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**SEÑOR
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISIÓN DE SIGNOS DISTINTIVOS**

E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 06-099780- -00005-0000

Fecha: 2008-06-09 16:08:31 Dep. 2020 NUECREACIONE
Tra. 2 PATENTES Eve: 1 REGDEPOSITO
Act. 518 NUEVAPUBLICAC Folios: 4

**Ref.: Expediente No.: 06-099.780 Patente de Invención: LINEA CELULAR VERO
ADAPTADA PARA CRECER EN SUSPENSIÓN.**

JAIME EDUARDO DELGADO VILLEGAS, Abogado, mayor y vecino de Bogotá, actuando en mi calidad de Apoderado de la sociedad ZELLTEK S.A., domiciliada en Santa Fe - Argentina, se dirige a su Digno Despacho para manifestarle y solicitarle lo siguiente:

1. Tal como lo puede apreciar su Despacho, en la página 458 de la Gaceta 591 del 30 de Abril de 2008, La patente de Invención de la referencia fue publicada.
2. Pese a lo anterior, observando la publicación, encontramos que existe una inconsistencia que determina que la misma no sea eficaz, como es la siguiente:
 - a) El título de la Patente de Invención es LINEA CELULAR VERO ADAPTADA PARA CRECER EN SUSPENSIÓN y no El título de la Patente de Invención es LINE CELULAR VERO ADAPTADA PARA CRECER EN SUSPENSIÓN, como figura en la publicación y en la Base de Datos de la Superintendencia de Industria y Comercio.

Teniendo en cuenta lo expuesto, solicito a su Digno Despacho volver a publicar dicha Patente de Invención en donde conste el verdadero nombre de la Patente de Invención.

Atentamente,


JAIME EDUARDO DELGADO VILLEGAS
C.C. No. 19'270.955 de Bogotá
T.P. No. 43.308 C.S.J.

PETITORIO

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
 Rad: 06-099760-00000-0000
 Feo: 2006-10-03 16:58:10 Dep. 2020 NUECREACIONES
 Tia. 2 PATENTES
 Eve: 1 REGDEPOSITO
 Act 411 PRESENTACION Folio: 57

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE

1 SOLICITUD DE: ☒ Patente de Invención ☐ Patente de Modelo de Utilidad			
2 SOLICITANTE (71)	Nombre: ZELLTEK S.A. Dirección: Lizandro de la Torre 3124 - Dpto 2, 3000 Santa Fe SANTA FE, ARGENTINA Nacionalidad o domicilio: ARGENTINA Lugar de Constitución:		IDENTIFICACIÓN C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual:
	Teléfono: Fax: E-mail:		Número:
3 REPRESENTANTE O APODERADO	Nombre: JAIME EDUARDO DELGADO VILLEGAS Dirección: Avenida 22 No. 37 - 31 OF. 202 Teléfono: 2444096 Fax: 2442496 E-mail:		IDENTIFICACIÓN C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cuál:
			Número: 19.270.955 TP 43.308 C.S.J. Bogotá
4 INVENTOR (ES) (72)	Nombre: MARCELO GUSTAVO DAELLI Dirección: Lizandro de la Torre 3124 - Dpto 2, 3000 Santa Fe, SANTA FE, ARGENTINA Teléfono: Fax: Nacionalidad o Domicilio: ARGENTINO		C.C.:
5 Título (54) LINEA CELULAR VERO ADAPTADA PARA CRECER EN SUSPENSIÓN			
6 Clasificación internacional (51)			
7 Prioridad SI ☒ NO ☐	(33) País de Origen US	(32) Fecha 04-10-2005	(31) Número de Solicitud 60/723377
8 Para publicar a partir de la fecha de la presente solicitud a los: 6 meses ☐ 12 meses ☐ 18 meses ☐ Otro ☐			
9 Comprobante de pago No. Fecha:			

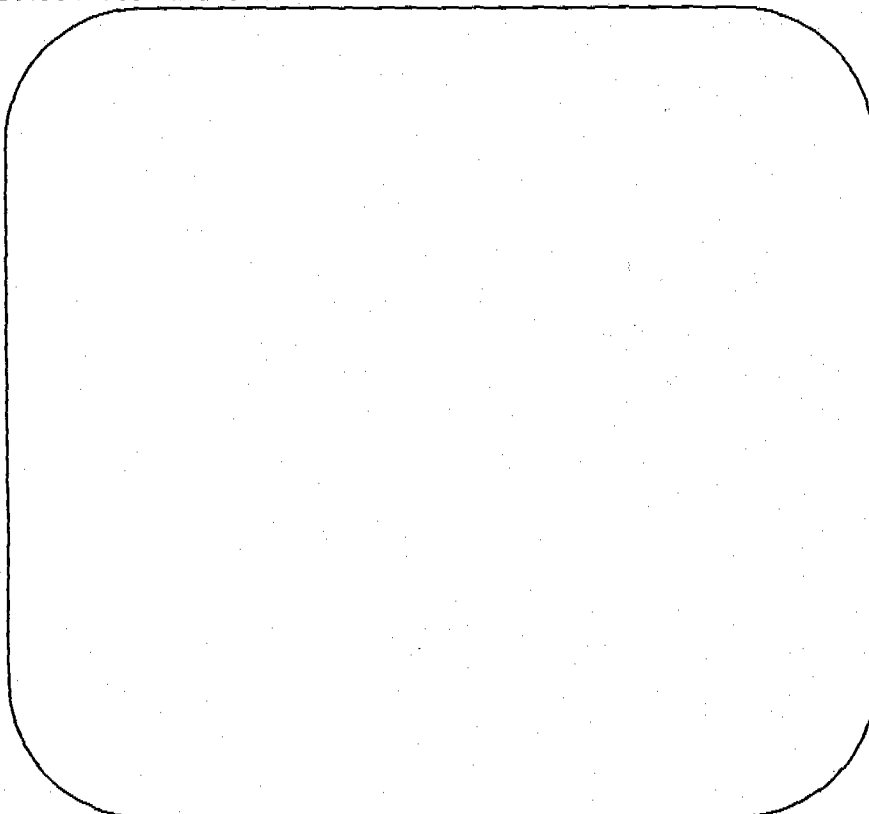
Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

10

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☐ Comprobante de pago de la tasa por concepto de excedente de palabras en la publicación.
- ☒ Comprobante de pago por reivindicación de prioridad.
- ☐ Certificado de existencia y representación legal cuando el solicitante sea persona jurídica.
- ☐ Poderes, si fuere el caso.
- ☐ Certificado de la fecha de presentación de la solicitud prioridad, expedida por la autoridad correspondiente y una copia certificada de la primera solicitud, si se reivindica prioridad.
- ☒ Traducción simple de la primera solicitud, si se reivindica prioridad.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Resumen
- ☒ Descripción de la invención.
- ☒ Una o más reivindicaciones.
- ☐ Dibujos y/o planos necesarios
- ☐ 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 12X12 y 6X6 cm.
- ☐ De ser el caso, información de otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas total o parcialmente con la invención de esta solicitud.

1 FIGURA CARACTERISTICA



12 Solicito la concesión de la patente,

NOMBRE: JAIME EDUARDO DELGADO VILLEGAS

FIRMA:

C.C. No. 19.270.955 Bogotá

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22
119

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
CONSULTA DE NUEVAS CREACIONES

Viernes 6 de Junio de 2008

Ver Afectaciones	Ver Actuaciones	Consulta Actos Administrativos	Cerrar Ventana
Datos de PATENTES DE INVENCION			
Expediente	6 99780	Fecha de solicitud	de 2006-10-03 16:58:10
Sector	BIOTECNOLOGIA	Clasificación	C12N 15/0 AC; lpc
lupac	-Nombre lupac	Linea celular VERO que crece en suspensión, sin necesidad de soporte no suero ni proteína.	
	-Nombre Comun	Linea Celualr VERO	
	-Indicación	producción de vacunas virales	
Título	LINE CELULAR VERO ADAPTADA PARA CRECER EN SUSPENSION		
Solicitante (s)	ZELLTEK S.A.		
Inventor(es)	MARCELO GUSTAVO DAELLI		
Gaceta	Numero	Fecha	NP
	591	30/04/2008	42
Estado	PUBLICACION 2008-04-30 00:00:00		
Resumen	Una línea celular VERO caracterizada por estar adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino y proteínas, y con la producción de vacunas virales usando dicha línea celular. Ésta relacionada con el establecimiento de una línea celular que puede crecer en un cultivo en suspensión sin la necesidad de que dichas células se adhieran a un material de soporte. Además la presente invención provee un proceso para obtener dicha línea celular Vero y un proceso para producir vacunas virales con dicha línea celular. También se refiere a los virus obtenidos por dicho proceso y las vacunas formuladas por dichos virus.		
REGISTRO INICIAL			
Expediente	6 99780	Secu even.0	Fecha de solicitud de 2006-10-03 16:58:10
Solicitante(s)	ZELLTEK S.A.		
Apoderado	JAIME EDUARDO DELGADO VILLEGAS		
Inventor(es)	MARCELO GUSTAVO DAELLI		
Actos Administrativos	OFICIO 14401 de 13/12/2006		
Estado	PUBLICACION 2008-04-30 00:00:00		
Ver Afectaciones	Ver Actuaciones	Consulta Actos Administrativos	Cerrar Ventana

REPÚBLICA DE COLOMBIA



Industria y Comercio

SUPERINTENDENCIA

PUBLICACIÓN DE RECTIFICACIÓN

EXPEDIENTE No. 06 099.780

De acuerdo a la solicitud de fecha 09/06/2008

Se publicó en la sección de rectificaciones gaceta No. 606
de fecha 31/07/2009

DIRECCIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 6-99780

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **591** DE

FECHA **30 DE ABRIL DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **30 DE JULIO DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO X

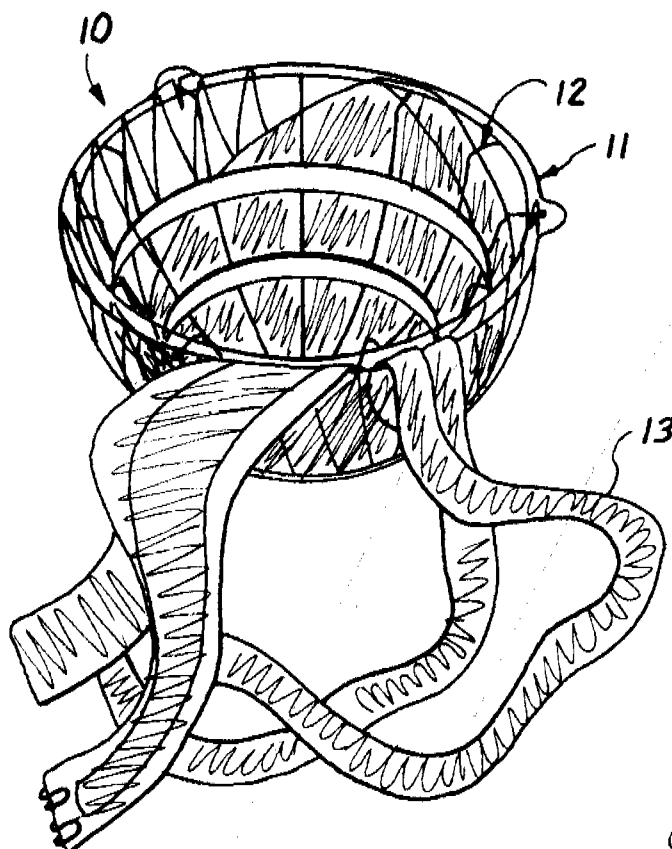


Fig. 1

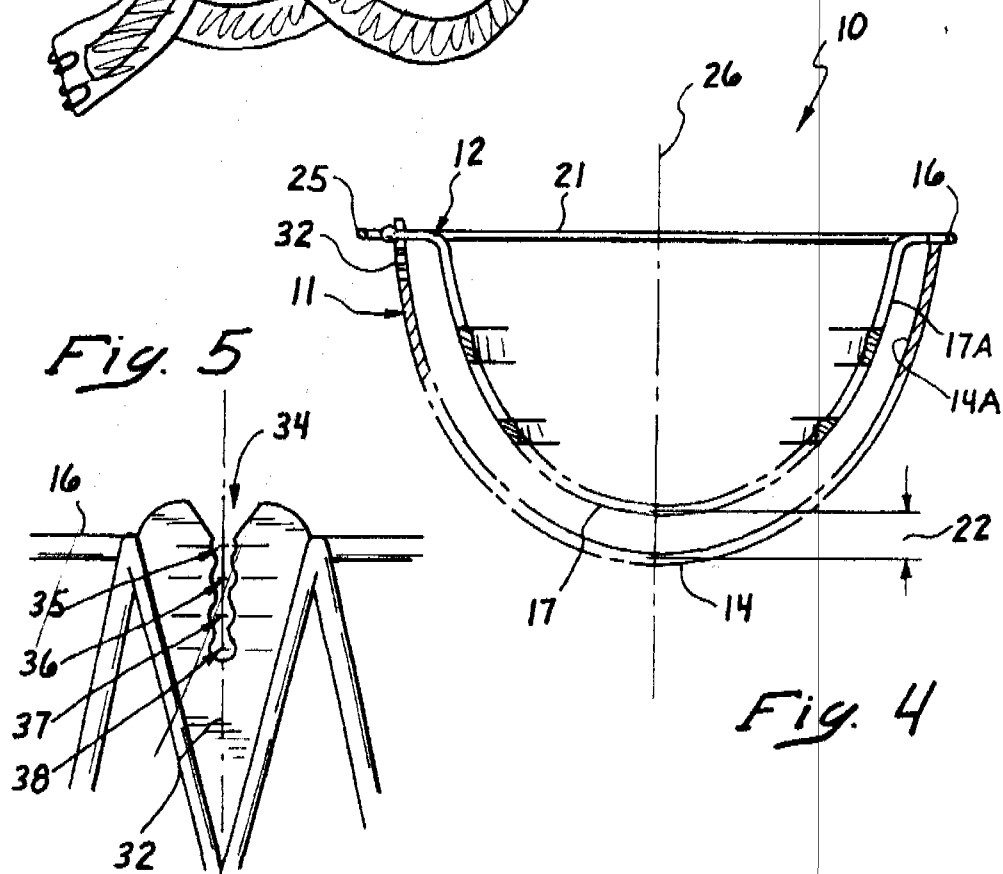


Fig. 5

Fig. 4

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174

3.4. Vaccine batch preparation	
3.5. Immunogenicity	
3.6. Stability	
4. Discussion	
Acknowledgement	
References	

1. Introduction

Rabies is a worldwide fatal disease and represents a severe public health problem in developing countries. Domestic animals, mainly dogs and cats, or wild animals (bats, monkeys and foxes) are important virus reservoirs and transmit this disease to human and animals of great economical interest, such as cattle, horses and others [1].

The World Health Organization notified that in 1988, in the whole world 32,451 human deaths due to rabies occurred and 7,576,323 people received post-exposure treatment [2]. In Brazil, from 1988 to 1997 there were 452 reported human rabies deaths, with a yearly mean of 342,830 people exposed to rabies suspect animals. The dog was the main transmitter of the disease (72.1%), followed by the bat (11.1%), cat (4.6%), fox (2%), monkey (1.5%), other animals (1.6%) and in 7.1% the transmitting species was not identified [3]. For post-exposure treatment and immunization of the professionals at risk (pre-exposure scheme) in this country approximately 2,500,000 vaccine doses are used per year. Fuenzalida & Palacios type [4] and cell culture vaccines.

The Butantan Institute started the production of human rabies vaccine in 1957 with the Semple-type [5]. The production of the vaccine in suckling mouse brain (Fuenzalida & Palacios type) was introduced in 1964 and the process was modified in 1987, with the introduction of a filtration to reduce the brain tissue present in the product [6]. This vaccine, although being immunogenic, may cause severe adverse neurological reactions, due to antibodies produced against myelin or other substances of the animal nervous tissue present in the product [7].

Since the appearance of the first rabies vaccine with Pasteur in 1885, many others were developed using different substrates for virus cultivation (animal brains; avian embryos; primary mammalian cells, human diploid cell or Vero cells), the antigen type and the route of administration [8], [9], [10], [11], [12], [13], [14], [15], [16], [17] and [18].

Among the vaccines for human use produced in cell cultures, recognized by the WHO, there is the Purified Vero Rabies Vaccine (Verorab) produced in Vero cells infected with rabies virus. These cells were established in Japan from the kidney of a normal adult African green monkey (*Cercopithecus aethiops*) [19]. A cell bank was established for the production of human vaccines in 1979 [10] and [20]. Extensive research was carried out to evaluate the oncogenic potential of the continuous lines. Vero cells showed to be free of oncogenic properties, not presenting risks to man when used as substrate in the production of vaccines [21], [22], [23] and [24]. The use of these cells allows also the large-scale production of vaccines using microcarriers and cultures in bioreactors [10] and [18].

Culture media, such as modified Eagle MEM, Leibovitz and others are used for the cultivation of Vero cells adhered to microcarriers in the production of viral suspensions. Supplementation of these media with fetal bovine serum, human or bovine albumin is necessary for the cellular growth in the bioreactor.

However, the use of serum in the cultures presents some disadvantages, such as the potential to induce hypersensitivity [25] and [26], the variability of the serum batches and the possible presence of contaminants (bacteria, fungi, mycoplasma, bovine viruses, etc.) and the high cost of a good quality serum [27]. There are some culture media formulated without any human or animal derived components, designed to support the serum-free growth of several cell lines of interest in virus production and biotechnology [28], [29], [30], [31], [32], [33] and [34].

Data obtained with serum-free VP-SFM medium (GIBCO) showed that its use increases the cellular density and rabies virus replication, decreases the possibility of contamination due to serum and reduces the time required for a production cycle in the bioreactor, thus minimizing the costs of the product [34].

In this paper we describe a methodology for the production of human rabies vaccine, prepared with rabies virus suspension obtained from infected Vero cells cultivated in a serum-free medium in the bioreactor.

2. Materials and methods

2.1. Cell lines

Cell banks (seed and working) used were made from Vero cells originated from passage 124 of the American Type Cell Collection (VERO-ATCC-CCL81), tested according to WHO requirements for cell lines [35]: the absence of fungi, bacteria, mycoplasmas [36] and other adventitious agents, determination of the karyotype and lack of tumorigenicity [37]. The banks were stored at -180°C .

2.2. Rabies virus

The seed and working banks were prepared with rabies virus suspensions obtained from Vero cells, cultivated in T-flasks (350 cm^2) and infected with fixed PV Pasteur virus (PV/RV/VERO Stock I-Pasteur, Paris). These banks were submitted to control tests such as viral identity, titration in BHK-21 cells [38], the absence of mycoplasma, fungi, bacteria and other adventitious agents [39].

2.3. Culture medium

Serum-free VP-SFM medium (GIBCO—Cat. no. 1168—Invitrogen Corporation) with 4 mM L-glutamine was used for the production of rabies virus in the bioreactor.

2.4. Cell culture system and viral infection

Cell cultures performed initially in 350 cm^2 T-flasks were inoculated into a bioreactor (Celligen-New Brunswick) of 3.7 l working volume, at a proportion of 16 cells per microcarrier (Cy todex 3-Pharmacia) with the initial seeding of $2.5 \times 10^5\text{ cell ml}^{-1}$. After 4 days of cellular cultivation in VP-SFM medium, the cells were infected with PV rabies virus (multiplicity of infection = 0.08).

ACTIVIDAD CELULASA)

- Respecto al documento D4 el solicitante afirma *"Se encuentran varias similitudes respecto al documento D3, toda vez que describe el uso de "acetatos de polivinilo" listados en la columna 2, líneas 25 a 36. Este documento tampoco revela acetatos de polivinilo hidrolizados y los pesos moleculares están por encima de 1.000.000 y 10.000.000"*.

El documento D4 es el documento más/cercano del estado de la técnica dado que muestra que ya se utilizan acetatos de polivinilo con peso moleculares superpuestos en el rango de esta solicitud como es 100.000 a 50.000.000 (Ver: D4: Col 1 líneas 65-66).

Respecto a que en el estado de la técnica no se mencione el grado de hidrólisis que se reivindica, se enfatiza que como bien se mostró en el análisis de nivel inventivo la persona del oficio normalmente versada en la materia técnica, conoce que dependiendo de la cantidad de grupos acetato del PVA que sean hidrolizados, el PVA se clasifica como de alta (99%), media (88%) y baja (78%) hidrólisis siendo el porcentaje molar de unidades repetitivas con grupos hidroxilo dentro de la totalidad de unidades repetitivas de la cadena y que el grado de hidrólisis es quizás la propiedad más importante del PVA ya que define su solubilidad en agua y su actividad superficial. También resulta de importancia el peso molecular del polímero, el cual se suele relacionar con la viscosidad de las soluciones acuosa que forma.

Por lo tanto, el porcentaje de hidrólisis y peso molecular en los acetatos polivinilo son dos variables obvias de estudio para la persona del oficio normalmente versada en la materia técnica que desee transportar composiciones viscosas como la de la presente solicitud.

En consecuencia y por todo lo expuesto anteriormente, se recomienda negar las reivindicaciones 1 – 32 (folios 213-217).

Bogotá, D. C.

Elaborado por:
Aprobado por:

The examination is being carried out on the following application documents:

Description, Pages

1-15 filed with entry into the regional phase before the EPO

Claims, Numbers

1-18 filed with entry into the regional phase before the EPO

Drawings, Sheets

1 filed with entry into the regional phase before the EPO

1. Cited documents

The following documents cited in the International Search Report or the Supplementary European Search Report are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

- D1: LITWIN J., CYTOTECHNOLOGY vol. 10, no. 2, 1992, pages 169 - 174
- D2: WO 2005 063970 A1
- D3: FRAZZATI-GALLINEA ET AL., VACCINE vol. 23, no. 4, 09 December 2004, pages 511 - 517
- D4: JINDRICH CINATL J.R., CELL BIOL. INT. vol. 17, no. 9, September 1993, pages 885 - 896
- D5: CHOI Y., VACCINE vol. 21, no. 17-18, 16 May 2003, pages 1867 - 1873
- D6: EP-A-1 260 581 (2002-11-27)
- D7: YUNG-SHYENG TSAO ET AL., CYTOTECHNOLOGY, KLUWER ACADEMIC PUBLISHERS, DO, vol. 37, no. 3, 1 November 2001 (2001-11-01), pages 189-198

2. Note has been taken of the International Preliminary Report on Patentability, drawn up for the present application in accordance with the PCT.

3. Subject-matter of the application:

Present application relates to a particular Vero cell line (DSM ACC2791) which has been adapted to growth in suspension, in the absence of supporting materials for its adherence and in a culture medium free of fetal calf serum. Said cell line is used for the production of viruses.

4. The amended claims filed with entry into the regional phase appear to be allowable under Article 123(2) EPC. Applicant filed also a new version of the description with entry into the regional phase before the EPO. The amendments made have however not been identified (Article 123(2) EPC, Guidelines E-II, 1). For the establishment of this opinion only the text of the description as originally filed has been taken into account.

5. Articles 83 and 84 EPC

5.1. The application fails to disclose the invention of claims 5-7 in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Article 83 EPC).

Claim 5 relates to a procedure to obtain the specific sVero p66 cell line. The examiner has serious doubts that the skilled person could obtain the specific sVero p66 cell line starting from just any anchorage-dependent Vero cell line (claim 5) in 30 days (claim 6) or even starting from Vero E6 cells (claim 7). The reasons therefore are the following: According to the application the sVero p66 cell line has been obtained starting from Vero E6 cells (passage 26, Banco Argentino de Celulas) and following a particular procedure which is incompletely described, involved moreover various undefined media and took 120 days (see example 1). The changes that occurred in the cell line during the weaning process and the adaptation to growth in suspension have not been disclosed. These changes are random events and hence unpredictable. The skilled person has thus no guarantee that following the procedure of Example 1 he would ever obtain sVero p66. As the successful performance of the invention is dependent on chance there is a fundamental insufficiency problem (see EPO Guidelines C-II, 4.11).

5.2. Applicant has not provided the deposit receipt issued by the depositary institution as proof of the deposit of sVero p66 (DSM ACC2791) in accordance with Rule 31 EPC (see EPO Guidelines, C-II 6.3).

Applicant's attention is drawn to the fact that if any of the requirements of Rule 31 EPC is not satisfied, the sVero p66 cell line cannot be considered as having been disclosed pursuant to Article 83 EPC by way of reference to the deposit. In this case the invention as claimed in claims 1-4 and 8-16 will also be objected to under Article 83 EPC.

5.3. Claim 8 violates Article 84 EPC because it is not clear whether the feature in the brackets is limiting or not (EPO Guidelines, C-III, 4.19).

6. Article 54 EPC

6.1. The present application does not meet the requirements of Article 52(1) EPC because the subject-matter of claims 17 and 18 is not new within the meaning of Article 54(1) and (2) EPC.

6.2. Claim 17 relates to a virus obtained by the procedure of claim 8.

According to established case law (T412/93, point 33 of the reasons): "the fact that a product is referred to in a claim as being the result of some process, does not automatically mean that the product is novel even if it is beyond dispute that the process referred to is new. The purpose of the reference to the process was to exclude those products which in the prior art were not obtained by the process. If,

on the evidence available, the process appears capable of producing every product meeting the characteristics of the product of the prior art, the reference to the process is not a limitation for the purpose of considering novelty. The process feature in a product claim can only be relied on for establishing novelty over the prior art, where use of that process necessarily means that the product has a particular characteristic and the skilled person following the teaching of the specification would inevitably achieve that characteristic, would be aware of that characteristic and would discard any products not having it." This is not the case here as the procedure according to claim 8 does not result in any particular characteristic of the thus obtained virus.

D3 discloses (abstract) Vero-cell rabies vaccine produced using serum-free medium and thus anticipates the subject-matter of claims 17 and 18.
D5 discloses inactivated hantaan virus vaccine derived from suspension culture of Vero cells and thus also anticipates the subject-matter of claims 17 and 18.
D6 discloses (paragraphs [0014]-[0020]) the production of various viruses including HSV1 and HSV2 in serum-free culture and their use in vaccine production and thus also anticipates the subject-matter of claims 17 and 18.

7. Article 56 EPC

- 7.1. The present application does not meet the requirements of Article 52(1) EPC because the subject-matter of claims 1-4 and 8-16 does not involve an inventive step within the meaning of Article 56 EPC.
- 7.2. Claim 1 relates to sVero p66, a Vero cell line adapted to grow in suspension, in the absence of supporting materials for its adherence and in a culture medium free of fetal calf serum.
According to the description as originally filed (page 8, lines 7-8) this cell line may grow in suspension as individual cells without forming aggregates (page 3, lines 15-22).

D3 discloses (abstract) the use of VERO-ATCC-CCL81 cells adhered to microcarriers, cultivated in a bioreactor in serum-free medium and infected with rabies for vaccine production.

The problem to be solved by the present invention may therefore be regarded as the provision of an alternative VERO cell line for use in viral vaccine production which needs no carrier in suspension culture.

The solution proposed in claim 1 of the present application cannot be considered to involve an inventive step (Articles 52(1) and 56 EPC), for the following reasons:

D6 discloses (abstract, paragraphs [0010]-[0020]) adaptation of Madin Darby Canine Kidney (MDCK) cells to suspension culture in serum-free medium for

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adhered to microcarriers cultivated in bioreactor or roller bottles) requires a culture medium supplemented with fetal bovine serum or albumin (human or bovine) for cell growth. The use of these components presents some disadvantages, such as potential to induce hypersensitivity [25] and [26], serum batch variability, possibility of contaminants, such as fungi, bacteria, mycoplasmas, bovine viruses, agent responsible for bovine spongiform encephalopathy (BSE) and the Creutzfeldt-Jacob disease in humans [44] and [45].

The development of serum-free culture media reduced these problems, facilitating the downstream process required for the obtention of the final purity [27]. There are different serum-free media for growth of several cell types (BHK-21, BSR, VERO, CHO, MDCK, and others). The MDSS₂ medium (Axcell Biotechnologies) is one of the most used in vaccine production [28], [29] and [30].

The serum-free VP-SFM medium, according to the manufacturer, was developed for Vero, BHK-21 and CHO cell growth. The use of this medium for Vero cells adhered to microcarriers, infected with PV rabies virus and cultivated in a Celligen bioreactor (New Brunswick), showed to be very efficient to increase the cell density and the viral multiplication [34]. Thus, this system demonstrated to be an excellent alternative for the obtention of viral suspensions for the preparation of rabies vaccine.

The potency of the vaccine batches prepared in VP-SFM medium showed to be much higher (5.8–13.6 IU per dose) than the minimum required for a human rabies vaccine (2.5 IU per dose) [39]. Correlation between the viral titers of suspensions utilized to prepare the batches and the results of potency was observed ($r = 0.87$) (Table 1).

In stability tests, all batches of rabies vaccine in serum-free medium maintained the minimum potency required at 37 °C after 30 days. The batches stored during 14 months at 2–8 °C presented potency ≥ 2.5 IU per dose. The same occurred after 12 h of storage at 45 °C. It is a very important factor in tropical countries such as Brazil with regions reaching approximately 41 °C. The increase in potency observed in some tests after a period of vaccine storage can be explained by the variability of NIH test [46].

Residual cellular DNA of the vaccines produced in Vero cells is one of the great concerns in the production of this immunobiological. The WHO recommends values ≤ 100 pg per dose. With the use of serum-free medium the downstream process was facilitated (one purification process by ion exchange chromatography) and the levels of residual cellular DNA obtained were lower than this value in all produced batches. Five of them presented values of 15 pg per dose, corresponding to the sensitivity of the test used for detection and two others 22.8 pg per dose.

The immunogenicity test of the vaccine performed in mice with a pre-exposure scheme (D₀, D₇ and D₂₈) presented excellent results when compared with the Verorab and HDCV vaccines. The statistical analysis demonstrated that there is correlation between the potency and the levels of neutralizing antibodies of the mice immunized ($r = 0.69$). However, independent of the potency value, all the animals immunized presented neutralizing antibodies titers higher than the minimum required after the second dose of vaccine.

The good results obtained with the rabies virus replication in the bioreactor and immunogenicity of this new vaccine produced in serum-free medium, permits the reduction of the costs of the process, in spite of the high price of serum-free media.

Thus, it is possible to obtain a high quality product with similar costs to the process that uses media with fetal bovine serum or bovine albumin for the production of rabies vaccine in Vero cells.

Acknowledgement

José Roberto Siqueira, Elaine Cristina Prado, Jovina Barbosa, Nete Teixeira and Carlos Alberto S. Penna for laboratory technical support.

References

- [1] C.E. Rupprecht, C.A. Hanlon and T. Hemachudha, Rabies re-examined, *Lancet Infect Dis*, **2** 6 (2002), pp. 327–343.
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(54) **CELL USABLE IN SERUM-FREE CULTURE AND SUSPENSION CULTURE AND PROCESS
FOR PRODUCING VIRUS FOR VACCINE BY USING THE CELL**

(57) A novel MDCK-derived cell line (B-702) is provided that can be grown in the absence of fetal calf serum and in suspension culture for allowing a tank culture on the production scale and needs no carrier in suspension

culture, as well as a process for producing virus for vaccine by using said cell line in a low-cost, highly safe and stable manner.

EP 1 260 581 A1

Description

TECHNICAL FIELD OF THE INVENTION

• [0001] The present invention relates to a cell line that can be grown in serum-free culture and in suspension culture without a carrier, and to production of a vaccine using said cell line. More particularly, the present invention relates to establishment of a cell line that can be grown without using serum and that can be grown in suspension culture without need of the cells to be adhered to any carrier, and a process for producing a vaccine with said cell line.

10 BACKGROUND OF THE INVENTION

[0002] In general, for production of a vaccine, there are widely used a growing chicken egg, a mouse brain, or primary cells or established cell lines from various animals such as chicken or ape. However, these conventional techniques involve various problems as mentioned below.

15 [0003] First, the use of a growing chicken egg necessitates management of chicken breeding, management of fertilized eggs to be adjusted to a vaccine production schedule, and laborious procedures including extensive purification for completely removing components derived from egg proteins while production. It is also problematic in view of animal protection.

20 [0004] Also in case of established cell lines, fetal calf serum as a cell growth factor must be added. However, its quality might vary among commercial products and it has a risk of contamination with mycoplasma, virus, or infectious proteins such as prion, and hence strict control of quality is required. Thus, a high-quality fetal calf serum such as one produced in New Zealand needs be used but is so costly that renders it unsuitable for production in industrial level.

25 [0005] Also among established cell lines where various kinds of viruses can be propagated, MDCK (Madin Darby Canine Kidney) cells are widely used in laboratory level (Proc. Soc. Exp. Biol. Med., 122, pp.931-935 (1966)). However, MDCK cells are much inclined to adhere to walls. Thus, the use of MDCK cells for an industrial large-scale culture is, due to its need for a large amount of culture medium, a culture vessel or a carrier for culture with a vast area, is disadvantageous in that: (1) it is extremely costly for equipment or a carrier; (2) it needs a stop for removing cells adhered to a carrier and thereby some loss of cells is produced while recovery; and (3) cells are damaged due to contact between carriers (beads) according to culture conditions. Thus, it is not practical to use directly MDCK cells for vaccine production in view of cost and equipment.

30 [0006] Consequently, there has been a demand for establishing a process for producing a vaccine in a low-cost, highly safe and stable manner.

DISCLOSURE OF THE INVENTION

35 [0007] Under the circumstances, in order to obviate the disadvantages of the prior art as stated above, the present inventors have kept on investigating. As a result, the present inventors have established a novel cell line which can be grown without fetal calf serum and in suspension culture for enabling tank culture on the production scale and does not need any carrier in suspension culture. The present inventors also established a novel process for producing a vaccine without fetal calf serum to thereby enable providing a vaccine with low cost and high safety, and thus the present invention has been completed.

BRIEF DESCRIPTION OF THE DRAWINGS

45 [0008]

Fig. 1 shows growth of B-702 according to the present invention.

BEST MODE FOR CARRYING OUT THE INVENTION

50 [0009] MDCK-derived cell line according to the present invention, which can be grown in serum-free culture and in suspension culture without a carrier, was prepared by the following steps:

- 55 (a) adapting MDCK cells to serum-free culture medium to prepare cells that can be grown in serum-free culture;
(b) applying the cells that can be grown in serum-free culture to suspension culture with a carrier to prepare cells that can be grown in suspension culture with a carrier; and
(c) applying the cells that can be grown in suspension culture with a carrier to suspension culture without a carrier to prepare cells that can be grown in suspension culture without need of a carrier.

[0010] More specifically, MDCK-derived cell line of the present invention was prepared in the following procedures.

[0011] MDCK cells were cultured in a monolayer in a 25 cm² culture flask containing Dulbecco Modified Eagle medium (hereinafter referred to as "D-MEM"; Gibco, Catalogue No. 11965-082) supplemented with 10% fetal calf serum (hereinafter referred to as "FCS") and non-essential amino acids (MEM Non-Essential Amino Acids Solution; Gibco, Catalogue No. 11140-050). After sufficient expansion of the cells, the cells were then cultured in the above medium containing 25% serum-free medium and subcultured upon confirming no abnormality in cells. A serum-free medium as used herein preferably contains no components from other animal species in view of safety. As an example, VP-SFM (Gibco, Catalogue No. 11681-020) was used herein. After subculture and sufficient expansion of the cells, the culture medium was replaced with D-MEM containing 50% VP-SFM. The procedures were repeated with a content of VP-SFM being increased to 75% and then 100% to adapt the cells to serum-free culture so that cells adapted to serum-free culture medium were ultimately established.

[0012] Then, the thus prepared cells adapted to serum-free culture medium were subjected to the following two approaches so that they can be grown in suspension culture without a carrier.

[0013] In the first approach, the cells adapted to serum-free culture medium were directly cultured in a spinner flask (Spinner Flask Complete, BELLECO, Catalogue No. 1965-00100) at 40 rpm with VP-SFM as a culture medium throughout the culture. When pH of the culture medium was lowered, a half of the medium alone was replaced with a fresh medium while leaving the cells to continue the culture. After about two-month culture, a population of cells was obtained that could be grown under this condition. However, a cellular density was at the most 5×10^5 cells/ml, but did not reach 1×10^6 cells/ml, the level necessary for industrialization.

[0014] In the second approach, the cells adapted to serum-free culture medium were adhered to a bead carrier Cytodex 1 (Pharmacia Biotech, Catalogue No. 17044801; bead concentration, 1 g (dry weight)/ml) and then cultured in a spinner flask (Spinner Flask Complete, BELLECO, Catalogue No. 1965-00100) at 40 rpm so that cells that can be grown under rotary shaking culture could firstly be produced. As a result, in about two months after initiation of culture, a population of cells was obtained that can be expanded and grown on the surface of the beads. Thereafter, a culture scale was enlarged to 250 ml, 500 ml and 1000 ml of a spinner flask (Spinner Flask Complete, BELLECO, Catalogue Nos. 1965-00250, 1965-00500, 1965-01000) while the cells were not treated with trypsin for release from the beads but a needed amount of VP-SFM medium and Cytodex 1 were added, to obtain a cell line that could be expanded to the culture scale. This cell line was then cultured in a spinner flask (Spinner Flask Complete, BELLECO, Catalogue No. 1965-00100) in the absence of a carrier Cytodex 1 with VP-SFM medium. As a result, after about a month, a cell line that could be grown in the absence of a carrier was obtained. This novel established cell line was named "B-702". B-702 is characteristic in that most of the cells are present in a unicellular state in suspension culture. Applicant has deposited B-702 at National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan under accession number FERM BP-7449 (transferred from FERM P-17582 (original deposit made on December 22, 1999); date of transfer to International Deposit, February 5, 2001).

[0015] B-702 was then estimated for its growth. In most cases of established cell lines, about 3-fold expansion in cell number is considered to be optimum for expansion culture. On the contrary, in case of B-702, it was found that an initial cellular density of 1×10^5 cells/ml could be expanded to 1×10^6 cells/ml within about a week, i.e. 10-fold expansion. In the end, after the 10-fold expansion was performed, this cell line could be expanded to 3×10^6 cells/ml in a 1000 ml spinner flask starting from a 100 ml spinner flask.

[0016] Then, B-702 was estimated for its capacity to produce viruses. Three different influenza vaccine strains that were used in the season from 1998 winter to 1999 spring (A/Beking/262/95(H1N1), A/Wuhan/359/95(H3N2), B/Mie/1/93) were inoculated at 0.001, 0.00003 and 0.0002 of M.O.I., respectively, into B-702 which were grown at a cellular density of 1×10^6 cells/ml in a 100 ml spinner flask. VP-SFM medium supplemented with 1% trypsin (Gibco, Catalogue No. 25200-072) was used for culture. After culture at 34°C for 3 days, 1×10^8 PFU of viruses were propagated in each culture supernatant, which propagation of viruses was equivalent to that of MDCK cells cultured in a growing chicken egg or 10% fetal calf serum. Thus, it was found that B-702 could produce viruses in the absence of fetal calf serum and a carrier for cells.

[0017] Besides, it was confirmed that B-702 had a capacity to produce viruses including DNA viruses such as human herpes virus type 1 and type 2, and RNA viruses such as measles virus, mumps virus and rubella virus, indicating that any viruses capable of adapting to MDCK cells could be produced by B-702.

[0018] Such viruses include any animal viruses, including families of Orthomyxoviridae, Paramyxoviridae, Togaviridae, Herpesviridae, Rhabdoviridae, Retroviridae, Reoviridae, Flaviviridae, Adenoviridae, Picornaviridae, Arenaviridae and Porviridae. Preferable viruses includes influenza virus, measles virus, mumps virus, rubella virus, HSV-1, HSV-2, rabies virus, RS virus, reovirus type 3, yellow fever virus, Japanese encephalitis virus, adenovirus types 1 to 47, poliovirus, Lassa virus and vacciniavirus. Most preferable viruses include influenza virus, human herpes virus type 1 and type 2, measles virus, mumps virus and rubella virus.

[0019] Thus, it was found that B-702 had wide utility as a cell line for virus production.

[0020] Viruses or viral antigens produced by MDCK-derived cell line, typically B-702, can be used as a source for preparing a vaccine.

[0021] B-702 according to the present invention can be grown without need of fetal calf serum and a carrier for cells and hence allows for production of various useful substances, including vaccine, with a cheap medium in a stable and safe manner. Especially in association with production of influenza virus, when global pandemicity of new type virus occurred, which is worldwide concern in recent years, currently no technique or equipment is provided in Japan for urgently producing a needed amount of vaccine in a large scale. Accordingly, production of vaccine using B-702 is a much prosperous means for this purpose.

[0022] The present invention is illustrated in more detail by means of the following Examples to bring about a better understanding of the present invention, but should not be construed to be limited thereto.

Example 1

[0023] B-702 was estimated for its growth with MDCK cells (ATCC CCL-34) as a control under the following conditions of culture medium, culture temperature, etc:

Culture medium: VP-SFM (Gibco, Catalogue No. 11681-020)
Additives: L-Glutamine (Gibco, Catalogue No. 25030-081), 2% (v/v)
Gentamicin solution (Sigma, Catalogue No. G-1397), 0.1% (v/v)
Culture condition: 37°C, 5% CO₂, moist
Rotation of spinner: 40 rpm

Cellular density at the beginning of culture: about 1×10^5 cells/ml

Subculture condition for expansion:

[0024] At the time when a cellular density reached about 1×10^6 cells/ml, a culture was scaled up from 100 ml to 250 ml, 500 ml, and 1000 ml. At each stage where a culture is to be scaled up, a starting cellular density was adjusted to 1×10^5 cells/ml.

[0025] The results are shown in Fig. 1 wherein when MDCK cells were cultured in the absence of fetal calf serum, and a carrier for cells, no increase in cell number was observed, the number of living cells drastically decreased on Day 3 and at the cells died out on Day 6. On the contrary, B-702 showed good increase in cell number even after scale-up of culture with increasing volume of a spinner flask.

Example 2

[0026] B-702 was used to propagate viruses. The viruses employed herein were three different influenza vaccine strains that were used in the season from 1998 winter to 1999 spring with M.O.I. as indicated in Table 1.

Table 1

Strain	M.O.I.
A/Beking/262/95(H1N1)	0.001
A/Wuhan/359/95(H3N2)	0.00003
B/Mie/1/93	0.0002

[0027] Culture conditions were as follows:

Cellular density: 1×10^6 cells/ml
Culture scale: 100 ml spinner flask
Culture medium: as described in Example 1 but supplemented with 1% trypsin (Gibco, Catalogue No. 25200-072)
Rotation of spinner: 40 rpm
Culture temperature etc.: 34°C, 5% CO₂, moist
Duration of culture: 3 days

[0028] It is commonly known that better viral propagation is observed at 34°C than at 37°C in culture of influenza virus and culture temperature currently used for vaccine production is also 34°C. Thus, viral culture in this test was

performed at 34°C. It is also commonly known that, in culture of influenza virus with established cell lines, a protease that cleaves hemagglutinin (HA) is necessary for viral infection (U.S. patent No. 4,500,513). Thus, trypsin was included in a culture medium as a protease. A virus titer was measured in accordance with a plaque counting method as used for measuring a titer of influenza virus (Practice compendium in microbiology, 2nd ed., The University of Tokyo, The Institute of Medical Science, alumni association ed., Maruzen K.K., p. 205-206). Specifically, commonly used MDCK cells (ATCC-CCL-34) are cultured on a 6 well culture plate, into which 1×10^6 diluent of a supernatant of the above viral culture is inoculated. After inoculation, agarose is overlaid onto the monolayer of the cells and standing culture is performed in CO₂ incubator for 4 days. After completion of culture, formalin is added to fix the cells. The agarose layer is then removed, the cells are dyed with trypan blue and a plaque number formed by the virus is counted.

[0029] In three-day culture, all the cells died out. A culture supernatant was recovered and a viral titer was measured. As a result, as shown in Table 2, all the three vaccine viral strains exhibited a virus titer of not less than 1×10^8 PFU/ml, equivalent to that of allantoic fluid when viruses were propagated in growing chicken egg.

Table 2

Virus	Virus titer (PFU/ml)
A/Beijing/262/95[H1N1]	6.3×10^8
A/Wuhan/359/95[H3N2]	2.5×10^8
B/Mier/1/93	1.2×10^8

Example 3

[0030] MDCK cells intrinsically allow for propagation of various viruses. In order to investigate whether B-702 cell line inherited this sensitivity, B-702 was inoculated with human herpes virus type 1 (HSV-1) and type 2 (HSV-2) as DNA virus. Culture condition was as described in Example 2 except that trypsin was not included in the culture medium. Culture was continued for five days. Inoculation was performed as indicated in Table 3.

Table 3

Inoculated Virus	Amount of inoculation
HSV-1 KOS strain	0.01 ml
HSV-2 MS strain	0.01 ml

[0031] On Day 5, all the cells died out. Culture supernatant was recovered and a virus titer (PFU/ml) was measured by diluting by 1000-fold the culture supernatant to Vero cells derived from the kidney of African green monkey, and counting the formed plaques. The results proved that B-702 had a good capacity to propagate DNA viruses as shown in Table 4.

Table 4

Inoculated virus	Virus titer (PFU/ml)
HSV-1	1.2×10^8
HSV-2	1.3×10^8

Example 4

[0032] In order to investigate whether MDCK cells are capable of propagating other RNA viruses than influenza virus, inoculation was then performed for measles virus, mumps virus and rubella virus. Culture conditions were as described in Example 3 and culture was continued for five days. Inoculation was performed as indicated in Table 5.

Table 5

Inoculated Virus	Amount of inoculation
Measles virus Ichinose strain (10^5 TCID ₅₀ /0.1ml)	0.01 ml
Mumps virus Enders strain (10^4 TCID ₅₀ /0.1ml)	0.01 ml
Rubella virus M33 strain (10^7 TCID ₅₀ /0.1ml)	0.01 ml

[0033] On Day 5, all the cells died out. Culture supernatant was recovered and a virus titer was measured. For

measurement of a virus titer, each of the above culture supernatant, diluted by 1,000-fold, 10,000-fold and 100,000-fold, was inoculated to each 6 wells of Vero cells cultured on a 24 well plate, which was then placed in a CO₂ incubator. After two-week standing culture, wells where cells were degenerated by viruses, i.e. CPE was demonstrated in the cells, were counted at each dilution, and 50% infection titer (TCID₅₀) was calculated by Kaerber's calculation method (Practice compendium in microbiology, 2nd ed., The University of Tokyo, The Institute of Medical Science, alumni association ed., Maruzen K.K., p. 205-206). The results proved that B-702 had a good capacity to propagate RNA viruses as shown in Table 6.

Table 6

Inoculated virus	Virus titer (TCID ₅₀)
Measles virus	$10^{4.5}$
Mumps virus	$10^{6.8}$
Rubella virus	$10^{6.6}$

Claims

1. A cell line that is derived from MDCK cells from canine kidney and can be grown in serum-free culture and in suspension culture without need of a carrier.
2. The cell line of claim 1 which does not contain components derived from animal species.
3. The cell line of claim 1 or 2 which is B-702.
4. The cell line of claim 1, 2 or 3 which is suitable for production of virus for vaccine.
5. The cell line of claim 4 wherein said virus is selected from the group consisting of Orthomyxoviridae, Paramyxoviridae, Togaviridae, Herpesviridae, Rhabdoviridae, Retroviridae, Reoviridae, Flaviviridae, Adenoviridae, Picornaviridae, Arenaviridae and Poxviridae.
6. The cell line of claim 5 wherein said virus is selected from the group consisting of influenza virus, measles virus, mumps virus, rubella virus, HSV-1, HSV-2, rabies virus, RS virus, reovirus type 3, yellow fever virus, Japanese encephalitis virus, adenovirus types 1 to 47, poliovirus, Lassa virus and vaccinia virus.
7. A process for preparing a cell line that is derived from MDCK cells and can be grown in serum-free culture and in suspension culture without a carrier, which comprises the steps:
 - (a) adapting MDCK cells to serum-free culture medium to prepare cells that can be grown in serum-free culture;
 - (b) applying the cells that can be grown in serum-free culture to suspension culture with a carrier to prepare cells that can be grown in suspension culture with a carrier; and
 - (c) applying the cells that can be grown in suspension culture with a carrier to suspension culture without a carrier to prepare cells that can be grown in suspension culture without need of a carrier.
8. The process of claim 7 wherein said serum-free culture medium used for serum-free culture does not contain components from animal species.
9. The process of claim 7 or 8 wherein said cell line derived from MDCK cells is B-702.
10. A process for preparing virus for vaccine, which comprises inoculating virus into a cell line that is derived from MDCK cells and can be grown in serum-free culture and in suspension culture without need of a carrier, and culturing said cell line in serum-free and suspension culture in the absence of a carrier.
11. The process of claim 10 wherein said serum-free culture medium used for serum-free culture does not contain components from animal species.
12. The process of claim 10 or 11 wherein said cell line derived from MDCK cells is B-702.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado

JAIME EDUARDO DELGADO VILLEGAS

ASUNTO Radicación 06-99780
 Trámite 002
 Evento 001
 Actuación 519
 Oficio
 Folios 015

15133

Patente de Invención

☒

Patente de Modelo de Utilidad

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Vía Nacional

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Vía PCT (fase nacional)

☐

Cap. I

☐

Cap. II

☐

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 30 a 50	03 Octubre 2006
Reivindicaciones:	Folios 51 a 54	03 Octubre 2006
Dibujos:	Folio 55	03 Octubre 2006
Prioridad:	US 60.723377	04 Octubre 2005

2. Objeto de la invención

Título de la solicitud: "Línea Celular VERO adaptada para crecer en suspensión"

El problema planteado en esta solicitud consiste en la necesidad de células VERO que puedan crecer en suspensión como células individuales son formar agregados en cultivos de alta densidad, además de no necesitar materiales de soporte para su adherencia.

Para resolver este problema técnico la solicitud en estudio proporciona una línea celular caracterizada por estar adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino y proteínas, útil para la producción a gran escala de vacunas.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C12N 5/00

3. No es una invención (Art 15 D 486 literal (b))

El artículo 15 en su literal b) señala que:

No se consideran invenciones el todo o parte de los seres vivos tal como se encuentran en la naturaleza, los procesos biológicos naturales, el material biológico existente en la naturaleza o aquel que pueda ser aislado inclusive genoma o germoplasma de cualquier ser vivo natural.

Los virus reclamados en la reivindicación 20 obedecen a material biológico que existe en la naturaleza, por lo que a menos que se especifique que dichos virus han sido modificados, no se puede considerar como una invención. Por lo anterior, la reivindicación 20 no puede ser considerada invención.

4. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

- Las reivindicaciones 1 a 3 que hacen referencia a una línea celular la cual es caracterizada por estar adaptada a diferentes condiciones de crecimiento, lo anterior corresponde a un resultado a alcanzar, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto.
- El número de depósito el cual caracteriza la línea celular reclamada en la reivindicación 4 debido a que es una característica técnica esencial de la invención debe estar dentro de la reivindicación principal (reivindicación 1).
- La Reivindicación 5 Indica el uso que puede darse a la línea celular reivindicada, el cual no es una característica técnica que defina a la invención. Se sugiere hacer la aclaración respectiva.
- El objeto de la reivindicación 6 que hace referencia a un procedimiento para que este adecuadamente caracterizado debe incluir todas y cada una de las condiciones y características específicas que se requieren para llegar a la formación de la línea celular, es así que se sugiere que dicha reivindicación incluya las características que se reclaman en las reivindicaciones 7 y 9.
- Las reivindicaciones 10 a 20 que hacen referencia a un procedimiento para producir virus, aunque involucran la presencia de una línea celular adaptada (sVEROp66) corresponden a un uso o a una aplicación de esta para la generación de virus ya conocidos en el estado de la técnica, es así que se sugiere al solicitante eliminar dichas reivindicaciones. Por otro lado, no se encuentra soporte en la descripción del uso de todos los virus reclamados en el presente capítulo reivindicatorio por lo cual se sugiere restringir a una generalización razonable de los virus teniendo en cuenta los ejemplos mencionados.
- Las reivindicaciones 20 y 21 definen el producto generado (virus y vacuna) por el procedimiento de obtención, lo anterior no es correcto puesto que dichos productos pueden caracterizarse por su naturaleza, composición, etc.

5. Unidad de invención (Art 25 D 486)

La solicitud de patente no tiene unidad de invención ya que comprende más de una invención o grupos de invenciones descritos a continuación:

Grupo inventivo 1: Reivindicaciones 1 a 9 relativas a la obtención de una línea celular VERO adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino.

Grupo inventivo 2: Reivindicaciones 10 a 20 relativas a un procedimiento para

producir un virus.
Grupo inventivo 3: Reivindicación 21 relativa a una vacuna formulada a partir de un virus.

Las características de las reivindicaciones independientes 1, 10 y 21 definen materia sin ninguna relación técnica y aunque se utilice un tipo de línea celular característico (en este caso línea sVEROp66), el procedimiento de generación de virus presentes en la naturaleza (D2, folio) y la generación de una vacuna de dichos virus es conocido (D3, folio). Por lo anterior se considera que dichos procedimientos corresponden a un uso o una aplicación del primer grupo inventivo; por lo cual, no pueden encontrarse elementos técnicos particulares correspondientes en las reivindicaciones.
En consecuencia, cada una de las reivindicaciones independientes 1, 10 y 21 representa una invención diferente. Por lo tanto, se considera que hay (3) invenciones en las reivindicaciones.

6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en fecha de presentación de la solicitud de prioridad (04 Octubre 2005) y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación
D1	Cytotechnology. 1992;10(2):169-74.	The growth of Vero cells in suspension as cell-aggregates in serum-free media.	1992
D2	Vaccine 2004; 23 (4). 511-517	Vero cell rabies vaccine produced using serum free medium	09 Diciembre 2004
D3	EP 1260581	Cell Usable in serum free cultura and suspensión cultura and process for produciong virus for vaccine by using the cell	27 Noviembre 2002

D1 (folio 125) divulga cultivos de células VERO que se presentan en suspensión y en un medio libre de suero fetal bovino. Dichas células no requieren un transportador o carrier para su desarrollo. También se divulgan procedimientos para lograr dicha línea celular.

D2 (folio 126 a 130) divulga producción de vacunas de virus de la rabia que se obtiene a partir del cultivo de este virus en línea celular VERO en un medio libre de suero fetal bovino y proteínas.

D3 (folio 131 a 133) divulga cultivos celulares en suspensión y libres de un carrier útiles para la producción de virus y vacunas.
URL:http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&adjacent=true&locale=en_gb&FT=D&date=20021127&CC=EP&NR=1260581A1&KC=A1

7. Examen de Patentabilidad (Art 14 D486)

Se hace el análisis del primer grupo inventivo

Novedad (Art 16 D486)

La reivindicación 1 consiste en una línea celular VERO caracterizada por estar adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Línea celular VERO	Línea celular VERO
Adaptada para crecer en: - Suspensión - en ausencia de materiales de soporte para su adherencia - en un medio de cultivo libre de suero fetal bovino	Adaptada para crecer en: - Suspensión - en ausencia de materiales de soporte para su adherencia - en un medio de cultivo libre de suero fetal bovino

Las características **esenciales** de la línea celular reclamada en la reivindicación 1 habían sido divulgadas previamente en D1, el cual da a conocer cultivos de la línea célula VERO. Dicha línea celular es capaz de ser cultivada bajo condiciones de suspensión celular y en un medio libre de suero fetal bovino, así mismo D1 revela que esta línea celular no requieren un transportador ó carrier para su desarrollo (folio 125, abstract).

En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 2, 5 y 6 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Nivel Inventivo (Art18 D486)

La **reivindicación 3** consiste en la línea de la celular VERO caracterizada porque dicha suspensión comprende células aisladas en ausencia de agregados de células.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2	D3
Línea celular VERO	Línea celular VERO	Línea celular VERO	Línea celular MDCK
Adaptada para crecer en: Suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino	Adaptada para crecer en: Suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino	Adaptada para crecer en: - Suspensión en un medio de cultivo libre de suero fetal bovino	Adaptada para crecer en: Suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino
Células aisladas en ausencia de agregados de células	Células presentes en forma de agregados celulares	Células colocadas en un carrier	Células aisladas en ausencia de agregados de células

Frente a D1 y D3

Los documentos D1 y D3 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 3 porque:

D1 divulga cultivos de la línea célula VERO. Dicha línea celular es capaz de ser cultivada bajo condiciones de suspensión celular y en un medio libre de suero fetal bovino, así mismo D1 revela que esta línea celular no requieren un transportador ó

carrier para su desarrollo puesto que se generan aglomerados de células con un tamaño que varía según las condiciones del medio (folio 125, abstract).

La diferencia entre la línea celular de la invención, definida en la reivindicación 3 y la línea celular divulgada en D1 es que las células son células aisladas y no se presentan en forma de agregados.

Por otro lado, **D3** hace referencia a una nueva línea celular derivada de MCDK la cual esta adaptada para crecer en suspensión, en ausencia de un carrier o un soporte de adherencia y en un medio de cultivo libre de suero fetal bovino y proteínas (folio 131, párrafo 007; folio 132, reivindicación 1). Por otro lado D3 describe el proceso para la obtención de dicha línea celular, el cual consiste en la adaptación de la línea celular a un medio de cultivo con bajo contenido de suero fetal bovino y posteriormente la aplicación de estas células a un medio sin la presencia de un soporte de adherencia (folio 131, párrafos 0011 a 0013; folio 132 reivindicación 7).

También se hace referencia a que este tipo de línea celular es susceptible a la adhesión en las paredes del recipiente que ocupan y esto ocasiona que su utilización a gran escala sea inconveniente (folio 131, párrafo 0005, línea 25 a 27).

La diferencia entre la invención, definida en la reivindicación 3 y lo divulgado en D3 consiste en que la línea celular corresponde a la línea celular VERO.

El hecho de que la línea celular VERO sea adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino, genera que dicha línea celular pueda ser utilizada para cultivos a gran escala y la generación de vacunas en reactores de fermentación estándares (ver folio 33).

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo modificar las líneas celulares VERO ya conocidas en el estado de la técnica tal como se presenta en D1 para conseguir que esta línea celular pueda ser utilizada para cultivos a gran escala y la generación de vacunas en reactores de fermentación estándares".

Sin embargo, las ventajas de un cultivo en suspensión de células individuales respecto a un cultivo de agregados celulares son conocidas en el estado de la técnica, ya que mejora la eficiencia en los sistemas de producción de virus que involucran bioreactores de alta densidad como es el caso de los cultivos de células MCDK dados a conocer en D3. Dicha línea celular ya divulgada, como se dijo anteriormente, presenta problemas de adhesión a superficies y conformación de aglomerados celulares que no permiten optimizar la producción de vacunas por lo cual se establecieron procesos de adaptación modificando las condiciones del medio llegando después de un manejo cuidadoso a una línea celular adecuada para crecer bajo condiciones específicas como son en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino y proteínas.

En consecuencia, la persona del oficio normalmente versada en la materia, que pretenda obtener una línea celular VERO modificada la cual este adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino estaría motivada con lo divulgado en el estado de la técnica a realizar un proceso de adaptación de la línea celular modificando las condiciones del medio de crecimiento para llegar así al objeto de la

reivindicación 3 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones 1, 2, 5 a 7 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Frente a D2 y D3

Los documentos D2 y D3 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 3 porque:

D2 divulga cultivos de una línea celular VERO modificada. Dicha línea celular es capaz de ser cultivada bajo condiciones de suspensión y en un medio libre de suero fetal bovino y proteínas (folio 127, párrafo 2.3), mediante la utilización de un microcarrier o soporte (folio 127, párrafo 2.3). La adaptación de estas células se lleva a cabo poniendo en contacto las células por 4 días en un medio adecuado (VP-SFM). Por otro lado se divulga el uso de dicha línea celular para la producción de vacunas del virus de la rabia (folio 128, párrafo 2.6).

La diferencia entre la línea celular de la invención, definida en la reivindicación 3 y la línea celular divulgada en D1 es que las células son células aisladas y no presentan la adición de un microcarrier.

D3 hace referencia a una nueva línea celular derivada de MCDK la cual esta adaptada para crecer en suspensión, en ausencia de un carrier o un soporte de adherencia y en un medio de cultivo libre de suero fetal bovino y proteínas (folio 131, párrafo 007; folio 132, reivindicación 1). Por otro lado D3 describe el proceso para la obtención de dicha línea celular, el cual consiste en la adaptación de la línea celular a un medio de cultivo con bajo contenido de suero fetal bovino y posteriormente la aplicación de estas células a un medio sin la presencia de un soporte de adherencia (folio 131, párrafos 0011 a 0013; folio 132 reivindicación 7).

También se hace referencia a que este tipo de línea celular es susceptible a la adhesión en las paredes del recipiente que ocupan y esto ocasiona que su utilización a gran escala sea inconveniente (folio 131, párrafo 0005, línea 25 a 27),

La diferencia entre la invención, definida en la reivindicación 3 y lo divulgado en D3 consiste en que la línea celular corresponde a la línea celular VERO.

El hecho de que la línea celular VERO sea adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia o microcarriers y en un medio de cultivo libre de suero fetal bovino, genera que dicha línea celular pueda ser utilizada para cultivos a gran escala y la generación de vacunas en reactores de fermentación estándares (ver folio 33).

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo modificar las líneas celulares VERO ya conocidas en el estado de la técnica tal como se presenta en D2 para conseguir que esta línea celular pueda ser utilizada para cultivos a gran escala y la generación de vacunas en reactores de fermentación estándares".

Sin embargo, las ventajas de un cultivo en suspensión de células individuales respecto a un cultivo que contenga un sistema de microcarrier ya es conocido en el estado de la técnica como es el caso de D3, ya que la sustracción de un componente dentro del medio de cultivo disminuye costos y posibles

contaminantes que puedan presentarse durante el desarrollo de la fermentación obteniéndose una mejora en la eficiencia de producción de virus que involucran bioreactores de alta, por lo cual se han establecido procesos de adaptación modificando las condiciones del medio de células que contienen microcarriers como las dadas a conocer en D2 llegando, después de un manejo cuidadoso, a una línea celular adecuada para crecer bajo condiciones específicas como son en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino y proteínas.

En consecuencia, la persona del oficio normalmente versada en la materia, que pretenda obtener una línea celular VERO modificada la cual este adaptada para crecer en suspensión, en ausencia de materiales de soporte para su adherencia y en un medio de cultivo libre de suero fetal bovino estaría motivada con lo divulgado en el estado de la técnica a realizar un proceso de adaptación de la línea celular modificando las condiciones del medio de crecimiento para llegar así al objeto de la reivindicación 3 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones 5 a 7 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

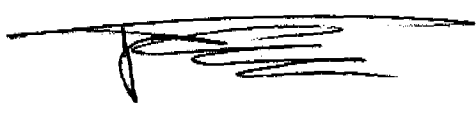
8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Cytotechnology. 1992;10(2):169-74.	1, 2, 5 y 6	Novedad
D2	Vaccine. 2004; 23 (4): 511-517	5 a 7	Nivel Inventivo
D3	EP 1260581	5 a 7	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

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



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

15 DIC. 2011

El anterior requerimiento fue notificado por fijación en lista, de fecha _____

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