Standard Diagnostic Procedures for Post-Vaccination Monitoring of Kemin Vaccines: AIV, NDV, IBV

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INTRODUCTION AND OBJECTIVE

Diagnostic tools are relevant to understand epidemiology dynamics on farms all over the world, by enabling detection and surveillance of those pathogens compromising welfare and productivity in the susceptible population leading to a significant economic impact.[1,2]

Beyond that, diagnostic tools and procedures can also allow us to effectively monitor the vaccine uptake in the immunized animals, thus increasing staff confidence and peace of mind. The aim of this technical review is to outline the standard diagnostic procedures implemented in the field to enable post-vaccination monitoring of Kemin vaccines containing Avian Influenza virus (AIV), Newcastle Disease virus (NDV) and Infectious Bronchitis virus (IBV).

FUNDAMENTALS OF VACCINE MONITORING

a. Proper vaccination

Correct application, storage and response can be subsequently assessed thanks to responses the vaccine elicits in the animals. This immune response would depend on the vaccine and tests that are available, as well as what antigen we are assessing, as some antigens will be able to be assessed by specific tests whilst others won't. For guidance on good vaccination practice (GVP) please refer to Kemin specific procedures applicable to hatchery or farm vaccination.

b. Sample collection

It is important for the bird samples to be correctly identified in the submission form and individually in the sample vial if appropriate. Adequate sample identification should include at least:

a. Flock identification and location
b. Owner contact information
c. Number birds in flock / shed
d. Number samples
e. Age of the flock
f. Type of production (layers, breeders, broilers, turkeys)
g. Genetics
h. Date of sample collection
i. Vaccination program
j. Flock history (health problems, previous outbreaks).

c. Laboratory tests

Procedures available to monitor vaccination with viral poultry vaccines include:

- Molecular tests: Intended to find either the antigens or evidence of their presence.

- Serological tests: Intended to detect antibodies produced against a specific antigen. Serological titers may take 4 to 20 days to be detected, evidencing the response of the animal to either contact with a certain antigen or vaccine. For serological studies, the samples should be blood samples collected by a qualified person (in most countries, it is a legal requirement for this to be carried out by a veterinarian surgeon).

d. Schedule

a. Breeders: 20 blood samples can be collected at 10 to 12 weeks of age, at the time of transfer to the production farm and then every 12 weeks afterwards.
b. Layers: one sampling of 20 blood samples can be collected prior to transfer to the lay farm, followed by samples collected every 12 weeks7. If samples are being collected to assess vaccine response, more frequent sampling may be needed, such as 3 weeks after the vaccination and then every 4 weeks, throughout the production cycle of the flock.
c. Broilers: one sampling of 20 blood samples can be collected 2 or 3 weeks post-vaccination, prior the transfer to the processing plant.

AVIAN INFLUENZA

When it comes to Avian influenza, diagnostic procedures also assist us to either detect field infection (pathogen surveillance) or vaccine antigen (vaccine monitoring).

a. Surveillance (field infection)

To assess if a vaccinated flock has been infected, sentinel birds must be left unvaccinated. Serum samples can be collected or swabs (cloacal and tracheal) in the case of clinical signs that can be attributed to Avian Influenza virus (AIV). In this context, the following is needed:

- Sentinel birds (non-vaccinated birds easily identified)
- Tracheal or cloacal swabs (or tissues) of sick animals if clinical signs are present in the flock that can be suspected and attributable to Avian Influenza. At least 15 swabs should be taken.
- Serum samples from sentinel birds to assess if the vaccinated flock was infected during the production cycle. At least 15 swabs should be taken.

b. Vaccine monitoring (vaccination uptake)

Intended to assess the protection level granted by the vaccine and verify that vaccination was carried out correctly.

b.1) Sampling: To assess vaccine uptake, correct vaccination and the immunity status of the flock (protected levels in case of a field challenge) serum samples are used. At least 15 samples should be taken from birds in different areas of the animal house and a higher number of samples may be needed depending on population size. For HI, the gold standard sampling is carried out with antigen specific to the vaccine used and sourced from Kemin, always ensuring that the antigen used in the HI test is compatible with the vaccine antigen. ELISA specific for Avian Influenza can also be used to assess if birds were correctly vaccinated but not if the birds are protected. To assess if birds were properly vaccinated, for both ELISA and HI, at least 80% of the birds should have seroconverted

(>80% positive samples). With ELISA, only vaccination compliance can be assessed, and >80 % of the animals must have seroconverted following vaccination.

b.2) Schedule: To assess the vaccination efficacy and protection, we should sample birds at least 3 weeks after vaccination expecting that seroconversion should be over 5 Log2 on HI. To ensure protection levels are maintained throughout the production cycle, HI test must be used and serum samples must be taken randomly from different birds in different points of the animal house. At least 15 samples should be taken but higher numbers may be needed. The schedule for sampling varies according to the production cycle:

- Breeders: at 35 and 45 weeks of age.
- Layers: at 35, 50, 60, 70, 80, 90 weeks of age.
- Broilers: at 21 days of age.

b.3) Thresholds: The titers recommended for adequate protection and recommendations on correct vaccination are based on experimental and field evidence, as outlined in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2012).1 A summary of the serological analysis carried out with HI (gold standard) can be seen on table 1.

Testing Method	Suitable Sample	Sample Size	Sampling Schedule	Critical Success Factors
Serology (e.g. HI titers)	Serum	>15 (depends on population and objective)	At least 3 weeks post vaccination. Follow up during production: • Breeders: at 35 and 45 weeks • Layers: at 35, 50, 60, 70, 80, 90 weeks of age	>80 % positive samples > 5 Log_2 on HI using specific antigen supplied by Kemin

Table 1: Summary of serological analysis with HI to assess vaccination compliance and protection

c. DIVA (differentiation from infected animals)

Viral RNA can be detected by PCR-based assays (gel-based reverse transcription–polymerase chain reaction (RT-PCR). Through regular PCR testing it is possible to characterize fragments of the genome in field viruses, thus enabling differentiation between infected animals from vaccinated animals. When the genetic material from the field virus is sufficiently available in sediment specimens, the positive PCR result will clearly indicate the presence of the field virus (positive result). The difference in genomic sequence therefore indicates whether the PCR result relates to the vaccine strain or the field strain.[3]

NEWCASTLE DISEASE

To monitor effective Newcastle disease (NDV) vaccination, it is possible to assess the antibody titer (serology) response that is considered protective. Unlike Avian Influenza, demonstration of freedom of natural infection is not important, as the virus is ubiquitous and widespread in all poultry producing countries. To assess the vaccine response and vaccination compliance, ELISA tests and Hemagglutination Inhibition (HI) antibody titers can be used. For both serology testing methods, knowledge of the history, vaccines used and regional epidemiology are key. The tests mentioned below for assessment of vaccination practices are valid for both live and inactivated NDV vaccines.

I. Enzyme-linked Immunosorbent Assay (ELISA)

To properly interpret the ELISA results, specific guidelines must be sought from the laboratories that produce the ELISA test kits, which consistently outline the threshold expected for each type of vaccine (live or inactivated), distinguishing from a field infection. The partner laboratory carrying out the test often also has the guidelines to interpret the results.

II. Hemagglutination Inhibition (HI)

The vaccine antigen should be used to carry out the test, and the samples to assess the response to vaccination should be collected at least 21 days after vaccination. Knowledge of the local NDV genotypes and strains is also important for the HI. For the HI test, the mean antibody titer should be above 9 $\log_2$ titer with the specific vaccine antigen and these should have a mean antibody titer measured by HI, using the specific vaccine antigen of 9 $\log_2$ titer.

A summary of the serological tests that can be carried out to monitor NDV vaccine antigens can be seen on table 2. Key considerations include:

a. Sampling

The samples needed for the serological tests are serum samples (blood samples). 25 to 30 samples should be sufficient to assess vaccination protection and also vaccination compliance. To demonstrate seroconversion post vaccination, paired samples can be collected a few weeks apart and analyzed.

		Testing Method	Suitable Samples	Sample Size	Sampling Schedule	Critical Success Factors
INACTIVATED or LIVE	1	Serology (e.g. **ELISA IgG titers**)	Serum	25-30 chickens	• Onset of lay • Peak of lay • After 60 weeks of age	• Sampling procedure • Baselines conditioned to commercial ELISA kits
	2	Serology (e.g. **HI titers**)	Serum	25-30 chickens	• Onset of lay • Peak of lay • After 60 weeks of age	• >80% positive • 9 $\log_2$ titer

Table 2: Summary of serological analysis with HI to assess vaccination compliance and protection

b. Schedule

The sampling times recommended to assess the immunity level granted by vaccination are:

- Breeders: at Onset of lay, Peak of lay and 60 weeks of age
- Layers: at Onset of lay, Peak of lay, 60 weeks and 90 weeks of age
- Broilers: at 21 days of age.

INFECTIOUS BRONCHITIS

For monitoring the proper vaccination with Infectious Bronchitis virus (IBV) vaccine, serology can be considered the gold standard testing method. Using this method, serum must be collected of at least 25 birds. As with Newcastle Disease, due to circulation of different wild IBV strains that can result in cross serological results in vaccinated chickens, knowing vaccination history, circulating IBV variants and pathogen surveillance scheme is key to balance the diagnostic. The seroconversion should be assessed mainly following the Inactivated vaccination, as the live vaccines may not lead to strong humoral immunity.

a. ELISA test

Commercial ELISA kits are often used for monitoring serum antibody responses derived from IBV vaccines. The antigens used in the kits are broadly cross-reactive among serotypes and allow for general serological monitoring of vaccinal responses and field challenges.

b. HI test

Method used for identifying serotype-specific responses to vaccination and field challenges especially in young growing chickens. Due to the co-circulation of different IBV genotypes or lineages in the same region, seroconversion with HI titers need to be analyzed with care and support of your veterinarian. Seroconversion to the vaccination can be monitored only if paired samples are collected a few weeks apart and analyzed either by a suitable commercial ELISA or HI test.

If the serological test chosen is HI, the specific vaccine antigen should be used. In contrast, if commercial ELISA kits are used, the guide for interpretation of the results must be obtained directly from the manufacturer of the commercial ELISA kit, in case of not being available at the laboratory carrying out the test.

A summary of the serological tests that can be carried out to monitor IBV vaccine antigens can be seen on table 3. Key considerations include:

c. Sampling

The samples needed for the serological tests are serum samples (blood samples). 25 to 30 samples should be sufficient to assess vaccination protection and also vaccination compliance. To demonstrate seroconversion post vaccination, paired samples can be collected a few weeks apart and analyzed.

d. Schedule

For serology blood samples must be collected and as before, it is important to remember that collection of samples too soon after vaccination or infection may lead to negative results. To assess if seroconversion occurred due to vaccination or field infection paired serology should be carried out to ascertain the antibody titers are rising:

- Breeders: at Onset of lay, Peak of lay and 60 weeks of age
- Layers: at Onset of lay, Peak of lay, 60 weeks and 90 weeks of age
- Broilers: at 21 days of age.

Given that IBV does not spontaneously agglutinate in chicken red blood cells, samples need to be treated with the enzyme neuraminidase before it can be used in the HI test.[2]

For serology, blood samples can be utilized to assess antibody response, for this ELISA kits are commercially available but HI can also be used and allows for serotype specific responses and assessment of vaccination (this can be difficult to do clearly in the field due to multiple infections of vaccine and field strains). 19, 20

Even though correlation with protection can be difficult in many cases we should expect a mean HI titer greater than 6.0 $\log_2$ to be a protective threshold. Due to the complexity of respiratory disease and conditions that may lead to reduced egg production and nephropathy, elimination of other diseases is needed when diagnosing IB.

ELISA tests have the advantage of being able to detect the serological response of the bird against all types of IBV infections while HI is more specific and allows to find specific serotype responses if the birds are exposed to particular serotypes in the field or from vaccination. When using ELISA tests, it is important to know what the cut off values are for the test used to enable proper interpretation.

		Testing Method	Suitable Sample	Sampling Size	Sampling Schedule	Critical Success Factor
INACTIVATED or LIVE	1	Serology (e.g. **ELISA IgG titers**)	Serum	25-30 chickens	• Paired serology at 3 weeks and 6 weeks post vacccination	• Baselines conditioned to commercial ELISA kits • NA > 80% positive
	2	Serology (e.g. **HI titers**) for IB Variant antigens	Serum	25-30 chickens	• Paired serology at 3 weeks and 6 weeks post vacccination	• >6 Log_2 • >80% positive (must be interpreted with Kemin technical advisor as circulating strains may impact)

Table 3: Summary of the serological tests that can be carried out to assess vaccination for IBV

CONCLUSION

Systematic post vaccination assessment allows poultry veterinarians to determine if the vaccination procedure was carried out correctly. With viral vaccines, assessing the number of birds that seroconverted to vaccination can be associated with immunity when a correlation between the antibody titers and protection is known. Knowing the standard diagnostic procedures to monitor Kemin Vaccines is of paramount importance because i) verifies the "good vaccination practice" for a particular flock, ii) helps the producer to understand the actual farm vaccination status and iv) supports the veterinarians in their decision-making process when upgrading vaccination programs.

References

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