X-OVO

Diagnostic Solutions

# X-OVO NEXT GENERATION INFECTIOUS BRONCHITIS PCR SEQUENCING

PROTECTED CHICK

IT ALL STARTS WITH EARLY VACCINATION

Protectotype

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MSD Animal Health

# WHAT IS X-OVO NEXT GENERATION SEQUENCING?

X-OvO, a laboratory in Scotland, has created a next generation IB PCR sequencing test, which evaluates a larger part of the IB virus genome, allowing for more accurate IB virus identification.

## The X-OvO database contains:

- Common commercial Infectious Bronchitis (IB) vaccines used in the field.
- Major and minor virus populations from a range of commercial vaccine vials (defined using Next Generation Sequencing (NGS) analyses).
- IB reference strains.

X-OvO has performed whole genome sequencing of the different commercial IB vaccine vials by means of NGS, identifying each amino acid mutation present in the different viral sub populations contained in each vaccine vial, giving us accurate IB reference strains.

These genome sequences can be considered as genetic fingerprints which allow us to differentiate viruses from one another.

X-OvO can CLEARLY diagnose different vaccine strains of which the below are a few examples:

- 4/91 from CR88 and 1/96 vaccines
- Ma5 from H120
- D388 QX from L1148 QX.

## X-OvO can definitively differentiate vaccine viruses from field viruses.

# X-OVO RESULTS IN BROILERS IN SOUTH AFRICA IN 2021

X-OvO IB PCR samples were taken at random and when required by clients wanting to monitor the Infectious Bronchitis situation on their farms. These results include IB surveillance samples that were taken from several commercial broiler farms from the beginning of 2021, up to and including July 2021. All the samples taken in this study were cloacal samples. The sample age ranged from 24 days of age up to 33 days of age. The birds in 21 of the 26 samples received only hatchery Infectious Bronchitis vaccine, no on farm hatchery IB vaccines were administered. The remaining 5 groups of birds received additional on farm IB vaccinations.

A total of 26 samples were included in this surveillance and the results obtained can be seen below:

## Broiler Infectious Bronchitis genotyping 2021

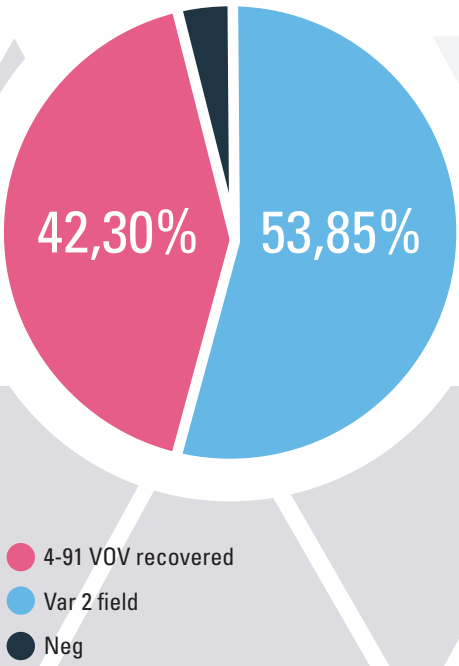



Figure 1. Results from 26 samples taken for X-OvO IB PCR in broilers on various vaccination programs.

## The results in numbers:

- 42,2 % of the samples tested positive for IB Var 2 field strain.
- 53,85 % of the samples tested positive for Nobilis® IB 4-91 vaccine or Nobilis® IB 4-91 vaccine origin virus.
- 0 – the number of the samples that tested positive for IB Var 2 field strain that were vaccinated with Nobilis® IB 4-91.
- 91,67 % of the samples that were taken from birds vaccinated with Nobilis® IB 4-91 tested positive for Nobilis® IB 4-91, the only exception was one sample which had a negative result.

The absence of a positive result may be due to:

**1. The flock not being well vaccinated.** Summary on the X-OvO report – 'The failure to administer vaccine properly resulting in negative PCR results occurs not infrequently in the field, hence this possibility should be reviewed on farm to confirm the situation'.

**2. A non-representative sample of the flock was taken.** Summary on the X-OvO report – 'On occasion, sampling of cull birds and birds at PM will yield negative results due to the degradation of RNA that results in a negative that is not a true reflection of the overall flock status'.

# X-OVO RESULTS IN BROILERS IN THE REST OF THE WORLD FROM 2008 TO 2019

The figure below depicts how frequently Nobilis® IB 4-91 vaccine or vaccine origin virus is isolated from broilers vaccinated with Nobilis® IB Ma5 + Nobilis® IB 4-91 at day old.

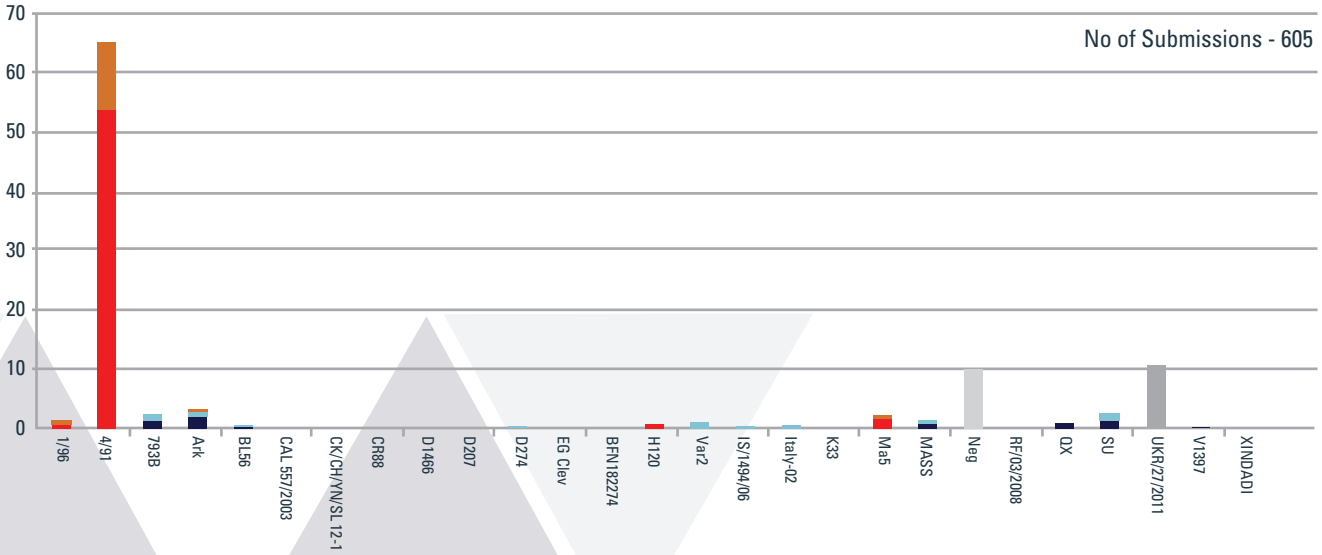



Figure 2. IB isolates from 605 samples from flocks in various parts of the world, where only Nobilis® IB Ma5 + Nobilis® IB 4-91 were applied to day-old chicks (2008 to 2019).

In the 605 submissions sent to the X-OvO lab represented above, we can see that in most of the sample results, Nobilis® IB 4-91 vaccine or Nobilis® IB 4-91 vaccine origin virus was isolated from these flocks. The low number of field viruses detected in these flocks demonstrates the dominance of Nobilis® IB 4-91 in well vaccinated flocks.

# THE IMPORTANCE OF GOOD QUALITY MASS STRAIN VACCINATION

Even though it has been demonstrated that Nobilis® IB 4-91 is such a dominant variant strain, the addition of a good quality Mass strain vaccination is crucial for broad spectrum IB protection. An example of this can be seen below from a farm in South Africa that was visited to take routine samples from 24 day old broilers. On inspection, the birds were showing significant respiratory signs including open mouth breathing and respiratory rales. These birds were vaccinated with a conventional H120 vaccine and Nobilis® IB 4-91 vaccine in the hatchery, no field vaccines for IB were administered. Cloacal samples were taken and sent to X-OvO for IB PCR sequencing.

**The result of this testing can be seen below:**
The molecular analysis revealed a significant quantity of IB viral RNA with a Ct (cycle threshold) value of 25.44. The significant quantity of viral RNA recovered at this sampling point supported the classification of a field virus.

| | |
|---|---|
| **Molecular Analysis:** | Molecular analysis identified a significant quantity of IB viral RNA (Ct = 25,44). |

## Comments

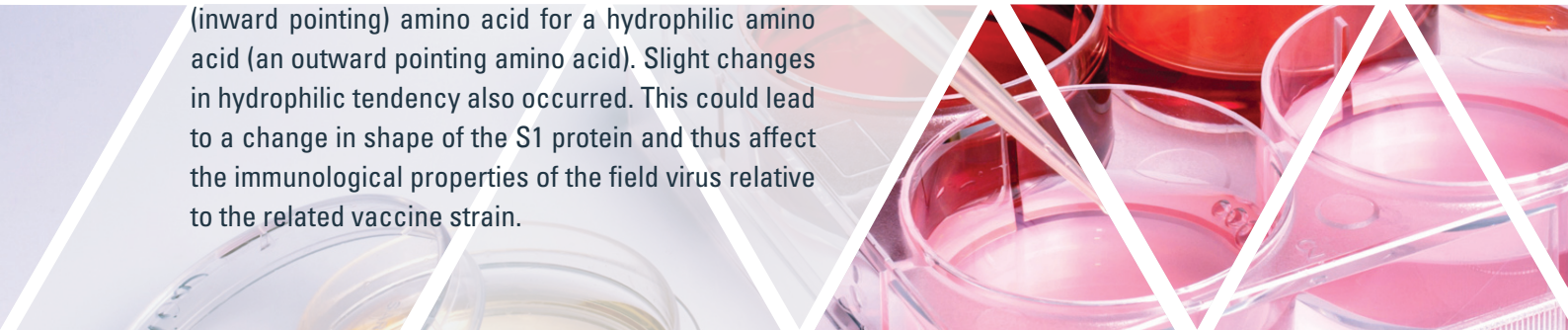
The analysis identified a Mass field virus with six amino acid substitutions different from the known H120 vaccine vial reference strain. One of the amino acid substitutions involved the change of a hydrophobic (inward pointing) amino acid for a hydrophilic amino acid (an outward pointing amino acid). Slight changes in hydrophilic tendency also occurred. This could lead to a change in shape of the S1 protein and thus affect the immunological properties of the field virus relative to the related vaccine strain.

## Conclusion

The analysis confirmed the presence of a Mass field virus in a cloacal FTA card sample from 24 day old broilers last vaccinated with Nobilis® IB 4-91 and H120 at 1 day of age.

Although the farm has a history of Mass live vaccination, the significant quantity of viral RNA recovered at this sampling point and the genetic sequence deviation are supportive of the classification of a Mass field virus. Please note that with the current vaccination programme, recovery of significant quantities of Nobilis® IB 4-91 vaccine would be considered normal in the absence of infectious bronchitis field virus infection. This result suggests that the field virus infection has arrived on farm in advance of the onset of immunity of the Mass vaccine. Since Ma5 vaccine offers more rapid replication in the face of maternal antibodies than other Mass-type vaccines, consideration of the inclusion of Ma5 may assist in the development of early onset mass type immunity.

This example demonstrates the importance of the inclusion of a highly immunogenic Mass strain vaccine such as Nobilis® IB Ma5 in the vaccination programme.



# WHAT ARE THE DIFFERENCES BETWEEN CONVENTIONAL H120 VACCINES AND NOBILIS® IB MA5?

- The Nobilis® IB Ma5 strains' S1 subunit of the spike gene was found to be structurally different compared to an H120 strain.
- The S1 subunit of the spike protein is directly involved in the stimulation of neutralising antibodies and thus, proteins.
- The difference found in the S1 subunit of the Ma5 strain, is the results of amino acid change that have actually helped the strain to become more immunogenic and more effective as a vaccine.
- Nobilis® IB Ma5 has the ability to break through high levels of MDA, meaning that it is highly effective even when administered at day-old, yet is also safe when given to chickens with low levels of MDA.
- Ma5 is proven to have a faster onset of immunity than H120.

References
1. X-OvO IB diagnosis algorithm, Dr Richard Currie
2. X-OvO broiler results South Africa January 2021 to July 2021
3. IB updates, October 2019, MSD Animal Health
4. A comparison Infectious Bronchitis (IB) live-attenuated vaccine strains Ma5 and H120, MSD Animal Health 2011.

X-OvO Next Generation Sequencing is a tool that allows you to identify your farm's IB strains more accurately and to monitor the success of your IB vaccination program.

FOR MORE INFORMATION CONTACT YOU MSD ANIMAL HEALTH KEY ACCOUNT MANAGER.