Critical success factors for in ovo vaccination

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In ovo vaccination is currently the standard procedure for hatchery applied Marek's disease vaccine in the United States. The laboratory concept of 'in the egg' vaccination has been expanded and developed into a commercially applied technology platform (Embrex Inovoject System) that is capable of placing antigens simultaneously into over 40,000 eggs per hour. Globally more than 15 billion eggs annually are injected in ovo in more than 30 countries.

Embryonic age

In order to maximise chick quality, chick performance, and immune status during the grow-out period, it is important to understand the embryonic stage of development. This is critical for timing of egg injection as measured by perfect hatch following in ovo application. Egg injection too early can reduce percent hatch by increasing the number of late dead and cull birds, as well as increase the incidence of missed vaccination.

Vaccination too late will create problems for vacuum transfer and increase loss of hatch due to egg breakage.

In general, the preferred time or 'window' for safely injecting the egg is from day 17 and 12-14 hours of incubation to day 19 and 2-4 hours of incubation, with time 'zero' being the normal egg set time.

Egg injection on 'early day 17' has been shown to reduce percent hatch by approximately 1-2% as compared to injection on 'day 18' (Table 1).

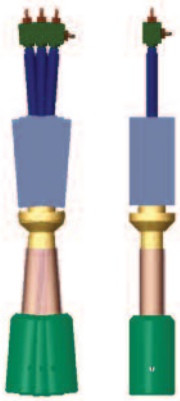
It should be noted that differences in day of transfer without in ovo vaccination (day 17 versus day 18) have also been shown to reduce percent hatch (Table 2).

Differences in percent hatch are due to dissimilar environmental conditions between the incubators and the hatchers

Flock age (weeks)	Day of transfer	No. of eggs	No. of sellable chicks	Hatch (%)	Cull (%)	Net (%)
< 31	17	422,676	342,784	82.82	1.75	81.10
	18	421,668	351,546	84.64	1.27	83.37
31 - 49	17	2,276,424	1,923,148	85.79	1.31	84.48
	18	2,454,048	2,112,552	87.17	1.08	86.08
> 49	17	1,270,116	903,824	72.63	1.47	71.16
	18	1,227,708	907,155	75.09	1.20	73.89

Table 1. Evaluation of embryonic age at the time of Inovoject System vaccination based on data collected from a commercial hatchery during a four month period (Brake, J. T. et al).

Fig. 1. A translational or sideways movement of the injector is used to 'find' the shell surface.



Critical factors for success

What makes in ovo vaccination technically successful? Here we will examine five interacting factors which are the keys to egg injection both in the laboratory and in the commercial poultry industry.

● Egg location.

Egg location is simply the ability to find the egg and place the injector device onto its surface. Furthermore, it is important to understand that the location needs to be correct both vertically and horizontally.

Once the eggs are placed on the tray they can change their orientation because of egg size and incubation turning, thus leaning them off centre in the incubation flat.

When an egg is leaning, the embryo will orient according to gravity, with the head pointing away from the direction of the tilt.

By providing translational or sideways movement of the injector to 'find' the shell surface, two things are accomplished. One, the injector is now aligned perpendicular to the shell surface (even though the egg is leaning), and two, the direction of the needle and punch is aimed at the centre of the interior of the egg, not perpendicular to the flat (Fig. 1).

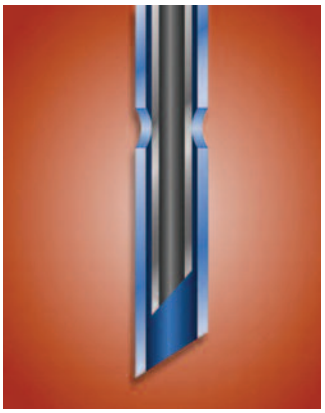


Fig. 2. Cross section of the 'needle inside a needle' concept.

● Shell penetration.

We cannot successfully access the embryo for vaccination without getting through the egg shell.

This sounds logical but there is a need to segregate this process in two actions – one being hole perforation and the other being embryo access – due to both the sterile and physiological requirements of vaccination of the embryo.

This is why the 'needle inside a needle concept' was developed with an external needle or 'punch' and internal needle or 'vaccinator' (Fig. 2).

The punch, or outer needle, was designed for shell penetration and has been tested for long term appli-

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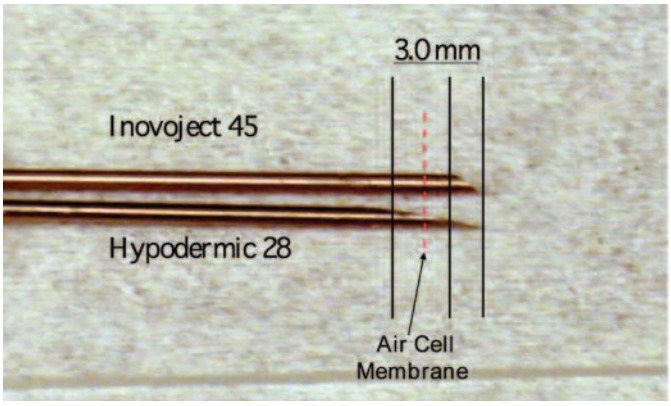
Fig. 3. Site of injection is affected by needle tip and size.



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cation. It typically lasts a year or more, individually piercing greater than one million eggs in its life.

The design of the punch includes exterior holes in the cannula itself to allow for sanitation, but the key aspect is the tip angle.

This angle provides a repeatable action of piercing the shell without breaking it, as well as minimising the deposition of shell debris on the inside or air cell membrane surface.

Site of injection.

Site of injection is affected by needle tip and size. The needle used to deliver vaccine via the Inovoject System (vaccinator needle) is a 20 gauge needle with a 45 degree straight cut bevel tip. The opening of the needle is less than 1mm.

In contrast, an ordinary disposable syringe has a 20 gauge needle with a standard B-bevel 28 degree tip that is approximately 3mm long.

If both needles simultaneously inject at 25mm depth into the egg, the site of injection could be very different (Fig. 3).

As discussed earlier, embryonic age interacts with the site of injection. Incubation time is not the only factor which can affect it, but also the incubator machine type, birds breed, and even prolonged egg storage time prior to incubation (>7 days). The relative embryo size and the presence or absence of extra-embryonic compartments in the egg affects the ability to target these compartments with in ovo injection.

Vaccine delivery.

Delivery aspects include not only the physical dynamics of vaccine integrity from bag to needle, but the whole process from frozen ampule through mixing and addition to the bag, to delivery to the embryo, and then development of immunity and ultimate defence against disease challenge.

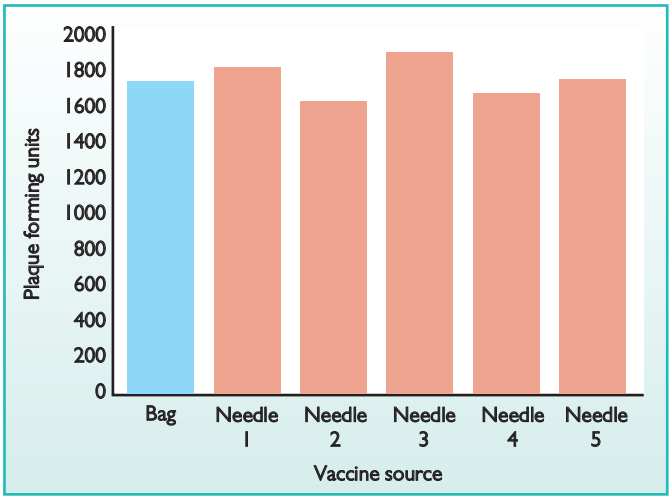

Fig. 4. Average titers (PFU) obtained with HVT per 0.05ml using a Jamesway Model Inovoject system (Marsh, T. E. et al).

For example, the quality of the vaccine as measured by plaque forming units (PFU) of HVT (Herpes Virus of Turkey) was delineated in the bag of vaccine and then directly out of the needles in five separate locations (Fig. 4).

As you can see, the samples taken from the needle are equal or greater than those taken directly from the bag. It is theorised that a slight increase in PFU from the needles is possibly due to the peristaltic pump action of the rollers which may separate small clumps of cells into individual cells, thus increasing the PFU measurements.

Sanitation.

Technical success of in ovo vaccination is highly dependant upon sanitation. Specific delivery of sanitation fluid helps provide optimal pathogen reduction to aid in the prevention of egg to egg contamination during normal operation.

Sanitation of the needle and punch is accomplished with a buffered chlorine solution which is delivered in a targeted fashion to all contact points between equipment and embryo.

The exterior of the needle and the interior and exterior of the shell punch are continually bathed in sanitation fluid between injection cycles. Sanitation of the vaccine delivery system daily (prior to and after the injection day) is also necessary for long term application success in the hatchery.

In conclusion, the ultimate goal is to have all birds properly vaccinated to help ensure immunity and to maximise production potential.

The method of choice throughout many countries is in ovo vaccination with the Embrex Inovoject System.

Using the five key technical components of this technology – egg location, shell penetration, site of injection, vaccine delivery and sanitation – helps to guarantee accurate vaccine delivery to the correct site of injection. ■

References are available from the authors on request

Table 2. Comparison of non-injected controls (C) and injected eggs from commercial trials with respect to different breeder flock ages and day of transfer/injection (Brake, J. T. et al).

Flock age (weeks)	Day of transfer	Treatment	No. of chicks	% hatch of total	% hatch of live
< 31	17	C	3,056	81.62	98.04
		I	3,101	82.83	97.52
	18	C	5,849	84.62	98.30
		I	5,957	86.18	98.51
31 - 49	17	C	30,240	88.24	98.56
		I	30,056	87.70	98.45
	18	C	20,069	89.34	98.62
		I	19,956	88.84	98.61
> = 50	17	C	5,243	79.15	97.91
		I	5,243	79.15	97.82
	18	C	8,697	83.88	98.23
		I	8,701	83.92	98.51
All flocks	17	C	38,539	86.33	98.43
		I	38,400	86.02	98.29
	18	C	34,615	87.09	98.47
		I	34,614	87.09	98.56