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	the Netherlands
(b)	Notification number	B/NL/08/005
(c)	Date of acknowledgement of notification	30/05/2008
(d)	Title of the project	"A phase II, double blind randomized study to evaluate the immunogenicity of the therapeutic HIV-1 vaccine NYVAC-B versus placebo in chronic HIV-1 infected patients successfully treated with HAART (TheraVac-03)".
(e)	Proposed period of release	From 01 September 2008 until 01 April 2010.

2. Notifier

Name of institution or company: Academic Medical Center of the university of Amsterdam, the Netherlands …

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)

Family: Poxviridae, Subfamily: Chordapoxviridae, Genus: Orthopoxviruses, species: Vaccinia, strain: NYVAC (New York Vaccinia Virus)

(c) Genetic stability – according to Annex IIIa, II, A(10)
Recombinant virus NYVAC-B was subjected to sequential passages in chicken embryo fibroblasts. The expression analysis of the HIV proteins *env*, *gag*, *pol and nef* was performed by westernblot analysis.

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

Yes (x) No (.)

If yes, insert the country code(s) DE, FR (and Switzerland, outside the EU).

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.
NYVAC-B is based on the live attenuteated vaccinia vector NYVAC. It has been shown that this vector cannot replicate in human cells. Complementation due the presence of other viruses dies not occur, prohibiting replication in this way. Spread of the GMo in the environment can only occur due to leakage out of the injection site short after vaccination. Other persons might be exposed to the GMO, thus infection of other persons cannot be excluded. The quantity of virus particles, however, will be much less than the quantity used during vaccination of the participating patients.

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)	not applicable
(ii)	genus	Orthopoxviruses
(iii)	species	Vaccinia
(iv)	subspecies	not applicable
(v)	strain	NYVAC (New York Vaccinia Virus)
(vi)	pathovar (biotype, ecotype, race, etc.)	not applicable
(vii)	common name	NYVAC-B

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 known.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
not applicable

5. (a) Detection techniques
Culture on chicken embryo fibroblasts

(b) Identification techniques
PCR

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (.)
If yes, specify
Related to Vaccine against smallpox (BSL 1)

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

If yes:

(a) to which of the following organisms:

humans	(x)
animals	(x)
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: unknown
(b) Generation time in the ecosystem where the release will take place:
Not applicable: the strain that will be used is replication incompetent.
(c) Way of reproduction: Sexual .. Asexual x

(c) Factors affecting reproduction:
NYVAC (New York Vaccinia; (vP866) is a highly attenuated vaccinia virus strain that was originally derived from a plaque-cloned isolate of the Copenhagen vaccinia vaccine strain (VC-2) by the precise deletion of 18 open reading frames (ORFs) from the viral genome(Tartaglia et al., 1992), which severely compromised its capacity to replicate in mammalian cells

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(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:
Temperature, humidity, UV radiation, and chemical disinfection.

10. (a) Ways of dissemination

Vaccinia can disseminate only via direct physical contact, but cases of dissemination via clothing, hands, nasopharynx of health care workers and via aerosols (reviewed in Sepkowitz, NEJM, 2003) have been described. People affected by (atopical) eczema seems to be more at risk tp a secondary infection with Vaccinia when someone in their neighbourhood is vaccinated with Vaccinia. In the live attenuated strain that will be used, both the C7L and the K1L host ranges genes are deleted, making it deficient for replication on human cell lines, as well as on cells derived from a number of other mammalian species (Tartaglia et al., 1992 and Perkus et al., 1990). Only short after vaccination with NYVAC, the vaccine could “leak out” form the injection site. Safety measures will be applied, as described in the protocol, to prevent this.

(b) Factors affecting dissemination
The attenuation of the viral vector, the hygienic/ safety procedures

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)
Not applicable.

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
The expression of the inserted HIV-1 genes, in order that the vaccinated person can develop an immune response to these antigens.

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 (x)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert
HIV-1 genes env and gag-pol-nef.

(b) Source of each constituent part of the insert
HIV-1 BX08 (which is a French R5 HIV-1 clade B isolate) for env, and HIV-1 IIIB (which is TCLA clade B) for gag-pol-nef.

(c) Intended function of each constituent part of the insert in the GMO
The expression of each of these inserted HIV-1 genes, in order that the vaccinated person can develop an immune response to these antigens.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (x)
- other, specify …

(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) not applicable

(ii)	family name for plants	not applicable
(iii)	genus	Retroviridae
(iv)	species	Human Immunodeficiency Virus type 1
(v)	subspecies	not applicable
(vi)	strain	BX08 (for env0; IIIB (for gag-pol-nef)
(vii)	cultivar/breeding line	not applicable
(viii)	pathovar	not applicable
(ix)	common name	HIV-1

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 (.)
plants (.)
other ..

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

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

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 Human immunodeficiency virus: Risk class BSL 3

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 …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (.) Unknown (.)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (x) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (x) Not known (.)
Specify …

2. Genetic stability of the genetically modified organism
Stable after 48 months at –20°C. Recombinant virus NYVAC-B was subjected to sequential passages in chicken embryo fibroblasts. The expression analysis of the HIV proteins env, gag, pol and nef was performed by westernblot analysis.

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
PCR

(b) Techniques used to identify the GMO
PCR plus sequencing

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The release is necessary to conduct a clinical trial part of the TheraVac project, financed by the EU (Fifth Framework Program, project number QLK2-CT-2002-01299). The objectives of TheraVac are the evaluation of the efficacy of two HIV-1 specific vaccines. One of these two vaccines is NYVAC-B. More specific, the release is necessary to conduct a phase-2

study to evaluate the immunogenicity of NYVAC-B. Secondary objectives include the evaluation of the safety of the vaccine. If the Immunogenicity and the safety will be shown to be encouraging, further studies will be scheduled to assess the clinical efficacy of the vaccine in chronically HIV-1 infected patients. If the vaccine is safe and effective, this is of the utmost importance, since the current available treatment for HIV-1 infection has severe drawbacks: metabolic complications, increased risk of cardiovascular disease and accumulation of resistance associated mutations. Strategies to limit the exposure to antiretroviral treatment are therefore necessary. However, simply interrupting therapy in chronically HIV-1 infected patients, even after a long period of successful antiretroviral treatment, is followed by a quick re-emergence of the virus. This reflects the lack of a protective immunity to HIV-1. A effective vaccine could evoke (more) protective immunity, so that (temporary) interruption of antiretroviral treatment will be possible.

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 natural reservoir of Vaccinia is unknown. However, it was used for the pox vaccination program in humans, until the global eradication of pox in 1978.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference): Amsterdam, the Netherlands

(b) Size of the site (m^2): (the entire AMC hospital), thousands of m^2

(i) actual release site (m^2): a room on the Special Investigation Unit in the Hospital: approx. 15 m^2, where the vaccine will administered in the upper arm (.,5 cm^2)

(ii) wider release site (m^2): (the entire AMC hospital), thousands of 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:

The lot that will be used in the TheraVac phase 1 and 2 clinical trials is NYVAC-HIV B vP2009 Lot # Z138, The batch size of the production lot was about 2775 mL of bulk clarified bulk. Of this batch in total 200 dosages are intended to be used for the TheraVac project.

(b) Duration of the operation:
Vaccination takes a few minutes.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

Only short after vaccination, vaccine could “leak” out of the injection site. In the protocol several safety procedures are included to prevent this. After vaccination, the injection site will be disinfected with alcohol and 10 minutes after vaccination, the injection site will be cleaned with a plaster that will remain at least 24 hours. All garbage will be kept separate from other hospitralk garbage, and will be destroyed appropriately.

5. Short description of average environmental conditions (weather, temperature, etc.)
Indoor, hospital (e.g. room temperature, all normal indoor circumstances).

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.

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)	not applicable
(ii)	family name for plants	not applicable
(iii)	genus	homo
(iv)	species	sapiens
(v)	subspecies	not applicable
(vi)	strain	not applicable
(vii)	cultivar/breeding line	not applicable
(viii)	pathovar	not applicable
(ix)	common name	human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
The development of an HIV-1 specific immune response against the HIV-1 genes env, gag, pol and nef which are in the GMO vaccine.

3. Any other potentially significant interactions with other organisms in the environment
Not applicable

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
NYVAC is an attenuated strain of Vaccinia. No signs of increased competitiveness, increased invasiveness for the GMO or illness related to these have been observed.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
Since NYVAC is replication incompetent, it cannot maintain itself in any ecosystem.

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
Not applicable.

(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	…

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Theoretically, recombination of “exogenous” HIV-genes from the NYVAC-B vaccine, en “endogenous” HIV-genes, part of the strain with which the patient is infected, could occur. This is very improbable, because it requires a contemporary infection of a certain cell with both the vaccine-virus and replicating autologous HIV-1. HIV-1 can replicate only in $CD4^+$ cells: $CD4^+$ T lymphocytes, dendritic cells and macrophages. In patients on successful treatment with antiretroviral treatment, and thus an undetectable plasma HIV-1 RNA (as the study participants), the replication rate in these cells is extremely low. If these cells might become infected with both vaccine virus and wild/type virus, messenger-RNA derived from the vaccine could recombinate with messenger RNA or genomic RNA of the “endogenous” HIV. Theoretically, this kind of recombination could occur also without the presence of the vaccine virus: between the “endogenous” HIV messenger-RNA and the genomic HIV-RNA. The possibility of obtaining a replication-competetent virus that can make the step via reverse-transcription to DNA and next to integration of this DNA in the host genomic DNA, and that is also able to compete with the present “endogenous” HIV-1, is theoretically very low. Considering that the HIV-genes in the vaccin-virus are “normal” HIV-genes, no additional risks of these genes (as compared to recombination within the “endogenous” virus as described above) are to be expected.

(b) from other organisms to the GMO:
Wild type vaccinia, able to recombinate with the GMO, is not prevalent. Therefore, this recombination risk can be neglected.

(c) likely consequences of gene transfer:
As described in the answer to (a), the possibility of gene transfer is very low. Since the HIV-1 genes in the vaccine are not targets of antiretroviral treatment, the susceptibility of a possible recombinated virus for antiretroviral treatment cannot be affected. Therefore, a recombinated virus would be suppressed by the treatment equally as the wild-type virus and thus would not have consequences.

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
The GMO itself will not be monitored, the patients in whom the GMO will be injected will. Patients will be monitored 2 hours in hospital after vaccination, and will have study visits at day 1, week 1, 2, 4 (= vaccination 2), 6, 8, 12, 24, 36, 48 after the first vaccination. During these visits general safety parameters will be measured/ collected (medical history, physical examination, standard safety laboratory, as well as plasma HIV-1 RNA and $CD4^+$ T cell count).

2. Methods for monitoring ecosystem effects
Not applicable.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
PCR/ sequencing.

4. Size of the monitoring area (m^2)
15 m^2

5. Duration of the monitoring
See answer to (1).

6. Frequency of the monitoring
See answer to (1).

I. Information on post-release and waste treatment

1. Post-release treatment of the site
All material used will be disposable and collected in a separate hospital garbage collection system. All surfaces that have been in contact with the GMO will be disinfected with alcohol or a chlorine solution.

2. Post-release treatment of the GMOs
The GMO will be injected in a patient. The empty vial will be treated as all other garbage possibly contaminated with the GMO.

3. (a) Type and amount of waste generated
Per vaccination (2 per study participant, 10 participants) the following disposable materials will be used: injection syringe + needle, vial, gloves, coat, hair cover, shoe covers, sterile covers for the injection site.

3. (b) Treatment of waste
Waste will be burnt, separate treatment from all other hospital waste,

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
   Vaccination procedure is designed to minimize the risk of unexpected dissemination: separate closed room, disposable material, disinfection after procedure. If vaccine might be spoilt, the affected areas will be disinfected with alcohol or chorine solution.

2. Methods for removal of the GMO(s) of the areas potentially affected
   Disinfection with alcohol or chorine solution.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   Surfaces will be disinfected with with alcohol or chorine solution. No plants or animals will be allowed to be present during the vaccination procedure.

4. Plans for protecting human health and the environment in the event of an undesirable effect
   In the unlikely case that, via unknown mechanisms, severe adverse events will occur or of the GMO might spread under humans, treatment with Vaccinia Immunoglobulines (VIG) might be applied. Another agent found to be effective against pox viruses is cidofovir. However, its value in the treatment of infections with poxviruses is unknown and it should be applied only n clinical studies in cases that VIG was not effective enough.