PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	*Netherlands*
(b)	Notification number	*B/NL/12/001*
(c)	Date of acknowledgement of notification	*01/08/2012*
(d)	Title of the project	
	MVA-based recombinant influenza vaccines encoding influenza virus hemagglutinins	
(e)	Proposed period of release	*From 01/09/2012 until 01/09/2020*

2. Notifier

Name of institution or company: *Erasmus Medical Center*

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)
Genus: *Orthopoxvirus*
Species: *Modified Vaccinia virus Ankara*

(c) Genetic stability – according to Annex IIIa, II, A(10)

The GMO was passaged five times at a low multiplicity of infection on chicken embryo fibroblasts (CEF cells). The virus harvest from the final passage was tested on genetic profile with PCR and antigen expression with immunocytochemistry and Western blot analysis. Previous genetic stability tests perfomed in this manner demonstrated that the virus was genetically stable.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (X)

If yes, insert the country code(s) …

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.

The only route by which the GMO could spread into the environment is by spillage from an open and intact vial or a damaged vial, needle sting accident, leakage from the injection site or exposure to contaminated waste. However the risk that another person actually becomes infected is minimal. In the test subject, residual virus may spread from the site of injection via blood or lymph. Data from the analysis of MVA distribution upon high dose inoculation into immune-suppressed non-human primates suggest that viral genomes can be detected in pharyngeal epithelial cells, PBMC and draining lymph nodes for up to two weeks post injection. However, the failure to re-isolate any viable MVA from the animals confirms that no virus replication takes place and the infection is strictly self-limiting.

Also in humans immunized with an MVA-based vaccine, no MVA virus could be detected in the blood 1 hour after immunization. Thus no viral shedding can take place from the test-subject apart from the injection site. Modifications and the cell substrate on which the GMO is grown do not alter this potential route of spreading.

B. **Information relating to the recipient or parental organism from which the GMO is derived**

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	*Poxviridae (subfamily: Chordopoxvirinae)*
(ii)	genus	*Orthopoxvirus*
(iii)	species	*Vaccinia virus*
(iv)	subspecies	*n.a.*
(v)	strain (MVA)	*Modified Vaccinica virus Ankara*
(vi)	pathovar (biotype, ecotype, race, etc.)	*n.a.*
(vii)	common name	*MVA*

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (X) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..

Macaronesian ..

(ii) No (X)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify *No natural host. MVA was derived from vaccinia virus and adapted to chicken embryo fibroblasts by loss of 30kb of its genome.(Mayr A et al. Infection 1975, 3:6-14) For Vaccinia virus the natural host is thought to be the cow or another bovine species.*

(b) If the organism is an animal: natural habitat or usual agroecosystem:
-

5. (a) Detection techniques
Modified Vaccinia virus Ankara (MVA) can detected by propagation of specimens in chicken embryo fibroblasts (CEF). MVA genome can be detected by PCR.

(b) Identification techniques

Recombinant Modified Vaccinia virus Ankara is identified by immunocytochemistry on infected cells (with an anti-MVA antibody), PCR analysis with primers for the six deletion sites in the MVA genome. Furthermore sequence analysis and restriction digest analysis can be used to identify the recombinant MVA virus.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (X) No (.)
If yes, specify
It is classified under Cogem CGM030519-06 and Cogem CGM030922-04 as a class 1 pathogen.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)

If yes:

(a) to which of the following organisms:

humans	(.)
animals	(.)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
In a replication competent environment the replication cycle of the GMO lies in the range of hours

(b) Generation time in the ecosystem where the release will take place:
Not applicable: In human cells the GMO is replication deficient

(c) Way of reproduction: Sexual .. Asexual X

(c) Factors affecting reproduction:
Loss of 30kb genetic material (during adaption of CVA on CEF cells) rendered the virus replication deficient is most cell types including most mammalian cells.

9. Survivability

(a) ability to form structures enhancing survival or dormancy: *Not applicable*.

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify …	

(b) relevant factors affecting survivability:
Environment: *Temperature, humidity, UV*
Disinfection: *UV, ethanol and other chemicals*

10. (a) Ways of dissemination
In humans and other mammals the virus is replication deficient and thus does not disseminate within the host. The only route by which the GMO could disseminate into the environment is by spillage from an open and intact vial or a damaged vial, needle sting accident, leakage from the injection site or exposure to contaminated waste.

(b) Factors affecting dissemination

The virus is replication deficient and thus an infection with the GMO is self-limiting. In case of accidental spilling, disinfection of the contaminated surface with 70% ethanol is sufficient to inactivate the virus.

11. *Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers) B/NL/06/009*

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

Insertion of a synthetic promoter (psynII) and an influenza virus hemagglutinin gene (HA) in deletion site III of the MVA virus genome. Upon infection of a cell by the recombinant MVA the HA gene is encoded and the HA protein is produced. Vaccination with the recombinant MVA will result in the induction of HA-specific antibodies that can protect against infection with a (homologous)_influenza virus.

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (.) No (X)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(.)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify …	

(b) Identity of the vector

…

(c) Host range of the vector

…

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify *homologous recombination by infecting chicken embryo fibroblasts with the wildtype MVA virus and subsequently transfecting the cells with the vector DNA (plasmid).*

6. Composition of the insert

(a) Composition of the insert
Synthetic promoter (psynII) that is poxvirus-dependent
influenza A virus HA gene
(b) Source of each constituent part of the insert
psynII promoter: synthetic…
HA gene: derived from an influenza A virus or ordered as a synthetic gene based on sequence information
(c) Intended function of each constituent part of the insert in the GMO
psynII promoter: facilitates expression of the HA gene in MVA-infected cells.
HA gene: encodes the HA protein to induce HA-specific immune responses.
(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: *integrated in the deletion III site of the MVA DNA genome*

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (X)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid	(.)
RNA virus	(X)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…
other, specify	…

2. Complete name

(i)	order and/or higher taxon (for animals)	*Orthomyxoviruses*
(ii)	family name for plants	*n.a.*
(iii)	genus	*Influenza viruses*
(iv)	species	*Influenza A virus*
(v)	subspecies	*n.a.*
(vi)	strain	*A/country/host (if not human)/isolate/year*
(vii)	cultivar/breeding line	*n.a.*
(viii)	pathovar	*n.a.*
(ix)	common name	*e.g. Influenza virus A/Netherlands/602/09 or A/NL/602/09*

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (X) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans	(X)
animals	(X)
plants	(.)
other	..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (X) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

The function of the influenza virus HA protein is binding of the virus particle to the host cell and membrane fusion of the viral membrane with the endosomal membrane. This function is the same for all 16 subtypes of HA and data from DNA vaccination trials show that there is no harmful effect of HA outside the context of an influenza.

In influenza virus particles, the envelope is completely derived from the cellular membrane of the host cell in which the viral surface proteins (HA and NA) are embedded. The HA protein is thus designed and meant to be incorporated in such a membrane. Poxviruses including MVA virus on the other hand have a different viral envelope. The primary intracellular mature virions are formed with one lipid membrane and acquire two additional membranes by wrapping in the trans-Golgi network and early endosome. These virions are termed intracellular enveloped virions (with three lipid membranes) and are transported along microtubules to the cell periphery, where their outermost membrane is lost when fusing with the plasma membrane, releasing the final double membrane enveloped virions from the cell. Thus, proteins integrated in the plasma membrane (such as HA) are not specifically targeted to or incorporated into the virus particles. Therefore the formation of pseudotyped virus (an MVA particle tropism change because of the influenza virus hemagglutinin) is impossible. Also the host range of the recombinant MVA (with the HA gene) will remain the same since that depends on the replication competence of the virus and that is not restored due to addition of the HA protein which is a structural protein of which it is highly unlikely that it will affect the block in morphogenesis.

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (X) No (.)

If yes, specify *Influenza viruses, depending or their pathogenicity, are classified as class 2 and 3 pathogens.*

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes (.) No (X) Not known (.)

Specify *MVA virus is most stable in a cell debris enclosed environment. In the MVA-vaccine preparations the virus is purified which reduces its stability. It is an enveloped virus and disinfection of contaminated surfaces with 70% ethanol is sufficient to inactivate the virus. In practice 70% ethanol will be used since it has been proven that it inactivates vaccinia viruses and there is no reason to assume that MVA (Modified Vaccinia virus Ankara) will not be susceptible to this inactivation method.*

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (.) No (X) Unknown (.)

Specify *The MVA-HA virus expresses the HA gene of which it is highly unlikely that it will affect the replication deficiency of the virus. This can be confirmed by HeLa Block assay. The principle behind this assay is that wildtype MVA virus can not replicate on HeLa cells due to accumulation of immature virions (IV) and the fact that no mature virions (MV) and extracellular enveloped virions (EV) can be formed. And the latter two types of virions are needed for replication. The assay thus is used to reassess the replication deficiency of recombinant MVA virus containing heterologous gene sequences with potential influence on MVA growth.*

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (X) Not known (.)

Specify *The host range will remain the same since that depends on the replication competence of the virus and that is not restored due to addition of the HA protein which is a structural protein of which it is highly unlikely that it will affect the block in morphogenesis. There is also no mechanism known through which the HA protein can be incorporated in the MVA virion by other means. Therefore it is highly unlikely that the HA protein constitutes a functional and/or structural part of the newly formed MVA virions in CEF cells. And even if the protein would be incorporated in the membrane of the MVA virus particle, the effect on virus tropism would remain unnoticed since the tropism is already extremely broad due to promiscuous receptor usage.*

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No (X) Not known (.)

Specify *MVA is avirulent and has shown to be safe for use in humans and mammals, even when they were immunocompromised. In contrast to normal vaccinia virus, MVA lacks many important viral immune evasion genes, e.g. it is readily recognized by the innate immune system and it cannot counteract antiviral functions of type I and type II interferon. In consequence, MVA is not able to replicate due to a block in morphogenesis because of which no new virus particles can be formed by an infected cell. In cells of human origin at least two mechanisms are in action in the block in morphogenesis, one has already been linked to the antiviral function of type I interferon but the molecular mechanism of the other remains to be elucidated. The pathogenicity of the recombinant virus has not altered, it is still replication deficient and not pathogenic in humans and mammals.*

…

2. Genetic stability of the genetically modified organism

The GMO was passaged five times at a low multiplicity of infection on chicken embryo fibroblasts (CEF cells). The virus harvest from the final passage was tested on genetic profile with PCR and antigen expression with immunocytochemistry and Western blot analysis. Previous genetic stability tests perfomed in this manner demonstrated that the virus was genetically stable.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Recombinant Modified Vaccinia virus Ankara (MVA) can detected by propagation of specimens in chicken embryo fibroblasts (CEF). MVA genome can be detected by PCR.

(b) Techniques used to identify the GMO
Recombinant Modified Vaccinia virus Ankara is identified by immunocytochemistry on infected cells (with two antibodies: anti-MVA, anti-HA), PCR analysis with primers for the six deletion sites in the MVA genome and HA-specific primers. Furthermore sequence analysis and restriction digest analysis can be used to identify the recombinant MVA virus.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Phase I clinical trial to assess safety and immunogenicity of the GMO as new influenza vaccine in young healty adults.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (X) No (.)
If yes, specify *The parental organism has no natural host thus the site of release is different because it is a new host.*

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference): Rotterdam, Erasmus Medical center, the Netherlands

(b) Size of the site (m^2): *Erasmus medical Centre, Central Location*
(i) actual release site (m^2): *+/- 48 m^2*

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable

4. Method and amount of release

(a) Quantities of GMOs to be released:
A maximum of 150 dosages per two weeks, divided over several administration rounds. Dosages are in a range of $10^6 – 10^8$ pfu.

(b) Duration of the operation:
Injection of the MVA and the involved hygienic measures take approximately minutes.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The injection site is disinfected with ethanol after administration of the GMO and subsequently covered with a waterproof bandaid that has to stay on for 24 hours. In case of accidental spillage of the GMO the material is disinfected with 70% ethanol.

5. Short description of average environmental conditions (weather, temperature, etc.)
Room temperature: 21-25 ^oC…
Humidity: 40-60%

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
Not applicable, there is only preclinical data available, studies within the current study are the first-in-man applications of MVA-HA.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	*Primates*
(ii)	family name for plants	*Hominidae*
(iii)	genus	*Homo*
(iv)	species	*Sapiens*
(v)	subspecies	*Sapiens*
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*Human*

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Due to the expression of the influenza HA gene in MVA-HA infected cells of the host, HA-specific antibodies and T cells will develop in the immunized human. Additionally, an MVA-specific immune response will be evoked.

3. Any other potentially significant interactions with other organisms in the environment
The possibility of potentially significant interactions with other organisms is minimal under the conditions of the proposed release of the replication deficient GMO. In the theoretical case of an interaction with the environment and more particular animals, due to infection via small wounds in the skin, this could result in local reactions as described also for humans. If mammals become infected, no virus replication can take place. There is a theoretical risk that hamsters are susceptible, due to the fact that MVA can replicate in BHK-21 (baby hamster kidney) cells. But since this is a highly conditioned cell line that has a crippled innate immune system and thus is more susceptible than normal immune competent cells, and the fact the MVA does not replicate in another hamster-derived cell line (CHO), replication of MVA in hamsters is highly unlikely. If chickens become infected MVA could possibly replicate in that host (the virus completely adapted to replication in Chicken Embryo Fibroblasts) however this does not have harmful effects as was demonstrated by Veits et al and Mayr (unpublished data) who both demonstrated that intentional infection of chickens with MVA does not result in disease in these animals.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details

The addition of the HA gene to the MVA virus does not result in selective advantage or disadvantage regarding competitiveness or invasiveness under the conditions of the proposed release.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
It is highly unlikely that the GMO will become persistent and invasive in other ecosystems. The virus is replication deficient and thus the infection in the test subjects is self-limiting. The only possible spread is by leakage from the injection site which is prevented by a waterproof band aid and if virus would leak anyway, the amount of virus is relatively low compared to the injected dose. Any spilled or leaked GMO can be effectively disinfected with 70% ethanol.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

Not applicable

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
The risk of recombination of the recombinant MVA with other poxviruses is almost zero. Variola virus that causes smallpox in humans has been eradicated since 1980. The only realistic candidates for recombination are monkeypox and cowpox. These viruses can infect humans, however only the latter is detected in the Netherlands sporadically in the past 50 years. Moreover, the event of a co-infection occurring in vivo is highly unlikely because of several reasons:
*1) The chance of a person in the Netherlands becoming infected with cowpox virus is very small (<1 cases of cowpox infections in humans in the Netherlands per year) The proportion of people taking part per study is 150/16,000,000. The chance that a person joins the study and becomes infected with cowpox is thus: 150 / 16,000,000 * 1/16,000,000 = $6*10^{-13}$*
2) Most human infections occur via the eye whereas the MVA vaccine is applied in the muscle of the upper arm. Hereby the two sites of infection are separated physically, making it virtually impossible for the two viruses to infect the same cell.

The MVA or MVA-HA virus infection is self-limiting (abortive replication) and thus the virus is only present for a short period of time until it has infected a cell. In the infected cell the MVA genes and recombinant gene are synthesized and expressed, resulting in production of MVA proteins and the influenza virus hemagglutinin glycoprotein. The MVA derived DNA is only present in the cytoplasm and thus cannot integrate in the human genome that is present in cell nucleus. After sometime the DNA is degraded by nucleases (enzymes in the cellular cytoplasm).

The risk of incorporation of the expressed influenza gene in wildtype influenza viruses is minimal. Reassortment (a process in which the wildtype influenza virus acquires a foreign influenza virus gene) of the MVA-encoded influenza virus gene with wildtype influenza virus is highly unlikely because:

1) Only the coding region of the gene is embedded in the MVA virus and not the non-coding regions (NCRs). These NCRs are necessary for packaging of the gene in the influenza virus particle and thus without them the gene will not be incorporated in the influenza virus.
2) There is a physical obstacle since the MVA virus is administered in the muscle of the upper arm and does not spread from thereon. Influenza viruses enter and reside in the respiratory tract and thus the viruses are present at two anatomically distinct locations.

(b) from other organisms to the GMO:
Highly unlikely for the same reasons as stated under (a)

(c) likely consequences of gene transfer:
In the highly unlikely event (as described under (a)) of a gene transfer to the GMO this will not render the virus replication competent since for that multiple specific recombinations have to take place. I the theoretical event of a double infection with a recombinant MVA virus and an influenza virus, the genetic material is present in two different compartments of the cell. The RNA (messenger RNA) of the MVA virus is

present in the cytoplasm whereas the positive sense vRNA (=the copy RNA that forms the gene segments) of the influenza virus is present in the nucleus.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
Not available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable

H. Information relating to monitoring

1. Methods for monitoring the GMOs
PCR to detect GMO genomic DNA

2. Methods for monitoring ecosystem effects
Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Sequence comparison of the genomic DNA of the other organism and the GMO.

4. Size of the monitoring area (m^2)
Not applicable

5. Duration of the monitoring
Not applicable

6. Frequency of the monitoring
Not applicable

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The room is cleaned and potentially contaminated surfaces are disinfected with 70% ethanol.

2. Post-release treatment of the GMOs
Vaccine vials are discarded according to protocol in a biosafety container.

3. (a) Type and amount of waste generated

The waste consists per vaccine administration of:
-band aid (potentially contains GMO) (disinfected with 70% ethanol)
-syringe with needle (contains GMO)
-vaccine vial (contains GMO)
-gloves (potentially contains GMO)
-surgerey sheets (potentially contains GMO)

3. (b) Treatment of waste

The waste will be disposed of in a biosafety container (SZA-vat) and will be treated as ML-I waste according to the procedures of the Erasmus MC based on Bijlage 9 of the Regeling GGO (2008). Band aids that are removed by test subjects, 24 hours after vaccination, outside the hospital will be disinfected with 70% ethanol, put in a sealable bag and discarded. (Suspected) contaminated surfaces will be disinfected with 70% ethanol which is wiped off after 10 minutes incubation. The wipes will be discarded as ML-I waste.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

Protocols, for stick and cut accidents and in case a spill has occurred, are in place. In case of spillage on clothing the textile will be disinfected with 70% ethanol (if spillage occurred in the size of droplets) before it will be washed.

In case of spillage on a surface it will be disinfected with 70% ethanol. Also when all handlings with the GMO have finished the surfaces that were used to work on (chairs, sinks and tables) will be disinfected and cleaned with 70% ethanol. Despite the fact that the unit is not a ML-I unit it will be treated as such.

In the highly unlikely case of leakage from the injection site onto clothing or bed linen of the test subject at home, this has to be washed; if the leakage spot is visible it should be disinfected with 70% ethanol before the textile is washed.

2. Methods for removal of the GMO(s) of the areas potentially affected

Contaminated areas/Surfaces will be disinfected with 70% ethanol solution.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

No plants, animals or soils shall be present on site during the release of the GMO.

4. Plans for protecting human health and the environment in the event of an undesirable effect

MVA and recombinant MVA-HA are apathogenic and have a strong host-range restriction. If, at all, the virus could spread after the proposed release to other humans or animal species, the infections will be self-limiting and thus will not result in an environmental impact. Undesirable effects thus are not to be expected. But in case changes in risk management occur, procedures will be adapted accordingly. A possible change is the occurrence of allergic reactions although the risk is low.