



CELLine Technical Report III

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Antibody Manufacture in the CELLine CL1000
Application: Murine hybridoma

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The successful production of antibody in vitro is dependent upon numerous factors. A variety of methods and devices for producing monoclonal antibody are available. One of the most important variables which impacts production rates and costs is the clone itself from which antibody is to be produced (1-3). Not only do the same clones perform differently under varied culture conditions, but different clones produce markedly different amounts of antibody when cultured under the same conditions (4,5).

For many manufacturers of antibody, a large number of different clones are routinely cultured to produce monoclonal antibody. The production amounts of antibody required may be only 100-200 mg. For these applications, a simple to use culture method which provides benefits associated with more expensive and complicated systems for larger production amounts is of benefit. The ability of the INTEGRA Biosciences CELLine culture flasks to meet the needs of the small scale manufacturing laboratory is provided below.

To assess the performance of the INTEGRA Biosciences CELLine culture devices in the production of antibody in vitro under manufacturing conditions; a large number of hybridoma clones (32 individual clones) were cultured in CL 1000 CELLine units by an independent manufacturing concern. The clones were selected at random and cultured by the independent commercial laboratory producing monoclonal antibody for use in research. The antibody produced was purified from culture supernatant by affinity chromatography. All produced antibody passed quality standards (purity, activity, specificity) except for one clone (see below) and was released as product.

The results indicate a range of production achieved using the CELLine CL 1000 in routine in vitro production of monoclonal antibody under manufacturing conditions. The manufacturing laboratory chose a reduced handling protocol to minimize labor and handling costs. Changes in

FBS supplementation and medium were evaluated during the process by the manufacturing laboratory. The protocol was not optimized for the individual clones.

Methods: Murine hybridoma cell lines 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. The hybridoma clones were obtained from sources around the world and included both clones obtained under license and clones generated by the manufacturer. The clones (fusion partners, isotypes) were randomly selected based on production needs. The cell lines cultured were derived from fusion partners which included 653, NS-1, SP20. Clones are coded by the manufacturer for confidential reasons.

Cell compartment medium: RPMI-1640 or DMEM; 2X L-glutamine (5 mM), penicillin G (66 mg/L), streptomycin sulfate (144 mg/l). Basal medium was supplemented with 10%, 15% or 20% FBS (Hyclone, Logan Utah). Additional supplementation of medium with an additional hybridoma growth supplement (0.1% Vitacyte, J. Brooks Irvine, CA)) was done for some cultures and not others.

Nutrient medium: RPMI-1640 or DMEM; 2X L-glutamine (5 mM) , penicillin G (66 mg/L), streptomycin sulfate (144 mg/l) with 0%, 0.8%, or 5% FBS. Additional supplementation of medium with an additional hybridoma growth supplement (0.1% Vitacyte) was used for some cultures.

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 2.0×10^6 cells/ml. Cells were removed from frozen stock initiated cultures and re suspended in fresh

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cell compartment medium prior to inoculation. Nutrient medium (1000ml) was supplied to the nutrient medium compartment and the devices placed into a 5% CO₂, 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 3-4 fold determined by cell numbers. Fresh cell compartment medium was added to the cell fraction (4-5 ml) to achieve a 20 ml volume 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 by pouring during cell compartment harvest. Devices were returned to incubator until next harvest. Devices were stacked atop of each other in incubator

Antibody purification: Culture supernatant was processed by eluting antibody from protein A affinity chromatography columns following affinity resin 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 Electrophoresis and Coomassie blue staining. Purified antibody was subjected to further labeling reactions to generate Fluorescein and Phycoerythrin conjugates. Labeled antibody was subjected to internal quality testing and released.

Results: As shown in [Table 1](#), a total of 33 individual murine hybridoma cultures were completed in the CELLine devices. Antibody isotypes included IgG1, IgG2a, IgG2b and Ig-M. A myeloma (AC.19 MOPC) producing IgG1 was also cultured. Cultures are presented in the sequence that they were run. Changes in serum supplementation, and medium were implemented by the laboratory as indicated. If cell growth was not established following inocu-

lation, increased serum was provided to either cell compartment or the nutrient medium as indicated.

The hybridoma clones were obtained from sources around the world and included clones obtained under license (majority) and clones generated by the manufacturer. The clones were randomly selected based on manufacturers production needs and represented a random sampling of isotypes and fusion partners. Many of the clones were newly received by the manufacturing laboratory and did not have extensive production records indicating expected production levels.

Clones AC 11,12,13 and 16 were difficult to establish initially but were successfully propagated after increasing inoculation densities and or providing increased serum concentrations in the nutrient medium. One clone AC.9 grew well in the devices but did not produce sufficient amounts of antibody. Sub-cloning (AC17) of clone AC.9 was not satisfactory in restoring antibody production to acceptable levels. The clone was unable to produce suitable product amounts when cultured in static culture flasks. Several clones (AC 18,31) were treated with an anti mycoplasma agent (enrofloxacin, Baytril) to treat suspected mycoplasma contamination. These clones were difficult to establish initially but grew and produced antibody at satisfactory levels following treatment. Cells were treated outside of CL 1000 flasks.

The total cell compartment supernatant volumes collected from each culture are shown in [Table 1](#) (Harvest Vol). AC15 was ran simultaneously in two units to increase production. The number of harvests are also shown indicating the total number of harvests/handling operations for each culture. Clone AC 2 was cultured in 3 CL 350 flasks. The harvest volume was determined from pooling the three flasks. Cell numbers are those counted from a single flask. The CL 350 has 1/3 the surface area as the CL 1000 and has 1/3 the capacity.

Maximum viable and maximum total cell numbers which were counted in supernatant from the cell compartment during harvest are also shown for cultures which were tracked by counting cells. The maximum cell counts reflect the highest numbers obtained during a harvest during the culture period and reflect the maximum cell capacities attained in the cell compartments during culture for the particular hybridoma. Representative growth curves from individual cultures are shown in [Fig. 1](#).



Cells proliferated throughout the interval between harvests as indicated by the accumulation of total cells. Maximum viable cell numbers ranged between 318 and 800 x 10⁶ cells per cell compartment for the different clones. The maximum number of total cells counted in a harvest from the cell compartment ranged between 648 and 3304 x 10⁶ cells for the different clones. The continued proliferation of cells after viable cell capacity was reached accounted for the large numbers of total cells recovered at harvest and contributed to overall antibody production.

Nutrient medium was exchanged during harvest and was usually depleted as evidenced by a decline in the total viable cell numbers in the cell compartment at harvest. Nutrient medium contained 0.8% FBS in the initial experiments, supplemented with 0.1% Vitacyte. Supplementation with 0.1% Vitacyte was done to duplicate culture conditions used in prior production runs in batch culture. Cell compartment medium was initially supplemented at 10% FBS. Subsequent experiments were ran with 0% FBS in the nutrient medium and 20% FBS in the cell compartment and no additional supplementation. The protocols were selected by the manufacturer. No significant difference in antibody production was reported by the manufacturing concern. The final protocol conditions were left at 20% FBS in the cell compartment and 0% FBS in the nutrient medium. The 20% FBS was chosen by the manufacturer to allow for prolonged intervals between handling to compensate for water flux into the cell compartment. Water flux into the cell compartment was observed in all cultures resulting in increased cell compartment volumes compared to inoculation volumes at harvest. The volume increase was influenced by duration between harvests, and cell numbers present at harvest.

A mean of 136 mg of purified antibody was recovered following affinity column purification of supernatant from the 30 individual cultures (Table 2).

The average culture duration was 36 days. The mean harvest volume was 144 ml of supernatant. The mean (purified) antibody concentration in the harvest supernatant was nearly 1 mg/ml. This was determined by dividing the harvest supernatant volume by the amount of antibody recovered following purification of the supernatant. The range of (purified) antibody concentration was between 2.21 mg/ml and 0.36 mg/ml for the individual cultures. The longest culture was 54 days (AC 18) which included a lack of initial growth which was subsequently established. Cell

lines AC29, AC9 and 17 were excluded from the determination of the mean values for the following reasons: AC 9 and AC 17 produced a partial antibody molecule which did not pass manufacturing criteria, AC17 was sub-cloned from AC9 and was also incapable of producing satisfactory antibody. AC29 was excluded due to poor growth. The manufacturer has reported that this cell does not appear to be producing in static culture and can not be considered a productive clone.

Summary: The CL 1000 proved to be a suitable production vessel for manufacturing limited amounts of monoclonal antibody. It provided cost savings through reduction of serum use, handling and processing. A concentrated product was obtained in a small volume of culture supernatant when compared to traditional batch cultures. The applicability of the CL 1000 was established in over 30 different hybridoma cell lines which comprised various immunoglobulin isotypes, and which were derived from various fusion partners.

The protocol was based on a continuous batch production method which reduced handling and was tailored by the manufacturing concern to its needs. The positive attributes of reduced overall costs, concentrated product and most significantly reduced labor were confirmed in this study. The data provides a range of production results from the culture of a large number of distinct murine hybridoma lines. It should be pointed out that multiple units can be employed for the same cell lines if increased production is desired. Scale up can be accomplished by operation of multiple flasks simultaneously. In conclusion, the CL 1000 flasks proved well suited for the small manufacturing laboratory and provided cost savings, reduced handling and ease of use when compared to prior batch methods used.



References:

1. Analysis of nutritional factors and physical conditions affecting growth and monoclonal antibody production of the Hybridoma KB-26.5 cell line. Sanfeliu A, Cairo JJ, Casas C, Sola C, Godia F. *Biotechnol Prog.*, 12 (2) 209-216, 1996
2. Fractional factorial study of hybridoma behavior. 1. Kinetics of growth and antibody production. Gaertner JG, Dhurjati P, *Biotechnol. Prog.*, 9 (3), 298-308, 1993
3. Role of metabolic waste products in the control of cell proliferation and antibody production by mouse hybridoma cell. Duval D, Demangel C, Miossec S, Geahel I, *Hybridoma* 11(3); 311-322, 1992
4. Physiology of cultured animal cells. Doverskog M, Ljunggren J, Ohman L, Haggstrom L. J. *Biotechnol*, 59 (1-2); 103-115, 1997
5. Effect of initial cell density on hybridoma growth, metabolism, monoclonal antibody production. Ozturk SS, Palsson BO, J. *Biotechnol.* 16 (3-4), 1990.

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Table 1: Cumulative Production Record CL 1000

Clone	Ig	Harvest Volume (ml)	mAB total (mg)	mAB conc. (mg/ml)	Duration (days)	Harvests	Nutrient Medium (%FBS)	Cell Compartment	0.1% Vitacyte	Medium	Maximum Viable cells (x 10 ⁶)	Maximum Total cells (x 10 ⁶)
AC.1	G1	120	89	0.74	47	5	0.8	10	Y	RPMI	616	1664
AC.2	G1	65	101	1.55	25	3	0.8	10	Y	RPMI	220	576
AC.3	G1	128	121	0.94	42	5	0.8	10	Y	RPMI	561	1701
AC.4	G1	45	33	0.73	22	3	0.8	10	Y	RPMI	543	1940
AC.5	G1	110	122	1.10	37	4	0.8	10	Y	RPMI	570	1476
AC.6	G1	100	80	0.80	30	4	0.8	10	Y	RPMI	n.a.	n.a.
AC.7	G2a	120	141	1.17	50	6	0.8	10	Y	RPMI	731	2080
AC.8	G2a	130	158	1.22	42	6	0.8	10	Y	RPMI	660	3304
AC.9	G1	104	23	0.22	38	5	0.8	10	Y	RPMI	682	1957
AC.10	G1	110	88	0.80	36	5	0.8	10	Y	RPMI	504	1120
AC.11	G1	108	95	0.88	35	4	10-5	15-20	Y	RPMI	800	1540
AC.12	G1	140	109	0.78	48	5	5-0.8	10-20	Y	RPMI/ DMEM	557	1425
AC.13	G1	144	71	0.49	28	4	2-0.8	20	N	RPMI	312	1172
AC.14	G2a	123	44	0.36	39	5	3	20	Y	RPMI/ DMEM	456	1707
AC.15	G1	190	165	0.87	39	6	0.8	20	N	RPMI	615	1660
AC.16	M	160	124	0.78	32	4	2-0.8	20	N	RPMI	300	992
AC.17	G1	n.d	n.d.	n.d.	38	4	5	15	N	DMEM	682	3000
AC.18	G1	130	138	1.10	54	5	5	10-20	Y	RPMI	535	1435
AC.19	G1	110	243	2.20	32	4	0.8	20	N	RPMI	644	1092
AC.20	M	135	95	0.70	30	4	0.8	20	N	RPMI	394	1160
AC.21	G1	180	120	0.70	29	5	0.8	20	N	RPMI	402	1608
AC.22	G2b	190	105	0.60	38	6	0.8	20	N	RPMI	444	1110
AC.23	G2b	180	123	0.70	29	5	0.8	20	N	RPMI	504	2102
AC.24	G1	150	179	1.20	31	5	0.8	20	N	RPMI	318	648
AC.25	G1	150	209	1.40	32	5	0	20	N	RPMI	n.a	n.a.
AC.26	G1	170	163	1.00	38	6	0	20	N	RPMI	n.a.	n.a
AC.27	G1	170	163	1.00	36	6	0	20	N	RPMI	n.a.	n.a
AC.28	G1	200	152	0.80	42	6	0	20	N	RPMI	n.a.	n.a
AC.29	G2a	168	24	0.14	31	5	0	20	N	RPMI	n.a.	n.a
AC.30	M	185	169	0.91	37	6	0	20	N	RPMI	n.a.	n.a
AC.31	G1	198	135	0.68	39	6	0	20	N	RPMI	n.a.	n.a
AC.32	G2b	144	197	1.40	27	5	0	20	N	RPMI	n.a.	n.a
AC.33	G1	220	176	0.80	38	7	0	20	N	RPMI	n.a.	n.a

n.a. = not available

Table 2

Mean (n=30) Harvest Volume (ml) Mean (n=30) Harvest Volume (ml)	Mean (n=30) mAb Total (mg)	Mean (n=30) mAb Concentration (mg/ml)	Mean (n=30) Duration (days)	Mean (n=30) Harvests
144 ml s=40	136.3 s=47.5	0.94 s=.26	36 s=7.5	5 s=1.0

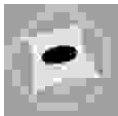


Figure 1: Growth curves of representative cultures: The number of viable cells and total cells present in the cell compartment of the CL 1000 during culture are shown. Viable cells are plotted with open circles, total cells are plotted with filled circles. The cell compartment was sampled on days indicated (usually at harvest) and the cells

counted. Cultures were split back as indicated and cell growth can be seen by increased numbers at next harvest. Cultures were split back by mixing cells with fresh cell compartment medium and reinoculating into the cell compartment.

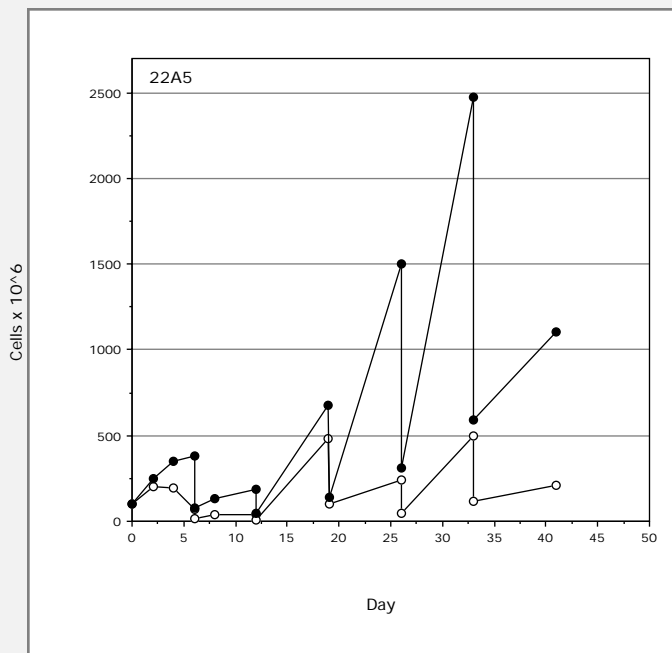


Figure 1: Cell line 22A5

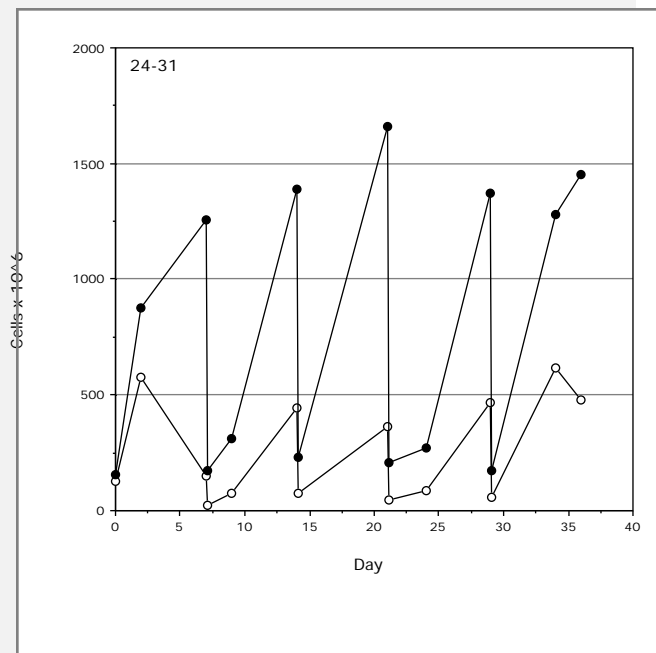


Figure 1: Cell line 24-31

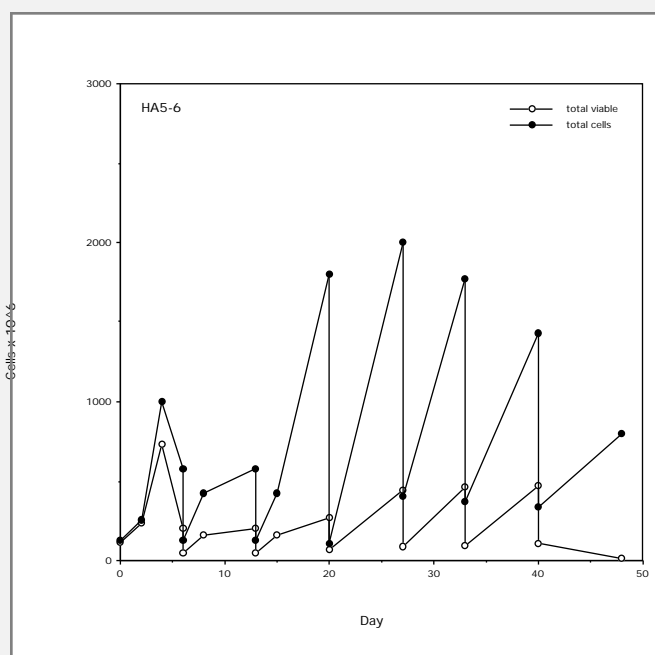


Figure 1: Cell line HA5-6

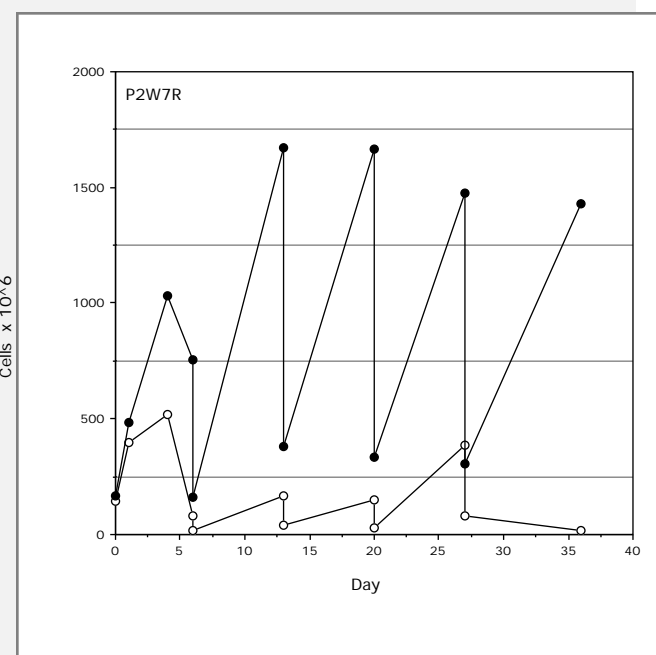


Figure 1: Cell line P2W7R