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

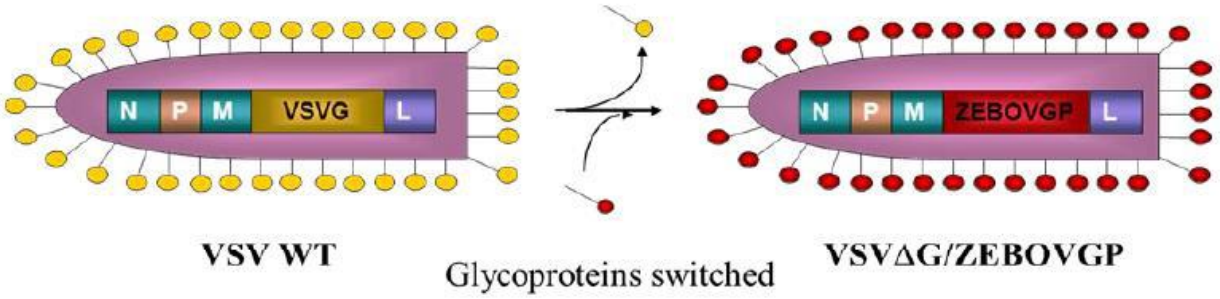
(a) Member State of notification: Belgium
(b) Notification number: N/A
(c) Date of acknowledgement of notification: 12/03/2019
(d) Title of the project
Importation of doses of V920 for Emergency Use
(e) Proposed period of release: From 01/05/2019 until 1/05/2020

2. Notifier

Name of institution or company: MSD Belgium BVBA/SPRL

3. GMO characterisation

The V920 vaccine candidate is a recombinant vesicular stomatitis virus (rVSV) which has the gene encoding for the VSV glycoprotein G deleted from its RNA and replaced with the Zaire Ebola virus (ZEBOV) glycoprotein (GP). The vaccine is a genetically engineered, replication-competent, attenuated live vaccine that induces immune responses after a single dose.



Attenuation of rVSVΔG-ZEBOV-GP is based principally on the reduction of viral replication and virulence linked to deletion of the VSV G gene, the viral determinant for neurotropism and pathogenicity [57] [7] [58], and replacement with Ebolavirus GP gene.

(a) Indicate whether the GMO is a:

viroid (.)
RNA virus (x)

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

specify phylum, class …

(b) Identity of the GMO (genus and species)

V920 comprises a single rVSV isolate (11481 nt, strain Indiana) in which the VSV envelope glycoprotein (G) has been deleted and replaced with the corresponding envelope GP of EBOV, Kikwit strain.

Besides replacement of the VSV-G by the ZEBOV-GP no additional sequences were added to the V920 genome. Sequences required for the construction of the V920 genome plasmid have not been integrated into the final V920 genome. In particular, there have not been any insertions of harmful sequences. There are no new traits besides replacement of the wt-VSV G with the EBOV GP sequence.

Attenuation of V920 seems to be based principally on a reduction of viral replication and virulence due to deletion of the VSV G gene, the viral determinant for neurotropism and pathogenicity, and replacement with Ebolavirus GP gene. Recombinant VSV with ZEBOV GP replacement has approximately 33% slower growth kinetics than wild-type VSV in Vero cell cultures, contributing to attenuation.

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

VSV's single-stranded, non-segmented RNA genome does not undergo reassortment. Furthermore, VSV replicates within the cytoplasm of infected cells and does not undergo genetic recombination or integration into the cellular genome.

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) …

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
Yes (x) No (.)
If yes:
- Member State of notification DE
Notification number IB16/160/14
Member State of notification ES
Notification number B/ES/15/09

Please use the following country codes:

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 V920 vaccine has been successfully administered to rodents (mice, rats, and hamsters), non-human primates, pigs, arthropods, and humans. Transmission of the vaccine virus from vaccines to other humans and to the environment appears to represent a negligible risk. If transmitted, the vaccine virus would retain its attenuated phenotype.
Persons vaccinated with V920 typically had low levels of virus in their blood for up to 1 week after vaccination, and all subjects assessed to date have cleared the virus from their blood by Day 28 postvaccination. Similar to other live viral vaccines, V920 vaccinated persons should not donate blood at least 1 month after vaccination.

Vesicular lesions of the skin appearing in the first 2 weeks after vaccination are rare, but are a potential source of virus infection; care should be taken to avoid contact spread from such lesions to others, including animals, by covering the vesicles until healing occurs.
Shedding of virus in adults, as measured to date in saliva or urine, is infrequent, at low levels, and appears to pose minimal, if any, risk of transmission to other persons. For children and adolescents, viremia is slightly higher than adults on Day 2, but decreases to zero by Day 7. Shedding in urine is similar to adults (low), but levels in saliva in adolescents are higher than adults, even at Day 7. Therefore, vaccine recipients should attempt to avoid close association with and exposure of high-risk individuals to blood and bodily fluids for up to 6 weeks following vaccination.

A study in arthropod vectors supports negligible risk for the vector-borne potential of the V920 vaccine. No replication was observed in *Anopheles* or *Aedes* mosquito, *Culicoides* biting midge, or *Lutzomyia* sand fly cells in culture, nor in live *Culex* and *Aedes* mosquitoes following exposure through intrathoracic inoculation or in a high-titer blood meal.
The risk of infection or disease in livestock animals is a theoretical concern due to the nature of the parental VSV virus. Transmission from humans to animals is unlikely due to the minimum levels of virus shedding by human vaccinees and management strategies to prevent direct contact of vaccinated individuals with animals during the period of potential shedding. While high doses of V920 have been shown to infect and cause limited clinical symptoms in pigs, exposure to doses of V920 e expected from shedding from vaccinated individuals, no clinical signs or transmission is expected. Cattle and horses are not expected to be susceptible to V920 infection.

In conclusion, risk to humans and the environment from exposure to the vaccine is expected to be negligible. However, risk management measures as described above should be implemented to limit exposure to V920 to the full extent possible.

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

other, specify …

The drug substance or GMO, is a live recombinant viral vaccine derived from the Vesicular Stomatitis Virus (VSV) backbone. This virus is modified by a deletion of the VSV-G envelope glycoprotein and substitution with the envelope glycoprotein of the Ebolavirus-Zaire Kikwit strain.

VSV is s single-stranded, negative-sense RNA encoding 5 transcriptional units: N (nucleoprotein), P (phosphoprotein), M (matrix), GP (glycoprotein), and L (polymerase). VSV belong to the family Rhabdoviridae, genus Vesiculovirus. These are bullet-shaped, singlestranded, negative-sense RNA viruses containing 5 genes, 1 of which is the viral GP.

2. Name
(i) order and/or higher taxon (for animals) Rhabdoviridae
(ii) genus Vesiculovirus
(iii) species …
(iv) subspecies …
(v) strain …
(vi) pathovar (biotype, ecotype, race, etc.) …
(vii) common name VSV

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

The VSV-related disease is limited to the Americas; however, it has been described in France (1915 and 1917) and in South Africa (1886 and 1897). The virus is maintained in nature in a cycle involving sandflies and rodent reservoirs. Rodents are the only known vertebrates that consistently develop a detectable viremia following VSV-NJ and VSV-I infection.
The frequency of naturally acquired overt disease with wt-VSV in humans is very lo
w. VSV
sensu strictu, is not present in Africa or in Europe. Infection of humans with wt-VSV can cause an influenza-like disease (usually without vesicle formation), incubation period 48 hrs, resolving in 3-5 days without complications. Mucosal ulceration, and lymphadenopathy are reported. Rare cases are severe enough to warrant hospitalization. Occupational exposure to wt-VSV or lab-adapted VSV strains (veterinarians, lab workers, agricultural workers) is known.

VSV disease in non-human primates under natural conditions is not known. Following intranasal inoculation, macaques shed minimal recombinant VSV (rVSV) in nasal washes for one day post-inoculation; viremia was not detected.

(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	

The virus is maintained in nature in a cycle involving sandflies and rodent reservoirs.

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

5. (a) Detection techniques

Rapid and reliable systems for the detection of VSV either through quantitative reverse transcription-PCR assay or enzyme-linked immunosorbent assays (ELISAs) are available for detection of antigen and antibody. National reference laboratories often identify VSV by complement fixation (CF) assays, electron microscopy, and virus isolation, as VSV is easily propagated in cell culture.

(b) Identification techniques
See 5(a)

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

VSV is assigned to risk group 2 according to DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 September2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC).

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (x) 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

Wild-type VSV (wt-VSV) causes significant disease in pigs, cattle, and horses, primarily manifesting as crusting and vesiculation of the mucous membranes and skin, and lameness due to involvement of the coronary bands of the hoof. Outbreaks caused by New Jersey strains (VSV-NJ) are more frequent and more severe than those caused by Indiana strains. V920 is based on a VSV Indiana strain (VSV-I).

VSV-NJ and VSV-I has been demonstrated to be transmitted between livestock by direct contact, likely including droplet spread and fomites, as well as mechanically by non-biting houseflies and face flies. Mechanical transmission by flies and animal-to-animal or animal to human transmission may occur through direct contact with vesicular lesions. Wild-type VSV has a broad tropism with VSV glycoprotein (G) and matrix (M) protein playing major roles. VSV G protein is responsible for VSV broad cell tropism. Antibodies to vesicular stomatitis viruses (VSV) have been found in many other species including deer, bats, raccoons, bears, turkeys and ducks.

The frequency of naturally acquired overt disease with wt-VSV in humans is very low. VSV *sensu strictu*, is not present in Africa or in Europe. Infection of humans with wt-VSV can cause an influenza-like disease (usually without vesicle formation), incubation period 48 hrs, resolving in 3-5 days without complications. Mucosal ulceration, and lymphadenopathy are reported. Rare cases are severe enough to warrant hospitalization. Occupational exposure to wt-VSV or lab-adapted VSV strains (veterinarians, lab workers, agricultural workers) is known.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The incubation period in animals is usually two to eight days; however, longer or shorter incubation periods have also been reported. In contrast, lesions or fever develop in 1-3 days in some experimentally infected horses and swine.

(a) Generation time in the ecosystem where the release will take place:

VSV virus can be transmitted to humans who come in close contact with infected animals. The incubation period is most commonly 3 to 4 days. The most common clinical manifestation is a limited, 3- to 5-day flu-like illness

…

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

(b) Factors affecting reproduction:

Not Applicable

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:

VSV is inactivated at low pH (1.5), and immediately upon heating to 60°C. VSV can survive for 3 to 4 days in infected saliva on milking pails, mangers and hay.

10. (a) Ways of dissemination

The virus is maintained in nature in a cycle involving sandflies and rodent reservoirs. Arthropods seem to respond to wt-VSV infection by induction of the autophagous system to eliminate virus. VSV is known to be able to persist in arthropod hosts over many generations.

(b) Factors affecting dissemination

See 10(a)

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)

According to sponsor information register there is no previous notifications of the GMO or recipient/parental organism made in country where the present notification is submitted.

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

1. Type of the genetic modification

(i) insertion of genetic material (x)

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

2. Intended outcome of the genetic modification

The cloning of the rVSV ZEBOV-GP virus has been described in the literature and consists of reverse genetics placing the ZEBOV envelope glycoprotein gene into the genome of the Indiana strain of Vesicular Stomatitis Virus as a substitution for the fusogenic VSV – G envelope glycoprotein. The reverse genetics system is based on 5 plasmids: one plasmid containing the complete genome of the desired recombinant VSV under the transcriptional control of T7 polymerase; a plasmid for each of the three nucleocapsid components (N, P and L), and a plasmid for expressing the T7 polymerase. This engineering strategy was taken from Lawson et al., 1985. All five plasmids are transfected into a cell where the coded proteins are expressed and assembled into virus particles, a process referred to as virus rescue. The rescued viruses were sequentially plaque purified and sequenced. One clone, P6PP5C4, was selected to become premaster virus.

The VSV XN2 –ZEBOV GP plasmid contains the entire VSV genome with the ZEBOV GP gene replacing the VSV – G gene. This constitutes the rVSVΔG-ZEBOV GP viral genome. The other 4 viral plasmids are helper plasmids encoding VSV proteins and the T7 RNA polymerase required to rescue the negative strand virus. Five plasmids were transfected for the rescue:

* pCAGGS-T7 (helper plasmid containing the T7 polymerase gene)
* pBS-N (helper plasmid containing VSV N protein gene)
* pBS-L (helper plasmid containing VSV L protein gene)
* pBS-P (helper plasmid containing VSV P protein gene)
* VSV XN2-ZEBOV GP (plasmid containing the entire VSV genome with the ZEBOV GP gene replacing the VSV G gene).

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 (x) No (.)

If no, go straight to question 5.

The cloning of the rVSV ZEBOV-GP consists of reverse genetics placing the ZEBOV envelope glycoprotein gene into the genome of the Indiana strain of Vesicular Stomatitis Virus as a substitution for the fusogenic VSV-G envelope glycoprotein.

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

(a) Type of vector

plasmid	(.)
bacteriophage	(.)
virus	(x)
cosmid	(.)

transposable element (.)
other, specify …

(b) Identity of the vector
Vesicular Stomatitis Virus (VSV)

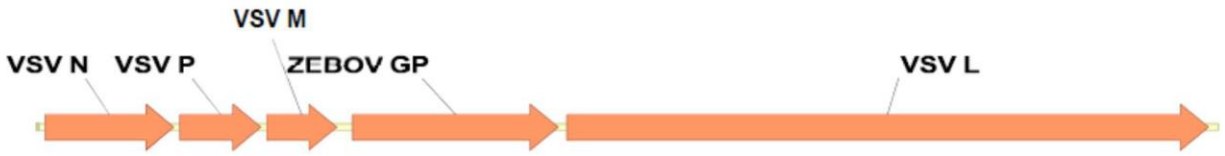
(c) Host range of the vector
The VSV-related disease is limited to the Americas; however, it has been described in France (1915 and 1917) and in South Africa (1886 and 1897). The virus is maintained in nature in a cycle involving sandflies and rodent reservoirs. Rodents are the only known vertebrates that consistently develop a detectable viremia following VSV-NJ and VSV-I infection.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (.) No (x)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
The rVSVΔG-ZEBOV-GP 11640 bp genome contains the entire VSV backbone with the exception of the VSV G gene which has been deleted, with the ZEBOV GP constituting the envelope of the chimeric virus, replacing the VSV G.



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

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

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 …

6. Composition of the insert

(a) Composition of the insert

The final genetic modified organism named rVSVΔG-ZEBOV GP is a live attenuated recombinant virus based on the complete structure of a single rVSV isolate replacing its VSV GP gene by an exogenous gene. The exogenous gene is the ZEBOV GP protein from the Kikwit strain (11481 nt, strain Indiana).

(b) Source of each constituent part of the insert

- The main constituent part is the complete genome of the VSV except the GP gene.
- The exogenous gene is the ZEBOV GP protein from the Kikwit strain (11481 nt, strain Indiana).

(c) Intended function of each constituent part of the insert in the GMO

- The complete VSV genome, except the GP gene, is providing the ability to produce a recombinant VSV virus. The recombinant virus is recued on cells transfected with VSVΔG/ZEBOV GP plasmids and VSV helper plasmids, and is fully infectious.

- The exogenous Kikwit strain ZEBOV GP protein gene will provide the genetically modified organism the property to produce Kikwit strain ZEBOV glycoprotein in achimeric VSV that will make possible a recombinant virus to act as attenuated vaccine for Kikwit strain ZEBOV.

…

(c) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify rVSV expressing ZEBOV GP

(d) 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)	Filoviridae
(ii)	family name for plants	…
(iii)	genus	Ebolavirus
(iv)	species	ZEBOV
(v)	subspecies	…
(vi)	strain	Kikwit
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Ebola virus

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):

Three virus species (Zaire, Sudan, and Bundibugyo EBOV) are responsible for the vast majority of human cases of Ebola hemorrhagic fever. Infection with EBOV has historically resulted in mortality rates up to 90% despite medical interventions.

Since 1976, there have been many EBOV outbreaks predominantly affecting small numbers of people in Central and East Africa, with two larger outbreaks in 1995 in Kikwit, Democratic Republic of the Congo and in Gulu, Uganda in 2000-2001. However, between March 2014 and March 2016, an unprecedented epidemic caused by the Zaire species of EBOV occurred in West Africa, exceeding all previous outbreaks with respect to geographic range, number of patients affected, and disruption of typical activities of civil society. Perhaps due to improved medical interventions, the case fatality rate in this outbreak was approximately 40%.

More recent outbreaks have occurred in the Democratic Republic of the Congo (DRC) in 2018. An outbreak occurred from May to July 2018 in Equateur Province; a total of 53 Ebola virus disease cases including 29 deaths (case fatality ratio: 55%). On July 24, 2018, WHO and the Ministry of Health for the DRC declared the end of this outbreak. Shortly thereafter, on August 1, 2018, another outbreak was declared in DRC's North Kivu province, which is still ongoing at the time of writing this document. As of 06 Jan 2019, per the WHO External Situation Report, a total of 625 cases of Ebola virus disease (among them 577 confirmed) and 377 deaths have been reported.

The EBOV glycoprotein (GP) is the only virally expressed protein on the virion surface and is critical for attachment to host cells and catalysis of membrane fusion. GP is not cytotoxic when expressed constitutively at a moderate level.

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 …

Ebola virus is assigned to risk group 4 according to DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 September2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC).

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

Yes (.) No (x) Not known (.)

Generally, the risk of genetic recombination of V920 with EBOV or VSV is believed to be non-existent. This is based on both the lack of integration of EBOV's or VSV's RNA genome into the host cell genome, the single-stranded, non-segmented nature of the genome, as well as attenuation of replication of V920 reducing the potential for co-infection.

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 (x) No (.) Not known (.)

Specify …

VSV derived vectors like other Rhabdoviruses are susceptible to mutation due to infidelity of the viral polymerase and to deletions, generating defective interfering (DI) particles. Studies of the V920 vaccine indicate that the genome sequence is stable during in vitro replication in cell cultures for production of virus seeds and vaccine.

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

Yes (x) No (.) Unknown (.)

Specify …

Attenuation of rVSVΔG-ZEBOV-GP is based principally on the reduction of viral replication and virulence linked to deletion of the VSV G gene, the viral determinant for neurotropism and pathogenicity, and replacement with Ebolavirus GP gene. Recombinant VSV with ZEBOV GP replacement has approximately 33% slower growth kinetics than wild-type VSV in Vero cell cultures, contributing to attenuation

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

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

Specify …

V920 has no selective advantage for replication, virulence or pathogenicity, neither over VSV nor over EBOV. Due to its function as a vaccine, it is supposed to confer to EBOV a selective disadvantage by providing protective immunity against EBOV in vaccinated individuals. V920 is attenuated regarding its pathogenicity compared to wt-VSV and, even if it was released into the environment in large numbers, it would not be expected to outcompete wt-VSV during co-infection of host species even if the release would happen in the Americas where VSV is endemic. There is no cross-immunity in humans for wt-VSV and V920. It is the goal of V920 to cause protective immunity against EBOV in individuals vaccinated with V920.

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

Yes (x) No (.) Not known (.)

Specify …

Data collected in the Phase 1, 2, and 3 studies to date have demonstrated V920 to be generally well-tolerated when administered to healthy, non-pregnant adults.

2. Genetic stability of the genetically modified organism

Studies of the V920 vaccine indicate that the genome sequence is stable during in vitro replication in cell cultures for production of virus seeds and vaccine.

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	(x)
animals	(x)
plants	(.)
other	…

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

The V920 vaccine has been successfully administered to rodents (mice, rats, and hamsters), non-human primates, pigs, arthropods, and humans. Transmission of the vaccine virus from vaccinees to other humans and to the environment appears to represent a negligible risk. If transmitted, the vaccine virus would retain its attenuated phenotype.

Persons vaccinated with V920 typically had low levels of virus in their blood for up to 1 week after vaccination, and all subjects assessed to date have cleared the virus from their blood by Day 28 postvaccination.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

Qualified real-time qRT-PCR assay was developed to specifically detect and quantitate the rVSVΔG-ZEBOV-GP recombinant virus in human urine, plasma, swabs, skin, and synovial fluid. The assay primer set and probes target the junction of the VSV and ZEBOV-GP sequences in the vaccine such that this assay is specific for the V920 vaccine and does not detect wild-type VSV or ZEBOV. The assay is composed of three principal steps: (1) Extraction of RNA from clinical specimens using the Roche MagNa Pure 96 total nucleic acid isolation kit; (2) one- step reverse transcription of mRNA to complementary DNA followed by amplification and detection of rVSVΔG-ZEBOV on the ABI QuantStudio™ 6; (3) results are reported as copies/mL using an external standard curve of rVSVΔG-ZEBOVGP calibrators. To verify RNA extraction from the specimen and successful RT-PCR amplification, an internal control (MS2 RNA phage) is spiked into each sample prior to RNA extraction and is amplified in parallel with the rVSVΔG-ZEBOV-GP target with each specimen for the entire assay procedure. The saliva matrix is currently being qualified in support of V920-016.

(b) Techniques used to identify the GMO

Refer to 4(a)

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
MSD is requesting to import V920 doses into Belgium for use in vaccination of health care workers traveling to Africa under Emergency Use conditions to support outbreak situations in Africa.

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
Vaccination will occur in a controlled clinical setting

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
CHU Saint-Pierre, Rue Haute 322, B1000 Bruxelles

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … 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
…
Transmission of rVSVΔG-ZEBOV-GP through close personal contact with susceptible farm animals is accepted as a theoretical possibility, with swine being the most relevant of livestock. Based on the data available from a study in pigs, clinical signs consistent with VSV infection were detected when a high dose of V920 was administered to pigs through intradermal and intranasal routes (4x107 pfu per animal). However, possible transfer of active rVSVΔG-ZEBOV-GP from urine, saliva, or vesicular lesions of the skin of vaccinated individuals that may occur during the first few weeks after vaccination are expected to contain much lower levels of rVSVΔG-ZEBOV-GP than levels used to inoculate pigs. Nonetheless, V920 recipients should attempt to avoid close association with and exposure of their blood and body fluids livestock animals for at least 1 month following vaccination to avoid the theoretical risk of spread of therVSVΔG-ZEBOV-GP vaccine virus.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Emergency use vaccination is expected to be for a limited number of healthcare workers. Each 1 mL dose contains ≥ 72 million plaque-forming units (pfu) of V920 at the time of use.

(b) Duration of the operation:

Based on clinical trial information, viremia with V920 (measured by the detection of rVSVΔG-ZEBOV-GP RNA in the blood) was common among vaccine recipients and resolved in a majority of subjects by 1 week.

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

Exposure scenario	Measure
Accidental breakage/spillage of V920 during transport or administration	Medical personnel involved in the administration are wearing personal protective equipment in order to minimize exposure. If breakage/spillage were to occur, disinfectants such as aldehydes, alcohols, and detergents are available to inactivate the vaccine through chemical disinfection and prevent release into the environment.
Accidental needle stick injury by medical personnel	Immediate disinfection of the injection site and cover the injection site, as advised for vaccinated individuals. Follow up for safety as a vaccinated individual.
Direct human contact with V920 shed by vaccinated individuals	Vaccinated individuals are informed about the potential for shedding and the need to avoid close association with and exposure of high-risk individuals to blood and bodily fluids for up to 6 weeks following vaccination. Individuals who develop vesicular rash after receiving the vaccine should cover the vesicles until they heal. Vaccinated individuals are required not to donate blood for one month following vaccination.
Direct contact of animals with V920 shed by vaccinated individuals	Vaccinated individuals will be requested to avoid exposure of livestock to blood and bodily fluids for at least 1 month following vaccination even though shedding of V920 is limited and the amount of V920 shed is likely too low to cause disease in animals. Individuals who develop vesicular rash after receiving the vaccine should cover the vesicles until they heal.
Unintended use / Misuse	Doses of V920 to be delivered to hospital centers for vaccination are well controlled and are handled as genetically modified microorganisms per local regulations. Only medical personnel trained to handle V920 have access to the vaccine.

5. Short description of average environmental conditions (weather, temperature, etc.)
Not Applicable

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.
The V920 vaccine has been successfully administered to rodents (mice, rats, and hamsters), non-human primates, pigs, arthropods, and humans. Transmission of the vaccine virus from vaccinees to other humans and to the environment appears to represent a negligible risk. If transmitted, the vaccine virus would retain its attenuated phenotype.

Persons vaccinated with V920 typically had low levels of virus in their blood for up to 1 week after vaccination, and all subjects assessed to date have cleared the virus from their blood by Day 28 postvaccination. Similar to other live viral vaccines, V920 vaccinated persons should not donate blood at least 1 month after vaccination.

Vesicular lesions of the skin appearing in the first 2 weeks after vaccination are rare, but are a potential source of virus infection; care should be taken to avoid contact spread from such lesions to others, including animals, by covering the vesicles until healing occurs.
Shedding of virus in adults, as measured to date in saliva or urine, is infrequent, at low levels, and appears to pose minimal, if any, risk of transmission to other persons. For children and adolescents, viremia is slightly higher than adults on Day 2, but decreases to zero by Day 7. Shedding in urine is similar to adults (low), but levels in saliva in adolescents are higher than

adults, even at Day 7. Therefore, vaccine recipients should attempt to avoid close association with and exposure of high-risk individuals to blood and bodily fluids for up to 6 weeks following vaccination.

A study in arthropod vectors supports negligible risk for the vector-borne potential of the V920 vaccine. No replication was observed in *Anopheles* or *Aedes* mosquito, *Culicoides* biting midge, or *Lutzomyia* sand fly cells in culture, nor in live *Culex* and *Aedes* mosquitoes following exposure through intrathoracic inoculation or in a high-titer blood meal.
The risk of infection or disease in livestock animals is a theoretical concern due to the nature of the parental VSV virus. Transmission from humans to animals is unlikely due to the minimum levels of virus shedding by human vaccinees and management strategies to prevent direct contact of vaccinated individuals with animals during the period of potential shedding. While high doses of V920 have been shown to infect and cause limited clinical symptoms in pigs, exposure to doses of V920 e expected from shedding from vaccinated individuals, no clinical signs or transmission is expected. Cattle and horses are not expected to be susceptible to V920 infection.

In conclusion, risk to humans and the environment from exposure to the vaccine is expected to be negligible. However, risk management measures as described above should be implemented to limit exposure to V920 to the full extent possible.

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)	Primate
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	Homo sapiens…
(v)	subspecies	…
(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)
Vaccination with resultant immune response

3. Any other potentially significant interactions with other organisms in the environment
No

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
V920, being pseudotyped with ZEBOV glycoprotein is more likely to be able to colonize ZEBOV-host species than VSV-host species. Based on the nature of the attenuating genetic structure of rVSVΔG-ZEBOV-GP, replication of rVSVΔG-ZEBOV-GP was highly restricted, and manifested as minimal, transient viremia, even in immunocompromised NHP (SHIV infected macaques).

According to results from pre-clinical trials, replication in arthropods has been demonstrated

to be absent and while pigs support replication following intranasal and intradermal inoculation with high numbers of vaccine particles, no transmission to sentinel pigs occurred. Due to the fact that horses and cattle are not host species for EBOV, it is expected that these species are less likely to be affected by V920 than pigs being hosts for both wt-VSV and EBOV.

No data are available for bats which are known as a reservoir for both wt-VSV and EBOV. However, these viruses do not seem to be highly pathogenic to bats. Therefore, V920 is not expected to be pathogenic to these species nor transmitted from these species to others.

Reversion of V920 back to a wild type VSV is not expected. There is no sequence for VSVG in the V920 product. A co-infection of permissive cells in humans or in an animal host for VSV is not expected as Europe is not an endemic area for VSV. Also, the absence of vesiculoviruses in Africa, and the restricted host relationships of rhabdovirus and filovirus groups used to construct rVSV-ZEBOV, limits opportunities for a host encountering two different viruses. Reversion back to a wild type EBOV is not considered a possibility as the only EBOV sequence in V920 is the ZEBOV GP.

In addition, homologous recombination of VSV strains or non-homologous recombination with other non-related RNA viruses is not believed to occur to any significant extent and as V920 does not cause long-lasting viremia in humans or animals, the probability of coinfection is further minimized. Thus, the generation of new chimeric viruses affecting new animal species is only a low probability theoretical possibility.

Gene transfer from V920 to other species is not expected. V920 is an RNA virus and does not contain homologous sequences with bacteria which would allow for such a transfer, even if reverse transcriptase would convert RNA in DNA.

V920 has no effect on biogeochemical processes, neither on its own nor through interactions with target or non-target organisms in the theoretical case interactions would occur.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

Based on clinical trial data, shedding is expected to be a rare occurrence. Even in the event of shedding in waste water no establishment in such a system can be expected due to the low volumes released, destruction of the virus by wastewater treatment techniques (e.g., temperature, chlorination).

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	…

None

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
V920 could be transmitted to natural hosts for both wt-VSV and wt-ZEBOV virus upon a high titer exposure, e.g. full vaccination doses. However, the studies conducted to date in a number of animal species including rodents, non-human primates, and insect vectors for wt-VSV and summarized in prior sections of the document demonstrate that rVSVΔG-ZEBOVGP has limited replication and/or is attenuated in all these species. Furthermore, the data suggest limited shedding, from vaccinated individuals or infected animals, limiting the probability of spread beyond the vaccinated individual.

(b) from other organisms to the GMO:
Not likely

(c) likely consequences of gene transfer:
Horizontal gene transfer to bacteria due to shedding of V920 from vaccinees and release into the waste water stream or through contact with the bacterial flora of vaccinees or contact persons is not expected as V920 is an RNA virus and is unlikely to contain homologous sequences with bacteria which would allow for such a transfer, even if reverse transcriptase would convert RNA to DNA

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.):
Specific stability in the environment of V920 is unknown. However, rVSVΔG-ZEBOV-GP is an enveloped virus which by its nature tends to be somewhat labile. The V920 vaccine has been shown to lose potency when held at 37oC (1.137 log10 pfu/ml potency loss per day) or 25oC (0.0790 log10 pfu/ml potency loss per day) and thus is expected to lose potency under ambient conditions in case of an inadvertent environmental release. Importantly, the ambient temperatures for regions where Ebola is endemic/epidemic and where V920 is most likely to
be used are closer to 37oC than 25oC suggesting that survival in that environment is likely to be short-lived.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
V920 has no effect on biogeochemical processes, neither on its own nor through interaction with target or non-target organisms.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Viremia and shedding of V920 in urine and saliva has been assessed in clinical trials using RT-PCR assays designed to detect the vaccine virus. As previously described viremia is low level and short lived. Shedding of vaccine virus in urine or saliva is infrequent, short lived, and at low levels in adults, with higher frequencies observed in adolescents and children. In addition, as previously described preliminary data suggests that V920 is not a threat for livestock species or for insect vector dissemination.

Following vaccination with V920, individuals may test positive for anti-Ebola glycoprotein (GP) antibody and/or Ebola GP nucleic acid or antigens, which are targets for certain Ebola diagnostic tests. Therefore, diagnostic testing for Ebola should target non-GP sections of the virus/genome.

2. Methods for monitoring ecosystem effects
See above

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable

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
Instruments, benches, surfaces, etc. should be decontaminated using disinfectants after the vaccination of individuals.

If breakage/spillage were to occur, disinfectants such as aldehydes, alcohols, and detergents are proven to reduce viral infection potential after only a few minutes (less than 5).

2. Post-release treatment of the GMOs
Not applicable

3. (a) Type and amount of waste generated
Empty vials and syringes. Follow local requirements for administration sites. Any unused vaccine or waste material should be disposed of in accordance with local requirements in order to achieve inactivation of V920.

3. (b) Treatment of waste
Delivery system components (injection needle and syringe) will be disposed of in a manner consistent with the standard practice of the institution for biohazardous sharps. In addition any disposable surgical instruments or other materials used during the administration procedure or collection of body fluids will be disposed according to standard biosafety practice of the institution. All non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity and then sterilized by autoclaving according to standard practice of the institution.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
If breakage/spillage were to occur, disinfectants such as aldehydes, alcohols, and detergents are proven to reduce viral infection potential after only a few minutes (less than 5).

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

Disinfectants such as aldehydes, alcohols, and detergents are proven to reduce viral infection potential after only a few minutes (less than 5).

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect
   Not anticipated based on data from clinical trials