SCIENTIFIC DISCUSSION

Invented name: Equilis Prequenza Te

Active substance/INN: A/equine-1/Praque/1/56
A/equine-2/Newmarket/1/93
A/equine-2/Newmarket/2/93
Tetanus toxoid

Target species: Horses

Therapeutic indication: Active immunisation of horses from 6 months of age against equine influenza to reduce clinical signs and virus excretion after infection, and active immunisation against tetanus to prevent mortality.

Withdrawal period: Zero days

Pharmaceutical form: Suspension for injection

ATCvet code: QI05AL01

Pharmaco-therapeutic group: Equine Influenza and tetanus vaccine

Marketing Authorisation Holder: Intervet International B.V.
Wim de Körverstraat 35
NL-5831 AN Boxmeer
The Netherlands
1. SUMMARY OF THE DOSSIER

Equilis Prequenza Te is presented in a 1 ml glass vial or a pre-filled glass syringe. The vaccine contains tetanus toxoid and purified haemagglutin subunits (HA) from three different equine influenza virus strains. The product is indicated for active immunisation of horses from 6 months of age against equine influenza to reduce clinical signs and virus excretion after infection, and active immunisation against tetanus to prevent mortality. The route of administration is intramuscular.

2. QUALITY ASSESSMENT

Composition

The vaccine contains tetanus toxoid 40 LF (flocculation equivalents)/ml and purified haemagglutin subunits (HA) from three different equine influenza virus strains; A/equine-1/Prague/1/56 100 AU (antigenic units)/ml; A/equine-2/Newmarket/1/93 50 AU/ml; A/equine-2/Newmarket/2/93 50 AU/ml. Standard excipients used as stabilisers or buffer components all comply with the Ph.Eur. Per dose the vaccine contains traces of thiomersal as a remnant of starting materials, as well as traces of formaldehyde.

Container

Either glass vials or pre-filled glass syringes are used. The vials are closed with a halogenobutyl rubber stopper and encapsulated with a coded aluminium cap. The ending of the plunger and the tip cap closing of the syringe are of halogenobutyl rubber. Both containers are sterilised.

Development Pharmaceutics

The choice of the three strains of equine influenza virus included in the vaccine was justified based on current outbreaks of influenza. The development of a subunit vaccine was justified. The subunit vaccine also reduces potential adverse effects by means of the production process.

The vaccine contains purified haemagglutinin and neuraminidase glycoproteins; however the neuraminidase content of the vaccine is not measured.

The influenza potency is determined in-vivo (guinea pigs Ph.Eur. 0249). The initially proposed release limits for the three influenza components (Prague/56, Newmarket-1 and Newmarket-2 respectively) were revised and higher limits with revised pass criteria agreed.

The tetanus component of Equilis Prequenza Te is a standard tetanus toxoid. It contains a fixed amount of purified tetanus toxoid (40 LF per dose of 1 ml) determined via flocculation testing. The final product is tested in the potency test as prescribed in the Ph.Eur. monograph 0697 by using a Toxoid Binding Inhibition (ToBI) assay.

The adjuvant for this vaccine is based on iscom-matrix technology. The iscom-matrix used here is an adjuvant formulation closely related to iscom but consisting of particles with a patented composition whose shape and appearance are as that of the iscom except for their lack of incorporated antigen. These iscom-matrix particles are formed by a HPLC-purified fraction of Quillaja saponins, cholesterol and phosphatidyl choline. The iscom-matrix is mixed with the antigen only. Thus, the iscom-matrix possesses no or drastically reduced haemolytic activity. Induction of high levels of serum antibodies against equine influenza and tetanus, which persisted for an unexpectedly long time were seen in studies and the matrix has an excellent safety profile in the target species. The amount of purified saponin in the vaccine has been set at 375 μg/ dose.
Clinical trials formulations

Composition of batches used in all efficacy and safety studies was provided. Full details and complete protocols of all batches were provided, and all batches were produced according to the standard methods as described in the dossier.

Method of manufacture

All production steps are performed in accordance with GMP. Where applicable sterile equipment and liquids are used.

Production of the equine influenza components

The production and purification of the monovalent influenza antigens is presented and includes information on the type of control tests performed. A detailed description of the manufacturing process is given. Each antigen is purified by affinity chromatography. After the purification process samples are collected for quality control tests including the determination of the amount of HA protein. The sterilised material itself is stored at 5 ± 3°C pending further processing.

Production of the tetanus component

Following an extension of the marketing authorisation in 2008 an additional tetanus toxoid supplier was introduced. A flow chart of the method of preparation of the fractionated tetanus toxoid concentrate and an in-depth description is provided for each tetanus toxoid supplier. The Tetanus Toxoid Concentrate (TTC) or the Crude Tetanus Toxoid is purified, and the bulk product obtained is Tetanus Toxoid Concentrate Fractionated (TTF) or Purified Tetanus Toxoid, which is then further processed to the final product. The methods used by each tetanus toxoid supplier are highly similar and any differences have been adequately justified. Hence the supplier of the tetanus toxoid does not impact on the final product.

Preparation of the finished product

The required amount of the three influenza antigens is prepared for production and the volume of this trivalent mixture is determined. As the antigen mixture is contained in a buffer containing part of the excipients, the extra amount of these excipients required to formulate a batch in which their concentration is standard, is calculated. The excipients are dissolved in Water for Injections, filtered and added to the bulk mixture. A sample is taken for the test on inactivation. The required amount of sterile filtered TTF or Purified Tetanus Toxoid, which is diluted with water for injections to the required concentration and adjuvant is added and the mixture is stirred until homogeneity and yields the final bulk product.

The product is produced in different batches sizes. The suspension is stored at 2-8 °C until filling. The last production steps are filling, using an automatic filling line and capping followed by quality control.

Validation data on the inactivation kinetics study of EIV Prague/1/56, Newmarket/2/93 and Newmarket/1/93, antigen-capture ELISA for influenza antigen qualification, potency test for the EIV-components, endotoxin test, inactivation control test on final product, HI antibody test, Toxoid Binding Inhibition test, sterility test on final product were presented and considered satisfactory. Validation data for the toxoiding process of the tetanus toxin from each supplier are presented and all batches satisfactorily passed the toxicity test thus demonstrating consistency in the manufacturing process.
Control of Starting Materials

Active substances

Production strains of equine influenza

All influenza master seeds were produced on SPF eggs, the required documentation of the SPF eggs used for the production for the NM-1 and NM-2 seed were provided, of the Prague seed (1986) these are not available. The eggs for the NM-1 and NM-2 seed were from SPF flocks that were controlled as specified by the Ph.Eur. at the time of testing. Data showing that all three influenza MSVs have been tested free of avian extraneous agents was provided.

A/equine-1/Prague/56

The original source of this stain was nasal washings from a horse in the CSSR in 1956 as mentioned in the ATCC form. After passages in 9-11 day old embryonated eggs the master seed virus (MSV) was established. The MSV is stored at –20° C. Control tests on the MSV for identity, freedom from bacterial and fungal contamination, exclusion of extraneous agents were conducted. The sterility of the MSV is tested according to the Ph.Eur. requirements. The MSV is found to be free from bacterial, fungal and mycoplasma contamination. An overview presenting the results for the absence of pestiviruses and the equine extraneous agents as listed in the Note for guidance III/3427/93 was provided. Further details of test performances of the specific tests and their validity concerning n-cpe pestiviruses, rabies virus, EAV, EIA virus and equine herpes virus was provided and deemed satisfactory.

A/equine-2/Newmarket/1/93 (US-Type)

The virus was isolated from nasopharyngeal swabs from horses during an influenza outbreak in 1993. After one passage in embryonated SPF hen’s eggs, the MSV stock was prepared by multiplying this first passage on 10 days preincubated embryonated SPF hen’s eggs. The MSV is stored at –20° C. Control tests on the MSV for identity, freedom from bacterial and fungal contamination, exclusion of extraneous agents were conducted. The sterility of the MSV is tested according to the Ph.Eur. requirements 2.6.1. and 2.6.7. The MSV is found to be free from bacterial, fungal and mycoplasma contamination. General tests of the MSV including the observation of any cytopathic effect or visible abnormality by Giemsa staining, haemadsorption and haemagglutinating activity were performed after inoculation of several cell lines with virus seed. Specific tests for the exclusion of non-cytophogenic pestiviruses and rabies virus have been performed with satisfying results. Tests to exclude any contamination with equine arteritis virus (EAV) and equine herpes viruses types 1, 2, 3 and 4 (EHV) were conducted.

A/equine-2/Newmarket/2/93 (EU-Type)

The virus was isolated from nasopharyngeal swabs from horses during an influenza outbreak in 1993. The master seed stock was prepared after one passage on 11 days pre-incubated embryonated SPF hen’s eggs. The harvested amnio-allantoic fluid was lyophilised and then stored at –20° C. Control tests on the MSV for identity, freedom from bacterial and fungal contamination, exclusion of extraneous agents were conducted. The sterility of the MSV is tested according to the Ph.Eur. requirements 2.6.1. and 2.6.7. The MSV is found to be free from bacterial, fungal and mycoplasma contamination. General tests of the MSV including the observation of any cytopathic effect or visible abnormality by Giemsa staining, haemadsorption and haemagglutinating activity were performed after inoculation of several cell lines with virus seed. Specific tests for the exclusion of non-cytophogenic pestiviruses and rabies virus have been performed with satisfying results. Tests to exclude any contamination with equine arteritis virus (EAV) and equine herpes viruses types 1, 2, 3 and 4 (EHV) were conducted.

Justification for freedom from some viruses has been based on freedom of the country of origin from these infections. Confirmation was provided that these viruses were not used in any laboratories where the virus stocks were handled.

Preparation and description of controls and tests carried out on Working Seed Virus (WSV)

Working seed virus stocks of EIV-Prague/1/56, EIV-Newmarket/1/93 and EIV-Newmarket/2/93 were produced on embryonated SPF chicken eggs up to passage level MSV+4 and freeze-dried. The vials are stored at –20° C. The infectivity titre of the WSV was determined by SPF egg titration. After collection of amnio-allantoic fluid the haemagglutination activity was determined and the titre
calculated as EID$_{50}$ or log 10 EID$_{50}$ per ml. The WSV is tested for sterility according to Ph.Eur. 2.6.1 and for the absence of any mycoplasma contamination according to Ph.Eur. 2.6.7. All starting materials of non-biological origin used during the production processes for the equine influenza antigens are used before purification of the antigens by affinity chromatography, and are not contained in the final product. Certificates of analysis for all materials used were presented showing compliance with the specifications set. Different buffers are used during the purification processes for the equine influenza antigens. Detailed information on the composition of the single buffers is given in the dossier.

**Clostridium tetani strain**

The production strain is a variant of the Harvard strain (ATCC Catalogue No. 10779). MS and WS were tested for purity and identity by morphology. A testing protocol for the actual tetanus WS was provided. Confirmation was provided that current raw material specification and testing for the tetanus component comply with the requirements of the current editions of Ph.Eur. Up-to-date specifications and certificates of analysis were provided for all starting materials.

**Eggs**

Embryonated eggs which derive from healthy non-SPF flocks are routinely used for the production of EIV-antigens for the final product. The fertilised hen’s eggs are subjected to a thorough control system that guarantees the absence of embryo-derived extraneous agents in the product and the flocks are certified to be free from pathogenic poultry diseases, including avian leucosis and reticuloendotheliosis.

**Adjuvant**

The adjuvant for this vaccine is based on iscom-matrix technology and consists of cholesterol, phosphatidyl choline and HPLC purified fraction of Quillaja saponins. Details regarding the manufacturing process, the quantitative composition as well as the quality control of the adjuvant allowing evaluation of the consistency of adjuvant batches produced by the supplier were provided. Stability data of one batch of iscom-matrix before use in the final product have been provided. A detailed evaluation of the risk of contamination of the adjuvant with extraneous agents according to Note for Guidance III/3427/93 was provided showing that with the production processes used and the control measures implemented, the risk of contamination of the adjuvant with extraneous agents is negligible.

**TSE-Risk assessment**

The TSE-Risk assessment was conducted according to Directive 1999/104/EC and Note for Guidance EMEA/410/01-Rev. 2. Statements of the manufacturers with respect to the source of the materials and copies of EDQM Certificates of Suitability (CoS) were provided. It can be summarised that only non-ruminant material, EDQM certified ruminant derived material and bovine milk derivatives coming from milk obtained from healthy animals in the same conditions as milk collected for human consumption are used in the production of this vaccine. Transmission of TSE to equines cannot be excluded but has not occurred so far under natural or experimental conditions. Thus, in respect of the information given, the risk that animal spongiform encephalopathy agents are transmitted by the use of this vaccine is estimated as minimal/negligible.

**Control tests during Production**

**Equine influenza components**

The main in-process controls conducted are; Inactivation control on at least 10 vaccine doses; Determination of antigen content; Endotoxin test; Sterility test. Results of in-process control tests on three consecutive production batches are provided and met the specifications.
**Tetanus Toxoid component**
Different sequence tests are performed during the production process of the tetanus component. In process controls performed during the production of the tetanus component and on the bulk product have been described. Results of in-process control tests on consecutive production runs of the tetanus component were provided and deemed satisfactory.

A test for filling volume is performed on each batch of finished product.

**Control tests on the finished product**

The description of the methods used for the control of the finished product (pH, visual appearance, potency and identity of influenza and tetanus components, sterility and safety tests, inactivation control tests for EIV) and the specifications were provided. The specifications proposed are appropriate to control the quality of the finished product.

Validation of the influenza batch potency test was performed in guinea pigs and horses. The final product test is also intended to check for the presence of a sufficient antigen amount and appropriate release limits were set. The setting of appropriate criteria provides additional assurance that a sub-standard batch will not be released. Validation of the Toxoid Binding Inhibition (ToBI) test was performed according to VICH GL 1 and 2. The parameters investigated were specificity, linearity, range, accuracy, precision, detection limit and robustness. A protective ToBI limit was set based on available data.

A test confirming the identity of the adjuvant in the final product is included as routine final product control test.

Safety tests are carried out on each batch. Confirmation was provided that the horses used for the final product batch safety test would have no equine influenza virus antibodies or, at most, a low level, and have not been vaccinated against equine influenza. Replacement of the batch safety test in guinea pigs as prescribed in Ph.Eur. monograph 0697 by a batch safety test in horses was justified.

The results of the analysis of three consecutive production runs of finished product vaccine were presented which complied with the required specifications.

**STABILITY**

**Stability of the bulk antigens**

Satisfactory stability data has been provided to justify a shelf life for the influenza bulk antigens and also for the tetanus toxoid bulk which is stored at 2-8°C.

**Stability of the finished product**

Stability studies have been conducted using batches of Equilis Frequenza Te stored at +2°C to +8°C. Based on the data provided, transportation need not be carried out under refrigerated conditions (+2°C to +8°C). The proposed shelf life of 24 months is justified.

**OVERALL CONCLUSION ON PART II**

The analytical part of the dossier is well described. Documentation and specifications reflecting the actual manufacturing and testing process for the tetanus component were provided. The production of the influenza virus antigen was described in detail. The method of manufacture was well described and the main in-process controls detailed in full. Compliance of starting materials of animal origin used during production with the requirements of the Note for guidance on minimising risk of transmitting animal spongiform encephalopathy agents via human and veterinary products was shown. Following
an extension of the marketing authorisation in 2008, the addition of another tetanus toxoid supplier was adequately justified.

Validation data for the ToBI test were provided and deemed satisfactory to differentiate between batches that pass or fail the batch potency test. Validation of the influenza virus batch potency test was addressed and appropriate pass criteria defined. The finished product and batch safety tests ensure a product of consistent quality is produced. Based on the stability data provided a shelf life of 24 months was justified for the finished product.
3. SAFETY ASSESSMENT

Equilis Prequenza Te is an inactivated adjuvanted vaccine indicated for the active immunisation of horses against the effects of an infection with wild type equine influenza virus of the subtypes A/equine-1 or A/equine-2, and infection with Clostridium tetani. The basic vaccination scheme consists of two 1 ml intramuscular injections, each a single dose, with an interval of 4 weeks. The minimum age for vaccination is 6 months. The safety studies were based on the relevant Ph.Eur. monographs and guidelines.

Five laboratory studies and 6 field studies have been conducted. Equilis Prequenza Te contains standard antigen content for all components. For the safety studies, vaccine batches produced and tested according to the production method and the standard release requirements described in Part II of the dossier were used.

LABORATORY TESTS

Safety of the administration of one dose/ an overdose/ the repeated administration of one dose

The studies are performed in line with the requirements of Ph.Eur. monograph 0249 (“Equine Influenza vaccine”). The adverse effects seen after administration of an overdose and repeated single dose to the foals were minor, transient, and limited to a minority of the foals in all studies performed independent of the time interval between the vaccinations. The time interval of 2 or 4 weeks between vaccinations does not influence the severity and character of adverse reactions after administration of the second vaccination. Based on all safety studies performed to assess the safety of a single, double and repeated single dose using batches of standard antigen content in horses of 2 to 4 months of age and older horses, it is concluded that vaccine will be well tolerated by horses of different ages.

Safety of Equilis Prequenza Te vaccine in foals 3 months of age - a double dose followed by a single dose

The objective of the GLP compliant study was to investigate the safety of the vaccine after intramuscular vaccination of young foals of approximately 3 months of age with an overdose and a repeated single dose.

Animals used in the study should have influenza antibody titres HI < 2^4 and not previously vaccinated against influenza or tetanus. On the day of vaccination half of the foals were seronegative (< 2^5) for equine influenza strains Prague/56, Newmarket-1/93, Newmarket-2/93. The other half showed low (≤2^4 or 2^5) antibody responses to one of the equine influenza strains. All horses had an antitoxin titre against tetanus toxoid of < 0.04 I.U./ml.

After vaccination all animals had induced antibody titres against all antigens present in the vaccine. Unvaccinated control horses did not develop antibodies to any of the antigens present in the vaccine.

Several parameters were examined in order to assess the safety of the product (rectal temperature, local and general reactions). The adverse effects seen after administration of an overdose and repeated single dose to the foals were minor, transient, and limited to a minority of the foals. After vaccination with the double dose one foal developed a warm, diffuse and painful swelling which disappeared within 24 hours and fever. In additional investigations no indication of an inflammatory response was found. The rectal temperature was increased in a further horse 5 hours after vaccination with a double dose, and in a few horses from three days after injection of a repeated single dose onwards, lasting one to three days. There was a mild depression in a few foals on the day of vaccination with the double dose. The vaccination with a double dose followed by a single dose is safe in foals 3±1 months of age. The observed reactions are acceptable. The SPC contains appropriate statements for a description of undesirable effects after single dose and overdose vaccination.
Safety of Equilis Prequenza Te vaccine in horses - double dose followed by a single dose

The objective of this GLP compliant study was to investigate the safety of the vaccine after intramuscular vaccination of horses of one year of age with an overdose and a repeated single dose. Only healthy horses 12 to 15 months of age were included in the study. Horses were vaccinated by intramuscular injection at day 0 of the study with a double dose (2 ml) and at day 14 with one dose (1 ml). One horse remained unvaccinated to serve as a control for concurrent field infection.

The results of the antibody tests showed that the horses can be declared as being seronegative for equine influenza and tetanus at first vaccination. The unvaccinated control horse did not develop antibodies to any of the antigens present in the vaccine.

Several parameters were examined in order to assess the safety of the product (rectal temperature, local and general reactions). The adverse effects seen in the horses after vaccination were limited and transient. After vaccination with the double dose a few horses had a local reaction at the injection site which disappeared within three days. The maximum size was 5 x 5 cm. After injection of a single dose several horses developed local reactions either as a diffuse swelling or a painful neck. The maximum size was 2 x 2 cm. All reactions disappeared within 24 to 48 hours. The rectal temperatures were increased in a few animals after vaccination with the double dose and in some horses after administration of a repeated single dose, lasting for one to two days. Systemic reactions were not observed after the two vaccinations. The safety studies showed that an increase in rectal temperature above the normal range could be observed for 24 hours, exceptionally for three days. The individual clinical data of the horses after the vaccinations were provided and confirmed the findings reported.

The vaccination with a double dose followed by a single dose is safe in yearlings. The observed reactions are acceptable. The SPC gives a description of undesirable effects after single dose and overdose vaccination and the nature of the local reactions was added.

Safety of Equilis Prequenza Te vaccine in horses – with emphasis on macroscopical examination of the injection site

The objective of the GLP compliant study was to examine post-mortem tissues surrounding the injection site for the occurrence of macroscopic lesions after intramuscular vaccination of horses of one year of age with an overdose and a repeated single dose. This study was conducted using horses that had already received a double dose and a single dose of vaccine within the preceding six weeks. The macroscopic and histopathological information provided in this study relates only to reactions occurring after repeated dosing.

The macroscopical and microscopical examination of the injection site did not reveal any vaccine remnants at 24 hours, 48 hours, 7 and 14 days after vaccination. Some oedema and slight inflammation were found. Even after three vaccinations within a short time period including a double dose, the vaccine is safe for the target animal with acceptable minor adverse effects. The vaccination with a double dose followed by a single dose is safe in yearlings.

At 24 hours post vaccination, a few horses that showed a swelling with a size of 1.5 x 1.5 cm were euthanised. At post-mortem examination of the injection site, an intramuscular oedema was visible in these horses. The size was approximately 10 x 3 x 4 cm and 8 x 4.5 x 3 cm, respectively. The muscle tissue itself did not show any macroscopical abnormalities. Vaccine remnants, abscessation, haemorrhages, calcification or necrosis of the tissue were not observed in any horse.

Examination of reproductive performance

The evaluation of the safety of Equilis Prequenza Te in pregnant thoroughbred mares before and after foaling

The objective of the GLP compliant study was to investigate the safety of the vaccine after intramuscular vaccination of pregnant thoroughbred mares in the last trimester of gestation, with a
double dose followed by a single dose 2 weeks later. The control mare did not develop anti-influenza antibody titres throughout the study.

Four hours after vaccination with a double dose 80% of mares showed a local minor reaction at the injection site reducing to 30 % after 24 hours. After injection of a repeated single dose 40% of mares showed a local minor reaction. The maximum size was 2 x 2 cm. Generally, the local reactions disappeared within 24 to 48 hours. There were no systemic reactions after both vaccinations and the rectal temperature was not increased above the normal level. No negative influence on gestation, foaling and offspring of the mares was observed.

The results of a separate field trial in which six pregnant horses were vaccinated during the first four months of gestation without any negative effect on the gestation, support the safety of the vaccine during the first trimester of pregnancy.

Examination of immunological functions

No specific studies were conducted. Equilis Prequenza Te is a vaccine containing inactivated viral protein and inactivated bacterial toxoid. Replication of vaccine virus or bacteria in any cell involved in the vaccinated animals’ immune system is therefore not applicable and subsequently impairment of the immune system is not to be expected. There is no reason to suspect any impact of vaccination with Equilis Prequenza Te on immunological functions.

Study of residues

Studies of residues were not presented. A withdrawal period of zero days was accepted. Equilis Prequenza Te is an inactivated vaccine.

Interactions

Safety and efficacy of concurrent administration of Equilis Prequenza Te vaccine and Tetanus Serum Intervet (tetanus antitoxin) in horses

The objective of the study was to investigate the safety and efficacy of the concurrent administration of the vaccines Equilis Prequenza Te and Tetanus Serum Intervet in horses.

Foals younger than one year of age obtained from an unvaccinated herd, were vaccinated with a single dose of Equilis Prequenza Te and received Tetanus serum from Intervet at the same time but at a different site, followed by a single dose of the vaccine four weeks later. In one of the horses a swelling of 2 x 2 cm in size was found at the injection site after first administration, which disappeared within 24 hours. No local reactions were observed at the injection site of the Tetanus serum. Fever was observed for one day in a few horses after first vaccination. Some horses developed diarrhoea and ocular discharge after the first vaccination. The reactions were mild and transient and disappeared within a few days without treatment. After second vaccination a local reaction of 1 x 1 cm was found in one horse and a diffuse swelling of 10 x 5 cm in another horse. The reactions had disappeared the next day. No systemic reactions and no fever were recorded after the second vaccination.

The reactions observed are mild and of only short duration which indicates the concurrent use of the vaccine and Tetanus serum can be considered to be safe for horses younger than one year of age. Therefore, the statement in the SPC section 5.8 is acceptable from safety point of view.

FIELD STUDIES

Field studies were performed in compliance with the VICH guidelines of “Good Clinical Practice” (GCP) and EC guideline III/3001/93 “Specific requirements for the production and control of Equine live and inactivated viral and bacterial vaccines”. A competitor product was used as positive control to enable comparison of the safety findings for Equilis Prequenza Te.
A positive controlled field safety and efficacy trial of Equilis Prequenza Te in foals

The objective of the study was to assess the safety of the Equilis Prequenza Te vaccine in foals kept and vaccinated under field conditions compared to a positive control product. Clinically healthy foals, were included in this multicentre study; ranging in age from 2 to 10 months old.

About one third of the horses showed a local reaction after each vaccination. The local reactions were characterised as a hard or soft swelling, mostly with a diameter of less than 2 cm and not painful. In general, the local reactions disappeared within 48 hours. From day three after vaccination onwards no local reactions were observed. One horse showed a local reaction (> 2 cm in diameter, soft) one day after the second vaccination. An increased rectal temperature (≥ 39° C) was observed in one third of the animals after first vaccination of both vaccines. After second vaccination none of the foals had a rectal temperature ≥ 39° C. Clinical signs like dullness, diarrhoea were not reported. The majority of the foals had swollen mandibular or retropharyngeal lymph nodes before and after vaccination at different days.

The reactions observed after vaccination of young horses under field conditions with Equilis Prequenza Te are mild and transient. These are acceptable reactions and are adequately reflected in the proposed SPC.

A positive controlled field safety and efficacy trial of Equilis Prequenza Te in pregnant mares

The objective of the study was to assess the safety of the Equilis Prequenza Te vaccine in pregnant mares kept and vaccinated under field conditions compared to a positive control product and to study the level of maternal antibodies of their offspring.

Clinically healthy mares were included in this multicentre study. The age of the group ranged from 2.8 to 24.5 years. The mares were pregnant between 3 and 9 months. At admission all mares which had a HI-influenza antibody titre of > 6 for all three strains received a single vaccination. The other mares, which had a titre of ≤ 6 for one or more strains, received a basic vaccination that consisted of two vaccinations with an interval of 4 weeks. All mares received a pre-foaling vaccination 4 to 5 weeks before the expected foaling date. Mares were vaccinated with the vaccine Equilis Prequenza Te or with the positive control product. 72 % of the Equilis Prequenza Te group and 73 % of the control group received a basic vaccination, the others a single vaccination. Four to 5 weeks before the expected date of foaling a pre-foaling vaccination was given to all but one horse of the Equilis Prequenza Te group. The mares were vaccinated intramuscularly. The mares were observed from admission until foaling.

All horses, vaccinated with both vaccines, remained healthy throughout the trial. Systemic reactions after vaccination were not observed. In both test groups several mares had swollen mandibular or retropharyngeal lymph nodes before and after vaccination on different days. Occasionally, slightly red mucous membranes, increased pulse and respiration or abdominal respiration were recorded. Generally after vaccination the rectal temperatures hardly increased and remained within the normal range (< 39° C). On day 28 two horses of the Equilis Prequenza Te group had fever (39.9° C and 40.0° C respectively).

About 60 % of the Equilis Prequenza Te group and 80 % of the positive control group showed a local reaction. The local reactions after vaccination mostly had a diameter smaller than 1 cm, were of soft nature and disappeared within two days after vaccination. Sometimes the local reactions were hard of nature, painful, 1-2 cm or > 2 cm in diameter and minimally swollen. Generally, the reactions disappeared within 24 to 48 hours. Fourteen days after vaccination no local reactions were observed in any of the animals. After the second and third vaccination no local reactions were reported for both vaccines.

In the Equilis Prequenza Te group 97 % of the mares gave birth to a healthy foal. One mare aborted 2 months before the expected foaling date, 129 days after second vaccination. Post mortem examination of the foal showed a congenital anomaly of the cardio-vascular system. No indication for an infection
was found. In the positive control group 97% of the mares gave birth to a healthy foal. One mare had a premature birth of twins 3 weeks before the expected foaling date, 149 days after first vaccination. In the majority of cases, the birth was finished within 15 to 30 minutes, the placenta came spontaneously within 2 hours and the foal stood and drank within 1-2 hours. The rectal temperatures of the foals were within the normal range during the first days of life.

There was no negative influence on gestation, foaling and offspring of the mares. The reactions observed after vaccination of pregnant mares were mild and transient.

**A field safety and efficacy trial of Equilis Prequenza Te and competitor product in horses**

The aim of the GCP study was to assess the safety and the efficacy of Equilis Prequenza Te in horses under field conditions by comparison with a positive control vaccine. Clinically healthy horses at an age of 5 - 41 months, belonging to two different farms, were randomly separated into three groups; vaccinated with one dose of Equilis Prequenza Te, one group was vaccinated with one dose of the competitor product and the third group was not vaccinated.

A quarter of the horses showed a local reaction after first vaccination and one third of horses after the second vaccination with a diameter of 1 cm or less.

**A positive controlled field efficacy trial of Equilis Prequenza Te in horses**

The aim of the study was to assess the efficacy of Equilis Prequenza Te in horses under field conditions compared to a competitor product. The trial was conducted in compliance with GCP guidelines using horses of 14 breeds housed on 4 sites, clinically healthy and at an age of 1.2 – 25 years, divided into three groups.

One group (46%) was vaccinated with one dose of Equilis Prequenza Te and the other group (44%) with one dose of the control product. Horses that had been vaccinated against influenza within the last twelve months received a single (booster) vaccination on day 0. If the previous vaccination was more than 12 months ago, the horses received a basic vaccination course consisting of 2 vaccinations with an interval of 28 days. In this study no adverse events were recorded. All animals remained healthy throughout the trial.

**A field trial to determine the sero-response with Equilis Prequenza and Equilis Prequenza Te in sero-negative horses**

The objective of the study was to determine and compare the sero-responses for influenza after vaccination with Equilis Prequenza and Equilis Prequenza Te in horses. Horses of different breeds and ages were divided into 3 groups. Group 1 (46%) received 2 vaccinations with Equilis Prequenza at an interval of 28 days. Group 2 (46%) were administered 2 vaccinations with Equilis Prequenza Te at an interval of 28 days. Group 3 (8%) served as controls.

No adverse events occurred and the general health was each time scored as “normal”.

**A field trial with Equilis Te and Equilis Prequenza Te to determine the serological response to tetanus toxoid in horses**

The aim of the study was to compare the sero-response to the tetanus component of Equilis Prequenza Te and Equilis Te in horses under field conditions. The trial was conducted in compliance with GCP guidelines on a stud farm in the EU. Clinically healthy horses at an age of 4 - 13.3 years, were separated into three groups.

Two groups were vaccinated with one dose of Equilis Te, one group with one dose of Equilis Prequenza Te. The horses were vaccinated with an interval of 4 weeks i.m. in the neck.
Some minor local reactions were observed. One day after first vaccination a local reaction of approximately 3 cm was observed in one horse. One day after second vaccination local reactions were observed in several horses. In some horses the reaction was hardly visible (< 1 cm). A few horses had a soft and not painful reaction of 2-3 cm. The general health was reported as being “normal”.

III.E. ECOTOXICITY

The product is an adjuvanted liquid vaccine containing inactivated viral antigens and sterile filtered purified tetanus toxoid as active components together with lactose and buffer salts. A phase 1 assessment was conducted and presented. On the basis of the results of the assessment of the hazards identified and the likelihood of their occurrence, it is concluded that the level of risk associated with each of the hazards is effectively zero. Therefore the Equilis Prequenza Te vaccine is judged to present no risk to the environment. On the basis of the phase 1 assessment, a phase 2 assessment to investigate the ecotoxicity of Equilis Prequenza Te is not necessary.

OVERALL CONCLUSION ON PART III

Laboratory studies were conducted to assess the safety of a single, double and repeated single dose using batches of standard antigen content in horses of 2 to 4 months of age, older horses and pregnant thoroughbred mares. The vaccine may induce local reactions in the horse. These local reactions are characterised by soft or sometimes hard swellings mostly with a diameter smaller than 2 cm. In rare cases the size was up to 5 cm in diameter and the injection site was painful. The reactions were transient and they disappeared normally within 24 to 48 hours. Sometimes an increase in rectal temperature above the normal range could be observed for 24 hours, exceptionally for 3 days. Other systemic reactions were not induced by the vaccine. That means that vaccine will be well tolerated by horses of different ages.

No negative influence on gestation, foaling and offspring of mares was observed after vaccination at different times during pregnancy. At the injection site, no remnants of the vaccine were found. The vaccine was administered at the same time, but at different sites with Tetanus serum. This administration was safe. An assessment of the ecotoxicity risks showed that the overall risk of the vaccine to the environment, humans and other animals is effectively zero.

As the adverse reactions following vaccination with a single dose, an overdose and a repeated single dose to foals and older horses as well as to pregnant mares were minor and transient in all studies the vaccine may be used safely in competition horses. Vaccination of horses just before or just after competition should be not performed. Additionally, the stress after vaccination, e.g. by training should be minimised.
4. EFFICACY ASSESSMENT

Equine influenza is an infectious respiratory disease caused by a virus belonging to the orthomyxovirus group. It is one of the most severe equine respiratory diseases and rapid spreading of the infection is typical. In an unprotected population morbidity rates of more than 80 % may occur. The disease is characterised by high fever and persistent severe cough. Infections caused by influenza virus often predispose to bacterial superinfection of the respiratory tract. This leads to a much more severe course of the disease than influenza infection itself. Strains belonging to the influenza subtypes A/Equi-1 and A/Equi-2 are responsible for the disease in horses. During recent years no A/Equi-1 field strains appeared. Strains belonging to subtype A/Equi-2 continue to circulate and cause large epidemics throughout horses world-wide (except for Australia and New Zealand). Vaccination is one of the accepted methods to prevent the disease. The protective antigenic proteins of the influenza virus correspond to the haemagglutinin and neuramidase contained in the membrane part of the viral envelope.

Tetanus is an acute, often fatal disease caused by the neurotoxin of Clostridium tetani, a slender, gram-positive, anaerobic rod that may develop terminal spores which are widely distributed in soil and intestines of animals and humans. The disease, which usually originates from contaminated wound, is characterised by generalised rigidity and convulsive spasms of skeletal muscles. The muscle stiffness usually involves the jaw (lockjaw) and neck and then becomes generalised. Most susceptible species to tetanus are horses and humans. Effective prophylaxis against this disease is only obtained by means of vaccination.

Laboratory trials
Establishment of a challenge model

Equine Influenza
The influenza challenges were performed in accordance with Ph.Eur. monograph 0249 in the target animal. The parameter used to assess the efficacy of Equilis Prequenza Te against equine influenza was the level of serum antibodies against the three influenza vaccine strains induced after vaccination. This was determined by means of a haemagglutination inhibition test (HI). The HI titre was identified in a twofold dilution series and specified as HI log2 titre. The vaccination was considered successful if a HI titre of $\geq 6$ log2 was induced and a titre increase of at least 2 log2 could be detected. The detected antibody levels in the trials against the H7N7 strain was satisfactory.

The second parameter was the comparison of the clinical signs and virus excretion induced by a challenge infection with strain A-equine/Kentucky/9/95 or A/Equine-2/South Africa/04/03 in a vaccinated group and a control group of animals. The vaccine was considered to be sufficiently effective if significant differences in the quantity of virus excreted and quantity and severity of the symptoms observed after 14 days could be identified in favour of the vaccines.

Tetanus
For ethical reasons, no challenge experiment has been performed against tetanus. The parameter used to assess the efficacy of Equilis Prequenza Te against tetanus was the level of serum antibodies against tetanus toxoid induced after vaccination, which was determined by means of a Toxoid Binding Inhibition (ToBI) test internally calibrated against the WHO tetanus antitoxin standard of the horse. Satisfactory validation data were provided for the ToBI test. A ToBI titre of 0.3 I.U./ ml has been shown to be protective in horses. All horses used for the laboratory trials were derived from a non-vaccinated herd free of antibodies against tetanus, seronegativity for tetanus was set at $< 0.05$ I.U./ ml.

Determination of the vaccine dose

No separate dose-response trials have been performed with regard to the EIV-components. Reviewing the data from the batch potency validation test performed in guinea pigs and the target animal a dose-dependant immunoresponse measured in HI antibody titres was demonstrated. Efficacy testing has been performed with vaccine batches with a standard amount of tetanus toxoid (fixed dose of 40
The amount of tetanus toxoid included in Equilis Prequenza Te is based on experience with existing Intervet vaccines.

**Onset of protection**

**Potency of Equilis Prequenza Te Vaccine in Horses against a Challenge with Influenza A/Equine-2/Kentucky/9/95**

Foals (2 – 7 months, influenza and tetanus naïve) were vaccinated twice with one dose of Equilis Prequenza Te vaccine at 4 weeks interval. Some animals were left unvaccinated to serve as controls. Four weeks after the second vaccination all horses were challenged by aerosol with A/equine-2/Kentucky/9/95 (H3N8, “American type”) virus. Information regarding the challenge strain was provided. The relevance of the strain for the current European field strain situation was supported. After challenge horses were monitored for clinical signs of influenza, rectal temperatures, virus excretion and serology. Clinical scores, total days of virus excretion and virus reisolation data from vaccinated and nonvaccinated animals were statistically compared.

Vaccination with Equilis Prequenza Te in foals, according to the recommended vaccination scheme, strongly reduces clinical signs and virus excretion when challenged with equine influenza virus strain A/equine-2/Kentucky/95. The challenge was performed by the oronasal route and the challenge model used in the studies described in the dossier is the only model described so far that enables a fairly accurate calculation of the challenge dose per horse.

**Equine Influenza**

The HI antibody titres against the three influenza strains were listed separately for each horse. At the beginning of the trial, all foals were seronegative against all three influenza strains. No anamnestic seroresponse was detected in the vaccinates one week after V 1. The vaccinates responded to the first immunisation with a slight development of antibody titres against all three strains. After the second immunisation a fast increase of the level could be detected. The titre in all controls remained negative until the day of challenge. The challenge clearly induced the development of antibodies against Newmarket/1/93. For Newmarket/2/93 the antibody titres increased to low levels 14 days after challenge. No control foal seroconverted against the Prague strain.

All observed parameters of the challenge were calculated for significant differences between the vaccinates and controls. After the basic vaccination course, the vaccinates developed high levels of HI antibody titres against all three influenza strains. After challenge with Kentucky/9/95 four weeks after the primary vaccination the vaccinates were not fully protected, but a significant difference in favour of the vaccinates could be demonstrated for all observed parameters. The clearest differences between the two groups could be observed in virus shedding, occurrence of coughing and development of fever.

**Tetanus**

Regarding the onset of protection against tetanus, serum samples obtained at the time of first vaccination, one week after first vaccination, at the time of second vaccination 4 weeks after first vaccination, and 2 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Whereas all vaccinated foals were seronegative to tetanus at the time of first vaccination (< 0.05 I.U. / ml), all foals clearly showed seroconversion 2 weeks after the second vaccination. All vaccinated foals were still seronegative to tetanus one week after the first vaccination and showed only low ToBI titres at the time of second vaccination thus lacking any anamnestic immune response.

Based on the results of this study, it is justified to conclude that the onset of protection against tetanus infection is 2 weeks after the second immunisation of basic vaccination.
The Influence of Maternal Antibody on the Efficacy of the Vaccine

The interference of maternal antibodies on the efficacy of inactivated vaccines against equine influenza and tetanus is well known. The data from the field trials presented in the dossier indicate that the humoral response of Equilis Prequenza Te may be impaired by interference with maternal antibodies against influenza. Development of a humoral response of Equilis Prequenza Te in the face of maternal antibodies against tetanus has not been demonstrated. Field data and laboratory data indicate that young animals at the age of 6 months that are without maternal antibody against influenza or tetanus respond adequately to vaccination. A complete vaccination response of seronegative foals at an age below 6 months could not be clearly demonstrated. Maternal antibodies to equine influenza and tetanus in the foal may persist up to 4-6 months of age depending on the amount of colostrum ingested shortly after birth and the immune status of the mare. In addition, it has also been recommended that foals born from mares vaccinated with Equilis Prequenza Te during the last 2 months of gestation should not be vaccinated before the age of 6 months. This has been taken into account in section 5.10 of the SPC.

Duration of Immunity

Duration of Protection achieved by Equilis Prequenza Te Vaccine in Horses against a Challenge with Influenza A/Equine-2/Kentucky/9/95, 6 Months after the Primary Vaccination Course

The aim of the study was to determine the duration of immunity of Equilis Prequenza Te after a basic vaccination course. Influenza and tetanus naive foals were administered twice a dose of a standard production batch of the test vaccine 4 weeks apart. Five months after the second dose the vaccinated foals were challenged together with 5 unvaccinated foals serving as control group with a recent field strain of Equine Influenza A of subtype H3N8 (Kentucky/9/95, “American” lineage). Blood samples were taken at several occasions throughout the study in order to control the development of the serum antibody titre against the 3 influenza strains and the tetanus component of the test vaccine. At the day of challenge, all vaccinates had a HI serum titre above $6.0 \log_2$ against all influenza strains of the vaccine.

Equine Influenza

At the beginning of the trial, all foals were seronegative against all three influenza strains. No anamnestic seroresponse was detected in the vaccinates one week after V1. After the basic vaccination course the vaccinates developed high levels of HI antibody titres against all 3 influenza strains. After the second immunisation, a fast increase of the serum titre could be detected. From that level a continuous small decrease of the antibody levels could be detected over the following weeks. Five months after V2, antibody titres indicate sufficient protection against influenza. After challenge all control animals developed clear clinical signs of influenza.

For the observed parameters after challenge a significant difference in favour of the vaccinates could be demonstrated. The clearest differences between the two groups could be observed in virus shedding, occurrence of coughing and development of fever.

Tetanus

Regarding the duration of protection against tetanus, serum samples obtained at the time of first vaccination, one week after first vaccination, at the time of second vaccination 4 weeks after first vaccination, as well as 2, 7, 12, 15 and 21 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Whereas all vaccinated foals were seronegative to tetanus at the time of first vaccination, all foals clearly showed seroconversion 2 weeks after the second vaccination. Afterwards, the serum tetanus antitoxin titres of vaccinated foals declined until 5 months after basic vaccination. All vaccinated foals were still seronegative to tetanus one week after the first vaccination and showed only low antitoxin titres at the time of second vaccination thus lacking any anamnestic immune response.

Based on the results of this study, it is justified to conclude that the vaccine offers protection against tetanus infection for at least 5 months after the second immunisation of basic vaccination.
Duration of Protection achieved by Equilis Prequenza Te Vaccine in Horses against a Challenge with Influenza A/Equine-2/Kentucky/9/95, 1 Year after the Third Vaccination

The aim of the study was to determine the duration of immunity of Equilis Prequenza Te against tetanus after the basic vaccination course and against influenza after the first revaccination in horses. Influenza and tetanus naive foals were administered twice a dose of a standard production batch of Equilis Prequenza Te 4 weeks apart. Five months after the second dose the vaccinated foals received a revaccination with one dose of a standard production batch of the influenza vaccine Equilis Prequenza. At 12 months after the third vaccination the foals of the vaccination group were challenged together with unvaccinated foals serving as control group with a recent field strain of Equine influenza A of subtype H3N8 (Kentucky/9/95, “American” lineage).

Equine influenza
At the day of challenge, all vaccinates had a HI serum titre above 6.0 log₂ against all influenza strains of the vaccine. The HI antibody titres against the 3 influenza strains are listed separately for each horse. The means of the group titres transformed in curve diagrams were presented. At the beginning of the trial, all foals were seronegative against all three influenza strains. No anamnestic seroresponse was detected in the vaccinates one week after V 1. The titre in all controls remained negative until the day of challenge. A fast and strong increase of antibodies against all strains could be observed after the revaccination at week 26 with the influenza vaccine. These titres decreased about 2 log₂ ranges during the next two months. Then, the titre decrease was continuous but slower and plateaued nearly half a year later.

At the day of challenge (twelve months after V 3) the group mean of the serum titres are identical with the titres obtained after bleeding 4 months earlier (week 61). Compared to the levels obtained at week 26 (V 3), these titres are identical for the Prague strain and 1 log₂ range higher for the two Newmarket strains.

After challenge the clinical signs of the vaccinates were significantly lower compared to the findings of the control group. The duration and amount of virus excretion was significantly reduced in the vaccinates compared to the controls. A sufficient protection against an influenza challenge 12 months after the first revaccination with Equilis Prequenza in foals, which have had a basic vaccination course with Equilis Prequenza Te, could be demonstrated. A yearly booster vaccination beginning with the second revaccination was supported.

In the vaccination group, nasal discharge was the only clinical sign which occurred. All foals developed serous nasal discharge at day 2. During the next days mild nasal discharge was observed in the majority of the vaccinates. Only one foal developed a marked mucopurulent discharge for 2 days. From day 8 until the end of the observation period this clinical sign was observed in very rare cases in the vaccinates. On day 14 all vaccinates seemed to have recovered completely.

Reduced appetite was observed in the control group on several days. One or 2 animals additionally developed malaise and depression. Nearly all controls were affected by nasal discharge from day 2 to 7, resulting sometimes in a mucopurulent quality. Beginning with day 9 the observed cases decreased. On day 14 no clinical signs could be found in the control animals. All parameters observed during challenge were calculated for significant differences between the vaccinates and controls.

Tetanus
Regarding the duration of protection against tetanus, serum samples obtained at the time of first vaccination, one week after first vaccination, at the time of second vaccination 4 weeks after first vaccination, as well as at regular intervals until 73 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Whereas all vaccinated foals were seronegative to tetanus at the time of first vaccination, all foals clearly showed seroconversion two weeks after the second vaccination. Afterwards, the ToBI titres of the vaccinated foals declined until 5 months after basic vaccination. During the following twelve months, the antibody titres continued to
decrease slowly. One and a half years after the first vaccination (at t = 77 weeks), the geometric mean of the ToBI titres was still sufficient for protection. All vaccinated foals were still seronegative to tetanus one week after the first vaccination and showed only low ToBI titres at the time of second vaccination thus lacking any anamnestic immune response. Based on the results of this study, it is justified to conclude that the vaccine offers protection against tetanus infection for at least 17 months after the second immunisation of the basic vaccination course as well as solid protection against equine influenza for at least one year after the third vaccination.

**Interactions- Safety and Efficacy of Concurrent Administration of Equilis Prequenza Te Vaccine and Tetanus Serum Intervet (Tetanus Antitoxin) in Horses**

The objective of the study was to investigate the safety and the efficacy of the concurrent administration of the vaccine Equilis Prequenza Te and Tetanus Serum Intervet in horses. Foals obtained from an unvaccinated herd received a single dose of the vaccine intramuscularly. At the same time the horses were injected intramuscularly with the prescribed dose of tetanus serum/kg bodyweight into the right side of the neck. Four weeks later a single dose of vaccine was administered to the horses at the left side of the neck. One horse was left unvaccinated to serve as control. All but one foal were between 6 to 12 months of age.

**Tetanus**

Serum samples obtained at the time of first vaccination, 4-6 hours, 24 hours, 48 hours, 72 hours, 7 days, 10 days, 14 days, 17 days and 21 days after the first vaccination, at the time of second vaccination four weeks after the first vaccination as well as two weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Serology data from vaccinated animals were used to demonstrate the ability of foals to create a humoral immune response in the face of passive immunisation against tetanus.

With regard to the efficacy after passive immunisation, as early as 4-6 hours after the concurrent administration of Equilis Prequenza Te and Tetanus-Serum Intervet, protective antitoxin levels against tetanus were found. Blood samples of three horses taken as early as 30 minutes after injection already showed antitoxin levels. These passively acquired antitoxin titres reached their maximum approximately two days after administration. Until 21 days after antiserum administration, the ToBI titres remained above the level indicative for protection for all horses in both serum groups. In 60% of the horses treated with Tetanus-Serum Intervet, the antitoxin titres against tetanus were above 0.1 I.U./ml until day 28 after treatment.

With regard to the efficacy after active immunisation, the average antitoxin titres measured at the moment of the second administration of the vaccine (day 28) were equal to that found in the other laboratory efficacy studies.

It should be noted that in both groups the antitoxin titres were significantly reduced compared to results obtained in the other laboratory efficacy studies at two weeks after the second vaccination.

**Equine Influenza**

Throughout the course of the study antibody titres against all influenza strains present in the vaccine were determined. At the start of the study no antibody titres against any of the influenza strains were found. At 7 days after vaccination low antibody titres could be detected. Two weeks after the second vaccination, the highest antibody titres were found. These titres were similar to those found in the other laboratory studies where horses received a basic vaccination. Furthermore, there were no significant differences between the groups of this study (p>0.05).

The unvaccinated control horse remained sero-negative against influenza throughout the observation period, indicating the absence of concurrent field infections with influenza virus. All vaccinated horses developed antibody titres against all influenza strains present in the vaccine far above the level required for clinical protection (HI = 6 log2) and comparable to those observed in earlier studies.
Based on the results presented in this study the following conclusions can be drawn with regard to protection against tetanus intoxication and influenza: Concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet will lead to a passive protection against tetanus for at least 21 days after concurrent administration. No negative influence on the development of HI antibody titres could be seen after concurrent administration of Equilis Prequenza Te and Tetanus Serum Intervet. Development of active immunity against tetanus is negatively influenced by concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet. An appropriate statement is included under section 5.8 “Posology and method of administration” of the SPC.

**Duration of Immunity against influenza and tetanus induced after vaccination with Equilis Prequenza Te and Equilis Prequenza**

The aim of the study was to assess the duration of immunity against tetanus and influenza induced by Equilis Prequenza-Te after the basic vaccination course and the first revaccination. Clinically healthy influenza- and tetanus-seronegative foals at an age of 5 – 9 months were included in the study. One group was i.m. vaccinated with one dose of Equilis Prequenza Te at time points = 0, 4 (second vaccination of basic vaccination course), 26 (first booster) and 129 (second booster) weeks. At time point= 78 weeks, they were i.m. vaccinated with one dose of Equilis Prequenza. Another group was left unvaccinated to serve as contact controls.

Before, 1, 2, 4 and 6 weeks after the first vaccination and at regular preset time points after the second vaccination, blood samples were taken from all animals to determine the serological response to influenza and tetanus. Antibody levels against tetanus were determined by means of the ToBI-test.

**Tetanus**

Prior to first vaccination and one week after first vaccination, no antibodies against tetanus were detected. Two weeks after the basic vaccination course, ToBI titres rose to high levels. These titres dropped to ToBI levels far above the limit required for clinical protection at the time of the first booster vaccination, but increased two weeks after the first booster vaccination. From two weeks after the first revaccination, peak antibody titres against tetanus started to drop to lower levels reaching plateau values several months prior to the second booster vaccination. The plateau value found was far above the level required for clinical protection. After the second booster vaccination, antibody titres against tetanus increased again. It was concluded that horses are protected against tetanus for at least 24 months after the basic vaccination and first revaccination course.

**Equine Influenza**

The detected antibody titres showed protective antibody levels for all three influenza strains beginning with week 6 onwards up to the end of the study at week 131. In the period between week 16 and week 26 (= V3) the number of vaccinates with titres (H3N8 subtype) near the minimum protective level increased.

**Efficacy of Equilis Prequenza Te in horses against a challenge with influenza A/Equine-2/South Africa/04/03 after the basic vaccination course**

The objective of the study was to test the efficacy of Equilis Prequenza Te against the A/Equine-2/South Africa/04/03 strain. Influenza- and tetanus-seronegative yearlings were included in the study. Horses were either vaccinated with two doses of Equilis Prequenza Te four weeks apart or served as untreated control animals. Three weeks after the administration of the second vaccine dose, all yearlings were challenged intranasally by aerosol with strain A/Equine-2/South Africa/04/03.

At the day of challenge, the average titre in the vaccination group was as high as seen in other studies. The control yearlings remained seronegative against influenza up to the day of challenge. In the vaccination group, only very mild signs of disease occurred after challenge. Within the group of controls all animals developed clear signs of influenza. Virus isolation from nasal swabs was successful over a period of one week with high titres within the controls. In contrast, in the vaccination group 70 % of vaccinates shed virus for only one day with low titres.
FIELD TRIALS

A Positive Controlled Field Safety and Efficacy Trial of Equilis Prequenza Te in Foals

The aim of the study was to determine the efficacy of Equilis Prequenza Te in foals of different breeds reared under field conditions on different farms by comparison of the test vaccine with a positive control vaccine. Foals aged 2 to 10 months were assigned to 2 groups and received a basic immunisation course by administering one dose of vaccine i.m. into the neck on day 0 and another dose of vaccine at day 28 of the trial. Group A received the Equilis Prequenza Te and group B the competitor vaccine.

Equine Influenza

Foals of group A developed high levels of antibody titres against all 3 influenza strains of the vaccine. The foals with maternal antibody titres against all influenza strains at the beginning of the trial did not reach the antibody levels of the seronegative foals where an antibody increase was found comparable to the induced antibody levels of the foals used for the laboratory trials. When comparing group A and B the antibody levels reached against the Prague strain were nearly identical. Regarding the antibody levels against the 2 Newmarket strains the antibody increase was higher in Group A compared to group B, but this could be related to different strains which are incorporated in the vaccines used. Descriptive statistics were used to summarise the data.

In the Equilis Prequenza Te group 63 % animals responded to vaccination; in the control vaccine group 43 % responded. All foals with an Influenza HI titre < 4.0 showed a response to vaccination. However, in some foals with high levels of maternal antibodies at the moment of the first vaccination an increase in HI influenza titre did not occur. In some cases foals without detectable maternally derived antibodies at the first immunisation responded poorly to the vaccination. The results obtained from the competitor product showed the same tendencies so the problems are not product-related.

Tetanus

Regarding the efficacy of Equilis Prequenza Te against tetanus in the field, serum samples obtained within 15 days before admission of the study, 7 days after the first vaccination, at the time of second vaccination 4 weeks after the first vaccination as well as 2 and 4 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test.

Not all serum samples were available. All foals were supposed to be seronegative to tetanus at the time of first vaccination, however at 7 days after the first vaccination some foals had low levels of antibodies against tetanus.

Two and 4 weeks after the second vaccination, individual ToBI titres showed a great variance, but were all far above the level indicative for protection against tetanus. The geometric mean titre reached two weeks after basic vaccination in the Equilis Prequenza Te group was significantly reduced compared to results obtained in the other laboratory efficacy studies 2 weeks after the second vaccination. In conclusion, Equilis Prequenza Te induces protective serum antitoxin titres to tetanus two weeks after basic vaccination in seronegative foals kept and vaccinated under field conditions.

Five additional field trials for the demonstration of efficacy of Equilis Prequenza Te under field conditions were presented:

A field safety and efficacy trial of Equilis Prequenza Te and competitor product in horses

The objective of the study was to assess the safety and efficacy of Equilis Prequenza Te in horses under field conditions in comparison with the positive control product. The trial was conducted in compliance with GCP guidelines. The study was not blinded, but determination of antibody titres was performed under blinded conditions. Clinically healthy horses at an age of 5 - 41 months, were randomly separated into 3 groups. One group was vaccinated with one dose of Equilis Prequenza Te, one group was vaccinated with one dose of the positive control and the third group was not vaccinated.
The horses were vaccinated i.m. in the neck on days 0 and 29 and were monitored for local and systemic reaction up to day 57.

**Tetanus**
Individual ToBI titres at admission in the three treatment groups varied and as most horses in each group were seropositive for tetanus, the study was not suitable for the evaluation of efficacy of Equilis Prequenza Te against tetanus in horses under field conditions.

**Equine Influenza**
HI titres of 4 and below were considered as negative. It is concluded from the data that Equilis Prequenza Te is an efficacious vaccine against equine influenza. The titre in the Equilis Prequenza Te group was higher compared to that detected in the control group. At the end of the basic immunisation course, the young foals of the trial showed a higher antibody titre against the influenza vaccine strains compared to the older animals used in the trial.

**Field Trial with Equilis Prequenza Te and Equilis Te**
The aim of the GCP compliant study was to compare the sero-response to the tetanus component of Equilis Prequenza Te and Equilis Te in horses under field conditions. The animal phase of the study was not blinded, but determination of antibody titres was performed under blinded conditions. Clinically healthy horses at an age of 4 - 13.3 years were used.

Two groups were vaccinated with one dose of Equilis Te, one group with one dose of Equilis Prequenza Te. The horses were vaccinated with an interval of 4 weeks i.m. in the neck. Blood samples were taken on day 0 just before first vaccination and on day 14, 28 (prior to second vaccination), 42 and 56 after the first vaccination. Prior to first vaccination, 57% of horses vaccinated with Equilis Te and 71% of horses vaccinated with Equilis Prequenza Te were seronegative to tetanus.

Fourteen days after the first vaccination, the ToBI titres of all horses were clearly above the level indicative for protection. After second vaccination, a rapid increase in ToBI titres was observed in seronegative animals of both test groups.

In horses that were seropositive to tetanus prior to first vaccination, Equilis Te and Equilis Prequenza Te induced comparable ToBI titres against tetanus. In horses that were seronegative to tetanus prior to first vaccination, the geometric mean ToBI titre of the horses vaccinated with Equilis Te was considerably higher compared to the geometric mean titre of the horses vaccinated with Equilis Prequenza Te.

**A positive controlled field efficacy trial of Equilis Prequenza Te in horses in The Netherlands**
The aim of the GCP study was to assess the efficacy of Equilis Prequenza Te in horses under field conditions compared to the competitor product. The animal phase of the study was not blinded, but determination of antibody titres was performed under blinded conditions. Clinically healthy horses of 14 breeds, and at an age of 1.2 – 25 years, were separated into 3 groups at 4 different sites. Per site, two animals remained unvaccinated to detect equine influenza field infections during the trial period (negative control group). The remaining horses were randomly assigned to 2 groups.

One group was vaccinated with one dose of Equilis Prequenza Te and the other group with one dose of the competitor control product.

Horses that had been vaccinated against influenza within the last 12 months received a single (booster) vaccination on day 0. If the previous vaccination was more than 12 months ago, the horses received a basic vaccination course consisting of 2 vaccinations with an interval of 28 days. Animals that received the basic vaccination course were bled on the following days: day 0 (just before first vaccination), day 7, 28 (prior to second vaccination), 42 and 56 after the first vaccination. Blood samples were taken on day 0 just before vaccination and on day 7, 14 and 28 for horses that received a single vaccination. Antibody levels against tetanus were determined by means of the ToBI test.
Results after basic vaccination course

At admission, the ToBI titres were similar in both vaccination groups. All horses showed an increase of ToBI titres after vaccination. Day 42 and day 56 after the basic vaccination, ToBI titres in the Equilis Prequenza Te group were significantly higher compared to the ToBI titres in the positive control group.

All horses that underwent the basic immunisation course responded to all influenza strains with protective antibody titres up to day 56. A significant difference between the two test vaccines could not be detected. Both vaccines induced protective levels of influenza antibodies in horses.

Results after single vaccination

All horses were seropositive prior to vaccination. In both vaccination groups, all but one horse showed an increase of ToBI titres after vaccination. ToBI titres in the Equilis Prequenza Te group did not differ significantly compared to the ToBI titres in the control group at all time points before and after vaccination.

The horses that received one booster vaccination responded with a mean influenza titre increase between 1.4 to 2.0 log₂ against all tested strains 14 days after the vaccination. A sufficient booster effect could be detected in adult influenza seropositive horses. A significant difference between the two test vaccines did not occur.

Both vaccines induced protective levels of influenza antibodies in horses. A sufficient booster effect could be detected in adult influenza seropositive horses. If the vaccine is administered to horses with high antibody titres, a further titre increase is induced.

It can be concluded that Equilis Prequenza Te is an effective vaccine against equine tetanus both in seronegative and seropositive horses.

A field trial to determine the seroresponse after vaccination with Equilis Prequenza and Equilis Prequenza Te in sero-negative horses

The objective of the study was to determine and compare the sero-responses for influenza after vaccination with Equilis Prequenza and Equilis Prequenza Te in horses. The serological response was measured against the influenza strains incorporated into the vaccines. At day 56, for two adult horses of the Equilis Prequenza Te group and one adult horse of the Equilis Prequenza group the level of protection for all strains was lower than seen in other studies. For strains Prague/56 and Newmarket/1/93 the induced antibody titres of the Equilis Prequenza group were higher compared to the Equilis Prequenza Te group.

It is concluded from the data provided that both vaccines are efficacious against influenza and that the titres induced by Equilis Prequenza are higher in comparison with Equilis Prequenza Te.

A Positive Controlled Safety and Efficacy Trial of Equilis Prequenza Te in Pregnant Mares

The aim of the study was to determine the efficacy of the vaccine against influenza in pregnant mares as well as the assessment of negative influences of vaccination on the ongoing pregnancy and foal delivery. Sufficient increase and the time of persistence of maternally derived influenza antibodies of the offspring was to be investigated under field conditions. Mares of different breeds and ages housed on different farms and pregnant for 3-9 months were randomly assigned to two groups; vaccinated with Equilis Prequenza Te or vaccinated with the control product. The mares which showed HI influenza antibody titres > 6 log₂ at the beginning of the trial received a single vaccination dose. All mares with lower titres were given two vaccination doses four weeks apart. All mares received an additional vaccination dose 4 to 5 weeks before the expected foaling date. The level of maternally derived antibodies in the foals was high. The decrease was very slow and a level of insufficient protection was reached from 5 months of age onwards.
Descriptive statistics were used to summarise the data. Differences in qualitative data (i.e. proportion of healthy newborn foals, incidence, nature and severity of local and systemic reactions) between the treatments were evaluated by non-parametric methods (Chi-square or Fisher exact test). Differences in antibody titres were evaluated by parametric methods (ANOVA).

The majority of the mares had a titre of < 6 against one or more strains at admission, and received a basic vaccination (i.e. two vaccinations with a 4 weeks interval). Within the Equilis Prequenza Te group, this was 72 % of the mares and 73 % within the control group. The remaining mares received a single vaccination.

HI-influenza antibody titres before first vaccination were significantly higher in the group of mares that received a single vaccination as compared to the mares that received a basic vaccination. After the pre-foaling vaccination the mares were analysed per treatment group because – within one treatment group - the HI influenza antibody titres in the single and basic vaccination groups were at the same level.

**Single vaccination**

Twenty-eight days after the first vaccination the mares of the Equilis Prequenza Te group showed significantly increased titres against all three strains compared to the titres on day 0. The mares of the control vaccine group showed a significant increase for Newmarket/1/93 on day 28 compared to the titre on day 0, but not for the other strains, Prague/56 and Newmarket/2/93. The HI-influenza antibody titres did not differ significantly between the Equilis Prequenza Te group and the control group.

**Primary vaccination**

Four weeks after the first vaccination (day 28) and 4 weeks after the second vaccination (day 42) the mares of both test groups showed a significant increase of titres against all three strains compared to the titres prior to vaccination on days 0 and 28. The HI-influenza antibody titres differed significantly between the Equilis Prequenza Te group and the control vaccine group on four occasions:

Two weeks after the pre-foaling vaccination the HI-influenza antibody titres of the mares of both test groups were significantly increased for all 3 strains compared to the titres prior to the pre-foaling vaccination. The HI-influenza antibody titres before and after the pre-foaling vaccination did not differ significantly between the Equilis Prequenza Te group and the control vaccine group.

The influenza HI titres of the foals in both treatment groups gradually declined with increasing age. The level of the HI-influenza antibody titres indicates that the mares were protected against influenza field infections during the whole trial period. It can be concluded that Equilis Prequenza Te is well tolerated and induces high HI-influenza antibody titres in pregnant mares.

The foals had high levels of maternally derived antibodies against equine influenza. At week 24 after birth, the age of first vaccination, in most of the foals the titres were still at a level that a sufficient active immunisation cannot be expected.

The main objective of the trial was the safety for the unborn foal. Therefore, the highest number of possible vaccinations was chosen. The information that a booster vaccination is administered 4 weeks before the expected foaling date, is relevant for the expected duration of maternally derived antibodies in the offspring of the dams which were treated in this manner.

The persistence of higher levels of maternal antibody that were found in 50-75% of the foals at the age of 5 months is directly related to the vaccination schedule applied. However, vaccination should not start earlier than 6 months of age unless levels of maternal antibody in foals have been determined. This is reflected in the SPC.

**Duration of immunity against tetanus under field conditions**

The natural log transformed data of the average concentrations found in the laboratory trials were plotted vs time. This was to address the question, whether duration of immunity against tetanus...
indicated to be at least 17 months after basic vaccination, will also be obtained for animals with a low tetanus antibody titre immediately after the basic vaccination course with Equilis Prequenza Te in the field, and to get some insight into the kinetics of the tetanus serum antibody concentration after vaccination. From this, it appeared that there was a biphasic decline. The half-life of the fast decline was approximately 4.7 weeks and of the slow decline approximately 33.3 weeks. The calculated half-lives allow a prediction of what for a given value at onset of immunity the final concentration at 72 weeks (i.e. point of 2nd revaccination) would be. For two serum samples from vaccinated foals with low ToBI titres after basic vaccination, the predicted decline was plotted until 76 weeks (17 months). Although both values are very low the data provide an indication that both foals are protected against tetanus until after 17 months after basic vaccination.

The prediction model was substantiated with additional ToBI titres of some horses used in other field efficacy studies. Thus, the duration of immunity against tetanus after the basic vaccination course (17 months) as well as the duration of immunity after the first booster vaccination (24 months) is considered to be demonstrated after vaccination with Equilis (Prequenza) Te under field conditions.

**Overall Conclusion on Part IV**

Efficacy studies have been carried out in the target species, the horse, by the recommended route of administration (intramuscular). All efficacy studies were performed with batches of Equilis Prequenza Te containing standard amounts of influenza antigens (A/equine-1/Prague/1/56 = 100 AU, A/equine-2/Newmarket/1/93 and A/equine-2/Newmarket/2/93 = 50 AU each), tetanus toxoid (40 Lf) and adjuvant (375 μg) in one dose of 1 ml.

**Equine Influenza components**

The influenza vaccine strains of Equilis Prequenza Te are in accordance with the actual recommendation of the OIE. The presented studies are undertaken in accordance with the Ph.Eur. monograph 0249. The test batches of the vaccine used in the efficacy trials were produced as for batches for the market. So the amount of antigen and adjuvant was not adjusted to minimum level. The use of batches with standard amounts of antigen and adjuvant is acceptable for the efficacy trials.

The development of antibodies and the outcome of the challenges undertaken demonstrate good efficacy of the vaccine. If any signs of equine influenza occur after infection, they are very mild and only a small amount of virus shedding is expected. Complete recovery of the horse will only take a few days. This is in accordance with the SPC. The chosen immunisation scheme for the vaccine with two vaccinations 4 weeks apart followed by a third vaccination 5 months later with yearly booster vaccinations afterwards is sufficient. The possibility of alternating revaccination with Equilis Prequenza Te and Equilis Prequenza is supported as indicated in the SPC section 5.8. According to the findings of the field trial undertaken in pregnant mares and their offspring the vaccine is fully efficacious in pregnant mares.

Should the vaccine product be changed, no renewed basic immunisation is necessary.

Foals born to mares with high influenza antibody titres after an immunisation in the late stage of pregnancy developed a high level of maternally derived antibodies. This provides an excellent protection for the first months of life. However, antibody levels can persist up to an age of 5 months, therefore the age of the first vaccination was fixed to 6 months. Foals born to unvaccinated mares or foals with known low levels of maternally derived antibodies are recommended to start the immunisation at 3 months of age.

The safety data have shown that Equilis Prequenza Te can be administered to foals aged 2-4 months old. The laboratory efficacy trials and the field trials have shown that foals when free or having low levels of antibodies against equine influenza, from the age of 3 months can develop the same humoral immune response as e.g. adult horses.

A strain exchange related to the A-Equi-2 American lineage was discussed and at the current time (April 2005), a strain update of the vaccine is not considered necessary.
**Tetanus toxoid component**

It was demonstrated that basic vaccination (consisting of two vaccinations 4 weeks apart) of seronegative foals at an age of 5 months onwards with Equilis Prequenza Te led to serum antitoxin levels against tetanus toxoid considered to indicate protection against tetanus two weeks after basic vaccination until at least 17 months after basic vaccination.

All foals used in the laboratory and field efficacy studies were seronegative to tetanus at the time of first vaccination. Thus, the influence of specific maternal antibodies on the efficacy of Equilis Prequenza Te against tetanus could not be assessed. As the presence of specific maternal antibodies to tetanus is known to have a significant inhibitory effect on the development of active immunity against tetanus in young foals after active immunisation, an appropriate minimum age of vaccination was fixed at 6 months.

Regarding the concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet, a passive protection against tetanus for at least 21 days after concurrent administration has been demonstrated. Nevertheless, the development of active immunity against tetanus is negatively influenced by concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet, as antitoxin titres obtained 2 weeks after the basic vaccination were significantly reduced compared to results obtained in the other laboratory efficacy studies 2 weeks after the second vaccination. As this might have a negative influence on the duration of immunity against tetanus, it is proposed to include a third vaccination at least 4 weeks later.

Duration of immunity of Equilis Prequenza Te against Tetanus after first (and further) booster immunisation was demonstrated. Results from a study to assess the duration of immunity against tetanus induced by Equilis Prequenza Te after the basic vaccination course and the first revaccination show that protective serum antibody titres against tetanus persisted for 24 months after the first revaccination (V3) with Equilis Prequenza Te, given at 5 months after the basic vaccination course.

The duration of immunity (17 months) after the basic vaccination course as well as the duration of immunity (24 months) after the first booster vaccination is considered to be demonstrated after vaccination with Equilis Prequenza Te under laboratory and field conditions.
5. RISK-BENEFIT

Equilis Prequenza Te is an aqueous subunit vaccine intended for horses from 6 months of age, in order to protect them against equine influenza and tetanus. The vaccine contains purified haemagglutinin (HA) of three different strains of equine influenza virus, and tetanus toxoid prepared from toxin produced by Clostridium tetani. The 3 influenza strains represent 2 subtypes of influenza A virus. Subtype A/equine 1 (H7N7) virus strains have not caused major outbreaks of equine influenza since the 1970s. Both H3N8 subtype vaccine strains are antigenetically distinguishable and it has been recommended by OIE to include them in current influenza vaccines for horses in Europe and US.

The haemagglutinin subunits and the tetanus toxoid are formulated with iscom-matrix, a new innovative adjuvant. The iscom-matrix contains a purified saponin. The adjuvant has excellent immune-inducing properties and a good safety profile.

The analytical part is correctly documented, especially with regard to the production and control of the antigens and the control of the raw materials.

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

The potential risks of the use of this inactivated adjuvated subunit vaccine against equine influenza and tetanus in horses may be that the vaccine causes abnormal local or systemic reactions in the target animal and the risk of self-injection administering the product. Both of these have been addressed and suitable warnings included in the SPC. No negative influence on gestation, foaling and offspring of mares was observed after vaccination at different times during pregnancy. The vaccine is safe in animals regarded as most sensitive. After administration of a double dose and the repeated administration of one dose no serious adverse systemic or local reactions were observed. Each vaccine batch is tested for safety in the target animal before batch release. Appropriate warnings are indicated in sections 5.4, 5.9 and 5.12 of the SPC.

An assessment of the ecotoxicity risks showed that the overall risk of the vaccine to the environment, humans and other animals is minimal.

Equine influenza is highly contagious and spreads rapidly between horses causing disease of high morbidity but low mortality. The clinical signs are typical for an acute respiratory disease and can consist of serous and/ or mucopurulent nasal discharges, a harsh dry cough and pyrexia. Normally the clinical signs end within 2-3 weeks post infection. Depending on the region, vaccine coverage of horses with regard to equine influenza is estimated between 50-70%. The efficacy of vaccines against Equine influenza is monitored closely and surveillance programmes to detect new subtypes of equine influenza have been implemented.

The causative agent for tetanus is Clostridium tetani, a gram-positive, anaerobic, spore-forming bacillus, which produces a potent toxin that can cause spasticity and tetany of the skeletal muscle. This potent neurotoxin is produced during the anaerobic growth of the bacterium in dead tissues, e.g. in dirty wounds. The bacterium is a common inhabitant of the intestinal tract of humans and animals and is abundant in soil. Horses have a much higher susceptibility to the tetanus neurotoxin than other animals. Immunity to tetanus toxin is induced only by immunisation; recovery from clinical tetanus does not result in protection against further attacks. Therefore, in general tetanus prophylaxis should be incorporated into all equine health maintenance programmes.

Equilis Prequenza Te is indicated for active immunisation of horses from 6 months of age against equine influenza to reduce clinical signs and virus excretion after infection, and active immunisation against tetanus to prevent mortality.
Vaccination with Equilis Prequenza Te provides protection against challenge at 2 weeks after basic vaccination, 5 months after basic vaccination, and at 12 months after revaccination for influenza as well as at 2 weeks until at least 17 months after the basic vaccination course, and until at least 24 months after revaccination for tetanus.

The interference of maternal antibodies on the efficacy of inactivated vaccines against equine influenza and tetanus is well known. The data from the field trials presented in the dossier indicate that also the humoral response of Equilis Prequenza Te may be impaired by interference with maternal antibodies against influenza. Development of a humoral response of Equilis Prequenza Te in the face of maternal antibodies against tetanus has not been demonstrated. Field data and laboratory data indicate that young animals at the age of 6 months that are without maternal antibody against influenza or tetanus respond adequately to vaccination. Maternal antibodies to equine influenza and tetanus in the foal may persist up to 4-6 months of age depending on the amount of colostrum ingested shortly after birth and the immune status of the mare. In addition, it has also been recommended that foals born from mares vaccinated during the last 2 months of gestation should not be vaccinated before the age of 6 months.

Concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet will lead to a passive protection against tetanus for at least 21 days after concurrent administration. Development of active immunity against tetanus is negatively influenced by concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet. No negative influence on the development of HI antibody titres could be seen after concurrent administration of Equilis Prequenza Te and Tetanus Serum Intervet. An appropriate statement has been proposed for point 5.8 of the SPC.

Based on the original and subsequent data presented, the Committee for Medicinal Products for Veterinary Use concluded by majority that the quality, safety and efficacy of Equilis Prequenza Te was considered to be in accordance with the requirements of Council Directive 2001/82/EC.