This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Bethesda, MD 20205

June 1, 1984

TO: Interested persons

SUBJECT: Points to consider in the characterization of cell lines used to produce biological products

FROM: John C. Petricciani, M.D.

A variety of tests have been applied in the characterization and monitoring of cell substrates since they were first used in the production of biological products several decades ago. Because advances in technology have led to the possibility of producing a wide variety of new products in other than the traditional cell substrates (e.g. primary cells and diploid cell lines), we have developed the attached draft of points which should be given consideration in the characterization of "new" cell substrates. The draft is being distributed in advance of the Workshop on Cell Substrates (scheduled for July 30-31) so that they can be taken into consideration during discussions on the acceptability of various types of cells.

In large part these "Points" were derived from what is currently required for primary or diploid cells, and the guidelines which were proposed by Jacobs et al for human diploid cells (J. Biol. Stand. 9: 331-342, 1981).

The first section of the "Points" describes general information which should be available on a cell line. Central to the use of cells for production is the cell seed system and the working cell bank. One of the main points on which we would like comments is the time at which cells should be taken for testing. As suggested in the "Points", the tests are recommended at 10 PDLs beyond the PDL used for production. Should more testing be done, and if so, what tests and when should they be done? The Jacobs guidelines, for example, state that all of the tests should be done on at least four occasions at approximately equal intervals (PDLs) during the lifespan of the diploid cell line.

Although extensive karyology background data and monitoring has been a hallmark of the requirements associated with the use of human diploid cells, these "Points" suggest that for other cell types only the general cytogenetic characteristics of the cell line are needed for characterization, and nothing is mentioned about chromosome monitoring during production. This obviously reflects the fact that many of the cell lines currently used for the production of ex erimental products are known to be chromosomally abnormal, and there would e little, if anything, to be gained from chromosomal studies. Comments on this area would be helpful, particulary with respect to how one might resolve the resulting disparity between what is currently required for human diploid cells and what is recommended for other cell lines.

The section on testing for viruses generally follows the Jacobs guidelines. We would, however, like comments on whether the tests should be limited to cell culture medium, or if they should also be done on a suspension of the cell line being characterized. Electron microscopy of at least 200 cells is recommended since that number should assure with 95% confidence that less than 10% of the cells are expressing virus particles. The viral interference test recommended in the Jacobs guidelines has been omitted in these "Points" because we believe the results from such tests are frequently so variable that it adds an unnecessary complication to the testing scheme, and because the other tests, when considered together, should be sufficient for the identification of a latent viral agent. Comments are especially sought on what specific types of tests might be considered for inclusion in "Other tests" at the end of the virus testing section of the "Points".

Please send your comments to me at the following address:

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8800 Rockville Pike
Bethesda, MD 20205

DRAFT

June 1, 1984

# Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals

Office of Biologics Research and Review
Center for Drugs and Biologics, FDA

This document is concerned with characterization of cell lines used to produce biologicals which are subject to licensure under the U.S. Public Health Service Act. Because advances in biotechnology are occurring at a rapid pace, information in this document is subject to change as new and significant findings become available. Accordingly, the discussion below should be interpreted as this Office's general expectations of manufacturers who produce biological products from cell lines and of points which they should consider during product development, in applications for investigational new drug exemptions (INDs), and in product license applications. Existing general regulations (21 CFR, 200 series and 600 series) are also broadly relevant and should be consulted. These points are not all-inclusive, and certain points may not be applicable to all situations. Therefore; the Office of Biologics Research and Review (OBRR) will review the adequacy of testing any cell line on a case-by-case basis.

All data and information submitted with or incorporated by reference in an IND, establishment or product license application or Master File are considered confidential according to 21 CFR 601.50 and 601.51. For further information about confidentiality of such submissions, the above cited CFR sections should be reviewed.

Storage of the cell seed and MWCB

Both the cell seed and the MWCB should be distributed to ampoules and stored in either the liquid phase or the vapor phase of liquid nitrogen. The location and identity of individual ampoules of cells should be thoroughly documented, and all other cells should be excluded from the area where the cell seed and MWCB are kept.

Culture medium

An accurate record of [redacted] of the cell culture medium used should be kept.

Growth characteristics in vitro

The growth pattern and [redacted] appearance of the cell line should be determined, and should be stable from the PDL of the cell seed to at least 10 PDLs beyond the PDL at which the cell line would be used in biologics production. If the cells have a finite life, the total number of PDLs through to senescence should be determined.

Time of sampling and testing of cells

Cells from the MWCB are propagated to the stages at which they are to be tested. The tests should be performed on pools prepared from cell suspensions removed from each culture vessel at least 10 PDLs beyond the PDL at which the cell line would be used in biologics production. In the case of diploid cell lines, the sample should comprise cells that are close to the stage of senescence.

Production and testing facilities

The cell should be propagated in premises in which no other cells are handled, and the testing of cells or supernatant fluids should be performed in separate premises or separate parts of the premises. Separate equipment and personnel should be employed in each of these areas, and no person who has been in contact with transmissible agents or experimental aninals, other than those connected with the cell being characterized, should enter the premises on the same day that any such contact has been made.

## 8. TUMORIGENICITY TESTING

Cells from MWCB should be examined for [redacted] In an acceptable in vivo testing, $10^6$ reference tumor cells inoculated subcutaneously or intramuscularly produce progressively growing tumors in at least 9 out of 10

animals, of which at least one shows evidence of netastases. HeLa, WiDr, KB, HT-1080, and others have been shown to be accpetable as reference tumor cell lines,

The systems shown to be suitable for this test include:

(a) nude (nu/nu) mice (1),

(b) newborn mice or hamsters that have been treated wfth antithymocyte serums (ATS) (2,3), and .

(c) thymectomized and irradiated mice that have been reconstituted with bone marrow from healthy mice (4).

A suitable test using newborn animals treated with ATS or ATG is to inoculate at least 20 animals with 0.1 mL of potent serum within 24 hours of birth. The injection is given either by the intramuscular or subcutaneous route and ,repeated on days 2, 7 and 14 of life. A potent ATS or ATG is one which suppresses the immune mechanisms of the animals such that the subsequent inoculation of $10^6$ reference tumor cells routinely produces progressively growing tumors and metastases. On the day of birth 10 of the 20 animals that have Seen given the antithymocyte preparation are inoculated with $10^6$ viable cells of the candidate cell line by the subcutaneous or intramuscular route at any site at which developing tunors can be palpatated (the base of the neck, the limbs, or the abdomen are suitable). The animals are observed for 21 days for evidence of nodule formation at the site of injection,. and measurements are made at suitable times to determine whether there has been progressive growth.

At the end of the 21' day observation period all animals are sacrificed and examined for gross evidence of tumor formation at the site of injection and in other organs such as the lymph nodes, lungs, kidneys and liver. All. tumor-like lesions and all inoculation sites are examined histopathologically. In addition, since some cell lines may form metastases without evidence of local tumor growth; the lungs and regional lymph nodes of all animals should be examined histologically.

For the purpose of this test, a progressively growing tumor is defined as a palpable nodule that increases in size over the 21 day observation period and that shows viable and mitotically active inoculated cells when examined histologically. The presence of microscopically viable cells in association with a stationary or regressing nodule is not considered a progressively growing tumor.

In addition, the cells may be tested for tumorigenicity in nonhuman primates (5). Such tests would include imunosuppression of the animals with a potent ATS or ATG, inoculation of candidate cells and reference tumor cells, observation for at least three weeks, and the histological examination of the inoculation sites as well as any potential metastatic lesions.

Organ cultures (6,7) or other in vitro tests nay be substituted for an animal test if the in vitro test has been shown to be as sensitive as an acceptable animal test.

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## C. KARYOLOGY

### INTRODUCTION

The [redacted] characterization and monitoring of human diploid cell lines proposed for use in the production of biological products has been an accepted test since the WI-38 cell line began to be used in the 1960s. The rationale for including this test was that gross aberrations in chromosome number or morphology night indicate:

(a) abnormalities intrinsic to the original fetal material;

(b) possibilities of mislabeling and/or contamination involving other cell populations;

(c) the presence of [redacted] such as viruses, mycoplasmas, etc;

(d) the inadvertent exposure of the cell line to chemical or physical mutagens which night similarly induce persisting chromosomal changes.

In 1978 an Ad Hoc Committee met to revise the karyology control recomendations, and the Committee's report was published in 1979, Of particular importance in that report were the following statements:

> "Although our experience in karyological control of human cell substrates was derived fron use of the fibroblast cell line WI-38, these revised requirgments do not specifically exclude any cell line on the basis of cell type or pattern of lifespan. It is recognized that human cell lines other than fibroblast-like lines nay be acceptable to control authorities even though their karyological characteristics can differ from those of human diploid fibroblast cell lines. The limits of acceptability recommended in this report should be viewed as explicitly applicable to all human diploid fibroblast cell lines, and as possibly useful in determining the acceptability of other human cell lines. At present, in addition to.M-38, only the hunan diploid fibroblast cell line MRC-5 meets these requirements for karyological control. Sinilarly, diploid cell lines derived from non-human species nay be acceptable to control authorities even though their karyological characteristics differ from those of human diploid fibroblasts, The characterization studies of such non-hunan cell lines should follow the general guidelines as presented for human cell lines.
>
> "The relevance to product safety to karyological monitoring of cell substrates in the production of non-replicating biological products such as interferon is open to question. The potential risks of using karyologically abnormal cells are related to the possible contamination of the product with cellular nucleic acid, Control authorities should therefore consider the purification procedures used in the manufacturing process and the confidence with which one can exclude the presence of cellular nucleic acid in the final product, Current technology may allow the elimination of cellular nucleic acids from non-replicating biological products produced in cell culture systems, in which case karyological monitoring of the cell substrate is considered unnecessary*

The detailed characterization and monitoring procedures described in 1979 (J. Biol. Stand. 7:397-404) apply specifically to the use of diploid cell lines for the production of live viral vaccines. Those procedures may be modified in the future in the light of new data and/or alternate methods to assure the safety of the cell substrate.

In the case of cell lines used for the production of biological products other than live virus vaccines, sufficient karyologic information should be provided to demonstrate the general cytogenetic characteristics of the cell line including a summary of any relevant published data.

## D. TESTS FOR THE PRESENCE OF MYCOPLASMAS

A pool of cells containing about 5 X $10^5$ cells per mL, suspended in the cell culture medium from which they were harvested, should be tested for the presence of mycoplasmas. Samples should be stored before use either (a) between 20C and 9°C for no longer than 24 hours or, (b) at -60°C or lower if stored for longer than 24 hours. The sample should be tested for the presence of mycoplasmas by using agar and broth media and by an indicator cell culture procedure. Each lot of agar and broth medium used should be free of antibiotics except for penicillin and examined for growth-promoting properties. The media used should be shown to be capable of supporting the growth of a standard inoculum of 100 colony-forming units (CFU) or 100 color-changing units (CCU) or less of at lest two known fastidious species of mycoplasmas, in not more than 15 passages from isolation, such as Mycoplasma pneumonia strain PI-1428 or equivalent strain and M. orale strain 1596 or equivalent strain. The indicator cell culture test procedure used should be pretested and shown to be capable of detecting known mycoplasma contaminants, such as the M. hyorhinis strain BTS-7; M. orale, strain 1596 or equivalent strains approved by the OBRR using an inoculum of 100 CFR or 700 CCU or less.

A 0.8 mL sample should be inoculated, in evenly distributed amounts, over the surface of 4 or more agar plates of at least two agar media, and 2 mL of the bulk lot sanple should be inoculated into a flask containing 20 mL of broth medium and incubated in 36 + 1°C. On 3, 7 and 14 days of incubation, 0.5 mL of the broth culture should be tested by subculture onto 4 or more agar plates. Two of the initial isolation plates and two each of the three subculture plates should be incubated aerobically in containers designed to prevent dessication and the remaining agar plates incubated in a 5 to 10% carbon dixoide in nitrogen and containing less than 0.5% oxygen. All culture plates should be incubated for 14 days and observed for growth of mycoplasma colonies microscopically at 100 times or more magnification. Each test should include two positive mycoplasma control cultures, one of which should be the Mycoplasma pneumoniae strain and the second species should be an arginine

hydrolyzer, such as the M. orale strain or equivalent strains. The inoculum of the-two positive mycoplasma control cultures should be 100 CFU or 100 CCU or less and used to test the efficacy of the media used. The presence of mycoplasmas should be determined by comparing the growth obtained from the test sample with that of the positive and negative control cultures with respect to colony size and morphology.

In addition, the sample should be tested for the presence of mycoplasmas by using an indicator-cell culture procedure. A Vero cell culture substrate should be used. An equivalent indicator cell substrate nay be acceptable whenever the manufacturer present evidence to demonstrate that such modification will provide equal sensitivity for the detection of known mycoplasma contaminants. One mL of the sample should be used to inoculate two or more indicator ce17 cultures grown on coverslips in dishes or equivalent containers. The inoculated indicator cell cultures should be examined by epifluorescence after 3 to 5 days of incubation at 36+ 1°C by a DNA-binding fluorochrome, such as bisbenzimadazole or equivalent strain. The test should include two positive mycoplasma controls, such as the M. hyorhinis and M. orale strains or equivalent strains, using an inoculum of 100 CCU or less and should also include a negative (non-infected) indicator cell culture control. (Marked cytopathic effects or nuclear chromatin fragmentation caused by viruses can affect interpretation of results. In such cases, specific neutralizing viral antiserum or nonpermissive cell culture substrate should be used,) The presence of mycoplasmas in the sample should be determined by microscopic examination of the inoculated indicator cell culture at 400 times magnification or greater. The microscopic appearance of the cell cultures inoculated with the sample should be compared with the appearance of the mycoplasma positive and the uninfected negative cell culture controls, The sample is satisfactory for further manufacture if none of the agar or broth media and none of the indicator cell culture procedures described show evidence for the presence of mycoplasmas.

## E. TESTS FOR THE PRESENCE OF BACTERIA AND FUNGI

A poll of cells containing about 5 x $10^5$ cells per mL, suspended in the cell culture medium from which they were harvested, should be tested for the presence of bacteria and fungi by following the sterility tests described in section 610.12 of Title 21 of the Code of Federal Regulations.

## F. TESTS FOR THE PRESENCE OF VIRUSES

1. Tests in cell cultures. The following types of cell cultures are suggested as a minimum in testing for the presence of viruses in a cell line,

   (a) the cell line being characterized;

(b) a normal human embryo cell line;

(c) a monkey kidney cell line.

Other cell cultures nay also be suggested depending on the cell type and its source. These cell cultures include:

(a) a HeLa cell line;

(b) a rabbit kidney ,cell line;

(c) others as indicated

The cells indicated above should be examined for normal morphology during an incubation period of at least 14 days after inoculation with a suspension of the cell being characterized. On days 3-5 and again after the 12th days at least 4% of the inoculated cultures of the cell lines indicated in 1(a), (b). and (c) should be washed and tested for hemadsorption using red cells from guinea pigs, chickens and from monkeys or humans, One-third of the cultures nay be used for erythrocytes derived from each of these species, or an equal mixture of erythrocytes from the three species nay be used. A serum and calcium-free salt solution should be used to wash the cells and to maintain then during the period of incubation. Tests should be read after the cultures have been incubated for 30 min at 3-4°C and again after 30 min at 34-37°C. If more than 20% of the cultures are discarded for accidental causes the tests should be repeated.

2. Prolonged cultivation and microscopy, The cells being characterized should be propagated serially to or beyond the production level and should be' pooled each time they are subcultured. During the period of cultivation the cells should be examined for any change in their cultural characteristics and any such changes should be recorded. When the cells reach 10 or more PDLs beyond the production level, or if they show earlier signs of degeneration, they should be harvested and a sample of at least $10^7$ cells should be used to prepare a pellet to be examined by transmission electron microscopy (TEM) for the presence of viruses or other nicrobial agents; at least 200 cells should be examined, Cell cultures at the same PDL should be examined by scanning electron nicroscopy to detect mycoplasma, fungi, and bacteria which night be resistant to growth in standard test media.

In addition, cells at the same PDL should be examined by TEM after treatment with an inducing agent such as BrdU in order to enhance the detection of latent retroviruses (1,2). A high speed pellet (100,000 x g for 90 minutes) of the cell-free supernatant fluid of cell cultures at the same

PDL should also be examined by TEM using phosphotungstic acid or uranyl acetate as an electron dense stain. The pellets should also be assayed for the presence of Rii-dependent DNA polymerase.

3. Tests in animals. Cells should be suspended in a suitable medium at a concentration of $10^7$ per mL and should be inoculated into groups of animals as described below.

At least 10 animals from at least two litters of suckling mice should be inoculated. Each animal should be inoculated with 0.1 mL intraperitoneally and 0.01 mL intracerebrally. The mice are observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, is necropsied and examined for evidence of viral infection. Such examination includes subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes, and daily observations are made for 14 days. In addition, a blind passage is made of a single pool of the emulsified tissue (minus skin and viscere) of all mice surviving the original 14-day test.

Ten adult mice should be inoculated. Each animal should be inoculated with 0.5 mL intraperitoneally and 0.03 mL intracerebrally.

Five guinea pigs should be inoculated. Each animal should be inoculated with 9 mL subcutaneously with 1 mL intradermally at multiple sites.

The animals should'be observed for four weeks and any that become sick or show any abnormality should be examined to establish the cause of illness.

Ten 10-day-old embryonated chicken eggs should be inoculated by the allantoic route using 0.1 mL per egg. Following incubation at 35°C for 72 hours, the allantoic fluids should be harvested, pooled and subpassed using the same route and methods into fresh embryonated eggs. Both the initial pool and the subpassage harvest should be tested with guinea pig and chick erythrocytes for the presence of hemagglutinin at 4°C and at room temperature (18-21°C). In addition, 10 embryonated eggs 6 to 7 days old should be inoculated by the yolk sac route using 0.1 mL per egg, Following incubation at 35° for at least 9 days, the yolk sacs are harvested and pooled. A 10%suspension of yolk sacs are subpassed by the same route into fresh embryonated eggs using the same route and methods, A11 embryos should be examined to confirm viability.

4. Other tests. Cells persistently or latently infected with one or more viruses may require special tests to characterize viral expression or replication so that elimination or inactivation, as appropriate, of the virus from the product can be rputinely demonstrated.

## References

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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# BIOTECHNOLOGY AND FDA REGULATION-OF HYBRIDOMA In Vitro DIAGNOSTIC PRODUCTS: List of Current Devices and Guidelines for Manufacturers

by

Kearby Fugate, Ph.D., S. Vadlamudi, Ph.D., D.V.M. and W.S. Stewart, M.S.

## Abstract

The incorporation of hybridana derived monoclonal antibodies in in vitro clinical laboratory devices presents a promising opportunity to improve existing immunochemical methods and to develop new analytical systems. 1 The impact upon the clinical laboratory has been comparable to that of automation only a few years ago. It is reasonable to expect that hybridana technology will create unique demands upon the regulatory responsibilities of the U.S. Food and Drug Administration (FDA).

Within the last two and a half years, the Center for Devices and Radiological Health (CDRH) has received numerous premarket applications for ~~in vitro~~ devices that utilize monoclonal antibodies, The CDRH anticipates an increased number of such submissions in the near future involving new devices, new [illegible] and the replacement of polyclonal antibody reagents in currently available test kits. For effective regulation of this new technology, during the last two and one-half years FDA has reviewed each submission independently, based upon the classification (Class I, II, III) of the analyte, rather than having developed a "hybridana class or group" approach, Experience gained by reviewing hybridana technology has contributed to improved performance specifications and will continue to facilitate the FDA development of hybridoma guidelines for manufacturers of diagnostic devices.

## Development and Characterization of Hybridoma Cell Lines

### A, General Concepts

Numerous satisfactory approaches to hybridoma production have been devised using a wide variety of immune and myeloma cell sources. Immunoglobulin products derived from these hybridomas are influenced both by genes derived from parent cells and by the environment imposed during ptoductiorr. Accordingly, both the origins and production procedures for monoclonal antibody-producing hybridomas should be described.

### B. Origins

To evaluate product consistency and stability, it is important to have information about the hybridoma cell line, for example:

I. Source, name, and characterization of the parent myeloma cell line with respect to any heavy or light chains which it synthesizes and/or secretes.

2. Murine strain of the immune cell, including identification of the immunogen and a general description of the immunization scheme.

3. A brief description of the cell cloning procedures,

4, In those instances in which the parental myeloma cell line is an immunoglobulin producer, characterization of the hybridoma cell line with respect to parental myeloma chains as well as immune cell [illegible] chain production.

5. Description of the seed lot system for establishing the primary and secondary lots. The seed cultures should be well characterized with respect to identify, stability, and any known contaminants and a description of how these seed cultures will be maintained should be included.

C. Production Procedure

The following information is helpful in evaluating manufacturing procedures;

1. A description of production, including an outline of the tissue culture procedure if production is in vitro. If production is in mice, their supplier(~),their genotype, their husbandry, the manner in which they are monitored for potentially pathogenic organisms, and a description of harvest and passage procedures.

2. Description of the steps that are taken to control viral, bacterial and mycoplasmal contamination.

3. Description of the acceptance criteria for tissue culture supernatants or ascites fluids intended for further manufacture.

4. Description of procedures used to purify the immunoglobulin.

5. Description of procedures used to prepare and fill final containers.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Bethesda MD 20205

DATE: June 20, 1983

TO: Manufacturers of Monoclonal Antibody Products and Other Interested Parties

FROM: Chairman, Hybridoma Committee,
Office of Biologics, NCDB, FDA

SUBJECT: ~~In Vitro~~ Monoclonal Antibody Products Which arc Identified as Biological Products

Most in vitro diagnostics are regulated by the Office of Medical Devices, NCDRH, however, those in vitro diagnostics commonly employed in blood banking come under the purview of the Office of Biologics and are subject to licensure under section 351 of the Public Health Service Act. These include Blood Grouping Sera, Anti-Human .Globulin and Antibody to Hepatitis B Surface Antigen.

In March of 1982, the Office of Biologics shared with manufacturers a draft document entitled "Points to Consider in the Manufacture of In Vitro Monoclonal Antibody Products". Manufacturers were asked to respond with their ideas, [illegible], and comments concerning the "Points to Consider" document, and 18 letters were received. Those letters have been thoroughly analyzed, and many of the points conveyed in the letters have influenced modifications in the March, 1982 draft.

Many manufacturers emphasized that hybridoma technology is a fast moving field and that the premature development of firm guidelines or requirements could adversely affect product development and ultimate product availability. It is for this reason that the Office of Biologics is now distributing a substantially revised second draft of .its March, 1982, "Points to Consider" document. We are again soliciting your comments, ideas and recommendations concerning criteria that may later be developed into guidelines or regulations for evaluating the effectiveness of in vitro diagnostics that contain monoclonal antibodies, and that are subject to licensure under the U.S. Public Health Service Act. We will greatly appreciate receiving your comments regarding the attached "Points to Consider" draft by September 1, 1983.

Some of the hiyhlights of the new draft include the following:

(1) The identification of information which the Office of Biologics needs to know in order to make informed regulatory judgements.

(2) A request for a description of those steps which have been taken to establish that the hybridoma secretion product remains unchanged rather than for steps to insure clonal stability.

(3) Deletion of the request in the earlier Draft for the determination of the percentage of specific secretor cells both in the seed population and a: the time of harvest.

(4) Deletion of the request in the earlier Draft for determination of the possible concurrent production of lymphokines by the hybridoma cell line.

(5) A provision which allows for non-sterile in vitro monoclonal antibody products, but stipulates that if the product is non-sterile, this should be indicated on the final container.

(6) Because subtle mutations may occur in either the constant or the variable portion of the immunoglobulin molecule and may affect either the stability or the specificity of the monoclonal antibody, the current version provides for stability testing of each product lot. It also allows biochemical and biophysical studies as an alternative to extensive specificity testing of each lot of in vitro diagnostic product. Frequently such biochemical and biophysical tests can effectively establish that the nonoclonal antibody in the lot under test is identical with the monoclonal antibody for which the license was granted.

(7) A provision that an in vitro monoclonal antibody product should have specificity and sensitivity comparable to those for traditional polyclonal antibody proaucts. If a similar licensed product exists, the specificity profile of the monoclonal product should be compared with that of the licensed product.

(8) A statement that tests should be done to establish a dating period under realistic field conditions, and that if six months of satisfactory stability data are available on the final product at the time of licensing, the remaining stability studies nay be performed concurrent with distribution.

Please address comments to:

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# Points to Consider in the Manufacture of *In Vitro* Monoclonal Antibody Products Subject to Licensure

Office of Biologics
National Center for Drugs and Biologics
Food and Drug Administration
Revised June, 1983

## INTRODUCTION

This document is concerned only with those few hybridoma-derived *in vitro* diagnostics which are subject to licensure by the Office of Biologics under the U.S. Public Health Service Act. At the present time, the *in vitro* diagnostics of this type are blood bank related products, namely blood grouping sera, anti-human globulin and hepatitis B surface antigen detection kits. Because hybridoma technology is a rapidly evolving field, the information in this document is subject to change as new and significant findings become available. Accordingly, the criteria discussed below should be interpreted as being what the Office of Biologics generally expects producers of such diagnostics to consider in product development and in their license applications. These criteria are not all-inclusive, and certain of them may not be applicable to all situations. The Office of Biologics reserves the right to review the adequacy of testing of any product on a case-by-case basis. Many aspects of manufacturing and of the production facility are not discussed here. Such specific details and the relevant concerns of interested manufacturers should more properly be discussed on an individual basis with the Office of Biologics and should be included in the specific product license application.

## GENERAL CONCEPTS

For *in vitro* diagnostic products, the primary concerns relate to the sensitivity, specificity, stability, potency and consistency of the product. The following points should be of help in preparing license applications for these products. In addition, pursuant to 21 CFR Part 600, there are certain standard requirements which will need to be addressed in a license application. Attention should be given, of course, to standards that now exist (e.g., Part 660.1 Antibody to Hepatitis B Surface Antigen) for already licensed products.

### I. Characterization of Cell Lines

A. Origins. To evaluate product consistency and stability, it is important to know about the hybridoma cell line. As an example:

1. Source, name, and characterization of the parent myeloma cell line with respect to any heavy or light chains which it synthesizes and secretes.

2. Source and species of the immune cell, including identification of the immunogen and a general description of the immunization scheme.

3. Ganeral description of the cell cloning and recloning procedures.

4. Description of the seed lot system for establishing the primary and secondary lots, if used. The seed cultures should be well characterized and a description of how the seed cultures will be maintained should be included. In the absence of using a seed lot system, the procedures used for propagating and characterizing the clone should be dcscribed.

5. Results of testing of the hybridoma cell line for possible concurrent production of additional Ig light or heavy chains which might interfere with the test.

B. Production Procedure

1. General description of production, including an outline of the tissue culture procedure if production is in vitro. If production is in mice, their genotype, and a description of harvest and passage procedures.

2. Description of the criteria which tissue culture supernatants or ascites fluids must meet in order to be acceptable for further manufacture.

3. Description of procedures used to prepare the [illegible] iron either the tissue culture or ascites fluids.

4. Cescription of the steps, if any, that are taken to control microbial contamination. If the product is non-sterile, this should be indicated on the final container.

II. Characterization of the Product

A. Immunologic Specificity and sensitivity. It has been demonstrated that spontaneous mutations affecting the immunoglobulin secretion products of hybridomas can occur at an unusually high rate. Accordingly, steps should be taken to establish that the hybridoma secretion product remains unchanged in successive product lots. The immunologic specificity and sensitivity requirements for a monoclonal antibody product should be comparable to those for a traditional polyclonal antibody product. If a siwilar polyclonal licensed product exists, the specificity prcfile of the monoclonal product should be compared with that of the polyclonal licensed product. Appropriate reaction conditions (tine, temperature, pH and protein concentration ranges, etc.) and the specific activity of the product (e.g. titer or mg/mL of antibody) should be established. Successive lots of the product should meet established product specifications.

B. Biochemical-Biophysical Studies. As an alternative to extensive specificity testing of each lot, abbreviated specificity testing may be performed when it is supplemented by appropriate biochemical and biophysical characterization of the monoclonal product. Such biochemical and biophysical studies would involve comparison of a sample of each lot to a retention sample of a prior bull; lot designated as a standard, using, where appropriate, the following types of tests:

1. The electrophoretic migration of the product in both the native and reduced states should be measured relative to the standard material on polyacrylamide gels.

2. The spectrotype of each lot should be compared with the reference material by isoelectric focusing.

3. Protein concentration should be estabished by a suitable assay as, for example, a Lowry determination.

4. The immunoglobulin class and, when appropriate, the subclass should be determined.

C. Stability of the Product. The final product will be subjected to a variety of conaitions when used in the field. Therefore, tests should be done to establish a dating period during which consistent results can be expectec under realistic field conditions. If six months of satisfactory stability data on the final container product are available at the tine of licensing, the Office of Biologics will consider whether the remaining stability studies may be performed concurrent with distribution.

A typical pre-licensing stability study would include tests at three month intervals throughout the maximum shelf life of the product. Serial titrations should be used when necessary to permit meaningful comparison of binding activities. Where possible, the sane source of antigen should be used throughout the study (frozen aliquots of reagent sera or cells are recommended). Moreover, because of the possibility that subtle mutations of monoclonal antibodies might affect their molecular integrity, the stability of each production lot should be checked by potency testing at the middle and end of its dating period. Because of the rapid evolution of hybridoma derived products, it is especially important that any significant changes in these products during storage be reported to the Office of Biologics upon discovery.