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MONOCLONAL ANTIBODIES AS DRUGS OR DEVICES: PRACTICAL AND REGULATORY ASPECTS

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1. Introduction

In 1975 Köhler and Milstein published their landmark paper entitled "Continuous cultures of fused cells secreting antibody of predefined specificity." Their research established a technique for the production of essentially unlimited quantities of homogenous, monospecific antibody of preselected

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specificity. The impact of monoclonal antibodies on many areas of biology and medicine has been recognized by the awarding of the Nobel prize in medicine to Kohler and Milstein. Now, ten years after Köhler and Milstein's pioneering work, more than 80 monoclonal antibody products are available commercially for *in vitro* diagnostic tests. Moreover, monoclonal antibodies and their derivatives are being intensely investigated for the treatment of cancer, autoimmune diseases, allergic conditions, drug overdose, viral and parasitic infections, and transplant rejection. It is highly probable that monoclonal antibodies for *in vivo* therapeutic applications will be licensed in the near future.

The increasing medical use of monoclonal antibodies has led to the development of more efficient and economical production methods. With the anticipated long term use of monoclonal antibodies in humans, attention has now been focused on the reproducibility of the production process and the purity, potency and safety of the final product. This review provides a conceptual background for understanding monoclonal antibody production and highlights recent developments in commercial scale production. As regulatory agencies are now concerned with identifying risk, in process and final product quality controls are

discussed with a review of aspects of monoclonal antibody products which pose particular safety concerns. The Food and Drug Administration has established criteria which may be developed into guidelines or regulations to be considered in the manufacture of monoclonal antibodies. These and other regulatory aspects of monoclonal antibody production are outlined. Finally, a brief summary of applications of monoclonal antibodies for therapy and diagnosis is provided to indicate the monoclonal antibody products that may be licensed in the near future. Currently licensed monoclonal antibody *in vitro* diagnostic agents are listed in the appendix to this review.

2. Theoretical Aspects of Monoclonal Antibody Production

Antibodies belong to that class of serum proteins called gamma globulins and are therefore also called immunoglobulins. An immunoglobulin consists of two types of polypeptide chains held together by disulphide bonds: heavy chains of approximately 55,000 daltons and light chains of approximately 25,000 daltons. The prototypic structure of an immunoglobulin molecule consists of two identical light chains and two identical heavy chains joined by disulphide bonds. Each heavy and light chain is divided into two functionally distinct

regions. The amino terminal 100 amino acids of the heavy (H) and light (L) chain is called the variable region. It contains a larger degree of amino acid sequence heterogeneity as well as the residues that make contact with antigen. The carboxy terminal constant portion of both heavy and light chains fall into one of a limited number of sequences: two types of light chains and eight types of heavy chains. There are five known classes, or isotypes, of immunoglobulin in humans: IgM, IgD, IgG, IgA and IgE. The immunoglobulin class is determined by the type of heavy chain, μ, δ, α, γ, ϵ, respectively, corresponding to the aforementioned classes. There are two types of light chain, κ, λ, for all immunoglobulin classes. The constant regions are responsible for antibody's role in eliminating foreign antigens by a variety of mechanisms including complement fixation, increased phagocytosis by macropnages and aggregation and precipitation of antigens.

IgG is the major immunoglobulin in mammalian serum and its structure can serve as a model for other immunoglobulins. On electron microscopy it can be seen to be a Y shaped molecule. If pure IgG is treated with the proteolytic enzyme papain, it splits into three approximately equal-sized fragments. Two of these fragments retain the ability to bind antigen and are called therefore antigen binding fragments (Fab). The

third fragment is sometimes crystallizable and is called the Fc fragment. The two Fab regions form the "arms" of the Y shaped molecule, the "tail" of the Y forms the Fc region. IgM is found in the second highest concentration in most mammals. Structurally, IgM is formed by 5 basic immunoglobulin subunits. As a large pentamer, IgM is confined to the blood vascular system. However, in a monomeric form IgM functions as antigenic receptors on B lymphocytes. IgA is normally a dimer and is found in body secretions where it is of critical importance in protecting body surfaces against invading microorganisms. IgE is unique in that it's Fc region binds to receptors on mast cells and basophils and, together with antigen, mediates the release of inflammatory agents from these cells. IgD is primarily a cell membrane immunoglobulin found on the surface of B cells in association with IgM. It is very susceptible to proteolysis as IgD has no interchain disulphide bonds.

The genetic basis for the heterogeneity of both light and heavy chains has now been elucidated and may be explained briefly as follows. The germ-line genome contains between a few hundred and a few thousand light chain variable region genes separated from the constant region gene by more than 10 kilobases. In the case of heavy chain genes, there are also a very large number of

variable region genes separated by a large insertion from a few constant region genes. For the production of a complete light chain, a single V gene segment is selected and becomes rearranged so that it is more closely associated with a constant region gene. The same selection and rearrangement process occurs for the heavy chain variable and constant gene segments to produce the complete heavy chain. Typically any given antibody producing cell produces immunoglobulins of a single class and light chain type, a phenomenon termed allelic exclusion.

Thus, the lymphoid stem cells in the bone marrow differentiate to produce B lymphocytes each of which is committed to respond to a single antigenic determinant. When an animal is injected with a heterogeneous antigenic mixture, B lymphocytes of the appropriate specificity bind to different molecules of the injected mixture, as well as different determinants (epitopes) on a single antigen molecule. The binding of antigen triggers the B lymphocytes to clonally proliferate and differentiate into antibody producing plasma cells. The antibody specificity is identical to that of the antigen receptor of the stimulated parent B lymphocyte. Each B lymphocyte and its progeny produces a single homogeneous antibody. However, since the serum antibody response is the sum of the responses of all the B lymphocyte clones

directed against the different antigenic determinants, the response is polyclonal (heterogeneous) instead of monoclonal (homogeneous).

Normal antibody producing cells cannot be maintained in continuous culture to produce commercially useful amounts of antibody. By contrast, myelomas are antibody forming cells which have undergone a malignant transformation and may be maintained in large scale, continuous culture. Importantly, they may secrete significant quantities of immunoglobulin. When cloned, the myeloma cell line can secrete monoclonal immunoglobulins of a single homogeneous amino acid sequence and binding specificity. However, the antigenic specificity of these monoclonal immunoglobulins is fixed and rarely known. Typically they are without diagnostic or therapeutic value.

In 1973 Cotton and Milstein studied the expression of immunoglobulin genes in hybrid cells obtained by fusion of two myelomal cell lines producing different immunoglobulins. The genetic information from both parents was codominantly expressed by a single hybrid cell. Heavy chains and light chains from each parent combined, but there was no scrambling of variable and constant regions. These results raised the possibility that fusion of a normal lymphocyte or plasma cell with

an indefinitely proliferating myeloma cell could result in a hybrid cell with the proliferative and secretory characteristics of the myeloma parent expressing the B lymphocytes antibody specificity.

Köhler and Milstein in 1975 demonstrated the validity of this concept. They mixed mouse myeloma cells with spleen cells immunized against sheep red blood cells. The myeloma cells and spleen cells were fused using inactivated Sendai virus to produce hybrid cells, or hybridomas. Successful cell fusions were identified by the use of a selective medium that will only allow growth of fused cells. It was found that fused cells secreted immunoglobulins from both parents and some of them secreted antibody against sheep red blood cells. They had for the first time developed continuous cultures of fused antibody secreting cells of preselected specificity.

3. Practical Aspects of Monoclonal Antibody Production

3.1. Choice of normal lymphocyte donor

Strong antibody responses are most easily obtained when the immunizing antigen differs structurally from the normal biologic components of the recipient. This is usually achieved by immunizing across a species barrier. In most situations *in vivo* immunization is carried out in the mouse or rat. This choice is based on convenience and the availability of appropriate

myeloma cell lines. In vitro immunizations are done when antigen is severely limiting or when a fusion with a human myeloma is required.

The choice of lymphocyte donor is not only governed by the ability to produce a good antibody response, the lymphocyte donor chosen, must, in most cases, be histocompatible with the strain in which the hybridoma will ultimately be grown. When mouse myeloma cell line of BALB/c origin are to be used, histocompatible BALB/c mice are then used for the initial immunization. Later, BALB/c mice can be used for carrying the hybridoma for bulk monoclonal antibody production. Rat myelomas suitable for fusion are of lou origin, and the hybridoma may then be carried by histocompatible lou rats.

Some success has been obtained in generating hybridomas from human lymphocytes (Olsson and Kaplan, 1980; Croce et al. 1980). These hybridomas relied on the identification of patients known to possess lymphocytes secreting antibody of a particular specificity. However, human B lymphocytes producing immunoglobulin represent a very small percentage of circulating lymphocytes compared with the spleen cells of a hyperimmunized mouse (Abrams, 1983). Enrichment for antigen specific lymphocytes is used to increase the relative proportion of specific antibody secreting lymphocytes. A variety of methods is available, many of

which involve labelling the required cells with antigen which has been coupled to a tracer, eg. erythrocytes or fluorescent dye, to facilitate separation of the mixture. In vitro immunization techniques have been successfully utilized for animal systems and it is hoped their application will greatly widen the scope of monoclonal antibodies that can be obtained from human x human hybridomas (Reading, 1982).

The need for histocompatibility of lymphocyte donor and hybridoma host may be overcome in some situations by the use of mice that have been sublethally irradiated to reduce their immunological reactivity. Alternatively, "nude" (athymic) mice or rats may be used as hybridoma nosts. Immunocompromised rodents however, are difficult to maintain free from infection. Thus, there is the concern that infective viral or bacterial products may contaminate the hybridoma cells and subsequently the monoclonal antibodies.

3.2. Immunization protocol

Many immunization protocols have been utilized. Experience has shown that long immunization schedules with multiple doses of antigen are rarely necessary. The yield of successful antibody producing cell fusions seems to be best when the time between the last immunization and the time of fusion is short i.e. several days. Immunogenicity is often increased when

the antigen is in an aggregated form. For water soluble antigens the use of an adjuvant is essential. Freund's complete adjuvant is most widely used. If the antigen is of low molecular weight such as some drug molecules; with little or no immunogenic potency, then it must be conjugated to an immunogenic carrier in order to elicit an antibody response. An alternate adjuvant system involves precipitation of protein antigen absorbed to alum (Chase, 1967). Intact living cells are highly immunogenic and do not usually require the use of adjuvants.

3.3 The Myeloma Cell

Not all cultured myelomas are capable of forming hybridomas. Even certain subcultures of known good lines have given poor results. Thus far the most well utilized mouse cell lines have been derived from MOPC-21 or MPC-11; both are murine plasmacytomas of BALB/c origin. These lines, which secrete IgG1 and IgG2b respectively, have been adapted to tissue culture for many years. Hybrid molecules will secrete all possible combinations of light and heavy chains. Thus, a fusion product of a secreting plasmacytoma with a spleen cell will produce a significant frequency of cell fusions producing the immunoglobulin of the plasmacytoma, not of the monoclonal antibody specificity desired.Variants of the MOPC-21 now exist in which partial or total loss of

synthesis of myeloma protein has occurred. P3-NS1-Ag4-1 has lost heavy chain synthesis but retains light chain production. Kearney et al., 1979, first described a subclone of P3-X63-Ag8 which does not express immunoglobulin heavy or light chains. This clone, X63-Ag8.653, can be used for efficient fusion with antibody forming cells to obtain hybrid cell lines producing pure monoclonal antibody. Lines which are nonsecretors facilitate selection of antibody forming clones since all immunoglobulin secretors are successful fusion products with antibody producing cells from the immunized animal.

Another widely used line is Sp 2/0-Ag-14 (abbreviated Sp2) which is another nonproductive variant selected from a hybridoma involving fusion of MOPC-21 and BALB/c spleen cells. This particular variant requires carefully controlled growth media. It is intolerant to alkaline medium in which it gives a low fusion frequency. A variant of Sp2 termed 'FO' has been produced by Fazeckas and Scheidigger (1980) with claims for particularly rapid growth, high fusion frequency and good cloning efficiency.

Rat myeloma cell lines which have been shown very useful for fusion are derived from the LOU/c strain of rats (Bazin, 1981). These include Y3-Ag-123 (abbreviated Y3) which secretes K (Kappa) chains

(Galfré, 1979) and the total non producer YB2/0, (Galfré and Milstein, 1981), which was derived from a fusion between Y3 myeloma cells and spleen cells from an AO rat. The advantage of rat myeloma cell lines is that larger amounts of antibody can be produced in ascitic fluid *in vivo* (Clark, 1983) Clark *et al.*, (1983), also report that rat x rat fusions result in 90-99% of hybrids secreting spleen cell derived antibody, compared with 60% of hybrids in mouse x mouse fusions. Rat x rat fusions yield a large number of antibodies which fix human complement, which may be of great importance for their therapeutic use. Despite these advantages of rat x rat fusions, several factors occasionally hamper *in vivo* production of rat monoclonal antibodies. Firstly, some transplanted hybridomas do not secrete the expected monoclonal antibody, and some hybridomas do not grow when transplanted into fully histocompatible recipients or athymic rats (Hirsch *et al.*, 1985).

Human myeloma lines have been disappointing in regard to hybrid formation, antibody secretion, and stability in comparison with their murine counterparts (Abrams *et al.*, 1983). A distinction needs to be made between Epstein Barr virus (EBV) transformed lymphoblastoid cell lines and a true myeloma. A true myeloma secretes large amounts of immunoglobulin of the same idiotype and heavy and light chain isotype as the

paraprotein in the patients serum. A true myeloma lacks also the EBV nuclear antigen. Human myeloma cell lines suitable for fusion include U-266 (SK-007) (Olsson and Kaplan, 1980) and Karpas 707 (Karpas, 1982). Lymphoblastoid cell lines which show high rates of hybrid formation are UC729-6 and UC729-HF2 (Abrams et al., 1983). An exciting recent development is the construction of chimeric antibodies in which the antigen recognizing variable regions of mouse antibodies are joined with the constant regions of human antibodies. These antibodies may be less likely to produce an immune response in humans than the all-mouse monoclonals. The production of chimeric antibodies involves transfection of immunoglobulin genes into lymphoid cells to produce transfectomas. By means of standard DNA techniques it is possible to attach any variable region to any heavy chain constant region. The chimeric gene is then transfected into a lymphoid cell and the transfected gene expressed (Morrison, 1985, Marx, 1985). Expression of Ig genes and synthesis of Ig molecules in bacteria provide an alternative to expression in lymphoid cells. Plasmids have been constructed that direct the synthesis of heavy chains and/or light chains of anticarcinoembryonic antigen antibody. However, assembly of the light and heavy chains into functional immunoglobulin molecules does not occur in bacterial

cells. (Cabilly, 1984). Conditions for in vivo assembly of heavy and light chains in eukaryotic cells are being investigated.

The myeloma cells from any source are usually cultured in Dulbecco's modified Eagle's medium (DME) or RPM 1-1640. The two media are essentially similar; RPM 1-1640 has no pyruvate, and DME has no asparagine. Neither of these ingredients is essential for hybridomas. Most culture media are buffered with HCO_3^- /CO_2. Streptomycin and penicillin at a dose of 100 µg/ml are often added. Tissue culture medium should be stored protected from direct sunlight or room fluorescent light, because light generates highly toxic photoproducts (Griffin, 1981). Fetal calf serum is usually added. The precise ingredients in the serum which make cells grow are poorly understood, but 10-15% fetal calf serum is generally added to the bulk medium and the batch of serum chosen is such that it is capable of supporting the growth of the myeloma cells at 1 cell per well. Some success has been obtained in growing hybridomas in serum free media, (Chang et al. 1980, Murakami et al. 1982), but the methods are not yet widely applicable.

It is important to store some representative vials of each batch of myeloma cells in liquid nitrogen to be used as safeguard resources in the event of

contamination or other problems which may be encountered during processing. It is also important to watch carefully the cell density of cultures. If cells become too dense, their viability decreases.

In addition to their immunoglobulin secreting character, myeloma cell lines must be chosen so that the hybrids may be selected from unfused myeloma and spleen cells. (This process is described in section 3.5.)

3.4 Fusion. In the original work of Köhler and Milstein, Sendai virus was used to promote cell fusion. Now, virtually all fusion protocols use polyethyiene glycol (PEG), which has a higher fusion frequency and greater reproducibility. PEG at a concentration of 30-50% is usually used. The molecular weight of PEG normally employed is 1000, 1500 or 4000. The limitation as to the molecular weight used is the high viscosity with higher molecular weight. It is important to verify the suitability of the PEG batch for good fusion and cell viability. Fusion frequency depends on pH and time of exposure to PEG. Sharon et al., 1980, showed maximal numbers of hybridomas were obtained at pH 8.0-8.2. The optimal time of exposure depends upon the PEG concentration, lower concentrations can be tolerated for longer periods of time before toxicity develops. It is important that myeloma cells are harvested for fusion

while in the logarithmic stage of growth. If viability is less than 98-99%, chances of success are diminished.

Other methods which may be useful for hybridoma production are those in which cells are fused in electric fields (Zimmerman et al., 1981, Van Brunt, 1985). There are now three main "electro"-technologies aimed at improving fusion efficiency: the Zimmerman electrofusion system, the Nova/Hopkins avidin-biotin bridge technique, and the plasmid-insertion system developed at Techniclone International (Santa Ana, CA). The Zimmerman system involves exposing cells to a nonhomogeneous, high frequency electric field. This alternating current field generates dipoles within the cells and attractions between the cells then orient the cells in a chain. A short direct current pulse then opens membrane pores in aligned cells and they fuse. Mouse and human cells need to be pretreated with enzymes such as pronase, neuramidase, or dispase to achieve membrane contact. The Nova/John Hopkins technique takes advantage of the specific immunoglobulin expressed on the surface of B cells. Myeloma and B cell pairs are aligned via an antigen-avidin biotin bridge; the fusion takes place after a series of short (5 microsec.), high voltage pulses. The "Electrogene" technique developed at Techniclone eliminates the myelomas cell by introducing immortality genes directly. The myc gene

from human malignant cells is cloned and inserted into a plasmid. A high voltage electric pulse introduces the plasmid into antibody producing lymphocyte. The resultant human cell line has been stable for more than eight months.

Only the Zimmerman system has been available long enough for critical assessment. The technique seems more successful for fusing plant cells. Difficulty has been experienced in growing human or murine cells after fusion. As these techniques are more widely applied and refined, their true potential will become apparent.

3.5 Hypoxanthine, Aminopterin and Thymidine (HAT) selection. If tumor cells are fused with normal cells, the culture will rapidly be overgrown by unfused tumor cells. A selection method is therefore required to allow growth of hybrids alone. The rationale for the almost universally used HAT by hypoxanthine, aminopterin and thymidine selection is as described below.

The main biosynthetic pathways for purines and pyrimidines can be blocked by the folic acid antagonist aminopterin. However, the cell can synthesize DNA via the "salvage" pathway, in which preformed nucleotides are recycled. These pathways depend on the enzymes thymidine kinase (TK) and hypoxanthine phosphoribosyl transference (HPRT). Thus, if the cell is provided with thymidine and hypoxanthine, DNA synthesis can occur,

provided the enzymes TK and HPRT are present. If these enzymes are lacking, cells may "pick up" the missing enzymes by fusion with another cell which possesses the relevant enzymes. If spleen cells, which possess TK and HPRT but die in culture, are fused with myeloma cells lacking TK and HPRT, only the hybrid cells will grow in medium containing hypoxanthine, aminopterin, and thymidine (HAT medium).

Mutant myeloma cells lacking TK or HPRT are produced by using toxic drugs. For example, thioguanine is incorporated into DNA via HPRT, resulting in death of HPRT positive cells. Only HPRT negative cells will survive thioguanine selection.

HAT selection is usually begun the day after fusion. After a few days of HAT selection, massive cell death will be observed and the medium will change from pink to yellow. After about 11 days, HT medium is added every 3 days to ensure adequate levels of hypoxanthine and thymidine while aminopterin is being removed by dilution.

One method being examined to increase the efficiency of myeloma spleen cell hybridization is to incorporate insulin into the HAT medium (Bartal, Feit and Hirsnaut, 1983). A significant rise in the number of antibody secreting clones was observed in the presence of HIAT compared to HAT medium alone. The

maximal effect of insulin on the above biological parameters ranged from 10^{-1} and 10^{-4} units/mL. This method has not been widely adopted. However, if insulin were to be employed in monoclonal antibody production, assurances would be needed that it is removed from the final product.

Areas where variation exists in the methodology concerns the feeding of cultures after fusion, and the use of feeder cells. Feeding involves removal of half the culture medium by suction, followed by its replacement with fresh medium. This process gradually dilutes out any antibody made by normal antibody-secreting cells and also removes waste products and replenishes nutrients.

Feeder cells are a slow growing or nongrowing population of cells which may be added to the culture medium. Although the mechanism is not understood, these cells are believed to make some factor needed for growth. Commonly used feeder cells are thymocytes, normal spleen cells or peritoneal cells. Endothelial growth cell supplement (ECGS) is now a popular alternative to the use of feeder cells. ECGS is a commercially marketed extract of bovine neural tissue which has been shown to be superior to murine peritoneal cells in supporting the growth of hybridoma colonies (Pintus et al., 1983).

After a week in HAT medium it is safe to assume that all the parental myeloma cells are dead, and any growing cells are hybrids.

3.6 Screening procedures

A rapid screening technique for antibody forming colonies is one of the essential requirements of monoclonal antibody production. Since newly formed hybridoma clones may be unstable, speed and economy of assay procedures are as important as accuracy and sensitivity. If cells are allowed to overgrow, the resulting selective pressure will often result in overgrowth of nonproducing cells. The screening assay should be set up and optimized well before fusion is started, because there is not enough time to eliminate problems later.

The most commonly employed screening assays are solid-phase radioimmunoassays, enzyme linked immunosorbent assays (ELISA), immunofluorescence screening and cytotoxicity assays. Initially, it is important to identify antibody secretion so a non antigen specific assay is used, such as a binding assay based on Staphylococcus aureus protein A which is selective for IgG class antibodies. Then, an assay selective for a particular antigen specificity is used.

The solid-phase radioimmunoassay (RIA) is based on the fact that polyvinyl surfaces will tightly adsorb

nanogram amounts of most proteins. The antigen is therefore bound to the solid surface and unreacted binding sites saturated with a large excess of an irrelevant protein, usually bovine serum albumin. The antibody is then added and left to bind the antigen. Finally, an ^{125}I labeled affinity purified antimmunoglobulin or ^{125}I labeled staphylococcal protein A is added which detects the presence of antibody bound to the antigen.

The ELISA is the most commonly used screening method. It is based on a principle similar to the RIA. In this case however an enzyme such as peroxidase, alkaline phosphatase or B-galactosidase is coupled to the anti-Ig antibody instead of the radiolabel. Thus, the anti-Ig antibody detects the binding of hybridoma antibody to the surface bound antigen. The amount of hybridoma antibody is then revealed by a color change upon addition of the appropriate substrate. The advantage of the ELISA is that no isotopes or scintillation counters are required, and positive wells may be detected by eye.

The presence of antibody may also be detected using fluorescein conjugated anti-Ig antibodies. Cells are then examined for antibody binding using a microscope with appropriate illumination and filters. A fluorescence activated cell sorter (FACS) may be used to

separate those positive populations of cells to which the fluorescein tagged antibody binds.

Cytotoxicity assays are used usually only in those instances where cytotoxic antibodies are of interest. A two step assay is often used in which antibody is bound to cells; unbound antibody is washed away, complement is added and cells are lysed. Cr-release is typically used as a marker of cell death.

3.7 Cloning and Preservation of Hybridomas

Once a cell culture supernatant is identified as positive for either antibody or immunoglobulin, it is essential to clone the producing cells. This step is important because nonproducers typically grow more rapidly than producing cells, and, if not removed, will eventually overgrow the culture. In addition, cloning insures that the final antibody will be homogeneous and monospecific.

Cells may be cloned in soft agar. The clones appear as small colonies since the agar prevents cell migration. Feeder cells may be added (Galfré and Milstein, 1981). Soft agar cloning is then followed by regrowth of the clone in liquid medium after which antibody production can be assessed. In contrast, cloning by limit dilution allows direct testing of supernatants, and is a preferred method of cloning. Typically hybridoma cells are cultured in a 96 well

plate, each well may contain 10^6 thymocytes as feeder cells. Ideally, the number of cells per well is chosen such that 30-50% of cells show no growth. At this concentration most positive wells will consist of a single clone. Each well must be assayed individually for antibody, but not all wells with growth will be active. The line should be recloned again to insure homogeneity. Periodic recloning is important if the culture is maintained by continuous passage.

A third method of cloning involves cell separation by use of the fluorescence activated cell sorter (Parks, 1979, Beverly, 1982). The instrumentation is expensive and thus available in only a few laboratories.

Once useful hybridomas have been identified and cloned, they must be grown in larger numbers for preservation of the cell line and for antibody production. The possibility of the spontaneous development of nonproducer variants never completely ceases. It is absolutely necessary therefore to prepare frozen stocks of hybridomas at the earliest possible time.

A suitable media in which to freeze the cells for long term storage can be prepared by adding dimethyl sulphoxide (DMSO) dropwise to ice cold fetal calf serum (100%) with shaking to a final concentration of 10% DMSO. The solution is then cooled back to $4^{\circ}C$ in ice.

The cells are spun down at 300 x gravity at 4°C and resuspended at 5 x 10^6 per ml in cold fetal calf serum. An equal volume of the DMSO/Fetal calf serum mixture is added and mixed with the cells and 1 ml aliquots pipetted into sterile freezing ampules. These should be immediately either frozen down in a controlled rate freezer at 1° per minute, or simply placed in a polystyrene box in a -70°C freezer overnight. The ampules are then maintained at -70°C. An ampule from the lot should then be thawed, regrown and tested to insure proper recovery of the frozen cell stocks. Such a system protects against events which might otherwise result in the complete loss of the hybridoma cell line.

Supernatants from continuously grown cells should be tested for antibody at frequent intervals. Typical antibody levels in a mature culture supernatant are 5-50 µg/ml, depending on the individual clone, cell density and time from the last media change. A falling antibody level should be taken as a signal of a problem with culture additives or of the need for recloning. Hybridoma cells may be frozen in liquid nitrogen and stored for several years with good recovery.

4.0 Large Scale Growth of Cells

4.1 Growth of Cells in Vitro

Large scale tissue culture techniques are the most important method of bulk production of monoclonal

antibodies. In vitro propagation methods can be better defined and lead to the production of better defined products than the in vivo technique. For human hybridomas growth in tissue culture overcomes the histocompatibility requirement for hybridoma growth in vivo. Large scale tissue culture techniques are also advantageous because of the need for daily care and handling of a large size mouse colony when very large quantities of monoclonal antibody are required. The size of the culture vessel can be scaled-up, allowing one to benefit from economies of scale, especially in relation to labor and capital costs. Large quantities of antibody are therefore produced more economically by this route (Duff, 1985). The risk of contamination by extraneous antibodies or adventitious agents of rodent origin is also reduced in cell culture.

Research to improve tissue culture techniques for monoclonal antibody production has three main aims.

a. to increase the yield per cell;
b. the suppression of extraneous proteins needed for cell growth in tissue culture;
c. production of antibodies in large culture vessels. (Bussard, 1984)

The production of monoclonal antibody by cells is variable, ranging from 5 to 100μg of antibody per 10^6 cells per 24 hours. This corresponds to a mean

synthesis of 200-4000 IgG molecules/cell/second. This figure is reasonably an underestimate as the synthetic rate per cell varies depending on the cells position in the cell cycle and not all cells synthesize IgG at a given time.

A typical growth medium for large scale culture is Dulbecco's modified Eagle's medium with high glucose, supplemented with 10-15% fetal calf serum. With a monoclonal antibody concentration of the order of 10-50 µg/ml, there will be 6000 µg/ml of total proteins and 300 µg/ml of immunoglobulin from the fetal calf serum. The monoclonal antibody thus represents only 3 to 15% of the total immunoglobulin in tissue culture fluid. The fetal calf serum is a complex mixture of components which will vary in its suitability for supporting cell growth and antibody production. Each lot must be individually assayed to insure its suitability before use. In order to eliminate this source of variability and to simpligy purification of the monoclonal antibody, efforts have been made to develop serum free culture media (Chang, 1980, Murakami, 1982).

Essential requirements for serum free growth appear to be insulin and transferrin at a physiological concentration of 5 µg/ml each. Murakami et al., 1982, also demonstrated a requirement for ethanolamine. They developed their media with the hybridoma line MPC11-BC.

After one step ammonium sulfate precipitation of the spent medium, more than 95% of the protein recovered was immunoglobulin, as shown by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE). Serum free media such as that described above, are being widely used. The advantages of a defined media arise from the simplification of the media production and the production of relatively pure antibody.

Tissue cultures of hybridomas may be expanded to very large sizes. Up to 200 ml cultures may be grown in flat flasks, but for larger cultures roller bottles are used. Spinner bottles with capacities of from 25ml to 15L are available for laboratory application, and large scale reactors (1000L) have been developed by modifying fermenters in which microorganisms are grown (Birch, 1985). These systems expose the hybridoma cell to a constantly changing environment with a gradual depletion of nutrients and an increase in metabolic products. Perfusion systems in which depleted medium is constantly removed and fresh medium added are therefore preferred. Perfusion reactors use special sensors, sampling ports, and input and output piping to maintain pH, oxygen, carbon dioxide, and nutrients at the proper level (de la Lera Moya et al., 1984). Such systems permit higher cell densities, which is important as lymphoid cells do not grow well at low densities. The concentration of

secreted monoclonal antibody is greater in perfusion reactors, having approached 200 µg/ml.

Recently, the application of microencapsulation technology to the culture of hybridoma cells has gained increasing recognition as a method for commercial scale production of monoclonal antibodies (Duff, 1988). Hybridomas are encapsulated under physiological conditions using mild chemicals and reactions. The microcapsules can be transferred to a reactor and nutrients and oxygen diffuse across the capsule membrane while contaminating proteins including nonspecific immunoglobulins are excluded. Using this method very high densities of hybridoma cells have been grown, resulting in high concentrations of monoclonal antibodies. Microencapsulation also permits purification of the monoclonal antibody before harvest. Before harvesting, culture solution that surrounds the microcapsules is replaced with a saline solution which both washes the outside of the capsules and removes contaminants inside the capsules by dialysis. The capsules are then opened physically to harvest the antibody. At that time, monoclonal antibody concentration is 45-80% of the total protein present - a much higher concentration of antibody than has been possible using other large scale culture methods.

Another technique under investigation for production of monoclonal antibodies involves entrapment of cells within agarose microbeads. In this case, immunoglobulin produced is exported through the microbeads into the culture medium. Compared with the conventional suspension cultures. This technique allows the construction of efficient systems which can produce higher concentrations of immunoglobin (Nilson, 1983). In contrast to the microencapsulation technique however, purification of the monoclonal antibody from the culture medium is required. The growth and monoclonal antibody production of human and murine hybridomas in an artificial capillary culture system (ACCS) have also been investigated (Weimann, 1983). These porous capillaries retain the hybridoma cells which release secreted monoclonal antibody into the extracapillary fluid. The grown of hybridomas in the ACCS results in yields of monoclonal antibody comparable with that obtained by in vivo culture (6-8 mg/ml). The monoclonal antibodies produced by this method will also be uncontaminated by murine proteins.

4.2. Growth of cells in vivo

The production of monoclonal antibodies in vivo by harvesting ascitic fluid in hybridoma implanted animals is a very common procedure due to the high concentration of monoclonal antibody obtainable. Antibody levels in

serum or ascitic fluid can typically reach 5-15 mg/ml. This represents 16 to 30% of the total mouse proteins and 50-70% of the mouse IgG.

Cells may be administered subcutaneously or intraperitoneally to a histocompatible host. A typical dose is 10^7 cells. Subcutaneous injection has the advantage that tumor growth is easily observed, but intraperitoneal injection is preferred. Implantation of the tumor is more successful and ascitis production is increased by injection of 0.5 ml pristane intraperitoneally, 1 week prior to injection of the cells. Tumor growth causes the production of ascites (5-50 ml per mouse) which needs to be drained every few days. Tumor overgrowth usually results in the death of the mice in one to three weeks.

Intraperitoneal ascites should be examined by electrophoresis on agarase or cellulose acetate strips for the presence of a "spike" of monoclonal antibody in the gamma globulin region. It is then stored in small aliquots, preferably at -70°c. It should not be subjected to multiple freeze-thaw cycles, because these may cause denaturation of some immunoglobulin classes, especially IgM.

In large scale in vivo production of monoclonal antibodies the only limit to the quantity of antibody that can be produced is the number of mice that can be

housed in a production facility at any one time. Hybridoma cells have an average generation time of 12 to 16 hours, so that propagation of sufficient numbers of cells for mouse innoculation is not difficult. Once it is successfully transferred *in vivo*, it can be passaged continuously. However, periodic *in vitro* cloning is important to insure that the line maintains its characteristics.

Growth of hybridomas *in vivo* is associated with problems such as lack of defined and well controlled conditions, the need for animal housing facilities and adequate attention to animal husbandry. The concentration of monoclonal antibody in ascites can easily be a thousand fold higher than in tissue culture supernatants. However, production methods which use hollow fibers or microencapsulation of hybridomas greatly increase the cell density and therefore monoclonal antibody concentration obtainable by *in vitro* culture. *In vitro* production has several distinct advantages which provide a strong impetous for continued improvement of efficient *in vitro* production methods.

4.5. *Factors which affect antibody yield.*

Cell hybrids almost always have a tendency to lose chromosomes. Sometimes the loss is preferentially from one parental cell, as is commonly the case in

interspecies hybridomas. Hybrids differ in stability so even with intensive care and repeated cloning, some lines will lose their capacity to produce antibody. Loss also depends on the isotype of the antibody produced. It is realistic to expect that 50-70% of all cultured cells which are initially positive with respect to antibody production will be lost (Goding, 1982). Recloning periodically will usually help insure the stability of a particular clone, but the risk of loss of production is never eliminated. Production loss may also result from inadequate growth conditions. It is therefore important to screen batches of fetal calf serum carefully to ensure proper growth support. Cell density should also be optimized for better yield. Cultures should be monitored carefully for overgrowth and expanded consistently with cell density requirements.

Mycoplasma, bacteria and fungi are known to have pathological effects on cells. Their presence will limit the yield from hybridomas as well as their conservation (Roseto et al., 1984). Mycoplasma contamination is particularly troublesome and elimination of the infection is not easy. Some random surveys show incidences of infection of greater than 50% in U.S. laboratories (Goding, 1983).

About 25 Mycoplasma and Acholeplasma species have been identified as cell culture contaminants but the most frequent are Mycoplasma hyorhinis, Mycoplasma orale, Mycoplasma arginini and Acholeplasma laidlaii (Marcus et al., 1980). These species are responsible for almost 85% of the contaminations in cell cultures. The current belief is that the contamination is adventitious. Primary sources are bovine (fetal calf serum) or porcine and not the tissue of origin of the cells (Goding, 1983). Better quality control of fetal calf serum by suppliers and routine inactivation of the serum (56°C for 1 hour) before use seems to reduce the incidence of contamination. Laboratory workers handling cultures are more likely to be a source of contamination. Strict personnel control and aseptic technique should be employed to avoid contamination, to minimize mycoplasma infection and to insure the sterility of the cell line as a whole.

Identification of a mycoplasma infection may be achieved by microscopic examination of cell culture (Roseto et al., 1984) or by staining with the DNA specific fluorescent dye, Hoechst 33258, (Russel et al., 1975). The Food and Drug Administration requires microscopic examination of cells used to produce biological products to detect mycoplasma contamination (CFR, Title 21, Subpart D, 610.30). Methods which have

been employed to try to eliminate mycoplasma infection include the use of antibodies, cultivation with macrophages (Schimmelpfeng et al., 1980), passage through nude mice, and selective DNA breakage with 5-bromouracil and visible light (Marcus et al., 1980). Treatment with anti-fungal agents often leads to the development of resistance and may mask low grade infection, so is best avoided. Passage of cells through mice or co-cultivation with macrophages is a laborious process not amenable to large numbers of contaminated hybrids.

The use of antifungal agents in tissue culture is not recommended because the toxic concentration is close to the therapeutic concentration, although some have found amphotericin B, 10 µg/mL, to be useful. Other investigators have found that cultures may be protected from airborne fungal spores by wrapping them in kitchen plastic wrap; apparently, sufficient gas exchange occurs through the plastic (Goding, 1982).

In general, prevention is preferable to cure. The factors which may adversely affect yield, described in this section, again emphasize the importance of maintaining adequate frozen stocks of cell lines.

4.4 Purification of monoclonal antibodies. The degree of purity required of a monoclonal antibody product depends upon its intended application. For example, if

fluorescein conjugated anti-immunoglobulin is used as a second step in indirect immunofluorescence experiments there is little reason to purify the first antibody. If monoclonal antibodies are required for direct immunoassays, for affinity chromatography, or for an *in vivo* application the demands for purity are greater.

The level of purity of the monoclonal antibody at the production stage depends on the method of production. Typically, crude tissue culture fluid could be used without further purification for immunoassays. This is common practice in many instances (Bussard, 1984). When hybridomas are grown *in vivo*, the presence of normal murine proteins as well as normal murine immunoglobulin usually necessitates further purification. The method chosen to purify the monoclonal antibody depends both on the antibody isotype and the antigenic specificity.

The simplest way to purify hybridoma antibodies is by affinity chromatography. If the antigen against which the antibody is directed is available in a suitable form, it may be coupled to cyanogen-bromide-activated Sepharose beads. Serum, ascites or tissue culture fluid may then be passed through the column and eluted with a variety of agents, such as acid, potassium thiocyanate, urea or guanidine hydrochloride. Where the immunizing hapten or a related

one is used to elute the antibody the purification process must be able to exclude the hapten ultimately from the final preparation.

Affinity chromatography requires that the antigen be present in an appropriate form and in sufficient quantities. Where the antigen itself is not suitable for affinity chromatography, a monoclonal antibody i.e., antiidotype against the second monoclonal antibody to be purified may be used. However, for large scale purification, the quantity of monoclonal antibody required for purification may still be too large to provide economical purification. It has been estimated that it would require 400 miced to produce enough antibody to load a one-liter column with a coupling density equal to 10 mgs of protein per ml (Van Brunt, 1985). Alternatively, antibodies of the appropriate class (IgG2a, IgG2b and IgG3 in the mouse) may be purified by affinity chromatography using a staphylococcal protein A Sepharose column. Binding to protein A is highly pH dependent, and it is worthwhile to optimize the conditions of elution.

Monoclonal antibodies may be purified by other methods indluding zone electrophoresis, ion exchange chromatography, chromatofocussing and gel permeation. Zone electrophoresis is now rarely used for immunoglobulin purification, because it is slow and has

a limited capacity. Ion exchange chromatography is a more popular method. It is a gentle technique with a good yield and a high level of purity is obtained. The most commonly used matrix consists of cellulose or agarose to which is attached ionizable diethylaminoethyl (DEAE) groups, which are anion exchangers. The antibodies are generally eluted by the addition of competing anions, with increasing concentrations of eluting anion, proteins are eluted in order of their isoelectric points. Gel filtration separates proteins according to their size. Its main role is as a crude preparative technique as it can not discriminate the monoclonal antibody from the bulk of normal murine immunoglobulins as do ion exchange or affinity chromatography.

For the production of monoclonal antibodies for therapeutic purposes the method of purification is not as important as validation of the purification process. The purification should be able to remove proteins which may cause an adverse reaction in a patient, and the process must be demonstrated to remove such contaminants.

5. Concerns in Assessment of the Safety of Monoclonal Antibodies.

The safety of a monoclonal antibody product for in vivo applications arises from two sources: the method of

production and the specificity of the antibody itself. The production method raises two general classes of safety issues (1) monoclonal antibodies are the products of malignant cells and so there is a theoretical risk of transferring in the product viruses or nucleic acids associated with malignancy, and (2) propagation of the hybridoma in vivo involves the use of animals which may harbor a number of microbial or viral agents, some of which could potentially produce diseases in humans. There is also the possibility that a virus, fungal or bacterial product might be introduced into the cell culture at any point in its in vitro processing.

5.1 Presence, detection and risk of residual cellular DNA. The chief concern regarding the application of continuous cell lines in the production of biologicals for human use, is that the final preparations might contain potentially oncogenic material derived from the substrate cells. In assessing the risk associated with residual cellular DNA the two key issues are (1) the amount of genetic material present in the product after the purification process, (2) the ability of the genetic material to cause a malignant transformation and (3) the amount of material which would make such a transformation likely.

The first issue is the simpler to assess. Many techniques are now available which allow validation of

the ability of the purification process to remove residual cellular DNA from the final product. The use of a sensitive test to validate removal of contaminating nucleic acids would minimize the theoretical risk associated with the introduction of DNA into the patient.

To show that the purification procedure removes DNA and RNA the hybridoma cell line may be incubated with radiolabeled nucleotides, such as ^{14}C-uridine or ^{14}C-thymidine (Spitler, 1984). After the defined purification procedure, radioactivity in the antibody fraction of the effluent would reveal DNA or RNA contamination. A more efficient procedure is the use of DNA probes generated by nick translation (van Wezel et al., 1981, Chou and Merigan, 1983). Nick translation is a simple enzymic reaction in which a piece of DNA is treated with DNase-1 to generate nicks randomly throughout the molecule. One then extends the nicks using DNA polymerase-1 from E.coli. The DNA is then radiolabeled in vitro by any one of the four ^{32}P - radiolabeled deoxynucleoside 5' triphosphates. These radiolabeled probes may be added to hybridoma cells and will reanneal with any homologous DNA present. Residual radioactivity tested after purification will indicate nucleic acid contamination.

Sensitive tests for polynucleotides which have been developed include dye-binding fluorescence enhancement assays (Kapúsiński and Skoczycas and James, 1977, Brunck, 1979, Labarca and Paigen, 1980), or hybridization using nick translated hybridoma cell DNA (van Wezel et al., 1982, Chou and Merigan, 1983). The former method is based on the finding that reaction of certain compounds with nucleic acids results in changes in their fluorescence. Recently, two new fluorchromes, DAPI (4',6' - diamidino-2-phenylindole) and compound Hoechst 33258 (2-[2-(4-hydroxy-phenyl)-6-benzimidazolyl]-6-(1-methyl-4piperazyl)-benzimidazol.3H-Cl), have been used with success for the fluorescent staining of DNA in cells. The fluorescence enhancement induced by these reagents is specific for DNA, although some intercalation with RNA occurs. Fluorescence of RNA is substantially higher using Hoechst H33258 than with DAPI, but the fluorescence enhancement relative to DNA is still below 1% (Brunk et al., 1979). DAPI and Hoechst 33258 binding to DNA appears to be highly specific for adenine-thyimidine base pairs (Kapúsciński and Skoczylas, 1977). Brunk et al., 1979, found that this technique could be used to measure nanogram levels of DNA in cellular homogenates using standard equipment. With a more sophisticated set up and purified DNA

Kapúsciński and Skoczylas, 1977, detected 5 pg/ml of DNA as a DAPI-DNA complex.

The fluorescence enhancement which is obtained by this method depends upon such factors as pH, ionic strength and temperature. These factors should therefore be defined for optimal sensitivity and reliability. The use of an internal standard allows reliable measurement and compensates for any fluorescence quenching due to cellular components or buffer.

Another sensitive method for detecting nucleic acids has recently been described (van Wezel et al., 1981, Chou and Merigan, 1983). This method involves immobilization of the nucleic acids on nitrocellulose filters and subsequent hybridization with radiolabeled, nick translated cellular DNA as a probe. Cellular DNA may be isolated from the parent cell line and radiolabeled by nick translation, as described earlier. Test samples containing DNA are then denatured by heating or treatment with alkali. Small amounts of samples (10 μL) are applied to nitrocellulose filters (dot blotting). Immobilization of nucleic acids is obtained by heating the filters for two hours at 80°C in a vacuum oven. The homologous, highly radiolabeled DNA probe is also denatured. The nitrocellulose filters are then incubated with a solution containing the

radioactive probe under hybridization conditions. Complementary strands will form stable hybrids, thus binding radioactive DNA to the filter. Radioactivity is determined by autoradiography or by liquid scintillation counting of filter pieces cut into separate dots of sample on the filter. Simultaneous dot blotting of solutions containing known quantities of DNA and of samples to be tested permits a quantitative determination of nucleic acids in the test samples. As well as cellular DNA, this technique may be adapted for the detection of viruses, such as Epstein-Barr virus, hepatitis B virus and cytomegalovirus (Chou and Merigan, 1983), and enterotoxigenic Escherichia coli (Mosley et al., 1982), as well as many other organisms of interest. Chou and Merigan, 1983, were able to detect 5 pg of DNA using ^{32}P labeled probe DNA. By increasing the specific activity of the probe levels as low as 1 pg can be detected.

There are some problems with the use of nick translated probes. The ability to generate nick translated probes with the greatest sensitivity is not totally reproducible. Thus, some batches of nick translated probes do not perform as well as others even though the same DNA was used. Optimization of the test requires the probes to be basically of the same length as the DNA. If the probes are too short, the

hybridization signal is low. If the probes are long, the hybridization signal is good but the background is high (G. Wahl, 1984). Despite these problems DNA nybridization is a powerful technique useful for process and final product quality control.

Techniques utilizing single stranded RNA probes, generated by the SP6 system overcome some of the problems associated with DNA hybridization. The SP6 system uses an RNA polymerase promoter which is derived from a Bacillus subtilis phage called SP6. This promoter is recognized only by a polymerase which is encoded by that phage. These agents are used to generate a single stranded RNA molecule complementary to a particular DNA sequence (Wahl, 1984). Since this is a synthetic reaction as opposed to a replacement reaction that one has in nick transaction, a net yield of RNA molecules is obtained, as opposed to just an equivalence of DNA molecules in the nick translation procedure. It is possible to increase the specific radioactivity of the probe and provide a more stable hybrid between RNA and DNA, so that the annealing conditions, or the nybridization conditions, can be more stringent so reducing the background and resulting in a greater signal to noise ratio (Martin, 1984).

The more difficult problem is to assess the amount of genetic material likely to be of transformation

significance. Given that sensitive methods for detecting nucleic acid contamination are available, how sensitive do these detection methods need to be to provide assurances that nucleic acids are not present at a level likely to produce a biological effect? In short, what is an acceptable level of nucleic acid contamination? A definitive answer to this question cannot yet be given in view of the gaps in scientific knowledge as to how a normal cell is transformed into a malignant cell. However, based on the present state of knowledge and experience, certain logical concepts can be applied.

The patient population likely to receive monoclonal antibodies for cancer therapy are not 'normal' individuals. These will be patients who have active tumors, and because such tumors have developed, these patients may be more susceptible to cellular DNA with the potential for malignant transformation.

Since the presence of residual cellular DNA from normal cells has already been accepted, it is only the abnormal DNA of non-normal cells that should be of concern in most cancers (Petriccian, 1985). The most worrisome component of the cellular DNA would therefore be that of oncogenes. Twenty oncogenes have so far been identified. There are approximately 3×10^9 base pairs of total DNA in human cells. Oncogenes range in size

from about 100 base pairs, the smallest, up to 1.6 kilobases. Although oncogenes have received a great deal of interest over the past few years, there is almost certainly not sufficient evidence to explain the entire process of tumorigenesis. There are other genetic factors which at least in some cases will be of significance, such as strong promoters, gene amplification, gene activation and gene suppression (Petricciani, 1985). Nevertheless, cellular oncogenes do represent specific sequences of some tumor cell DNAs that have transforming potential, and so are identifiable pieces of abnormal DNA from abnormal cells. As such, they can be useful for assessing the biological significance of various residual cellular DNA levels.

By processes which have previously been described, new biotechnology products contain about 10 pg or less of residual cellular DNA per dose. This is equivalent to about one mammalian genome. Although specific data are not available, it is reasonable to assume that the residual cellular DNA contains random portions of the genome and that various sizes of DNA are present, without either selective elimination or selective concentration of any specific gene sequences. A "worst case" analysis may be made to assess the possible risk of 10 pg of residual cellular DNA (Petricciani, 1984, 1985). It may be assumed that atleast some of the

residual cellular DNA sequences are large enough to retain the potential for biological activity and there is a single cellular oncogene of 1 kilobase in the 10 pg of residual cellular DNA. One copy of a 1 kilobase cellular oncogene is equivalent to about 10^{-6} picograms of DNA. A variety of studies have shown that under optimal experimental conditions in vitro, purified cellular oncogene DNA has a transforming efficiency of 10^{4} focus forming units per microgram. This means that 100 pg of purified cellular oncogene DNA are needed to result in one focus of transformation in the 3T3 system. Going back to residual cellular DNA with one cellular oncogene copy, there is then a factor of 10^{-8} between a minimally effective DNA transformation dose in vitro and and the amount of cellular oncogene DNA which might theoretically be in a product. If one does similar calculations based on recent in vivo data showing that 2 micrograms of cloned viral oncogenes of DNA were required to induce tumors in chickens, the factor becomes 10^{-12}.

The anlysis of the issue of acceptable residual cellular DNA was the subject of a recent workshop on the use of abnormal cells in the production of new products (Workshop on abnormal cells, new products and risk, July 1984, DHHS, FDA). It was concluded that if the manufacturing process consistently yields a product that

contains a particular amount of DNA in the pg range per dose, it should be safe. It should be noted that 10 pg/dose limit has already been accepted for polio myelitis vaccine produced by Vero cell lines (van Metre, 1984). It was concluded that if the manufacturing process results in loss of biologically active DNA in the final product, again the product should be safe. In this case there is no upper limit to the amount of DNA that can be present, as long as it has been shown to be biologically inactive.

5.2 Viruses. The other major safety issue is the presence of endogenous and adventitious viruses. As hybridomas are often grown in mice, and the parent myeloma is usually of murine origin, all the viruses which commonly affect these laboratory animals should be considered as possible contaminants. Viruses such as Sendai virus, pneumonia virus of mice (PVM), polyoma, mouse hepatitis virus (MHV), ectromelia, lymphocyte choriomeningitis (LCM) and minute virus of mice are frequently found in mouse colonies. PVM, Sendai, MVM and MHV viruses are the most prevalent occurring in over 80% of the tested colonies. The mice used should be certified free of murine viruses. The Office of Biologics of the Food and Drug Administrations, Center for Drugs and Biologics, outline quality assurance tests for possible viral contamination in their "Points to

consider in the manufacture of monoclonal antibodies for human use". The purpose and content of this document is discussed in section 10.1.

The main concern, however, is the contamination of the final product by retroviruses. The majority of mammalian cells contain in their chromosomes genetic information related to retroviruses. In particular, the inbred strains of mice used in laboratory studies, have a number of different types of these viruses and murine cell lines frequently express virus. Retrovirus production has been noted in a number of myeloma cell lines and in hybridomas prepared from them. Therefore crude monoclonal antibody preparations may contain large numbers of these viruses.

Retroviruses have been shown to be capable of activating or of acquiring oncogenes from cells. They may also recombine with endogenous leucosis virus to produce a recombinant virus with pathogenic properties. Comparison of endogenous cell DNA and retrovirus sequences has shown that retroviruses are capable of crossing genus and even class barriers. Retroviruses that are harmless in one species may be pathogenic in another (Thornton and Nicholas, 1984, for a detailed review).

From the point of view of public health and safety, there has been room for the use of cells that contain

endogenous viruses (Petricciani, 1985). Until recently all yellow fever vaccines were produced in hen's eggs which were infected with avian leukosis virus. A large restrospective study indicated no increased health risk for cancer in the recipients of the vaccine. Nevertheless, even though safety was not an issue, the current preparations of yellow fever seed virus is avian leukosis free.

Influenza vaccine is produced in fertilized hen's eggs which contain avian leukosis viruses, but the licensed influenza vaccines are inactivated, (in contrast to the yellow fever vaccine, which contains live virus). The avian leukosis virus is killed before being administered to humans in the form of vaccine. Thus the threat posed by the potential presence of a retrovirus will depend also on the processing to which the product is subjected.

Nevertheless, because of the theoretical risk associated with introducing retroviral particles into a patient via a monoclonal antibody product,the quality assurance testing and final product quality control should take into account retroviruses as possible contaminants. As genetic information coding for retroviruses is present in all living cells, it is not possible to preclude viral production when monoclonal antibody is manufactured by the conventional procedure.

Retrovirus production can be detected in cultures of myeloma and hybridomas by electron microscopy or reverse transcriptase assay. Reverse transcriptase is an enzyme unique to retroviruses. Normal procedures for purification of monoclonal antibody are likely to destroy or remove retroviruses (Goldstein, 1984, Thornton and Nicholas, 1984). The final product may be tested by cultivation in a range of cells followed by electron microscopy or reverse transcriptase assay. Alternatively nucleic acid estimations may be conducted as described earlier.

In determining whether a monoclonal antibody product is safe for clinical trials, the FDA considers the methods that have been used to exclude nucleic acids, both cellular DNA and viral nucleic acid and whether the purification process has removed nucleic acids or has deactivated viruses. Again, risk will be weighed against potential benefit.

5.3 Protein contaminants. Monoclonal antibody production methods introduce the possibility of protein contaminants. Residual cellular proteins may come from a variety of sources which include media constituents, such as fetal calf serum, and proteins arising from affinity columns used for purification. These proteins are a potential source of risk because they may be recognized as antigens by the patient receiving the

monoclonal antibody product and stimulate immune responses. They could potentially induce tissue damage by various allergic mechanisms mediated by IgE, complement, IgG and cellular factors. Bioactive proteins or peptides derived from the cells used in the culture or from contaminating bacteria may be clinically significant. The importance of bacterial endotoxin is well recognized. A fairly large number of continuous cell lines have been found to produce one or more cytokines. These cytokines, for example, interleukein 2, if present in sufficient quantity could have direct biological effects if a patient receives therapeutic or diagnostic monoclonal antibody.

A rough guideline for the purity of the product has been adopted for the newer recombinant DNA derived products from E. coli. Approximately 100 parts per million of the protein in the product could consist of contaminants, but the majority of the protein must be the actual biological substance itself. This level of contamination has been established as a upper limit of acceptability for a very potent immunogen which had previously contaminated a number of biologics, i.e., fetal calf serum. Experience over the years has demonstrated that contamination below this level with the potent antigen has not resulted in unsafe products. This gives assurance that this is a reasonable level of protein contaminant concomitant with safety. In

addition a level of contamination of 100 parts per million is in many instances readily achievable, and is not usually an undue burden on manufacturers.

The problem with many protein contaminants is that they are difficult to measure, in so far as it may not be known exactly which proteins are likely to be present in the biologic itself so one can set up an immunoassay. The clinical significance of the protein contaminants may be generally unknown until the individual product is tested clinically. For monoclonal antibody products, this assessment is complicated by the fact that monoclonal antibodies of murine origin may themselves be the overwhelming antigen and induce an allergic response.

It is important, however, to be aware of the type of protein contaminants and to monitor the production process and the final product in an appropriate fashion to make sure that the levels are in the appropriate range. Tests for animal safety, bacterial contamination, endotoxin, bovine serum, blood group antigen and animal proteins, such as mouse and standard mouse hybridoma products, should be applied (van Metre, 1984).

6. In Process Quality Control of Monoclonal Antibody Production

Having discussed the methods of production of monoclonal antibody and their likely contaminants,

principles for assuring the quality of a final monoclonal antibody product may be established. In developing tests for in process quality assurance of large scale production of monoclonal antibodies, a manufacturer must bear in mind that the product must conform to FDA standards. As biologic drugs, monoclonal antibodies must be manufactured in compliance with applicable Drug Good Manufacturing Practices, Biologic Good Manufacturing Practices and certain manufacturing and quality control guidelines which have been recommended for monoclonal antibody production. The fundamental requirement of the in process quality assurance tests are that the test must demonstrate that the production process yields a product which is pure effective and safe for its intended application.

6.1 Characterization of the hybridoma cell line. To evaluate product consistency and stability it is important to establish and document the lineage of the antibody secreting cell lines. This information should include the source, name, and characterization of the parent myeloma cell line with respect to any heavy or light chains which it synthesizes and secretes. The source and species of the immune cell as well as the purity of the immunogen and the immunization protocol should also be documented.

A stable clone of antibody secreting cells is required for large scale production of monoclonal antibodies. Recloning procedures which help maintain clone stability should be documented. A cell seed lot system is established to insure survival of the line. The manufacturer should document the quantity and concentration of cells stored in this way as well as conditions under which they are stored.

Before cell lines are put into production a number of specific quality control tests should be made. As hybridoma cells may be genetically unstable, hybridoma cells should be routinely tested at weekly intervals by a suitable immunoassay to ensure that the antibody secretion remains consistent. The hybridoma product should also be characterized by biochemical and biophysical tests to check the clonal stability. These tests should include electrophoretic characterization of the secreted immunoglobulin. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and isoelectric focusing will also identify any immunoglobulin light or heavy chains which the hybridoma secretes. High pressure liquid chromatography (see chromatography <621>, USP XXI, p. 1226), may be used to determine the purity of the hybridoma secretion product. The protein concentration produced relative to cell volume should be

determined using an appropriate protein assay such as the Lowry test.

Hybridoma cell lines used in monoclonal antibody production should be routinely monitored for the presence of microbial contamination, especially those contaminants which are difficult to detect microscopically, such as mycoplasmas and murine viruses. Additionally, it is important to screen them for bacterial and fungal contamination. Methods for detecting mycoplasma contamination have been discussed (see section 4.5). Murine viruses can be detected with a defined mouse antibody production test. This test measures the presence in mouse sera of antibody to a panel of commonly found murine viruses. To conduct the test naive mice (mice that are immunologically naive with respect to the common murine viruses found in most laboratory animals) are injected with a small dose of the hybridoma cells being tested. The mice are subsequently isolated for 28 days in an aseptic environment, after which tests for the production of antibodies against murine viruses are conducted. In addition to a mouse antibody production test an appropriately controlled test for lymphocytic choriomeningitis (LCM) virus should be performed by intracerebral inoculation into several healthy, weanling, LCM-negative mice.

As indicated in section 5 of this report, the hybridoma may be contaminated with nucleic acids of viral or cellular origin. Sensitive methods such as the fluoresence enhancement assays or hybridization analysis previously described should be performed to establish the level of nucleic acid contamination. The determination of nucleic acid contamination of the hybridoma may indicate a level of contamination likely in the final product. A nucleic acid determination at this stage can also be used to establish whether the subsequent purification process removes nucleic acids.

6.2 Quality of mouse colonies for in vivo monoclonal antibody production. For a good yield of high quality monoclonal antibody from ascitic fluid, it is important to ensure initial and continued good health of the mice employed. Mice should be from a colony whose genetic composition is routinely monitored. They should be determined to be free of ectoparasites and endoparasites, murine viruses and bacterial pathogens. The size and age of mice should be given careful consideration. Animals 6 to 8 weeks old and weighing 15 to 20 g are a good choice. Younger animals are too small and their serum cannot be monitored for the presence of antibody to pathogens, since they are not fully immunocompetent. Although there are generally no

differences in ascitic fluid production between male and female mice, females are preferred because they show no territorial aggressiveness and can thus be caged in larger groups (de la Llera Moya et al., 1984).

To ensure that the mice stay healthy there should be a program to monitor their health routinely. If rats are used for hybridoma production they should be of equivalent quality. (The manufacturer should meet the requirements detailed in CFR, Title 21, subchapter F, subpart B. 600.11, as to the care and quarantine of animals used in the manufacture of biologics). The use of a diseased animal is unacceptable in the production of a biologic product. If an unhealthy animal were used for the production of a monoclonal antibody and its ascitic fluid pooled with the fluid from other healthy animals, then the entire batch is rendered unacceptable for further processing. It is therefore in the best interests of the manufacturers to ensure that mouse colonies, or other animals used for in vivo production of monoclonal antibodies are in good health.

6.3 Quality of pooled ascites and tissue culture fluids. The ascitic fluid from many mice and the supernatants from tissue culture vessels are pooled prior to further processing. This pooled fluid will in most cases constitute a single pooled bulk. Each

manufacturer should set up criteria which tissue culture supernatants or ascitic fluids must meet in order to be acceptable for further manufacture. These criteria may depend on levels of microorganism contamination, antibody specificity or affinity in the pooled bulk, or concentration of antibody per ml of fluid.

The pooled ascitic or tissue culture fluids should again be tested for the presence of nucleic acid and viral contamination. When hybridoma propagation is *in vitro*, all bovine serum additives should be demonstrated free of bovine diarrhea virus. Tests should also be performed for bacterial and fungal contamination.

The General Biologic Products Standards (CFR, Title 21, Subchapter F, subpart D, 610.30) requires that prefiltered bulks of pooled ascitic and tissue culture fluids should be free of mycoplasma. Appropriate testing for mycoplasmal contamination is described therein.

6.4 *Sterility assurance of monoclonal antibody production*. Monoclonal antibody products for *in vivo* use must be sterile. Monoclonal antibodies cannot be terminally sterilized and therefore must be produced by aseptic processing. This requires the manufacturing process and production facility to be designed to minimize the risk of microbial contamination of the raw materials and final product.

To control microbial contamination in the processing or filling environment, the air should be filtered to remove airborne microorganisms. Personnel should be appropriately equipped and gowned to avoid introducing contamination. A positive pressure differential between the controlled aseptic working area and other work areas is established. The positive pressure in the aseptic processing area will insure that the air from other working area does not enter the controlled environment.

The in process testing of monoclonal antibody production must include microbial environmental monitoring at the time a batch is prepared. In order to establish that the controlled environment remains suitable for aseptic processing, periodical environmental filter examination should be performed. To ensure that personnel continue to use correct asceptic technique, periodic sterile culture medium processing should be performed. (USP XXI, Sterilization and Sterility Assurance <1211>.)

6.5 Quality Control of the Final Monoclonal Antibody Product. The final monoclonal antibody product should be pure and potent. By pure, it is meant that it should be free from protein, viral and nucleic acid contamination. If the antibody is intended for in vivo use it must also be sterile

The ability to bind strongly and specifically to a target cell or molecule determines the potency of the monoclonal antibody. The immunologic specificity and sensitivity requirements for a monoclonal antibody product should be comparable to those for a traditional polyclonal antibody product. If a similar polyclonal licensed product exists, the specificity profile of the monoclonal product should be comparable at least with that of the polyclonal licensed product. Appropriate reaction conditions (time, temperature, pH and protein concentration ranges) and the specific activity of the product should be established. Successive lots of the product should meet established product specifications ("Points to Consider in the Manufacture of In Vitro Monoclonal Antibody products," Office of Biologics, FDA, June 20th, 1983). If no similar licensed product exists, specificity for the target molecule and sensitivity of the monoclonal antibody assay system should be equivalent to that seen for a well characterized antibody from a previous lot which has been designated a reference standard by the FDA.

It is therefore important in the investigational stage of the monoclonal antibody product development to establish tissue and cellular reactivity of the monoclonal antibody reference standard. The antigenic determinant against which the monoclonal antibody is

directed may be demonstrated on human cells or tissues other than the intended target tissue. Laboratory tests should be set up which allow an assessment of cross reactivity with sites other than the intended target. The tests should also evaluate the resultant risk or hazard of the cross reactivity to potential recipients.

As an alternative to extensive specificity and cross reactivity testing of each lot, abbreviated specificity testing may be performed when supplemented by appropriate biochemical and biophysical characterization of the monoclonal antibody product. Therefore, following purification procedures, the manufacturers should establish gel electrophoresis and isoelectric focusing patterns of the monoclonal reagent.

High pressure liquid chromatography analysis may be used to quantitate the level of protein contamination. The final product should also be tested for the presence of viral and nucleic acid contamination. The Food and Drug Administration ("Points to consider in the Manufacture of Monoclonal Antibody Products for Human Use," Office of Biologics, July 25, 1983), has indicated that it may consider as an alternative to routine final product testing, on a case by case bais, validation procedures which establish that viruses and nucleic acids are removed during the purification process.

Any monoclonal antibody product for parenteral use is also subject to the requirements for injectable preparations. To date, most monoclonal reagents for *in vivo* use have been supplied in liquid form, either in sterile filled ampules or with appropriate preservatives. The product must meet The USP specifications, (USP XXI, Injections <1>). Injections meet the requirements under USP XXI, Sterility Tests <71>. In an absolute definition of sterility, a specimen would be deemed sterile only when there is complete absence of viable microorganisms from it. Absolute sterility cannot be practically demonstrated without complete testing of every finished article. The USP points out that "the sterility of a lot purported to be sterile is therefore defined in probablistic terms, where the likelihood of a contaminated unit or article is acceptably remote." Hence sterility of the final product depends on the adequacy of the aseptic processing and cannot be assured solely by reliance on sterility testing.

Monoclonal antibody products must also meet the requirements of the USP XXI General Safety Test <157>, and the Pyrogen Test <151>, or Bacterial Endotoxins Test <85>. The General Safety Test is intended to detect extraneous toxic contaminants which may have been introduced in the manufacturing process. It is not a

test for intrinsic toxicity of the product. The Pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection of the product concerned. The limulus amebocyte lysate (LAL) assay (USP XXI Bacterial Endotoxins Test <85>) may be used where a more sensitive test is required and it can be validly used without inhibition or enhancement by interferring substances. (See Guidelines for Use of the Limilus Amebocyte (LAL) Test from Dockets management Brancn (HFA, -305), FDA, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857). Additionally, if the monoclonal reagent is derived from human blood cells, there may be a requirement for performance of a Hepatitis B Surface antigen Test. The label on the final container should indicate whether or not the test has been performed and, if performed, the negative result. The label should also state that although the test has been performed, there is no test available which provides a complete assurance that Hepatitis B virus is not present. The final product should also be tested for the presence of viral and nucleic acid contamination by methods which have already been discussed.

If the monoclonal antibody product is in the form of a dried powder, then tests must be performed to establish the residual moisture content. The tests

described under USP XXI, Water Determination <921>, may be appropriate methods.

7. Regulatory Aspects of Monoclonal Antibody Production

7.1 The Food and Drug Administration (FDA). The Food and Drug Administration is responsible for assuring that each new monoclonal antibody product meets required standards of safety, purity, potency, and efficacy. The novelty of the hybridoma technology poses unique challenges for those responsible for the licensing and release of these products. The Food and Drug Administration drew from their experience in setting standards for vaccines and recombinant DNA products when developing guidelines for the licensing of monoclonal antibodies. Certain established standards for particular classes of medicinal products also apply to them. The Office of Medical Devices, Center for Medical Devices and Radiological Health of the FDA regulates monoclonal antibody products intended for in vitro diagnostic use. The exceptions are monoclonal antibody based diagnostics that are used in conjunction with an already FDA-licensed biologic product; these fall under the jurisdiction of the Office of Biologics, Center for Drugs & Biologics of the FDA. The Office of Biologics also regulates monoclonal antibody products intended for therapeutic use. Those monoclonal antibody products regulated by the Office of Biologics are designated

biological products and are subject to licensure under the Public Health Service Act. Section 351(a) of the Public Health Service Act [42 U.S.C. 262(a)] requires, in part, that any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative applicable to the prevention, treatment, or cure of diseases or injuries of humans must be propagated or manufactured and prepared at an establishment holding an unsuspended and unrevoked license.

A monoclonal antibody product is analogous to both an antitoxin and a therapeutic serum and so any monoclonal antibody product intended either for in vivo use or for in vitro testing of a licensed biological product is a biological product subject to the licensing provisions of the Public Health Service Act. The types of in vitro diagnostic monoclonal antibody products which fall into this category include Blood Grouping Sera, Anti-Globulin Sera and Antibody to Hepatitis B surface antigen. As biological products they must meet the requirements of Subchapter F, Parts 600-680, of Title 21, Code of Federal Regulations, as they relate to biologics in general.

Two forms of licenses are required for the manufacture of biologics. First, an establishment license is required. An establishment license is issued only after inspection of the establishment by an

inspector from the Food and Drug Administration has determined that the establishment complies with applicable standards prescribed in the regulations in Subchapter F, Title 21, CFR 600.10-600.15. A product license is required for every biological product manufactured at the licensed establishment.

In order to assist manufacturers of monoclonal antibodies in product development and during their investigational new drug studies, the Office of Biologics, FDA, has prepared two documents titled "Points to Consider in the Manufacture of Monoclonal Antibody Products for Human Use," dated July 25, 1983, and "Points to Consider in the Manufacture of In Vitro Monoclonal Antibody Products Subject to Licensure," dated June 20, 1983. The FDA may develop these documents into guidelines or regulations to ensure the safety, purity, potency, and effectiveness of monoclonal antibodies which are biological products. These draft documents provide details of the criteria that the Office of Biologics expects producers of monoclonal antibody biologic products to consider in their product development and their license applications.

Because monoclonal antibody production is a new technology, The Food and Drug Administration aims not to prematurely develop firm guidelines or requirements that might adversely affect product development and ultimate

product availability. The agency, therefore, stresses that these documents are not all-inclusive and certain points may not be applicable to all situations. The Food and Drug Administration is therefore soliciting comments on the content of their "Points to Consider" documents from manufacturers and others concerned with setting standards for monoclonal antibody products. The FDA will also consider individual manufacturers' evaluations of their monoclonal antibody product on a case by case basis.

The "Points to Consider in the Manufacture of Monoclonal Antibody Products for Human Use" document provides material applicable only to monoclonal antibodies of murine-murine origin. Monoclonal antibodies obtained from hybridomas of human-human origin or interspecies origin and monoclonal antibodies coupled with drugs or toxins must be discussed with the Office of Biologics on an individual basis. The "Points to Consider" document of July 25, 1983, has been revised and will be available in the near future.

For monoclonal antibodies for *in vitro* diagnostic products the primary concerns relate to sensitivity, specificity, stability, potency and consistency of the product. The Office of Biologics' guidelines therefore outline the information which they would require in order to establish that the *in vitro* diagnostic

monoclonal antibody possesses these characteristics. This information includes characterization of the cell line, documentation describing the production procedure, biochemical and biophysical studies of the final product and potency testing compared to a designated reference standard or licensed polyclonal antibody product.

For monoclonal antibodies for human use, there is obviously a greater concern for safety. The guidelines for developing monoclonal antibodies for human use therefore emphasizes the purity of monoclonal antibodies in addition to those characteristics desirable for *in vitro* monoclonal reagents. The Office of Biologics suggests suitable testing for murine viruses and nucleic acid contamination, purification procedures, and appropriate preclinical animal testing of monoclonal antibody products. The guidelines also emphasize appropriate quality control and final container testing. Recommended quality control of the final product includes biochemical and biophysical studies, sterility testing and other tests which are generally applicable to injectable preparations.

For both *in vivo* and *in vitro* monoclonal antibody products studies should be performed to establish an expiration dating period of the final formulations in their probable market packaging. Because of the possibility of subtle denaturation of monoclonal

antibodies which might affect their utility, the stability of each production lot should be checked by tests for fragmentation, aggregation and potency at intervals throughout the dating period. ("Points to Consider in the Manufacture of Monoclonal Antibody Products for Human Use," Office of Biologics, FDA, July 25, 1983).

In addition to the FDA guidelines which apply particularly to monoclonal antibodies, the product should comply with general biological products standards (CFR, Title 21, Subchapter F, part 610). When a monoclonal antibody has been approved and a product license obtained, specifications for that product are established. The regulations stipulate that "no lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to such product." Samples of the monoclonal antibody are also submitted to the FDA. When each subsequent production lot has been prepared samples must be sent to the FDA as well as documentation to show that all the applicable tests have been performed and the results of these tests. The FDA will review these data and may select certain tests that are repeated at the FDA laboratories; they often repeat the test for potency. The preferred tests involve comparison of the product with a potency standard. The

potency standard is used solely for potency testing and may have been obtained from the manufacturer, or prepared by the FDA, or in the case of monoclonal antibodies, the potency standard may be an existing polyclonal antibody product. The batch to batch consistency is also an important parameter. For polio virus vaccine, for example, there used to be a requirement for 5 consecutive batches to meet product specifications (this requirement does not apply to batches actually used in clinical trials). If one batch fails, then five more may be needed to be prepared and meet specifications before all the batches may be released. The FDA may also decide to set up its own stability testing of the product.

The reference standards for biological products are held by the Office of Biologics, FDA. These reference standards are not generally available and may only be supplied to the manufacturer of the licensed product.

7.2 The United States Pharmacopeia (USP)

The primary purpose of the USP is "to provide authoritative standards and specifications for materials and substances and their preparations that are used in the practice of the healing arts." The USP makes no distinction between products which are diagnostic agents and those used for therapy. The USP develops standards of identity, quality, strength, purity, packaging,

labeling, and, where applicable, bioavailability, stability, and procedures for proper handling and storage of all commonly used medicinal products.

As monoclonal antibody products become widely used, standards will be developed and included in the USP. As biological products, monoclonal antibodies are regulated by the Office of Biologics and the Office of Medical Devices of the FDA. The FDA holds reference standards for biological products. However, certain provisions of the USP are generally applicable to biologic products including tests for potency, general safety, sterility, purity, water (residual moisture), pyrogens, identity and constituent materials (CFR, Title 21, Subchapter F, parts 620.10 to 610.15 and see USP XXI, General Safety <157>, Sterility Tests <71>, Water Determination <921>, and Pyrogen Test <151> as well as Bacterial Endotoxins Test <157>). Constituent materials such as preservatives and diluents should meet compendial standards.

For biological products, Pharmacopeial monographs reflect the current Food and Drug Regulations. However, they cover those aspects of identity, quality, purity and potency, and packaging and storage that are of particular interest to pharmacists and physicians responsible for the purchase, storage, and use of biologics.

The development of monoclonal antibodies have three main implications for the United States Pharmacopeia. Firstly, monographs for those products prepared by traditional methods will need to be revised to account for the equivalent product produced by hybridoma technology. Secondly, standards will be developed for new monoclonal antibodies for which no equivalent licensed traditional product presently exists. Finally, monoclonal antibodies may provide reagents for improved assays. For example, monoclonal antibodies have been developed against the E. Coli enterotoxin and may provide reagents for sensitive assays of pyrogens (Hemelhof et al., 1984).

8. Applications of Monoclonal Antibodies

8.1 Monoclonal antibodies as in vitro diagnostic agents. As homogeneous, monospecific agents capable of being produced in essentially unlimited quantities, the value of monoclonal antibodies in diagnosis and clinical investigation is clear. It is also clear that monoclonal antibodies as in vitro diagnostic agents raise fewer safety concerns. For these reasons there has been rapid development of monoclonal antibody diagnostics and more than 89 products have been licensed since 1981. A listing of currently licensed in vitro diagnostic monoclonal reagents is appended to this report.

Monoclonal antibodies to blood group antigens A and B have been developed with significant advantages over the conventional antisera. Blood typing sera is conventionally obtained from human donors. The donated serum must be carefully screened for the presence of unwanted antibodies whose activity might obscure the anti A or anti B reaction. Hyperimmunization of the human donor with red blood cells of the appropriate group is the procedure employed in some countries, for example in the U.K. This potentially hazardous procedure is avoided if monoclonal reagents are used. In addition to typing red blood cells for blood transfusions, it is hoped that monoclonal antibodies against histocompatibility antigens will provide standard reagents for typing tissue for transplantation.

The large quantities in which these antibodies may be produced has resulted in monoclonal reagents slowly replacing conventional antiserums in standard kits for immunoassays, and many such assay kits are commercially available. Several companies have produced pregnancy testing kits using monoclonal antibodies against human chorionic gonadotrophin. Assay kits for testing serum concentrations of drugs with low therapeutic indices such as phenytoin, theophylline and phenobarbital have also become available. There is the obvious potential therefore for the development of monoclonal antibodies

to other drugs to provide reagents for rapid and convenient serum analyses.

Monoclonal antibodies have had a tremendous impact on the diagnosis of infectious diseases. For identification of specific pathogens in clinical specimens incubation with fluorescein conjugated monoclonal antibodies allows diagnosis by fluorsecence microscopy (Engleberg and Elsenstein, 1984). In the past this approach has not been practical because of unacceptable levels of background fluorescence associated with polyclonal antisera. The monoclonal reagents, even at high titre, do not produce significant nonspecific fluorescence.

Monoclonal antibodies have now been developed which, by means of immunofluorescence microscopy, provide rapid diagnosis of gonococcal, chlamydial and herpes virus infections (Nowinski et al., 1983). With immunofluorescence being used as the assay system, the monoclonal antibodies demonstrated sensitivities of 94 to 99 percent for culture confirmation and 85 to 90 percent for direct diagnosis of specimens smeared on microscope slides. Since the direct diagnostic tests only require 15 to 20 minutes to perform, they represent a major advance in the rapidity of diagnosis of these sexually transmitted infections. Previous assays required 3 to 6 days to perform.

Monoclonal antibodies can also be used to improve the accuracy of serological methods aimed at quantifying pathogen-specific antibodies in patients serum (Engleberg and Eisenstein, 1984). Employing labeled monoclonal antibodies, rather than standard antiserums has two advantages; specificity and convenience, as the antigen need not be highly purified. Because of these advantages the use of monoclonal antibodies is facilitating the development of improved serologic tests for certain mycobacterial, protozoal and helminthic infections (Engleberg and Eisenstein, 1984).

In the field of tumor immunology monoclonal antibodies provide hopes of improved diagnosis and therapy of cancer. Although there is not yet conclusive evidence of a tumor specific antigen, certain tumor associated antigens have been identified. The quantitative difference between the expression of these antigens on tumors and normal cells means that monoclonal antibodies specific for tumor associated antigens may be used for diagnosis and classification of tumours. High plasma levels of carcinoembryonic antigen (CEA) has been reported to be an indicator of the possible presence of metastatic disease in patients with cancers of the digestive system, breast, and lung (Colcher, 1984). In the diagnosis of leukemia and lymphoma, monoclonal antibodies have been developed

which allow phenotyping of T cells at various stages of development. The "T" series of monoclonal antibodies produced by Ortho Diagnostic Systems, Inc. (OKT) or Beckton Dickinson (Leu) may be used to subcategorize T cells as to type (T4 or T8) and as to the stage in development in which they were arrested and continue to proliferate in T cell malignancies. Effective treatment of these malignancies requires selecting the proper treatment regimen which is most effective against cancer cells and least dangerous to the patient. Monoclonal antibodies against T cells, monocytes and B cell subsets permit more accurate classification with a view to better selection of patient treatment (Hoffman, 1985, Vodinelick, 1984).

The "T" antibodies may also be used to assess immune status in diseases of the immune system. Many immune deficiencies are of T cell origin (Hoffman, 1985) and identification of the defective subset may help classify the disease, optimize treatment, and monitor the effect of treatment. AIDS is an example of where the "T" series of antibodies has been very useful. These antibodies may similarly be used to monitor immunosuppressive therapy after organ transplantation.

In clinical investigation monoclonal antibodies have been, and continue to be, valuable tools in increasing our knowledge of biological systems and

disease processes. The rapid and accurate diagnostic techniques which have emerged from the hybridoma technology are important links to improved therapy. No doubt monoclonal antibodies will continue to be beneficially exploited as in vitro diagnostic agents with many more becoming commercially available.

8.2 Monoclonal antibodies in therapy.

The potential of monoclonal antibodies in diagnosis, as clinical reagents, and as research probes is well established. However, the therapeutic potential of monoclonal antibodies has yet to be realized. Two broad categories of application may be viewed for monoclonal antibodies in therapy. Firstly, the antibody itself is a therapeutic agent in many cases. The antibody may also be used as a carrier molecule to bring therapeutically active molecules to a particular target site.

8.2.1 Monoclonal antibodies as drugs.

8.2.1.1 Vaccines.

Perhaps one of the most obvious roles of monoclonal antibodies is in passive immunization. This approach to the treatment of bacterial, viral and parasitic infections is actively being pursued.

Malaria persists as a major disease in the tropical world. Because of the decreasing effectiveness of insecticides and antimalarial drugs, efforts are now

being made to develop vaccines directed against different stages of the malarial parasites life cycle. Theoretically antibodies can block merozoite invasion of erythrocytes either by interacting with the merozoite receptor for the red blood cell or by agglutinating merozoites as they emerge from the rupturing schizont. Two hybridomas have been identified (Epstein et al., 1981) against merozoites of P. knowelsi, a species causing malaria in primates, that block invasion of erythrocytes by agglutinating merozoites. Protective immunity in mice to P. yoelli has been shown to be dependent on B lymhocytes and their products, making this an ideal system to study the role of antibodies in vivo. A monoclonal antibody specific for determinants on antigens of intraerythrocytic stages of P. yoelli have been reported (Mjarian et al., 1984). Passive administration of this antibody prior to infection of mice with a lethal strain of P. yoelli prevented death in these animals. Vaccines against malaria will probably be derived from antigens identified with monoclonal antibodies. Multivalent vaccines might combine sporozoite and asexual components to provide temporary protection to the host which could be used in conjunction with antimalarials.

Schistosomiasis, or bilharzia, is another major health problem in many tropical countries. The

immunopathological basis of schistosomal disease has been appreciated for some time, but progress has been hampered by the lack of identification of biologically relevant antigens, as well as difficulties inherent in the purification of individual antigens from an organism whose propagation depends upon passage in a mammalian host. Three monoclonal antibodies have been developed (Strand et al., 1982) to identify and characterize glycoproteins in different schistosomal development stages. Future work will be aimed at applying the monoclonal antibodies to clinical diagnosis and treatment of schitosomal infection.

8.2.1.2 Monoclonal antibodies as drugs in cancer therapy.

It has long been hoped that antibodies directed against tumor cell surfaces could be exploited in tumor therapy. The therapeutic application of monoclonal antibodies depends on their ability to localize to tumor cell and to kill these cells in vivo.

The immunotherapeutic application of IgG2a cytotoxic antibody to p97, a melanoma associated antigen, has been assessed. This monoclonal antibody can kill melanoma cells in vitro in the presence of rabbit complement, and cytotoxicity is greatly enhanced with two antibodies to two epitopes of p97 (Hellström et al., 1981). Presumably closely adjacent antibody

molecules at the cell surface are particularly efficient at activating complement mediated cytolysis.

Tumors of the B lymphocyte lineage offer a special opportunity for the production of antibodies to tumor associated antigens. Each B cell produces a different immunoglobulin molecule with a unique idiotype that is common to all members of the malignant clone and different from virtually all normal B cells of the host. The idiotype of the tumor cell surface immunoglobulin represents the closest approximation of a tumor specific antigen available. Non-secreting B cell malignancies are the prime candidates for anti idiotype therapy, since they are not associated with high serum levels of idiotype-bearing protein, which would block the effects of the antibody. Diseases in this category include follicular lymphoma, Burkitt's lymphoma, diffuse large cell lymphoma, and chronic lymphocytic leukemia. A dramatic clinical result has been reported in a single patient with follicular lymphoma with monoclonal anti-idiotype antibody (Levy and Miller, 1983).

A major difficulty with the use of antibody alone appears to be that of antigenic modulation, where apparent loss of the surface antigen can occur within a few minutes to an hour or two after exposure to some of the monoclonal antibodies. In some cases, modulation appears to be a temporary problem, with return of the

surface antigen within 24-36 hours. Moreover, modulation does not occur with all antibodies. A possible solution to antigenic modulation is to use two monoclonal antibodies directed against different epitopes on the same tumor cell. Another problem implicit in the use of antibody alone is the release of free antigen which forms immune complexes with the infused monoclonal, blocking its effects on the tumor cell.

Bone marrow toxicity represents the limiting factor in most of the effective therapy for leukemia and lymphoma. The patient's own bone marrow may be harvested prior to therapy, cryopreserved and used to reconstitute hemopoietic function after supralethal therapy. Antibodies can be used to eliminate tumor cells from such bone marrow specimens. The use of monoclonal antibodies in autologous bone marrow transplantation in this way has many advantages over the use of antibodies in in vivo therapy. Incubations can be performed under conditions that do not allow antigenic modulation, such as reduced temperatures. Circulating blocking factors can be removed. Heterologous complement can be added to lyse antibody-coated cells and multiple treatments can be used to enhance the elimination of tumor cells. Reports of clinical trials employing this technique are very

encouraging. The results of anti-B1 monoclonal antibody and complement treatment in autologous bone marrow transplantation for relapsed non-Hodgkins lymphoma have been reported (Nadler et al., 1984). Eight patients were treated with intensive chemoradiotherapy and reconstituted with autologous bone marrow rendered free of tumor cells by the B cell specific monoclonal antibody anti-B1 and complement. All patients treated achieved a complete clinical response and had stable haematological engraftment by eight weeks. No significant acute or chronic toxic effect occurred.

In cancer therapy to insure a complete tumor kill, it is envisaged that more than one procedure will have to be used. Ultimately the relative fragility of the hematopoietic stem cells may be the limiting factor with regard to the number of manipulations that can be tolerated.

8.2.1.3 Monoclonal antibodies in the treatment of transplant rejection.

During the rising success of transplantation as a clinical treatment over the past two decades, the control of rejection has depended on nonspecific suppression of the immune response by a number pharmacological agents, especially azathioprine cyclosporin A, and prednisone as well as polyclonal antilymphocyte sera. These agents have undesirable

effects on cells outside the immune system and they affect too broad a spectrum of cells within the immune system itself. Accordingly, many recent efforts to improve immunosuppressive treatment have included a search for agents that will have a more precise and predictable impact. Monoclonal antibodies have been used to treat transplant rejection in non-human primates and in patients. Patient trials have so far been confined to the use of the pan-T-cell antibody OKT3 (Russel et al., 1984). This has been shown to be the most effective agent available for the reversal of acute cellular rejection in human kidney transplants. The appearance of antibodies to the injected monoclonal reagent can interfere with continuing effects, but did not appear to introduce serious dangers for the trial patients.

A new rat anti-human lymphocyte antibody, which lyses cells with autologous human complement, for the depletion of T lymphocytes from human bone marrow allografts in vitro has also been reported. (Waldman et al., 1984). This procedure is aimed at protecting the marrow recipients from a Graft versus Host response. Graft versus Host disease (GVHD) is one of the major obstacles to successful transplantation because it causes a high rate of morbidity and mortality despite post transplant immunosuppressive regimens. The trial

conducted involved 11 patients with histocompatible siblings as marrow donors. T cell depletion was substantial after treatment of the marrow with the monoclonal antibody. No signs of GVHD developed in any of the patients, who were observed for a period of 12 months.

8.2.1.4 Monoclonal antibodies for the reversal of drug toxicity.

Digitalis glycosides are of great importance in the treatment of congestive heart disease. Unfortunately, due to their narrow therapeutic ratio, digitalis intoxication is one of the most frequent adverse reactions reported. There is no specific antidote and the cardiac arythmias that are a feature of digitalis intoxication are commonly fatal. Butler and Chen, (Butler, 1967, 1970, 1973) first prepared antibodies capable of specific binding of digoxin by immunizing rabbits with a synthetic protein-digoxin conjugate. They pointed out the possible value of digoxin antibodies in the treatment of digoxin toxicity. If an antibody has a higher affinity for the drug than for the physiological receptor, it would be possible to transfer the ligand from the receptor to the antibody simply by means of mass action. Three high affinity antibodies specific for digoxin have now been successfully produced (Haber, 1982). In many years of study of the

conventionally obtained digoxin specific antisera, the kind of molecular discrimination provided by these monoclonal antibodies had not previously been observed. One of the monoclonal antibodies has an affinity 5×10^9 for digoxin and a nearly equal affinity for digitoxin. This antibody, which can be provided in unlimited quantities, has been shown to reverse digoxin toxicity in experimental animals and in man.

This approach could be applied to many other drugs or toxins.

8.2.1.5 Monoclonal antibodies in the treatment of autoimmune disease. Many autoimmune diseases are characterized by the production of auto antibodies. Monoclonal antibodies may be used first to characterize the auto antibodies associated with a particular autoimmune disease and possible monoclonal anti-autoantibodies could be used in treatment.

Monoclonal anti-DNA antibodies have been prepared by the hybridoma technique and used in the analysis of idiotype of anti-DNA antibodies in systemic lupus erythematosus (SLE) (Isenberg et al., 1984). Concordance was found between idiotype levels and clinical activity in eight of twelve patients. Measurement of idiotypes of anti-DNA antibodies may provide information valuable in monitoring the clinical

course of patients with SLE. These findings also raise the possibility of anti-idiotype therapy of the disease.

In addition, in animal models of systemic lupus erythematosus, multiple sclerosis and arthritis. Anti Ia antibodies and anti L3T4 antibodies (helper T cell antibodies) have been shown to inhibit onset and progression of disease. Antibodies against the human equivalent targets may be expected to be tried in therapy.

8.2.2 Monoclonal antibodies as carriers.

As yet the mechanisms by which a monoclonal antibody alone may bring about cell destruction *in vivo* are poorly understood. Despite the limited success obtained using the monoclonal antibody alone, it is likely that the use of antibody conjugated to drugs, toxins or radioisotopes offer the best hope for the development of targeted cancer-specific cytotoxic reagents.

8.2.2.1. Immunotoxins.

The term immunotoxin is used to designate a hybrid molecule formed by an antibody linked to a toxic moiety. The toxins that have been used most frequently in this respect are ricin, abrin, gelonin and diptheria toxin. An example of the toxicity of these agents is provided by the finding that one molecule of ricin entering the cytoplasm of the cell is sufficient to kill it.

There have been a number of reports that indicate immunotoxins containing ricin or the abrin A chain, administered shortly after injection of tumor cells in rodents can significantly delay the onset of disease and increase the number of survivors. Effective killing of up to 10^5 tumor cells has been described using these immunotoxins. In a study involving an established murine B cell leukemia (BCL1), long term remissions were induced in animals with large tumor burdens by non specific cytoreductive therapy (total lymphoid irradiation and splenectomy) followed by immunotoxins containing anti-idiotype or anti-delta A chain (Vitetta & Uhr, 1984, Vitetta, 1983). The results of such studies suggest that immunotoxins, in conjunction with nonspecific cytoreductive therapy, may bring tumor burdens to levels that can be kept in check by immune mechanisms.

The problems associated with *in vivo* use of immunotoxins are related to their high level of toxicity, and problems associated with the use of antibodies *in vivo*. The toxins derived from plants or bacteria usually consist of a toxic polypeptide (A chain) that is disulphide bonded to a cell binding polypeptide (B chain). In general immunotoxins containing the A chain are not highly toxic, although some damage is caused by non specific uptake by the

cells of the reticuloendothelial system. Antibody formation against the immunotoxin might necessitate the use of a prior regimen for inducing tolerance. The stability of the conjugate is very important, requiring investigation of several types of cross links for binding antibodies to toxin. It is unclear whether currently used immunotoxins will be able to penetrate effectively into extracellular spaces and solid tumors. Finally, cross reactivity between the monoclonal antibody directed to tumor cells and antigen on normal cells may occur. With each tumor it will be necessary to determine the extent to which it's tumor associated antigens are present on normal cells and the roles of these normal cells in maintenance of physiological processes.

8.2.2.2. Antibodies as carriers of cytotoxic drugs and biological response modifiers.

A safer approach than toxins is the use of conventional anti-cancer drugs which are already acceptable in clinical practice. The use of adriamycin coupled to a monoclonal antibody has been reported to have therapeutic effects against a rat mammary carcinoma (Baldwin and Pim, 1983). Conjugates of the phase specific anti-mitotic drug vindesine and polyclonal antibodies have been shown in vitro to have cytotoxic effects on human tumor cells. Selective action against

certain tumor target cells of vindesine coupled to a monoclonal antibody raised against an osteogenic sarcoma cell line has also been shown (Embleton et al., 1983).

The concept of targeting antitumor agents applies not only to cytotoxic drugs, but also to immunotherapy with biological response modifying agents (BRM). Early studies with bacterial vaccines including BCG and C.Parvum, and more recently with agents such as muramyl dipeptide and interferon (Baldwin and Pim, 1983) demonstrate that systemic treatment augments natural killer cell (NK) and macrophage mediated activities. However, for these cells to exert anti-tumor effects they have to traffic to and become localized within tumors. Although the role of NK cells in tumor rejection is not clearly established, this does not compromise the possible beneficial effects of a targeted immunomodulator, because these agents may have a stimulatory action on a diverse range of effector cells.

8.2.2.3 Monoclonal antibodies as carriers of radionuclides.

Monoclonal antibodies may also be used as delivery vehicles for radioisotopes. Until now however they have been used mainly for radioisotopic imaging. When larger amounts of radiation are employed, antitumor therapy is possible. Very early clinical investigations are getting underway to explore the potential of antibody-

radioisotope therapy (Carrasquillo et al., 1984, Baldwin and Pim, 1983). Ettinger et al (1982) used ^{131}I labelled antiferritin IgG and ^{131}I-labelled anti-CEA IgG as part of an integrated treatment program for primary hepatic malignancies. The use of a combination therapy made it difficult to assess the toxicity and therapeutic benefits of the radiolabelled antibody. Nonetheless the results of this and similar studies are encouraging (Carrasquillo, 1984).

A major problem often encountered with monoclonal antibody-radioisotope conjugates is the instability of the conjugate. There are now many documented instances of iodine labelled monoclonal antibodies that are highly unstable because of enzymic degradation once they have been administered. Labelling with ^{111}In (Halpern et al., 1983) avoids the dehalogenation problem and has improved the tumor uptake of the radiopharmaceutical. Use of the Fab conjugate rather than the intact antibody molecule facilitates selective targeting at the tumor antigen and diminishes the otherwise large non specific uptake by the reticuloendothelial system.

9. In Vivo Administration of the Monoclonal Antibodies.

Monoclonal antibodies have a bright future in the development of vaccines, for in vitro procedures to "cleanse" bone marrow, for the reversal of drug toxicity and in the study and possible treatment of auto-immune

disease. They have exciting potential in the treatment of cancer, either as antibody alone or as an immune conjugate. Several problems have arisen with the use of monoclonal antibodies in vivo, particularly in the area of cancer therapy. However, these problems should not cast a pessimistic shadow over the therapeutic potential of monoclonal antibodies, rather they should provide the impetus for further animal and clinical studies to try to resolve what are likely to be temporary obstacles.

The review of applications provides examples of some of the monoclonal antibodies presently under investigation. A central data bank has been established to keep track of monoclonal antibodies which have been developed. Further information about monoclonal antibodies presently prepared and being used for investigation may be obtained from:

AMERICAN TYPE CULTURE COLLECTION
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Rockville, MD 20852 (USA)

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GLOSSARY

Clone.--Progeny derived by asexual reproduction from a single individual. Unless there has been mutation or other sporadic change, every individual within a clone is identical to every other.

Epitope.--The small portion of the antigenic molecule specifically recognized by an antibody.

Histocompatibility.--Histocompatibility antigens are found on the surface of nucleated cells that provoke allograft rejection and regulate immune responses. Each individual animal possesses its own characteristic set of histocompatibility antigens.

Idiotype.--Idiotopes are antigenic determinants on immunoglobulins which result from the unique structures generated within the antigen binding site. The collection of idiotopes on an immunoglobulin moleucle is its idiotype.

Isotype.--Antigenic determinants found on immunoglublins of all animals of a species. Isotypic determinants are found on the heavy chains of all molecules of a specific isotype. On the heavy chains these determine the immunoglobulin class or subclass. On the light chain, these determine the subtype.

Lymphoblastoid Cell Line.--Cells that are derived from B lymphocytes and that have the capacity to grow continuously in culture following infection with Epstein Barr Virus.

Myeloma.--Operationally synonymous with plasmacytoma. Malignant proliferation of B lymphocytes or plasma cells often producting monoclonal populations of immunoglobulins or immunoglobulin fragments.

Oncogene (ONC GENE).--A gene is the genome of some retroviruses that is not necessary for viral replication but is necessary for the ability of the viruses to cause cell transformation *in vitro* and rapid induction of tumors *in vivo*.

Recombinant DNA.--A segment of DNA that has been cleaved from a larger DNA structure and then inserted into the DNA of a vector that is capable of self replication.

Retrovirus.--Small RNA virus consisting of virally coded glycoprotein in a lipid membrane derived from host cell membranes and on RNA nucleoprotein core. The small genome of these viruses contain a gene coding for reverse transcriptase (that directs the production of a DNA copy of RNA), virion proteins and often an oncogene.

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APPENDIX

Table 1. Monoclonal Antibody In Vitro Diagnostic Products Approved by the Office of Medical Devices, FDA.*

	Manufacturer	Product	Date Approved
1. Polypeptide Hormones	Hybritech Inc.	Tandem Qualitative HCG Kit	10/13/81
		Tandem HCG Kit	4/23/82
Human Chorionic Gonadotrophin		Tandem HCG Immunoenzymetric Assay Kit	7/20/82
		Tandem-E HCG Visual End Point Immunoenzymetric Assay	2/4/83
		Tandem R Total B-HCG Kit	
		Tandem Visual HCG Immunoenzymetric Assay	1/27/84

(Table 1 continued)

	Manufacturer	Product	Date Approved
		Tandem Fast Visual HCG Assay Kit Immunoenzymetric Assay	8/23/84
		Encore HCG Reagent	10/4/84
	Biogenex Laboratories	Riogen B-HCG RIA	7/13/82
	Monoclonal Antibiodies, Inc.	Model HCG Assay	9/24/82
		Serum HCG Assay	9/2/83
		Pregnastick™ Urine HCG Kit	1/9/84
		OVU Stick™ Urine HCG Kit	4/12/84

Manufacturer	Product	Date Approved
Carter Wallace, Inc.	Genesis B-HCG	1/20/83
	HCG Beta Slide Monoclonal	8/4/83
Warner Lambert Co.	E.P.T. Plus™	6/22/83
	Early in Home Pregnancy Test	9/12/83
Syncor International Corp.	Star HCG Monoclonal Immunorad	10/21/83
Ventrex, Inv.	Ventrezyme Beta-HCG Immunoassay HCG	5/3/84
Organon Inc.	Oreia II B-HCG, Enzyme Immunoassay	11/28/83
	Oreia II B-HCG Pregnancy Test Kit	5/30/83

(Table 1 continued)

(Table 1 continued)

	Manufacturer	Product	Date Approved
		Duoclon™ Color-Cept	8/24/84
	Travenol-Genetech Diag.	CA-1511, *in vitro* Radioimmunoassay Test Gammadab® M HCG Radio-Immunoassay Kit	6/12/84
	Stanbio Laboratory, Inc.	Stanbio Quick-Tell Monoclonal Indirect Pregnancy Test	9/21/84
	Ortho Diagnostic Systems	Ortho B-HCG Slide Test	11/17/84
	Quidel	Quidel HCG EIA Kit	11/10/83
		Early Pregnancy Detection Test Kit	11/16/83
	International Diagnostics	Preg Beta Plus Slide	5/29/84

	Manufacturer	Product	Date Approved
	Fisher Diagnostics	Pregnallone™ Tube Pregnancy Test Kit	1/17/85
Thyroid Stimulating Hormone	Hybritech, Inc.	Tandem T Thyroid Stimulating Hormone	10/8/82
	Terumo Medical Corporation	Terumo(R) Sensi-bead EIA TSH Kit	3/1/85
Prolactin	Hybritech, Inc.	Tandem Prolactin Kit	6/10/82
Human Growth Hormone	Hybritech, Inc.	Tandem HGH Kit	6/8/82
2. Tumor Markers			
CEA	Abbott Labs	CEA	3/29/82
		CEA-EIA	7/26/84
	Hybritech, Inc.	Tandem-R CEA	1/16/85

(Table 1 continued)

(Table 1 continued)

	Manufacturer	Product	Date Approved
Prostatic Acid Phosphatase	Hybritech, Inc.	Abbott PAP-EIA	1/19/82
	Abbott Labs	Tandem PAP Kit	9/1/81
3. Cell Surface Antigens			
T Cells	Ortho Diagnostic Systems	Ortho Spectrum III and Ortho-mune OKT-11	4/6/82
T and B Cells	Hybritech, Inc.	Cytotag T & B Cell Kit	7/26/82
4. Allergy			
IgE	Hybritech, Inc.	Tandem Dem-IgE Kit	5/29/81
		Tandem-E IgE Immunoenzymetric Assay	12/23/82
	Allergenetics	IgE Fast Test	11/10/82
		Total IgE Fast Test	1/13/83

	Manufacturer	Product	Date Approved
		Labelled ^{125}I Anti-IgE	2/3/84
Cymopapain Sensitivity		Cymofast Test	11/22/83
5. Therapeutic Drug Monitoring			
Theophylline	Miles Laboratories	Amwa TDA Theophylline	6/21/83
		Seralyser Theophylline Reagent Strips	10/7/83
	E.I. Dupont Denemours	ALA™ Theophylline Test Pack	8/25/83
	American Dade	Stratus Theophylline Assay	8/22/83
Gentamicin	Miles Laboratories	Ames TDA Gentimicin Test	12/14/82

(Table 1 continued)

(Table 1 continued)

	Manufacturer	Product	Date Approved
Phenytoin	Dupont Denemours & Co.	aca^{R} Phenytoin Analytical Test Pack	10/10/84
	Syva Co.	Syva Advance Emit Phenytoin Assay	1/17/85
		Emit Phenytoin Assay	1/17/85
		Emit$^{(R)}$ QST^{TM} Phenytoin Assay	3/15/85
Phenobarbital	Miles Laboratories	Ames TDA Phenobarbital	8/14/84
Sisomicin	Miles Laboratories	Ames TDA Sisomicin Test Control Serum	9/26/83
6. Infectious Disease			
Chlamydia	Syva Co.	Micro Trak Chlamydia Trachomatis Culture	12/11/82

	Manufacturer	Product	Date Approved
		Micro Trak Chlamydia Trachomatis Direct	9/26/83
Rabies	Centocor	F'TC Anti-Rabies Monoclonal Globulin	4/16/82
Rubella	Orthodiagnostic Systems	Ortho Rubella Elisa Test	3/17/83
Herpes	Syva Co.	Micro Trak HSV 1 & 2 Culture Confirmation	6/22/83
	Electronucleonics Inc.	Herpes ID and Typing Test HITT	9/20/83
Varicella	Ortho Diagnostic Systems	Ortho Varicella Zoster Virus Identification Reagent	5/30/84

* The information in this table was obtained from the Office of Medical Devices, Division of Clinical Laboratory Devices' listing of Disclosable Submissions which use hybridoma technology. This is not a complete listing of FDA approved products as of 3/21/85. The inclusion of any particular manufacturer's product does not signify that the authors of this review or the U.S.P. endorse that product.

Table 2. Monoclonal Antibody In Vitro Diagnostic Products Approved by the Office of Biologics, FDA*

Product	Manufacturer	Date of License
Anti-Human Serum Anti-Complement C3d	Ortho Diagnostics	3/26/81
Anti-Human Serum Anti-C3b-C3d	Ortho Diagnostics	10/7/81
Antibody to Hepatitis B Surface Antigen	Centocor, Inc.	1/28/83
Anti-Human Serum	Ortho Diagnostics	2/2/83
Blood Grouping Serum, Anti M	Gamma Biologicals, Inc.	1/12/84
Blood Grouping Serum Anti-A Anti-B Anti-Le^a Anti-Le^b	Chembiomed Ltd.	5/17/84

Product	Manufacturer	Date of License
Blood Grouping Serum Anti-A Anti-B Anti-Le^a Anti-Le^b	Immucor, Inc.	5/19/85
Blood Grouping Serum Anti-A Anti-B	Biotest	3/27/85
Antibody to Hepatitis B Surface Antigen	Abbott	4/1/85

* Taken from FDA document 2271H of 4/16/85