CELLine Technical Report II

Comparison of batch vs. CELLine culture for production of monoclonal antibody in vitro as alternatives to ascites.
Application: Murine Hybridoma

Martin Wolf Ph.D. • Wilson Wolf Corp. • Minneapolis • USA

As an alternative to ascites, in vitro production of monoclonal antibody can be accomplished with a variety of methods (1-5). Comparison of in vitro methods indicates not all methods are equally cost effective or user friendly. The ascites method has a number of perceived advantages over most in vitro methods, primarily a low cost of production based on mg of antibody produced and a concentrated product which eases down stream processing requirements.

Many systems and instruments (Bioreactors) designed specifically to produce mAb in vitro are burdened by capital instrument expense and additional culture-ware costs. These systems also require a learning curve for successful use. For small scale production, many of these systems are not cost effective. These systems become more cost effective as production increases, due to amortization of costs over larger amounts of produced antibody. In contrast, the ascites method does not become less expensive as the amount of antibody to be produced increases. Ascites production costs are generally linearly related to the amount of antibody required (number of mice).

For small production runs, ascites has been considered more cost effective than most other methods, as it can be readily scaled to small production needs. A single mouse can produce milligram amounts of antibody and provide it in a concentrated form. To effectively compete with ascites for production of modest amounts of monoclonal antibody, in vitro methods must have no capital costs and provide additional cost saving benefits. For example, production of concentrated antibody, reduced serum use, and reduced labor are benefits which make in vitro methods competitive to ascites for production of modest amounts of antibody. These benefits are unfortunately available only with more costly and complicated systems and are not cost effective for producing smaller amounts of antibody.
The INTEGRA Biosciences CELLine culture devices have no capital equipment costs, a low purchase cost, provide concentrated product, reduce serum use, and reduce handling. Importantly all of these attributes are available in a device as simple to use as a standard tissue culture flask. The CELLine CL 1000 was used to produce modest amounts of monoclonal antibody in a manufacturing laboratory. The CL 1000 is a member of a product family of high density cell culture devices available from INTEGRA Biosciences (Ijamsville, MD). The CL 1000 has a reservoir volume of 1 liter with a cell compartment capacity of 20-30 ml. The results obtained with the INTEGRA Biosciences CELLine CL 1000 are presented to provide a demonstration of the cost savings and additional benefits obtained by producing antibody in these devices.

The results from 7 different murine hybridoma cultures carried out in the CL 1000 are presented. The cultures were carried out by a commercial manufacturing laboratory which produces and sells antibody reagents to the research community. A minimal handling protocol (continuous batch process) was employed by the manufacturing laboratory to reduce the handling of the cultures. The hybridoma cells were maintained in the cell compartment of the CL 1000 and harvested at approximately 7 day intervals. During harvest, cells and supernatant were collected from the cell compartment, a fraction of the harvest was reinoculated into the cell compartment with fresh medium. Nutrient medium was removed and replaced with fresh medium on day of harvest. Results obtained from cultures in the CL 1000 are compared to previous results obtained with the same cell lines cultured in a batch method in traditional tissue culture flasks at the facility.

Methods: Murine hybridoma cell lines (Table 1) were thawed from frozen stocks and expanded in static culture (RPMI-1640, 10-15% FBS, 2X L-Glutamine, Pen-Strep). After demonstration of consistent cell doubling in static culture, cells were inoculated into the CL 1000 devices.

Table 1

Hybridoma	Isotype	Fusion Partner	Inoculation Concentration Viable Cell/ml
AC.1	IgG1	Ns-1	7.5 x 10^6
AC.2	IgG2a	653	6.8 x 10^6
AC.3	IgG1	Ns-1	3.7 x 10^6
AC.4	IgG2a	Sp20	5.6 x 10^6
AC.5	IgG1	Sp20	7.2 x 10^6
AC.6	IgG1	n.k.	7.2 x 10^6
AC.7	IgG1	n.k.	5.5 x 10^6
		n.k. = not known	

Cell compartment medium: RPMI-1640; 2X L-glutamine (5 mM), penicillin G (66 mg/L), streptomycin sulfate (144 mg/l). Basal medium was supplemented with 10% FBS (Hyclone, Logan Utah). Additional supplementation of medium with a hybridoma growth supplement (0.1% Vitacyte, J. Brooks Irvine, CA)) was done to remain consistent with prior batch production runs of the same cell lines in traditional flasks.

Nutrient medium: RPMI-1640; 2X L-glutamine (5 mM) , penicillin G (66 mg/L) streptomycin sulfate (144 mg/l) with 0.8% FBS, 0.1% Vitacyte.

Inoculation: Cells were inoculated from static culture at Day 0 in a 20 ml volume into the cell compartment of the CL 1000 devices. Inoculation density was maintained above 3.0 x 10^6 cells/ml. Cells were removed from frozen stock initiated cultures and re suspended in fresh cell compartment medium prior to inoculation. Nutrient medium (1000ml) was supplied to the nutrient medium compartment and the devices placed into a 5% CO_2, 37° C. humidified tissue culture incubator.

Harvest: At harvest, the total cell compartment volume was removed from the CL 1000 units by pipette. Cell numbers were determined by diluting and counting samples using a standard hemacytometer. Viable cells were discriminated from non-viable cells by trypan blue staining and phase contrast microscopy. Cell compartment contents were split back between 3-5 fold determined by cell numbers in the cell compartment at time of harvest. Fresh cell compartment medium (17-15 ml) was added to the cell fraction (3-5 ml) to achieve a 20 ml volume and the cell suspension returned to the cell compartment. The harvested cell containing supernatant fraction was kept sterile and stored at 4 °C until purification by affinity chromatography. Nutrient medium (1000ml) was removed and replaced with fresh medium at the time the cell compartment was harvested. Devices were returned to incubator until next harvest. Devices were stacked atop of each other in the incubator.

Antibody purification: Culture supernatant was processed by eluting antibody from protein A affinity chromatography columns following manufacturers protocol. Eluted antibody fractions were collected, pooled and antibody quantified by spectrophotometer and ELISA. Sandwich ELISA was performed with polyclonal goat anti-mouse IgG or IgM capture antibody and polyclonal anti-mouse IgG or IgM antibody labeled with peroxidase. Color was developed with ABTS. Antibody purity was assessed by SDS-PAGE and Coomassie blue staining. Purified antibody was subjected to further labeling reactions to generate Fluorescein and Phycoerythrin conjugates. Purified antibody was subjected to internal quality testing and released.

Results: A representative cell growth curve is shown in Fig. 1. The total cells and total viable cells are plotted against day of culture. The cell compartments were split back at harvest and growth of the reinoculated cells resumed. The semi continuous batch process generated over 1x10^9 total cells within the cell compartment at harvest. The total viable cell numbers obtained prior to or at day of harvest indicate the maximum capacity for viable cells in the cell compartment. The maximum cell numbers counted from a single harvest during the entire culture period for the individual cultures are shown in Table 2.

Table 2

Hybridoma	Maximum Viable Cells (cell compartment)	Maximum Total Cells (cell compartment)
AC.1	561 x 10^6	1701 x 10^6
AC.2	660 x 10^6	3304 x 10^6
AC.3	520 x 10^6	2140 x 10^6
AC.4	731 x 10^6	2080 x 10^6
AC.5	682 x 10^6	1957 x 10^6
AC.6	570 x 10^6	1476 x 10^6
AC.7	504 x 10^6	1120 x 10^6

The production results for the individual cultures are shown in Table 3. The mean number of harvests for the 7 cultures was 5. A mean harvest volume of 114 ml was processed for antibody purification. Clone AC.5 had an unusually low yield of antibody and analysis indicated that the majority of the antibody present was not intact (SDS-PAGE; size analysis). Supernatant from batch culture of this clone in traditional flasks also contained partial antibody fragments. Results from this clone were not included in the following mean values as it was considered to be a non productive clone. It should be noted that a modest amount of recoverable antibody was obtained from the CELLine culture while supernatant from batch cultures were unable to be used.

A mean of 118 mg of purified antibody per culture was obtained. The mean duration for the 6 cultures was 39 days. The mean antibody concentration recovered from harvested supernatant was 1.02 mg/ml. This concentration was calculated by dividing the volume of processed supernatant (116 ml) by the amount of antibody which was recovered following purification (118 mg). The mg of antibody produced per liter of nutrient medium consumed was 23.7 mg/l. The mean antibody concentration obtained from the standard batch culture method used previously in production for the 6 different clones was 31 mg/l. The batch cultures were maintained until viable cell numbers were nearly exhausted.

The CELLine 1000 devices consumed significantly less serum to produce antibody when compared to results from the traditional batch cultures. Nutrient medium in the CELLine CL 1000 was supplemented with only 0.8% FBS and the cell compartment was supplemented with 10% FBS. About 50-60 ml of FBS was consumed for an individual culture. In contrast a batch culture consumed approximately 380 ml of FBS to produce equivalent amounts of antibody. Supplementation of the nutrient medium in the CELLine CL 1000 with FBS has been demonstrated not to be necessary for many hybridoma clones.

Purification of the culture supernatant from the CELLine CL 1000 resulted in both time and labor savings when compared to traditional batch culture. Instead of concentrating more than 3 liters of medium to recover comparable amounts of antibody, the CELLine supernatant (mean:116 ml) was centrifuged and applied directly to the affinity purification columns. Consequently, there was no simultaneous concentration of serum protein in the supernatant which can lead to purification difficulties associated with concentrating traditional culture supernatant.

Table 3

Hybridoma	Number of Harvests	Total harvest volume ml	Mg Ab Total mg	Culture Duration days	mAb concentration mg/ml	mAb mg/liter nutrient medium
AC.1	5	128	121	42	1.06	24.2
AC.2	6	130	158	42	1.21	26.3
AC.3	4	100	80	30	0.8	19.9
AC.4	6	120	141	50	1.17	23.5
AC.5	5	104	23	38	0.22	4.6
AC.6	4	110	121	37	1.1	30.4
AC.7	5	110	87	36	0.8	17.6
Mean n=6	5.0	116.3	118.1	39.5	1.02	23.7

Summary: The results obtained from hybridoma cultures in INTEGRA Biosciences CELLine CL 1000 culture flasks indicate the following:
1. Cell growth was obtained in all of the 7 cultures (One clone AC.5 was not considered a productive clone and excluded from the mean values derived for the cultures). 2. The mean production of the remaining 6 clones was 118 mg of purified antibody over a mean culture duration of 39 days.
3. Significant reductions in serum consumption was obtained compared to traditional batch cultures of the same cell lines.
4. Nutrient medium consumed per mg of antibody produced in the CELLine CL 1000 was slightly greater than that obtained in batch culture, 23.7 mg/l compared to 31 mg/l. The nutrient medium in the CL 1000 was less expensive when compared to that used in batch culture as it contained only 0.8% FBS. The lower efficiency in nutrient medium consumption was more than offset by reduced nutrient medium costs by significant reduction in consumption of FBS.
5. A concentrated supernatant (1 mg/ml; mean) was obtained from the CL 1000 cultures when compared to prior batch cultures, reducing downstream processing requirements and eliminating the need for a concentrating step prior to antibody purification.

The results indicate that a single CL 1000 can produce greater than 100 mg of purified antibody in approximately 39 days while requiring only 5 harvests. The semi-continuous batch method allowed for sequential harvests and reduced handling. The CL 1000 units were no more difficult to handle than traditional tissue culture flasks and did not require syringes to access cells or medium. The CL 1000 units were stacked in the incubator and did not take up extra incubator space or require any support systems such as a roller apparatus or perfusion pump. A concentrated product was obtained from all of the cultures which simplified volume handling and processing downstream of the culture, eliminating any concentration steps normally associated with processing traditional culture supernatant.

In conclusion; the CELLine CL 1000 units are considered cost competitive to ascites as an in vitro method for producing antibody. The operating costs (medium and serum) were significantly less than those associated with traditional in vitro batch methods using standard tissue culture flasks or gas permeable bags. Additional benefits such as concentrated product, reduced handling, and simplified downstream processing were also obtained with the CL 1000 devices. Most importantly, the ease of use and reduced handling needed to achieve the above mentioned benefits make the CELLine culture flasks a viable alternative to ascites for producing monoclonal antibody.

References:

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http://www.cell@integra-biosciences.com

Tel.: +1-800-886-8675
CH Tel.: +41-1-877 46 46
D Tel.: +49-6404 809-0
F Tel.: +33-1-34 46 71 71
GB Tel.: +44-1462 48 65 48

Figure 1: Growth Curve (AC.5). The number of total cells and viable cells which were recovered from the cell compartment of the CL 1000 during culture are shown. The cells were counted using a standard hemacytometer and non viable cells determined by trypan blue staining. The culture was harvested at times indicated by the arrows. The cell numbers were split back as indicated and cell growth between harvests is shown. The semi continuous batch process; harvests followed by resumed cell expansion of the inoculum is readily seen. The viable cell capacity of the CL 1000 was demonstrated during the initial 5 days of culture. Note, that the number of viable cells did not continue to increase, but declined as nutrient medium was consumed. Addition of fresh nutrient medium does not lead to increased viable cell numbers within the cell compartment beyond its capacity. The viable cells do continue to proliferate at maximum capacity and lead to the very high total cell numbers seen at harvest. Splitting back of the cell compartment allowed for continued cell proliferation and removal of excess cells. The growth of all the hybridoma clones was similar to the growth shown for AC.5.

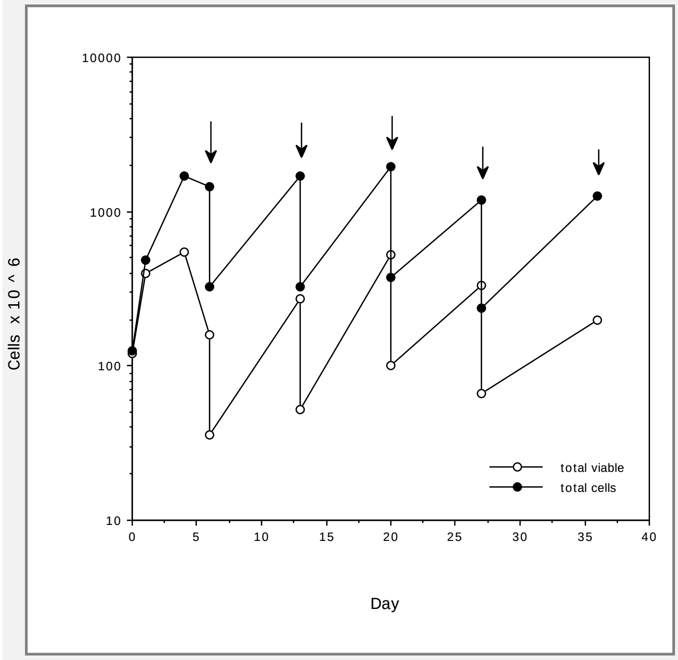

Figure 1