

# POTENTIATION OF DNA VACCINATION USING AN INTEGRATED APPLICATION SYSTEM FOR ELECTROPORATION-MEDIATED INTRAMUSCULAR DELIVERY

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## ABSTRACT

Preclinical studies in animal models indicate that the intramuscular administration of plasmid DNA (pDNA) encoding antigenic sequences is an effective immunization strategy. Vaccines based on pDNA represent an appealing immunization strategy since such vaccines would be easy to produce, standardize and store, would raise fewer safety concerns than viral vectors, and would be suitable for multiple immunizations (in distinction with viral vectors). However, it has been more difficult to induce immune responses to pDNA vaccines in larger animals and humans than in mice. As a result, an effective delivery method is generally considered necessary for the clinical development of pDNA vaccines usable in humans. Electroporation (EP), a potent non-viral delivery technique utilizing the *in vivo* application of electrical fields dramatically enhances intracellular uptake of nucleic acids and ensuing gene expression. This technique might provide a means to overcome some of the traditional hurdles associated with the use of pDNA vaccines in larger species.

A platform for DNA-based immunization in the clinical setting must provide effective and reproducible delivery from subject to subject. Ichor's proprietary TriGrid™ intramuscular delivery technology addresses these issues by providing an integrated automated system that controls the intramuscular DNA administration and subsequent application of electrical fields. In this manner, effective and reliable administration can be achieved. The application system suitable for application in large species (i.e., >2kg) consists of a reusable Integrated Applicator containing mechanisms to automatically implant the electrodes and administer the vaccine and a single use Application Cartridge that encloses the electrode array and accommodates an off the shelf syringe and features automatic sharps protection.

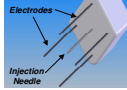
The integrated TriGrid™ delivery technology was capable of consistently inducing potent immune responses to DNA vaccination in mice as well as larger animals against a variety of antigens, including bacterial and viral antigens. Particularly notable are the following observations:

- Immune responses were induced by EP-mediated vaccination even with antigens that did not induce immune responses following direct injection of naked DNA in larger species. This suggests that EP is effective in multiple species.
- EP-mediated pDNA vaccination provided one to two orders of magnitude enhancement in antibody responses and up to 5-10 fold enhancement in the magnitude of cellular responses as compared to direct injection of naked pDNA.
- Immune responses following EP-based vaccination were observed over a broad range of DNA doses, including doses that approximate, on a body mass basis, vaccines doses used in clinical trials.
- EP-based vaccination resulted in sustained immune responses after few immunizations.

These characteristics reflect the properties of EP as a potent *in vivo* gene delivery method and suggest that the technology may overcome an important hurdle hindering the clinical development of DNA vaccines.

## THE TRIGRID™ ELECTROPORATION TECHNOLOGY

### TriGrid™ Array Configuration for Intramuscular Delivery



### A Potent Technology Platform for *In Vivo* Delivery of Plasmid DNA

*In vivo* electroporation (EP) utilizes application of electrical fields in tissue to enhance intracellular delivery of therapeutic agents. The key requirement for effective EP-mediated agent delivery is localized propagation of threshold level electrical fields at the target site in the presence of the agent of interest. Ichor's TriGrid™ EP system is designed for safe, effective, and reproducible application of EP in a manner compatible with the clinical setting. A TriGrid™ application array consists of slender penetrating electrodes arranged in interlocking equilateral triangles. The number and spacing of electrodes are selected to propagate EP inducing electrical fields throughout the target tissue and insure that these fields are co-localized with the distribution of the agent of interest.

Key features of the TriGrid™ system include:

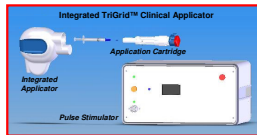
- EP-inducing electrical fields confined to a well defined site of administration
- Array adaptability allowing for complete coverage of a target region of tissue
- Efficient electrical field propagation minimizing tissue damage and facilitating safe, consistent and rapid delivery



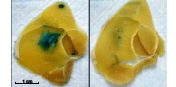
### Development of a Clinically Suitable Vaccine Applicator

Ichor's proprietary TriGrid™ intramuscular vaccine applicator has been designed for effective, reproducible delivery while minimizing complexity and expense associated with the procedure. The system consists of three components:

- A portable **Pulse Stimulator**, which controls the administration sequence and generates the electrical signals necessary to enhance the intracellular delivery of the vaccine;
- An **Integrated Applicator**, which consists of a reusable hand-held device that contains mechanisms to automatically implant the electrodes and administer the vaccine;
- A single use **Application Cartridge** that encloses the TriGrid™ Electrode Array, accommodates an off-the-shelf syringe and features automatic sharps injury prevention.

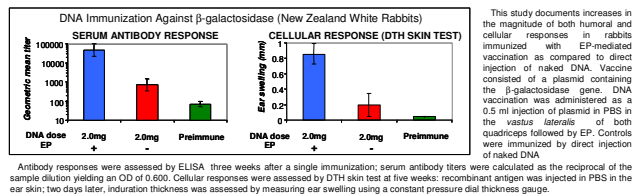


Distribution of expression of reporter gene  $\beta$ -galactosidase in rabbit quadriceps following intramuscular gene transfer



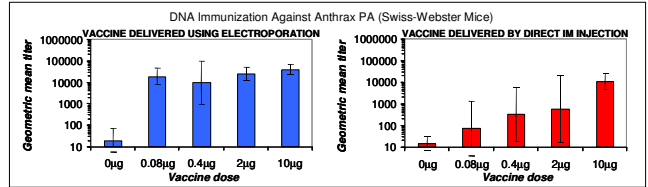
## INDUCTION OF BROAD IMMUNE RESPONSES BY DNA VACCINES DELIVERED USING TRIGRID™ ELECTROPORATION

### Induction of Both Humoral and Cellular Immune Responses

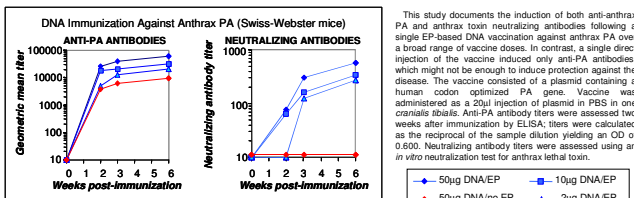


## TIME COURSE AND DOSING STUDIES

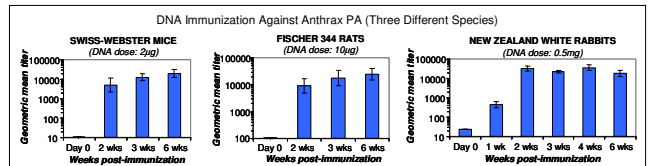
### Dose Efficiency



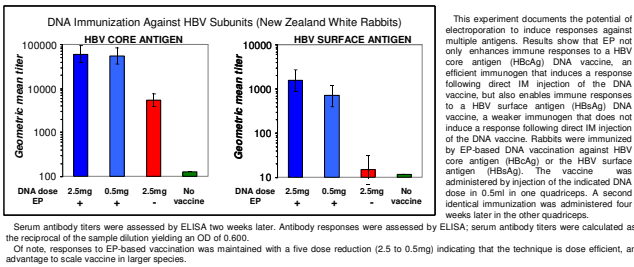
### Induction of Multispecific Immune Responses



### Rapid Onset of Immune Responses



### Ability to Generate Immune Responses Against Immunogens of Varied Potency



## KEY FINDINGS SUPPORTING THE USE OF THE TRIGRID™ DNA DELIVERY TECHNOLOGY FOR CLINICAL DNA VACCINE

**Quantitative improvement of immune responses.** EP-based DNA vaccination dramatically enhances the magnitude of humoral and cellular responses. In addition, the procedure is particularly dose efficient and induces rapid responses in multiple species. This suggests that Ichor's TriGrid™ electroporation technology may overcome an important hurdle hindering the clinical development of DNA vaccines.

**Qualitative improvement of immune responses.** Electroporation-based delivery induces broader immune responses to DNA vaccines than direct IM injection. Particularly notable features include the induction of both antibody and T cell responses, the induction of multispecific responses and the induction of responses to both strong and weaker immunogens.

**A clinically suitable vaccine delivery system.** Ichor has developed a clinically suitable, cost-effective method for intramuscular delivery of DNA vaccines. Key attributes of the system include effective and reproducible delivery achieved using a rapid application sequence and minimal operator training.