

LYMPHOCYTE CULTURE CENTER
The School of Medicine

Dear Investigator:

The Lymphocyte Culture Center (LCC) has been a research support facility of the University of Virginia School of Medicine since January 1983. The primary function of the LCC is to make available to researchers the most current technology and expertise for the construction and selection of lymphocyte-myeloma hybridomas for the production of monoclonal antibodies. The LCC can also provide expertise in the use of these reagents in basic research programs. Services are customized to the specific requirements of individual investigators to optimize the recovery of appropriate antigen specific monoclonal antibodies. The LCC has also provided research support services to other academic institutions and companies, and to federal agencies such as the FBI and USAMRIID.

The center occupies an approximately 1270 square foot modern laboratory equipped with cell culture incubators, laminar flow hoods, centrifuges, inverted and standard microscopes, automated microplate washer and ELISA plate readers, automated HPLC systems, etc. We also have facilities for the cryopreservation and long-term storage of established hybridomas and myeloma cell lines in liquid nitrogen cell banks. The LCC is currently staffed by myself, three laboratory specialists advanced.

The LCC will immunize animals, collect sera and develop ELISA assay strategies appropriate for screening large numbers of hybridoma culture supernatants and animal sera. Once an appropriate assay system has been developed (either in the LCC or in the individual researcher's laboratory) to demonstrate the successful immunization of the necessary animals, fusions are scheduled on a first come first serve basis. The facility conducts all aspects of cell fusion, assaying by ELISA for specific antibody positive cultures, culturing, cloning, freezing, and recovery of specific antibody producing clones. Investigators receive, upon request, hybridoma culture supernatants and the individual hybridoma clones. The center also provides other extended services including bulk expansion of clones, isotyping and sub-isotyping of monoclonal antibodies, bulk monoclonal antibody production *in vitro* using single use bioreactors, and monoclonal and polyclonal antibody purification by affinity chromatography on recombinant Protein G columns. The LCC also serves as a repository, production facility and distribution point for intellectual property (hybridomas and monoclonal antibodies) licensed by the University of Virginia Patent Foundation.

Our standard mouse fusion protocol [M.D. Chapman, W.M. Sutherland, and T.A.E. Platts-Mills (1984), *J. Immunol.* 133:2488-2495; J-H. Chang, W.M. Sutherland, and S.J. Parsons (1995), *Methods in Enzymology* 254:430-445] is modified from published procedures (V.T. Oi and L.A. Herzenberg in *Selected Methods in Cellular Immunology*, ed. B.B. Mishell and S.M. Shiigi, W.H. Freeman & Co., 1980, p. 351). Briefly, spleen cells from an immunized mouse are washed once in Iscove's MDM and mixed with washed Sp2/0-Ag14 myeloma cells [Shulman, et al. (1978), *Nature* 276:269] at a 5:1 ratio (spleenocyte to myeloma). The cells are pelleted and the medium aspirated. Cell fusion is accomplished by the stepwise addition of 50% polyethylene glycol (PEG 4,000, Gibco) over one minute. The PEG is then diluted dropwise with Iscove's MDM and the cells pelleted and gently washed once in Iscove's MDM containing 15% selected fetal bovine serum, hypoxanthine (H), and thymidine (T). The cells are resuspended in HT medium, transferred to a petri dish and incubated at 37°C in a humidified atmosphere of 5% CO₂/95% air for one hour. The cells are resuspended in HT medium and plated into 96-well tissue culture plates (Costar, Cambridge, Mass.) at a density of approximately 2-4 x 10⁵ cells/well. The cultures are fed 24 hours later with HT medium containing aminopterin (HAT medium) and maintained in this medium for two weeks with periodic feeding. Macroscopic colonies usually appear within 7-10 days following the fusion and supernatants are screened for specific antibody production. Antibody positive hybridomas are cloned twice by limiting dilution, and frozen cell stocks are stored in liquid nitrogen cell banks at each stage of hybridoma selection (parental, primary, and secondary clones – we currently store over 25,000 vials of cells).

Please contact me if I can be of further assistance to you in meeting the goals of your research projects, or if further details are needed concerning the research services provided by the LCC.

Sincerely,

Bill

William M. Sutherland, Ph.D.
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Resource Director
Lymphocyte Culture Center