DEVELOPMENT OF SAFE AND EFFICIENT POULTRY VACCINES: A LONG TERM COLLABORATION BETWEEN NPP “AVIVAC” AND SEPPIC

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NPP “AVIVAC” and SEPPIC have built over the years a long-term relationship to develop and produce new safe, stable and efficient adjuvanted poultry vaccines. This fruitful collaboration has led to the launch of NPP “AVIVAC” vaccines that confer a high protection to avian species against diverse diseases.

SEPPIC is a French company which is a recognized actor in the fields of polymers, surfactants, emulsion technologies for the cosmetics and pharmaceutical industry. SEPPIC is a global leader in veterinary vaccine adjuvants and a key player in injectable excipients, solubilizers and active ingredient carriers. SEPPIC produces the Montanide™ range of veterinary adjuvants which has shown to be highly efficient in multiple animal species.

Vaccine adjuvants are added to vaccine formulations for multiple purposes, such as increasing the protection given to animals by veterinary vaccines, enhancing the protection duration of the vaccine, reducing the number of injections needed, reducing the antigen load in the vaccine, or orienting the immune response conferred by the vaccine. Diverse adjuvants technologies for veterinary vaccines have been developed in the last decades [1].

Water-in-mineral-oil (W/O) emulsion adjuvants are extensively used for the formulation of inactivated avian vaccines. These strong adjuvants induce long-term efficacy of poultry vaccines. They consist of a mix of a specific mineral oil of injectable grade and a surfactant system to stabilize the vaccine emulsion. It should be noted that a specific care has to be given to the quality of surfactants as well as to the nature of the oil to control safety issues [2]. The Montanide™ range of veterinary adjuvants include emulsion, micro-emulsion and polymeric adjuvants and have been used extensively for prophylactic veterinary vaccination in farm and companion animals [3]. Montanide™ W/O adjuvants for poultry are ready to use adjuvants that induce long lasting protection in chickens and other avian species.

As part of NPP “AVIVAC” and SEPPIC on-going collaboration, different trials have been performed in the last 3 years: Optimization of vaccine manufacturing processes at industrial scale, optimization of vaccine formulations using innovative adjuvants, safety and immunological performance tests in chickens.

1. Optimization of vaccine emulsification industrial process:

Vaccine formulation

In order to confer a long term protection to poultry, NPP AVIVAC manufactures specific vaccines with SEPPIC adjuvant Montanide™ ISA 70 VG. This highly efficient adjuvant forms water in oil emulsions, with a homogenous repartition of antigen droplets in an oil phase. This improves the efficacy of the vaccine by providing a depot effect of the oil at the injection site and allows slower antigen release for a longer immune response.

Water in oil emulsion manufacture

In order to manufacture water in oil vaccines (Figure 1), emulsification process requires a strong energy to ensure a small size of antigen droplet and long term stability of the vaccine. This high shear energy is provided by a rotor stator mixer, which consists of a central rotating part (rotor) and a static ring surrounding the rotor (stator). After creating large droplets of antigen in the adjuvant (~10-20µm wide), the pre-emulsion is fed in a rotor stator mixer by pumping effect. Centrifugal force is then propelling the antigen droplets at very high speed towards the rotor periphery. At the narrow gap between rotor and
stator, and through the stator perforations, the droplets undergo a sudden drop of velocity and high shear stress that splits them into smaller droplets (Figure 2). A repetition of this cycle will help to reach a droplet size that enables good vaccine stability (~1µm).

Figure 1: Emulsion manufacture theory

**Process optimisation**
To guaranty the best vaccine quality, NPP AVIVAC and SEPPIC performed a process optimisation for optimal vaccine stability and efficacy. Several parameters were assessed: Speed, gap size, process time and process type. The target was to achieve a droplet size allowing for good stability (Table 1).

<table>
<thead>
<tr>
<th>Process</th>
<th>Droplet size (D_{[0.9]})</th>
<th>Granulometry Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inefficient process</td>
<td>15µm</td>
<td><img src="granulometry1.png" alt="Granulometry" /></td>
</tr>
<tr>
<td>Partly efficient process</td>
<td>4µm</td>
<td><img src="granulometry2.png" alt="Granulometry" /></td>
</tr>
<tr>
<td>Efficient industrial process</td>
<td>1µm</td>
<td><img src="granulometry3.png" alt="Granulometry" /></td>
</tr>
<tr>
<td>Laboratory scale control</td>
<td>1µm</td>
<td><img src="granulometry4.png" alt="Granulometry" /></td>
</tr>
</tbody>
</table>

Table 1: Optimization of vaccine emulsification process: droplet size

**Vaccine control**
Quality of the process is checked by a galenic control of the vaccines (viscosity, droplet size and resistance to centrifugation at 2570G). A stability program at 4°C, 20°C and 37°C follows the aspect of the vaccines for up to two years. Oil layer and sedimentation are non-critical defects, as hand shaking before injection gives back the initial homogenous vaccine. Breakage (water layer at the bottom) is a non reversible critical defect of the vaccine: high shear energy is necessary to go back small droplets, hand shaking is not sufficient, so broken vaccines should not be injected.

Results: Optimized process
SEPPIC and NPP AVIVAC collaborated to optimize the vaccine production industrial process. A batch of inactivated Newcastle Disease vaccine was manufactured at 115l scale by recycling process on a Silverson 275LS at 3000rpm with Montanide™ ISA 70 VG. A longer process helped to reduce the
antigen droplet size (Table 1). After optimization of the process the vaccine shows no critical defects and has more than one year of stability at 4°C.

Avivac conducted a vaccination trial with the process optimized batch of ND vaccine.
- **Safety** was assessed by observation at injection site after 2ml dose injection. No moderate local reactions were observed.
- **Efficacy** was assessed by vaccinating ten 80day-old-chickens with 0.5ml dose by subcutaneous injection in the middle third of the neck. Negative control group was not vaccinated. The chickens were kept for 28 days in isolated cages. Blood samples were taken at day 0 and day 21 after vaccination. Specific antibody titers against inactive NDV were measured by HI test. This batch of ND vaccine induced very high antibody titer level against NDV in chickens at day 21 (Table 2).

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Days after vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND Montanide ISA 70 VG</td>
<td>D+0: 6.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>D+21: 13.6 ± 0.8</td>
</tr>
<tr>
<td>Control (not vaccinated)</td>
<td>D+0: 5.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>D+21: 5.6 ± 0.7</td>
</tr>
</tbody>
</table>

Table 2 : HI antibody titers after vaccination (log2)

**Conclusion**

Throughout vaccine development, process optimization and routine control of the produced commercial vaccines, Avivac ensures to have robust and reproducible vaccine manufacture processes, in order to provide consistently efficient and safe vaccines.

2. Development of Newcastle disease vaccines using a new robust adjuvant:

Chickens receive multiple vaccinations during their life-time. Multivalent vaccines containing multiple antigens are therefore used frequently in the field to limit the number of injections. These vaccines can induce protection against diverse strains of the same pathogen, or against multiple pathogens. Multivalent antigenic media are complex media that contain multiple biological components that can influence the stability of vaccine emulsions. Evolution and destabilization of vaccine emulsions during storage have indeed been observed and can be linked to high concentration of enzymes such as lipases and esterases in antigenic media, and to stressing storage conditions. Robust formulations are therefore needed to ensure stability of emulsions even in destabilizing conditions, and with flexible ratio of oil and antigenic media in the vaccine. These features are therefore especially important for the formulation of multivalent vaccines.

Montanide™ ISA 71R VG is an innovative emulsion adjuvant dedicated to poultry vaccines that has been developed by SEPPIC to resist to destabilizing antigenic media and storage conditions. A collaborative study performed by SEPPIC and NPP “AVIVAC” showed that Montanide™ ISA 71R VG allows the formulation of safe and efficient inactivated vaccines against Newcastle disease.

Inactivated Newcastle disease virus (NDV, La Sota strain, produced by NPP Avivac) was formulated either with reference W/O adjuvant Montanide™ ISA 70 VG (used at 70%), with robust adjuvant Montanide™ ISA 71R VG (used at 60%) or in PBS. All vaccines contained the same amount of antigen. Formulation with Montanide™ adjuvants was performed at high shear using a IKA 25T mixer and following SEPPIC’s recommendations. 80 1-day old chickens (Hysex white) were randomly separated in 4 groups of 20 animals, and received at day 0 subcutaneously in the neck 0.1ml of the following vaccines:

- **Group 1- ISA 71R**: NDV Antigen + Montanide™ ISA 71R VG (60% adjuvant / 40% aqueous, weight/weight)
- **Group 2- ISA 70**: NDV Antigen + Montanide™ ISA 70 VG (70% adjuvant / 30% aqueous, weight/weight)
- **Group 3- No adjuvant**: NDV antigen + PBS, No adjuvant
- **Group 4- Not vaccinated**
All birds were slaughtered at D21, and local reactions at the injection site were assessed at slaughter. Blood samples were taken at D14 and D21, and antibody titers in blood serum were assessed by hemagglutination inhibition test (HI).

In adjuvanted groups, small granulomas (smaller than 2mm, Figure 4) were observed in some animals. This type of local reactions is minor and acceptable for poultry production. No significant difference was observed between robust and reference adjuvants. These results show that all tested vaccines were safe in chickens.

![Figure 4](image)

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Frequency of acceptable local reactions at slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISA 71R VG Resisting W/O</td>
<td>13/20</td>
</tr>
<tr>
<td>ISA 70 VG Reference W/O</td>
<td>11/20</td>
</tr>
<tr>
<td>No adjuvant</td>
<td>0/20</td>
</tr>
</tbody>
</table>

**Figure 4:** a/ Frequency of local reactions observed in chickens. b/ Representative local reaction observed with adjuvanted vaccines.

Specific antibody titers against inactivated NDV were measured by HI titration. Results are presented in Figure 5. Maternal immunity was still detectable at D14 post vaccination but had disappeared at D21. At D21, significantly higher titers were observed in groups vaccinated with adjuvanted vaccines, compared to non adjuvanted vaccinated birds or non vaccinated birds. Both adjuvants induced comparable antibody titers. It should be noted that W/O reference adjuvant Montanide™ ISA 70 VG is used at 70% in the vaccine, whereas Montanide™ ISA 71R VG is used only at 60% in this trial.

![Figure 5](image)

**Figure 5:** HI antibody titers after NDV vaccination

These results show that this resisting adjuvant can be used to develop robust, safe and efficient Newcastle Disease vaccines for poultry. Results of this collaborative trial have been presented in the SEPPIC symposium during World Veterinary Poultry Association congress in Nantes, France, in 2013.

**References**
