**Department for Environment, Food and Rural Affairs**

# Application for consent to release a GMO – organisms other than higher plants

## Part A1: Information required under schedule 2 of the Genetically Modified organisms (Deliberate Release) Regulations 2002

### Part I: General information

**1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.**

Head of Exotics Disease Policy,
Department for Environment, Food and Rural Affairs (Defra), Seacole Building, 2 Marsham Street, London SW1P 4DF

Senior Responsible Officer,
APHA Woodham Lane, Addlestone, Surrey KT15 3NB
Authorisation to conduct study

Project Lead,
APHA Woodham Lane, Addlestone, Surrey KT15 3NB
Day-to-day study leadership

Project Investigator,
APHA Woodham Lane, Addlestone, Surrey KT15 3NB
Conduct of study according to protocol. Investigator responsibilities including management of study sites, control and storage of IVP, health and welfare of animals.

Project Investigator,
APHA Crewe, Hornbeam House, Electra Way, Crewe, Cheshire, CW16GJ
Co-ordinating monitoring activities, data QC and monitoring, assuring overall compliance with VICH GCP and adherence with study protocol.

**2. The title of the project.**

Poultry trial to assess vaccination regiment for high pathogenicity avian influenza (HPAI) in Turkeys.

### Part II: Information relating to the organisms

**Characteristics of the donor, parental and recipient organisms**

**3. Scientific name and taxonomy.**

**Donor:**

Birnaviridae family, genus *Avibirnavus*, Infectious Bursal disease virus, Faragher 52/70 strain

Orthomyxoviridae, Influenza virus, type A

**Recipient:**

Herpesvirales, Mardivirus, Meleagrid herpesvirus 1, FC 126

**4. Usual strain, cultivar or other name.**

The parental organism is Herpesvirus of Turkeys (HVT) strain FC-126. The strain has been isolated from the kidney of a commercial turkey in the US (Indiana) in the late sixties (Witter et al, 1970). The virus has been characterized as non-pathogenic. HVT FC-126 has been used as a vaccine strain for vaccination against Marek's Diesase (MD) for more than 45 years. HVT is a fully apathogenic virus and its host range is limited to avian species. HVT causes no clinical disease in chickens and contact spread rarely occurs.
The VP2 insert was derived from a classical strain of Infectious Bursal Disease (IBD) virus known as Faragher 52/70 strain.
Low pathogenic avian Influenza (LPAI) virus originates from 1. mute swan /Hungary/4999/2006 and 2. A computationally optimized sequence of LPAI (Computationally Optimized Broadly Reactive Antigen (COBRA)) based upon known Highly pathogenic avian influenza (HPAI), Strain H5N1 Haemoglutinin A (HA) sequences to an artificial HA construct.
.

**5. Phenotypic and genetic markers.**

The phenotypic characteristics (observable characteristic) of the HVT-FC126 strain are:
- It is a fully apathogenic virus
- The virus is strongly cell-associated
- The virus causes a typical cytopathic effect (CPE) in chicken embryo fibroblasts (CEF)
- Its host range is limited to avian species
- In its natural host – turkeys- the virus spreads
- In the target species for its use as a vaccine – chickens- no spreading was observed

- It can be applied safely to chickens at a very early age (in ovo or at one day of age). Its replication is self-limiting, remains latent through the life of the bird and antibodies persist for life (Schat, K.A, Diseases of Poultry, 12th edition, page 491).
- HVT FC-126 strain has been used worldwide since 1972 to vaccinate chickens against MD (Witter and Schat 2003).
- Its complete genome sequence is available (Genbank accession # AF291866; Afonso, 2001).

The FC126 strain does not contain any known genotypic markers i.e. it cannot be differentiated from other herpes virus of turkey (HVT) strains on genetic level.

**6. The degree of relatedness between the donor and recipient or between parental organisms.**

According to the Baltimore classification of viruses the donors and the recipient viruses belong to three distinct classes. The first donor virus, IBD is a double-stranded RNA virus, belonging to class III. The second donor virus (Influenza A) belongs to class V (negative sense single stranded RNA virus) and the recipient (HVT) virus belongs to class I (double stranded DNA viruses).

**7. The description of identification and detection techniques.**

The virus can be grown in primary or secondary cultures of chicken cells such as embryonic fibroblasts and causes a typical cytopathic effect (CPE). These plaques can be seen macroscopically or visualized by Giemsa-, Naphtalene black- or serospecific staining. HVT in blood samples from infected chickens can be identified by plating lymphocytes on monolayers of primary or secondary chicken cells. Detection can also be performed on DNA extracted from the virus using the polymerase chain reaction (PCR).

HVT virus can be identified further by labeling viral foci with the aid of the immuno fluorescence method using specific anti-HVT antibodies. Alternatively, detection can be performed on DNA extracted from the infected cells/virus using the polymerase chain reaction (PCR). A primer set composed of HVT genome specific primers can be used to specifically detect HVT.

**8. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.**

Detection of virus by plating in chicken cell cultures is very sensitive, even single infected cells can be detected. The reliability of the method is very high based on experiences with similar HVT-based vaccines (e.g. Vectormune HVT AIV). Maximum specificity can be obtained by confirming the results in tissue culture with immuno-fluorescence techniques using monoclonal antibodies.

**9. The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey, parasites and competitors, symbionts and hosts.**

HVT is indigenous to EC countries. HVT is a ubiquitous apathogenic virus and the

natural host is the turkey. The virus is host-restricted and has only been described as infecting turkeys by natural routes. Other avian species like chickens, quail and pheasants can be infected experimentally by injection. This feature is the basis for parenteral vaccination of chickens with HVT based vaccines to protect them against Marek's Disease (MDV) and other viruses.

**10. The organisms with which transfer of genetic material is known to occur under natural conditions.**

Transfer or exchange of genetic material with other organisms has never been observed for HVT. HVT is commonly present in vaccinated chickens that become "superinfected" with virulent MDV. Further, serotype 3 (HVT) is often given with serotype 2 and/or serotype 1 strains as a polyvalent vaccine. As there have never been reports on the recombination of HVT with either the virulent MDV or the serotype 2, this possibility can be considered extremely small.

**11. Verification of the genetic stability of the organisms and factors affecting that stability.**

Witter et al. (1970) isolated the HVT FC-126 strain from a commercial turkey flock and found it apathogenic for both turkeys and chickens. Hence, the recipient/parenteral strain used for rHVT/IBDVP2/AI-H5 was not obtained by attenuation of a pathogenic field isolate by (gradually) forcing genetic change to obtain the desired attenuation. As no selection pressure was applied, the possibility for genetic change in this DNA virus is remote. This is confirmed by the 45 years worldwide use as a vaccine strain, during which no reports have been issued regarding genetic change.

**12. The following pathological, ecological and physiological traits:**

**a. the classification of hazard according to existing Community rules concerning the protection of human health and the environment;**

The recipient is not a hazard. HVT is not indicated in the EU Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work and is not considered as a zoonosis. No other species than avian are known to be susceptible to HVT virus infection. Similarly, the donor IBD virus is also not indicated as a hazard.
The Second donor is: according to EU Directive 2000/54/EC, Avian Influenza can be considered as a class 2 organism. Exposure of humans to infected birds can cause respiratory symptoms. As a complication mortality can occur, depending on the adaption of the AI strain to humans.

**b. the generation time in natural ecosystems, and the sexual and asexual reproductive cycle;**

The natural host is turkey. The generation time in the natural host and in chickens can be estimated between 12 and 48 hours. HVT causes a persistent infection and can be detected during the whole life of the animal. Spreading of the virus between turkeys occurs through the shedding/release of dust particles from feather follicles.

By contrast, spreading of virus between chickens can only take place for a short period of time post vaccination.

**c. information on survivability, including seasonability and the ability to form survival structures, including seeds, spores and sclerotia;**

HVT vaccine viruses are being produced in chicken embryo fibroblast (CEF) cells and stored in liquid nitrogen. The virus can only survive in viable CEF cells. Factors that influence the survival of CEF cells (high temperatures, desiccation, pH, etc.) also affect the stability of the virus. After vaccination of chickens the virus could be shed via dust from the feather follicles. These dust particles can be relatively stable but are not infectious for chickens. However, as stated previously only turkeys can become naturally infected, any other avian species require experimental injection to get infected
The HVT virus does not form survival structures.

**d. pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms;**

None.

**e. antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;**

Not applicable.

**f. involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration.**

Not applicable.

**13. The sequence, frequency of mobilisation and specificity of indigenous vectors and the presence in those vectors of genes which confer resistance to environmental stresses.**

Not applicable.

**14. The history of previous genetic modifications.**

HVT strain FC-126 has been used as the vector in a number of recombinant vaccines for poultry, with a number of products from different manufacturers having received marketing authorisation in the EU/GB, including under the Vectormune, Innovax and Vaxxitek brands (see section 9 for further explanation as to the suitability of this vector).

**Characteristics of the vector**

**15. The nature and source of the vector.**

To generate the GM virus, the homologous recombination of cloned overlapping sub-genomic fragments using E. coli plasmids (derived from the pUC18 vector plasmid) was performed in HVT-infected embryo fibroblasts. Therefore the entire HVT genome was cloned into bacterial vectors as several large overlapping sub-genomic fragments.

**16. The sequence of transposons, vectors and other non-coding genetic segments used to construct the genetically modified organisms and to make the introduced vector and insert function in those organisms.**

These were pUC18 derived vectors, see van Zijl et al., 1988 for their derivation and sequence references.

**17. The frequency of mobilisation, genetic transfer capabilities and/or methods of determination of the inserted vector.**

These vectors are restricted in their mobilisation and transfer capabilities. Once the overlapping HVT fragments are transfected into CEF cells, the complete HVT genome is then obtained by homologous recombination and the inserted HA cassette thus integrated into the HVT genome. It could not then be further mobilized or transferred. Repeated passage through CEF cells was followed by sequencing to confirm stability of the insert.

**18. The degree to which the vector is limited to the DNA required to perform the intended function.**

These bacterial vectors are limited as to their functions as set out above.

**Characteristics of the modified organisms**

**19. The methods used for the modification.**

The vaccine contains the HVT FC126 strain with an insertion of the VP2 gene of infectious bursal disease, into which an insertion of HA H5 construct is added.

The VP2 open reading frame was inserted at the locus of the small subunit of ribonucleotide reductase gene (HSV-1 UL40 homolog) without any exogenous promoter in vHVT001; to make Vaxxitek HVT-IBD. (Darteil et al., 1995).

**20. The methods used:**

**a. to construct inserts and introduce them into the recipient organism;**

The construction of rHVT/IBDVP2/H5 followed the method used as a standard procedure for the construction of herpesviruses and previously published in research papers (e.g. van Zijl et al.,1988). This in essence involved generating the GM virus

by way of the homologous recombination of cloned overlapping sub-genomic fragments using E. coli plasmids (derived from the pUC18 vector plasmid) in HVT-infected embryo fibroblasts. This began with the entire HVT genome being cloned into bacterial vectors as several large overlapping sub-genomic fragments.

**21. The description of any insert and/or vector construction.**

The inserted DNA is composed of HA of a LPAI H5 construct and cytomegalovirus (CMV) and polyadenylation sequences.

**22. The 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 inserted HA H5 is a synthetic construct with optimized broadly reactive antigenic properties for HA.

**23. The methods and criteria used for selection**

Passage in CEF cells after the process described in section 17 to select for the recombinant HVT was followed and stability of the recombinant HVT was confirmed by sequencing.

**24. The sequence, functional identity and location of the altered, inserted or deleted nucleic acid segments in question and, in particular, any known harmful sequence.**

The insert contains the HA-gene construct and promoter and terminator sequences. The function of the promoter sequences is to facilitate the transcription of the genes and the function of the terminator sequences is to facilitate termination of transcription

**Characteristics of the genetically modified organisms in their final form**

**25. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed.**

The inserted HA-gene construct of LPAI will be transcribed and express the HA-protein, after vaccination with the HVT recombinant. Similarly, the VP2 gene will be transcribed and express the VP2 protein. Thus, there will be stimulation of an immune response to these two antigens, along with that to the HVT vector itself.

**26. The structure and amount of any vector or donor nucleic acid remaining in the final construction of the modified organisms.**

No vector was left in the recombinant HVT vaccine.

**27. The stability of the organism in terms of genetic traits.**

The genetic stability of the recombinant HVT was confirmed by sequencing and confirmation of the expression of the inserted genes following repeated passage in CEF cells.

**28. The rate and level of expression of the new genetic material in the organisms and the method and sensitivity of measurement of that rate and level.**

The inserted genes are expected to be expressed at the same rate as the other genes within the vector, as measured by methods described previously in sections 7 and 8.

**29. The activity of the gene product.**

Insertion of the genes does not change the non-pathogenic nature of the HVT FC-126 recipient. HVT FC-126 is a naturally non-pathogenic virus.

**30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector**.

The virus can be grown in primary or secondary cultures of chicken cells such as embryonic fibroblasts and causes a typical cytopathic effect (CPE). These plaques can be seen macroscopically or visualized by Giemsa-, Naphtalene black- or serospecific staining. HVT in blood samples from infected chickens can be identified by plating lymphocytes on monolayers of primary or secondary chicken cells. Detection can also be performed on DNA extracted from the virus using the polymerase chain reaction (PCR).

HVT virus can be identified by labeling viral foci with the aid of the immuno fluorescence method using specific anti-HVT antibodies. Alternatively, detection can be performed on DNA extracted from the infected cells/virus using the polymerase chain reaction (PCR). A primer set composed of HVT genome specific primers can be used to specifically detect HVT. Specific primers in the HVT genome HA gene have also been selected for additional confirmatory detection purposes.

**31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.**

Detection of virus by plating in chicken cell cultures is very sensitive, even single infected cells can be detected. The reliability of the method is very high based on experiences with similar HVT-based vaccines ( e.g. Vectormune HVT AIV). Maximum specificity can be obtained by confirming the results in tissue culture with immuno-fluorescence techniques using monoclonal antibodies.

**32. The history of previous releases or uses of the organisms.**

The GMO has been previously released in the EU, see Part A2 for details.

**33. In relation to human health, animal health and plant health**

The HVT vector is only able to naturally infect Turkeys, therefore the answers to this section reflect this.

**a. the toxic or allergenic effects of the non-viable organisms and/or their metabolic products,**

None identified. The GMO only contains one genetic element of the HPAI virus and so beyond triggering an immune response will not cause any other effects.

**b. the comparison of the organisms to the donor, recipient or (where appropriate) parental organism regarding pathogenicity,**

The recipient vector is apothogenic to turkeys; the two genetic elements inserted into it will not be expected to alter this, as evidenced by results from the use of other, similarly constructed vaccines.

**c. the capacity of the organisms for colonization, and**

The in vivo growth characteristics, including colonisation, of the GMO in turkeys are the same, or slightly reduced, compared to the recipient.

**d. if the organisms are pathogenic to humans who are immunocompetent –**

Not applicable

**i. diseases caused and mechanisms of pathogenicity including invasiveness and virulence,**

Not applicable.

**ii. communicability,**

Not applicable.

**iii. infective dose,**

Not applicable.

**iv. host range and possibility of alteration,**

Not applicable.

**v. possibility of survival outside of human host,**

Not applicable.

**vi. presence of vectors or means of dissemination,**

Not applicable.

**vii. biological stability,**

Not applicable.

**viii. antibiotic-resistance patterns,**

Not applicable.

**ix. allergenicity**

Not applicable.

**x. availability of appropriate therapies**

Not applicable.

**e. Other product hazards**

None.

**Part III: Information relating to the conditions of release**

**The release**

**34. The description of the proposed deliberate release, including the initial purpose or purposes of the release and any intention to use the genetically modified organisms as or in a product in the future**.

The proposed veterinary trial will be field based, involving 1000 turkeys, and conducted over a approximately 168-day period. The trial will compare three recombinant vaccines in total; two of these already have marketing authorisation within GB (Innovax-ND-H5 and Vectormune rHVT-HA5) and are therefore not part of this GM release application. There is a further booster vaccine, which is an inactivated subunit antigen, derived from the GMO described in this application. One day old poults will be vaccinated: 300 in total for each of the three viable recombinant vaccines, with 100 serving as unvaccinated controls, and all birds then transported to a brooding site. Following this at 42 days old 150 birds from each vaccine group will receive the booster vaccine. All the birds (except the sample numbers to be challenge tested as described in section 35 below) will then move to the rearing /finishing sites to complete a regular production period of 168 days.

**35. The intended dates of the release and time planning of the experiment including frequency and duration of releases.**

Late February 2026. The GMO will be released as a single administered dose to a total of 300, one day old turkey poults. The trial will last 168 days in total. However, there will be two challenge studies conducted with a subset (20 per group) of each

treatment (vaccine+/- booster) at 106 days and a further subset at 148 days to evaluate the impact of age on infection and vaccination status. Challenge tests will be conducted in containment animal facilities at APHA with a contemporary UK isolate of H5N1 clade 2.4.4.4b HPAI virus.

**36. The preparation of the site before the release.**

Enhanced biosecurity measures will be applied at the hatchery, brooding and rearing/finishing sites in line with those that are required in an avian influenza prevention zone (AIPZ) (a zone declared by government, where strict biosecurity and hygiene measures are mandatory for all bird keepers to prevent the spread of avian influenza). Measures include only essential movements of people, vehicles and equipment to and from the areas where the poultry are kept; cleansing and disinfection of equipment, vehicles and footwear; and, storing feed, water and bedding under cover.

**37. The size of the site.**

The number of turkeys included in the trial, 1000, is comparable to a small size commercial turkey business, therefore the site(s) housing them correspond to this requirement.
[Type in font 'Arial', size 12, left-hand justify, and do not underline the text]

**38. The method or methods to be used for the release.**

Trial birds will be transported in sealed vehicles, supervised by qualified vets. No other animals will be present in the vehicles. Vehicles will be cleansed and disinfected before and after use. On farm, turkeys will be kept according to standard poultry industry practices. At the brooding and rearing sites, trial turkeys will be housed in seven mixed-sexed groups according to vaccine regime. The space per animal will be equal to or greater than the guidelines from Codes of recommendations for the welfare of livestock - turkeys - GOV.UK. No other poultry will be kept at the premises during the trial.

**39. The quantity of organisms to be released.**

The inoculation of each turkey will be that recommended by the vaccine manufacturer: 3-6-4.4log10 plaque forming units (PFU) per dose. This will be given as a single subcutaneous injection of 0.2 ml thawed vaccine.

**40. The disturbance of the site, including the type and method of cultivation, mining, irrigation, or other activities.**

Beyond ensuring all sites adhere to the enhanced biosecurity measures described in section 36, there will be no other disturbances or alterations to the sites.

**41. The worker protection measures taken during the release.**

In case of accidental self-injection, workers should seek medical advice immediately and show the Summary of Product characteristics (SPC) to the physician. In addition, any incidents must be reported to the Investigator immediately who in turn

will notify the designated qualified person for pharmacovigilance (QPPV) within 24 hours of learning of it.
The GMO will be supplied as a concentrate stored and transported in liquid nitrogen. Workers will be instructed to wear protective gloves, spectacles and boots when handling the veterinary medicinal product, before withdrawing from liquid nitrogen, and during both the ampoule thawing and opening operations. This is to guard against the possibility that frozen glass ampoules may explode during sudden temperature changes.

**42. The post-release treatment of the site.**

The trial birds will be housed in five different groups at the brooding and rearing / finishing sites. No other poultry will be kept at the premises during the trial. This and the imposition of enhanced biosecurity measures at the site(s) during the trial will ensure no further post-release treatment is required.

**43. The techniques foreseen for elimination or inactivation of the organisms at the end of the experiment or other purposes of the release.**

Birds will not come into contact with any other birds or animals. Birds will be immediately culled at the slaughtering facility. This will take place at the end of the day after which the slaughterhouse will undergo cleansing and disinfection. Those birds taken for the challenge study will be stored, used and disposed of within Specified Animal Pathogen Order (SAPO) Level 4 and Advisory Committee on Dangerous Pathogens (ACDP) Level 3 containment facilities.

**44. Information on, and the results of, previous releases of the organisms and in particular, releases on a different scale or into different ecosystems.**

No previous release in the UK.

**The environment (both on the site and in the wider environment)**

**45. The geographical location and national grid reference of the site or sites onto which the release**

See Part A5.

**46. The physical or biological proximity of the site to humans and other significant biota.**

The trial will be partially in the field. There will be proximity to humans who are looking after the birds or involved in the trial. Stringent biosecurity will be applied at all times, limiting likelihood of physical or biological proximity to other significant biota. The trial will be GCPv compliant.

**47. The proximity to significant biotopes, protected areas or drinking water supplies.**

The birds involved in the trial will have proximity to other birds in the trial, and to their own drinking water supplies.

**48. The climatic characteristics of the region or regions likely to be affected.**

The birds will be kept in climate-controlled indoor conditions at all times; whether in the lab, in the field or in transit.

**49. The geographical, geological and pedological characteristics.**

No characteristics of relevance to note.

**50. The flora and fauna, including crops, livestock and migratory species.**

Birds will not come into contact with any other birds or animals. Birds will be immediately culled at the facility. This will take place at the end of the day after which the slaughterhouse will undergo cleansing and disinfection.

**51. The description of target and non-target ecosystems likely to be affected.**

No other poultry will be kept at the premises during the trial. The hygiene and biosecurity rules to be applied while the birds are in transit and at the rearing sites are outlined in section 36 above. It is imperative that the field trial birds do not get naturally infected with HPAI from the environment. If this happens, the standard response procedure to a confirmed infected premises will be stood up, disease control zones will be put in place and all birds on the infected premises will be humanely culled.

**52. The comparison of the natural habitat of the recipient organisms with the proposed site or sites of release.**

Turkeys are farmed animals. The number of animals to be included in the trial is justified by the housing density of the farms, the rate of early withdrawal/premature mortality of the trial species and the number of experimental units required to perform statistical analyses undertaken.

**53. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.**

None.

**Part IV: Information relating to the interactions between the organisms and the environment**

**Characteristics affecting survival, multiplication and dissemination**

**54. The biological features which affect survival, multiplication and dispersal.**

The natural host of HVT is Turkeys. HVT causes a persistent infection and can be detected during the whole life of the animal. Spreading of the virus between turkeys occurs through the shedding/release of dust particles from feather follicles.

**55. The known or predicted environmental conditions which may affect survival, multiplication and dissemination, including wind, water, soil, temperature and pH.**

Outside of the turkey host, the virus can only survive in viable CEF cells. Factors that influence the survival of CEF cells (high temperatures, desiccation, pH, etc.) also affect the stability of the virus.

**56. The sensitivity to specific agents.**

The GM strain is no less susceptible to antiviral chemical and other agents than the wild type, and so is easily killed.

**Interactions with the environment**

**57. The predicted habitat of the organism.**

The only known natural host of HVT is Turkeys.

**58. The studies of the behaviour and characteristics of the organisms and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses.**

Inoculation of turkeys with HVT results in a persistent, lifelong infection. The recombinant HVT is being trialled to evaluate if immunity to HPAI is also afforded for the (commercial) production life of the turkeys.

**59. The capability of post-release transfer of genetic material-**

**a. from the genetically modified organisms into organisms in affected ecosystems,**

The HVT vector has no other natural host, and is a very stable vaccine vector with no evidence of genetic transfer seen in over four decades of use and the inserted genes do not change.

**b. from indigenous organisms to the genetically modified organisms.**

The vector HVT strain has been used for c.45 years with no evidence of recombination with other (micro-) organisms; the inserted genes do not increase this likelihood.

**60. The likelihood of post-release selection leading to the expression of unexpected or undesirable traits in the genetically modified organisms.**

The stability of the recombinant virus and expression of its genetic material has been evaluated by passage in CEF cells, and by veterinary trials in EU countries.

**61. The measures employed to ensure and to verify genetic stability, the description of genetic traits which may prevent or minimise dispersal of genetic material and methods to verify genetic stability.**

This has been described above, and involved serial passage and results from trials.

**62. The routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact and burrowing.**

The natural host is turkey. The generation time in the natural host and in chickens can be estimated between 12 and 48 hours. HVT causes a persistent infection and can be detected during the whole life of the animal. Spreading of the virus between turkeys occurs through the shedding/release of dust particles from feather follicles.

**63. The description of ecosystems to which the organisms could be disseminated.**

None, beyond the trial sites. The GM virus is no more capable of dissemination than the wild type, outside of the host it dies rapidly.

**64. The potential for excessive population increase of the organisms in the environment.**

The GMO does not multiply any faster than that of the wild type (in fact this, like other recombinant HVT vaccines has been reported as being slightly slower in this aspect by comparison to the wild type).

**65. The competitive advantage of the organisms in relation to the unmodified recipient or parental organism or organisms.**

Dissemination of the GMOs has been studied in chickens in comparison to the recipient HVT FC-126 strain; no apparent qualitative differences between the GMOs and HVT FC-126 in terms of virus localization and chronology of virus appearance in tissues tested except for reduced replication are acceptable. Therefore, the dissemination rate of the GMOs from vaccinated chickens is the same or decreased compared to the recipient virus.

**66. The identification and description of the target organisms if applicable.**

The target organism is Turkeys Due to restrictions at the challenge phase, the 'Roly Poly' Bronze breed will be utilised for the study. These reach a lower breed weight and therefore enable maximisation of birds that can be challenged in high containment whilst ensuring robust statistical power.

**67. The anticipated mechanism and result of interaction between the released organisms and the target organisms if applicable.**

It may be possible for vaccinated birds to shed the vaccine HVT but birds will be separated based on vaccine regimen stated in the protocol and as such there won't be a mechanism for undesired interactions between vaccinated birds and non-target birds. Further, the vehicle for these vaccines is already in use to deliver proteins from other viral agents and is used both across the UK and globally.

**68. The identification and description of non-target organisms which may be adversely affected by the release of the genetically modified organisms, and the anticipated mechanisms of any identified adverse reaction.**

Housing of vaccinated birds and biosecurity will prevent any risk of transmission of the GMO to non-target organisms. Further, the vehicle for these vaccines is already in use to deliver proteins from other viral agents and is used both across the UK and globally.

**69. The likelihood of post-release shifts in biological interactions or in the host range.**

The vehicle for these vaccines is already in use to deliver protein from other viral agents and is used both across the UK and globally. There is no predicted post-release shift in biological interactions or host range.]

**70. The known or predicted interactions with non-target organisms in the environment, including competitors, prey, hosts, symbionts, predators, parasites and pathogens.**

There are no predicted interactions with non-target organisms

**71. The known or predicted involvement in biogeochemical processes.**

There are no predicted interactions with biogeochemical processes

**72. Any other potentially significant interactions with the environment.**

There are no predicted interactions with the environment

**Part V: Information on monitoring, control, waste treatment and emergency response plans**

**Monitoring techniques**

**73. Methods for tracing the organisms and for monitoring their effects.**

Antibody responses will be used post vaccination to monitor the effect of vaccination. Excretion of vaccine will not be monitored. Further, the vehicle for these vaccines is already in use to deliver proteins from other viral agents and is used both across the UK and globally.

**74. Specificity (to identify the organisms and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques.**

Antibody responses will be monitored comparing vaccinated birds with unvaccinated birds.

**75. Techniques for detecting transfer of the donated genetic material to other organisms.**

Vaccinated birds will be housed with no opportunities for transfer of the donated genetic material to other organisms

**76. Duration and frequency of the monitoring.**

Antibody responses will be assessed at several timepoints post vaccination to monitor the effect of vaccination. From the protocol:

"At Day 0, twenty unvaccinated day-old poults enrolled onto the study will be euthanized by the poultry vet on farm, and heart blood samples obtained. The blood samples will be placed in the transportation container (provided by APHA) as soon as possible to ensure the required temperature range (22°C +/- 5°C) is maintained and sent to APHA for serology testing to determine baseline HPAI immunity.

At Days 28, 56, 84, 112 and 140, blood samples (approximately 1 mL) will be taken on-farm by the poultry vet for the routine testing of poultry diseases (including *Mycoplasma spp.* and *Ornithobacterium rhinotracheale*) from the (wing) brachial vein, into a whole blood vacutainer, pre-labelled with a unique sample identifier. Monthly blood samples are also required to comply with EU surveillance requirements described in Annex XIII Part 5 of regulation 2023/361. Blood collected at monthly timepoints for routine veterinary practices will also be used to assess antibody longevity across the different vaccination groups. Monthly blood assessment will be undertaken for antibody response post vaccination using both World Organisation for Animal Health (WOAH) and locally adopted serological assays. Use of these routine diagnostic samples for scientific analysis is justified under the guidance provided by the RCVS / BVA Ethical Review Working Party Report (2013), *Practical considerations of the interface between clinical research and ASPA requirements*.

At the end of the study, all remaining unchallenged trial birds on farm will be transferred directly to the named slaughterhouse, where they will be immediately slaughtered and a poultry vet will collect terminal blood samples from all remaining birds for further analysis at APHA.

**Control of the release**

**77. Methods and procedures to avoid and/or minimise the spread of the organisms beyond the site of release or the designated area for use.**

Enhanced biosecurity measures will be applied at the hatchery, brooding and rearing/finishing sites in line with those that are required in an avian influenza prevention zone (AIPZ) (a zone declared by government, where strict biosecurity and hygiene measures are mandatory for all bird keepers to prevent the spread of avian influenza). Measures include only essential movements of people, vehicles and equipment to and from the areas where the poultry are kept; cleansing and disinfection of equipment, vehicles and footwear; and, storing feed, water and bedding under cover.

**78. Methods and procedures to protect the site from intrusion by unauthorised individuals.**

Enhanced biosecurity measures will be applied at the hatchery, brooding and rearing/finishing sites in line with those that are required in an avian influenza prevention zone (AIPZ) (a zone declared by government, where strict biosecurity and hygiene measures are mandatory for all bird keepers to prevent the spread of avian influenza). Measures include only essential movements of people, vehicles and equipment to and from the areas where the poultry are kept; cleansing and disinfection of equipment, vehicles and footwear; and, storing feed, water and bedding under cover.

**79. Methods and procedures to prevent other organisms from entering the site.**

Enhanced biosecurity measures will be applied at the hatchery, brooding and rearing/finishing sites in line with those that are required in an avian influenza prevention zone (AIPZ) (a zone declared by government, where strict biosecurity and hygiene measures are mandatory for all bird keepers to prevent the spread of avian influenza). Measures include only essential movements of people, vehicles and equipment to and from the areas where the poultry are kept; cleansing and disinfection of equipment, vehicles and footwear; and, storing feed, water and bedding under cover. No other poultry will be kept at the premises during the trial.

**Waste treatment**

**80. Type of waste generated.**

This will consist of turkey carcasses.

**81. Expected amount of waste.**

The GMO will be inoculated into 300 turkeys, but all 1000 turkeys used in the trial will either be culled in containment and disposed of as clinical waste following challenge or be slaughtered and any animal by-products disposed of under appropriate health and food safety regimes.

**82. Description of treatment envisaged.**

The subset of birds used in the challenge test will be disposed of under appropriate containment level biosecurity measures, as they will be contaminated with live HPAI virus. The remainder of the trial turkeys will not require further treatment after slaughtering; but all premises will be disinfected as a precautionary measure.

**Emergency response plans**

**83. Methods and procedures for controlling the organisms in case of unexpected spread.**

This will be controlled because enhanced biosecurity measures will be applied at the hatchery, brooding and rearing/finishing sites in line with those that are required in an avian influenza prevention zone (AIPZ) (a zone declared by government, where strict biosecurity and hygiene measures are mandatory for all bird keepers to prevent the spread of avian influenza). Trial birds will be transported in sealed vehicles, supervised by qualified vets. No other animals will be present in the vehicles. Vehicles will be cleansed and disinfected before and after use.

**84. Methods, such as eradication of the organisms, for decontamination of the areas affected.**

Measures include only essential movements of people, vehicles and equipment to and from the areas where the poultry are kept; cleansing and disinfection of equipment, vehicles and footwear; and, storing feed, water and bedding under cover. Trial birds will be transported in sealed vehicles, supervised by qualified vets. No other animals will be present in the vehicles. Vehicles will be cleansed and disinfected before and after use.

**85. Methods for disposal or sanitation of plants, animals, soils and any other thing exposed during or after the spread.**

See section 84 for details.

**86. Methods for the isolation of the areas affected by the spread.**

This will be achieved by the enhanced biosecurity measures applied at the hatchery, brooding and rearing/finishing sites in line with those that are required in an avian influenza prevention zone (AIPZ) (a zone declared by government, where strict biosecurity and hygiene measures are mandatory for all bird keepers to prevent the spread of avian influenza).

**87. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.**

Enhanced biosecurity measures will be applied at the hatchery, brooding and rearing/finishing sites in line with those that are required in an avian influenza prevention zone (AIPZ) (a zone declared by government, where strict biosecurity and hygiene measures are mandatory for all bird keepers to prevent the spread of avian influenza).

**Part VI: A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this schedule, and the name of the body or bodies responsible for carrying out the studies.**

[Type in font ‘Arial’, size 12, left-hand justify, and do not underline the text]

**References**

Raphaël Darteil, Michel Bublot, Eliane Laplace, Jean-François Bouquet, Jean-Christophe Audonnet, Michel Rivière. (1995) Herpesvirus of Turkey Recombinant Viruses Expressing Infectious Bursal Disease Virus (IBDV) VP2 Immunogen Induce Protection against an IBDV Virulent Challenge in Chickens, Virology, **211**, Issue 2, Pages 481-490,
https://doi.org/10.1006/viro.1995.1430

Germeraad, E. A., Bouwman, K. M., Jansen, C. A., Gonzales, J. L., Augustijn-Schretlen, M., Fabri, T., de Wit, M. K., Stegeman, J. A., Velkers, F. C., de Wit, J. J., de Jong, M. C. M., & Beerens, N. (2024). Progress report: transmission study testing HVT-based H5 vaccine against highly pathogenic avian influenza (HPAI) H5N1 virus (clade 2.3.4.4b): First report, 8-weeks post vaccination with VAXXITEK HVT+IBD+H5. (Wageningen Bioveterinary Research report). Wageningen Bioveterinary Research. https://doi.org/10.18174/662098

van Zijl M, Quint W, Briaire J, de Rover T, Gielkens A, Berns A. (1988). Regeneration of Herpesviruses from Molecularly Cloned Subgenomic Fragments. Journal of Virology 62: 2191-2195.

Witter RL, Nazerian K, Purchase HG, and Burgoyne GH. (1970) Isolation from Turkeys of a Cell-Associated Herpesvirus antigenically related to Marek's Disease Virus. Am.J.Vet.Res. 31 (3) 525-538.

**Application for consent to release a GMO**

**Part A2: data or results from any previous releases of the GMO**

**Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].**

The GMO, Vaxxitek HVT-IBD-H5, was subject to a release application under 2001/18/EC to the Netherlands (ref: B/NL/23/005), with starting date of 01/06/2023 and finishing date of 30/01/2030. A progress report on this trial was published: https://doi.org/10.18174/662098

## Part A3: Details of previous applications for release

**Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.**

The GMO, Vaxxitek HVT-IBD-H5, was subject to a release application under 2001/18/EC to the Netherlands (ref: B/NL/23/005), with starting date of 01/06/2023 and finishing date of 30/01/2030. A progress report on this trial was published: https://doi.org/10.18174/662098

## Part A4: Risk assessment and a statement on risk evaluation

As the HVT recombinants are nonpathogenic, the level of risk for both humans and the environment can be considered as effectively zero.

### Risk Assessment: environmental impact of the release of the GMOs

No environmental impact is expected. - HVT is a naturally non-pathogenic virus. Its natural host is the turkey, but the virus can also replicate in chickens, but only after an intramuscular or subcutaneous application. Replication in other avian species is very unlikely. HVT causes no clinical disease in turkeys, chickens and other avian species. Genetic modifications made by introducing either the IBD VP2 or the HA H5 genes does not change the non-pathogenic phenotype of the parent virus and the recombinants are therefore still non-pathogenic.

### Risk assessment: factors affecting dissemination

The virus can spread via inhalation of dust particles shed from the skin from infected (or vaccinated) birds to turkeys but spreading to chickens is highly unlikely. Shedding from vaccinated chickens is limited and transient in nature; but more prolonged in Turkeys.

### Risk assessment: human health impact

HVT and recombinant HVTs are not capable of replicating in mammalian cells and cannot infect humans.

### Risk assessment: environmental impact

HVT and recombinant HVTs are not capable of replicating in mammalian cells and cannot infect humans.

### Risk assessment: monitoring the GMO

No specific monitoring will occur.

**Risk assessment: emergency response**

Not applicable.

## Part A5: Assessment of commercial or confidentiality of information contained in this application.

**Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.**

The exact geographical locations of the hatchery, brood site(s), finishing site and slaughterhouse used by the applicant have been kept from the public register on the grounds of confidentiality with respect to their ongoing commercial enterprise subsequent to this GMO release trial. However, these details will be made available to Inspectors acting on behalf of the competent authority.

## Part A6: Statement on whether detailed information on the description of the GMO and the purpose of release has been published

**Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.**
**This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.**

This vaccine is derived from that of one already approved for use in GB; Vaxxitek HVT+IBD, and Vaxxitek HVT+IBD+H5 has received EMA approval for marketing in the EU. Therefore, Summary Product Characteristics (SPC) are available for both vaccines; but detailed molecular sequences are not included in such information.