VACCINE COMPOSITION

The invention relates to the field of vaccines and in particular, attenuated live vaccines. More precisely, the invention relates to a tetravalent vaccine composition against Dengue in which the quantity of Dengue virus of serotype 3 is reduced compared with the quantity of virus of the other serotypes.

Dengue, as well as its severe form, haemorrhagic fever, is a tropical disease of viral origin. The virus responsible for it is part of the Flaviviridae family (genus Flavivirus), which also comprises the yellow fever virus as well as the Japanese encephalitis virus. Four serotypes capable of causing Dengue are known. Whereas this disease is currently spreading worldwide, no vaccine is currently commercially available. Indeed, in order to be able to be offered with every chance of effectively preventing Dengue, a vaccine, while providing guarantees of safety, must be capable of inducing in the individual to which it is administered an immune-system response capable of protecting them during subsequent infection with the wild-type virus. The vaccine should preferably be effective irrespective of the serotype of the wild-type virus. However, up until now, none of the candidate vaccines studied has exhibited all the necessary qualities.

A tetravalent vaccine containing the 4 strains deposited at the CNCM of the Pasteur Institute of Paris under the references I-2480, I-2481, I-2482 and I-2483, which are attenuated Dengue strains of the serotypes 1, 2, 3 and 4, has already been tested on human beings, as has been described by Natth BHAMARAPRAVATI and Sutee YOKSAN in Chapter 17, pages 373-374 of the book DENGUE AND DENGUE HEMORRHAGIC FEVER published by D.J. Gubler and G. Kuno, CAB INTERNATIONAL, 1997. Although according to this prior art all of the subjects in the

clinical trial were seroconverted overall, only suffering mild secondary reactions, it was wished to improve the formulation of the vaccine composition in order to optimize it. It was indeed wished to have a vaccine composition which can be administered to human beings safely while inducing a seroconversion with respect to each of the serotypes of a maximum number of vaccinated subjects; the vaccine composition must in addition be able to be prepared under industrial conditions at a reduced cost so as to render the vaccination accessible to all, and in particular to the poorest countries which are among those most affected by this disease.

To achieve this aim, the invention provides a tetravalent vaccine composition against Dengue, comprising viruses of each of the attenuated strains deposited at the CNCM at the Pasteur Institute of Paris under the references I-2480, I-2481, I-2482 and I-2483, characterized in that the relative quantity of virus of each of the serotypes is such that the quantity of virus of serotype 3 is less than the quantity of virus of the other serotypes.

According to one characteristic of the invention, the quantity of virus, per vaccine dose, of serotype 3 is at least 1 unit less than the quantity of virus of the other serotypes when the viral titre is expressed as log10 of the dilution of virus necessary to infect 50% of the cells.

According to one embodiment of the invention, the viruses of the serotypes other than serotype 3 may be present in equal quantities, or in proportions such that the quantity of virus, per vaccine dose, of serotype 1 is at least 1 unit greater than the quantity of virus of the other serotypes when the viral titre is expressed as log10 of the dilution of virus necessary to infect 50% of the cells.

Surprisingly, it has been observed that with such formulations, the immune responses induced in vaccinated persons were completely satisfactory,

whereas the secondary reactions were very highly reduced.

According to a specific embodiment of the invention, the quantity of virus of each of the serotypes 1 to 4, per vaccine dose, corresponds to one of the following formulations: 3,2,1,2 or 3,3,1,3 in which the figures express the values of the logs10 of the dilution of virus necessary to infect 50% of the cells.

According to one characteristic of the invention, the vaccine composition is intended to be administered by subcutaneous injection.

According to a particularly advantageous embodiment, the administration of the vaccine composition is carried out in 2 doses separated by at least 1 month. Indeed, it has been observed that an increased antibody response and, quite unexpectedly, a lower frequency of secondary reactions are then obtained.

It has in addition been observed that the administration of a second dose made it possible to "supplement" the response induced during the first dose, in the sense that individuals who did not seroconvert with respect to a certain serotype during the 1st dose have the capacity to do so during the 2nd dose; this observation, combined with the presence of a cellular-type immune response demonstrated during previous clinical trials with vaccine compositions comprising the same attenuated live strains as those of the present invention, suggests that the vaccine composition provided by the present invention effectively makes it possible to prevent the Dengue disease.

The subject of the present invention is also the use of a viral composition comprising viruses of each of the attenuated strains deposited at the CNCM at the Pasteur Institute of Paris under the references I-2480, I-2481, I-2482 and I-2483, in which the relative quantity of virus of each of the serotypes is

such that the quantity of virus of serotype 3 is less than the quantity of virus of the other serotypes, for the manufacture of a vaccine composition against Dengue.

The subject of the invention is also a method of vaccination against Dengue, according to which there is administered to a human being a tetravalent vaccine composition against Dengue, comprising viruses of each of the attenuated strains deposited at the CNCM at the Pasteur Institute of Paris under the references I-2480, I-2481, I-2482 and I-2483, characterized in that the relative quantity of virus of each of the serotypes is such that the quantity of virus of serotype 3 is less than the quantity of virus of the other serotypes.

The virus strains according to the present invention were all obtained from the serum of patients who had been infected with Dengue in South East Asia. The isolates were passaged over mammalian cells and then inoculated by the intrathoracic (IT) route into farmed mosquitoes (*Toxorhynchites* or *Aedes aegypti*). This step made it possible to eliminate potential contaminating viruses which might not be arboviruses; indeed, only arboviruses migrate into the salivary glands of mosquitoes after intrathoracic administration; it was therefore necessary to separate the head from the rest of the mosquito's body and grind it in order to obtain the purified virus strain.

Next, the viruses of serotypes 1, 2 and 4 were passaged over primary dog kidney cells (PDK), at 32°C, every 5 to 10 days, whereas the virus of serotype 3 was attenuated by passage over monkey kidney cells (PGMK) and then cultured on foetal Rhesus pulmonary cells (FRhL).

The attenuated strains which are obtained then have the following characteristics:

- When titrations are performed on mammalian cells, for example on LLC-MK2 cells which are cells derived from monkey kidney, the attenuated strains

give small plaques compared with the parental strains from which they are derived.

- In new born mice, when they are inoculated by the intracerebral route, they have a low virulence.
- In monkeys, when they are inoculated by the subcutaneous route, they cause a low (or even undetectable) viraemia.
- They are sensitive at a temperature close to 40°C.

According to the present invention, strain derived from the strains which are the subject of the deposits at the CNCM I-2480, I-2481, I-2482 and I-2483 is understood to mean viral strains obtained from these strains but which have been subjected to additional passages on cells permissive to the viruses, while preserving the properties mentioned above.

The strain 16007, of serotype 1, was isolated in 1964 from a patient suffering from Dengue associated with a shock syndrome in Thailand. The original viraemic serum was passaged over tissue cultures before being inoculated into the mosquito *Toxorhynchites amboinensis*. Next, the virus obtained was passaged over PGMK cells in order to prepare the stock viral seed. The seed is then passaged over PDK cells, up to 43 times. The strain obtained after 13 passages over PDK cells has all the attenuation characteristics for use as a vaccine; it is the one which is the subject of the deposit I-2480 at the CNCM of the Pasteur Institute in Paris.

Although this strain is the one preferred for the vaccine composition according to the invention, it is quite possible to use a strain derived therefrom, and in particular a strain which has been subjected to a few additional passages over PDK cells, or a strain which has been subsequently cultured on cells allowing it to preserve its immunogenic character while at the same time not increasing its virulent character. Thus, strains which have been cultured on VERO cells could also be suitable. Any strain derived from the strain I-

2480 is considered as forming part of the present invention.

The strain 16681, of serotype 2, was isolated from a patient representing a fatal case of Dengue in Thailand in 1964. This strain was passaged twice in *Toxorhynchites amboinensis* mosquitoes and once over monkey kidney cells in order to produce the stock seed. The strain 16681 PDK 53 which is suitable for the purposes of the invention and which is the subject of the deposit I-2481 at the CNCM was obtained after 53 successive weekly passages over PDK cells.

Although this strain is the one preferred for the vaccine composition according to the invention, it is quite possible to use a strain derived therefrom, and in particular a strain which has been subjected to a few additional passages over PDK cells, or a strain which has been subsequently cultured on cells allowing it to preserve its immunogenic character while at the same time not increasing its virulent character. Thus, strains which have been cultured on VERO cells could also be suitable. Any strain derived from the strain I-2481 is considered as forming part of the present invention.

The strain 16562, of serotype 3, was isolated from a patient suffering from Dengue in Manila in 1972. It was attenuated by 30 successive passages over PGMK cells and then cultured on FRhL cells. The vaccine strain 16562 PGMK 30 FRhL 3, which is the subject of the deposit I-2482 at the CNCM and suitable for the purposes of the invention, was subjected to 3 successive passages over FRhL cells after the 30 passages over PGMK cells.

The strain 1036, of serotype 4, was isolated from a child suffering from classical Dengue in Indonesia. This strain was attenuated by successive passages over PDK cells. The strain 1036 PDK 48, suitable for the purposes of the invention and the subject of the deposit I-2483 at the CNCM, was passaged 48 times over PDK cells.

Although this strain is the one preferred for the vaccine composition according to the invention, it is quite possible to use a strain derived therefrom, and in particular a strain which has been subjected to a few additional passages over PDK cells, or a strain which has been subsequently cultured on cells allowing it to preserve its immunogenic character while at the same time not increasing its virulent character. Thus, strains which have been cultured on VERO cells could also be suitable. Any strain derived from the strain I-2483 is considered as forming part of the present invention.

According to the invention, the quantity of virus of each of the serotypes is preferably obtained according to the method described in patent application WO98/45709 according to which the virus is propagated on cells which are permissive to the virus but which at the same time do not induce interference between the various serotypes, such as the VERO cells, and then the quantity of virus of each of the serotypes is determined by means of a monoclonal antibody specific for the serotype which it is desired to titrate. The viral titre is determined by the Spearman and Karber method and expressed as dose infecting 50% of the cell cultures ($CCID_{50}$).

Any other method equivalent to this method, and in particular the plaque method whose results are expressed in pfu, may also be used.

Likewise, any method (such as inoculation into live mosquitoes) whose results would be expressed in a unit which is completely different, but for which it would be possible to make the correspondence with the method described in application WO98/45709, may also be used.

According to the invention, vaccine dose is understood to mean the quantity of vaccine composition administered all at once to a person whom it is desired to vaccinate. This quantity may be in particular 0.5 ml during a subcutaneous injection.

The vaccine compositions according to the invention may comprise all the components usually present in a vaccine, and in particular stabilizers, preservatives and adjuvants. However, good results have been obtained without a vaccine adjuvant.

The example which follows illustrates a specific embodiment of the present invention.

Tetravalent compositions having the following formulations, in which the titres of each of the serotypes are expressed as $\log_{10}$ of the dilution of virus necessary to infect 50% of the cells (i.e. as $CCID_{50}$/dose, are prepared from bulk viral suspensions comprising the strains 16007 PDK13, 16681 PDK53, 16562 PGMK30FrhL3 and 1036 PDK48.

Group	DENGUE 1	DENGUE 2	DENGUE 3	DENGUE 4
A	4	4	4	4
B	3	2	1	2
C	4	4	1	4

The vaccine compositions are prepared in the form of 0.5 ml doses.

The subjects in the trial are Thai adults aged between 18 and 40 years.

The number of subjects for group A is n = 11, for group B n = 5, for group C n = 6.

The administration of the vaccine composition is made by subcutaneous injection into the deltoid region, in a quantity of 0.5 ml for the 1st dose and in a quantity of 0.5 ml for the second dose which is administered 6 months later.

Blood samples are collected in order to carry out assays of neutralizing antibodies, detections of viraemia as well as assays of various biological parameters.

Moreover, all the symptoms relating to the undesirable reactions during the vaccination were observed and are reported in Table 1 below in the form of a tolerance index expressed in terms of a mean +/-

standard deviation with, in parentheses, the minimum and maximum values, either after the 1st dose or after the 2nd.

The tolerance index is calculated from the systemic reactions in each subject. The product of the duration and the severity is calculated for the following systemic reactions: fever, shiver, discomfort, cephalalgia, myalgia, arthralgia, rash, pruritus, ocular pain, photophobia and conjunctivitis. Then for each subject, the maximum value between fever and shiver, myalgia and arthralgia, rash and pruritus, ocular pain, photophobia and conjunctivitis is selected and added to the value for discomfort and cephalalgia. Finally, the mean of the values for the subjects (+/- standard deviation) is calculated for each group. This index therefore makes it possible, for each subject, to evaluate the tolerance taking into account the number of reactions, their severity and their duration.

Table 1: Tolerance index

	Group A	Group B	Group C
1st dose	27.4 +/- 18.7 (3-63)	11 +/- 4.06 (4-14)	9.2 +/- 9.2 (0-24)
2nd dose	2.36 +/- 2.06 (0-6)	0.6 +/- 0.89 (0-2)	0.5 +/- 0.55 (0-1)

These results clearly show that the vaccine composition according to the invention (Groups B and C) leads to a lower index than the vaccine composition in which all the serotypes are present in an equal quantity.

It can also be seen that the undesirable reactions are much fewer, and even practically non-existent, during the 2nd dose.

The results relating to the neutralizing antibodies for each of the serotypes administered, expressed as GMT, are represented in Tables 2 and 3 below. The results were obtained by the plaque

reduction test according to the method described by Russel et al., *"A plaque reduction test for dengue virus neutralizing antibodies"*, Journal of Immunology, 1967;99:285-290, and are expressed as the reciprocal of the dilution which leads to a reduction of 50% of the lysis plaque on LLC-MK2 cells. This method consists in bringing into contact dilutions of sera with a defined number of viral particles which, inoculated into the permissive cells LLCMK2, give lysis plaques, that is to say a partial localized destruction of the cellular lawn. When the test is carried out under standard conditions, the presence of neutralizing antibodies leads to the reduction in the number of lysis plaques. The result is expressed as the last dilution of serum still giving 50% reduction in the total number of plaques, assessed in comparison with a test carried out with a negative reference serum.

Table 2: Results of the neutralizing-antibody titres (geometric mean) for serotypes 1 and 2.

	DENGUE 1					DENGUE 2				
	1st Dose			2nd Dose		1st Dose			2nd Dose	
	D0	D28	D60	D0	D28	D0	D28	D60	D0	D28
Group A	5	45.3	43.3	19.9	137	5	39.5	35.1	25.2	189
Group B	5	30.2	21.6	13.5	30.8	5	55.3	54.4	26	91.9
Group C	5	5.0	8.13	15.6	60.3	5	48.6	103	112	353

A titre of 5 corresponds to a negative result.

Table 3: Results of the neutralizing antibody titres (geometric mean) for serotypes 3 and 4.

	DENGUE 3					DENGUE 4				
	1st Dose			2nd Dose		1st Dose			2nd Dose	
	D0	D28	D60	D0	D28	D0	D28	D60	D0	D28
Group A	5	489	338	240	324	5	22.7	24.2	18.2	101
Group B	5	823	780	109	264	5	27.6	15.3	6.98	15.2
Group C	5	224	206	101	205	5	5	5	5	55

These results show that, with the vaccine compositions according to the present invention, the immune response for neutralizing antibodies is satisfactory with an effect of supplementation of this immune response with the 2nd dose.

If these results are expressed as seroconversion levels, i.e. as a percentage of subjects having a neutralizing-antibody titre greater than or equal to 10, it is observed that all the subjects seroconverted with respect to 3 serotypes, and that 75% of the subjects who received a vaccine composition according to the invention seroconverted with respect to the 4 serotypes.

Thus, it is possible, by virtue of the present invention, to have vaccine compositions against Dengue having an improved safety profile but with a preserved immunogenic character.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

ADDRESSEE:

AVENTIS PASTEUR
2, Avenue du Pont Pasteur
69007 LYON

NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE EVENT OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by
the INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number issued by the INTERNATIONAL DEPOSITARY AUTHORITY:
16007 PDK 13/S3236	I - 2480

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified in section I was accompanied by:

☒ a scientific description

☒ a proposed taxonomic designation

(Indicate as applicable)

III. RECEIPT AND ACCEPTANCE

The present International Depositary Authority accepts the microorganism identified in section I, which it received on 25 May 2000 (date of the original deposit)[1]

IV. RECEIPT OF A REQUEST FOR CONVERSION

The present International Depositary Authority received the microorganism identified under section I on (date of the original deposit) and received a request for conversion of the original deposit conforming to the Budapest Treaty on (date of receipt of the request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: CNCM
Collection Nationale
de Cultures de Microorganismes

Address: INSTITUT PASTEUR
28, Rue du Docteur Roux
F-75724 PARIS CEDEX 15

Signature(s) of the person(s) having the power to represent the International Depositary Authority, or of authorized official(s): Georges WAGENER

(signature)

Date: Paris, 23 October 2000

[1] If Rule 6.4.d) applies, this date shall be the date on which the status of the international depositary authority was acquired.

Form BP/4 (single page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

ADDRESSEE:

AVENTIS PASTEUR
2, Avenue du Pont Pasteur
69007 LYON

NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE EVENT OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by
the INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number issued by the INTERNATIONAL DEPOSITARY AUTHORITY:
16681 PDK 53/S3239	I - 2481

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified in section I was accompanied by:

☒ a scientific description

☒ a proposed taxonomic designation

(Indicate as applicable)

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Name: CNCM
Collection Nationale
de Cultures de Microorganismes

Address: INSTITUT PASTEUR
28, Rue du Docteur Roux
F-75724 PARIS CEDEX 15

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(signature)

Date: Paris, 23 October 2000

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Form BP/4 (single page)

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FOR THE PURPOSES OF PATENT PROCEDURE

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AVENTIS PASTEUR
2, Avenue du Pont Pasteur
69007 LYON

NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE EVENT OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by
the INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number issued by the INTERNATIONAL DEPOSITARY AUTHORITY:
16562 PGMK 30 FRhL3/S3242	I - 2482

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified in section I was accompanied by:

☒ a scientific description

☒ a proposed taxonomic designation

(Indicate as applicable)

III. RECEIPT AND ACCEPTANCE

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V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: CNCM
Collection Nationale
de Cultures de Microorganismes

Address: INSTITUT PASTEUR
28, Rue du Docteur Roux
F-75724 PARIS CEDEX 15

Signature(s) of the person(s) having the power to represent the International Depositary Authority, or of authorized official(s): Georges WAGENER

(signature)

Date: Paris, 23 October 2000

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Form BP/4 (single page)

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ADDRESSEE:

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2, Avenue du Pont Pasteur
69007 LYON

NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE EVENT OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by
the INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number issued by the INTERNATIONAL DEPOSITARY AUTHORITY:
1036 PDK 48/S3262	I - 2483

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified in section I was accompanied by:

☒ a scientific description

☒ a proposed taxonomic designation

(Indicate as applicable)

III. RECEIPT AND ACCEPTANCE

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IV. RECEIPT OF A REQUEST FOR CONVERSION

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V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: CNCM
Collection Nationale
de Cultures de Microorganismes

Address: INSTITUT PASTEUR
28, Rue du Docteur Roux
F-75724 PARIS CEDEX 15

Signature(s) of the person(s) having the power to represent the International Depositary Authority, or of authorized official(s): Georges WAGENER

(signature)

Date: Paris, 23 October 2000

[1] If Rule 6.4.d) applies, this date shall be the date on which the status of the international depositary authority was acquired.

Form BP/4 (single page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

ADDRESSEE:

Mr. Hervé TAINTURIER
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2, avenue du Pont Pasteur
69367 LYON CEDEX 07

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AVENTIS PASTEUR Address: 2, Avenue du Pont Pasteur 69007 LYON	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: I - 2480 Date of the deposit or of the transfer[1]: 25 May 2000

III. VIABILITY STATEMENT

The viability of the microorganism identified under section II above was tested on 20 June 2000[2]. On that date, the said microorganism was

☒[3] viable

☐[4] no longer viable

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

Form BP/9 (first page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

ADDRESSEE:

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VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AVENTIS PASTEUR Address: 2, Avenue du Pont Pasteur 69007 LYON	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: I - 2481 Date of the deposit or of the transfer[1]: 25 May 2000
III. VIABILITY STATEMENT	
The viability of the microorganism identified under section II above was tested on 20 June 2000[2]. On that date, the said microorganism was ☒[3] viable ☐[4] no longer viable	

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

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VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AVENTIS PASTEUR Address: 2, Avenue du Pont Pasteur 69007 LYON	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: I - 2482 Date of the deposit or of the transfer[1]: 25 May 2000

III. VIABILITY STATEMENT

The viability of the microorganism identified under section II above was tested on 20 June 2000[2]. On that date, the said microorganism was

☒[3] viable

☐[4] no longer viable

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

Form BP/9 (firtst page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

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VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AVENTIS PASTEUR Address: 2, Avenue du Pont Pasteur 69007 LYON	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: I - 2483 Date of the deposit or of the transfer[1]: 25 May 2000

III. VIABILITY STATEMENT

The viability of the microorganism identified under section II above was tested on 20 June 2000[2]. On that date, the said microorganism was

☒[3] viable

☐[4] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

Form BP/9 (first page)

CLAIMS

1. Tetravalent vaccine composition against Dengue, comprising viruses of each of the attenuated strains deposited at the CNCM at the Pasteur Institute of Paris under the references I-2480, I-2481, I-2482 and I-2483, characterized in that the relative quantity of virus of each of the serotypes is such that the quantity of virus of serotype 3 is less than the quantity of virus of the other serotypes.

2. Vaccine composition according to Claim 1, in which the quantity of virus, per vaccine dose, of serotype 3 is at least 1 unit less than the quantity of virus of the other serotypes when the viral titre is expressed as log10 of the dilution of virus necessary to infect 50% of the cells.

3. Vaccine composition according to one of the preceding claims, in which the quantity of virus, per vaccine dose, of serotype 1 is at least 1 unit greater than the quantity of virus of the other serotypes when the viral titre is expressed as log10 of the dilution of virus necessary to infect 50% of the cells.

4. Vaccine composition according to one of the preceding claims, according to which the quantity of virus of each of the serotypes 1 to 4, per vaccine dose, corresponds to one of the following formulations: 3,2,1,2 or 3,3,1,3 in which the figures express the values of the logs10 of the dilution of virus necessary to infect 50% of the cells.

5. Vaccine composition according to one of the preceding claims, characterized in that it is intended to be administered twice separated by a time interval of at least 1 month.

6. Vaccine composition according to one of the preceding claims, characterized in that it is intended for subcutaneous administration.

7. Use of a viral composition comprising viruses of each of the attenuated strains deposited at the CNCM at the Pasteur Institute of Paris under the references I-2480, I-2481, I-2482 and I-2483, in which the relative quantity of virus of each of the serotypes is such that the quantity of virus of serotype 3 is less than the quantity of virus of the other serotypes, for the manufacture of a vaccine composition against Dengue.