

REPORT OF THE COMMITTEE ON BIOLOGICS AND BIOTECHNOLOGY

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The Committee on Biologics and Biotechnology met on Wednesday, October 27, 2004, from 8:00 am to 12:00 pm. Four members and 15 guests were present. Chair Robert Tully welcomed the participants to Greensboro and the Committee meeting. The agenda for the meeting and last year's Committee report were reviewed and attendees introduced themselves.

Dr. Richard Hill, Director, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS), Veterinary Services (VS), Center for Veterinary Biologics (CVB) reviewed a number of CVB activities that have occurred over the last year. They include:

- a) Review of CVB Output (see charts on next page)
- b) Discussion of international harmonization efforts in a talk entitled "VICH – International Cooperation on Harmonization of Technical Requirements for the Registration of Veterinary Biologics." (see <http://vich.eudra.org/>). VICH is a trilateral (EU-Japan-USA) program aimed at harmonizing technical requirements for veterinary product registration. VICH was officially launched in April 1996. Dr. Hill presented the background, objectives, history and process of the International Harmonization of Standards for Veterinary Biologics. He spoke about the 15th steering committee meeting in Berlin, Germany that he returned from this month. At this meeting the charter was re-authorized with some modifications to the VICH process and work plan after 2005. The next meeting will be in Washington, DC May 24-25 and 28, 2005.

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CVB FY 2004 Licensing Summary			
	FY02	FY03	FY04
Submissions	7893 (+188)	7907 (+14)	6945 (-962)
Licenses Issued	93 (-20)	106 (+13)	89 (-17)
Total Active Products	2512 (+31)	2535 (+23)	2518 (-17)
Biotech. Licensed	3 (-1)	6 (+3)	24 (+18)
Unique Product Licensed	19 (-17)	13 (-6)	17 (+4)
Permits	163 (-83)	153 (-12)	181 (+28)
Response Time	Steady (-3%)	Steady (+3%)	Steady (-3%)

CVB FY 2003 Testing Summary			
	FY02	FY03	FY04
Master Seed/Cells	64	64	122
Serials Eligible	12,047	11,394	11,339
Prelicense/Outline tests	543	434	483
Problem/Reprocess tests	73	84	192
Check/Test & Release tests	248	134	968
Stability tests	12	45	24
Unsatisfactory Test Range	0.84-20	0-33.3	0-40.0
% tested	1.8	.91	6.71
% satisfactory	99	99	94.38
Components eligible	25,943	24,288	23,349
% tested	0.03	0.03	0.03

He then reviewed accomplishments and problem areas VICH has experienced up to this time. He feels a major outcome at this point is that Japan is going to change regulatory direction of veterinary biologics by adopting the Master Seed Concept.

c) Dr. Hill presented an overview on licensing plant-derived vaccines in a talk entitled "CVB Licensing Guidelines for Plant Derived Biologics." Dr. Pat Foley and Louise Henderson developed the presentation as a result of their involvement with the Food and Drug Administration (FDA) on this subject. This presentation was an update on dialogue between USDA-APHIS-CVB and FDA regarding development of regulatory guidelines and jurisdictional authority for future plant derived biotech products. This dialogue has occurred over the past 2 or 3 years. The presentation highlighted the differences, similarities and

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challenges of applying this technology to commercial products while protecting animals, man and the environment.

Dr. Lawrence Elsken, Section Leader, Compliance with the Center for Veterinary Biologics-Inspection and Compliance (CVB-IC) reported that CVB-IC activities in fiscal year 2004 have resulted in continued compliance with the regulations and standards promulgated under the authorities in the Virus-Serum-Toxin Act (VSTA). CVB-IC monitors 122 active licensees and permittees at nearly 175 sites. CVB conducted 33 in-depth inspections, 2 follow-up inspections and 54 special inspections. The majority of special inspections were conducted for pre-licensing or new facilities and to inspect licensed manufacturers for compliance to the Select Agent regulations as part of the registration process under the Agriculture Bio-terrorism and Preparedness Act of 2002.

In Fiscal Year 2004, CVB processed 524 requests for Export Certificates (serial) and nearly 3,000 Certificates of Licensing and Inspection (product). Export activities by serial more than doubled this fiscal year and export activities by product reduced by approximately 24%. The reduction in product exports was due mainly to the positive case of Bovine Spongiform Encephalopathy (BSE) in the state of Washington. Serials reviewed and processed by CVB were reported and summarized as 16,214; 15,789 serials were released for marketing. In addition, a pilot study was conducted on the new Administrative Inspection Review program and was reported. This new inspection process will be implemented with all licensees and will reduce the administrative time required for licensees to gather information during an inspection and allow the licensees to conduct these reviews and submit information to CVB on a scheduled basis.

Quality Assurance (QA) activities were reported including assignment of QA Leads within each CVB-Product Evaluation and Licensing laboratory section and development of a QA Vision Statement by the CVB Directors. This vision statement will be reviewed annually and updated to reflect ongoing changes as appropriate. Interactive audits at the laboratory have also been conducted utilizing Inspection and Compliance Inspectors and have been extremely useful for the laboratories.

Compliance activities reported included updates on investigation numbers for CVB (35 opened, 42 closed). The breakdown of investigations opened was 12 for unlicensed entities and 23 opened on licensed biologics firms. The licensed firms investigations opened included false and misleading advertising, promotions and/or product labeling. In addition, information was provided on compliance concerns related to animal owner exemptions and autogenous products under 9 CFR Part 107. An update on pharmacovigilance activities was also provided and progress within VICH and publication of a proposed rule

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for CVB continues. Voluntary reports of adverse events continue to decrease due primarily to the dissolution of the Veterinary Practitioners Reporting network and unfamiliarity of reporting adverse events to CVB. 259 reports were received in fiscal year 2004. These reports were summarized by species and event type and provided to attendees.

Progress continues toward development of the Licensing, Serial Release and Testing Information System (LSRTIS). This is the CVB portion of the VS Ames Automated Information Management System. Phase I was completed in 2003 and progress is now beginning on phase II which will include the laboratory sample receipt and test result, as well as the serial release components.

A time-specific Committee paper entitled "Development of Plant Cell Produced Vaccines for Animal Health Applications" was presented by Charles A. Mihaliak, Dow AgroSciences. The complete text of this paper is included in these proceedings.

The Committee discussed revision to the Committee Mission Statement. Several years ago, the USAHA Committee on Biologics was combined with the USAHA Committee on Biotechnology to create the Committee on Biologics and Biotechnology. To complete this merger, a new mission statement was adopted in order to reflect the objectives of the new committee. That new mission statement is: "The purpose of the Committee on Biologics and Biotechnology is to monitor 1) new developments in veterinary biologics, 2) regulation of the manufacture, distribution and use of veterinary biologics, and 3) needs of the livestock industries for new biological products. The Committee has the responsibility of keeping abreast and advising USAHA of new biotechnology, products and regulations that may have profound economic implications on animal health. Further, the Committee provides a forum to focus on issues and developments in the field of biotechnology that are designed to provide protection to man, animals and the environment. Committee action may be in the form of recommendations to the USAHA President for action or, in the case of major issues, resolutions to be considered by the General Session."

The Committee expressed their dissatisfaction with the scheduled meeting time. Scheduling of the Committee meeting late in the USAHA meeting schedule likely contributed to the poor attendance. Additionally, this year's meeting time overlapped with the meetings of the Committee on Public Health and Rabies, and the Committee on Pharmaceuticals, both of which have the potential for agenda items that impact individuals on this Committee. The group encouraged the chair to work with the USAHA leadership to find a more suitable time for holding the Committee meeting.

A resolution was approved supporting USDA-APHIS-CVB to assume the jurisdiction for animal disease vaccines that also have a pub-

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lic health benefit.

A discussion of the issues surrounding the worldwide availability of fetal bovine serum and previous USDA actions in 1992, 1994 and 1998 concerning Fetal Bovine Serum (FBS) was presented by Percy Hawkes. As a result of the discussion, a resolution was approved requesting USDA, APHIS, VS to re-propose the use of gamma irradiation for the importation of Fetal Bovine Serum from countries or regions that are free of BSE but that have restrictions because of other pathogens.

Both resolutions were forwarded to the Committee on Nominations and Resolutions for approval by the general membership.

DEVELOPMENT OF PLANT CELL PRODUCED VACCINES FOR ANIMAL HEALTH APPLICATIONS

Charles A. Mihaliak¹, Steven Webb¹, Timothy Miller², Matt Fanton²,
Dwayne Kirk³, Guy Cardineau³, Hugh Mason³, Amanda Walmsley³,
Charles Arntzen³ and Joyce Van Eck⁴

¹ Dow AgroSciences LLC, Indianapolis IN,

² Benchmark Biolabs, Lincoln NE,

³ Arizona State University, Tempe AZ,

⁴ Boyce Thompson Institute, Ithaca NY

Rapid advancements in the field of vaccines made from plants over the past decade have provided evidence that the technology may be able to address numerous animal disease issues. Expression of recombinant proteins in plants has become a well-established practice leading to many commercially successful applications. Advances in recombinant DNA technology and plant cell transformation also allow introduction of antigen genes derived from viral and bacterial diseases into plant cells and provides the basis for new vaccine technology developments in Animal Health (Curtiss and Cardineau 1988).

Several laboratories have explored the production of immunogens in recombinant plant systems. Arntzen and colleagues demonstrated that the hepatitis B surface antigen produced in transgenic tobacco antigen was indistinguishable from the native antigen (Mason et. al, 1992). Several other antigens have subsequently been produced in plant systems including, E. coli heat labile enterotoxin (Haq et. al., 1995), the Norwalk virus capsid protein, (Mason et. al, 1996) and the rabies virus glycoprotein (McGarvey et. al., 1995). Since these early investigations, over 20 additional antigens have been expressed in a variety of plant systems, further demonstrating the feasibility of the approach and representing a basis for the development of commercial plant-made vaccines for the animal health industry (Walmsley and Arntzen. 2003).

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Plant cell produced vaccines have the potential to combine the convenience of existing vaccine products with improved efficacy and safety attainable through delivery of potent, mucosally active antigens without using animal origin materials during production. The potential for plant cell produced vaccines to deliver safe, convenient and efficacious products to improve disease control is unparalleled when compared with existing and emerging technologies. The plant cell produced vaccine production system is based on the use of a recombinant plant cell line. Vaccine production occurs when a recombinant cell line expressing an antigen is grown as a suspension culture in a conventional bioreactor system. Large quantities of vaccine can be produced in a bioreactor system in a relatively short time period (weeks). Minimal processing of the harvested cells is necessary to extract and prepare the antigen for formulation into the final vaccine.

The current study was undertaken to demonstrate the validity of the plant cell produced vaccine system for animal health applications. Proof of concept research for plant-cell produced vaccines has been conducted using a poultry model for Newcastle Disease Virus (NDV).

NDV is a global pathogen in poultry best controlled through vaccination. Effective vaccination programs are well developed for the control of NDV (Beard and Brugh, 1975) and are widely used in commercial poultry operations. In chickens, Newcastle Disease (ND) is characterized by lesions in the brain or gastrointestinal tract, morbidity rates near 100 percent, and mortality rates as high as 90 percent in susceptible chickens. A single, dominant viral surface antigen, haemagglutinin/neuraminidase (HN), is known to provide protection against NDV. A well-defined disease challenge model is established for NDV. This model is based on a standard challenge test defined by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Center for Veterinary Biologics (Torres, 1999) using the Texas GB strain of NDV. Standardized hemagglutination inhibition analyses have also been developed which allow for measurement of antibody titers as an indicator of disease protection.

Recombinant plant cells lines were generated via *Agrobacterium* transformation to express a gene encoding for the HN antigen. Proof-of-Concept studies were conducted to demonstrate the technical feasibility of the production system at laboratory scale. The study was designed to address critical question during the technical proof of concept stage: including whether vaccine antigens be expressed in the plant cell lines and whether target animals immunized with the plant-made antigen be immunologically protected against a disease challenge.

A recombinant plant cell line which has been engineered to express the gene encoding for the HN protein from NDV has been created. Expression and in-vitro activity of the plant cell expressed HN

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antigen were verified by Enzyme Linked Immunosorbent Assay (ELISA) and hemagglutination inhibition, respectively. A master seed established from the cell line was then used to produce vaccine containing the HN antigen. Vaccine was prepared from a pool of bulk antigen after growth of the plant cell suspension in a bioreactor culture derived from the master seed.

A disease challenge study was conducted to determine whether the plant cell expressed HN antigen could successfully protect chickens from an NDV disease challenge. Four dose levels of the vaccine were prepared as well as a control vaccine derived from non-transformed plant cells. Formulated vaccines were analyzed for HN concentration prior to each vaccination using an HN-specific ELISA. The reference antigen used was a semi-purified HN preparation derived from a LaSota NDV strain.

After an 8-day acclimation period, specific pathogen free chicks were assigned to each treatment group. On Day 0 of the trial, each chick was vaccinated subcutaneously into the loose skin of the neck area with 0.5 mL of either the plant cell produced vaccine or a control cell lysate. On Day 14, birds were vaccinated subcutaneously with a second dose of vaccine or control cell lysate. On Day 28 of the trial, all birds except for the unchallenged controls were challenged with NDV Texas GB strain by intramuscular injection into the right breast. Each 0.5 ml dose contained approximately 1×10^2 ELD₅₀.

Individual blood samples were collected from each bird on Day 24. Antibody specific response to vaccination was determined by a standardized hemagglutination-inhibition assay using the LaSota NDV strain. Clinical observations were recorded daily for 14 days post-challenge to measure the ability of the vaccine to protect against disease challenge.

Results from this study demonstrated that birds vaccinated subcutaneously with a non-replicating, subunit HN antigen from NDV, derived from recombinant plant cell culture can protect against lethal challenge to NDV (Table 1). Serologic response to vaccination was measurable in birds from all treatment groups. The dose response capable of greater than 90% protection ranged between 3 and 33 mg/dose with overall protection of 95%. This study has demonstrated that plant-cell produced vaccines are capable of providing protective immunity against NDV. Further, these data positively answered the proof of concept objectives of demonstrating antigen expression in plant cells, serologic conversion and protection from disease challenge.

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Table 1. Summary of results of a disease challenge trial to demonstrate potency at different dose levels of a plant cell derived HN antigen against a virulent Newcastle disease virus strain.

^a For the purposes of calculating the HAI average, titers of <8 were assigned a value of 1

Treatment	Antigen Dose (mg HN)	Challenge	# of Birds/ # Protected	HAI Average ^a	% Protection
Control	0.0	Unchallenged	14/14	<8	100
Control	0.0	Challenged	0/14	<8	0
Plant cell HN	33	Challenged	14/14	77	100
Plant cell HN	11	Challenged	12/14	19	86
Plant cell HN	5	Challenged	14/14	8	100
Plant cell HN	3	Challenged	13/14	3	93

Similar results have also been obtained in disease challenge studies using Avian Influenza and other poultry diseases after chickens were immunized with plant cell produced antigens. Preliminary studies have also provided promising data to suggest that mucosal stimulation is feasible with low doses of plant-cell produced vaccines.

The successful demonstration of the utility of plant cell produced vaccines positions this system to begin providing solutions to many of the existing and emerging animal disease challenges. Potent and effective plant cell produced antigens are demonstrated to be effective in protecting against disease challenge and are amenable to delivery via multiple routes. There is no risk of shedding or spreading of the disease, or of “environmental escape” of the vaccine vector. Once harvested, the entire production process occurs in fully contained facilities, the final product is non-replicating and the plant material can no longer replicate. No animal origin materials are required for vaccine production and studies have demonstrated that the vaccines can be prepared to be free of mycoplasma and other extraneous agents. Freeze dried preparations of the antigen can be stored for long periods (years) at room temperature. Plant cell produced vaccines also offer the opportunity to easily developed diagnostics which allow differentiation of diseased and vaccinated animals since only a single antigen is expressed. The vaccine is highly specific to the disease agent; thus, diagnostics tools can be tailored specifically for the purpose of differentiation.

Effective detection, control and prevention of animal disease are of

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the utmost importance to maintaining the health of world poultry and livestock product markets. An ideal vaccine would induce mucosal immunity specific to the infection, have long duration, require minimal or no boosters, have impeccable safety, would not induce adverse reactions, and would be easy to administer. The desire to meet these criteria, and especially safety, necessitate development of vaccines that do not depend on the use of viable disease agents (Bowerstock and Martin, 1999). In addition, any new vaccine must be designed to allow for differentiation of vaccinated and infected animals (DIVA strategy). Plant-cell produced vaccines have the potential to meet the desired criteria of an ideal vaccine.

Therefore, it is highly desirable to employ vaccines which induce protective mucosal immune responses. A major barrier to inducing mucosal immunity is delivery of safe vaccines to the mucosal site. Practical difficulties in delivering mucosal vaccination have been primarily due to the limited availability of efficacious mucosal vaccines. As a result, most vaccines only stimulate production of circulating antibodies that do not necessarily cross to mucosal sites (Bowerstock and Martin, 1999).

References

- Beard, C.W. and Brugh, M. (1975) "Immunity to Newcastle Disease." *American Journal of Veterinary Research*. 36:509-512.
- Bowerstock, T.L. and Martin S. (1999) "Vaccine delivery to animals." *Advanced Drug Delivery Reviews* 38:167-194.
- Curtiss, Roy III, and Guy A. Cardineau. (1988) Oral Immunization by transgenic plants. US patent 5686079. Priority date Sept 6. 1988
- Haq T A; Mason H S; Clements J D; Arntzen C J. (1995) "Oral immunization with a recombinant bacterial antigen produced in transgenic plants." *Science* 268:714-716.
- Mason, H. S., Lam, D. M. K., Arntzen, C. J. (1992) "Expression of hepatitis B surface antigen in transgenic plants." *Proceedings of the National Academy of Sciences of the United States of America* 89:11745-11749.
- Mason, H. S., Ball, J. M., Shi, J. J., Jiang, X. Estes, M. K., Arntzen, C. J. (1996) "Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice." *Proceedings of the National Academy of Sciences of the United States of America*. 93(11): 5335-5340
- McGarvey, P. B., Hammond, J., Dienelt, M. M., Hooper, D. C., Fu, Z. F.; Dietzschold, B., Koprowski, H., Michaels, F. H. (1995) "Expression of the rabies virus glycoprotein in transgenic tomatoes." *Bio/Technology*, 13(13):1484-1487.
- Torres, A (1999), "GB Texas Newcastle Disease Challenge Virus." USDA Veterinary Services Memorandum No. 800.95. <http://>

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aphisweb.aphis.usda.gov/vs/cvb/memos/memo800_95.pdf

Walmsley, A. M., Arntzen, C. J. (2003) "Plant cell factories and mucosal vaccines." *Current Opinion in Biotechnology*, 14(2), 145-150.