


The Road to Licensure of a DNA Vaccine for Atlantic Salmon

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Presentation Overview

- The incentive to commercialize and brief description of Apex-IHN[®]
- Prior to commercialisation: communication plan
- The Canadian regulatory system and licensing requirements
- Product safety: target species, humans and the environment.

The Incentive to Commercialize Apex-IHN®

Infectious haematopoietic necrosis (IHN) virus in Western Canada

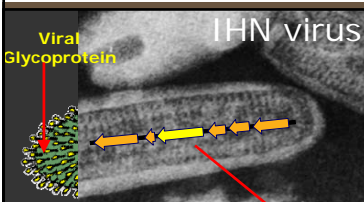
- Outbreaks occurred in 1992, 1995, 1996, 1997
- Between 2001-2003
36 farm sites were affected
70% mortality in < 1 Kg fish
40-50% mortality in > 1Kg fish
- \$ 200 Million lost in sales was devastating to the Canadian salmon aquaculture industry.
- No efficacious licensed treatments or vaccines were available (i.e conventional vaccine)



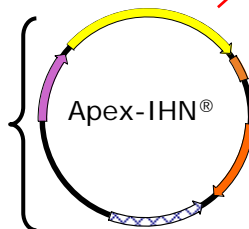
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About Apex-IHN®



- Glycoprotein gene
- CMV promoter
- BGA polyA signal
- Replication origin
- Selection marker



DNA Vaccination

- Pure plasmid DNA in sterile saline solution
- Intramuscular injection (0.05 mL)
- Single injection of 10 ug
- No adjuvant

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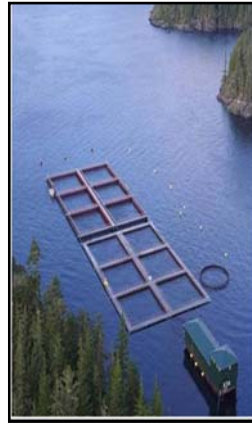


Apex-IHN®

Time lines of the registration process

- Apex-IHN was the first commercial DNA vaccine licensed in Canada in 2005.
 - 2002 (Dec) – Dossier Submitted
 - 2003 (Oct) – Conditional License (310 days)
 - 2004 (Sept) – Field Trial Permit Issued
 - 2005 (July) – Full Product License Issued

- Total Review Time = 2.5 years



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Prior to Commercialization

Establishment of a strategic communication plan

- A communication team composed of specialists in Public Relations, Regulatory Affairs, Marketing, Veterinarians, Scientists, customers, and external consultants was created.
- Team members receive scientific training on the specifics of recombinant DNA technology in an open forum format.
- The communication team identified the key stakeholders, gaps and potential forthcoming difficulties to be addressed and developed a strategic communication plan.

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Prior to Commercialization

the resulting communication plan

- *Adopt a fully transparent communication approach including involvement of all key stakeholders (customers, local veterinarians, environmental groups, the general public).*

- Factual presentation on the scientific bases of DNA vaccination were given to stakeholders to forestall against uninformed backlash and a negative public perception.
- A Question & Answer document was made publicly available.

The Canadian Regulatory System

for Veterinary Biologics

- The Veterinary Biologics Section (VBS), Animal Health and Production Division, Canadian Food Inspection Agency (CFIA) is responsible for licensing veterinary biologics for use in Canada.
- CFIA has responsibility for regulating the manufacture, testing, import, distribution and sale of veterinary biologics.
- The registration of new products involves review and approval of facility documents, product manufacturing & testing, as well as inspection of facilities & finished product testing.

Licensing Requirements for DNA Vaccines

Licensing requirements for nucleic acid (i.e. DNA) vaccines are the same as another veterinary vaccine and require fulfilment of:

Purity – Potency – Efficacy - Safety

- Purity: based on biochemical tests for by-products (proteins, endotoxins) and microbiological evaluations for sterility.
- Potency: *in vivo* measurement of immunological responses or protection following animal inoculation and *in vitro* methods (e.g. detection of the expressed gene in transfected cells).
- Efficacy: is demonstrated by a vaccination-challenge study conducted in target animals as used in the final formulation.
- Safety: a broader definition of safety including specific examination of animal, human, and environmental safety.

Product Safety

Product safety evaluation is on a risk-based approach

- CFIA uses a risk-based approach to evaluate the safety of the product in target and non-target species, humans and the environment.
- Since no benchmark safety test was available for DNA vaccines, consultation with the CFIA to develop pre-agreed protocols for the documentation of special safety aspects was necessary.
- Undefined threshold values for interpretation of the special safety test results was an added source of uncertainty (no reference to what “normal” would look like).

Environmental Risk Assessment

Required content

Part of the requirements for licensing Apex-IHN was to produce an environmental risk assessment, a public document contains information on:

- *The molecular and biological characteristics of the recombinant DNA vaccine*
- *Safety in target animal and non-target animal*
- *Human safety*
- *Environmental considerations*
- *Risk mitigation measures*

The Environmental Assessment document is distributed to stakeholders for comment during the development, and made available to the public when finalized.

www.inspection.gc.ca/english/anima/vetbio/eaaa/vbeaihnve.shtml

Environmental Risk Assessment

A multifaceted approach

In order to complete the environmental risk assessment, multiple avenues to generate the required information necessitated:

- *A full understanding of the logistics behind fish vaccination and the environmental surroundings.*
- *The application of logical thinking and common sense played an important role.*
- *The development and approval of new protocols providing the answers to the best of our ability.*
- *Extensive literature search to substantiate or support findings.*

Safety in Target Species

Routine and special safety

- Evaluation in laboratory and field safety studies
 - Mortality, behavioral changes, local and systemic reactions in animals to vaccination were monitored according to predetermined criteria.
- Potential safety considerations:
 1. Integration of injected plasmids into host genome (cancer risk)
 2. Germline transmission (risk of generating GMO)
Test 1 and 2 required special equipment and validated protocols.
Needed to be outsourced at the time. Very costly.
 - No threshold values or acceptance criteria were defined.
 - Recently, FDA set the acceptable residual plasmid copy number to 30,000 copies/ug host genomic DNA.
(<http://www.fda.gov/Cber/gdlns/plasdnvac.htm>)

Apex-IHN[®] Safety Profile in Target Species

- The risk associated with the integration of up to 50 plasmid copies is significantly less (appr. 43 X) than the risk associated with spontaneous mutation.
- The lack of plasmid detection in the gonads indicates that the risk of germline transmission is low and unlikely.
- We have determined that the residual plasmid copy number at the site of injection one year later to minimal at 0.01% of the initial dose.
- Identical percent mortality (0.11%) between vaccinates and controls in field safety confirmed the safety of APEX IHN[®] at dose.
- The absence of skin darkening, lethargic behaviour and the normal return to feed following vaccination as well as the absence of myopathy with little or no inflammation and no morphological difference further demonstrated safety.

Human Safety

- Assessment by Health Canada
 - Following the review of the Human Health Risk Assessment, the Bureau of Microbial Hazards, Microbiology Evaluation Division, Health Canada indicated that there is no risk to the safety of Canadians in regard to exposure to food products for human consumption derived from fish immunized with this vaccine.
- Previous Safe Use
 - Plasmid based vaccines have been used in human phase I clinical trials to vaccinate against a range of diseases with no adverse reactions reported attributable to the vaccine, for example MacGregor *et al.*, 2002, McConkey *et al.*, 2003, Wang *et al.*, 2001.
 - The amount of plasmid DNA administered in a single human dose is at least 100-300 µg, which is 10X-30X the dose given to the fish.

Human Safety

- Consumption of Food Products
 - Less than 0.01% of the injected vaccine remains after one year. In addition, plasmid DNA is rapidly degraded in the stomach and duodenum due to gastric acid, and pancreatic and bile secretions respectively (Maturin and Curtiss, 1977).
- Self-injection by the Operator
 - Safety studies carried out in mice using the recommended dose or a 10X dose by the company demonstrated that the vaccine was safe without any adverse effects.
 - The probability of human exposure is limited to the administration of the vaccine by experienced operators (vaccinators). Past studies have documented that self-injection can occur once for every 480,000 fish injected (Dyrkorn *et al.*, 1993, cited in Leira and Baalsrud, 1997).

Environmental Consequences

- The vaccine is administered to Atlantic salmon at the hatchery, in artificial tank based rearing systems. By law, all hatchery effluent water in BC has to be treated; it cannot be released directly to fish-bearing waters. Consequently, the risk to the environment is considered negligible.
- *E. coli*, the most likely non-target organism that could be implicated in transmitting the plasmid outside the target species, is not considered a natural component of the gut flora of salmonids under cultured conditions (Del Rio-Rodriguez *et al.*, 1997) and is absent from the intestinal content of cultured fishes (Gonzalez *et al.*, 1999).

Environmental Consequences

- Internal study conducted on the risk of shed and spread of the plasmid DNA demonstrated that the vaccine is not shed from vaccinated fish.
- The disease agent IHNV is endemic to the British Columbia coast and expression of the IHNV glycoprotein already occurs in the environment naturally.
- The vaccine contains no additives, inactivating agents or adjuvants, and therefore presents no contamination risk from these ingredients.
- The DNA vaccine is a non-virulent, non-replicating agent, therefore reversion to virulence is not a risk factor as compared to inactivated vaccines.

Licensing Decision at the CFIA

Is based on risk analysis

- The essential components of risk analysis are:
 - risk assessment
 - risk management
 - risk communication
- The process of identifying a hazard and characterizing or estimating the risk presented by that hazard was traditionally performed using *qualitative methods*.
- More recently, quantitative risk assessment using '*scenario tree analysis*' to predict the likelihood of various outcomes and their respective impacts in the development of licensing decisions has become available at the CFIA. (<http://www.inspection.gc.ca/english/anima/vetbio/info/vb323e.shtml>)

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Guides for Licensing Nucleic Acid Vaccines in Canada

Key internet links

- Health of Animals Act - <http://laws.justice.gc.ca/en/H-3.3>
- Health of Animals Regulation - <http://laws.justice.gc.ca/en/C.R.C.-c.296>
- Canadian Food Inspection Agency – Main Website
www.inspection.gc.ca
- The Regulation of Veterinary Biologics in Canada
<http://www.inspection.gc.ca/english/anima/vetbio/ref/vb410e.shtml>
- Licensing Requirements for Veterinary Biologics in Canada
<http://www.inspection.gc.ca/english/anima/vetbio/ref/vb405e.shtml>
- Veterinary Biologics Guideline 3.2E – Guideline for Regulation of Veterinary Biologics produced by Biotechnology
<http://www.inspection.gc.ca/english/anima/vetbio/info/vb302e.shtml>
- Veterinary Biologics Guideline 3.23E – Guidelines for Licensing Nucleic Acid Vaccines
www.inspection.gc.ca/english/anima/vetbio/info/vb323e.shtml

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Thank You For Your Attention...



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