Evaluation of Veterinary Biologics (Feedlot)

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Veterinary Biological Products of Bacterial Origin

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The Bacteriology Laboratory is one of the main units within APHIS Veterinary Services' Biologics Laboratories. The Bacteriology Laboratory's responsibilities include the development of valid methods to test biological products for potency or efficacy, safety, purity, and stability. For certain products the need for other factors, i.e., spore counts, cell counts, dissociation, sensitivity, hydrogen ion concentration and preservative content must also be determined.

References and reagents evaluated by Veterinary Services are used in testing by both commercial producers of biologics and in Biological Laboratories. Both reagents and reference preparations must be produced and evaluated and some must be standardized with available International Standards. Reagents must be used as an integral part of a test system whereas the use of reference preparations is optional.

The final step in the development of materials and methods is the comparison of potency tests, usually developed first using small laboratory animals, with potency tests conducted using host animals. In some cases the laboratory and host animal may be the same species. A biological product which after vaccination produces only marginal protection against challenge in laboratory animals may also produce marginal protection in host animals, using the same materials and methods. Comparison of this type must satisfy biometrical requirements, i.e., adequate numbers of both small and host animals, approved work plans prior to the initiation of experiments, valid criteria for comparison, i.e., live-dead results after vaccination and challenge plus serum agglutination titers and the use of sufficiently lethal challenge preparations.

Generally, the required materials (U.S. Standards) and methods are defined either in documents called Standard Requirements (S.R.) or in the Code of Federal Regulations. Both have the force of law. In addition, Biologics Laboratories issued Supplemental Assay Methods (S.A.M.) which lists materials and methods used by Biologics Laboratories in greater detail. Supplemental Assay Methods do not have the force of law.

It should be understood that Standard Requirements are minimum standards. This in no way debases any biological product since most exceed S.R.'s considerably. Commercial laboratories may dilute a lot containing excess antigens to that point where they just pass the required level of S.R.'s. In some cases, it may be cheaper for a commercial laboratory to use whole culture bacterins as produced rather than to dilute these. Dilution may require the use of more test animals in possible repeat tests to assure efficacy.

We have occasionally been asked, "Why don't you grade biologics?" It would be impossible to grade most biologics of bacterial origin, since life versus death is an "all or none" reaction. In many cases the use of clinical signs is not adequate; in other cases they must be used in the absence of more definitive criteria. Grading would be very subjective rather than objective and controversies with quality control representatives of commercial laboratories would repeatedly result. More important, grading would only deter the Bacteriology Laboratory from conducting sufficient potency tests which would assure the livestock industry and veterinary practitioners that biologics of bacterial origin on the market are efficacious, safe, and pure.

With this bit of introduction let us move on to those items which I particularly want to discuss today. These items are (a) three of the trends of the time in the production of biologics of bacterial origin, (b) how these three trends may affect the immunological responses of vaccinated animals, (c) what advantages and disadvantages may result due to these trends, and (d) some information on each of the most important bacterial organisms, both aerobic and anaerobic, used in the production of biologics.

In the past few years an increasing number of biological products have been licensed which contain the antigens of three to six or more bacterial species. As the number of components have increased the volume of the vaccination dose has decreased with this applying generally to most biologics whether they be single antigen or multi-antigen products. The combined effects of these two trends is to sometimes make the production of biologics technically more difficult. The third trend, the use of fermentors in production, has helped alleviate some of the problems caused by the first two trends.

Particulate antigen production can be increased markedly using fermentors. Concentration and purification can be accomplished. Soluble antigens, often necessary to produce the most efficacious bacterial biologics, are more difficult to produce, concentrate, and purify in fermentors.

How may these technical difficulties affect veterinary biologics? In multi-component products containing many antigens, physical limitations prevent a great excess of antigenic mass for any individual component. The probability of any component being substandard for potency increases as the number of components in the biologic increases. It should be understood that the Bacteriology Laboratory can at present test only a certain percent of most of the total number of serials produced. The exception to this rule is biologics used in national eradication campaigns which are tested 100 percent.

At this point, I want to emphasize that the Bacteriology Laboratory must assume a strictly neutral attitude concerning the development of multi-component biologics. However, ethical considerations dictate that veterinary practitioners, farmers, and ranchers be informed that new developments in the production of biologics have both advantages and disadvantages.

Interference between antigenic components may occur. This interference may be (a) physical, (b) immunological, or both. Physical interference, for lack of a better term, has been detected when *Clostridium perfringens* Type C and Type D toxoids are combined. Type C antigenic mass, in combination, must be increased to produce the same immunological response produced by lesser quantities of Type C antigen alone. This interference occurs regardless of the extent that experimental animals have previously been in contact with, or inoculated with, either or both of these antigens.

Immunological interference has been hypothesized but to the best of my knowledge not documented in the field of veterinary medicine. This may be due to the costs necessary to prove its occurrence. This type of interference may occur in animals previously exposed to or vaccinated with certain antigens to a degree that a threshold or basal immune response has occurred. Thereafter, upon vaccination with a multi-component product, the animal may respond anamnestically to those antigens to which it has been previously exposed but fail to respond or respond poorly to the other antigens in the biologic. This possibility appears particularly pertinent when dealing with organisms, i.e., *Pasteurella multocida*, *Escherichia coli*, or *Clostridium septicum* which are widespread but from which, considering our present state of technical knowledge, the protective response may be inadequate.

Now, I would briefly like to comment on the various organisms incorporated into bacterial biologics. Aerobic organisms (A) or biologics include:

A. 1. *Leptospira pomona* bacterins.

Extensive experimental studies both contractual and in the Bacteriology Laboratory involving hamsters and cattle have been conducted. It is hoped that these studies with *L. pomona* bacterins can serve as a model for other Leptospira serotypes. However, it is recognized that inter-

polation of data obtained using this one serotype may not be valid for other serotypes.

Products which pass the current potency test requirements should provide protective immunity for at least one year in immunologically competent, vaccinated cattle. Correlation has been attained between the immune response produced in vaccinated hamsters and that produced in vaccinated cattle when a Reference Bacterin of satisfactory potency was used.

A revised S.R. utilizing a two-stage potency test with 10 hamster vaccinates required per stage is to become effective soon.

2. Combined *L. canicola* — *L. icterohaemorrhagiae* bacterins.

These serotypes were originally licensed for use in dogs; later their use was permitted in cattle and swine. Priorities requiring the use of animal testing space for other products plus the costs involved when using cattle or swine have prevented efficacy studies in these species. Efficacy studies are being conducted by Biologics Laboratories in dogs.

3. Other Leptospira serotype bacterins.

Some demands have been expressed recently to permit the licensing and interstate shipment of *L. grippotyphosa* and *L. hardjo* bacterins. The problem seemingly has been that commercial laboratories are unable to foresee sufficient economic returns from the future sales of these bacterins to balance the developmental costs necessary to obtain a license. Cost-benefit is not a new concept. Many commercial laboratories removed unprofitable "service products" from their product lines more than 15 years ago. Standard Requirements to assure efficacious biologics appear to be desired by most livestock raisers and veterinary practitioners.

Experimental studies which are included in Veterinary Services' Fiscal Year 1973 goals are now being conducted on these two serotypes. Every effort consistent with present requirements will be made by the Bacteriology Laboratory to evaluate these bacterins when and if they are presented to support an application for license.

4. *Leptospira spp.* Killed Diagnostic Antigens.

Killed diagnostic antigens for *L. pomona*, *L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, and *L. hardjo* are now available commercially from a U.S. Department of Agriculture licensed establishment. These are the same serotypes which will be permitted in the production of commercial bacterins in single component to not greater than triple-component bacterins.

Livestock raisers and veterinary practitioners are respectfully urged to encourage greater use of these antigens by veterinary diagnostic laboratories so that the incidence of those serotypes causing leptospirosis can be more accurately determined.

5. *Brucella abortus* vaccine and stained antigen.

Several years ago a projection was made by some of our people that Strain 19 *Brucella abortus* vaccine would be phased out of use by 1972. The number of serials produced in Fiscal Years 1970, 1971, and 1972 have remained about the same. However, this number of serials is markedly less than the number of serials produced in the mid-1960's.

6. *Vibrio fetus* bacterin.

A potency test to determine the efficacy of this product is being developed using the serums of vaccinated small laboratory animals and cattle. It appears that the best measure of potency remains to be determined.

7. Bacterial organisms used in the production of Mixed Bacterins or Antibacterial Serums.

Commercial laboratories have been notified that valid potency assays must be developed for each of the bacterial species used in the production of Mixed Bacterins and Antibacterial Serums. The Animal and Plant Health Inspection Service has placed high priorities on the development of test methods for antigens not now being assayed for efficacy because valid potency test procedures are lacking.

Commercial and Biologics Laboratories are now engaged in cooperative experimental projects with *Pasteurella multocida* and *Salmonella typhimurium*. Preliminary results indicate a reasonable probability for success in developing potency assays to measure the antigens of these two organisms. Biologics Laboratories will later attempt to develop a potency assay to measure the antigens of *Escherichia coli* using calves.

Generally the development of potency assays for the remainder of the antigens of bacterial organisms used in the production of Mixed Bacterins or Antibacterial Serums will be left to the Commercial Laboratories. This will include the Staphylococci-Streptococci Mastitis Bacterins or Bacterin-Toxoids.

Anaerobic organisms (B) or biologics include:

B. 1. *Clostridium chauvoei* bacterin.

This clostridial organism causes considerable

economic losses in cattle. It was chosen as the first organism for the development of materials and methods used in potency assays to measure the efficacy of several clostridial antigens. About 250 head of cattle were used in experimental studies. Correlation was obtained between the immune response of guinea pigs vaccinated with experimental or commercial bacterins of marginal potency as compared to the immune response in cattle vaccinated with the same bacterins. The method uses double vaccination and challenge with 10 to 11 days between the first and second vaccination, as well as, between the second vaccination and challenge in the Bacteriology Laboratory. Criteria for correlation were life versus death of challenged vaccinates, as well as comparison of serum agglutination titers. Further, it required that a majority of unvaccinated controls also die after challenge.

Experimental studies extended from 1963 thru 1965. As a result, potency requirements were increased. It is believed that potency requirements are adequate now to protect vaccinated cattle against most conditions encountered in the field. It should be understood that while the antigens of *C. chauvoei* are very immunogenic that one vaccination of a week old calf should not be considered adequate nor should protection of 100 percent of vaccinated cattle be expected. I particularly want to emphasize this since in my opinion no other bacterial antigen is as strongly immunogenic as *C. chauvoei* in that it will protect a relatively high percent of vaccinated, immunologically competent cattle. Based upon the results of many guinea pig and cattle potency tests it would appear that the necessary antigens are approximately 75 percent particulate and 25 percent soluble. Commercial laboratories must monitor production on a serial by serial basis or else potency may quickly be lost.

2. *Clostridium novyi* toxoid or bacterin-toxoid.

Correlation of the immune response between guinea pigs vaccinated with toxoid or bacterin-toxoids of marginal potency and 191 head of sheep vaccinated with the same products has been obtained.

Both Type A and Type B cause disease in cattle. Commercial laboratories have been advised that stock cultures of both types should be used in the production of biologics. This recommendation is based upon the markedly different gross pathological lesions caused by the two types apparently due to the minor toxins elaborated.

Necessary antigens appear to be approximately 90 percent soluble and 10 percent particulate. The beneficial effect of small amounts of particulate antigens was demonstrated in both vaccinated cattle and sheep inoculated with a whole culture-spore challenge preparation. In those animals in which protection from soluble antigen appeared marginal the presence of small amounts of particulate antigens appeared to tip the balance for survival. The necessary antigens of *C. novyi* appear to be relatively easy to produce commercially.

3. *Clostridium haemolyticum* bacterin.

Positive correlation of the protective immune response between guinea pigs vaccinated with a Reference Bacterin using graded doses and the protective immune response in cattle has been obtained. This comparison required the use of an intrahepatic challenge in cattle contained in recently autoclaved 40 percent calcium chloride. The necessary antigens appear to be primarily particulate. However, soluble antigens are of some importance in the immune response.

The most pertinent problem with *C. haemolyticum* bacterins appears to be a relatively short duration of immunity in endemic areas ranging from three to six months. Biologics Laboratories is now checking duration of immunity in vaccinated and challenged cattle. It would appear that the relatively short duration of immunity must be accepted until more is learned about *C. haemolyticum* antigens.

4. *Clostridium sordelli* bacterin.

A Standard Requirement for potency using guinea pigs has been developed. The challenge preparation inoculated intramuscularly appears to be consistently lethal for cattle. Correlation of the immune response between guinea pigs and cattle appears possible.

The pertinent problem with *C. sordelli* is the determination of its incidence and economic importance. While isolations are occasionally reported in the mid and far west, it also appears that misidentification with *C. novyi* occurs.

5. *Clostridium septicum* bacterin.

At the present time no Standard Requirement for potency exists for this clostridial antigen. In the past 10 years few reports have been received by Biologics Laboratories in which losses of more than single cows after calving were involved.

Preliminary studies indicate that rabbits and sheep can be protected after two vaccinations and a challenge. However, the level of protection would appear to be extremely low since no more than 1 to 5 LD*50* of challenge can be used. Valid potency

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

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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