SCIENTIFIC DISCUSSION

PRODUCT PROFILE

Product Tradename:	ProteqFlu-Te
Product type:	Immunological
Applicant company (Name and Address):	Merial S.A.S., 29 Avenue Tony Garnier, 69007 Lyon, France
Applicant contact point (Name, telephone, e-mail, fax):	Dr. Jacques Lechenet, tel : 00 33 472 72 33 89, jacques.lechenet@merial.com, fax : 00 33 472 72 33 68
Active substances:	vCP2242 virus and vCP1533 virus and *Clostridium tetani* toxoid
Pharmaceutical form:	Suspension for injection
Strength:	5.3 log10 $FAID_{50}$ to 7.66 log10 $FAID_{50}$ for both vCP2242 and vCP1533 and ≥ 30 IU/ml for tetanus
Packaging and Package size:	Type I glass bottle, butyl elastomer closure and aluminium cap for the bottle
Route of administration:	Intramuscular use
Target species:	Horses
Withdrawal period:	Zero days
Therapeutic indication:	Active immunisation of horses of 4 months of age or older against equine influenza to reduce clinical signs and virus excretion after infection, and against tetanus to prevent mortality.

SCIENTIFIC DISCUSSION

I. Summary of the dossier

ProteqFlu-Te is a vaccine intended to protect against equine influenza and tetanus. It is a liquid vaccine (suspension for injection) containing containing 2 recombinant canarypox viruses expressing the haemagglutinin HA gene from the equine influenza virus strains A/equi-2/Ohio/03 (USA representative) and A/equi-2/Newmarket/2/93 (European representative), respectively, the tetanus toxoid and a carbomer adjuvant.

II.A. Qualitative and quantitative composition

II.A.1 – II.A.3 Qualitative and quantitative particulars

For 1 ml dose:

Active ingredients:
Influenza A/equi-2/Ohio/03 [H_3N_8] recombinant Canarypox virus (vCP2242)) ≥ 5.3 log_{10} $FAID_{50}$
Influenza A/equi-2/Newmarket/2/93 [H_3N_8] recombinant Canarypox virus (vCP1533) ≥ 5.3 log_{10} $FAID_{50}$
*Fluorescent assay infectious dose 50%

Clostridium tetani toxoid ≥ 30 IU/ml
** antitoxic antibody titre induced after repeated vaccination in guinea pig sera according to Ph. Eur.

Adjuvant:
Carbomer 4 mg

Container:
Type I glass bottle

Closure:
Butyl elastomer closure, sealed with an aluminium cap.

II.A.4 Development pharmaceutics

Choice of the strains:
Effective vaccination needs regular update of influenza vaccine strains. Two international surveys indicate that influenza A/equi-1 viruses have not been isolated anywhere in the world since 1980. Severe outbreaks of influenza in horses caused by influenza A/equi-2 viruses currently occur worldwide.

The following recommendations were made at the last WHO/OIE meeting in September 1995:
Influenza A/equi-2/Newmarket/2/93-like viruses should be included in the vaccines to represent the European group of H_3N_8 viruses.
Influenza A/equi-2/Kentucky/94-like viruses should be included in the vaccines to represent the American group of H_3N_8 viruses.

Such strains were selected by Merial at the time of first registration. However in 2004, the Expert Surveillance Panel recommended to replace the influenza A/equi-2/Kentucky/94 strain (American lineage) by influenza A/equi-2/Ohio/2003 or A/equi-2/South Africa/4/2003. The recommendation concerning the European lineage remained unchanged.

In accordance with this new recommendation, vCP1529 was substituted by the recombinant vCP2242 construct expressing the haemagglutinin gene from influenza virus A/equi-2/Ohio/03.

The use of a live vectored recombinant canarypoxvirus allows *in vivo* expression of the influenza inserted gene, without replication of the vector in mammalian species.

Toxoid component:
The *Clostridium tetani* strain, used to produce the toxoid component, has been used for many years for human and animal vaccines.

Production process:
The production process includes a purification step of the active ingredients, to improve safety of the product.

Choice of the antigen quantification method:
Quantification of the influenza active ingredients is achieved by an indirect immunofluorescence assay using monoclonal antibodies and expressed in $FAID_{50}$.
The method of quantification of the tetanus toxoid is the WHO method described in the manual for production and control of vaccines. It is based on determination of the flocculating titre.

Choice of the antigen concentration:
Influenza active ingredients:
Minimum protective titre: $10^{5.3}$ $FAID_{50}$ per strain
Maximum release titre: $10^{7.6}$ $FAID_{50}$ per dose and per strain

Tetanus toxoid:
Minimum protective content / titre: 30 IU

Choice of the adjuvant:
Carbomer is a classical and well tolerated adjuvant for equine vaccines. It also contributes to the stability of the vaccine.

II.B Description of the method of preparation of the finished product

The buffered physiological saline pH 7.1 is prepared by weighing, mixing and homogenisation of the different components, sodium chloride, disodium phosphate, monopotassium phosphate and water for injections and sterilised,
The appropriate volume of each active ingredient (vCP2242, vCP1533 and tetanus toxoid),determined from the titres measured at the end of production, is added to the buffered physiological salineand then mixed and stirred.
The carbomer solution is prepared by weighing, mixing and homogenisation of the different components: Carbomer, Sodium chloride and Water for injections. The solution is sterilised.
The necessary volume of this carbomer solution is slowly added to the blend buffered physiological saline/active ingredients, The bulk obtained is stored between +2°C and +8°C. Sterile bottles are filled, closed, capped and packaged.

II.C Production and control of starting materials

II.C.1 Starting materials listed in a pharmacopoeia

Starting material
Carbomer
Dipotassium phosphate
Disodium phosphate dihydrate
Disodium phosphate dodecahydrate
Embryonated eggs from a SPF flock
Formaldehyde
Gentamicin sulphate
Glutamic acid
Glycine
Lactose monohydrate
Potassium chloride
Potassium dihydrogen phosphate
Potassium hydroxide Purified water Sodium chloride
Sodium citrate
Sodium dihydrogen phosphate dihydrate Sodium hydrogen carbonate Trometamol (Trisaminomethane) Water for injections Glass containers for pharmac. use Butyl elastomer closure

II.C.2 Starting materials not in a pharmacopoeia

1) Starting materials of biological origin

Starting materials
AI: vCP2242
AI: vCP1533
AI: Clostridium tetani toxoid
Calf serum
Adult bovine serum
Pronase
SPF chicken embryo cells
TPB
Beef heart extract
Tryptone V
Casein peptides N3
Modified thioglycolate medium - Pancreatic digest of casein
Thioglycolate medium - Pancreatic digest of casein
Skimmed milk

Recombinant canarypoxvirus vCP2242:

Genetic engineering:
Starting materials:
Parental canarypoxvirus:
The parent strain was isolated in Germany and attenuated by passages in chicken embryo fibroblasts. At this stage, the strain is currently used in a vaccine (KANAPOX). It was then purified through successive plaque passages. One plaque isolate was selected and amplified through additional passages. This clone is not able to replicate in tissue culture cells derived from non-avian species. In particular, this virus is not able to multiply in equine cells.

Donor organisms:
Equine Influenza virus A/equi-2/Ohio/03,provides the haemagglutinin gene of interest.

Plasmids:
The structure of the plasmid is described as follows:
flanking arm C5R + H6 promoter + HA gene + flanking arm C5L

The construction of plasmid can briefly be described as follows:
The HA is a synthetic gene derived from the HA native sequence of the equine influenza virus (EIV) H3N8 Ohio/03 strain. The gene is ligated into the donor plasmid at the locus containing the right flanking arm, the promoter and the left flanking arm. The sequence of the insert is confirmed. The resulting plasmid is the donor plasmid.

Construction of the recombinant virus:
In vitro homologous recombination was used to generate the recombinant virus vCP2242. The recombination was performed by transfection of CEF with with plasmid subsequently infected with the parental ALVAC virus. The generated recombinant plaques were selected on the basis of hybridisation. One representative plaque was selected and amplified through passages in primary chick embryo cells. The selected recombinant was confirmed to be 100% positive for the synthetic HA insert and 100% negative for the C5 ORF (the latter indicating that no parental virus is present in the population).

Description :
The recombinant virus vCP2242 is a canarypoxvirus containing and expressing the HA coding sequence of equine influenza virus A/equi-2/Ohio/03 strain, inserted in each of its C5 locus, under the control of the H6 promoter from the vaccinia virus.

Control of homogeneity of the recombinant virus:
The control is performed using the immunoplaques assay technique.

Control of expression:
Appropriate expression of the HA gene product was checked by Western Blot technique using a pool of mouse monoclonal anti-EIV HA antibodies and peroxidase-conjugated goat anti-mouse antiserum.

Control of genetic stability of the recombinant virus:
Orthopoxvirus is known to be stable. Genetic and biological stability were studied for ALVAC-based vectors in permissive and non-permissive hosts. From the results obtained with ALVAC-based vectors, the ALVAC vector is stable.
Also sequence data showed a 100% homology to the original vCP2242 strain.

Controls on the Master Seed Virus:
Identity by using 2 monoclonal antibodies, one specific for the canarypoxvirus, and the other for the haemagglutinin from the.A/equi-2/Ohio/03 strain. Results were positive for canarypoxvirus and for the haemagglutinin from the Ohio strain, but negative for the haemagglutinin from the Newmarket/2/93 strain.

Viral purity:
Mammalian extraneous agents:
Cultures of various cells susceptible to equine, ruminant and porcine viruses, pestiviruses and rabies virus were performed. After amplification runs, no CPE (cytopathogenic effect) was observed at each passage and after staining at the end of the last passage; no haemadsorbing agent or haemagglutination was observed at the end of the last passage; no virus was detected by Immunofluorescence (IF).

Avian extraneous agents:
SPF chicks were inoculated with MSV and sera were collected and examined for the presence of antibodies. No seroconversion was observed. There was an absence of mycoplasma contamination in compliance with Ph. Eur. 2.6.7. . Absence of bacterial and fungal contamination in accordance with the Ph. Eur. test 2.6.1. Appropriate certificates of analysis were provided.

Recombinant canarypoxvirus vCP1533:

Starting materials:
Parental canarypoxvirus: see above, in paragraph dealing with vCP2242 component.
Donor organisms:
Equine influenza virus A/equi-2/Newmarket/2/93 provides the haemagglutinin gene of interest.
The flanking arms are provided by the plasmids, necessary to achieve *in vitro* homologous recombination.

Plasmids:
The structure of the plasmid is described as follows:
flanking arm C5R + H6 promoter + HA gene + flanking arm C5L
The construction of plasmid is described as follows: The HA gene is derived from the HA native sequence of the equine influenza virus (EIV) H3N8 A/equi-2/Newmarket/2/93 strain. The plasmid containing the HA native gene is digested and the isolated HA gene fragment is then ligated into the donor plasmid for the C5 locus containing the right flanking arm, the promoter and the left flanking arm. The sequence of the insert (promoter + HA gene) is confirmed. The resulting plasmid is then used as the donor plasmid for generating vCP1533.

Construction of the recombinant virus:
In vitro homologous recombination was used to generate the recombinant virus vCP1533. The recombination was performed by co-infection of CEF with parental virus ALVAC and plasmid linearised. During this process, insertion of the expression cassette and deletion of the corresponding open reading frame occurred. The generated recombinant plaques were selected on the basis of hybridisation. One representative plaque was selected and amplified through passages in primary chick embryo cells. Due to the fact that the genome structure of the poxvirus contains 2 loci, vCP1533 carries subsequently 2 copies of the expression cassette.

Description:
The recombinant virus vCP1533 is a canarypoxvirus containing and expressing the HA coding sequence of Equine influenza virus A/equi-2/Newmarket/2/93 strain.

Control of homogeneity of the recombinant virus:
vCP1533 was cultivated on CEF monolayers. The infected monolayers were processed by plaque immunoassay for detection of expression of HA.

Control of expression:
Appropriate expression of the HA gene product was checked out by immunoprecipitation analysis using a monoclonal antibody**.**

Control of genetic stability of the recombinant virus:
The genomic stability of vCP1533 was controlled by sequencing the HA expression cassette and insertion locus flanking arms, cultivated on CEF monolayers. Virus vCP1533 was propagated in CEF

cells. Viral genomic DNA was isolated and the region of interest amplified by PCR. Sequence analysis revealed that the vCP1533 is identical to the original vCP1533.

Controls on the Master Seed Virus:
Identity by using monoclonal antibodies, for the canarypoxvirus, for the haemagglutinin from the A/equi-2/Newmarket/2/93 strain, and from the A/equi-2/Ohio/03 strain was carried out.

Viral purity:
Mammalian extraneous agents:
Cultures of various cells susceptible to equine, bovine and porcine viruses, pestiviruses and rabies virus were performed, in the same way as for the Kentucky strain. After amplifications runs, no CPE was observed at each passage and after staining at the end of the last passage; no haemadsorbtion or haemagglutination was observed at the end of the last passage, no virus was detected by IF or IP.

Avian extraneous agents: SPF chicks were inoculated with MSV and sera were collected and examined for the presence of antibodies in the same way as for the Kentucky strain. No seroconversion was observed.

Absence of bacterial and yeast contamination was shown by general and specific tests. There was absence of mycoplasmic contamination.

Appropriate certificates of analysis were provided for the Master Seed Virus and the Working Seed Virus.

Controls on the Active Ingredient:
By determination of the $FAID_{50}$ titre (Fluorescent Assay Infectious Dose 50%) in infected chicken embryo cells, through detection of the specific hemagglutinin (HA) by an indirect immunofluorescence assay using monoclonal antibodies. Absence of bacterial and yeast contamination was shown in accordance with the Ph. Eur. test 2.6.1. Appropriate certificates of analysis were provided.

Tetanus toxoid:
The following details are given:
Origin and history: the current strain is derived from the Harvard 49205 strain, a highly toxigenic strain, initially isolated in 1943.
Controls: they can be summarised as follows:

Level of production	**Type of controls**
On the Master Seed Bacteria	Purity Identity
On the Working Seed Bacteria	Purity Identity
Industrial culture	Purity
Purified tetanus toxoid	Free formaldehyde assay Protein nitrogen assay Flocculating titre Antigenic purity Sterility test Specific toxicity Irreversibility of tetanus toxoid

Additional information of interest :
Purity and identity are checked through growth characteristics, staining and biochemical characteristics of the strain.

The conditions of culture comply with the Ph. Eur. monograph No. 0452.

Flocculating titre (toxoid content): a calibrated anti-tetanus serum is used, in compliance with WHO standards.

Certificates of analysis were provided for all substances.

Beef heart extract, **Casein peptides N3**, **Tryptone V:**
Certificates were provided for each of the substances.

Bovine serum:
One certificate of analysis was provided and was in accordance with the corresponding monograph.

Pronase:
A certificate of analysis is provided and was in accordance with the monograph.

SPF chicken embryo cells:
The following details were given: Origin: SPF flock. A certificate is not necessary because cell concentration is only an industrial matter.

Tryptose phosphate broth:
A certificate of analysis was provided, in accordance with the monograph.

2) Starting materials of non-biological origin

Starting material
Ammonium sulphate
Buffered physiological saline pH7.1
antifoam soluble
Hydrochloric acid
Lactoglutamate buffer
Modified thioglycolate medium
PBS
Stabiliser
Thioglycolate medium
TRIS buffer pH 9
F10-199 medium
Massachusetts medium

Ammonium sulphate, antifoam soluble:
Certificates were provided for each of the substances.

Hydrochloric acid 1N:
A certificate of analysis was provided, in accordance with the monograph.

Thioglycolate medium:
Composition is given. Certificates of analysis were provided.

F10-199 medium:
Composition and preparation are given.

Massachusetts medium:
Composition is given.
No certificate of analysis is provided.

Modified thioglycolate medium:
Composition is given. Certificates of analysis are provided.

Buffered physiological saline pH 7.1:
Composition and preparation are given.
A certificate of analysis is provided, in accordance with the monograph.

TRIS buffer pH 9:
Composition and preparation are given.

Lactoglutamate buffer:
Composition and preparation are given.
A certificate of analysis is provided, in accordance with the monograph.

PBS (without Calcium and Magnesium):
Composition and preparation are given.
A certificate of analysis was provided, in accordance with the monograph.

Stabiliser:
Composition and preparation are given.
A certificate of analysis is provided, in accordance with the monograph.

II.C.3 Production

vCP2242 and vCP1533:
Each virus (vCP2242 and vCP1533) is cultivated in the same manner. The recombinant clone is directly used for the preparation of the Master Seed Virus (MSV). The MSV was obtained by passage on SPF chicken embryo cells. The Working Seed Virus (WSV) was obtained by culture of MSV on SPF chicken embryo cells. It corresponds to the 4th passage at most from the MSV. A seed lot system is used for the preparation of the active ingredient. Batches of active ingredient consist of the 5th passage at most from the MSV, cultured on SPF chicken embryo cells. The active ingredients can be stored for at most 24 months.

Tetanus toxoid:
Obtention of the inoculum: medium is inoculated with 1 ampoule of Working Seed Bacteria (WSB) and cultured; subcultures are prepared every day for passages, to enhance the multiplication capacity of the strain.

Scaling-up process from inoculum to industrial culture: a tube culture is used to inoculate a vial of medium, which is used to inoculate the other medium (2^{nd} and 3^{rd} precultures).

Industrial culture: the medium is used to inoculate fermentors; culture lasts for several days; at the end of the culture time, fermentors are cooled and solutions are added under stirring to allow extraction of the toxin.

Preparation of toxin: after extraction, the toxin is clarified; the supernatant is collected and the toxin is concentrated.

Detoxification: formalin is, under intermittent stirring during several days; then temperature is raised up for several weeks, which allows detoxification. After detoxification, storage can last for several weeks before further processing.

Purification of the toxoid: it is achieved through precipitation of the toxoid after concentration; the precipitate is collected by centrifugation.

Obtaining of the active ingredient: the precipitate is dissolved, and clarified by filtration. The volume is adjusted; pH is adjusted; the solution is finally sterilised by filtration. The tetanus toxoid can be kept at 5°C.

Possible reprocessing: if the specific toxicity test or the toxicity reversal test are not satisfactory, a new detoxification step is processed, the bulk is reprocessed through a complete purification process as described above.

II.D Specific measures to prevent TSE risk

A risk-benefit analysis for the whole product with regard to TSE is provided. The information is also summarised below. The updated table of starting materials not listed in a Ph. Eur. and of biological origin is shown below.

Starting materials of animal origin
AI: vCP2242
AI: vCP1533
AI: Clostridium tetani toxoid
Calf serum
F10-199 medium - Cholesterol
Adult bovine serum
Pronase
SPF chicken embryo cells
Tryptose Phosphate Broth (TPB)
Beef heart extract
Tryptone V
Casein peptides N3
Modified thioglycolate medium - Bacteriological meat extract - Pancreatic digest of casein
Thioglycolate medium - Pancreatic digest of casein
Skimmed milk

The risk assessment of the vCP2242, vCP1533 and *Clostridium tetani* seed lots was provided. From the analysis performed it can be concluded that the risk of transmitting TSE through the vCP2242, vCP1533 and *Clostridium tetani* strains can be considered as extremely low.

a. Starting materials of ruminant origin in the scope of TSE assessment

- Calf serum and adult bovine serum: the compliance with regard to TSE is shown through the certificate of suitability and the scientific documentation (with complementary information).
- Massachusetts medium is prepared locally with Beef heart extract. The compliance with regard to TSE is shown through the certificate of suitability.
- Modified thioglycolate medium is bought ready-made and contained Bacteriological meat extract. The compliance with regard to TSE is shown through the certificate of suitability

b. Starting materials of ruminant origin out of the scope of TSE assessment

- F10-199 medium contains cholesterol: obtained from whole grease, classified as a tissue with no detectable infectivity. The wool is collected from healthy sheep and is then chemically processed.
- TPB is derived from milk suited for human consumption.

- Massachusetts medium is prepared locally with 2 milk derivatives:
  → Casein peptides N3 are derived from milk suited for human consumption.
  → Tryptone V is derived from milk suited for human consumption.
  Finally, skimmed milk is derived from milk suited for human consumption.
- Modified thioglycolate medium is bought ready-made and contains Pancreatic digest of casein which is derived from milk suited for human consumption.
- Thioglycolate medium is bought ready made and contains only Pancreatic digest of casein derived from milk suited for human consumption.

c. Other starting materials of non-ruminant origin
- Collagen hydrolysate is of porcine origin.
- Pronase originates from Streptomyces griseus.
- SPF chicken embryo cells are prepared from SPF flocks not at risk. The preparation of the SPF chicken embryo cells uses calf serum and pronase.

The TSE risk can, therefore, be considered as minimal.

Conclusion on TSE risk
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) and Commission Directive 2001/82/EC as amended.

II.E In Process Control Tests

The results of the control test during preparation of batches vaccinesare presented.

II.F Control tests on the finished product

1) General characteristics of the finished product

The tests include a check of the appearance, the volume; and measurement of the pH.

2) Identification and assay of the active ingredient(s)

vCP2242 and vCP1533:
By determination of the $FAID_{50}$ titre (Fluorescent Assay Infectious Dose 50%) in infected chicken embryo cells, through detection of the specific hemagglutinin (HA) by an indirect immunofluorescence assay using monoclonal antibodies.

Tetanus toxoid:
In compliance with the assay "potency" of the Ph. Eur. monograph 697 - "Vaccinum tetani ad usum veterinarium", test A.

3) Identification and assay of the adjuvants

Identification of adjuvant.

4) Safety tests

In compliance with Ph. Eur. monograph 697.

5) Sterility and purity tests

Bacterial, fungal and mycoplasmic sterility, in compliance with Eur. Ph. monographs 2.6.1 and 2.6.7.
Viral purity.

6) Inactivation

Not applicable.

7) Residual humidity

Not applicable.

8) Batch-to-batch consistency

The results of 3 batches were presented.

II.G Stability studies

Batches, complying with the acceptance criteria on finished product, were tested for stability. The stability study is still ongoing and results will be provided upon completion. Thus, a provisional shelf-life of 18 months was accepted.

II.H GENETICALLY MODIFIED ORGANISMS

H. I - INFORMATION RELATING TO THE GMO

A - CHARACTERISTICS OF (A) THE DONOR, (B) THE RECIPIENT OR (C) PARENTAL ORGANISM(S)

1 - scientific name
Canarypox virus (cPV). ALVAC is the designation given to a plaque-purified clone of an attenuated licensed vaccine against poxvirosis of canaries derived from Canarypox virus.

2 - taxonomy
Family : Poxviridae
Subfamily : Chordopoxviridae
Genus : Avipoxvirus
Species : Canarypox virus
Clone : ALVAC

3 - other names (usual name, strain name, etc.),
ALVAC vector is also designated CPpp (CanaryPox plaque purified).

4 - phenotypic and genetic markers
Poxviruses are distinguished mainly by their morphology (large size 220-300 nm x 140-170 nm, and a characteristic ovoid or brick-like shape of the virion), large double-stranded DNA genome and cytoplasmic site of replication. ALVAC vector is a highly attenuated canarypox virus. ALVAC vector exhibits a restricted host range for productive replication to avian species. The ALVAC vector and the derivative recombinants were shown not to replicate in tissue culture cells derived from non-avian species and attempts to adapt the virus for replication by serial passages were unsuccessful, confirming the historical classification of avipoxviruses based on restricted host range.

5 - degree of relatedness between donor and recipient or between parental organisms
The haemagglutinin (HA) genes inserted in the vCP2242 and vCP1533 recombinant viruses are derived from the A/equi-2/Ohio/03 and the A2/Newmarket/2/93 (H_3N_8) equine influenza viruses, respectively. There is no evidence of any relationship between ALVAC vector (Poxviridae) and equine influenza viruses (Orthomyxoviridae). Moreover, the genome of Poxviridae is DNA whereas the genome of Orthomyxoviridae is RNA.

Concerning the promoter inserted in the vCP2242 and vCP1533 recombinant viruses to drive expression of the HA gene, the greatest homology detected was around the region which is not located within the fragment studied.

6 - description of identification and detection techniques

A number of techniques can be used to detect and identify Poxvirus. Biological characteristics based on host-range, morphology and ceiling temperature of the pock produced on the chorioallantoic membrane of the developing chick embryo, provide a first means to detect and identify isolates of poxvirus. In particular, the large size of the poxvirus particle and the characteristic cytoplasmic inclusion bodies observed in sections of poxvirus infected cells provide criteria for virus identification. Thus, the technique based on the detection of a characteristic cytopathic effect caused by the canarypox virus in chicken embryo cells can be used to calculate the infective titre. Moreover, a number of tests such as restriction endonuclease mapping of the viral DNA, Polymerase Chain Reaction (PCR), ELISA or immunofluorescence using specific monoclonal antibodies and western blot provide an unequivocal way of identifying isolates. In Merial, release titration method can also be used to identify canarypox virus since it corresponds to an immunofluorescence assay using monoclonal antibodies, one being specific for the canarypox virus.

7 - sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques

The techniques of detection and identification used were shown to provide sensitive, reliable and specific identification of the virus.

8 - description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts

Avipoxviruses were detected in a variety of birds over the world. The host range of the canarypox virus seems limited to the canary and to some passerine birds (some members of the Fringillidae family). Moreover, the ALVAC vector itself was used to vaccinate cage birds and, is non pathogenic in the canary (the permissive host of the canarypox virus).

9 - organisms with which transfer of genetic material is known to occur under natural conditions

9.1 - Potential for genetic transfer and exchange between poxviruses: submitted elements confirm that potential for genetic transfer and exchange between poxviruses is negligible.

9.2 - Potential for genetic transfer and exchange with a virus related to the donor organism:
There are several levels of prohibition for recombination between a canarypox virus and an influenza virus. In conclusion, genetic transfer and exchange involving the GMOs with other organisms are highly unlikely.

10 - verification of the genetic stability of the organisms and factors affecting it

Orthopoxvirus is known to be stable. Genetic and biological stability were studied for ALVAC-based vectors in permissive and non-permissive hosts. From the results obtained with ALVAC-based vectors, the ALVAC vector is stable.

11 - pathological, ecological and physiological traits:

11.a - classification of hazard according to existing Community rules concerning the protection of human health and/or the environment

In France, the ALVAC vector was classified class 1, group I, according to Directive 90/219/EEC by the "Commission du Génie Génétique". In the USA, due to the safety profile of the ALVAC strain, the Recombinant DNA Advisory Committee (RAC) of the National Institute of Health (NIH) reduced the biological containment condition for this virus from BL-2 to BL-1.

11.b - generation time in natural ecosystems, sexual and asexual reproductive cycle
Virulence is highly reduced in canaries and the virus undergoes an abortive replication in non-avian species.

11.c - information on survival, including seasonability and the ability to form survival structures
Since the ALVAC vector (highly attenuated) does not replicate in non-avian species, its survival in mammals is short and its ability to form survival structures is excluded. The chances of survival of the ALVAC vector in permissive or nonpermissive hosts are negligible.

11.d – pathogenicity, infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms.
Observations demonstrate the absence of pathogenicity of the ALVAC vector.

11.e - antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy
Not applicable.

11.f - involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.
Not applicable.

12 - Nature of indigenous vectors: a) – sequence, b) frequency of mobilisation, c) specificity, d) presence of genes which confer resistance
Not applicable.

13 - History of previous genetic modifications
ALVAC is a naturally attenuated vaccine and is also the recipient for vaccines already licensed. Moreover, as already mentioned, a number of ALVAC-based vaccine candidates have been tested in animals and/or human volunteers.

B. CHARACTERISTICS OF THE DONOR

vCP2242 and vCP1533, both consisting of a recombinant canarypox virus expressing the haemagglutinin gene from equine influenza virus A/equi-2/Ohio/03 (H_3N_8) and equine influenza virus A2/Newmarket/2/93, respectively.

1 - Equine Influenza viruses

1.1 - nature and source of the vector

Family	: Orthomyxoviridae
Genus	: Influenza virus
Species	: equine influenza virus
Strain for vCP2242	: A/equi-2/Ohio/03 (H_3N_8)
Strain for vCP1533	: A2/Newmarket/2/93 (H_3N_8)

Equine influenza virus is a replication-competent virus consisting of an envelope with large peplomers surrounding a helical nucleocapsid. Two proteins are found in the envelope, the haemagglutinin (HA) and the neuraminidase (NA). The HA-encoding gene is the one (and only one) used here.

C. CHARACTERISTICS OF THE MODIFIED ORGANISM

vCP2242 and vCP1533, both consisting of a recombinant canarypox virus expressing the haemagglutinin gene from equine influenza virus A/equi-2/Ohio/03 and equine influenza virus A2/Newmarket/2/93, respectively.

1 - Information relating to the genetic modification:

1.a - methods used for the modification, 1.b - methods used to construct and introduce the insert(s) into the recipient or to delete a sequence, 1.c - description of the insert and/or vector construction
These methods are described in part.1.d - purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function.

1.d - purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function
The donor plasmids are limited to the expression cassette surrounded by flanking arms of canarypox origin. The identity of the donor plasmid used was analysed by restriction endocnuclease mapping and sequencing. Homogeneity of the population was amongst other tests confirmed by an immunological assay, which detects expression of the inserted gene at the plaque level. The results showed that the stock is homogenous for the expression of HA gene product. Restriction endonuclease mapping and Southern blotting analyses of viral DNA indicated appropriate insertion of HA coding sequence.
The correct sequence of the gene inserted into vCP2242 and vCP1533 was reconfirmed by sequence analysis.

1.e - methods and criteria used for selection
See 1.d

1.f - sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question with particular reference to any known harmful sequence
For both vCP2242 and vCP1533, the inserted fragment corresponds to the expression cassette constituted by the HA gene placed under the control of the promoter. Its correct sequence was reconfirmed by sequence analysis.

2 - Information on the final GMO:

2.a - description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed
vCP2242 : Recombinant virus vCP2242 carries the haemagglutinin gene from A/equi-2/Ohio/03 equine influenza virus. Appropriate processing of the haemagglutinin gene product was confirmed.

vCP1533: Recombinant virus vCP1533 carries the haemagglutinin gene from A2/Newmarket/2/93 equine influenza virus. Appropriate processing of the haemagglutinin gene product was confirmed.

2.b - structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism
Not applicable for both vCP2242 and vCP1533: no plasmid sequences were transferred in the final GMOs.

2.c - stability of the organism in terms of genetic traits
Both recombinant viruses retained the inserted haemagglutinin gene with high stability along *in vitro* passages.

2.d - rate and level of expression of the new genetic material. Method and sensitivity of measurement
The rate and level of expression of each HA glycoprotein, is sufficient to induce an immune response, which is protective against an equine influenza virus challenge.

2.e - activity of the expressed protein(s)
Both newly expressed HA glycoproteins act as immunogen and induce a protective immune response against an equine influenza virus infection. This was shown following challenge in ponies vaccinated with vCP1533. Efficacy conferred by vCP1529 was adequately demonstrated by serology and challenge. vCP2242 being very close to vCP1529, a similar behaviour is expected. This was confirmed by s bioequivalence efficacy study.

2.f - description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector
Detection of a characteristic cytopathic effect. Indirect immunofluorescence using specific monoclonal antibodiesand the HA transgenes and the canarypox virus backbone.
Expression of the different HA glycoproteins can be specifically identified and quantitated using monoclonal antibodies.

2.g - sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques
Technique was shown as sensitive, reliable and specific.

2.h - history of previous releases or uses of the GMO
vCP2242 and vCP1533 were experimentally used in a number of species to determine safety and efficacy. vCP2242 and vCP1533 were used for field trials.

2.i - considerations for human health and animal health, as well as plant health

2.i.(i) - toxic or allergenic effects of the GMOs and/or their metabolic products
Not applicable: vCP 2242 and vCP 1533 have no toxic or allergenic effects.

2.i.(ii) - comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity
ALVAC vector was demonstrated to be safe in a number of species: human, canary bird, duck, chicken, cat, dog, cow or cattle, horse, pig, mouse, guinea-pig, rabbit. The vCP2242 expressing the HA gene from A2/Kentucky/94 was demonstrated safe in all tested species,. As the only difference between vCP1529 and vCP2242 is the haemagglutinin expressed, it is expected that both vCP have the same safety profile. This has been confirmed by additional studies carried out with the vCP2242 in horses, canary birds and dogs.

2.i.(iii) - capacity for colonisation
Not applicable: ALVAC and ALVAC-based vectors including vCP1529 and vCP1533 are not replicative in non-avian species.

2.i.(iv) - if the organism is pathogenic to humans who are immunocompetent
Not applicable: ALVAC and ALVAC-based vectors including vCP2242 and vCP1533 are not replicative in humans. Moreover, a number of ALVAC-based vaccine candidates have been inoculated into human volunteers.

2.i.(v) – other product hazards
Considering the submitted information the likelihood of hazards occurring is therefore negligible.

H. II - INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT

A - INFORMATION ON THE RELEASE

vCP2242 and vCP1533 are intended to protect domestic horses against A/equi-2/Ohio/03 and A2/Newmarket/2/93 equine influenza virus infection, respectively. Inoculation into the target organism is performed by the intramuscular route. Results obtained from experimental studies

including vaccination/challenge trials demonstrated the protective efficacy of vCP1533 against influenza in domestic horses. Together with the inherent safety properties associated with the ALVAC vector, vCP2242 and vCP1533 included in vaccine range may provide a safe and effective influenza vaccine.

B - INFORMATION ON THE ENVIRONMENT (BOTH ON THE SITE AND IN THE WIDER ENVIRONMENT)

Not applicable.

H. III - INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT

A - CHARACTERISTICS AFFECTING SURVIVAL, MULTIPLICATION AND DISSEMINATION

1 - biological features which affect survival, multiplication and dispersal
vCP2242 and vCP1533 recombinant viruses do not replicate in non-avian cells, as demonstrated. Therefore, in the absence of replication, vCP2242 and vCP1533 cannot disseminate within the vaccinated horse, cannot disseminate from a vaccinated horse to a non-vaccinated contact or cannot contaminate the general environment, thus providing a significant safety margin. This was confirmed in a number of studies.

2 - known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.)
Predicted environmental conditions (wind, water, soil, temperature, pH ...) should not affect survival, multiplication and dissemination.

3 - sensitivity to specific agents
Poxviruses and therefore ALVAC-based viruses are readily inactivated by chlorhexidine, benzalkonium chloride, formaldehyde and bleach.

B - INTERACTIONS WITH THE ENVIRONMENT

1 - predicted habitat of the GMOs,
Domestic horses. However, there is no persistence in horses.

2 - studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses
In vitro, the lack of replication of vCP2242 and vCP1533 recombinant viruses in mammalian cells was demonstrated. *In vivo*, safety was demonstrated. Absence of spread was demonstrated.

3 - genetic transfer capability
The assessment of a genetic transfer is detailed in part A9.

4 - likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism
Likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism is negligible.

5 - measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability
Dispersal of genetic material is highly unlikely.

6 - routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.
vCP2242 and vCP1533 recombinant viruses do not replicate in non-avian species so that dissemination into non-avian species including horses cannot take place.

7 - description of ecosystems to which the GMOs could be disseminated
Although very unlikely as discussed above, the GMOs could be disseminated to canary (the only permissive host).

8 - potential for excessive population increase in the environment
Considering the submitted information the potential for excessive population of the GMOs in the environment should be excluded.

9 - competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s)
Considering the data listed above (in § B.8), and the results of an experimental study, a competitive advantage of the GMOs in relation to the unmodified parental organism is not expected.

10 - identification and description of the target organisms if applicable
The target organism is the domestic horse.

11 - anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable
Following inoculation into the domestic horse, vCP2242 and vCP1533 recombinant viruses undergo abortive replication.

12 - identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction
Because of the restricted host range to avian species of vCP2242 and vCP1533 recombinant viruses for productive replication, birds and particularly canary birds are the only, non-target organisms which may be affected unwillingly. However, this is highly unlikely since in the final vaccine, vCP2242 and vCP1533 recombinant viruses are submitted to multiple degrees of containment:

13 - likelihood of post-release shifts in biological interactions or in host range
Likelihood of post-release shifts in biological interactions or in host range is excluded in the absence of productive replication and in the presence of the multiple degrees of containment listed above.

14 - known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens
The chances that non-target organisms may be affected unwillingly is highly unlikely, because of the remarks listed above.

15 - known or predicted involvement in biogeochemical processes,
Not applicable, vCP2242 and vCP1533 are not involved in any biogeochemical process.

16 - other potential interactions with the environment.
None.

H. IV - INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

A - MONITORING TECHNIQUES

1 - methods for tracing the GMOs, and for monitoring their effects

The vaccine containing the GMOs will be prescribed by a veterinary surgeon only. If necessary, the GMO may be detected by culture on chicken embryo cells and inserts identified by immunofluorescence. Moreover, expression of the different HA glycoproteins can also be specifically identified and quantitated using monoclonal antibodies.

2 - specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques
The described techniques are specific, sensitive and reliable.

3 - techniques for detecting transfer of the donated genetic material to other organisms
If necessary, the GMOs may be detected by culture on chicken embryo cells and identified by indirect immunofluorescence assay.

4 - duration and frequency of the monitoring.
The vaccine containing the GMOs will only be prescribed by veterinary surgeons, which restricts any potential for release into the environment. Should the need for monitoring arise, this would be orchestrated by the competent authorities under Directive 2001/18/EC.

B - CONTROL OF THE RELEASE

1 - methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of release or the designated area for use
The vaccine containing vCP2242 and vCP1533 recombinant viruses is for animal use only (under prescription and surveillance of a veterinary surgeon). Moreover, it is submitted to several containment levels:
The vaccine is stored in hermetically sealed glass bottles.
Since the virus is replication-defective the intramuscular administration route provides a further natural containment barrier.

In conclusion, spread beyond the site of use or areas of designated release is, therefore, highly unlikely.

2 - methods and procedures to protect the site from intrusion by unauthorised individuals
Not applicable.

3 - methods and procedures to prevent other organisms from entering the site
Not applicable.

C - WASTE TREATMENT

1 - type of waste generated
Glass bottles and material used for injection
2 - expected amount of waste
1 vial per vaccinated horse.

3 - description of treatment envisaged
Waste material should be disposed off in accordance with local requirements.

D - EMERGENCY RESPONSE PLANS

1 - methods and procedures for controlling the GMOs in case of unexpected spread
Considering the data discussed previously, the probability of unexpected spread is very low. However, in case of unexpected spread, vaccination of horses could be suspended and previous vaccinated animals carefully looked after.

2 - methods for decontamination of the areas affected, for example eradication of the GMOs
In case of accident (broken syringes or bottles) or accidental contamination of surfaces (walls, ceilings, etc.), disinfection is carried out with bleach. In case of contamination of the skin of the animal or persons in charge of its restrain during the injection, the contaminated area will be treated, as the injection site, with classical disinfectants (70° ethanol solution, bleach).

3 - methods for disposal or sanitation of plants, animals, soils, etc., that were exposed during or after the spread
Due to the absence of spread, disinfection is sufficient.

4 - methods for the isolation of the area affected by the spread
Not relevant.

5 - plants for protecting human health and the environment in case of the occurrence of an undesirable effect
In case of accidental injection to man, a doctor will be notified for monitoring, if necessary. Indeed, the ALVAC vector by itself or as a recombinant virus was already used in humans (trials, at the phase I and III without observing any safety problem, even after several doses. A medical data system (human and veterinary) will be implemented. All events will be recorded and analysed in detail as well as their monitoring if any.

Conclusion
The analytical part is correctly documented, especially with regard to the biotechnological aspect and the control of the raw materials.

III. - SAFETY

III.A Introduction

Initial safety studies were conducted in the target species, in laboratory tests and in field trials, of various ages (from 3.5 months to 13 years in the laboratory, from 1 month to 40 years in the field) and physiological status (foals, non-pregnant female and male adults, pregnant mares). In particular, safety of the vaccine was tested on pregnant mares at various stages of pregnancy.

Three formulations of the vaccine corresponding to different levels of purification and different stabilisers were used in the laboratory and field trials. Equivalence of these formulations has been established. Various batches for equine influenza components and for tetanus toxoid were used, many of which were at very high titres.

Additional safety studies were conducted in the target species supporting the safety profile of the new formulated product following the substitution of the vCP1529 with the vCP2242 strain.

Although ALVAC-vectors are known to be non replicative in mammals, studies were undertaken to assess the capacity of the live viruses to spread, to disseminate, to replicate on the place of inoculation, to multiply *in vitro* and to recombine with another virus.

Ecotoxicity was assessed in compliance with the requirements of the Annex to European Directive 2001/18/EC, supported by several trials. In particular, safety studies were conducted in non-target species, involving the canary, duck, chicken, pig, mouse and guinea-pig.

III.B Laboratory tests

III. B.1 - 3 Safety of the administration of one dose, of one administration of an overdose, and of the repeated administration of one dose

Brief description of the first trial design:
Sixteen susceptible foals of approximately 4 months of age (3 ½ to 4 ¾ months) were used. Three different formulations of ProteqFlu-Te vaccines were used.

Examinations:
Blood sampling was performed on days D1 and D42. Clinical monitoring took place from day D1 to D42 and included general condition, local reaction, body temperature, and histology of the injection site.

Results:
Whatever the vaccination period, some animals showed local reactions at the site of vaccination of ≤ 1 cm for a maximum period of 4 days. No stiff neck, no pruritis, no cutaneous heat and no pain were recorded, except for 2 animals after the third vaccination period. No systemic reaction was registered, except for 1 animal in group (adjuvant only). On some occasions, a temperature increase > 39 °C (maximum of 40.5 °C in 1 animal) was registered mainly during the three days following vacination. The autopsy samples examined did not show any obvious abnormality, except in 1 case where a limited focally necrotic granulomatous myositis was observed.

Brief description of the second trial design:
Batch safety testing was used as follows:

Number of horses per batch testing	Vaccination scheme (IM administration)	Neck side	Number of doses injected
2	D0: overdose	Left Right	1 dose 1 dose
	D14: single dose	Left	1 dose

Of a total of 27 horses, older than 6 months, 13 were used several times, leading to repeated administration of overdoses/doses.

20 pilot batches of ProteqFlu-Te were used:

Examinations:
Clinical monitoring (general and local reaction) was carried out from day D1 to D3, from day D6 to D7, from day D14 to D17, from day D20 to D21 and on day D24. No rectal temperature measurements were performed.

Results:
Whatever the vaccination period, some animals showed mild and transient swelling at the injection site, which had mostly disappeared by day 3 post vaccination. No systemic reactions were recorded. Repeated administration of a dose/overdose did not increase the rate of adverse reactions. The virus titre seems to be correlated with the occurrence of local reactions, whereas the tetanus toxoid content seems to have no effect. The results show that the less purified vaccine formulation gave rise to a somewhat higher incidence of local reactions than the more purified one.

Brief description of the third trial design:
Vaccination with ProteqFlu-Te of 2 x 3 horses (5 females and 1 male) from 2 to 11 years of age, divided into 2 groups, as follows:

Group	Posology (IM administration)
1	Double dose (in one site) at D0
2	Single dose at D14

Examinations:
Blood sampling was performed on days D0 and D28. Clinical monitoring (including rectal temperature measurement) from D0 to D28, just before injection, 4 hours after injection and each of the following day: general and local reactions (scoring).

Results:
One animal of group 1 showed a very mild and transient swelling after first injection for one day; all three horses of group 1 showed mild and transient swelling after second injection for two days, whereas none of the second group showed any local reaction. The maximum swelling registered was 5.0 x 3.0 x 0.5 cm^3.
No systemic reactions were registered.
Brief description of an additional study performed with the new product formulation containing the vCP2242 strain instead of the vCP1529.
12 horses from 2 to 5 years of age were used in two groups: Group A consisted of 10 horses that were vaccinated on day 0 with 2ml, on day 28 with 1 ml and day 42 with 1ml. The total dose of recombinant virus was respectively 20 times the maximum global dose on D0and 10 times the maximum global dose on D28 and D42. . Group B consisted of 2 animals, which were not vaccinated.

Examinations:
Blood sampling was performed on days D0, D28, D42 and D56. Clinical monitoring on D0, D0+5/6h, D1 to D14, D28, D28+5/6h, D29 to D41, D42, D42+5/6h, D43 to D56 : general and local reactions (scoring).
Virus isolation was performed to whole blood samples from days D0, D0+5/6h, D1 and to urine, faeces, nasal secretion and saliva samples from days D0, D2, D4.

Results:
Moderate hyperthermia was recorded in 6 horses the day after the first injection. No hyperthermia was recorded after the second injection. One horse presented slight hyperthermia during 1 day after the third injection. No other general reaction was registered. Mild and transient swelling (not painful) were seen in 5 horses for up to 1 day after first injection, in 6 horses for 1 to 4 days after the second

injection, and in all horses for 2 days after the third injection. No other local reaction was registered; no virus isolation could be achieved. Serology: all horses were seronegative before vaccination. On D42 and D56, all vaccinated horses had in average high ELISA antibody titres against tetanus. On D56, all vaccinated horses had high SRH antibody titres against influenza. The controls remained seronegative to both tetanus and influenza.

Examination of reproductive performance

Brief description of the first trial design:
14 mares of approximately 1.5 to 12 years of age were used. They were vaccinated IM with ProteqFlu-Te at 8-9 months of pregnancy.

Examinations:
Blood sampling of the mares on days D0 and D14.
Blood sampling of the foals on days after foaling DF0 and DF30.
Clinical monitoring of the mares from D0 to D14, just before injection, 4 hours after injection and each of the following days: general and local reactions (scoring); temperature was recorded for 4-6 days after injection.
Close observation of the mares around the time of foaling.
Clinical monitoring (any adverse reaction) from DF1 to DF30 of the mares and the foals.

Results:
No general reaction was registered, except for 1 mare of group 2 which showed hyperthermia (39.3°C at D1). One mare of each group showed a mild and transient local reaction (maximum of 1.5 cm of diameter, 2 mm thick, lasting for 1 day - except for the mare of group 3 which showed a palpable and persistent, but hardly visible and not painful, local reaction). All foals were normal, except for 1 stillborn foal of group 3, dead probably because of aspiration of amniotic fluid (foal found covered by its amniotic sac). No macroscopic or histopathological lesions could be found. During the follow-up period DF1-DF30, 1 foal was unable to stand up on days DF8 and DF9, and died on DF10. A histopathological examination was carried out but proved to be inconclusive.

Brief description of the second trial design:
Twenty-one mares of approximately 2 to 13 years of age were used. Six of them were vaccinated IM with ProteqFlu-Te at more than 7 months of pregnancy and 15 at less than 7 months of pregnancy. Two different formulations of vaccines were used.

Examinations:
Blood sampling of the mares on days D0 and D14: Newmarket antibody titration on D-1 and D14 and tetanus toxoid antibody titration on D0.
Clinical monitoring of the mares from D0 to D14, just before injection, 4 hours after injection and each of the following days: general (scoring) and local reactions (scoring);
Temperature was recorded 4 hours after injection and every day during the 14 days after vaccination.
Close observation of the mares around the time of foaling.
Clinical monitoring (any adverse reaction) from DF1 to DF30 of the mares and the foals.

Results:
No general reaction was registered, except for 1 mare of group 2 which showed hyperthermia (38.9°C at D2, normal on next day). Four mares of group 1 (one at D0+4h and three at D1) and one mare of group 3 (on D1 and D2) showed a very mild local reaction ($\leq$ 5x5 cm^2). All foals were normal, except for 1 stillborn foal of group 1 (due to dystocia) and for 1 foal of group 2 found dead (probably due to dystocia). They both came from mares of category "< 7 months of gestation". No macroscopic or histopathological lesions could be found. During the follow-up period DF1-DF30, 1 foal of group 1 was recorded as being blind; 1 foal of group 1 was rejected by its mare and died from dehydration and malnutrition. Histopathological examination did not reveal any obvious cause, but an early septicemic process appeared to be the most likely cause of blindness.

Examination of immunological functions
The canarypox vector is not replicative. This vector, as well as all the other components of the vaccine, are not known for any adverse effect on immunogenicity.

Specific requirements for live vaccines

Spread of the vaccine strain:
In the studies presented below, no re-isolation of virus was possible from samples following vaccination. Thus, no spread is expected.

Dissemination in the vaccinated animal:

Brief description of the first trial design :
Four foals of approximately 9-10 months of age and 1 adult horse of 4 years of age were used.

Examinations:
Blood sampling was performed on days D0 and D35 to test for the presence of ELISA antibodies against the canarypox vector and IHA antibodies against both Influenza HA inserts.
Clinical monitoring: general and local reactions (scoring); temperature was recorded.
Tests for the presence of canarypox virus were carried out.

Results:
Hyperthermia was seen on some occasions in both groups 1 and 2 (around 39.2°C), without impairment of general condition; no other systemic reaction was observed. No local reaction was observed. All the samples from all the horses were found negative for virus isolation.

All the above supports the conclusion that canarypoxvirus does not disseminate.

Brief description of the second trial design:
Eight foals of 9 to 20 weeks of age were used.

Examinations:
Blood sampling was performed on days D0 and D35, to test for the presence of ELISA antibodies against the canarypox vector and IHA antibodies against both Influenza HA inserts.
Clinical monitoring: general and local reactions (scoring) just before injection, at 4-6 hours after injection and on days D1, D2, D3, D4, D7 and D14; temperature was recorded just before injection, at 4-6 hours after injection and on days D1, D2, D3, D4, D7 and D14.
Tests for the presence of canarypox virus were carried out.

Results:
All animals had high antibody titres at D0 against Influenza, declining during the course of the study despite vaccination. Hyperthermia was seen on some occasions in both groups 1 and 2 (around 39.2°C), without impairment of general condition; no other systemic reaction was observed. No local reaction was observed. All the samples from all the horses were found negative for virus isolation.

The above results support the conclusion that canarypoxvirus does not disseminate.

Additional studies were conducted, following the substitution of vCP1529 strain with the vCP2242 strain to support the conclusion that canarypoxvirus does not disseminate.

i) Study on the *in vitro* replication of vCP2242 in avian and mammalian cells:
It was shown that vCP2242 cannot replicate in canine and equine cells, either primary cells or cell lines. Replication of vCP2242 was observed in primary CEC cells (avian cells).

ii) Evaluation of the safety of the viral recombinant canarypox equine influenza strain A/equi-2/Ohio/03 (vCP2242) in canaries. It was shown that recombinant virus isolation was possible on skin and in internal organs of canaries, but probably not in excess with regard to the parental virus.

iii) Safety in horses of the administration of a single overdose and two repeated doses of the vaccine. Diffusibility of the recombinant canarypox virus vCP1533 and vCP 2242: This trial is described in section III.B. It was shown that vCP2242 and vCP1533 could not be reisolated in horses (on whole blood, urine, faces, nasal secretions, saliva) even when administered at high dose.

Reversion to virulence:
As Alvac-canarypox vector is not replicative in mammalian cells, the Applicant considers reversion to virulence cannot occur. Thus, no such test was undertaken. The same view was already shared by the CVMP in scientific advice given to the company previously for a recombinant FeLV-canarypox vaccine, using the same vector as for these influenza-canarypox strains.

Biological properties of the vaccine strain:

Brief description of the first trial design :
Two foals of 4-5 months of age and two adult horses of 3 and 10 years of age were used. The skin of the left side of their necks was scarified with either a preparation containing both influenza viruses, or with the reconstituted vaccine. On the same date, an IM injection of the vaccine was administered on the right side of the neck of each horse.

Examinations:
Blood sampling was performed on days D0 and D22, to test for the presence of ELISA antibodies against the canarypox vector.
Biopsies were performed from the centre of the randomly chosen scarification site.

Results:
The 2 foals were seronegative and the 2 adult horses showed antibodies against canarypox virus before vaccination; all animals seroconverted after vaccination. On the scarified skin, virus could be reisolated from the samples up to D4; after IM injection, virus isolation was negative, but positive for 1 horse on day D1. The above results support the conclusion that canarypoxvirus does not replicate at the site of inoculation.

Brief description of the second trial design:
The possibility of *in vitro* multiplication of the 2 virus strains was tested on 3 different cell types: chicken cells, foal cells and horse cells.

Examinations:
Viral titration in order to detect viral multiplication.

Results:
Titre of both viruses increased on chicken cells only. The results support the conclusion that canarypoxvirus does not replicate in mammalian cells.

Recombination or genomic reassortment of strains:

General considerations:
Potential for genetic transfer and exchange between poxviruses: Only the recombination between the ALVAC-derived recombinant (such as vCP2242 and VCP1533 strains) and another poxvirus is theoretically possible. This is highly unlikely to happen in the conditions of dissemination. The potential for genetic transfer and exchange between poxviruses is negligible.

Potential for genetic transfer and exchange with a virus containing the genes inserted into the vCP2242 and vCP1533: recombination between a canarypox virus (DNA virus) and an influenza virus (retrovirus) is highly unlikely to happen because of the different nature of the nucleic acids and because of the different replication sites (the cytoplasm for poxvirus and the nucleus for the retrovirus).

In conclusion, genetic transfer and exchange involving an ALVAC vector with other organisms are highly unlikely.

Brief description of the first trial design:
Canaries were divided into cages. Mixed infection was administered by cutaneous route with ratios of ALVAC-recombinant and wild type strain. On days D8 and D15, four birds were euthanatised. The inoculation site was taken and prepared for infectivity titration, and reinfection through serial passages. From the second to the last passage, all the birds of each group were euthanatised on day D8 after inoculation and treated as described for the first passage

Examinations:
Weighing was carried out on the inoculation day and on the day of sacrifice. Titres were checked, the animals inoculated and virus harvested per animal along the *in vivo* passages.

Results:
Viral titres of the other 4 groups are in general comparable to those of the group with the pure wild type population of canarypoxvirus. Thus, it can be concluded that no population with a higher virulence than the wild type emerged from the mixtures used in this trial.

Study of residues:
Not applicable.

Interactions:
No known interaction.

Field trials

Brief description of the first trial design:
The aim of this study was to investigate the safety of a primary course of two vaccinations under field conditions. Efficacy aspects of this trial are described in the efficacy part of the report. One hundred and thirteen foals between 1 and 9 months of age and of various breeds including thoroughbreds, coming from 19 different sites, were used. All foals were seronegative or had low levels of residual maternally derived antibodies. They were divided into 3 groups. Age distribution amongst the groups was as follows:

Group	**Age of foals**			
	< 4 months	**4 to 7 months**	**> 7 months**	Total
A1	6	28	4	38
A2	1	34	4	39
B	2	30	4	36
Total	9	92	12	113

No difference of the age distribution existed between the groups. Each horse was vaccinated IM twice, on days D0 and D35. Various batches with different Influenza titres, tetanus toxoid contents and purity levels were used. Horses of group B were vaccinated with a standard vaccine already registered in many countries.

Examinations:
Clinical monitoring (general and local reaction) 3 to 5 hours after injection, daily between 1 and 4 days, then at D7 and D14. In some randomly chosen foals, rectal temperature was recorded according to the same chronology.

Results:
No general reaction was observed following the first injection. A few foals showed a general reaction following the second injection. Two horses of group A1 and 2 of group A2 showed nasal discharge and/or swelling of the submandibular lymph nodes. These horses originated from the same site; for some of them, these symptoms were present before the first injection and lasted for more than 14 days.

These animals recovered uneventfully without any further treatment. One foal of group A2 showed apathy and anorexia on day D39. It lasted for a few days and the animal recovered uneventfully. One foal of group B showed apathy on the day following the second injection. Transient and slight hyperthermia was observed in foals of each group throughout both post-vaccination monitoring periods.

Local reactions:
After first injection: 1 foal of group A1 showed swelling and heat on days D2 and D3, 1 foal of group A2 showed swelling 4 hours after injection and on day D1, and 1 foal of group B showed swelling 4 hours after injection, up to day D7. Except for the foal of group B, swelling was never greater than 5 cm.
After second injection: no foal of group A1 and A2 showed any local reaction.

Brief description of the second trial design:
The aim of this multicentric study was to assess the safety of a booster injection in primed adult horses. Nine hundred and ninety-six horses between 1 and 40 years of age and of various breeds, coming from 8 different sites, were used. They were divided into 3 groups, as follows:

Group	Vaccine used	Number of horses
A1	ProteqFlu-Te	337
A2	ProteqFlu-Te	334
B	Tetagripiffa	325

Each horse was vaccinated once IM. Various batches with different Influenza titres, tetanus toxoid contents and purity levels were used. Horses of group B were vaccinated with a standard vaccine TETAGRIPIFFA (a vaccine manufactured by Merial) already registered in many countries, including France.

Examinations:
Clinical monitoring (general and local reaction) 3 to 5 hours after injection, daily between 1 and 4 days, then on some animals at D7 and D14. In some randomly chosen horses, rectal temperature was recorded according to the same chronology.

Results:
Few general reactions were observed following the injection: depression of 1 horse in group A1 was depressed on day D14 and of 1 horse in group B on days D2 and D3. A few other general reactions were recorded, apparently not considered to be linked to the use of the vaccine. The number of horses with systemic reactions can be summarised as follows:

Group	Number of horses	General condition (%)	Anorexia (%)	Other (%)
A1	337	1 (0.3)	0	6 (1.8)
A2	334	0	0	10 (3.0)
B	325	1 (0.3)	0	4 (1.2)

No hyperthermia was observed in horses of each group throughout post-vaccination monitoring period. An apparent slight rise in temperature (0.2°C) was, however, observed on the day following injection, as well as differences between sites between D0 and D1.

Local reactions were observed in 10.3 % of horses. They were mainly transient and recorded on day D1. The main reaction was swelling, and pain, local hyperthermia and stiffness of the neck were sometimes recorded.

The total number of horses with local site reactions was as follows:

Group	Number of horses	Injection site reaction (%)
A1	337	43 (12.8 %)
A2	334	31 (9.3 %)
B	325	29 (8.9 %)
Total	996	103 (10.3 %)

The following two trials were performed to demonstrate the safety of the vaccine in pregnant mares under field conditions.

Brief description of the third trial design:
One hundred and forty-three mares between 1 and 28 years of age and of various breeds including thoroughbred and draught horses, coming from 20 different sites in France and Belgium, were used. They were at different stages of pregnancy when vaccinated (between 2 and 10 months of pregnancy). They were divided into 3 groups, as follows:

Group	Vaccine used	Number of horses		
		≤ 10 years	> 10 years	Total
A1	ProteqFlu-Te	28	18	46
A2	ProteqFlu-Te	27	22	49
B	Tetagripiffa	28	20	48
	Total	83	60	143

Each horse was vaccinated once IM. Various batches with different influenza titres, tetanus toxoid contents and purity levels were used. Horses of group B were vaccinated with a standard vaccine already registered in many countries, including France.

Examinations:
Clinical monitoring (scoring of general and local reactions) took place 3 to 5 hours after injection, daily between 1 and 4 days, then at D7 and D14. In some randomly chosen horses, rectal temperature was recorded according to the same chronology. Foaling and post-foaling monitoring also took place. Serology was undertaken on days D0 and D14. All sera were tested for IHA antibodies to A/equi-2/Newmarket/2/93.

Results:
Few general reactions were observed following the injection: 1 mare of group A1 (no effect on foaling and on the foal) was depressed after the injection during 1 day; anorexia on D1 in 1 mare of group A1 and 1 mare of group A2; nasal discharge on days D2 and D3 in 2 mares of group B. No hyperthermia was observed in 67 horses of each group throughout post-vaccination monitoring period.

Few local reactions were observed following the injection, similar in all groups: 3 mares of group A1 (6.5%), 4 mares of group A2 (8.2%) and 5 mares of group B (10.4%) showed slight and/or transient reactions, except for 1 mare of group B which showed swelling from D3 to D14.
Foaling monitoring: the data can be summarised as follows:

Group	Not pregnant or embryonic resorption	Abortion	Dystocia	Stillbirth	Abnormal foal	Total
A1	3	1	2	2	1	9
A2	5	1	4	2	1	13
B	3	4	3	3	2	16
Total	11	6	9	7	4	37

Post-foaling monitoring: 2 foals of group A2 died, one because it was not able to feed (because of an abnormal flexion of the fore leg), the other because of a severe pneumonia. Four other foals experienced adverse events (broken leg, hock joint arthritis), but recovered uneventfully. No vaccine related effect was suspected.
Serology: in general, mares had high titres against influenza before vaccination. On D0, 9 mares of group A1, 6 mares of group A2 and 4 mares of group B were however seronegative. On D14, only 1 mare of group B was still seronegative. A booster effect was seen following injection on the other mares.

Brief description of the fourth trial design :
Sixty mares between 4 and 19 years of age and of various breeds, coming from 2 different sites in Hungary, were used. They were at different stages of pregnancy when vaccinated (between 6 and 9 months of pregnancy), and divided into 3 groups of 20 mares each. Each horse was vaccinated once IM. Different formulations were used
Group B horses were vaccinated with a standard vaccine TETAGRIPIFFA already registered in many countries.

Examinations:
Clinical monitoring (scoring of general and local reactions) was undertaken 3 to 5 hours after injection, daily between 1 and 4 days, then at D7 and D14. Rectal temperature was recorded according to the same chronology. Foaling and post-foaling monitoring took place. Serology was undertaken on day D0 and D14. All sera were tested for IHA antibodies to A/equi-2/Newmarket/2/93.

Results:
No general or local reactions were observed following the injection.
General reactions: one mare showed hyperthermia (39.9°C) 3-5 hours after injection, which returned to normal afterwards. During the period after foaling, some mares showed some clinical signs including kerato-uveitis, dermatitis and corneal injury, which were not vaccine-related. Monitoring of clinical signs during and post foaling is summarised as follows:

	Group		
Events	**A1**	**A2**	**B**
Depression	3 (3/5 h)	-	-
Abortion	1 (hydrocephalus)	-	1 (twin pregnancy)
Diarrhoea	1 (1 week)	1 (1 week) + 1 (4 weeks)	-
Conjunctivitis	-	1 (3 weeks)	-
Hyperthermia	-	-	1 (2 weeks) + 1 (3 weeks)
Hock junction arthritis	-	-	1
Navel hernia	1	-	-

All the other mares foaled uneventfully. All the other foals remained healthy.
Serology: in general, mares had high titres against influenza before vaccination. On D0, 4 mares of group A1, 8 mares of group A2 and 6 mares of group B were however seronegative. On D14, 4 mares of group B were still seronegative. A booster effect was seen following injection on the other mares.

An additional study for the evaluation by serology of the bio-equivalence between vCP1529 and vCP2242 at the minimum protective dose, was presented. This was a field trial on foals.
This trial is described in detail section IV. A secondary objective of this study was to investigate under field conditions the safety of the new formulation when administered according to the recommended vaccination schedule. Animals were vaccinated at D0, D35 and D182. Clinical monitoring was done on D1 to D4, D36 to D39 and D183 to D186. Rectal temperature recording was done on D1, D36 and D183. Neither local nor general reactions were observed.
The results of the study support the fact that this vaccine is well tolerated.

III.C Ecotoxicity

With regard to ecotoxicity, the characteristics of the parental donor organisms, of the vector and of the modified organisms vCP2242 and vCP1533 are correctly described in the analytical part, chapter C2.1 - "Starting materials not in a pharmacopoeia - Starting materials of biological origin". Homogeneity and genetic stability of the recombinant viruses were shown in part II H. After inoculation, the viruses do not multiply in the horse but express the protective proteins. Considering that the vaccine is administered by individual intramuscular injections, that the recombinant canarypoxviruses are safe constructs, do not replicate at the site of inoculation, and that they do not disseminate from animals to animals, there is no environmental risk which can be identified through the use of this vaccine.

Conclusion
It can be concluded that the product is safe at very high doses in the target species, as demonstrated in laboratory or field conditions, in a large number of horses (about 2000), of all ages (from 1 month to 40 years) and physiological status (foals, non-pregnant and pregnant female adults and male adults). Safety of the vaccine in pregnant mares was shown, whatever the stage of pregnancy. On some occasions, a transient and in general mild temperature increase, some transient and moderate swelling, pain and local hyperthermia at the injection point, as well as apathy and reduced appetite were observed. These aspects are addressed with appropriate statements in the SPC.

It was also shown that, when used in the horse, the live viruses contained in the vaccine do not spread, do not disseminate, do not replicate on the place of inoculation and do not recombine with another virus. They multiply *in vitro* only in avian cells (not in mammalian cells), or *in vivo* in avian species.

The product is safe in non-target species, such as the canary (where the recombinant virus might spread), duck, chicken, pig, mouse and guinea-pig.
With regard to ecotoxicity it can be concluded that there is no environmental risk, which can be identified through the use of this vaccine.
Additional laboratory and field studies with the new product formulation containing the vCP2242 strain showed that the safety profile of the product remains unchanged.

IV – EFFICACY

IV.A INTRODUCTION

All efficacy studies presented were conducted in the target species and for each test, non-vaccinated animals or animals treated with other vaccines were included. In the laboratory tests, efficacy was studied in seronegative ponies (6 months to 3 years of age). In the field trials, mixed breeds of horses (1 month to 40 years of age) were vaccinated. Three formulations of the vaccine corresponding to different levels of purification and different stabilisers were used in the laboratory and field trials. The applicant demonstrates that these formulations can be considered equivalent for the induction of protection. The minimum dose for equine influenza components ($10^{6.5}$ $FAID_{50}$) was used in two studies. The minimum antigen content for tetanus toxoid was used in all but one study. The efficacy of vaccination against influenza with the finished product was assessed by controlled challenge experiment in laboratory studies. The challenge was performed by exposure to an aerosol of viral suspension of influenza A/equi-2/Sussex/89 (European type), which can be considered a good way to reproduce the natural infection. The protection against influenza 5 months after the primary vaccination and one year after the third vaccination was also assessed by a challenge experiment in the laboratory. The efficacy of vaccination against tetanus with the finished product was assessed by serological studies (toxin neutralisation test and ELISA). In order to avoid tetanus challenge in the horses, the applicant provided publications establishing protective levels of neutralising antibodies to tetanus toxin. This approach is acceptable.
ProteqFlu Te was initially formulated as a powder to be reconstituted with a solvent. A liquid form (suspension for injection) was further developed. An additional field study was conducted to support the efficacy of the new formulation containing the vCP2242 strain. The efficacy of the new formualtion was studied by serology (for both influenza components and tetanus component) and compared to the one of the vaccine containing the vCP1529 strain. It was concluded that both formulations resulted in identical serology against influenza and tetanus. Thus the replacement of vCP1529 by vCP2242 had no impact on the efficacy of the product.

The trials can be summarised as follows:

Trial	**Animal: number, age**	**Animal: immunological status**	**Vaccine**	**Challenge: day after the first injection**	**Serological monitoring until day**
Laboratory trials					
	6, 25–41 weeks of age	No previous history of vaccination against influenza and tetanus	Diluents in combination with a cocktail of canarypox influenza recombinants	-	D 92 Tetanus D 385
	15, 7-8 months	Seronegative and unprimed for influenza	Recombinant Newmarket/2/93	+ D49	D63
	30, 12 months	Seronegative and unprimed for influenza	Recombinant - Newmarket/2/93 - Kentucky/94 - Prague/56	-	D160
	15, 1-3 years	Seronegative and unprimed for influenza	Recombinant Newmarket/2/93	+ D49	D63
	5, 12 months	Seronegative and unprimed for influenza	Recombinant Newmarket/2/93	+ 1 year	1 year (D420)

Trial	Animal: number, age	Animal: immunological status	Vaccine	Challenge: day after the first injection	Serological monitoring until day
	24, 2 years	Seronegative and unprimed for influenza, vaccinated against tetanus	ProteqFlu-Te	+ D49	D63 tetanus D35
	35, 16 months	Seronegative and unprimed for influenza, all but four seronegative and unprimed for tetanus	ProteqFlu-Te and Diluent	+ 5 months post V2 + 1 year post V3	D546 (D371 post V3) Tetanus : D511 (D336 post V3)
	16, 20 months	Seronegative and unprimed for influenza	ProteqFlu-Te	-	D175
Field trials					
	53, 4-7 months	No previous vaccination against tetanus and influenza; foals were born to vaccinated mares	ProteqFlu-Te	-	D70 D 267 (14 days post V3)
	77, 1-9 months	No previous vaccination against tetanus and influenza;	ProteqFlu-Te	-	D49
	395, 2-40 years	Regularly vaccinated against influenza and tetanus	ProteqFlu-Te	-	D49
	6-12 months	Seronegative to equine influenza and to tetanus	ProteqFlu-Te old formulation (with vCP1529 strain) Proteq Flu- Te new formulation (with vCP2242 strain)	-	D196

IV.B LABORATORY TRIALS

1) Safety and efficacy in the horse of the recombinant canarypox virus vCP1533: a feasibility study.

Brief description of the first trial design:
Twenty-six male ponies between 7 and 8 months of age, free from antibodies against influenza, were included in the study and received 2 vaccinations administered by intra-muscular route at a 35-day interval with a single dose (1 ml):

Group	Number of horses	Vaccine	Adjuvant
A	5	vCP1533	None (sterile water)
B	5	vCP1533	Carbomer (4 mg/ml)
C	5	competitor vaccine against equine influenza strains Prague/56, Miami/63 and Suffolk/89 as well as tetanus	Carbomer and aluminium hydroxide
D	6	Tetagripiffa	Aluminium hydroxide
E Control	5		Carbomer (4 mg/ml)

Challenge: on D49 (2 weeks after the second vaccination), each pony was infected by exposure to an aerosol of viral suspension of (H_3N_8) influenza A/equi-2/Sussex/89.

Examinations:
Clinical examination during 10 days after challenge: rectal temperature, including scoring for influenza-related symptoms (coughing, nasal and ocular discharge, swelling and/or pain reactions upon palpation of submandibular lymph nodes).
Nasal swabs and titration of influenza virus from D1 to D8 post-challenge.
Serologic monitoring on D0, D7, D15, D35, D49, D56 and D63:
Titration of antibodies against influenza A/equi-2/Newmarket/2/93 with Single Radial Haemolysis (SRH) method.
Titration of antibodies against influenza A/equi-1/Prague/56 for sera of groups C, D and E with Single Radial Haemolysis (SRH) method.

Results:

Clinical responses:
Temperature: a significant difference between vaccinates and controls was observed; all the controls developed pyrexia with a mean duration of 4.4 days; none of the ponies in groups B and C, one pony in group A and 3 ponies in group D developed slight rises in temperature.

Coughing: no coughing was observed in group A, B and C; all controls of group E developed coughs (mean duration: 4.3 days) and in group D, 4 ponies developed coughs (mean duration: 3.5 days).

Nasal and ocular discharge: occasional discharges were observed in group A, B and C; 4 controls of group E developed nasal discharges (mean duration : 4 days) and in group D, 4 ponies developed nasal discharges (mean duration : 1.33 days).

Clinical score:
The clinical scores were reduced in all vaccinated groups compared to the other groups.
Virus excretion: no virus shedding was observed in group B; virus shedding (amount and frequency) was significantly reduced in groups A and C on day 2 to 5 post-challenge when compared to the controls.

Serologic responses:
Prague/56: negative serology results at day 7 for groups C, D and E. Three animals of groups C and D developed detectable SRH antibodies after the first vaccination and after the second vaccination. All ponies of these groups developed enhanced responses against Prague /56. Group E ponies remained negative.

Newmarket/2/93: negative serology results at day 7 for all the animals. Group E ponies remained negative up to challenge. The serological responses in groups C and D were typical for inactivated whole virus vaccines. All ponies in groups A and B produced consistently high SRH levels following the second vaccination and the levels were exceptionally high for group B (> 131 mm^2).

Conclusion:
Groups A and C both provided good clinical protection with little difference in amounts and duration of virus excretion. Group B produced excellent responses to vaccination, which provided protection against disease and infection in all 5 ponies.

2) Efficacy study on recombinant canarypox-influenza vaccines in horses – adjuvant-dose effect

Brief description of the second trial design:
Thirty ponies 12 months old, free from antibodies against influenza, were included in the study and received 2 intra-muscular vaccinations at 35-day interval with a single dose (1 ml):

Group	Number of horses	Strains	Amount of carbomer
A	5	vCP1533	Low
B	5	vCP1533	Medium
C	5	vCP1533	High
D	5	vCP1533	medium
E	5	vCP1529, vCP1533	Low
F	5	Control	Low

Examinations:
Blood sampling was carried out on days D0, D7, D14, D35, D42, D49, D63, D85, D113 and D160. All sera were tested for the presence of SRH antibody titres to influenza A/equi-2/Newmarket/2/93. Sera from groups A, E and F were tested for the presence of SRH antibody titres to influenza A/equi-1/Prague/56.

Results:

Serologic responses:
Prague/56: negative serology results at day 7 for groups A, E and F. Group A ponies remained negative. One week after the second vaccination, a peak level of SRH antibodies was observed. The antibody levels declined significantly more rapidly in group E compared to group F.

Newmarket/2/93: negative serology results at day 7 for all the animals. Group F ponies remained negative. No significant differences between the antibody responses in groups A, B and C were observed although the response in group A (low Carbomer) was lower after the first vaccination. For group D (lower dose), the level of antibodies after one injection was significantly weaker than for group B; however after 2 doses the antibody response was equally strong. The animals of group E (combination of strains) presented a strong response after the first injection and a very high response after the second injection.

Conclusion:
The canarypox-influenza vaccine should contain at least 4 mg of Carbomer. The antibody responses to Newmarket/2/93 were not impaired in a combination environment (i.e. a combination of strains).

3) Choice of the minimal titres for Influenza components:

a) Dose/response efficacy study on vaccine vCP1533 in horses

Brief description of the first trial design:
Twenty-six ponies between 1 and 3 years of age, free from antibodies against influenza, were included in the study and received 2 intra-muscular vaccinations at 35-day interval with a single dose (1 ml) :

Group	Number of horses	Strains or vaccines	Adjuvant
A	5	vCP1533	Carbomer 4 mg
B	5	vCP1533	Carbomer 4 mg
C	5	vCP1533	Carbomer 4 mg
D	6	Equiffa	oil
E	5		Carbomer 4 mg

Equiffa = inactivated whole virus vaccine containing **Prague/56, Newmarket/2/93** and EHV-1 glycoproteins (manufactured by Merial) ;
vCP132 = recombinant canarypox virus expressing gB, gC and gD genes of EHV-1 (strain Kentucky D) ;
vCP1533 = vaccine containing recombinant canarypox expressing HA gene of influenza **Newmarket/2/93**.

Challenge: on D49 (2 weeks after the second vaccination), each pony was infected by exposure to an aerosol of viral suspension of influenza A/equi-2/Sussex/89.

Examinations:
Clinical examination during 10 days after challenge: rectal temperature including scoring for influenza-related symptoms (coughing, nasal and ocular discharge, swelling and/or pain reactions upon palpation of submandibular lymph nodes).
Nasal swabs and titration of influenza virus from D1 to D9 post-challenge.
Serologic monitoring: titration of antibodies against influenza A/equi-2/Newmarket/2/93 on days D0, D7, D14, D35, D49, D56 and D63 with Single Radial Haemolysis (SRH) method; titration of antibodies against influenza A/equi-1/Prague/56 for sera of group A, D and E.

Results:

Clinical responses:
Temperature: in groups B, C and D, no pyrexia was observed; in group A, one animal presented transient pyrexia of one day duration; all the controls developed pyrexia with a mean duration of 5.2 days.
Coughing: no coughing was observed in group A and C; all controls of group E developed coughs; in group D, 2 ponies developed coughs and in group B, one pony developed coughs.

Nasal and ocular discharge: 4 controls of group E developed nasal discharges and in group D, 5 ponies developed nasal discharges The groups A, B and C had scores, which increased as the titre of the vaccine decreased.

Clinical score:
The clinical scores were reduced in all vaccinated compared to controls. The clinical scores revealed some dose-response effect between groups A, B and C, although differences were not significant.
Virus excretion: no virus shedding was observed in groups A and B; virus shedding was reduced in groups C and D as compared to the controls of group E.

Serologic responses:
Prague/56: negative serology results at day 7 for groups A, D and E. Four animals of group D developed detectable SRH antibodies after the first vaccination and after the second vaccination, all

ponies of this group developed enhanced responses against Prague/56. Groups A and E ponies remained negative.

Newmarket/2/93: negative serology results at day 0 for all the animals. Group E ponies remained negative up to challenge. At day 7, SRH antibodies were detectable in 4 out of 5 ponies in group A, in 2 out of 5 ponies in group B and in 2 out of 5 ponies in group D. At day 49 (14 days post-second vaccination), groups A and B had a similar mean SRH levels. Group C mean level was slightly lower. SRH levels of groups A, B and C were significantly higher than those of group D.

Conclusion:
There is strong dose-related serological response after first vaccination; this dose-response relationship disappeared after the second vaccination. No differences in protection were observed between the different doses of vaccine.

4) Duration of protection provided by vCP1533 at one year after a primary course of two vaccinations

Brief description of the third trial design:
This study was an extension of study presented above. Ten ponies 12 months old, free from antibodies against influenza, were included in the study and received 2 intra-muscular vaccinations at 35-day interval with a single dose (1 ml):

Group	Number of horses	Strains	Carbomer content / dose
B	5	vCP1533	medium
F	5	vCP1502	low

vCP1502 = recombinant canarypox expressing HA gene of influenza **Prague/56**
vCP1533 = vaccine containing recombinant canarypox expressing HA gene of influenza **Newmarket/2/93.**

Challenge: One year after the second vaccination, each pony was infected by exposure to an aerosol of viral suspension of influenza A/equi-2/Sussex/89.

Examinations:
Clinical examination during 10 days after challenge: rectal temperature, including scoring for influenza-related symptoms (coughing, nasal and ocular discharge, swelling and/or pain reactions upon palpation of submandibular lymph nodes). Daily clinical score including the scores for rectal temperature, coughing, general condition and anorexia.
Nasal swabs and titration of influenza virus from D1 to D10 post-challenge.
Serologic monitoring: titration of antibodies against influenza A/equi-2/Newmarket/2/93 with Single Radial Haemolysis (SRH) method at regular intervals until 2 weeks after challenge.

Results:

Clinical responses:
Temperature: all the animals developed pyrexia with a mean duration of 4.0 days for group F and of 1.6 days for group B.

Coughing: all controls of group F developed coughs (mean duration: 6 days) whereas only 3 out of 5 vaccinates coughed for a significantly shorter period (mean duration : 2.2 days).

Nasal and ocular discharge: no significant difference was observed between vaccinates and controls.

Clinical score:
The total clinical score (temperature, coughing, general condition) was significantly lower in the vaccinates than in the controls.

Virus excretion: virus shedding (amount, duration) was significantly reduced in group B as compared to group F.

Serologic responses to Newmarket/2/93: after the two injections of vaccine, the highest level of antibodies was induced in group B at day 49. This level gradually declined over the 1 year observation period and reached a low value at the time of the challenge. Animals of group F were still seronegative at the time of the challenge.

Conclusion:

A primary course of two vaccinations with vCP1533 provides a duration of immunity of at least one year against A/equi-2.

5) Tetanus component:

A comparative study on the duration of immunity provided by a primary vaccination course of two injections: tetanus toxoid

Brief description of the trial design:
Fourteen foals, between 25 and 40 weeks of age, with no previous history of vaccination against influenza or tetanus, were included in the study and vaccinated by intra-muscular injection according to the following schedule:

Group	Number of horses	Vaccines	Injection time
A	8	Tetagripiffa	D0, D35, D371
B	5	Tetanus toxoid with 4 mg carbomer	D0, D35, D371
		vCP1502, vCP1529, vCP1533 with 4 mg carbomer	D0[#], D35[#]
		vCP1533 with 4 mg carbomer	D204[#], D371[#]
C	1	Tetanus toxoid with 4 mg carbomer	D0, D35, D371

Tetagripiffa = inactivated whole virus vaccine containing **Prague/56, Newmarket/2/93** and tetanus (Merial); vCP1502 = recombinant canarypox expressing HA gene of influenza **Prague/56**; * titres not given.
vCP1533 = vaccine containing recombinant canarypox expressing HA gene of influenza **Newmarket/2/93.**
[#]vaccinated at a separate site

Examinations:
Blood sampling at regular intervals until day 399. Selected sera were tested for the presence of ELISA antibodies against tetanus. Day 371 sera were also tested in the mouse Toxin Neutralisation test for titration of tetanus antitoxins.
Selected sera were tested for the presence of SRH antibody titres to influenza Newmarket/2/93 and Prague/56 at days D0, D7, D14, D35, D49, D63 and D92.

Results:
Tetanus:
ELISA: At day 0, all foals were seronegative. At day 49 (2 weeks after the second injection), the highest ELISA antibody levels were observed and the titres gradually decreased in time. On day 371, the titres were either very low or absent. A strong booster response was observed after the third dose given one year after second vaccination.
Mouse TN test: All sera still contained protective levels of antitoxin at 12 months after the vaccination.
Influenza: all foals (except group C) responded serologically to influenza Prague/56 and Newmarket/2/93.

Conclusion:
A primary course of two vaccinations with ProteqFlu-Te provides a duration of immunity of at least one year against tetanus.

6) Efficacy demonstrated by challenge:

a) Comparison of three different formulations - a study on the potency of a recombinant canarypox-flu vaccine according to the requirements of monograph 249 of the European Pharmacopoeia

Brief description of the first trial design:
Three formulations of vaccine were tested. Twenty-four ponies, 2 years old, seronegative and unprimed to influenza A/equi-2, but with a history of vaccination against tetanus were included in the study. They were vaccinated by the intramuscular route twice at a 35-day interval with a single dose (freeze-dried pellet resuspended in its diluent at a ratio of 1:2), as follows:

Group	Number of horses	Formulation
A	6	vaccine
B	6	vaccine
C	6	vaccine
D	6	diluent only (1 ml)

Challenge: on D49 (2 weeks after the second vaccination), each pony was infected by exposure to an aerosol of viral suspension of influenza A/equi-2/Sussex/89.

Examinations:
Clinical examination during 14 days after challenge: rectal temperature, including scoring for influenza-related symptoms (general condition and anorexia, coughing, nasal and ocular discharge, swelling and/or pain reactions upon palpation of submandibular lymph nodes).
Nasal swabs and titration of influenza virus from D1 to D14 post-challenge.
Serologic monitoring : titration of antibodies against influenza A/equi-2/Newmarket/2/93 and A/equi-2/Newmarket/1/93 (closely related to Kentucky/94) on days D0, D7, D14, D35, D49, D56 and D63 with Single Radial Haemolysis (SRH) method. Titration of antibodies against tetanus by ELISA for selected sera obtained on days D0 and D35.

Results:

Clinical responses:
Temperature: in groups A, B and C, no pyrexia was observed; 5 controls developed pyrexia with a mean duration of 5.3 days.

Coughing: no coughing was observed in group A, B and C; 5 controls developed coughs with a mean duration of 3 days.
Nasal and ocular discharge: 5 controls of group D developed persistent nasal discharges; 2 ponies in group A and 1 in group C presented a slight serous discharge on one day.

Clinical score:
The clinical scores were reduced in all vaccinated groups compared to controls.
Virus excretion: no virus shedding was observed in groups A, B and C; the mean duration in the control group was 4.2 days.

Serologic responses:
Newmarket/2/93: negative serology results at day 0 for all the animals. Group D ponies remained negative up to challenge. All ponies in groups A, B and C developed consistently high SRH antibodies.

Newmarket/1/93: negative serology results at day 0 for all the animals. Group D ponies remained negative up to challenge. All ponies in groups A, B and C developed consistently high SRH antibodies.

Tetanus: at the time of the first vaccination, all ponies were seronegative or had low amount of antibodies. The first injection induced high, although variable, antibody levels showing that the ponies were primed against tetanus.

Conclusion:

The vaccine ProteqFlu-Te complies with the requirements on efficacy of monograph 249 of the Ph. Eur.

b) Duration of immunity in foals evaluated five months post second injection by challenge and one year post second injection by serological monitoring

Brief description of the second trial design:
Two formulations of vaccine were tested. Thirty-five ponies, 16 months old, seronegative and unprimed to influenza A/equi-2 were used. The 23 out of 35 ponies seronegative and unprimed to tetanus, were randomly assigned to 3 treatment groups (A, B, C) of 7 ponies each. The remaining ponies were randomly assigned to 2 treatment groups (D, E) of 7 ponies each. They were vaccinated IM with a single dose each time (freeze-dried pellet resuspended in its diluent, as follows:

Group	Formulation	Injection time
A	Vaccine	D0, D35
B	Vaccine	D0, D35
C	Vaccine	D0, D35, D175
D	Diluent only	D0, D35
E	Diluent only	D0, D35, D175

Challenge: 26 weeks after the first vaccination (day 182), each pony in groups A, B and D was infected by exposure to an aerosol of viral suspension of influenza A/equi-2/Sussex/89.

Examinations:
Clinical examination during 10 days after challenge: rectal temperature, including scoring for influenza-related symptoms (general condition and anorexia, coughing, nasal and ocular discharge, swelling and/or pain reactions upon palpation of submandibular lymph nodes).
Nasal swabs and titration of influenza virus from D1 to D10 post-challenge.
Serologic monitoring for all groups : titration of antibodies against influenza A/equi-2/Newmarket/2/93 and A/equi-2/Newmarket/1/93 (closely related to Kentucky/94) at regular intervals up to day 371 with Single Radial Haemolysis (SRH) method. Titration of antibodies against tetanus by ELISA for selected sera. A total of 19 (Day 175) sera from groups A, B and C (and one pony in group D) having low ELISA antibody titre were also tested in the mouse toxin Neutralisation test.

Results:

Clinical responses:
Temperature: 6 controls of group D developed pyrexia for 5-7 days; 9 vaccinates (4 in group A and 5 in group B) developed pyrexia for 1-3 days.
Coughing: 6 controls of group D developed coughs for 5-7 days; 2 vaccinates (1 in group A and 1 in group B) developed coughs for 1-4 days.

Nasal and ocular discharge: 6 controls of group D developed nasal discharges but vaccinates presented this symptom only sporadically.

Clinical score: they were significantly different between the control group and the vaccinated groups. No difference was found between groups A and B.
One control pony died on day 9 post-challenge probably as a result of a bacterial infection.

Virus excretion: all control ponies shed virus for 4-6 days, with a mean duration of 5.1 days. The mean duration of shedding in the vaccinated groups was 3.2 days. The total amount of virus recovered from the vaccinates was significantly lower than in the controls. No difference was found between groups A and B.

Serologic responses:
Newmarket/2/93: negative serology results at day 0 for all the animals. Group D ponies remained negative up to challenge and group E ponies up to the end of the study. Two weeks after the second vaccination (D49), mean antibody titres peaked. For groups A and B (two dose regime), antibodies were still detectable at time of challenge. For group C, after the third dose, all ponies developed a strong booster response. Antibody titres declined more slowly than after the second dose and 6 months after the third dose, the mean titre was still higher.

Newmarket/1/93: negative serology results at day 0 for all the animals. Group D ponies remained negative up to challenge and group E ponies up to the end of the study. Two weeks after the second vaccination, mean antibody titres peaked. For groups A and B (two dose regime), antibodies were still detectable at time of challenge. For group C, after the third dose all ponies developed a strong booster response. Antibody titres declined more slowly than after the second dose and 6 months after the third dose, the mean titre was still high.

Tetanus: at the time of the first vaccination, ponies of groups A, B and C were seronegative. Ponies of group D and E were seronegative or had low amount of antibodies. The primary course of two vaccinations induced high, but variable, levels of ELISA antibodies in all of 27 unprimed ponies and very strong booster responses in all primed ponies 14 days after the second vaccination. Antibodies declined over the 5 months observation period.
At day 175, 18 out of 19 ponies tested via TNT had protective levels of antibodies. The third dose of vaccine administered to groups C and E induced a booster response.

Conclusion:
A primary course of two doses of vaccines provided significant protection against a challenge with influenza 5 months after the second dose and provided immunity against tetanus of at least 5 months.

7) Efficacy demonstrated by serology:

a) Duration of immunity in foals – Bio-equivalence between two dose levels of canarypox influenza

Brief description of the first trial design:
Twenty four ponies 20 months old, seronegative and unprimed to influenza A/equi-2, were included in the study and received intra-muscular vaccinations with a single dose:

Group	Number of horses	Vaccines	Injection time
A	7	ProteqFlu-Te	D0, D35
B	9	ProteqFlu-Te	D0, D35
C	8	Diluent only (1 ml)	D0, D35

Examinations:
Serologic monitoring for all groups : titration of antibodies against influenza A/equi-2/Newmarket/2/93 and A/equi-2/Newmarket/1/93 (closely related to Kentucky/94) at week 0, 1, 2, 5, 7, 9, 11, 13, 17, 21 and 25 (day 175) with Single Radial Haemolysis (SRH) method.

Results:
Newmarket/2/93: negative serology results at day 0 for all the animals. Group C ponies remained negative up to the end of the study. Two weeks after the second vaccination (D49), mean antibody titres peaked. For groups A and B, antibodies were still detectable at 5 months after the second dose.
Newmarket/1/93: negative serology results at day 0 for all the animals. Group C ponies remained negative up to the end of the study. Two weeks after the second vaccination (D49), mean antibody titres peaked for groups A and B. For both groups, antibodies were still detectable at 5 months after the second dose.

Conclusion:
A primary course of two doses of vaccines stimulated high levels of antibody being sustained for at least 5 months after the second vaccination. The two dose levels of vaccine were bio-equivalent.

IV.C FIELD TRIALS

1) Primary vaccination in non primed foals

a) A comparative efficacy study in foals under field conditions. Primary course vaccination. Clinical trial

Brief description of the trial design:
The study was a multicentric trial carried out in Hungary. It was conducted in three sites and included 118 foals, aged between 4 (a total of 11 foals) and 7 months. Foals were seronegative with no history of vaccination against influenza or tetanus, and were born to vaccinated mares. At each site, the animals were randomly allocated to 5 different treatment groups as follows:

Group	Number of horses	Vaccine (formulation)	Injection time
I	39	ProteqFlu-Te	D0, D35 D253
II	38	ProteqFlu-Te	D0, D35 D253
III	19	Competitor vaccine to ProteqFlu-Te*	D0, D35 D253
IV	19	Competitor vaccine to ProteqFlu-Te**	D0, D35 D253
V	3	None	-

*Inactivated vaccine containing influenza Newmarket, Brentwood, Borlang/91 with tetanus toxoid adjuvanted with aluminium phosphate and ISCOM.
**Inactivated vaccine containing influenza Prague/56, Miami/63, Suffolk/89 with tetanus toxoid adjuvanted with carbomer and aluminium hydroxide.

Examinations:
Blood samples were collected at regular interval until 2 weeks after the third vaccination (D 267). Sera were tested for antibodies against influenza A/equi-2/Newmarket/2/93 and A/equi-2/Kildare/92 (closely related to Kentucky/94) with Single Radial Haemolysis (SRH) method and antibodies against tetanus by ELISA.

Results:

Serologic responses:
Influenza: Seven weeks before the beginning of the study, a few number of foals (16 on the 3 studs) had pre-existing antibodies against influenza Newmarket/2/93; the HI titres varied from 4 to 16 and

probably represented residual maternally derived antibodies. At D0, 3 animals had antibodies against influenza Newmarket/2/93. The 3 control foals did not seroconvert to influenza during the study.

For Newmarket/2/93, at 2 weeks after the second vaccination (D49) and at day 70, no significant differences were observed between the 4 groups.

For Kildare/92, at 2 weeks after the second vaccination (D49) no significant differences were observed between the 4 groups. At day 70, statistical analysis showed a significant group effect due to a group II versus group III difference (II<III).

Tetanus: a total of 91 sera were available for statistical analysis of the antibody response to tetanus. All foals responded serologically to tetanus after the two vaccinations. Titres were highly variable, but present. No difference was found between the two formulations of vaccine PROTEQFLU Te. A significant difference was observed at D49 between the vaccinated groups with lower titres in group I than in group III. At D70, a significant difference was observed between groups I and II on the one hand and group III on the other hand. At the time of V3 (D 253), a lot of animals were seronegative regarding tetanus.
Fourteen days post V3, all antibody titres were largely protective.

Conclusion:

A primary course of two doses of vaccines is efficacious under field conditions in foals at the minimum age recommended for vaccination. The vaccine induced high antibody titres against both influenza antigens and tetanus toxoid. The results also show that a booster vaccination with a single dose of ProteqFlu-Te at more than 6 months after the primary vaccination course induced high antibody titres against both influenza antigens and tetanus toxoid in foals previously vaccinated with ProteqFlu-Te or with two commercially available vaccines.

2) Primary vaccination in foals

a) Field efficacy and safety study in foals- A multicentric field trial in France and Belgium

Brief description of the trial design:
Already detailed in the Safety part.

Examinations:
Blood samples were collected on days D0, D35 and D49. All sera were tested for SRH antibodies to A/equi-2/Newmarket/2/93 and A/equi-2/Kildare/92 or A/equi-2/Newmarket/1/93. They were also tested for ELISA antibodies against tetanus toxoid.

Results:
Serology against influenza: on day D0, some foals showed low titres (3 foals of group A1, 6 foals of group A2, 7 foals of group B against A/equi-2/Kildare/92 or A/equi-2/Newmarket/1/93; 3 foals of group A1, 6 foals of group A2, 5 foals of group B against A/equi-2/Newmarket/2/93), due to the persistence of maternally derived antibodies. Following the second injection, a booster effect was shown in most of the foals. No statistically significant difference on the mean antibody titres on D49 existed between the groups.
Serology against tetanus: on day D0, some foals showed low titres (2 foals of group A1, 4 foals of group A2, 3 foals of group B), due to the persistence of maternally derived antibodies. Following the second injection, a booster effect was shown in most of the foals.
The mean antibody titres on D49 was significantly higher in group B compared to the others. After 2 injections, the antibody titre was protective in all animals except in 1 foal of group A1 and 1 foal of group A2. The mean titre on D49 was significantly higher in group B when compared to groups A1 and A2.

Conclusion:

A primary course of two doses of vaccines is efficacious under field conditions in foals at the minimum age recommended for vaccination. The vaccine induced high antibody titres against both influenza antigens and tetanus toxoid in almost all foals.

3) Booster vaccination in primed horses

a) Field efficacy study in adult horses of booster vaccination

Brief description of the trial design:
Already detailed in the Safety part.

Examinations:
Blood samples were collected at D0, D14 and D49 (some horses). Sera were tested for antibodies against influenza A/equi-2/Newmarket/2/93 and A/equi-2/Kildare/92 (closely related to Kentucky/94) with HI method and for antibodies against tetanus by ELISA.

Results:
Influenza: There was a clear booster effect with all 3 vaccines giving a rise in antibody titres by 14 days after injection. Only one animal from group A1 was still negative to Kildare/92 and Newmarket/2/93 on D14.

Tetanus: A total of 601 Day 0 samples were analysed for tetanus antitoxin levels.

On D0, 27 horses had very low titres , 14 of them being seronegative. Only the sera from these 27 horses were analysed at D14 and 49.
Individual responses were very variable. Apart from two horses in group B and one horse in group A1 (all probably non-responders), no horses remained negative following the vaccination.

Conclusion:
An injection of vaccine gave a good booster effect in previously vaccinated horses under field conditions.

Field efficacy study of the new product formulation by serology to support the bio-equivalence between vCP1529 and vCP2242 at the minimum protective dose

Brief description of the trial design:
The study included 23 susceptible foals, aged between 6 and 12 months. The animals were randomly allocated to following treatment groups: Group C (9 horses) treated with new formulation, Group D (10 horses) treated with old formulation and Group S (4 horses) no treatment. All animals were vaccinated on D0, D35 and D182.

Examinations:
The animals were clinically monitored on D1 to D4, D36 to D39 and D183 to D186. The rectal temperature recording was achieved on D1, D36 and D183. Finally blood samples were collected at D-28, D0, D7, D14, D35, D49, D70, D119, D154, D182 and D196. Sera were tested for antibodies against influenza A/equi-2/Kildare/92 (homologous antigen to A/equi-2/Kentucky/94),A/equi-2/South Africa/4/03 (homologous antigen to A/equi-2/Ohio/03) and A/eq/Newmarket/2/93.

Results:
No local or general reactions were registered. The non-inferiority test performed on the SRH titres to influenza A/eq/Newmarket/2/93 induced by both vaccines, from D35 to D196, showed that the titres were equivalent on each time point.; the non-inferiority test performed on the SRH titres to influenza A/eq/Kildare/92 and A/eq/South Africa/4/03 induced by each respective vaccine, from D35 to D196, showed that the titres were equivalent on each time point. All the foals were seronegative to tetanus before the beginning of the study. The non-inferiority test performed on the ELISA titres to tetanus

induced by both vaccines, from D49 to D196, showed that the titres were equivalent on each time point.

Conclusion:

Both vaccinated groups developed identical serology against influenza and tetanus. The replacement of vCP1529 by vCP2242 had no impact on efficacy.

V. RISK-BENEFIT ASSESSMENT

ProteqFlu-Te is a vaccine intended to protect against influenza and tetanus. It is a suspension for injection and contains 2 recombinant canarypox viruses expressing the haemagglutinin HA gene from the equine influenza virus strains A/equi-2/Ohio/2003 (USA representative) and A/equi-2/Newmarket/2/93 (European representative), respectively, tetanus toxoid and carbomer as an adjuvant.

The Quality dossier is correctly documented, particularly with regard to its biotechnological aspects 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.1) and Commission Directive 1999/104/EEC.

It can be concluded that the product is safe at very high doses in the target species, as demonstrated in laboratory or field conditions, in a large number of horses (about 2000), of all ages (from 1 month to 40 years) and physiological status (foals, non-pregnant and pregnant female adults and male adults). Safety of the vaccine in pregnant mares was shown, whatever the stage of pregnancy. On some occasions, a transient and in general mild temperature increase, some transient and moderate swelling, pain and local hyperthermia at the injection point, as well as apathy and reduced appetite were observed. These aspects are correctly addressed in the SPC.

It was also shown that, when used in the horse, the live viruses contained in the vaccine do not spread, do not disseminate, do not replicate at the site of inoculation and do not recombine with other viruses. The viruses multiply *in vitro* only in avian cells (not in mammalian cells), or *in vivo* in some avian species. The product is safe in non-target species, such as the canary (where the recombinant virus might spread), duck, chicken, pig, mouse and guinea-pig.

With regard to ecotoxicity, the characteristics of the parental donor organisms, of the vector and of the modified organisms vCP2242 and vCP1533 are correctly described. Homogeneity and genetic stability of the recombinant viruses were shown. After inoculation, the viruses do not multiply in the horse but express the protective proteins. Considering that the vaccine is administered by individual intramuscular injections, that the recombinant canarypox viruses are safe constructs, that they do not replicate at the site of inoculation, and that they do not disseminate from animal to animal, there is no environmental risk, which can be identified through the use of this vaccine.

Both influenza recombinant Canarypox viruses are [H_3N_8]: thus it is not possible to have specific efficacy data on each virus separately, because of cross-reactions between them. Moreover, if the use of one strain (ie vCP1533) can be demonstrated as being efficacious, the addition of a second similar strain (ie vCP2242) is likely to enhance efficacy.
Antibody titres are considered as being good markers for tetanus and influenza diseases. Thus, challenges are not absolutely necessary. Thus, if the previously stated rationale is accepted, it can be concluded that immediate protection was addressed through serology, and that a duration of protection was addressed through challenge for influenza (for about of 5 months after second vaccination and one year after third vacination), and through serology (for about 1 year) for influenza and tetanus. Thus, protection is globally demonstrated through the different trials provided.

Following the recommendation of the Expert Surveillance Panel in 2004 to replace the influenza A/equi-2/Kentucky/94 strain (American lineage) by influenza A/equi-2/Ohio/2003 or A/equi-2/South Africa/4/2003, Merial applied to substitute the vCP1529 construct by the recombinant vCP2242 construct expressing the haemagglutinin gene from influenza virus A/equi-2/Ohio/03. The provided quality data were found to be satisfactory. It was shown that both constructs vCP1529 and vCP2242 are very similar. Based on the provided safety data it was concluded that the newly formulated product is safe in the target species and in line with the current SPC. It was also shown that there is no environmental risk which can be identified through the use of the new formulation of the vaccine. Finally, the efficacy of the new formulation was considered equivalent to the old one, and sufficiently demonstrated

Based on the original and subsequent data on quality, safety and efficacy presented, the CVMP considered by consensus that the benefit/risk profile of ProteqFlu-Te was favourable in the active immunisation of horses of 4 months of age or older against equine influenza to reduce clinical signs and virus excretion after infection, and against tetanus to prevent mortality, in accordance with the requirements of Council Directive 2001/82/EC.