tests with reproducible correlation between commercial laboratories and Biologics Laboratories may be difficult. The need to use sheep to evaluate this product appears quite likely.

6. *Clostridium perfrigens* toxoid or bacterin-toxoid—Types C and D.

In calves the need for protection provided by Type C antigens has been well documented. However, the need for Type D antigens in either calves or adult cattle is based mostly upon clinical observations with little immunological support. Cattle appear to respond poorly, if at all, to vaccination with Type D antigens.

Potency requirements for Type D toxoids or bacterin-toxoids are based upon the needs in feeder lambs. Even with this need for a relatively short duration of immunity it is advisable to double vaccinate lambs under certain conditions of feedlot husbandry.

These two types of *C. p fringens* toxoids appear to be a prime choice for higher potency requirements, particularly with their increasing amount of use in multi-component biologics where interference may occur. These two toxoids are the only bacterial origin biologics having a potency test for which I personally believe this recommendation necessary.

7. Anthrax Spore Vaccine, Non-escapsulated.

The work of Dr. Max Sterne amply demonstrated correlation of the immune response in guinea pigs as compared to cattle vaccinated with the same vaccine.

Efficacy of serials produced in recent years has been proven in guinea pigs by Biologics Laboratories. The only apparent problem encountered after vaccination of some cattle in the field with this product is the development of progressive edema from the vaccination site. Injections of penicillin control this condition.

It should be understood that with this biologic an immune state in cattle is not achieved until seven to ten days after the second vaccination.

In conclusion I would like to state that bacterial veterinary biologics on the market today appear to be a real bargain for the final consumer—the livestock raiser. Why? Simply because efficacy can be assured for most of these bacterial biological products. For those few where no efficacy has been definitely established either commercial laboratories or Biologics Laboratories are conducting developmental work to prove or disprove efficacy.

Veterinary Biological Products of Viral Origin

C. E. Phillips, *D. V.M.*
Chief Veterinarian
Virology/Laboratory
Biologics Laboratories
Ames, Iowa

The responsibility of the Virology Laboratory of Veterinary Services is to evaluate veterinary virus biologics licensed for distribution or presented for licensing.

The first modified live virus infectious bovine rhinotracheitis (IBR) vaccine was licensed in 1958 for use in feedlots. In the rapidly expanding feedlots, IBR infection was a major problem with up to 100% morbidity, and the initial vaccine was readily accepted. These vaccines were of relatively low modification serving to protect calves against infection in feedlots where the disease was endemic. As the use of these vaccines increased and their use outside of feedlots became more prevalent, reports of unwanted abortions were reported. It was recognized that early IBR vaccines did cause abortion in pregnant heifers and was actually used as an abortifacient. A sequel to this problem was a Veterinary Biologics Division label requirement, "Do not vaccinate pregnant cattle," which became mandatory in 1960.

In an effort to avoid problem herd trouble cases, additional cell passages were made by biological producers. These cell passages ranged from 20 to 130 passages in homologous and heterologous cells when the master seed virus requirement was established in 1969 (Graph No. 1). The cell passage modification of seed viruses for bovine virus diarrhea vaccines ranged from four to 102 passages (Graph No. 2). The cell passage modification of seed viruses for parainfluenza 3

vaccines ranged from three to 100 passages (Graph No. 3).

The effect of high level attenuation, particularly in heterologous cells, has not been completely evaluated for IBR virus. An analogous situation existed several years ago when it was demonstrated that the high passage Onderstepoort egg adapted distemper virus required a tenfold higher log titer to immunize dogs than comparative lower passage strains.

In order to re-establish safety and potency of IBR vaccines, a master seed virus requirement was established (Tables 1A and B). The master seed virus tests established the amount of virus required to immunize cattle against an effective challenge measured by serological and systemic response.

The requirements to establish the antigenicity portion of the master seed for IBR are (1):

"Antigenicity tests (potency evaluation in calves) shall be conducted on vaccine produced according to the outline of production from seed virus of the highest passage level to be used for production. Licensees may use previous experience to select a suitable virus dose for the antigenicity test. A geometric mean titer of the dried vaccine produced from the highest passage of the virus shall be established before a potency test is conducted in calves. The minimum acceptable titer for each serial of vaccine shall be not less than $10^{4.2}$ $TCID_{50}$ per dose.

"The antigenicity of the selected virus dose shall be established by inoculating 20 IBR susceptible calves with the predetermined quantity of virus or vaccine simultaneously tested for virus titer. A virus titration shall be conducted on a sample of the vaccine virus dilution used in this test. Prior to vaccination, individual serums from these animals shall show no neutralization in a final dilution of 1:2 (constant virus, 100 to 500 $TCID_{50}$ varying serum). Virus neutralization tests shall be conducted on individual serum samples collected 14 to 28 days postvaccination.

"The test virus shall be 100 to 500 $TCID_{50}$/0.1 ml. The individual serum samples from 19 or more of the 20 calves shall show neutralization in all tubes at the 1:2 dilution.

"After test animals have been bled for postvaccination serum neutralization tests, all vaccinated calves and five susceptible control calves shall be challenged with virulent virus (2). The rectal temperature of each animal shall be taken and the presence or absence of respiratory or other clinical signs observed and recorded daily for 14 days.

"Four of five (80%) of the control calves shall show clinical signs of IBR and a marked temperature rise (104.5 or greater) postchallenge.

"The seed virus meets antigenicity requirements if 19 of the 20 or 20 of the 20 postvaccination serums show neutralization in all five tubes of the 1:2 dilution and no more than one of the vaccinated calves shall show a temperature of 103.5°F or exhibit respiratory or other clinical signs of IBR postchallenge."*

Safety. The vaccinates are observed throughout the prechallenge period. The vaccinated calves are observed and temperatures taken and recorded during the prechallenge period for vaccine safety.

The test may be repeated. Actually, some producers have repeated this test several times. In some cases, to gain experience and information to improve their vaccines; in other cases, vaccines have been removed from the market by the licensee because they would not meet the requirements.

Providing the seed virus has been shown to be antigenic and safe, the producer must maintain the established virus content per dose throughout the dating period. In order to maintain this virus content, an excess of virus is required in the vaccine as produced and titrations are conducted at the expiration date to assure required virus content.

Similar requirements have been established for bovine virus diarrhea (BVD) and bovine parainfluenza 3 (PI_3) vaccines with acceptable criterion variation applicable to the virus (3 and 4).

Acceptable criteria for BVD vaccines include requirements that vaccinated calves must have a serum dilution vaccination ratio of 1:8 or higher.

"The white cell count shall be determined on all vaccinates and controls daily from the second through the eighth day postchallenge.

"A significant leukopenia shall be required in at least four of the five control calves when compared with the vaccinates."*

Acceptable requirements for PI_3 vaccines include:

"Three to four weeks after vaccination, all calves shall be bled for serum antibodies and nasal specimens shall be collected for PI_3 virus isolation.

"On the same day, all vaccinated calves and the controls shall be given acceptable challenge PI_3 virus titrating at least 10^7 $TCID_{50}$ per ml. The challenge may be performed by instilling 2 ml in each nostril or by inhalation of an aerosol suspension over a given period of time. Upon request, challenge virus and instructions shall be furnished by Veterinary Services.

"Postchallenge Observation. Each day during a 14-day postchallenge period, each animal shall be examined for clinical signs of respiratory disease and the body temperature recorded.

"Each day for at least the first ten days postchallenge, nasal specimens for virus isolation attempts shall be taken.

"All animals shall be bled on day 6 $\pm$ 2 days postchallenge, and all animals shall be bled on day 28 $\pm$ 2 days postchallenge for serum antibody studies.

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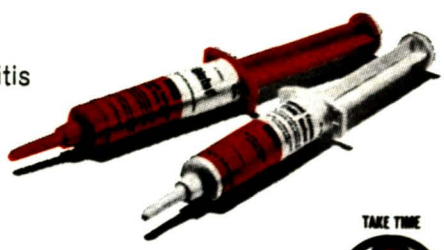

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TAKE TIME

OBSERVE LABEL DIRECTIONS

from the strong red line of **Upjohn** *Veterinary Products, Kalamazoo, Michigan 49001*

"Satisfactory Test Criteria. Before vaccination, all calves shall be negative for PI_3 neutralizing antibodies at the 1:2 final dilution and for PI_3 virus recovery from nasal specimens.

"All serum neutralization tests shall be with 100 to 500 $TCID_{50}$ of PI_3 virus per tube.

"All virus isolation attempts shall be by culture and at least one subculture in PI_3 susceptible cells for a total of at least 14 days.

"Three to four weeks after vaccination, 19 of the 20 or 20 of the 20 vaccinated calves shall have PI_3 neutralizing antibody titers of at least 1:4 and all five control calves shall be negative at 1:2 dilution. None of the postvaccination serums collected from the vaccinated animals on day 6 ± 2 days shall reveal (anamnestic) SN antibody titers of 1:32 or greater based upon final dilution. No more than five alternates shall be allowed for the vaccinate group.

"Satisfactory resistance to challenge by vaccinated animals shall be determined by a significant difference between virus isolation rates from vaccinates and controls. The virus neutralization titers of postchallenge serums from the vaccinates and the controls shall be considered in the evaluation of the test validity."*

Concurrent with the master seed virus concept, testing procedures were developed to assure that substrates were free from adventitious agents. Isolation of BVD virus from IBR vaccines and subsequent confirmation that this virus came from fetal calf kidneys and serums has been completed; 2,682,820 doses of IBR vaccine were withheld from the market for BVD contamination from July 1, 1971, to June 30, 1972. Isolation of BVD virus from IBR vaccines resulted in a study of other possible contaminants and their source.

Methods were developed and made mandatory for the testing of cell lines (5) (Tables 2A and B), primary cells (6) (Table 3), and other substrates (7) (Table 4) used in vaccine production for extraneous viruses. Since the inception of these mandatory requirements for primary cells, BVD, IBR, Adeno, and Entero viruses have been identified and other cells have been discarded without virus identification when aberrations in cell growth occur. Aberrations such as hemadsorption, nonspecific cytopathology, positive fluorescent against contaminant viruses are cause for discarding the cell. Licensees are encouraged to make a complete study of primary cells, freeze these in aliquots, and expand them up to ten passages for vaccine production.

Licensees are encouraged to develop and/or use available cell lines that have been approved by APHIS for vaccine production. In addition to all the tests required for primary cells to demonstrate freedom from extraneous agents, the cell line must be tested for tumorigenicity, oncogenicity, and species homogenicity. Vaccines and vaccine substrates must be free from mycoplasma spp. (8).

Selection of rabies vaccines for cattle has been predicated upon the high degree of susceptibility of cattle to rabies. Safety and ability to immunize cattle has to be established before a modified live virus vaccine is accepted for licensing. Available rabies vaccine for cattle immunization and available information is presented in Table 5A for modified vaccines and Table 5B for inactivated vaccines.

Wart vaccines available for bovine inoculation are produced by 14 companies (Table 6). Some vaccines and bacterins including wart vaccines were licensed and marketed without adequate efficacy tests. Veterinary Services now requires that adequate tests be developed to demonstrate efficacy of each product and eventually assay potency of each serial. These developmental projects are in progress at this time.

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Table 1A

Requirements for Establishing an Approved Master Seed Stock for IBR, BVD, or PI_3

A. Confirm IDENTITY of virus stock by demonstrating:
   1. Typical Fluorescence using fluorescent antibody procedures.
   2. Neutralization of a specific reference antiserum.
B. Confirm ABSENCE OF EXTRANEOUS AGENTS by demonstrating:
   1. No bacteria, yeasts, fungi, including mycoplasms and Brucella spp.
   2. No contaminating hemadsorbing viruses (Had test).
   3. No contaminating cytopathic virus (C.P.E. observation).
   4. No fluorescence against contaminating IBR, BVD, PI_3, Reo 1, Bovine Adenovirus I and V, Bovine Enteroviruses I, II, III, and IV, or Hog Cholera and other possible species contaminants.

Table 1B

C. Confirm SAFETY of virus seed stock by demonstrating:
   1. No reaction in at least 20 seronegative calves over 14 to 28 day observation period.
D. Confirm IMMUNOGENICITY of virus stock by demonstrating:
   1. Resistance to challenge in a statistically significant number of vaccinated calves (at least 19/20, $P < .05$) using adequate challenge control animals.
   2. Acceptable serum conversion in the vaccinates for each virus.
E. Limit PASSAGES of approved master seed for vaccine production to ten passages from the master seed.
F. All results must be acceptable to APHIS and licensee results may be confirmed in APHIS laboratories.

Table 2A

Requirements for Approval of an Established Cell Line for Vaccine Production

A. Record of HISTORY of the cell line demonstrating:
   1. That the line is derived from normal tissues of a healthy animal.
B. Evidence that the line demonstrates NORMAL CELL CHARACTERISTICS by:
   1. Examining 50 mitotic cells at lowest and highest allowable passage levels with no more than ± 15% change in modal number.
   2. Monitoring stability of microscopic appearance, growth rate, acid production, etc.
   3. Confirming the species of cell origin is in agreement with cell history.

Table 2B

C. Evidences that the line is FREE OF EXTRANEOUS AGENTS by demonstrating:
   1. Absence of bacteria, yeasts, fungi, and mycoplasms.
   2. Absence of Hemadsorbing Viruses.
   3. Absence of CPI producing viruses during a 14-day observation period.
   4. Absence of contaminant viruses when stained by fluorescent conjugates against IBR, BVD, PI_3, Reo 1, Bovine Adenoviruses I and V, Bovine Enteroviruses I, II, III, and IV, Hog Cholera or other conjugates applicable to the cell origin.
   5. Absence of evidences of tumorigenicity using conditioned lab animals, hamster cheek pouch inoculation, or host animals.
D. CELL PASSAGES not to exceed 20 from master seed stock to cells used for vaccine production.

Table 3

Requirements for Use of Primary Cells in Vaccine Production

A. Record of HISTORY of the tissue demonstrating:
   1. That the cells were derived from normal tissues of healthy animals.
B. Cells to be used for vaccine production are limited to ten passages.
C. Evidences that the cells are FREE OF EXTRANEOUS AGENTS by demonstrating:
   1. Absence of bacteria, yeasts, fungi, and mycoplasms (and Brucella).
   2. Absence of CPE producing agents during 14-day observation period.
   3. Absence of fluorescence with stained conjugates chosen to reflect virus diseases that may be associated with the species of origin of the cell.

Table 4

Requirements for Nonsterilizable Additives Used in Vaccine Production (Serum, Albumin, Trypsin, etc.)

A. Evidences that the additives are FREE OF EXTRANEOUS AGENTS by demonstrating:
   1. Absence of bacteria, yeasts, fungi, and mycoplasms.
   2. Absence of contaminant hemadsorbing viruses.
   3. Absence of contaminant CPE producing viruses.
   4. Absence of fluorescence against potential contaminating viruses using conjugates selected for the substrate origin.
B. Evidences that the Additives are FREE OF INHIBITORS by demonstrating:
   1. Mandatory requirements that preservative antibiotic levels be within prescribed limits.
   2. Absence of virucidal properties in applicable combination vaccines.

Table 5A

Rabies Vaccines Available for Cattle Immunization

Live Vaccines Licensed in U.S.A.

Type	Dose	Age	Route	Booster	Duration of Immunity
TCO, Canine Kidney (HEP), (FL)	2 Doses of 1 ml each 6 weeks apart	As required	IM	1 ml Annually	1 Year
Porcine Kidney (ERA)	1 dose of 2 ml	4 mos.	IM	2 ml every 4 Years	4 Years

Table 5B

Rabies Vaccines Available for Cattle Immunization

Inactivated Vaccines Licensed in U.S.A.

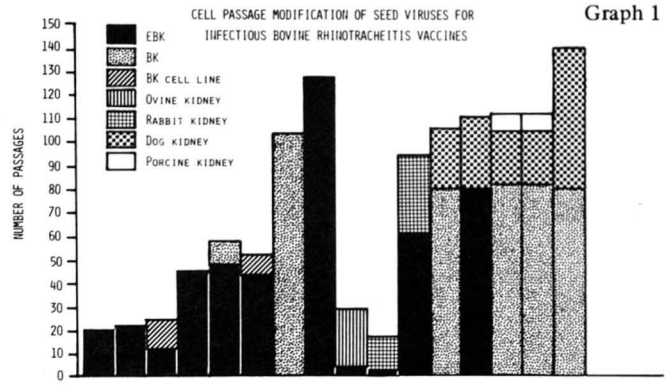
Type	Dose	Age	Route	Booster	Duration of Immunity
TCO, Primary Hamster Kidney (Fixed Virus) With Adjuvant	(2 doses) 4 ml/dose	As Indicated	SC or IM	4 ml Annually	1 Year
TCO, Primary Hamster Kidney (Fixed Virus) Without Adjuvant	(2 Doses) 10-15 ml/ Dose	As Required	SC or IM	10 ml Annually	1 Year
Tissue Origin, Caprine Nervous Tissue (Fixed Virus)	1 Dose 50 ml	As required	SC or IM	50 ml Annually	1 Year

(Vaccine compendium developed by Rabies Subcommittee, Animal Health Committee, National Research Council-National Academy of Sciences, 1971-1972)

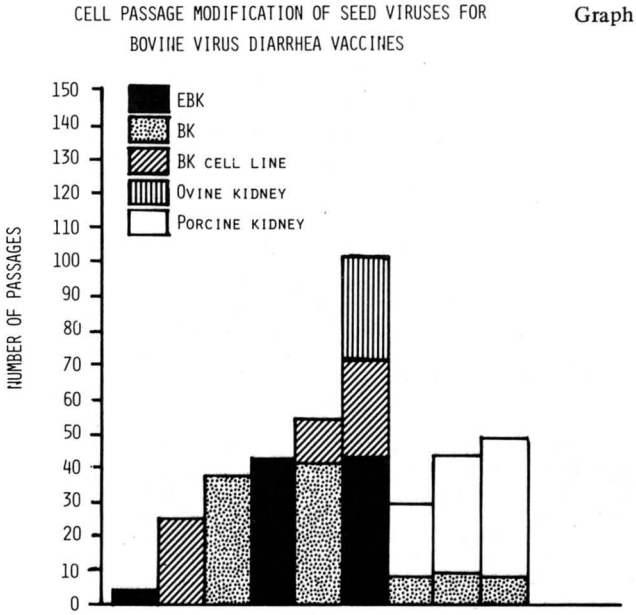
Table 6

WART VACCINES PRODUCED

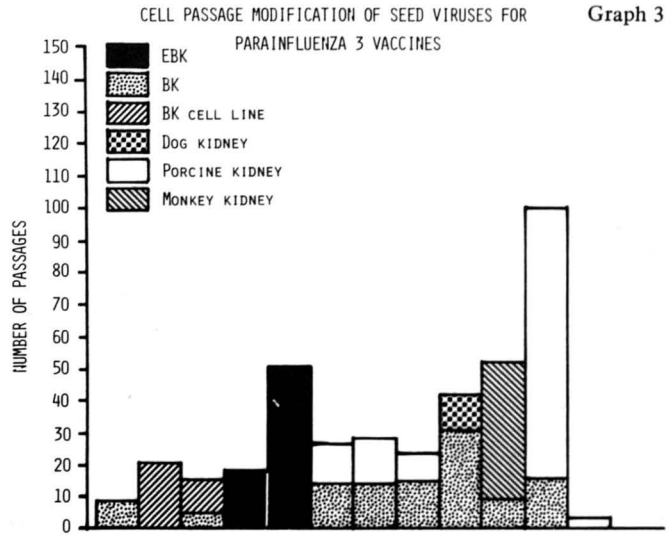
Bovine Wart Origin, Killed Virus	Ten Companies
Chick Embryo Origin, Killed Virus	Four Companies



Graph 1



Graph 2



Graph 3

Production of Biologicals Under New Government Regulations

Busch Meredith, *D. V.M.*
Vice President, Manufacturing
Diamond Laboratories, Inc.
Des Moines, Iowa

The Federal Government regulates the veterinary biologics industry by a law (often called the Serum-Virus-Toxin Act) enacted in March 1913. This law gives the Secretary of Agriculture authority to control the interstate movement of products for veterinary use and to insure that these products are not worthless, contaminated, dangerous, or harmful under the Act.

The Secretary of Agriculture has presently delegated the authority for biological products to the Animal and Plant Health Inspection Service. The Biologics Staff of Veterinary Services of this agency establishes conditions for licensing for both the production facility and individual products. Any person, corporate or individual, technically qualified, who can provide adequate laboratory facilities for production and testing, and with sufficient data to license at least one biological product, can become a producer of veterinary biologicals. Before you rush to Washington for a U.S. veterinary license, let us consider some of the detail that is required today in veterinary biological production.