

Basic principles of *in ovo* vaccination



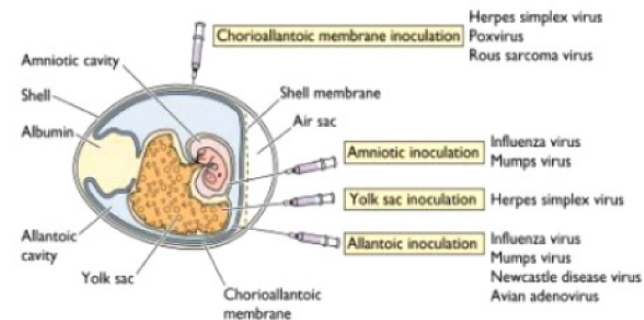
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***In ovo* vaccination is the procedure of administering a vaccine inside the egg when the embryo is at a late stage of development, targeting those specific embryonic structures where the vaccine is able to stimulate an immune response.**

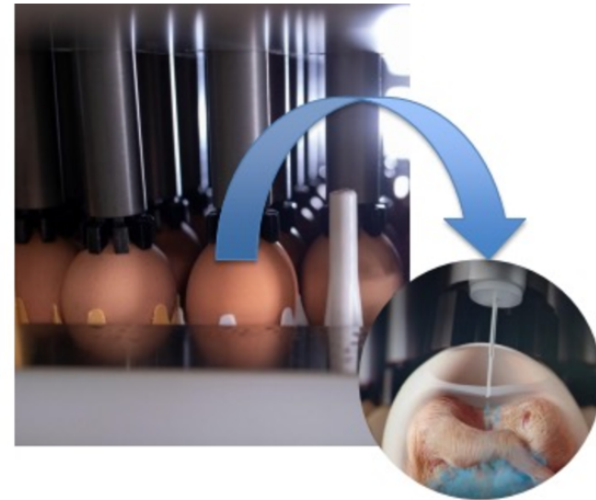
In the United States, it is the standard hatchery procedure for the application of Marek's disease vaccines and its different recombinant vaccines variations.





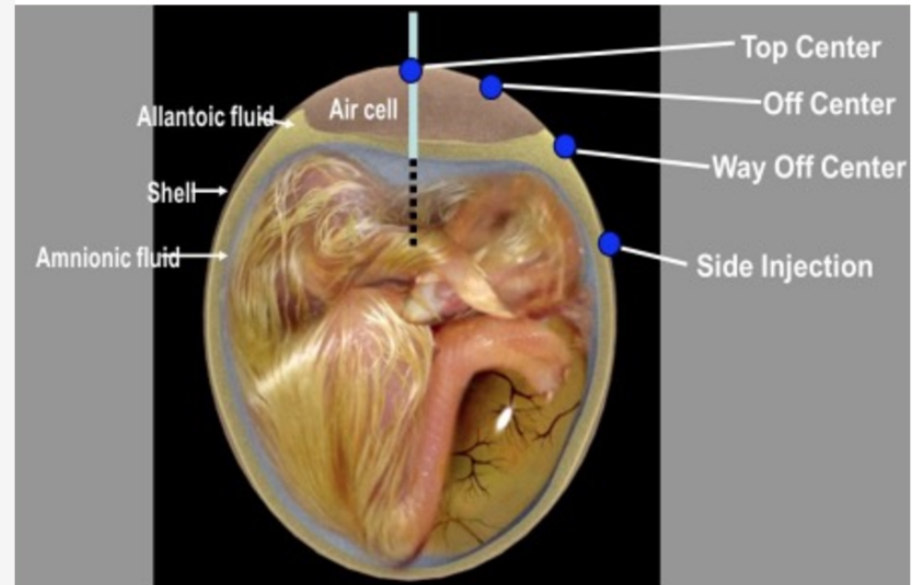
The laboratory concept of “inside the egg” vaccination has been expanded and developed into a technological platform that on a commercial scale can place several antigens simultaneously on more than 40,000 eggs per hour. Globally an estimated 21 billion eggs are injected *in ovo* in more than 30 countries.

What makes an *in ovo* vaccination technically successful? Here, we will examine **five different factors that are key to commercial-scale egg injection in the poultry industry.**



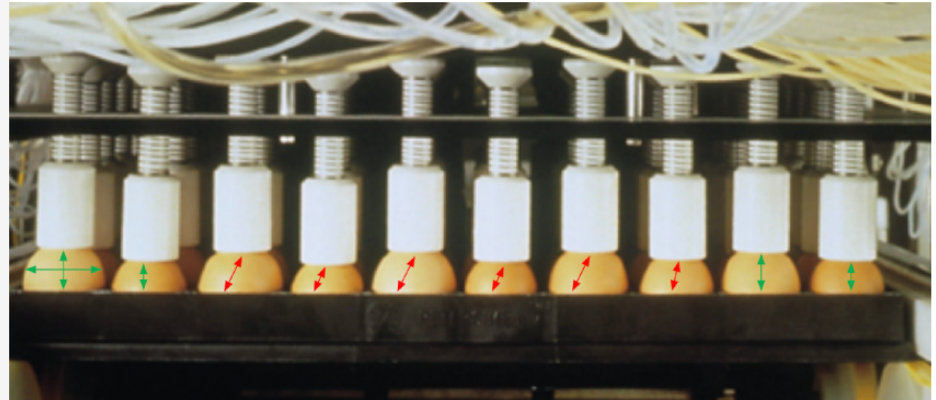
1 | Adaptation to the egg

It is the ability to locate the egg and place the injector toolings on the top of the eggs in relation to their longitudinal axis. Once the eggs are placed in the tray, they can change their orientation due to their size and the constant turning inside the incubator so they can tilt out of the center of the tray's alveoli.



When an egg is tilted, the embryo will orient itself according to gravity with the head pointing in the opposite direction to the inclination. With the ability to make translational and rotational movements **the injector can “find” the top of the shell and two things are achieved:**

- ▶ **One:** the injector is now aligned perpendicular to the surface of the shell (even though the egg is tilted)
- ▶ **Two:** the direction of the perforator and the vaccination needle point to the center of the inside of the egg where the embryo is.

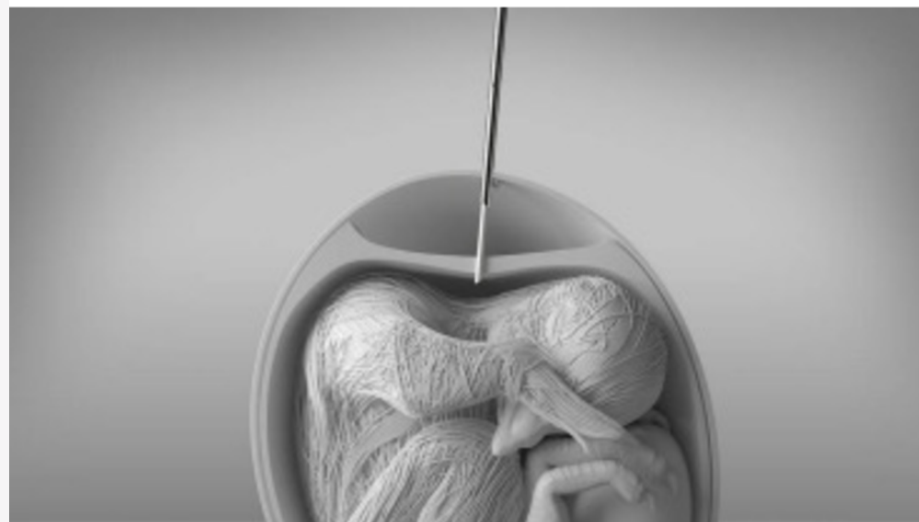


Eggs seldom arrived for injection in a vertical position, sitting correctly in the egg-tray.

2| Shell perforation

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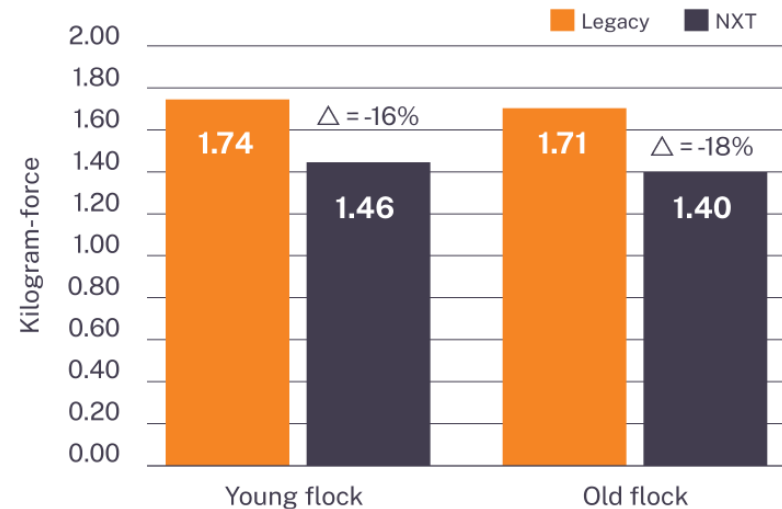


We cannot successfully access the embryo for vaccination without passing through the eggshell.

This sounds logical, but **there is a need to segregate this process into two actions** since it is necessary to maintain the integrity of the shell and minimize the deposition of shell debris over the chorio-allantoic membrane in the lower part of the air cell to prevent contamination by bacteria and fungi.

- ▶ First is the **perforation of the hole.**
- ▶ Second is **the access to the embryo.**


The perforation without breaking of the shell is influenced by the design of the punch needle and the speed with which it comes in contact with the shell. It has been seen that with an increase in the speed of the perforator from 1.5 inch/sec to 25 inches/sec the impact force is decreased between 16 to 18% depending on the quality of the eggshell.



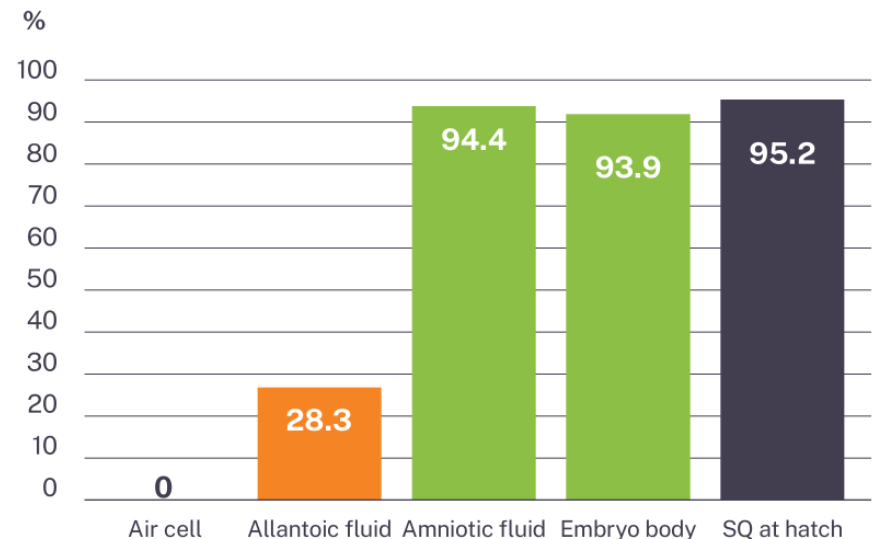
Zoetis Test Report: 7RJFJ, GELTD-1188, July 2020.

3 | The site of injection

The efficacy of a vaccine and the physical integrity of the embryo may be affected by the embryonic compartment in which it is deposited. The degree of embryonic development interacts with the injection site and factors affecting embryo development such as incubation time, type of incubator machines (multistage or single stage), breed and egg storage time eggs (> 7 days) will also impact the injection site.

 **Physiological age not only determine the actual size of the embryo, but also impacts the size and extension of extraembryonic compartments in the egg, influencing the site where the vaccine will be deposited.**

Injections into the amnion and body of the embryo are effective. Injections into the allantoic, air cell, or yolk are much less effective.



Wakenell PS, et al. Effect of *in ovo* Vaccine Delivery Route on Herpesvirus of Turkey/SB-1 Efficacy and Viremia. *Avian Diseases* 46:274-280, 2002.

4 | Vaccine delivery

The gentle pumping of the vaccine from the bag to the tip of the vaccinating needles, that guarantees the physical integrity of the cells containing the vaccine viruses is not the only element to consider in the proper administration; the process from thawing the ampoules to mixing and incorporating other additives also has its impact on delivery a good quality product to the embryo and the subsequent development of immunity and the final protection against the local disease challenge prevalent in the production area.

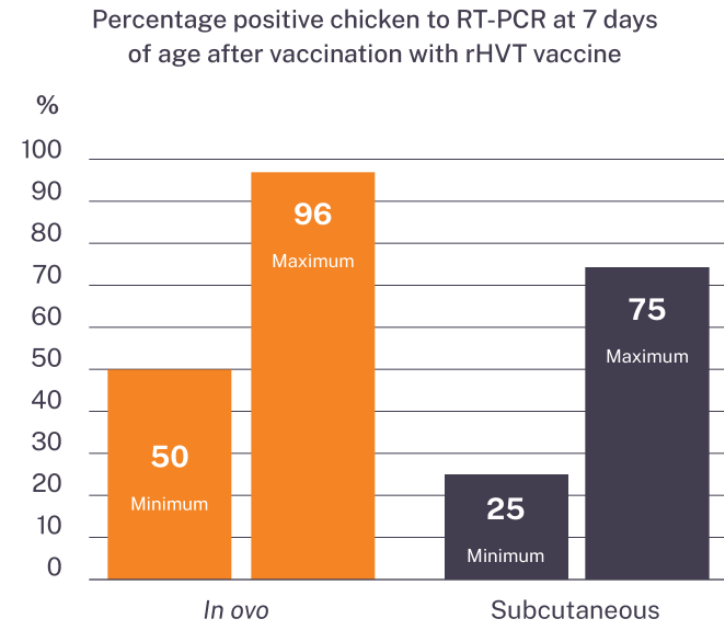
**Two types of pumps are currently used:
Peristaltic and Solenoid:**



Both types have in common that they are gentle and precise in the application of the force with which they deliver the volume of the vaccine.

The **solenoid pumps allow for selective vaccination** by activating only the pumps on those positions where viable eggs have been detected, while the **peristaltic pump will dispense the vaccine to all injectors that are in the head.**

The level of positivity at 7 days of age using RT-PCR in birds vaccinated with recombinant vaccines ranged from 50-96% in animals vaccinated *in ovo*, while those vaccinated subcutaneously fluctuates from 25-75%, suggesting a safer administration via *in ovo*.



A.L. Cortes, et al. Establishment of reference values for monitoring vaccination with a recombinant HVT by real-time PCR: Experiment data and seven years survey of field cases. AAAP July 2018. Denver.

5 | Constant disinfection

The technical success of *in ovo* vaccination on a commercial scale largely depends on the constant needles disinfection.

It is well known that **poor hygiene during the vaccination process will compromise the quality of day-old chicks and lead to poor performance in the future.**

A **dual-needle design**, where the vaccinating needle is inside the punching needle **allows for more efficient disinfection of the contact points between the injection equipment and the eggs**, leading to optimal pathogen reduction and prevent the transfer of harmful microorganisms from egg to egg during normal operation.

The outside of the vaccinating needles and the inside and outside of the punching needles are continuously bathed in a disinfectant liquid with 5,000 or 2,500 PPM of chlorine depending on the chlorine source used.

Sanitizer type	Chlorine (ppm)	pH	% HOCl-	HOCl- (Hypochlorous acid)
Inovotabs® (Sodium dichloroisocyanurate)	2,500	7.0-7.2	80%	2,000
Hand mix (Sodium hypochlorite)	5,000	8.5-9.0	10%	500



Summary

The *in ovo* vaccination technology has been used in the commercial poultry industry for more than 30 years and some of its advantages include early immunity against disease, reliable vaccine delivery, efficient processing, and less bird stress.

The **5 critical factors for successful *in ovo* vaccination** are based on decades of research and development, still valid, and should be considered when evaluating *in ovo* equipment.

An *in ovo* vaccination equipment must:

- ▶ Have the flexibility to adapt to different eggs sizes and their positions in the tray.
- ▶ Penetrate the shell without causing breaks.
- ▶ Apply the vaccine in the embryonic compartment where an immune response is generated.
- ▶ Administer the vaccine without affecting its titer.
- ▶ Have constant disinfection of the needles and elements that come in contact with the eggs and embryos.

Thank you!

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