

---

# **Guidance for Industry**

## **Monoclonal Antibodies Used as Reagents in Drug Manufacturing**

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)  
March 2001**

---

# Guidance for Industry

## Monoclonal Antibodies Used as Reagents in Drug Manufacturing

Additional copies are available from:

Drug Information Branch, HFD-210  
Center for Drug Evaluation and Research (CDER)  
5600 Fishers Lane  
Rockville, Maryland 20857  
(Tel) 301-827-4573  
(Internet) <http://www.fda.gov/cder/guidance/index.htm>

or

Office of Communications  
Training and Manufacturers Assistance, HFM-40  
Center for Biologics Evaluation and Research (CBER)  
1401 Rockville Pike  
Rockville, Maryland 20852-1448  
(Fax) 888-CBERFAX or 301-827-3844  
(Voice Information) 800-835-4709 or 301-827-1800  
(Internet) <http://www.fda.gov/cber/guidelines.htm>

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)  
March 2001**

## TABLE OF CONTENTS

|      |                                                                       |   |
|------|-----------------------------------------------------------------------|---|
| I.   | INTRODUCTION.....                                                     | 1 |
| II.  | BACKGROUND.....                                                       | 2 |
| III. | PRODUCTION OF MONOCLONAL ANTIBODY REAGENTS .....                      | 2 |
| IV.  | MONOCLONAL ANTIBODY REAGENTS IN DRUG MANUFACTURING.....               | 4 |
| A.   | PURIFICATION OF DRUG SUBSTANCE .....                                  | 4 |
| B.   | COMPARABILITY.....                                                    | 5 |
| V.   | SPECIFICATIONS FOR MONOCLONAL ANTIBODY REAGENTS.....                  | 5 |
| A.   | TESTING OF UNCONJUGATED MONOCLONAL ANTIBODY REAGENTS .....            | 6 |
| B.   | TESTING OF MONOCLONAL ANTIBODY REAGENTS LINKED TO SOLID SUPPORT ..... | 6 |
| VI.  | STABILITY OF MONOCLONAL ANTIBODY REAGENTS .....                       | 6 |
|      | REFERENCES .....                                                      | 8 |

# GUIDANCE FOR INDUSTRY<sup>1</sup>

## Monoclonal Antibodies Used as Reagents in Drug Manufacturing

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any 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 statutes and regulations.

### I. INTRODUCTION

This guidance is intended to provide recommendations to sponsors and applicants on the use of monoclonal antibodies (mAbs) as reagents in the manufacture of drug substances<sup>2</sup> that are regulated by the Center for Drug Evaluation and Research (CDER) or the Center for Biologics Evaluation and Research (CBER). The guidance focuses on the chemistry, manufacturing, and control (CMC) issues that should be addressed in new drug applications (NDAs), abbreviated new drug applications (ANDAs), biologics license applications (BLAs), supplements to these applications, or investigational new drug applications (INDs).

This document presents issues associated with and recommendations on the documentation to support the use of mAb reagents generated by hybridoma technology or production of recombinant mAb or their fragments in bacteria, including phage display technology, fungi (yeasts and molds), and nonprimate animal-derived transfected cell lines. Monoclonal antibodies or their fragments generated by other methods can present additional concerns. The recommendations provided in this document should be considered when such materials are used; however, the guidance does not address the particular method of production of the mAbs or their fragments.

This document does not provide recommendations on mAbs that are used as diagnostics, radiolabeled imaging agents, or therapeutic products. For a discussion of mAb products for human therapeutic or diagnostic use please refer to the *Points to Consider in the Manufacture and Testing of Monoclonal*

---

<sup>1</sup> This guidance has been prepared by the Monoclonal Antibodies Working Group of the rDNA Reagent Technical Committee of the Complex Drug Substances Coordinating Committee (CDS CC) in the Center for Drug Evaluation and Research (CDER), with input from the Center for Biologics Evaluation and Research (CBER), at the FDA.

<sup>2</sup> The term *drug substance*, which is used throughout the text, is intended to include biological products as defined in 21 CFR 600.3(g).

*Antibody Products for Human Use* (PTC 1997).<sup>3</sup> The recommendations for characterization and testing for mAbs used as parenteral pharmaceuticals are by necessity stringent, and not all of them are applicable to mAbs that are used as reagents in drug manufacturing.

## II. BACKGROUND

Monoclonal antibodies are immunoglobulin molecules secreted from a population of identical cells (i.e., cloned cells). They are homogeneous in structure and binding specificity. In the context of this guidance, *mAb reagents* refers to monoclonal antibodies used as reagents in a drug substance manufacturing process.

The issues related to mAbs used as reagents are somewhat different from those of mAbs used as parenteral therapeutic agents. For mAb reagents, the primary emphasis is on assessment of the following:

- C Biological safety, in particular the assessment of contamination of the mAb reagent with adventitious agents and/or process-related impurities from the cell substrate or cell line sources.
- C Performance characteristics of the mAb reagent during drug substance manufacture (e.g., avidity and specificity for the target molecule).
- C Potential presence of residual amounts of the mAb reagent in the final drug substance and/or drug product.

The recommendations in this guidance apply to the use of mAb reagents in the drug substance manufacturing process where the mAb reagent is used to purify the drug substance. The extent of characterization required for the mAb reagent depends on the nature of the steps that follow use of the mAb, and thus will vary among submissions. While many CMC concerns regarding the use of mAb reagents are unique to biotechnology-produced reagents, the general concepts expressed in the *FDA Guideline for Submitting Supporting Documentation in Drug Applications for the Manufacture of Drug Substances* (FDA 1987) also apply. An early and continued dialogue between the applicant and the Agency is encouraged to discuss the data that should be submitted to support the use of the mAb reagent.

## III. PRODUCTION OF MONOCLONAL ANTIBODY REAGENTS

---

<sup>3</sup> This document is available on the Internet at <http://www.fda.gov/cber/points.htm>.

The sponsor or applicant should submit information (e.g., production process, specification) to support the use of the mAb reagent or a letter of authorization (LOA) to a drug master file (DMF) that contains this information.

A description of the mAb manufacturing process should be provided. The description is used to assess the potential impact on the biological safety, quality, and purity of the drug substance and/or drug product. The mAb reagent should be adequately characterized and its identity, purity, and structural integrity should be assessed, as these factors are vital to its efficient and uninterrupted performance during production of drug substances (see section IV). Reagents that have not been fully characterized for viral safety should not be introduced into facilities where biologics and drugs from mammalian cell culture are produced because of the potential for cross-contamination. Additional recommendations relating to mAb reagents are:

- For mAb reagents prepared using hybridoma propagation, serum additives in culture media should be free of contaminants and adventitious agents.
- Manufacturers should use bovine-derived materials only from cattle that were born, raised, and slaughtered in countries that are free of BSE (bovine spongiform encephalopathy).<sup>4</sup>

The predominant concern with the use of mAb reagents in drug substance manufacture is the introduction of adventitious agents (e.g., viruses, bacteria, fungi, mycoplasma) and/or process-related impurities (e.g., protein and DNA contaminants, column leachables, media components) into the drug substance. Of particular concern are those that are not removed during drug substance manufacture steps after the introduction of the mAb reagent. In many instances, the extent of the cell bank safety characterization and the clearance studies for adventitious agents and/or process-related impurities should follow the established standards for mAbs intended for human use (see PTC 1997, sections II.B and C). A reduced level (i.e., less than recommended in PTC 1997) of testing of cell banks and/or validation of the procedures used to remove or inactivate adventitious agents and/or process-related impurities during purification of the mAb may be appropriate under certain circumstances, with justification. Early dialogue with the Agency is encouraged when a reduced level of testing and/or validation is planned. A reduced level can be justified when, for example:

C The drug product is terminally sterilized.

---

<sup>4</sup> A list of countries affected by BSE or those that have a substantial risk associated with BSE (due to a lack of implementation of an adequate surveillance program) can be found on the Internet at <http://www.aphis.usda.gov/NCIE/country.html>.

- C The use of the reagent is followed by adequate steps for the removal and/or inactivation of the adventitious agents and/or process-related impurities. In this instance, the overall assessment of the removal and/or inactivation process can take into account validation data from steps in the manufacture of the reagent and manufacture of the drug substance.
- C Processing steps downstream of the reagent include extremes of pH or organic solvents, and there are reliable data in the scientific literature that the extremes remove and/or inactivate adventitious agents and/or process-related impurities.
- C The mAb reagent is produced in an expression system in which human infectious agents do not propagate (e.g., plants, bacteria, fungi, insect cultures).

#### **IV. MONOCLONAL ANTIBODY REAGENTS IN DRUG MANUFACTURING**

A major use of mAb reagents is in the purification of drug substance by mAbs attached to a solid support (e.g., immunoaffinity chromatography). Issues relating to and recommendations on the information to submit in support of the use of mAb reagents in the purification process are discussed below. The information that should be submitted to support other uses of mAb reagents in drug manufacture will depend on the use and are not discussed in this guidance. Sponsors or applicants with questions on documentation to support other uses of mAb reagents are encouraged to contact the Agency.

##### **A. Purification of Drug Substance**

The drug substance purification processes should be described in the application. The drug substance manufacturer should establish a specification for the incoming mAb reagent, and perform testing before using the reagents in the manufacturing process. In addition to identity testing for the incoming mAb reagent, drug substance manufacturers should carry out additional testing (e.g., binding activity, adventitious agents) to ensure that the reagent will perform as intended. Affinity and specificity studies are recommended to assess whether the characteristics of a mAb reagent are optimal for targeted binding to the appropriate substrate during the manufacture of the drug substance.

Leaching of mAb or impurities from the solid support into the final product should be considered when specifications are established for the drug substance. The amount of column leachables is not uniform over the column lifespan and depends on several factors (e.g., length of storage, solutions used in the regeneration and/or sanitization steps, column operating parameters). A variety of methods can be used to test for leachables such as sampling the buffer flow-through prior to the load of the drug substance intermediate, in-process testing of the intermediate bulk, or testing the final drug substance. Alternatively, if documentation is

available that the production steps that follow the use of the reagent mAb reduce the maximum amount of column leachables to appropriate levels, this documentation can be provided in lieu of routine testing for leachables.

Data on the ability of the affinity column to achieve the intended purity under specified working conditions should be submitted. The stability of the mAb reagent during use, the column performance, and the microbial contaminants should be monitored during production of drug substance and documented by the drug substance manufacturer. Tests and acceptance criteria for residual mAb should be included in the specifications for drug substances processed with mAb reagents. Residual mAb should be monitored by sensitive and specific assay (e.g., enzyme-linked immunosorbent assay (ELISA)).

## **B. Comparability**

Changes in the mAb supplier or changes in the manufacturing process of mAb or solid support are considered to be drug substance manufacturing process changes that can have an effect on the biological safety and effectiveness of the drug substance and, consequently, the final product. In cases where significant changes have been implemented in the mAb manufacturing process that may change the purity or the performance of the reagent (e.g., specificity, avidity, microbiological safety), appropriate product comparability testing should be performed. The guidance document entitled *FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products* (1996) contains a discussion of comparability testing for mAbs used parenterally. Comparability testing for mAb reagents should focus mainly on the performance characteristics of the reagent and its purity and stability. This is particularly important when changes in the reagent manufacture are likely to have an impact on the biological safety, purity, quality, or stability of the drug substance and/or drug product.

## **V. SPECIFICATIONS FOR MONOCLONAL ANTIBODY REAGENTS**

Specifications for the mAb reagents should be provided. A certificate of analysis (COA) should be available for each individual reagent lot. For monoclonal antibodies linked to a solid support, COAs should be provided for both forms, unconjugated and linked. A copy of a representative COA should be provided.

The COA should provide the test results, including those for adventitious agents, expiration date, and a disclaimer statement in large bold lettering: **REAGENT USE ONLY; NOT INTENDED FOR HUMAN USE.**

### **A. Testing of Unconjugated Monoclonal Antibody Reagents**

Tests to adequately characterize the unconjugated mAb reagent typically include:

- C Identity (e.g., reducing and nonreducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) pattern, isoelectric focusing (IEF) profile)
- C Purity (e.g., high performance liquid chromatography (HPLC), SDS-PAGE, capillary electrophoresis)
- C Protein concentration
- C Binding to the target molecule
- C pH
- C Microbial and/or bacterial endotoxin limits, as appropriate
- C Preservatives, as appropriate

### **B. Testing of Monoclonal Antibody Reagents Linked to Solid Support**

Tests for mAb reagents linked to solid support should include, at minimum, the following:

- C Physical characteristics (e.g., mean particle size, matrix structure)
- C Concentration of mAb (e.g., milligrams of mAb per gram of resin)
- C Specific binding capacity at recommended temperature and buffer ranges
- C Amount of leaching of mAb
- C Microbial and/or bacterial endotoxin limits, as appropriate
- C Preservatives, as appropriate

## **VI. STABILITY OF MONOCLONAL ANTIBODY REAGENTS**

The mAb manufacturer should perform real-time stability studies of unconjugated and conjugated mAb. Based on these studies, the mAb manufacturer should determine and provide an expiry date for each lot of mAb reagent. Stability indicating tests should focus on performance and physical integrity of the mAb reagent. Either the drug substance manufacturer or the reagent manufacturer should provide data supporting the in-use chemical stability of the column and mAb reagent using the recommended storage buffer, regeneration and/or cleaning solutions under specific time and temperatures.

## REFERENCES

*Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use*, FDA, 1997 (PTC 1997).

*Guideline for Submitting Supporting Documentation in Drug Applications for the Manufacture of Drug Substances*, FDA, 1987.

FDA guidance for industry on *Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products*, FDA, 1996.