

Report of the Workshop on Monoclonal Antibodies

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The development of monoclonal antibodies (mAbs) revolutionised clinical treatment and biomedical research. The original possibility of using this technique for replacing uses of laboratory animals often continues to be overlooked or ignored. In the decades following the original discovery, millions of animals died while available humane alternatives were under-utilised. Speakers in this workshop provided comprehensive information on state-of-the-art current best practices for *in vitro* mAb production in commercial and research settings; a review of small-scale *in vitro* mAb production methods; consideration of the pros and cons of using *in vitro* methods, and a case study detailing one large pharmaceutical company's switch to using only *in vitro* methods.

Dr Coenraad Hendriksen (Netherlands Centre on Alternatives to Animal Use (NCA) and National Institute of Public Health and the Environment [RIVM], The Netherlands) set the stage for the workshop with a concise, comprehensive review of the processes involved in the creation and production of mAbs; the pros and cons of both *in vivo* and *in vitro* methods (with an emphasis on the animal welfare concerns associated with the former); and consideration of the legal status of *in vivo* ascites production in Europe and other countries.

Dr Frances Weis-Garcia (Memorial Sloan-Kettering Cancer Center, USA), discussed small scale *in vitro* mAb production, summarising in considerable detail what products are currently available and what issues need to be considered when selecting a mAb production system, both for meeting the needs of individual laboratories and core facilities. She noted that, with few exceptions, there is no longer a need to use animals for ascites production of mAbs.

Based on the available literature and her personal experiences with running a major core production facility, Dr Weis-Garcia reviewed the basic attributes, advantages and limitations of examples of stationary (i.e. standard flasks, gas permeable bags, CELLline flasks and hollow fibre capillary systems) and suspension (i.e. roller bottles, gas permeable bags on rockers and the miniPERM) mAb *in vitro* production methods (Table 1). She also addressed actual performance characteristics of the most commonly utilised systems (Table 2). Since several of these approaches required adaption of the hybridoma cells to low fetal bovine serum (FBS) or serum-free conditions, this presentation included specific protocols to accomplish that goal.

Dr Weis-Garcia's contribution then focused on a detailed consideration of two specific *in vitro* sys-

Table 1: Things to consider

System	Labour (time)	Relative cost	Technical skill	More than standard tissue culture	Multiple harvests	mAb scale
Traditional flasks	+	++	+	No	No	< 25mg
Gas permeable bags	+	++	+	No	No	5–100mg
Bioreactor flasks	+	++	++	No	Yes	5–1000mg
Hollow fibre	++++	++	+++	System	Yes	> 1000mg
Spinner flasks	+	++	++	No	No	< 50mg
Roller bottles	+	++	+	Roller bottle		
				Incubator	No	5–100mg
Bioreactor roller bottles	+++	++	+++	Roller	Yes	5–1000mg

Sources: Survey of Academic mAb Core Facilities and Jackson et al. (1999). Lab Animal 28, p. 38.

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Table 2: What systems are being used?

System	Core usage	Medium	FBS	Success rate	mAb concentration (mg/ml)	Advantages	Disadvantages
T-150/225 (Corning, Falcon, etc.)	4/10	DME (HG) RPMI-1640	10% 5-10%	> 95%	0.01-0.10 0.05-0.17	Technical ease No adaption	Low yield
CELLine flasks (Integra and BD)	7/10	BD Cell MAb-Basal BD Cell MAb-SF BD Cell MAb-AF	10% 10% 0.5%	100% = M/M 95% = R/M	1.0-3.0 0.5-3.0 0.2-4.0	Technical ease Good yield Minimal labour	Media adaption
Fibre cell (Bellco)	1/10	Serum free	0%	?	0.5-5.0		Media adaption
Spinner cultures (Corning, Falcon, etc.)	1/10	DME/RPMI Serum free	10% 0	100% = M/M 99% = R/M	0.01-0.05	Technical ease No adaption	Low yield
Roller bottles (Corning, Falcon, etc.)	2/10	DME Serum free (in-house)	10% 0	> 95%	0.01-0.05 0.02-0.16	Technical ease No adaption	Low yield
miniPERM (IVSS and Vivascience)	3/10	DME (HG) Serum free	10% 0.5%	> 99%	0.2-0.5 0.2-2.0	Good yield	Media adaption Contamination

AF = animal free; BD = BD Diagnostic Systems; DME = Dulbecco's modified Eagle's medium; FBS = fetal bovine serum; HG = high glucose; IVSS = In Vitro Systems and Services; M/M = hybridoma from fusion of mouse B cell and mouse myeloma; R/M = hybridoma from fusion of rat B cell and mouse myeloma; RPMI = RPMI medium 1640; SF = serum free.

tems. Both the miniPERM (MP) and CELLline (CL) require adaptation of the cells and utilise serum-free media that may be supplemented with 0.5% low Ig FBS. mAb production is monitored by glucose consumption in the MP and viable cell number with the CL. The MP requires minimal effort for cell adaptation, significant labour for production and works with all types of hybridoma lines. In contrast, the CL is associated with more effort to adapt the cells, minimal effort for production and works with more than 95% of all hybridoma cell lines.

She ended her presentation by considering the production results for a representative sample of mouse and rat hybridoma lines in both the miniPERM and CELLline systems (Table 3). These data reinforced her earlier conclusion that *in vitro* methods can replace all but a few uses of ascites for the production of mAbs.

Dr Vincent Dewar (GlaxoSmithKline Biologicals [GSKB], Belgium), discussed the implementation of *in vitro* methods for mAb production — a case study. GSKB produces mAbs for immunotools and test reagents (e.g. ELISA, Western blot), but not for immunotherapeutic purposes. In 1997, 100% of mAb production was by the ascites method, involving 64 production runs, more than 4g of mAbs and 350 mAb users. By 2001, they had switched 100% to *in vitro* methods, with 73 production runs resulting in more than 4g of mAbs for 500 users. This conversion included hiring one technician dedicated full-time to monoclonal production.

Initial concerns for GSKB focused on which system to choose; would all of their hybridomas pro-

duce?; what would be the secretion rates and production yields?; and what would be the costs of their manpower needs? They were also concerned about the characterisation and properties of the mAbs produced by *in vitro* methods.

GSKB tested several systems: miniPERM (roller bioreactor), CELLline (mini-bioreactor with two chambers utilising membrane technology), Technomouse and Cellpharm (both based on hollow fibre devices). Based on this experience, they selected the CELLline 1000 for ease of use and good production yields for the range of 10–50mg, and the CP100 as an independent unit that can be scaled up (using CP2500) for yields of 150–200mg. The miniPERM method is kept as a back-up system.

Dr Dewar presented production data and noted that the results exceeded their initial expectations. Since 1998, they have never failed to grow a hybridoma when using the CL1000 or CP100 systems. They ran in parallel *in vivo* and *in vitro* productions to compare the ELISA reactivity tests of old ascites batches and new *in vitro* mAbs. In both cases, no differences were noted. They also noted no differences in affinity-based checkpoints performed by surface plasma resonance (biacore). As a result of this experience, ascites production was stopped in June 1999.

Dr Dewar reviewed some of the insights gained from their efforts: practical experience in cell culture methods is essential; do not re-use the systems; use gamma-irradiated material if no specifically dedicated autoclave is available for the miniPERM; equilibrate the CP100 system

Table 3: miniPERM vs CELLline production

Clone name	Hybridoma		miniPERM HSFM + 0.5% FBS			CELLline BDAF + 0.5% FBS		
	B cell	Myeloma	Days	Ave. [mAb] (mg/ml)	Ave. mg/ day/BR	Days	Ave. [mAb] (mg/ml)	Ave. mg/ day/BR
HECD-1	Mouse	??	27	0.3	2.6	20	0.6	4.4
200-3G6-4 (cl. 1FD5)	Mouse	P3X63.Ag8	17	0.2	1.7	32	0.7	4.7
OKT3	Mouse	P3X63.Ag8	34	0.3	2.5	27	0.7	4.3
32B9/B4	Mouse	SP2/0	35	0.5	4.2	24	0.6	4.5
KcsA-1	Mouse	Sp2/0	17	0.7	5.3	20	1.0	7.3
PK136 (cl. 30)	Mouse	Sp2/0	21	1.3	8.7	41	0.5	3.0
STCLC10C3/G4	Mouse	Sp2/0	27	0.8	6.5	34	1.5	9.8
53-6.72	Rat	NS-1	27	0.9	8.3	34	1.9	12.4
2.4G2	Rat	P3U1	20	1.0	7.8	20	1.2	8.1
GK1.5	Rat	Sp2/0	27	0.6	6.0	27	1.3	6.5
R210F6	Rat	Sp2/0	27	0.4	3.3	17	0.7	2.9

BDAF = BD Cell MAb, animal-free media (BD Diagnostic Systems); BR = bioreactor; FBS = fetal bovine serum; HSFM = hybridoma serum-free media (Gibco/Invitrogen).

Note added in proof: HSFM has replaced BDAF in the CELLline system.

three days before inoculating cells; and pay attention to potential membrane leakage when inoculating and harvesting cells with the CELLline systems.

His take-home message for the workshop participants was that *in vitro* works in more cases than they had expected. He concluded with several recommendations and observations to facilitate the

conversion from *in vivo* to *in vitro* mAb production: develop and centralise the expertise in production units with specialised staff working for several groups or internal customers; the type of material selected is less important than the experience in working with it; harmonise for production protocols within a company or university; and seek out expertise when needed.