

December 4, 1984

VETERINARY SERVICES MEMORANDUM NO. 800.68

Subject: New Biotechnology for Preparation of Animal Biological Products

To: Biologics Licensees and Applicants  
National Veterinary Services Laboratories  
Regional Directors  
Biologics Specialists  
Area Veterinarians in Charge

I. PURPOSE

The purpose is to establish policies and procedures for use of new biotechnological procedures for preparation of veterinary biological products subject to licensure under the provisions of Title 9, Code of Federal Regulations (9 CFR), Parts 101 to 117.

II. POLICIES AND PROCEDURES

A. General

Veterinary biological products prepared using new biotechnological procedures such as recombinant DNA, chemical synthesis, or hybridoma technology will be treated as analogous to products prepared by conventional techniques. A separate license will be required for each such product even if the product is identical in molecular or chemical structure to a naturally occurring substance or a conventionally prepared product.

The unlimited number and kinds of products that may result from these new biotechnological procedures make it impossible to define all requirements in specific terms. Each product must be evaluated individually to determine what will be necessary to establish its purity, safety, potency, and efficacy. Special assays, preferably using in vitro methods, may be required for potency and stability determinations. Additional tests may be required to assure safety, especially when live microorganisms are present in the biological product.

## B. Recombinant Products

### 1. Guidelines

The United States Department of Agriculture (USDA) requires recipients of research grants and manufacturers of veterinary biological products using recombinant DNA technology to follow guidelines provided by the National Institutes of Health (NIH). These guidelines are also appropriate for development and production of animal biological products.

"Guidelines for Research Involving Recombinant DNA Molecules" and "Recombinant DNA Research; Physical Containment Recommendations for Large-Scale Use of Organisms Containing Recombinant DNA Molecules" have been published and are periodically updated in the Federal Register. These two documents, along with "Administrative Practices Supplement to the NIH Guidelines for Research Involving Recombinant DNA Molecules" published by NIH, outline the current policy of NIH concerning recombinant DNA research and the procedures required to contain recombinant microorganisms when used in large-scale production. Copies of these documents may be obtained upon request from the Office of Recombinant DNA Activities, NIH, Room 4A52, Building 31, Bethesda, MD 20205.

### 2. Recombinant DNA Technology

The technology encompasses the isolation, characterization, and insertion of foreign DNA into vectors for the production of foreign gene products in suitable expression systems.

#### a. Foreign DNA

The genetic information coding for the product of interest is referred to as foreign DNA. It consists of a naturally occurring or chemically synthesized nucleotide sequence known to code for a functional gene product. The specific cloned nucleotide segment coding for the desired product must be defined in data supporting each license application. These data must also include a description of antigen or DNA source material, the nucleotide sequence, and the restriction endonuclease digestion map.

#### b. Vectors

A vector is a cloning vehicle which provides a suitable origin of replication necessary for production of foreign DNA. Such replicons include plasmids, bacteriophage, or viruses such as vaccinia, bovine papillomavirus, adenoviruses, or SV40. A restriction endonuclease map of the vector construct describing structural genes, regulatory or promoter regions, insert orientation, and a description of readily detectable phenotypic traits on host cells will be required as supporting data.

### c. Expression Systems

Production of functional gene products depends on the efficient expression of cloned DNA-vector complexes in suitable host microorganisms such as *Escherichia coli*, *Bacillus subtilis*, or *Saccharomyces cerevisiae*. Tissue culture cells may also be used as expression systems for replication of vectors. The mechanism of transfer, copy number, and the physical state of the constructed vector inside the host cell, integrated or extrachromosomal, should be described.

### 3. Chemically Synthesized Antigens

When the product consists of chemically synthesized polypeptides, the appropriate amino acid sequences will mimic the antigenic site or epitope found in the native antigen. Supporting data shall include type, degree, and persistence of the immune response following administration of the synthetic peptide. Procedures used to increase and prolong the antibody response such as coupling to carrier proteins or addition of adjuvants, must also be described.

### 4. Master Seeds

a. Recombinant derived bacterial or viral seed stocks used to prepare veterinary biological products must meet established procedures used to certify Master Seeds for conventionally prepared biological products (9 CFR 101.7).

b. Tissue culture-propagated cells from vertebrate animals used for vector propagation and antigen production must meet the requirements of 9 CFR 113.51 or 113.52.

c. If the gene product in any preparation has been adequately characterized, use of newly constructed Master Seed vectors may be permitted under abbreviated immunogenicity test procedures, subject to reevaluation for safety.

### 5. Product and Serial Release

Each Outline of Production shall be prepared in accordance with CFR 114.9. Outlines must include procedures to ensure consistency in production and recovery of specific antigenic material. Recovery procedures must include the removal of excessive antibiotic levels (9 CFR 114.10) and undesirable fermentation byproducts such as bacterial endotoxins.

In addition to the serial release tests for purity, safety, and potency, additional antigenic characterization such as electrophoretic mobility or immunoelectrophoresis may be required to demonstrate consistent gene expression.

C. Monoclonal Antibody Procedures

1. The specificity and potency of monoclonal antibody products will be compared with that of similar polyclonal antibody products. The specificity and sensitivity of monoclonal antibody products must be at least equal to that of antibody products of traditional polyclonal nature.

2. Monoclonal antibody products must be derived from Master Cell Stocks which meet the requirements of 9 CFR 113.52. Description of cell cloning procedures and preparation and characterization of cell passages must also be provided.

3. The Outline of Production must provide a description of all processes including scale-up, ascites fluid or cell culture supernate preparation, purification, concentration, and inactivation. Mouse colonies must be screened to demonstrate freedom from adventitious agents, especially those detected by the mouse antibody production (MAP) test. If the MAP test discloses the presence of murine leukemia viruses or other adventitious agents, the product shall not be released unless inactivation procedures approved by Veterinary Services have been conducted.

The immunologic specificity of the monoclonal antibody product must be fully described, and methods used to characterize the product and to evaluate all production serials prior to release must be included in the filed Outline of Production.

III. ENVIRONMENTAL CONSIDERATIONS

Under the present NIH guidelines, the deliberate release of any organism containing recombinant DNA into the environment is subject to review and approval by appropriate Federal agencies. In normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment. In the event that a veterinary biological product would be considered to be released into the environment, the issuance of a license or import permit may require an environmental impact statement and interagency approval.

/s/

K. R. Hook  
Acting Deputy Administrator  
Veterinary Services