psi or >4.1 kPa) during operation of rocker.

### CHO Experiment

A cell growth experiment was executed to monitor viable cell count and cell viability (%) over 7 days. Non-adherent Chinese Hamster Ovary (CHO) cells were seeded in shaker flasks. A CD-CHO media from GIBCO® requiring added glutamine was prepared. A CHO working cell bank was suspended and then added to media in shaker flasks, then incubated in a 5% CO<sub>2</sub> and 37°C environment at a shaker speed of 110 rpm for 2 days. After 2 days, viable cell count and cell viability was 0.39 x 10<sup>6</sup>/ml and 98%, respectively. Additional media was added, and shaker flasks were placed back into incubator for 72 hrs at 125 rpm. After 72 hrs, viable cell count and viability were 4.10 x 10<sup>6</sup>/ml and 96.8%, respectively. Prior to inoculation of bag, flasks with seeded CHO culture were placed into incubator for 72 hrs at a speed of 125 rpm.

A gamma sterilized 10L Charter Clear-Pak® bag with the Table 1 modifications was placed on the rocker (same BIOSTAT® unit from venting experiment) and 4.6L of media and 400ml of CHO culture (from shaker flasks) were pumped into bag. Rocker settings are listed in Table 2. Initial (t=0) viable cell count and cell viability for the cell culture bag were 0.38 x 10<sup>6</sup> and 99.4%, respectively. The bag was tested for 7 days and sampled daily for viable cell count and % viability. Nutrients and pH were monitored from daily sampling, but no feedback controls were used; therefore, no adjustments to the cell culture were made during the 7 days.

**FIGURE 2**



Time (h)	Viable cell (10 <sup>6</sup> )	Viability (%)
0	0.38	99.4
24.5	0.75	98.3
48	1.81	98.6
72	3.92	99.6
96	7.43	99.8
120	9.42	99.5
144	7.96	98.8
168	8.73	88.7

As shown in Figure 2, the cell growth study showed acceptable performance of the Charter cell culture bag design. Acceptable performance was defined by the high viable cell count after 7 days and high cell viability for the duration of the test. The drop in cell viability at 7 days (160 hrs) is to be expected based on depletion of cell nutrients in the media and slowing of cell growth. Back pressure was also monitored and was equal to 0.1 psi during the 7 days.

### Conclusions

Data collected from the testing provide evidence that the Charter Clear-Pak® cell culture bags produced optimal performance for the cultivation of non-adherent CHO animal cells.