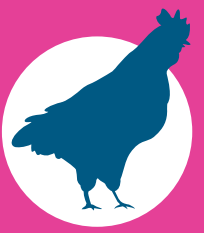
# Vaxsafe® ND
## (Strain V4)


BIOPROPERTIES
The Vaccine Innovators

# Features of Vaxsafe® ND Vaccine (living)

- Produced from a low passage isolate of V4 held by CSIRO since isolation in 1966
- Seedlot development included bird-to-bird passages to ensure high bird infectivity
- Highly transmissible ensuring uniform flock protection
- Able to induce protection in the face of a low level of maternal antibody
- Compatible with other live viral vaccines including HVT, IB and IBD
- Proven safety and efficacy under field conditions



## Product Development

Newcastle disease (ND) virus, strain V4 was first isolated at the Queensland Veterinary Research Institute in 1966. The strain was stored by CSIRO before supply to BIOPROPERTIES for vaccine development. BIOPROPERTIES undertook extensive testing in conjunction with Australian Animal Health Laboratory (AAHL), a division of CSIRO, to develop a seed lot that would provide a stable finished vaccine. BIOPROPERTIES then conducted the required regulatory testing of the vaccine under laboratory and field conditions. Vaxsafe® ND Vaccine (living) (Vaxsafe® ND) was registered in 2005 for vaccination of broiler chickens. The finished product consists of a freeze-dried suspension of live ND virus in SPF embryo allantoic fluid.

## Biological and Molecular Characteristics of the Vaccine Seed

The precise identification of the seed lot used to produce Vaxsafe® ND was undertaken with AAHL. These studies confirmed that the master seed had biological and molecular characteristics consistent with those of reference and historical isolates of ND V4. In the region of the genome identified as the major virulence determinant, the fusion protein cleavage site, the master seed and finished product passage level were shown to have molecular characteristics consistent with other lentogenic ND strains.


## Safety in Chickens

A number of experiments have been undertaken in chickens under laboratory and field conditions to prove the safety of the vaccine.

### Pathogenicity testing in SPF chickens

Fourteen-day-old SPF chickens were vaccinated orally with a standard field dose, 10 times standard dose and 100 times standard dose of Vaxsafe® ND. Another group was vaccinated with 10 times standard dose on three occasions at 28 day intervals. No adverse reactions were detected in any of the groups up to 70 days of age (up to 84 days of age in the repeat vaccinated group). No pathological lesions were evident and the body weight of the chickens in all groups did not differ significantly from that of a group of unvaccinated chickens or from a group vaccinated with another registered live ND V4 vaccine.

### Safety in broiler chickens

When 7, 12 and 17-day-old broiler chickens with maternal antibody to ND virus were vaccinated orally with Vaxsafe® ND, no adverse reactions were observed and there were no significant differences in body weight between the vaccinated and control groups. In addition, when Vaxsafe® ND was administered at 17 days of age to chickens previously vaccinated by coarse-spray with Vaxsafe® ND vaccine at 1-day-old, no adverse reactions were observed up to 42 days of age.

### Comparative safety studies with another registered live ND V4 vaccine

In three comparative studies, Vaxsafe® ND showed safety characteristics equivalent to those of another registered live ND V4 vaccine. Both vaccines met the requirements of the Australian standard for safety.

### In-contact transmission of Vaxsafe® ND

To assess the horizontal transmissibility of Vaxsafe® ND, vaccinated chickens were placed in close contact with unvaccinated chickens under laboratory conditions. The study was conducted using SPF and commercial broiler chickens. Evidence of rapid spread of vaccine virus to the in-contact birds was demonstrated by serological testing such that in-contact birds had a mean serum antibody level that was not significantly different from vaccinated birds at 14 days after vaccination. No adverse clinical reactions were observed and there was no significant difference in body weight between the vaccinated and the in-contact groups.

### Interaction with other vaccines

Vaxsafe® ND was administered to broiler chickens in a large-scale field trial at 10 days of age following the use of Herpesvirus of turkeys (HVT) vaccine *in ovo* and Infectious bronchitis (IB) vaccine at day-old. No adverse reactions were reported. In addition, Vaxsafe® ND has been given to SPF chickens at three weeks after the administration of Vaxsafe® IBD without any adverse effects or reduction in ability to stimulate production of ND Haemagglutination Inhibition (HI) antibody.

# Efficacy of Vaxsafe® ND

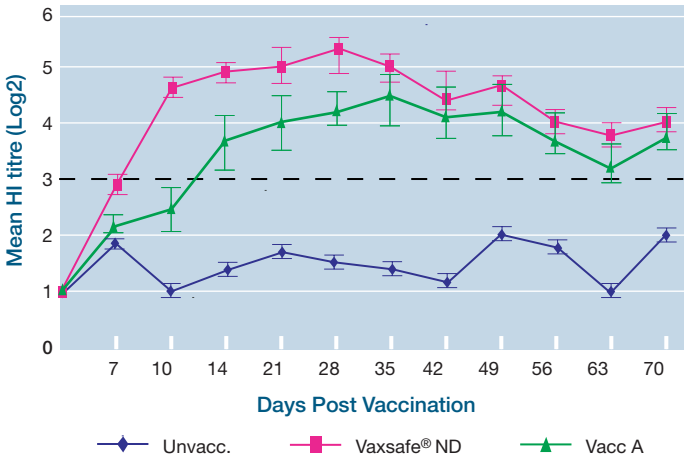
As there is a high correlation between ND HI antibody response and protection against virulent ND virus challenge, all studies on the efficacy of Vaxsafe® ND were undertaken without challenge with virulent ND virus.

## Laboratory efficacy studies

Four laboratory efficacy studies were undertaken using both SPF White Leghorn chickens and commercial broiler chickens. In SPF chickens, and in ND maternal antibody negative commercial broilers, Vaxsafe® ND, with a titre equivalent to the minimum allowable at the end of its shelf life, induced HI antibody responses well in excess of those required for protection against virulent challenge (Figures 1 & 2 respectively).

It was also found that in a dose response study involving SPF chickens, Vaxsafe® ND had a wide dosage margin. This wide margin gives confidence that other external factors such as ND maternal antibody or poor storage conditions will have less of an effect on the performance of the vaccine. When required, it was found that a further vaccination with Vaxsafe® ND, 4 weeks later could further boost HI antibody levels.

**Figure 1. Serum antibody response after oral vaccination of 14-day-old SPF chickens with Vaxsafe® ND to 10 weeks post vaccination.**

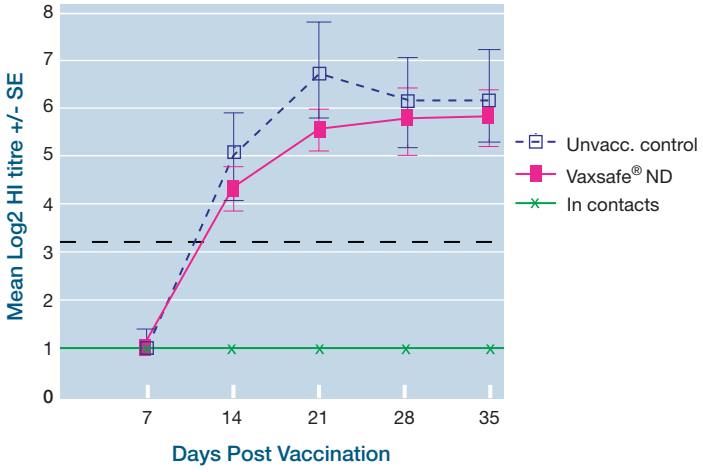



Notes: Vaxsafe® ND administered orally to SPF chickens at 14 days of age. Dotted line indicates minimum mean HI titre to comply with national ND vaccination SOPs. Vacc A is an existing registered V4 vaccine.

## Efficacy in broiler chickens without ND maternal antibody

When maternal antibody negative broiler chickens were vaccinated with Vaxsafe® ND at 7 days of age, all birds were considered positive (HI titre $\geq 2^3$) at 14 days post vaccination (Figure 2). Unvaccinated hatch mates developed an antibody response that was not significantly different from vaccinated birds, with all birds positive within 14 days after contact with vaccinated birds, indicating a high level of transmission.

**Figure 2. Serum antibody response after oral vaccination of 7-day-old maternal antibody free broiler chickens with Vaxsafe® ND to 5 weeks post vaccination.**

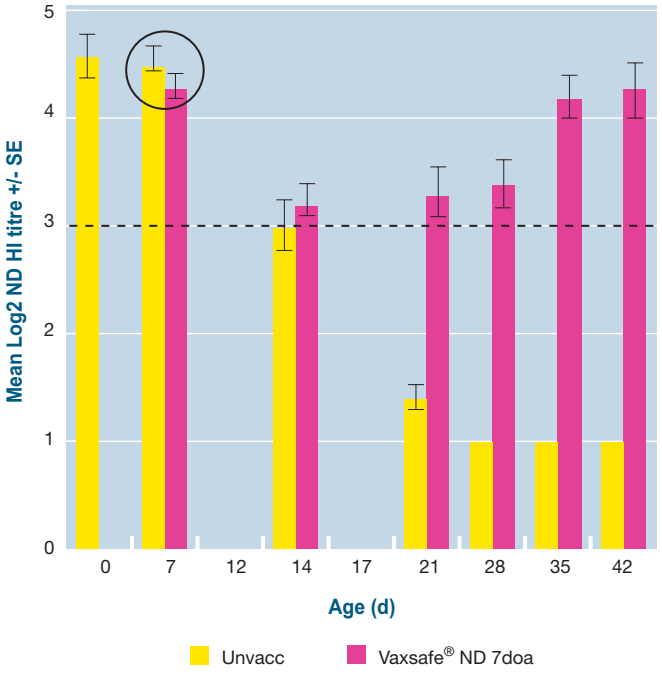



Notes: Solid pink line indicates antibody response in vaccinates; purple dotted line indicates antibody response in unvaccinated, in contact hatch mates. Black dotted line at $2^3$ HI units indicates minimum mean HI titre to comply with national ND vaccination SOPs.

## Efficacy in broiler chickens with ND maternal antibody

When 7, 12 and 17-day-old broiler chickens with high to low levels of ND maternal antibody to ND virus, respectively were vaccinated orally with Vaxsafe® ND, all age groups produced protective levels of ND HI antibody. However, the broiler chickens vaccinated at 7 days of age had the most consistent protective levels of antibody (Figure 3). Vaxsafe® ND induced an active antibody response significantly earlier than another registered live ND V4 vaccine. When Vaxsafe® ND was administered at 17 days of age to chickens previously coarse-spray vaccinated with ND V4 vaccine at 1-day-old, it did not induce antibody levels as high as a single dose given at 17 days of age when passive antibody levels had fallen. However, both vaccination programs induced HI antibody responses well in excess of the minimum standards required under the current Australian Government ND control program.

**Figure 3. Comparison of mean ND HI titres to 42 days of age in maternal antibody positive commercial broilers vaccinated with Vaxsafe® ND at 7 days of age and similar unvaccinated chickens.**




Notes: Vaxsafe® ND administered (oral) to commercial maternal antibody positive broiler chickens at 7 days of age. Dotted line indicates minimum mean HI titre to comply with national ND vaccination SOPs. Circle indicates age of vaccination.

## Onset and duration of protection

In SPF chickens, a protective antibody response was achieved as early as 7 days after oral vaccination with Vaxsafe® ND. However, in the presence of maternal antibody in broiler chickens, protective levels were delayed until 10-11 days post vaccination. As indicated above, Vaxsafe® ND induced superior efficacy characteristics in terms of earlier onset of immunity, higher protective titre and a longer duration of immunity. Overall, the duration of protection induced by Vaxsafe® ND would adequately cover chickens to the age at which the majority of broiler chickens are slaughtered.

# Vaxsafe® ND Vaccine (living)

## Safety under Field Conditions

The safety of Vaxsafe® ND was demonstrated in a large-scale field trial involving over two million broiler chickens across two States of Australia. Broilers were vaccinated by means of the drinking water at 10 days of age and observed for evidence of any adverse effects as well as measured against standard broiler performance parameters. The study found that there were no adverse effects from Vaxsafe® ND and flocks vaccinated with Vaxsafe® ND performed to the same production standards as those vaccinated with another registered live ND V4 vaccine (Table 1). It was also shown that Vaxsafe® ND could be administered safely in the presence of ND maternal antibody in the field.

Additionally, all production parameters recorded were not significantly different ($p>0.05$) in flocks vaccinated with Vaxsafe® ND compared to those vaccinated with another registered live ND V4 vaccine (Table 1).

**Table 1. Comparative productivity data: Differential between mean data at slaughter age for 36 flocks vaccinated at 10 days of age with Vaxsafe® ND and 10 flocks vaccinated at the same age with another registered live ND V4 vaccine.**

| Productivity Parameter | Vaxsafe® ND | Level of Significance |
|---|---|---|
| % Mortality | -0.27* | 0.495 |
| Age to Slaughter | -0.6* | 0.451 |
| PIF[&] | +6.0* | 0.116 |

* Differences were not significant ($p>0.05$)
[&] Performance Indicator Factor

## Vaccine Use

A full description of the storage, handling and method of administration of Vaxsafe® ND is provided in the leaflet that accompanies the vaccine.

### Vaccine presentation

Vaxsafe® ND is supplied as a freeze-dried product in 2000, 3000, 4000, 5000 and 10,000 dose presentations in a 3mL and 10mL glass vial with a rubber stopper and tear-off aluminium seal.

### Vaccine administration

Vaxsafe® ND should be diluted for drinking water administration as described in the leaflet accompanying the vaccine.

### Vaccine storage

Vaxsafe® ND should be held at -18°C or lower until required for use.

### Vaccine ordering

ND vaccination, while compulsory in several states of Australia, must only be carried out after obtaining approval (permit) from the respective state government. This permit must be sent to your vaccine supplier before an order can be processed.

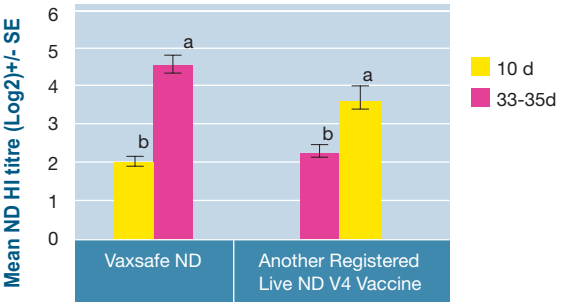
### Vaccination program

Broiler chickens should be vaccinated in accordance with the current Australian Government ND control program. The preferred vaccination program is a single dose at 7 to 14 days of age. Each broiler flock must achieve a mean HI antibody titre of $2^3$ at 35 days of age with at least 67% of samples reaching that titre. A single vaccination with Vaxsafe® ND has been shown to be able to induce protective levels of antibody in the face of low levels ($<2^3$) of ND maternal antibody. However, where ND maternal antibody levels may be very high, it is recommended to delay vaccination until the levels drop to less than $2^3$ or to apply a repeat dose of Vaxsafe® ND.

## Efficacy under Field Conditions

The efficacy of Vaxsafe® ND was further assessed under field conditions. Over two million broiler chickens were vaccinated in two States of Australia at 10 days of age. The field efficacy of Vaxsafe® ND was evaluated by measuring the ND HI antibody response to the vaccine as well as measuring standard broiler performance parameters. The study found that Vaxsafe® ND stimulated antibody levels approximately two-fold higher than another registered live ND V4 vaccine ($p=0.06$) as shown in Figure 4.

**Figure 4. Broiler field trials: Comparison of mean ND HI antibody titres at 10 days (day of vaccination) and 33-35 days of age (according to National ND SOPs) following vaccination with either Vaxsafe® ND (36 flocks) or another registered live ND vaccine (10 flocks).**




Note: Difference between treatment groups at 33-35 days of age = 1.0 $Log_2$ ($p=0.06$)

In addition, over 90% of broiler flocks exceeded protective HI antibody levels at 35 days of age compared to only 57% with another registered live ND V4 vaccine (Table 2).

**Table 2. Broiler field trials: Comparative immunogenicity* of Vaxsafe® ND (36 flocks) and another registered live ND V4 vaccine (10 flocks)**

| Location | Vaxsafe® ND | Another Registered Live ND V4 Vaccine |
|---|---|---|
| Victoria | 92% | 43% |
| South Australia | 89% | 71% |

* Immunogenicity was based on the percentage of broiler flocks whose mean ND HI titre, when sampled at 35 days of age, was > $2^3$.

### Compatibility with other vaccines

Vaxsafe® ND has been administered to broiler chickens in large-scale field trials from 10 days of age following the use of Herpesvirus of turkeys (HVT) vaccine *in ovo* and Infectious bronchitis (IB) vaccine at day-old. No evidence was found that Vaxsafe® ND had any deleterious effect on the protection induced by the other vaccines.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