SCIENTIFIC DISCUSSION

Product name:	Nobilis IB 4-91
Procedure No.:	EMEA/V/C/036
Applicant company :	Intervet International B.V., Wim de Körverstraat 35, 5831 AN Boxmeer, The Netherlands
Active substances and strengths: **(ATCvet code)**	Live attenuated avian Infectious Bronchitis virus variant strain 4-91 (QI01AD07)
Proposed International Non-proprietary Name:	N/a
Pharmaceutical form:	Lyophilised vaccine pellet for reconstitution.
Strength	At least 3.6 $\log_{10}$ EID_{50} per dose. (EID_{50} = 50% Embryo Infective Dose)
Presentation:	10 ml glass vial containing 500, 1000, 2500, 5000 or 10000 doses of lyophilised vaccine.
Package size:	10 ml glass vial; 1 or 10 vials per pack
Target species:	Chickens.
Withdrawal period:	Zero days
Routes and method of administration:	Respiratory, oral, intranasal/ocular by spray, in the drinking water or by dropper.
Product type:	Immunological
Therapeutic indication:	Active immunisation of chickens to reduce the respiratory signs of Infectious Bronchitis caused by the variant strain IB4-91.

SCIENTIFIC DISCUSSION

1. INTRODUCTION

Nobilis IB 4-91 is a live attenuated viral vaccine containing Infectious Bronchitis virus variant strain 4-91 (793B) for the active immunisation of chickens to reduce the respiratory signs of disease caused by the variant strain IB 4-91 (793B). It is presented as a freeze-dried pellet for reconstitution for intranasal/ocular use, spray or administration via the drinking water.

Each vial contains at least 3.6 $\log_{10}EID_{50}$ of live attenuated avian Infectious Bronchitis Virus variant strain 4-91 in stabiliser. The freeze-dried pellet is presented in 10 ml glass vials of Type II hydrolytic glass containing 500, 1000, 2500, 5000, or 10000 doses.

Nobilis IB4-91 qualifies for the centralised system under Part B of the Annex to Council Regulation (EEC) No. 2309/93 as it contains a new variant which was not authorised for use by any Member State for use in food-producing animals and complies with the definition of a new active substance in accordance with the CVMP position paper. A Provisional Marketing Authorisation had, however, been issued by the Veterinary Medicines Directorate in the UK and an Authorisation for Temporary Usage had been granted by the Agence Nationale du Médicament Vétérinaire .

2. OVERVIEW OF PART II OF THE DOSSIER: QUALITY ASPECTS

2.1 QUALTATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

The product contains*:*

Active Component:

For 1 dose:
Live Infectious Bronchitis virus variant strain 4-91, at least 3.6 $\log_{10}EID_{50}$

Container:

The containers are of Type II hydrolytic glass with bungs made of halogenobutyl rubber and sealed with a tear-off coded aluminium cap.

Product Development Studies

In recent years, outbreaks of IB have been seen in flocks vaccinated with the Massachusetts strain of IB virus (IBV). Isolates of IBV from these outbreaks were found to be serologically related but distinct from the 30 strains previously described. One of these isolates, IB4-91, was used for the development of this vaccine. The report of a survey performed in 1992/3 to determine the incidence of neutralising antibodies to IB due to IBV variant strain 4-91 in UK breeder and commercial layer flocks showed that, of the 25 flocks sampled, over 80% had antibodies to this serotype. IB due to IBV variant strain 4-91 has also been described in France, the Netherlands, Italy and Belgium. Specific neutralising antibodies against IB 4-91 in sera have also been demonstrated in broiler, breeder and layer flocks in other Member States, such as Spain, Germany and Greece.

2.2 METHOD OF PREPARATION

SPF eggs are inoculated with seed virus and incubated and the allantoic fluid harvested. The stabiliser is prepared and added to the harvest material. The product is then filled and freeze-dried. The freeze-dried vials of final product are stored at - 20 ^{o}C.

2.3 CONTROL OF STARTING MATERIALS

The skimmed milk powder used in the batches of vaccine is selected from batches of powder which are produced in the Netherlands.

The skimmed milk powder is heat treated at 100°C for 1 minute. The tryptose is obtained from selected suppliers. The species of origin is listed as being both bovine and non-bovine. Other starting materials of animal origin are obtained from selected suppliers. Bovine material is sourced from New Zealand and non-bovine material from the USA and is in compliance with the recent Commission Decision 97/534/EC and with CVMP Guidelines. Sterilisation is performed by Intervet by autoclaving at 121°C for 30 minutes.

2.4 CONTROL AT INTERMEDIATE STAGES OF MANUFACTURE

Before use in the finished product each aliquot is tested for its bioburden. Limits of acceptance are "no organisms detected after a minimum of 24 hours incubation". Checks on fill volume and the monitoring of the freeze-drying process are performed. The in-process results for three consecutive batches of product have been presented.

2.5 CONTROL OF THE FINISHED PRODUCT

Safety testing and virus titration are carried out according to the European Pharmacopoeia (Ph. Eur.) monograph “freeze-dried avian infectious bronchitis live vaccine- 442”. The vaccine must contain at least 3.6 $\log_{10}$ EID_{50} per bird dose at release.

The test for extraneous agents in chicks (V.2.1.3.5) is performed as required by the Ph. Eur. monograph (442). The Company are also to carry out the three extraneous agent tests which are described in this monograph on at least the first ten batches. The potency test described in the Ph Eur monograph (442) has been omitted from the routine finished product tests. This test has been carried out on prototype batch MSV+5 (batch 001).

A test for residual moisture is carried out using the Karl Fischer method with the specification that the residual moisture may not exceed 5%. Before shipment all vials must pass the vacuum test and must have the right code. The full batch protocols for three batches of finished product have been presented.

2.6 STABILITY

Stability of the freeze-dried product

The stability data for one batch only supports a shelf-life of 12 months at –20 ºC followed by 12 months at
2 - 8 ºC. Until such time as further stability data is provided, the shelf-life will be 9 months at 2 – 8 ºC (following 12 months storage at –20 ºC).

Stability of the reconstituted product

Vaccine stability is poor in tap water alone but improves if 0.2% skimmed milk powder is added to the diluent. The study was adequate to support an in-use shelf-life of two hours when reconstituted in tap water plus 0.2% skimmed milk powder. Advice to this effect has been placed on the SPC and product literature regarding the incorporation of skimmed milk powder during the reconstitution of the vaccine.

FOLLOW-UP MEASURES - QUALITY

The Applicant has indicated the modifications which are required to certain of the SOPs for the tests performed on starting materials and final batch tests to the satisfaction of the CVMP. All three suppliers of SPF eggs are in conformity with the Ph Eur. providing that the AGP test for adenovirus is replaced by an ELISA or IFT test. The Applicant has given an undertaking to do so.

The Applicant has agreed to carry out the tests for extraneous agents specified in the Ph. Eur. which include the extraneous agent test in chicks including the tests for antibodies to Avian Leukosis Virus and Avian Influenza A. Each batch of finished product will also be tested for, in addition, Avian Leukosis Virus as this is the only test which will detect contamination with the Subgroup J virus.

The Applicant will provide the results of the extraneous agent testing on the first ten batches of products to be marketed, for consideration by the CVMP.

3. OVERVIEW OF PART III OF THE DOSSIER: TOXICOLOGICAL AND PHARMACOLOGIAL ASPECTS

3.1 SAFETY

Safety data have been presented for the recommendations for use currently proposed for the product. These data show the product to be safe but that the virus will spread. Compatibility has been shown between this product and vaccines containing Gumboro strains D78 and 228E, IBV strains Ma5 and H120 and Newcastle disease virus Clone 30. There may, however, be an incompatibility between this product and Turkey Rhinotracheitis (TRT) vaccines if given within 7 days of each other. Some evidence has been presented with respect to the safety of this product in laying hens and chicks that subsequently become layers but this is insufficient to demonstrate the safety of this product in layers. Appropriate wording has therefore been included in the SPC to state that the vaccine is not to be used in future layers and breeders and chickens in lay.

The dose per bird is stated on the SPC as at least 3.6 $\log_{10}$ EID_{50} as measured by ELISA. This corresponds to the 3.0 $\log_{10}$ EID_{50} as read by the macroscopic read out method quoted in the safety studies.

<u>LABORATORY TESTS</u>

3.1.1 Safety of the Repeated Administration of One Dose

The results presented show that the product is safe when given at maximum release titre to chickens of day-old by eyedrop or coarse spray and to chickens of seven days-old in the drinking water.

3.1.2 Safety of One Administration of an Overdose

The results showed that just less than 10 times the recommended dose of IB 4-91 MSV+1 (6.0 $\log_{10}$ EID_{50}) was safe when administered to day-old chicks by coarse spray and that just less than 100 times the maximum recommended dose (6.9 $\log_{10}$ EID_{50}) was safe when administered to 7-day old chicks via the drinking water. Administration of the vaccine at up to 20 times the maximum recommended dose by eyedrop to day-old chicks was shown to be safe.

3.1.3 Safety of the Repeated Administration of One Dose

The results showed that two administrations of the maximum recommended dose by any of the recommended routes of administration were safe in chicks in which the first dose was given at day-old.

3.1.4 Examination of Reproductive Performance

Two studies were presented. Both studies provide some evidence that the product may be safe for use in layers but the first study included chickens that had been used in a previous TRT challenge study and neither study included appropriate controls. Consequently the contra-indication for use in future layers and breeders and chickens in lay has been made in the SPC and the product literature.

3.1.5 Examination of Immunological Functions

The company stated that IB is not known to affect any of the immunological functions in chickens. The company have also performed studies to demonstrate that there was no interference observed when chicks were vaccinated with vaccines containing Gumboro strains D78 and 228E, and IBV strains Ma5, H120 and Newcastle disease virus Clone 30.

3.2 FIELD STUDIES

Combined safety and efficacy field trials have been conducted in the UK and France. These are discussed under Efficacy.

3.3 SPECIAL REQUIREMENTS FOR LIVE VACCINES

3.3.1 Spread of the Vaccine Strain

From the overdose study provided it was possible to conclude that whichever route of vaccination was used the vaccine virus spreads to in-contact animals. An appropriate warning has been placed on the SPC and product literature.

3.3.2 Dissemination in the Vaccinated Animal

No studies have been performed but a brief general statement about dissemination of IBV has been made. There is no reason to believe that the strain of IBV in the vaccine should behave differently to other IBV strains. It is likely to be able to be recovered from the respiratory tract, the intestinal tract and the faeces for some time after vaccination.

3.3.3 Reversion to Virulence of Attenuated Vaccines

A reversion to virulence study has been carried out according to the requirements of the Ph Eur monograph. The results of the study compare favourably with those of the report of a similar study carried out by the same workers on the H120 vaccine strain suggesting that the risks of reversion to virulence of the current product are comparable to those of products already on the market in the EU.

3.3.4 Biological Properties of the Vaccine Strain

(discussed under Part 2)

3.3.5 Recombination or Genomic Reassortment of Strains

IB is a single-stranded, non-segmented RNA virus and is, therefore, not subject to genomic reassortment. Since the virus is readily transmissible and fairly persistent in the environment recombination with wild strains is theoretically possible and cannot be ruled out. As the original virulent IB 4-91 variant strain has been identified in the EU and several countries throughout the world the vaccine does not pose any risk beyond that which is already present in the field.

3.3.6 Study of Residues

No specific studies have been performed but IBV is not zoonotic and there are no excipients of any significance in the vaccine. A withdrawal period of zero days is appropriate.

3.3.7 Interactions

Studies have been performed in which the safety and efficacy of Nobilis IB 4-91 administered alone or in combination with vaccines containing Gumboro strains D78 and 228E, IBV strains Ma5 and H120 and NDV Clone 30 were compared. Within the limits of the trials performed, the compatibility level between the different vaccines tested can be considered as acceptable. Several studies have been performed to assess the compatibility of IBV 4-91 with TRT vaccines. One study has shown that when Nobilis IB 4-91 is given at day-old it may adversely affect the efficacy of TRT vaccine when given within 7 days of the IB vaccine. An appropriate warning has been included on the SPC and product literature.

3.4 ECOTOXICITY

A phase 1 ecotoxicity assessment has been carried out and this indicates that no hazard arises from use of the product.

4. OVERVIEW OF PART IV OF THE DOSSIER: CLINICAL ASPECTS

Nobilis IB 4-91 is indicated for the vaccination of chickens against Infectious Bronchitis virus variant strain IB 4-91. The vaccine is recommended for administration to chicks (day-old and older) by coarse spray or by the intranasal/ocular route and to chicks of 7 days and older via the drinking water. Advice is given in the SPC to re-vaccinate every 6 weeks after the initial administration

Efficacy data has been submitted to support the claims being made for the product in the SPC and product literature provided by the applicant and a marketing authorisation can be issued on the efficacy data provided. Data have been provided to show a duration of immunity of up to 6 weeks following vaccination of day-old chicks. Such a duration of immunity would be sufficient to protect broilers for life.

A general statement is provided regarding the choice of the minimum effective dose. The dose of 3.0 $\log_{10}$ EID_{50} by macroscopical read out, quoted in the study reports, corresponds to 3.6 $\log_{10}$ EID_{50} by the ELISA method, as used as the minimum limit of acceptance in the finished product tests.

4.1 LABORATORY STUDIES

4.1.1 Determination of the minimum protective dose

The minimum protective dose was determined for each of the routes of administration (eyedrop and spray to day-old chickens and by drinking water to 7-day old chickens). The experiments fulfilled the requirements of the Ph Eur monograph for Live IB vaccines except that the challenge dose used for the groups vaccinated by eyedrop and drinking water was much lower than intended. Nevertheless, unvaccinated controls showed high ciliostasis scores and were 100% positive by virus re-isolation.

4.1.2 Efficacy of Nobilis IB 4-91 against a French isolate of the 4-91 strain

The results showed that, despite low challenge doses, the two control groups both had high ciliostasis test scores following challenge with virulent IB 4-91 or the French isolate respectively whereas both groups of vaccinates were protected. The study showed that, at the minimum recommended dose, the vaccine protected against challenge with either virulent IB 4-91 or a French field isolate 3 weeks after vaccination.

4.1.3 Efficacy of Nobilis IB 4-91 vaccine against challenge with a Dutch isolate of IB 4-91.

The study showed that, at the minimum recommended dose, the vaccine protected against challenge with either virulent IB 4-91 or a Dutch field isolate 3 weeks after vaccination.

4.1.4 Efficacy of Nobilis IB 4-91 vaccine against a Massachusetts challenge

Two groups of 40 SPF chicks were vaccinated by eyedrop at day-old with either the IB 4-91 vaccine (3.5 $\log_{10}$ EID_{50} per bird) or a standard Ma5 vaccine (4.0 $\log_{10}EID_{50}$ per bird). A further group of twenty were kept as unvaccinated controls. All chickens were challenged 3 weeks later with IB M41 (2.6 $\log_{10}EID_{50}$ per bird) by eyedrop. Ciliostasis testing was carried out in half the chickens 4 days after challenge and at 6 days after challenge in the other half. The results of this study showed that chickens vaccinated with IB 4-91 were not fully protected against Ma5 challenge whereas complete protection was seen in those chickens vaccinated with M41.

4.1.5 Efficacy of the combination of IBV 4-91 vaccine and Massachusetts vaccines (H120 and Ma5) against a challenge with Italian strains of IB 4-91

In this report three groups of approximately 25 SPF chicks were vaccinated at day-old with an IB H120 vaccine by coarse spray (3.9 $\log_{10}$ EID_{50} per dose). Two of these groups were then revaccinated at 14 days old by coarse spray with either an Ma5 vaccine (2.9 $\log_{10}$ EID_{50} per dose) or IB 4-91 vaccine (2.8 $\log_{10}$ EID_{50} per dose). A fourth group was kept as unvaccinated controls. Chickens from each group were challenged at 5-6 weeks old with one of two Italian isolates of IB 4-91 (strain 710 at a titre of 4.5 $\log_{10}$ CD_{50} per dose or strain 2149 at a titre of 4.2 $\log_{10}$ CD_{50} per dose). There were 5 deaths during the study, none were attributable to vaccination. Antibody titres (ELISA) prior to challenge were presented. All vaccinated groups showed higher titres than the controls. The highest titres being seen in the group that received vaccination with H120 at day-old and then IB 4-91 at 14 days old.
Protection, as assessed by ciliostasis scores, was as follows:

Challenge strain	% Chickens Protected (in each group). vaccine		
	H120(day-old)	H120(day-old)+ Ma5(14 days)	H120(day-old)+4-91(14 days)
710	6/11= 54.5%	6/12 chickens = 50%	12/13 chickens = 92.3%
2149	8/11= 72.7%	8/11 chickens = 72.7%	12/12 chickens = 100%

The best protection against both strains was therefore seen in those groups of chickens given the H120, 4-91 combination of vaccines.

4.1.6 Duration of immunity: SPF chickens

A group of 8 SPF chicks were vaccinated at day-old by eyedrop with IB 4-91 vaccine (3.5 $\log_{10}EID_{50}$/bird). Ten chicks remained unvaccinated as controls. At 6 weeks after vaccination the vaccinates were bled and, together with the unvaccinated controls, were challenged by eyedrop with a dose of virulent IB 4-91 (1.9 $\log_{10}EID_{50}$/bird). Protection against challenge was assessed by the ciliostasis test on tracheal rings taken 5 days post challenge. Serum samples taken 3 weeks post vaccination showed that chickens had seroconverted with a mean HI titre of 8.3 $\log_2$. At the time of challenge (6 weeks post vaccination) the mean HI antibody titre of the 8 chickens tested was 8.5 $\log_2$ indicating that vaccinal antibody had persisted for at least 6 weeks. All of the chicks vaccinated 6 weeks earlier by eyedrop were protected (as assessed by the ciliostasis test) from the effects of challenge with virulent homologous virus. The experiment showed that both the serum antibody titre and the degree of protection are maintained for at least 6 weeks after vaccination by eyedrop with Nobilis IB 4-91 in SPF chickens.

4.1.7 Duration of immunity - protection of future breeders and layers

Two groups of two week old SPF chicks were kept in separate isolators and vaccinated by eyedrop with IB 4-91(MSV+5 batch number 001, titre 2.3 $\log_{10}EID_{50}$/bird by macroscopical read-out). At

eight weeks of age one of the two groups were re-vaccinated with IB 4-91 again by eyedrop (3.9 $\log_{10}$EID$_{50}$/bird as read by ELISA test). A third group were kept as unvaccinated controls (these were kept in a separate isolator at the same site as the vaccinates) and a fourth group (also unvaccinated controls) were held at a different site. Serological responses to vaccination were assessed at intervals by ELISA (using M41 as the coating antigen). At 16 weeks of age all of the chickens were challenged with virulent IB 4-91 (by eyedrop and intranasally at 2.9 $\log_{10}$EID$_{50}$/bird). Protection was assessed by ciliostasis scores at 5 and 7 days post challenge. Three chickens died during the study and one bird was culled. No deaths were attributed to IBV vaccination. Antibody titres of $\geq$9 $\log_2$ were seen in all vaccinates at 16 weeks of age and there was very little difference between the titres of the two vaccinated groups. A rise in antibody titre was seen in both groups of controls between 14-16 weeks of age.

The possibility of a challenge having occurred was investigated and the control chickens were all shown to be negative for IB antibodies (by an HI test using M41 as antigen) at 16 weeks of age. The vaccinates were also shown to be negative. The results of the challenge showed that 100% of the vaccinates and 0% of the controls were protected at 16 weeks of age.

4.1.8 Efficacy of Nobilis IB 4-91 in chickens with maternal immunity

From a study in commercial broiler chicks with high levels of maternally-derived IB 4-91 neutralising antibody (reciprocal neutralising antibody titres of 20, 35, 45, 55 and 130), it can be concluded that the vaccine can protect chickens with very high levels of maternally-derived immunity from challenge with virulent IB 4-91 three weeks post vaccination but studies did indicate that the protection seen in commercial chickens is less than that seen in SPF chickens.

4.1.9 Duration of immunity: chickens with maternal immunity

Commercial broilers with high levels of maternally-derived antibody were vaccinated at day-old with Nobilis IB 4-91 by spray (3.4 $\log_{10}$ EID$_{50}$ per bird) and then challenged along with a second group of ten unvaccinated control chicks 5 weeks later with virulent IB 4-91 (2.0 $\log_{10}$ EID$_{50}$ per bird). The study showed that both the serum antibody titre and the degree of protection are maintained for at least 5 weeks after vaccination with Nobilis IB 4-91 in chickens with high levels of maternally-derived antibody at the time of vaccination.

4.1.10 Nobilis IB 4-91 as a primer for inactivated Ma5 vaccines

Although the evidence is limited by the numbers of chickens (especially the small number of challenge test controls) used in this study, it can be concluded that vaccination with IB 4-91 at 2 weeks of age may act as a primer for vaccination with a product containing the inactivated Ma5 antigen.

4.2 FIELD STUDIES

4.2.1 Field trials in the UK

The Applicant provided the results of a series of field trials performed at a total of 26 broiler integration sites. Three vaccination regimes were used for different groups of chickens. Chickens were either vaccinated at day-old with IBV H120 vaccine and then 2 weeks later with IBV 4-91 (group 1) or at day-old and again 2 weeks later with H120 (group 2) or given just one vaccination of H120 at day-old (group 3). All vaccines were given by coarse spray.

Mortality figures, site rejects and profit per square metre per week were compared between vaccination groups and between sites. If sites that experienced disease problems other than Infectious Bronchitis (such as Infectious Bursal Disease, coccidiosis or leg problems) are excluded from the figures then there was no significant difference between the mean mortality figures for the different vaccination regimes. Comparison of the average site % factory rejects did not show any significant

difference between the different vaccination regimes. Financial margins (with the sites that experienced other disease problems excluded) show an increase in group 1 over those in group 2. Group 2 showed an improvement during the course of the trial. Both group 1 and group 2 profit per square metre per week in each round was greater than those of group 3.

Serology (IB 4-91 specific neutralising antibodies) showed a response to vaccination in all of the IB 4-91 vaccinated flocks. Specific neutralising antibodies to IB 4-91 were also found in some of the chickens in the other groups. It was, therefore, assumed that there may have been a field challenge during the trial. It is also possible that there may have been some spread of the vaccine virus. The presence of maternally derived antibodies in chickens at 12 of the sites was also demonstrated.

Some of the chickens that had received H120 vaccination at day-old followed by IB 4-91 vaccination at 14 days old were challenged, under laboratory conditions, with virulent IB 4-91 at 3 and 5 weeks post vaccination. It is stated that the IB 4-91 vaccine was used at an estimated dose of 3.0 $\log_{10}EID_{50}$/bird. Ciliostasis scores were compared with those of controls (chickens that had received H120 vaccination at day old but had been given no second vaccination). The IB4-91 vaccinated group showed 100% protection and the controls 17% protection 3 weeks post vaccination. At 5 weeks post vaccination 88% protection was seen in the IB4-91 group. Although the challenge resulted in only 78% of controls with clinical scores of < 20, the serology showed clear differences in the HI and ELISA titres to IB 4-91 between the IB 4-91 vaccinated group and the controls at both 3 and 5 weeks post vaccination.

4.2.2 Field trials in France

Flocks on various sites in France that had been vaccinated at day-old with a vaccine containing H120 were vaccinated at 14-20 days of age with either H120 vaccine or IB 4-91 vaccine. Some of the chickens also received Gumboro vaccination at various time points.

The total percentage of post vaccination reactions (PVR) was similar in both groups of chickens. The most common PVR in both groups being respiratory signs. Some intestinal signs were reported. The report suggests that these may have been due to problems with the feed. No significant differences were seen in the mortality rates between the two groups but the average site rejects (%) was higher for the IB 4-91 group at 3 out of 4 of the sites. These differences were, however, found to be not significant. The incidence of respiratory problems was found to be greater in the group that had received two H120 vaccinations. Some of the performance data appears better for the group given two H120 vaccinations. It is notable that at 3 out of 4 sites the mean daily growth rates were lower for chickens given IB 4-91 than those given two H120 vaccinations but, with the exception of the chickens at one site, the age at slaughter was lower for the IB 4-91 vaccinated groups. Serology results indicated that it was unlikely that there had been any natural challenge with IB 4-91 during the trial.

5. RISK-BENEFIT ASSESSMENT AND CONCLUSIONS

Based on the original and complementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC and supported the claims proposed by the Applicant.

Some minor quality points still need to be clarified, however the Committee agreed that these could be addressed on an on-going basis by the Applicant without delaying the authorisation process. The Applicant has given a commitment to address these points.