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Autologous Biologicals – Science/Regulations

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REASONS FOR INCREASED INTEREST IN AUTOLOGOUS/TARGETED BIOLOGICALS

There has been increased interest in Targeted Biologicals in the United States for several reasons. First, new technology is available to facilitate science-based isolate selection and manufacture. The science of Autologous/Targeted Biologicals will be discussed later in this presentation. A second reason for the increased interest in Targeted Biologicals is the fact that the livestock production methods have changed to favour herd/flock specific products controlled by a veterinarian. There have been changes in management methods such as segregated early weaning in swine, accelerated feeding and early weaning of dairy calves, and forced moulting in poultry. Herds and flocks have increased in stocking density and size. These factors stress animals and may facilitate mutation, strain variation and increased virulence of pathogens. Under these conditions, Traditional Biologicals may not be relevant to current field isolates. Many veterinarians favour vaccinating only for pathogens isolated from a herd/flock. A third reason for the increased interest in these products is that the licensing procedure for Targeted Biologicals is responsive to the needs of livestock producers. The process is abbreviated, requiring much less time and money. This allows for a more rapid response to emerging pathogens, strain variations and mutations, while facilitating the development of monovalent and multivalent products for limited markets or those for minor species.

GOVERNMENT REGULATIONS

The licensing procedure for Targeted Biologicals does not allow the sale of live attenuated or recombinant products because these would require more extensive safety testing. Government regulations are the same for Traditional and Targeted Biologicals regarding licensing of facilities, approval of personnel, product licence

documentation and unannounced USDA inspections. They are also the same for testing primary cells and cell lines [1]. Cell line testing regulations include karyology, purity, adventitious agents (confirmed by government), mycoplasma and animal origin ingredient testing. Regulations relating to documentation of Master Seed differ in some aspects. The isolation and passage history is necessary for both product types, as is identification except, in the interest of a timely response to the veterinarian/producer, for the first serial of Targeted Biologicals from a given isolate. Extensive purity testing is performed on Master Seeds of Traditional Biologicals, but not the seeds of Targeted Biologicals. The Master Seeds for the latter are animal isolates. Typically, there are minimal in vitro passages made from the isolate, helping to assure retention of herd-relevant antigens. Often the seeds of Traditional Products are passaged numerous times to obtain adequate stocks of Master and Working Seeds to last possibly for decades. To use an isolate beyond 15 months but no more than 24 months, manufacturers of Targeted Biologicals are required to document efficacy and lack of adverse reactions in the field, provide an assessment of herd health status, and document a veterinarian/client/patient relationship. Manufacturers of Traditional Biologicals are not required to provide proof of any of the above and may continue the use of a particular isolate in a herd or flock indefinitely.

Manufacturers of Traditional Products predict field performance by conducting a host animal immunogenicity test, which may use an artificial challenge method with an isolate that is not necessarily characteristic of current field pathogens. The results may not reflect a product's performance under actual field conditions. They are also required to perform a host animal safety study before licensing. Manufacturers of Targeted Biologicals, on the other hand, anticipate field performance by incorporating isolates that are relevant to the disease problems in a herd or flock and by minimizing in vitro passages.

Government regulations for production documentation are the same for both product types regarding Outlines of Production, Special Outlines, extraneous agent testing of animal origin ingredients, batch records and product labelling.

Quality Control regulations that are the same for Traditional and Targeted Biologicals are formaldehyde determination, testing of ingredients for mycoplasma contamination, government retention samples, government release of serials, sterility and safety testing. However, the first serial of a Targeted Biological made with a given isolate may be released after three days of testing for sterility and safety, provided the tests are satisfactory at that time. The tests are continued and serials must be recalled if a problem develops. Regulations relating to potency testing differ in that a serial release potency test is required for Traditional Biologicals, while Targeted Biologicals must contain isolates from sick or dead animals, giving them herd/flock relevance.

Duration of immunity studies may be required for new entities and special cases of Traditional, but not Targeted products. Retrospective stability testing is required for Traditional Biologicals because they are manufactured months to years before use, whereas Targeted Biologicals are made to order and typically are to be used quickly.

SELECTION OF SUITABLE ISOLATES

New molecular technologies such as PCR and DNA sequencing have become increasingly important methods for diagnostic laboratories and manufacturers of

Targeted Biologicals. These techniques provide a rapid means to identify, characterize and select the most suitable isolates for use in such products. The manufacture of Targeted Biologicals requires a close collaboration between a state-of-the-art diagnostic laboratory and the herd/flock veterinarian. The veterinarian sends appropriate tissues and/or body fluids from diseased animals to the diagnostic laboratory that identifies and characterizes potential pathogens. The laboratory findings are discussed and a determination is made regarding which isolates are most suitable to address the herd/flock health problems.

Figures 1 to 4 demonstrate the importance of molecular methods for use in selecting suitable isolates for incorporation into Targeted Biologicals [2]. Figure 1 shows the PCR amplification pattern of the R1 repeat region of the p97 adhesin gene from some field isolates of *Mycoplasma hyopneumoniae* received by MVP Laboratories over a period of a year as compared with the same region from DNA extracted from seven Traditional *M. hyopneumoniae* products. The sizes of the p97 R1 repeat gene are ≥ 300 bp. In contrast, the size of the same gene region from the field isolates is < 280 bp. This variation may seem trivial. However, Hsu and Minion have reported that the cilium binding site of *M. hyopneumoniae* is located in this p97 gene sequence [3]. More specifically, Minion et al published that only three repeat units (15 amino acids) are needed for defining the p97 binding epitope [4]. Therefore, it can be postulated that any change in the R1 repeat region of this gene may prevent the binding of antibodies that are specific for the original epitope. These seemingly small variations in the p97 gene of the field isolates, as compared with that region of the isolates found in the Traditional Biologicals, may explain the lack of protection often observed with such products. Targeted Biologicals would include isolates containing the relevant p97 gene sequence. Figure 2 demonstrates the use of PCR to determine the species of *Mycoplasma* isolated from two separate cases. In a rapid test that requires only a couple of hours, it can be determined that

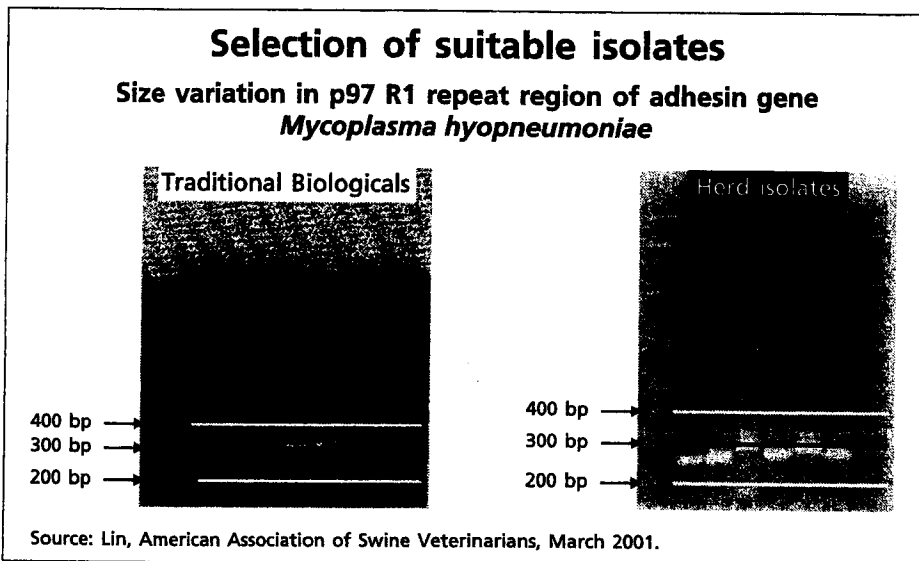
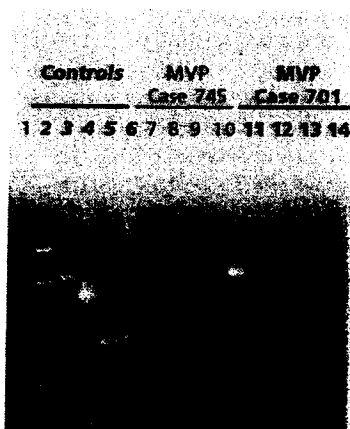


Fig. 1: Comparison of the R1 repeat region of p97 gene of *Mycoplasma hyopneumoniae* from herd isolates with strains from Traditional Biologicals.

Selection of suitable isolates

Use of PCR for identification of mycoplasma genus and species



1. Blank
2. *DNA ladder*
3. *M. hyopneumoniae*
4. *M. hyorhinis*
5. *M. hyosynoviae*
6. *M. flocculare*
7. Unknown isolate MVP case 745
8. Unknown isolate MVP case 745
9. Unknown isolate MVP case 745
10. Unknown isolate MVP case 745
11. **Unknown isolate MVP case 701**
12. **Unknown isolate MVP case 701**
13. **Unknown isolate MVP case 701**
14. **Unknown isolate MVP case 701**

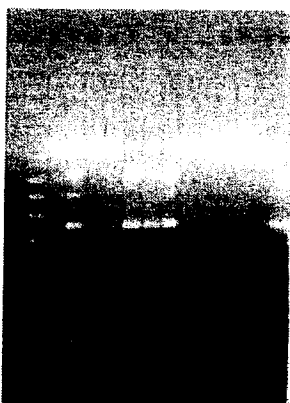
Source: MVP Laboratories, Inc.

Fig. 2: Use of PCR for identification of the genus and species of *Mycoplasma hyopneumoniae* isolates.

Selection of suitable isolates

PCR characterization of *Haemophilus parasuis*

RFLP genotype



Lane	Serotype	RFLP
3	NT	1A
4	13	3A
5	4	1A
6	4	1B
7	4	2A
8	4	3A
9	4	2B
10	NT	1A
11	2	1A
12	4	1A
13	NT	1A
14	4	1A
15	4	1B

NT = Nontypeable

Source: Lin, American Association of Swine Veterinarians, March 2003.

Fig. 3: Use of PCR to characterize *Haemophilus parasuis* isolates to differentiate nontypeables and isolates within serotypes.

the isolates from cases 745 and 701 are *M. hyopneumoniae* [2]. Figure 3 shows the added value of PCR when evaluating isolates of *Haemophilus parasuis* [5]. Isolates of this organism are normally identified by serotyping. Fifteen serotypes are known. However, there are numerous isolates obtained that are non-typable (NT). By using PCR genotyping, the non-typable isolates can be differentiated. Additionally, differentiation within a single serotype can be made (as represented by serotype 4 in the slide). Figure 4 represents the importance of using molecular techniques to select a suitable isolate for a Targeted Biological containing *Clostridium difficile*. Two different isolates were obtained from a single case. Both isolates were identified as *C. difficile* by normal biochemical identification procedures. However, after PCR analysis, it was determined that isolate #1 contained the genes for both the A and B toxin while isolate #2 contained genes for neither toxin. Isolate 1 was chosen for incorporation into the Targeted Biological. If isolate 2 had been chosen for the product, the critical protective toxoid could not have been prepared and the herd probably would not have been protected.

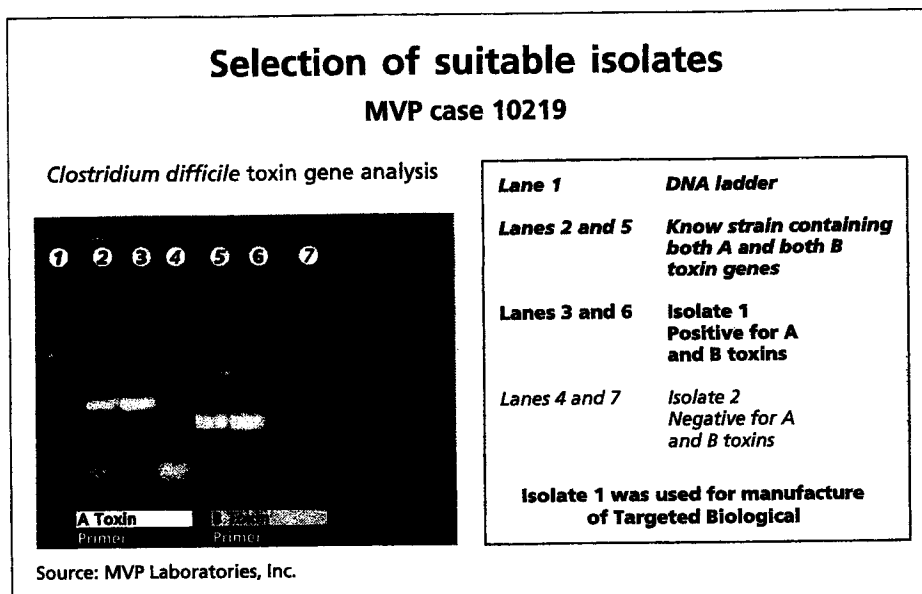


Fig. 4: Use of PCR to select the correct isolate from two *Clostridium difficile* organisms obtained from the same tissue sample.

QUALITY AND CONSISTENCY OF PRODUCTION

After the selection of suitable isolates, the process of production is begun. This includes customizing the formulation to the herd of origin by further discussing the herd problems with the veterinarian to determine the combinations, proportions, dose and vial size. Adjuvant selection is based on the diseases requiring protection and is critical because Targeted Biologicals are inactivated. If protection is antibody-dependent, an adjuvant such as Emulsigen® may be used. If protection is

T-cell dependent, an adjuvant incorporating a T-cell stimulating component may be selected (Polygen™ or Carbigen™). After selection of the adjuvant system to use, there is a discussion and decision on the most appropriate vaccination schedule, which is included on the product label.

Although there may not be specific requirements for characterization of growth, harvest, inactivation and adjuvants for various pathogens, any manufacturer interested in producing quality products that will perform well in the field will conduct such studies. Product quality is, at least in part, a reflection of the manufacturer's integrity. Figures 5 to 9 demonstrate the types of evaluations that MVP and other ethical manufacturers conduct, on a periodical basis, in order to manufacture immunogenic biologicals consistently. Figure 5 illustrates that it is important to evaluate media ingredients for their growth-promoting characteristics. In the case of yeast extract, MVP has determined that, in most cases, better yields of organisms can be obtained with fresh yeast extract produced in-house rather than powdered yeast extract purchased from a third party. Figure 6 shows a growth curve for *Streptococcus suis*, used to establish the optimum harvest time. This helps to ensure that there is adequate antigenic mass for each isolate. Figures 7 and 8 indicate an additional method conducted to select the optimum harvest time for antigen production, as well as the optimum inactivation method. These slides relate to the retention of antigens known to be immunogenic for *Mycoplasma bovis*. It is noted in Figure 7 that the important antigens decrease slightly after 24 hours of growth, probably due to a protease, so the harvest time for this organism is

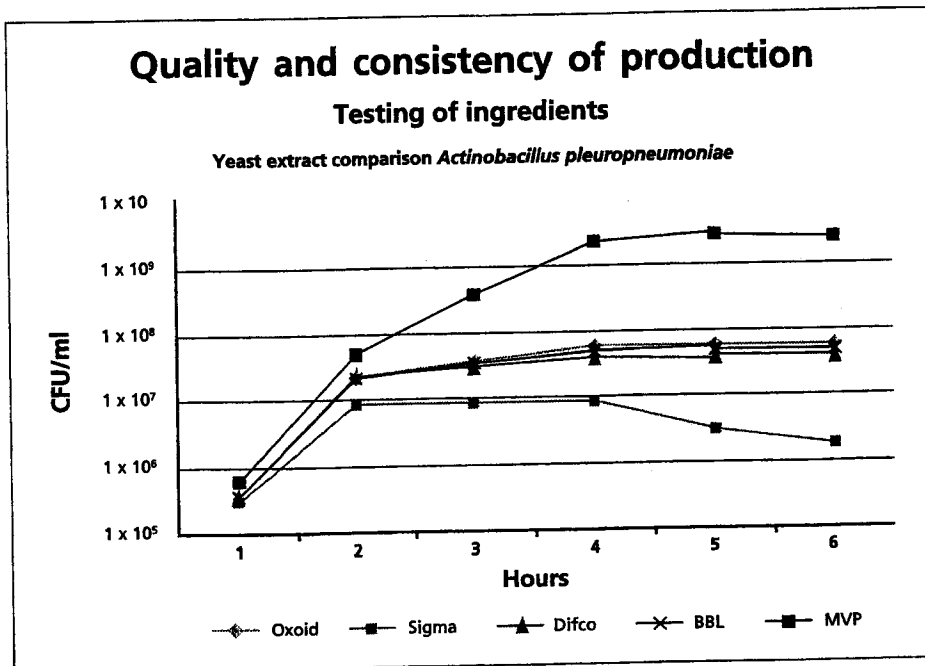


Fig. 5: Testing of ingredients such as yeast extract is important to obtain the highest antigenic mass for the product.

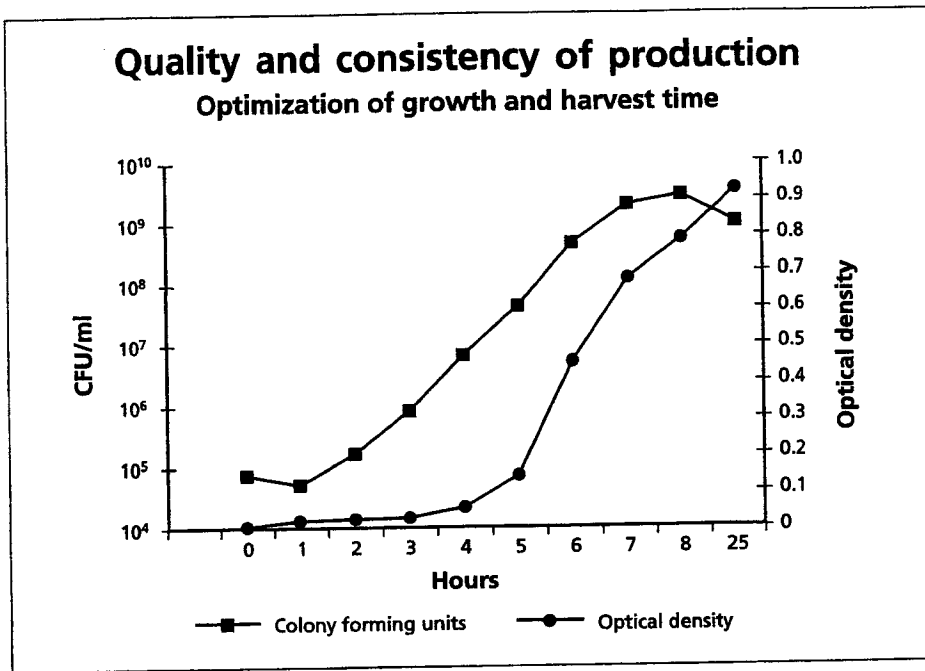


Fig. 6: Optimization of growth and harvest time base on optical density and colony forming units for *Streptococcus suis*.

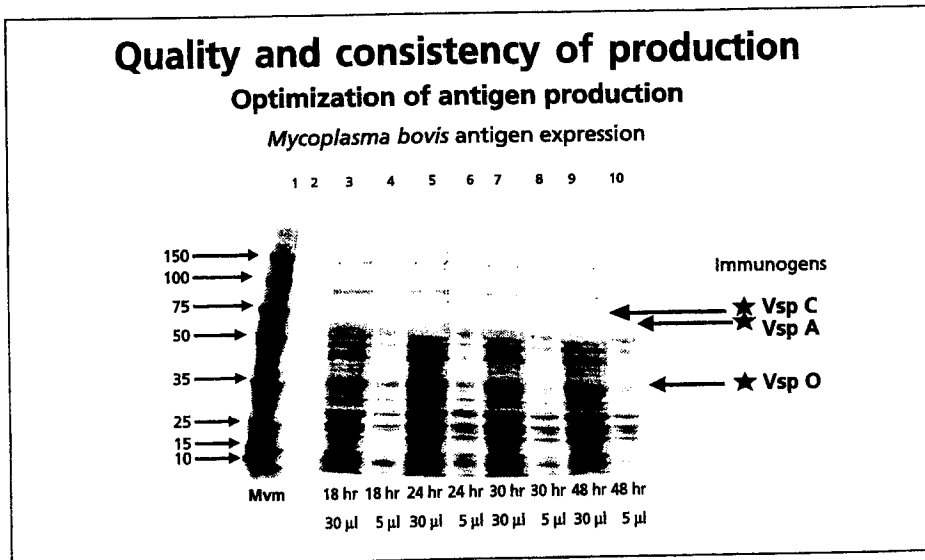


Fig. 7: Optimization of antigen production to ensure the presence of immunogens.

Quality and consistency of production

Optimization of inactivation methods

Mycoplasma bovis – Retain Vsp antigens

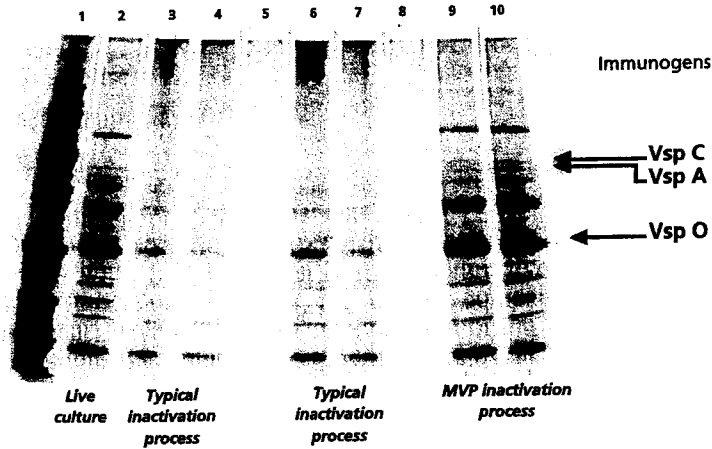


Fig. 8: Optimization of inactivating agent so as to retain important antigens.

Quality and consistency of production

Optimization of adjuvant

Mycoplasma bovis – Adjuvant evaluation in feedlot cattle

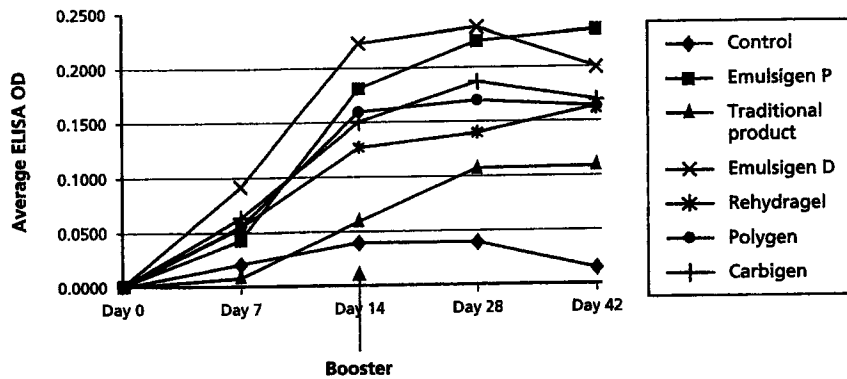


Fig. 9: Optimization of adjuvant to antigen is critical in ensuring performance of product.

optimally 18 hours. From Figure 8 it is apparent that certain inactivating agents totally destroy the important antigens. Without harvest time determination and use of the correct inactivating agent, the Targeted Biological would probably not protect. Figure 9 demonstrates the importance of evaluating adjuvants and their effectiveness with each antigen. Such studies are not required for either Traditional or Targeted Biologicals. Targeted Biologicals have the advantage of using the best adjuvant for the product if the manufacturer has multiple adjuvants approved.

ADVANTAGES OF TARGETED BIOLOGICALS

There are numerous advantages of Targeted Biologicals over Traditional Biologicals. These include: (i) providing rapid response to new pathogens and relevant mutations; (ii) inclusion of isolates in the product that are specific for the herd pathogens; (iii) allowing selection of adjuvants most appropriate for the pathogen; (iv) providing products for limited markets; (v) requiring collaboration with a licensed veterinarian; and (vi) addressing customer demand for selection of antigens and control of prices.

CONCLUSION

The reality is that there is increased interest from veterinarians and livestock producers in Targeted Biologicals in the U.S. This increased interest is led by the development of new molecular technologies that facilitate science-based selection of isolates and manufacture of Targeted Biologicals. Finally, it takes both government regulators and manufacturer integrity to ensure that quality products are available to the veterinarians and producers, whether these products are Traditional or Targeted Biologicals.

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