MERCK ANIMAL HEALTH

TECHNICAL SERVICES BULLETIN

MILDVAC®-MA5:

Mildvac®-Ma5 Protection: Rapid Growth and Better Antigenicity

Gene sequencing of the **Mildvac®-Ma5** vaccine S1 spike protein reveals 99.7% homology (genetic similarity) with H120 vaccine and 96.7% homology with Massachusetts M41 vaccine, but sequence homology does not predict the ability of a vaccine to induce a strong immune response. The ability of an infectious bronchitis (IB) vaccine to protect against a challenge depends upon several characteristics. Two important characteristics are the ability of the vaccine to replicate quickly (the driving force to produce a rapid onset of immunity) and the antigenicity of the vaccine. Antigenicity refers to the ability of a vaccine to stimulate the production of antibody against the key amino acids that enable a virus to bind to the host tissue.

Figure 1 Percent of positive tracheal swabs **Mildvac-Ma5** vs. H120 (qRT-PCR)

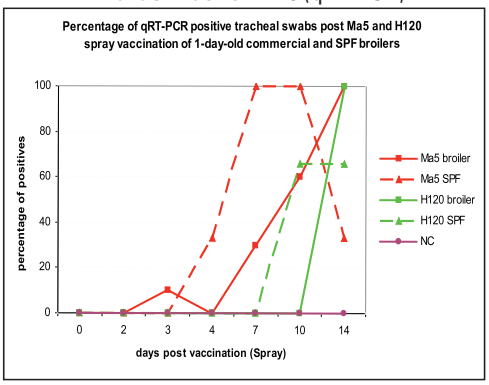
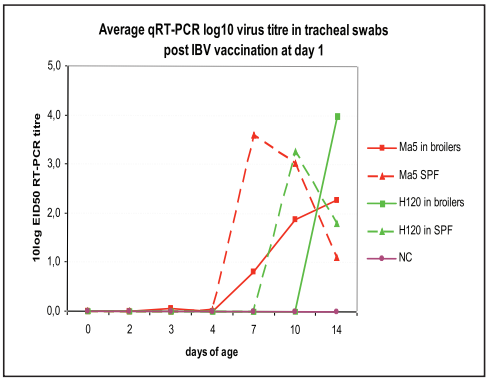


Figure 2 Average virus titer (qRT-PCR log 10) **Mildvac-Ma5** vs. H120



RAPID REPLICATION

Mildvac-Ma5 has demonstrated a faster replication pattern than H120 vaccine based upon quantitative real-time polymerase chain reaction (qRT-PCR) techniques (DeWit 2011; Figures 1 and 2). This important feature results in earlier protection, as well as ensuring that vaccination reactions are felt early in the growth curve, with no negative impact on final processing weight and feed efficiency.

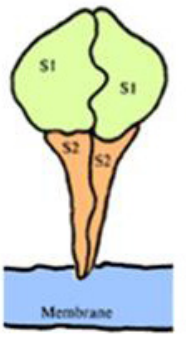
CONCLUSION

- **Mildvac®-Ma5** demonstrates rapid replication, making it a highly effective driver of an early onset of immunity.
- **Mildvac®-Ma5** demonstrates higher antigenicity compared to mild Mass 41-type vaccine at two of the four key amino acid positions shown to be critical to host tissue binding.
- Superior replication characteristics and superior antigenicity at specific receptor binding sites combine to explain why **Mildvac®-Ma5** is a uniquely protective IB vaccine capable of "Protectotype" cross-protection against non-homologous IB strains as demonstrated by a reduction in ciliostasis.
- These characteristics of **Mildvac®-Ma5** may allow companies to reduce the use of Arkansas-DPI vaccines in broilers.

ANTIGENICITY

Determination of infectious bronchitis antigenicity has focused on the S1 subunit of the spike glycoprotein (see figure 3). Parts of the S1 subunit contain hypervariable regions of amino acid sequences, which define the particular IB virus type (genotype). These hypervariable regions have also been shown to include the "receptor binding domain" - the part of the S1 protein that allows the IB virus to attach to the host tissues (Promkuntod et al, 2013). Those hypervariable regions within S1 are antigenic and stimulate a protective antibody response in the chicken, but the level of antigenicity can vary among proteins.

Figure 3 IB Spike Glycoprotein



There are several predictors of protein antigenicity including the presence or absence of specific amino acids, the sequence of those amino acids and how those amino acids influence protein folding (Jameson-Wolf Antigenic Index, 1988*).

Four specific amino acid positions in the mapped sequence of the hypervariable region of the S1 subunit are of particular importance to receptor binding: N38, H43, P63 and T69. Although the entire hypervariable region (N-terminal comprising 253 amino acids) was involved in host cell binding, the mutation of any of these four amino acids prevented binding.

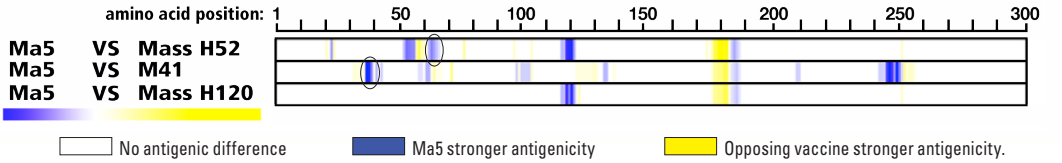
Dr. Mark Jackwood and Dr. Brian Jordan at the Poultry Diagnostic and Research Center, Athens, GA, have used the Jameson-Wolf index to compare the antigenicity of **Mildvac®-Ma5** to three other prominent IB vaccines (Figure 4). In each vaccine comparison band, the blue lines indicate a predicted superior antigenicity for **Mildvac®-Ma5**. White color means the vaccines are predicted to have the same antigenicity. Yellow lines indicate the comparison vaccine has a predicted superior antigenicity at that site. The darker the line, the greater the antigenic differences between compared vaccines.

The most critical difference in this plot comparison is for **Mildvac®-Ma5** vs. M41: the dark blue lines falling between positions 37 and 43 (circled) incorporate critical antigen position N38 and H43, both of which are key positions for receptor binding. In the comparison of **Mildvac®-Ma5** vs. Mass H52, a lighter blue line is present at position 62 to 66 (circled), with P63 as the greatest difference (darkest) in this band, showing a region of stronger antigenicity for **Mildvac®-Ma5**. Additional dark blue lines outside the key amino acid positions can also be observed in each of the comparisons, likely contributing to the overall antigenicity of **Mildvac®-Ma5**.

Virus replication and antigenic differences provides data demonstrating why these three genetically similar Mass-type vaccines may provide different levels of protection against a non-homologous strain. **Mildvac®-Ma5** not only replicates rapidly, it demonstrates superior antigenicity at several key amino acid positions.

* The Jameson-Wolf Antigenic Index (1988) is an algorithm that predicts the surface accessibility of amino acids: it provides a topographical map of the protein structure. Greater accessibility is equated to greater antigenicity: a better ability to stimulate a specific immune response.

Figure 4 Predicted antigenicity of **Mildvac®-Ma5** vs. H 52, M41 or Mass H120 for the N-terminal 253 amino acids associated with host tissue binding.



REFERENCES

Promkuntod, N., R.E.W. van Eijndhoven, G. de Vrieze, A. Gröne, M.H. Verheije (2014) Mapping of the receptor binding domain and amino acids critical for attachment in the spike protein of avian coronavirus infectious bronchitis virus. Virology 448 (26-32)

Jameson, B.A. and H. Wolf. (1988) The antigenicity index: a novel algorithm for predicting antigenic determinants. Cabios. 4 (1) 181-186

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