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PCT

Industria y Comercio
SUPERINTENDENCIA

10-74461

DELEGATURA DE PROPIEDAD INDUSTRIAL

DIRECCIÓN DE NUEVAS CREACIONES



SOLICITUD DE PATENTE DE
INVENCION DIVISIONAL

TITULO: BACTERIAS TRATADAS TERMICAMENTE Y VACUNAS DE
EMULSION PREPARADAS A PARTIR DE TALES BACTERINAS TRATADAS
TERMICAMENTE

SOLICITANTE: PFIZER INC

DIRECCIÓN: 235 East 42nd Street, New York, NY 10017, E. U. A.

APODERADO: CARLOS R. OLARTE

BOGOTÁ 17 de Junio de 2010

Car. 6.1.1.1
21-03-2010



No. 10-074461- -00000-0000

Fecha: 2010-06-21 17:01:51 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 65

PETITORIO - FORMULARIO ÚNICO

PATENTE PCT

ENTRADA EN FASE NACIONAL

P2010/000078

SOLICITUD DE:

1

☒

Patente de Invención.

☐

Patente de Modelo de Utilidad.

☒

Capítulo I.

☐

Capítulo II.

2

SOLICITANTE
(71)

Nombre: PFIZER INC

Dirección: 235 East 42nd Street, New York, NY 10017 E.U.A.

Nacionalidad o Domicilio: Estadounidense

Lugar de Constitución: Estados Unidos de America

Teléfono: _____ **Fax:** _____

E-mail: _____

IDENTIFICACIÓN

C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número _____

REPRESENTANTE
O APODERADO

Nombre: CARLOS R. OLARTE

Dirección: Diagonal 34 No. 5-28 Bogotá, Colombia

Teléfono: 601-7700 **Fax:** 601-7799

E-mail: office@olarteraisbeck.com

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C.C. ☒ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número 79.782.747Bta
TP 74.295

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(72)

Nombre: 1) Mark Davis GOODYEAR; 2) Michel John HUETHER; 3) Richard Lee KREBS; 4) Nancee L. OIEN

Dirección: 1 y 4) 7000 Portage Road, Kalamazoo, MI 49001 EUA; 2 y 3) 601 West Cornhusker Highway, B771, Lincoln, Nebraska 68521; 4) 01

Nacionalidad o Domicilio: Estadounidenses

5

PCT (86)

Solicitud Internacional No. PCT/IB2008/003369

Fecha Diciembre 4, 2008

Publicación Internacional No. WO 2009/090461

Fecha Julio 23, 2009

6

Título (54) ~~BACTERINAS TRATADAS TERMICAMENTE Y VACUNAS DE EMULSIÓN PREPARADAS A PARTIR DE TALES BACTERINAS TRATADAS TERMICAMENTE~~

UNA VACUNA QUE COMPRENDE UNA EMULSIÓN Y UNA BACTERINA TRATADA CON CALOR QUE COMPRENDE UNA SUSPENSIÓN DE LA BACTERIA MUERTA

Clasificación Internacional (51) A61K 39/02

Prioridad

SI ☒ NO ☐

(33) País de Origen

US

(32) Fecha

Diciembre 21, 2007

(31) Número de Solicitud

60/015.718

9

Comprobante de pago No. 10-

Fecha Junio de 2010



ANEXOS

2



Comprobante de pago de la tasa de presentación de la solicitud.



Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.



Poderes, si fuere el caso. Adjunto copia simple ver expediente No. 06-029.042



Documento de cesión del inventor al solicitante o a su causante.



Declaraciones.



Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).



Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).



Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).



Traducción del examen preliminar internacional efectuado por la IPEA.



De ser el caso, copia del contrato de acceso.



De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.



De ser el caso, certificado de depósito del material biológico.



Arte final 12 x 12.



De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.



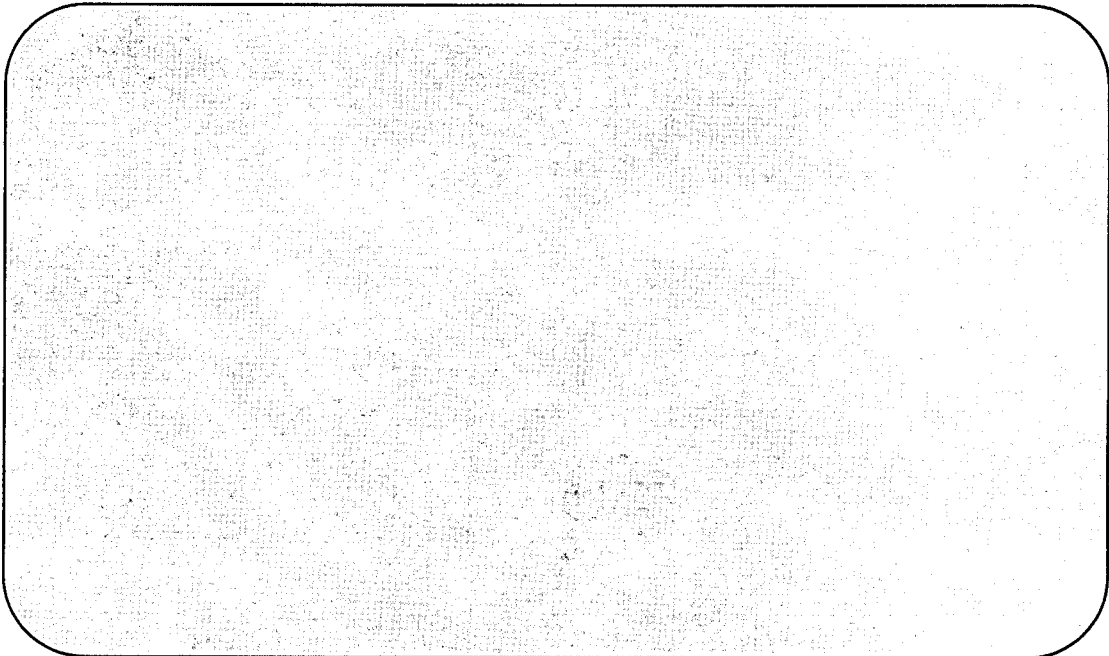
De ser el caso, modificaciones efectuadas a la solicitud internacional.



Otros. Búsqueda Internacional

11

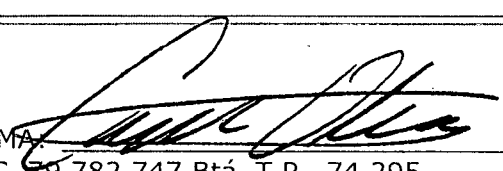
FIGURA CARACTERÍSTICA:



12

Solicito la concesión de la patente,

NOMBRE: CARLOS R. OLARTE

FIRMA: 

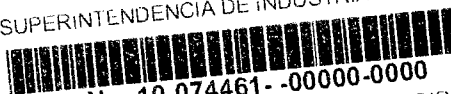
C.C. 79.782.747 Btá T.P. 74.295

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176029-2

RECIBO OFICIAL DE CAJA : 10 - 49,669
FECHA : JUNIO 21 DE 2010

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-074461-00000-0000

Fecha: 2010-06-21 17:01:51 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eva: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 65

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
CLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	174689286	21/06/2010	7,858,000.00

***** CONCEPTO *****

CANT. ENTREGADO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	
	1 TRAMITES DE SOL. DE PATENTE DE IN	554,000.00
	TOTAL :	\$ 554,000.00

SON: QUINIENTOS CINCUENTA Y CUATRO MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

4

Traducción oficial del Inglés por Marlén Neira Castro, resolución
2117 del Ministerio de Justicia de Colombia

APOSTILLA EN PODER DE REPRESENTACIÓN DE
PFIZER INC.

Bilingüe inglés- español otorgado en Nueva York, Estados Unidos el 14 de julio
de 2003 y firmado por J. Trevor Lumb, Vicepresidente de Pfizer Products Inc.

CERTIFICACION NOTARIAL

Bilingüe inglés- español, el 14 de julio de 2003 el suscrito notario Kenneth
Greenwood certifica que J. Trevor Lumb, domiciliado en Nueva York ejercía
funciones de Asistente General Consejero de Patentes la Compañía Pfizer Inc., con
sede en Nueva York, Nueva York, Estados Unidos.

Firma del notario: Kenneth Greenwood

Sello notarial: certificado, verificado y autenticado en la Localidad de Egham
en Surrey, 14 julio, 2003 por el Notario Kenneth Greenwood-Horne Engall & Freeman

Sello Notario Público en alto relieve rojo.

APOSTILLA

REINO UNIDO DE GRAN BRETAÑA E IRLANDA DEL NORTE

1. País :Reino Unido de Gran Bretaña e Irlanda del Norte
Este documento Público
2. ha sido firmado por K. Greenwood
3. ejerce funciones de Notario Público
4. Lleva el sello de dicho Notario Público

CERTIFICADÓ

5. En Londres 6. El 17 de julio de 2003
7. Por el Secretario de Estado Principal de su Majestad para Asuntos
exteriores y de la mancomunidad
8. No. G210434
9. Sello del Secretario de Estado Principal de su Majestad para Asuntos
exteriores y de la mancomunidad
10. Firma de R. Bawden - Por Secretario de Estado

Si el documento se usa en un país que no es parte de la convención de la Haya del 5 de octubre 1961, debe
presentarse en sección consular de la misión que representa el país.

Es traducción fiel del inglés, con copia archivo para confrontación. Bogotá 25 de julio-2003. Marlén Neira C

Marlén Neira C
MARLEN NEIRA CASTRO
Traductor e Intérprete Oficial
Inglés - Español - Inglés
Resolución No. 2117

OLARTE RAISBECK

OLARTE RAISBECK & FRIERI, LTDA.
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Power of Attorney

The undersigned,

domiciled in

235 East 42nd Street, New York, NY 10017 USA

by means of these presents hereby grants to **Carlos R. Olarte, J. Ian Raisbeck and Ana María Frieri** full and sufficient Power of Attorney to file, prosecute, obtain, defend and enforce any and all of the company's Patents, Utility Models and Industrial Designs in the Republic of Colombia, to which end it empowers them to take all steps necessary before any administrative or judicial authority of any class for the objects stated, to file petitions, appeals, replies and oppositions, pay taxes and annuities, request testimonies, receive documents and securities, abandon applications and collect refunds. Sufficient power is hereby granted to receive notifications on behalf of the Company, to confer powers on those who are to represent the company judicially and extrajudicially, to answer in court in the matter of all claims and demands that may be presented in connection with the objects stated, giving them likewise power to substitute these presents and revoke such substitutions.

Signature/Firma: _____

Date/Fecha: July 14, 2003

City, Country/Ciudad, País: New York, USA

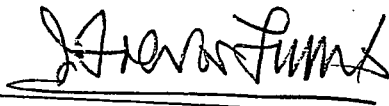
Poder

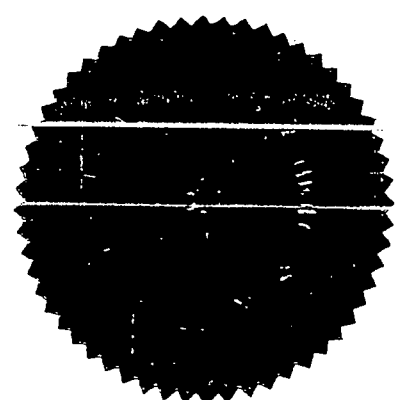
El suscrito,

Pfizer Inc.

domiciliado en

por medio del presente otorga a **Carlos R. Olarte, J. Ian Raisbeck y Ana María Frieri** poder especial, amplio y suficiente para presentar, tramitar, obtener, defender y hacer respetar todas y cualquiera de Patentes, Modelos de Utilidad y Diseños Industriales de la compañía en la República de Colombia, a cuyo efecto los faculta para dar todos los pasos necesarios ante cualquier autoridad administrativa o judicial de cualquier jerarquía para cumplir con lo encomendado, elevar solicitudes, apelaciones, respuestas y oposiciones, pagar impuestos y anualidades, solicitar testimonios, recibir documentos y valores, desistir y percibir. Se concede poder bastante para recibir notificaciones a nombre de la compañía, otorgar poderes a quienes vayan a representar a la compañía judicial y extrajudicialmente, responder en juicio a todas las reclamaciones o demandas que por motivo de lo encomendado se presentaren, dándoles asimismo facultad para sustituir el presente y revocar dichas sustituciones.





OLARTE RAISBECK

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Notarial Certification

On this date,
July 14, 2003

The undersigned Notary Public certifies:

1. That J. Trevor Lumb

of legal age and domiciled in

New York, USA
signed the foregoing document.

2. That the grantor has executed the foregoing document as Assistant General Patent Counsel of Pfizer Inc.

3. That Pfizer Inc.,
having its principal place of business located in New York, New York, U.S.A.
is duly incorporated, is currently in existence, and that the purposes for which the Power of Attorney is granted are within the scope of its corporate purposes.

4. That the grantor has the authority to execute the Power of Attorney and that his/her representation is legal.

5. The foundation for my certifications contained in points 2, 3 and 4 was the exhibition of the following authentic document(s):

Certificación Notarial

En esta fecha,

el Notario Público suscrito certifica:

1. Que

J. Trevor Lumb

mayor de edad y domiciliado

New York

suscribió el documento que antecede.

2. Que el otorgante ha suscrito el referido documento en su carácter de
de

4. Que

con sede principal ubicada en

está legalmente constituida, que tiene existencia legal vigente y que los propósitos para los cuales se otorga el poder se encuentra dentro de los que comprenden su objeto social.

4. Que el otorgante tiene la representación para suscribir el poder, y que ésta es legítima.

5. El fundamento de mis certificaciones contenidas en los puntos 2, 3 y 4 fue la exhibición de los (del) siguiente(s) documento(s) auténtico(s):

NOTARY PUBLIC (Signature):

EXECUTION
Certified Verified and Authenticated
In The Town Of Exbury in Surrey

This 14th

Day Of July

2003

KENNETH GREENWOOD
NOTARY PUBLIC
HORNBEINGALL AND FREEMAN



7

APOSTILLE
(Hague Convention of 5 October 1961 / Convention de La Haye du 5 octobre 1961)

UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

1. Country: United Kingdom of Great Britain and Northern Ireland
Pays: Royaume-Uni de Grande-Bretagne et d'Irlande du Nord

This public document / Le présent acte public

2. Has been signed by K Greenwood
a été signé par

3. Acting in the capacity of Notary Public
agissant en qualité de

4. Bears the seal/stamp of The Said Notary Public
est revêtu du sceau/timbre de

5. at London/à Londres

Certified/Attesté
6. the/le 17 July 2003

7. by Her Majesty's Principal Secretary of State for Foreign and Commonwealth Affairs /
par le Secrétaire d'Etat Principal de Sa Majesté aux Affaires Etrangères et du Commonwealth.

8. Number/sous No

G210434

9. Stamp:
timbre:

10. Signature: R. Bawden



For the Secretary of State / Pour le Secrétaire d'Etat

If this document is to be used in a country which is not party to the Hague Convention of 5 October 1961,
it should be presented to the consular section of the mission representing that country.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 July 2009 (23.07.2009)

PCT

(10) International Publication Number
WO 2009/090461 A1

(51) International Patent Classification:
A61K 39/02 (2006.01)

68521 (US). **OIEN, Nancee L.** [US/US]; Pfizer Global Research & Development, 7000 Portage Road, Kalamazoo, MI 49001 (US).

(21) International Application Number:

PCT/IB2008/003369

(74) Agent: **FULLER, Grover, F., Jr.**; Pfizer Inc., 150 East 42nd Street, NY, New York, 10017 (US).

(22) International Filing Date:

4 December 2008 (04.12.2008)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/015,718 21 December 2007 (21.12.2007) US

(71) Applicant (for all designated States except US): **PFIZER INC.** [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(72) Inventors; and

(75) Inventors/Applicants (for US only): **GOODYEAR, Mark Davis** [US/US]; Pfizer Global Research and Development, 7000 Portage Road, Kalamazoo, MI 49001 (US). **HUETHER, Michael John** [US/US]; Pfizer Global Research and Development, 601 West Cornhusker Highway, B771, Lincoln, Nebraska 68521 (US). **KREBS, Richard, Lee** [US/US]; Pfizer Global Research and Development, 601 West Cornhusker Highway B771, Lincoln, Nebraska

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(54) Title: HEAT TREATED BACTERINS, AND EMULSION VACCINES PREPARED FROM SUCH HEAT TREATED BACTERINS

(57) Abstract: Heat treated bacterins, a method of producing heat treated bacterins, and porcine emulsion vaccines prepared from such heat treated bacterins are disclosed.

WO 2009/090461 A1

HEAT TREATED BACTERINS, AND EMULSION VACCINES PREPARED FROM SUCH HEAT TREATED BACTERINS

FIELD OF INVENTION

5 This invention relates generally to the field of vaccines and to methods of stabilizing emulsion vaccines. In particular, this invention relates to heat treated bacterins, a method of producing heat treated bacterins, and to porcine emulsion vaccines prepared from such heat treated bacterins.

10 BACKGROUND OF THE INVENTION

Vaccination is increasingly used to control the infectious diseases in animals. Adjuvants are frequently used in vaccines because they are able to increase the humoral and/or cellular immune response to an antigen.

15 Vaccines are often formulated as emulsions because the emulsion can act as an adjuvant, and has the property of retaining the antigen as a depot at the site of injection. Emulsifiers are commonly used in emulsion vaccines. Besides using emulsifiers, the stability of the emulsion vaccines may also be achieved through reducing the droplet size of the emulsion by mechanical means.

20 U.S. Patent No. 5,084,269 relates to an adjuvant formulation containing lecithin in combination with mineral oil, which produces less irritation within the host animal, and simultaneously induces increased systemic immunity. Compositions according to US Patent 5,084,269 are in commercial use under the trade name AMPHIGEN®, a trademark of Pfizer, Inc.

25 Generally, bacterial antigens are unstable when heated and even brief exposure to elevated temperatures can reduce the activity of the antigens. For example current anthrax vaccines can lose all biological activity with 48 hours at 37°C (S. Sing, N. Ahuja, V. Chauhan, E. Rajasekaran, W. S. Mohsin, R. Bhat, and R. Bhatnagar; Bioche. Biophys. Res. Commun. 2002 Sep. 6; 30 295(5):1058-62).

-2-

SUMMARY OF INVENTION

This invention relates to heat treated bacterins, a method of producing heat treated bacterins, and porcine emulsion vaccines prepared from such heat treated bacterins. The method comprises heating the bacterin to a
5 temperature of about 35 to about 80°C to form a heat treated bacterin.

DETAILED DESCRIPTION

DEFINITIONS

10 Acceptable antigenic activity – The term “acceptable antigenic activity” means the ability to induce a protective immune response in vaccinated animals after being challenged or by passing a codified potency test with homologous live organism.

Bacterin – The term “bacterin” means a suspension of killed bacteria
15 which may be used as a component of a vaccine.

Emulsifier – The term “emulsifier” means a substance used to make an emulsion more stable.

Emulsion – The term “emulsion” means a composition of two immiscible liquids in which small droplets of one liquid are suspended in a
20 continuous phase of the other liquid.

Heat treated bacterin – The term “heat treated bacterin” means a bacterin that has been heat treated and which has a lipase activity of 50% or less than the lipase activity before the heat treatment, and has acceptable antigenic activity.

25 Invert Emulsion – The term “invert emulsion” means a water in oil emulsion.

Lipase – The term “lipase” means enzymes, esterases, lipases, and phospholipases, which can cause breakdown of an emulsifier in an emulsion vaccine.

30 Normal Emulsion – The term “normal emulsion” means an oil in water emulsion.

Oil in Water Emulsion – The term “oil in water emulsion” means an emulsion in which small droplets of oil are suspended in a continuous water phase.

Room Temperature – The term “room temperature” means a
5 temperature from 18 to 25°C.

Water in Oil Emulsion – The term “water in oil emulsion” means an emulsion in which droplets of water are suspended in a continuous oil phase.

DESCRIPTION

10 This invention relates to bacterins with reduced lipase activity, porcine emulsion vaccines prepared from such bacterins, and a method of reducing the lipase activity of bacterins. In addition to antigenic components, some bacterins have lipase activity. When bacterins with lipase activity are incorporated into an emulsion, the lipase may break down the emulsifiers
15 used to create the emulsion. Emulsion vaccines that contain bacterins having high lipase activity tend to be unstable emulsions, and those that contain bacterins having low levels of lipase tend to be stable. Examples of bacteria which may, when killed, produce bacterins having lipase activity include *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, *Escherichia coli*,
20 *Mycoplasma hyopneumoniae*, and *Leptospira* species, such as the known pathogens *Leptospira canicola*, *Leptospira grippotyposa*, *Leptospira hardjo*, *Leptospira icterohaemorrhagiae*, *Leptospira bratislava* and *Leptospira pomona*. These bacteria can cause diseases in pigs, and vaccination against these diseases is desirable. The *Leptospira* bacterins are more likely to have
25 a high lipase activity while an *Erysipelothrix rhusiopathiae* bacterin may have a lower, more manageable lipase activity.

The lipase, which can break down the emulsifiers used to create the emulsion, and thus cause emulsion instability and breakdown, may include one or more emulsion breaking enzymes such as esterases, lipases, and
30 phospholipases. Collectively these enzymes, esterases, lipases, and phospholipases are referred to as lipase. The lipase activity of a bacterin may be measured using a synthetic substrate called O-pivaloyloxymethyl umbelliferone (C-POM). The rate of hydrolysis caused by the lipase is the

measure of the lipase activity. The reaction rate of the hydrolysis caused by the lipase in this reaction is monitored by an increase in the fluorescence intensity of the product of the lipase activity. The reaction rate is dependent upon the exact hydrolysis test conditions chosen, so that comparisons of lipase activity levels, as measured by hydrolysis rates, should be made using data produced by the same test conditions. Literature methods are disclosed in several articles, including Kurioka S. and Matsuda M. (1976) *Ana. Biochem.* 75: 281-289, De Silva N.S. and Quinn P.A. (1987) *J. Clin. Microbiol.* 25: 729-731, and Grau A. and Ortiz A. (1998) *Chem. Phys. of Lipids.* 91: 109-118.

10 In an emulsion vaccine, the break down of the emulsion causes phase separation of the components. This is undesirable because when there is phase separation the individual doses removed from the container may not contain the same level of the vaccine components. In addition, the loss of emulsion can lead to a loss of the adjuvant activity of the emulsifier and lead to a reduction in the antigenic effect of the vaccine.

20 Attenuated live viruses are frequently included in vaccines along with bacterins. Such vaccines are useful because a single vaccine can be used to create immunity to different diseases with one vaccine. If the lipase activity is present in the bacterin, it will cause release of the emulsifier from the emulsion. This free emulsifier can disrupt and inactivate the live vaccine viruses, thereby leading to a loss of viral infectivity.

25 A bacterin useful in vaccines may be formed by culturing the bacterium of interest, and then killing the bacteria to produce a bacterin containing a variety of bacterial components, including cell wall components. The bacteria may be killed by a variety of methods including exposing them to a compound such as merthiolate, formalin, formaldehyde, diethylamine, binary ethylenamine (BEI), beta propiolactone (BPL), and glutaraldehyde. Combinations of these compounds may be used. In addition, it is possible to kill the bacteria with sterilizing radiation.

30 It has now been found that the lipase activity of a bacterin having such lipase activity may be reduced by heat treatment. Specifically, the lipase activity of a bacterin may be reduced by heating the bacterin to a temperature of about 35 to about 80°C to form a heat treated bacterin, which has

-5-

acceptable antigenic activity. The heat treatment is conducted for a period of time sufficient so that the lipase activity of the heat treated bacterin is 50% or less than that found in the bacterin prior to the heat treatment. For good emulsion vaccine stability it is not necessary that the lipase activity be
5 reduced to zero. We have found that vaccines having a good shelf life may be prepared from heat treated bacterins having lipase activity level that is 50% or less than of the lipase activity level before the heat treatment.

When a rate of hydrolysis of a test substrate has been used as a measure of the lipase activity of a bacterin, then the rate of hydrolysis of the
10 test substrate before the heat treatment is compared to the rate of hydrolysis after the heat treatment. The heat treatment is conducted so as to reduce the rate of hydrolysis to 50% or less than the rate of hydrolysis that is observed for the fresh bacterin.

The exact method of measuring the lipase activity level is not critical as
15 long as the same method is used to measure the activity before the heat treatment and the activity after the heat treatment. For example, if the rate of hydrolysis of a test substrate is measured using one substrate, a different substrate might produce a different rate. However, if the same substrate is used for the initial activity determination and the activity determination after
20 treatment, the relative rates will still show the effect of the heat treatment.

There are codified tests for antigenic activity for *Leptospira Pomona* Bacterin (9 CFR §113.101), *Leptospira Icterohaemorrhagiae* Bacterin (9 CFR §113.102), *Leptospira Canicola* Bacterin (9 CFR §113.103), *Leptospira Grippotyphosa* Bacterin (9 CFR §113.104), and *Leptospira Hardjo* Bacterin (9
25 CFR §113.105) (9 CFR §113.101, §113.102, §113.103, §113.104, and §113.105). For these species acceptable antigenic activity may be defined as the ability to induce a protective immune response in vaccinated hamsters such that when hamsters are challenged with homologous live bacteria, at least 75% of the vaccinated hamsters survive in a model where at least 80%
30 of the non-vaccinated hamsters do not survive. In the case of the antigen, *Leptospira hardjo*, acceptable antigenic activity may be defined as the ability of a vaccine to induce a serological agglutination geometric mean titer against *Leptospira hardjo* of ≥ 40 in calves that have been vaccinated with a vaccine

comprising the bacterial antigen, *Leptospira hardjo*. For other bacterins acceptable antigenic activity is defined as the ability to induce a protective immune response in vaccinated animals after being challenged or by passing a potency test with homologous live organism.

The heat treatment may be conducted over a range of temperatures, and for a variable length of time. Generally, the heating may be done at a temperature of about 35 to about 80°C for about 20 minutes to about 24 hours. When the bacterin is heated to a higher temperature, such as about 75 to about 80°C, the time of heating is at the short end of the time range. When the heating is done at a lower temperature, the heating is done for a longer period of time. Another combination of temperature and time is heating at a temperature of about 60 to about 70°C for about 9 to about 10 hours. Another combination of temperature and time is heating at a temperature of about 65 to about 70°C for about 5 to about 8 hours. Another combination of temperature and time is heating at a temperature of about 65 to about 70°C for about one hour. Another combination of temperature and time is heating at a temperature of about 55 to about 65°C for about 5 to about 8 hours.

The bacterins, after the heat treatment, have a lower lipase activity than freshly prepared bacterins but otherwise may be formulated in the same manner as freshly prepared bacterins. Accordingly, the heat treated bacterins may be incorporated into vaccines by ordinary methods of producing vaccines. These methods are well known in the art.

Emulsion vaccines may be formed by combining the desired bacterin with an oils phase and an emulsifier, or emulsifiers. The combination is then subjected to intense agitation to form an emulsion. Suitable agitation methods include homogenizing and subsequently microfluidizing. Preservatives and excipients may also be included in the combination prior to emulsification.

Vaccines may include both bacterins and viral antigens. In preparing a vaccine that includes bacterins and viral antigens, the bacterins, any viral antigens to be included, the emulsifier, or emulsifiers, and optionally preservatives and excipients are combined with an oil phase, and emulsified. Following emulsion formation, the pH of the formulations may be adjusted to

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an appropriate pH using either solutions of NaOH or HCl. For vaccine use, it is generally desirable that the pH be close to neutral to avoid irritation at the injection site. A pH of about 7.0 to about 7.3 is common.

Suitable oil phases for emulsion vaccine formation include non-
5 metabolizable oils and metabolizable oils. The non-metabolizable oils include mineral oils, such as white mineral oil, and light mineral oil. The metabolizable oils include vegetable oils, fish oils and synthetic fatty acid glycerides.

Examples of emulsifiers that may be used in preparing emulsion
10 vaccines of this invention are phospholipids, sorbitan esters, polyethoxylated sorbitan esters, and mannitol derivatives which are common vaccine emulsifiers. Phospholipid emulsifiers include lecithin, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and lecithin, (e.g. such as AMPHIGEN®). Sorbitan ester emulsifiers include
15 sorbitan monolaurate, (e.g. Span® 20 and Arlacel® 20), sorbitan monooleate (e.g. Span® 80 and Arlacel® 80), sorbitan monopalmitate (e.g. Span® 40 and Arlacel® 40), and sorbitan monostearate (e.g. Span® 60 and Arlacel® 60). Polyethoxylated sorbitan esters include polyethoxy sorbitan monolaurate (e.g. Tween® 20 and Tween® 21), polyethoxy sorbitan monooleate (e.g. Tween®
20 80), polyethoxy sorbitan monopalmitate (e.g. Tween® 40), and polyethoxy sorbitan monostearate (e.g. Tween® 60). Mannitol derivative emulsifiers include mannitol octadecanoic ethers. Span®, Arlacel®, and Tween® are trademarks of ICI Americas. AMPHIGEN® is a trademark of Pfizer, Inc. Generally, vaccines are formulated as normal oil in water emulsions, although
25 it is possible to prepare invert water in oil emulsions.

A variety of adjuvants, such as Quil A, cholesterol, aluminum phosphate, and aluminum hydroxide, and preservatives such as merthiolate may be used in vaccines. Quil A is purified mixture of quillaja saponins extracted from the bark of the South American tree Quillaja Saponaria Molina.
30 Quil A acts directly on the immune system to activate a generalized state of sensitivity. In doing so, it induces both humoral and cell-mediated responses. The lipophilic chain allows interaction of antigen and adjuvant to be delivered into the cytosol for processing in an endogenous pathway. Quil A is often

used with cholesterol because cholesterol eliminates the less desirable side effects when added in the appropriate proportions. Cholesterol forms insoluble complexes with Quil A that form helix-like structures as the cholesterol binds with Quil A, thus exposing the molecule's sugar units that help stimulate the immune response.

It is common to add porcine viral antigens to vaccines containing bacterins. One advantage of this approach is that one vaccine may be used to create immunity to several diseases instead of requiring dosages of several different vaccines to achieve the same result. Both killed viruses and attenuated live viruses may be used in vaccines. Among the porcine disease causing viruses that may be used are Porcine Adenovirus, Porcine Circovirus, Porcine herpes viruses, Pseudorabies virus, Classical swine fever virus, Porcine epidemic diarrhea virus, Porcine hemagglutinating encephalomyelitis virus, Porcine parvovirus, Porcine Respiratory Corona virus, Porcine Reproductive and Respiratory Virus, Swine Influenza, Transmissible gastroenteritis virus, and Vesicular stomatitis virus.

If lipase activity is present in the bacterin, it may cause release of the emulsifier from the emulsion. This free emulsifier may disrupt the live virus envelope, and inactivate the live vaccine viruses, thereby leading to a loss of viral infectivity. Accordingly, heat treatment of the bacterin serves to stabilize the emulsion, and preserve its adjuvant effect, as well as preserving the viral infectivity of the viruses.

The following examples are provided for the purpose of further illustration and are not intended to limit the scope of the claimed invention.

PROCEDURES

Procedure 1 Determination of Turbidity

Turbidity is determined in Nephelometric Units (NU) by a light scattering method. The intensity of light scattered by the sample under defined conditions is compared to the intensity of light scattered by a standard reference suspension. The higher the intensity of the scattered light, the higher the turbidity of the sample. A light source is directed into the sample and the light scatter is measured at 90° to the direction of the light source.

The instrument is calibrated by measuring the light scatter from a formazin suspension.

Calibration Of The Nephelometer Instrument

Ultra-filtered water is prepared by filtering distilled water through a
5 membrane filter having a pore size of 0.2 µm. A first solution is prepared by
dissolving 1.00 g hydrazine sulfate, (NH₂) H₂SO₄, in ultra-filtered water and
diluted with ultra-filtered water to 100 ml, in a volumetric flask. A second
solution is prepared by dissolving 10.00 g. of hexamethylenetetramine in ultra-
filtered water and diluting with ultra-filtered water to 100 ml, in a volumetric
10 flask. A formazin suspension is prepared by mixing 5.0 ml of the first solution
with 5.0 ml of the second solution. The mixture is allowed to stand for 24
hours at approximately 24°C. The mixture is diluted to 100ml with ultra-
filtered water to form a stock turbidity suspension having a turbidity of 400 NU.
A 40 NU formazin turbidity suspension is prepared by diluting 10.00 ml of the
15 stock turbidity suspension to 100 ml with ultra-filtered water. Further
calibration solutions are prepared by diluting the stock solution.

Measurement of Turbidity

The sample to be measured is diluted with ultra-filtered water so that
20 the turbidity falls within the calibrated range of the nephelometer. The
turbidity is measured and the original turbidity is calculated using the following
equation:

$$\text{Original Turbidity in NU} = \frac{M \times (D + O)}{O}$$

25 where: M is the turbidity of the diluted sample in NU
D is the volume of dilution water, in mL
O is the original sample volume, in mL

Procedure 2 Lipase Analysis

30 Lipase activity was determined using O-pivaloxymethylumbelliferone as
a fluorogenic substrate. Lipase catalyzed hydrolysis of this non-fluorescent
substrate produces a hydroxymethylether, which is unstable under aqueous
conditions. The decomposition of the unstable hydroxymethylether generates

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formaldehyde and the fluorescent product umbelliferone. Monitoring the fluorescence intensity of umbelliferone produced, as a function of time, provides a sensitive kinetic measurement of the lipase enzymatic activity.

O-pivaloxymethylumbelliferone (Molecular Probes product no. P35901) solutions were prepared in neat DMSO, at a stock concentration of 5 mM; unused solution was stored at -20°C, protected from light. The 5 mM O-pivaloxymethylumbelliferone solution was diluted to 750 µM using 58 mM TRIS-HCl buffer (pH 8.0), and the resulting solution pre-warmed to 37°C. The bacterin sample or the control buffer/medium was centrifuged for 10 minutes at room temperature at 6500 X gravity to form a pellet and a supernatant. Reactions were performed by combining 15 µL of 100 mM TRIS-HCl buffer (pH 8.0) with 15 µL of the supernatant at room temperature from the bacterin sample or the control buffer/medium, in assay wells of low volume 96 well plates (Corning 3393, black polystyrene non-binding surface, half area); pre-incubating for 10 minutes at 37°C; then initiating the reaction by the addition of 20 µL of 750 µM O-pivaloxymethylumbelliferone or the control buffer/medium. The resulting reaction mixtures contained 53 mM TRIS-HCl buffer (pH 8.0) and 0 or 300 µM O-pivaloxymethylumbelliferone. Fluorescence intensity was measured at 30-45 second intervals over a one-hour period (Spectramax Gemini XS, 37 °C, $\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$, PMT sensitivity setting 'medium', 6 reads per well). The reaction rate was determined from the slope of the resulting progress curve.

Procedure 3 Measurement Of The Turbidity Of An *Erysipelothrix rhusiopathiae* Preparation

The turbidity of an *Erysipelothrix rhusiopathiae* preparation is measured spectrophotometrically at a wavelength of 600 nm. The result is reported in optical units (OU).

EXAMPLES

Example 1 Reduction Of Lipase Activity By Heat Treatment

A pool of merthiolate killed leptospira containing the following species *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, *Leptospira*

grippotyphosa, *Leptospira hardjo*, and *Leptospira pomona* was prepared to from individual bacterins. Six samples of the combined bacterins were stored overnight (approximately 12 hours) at 4°C, 37°C, 45°C, 56°C, 65°C, and 80°C. The sample stored at 4°C served as the non-treated control. The
5 samples stored for 12 hours at 37°C, 45°C, 56°C, 65°C, and 80°C were heat treated samples. After storage, the rate at which a test substrate hydrolysed in the presence of each bacterin was measured according to the method of Procedure 2. The rate of hydrolysis for a sample divided by the rate of hydrolysis of the sample stored at 4°C multiplied by 100 is the percentage of
10 the original lipase activity of each bacterin that remains after storage. The following chart shows the temperature of storage and the percentage of the original lipase activity that remains after storage.

Storage Temperature (12 hours)	4°C	37°C	45°C	56°C	65°C	80°C
Percent of Original Lipase Activity	100%	55.4%	32.5%	15.7%	10.8%	8.4%

15 Two *Leptospira Bratislava* serials were prepared. The serials were inactivated with merthiolate and then subjected to heat treatment at 65°C for 8 hours. Samples were pulled pre-treatment and every two hours during treatment. Lipase activity was determined at each time point according to the method of Procedure 2. When a sample was taken, the rate at which a test
20 substrate hydrolysed in the presence of each sample was measured. The rate of hydrolysis for a sample divided by the rate of initial hydrolysis rate multiplied by 100 is the percentage of the original lipase activity of each bacterin that remains after heat treatment. The following chart shows the sample time and the average percentage of the original lipase activity that
25 remains at that time.

Sample time (hours)	Initial	2	4	6	8
Percent of Original Lipase Activity	100%	41%	34%	28%	24%

Example 2 Preparation of Experimental Vaccine Formulations

5 Cultures of *Leptospira canicola*, *Leptospira icterogorrrhagiae*,
Leptospira grippotyphosa, *Leptospira hardjo*, *Leptospira pomona*, *Leptospira Bratislava*, *Erysipelothrix rhusiopathieae*, and *Porcine parvovirus* were grown. The turbidity of each *Leptospira* culture was measured in nephelometric units (NU). The turbidity of the *Erysipelothrix rhusiopathieae* culture was measured
10 in optical units (OU). The bacteria were killed with merthiolate to form bacterins. Each *Leptospira* bacterin was heat treated at 65°C for 8 hours to reduce the lipase activity. The *Erysipelothrix rhusiopathieae* bacterin was not heat treated. The *Leptospira* bacterins were combined with killed *Porcine parvovirus* and killed *Erysipelothrix rhusiopathieae* then mixed with
15 AMPHIGEN®, adjuvants, preservatives, and diluting buffer so that each 2 ml dose of the vaccine contained the components set forth in the chart below.

Concentrations of Antigens

Component	Concentration of Component/Dose
<i>L. canicola</i>	1200 NU/2 ml dose
<i>L. icterohaemorrhagiae</i>	1200 NU/2 ml dose
<i>L. grippotyphosa</i>	1200 NU/2 ml dose
<i>L. hardjo</i>	2400 NU/2 ml dose
<i>L. pomona</i>	1200 NU/2 ml dose
<i>L. Bratislava</i>	1200 NU/2 ml dose
<i>Erysipelothrix rhusiopathieae</i>	14 OU/2 ml dose
<i>Porcine parvovirus</i>	17,920 HA/.05ml

Example 3 Potency Testing in Hamsters and Pigs

The vaccine of Example 2 was administered to hamsters and rabbits to test for potency using standard lab animal models. The test hamsters were then challenged with a dose of *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, *Leptospira grippotyphosa*, *Leptospira Bratislava*, or *Leptospira pomona* to test potency of the vaccines. The numbers of survivors were measured as a demonstration of efficacy. Rabbit microscopic agglutination titers were measured against *Leptospira hardjo* to demonstrate the potency of that fraction of the vaccine. The table below shows that vaccines prepared from heat treated *Leptospira* bacterins are capable of producing an antigenic response that passes efficacy criteria.

Leptospira Thermal Conditioning	HAMSTER SURVIVORS					Rabbit-SEROLOGY
	Canicola	Bratislava	Ictero	Grippo	Pomona	Hardjo
65 °C (8 hours)	10/10	10/10	10/10	10/10	10/10	Pass
Untreated	10/10	10/10	10/10	10/10	10/10	Pass

Erysipelothrix rhusiopathiae was tested in rabbits by comparing the vaccine serological titer to the titer of a reference vaccine. The vaccine had an RP (relative potency) of 3.0. PPV was tested in the hemagglutination assay and had an HA titer of 1024 HA/.05ml. A titer of 320 HA/.05ml is an acceptable value for a vaccine.

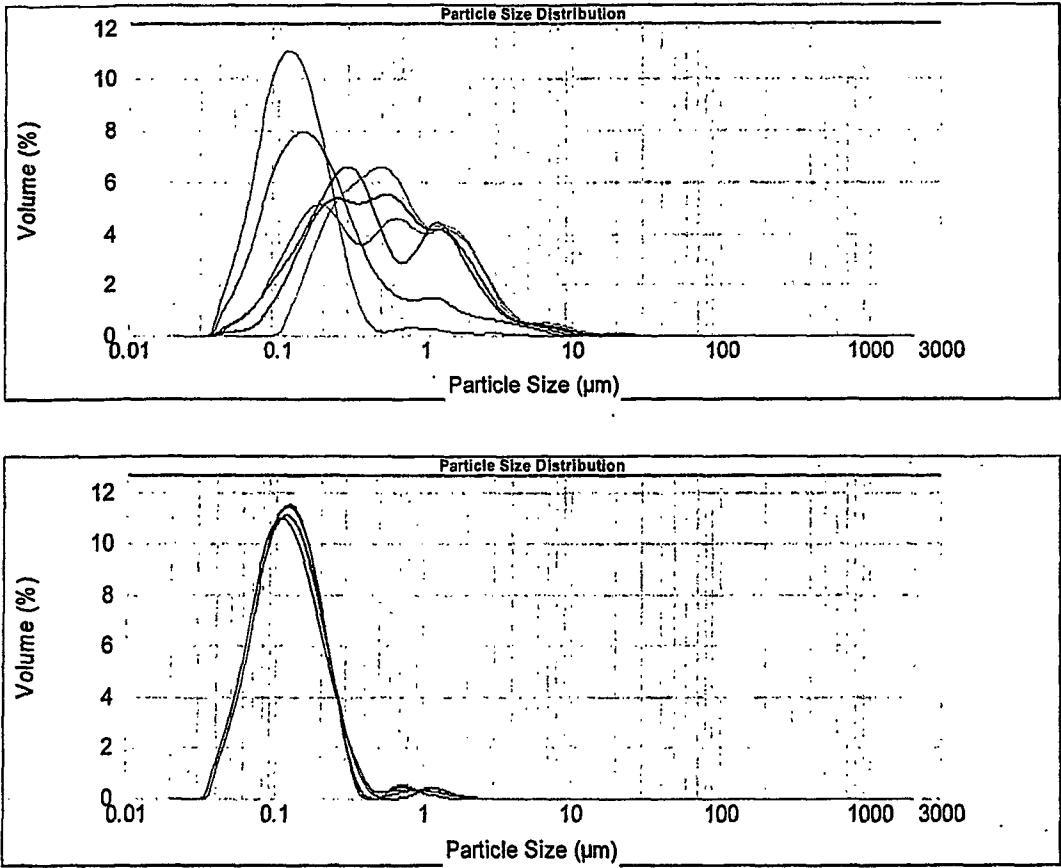
Example 4 Physiochemical Testing Of Vaccines

A vaccine was prepared with heat treated *Leptospira* bacterins and other components according to the formulation listed in Example 2. A similar vaccine was prepared from non-heat treated *Leptospira* bacterins according to the method of Example 2. Both vaccine formulations were stored at 4°C for 0, 6, 12, 15 and 18 months of age. Particle size analysis was done for each vaccine at each time point using a laser diffractometer.

The charts shown below show particle size distributions for each vaccine over several months of monitoring (0, 6, 12, 15 and 18 months).

Particle size analysis of freshly prepared vaccine containing heat treated *Leptospira* bacterins day 0

5



The vaccine prepared from non-heat treated *Leptospira* bacterins (upper graph) shows an increase in particle size indicating emulsion breakdown. The vaccine prepared from heat treated *Leptospira* bacterins (lower graph) shows particle size retention through 18 months of age indicating emulsion stability.

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Example 5 PPV Hemagglutination Assay (HA)

The vaccine formulations listed in Example 2 (the vaccines in the list in Example 2 contained all of the antigens listed – same vaccines for all work) prepared from non-heat treated *Leptospira* bacterins and heat treated *Leptospira* bacterins were initially tested for HA titer and hemolysis titer. The stability of HA titers at various time points. The HA assay was performed by

15

adjusting a sample to pH 11-11.2 to extract the PPV (Porcine parvovirus) virus from the aluminum hydroxide gel. The sample was then centrifuged and the supernatant collected for use in the assay. Guinea pig red blood cells were added to a 96 well plate to serve as the agglutination indicator. The sample supernatant was diluted 2 fold across duplicate rows with a starting dilution of 1:5. The plate is incubated at $5 \pm 3^{\circ}\text{C}$ for 16-24 hours. The degree of hemagglutination is scored from 0-4 for each well. The titer is recorded as the last dilution containing a score of 2 or above. During the test of vaccines, which were not heat treated, it was observed that the vaccine was causing hemolysis. The hemolysis titer was the highest dilution at which hemolysis was observed. Heat treated vaccines did not produce hemolysis.

The chart below shows the average HA titers and hemolysis titers over time. CTC is a vaccine with heat treated *Leptospira* bacterins. OOP is a vaccine without heat treated *Leptospira* bacterins.

15

Chart 1: PPV HA Titers Over Time

[illegible]

CLAIMS

1. A vaccine comprising an emulsion, a heat treated bacterin comprising a suspension of killed bacteria, wherein the killed bacteria are of the species *Leptospira Bratislava* and 1 to 13 porcine disease causing viruses selected
5 from the group consisting of Porcine Adenovirus, Porcine Circovirus, Porcine herpes viruses, Pseudorabies virus, Classical swine fever virus, Porcine epidemic diarrhea virus, Porcine hemagglutinating encephalomyelitis virus, Porcine parvovirus, Porcine Respiratory Corona virus, Porcine Reproductive and Respiratory Virus, Swine Influenza, Transmissible gastroenteritis virus,
10 and Vesicular stomatitis virus.
2. A vaccine according to claim 1 further comprising a lecithin preparation, and alum based adjuvant.
- 15 3. A vaccine according to claim 2 further comprising a lecithin in oil preparation.
4. A vaccine according to claim 1 further comprising heat treated bacterins comprising a suspension of killed bacteria, wherein the killed
20 bacteria are one to six bacteria species selected from the group consisting of *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira Pomona*, and *Erysipelothrix rhusiopathiae*.
- 25 5. A vaccine according to claim 4 further comprising a lecithin preparation, and alum based adjuvant.
6. A vaccine according to claim 5 further comprising a lecithin in oil preparation.

BACTERINAS TRATADAS TÉRMICAMENTE Y VACUNAS DE EMULSIÓN
PREPARADAS A PARTIR DE TALES BACTERINAS TRATADAS
TÉRMICAMENTE

5 CAMPO DE LA INVENCION

Esta invención se refiere generalmente al campo de las vacunas y a procedimientos para estabilizar vacunas de emulsión. En particular, esta invención se refiere a bacterinas tratadas térmicamente, a un procedimiento para producir bacterinas tratadas térmicamente y a vacunas de emulsión porcinas preparadas a partir de tales bacterinas tratadas térmicamente.

● ANTECEDENTES DE LA INVENCION

La vacunación se usa cada vez más para combatir las enfermedades infecciosas en animales. Se usan frecuentemente adyuvantes en vacunas debido a que son capaces de aumentar la respuesta inmune humoral y/o celular contra un antígeno. Las vacunas se formulan a menudo como emulsiones ya que la emulsión puede actuar como un adyuvante y tiene la propiedad de retener el antígeno como un depósito en el sitio de inyección. Se usan comúnmente emulsionantes en vacunas de emulsión. Además de usar emulsionantes, la estabilidad de las vacunas de emulsión también se puede conseguir reduciendo el tamaño de gota de la emulsión por medios mecánicos.

● La Patente de Estados Unidos N° 5.084.269 se refiere a una formulación de adyuvante que contiene lecitina en combinación con aceite mineral, que produce menos irritación en el animal huésped e induce simultáneamente una inmunidad sistémica aumentada. Las composiciones de acuerdo con la Patente

de Estados Unidos 5.084.269 están en uso comercial con el nombre comercial AMPHIGEN[®], una marca comercial de Pfizer, Inc.

Generalmente, los antígenos bacterianos son inestables cuando se calientan e incluso una exposición breve a temperaturas elevadas puede disminuir la actividad de los antígenos. Por ejemplo, las vacunas de carbunco actuales pueden perder toda la actividad biológica en 48 horas a 37°C (S. Sing, N. Ahuja, VI Chauhan, E. Rajasekaran, W. S. Mohsin, R. Bhat, y R. Bhatnagar, Broche. Biophys. Res. Commun. 2002 Sep. 6; 295 (5): 1058-62).

10 SUMARIO DE LA INVENCION

Esta invención se refiere a bacterinas tratadas térmicamente, a un procedimiento para producir bacterinas tratadas térmicamente y a vacunas de emulsión porcinas preparadas a partir de tales bacterinas tratadas térmicamente. El procedimiento comprende calentar la bacteria hasta una temperatura de aproximadamente 35 a aproximadamente 80°C para formar una bacteria tratada térmicamente.

DESCRIPCIÓN DETALLADA

DEFINICIONES

20 Actividad antigénica aceptable – La expresión “actividad antigénica aceptable” se refiere a la capacidad de inducir una respuesta inmune protectora en animales vacunados después de ser provocados o pasando un ensayo de potencia codificado con organismos vivos homólogos.

Bacteria – El término “bacteria” se refiere a una suspensión de bacterias muertas que se puede usar como un componente de una vacuna.

Emulsionante – El término “emulsionante” se refiere a una sustancia usada para hacer que una emulsión sea más estable.

Emulsión – El término “emulsión” se refiere a una composición de dos líquidos inmiscibles en la que gotas pequeñas de un líquido están suspendidas en una fase continua del otro líquido.

Bacterina tratada térmicamente – La expresión “bacterina tratada térmicamente” significa una bacterina que se ha tratado térmicamente y que tiene una actividad de lipasa del 50% o menor que la actividad de lipasa antes del tratamiento térmico y tiene una actividad antigénica aceptable.

Emulsión Invertida – El término “emulsión invertida” se refiere a una emulsión de agua en aceite.

Lipasa – El término “lipasa” se refiere a enzimas, esterasas, lipasas y fosfolipasas, que pueden causar la degradación de un emulsionante en una vacuna de emulsión.

Emulsión normal – El término “emulsión normal” se refiere a una emulsión de aceite en agua.

Emulsión de aceite en agua – El término “emulsión de aceite en agua” se refiere a una emulsión en la que pequeñas gotas de aceite están suspendidas en una fase acuosa continua.

Temperatura ambiente – El término “temperatura ambiente” significa una temperatura de 18 a 25°C.

Emulsión de agua en aceite – El término “emulsión de agua en aceite” significa una emulsión en la que están suspendidas gotas de agua en una fase oleosa continua.

DESCRIPCIÓN

Esta invención se refiere a bacterinas con actividad de lipasa reducida, vacunas de emulsión porcinas preparadas a partir de tales bacterinas y a un procedimiento para disminuir la actividad de lipasa de bacterinas. Además de
5 componentes antigénicos, algunas bacterinas tienen actividad de lipasa. Cuando las bacterinas con actividad de lipasa se incorporan a una emulsión, la lipasa puede degradar los emulsionantes usados para generar la emulsión. Las vacunas de emulsión que contienen bacterinas que tienen una actividad de lipasa elevada tienden a ser emulsiones inestables, y las que contienen
10 bacterinas que tienen niveles reducidos de lipasa tienden a ser estables. Los ejemplos de bacterias que cuando se destruyen pueden producir bacterinas que tienen actividad de lipasa incluyen *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, *Escherichia coli*, *Mycoplasma hyopneumoniae* y especies de *Leptospira*, tales como los patógenos conocidos *Leptospira canicola*,
15 *Leptospira grippotyposa*, *Leptospira hardjo*, *Leptospira icterohaemorrhagiae*, *Leptospira bratislava* y *Leptospira pomona*. Estas bacterias pueden causar enfermedades en cerdos y es deseable la vacunación contra estas enfermedades. Las bacterinas de *Leptospira* tendrán más probablemente una actividad de lipasa elevada, mientras que una bacteria de *Erysipelothrix*
20 *rhusiopathiae* puede tener una actividad de lipasa inferior, más manejable.

La lipasa, que puede degradar los emulsionantes usados para generar la emulsión y, por tanto, causar la inestabilidad y degradación de la emulsión, puede incluir una o más enzimas de degradación de la emulsión, tales como esterases, lipasas y fosfolipasas. En conjunto, estas enzimas, esterases,
25 lipasas y fosfolipasas se denominan lipasa. La actividad de lipasa de una

bacterina se puede medir usando un sustrato sintético denominado O-pivaloiloximetil umbeliferona (C-POM). La velocidad de la hidrólisis causada por la lipasa es la medida de la actividad de lipasa. La velocidad de reacción de la hidrólisis causada por la lipasa en esta reacción se controla por un aumento en la intensidad de fluorescencia del producto de la actividad de lipasa. La velocidad de reacción depende de las condiciones de ensayo de hidrólisis exactas elegidas, de tal forma que se deben realizar comparaciones de los niveles de actividad de lipasa, medidos por las velocidades de hidrólisis, usando los datos producidos por las mismas condiciones de ensayo. Se describen procedimientos de la bibliografía en varios artículos, incluyendo Kurioka S. y Matsuda M. (1976) *Ana. Biochem.* 75: 281-289, De Silva N. S. y Quinn P. A. (1987) *J. Clin. Microbiol.* 25: 729-731, y Grau, A. y Ortiz A. (1998) *Chem. Phys. Of Lipids.* 91: 109-118.

En una vacuna de emulsión, la degradación de la emulsión produce la separación de fases de los componentes. Esto es indeseable ya que cuando hay separación de fases, las dosis individuales retiradas del recipiente pueden no contener el mismo nivel de los componentes de vacuna. Además, la pérdida de emulsión puede conducir a una pérdida de la actividad adyuvante del emulsionante y conducir a una disminución del efecto antigénico de la vacuna.

Los virus vivos atenuados se incluyen con frecuencia en vacunas junto con bacterinas. Tales vacunas son útiles porque se puede usar una sola vacuna para generar inmunidad para diferentes enfermedades con una vacuna. Si la actividad de lipasa está presente en la bacterina, causará la liberación del emulsionante de la emulsión. Este emulsionante libre puede alterar e inactivar los virus de la vacuna viva, conduciendo de este modo a una pérdida de

infectividad viral.

Una bacterina útil en vacunas se puede formar por cultivo de la bacteria de interés y después destrucción de las bacterias para producir una bacterina que contiene una diversidad de componentes bacterianos, incluyendo
5 componentes de la pared celular. Las bacterias pueden destruirse mediante una diversidad de procedimientos que incluyen exposición de las mismas a un compuesto tal como mertiolato, formalina, formaldehído, dietilamina, etilenamina binaria (BEI), beta propiolactona (BPL) y glutaraldehído. Se pueden usar combinaciones de estos compuestos. Además, es posible destruir las
10 bacterias con radiación esterilizante.

Ahora se ha descubierto que la actividad de lipasa de una bacterina que tiene tal actividad de lipasa se puede reducir por tratamiento térmico. En concreto, la actividad de lipasa de una bacterina se puede reducir calentado la bacterina hasta una temperatura de aproximadamente 35 a aproximadamente
15 80°C para formar una bacterina tratada térmicamente, que tiene una actividad antigénica aceptable. El tratamiento térmico se realiza durante un periodo de tiempo suficiente de tal forma que la actividad de lipasa de la bacterina tratada térmicamente es del 50% o menor que la observada en la bacterina antes del tratamiento térmico. Para una buena estabilidad de la vacuna de emulsión no
20 es necesario que la actividad de lipasa se reduzca a cero. Se han descubierto vacunas que tienen una buena vida útil que se pueden preparar a partir de bacterinas tratadas térmicamente que tienen un nivel de actividad de lipasa que es del 50% o menor que el nivel de actividad de lipasa antes del tratamiento térmico.

25 Cuando se ha usado una velocidad de hidrólisis de un sustrato de

ensayo como una medida de la actividad de lipasa de una bacterina, entonces la velocidad de hidrólisis del sustrato de ensayo antes del tratamiento térmico se compara con la velocidad de hidrólisis después del tratamiento térmico. El tratamiento térmico se realiza para reducir la velocidad de hidrólisis hasta el
5 50% o menos de la velocidad de hidrólisis que se observa para la bacterina fresca.

El procedimiento exacto para medir el nivel de actividad de lipasa no es crítico siempre que se use el mismo procedimiento para medir la actividad antes del tratamiento térmico y la actividad después del tratamiento térmico.
10 Por ejemplo, si la velocidad de hidrólisis de un sustrato de ensayo se mide usando un sustrato, un sustrato diferente puede producir una velocidad diferente. Sin embargo, si se usa el mismo sustrato para la determinación de la actividad inicial y la determinación de la actividad después del tratamiento, las velocidades relativas todavía mostrarán el efecto del tratamiento térmico.

15 Hay ensayos codificados para actividad antigénica para la bacterina de *Leptospira pomona* (9 CFR §113.101), bacterina de *Leptospira icterohaemorrhagiae* (9 CFR §113.102), bacterina de *Leptospira canicola* (9 CFR §113.103), bacterina de *Leptospira grippotyphosa* (9 CFR §113.104) y bacterina de *Leptospira hardjo* (9 CFR §113.105) (9 CFR §113.101, §113.102,
20 §113.103, §113.104 y §113.105). Para estas especies se puede definir una actividad antigénica aceptable como la capacidad de inducir una respuesta inmune protectora en hámsteres vacunados de tal forma que cuando se provocan los hámsteres con bacteria viva homóloga, al menos el 75% de los hámsteres vacunados sobreviven en un modelo mientras que al menos el 80%
25 de los hámsteres no vacunados no sobreviven. En el caso del antígeno,

Leptospira hardjo, se puede definir una actividad antigénica aceptable como la capacidad de una vacuna de inducir un título de media geométrica de aglutinación serológica contra *Leptospira hardjo* de ≥ 40 en terneros que se han vacunado con una vacuna que comprende el antígeno bacteriano, *Leptospira*
5 *hardjo*. Para otras bacterinas, la actividad antigénica aceptable se define como la capacidad de inducir una respuesta inmune protectora en animales vacunados después de provocarlos o pasar un ensayo de potencia con organismo vivo homólogo.

El tratamiento térmico se puede realizar a lo largo de un intervalo de
10 temperaturas y durante una extensión de tiempo variable. Generalmente, el calentamiento se puede realizar a una temperatura de aproximadamente 35 a aproximadamente 80°C durante aproximadamente 20 minutos a aproximadamente 24 horas. Cuando la bacterina se calienta a una temperatura mayor, tal como de aproximadamente 75 a aproximadamente 80°C, el tiempo
15 de calentamiento está en el extremo corto del intervalo de tiempo. Cuando el calentamiento se realiza a una temperatura inferior, el calentamiento se realiza durante un periodo de tiempo más prolongado. Otra combinación de temperatura y tiempo es el calentamiento a una temperatura de aproximadamente 60 a aproximadamente 70°C durante aproximadamente 9 a
20 aproximadamente 10 horas. Otra combinación de temperatura y tiempo es el calentamiento a una temperatura de aproximadamente 65 a aproximadamente 70°C durante aproximadamente 5 a aproximadamente 8 horas. Otra combinación de temperatura y tiempo es el calentamiento a una temperatura de aproximadamente 65 a aproximadamente 70°C durante aproximadamente
25 una hora. Otra combinación de temperatura y tiempo es el calentamiento a una

temperatura de aproximadamente 55 a aproximadamente 65°C durante aproximadamente 5 a aproximadamente 8 horas.

Las bacterinas, después del tratamiento térmico, tienen una actividad de lipasa menor que las bacterinas recién preparadas pero, por lo demás, se pueden formular de la misma manera que las bacterinas recién preparadas. En consecuencia, las bacterinas tratadas térmicamente se pueden incorporar en vacunas mediante procedimientos normales de producción de vacunas. Estos procedimientos se conocen bien en la técnica.

Las vacunas de emulsión se pueden formar combinando la bacteria deseada con una fase oleosa y un emulsionante o emulsionantes. Después, la combinación se somete a agitación intensa para formar una emulsión. Los procedimientos de agitación adecuados incluyen homogeneizado y, posteriormente, microfluidificación. También se pueden incluir conservantes y excipientes en la combinación antes de la formación de la emulsión.

Las vacunas pueden incluir tanto bacterinas como antígenos virales. Al preparar una vacuna que incluye bacterinas y antígenos virales, las bacterinas, cualquier antígeno viral que se vaya a incluir, el emulsionante o emulsionantes y, opcionalmente, conservantes y excipientes se combinan con una fase oleosa y se emulsionan. Después de la formación de la emulsión, el pH de las formulaciones se puede ajustar a un pH de apropiado usando soluciones de NaOH o HCl. Para el uso en vacunas, generalmente es deseable que el pH sea próximo al neutro para evitar la irritación en el sitio de inyección. Es común un pH de aproximadamente 7,0 a aproximadamente 7,3.

Las fases oleosas adecuadas para la formación de una vacuna de emulsión incluyen aceites no metabolizables y aceites metabolizables. Los

aceites no metabolizables incluyen aceites minerales, tales como aceite mineral blanco y aceite mineral ligero. Los aceites metabolizables incluyen aceites vegetales, aceites de pescado y glicéridos de ácidos grasos sintéticos.

Los ejemplos de emulsionantes que se pueden usar para preparar

5 vacunas de emulsión de esta invención son fosfolípidos, ésteres de sorbitán, ésteres de sorbitán polietoxilados y derivados de manitol que son emulsionantes de vacunas comunes. Los emulsionantes fosfolipídicos incluyen lecitina, fosfatidiletanolamina, fosfatidilinositol, fosfatidilserina y lecitina (por ejemplo, tal como AMPHIGEN®). Los emulsionantes de éster de sorbitán

10 incluyen monolaurato de sorbitán (por ejemplo, Span® 20 y Arlacel® 20), monooleato de sorbitán (por ejemplo, Span® 80 y Arlacel® 80), monopalmitato de sorbitán (por ejemplo, Span® 40 y Arlacel® 40) y monoestearato de sorbitán (por ejemplo, Span® 60 y Arlacel® 60). Los ésteres de sorbitán polietoxilados incluyen monolaurato de polietoxisorbitán (por ejemplo, Tween® 20 y Tween®

15 21), monooleato de polietoxisorbitán (por ejemplo, Tween® 80), monopalmitato de polietoxisorbitán (por ejemplo, Tween® 40) y monoestearato de polietoxisorbitán (por ejemplo Tween® 60). Los emulsionantes derivados de manitol incluyen éteres octadecanoicos de manitol. Span®, Arlacel® y Tween® son marcas comerciales de ICI Americas. AMPHIGEN® es una marca

20 comercial de Pfizer, Inc. Generalmente, las vacunas se formulan como emulsiones normales de aceite en agua, aunque es posible preparar emulsiones invertidas de agua en aceite.

Se pueden usar una diversidad de adyuvantes, tales como Quil A, colesterol, fosfato de aluminio e hidróxido de aluminio y conservantes tales

25 como mertiolato en vacunas. Quil A es una mezcla purificada de saponinas de

Quillaja extraídas de la corteza del árbol sudamericano *Quillaja saponaria* Molina. El Quil A actúa directamente sobre el sistema inmune para activar un estado generalizado de sensibilidad. Al hacerlo, induce respuestas tanto humorales como mediadas por células. La cadena lipófila permite la interacción
5 del antígeno y del adyuvante que se van a suministrar al citosol para el procesamiento en una ruta endógena. A menudo se usa Quil A con colesterol ya que el colesterol elimina los efectos secundarios menos deseables cuando se añade en las proporciones apropiadas. El colesterol forma complejos insolubles con Quil A que forman estructuras de tipo hélice cuando el colesterol
10 se une a Quil A, exponiendo de este modo las unidades de azúcar de la molécula que ayudan a estimular la respuesta inmune.

Es común añadir antígenos virales porcinos a vacunas que contienen bacterinas. Una ventaja de esta estrategia es que se puede usar una vacuna para generar inmunidad para varias enfermedades en vez de requerir
15 dosificaciones de varias vacunas diferentes para conseguir el mismo resultado. Se pueden usar tanto virus inactivados como virus vivos atenuados en vacunas. Entre los virus que causan enfermedades porcinas que se pueden usar están el adenovirus porcino, circovirus porcino, herpesvirus porcino, virus de la pseudorrabia, virus de la fiebre porcina clásica, virus de la diarrea
20 epidémica porcina, virus de encefalomiелitis hemaglutinante porcina, parvovirus porcino, coronavirus respiratorio porcino, virus del síndrome reproductor y respiratorio porcino, influenza porcina, virus de la gastroenteritis transmisible y virus de la estomatitis vesicular.

Si la actividad de lipasa está presente en la bacterina, puede causar la
25 liberación del emulsionante de la emulsión. Este emulsionante libre puede

alterar la envuelta del virus vivo e inactivar los virus de vacunas vivas, conduciendo de este modo a una pérdida de infectividad viral. En consecuencia, el tratamiento térmico de la bacterina sirve para estabilizar la emulsión y conservar su efecto adyuvante, así como para conservar la infectividad viral de los virus.

Los siguientes ejemplos se proporcionan con el propósito de una ilustración adicional y no tienen por objeto limitar el alcance de la invención que se reivindica.

10 PROCEDIMIENTOS

Procedimiento 1 Determinación de turbidez

La turbidez se determina en unidades nefelométricas (UN) mediante un procedimiento de dispersión de la luz. La intensidad de la luz dispersada por la muestra en condiciones definidas se compara con la intensidad de la luz dispersada por una suspensión de referencia patrón. Cuando mayor sea la intensidad de la luz dispersada, mayor es la turbidez de la muestra. Se dirige una fuente de luz hacia la muestra y se mide la dispersión de la luz en un ángulo de 90° a la dirección de la fuente de luz. El instrumento se calibra midiendo la dispersión de la luz de una suspensión de formazina.

20 Calibrado del instrumento nefelómetro

Se prepara agua ultrafiltrada filtrando agua destilada a través de un filtro de membrana que tiene un tamaño de poro de 0,2 µm. Se prepara una primera solución disolviendo 1,00 g de sulfato de hidrazina, $(\text{NH}_2)_2\text{H}_2\text{SO}_4$, en agua ultrafiltrada y se diluye con agua ultrafiltrada hasta 100 ml en un matraz volumétrico. Se prepara una segunda solución disolviendo 10,00 g de

hexametilenotetramina en agua ultrafiltrada y diluyendo con agua ultrafiltrada hasta 100 ml en un matraz volumétrico. Se prepara una suspensión de formazina mezclando 5,0 ml de la primera solución con 5,0 ml de la segunda solución. Se deja reposar la mezcla durante 24 horas a aproximadamente 24°C. La mezcla se diluye hasta 100 ml con agua ultrafiltrada para formar una suspensión de turbidez madre que tiene una turbidez de 400 UN. Se prepara una suspensión de formazina de una turbidez de 40 UN diluyendo 10,00 ml de la suspensión de turbidez madre con 100 ml de agua ultrafiltrada. Se preparan soluciones de calibrado adicionales diluyendo la solución madre.

10 Medición de turbidez

La muestra a medir se diluye con agua ultrafiltrada de tal forma que la turbidez entre dentro del intervalo calibrado del nefelómetro. La turbidez se mide y se calcula la turbidez original usando la siguiente ecuación:

$$\text{Turbidez original en UN} = \frac{M \times (D + O)}{O}$$

donde: M es la turbidez de la muestra diluida en UN

15 D es el volumen de agua de dilución, en ml

O es el volumen de muestra original, en ml

Procedimiento 2 Análisis de lipasa

La actividad de lipasa se determinó usando O-pivaloximetilumbeliferona como sustrato fluorogénico. La hidrólisis catalizada por lipasa de este sustrato no fluorescente produce un hidroximetiléter que es inestable en condiciones acuosas. La descomposición del hidroximetiléter inestable genera formaldehído y el producto fluorescente umbeliferona. El control de la intensidad de fluorescencia de la umbeliferona producida, en función del tiempo, proporciona

una medición cinética sensible de la actividad enzimática de lipasa.

Se prepararon soluciones de O-pivaloximetilumbeliferona (Molecular Probes producto nº P35901) en DMSO puro a una concentración de solución madre de 5 mM; se almacenó solución sin usar a -20°C, protegida de la luz. La solución de O-pivaloximetilumbeliferona 5 mM se diluyó hasta 750 µM usando
5 tampón TRIS-HCl 58 mM (pH 8,0) y la solución resultante se precalentó hasta 37°C. La muestra de bacterina o el tampón/medio de control se centrifugó durante 10 minutos a temperatura ambiente a 6500 X gravedad para formar un sedimento y un sobrenadante. Se realizaron las reacciones combinando 15 µl
10 de tampón TRIS-HCl 100 mM (pH 8,0) con 15 µl del sobrenadante a temperatura ambiente a partir de la muestra de bacterina o del tampón/medio de control, en pocillos de ensayo de placas de 96 pocillos de volumen reducido (Corning 3393, superficie de no unión de poliestireno negra, semiárea); preincubando durante 10 minutos a 37°C; iniciando después la reacción
15 mediante la adición de 20 µl de O-pivaloximetilumbeliferona 750 µM o el tampón/medio de control. Las mezclas de reacción resultantes contenían tampón TRIS-HCl 53 mM (pH 8,0) y O-pivaloximetilumbeliferona 0 ó 300 µM. Se midió la intensidad de fluorescencia a intervalos de 30-45 segundos a lo largo de un periodo de una hora (Spectramax Gemini XS, 37°C, λ_{ex} = 360 nm
20 λ_{em} = 460 nm, "medio" de ajuste de sensibilidad PMT, 6 lecturas por pocillo). La velocidad de reacción se determinó a partir de la pendiente de la curva de progresión resultante.

Procedimiento 3 Medición de la turbidez de una preparación de

Erysipelothrix rhusiopathiae

25 Se mide espectrofotométricamente la turbidez de una preparación de

Erysipelothrix rhusiopathiae a una longitud de onda de 600 nm. El resultado se describe en unidades ópticas (UO).

EJEMPLOS

Ejemplo 1 Disminución de la actividad de lipasa por tratamiento térmico

5 Se preparó una combinación de leptospiras destruidas con mertiolato que contenía las siguientes especies: *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, *Leptospira grippotyphosa*, *Leptospira hardjo* y *Leptospira pomona* a partir de bacterinas individuales. Se almacenaron seis muestras de las bacterinas combinadas durante una noche (aproximadamente 12 horas) a
10 4°C, 37°C, 45°C, 56°C, 65°C y 80°C. La muestra almacenada a 4°C sirvió como el control sin tratar. Las muestras almacenadas durante 12 horas a 37°C, 45°C, 56°C, 65°C y 80°C eran muestras tratadas térmicamente. Después del almacenamiento, la velocidad a la que se hidrolizaba un sustrato de ensayo en presencia de cada bacterina se midió de acuerdo con el procedimiento del
15 Procedimiento 2. La velocidad de hidrólisis para una muestra dividida por la velocidad de hidrólisis de la muestra almacenada a 4°C multiplicada por 100 es el porcentaje de la actividad de lipasa original de cada bacterina que se conserva después del almacenamiento. El siguiente cuadro muestra la temperatura de almacenamiento y el porcentaje de la actividad de lipasa
20 original que se conserva después del almacenamiento.

Temperatura de almacenamiento (12 horas)	4°C	37°C	45°C	56°C	65°C	80°C
Porcentaje de actividad de lipasa original	100%	55,4%	32,5%	15,7%	10,8%	8,4%

Se prepararon dos series de *Leptospira bratislava*. Las series se inactivaron con mertiolato y después se sometieron a tratamiento térmico a 65°C durante 8 horas. Las muestras se sacudieron antes del tratamiento y cada dos horas durante el tratamiento. Se determinó la actividad de lipasa en cada momento de acuerdo con el procedimiento del Procedimiento 2. Cuando se tomaba una muestra, se medía la velocidad a la que se hidrolizaba un sustrato de ensayo en presencia de cada muestra. La velocidad de hidrólisis para una muestra dividida por la velocidad de la velocidad de hidrólisis inicial multiplicada por 100 es el porcentaje de la actividad de lipasa original de cada bacterina que se conserva después del tratamiento térmico. El siguiente cuadro muestra el tiempo de muestra y el porcentaje promedio de la actividad de lipasa original que se conserva a ese tiempo.

Tiempo de muestra (horas)	Inicial	2	4	6	8
Porcentaje de actividad de lipasa original	100%	41%	34%	28%	24%

Ejemplo 2 Preparación de formulaciones de vacuna experimental

Se cultivaron cultivos de *Leptospira canicola*, *Leptospira icterogorrrhagiae*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira pomona*, *Leptospira bratislava*, *Erysipelothrix rhusiopathieae* y parvovirus porcino. Se midió la turbidez de cada cultivo de *Leptospira* en unidades nefelométricas (UN). Se midió la turbidez del cultivo de *Erysipelothrix rhusiopathieae* en unidades ópticas (UO). Las bacterias se destruyeron con mertiolato para formar bacterinas. Cada bacterina de *Leptospira* se trató térmicamente a 65°C durante 8 horas para reducir la actividad de lipasa. La bacterina de *Erysipelothrix rhusiopathieae* no se trató térmicamente. Las

bacterinas de *Leptospira* se combinaron con parvovirus porcino inactivado y *Erysipelothrix rhusiopathieae* destruida y después se mezclaron con AMPHIGEN®, adyuvantes, conservantes y tampón de dilución de tal forma que cada 2 ml de dosis de la vacuna contenían los componentes indicados en el

5 cuadro siguiente.

Concentraciones de antígenos

Componente	Concentración de componente/dosis
<i>L. canicola</i>	1200 UN/dosis de 2 ml
<i>L. icterohaemorrhagiae</i>	1200 UN/dosis de 2 ml
<i>L. grippotyphosa</i>	1200 UN/dosis de 2 ml
<i>L. hardjo</i>	2400 UN/dosis de 2 ml
<i>L. pomona</i>	1200 UN/dosis de 2 ml
<i>L. bratislava</i>	1200 UN/dosis de 2 ml
<i>Erysipelothrix rhusiopathieae</i>	14 UO/dosis de 2 ml
Parvovirus porcino	17.920 HA/0,05 ml

Ejemplo 3 Ensayo de potencia en hámsteres y cerdos

La vacuna del Ejemplo 2 se administró a hámsteres y conejos para
10 ensayar para potencia usando modelos animales de laboratorio
convencionales. Los hámsteres de ensayo se provocaron después con una
dosis de *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, *Leptospira*
grippotyphosa, *Leptospira bratislava* o *Leptospira pomona* para ensayar la
potencia de las vacunas. Se midió el número de supervivientes como una
15 demostración de eficacia. Se midieron los títulos de aglutinación microscópica

de conejo frente a *Leptospira hardjo* para demostrar la potencia de esa fracción de la vacuna. La siguiente tabla muestra que las vacunas preparadas a partir de bacterinas de *Leptospira* tratadas térmicamente son capaces de producir una respuesta antigénica que pasa los criterios de eficacia.

Acondicionamiento térmico de <i>Leptospira</i>	HÁMSTERES SUPERVIVIENTES					SEROLOGÍA de conejo
	canicola	bratislava	ictero	grippe	pomona	hardjo
65°C (8 horas)	10/10	10/10	10/10	10/10	10/10	Pasa
Sin tratar	10/10	10/10	10/10	10/10	10/10	Pasa

5

Se ensayó *Erysipelothrix rhusiopathiae* en conejos comparando el título serológico de la vacuna con el título de una vacuna de referencia. La vacuna tenía una RP (potencia relativa) de 3,0. Se ensayó PPV en el ensayo de hemaglutinación y tenía un título de HA de 1024 HA/0,05 ml. Un título de 320

10 HA/0,05 ml es un valor aceptable para una vacuna.

Ejemplo 4 Ensayo fisicoquímico de vacunas

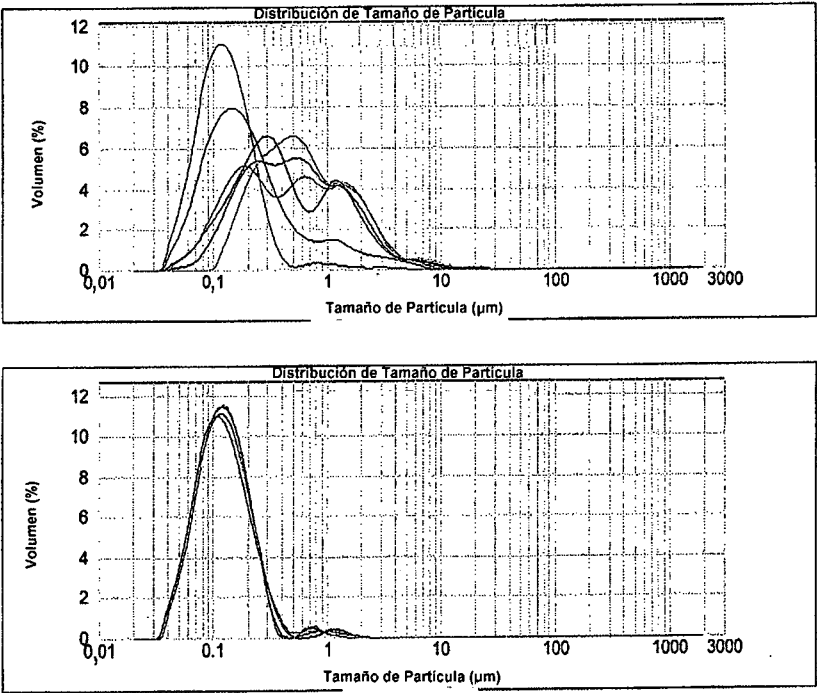
Se preparó una vacuna con bacterinas de *Leptospira* tratadas térmicamente y otros componentes de acuerdo con la formulación enumerada en el Ejemplo 2. Se preparó una vacuna similar a partir de bacterinas de

15 *Leptospira* sin tratar térmicamente de acuerdo con el procedimiento del Ejemplo 2. Ambas formulaciones de vacuna se almacenaron a 4°C durante 0, 6, 12, 15 y 18 meses de permanencia. Se realizó el análisis de tamaño de partícula para cada vacuna en cada momento usando un difractómetro láser.

Los gráficos mostrados a continuación muestran las distribuciones de

20 tamaño de partícula para cada vacuna a lo largo de varios meses de control (0, 6, 12, 15 y 18 meses).

Análisis del tamaño de partícula de vacunas recién preparadas que contienen bacterinas de *Leptospira* tratadas térmicamente a día 0



- 5 La vacuna preparada a partir de bacterinas de *Leptospira* sin tratar térmicamente (gráfico superior) muestra un aumento en el tamaño de partícula indicando degradación de la emulsión. La vacuna preparada a partir de bacterinas de *Leptospira* tratadas térmicamente (gráfico inferior) muestra la conservación del tamaño de partícula a lo largo de 18 meses de almacenaje,
- 10 indicando la estabilidad de la emulsión.

Ejemplo 5 Ensayo de hemaglutinación (HA) de PPV

Las formulaciones de vacuna enumeradas en el Ejemplo 2 (las vacunas en la lista en el Ejemplo 2 contenían todas los antígenos enumerados — mismas vacunas para todo el trabajo) preparadas a partir de bacterinas de

15 *Leptospira* sin tratar térmicamente y bacterinas de *Leptospira* tratadas

térmicamente se ensayaron inicialmente para el título de HA y el título de hemólisis. La estabilidad de los títulos de HA en diversos momentos. El ensayo de HA se realizó ajustando una muestra a un pH de 11-11,2 para extraer el virus PPV (parvovirus porcino) del gel de hidróxido de aluminio. Después se

5 centrifugó la muestra y se recogió el sobrenadante para usar en el ensayo. Se añadieron glóbulos rojos de cobaya a una placa de 96 pocillos que sirve como el indicador de aglutinación. El sobrenadante de la muestra se diluyó 2 veces a lo largo de filas por duplicado con una dilución de partida de 1:5. La placa se incubó a $5 \pm 3^{\circ}\text{C}$ durante 16-24 horas. El grado de hemaglutinación se puntúa

10 de 0-4 para cada pocillo. El título se registra como la última dilución que contiene una puntuación de 2 o superior. Durante el ensayo de vacunas que no estaban tratadas térmicamente, se observó que la vacuna estaba causando hemólisis. El título de la hemólisis era la mayor dilución a la que se observó hemólisis. Las vacunas tratadas térmicamente no produjeron hemólisis.

15 El siguiente cuadro muestra los títulos de HA y los títulos de hemólisis promedio a lo largo del tiempo. La CTC es una vacuna con bacterinas de *Leptospira* tratadas térmicamente. La OOP es una vacuna sin bacterinas de *Leptospira* tratadas térmicamente.

Cuadro 1: Títulos de HA de PPV a lo largo del tiempo

				Seguimiento de HA de PPV de OOP y CTC a lo largo del tiempo						
				1	2	3	4	5	7	10
INICIAL				mes	meses	meses	meses	meses	meses	meses
	1	2	3							
OOP	160	1280	1280	1280	1280	1280	640	1280	1280	1280

Habiendo descrito la invención como antecede, se declara como propiedad lo contenido en las siguientes

REIVINDICACIONES

- 5 1. Una vacuna que comprende una emulsión, una bacterina tratada
térmicamente que comprende una suspensión de bacterias muertas, en la que
las bacterias muertas son de las especies *Leptospira Bratislava*, y de 1 a 13
virus causantes de enfermedad porcina seleccionados del grupo constituido por
adenovirus porcino, circovirus porcino, herpesvirus porcino, virus de la
10 pseudorrabia, virus de la fiebre porcina clásica, virus de la diarrea epidémica
porcina, virus de encefalomiелitis hemaglutinante porcina, parvovirus porcino,
coronavirus respiratorio porcino, virus del síndrome reproductor y respiratorio
porcino, influenza porcina, virus de la gastroenteritis transmisible y virus de la
estomatitis vesicular.
- 15 2. Una vacuna de acuerdo con la reivindicación 1, que comprende
adicionalmente una preparación de lecitina y un adyuvante a base de alumbre.
3. Una vacuna de acuerdo con la reivindicación 2, que comprende
20 adicionalmente una lecitina en una preparación de aceite.
4. Una vacuna de acuerdo con la reivindicación 1, que comprende
adicionalmente bacterinas tratadas térmicamente que comprenden una
suspensión de bacterias muertas, en la que las bacterias muertas son de una a
25 seis especies de bacterias seleccionadas del grupo constituido por *Leptospira*

canicola, Leptospira icterohaemorrhagiae, Leptospira grippotyphosa, Leptospira hardjo, Leptospira pomona y Erysipelothrix rhusiopathiae.

5. Una vacuna de acuerdo con la reivindicación 4, que comprende
5 adicionalmente una preparación de lecitina y un adyuvante a base de alumbre.

6. Una vacuna de acuerdo con la reivindicación 5, que comprende
adicionalmente una lecitina en una preparación de aceite.

PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

PCT

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PFIZER INC.

MAY 12 2009

PATENT DEPARTMENT
LEGAL DIVISION - NYC

NOTIFICATION OF TRANSMITTAL OF
 THE INTERNATIONAL SEARCH REPORT AND
 THE WRITTEN OPINION OF THE INTERNATIONAL
 SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference PC33709A	Date of mailing (day/month/year) 07/05/2009
International application No. PCT/IB2008/003369	International filing date (day/month/year) 04/12/2008
Applicant PFIZER INC.	

DOCKETED

Due Date 7/7/09

OFFICE NEW YORK

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 338.82.70

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority	Authorized officer
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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Volume I/A, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

If a demand for international preliminary examination is made, the written opinion of the International Searching Authority will, except in certain cases where the International Preliminary Examining Authority did not act as International Searching Authority and where it has notified the International Bureau under Rule 66.1 b/s(b), be considered to be a written opinion of the International Preliminary Examining Authority. If a demand is made, the applicant may submit to the International Preliminary Examining Authority a reply to the written opinion together, where appropriate, with amendments before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later (Rule 43b/s.1(c)).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PC33709A	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, Item 5 below.	
International application No. PCT/IB2008/003369	International filing date (day/month/year) 04/12/2008	(Earliest) Priority Date (day/month/year) 21/12/2007
Applicant PFIZER INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☐ This international search report has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☐ Certain claims were found unsearchable (See Box No. II)

3. ☐ Unity of invention is lacking (see Box No. III)

4. With regard to the title,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the drawings,

a. the figure of the drawings to be published with the abstract is Figure No. _____

- ☐ as suggested by the applicant
☐ as selected by this Authority, because the applicant failed to suggest a figure
☐ as selected by this Authority, because this figure better characterizes the invention

INTERNATIONAL SEARCH REPORT

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International application No
PCT/IB2008/003369

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANONYMUS: "Pfizer Material Safety Data Sheet for Product "FarrowSure B""[Online] 10 May 2004 (2004-05-10), pages 1-7, XP002524425 Retrieved from the Internet: URL: http://www.pfizerah.com/PAHimages/msds_us/FW.pdf [retrieved on 2009-04-20] page 1	1-6
Y	US 5 084 269 A (KULLENBERG FRED W [US]) 28 January 1992 (1992-01-28) the whole document	1-6
Y	US 2006/233830 A1 (WONG TUEN-YEE [AU] ET AL) 19 October 2006 (2006-10-19) paragraphs [0119], [0124], [0144] ----- -/--	1-6

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

21 April 2009

Date of mailing of the international search report

07/05/2009

Name and mailing address of the ISA/

Authorized officer

INTERNATIONAL SEARCH REPORT

54

International application No

PCT/IB2008/003369

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZEIGLER J A ET AL: "Immunization against leptospirosis: vaccine trials with heat-killed whole cell and outer envelope antigens in hamsters" BULLETIN OF THE PAN AMERICAN HEALTH ORGANIZATION, PAN AMERICAN SANITARY BUREAU, WASHINGTON, US, vol. 10, no. 2, 1 January 1976 (1976-01-01), pages 126-130, XP008089205 ISSN: 0085-4638 the whole document	1-6
A	----- WO 2006/038115 A (PFIZER PROD INC [US]; FRANTZ JOSEPH [US]; NEWBY THOMAS JACK [US]; TUCK) 13 April 2006 (2006-04-13) abstract; claim 16	1-6
A	----- BURNS K F ET AL: "Evaluation of the effect of heating an oil-emulsion Pasteurella multocida bacterin on tissue reaction and immunity" AVIAN DISEASES, AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS, KENNET SQ., PA, US, vol. 47, no. 1, 1 January 2003 (2003-01-01), pages 54-58, XP008089224 ISSN: 0005-2086 the whole document	
A	----- MILLER D A ET AL: "Porcine leptospirosis in Iowa." JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION : OFFICIAL PUBLICATION OF THE AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS, INC JUL 1990, vol. 2, no. 3, July 1990 (1990-07), pages 171-175, XP002523326 ISSN: 1040-6387 abstract	
A	----- SRIVASTAVA S K: "Prospects of developing leptospiral vaccines for animals." INDIAN JOURNAL OF MEDICAL MICROBIOLOGY OCT 2006, vol. 24, no. 4, October 2006 (2006-10), pages 331-336, XP002523327 ISSN: 0255-0857 the whole document	

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2008/003369

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	MARTINS SOTO F R ET AL: "Evaluation of colostrum immunity in swine with commercial anti-leptospira polyvalent whole-bacteria vaccine" COMPARATIVE IMMUNOLOGY, MICROBIOLOGY AND INFECTIOUS DISEASES, PERGAMON PRESS, OXFORD, GB, vol. 31, no. 4, 1 July 2008 (2008-07-01), pages 327-335, XP022688791 ISSN: 0147-9571 [retrieved on 2008-05-27] -----	
P,X	WO 2008/106393 A (WYETH CORP [US]; GILL MICHAEL A [US]) 4 September 2008 (2008-09-04) the whole document -----	1-6
P,X	WO 2008/032158 A (PFIZER PROD INC [US]; GOODYEAR MARK DAVIS [US]; HUETHER MICHAEL JOHN []) 20 March 2008 (2008-03-20) the whole document -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2008/003369

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5084269	A	28-01-1992	NONE	
US 2006233830	A1	19-10-2006	WO 2004026336 A1 CA 2497878 A1 CN 1691962 A EP 1542717 A1 JP 2006503830 T KR 20050084575 A RU 2339400 C2	01-04-2004 01-04-2004 02-11-2005 22-06-2005 02-02-2006 26-08-2005 27-11-2008
WO 2006038115	A	13-04-2006	AU 2005290921 A1 BR PI0516253 A CA 2583689 A1 CN 101035558 A EP 1799253 A1 JP 4163245 B2 JP 2008515873 T JP 2008239627 A KR 20070050499 A KR 20080096610 A	13-04-2006 26-08-2008 13-04-2006 12-09-2007 27-06-2007 08-10-2008 15-05-2008 09-10-2008 15-05-2007 30-10-2008
WO 2008106393	A	04-09-2008	NONE	
WO 2008032158	A	20-03-2008	AR 062758 A1 AU 2007297287 A1 CL 26212007 A1 US 2008112970 A1	03-12-2008 20-03-2008 14-03-2008 15-05-2008

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/IB2008/003369

International filing date (day/month/year)
04.12.2008

Priority date (day/month/year)
21.12.2007

International Patent Classification (IPC) or both national classification and IPC
INV. A61K39/02

Applicant
PFIZER INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office

Date of completion of
this opinion

Authorized Officer



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**International application No.
PCT/B2008/003369

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ on paper
 - ☐ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in electronic form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/IB2008/003369

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>1-6</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	
	No: Claims	<u>1-6</u>
Industrial applicability (IA)	Yes: Claims	<u>1-6</u>
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)

and /or

2. Non-written disclosures (Rules 43bis.1 and 70.9)

see form 210

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/IB2008/003369

Ad Section V: Reasoned statement with regard to novelty, inventive step or industrial applicability

1) Documents

D1...Pfizer Material Safety Data Sheet of FarrowSure^{RB}

D2...US 5 084 269

2) Novelty and Inventive step

The present application relates to a vaccine comprising an emulsion, a heat treated bacterin, wherein the killed bacteria are of the species *Leptospira Bratislava*, and 1 to 13 porcine disease causing viruses. The application discloses that heat treatment of the killed bacteria reduced the lipase activity in a mixture of merthiolate killed leptospira and that such formulation had an increased stability.

2.1) As the prior art does not disclose a vaccine as defined in claim 1 novelty can be acknowledged for the current set of claims.

2.2) The claims, however, are not considered to involve an inventive step for the following reasons:

D1 which discloses a porcine combination vaccine comprising several *Leptospira* strains and porcine parvovirus is considered to represent the closest prior art.

The difference between D1 and the present claims appears to lie in the formulation of the vaccine.

The problem to be solved by the claim can thus be seen in the provision of a vaccine with improved antigenicity.

D2 discloses that the therapeutic effectiveness of antigens is improved by adjuvants comprising a mixture of lecithin and edible or non-edible oils.

The provision of a vaccine as defined in claims 1-6 is therefore considered obvious for the skilled person.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/IB2008/003369

Consequently, **claims 1-6** do not meet the requirements of Art. 33(3) PCT.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2008/003369

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANONYMUS: "Pfizer Material Safety Data Sheet for Product "FarrowSure B""[Online] 10 May 2004 (2004-05-10), pages 1-7, XP002524425 Retrieved from the Internet: URL: http://www.pfizerah.com/PAHimages/msds_us/FW.pdf [retrieved on 2009-04-20] page 1	1-6
Y	US 5 084 269 A (KULLENBERG FRED W [US]) 28 January 1992 (1992-01-28) the whole document	1-6
Y	US 2006/233830 A1 (WONG TUEN-YEE [AU] ET AL) 19 October 2006 (2006-10-19) paragraphs [0119], [0124], [0144]	1-6
	----- -/--	

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

21 April 2009

Date of mailing of the international search report

07/05/2009

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Kalsner, Inge

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2008/003369

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZEIGLER J A ET AL: "Immunization against leptospirosis: vaccine trials with heat-killed whole cell and outer envelope antigens in hamsters" BULLETIN OF THE PAN AMERICAN HEALTH ORGANIZATION, PAN AMERICAN SANITARY BUREAU, WASHINGTON, US, vol. 10, no. 2, 1 January 1976 (1976-01-01), pages 126-130, XP008089205 ISSN: 0085-4638 the whole document	1-6
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A	BURNS K F ET AL: "Evaluation of the effect of heating an oil-emulsion Pasteurella multocida bacterin on tissue reaction and immunity" AVIAN DISEASES, AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS, KENNET SQ., PA, US, vol. 47, no. 1, 1 January 2003 (2003-01-01), pages 54-58, XP008089224 ISSN: 0005-2086 the whole document	
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2008/003369

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	MARTINS SOTO F R ET AL: "Evaluation of colostral immunity in swine with commercial anti-leptospira polyvalent whole-bacteria vaccine" COMPARATIVE IMMUNOLOGY, MICROBIOLOGY AND INFECTIOUS DISEASES, PERGAMON PRESS, OXFORD, GB, vol. 31, no. 4, 1 July 2008 (2008-07-01), pages 327-335, XP022688791 ISSN: 0147-9571 [retrieved on 2008-05-27]. -----	
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P,X	WO 2008/032158 A (PFIZER PROD INC [US]; GOODYEAR MARK DAVIS [US]; HUETHER MICHAEL JOHN [US]) 20 March 2008 (2008-03-20) the whole document -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2008/003369

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-074461- -00000-0000

Fecha: 2010-06-21 17:01:51 Dep. 2020 DIR.NUEVASCR
Tra 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 65

PATENTE DE INVENCION ☐

MODELO DE

☐	Indicación que se solicita una patente.
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Descripción de la invención
☐	Dibujos de ser estos pertinentes
☐	Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)
Completa ☐ Incompleta ☐	

PATENTE DE INVENCION PCT ☒

MODELO DE UTILIDAD PCT ☐

Art.33 Decisión 486/00, Circular Única

☒	Indicación que se solicita una PCT
☒	Copia de la solicitud en español, tal como fue presentada inicialmente (capítulo descriptivo, reivindicatorio, resumen)
☐	Dibujos de ser estos pertinentes
☒	Comprobante de pago de las tasas establecidas (de ser el caso formato de descuento)
Completa ☐ Incompleta ☐	

DISEÑO INDUSTRIAL ☐

(Art. 119 Decisión 486/00)

☐	Indicación que se solicita Diseño industrial
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Representación gráfica y fotográfica del Diseño industrial o muestra del material que incorpora el diseño
☐	Comprobante de pago de las tasas establecidas
Completa ☐ Incompleta ☐	

ESQUEMA DE TRAZADO ☐

(Art. 92 Decisión 486/00)

☐	Indicación que se solicita un esquema de trazado
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Representación gráfica de un esquema de trazado
☐	Comprobante de pago de las tasas establecidas
Completa ☐ Incompleta ☐	



Bogotá D.C.,
2020



Industria y Comercio
SUPERINTENDENCIA

Doctor(a)
OLARTE GARCIA CARLOS REYNALDO
Apoderado(a) y/o Representante de
PFIZER INC.

REFERENCIA	Radicación No.	10 074461
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	<u>09452</u>

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. El solicitante debe presentar un nuevo título que permita identificar la materia de la cual trata la solicitud, ya que el título relacionado no se encuentra con la precisión necesaria para identificar claramente el objeto de la solicitud de patente. (Regla 4.3 PCT, Art 26-27 D-486, Circular única 5.2.9)

2. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante, conforme a lo establecido por el artículo 26 literal k) de la Decisión 486 de la Comisión de la Comunidad Andina. Cuando se trate de documentos otorgados en el extranjero, estos deberán legalizarse de conformidad con lo dispuesto por los artículos 259 y 260 del Código de Procedimiento Civil. Cuando se trate de las declaraciones a que hace referencia la Regla 4.17 ii) del Tratado de Cooperación en Materia de Patentes PCT., el alcance de las tales declaraciones se determinará según indiquen claramente una cesión de derechos del inventor al solicitante o su causante, de conformidad con lo dispuesto en el numeral 5.2.15 del Capítulo Quinto, Título X de la Circular Única de Superintendencia de Industria y Comercio.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Continúa en la página siguiente...

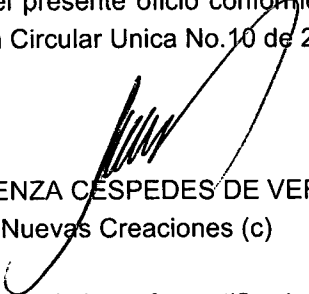


Continuación radicado No: 10 074461



Industria y Comercio
SUPERINTENDENCIA

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Directora de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista de fecha 30 JUL 2018
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