ITP

Informe Tecnológico de Patentes

N/Ref.: V6208

Título

Métodos de diagnóstico de fiebre aftosa basados en análisis del material genético aplicables a todos los serotipos inmunológicos.

Realizado para

Fecha:

18 de febrero de 2013

Elaborado por:

Técnico superior examinador de patentes

Oficina Española de Patentes y Marcas

MINISTERIO DE INDUSTRIA, ENERGÍA Y TURISMO

Oficina Española de Patentes y Marcas

Unidad de Información Tecnológica

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Finalidad

Este informe se ha realizado para identificar los documentos más próximos al objeto o tecnología descrito por el cliente, con el fin de tener una visión amplia del estado de la técnica en relación con dicho objeto.

La finalidad del presente informe es la recuperación de información tanto de patentes como de literatura no patente.

Documentación de partida

El cliente ha aportado como base para el análisis una memoria técnica en la que se realiza una descripción de la cuestión de interés y se proponen una serie de palabras clave en relación con la invención

En adelante nos referiremos a esta documentación como "documentación de partida" .

De acuerdo con dicha documentación, el objeto técnico propuesto consiste en métodos de diagnóstico de fiebre aftosa basados en el análisis del material genético del virus que se puedan aplicar al diagnóstico de todos los serotipos conocidos.

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Estrategia de búsqueda

Características técnicas en las que se ha centrado la búsqueda

La búsqueda se ha centrado en la localización de documentos que incluyan el siguiente conjunto de características técnicas:

Métodos universales para detectar los virus correspondientes a los 7 serotipos inmunológicos distintos: A,O,C, SAT1, SAT2, SAT3 y Asia1 procedentes de muestras de tejidos o fluidos tanto de animales, alimentos o cualquier producto susceptible de haber estado en contacto con el virus.

Bases de Datos utilizadas

En función del objeto de la invención, se ha realizado la búsqueda en las siguientes bases de datos:

Bases de datos de patentes

WPI, EPODOC, INVENES

Bases de datos de literatura no patente

BIOSIS, MEDLINE, EMBASE, NPL, XPESP

Clasificaciones y palabras clave empleadas en la búsqueda

Para consultar las mencionadas bases de datos, se han empleado los siguientes criterios de búsqueda:

Códigos de la CIP

C12Q 1/68

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Códigos de la CPC

C12Q1/6888

Palabras clave

En español

Aftosa, glosopeda, aphtovirus, picornavirus, polivalente, universal, serotipo , PCR, cebador, sonda.

Otros idiomas

FDMV, foot and mouth, aphtovirus, picornavirus, serotype, universal, PCR, primer, probe

Vea nuestro apartado de *Información Tecnológica* en la web de la OEPM para más detalles sobre la metodología seguida para la realización del informe, las bases de datos, la estrategia de búsqueda y la terminología de patentes

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Análisis de los resultados

Documentos representativos

De entre todos los documentos referenciados e incluidos en la sección final "Listados de referencias" , se han seleccionado los más representativos en relación con la tecnología u objeto técnico descrito por el cliente.

A continuación se identifican dichos documentos más representativos:

Literatura Patente

WO2011150115 (LAWRENCE LIVERMORE NATIONAL SECURITY, LLC) 01.12.2011

EP1583556 (E.I. DU PONT DE NEMPOURS AND CO.) 12.10.2005

WO02095074 (TETRACORE INC.) 28.11.2002

Literatura no Patente

Hoffmann et al. A review of RT-PCR technologies used in veterinary virology and disease control: Sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health. Veterinary Microbiology Oct.2009, vol 139, pp. 1-23 ISSN 0378-1135

http://www.idpublications.com/journals/PDFs/VM/VM_MostDown_1.pdf

McKillen John etr al. Pan-serotypic detection of foot-and-mouth disease virus using a minor groove binder probe reverse transcription polymerase chain reaction assay. Journal of Virological Methods Jun 2011, vol 174 (1-2), pp. 117-119 ISSN 0166-0934

http://www.ncbi.nlm.nih.gov/pubmed/21419170

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Lau Lok-Ting et al. Detection of foot-and-mouth disease virus by nucleic acidsequence-based amplification (NASBA). Veterinary Microbiology. Jan 2008, vol 126 (1-3), pp. 101-110 ISSN 0378-1135

http://www.ncbi.nlm.nih.gov/pubmed/17728080

Análisis del contenido de los documentos representativos en relación con el objeto técnico descrito por el cliente

El documento EP1583556 (E.I. DU PONT DE NEMOURS AND COMPANY): se refiere a un método para detectar la presencia del virus de la fiebre aftosa (FMDV "foot and mouth disease virus) en una muestra mediante RT-PCR utilizando varios pares de cebadores basados en 5 secuencias diferentes dirigidas a **distintas regiones especificas** del genoma del virus (**2A, 2B, 2C)**. Con esta técnica se detectan los distintos serotipos y variaciones debidas a mutaciones de los mismos. Tambien se reivindica el KIT de detección.

El documento WO02095074 (TETRACORE INC): describe un método de diagnostico con RT-PCR utilizando sondas y cebadores dirigidos a la **region altamente conservada 3D** para el diagnostico de todos los serotipos de fiebre aftosa.

El documento WO2011150115 (LAWRENCE LIVERMORE NATIONAL SECURITY LLC.): describe un método y dispositivo para la deteccion de acidos nucleicos en una muestra utilizando **amplificaciones isotermicas** para diagnosticos "in situ" . En el ejemplo 3 se refiere concretamente al FMDV y la diana utilizada es **la región 3D**. (Ver ejemplo 3).

El documento Hoffmann el al es una publicación de una revisión de las distintas técnicas RT-PCR para la detección de virus de fiebre aftosa (ver, paginas 5-7). En la tabla 1 se hace referencia a los métodos publicados para la detección de todos los serotipos (regiones IRES, 3D, 2B) con sondas taqMan,

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con sondas de hibridacion duales, con ligandos del surco menor (MGB) o mediante transferencia de energía con cebadores sonda (PriProET).

El documento Lau et al describe la utilización del método de "amplificación isotérmica de ácidos nucleicos" (NASBA) para la detección de todos los serotipos de fiebre aftosa **utilizando cebadores conocidos** (region **IRES**) y muestras procedentes del laboratorio de referencia de la FAO. La eficacia de esta técnica resulta similar a la RT-PCR, y se trata de un método menos costoso y mas sencillo de utilizar.

El documento McKillen et al describe un método de detección de todos los serotipos de fiebre aftosa con ligandos del surco menor (MGB) con la región 3D como diana.

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Conclusión

A la vista de la información recuperada, podríamos concluir que la tendencia actual en relación con el diagnóstico de la fiebre aftosa por técnicas de análisis genético del virus parece ir dirigida a:

• la identificación de las regiones diana conservadas en el genoma de los 7 serotipos

• la utilización simultánea de más de una región diana del genoma del virus

• la utilización de un panel de ensayos para detectar la posibles mutaciones de las zonas de unión de las sondas

• la utilización de nuevas técnicas de diagnóstico diferencial que resulten más rápidas, económicas y específicas, como LAMP y NASBA que se realizan a temperatura constante. Se trata de ensayos menos costosos y que se pueden aplicar "in situ", con dispositivos portátiles.

Si el solicitante desea aportar información más concreta sobre una invención, y en especial si ésta se estructura en forma de reivindicaciones, que son las que delimitan la protección que otorga una patente, en un nuevo Informe se podría realizar una comparación detallada de esa tecnología concreta con el Estado de la Técnica relevante. En este caso, se debe evitar realizar reivindicaciones de carácter general y hacer incidencia exclusivamente en los aspectos novedosos de la invención desarrollada.

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Observaciones generales

Desde la realización de este informe hasta la presentación oficial de una posible solicitud de patente o de modelo de utilidad pueden aparecer nuevas publicaciones relevantes, por lo que, en caso de demorarse la presentación de la solicitud, puede ser conveniente realizar una vigilancia tecnológica periódica en bases de datos nacionales e internacionales, utilizando, entre otras, las clasificaciones y palabras claves propuestas en la sección "Estrategia de búsqueda" de este informe.

También es importante recordar que no sólo las publicaciones de terceros anteriores a la fecha de solicitud destruyen su novedad, sino que también las propias acciones de divulgación y/o publicación anterior (artículos en revistas, exposición en ferias no oficiales, documentos técnicos departamentales de acceso general, etc.) de los mismos solicitantes de este informe pueden afectar al cumplimiento de los *requisitos de patentabilidad -novedad y actividad inventiva-* por la invención propuesta.

Por todo lo anterior, una vez redactada la posible solicitud de patente o modelo de utilidad y antes de presentarla al registro, sería recomendable un nuevo estudio para evaluar el cumplimiento de los requisitos de novedad y actividad inventiva en función del juego de reivindicaciones que se proponga.

Por último, resaltar que se pueden obtener deducciones fiscales por actividades de investigación, desarrollo e innovación tecnológica. En caso de que el objeto del proyecto se corresponda con el de un Informe Tecnológico de Patentes (ITP) realizado por la OEPM, este podrá aportarse a la entidad certificadora acreditada por ENAC para la realización de informes técnicos relacionados con los informes motivados vinculantes que establece el Real Decreto 1432/2003 de 21 de noviembre, teniendo ésta el compromiso de realizar un descuento fijo equivalente al 50% del precio del Informe Tecnológico de Patentes. (Convenio de colaboración suscrito entre el Ministerio de Ciencia e Innovación, la Oficina Española de Patentes y Marcas y las Entidades certificadoras) Para más información puede acceder a través del siguiente enlace.

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NOTA: *El presente Informe se ha realizado con el máximo rigor, de acuerdo con una metodología consolidada y tratando de ceñirse estrechamente a las necesidades del cliente. Este Informe* ***no vincula*** *a la OEPM en lo que se refiere a los resultados que puedan obtenerse de una subsiguiente solicitud formal de registro en alguna de las modalidades de propiedad industrial.*

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Listados de referencias

Literatura Patente

1/29	@ WPI / Thomson
PN	WO2012151111A1 2012-11-08 DW201276 CA2834976A1 2012-11-08 DW201401 AU2012251027A1 2013-12-19 DW201402 EP2705165A1 2014-03-12 DW201419 US2014087954A1 2014-03-27 DW201424 CN103649335A 2014-03-19 DW201432 JP2014512838A 2014-05-29 DW201435 US8741564B2 2014-06-03 DW201436 US2014235460A1 2014-08-21 DW201455 AU2012251027B2 2015-03-26 DW201523 AU2015203545A1 2015-07-23 DW201555 CN103649335B 2015-11-25 DW201603 CA2834976C 2016-03-15 DW201622
TI	Determining sequence of target nucleic acid molecule e.g. DNA or RNA in a sample involves contacting sample with nuclease protection probe comprising flanking sequence under conditions to specifically bind to target nucleic acid molecule
PA	(HIGH-N) HIGH THROUGHPUT GENOMICS INC(HTGM-N) HTG MOLECULAR DIAGNOSTICS INC
ICAI	C12P19/34; C12Q1/68;
AB	- NOVELTY : Determining (M1) sequence of at least one target nucleic acid molecule (200) in a sample involves a) contacting sample with at least one nuclease protection probe comprising a flanking sequence (NPPF) (202) under conditions for NPPF to specifically bind to target nucleic acid molecule, where NPPF comprises a 5'-end and 3'-end; a sequence complementary to a region of target nucleic acid molecule permitting specific binding between the NPPF and the target nucleic acid molecule; and a flanking sequence located 5' and/or 3', to the sequence complementary to target nucleic acid molecule. - DETAILED DESCRIPTION : Determining (M1) a sequence of at least one target nucleic acid molecule (200) in a sample involves a) contacting the sample with at least one nuclease protection probe comprising a flanking sequence (NPPF) (202) under conditions for the NPPF to specifically bind to the target nucleic acid molecule, where the NPPF comprises: a 5'-end and a 3'-end; a sequence complementary to a region of the target nucleic acid molecule, permitting specific binding between the NPPF and the target nucleic acid molecule; a flanking sequence located 5' and/or 3', to the sequence complementary to the target nucleic acid molecule, where the flanking sequence comprises at least 12 contiguous nucleotides not found in a nucleic acid molecule present in the sample, providing a universal amplification sequence, and the flanking sequence is complementary to at least a portion of an amplification primer; b) contacting the sample with a nucleic acid molecule comprising a sequence complementary to the flanking sequence (CFS) (204) under conditions for the flanking sequence to specifically bind to the CFS; c) contacting the sample with a nuclease specific for single-stranded nucleic acid molecules under conditions to remove unbound nucleic acid molecules, thus generating a digested sample comprising NPPFs hybridized to the target nucleic acid molecule and CFSs; d) amplifying NPPFs in the digested sample with the amplification primer (208), thus generating NPPF amplicons (210), where at least one amplification primer comprises a region that is complementary to the flanking sequence of the NPPF; and e) sequencing at least a portion of the NPPF amplicons, thus determining the sequence of the at least one target nucleic acid molecule in the sample. An

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INDEPENDENT CLAIM is included for detecting (M2) at least one target nucleic acid molecule in a sample involving carrying out steps (a)-d), and detecting the NPPF amplicons, thus detecting at least one target nucleic acid molecule in the sample.
- USE : For determining a sequence of, or detecting at least one target nucleic acid molecule in a sample (claimed); to detect DNA or RNA, mutations such as gene fusions, insertions or deletions, tandem repeats, single nucleotide polymorphisms (SNPs), and DNA methylation.
- ADVANTAGE : The method sequences or detects at least one target nucleic acid molecule in a several of samples simultaneously. The method can be performed on several of samples and at least two target nucleic acid molecules are detected in each of the samples. The method greatly improves prior quantitative nuclease protection assay (qNPA) and quantitative nuclease protection sequencing (qNPS) methods and represents an improvement to current nucleic acid detection and sequencing methods. Because the methods require less processing of the target nucleic acid molecules, bias introduced by such processing can be reduced or eliminated. The methods permit one to analyze a range of sample types not otherwise amenable to detection sequencing. In addition, this results in less loss of the RNA from the sample, providing a more accurate result. It also reduces enzyme bias. The methods also provide for targeted detection and sequencing of a desired nucleic acid molecule. This greatly simplifies data analysis. The amount of data generated is simplified, as only a portion of the target needs to be detected or sequenced. Long reads of nucleotides are not required, nor do fragments of sequences need to be properly aligned to a reference sequence. In addition, the results can be simply counted, without the need for complicated bioinformatics analysis. The use of NPPF permits multiplexing, and conserves the stoichiometry of the detected or sequenced target nucleic acid molecule, because the flanking sequences on the probe permit universal primer binding sites for amplification and permit addition of sequencing adapters and experimental tags (at either the 3'- or the 5'- end, or at both ends to increase multiplexing), without destroying the stoichiometry. As the flanking sites can be universal, the same primers can be used to amplify any NPPF for any target sequence, thus allowing for multiplexing and conservation of stoichiometry. By amplifying from both ends of the NPPF, the methods provide greater specificity than prior art qNPA and qNPS methods.
- DESCRIPTION OF DRAWINGS : The figure shows a schematic diagram showing the initial steps of a method of using the nuclease protection probe comprising a flanking sequence (NPPFs) to detect or sequence a nucleic acid molecule.
200 : Target nucleic acid
202 : Nuclease protection probe comprising a flanking sequence (NPPFs)
204 : Complementary to flanking sequences (CFS)
208 : Primer
210 : Amplicons
- BIOTECHNOLOGY : Preferred Method: In the method (M1) or (M2), at least one target nucleic acid molecule is fixed, cross-linked, or insoluble; and the sample is fixed. The method sequences or detects at least two target nucleic acid molecules, where the sample is contacted with at least two different NPPFs, each NPPF specific for a different target nucleic acid molecule, and at least one NPPF is specific for a miRNA target nucleic acid molecule. The sequencing involves Solexa sequencing, 454 sequencing, chain termination sequencing, dye termination sequencing, or pyrosequencing (preferably single molecule sequencing). The methods further involve lysing the sample; comparing NPPF sequence obtained to a reference sequence database; and determining a number of each identified NPPF sequences. Detecting the NPPF amplicons involves: contacting the NPPF amplicons with a surface comprising multiple spatially discrete regions, each region comprising at least one anchor in association with a bifunctional linker, where the bifunctional linker comprises a first portion which specifically binds to the anchor and a second portion which specifically binds to at least a portion of one of the NPPF amplicons, under conditions for the NPPF amplicons to specifically bind to the second portion of the bifunctional linker; or contacting the NPPF

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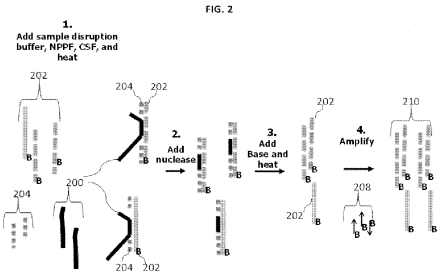

amplicons with a surface comprising multiple spatially discrete regions, each region comprising at least one nucleic acid anchor having a region complementary to at least a portion of one of the NPPF amplicons, under conditions for the NPPF amplicons to specifically bind to the nucleic acid anchor; or contacting the NPPF amplicons with a population of surfaces, where the population of surfaces comprises subpopulations of surfaces, and each subpopulation of surfaces comprises at least one anchor in association with a bifunctional linker comprising a first portion which specifically binds to at least a portion of one of the NPPF amplicons, under conditions for the NPPF amplicons to specifically bind to the second portion of the bifunctional linker; or contacting the NPPF amplicons with a population of surfaces, where the population of surfaces comprises subpopulations of surfaces, and each subpopulation of surfaces comprises at least one nucleic acid anchor having a region complementary to at least a portion of one of the NPPF amplicons, under conditions for the NPPF amplicons to specifically bind to the nucleic acid anchor. In the method, second portion of the bifunctional linker is complementary to the NPPF region complementary to the region of the target nucleic acid molecule, thus permitting specific binding between the NPPF amplicon and the bifunctional linker. Detecting the NPPF amplicons involves contacting the NPPF amplicons with avidin or streptavidin conjugated to horseradish peroxidase or alkaline phosphatase. Preferred Components: The NPPF comprises a DNA molecule having 35-150 nucleotides. The sequence complementary to a region of the target nucleic acid molecule is 10-60 nucleotides in length. The flanking sequence is 12 to 50 nucleotides in length. The NPPF comprises a flanking sequence at the 5'-end and the 3'-end, where the flanking sequence at the 5'-end differs from the flanking sequence at the 3'-end. At least one amplification primer further comprises a sequence that permits attachment of an experimental tag or sequencing adapter to the NPPF amplicon during the amplification step. The flanking sequence further comprises an experimental tag and/or sequencing adapter. The experimental tag comprises a nucleic acid sequence that permits identification of a sample, subject, treatment or target nucleic acid sequence. The sequencing adapter comprises a nucleic acid sequence that permits capture onto a sequencing platform. The experimental tag or sequence tag is present on the 5'-end or 3'-end of the NPPF amplicon. The nuclease comprises an exonuclease and/or an endonuclease. The nuclease specific for single-stranded nucleic acid molecules comprises S1 nuclease. The population of surfaces comprises a population of beads. The NPPF amplicon comprises a detectable label. The detectable label comprises a hapten, a fluorescent molecule, an enzyme, a radioisotope, or biotin. At least one NPPF comprises at least 10 NPPFs, and the sample is formalin fixed.EXAMPLE : No suitable example given.

2/29	@ WPI / Thomson
PN	WO2012055408A1 2012-05-03 DW201232 CA2815259A1 2012-05-03 DW201337 EP2633074A1 2013-09-04 DW201358 US2013230856A1 2013-09-05 DW201358 KR20130113476A 2013-10-15 DW201370 JP2013544507A 2013-12-19 DW201401 SG189513A1 2013-05-31 DW201413 CN103517991A 2014-01-15 DW201420 HK1188259A0 2014-04-25 DW201432
TI	Capturing target polynucleotide from sample, used e.g. to diagnose e.g. cancer, comprises removing a base (e.g. adenine) from target polynucleotide to generate abasic site, and capturing the target polynucleotide with a complementary probe

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ICAI C12N15/11; C12Q1/68; G01N33/53; G01N37/00;

AB - NOVELTY :

Capturing target polynucleotide from a sample, comprises: removing at least one type of base comprising adenine, thymine, uracil, cytosine or guanine, 5-hydroxymethyl-deoxycytidine, 5-methylcytosine, pseudouridine, dihydrouridine, inosine, 7-methylguanosine, hypoxanthine, xanthine or their 2'-O-methyl-derivatives and/or N-methyl-derivatives from the target polynucleotide, to generate at least one abasic site; and capturing the target polynucleotide with a complementary probe comprising at least one intercalator molecule inserted into the backbone structure of a polynucleotide probe.

- DETAILED DESCRIPTION :

Capturing target polynucleotide from a sample, comprises: removing at least one type of base comprising adenine, thymine, uracil, cytosine or guanine, 5-hydroxymethyl-deoxycytidine, 5-methylcytosine, pseudouridine, dihydrouridine, inosine, 7-methylguanosine, hypoxanthine, xanthine or their 2'-O-methyl-derivatives and/or N-methyl-derivatives from the target polynucleotide, to generate at least one abasic site; and capturing the target polynucleotide with a complementary probe comprising at least one intercalator molecule inserted into the backbone structure of a polynucleotide probe, where the intercalator molecule(s) fits morphologically into the abasic sites of a complementary polynucleotide target sequence. The target polynucleotide may be made of naturally occurring nucleotides and/or of nucleotides which are not known to occur naturally, where the target polynucleotide may thus e.g. be made of nucleotides (N1) comprising RNA, alpha -L-RNA, beta -D-RNA, 2'-R-RNA, DNA, locked nucleic acid (LNA), peptide nucleic acid (PNA), phosphorodiamidate morpholino oligo (PMO), threose nucleic acid (TNA), glycol nucleic acid (GNA), oligonucleotide N3'-P5' phosphoramidates, bicyclic nucleic acid (BNA), alpha -L-LNA, hexitol nucleic acid (HNA), mannitol nucleic acid (MNA), altritol nucleic acid (ANA), CAN, INA, cyclohexenyl nucleic acid (CeNA), (2'-NH)-TNA, (3'-NH)-TNA, alpha -L-ribo-LNA, alpha -L-xylo-LNA, beta -D-ribo-LNA, beta -D-xylo-LNA, [3.2.1]-LNA, bicyclo-DNA, 6-amino-bicyclo-DNA, 5-epi-bicyclo-DNA, alpha -bicyclo-DNA, tricyclo-DNA, bicyclo[4.3.0]-DNA, bicyclo[3.2.1]-DNA, bicyclo[4.3.0]amide-DNA, beta -D-ribopyranosyl-NA, alpha -L-lyxopyranosyl-NA, 2'-OR-RNA and/or 2-AE-RNA. INDEPENDENT CLAIMS are also included for:

(1) diagnosing at least one disease comprising using the above method;

(2) a polynucleotide probe suitable for interaction with a polynucleotide target, where the polynucleotide probe comprises exactly 1 intercalator molecule; and

(3) a polynucleotide probe suitable for interaction with a complementary polynucleotide target, where the polynucleotide probe comprises at least two intercalator molecules.

- USE :

The method is useful: for capturing target polynucleotide from a sample, for diagnosing a disease, which is a genetic i.e. hereditary disease comprising cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) syndrome, multiple, late-onset carboxylase deficiency, familial cerebelloretinal angiomatosis, fibrostenosing Crohn's disease, phenylalanine hydroxylase deficiency disease, Fabry's disease, hereditary coproporphyria, incontinentia pigmenti, microcephaly, polycystic kidney disease, Siderius X-linked mental retardation syndrome caused by mutations in the plant homeo domain finger protein 8 gene or achondroplasia, cancer, infectious disease, headache or a fetus disorder; for quantitation of target RNA (i.e. quantitative RNA analysis); for detecting at least one target polynucleotide from a sample (feed, soil, food or drinking water); in prenatal diagnosis; in monitoring antisense therapy; for identifying familial relatives; in personalized medicine and forensic genetics; for detection of microorganisms; and in archaeology and paleopathology, food contamination and environmental pollution (all claimed).

- ADVANTAGE :

The method: utilizes the complementary probe comprising intercalator molecule, which increases the stability of the target polynucleotide duplex structure; and is capable of capturing 1-1000 and/or more than 1000 different target polynucleotide sequences.

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- BIOTECHNOLOGY :
Preferred Method: The capturing method comprises: providing double stranded target polynucleotide which may be made of naturally occurring nucleotides and/or nucleotides which are not known to occur naturally e.g. DNA; destabilizing the double stranded target polynucleotide by removing the base from it, to generate abasic site; denaturing the destabilized double stranded target polynucleotide to single stranded target polynucleotide; and capturing the single stranded target polynucleotide with a complementary polynucleotide probe, e.g. a DNA probe, comprising the intercalator molecule inserted into the backbone-structure of a polynucleotide probe fitting morphologically into the abasic site of the complementary polynucleotide target sequence. The capturing method further comprises: at least one washing step to remove unbound nucleotide material which may be made of naturally occurring nucleotides and/or nucleotides which are not known to occur in nature; converting the base in the double stranded target polynucleotide to another chemical entity; destabilizing the double stranded target polynucleotide by removing at least one chemical entity from it; converting at least one cytosine in the target polynucleotide to at least one uracil, where the conversion of cytosine to uracil is preformed by bisulfite treatment; using a complementary detection probe comprising at least one label; and a washing step prior to and/or after addition of the detection probe. In the capturing method: adenine, thymine, cytosine, guanine or uracil is removed from the double stranded target polynucleotide and/or single stranded polynucleotide by at least one enzyme, physical stress and/or temperature change, where the removal of uracil is performed by using uracil dehydrogenase, and the removal of adenine is performed by adjustment of the pH value; 1-3 types of bases are removed from the target polynucleotide; and the total number of bases that are removed from the target polynucleotide is 1-20 or more than 20. The diagnosis comprises detecting: target polynucleotide from the genome of individual that is tested; target polynucleotide which is not derived from the genome of the individual that is tested; or target polynucleotide from bacteria, virus, fungus, prions, protozoa and/or plant. In the diagnosing method, the disease to be diagnosed is at least one genetic i.e. hereditary disease, cancer (preferred), infectious disease, headache or other disease, where the cancer is characterized by: at least one mutation in at least one gene encoding the proteins comprising e.g. tumor suppressor protein 101F6, active breakpoint cluster region-related protein, adenosine diphosphate ribosyltransferase-like 3, acidic (leucine-rich) nuclear phosphoprotein 32 family, member C (ANP32C), ANP32D, adenomatosis polyposis coli 2 (APC2), APC, rho-guanosine triphosphatase (GTP) activating protein 8, aplasia Ras homolog member I, Ataxia telangiectasia mutated, ATPase aminophospholipid transporter class I type 8A member 2, beclin-1, bridging integrator 1, breast cancer 1, early onset (BRCA1), BRCA2, B-cell translocation gene 1 (BTG1), BTG2, adenylate cyclase-associated protein 1, cysteinyl-transfer RNA synthetase, caveolin 1, cluster of differentiation 81, cell division cycle 23 homolog, cyclin-dependent kinase (CDK) 2 associated protein 1, CDK inhibitor 1A, CDK inhibitor 1C, CDK inhibitor 2C, CDK inhibitor 2B, calcium-activated chloride channel regulator 2, cyclic adenosine monophosphate responsive element binding protein-like 2, catenin (cadherin-associated protein) alpha 1, cullin 2, disabled homolog 2, deleted in breast cancer 2, deleted in colorectal carcinoma, deleted in esophageal cancer 1, deleted in liver cancer 1, deleted in lung and esophageal cancer 1, deleted in lymphocytic leukemia (DLEU)1, DLEU2, disks large homolog 1, deleted in malignant brain tumors 1, early growth response 1, fatty acid binding protein 3, fibrinogen-like 1, fragile histidine triad, forkhead box (FOX) D1, FOXP1, cyclin G-associated kinase, growth arrest-specific (GAS)-1 and GAS11; at least one tumor antigen comprising alpha -actinin-4, v-raf murine sarcoma viral oncogene homolog B1, caspase 5 apoptosis-related cysteine peptidase (CASP-5), CASP-8, beta -catenin, cell division cycle 27, CDK4, CDK inhibitor 2A, human acetyl-CoA carboxylase 1, dek-can fusion protein, elongation factor Tu GTP binding domain containing 2, elongation factor 2, internal tandem duplications of fms-like tyrosine kinase 3 (FLT3-ITD), fibronectin 1, glycoprotein NMB (GPNMB), low density lipoprotein receptor-fucosyl transferase AS fusion protein, human leukocyte antigen (HLA)-A2(d),

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HLA-A11(d), heat shock 70kD protein 2, homolog of melanoma antigen recognized by T cells 2 (MART2), malic enzyme 1, melanoma associated antigen (mutated) (MUM)-1 MUM-2, MUM-3, polynucleotide adenylyltransferase gamma (neo-PAP), myosin class I, nuclear transcription factor Y, gamma (NFYC), OGT, osteosarcoma amplified 9 endoplasmic reticulum lectin (OS-9), protein 53, pml-RARalpha fusion 5 protein, peroxiredoxin 5, receptor-type tyrosine-protein phosphatase kappa, K-ras, N-ras, retinoblastoma protein associated factor 600, sirtuin-2, small nuclear ribonucleoprotein D1, triosephosphate isomerase, B melanoma antigen 1 (BAGE-1), kitakyushu lung cancer antigen 1, coiled-coil domain-containing protein 110 (KM-HN-1), melanoma-associated antigen 1 (MAGE-A1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-A10 and MAGE-A12; or at least one e mutation in at least one gene, which is ras association domain family member 2 (RASSF2) or secreted frizzled-related protein 2, or tissue factor pathway inhibitor 2, N-myc downstream regulated gene family member 4, GATA4 or GATA5. Preferred Components: The complementary probe is made of polynucleotide which may be made of naturally occurring nucleotides and/or nucleotides which are not known to occur in nature, where the complementary probe may thus e.g. be made of above nucleotides (N1). The total number of intercalator molecules in the complementary probe is 1-20, or more than 20. The intercalator molecule has been inserted into 10-100% of the abasic sites in the target DNA and/or RNA, where the insertion of the intercalator molecules results in increased melting temperature of the polynucleotide duplex comprising the target polynucleotide and the complementary probe. The ratio of intercalator molecules to the total number of bases in the complementary probe is 1:50-1:2, including 1:50-1:40, 1:40-1:30, 1:30-1:20, 1:20-1:10, 1:10-1:5 and/or 1:5-1:2. The complementary probe: comprises more than one type of intercalator molecules, preferably 2-5 or more than 5 different types of intercalator molecules; and is connected to a support. The support is a solid support comprising microtiter plate or other plate formats, reagent tubes, glass slides or other supports for use in array or microarray analysis, tubings or channels of micro fluidic chambers or devices and Biacore(RTM: Optical biosensor) chips. The label is biotin, a fluorescent label, 5-(and 6)-carboxy fluorescein, 5-or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate, rhodamine, tetramethylrhodamine, dyes, cyanine 2, cyanine 3 and cyanine 5, peridinin chlorophyll protein (PerCP), phycobiliproteins, R-phycoerythrin, allophycoerythrin, Texas red, Princeton red, green fluorescent protein or thier analogs, conjugates of R-phycoerythrin or allophycoerythrin, inorganic fluorescent labels based on semiconductor nanocrystals (quantum dot and Qdot(RTM: Fluorophore) nanocrystals), time-resolved fluorescent labels based on lanthanides like europium(III) and samarium(III), haptens, dinitrophenol, digoxigenin, enzymic labels, horse radish peroxidase, alkaline phosphatase, beta -galactosidase, glucose-6-phosphate dehydrogenase, beta -N-acetylglucosaminidase, (beta -glucuronidase, invertase, xanthine oxidase, firefly luciferase and glucose oxidase, luminescence labels, luminol, isoluminol, acridinium esters, 1,2-dioxetanes, pyridopyridazines, radioactivity labels, isotopes of iodide, isotopes of cobalt, isotopes of selenium, isotopes of tritium, or isotopes of phosphorus. The biotin is detected by use of streptavidin-R-phycoerythrin. The complementary detection probe comprises at least one intercalator molecule, where the total number of intercalator molecules is 1-20 or more than 20 different or identical intercalator molecules. The target polynucleotide (RNA and/or DNA) is: derived from a human, an animal, bacteria, virus, fungus, prions, protozoa and/or plant; isolate from a sample from a human or animal body; isolate from humans, animals, birds, insects, plants, algae, fungi, yeast, viruses, bacteria and phages, multi-cellular and mono-cellular organisms, preferably isolate from feces, blood, semen, cerebrospinal fluid, sputum, vaginal fluid, urine, saliva, hair, other bodily fluids, tissue samples, whole organs, sweat, tears, skin cells, hair, bone, teeth or appropriate fluid or tissue from personal items (e.g. toothbrush and razor) or from samples (e.g. sperm or biopsy tissue or liquid) or other substructures of humans or animals, where the bacteria is e.g. Acinetobacter baumannii, Actinomyces canis, Bacillus brevis, Bordetella avium, Chlamydia trachomatis, Clostridium botulinum,

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Corynebacterium parvum, Delftia acidovorans, Dickeya sp., Eikenella corrodens, Enterobacter cloacae, Flavobacterium aquatile, Fusobacterium nucleatum, Gardnerella vaginalis, Gemella morbillorum, Haemophilus avium, Hafnia alvei, Klebsiella mobilis, Kingella kingae, Lactobacillus agilis, Legionella anisa, Leuconostoc ficulneum, Microbacterium multiforme, Mycoplasma pneumoniae, Micrococcus luteus, Moraxella caprae, Neisseria meningitidis, Nocardia brasiliensis, Obesumbacterium, Oxalobacter formigenes, Pantoea ananatis, Pasteurella ureae, Pseudomonas septica, Proteus mirabilis, Ralstonia basilensis, Rickettsia typhi, Salmonella enterica, Shigella flexneri, Streptococcus pyogenes, Treponema carateum, Tropheryma whipplei, Tatumella, Ureaplasma urealyticum, Vibrio cholerae, Vogesella indigofera, Wigglesworthia sp., Wolbachia sp., Xenorhabdus sp., Yersinia enterocoliticaand Yokenella sp., and the virus is Abelson murine leukemia virus, adenovirus, baculovirus, borna virus, bunyavirus, calicivirus, camelpox virus, Cardiovirus, Densovirus, duck hepatitis B virus, encephalitis virus, endogenous retrovirus, Epstein-Barr virus, Flavivirus, feline parvovirus, Furovirus, geminivirus, goatpox virus, Guanarito virus, hepatitis A virus, herpes virus, human foamy virus, ichnovirus, Ilarvirus, iridovirus, Japanese B virus, Junin virus, Kaposi's sarcoma-associated herpesvirus, Kemerovo virus, Lassa fever virus, leukovirus, Mapuera virus, Mayaro virus, Nariva virus, nuclear polyhedrosis virus, oncornavirus, papovavirus, picornavirus, Qalyub virus, Queensland fruitfly virus, rabies virus, Riley's virus, San Perlita virus, Shuni virus, tobacco rattle virus, ulcerative disease rhabdovirus, variola virus, West Nile virus, Xingu virus, yellow fever virus and zika virus. The polynucleotide probe is made of nucleotides (N1), preferably a DNA probe, a RNA probe, a LNA probe or a PNA probe.

[Image]ORGANIC CHEMISTRY :

Preferred Components: The intercalator molecule: is twisted intercalating nucleic acid (TINA), INA, ortho-TINA, para-TINA or di-DNA substituted 2-phenyl-1H-phenanthro[9,10-d]imidazole compound of formula (I) (AMANY); and has a size of 20-400 Å (20-40 Å , 40-60 Å , 60-80 Å , 80-100 Å , 100-120 Å , 120-140 Å , 140-160 Å , 160-180 Å , 180-200 Å , 200-220 Å , 220-240 Å , 240-260 Å , 260-280 Å , 280-300 Å , 300-320 Å , 320-340 Å , 340-360 Å , 360-380 Å and/or 380-400 Å). The intercalating unit of the intercalator preferably comprises a chemical group comprising polyaromates and heteropolyaromates, where the polyaromates or heteropolyaromates comprises at least 2 aromatic rings, preferably more than 8 aromatic rings. The heteropolyaromates contain: at least one aromatic ring in which at least one carbon atom is replaced by a heteroatom comprising nitrogen or oxygen; at least 2 heteroatoms, preferably more than 5 heteroatoms; and nitrogen and/or oxygen as the heteroatoms. The polyaromates or heteropolyaromates are substituted with at least one substituent comprising hydroxy, halo, mercapto, thio, cyano, alkylthio, heterocyclyl, aryl, heteroaryl, carboxy, carboalkoyl, alkyl, alkenyl, alkynyl, nitro, amino, alkoxy and amido, or two adjacent substituents may together form N=C-CH or C=C. The intercalating unit of the intercalator is polyaromate or heteropolyaromate capable of increasing the stability of the polynucleotide duplex structure, where the intercalating unit: comprises bi-cyclic aromatic ring systems, tricyclic aromatic ring systems, tetracyclic aromatic ring systems, pentacyclic aromatic ring systems or their heteroaromatic analogs or substitutions, preferably phenanthroline, phenazine, phenanthridine, pyrene, anthracene, naphthalene, phenanthrene, picene, chrysene, naphthacene, benzanthracene, stilbene or porphyrin, phenanthroimidazole or naphthalimide; or is a modified nucleobase, which is MPyU, AMPyU, oxo-pyrene labeled uracil (oxoPyU) or their analogs where uracil (U) is replaced with any of the other nucleobases.POLYMERS :

Preferred Components: The support is polyether ether ketone, polypropylene, polyethylene, polyethylene terephthalate, PVC, polyamide/nylon, polycarbonate, cyclic olefin copolymer, filter paper, cotton, cellulose, poly(4-vinylbenzyl chloride), polyvinylidene fluoride, polystyrene, Toyopearl(RTM: Chromatography resin), hydrogels, polyimide, 1,2-polybutadiene, liquid silicon rubber, polydimethylsiloxane,

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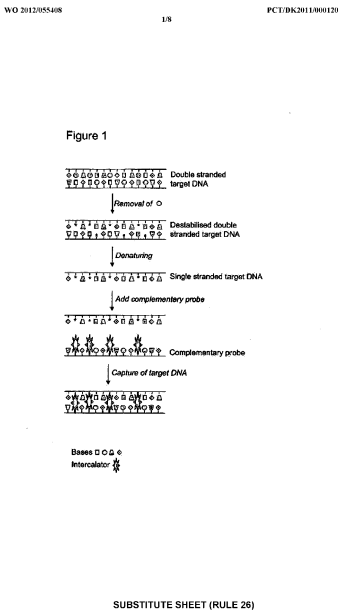
fluoropolymers and copolymers (e.g. polytetrafluoroethylene, perfluoroethylene propylene copolymer and ethylene tetrafluoroethylene), poly(methyl methacrylate), nanoporous materials, membranes, mesostructured cellular foam, single wall or multiwall carbon nanotubes, particulate matters, beads, magnetic beads, non-magnetic beads, polystyrene beads, magnetic polystyrene beads, Sepharose(RTM: Crosslinked polysaccharide polymer material extracted from seaweed) beads, Sephacryl(RTM: Covalently crosslinked allyl dextrose gel) beads, polystyrene beads, agarose beads, polysaccharide beads, or polycarbamate beads.



3/29	@ WPI / Thomson
PN	CN101870980A 2010-10-27 DW201082 CN101870980B 2013-05-29 DW201367
TI	New Asia type 1 foot and mouth disease virus 3A anti-structure protein epitope deletion infectious complementary DNA (cDNA), useful for diagnosing natural infectious animal
PA	(UYNE-N) UNIV NORTHEAST AGRIC
ICAI	C12N15/09; C12N15/42; C12N15/66;
AB	- NOVELTY : An Asia type 1 foot and mouth disease virus 3A anti-structure protein epitope deletion infectious complementary DNA (cDNA) comprising fully defined 8154 bp (SEQ ID NO. 1) given in the specification, is new. - DETAILED DESCRIPTION : An INDEPENDENT CLAIM is a method for producing Asia type 1 foot and mouth disease virus 3A anti-structure protein epitope deletion infectious cDNA. - USE : The cDNA is useful for diagnosing natural infectious animal. - ADVANTAGE : The invention solves the problem that it is difficult to monitor the current inactivated vaccine purification process, and the residual anti-structure protein may interfere the diagnosis to the natural infectious animal, and thus, the accuracy of diagnosis may be reduced. - BIOTECHNOLOGY : Preparation (claimed): Producing Asia type 1 foot and mouth disease virus 3A anti-structure protein epitope deletion infectious cDNA comprises: (a) cloning 3A14L and 3A14R of 91-104-bit amino acid of 3A gene from pBSAs overall-length recombinant plasmid to obtain 3A14L PCR product and 3A14R PCR product, and then blending PCR and recycling the blended products; and (b) performing double digestion for the pBSAs overall-length recombinant plasmid and blended products by using EcoRT22I and SmaI in several steps, and then connecting and constructing the recombinant plasmid pBSAs-3A14D to finish the construction of Asia type 1 foot and mouth disease virus 3A anti-structure protein epitope deletion infectious cDNA. Cloning 3A14L of 91-104-bit amino acid of 3A gene uses the following reaction system with the following components: 3 mu l of 500x of the pBSAs overall-length recombinant plasmid that are diluted and used as a template, 1 mu l of 20 microns of primer L1 5'-tcatcaaactcctgagcccgc-3' (SEQ ID NO. 2), 1 mu l of 20 microns of primer comprising fully defined 39 bp (SEQ ID NO. 3) given in

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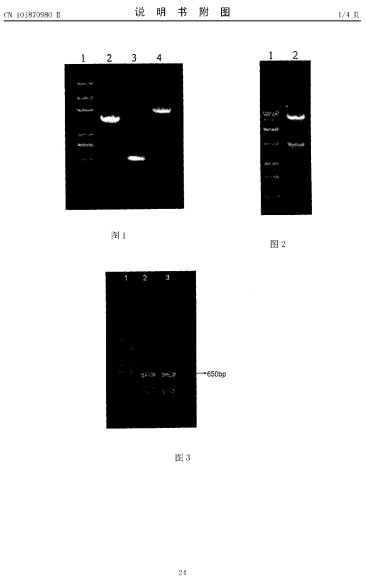

the specification, 10 mu l of 5x PrimeSTAR Buffer, 4 mu l of 2.5mM deoxynucleotide triphosphate (dNTP), 0.5 mu l of PrimeSTAR HS DNA polymerase, and distilled water (dH 2O) that is used to compensate to 50 mu l; and the condition is as follows: denaturation is performed at the temperature of 94[deg] C for 3 minutes, 94[deg] C of 20 seconds, 57[deg] C for 30 seconds, and 72[deg] C for 1 minute, and 25 circles are included, and the reactant is extended for 7 minutes at the temperature of 72[deg] C. Cloning 3A14R of 91-104-bit amino acid of 3A gene uses the following reaction system with the following components: 3 mu l of 500x of the pBSAs overall-length recombinant plasmid that are diluted and used as a template, 1 mu l of 20 microns of primer L1 comprising fully defined 39 bp (SEQ ID NO. 4) given in the specification, 1 mu l of 20 microns of primer R2 5'-tgacgaggcacgaggtaggc-3' (SEQ ID NO. 5), 10 mu l of 5X PrimeSTAR Buffer, 4 mu l of 2.5 mM dNTP, 0.5 mu l of PrimeSTAR HS DNA polymerase, and Dh 2O that is used to compensate to 50 mu l; and the condition is as follows: denaturation is performed at the temperature of 94[deg] C for 3 minutes, 94[deg] C of 20 seconds, 57[deg] C for 30 seconds, and 72[deg] C for 1 minute, and 25 circles are included, and the reactant is extended for 7 minutes at the temperature of 72[deg] C. Blending PCR in (a) is performed in two steps, the first step of blending PCR comprises the following reaction system with the following components: 10 mu l of 5xPrimeSTAR Buffer, 4mu l of 2.5 mM dNTP, 5 mu l of 3A14L PCR products, 5 mu l of 3A14R PCR products, and 0.5 mu l of PrimeSTAR HS DNA polymerase, and the condition is as follows: denaturation is performed at the temperature of 94[deg] C for 3 minutes, 94[deg] C of 20 seconds, 50[deg] C for 30 seconds, and 72[deg] C for 1 minute, and 10 circles are included, and the reactant is extended for 7min at the temperature of 72[deg] C; the second step of blending PCR comprises a reaction system with the following components: 5 mu l of 10x ExTaqBuffer, 4 mu l of 2.5 mM dNTP, 1 mu l of 20 mu M of primer L1, 1 mu l of 20 mu M primer R2, 3 mu l of the PCR products in (a), 0.5 mu l of ExTaqDNA polymerase, and dH 2O that is used to compensate the reactant to 50 mu l; and the condition is performed at the temperature of 94[deg] C for 3 minutes, 94[deg] C of 15 seconds, 60[deg] C for 30 seconds, and 72[deg] C for 2 minutes, and 25 circles are included, and the reactant is extended for 7 minutes at the temperature of 72[deg] C and then stored at 4[deg] C. The system of the double digestion reaction in step two comprises the following system: 30.0 mu l of pBSAs overall-length recombinant plasmid, 2.5 mu l of EcRT22I, 5 mu l of 10xH Buffer, and 12.5 mu l of sterile water. The system of the double digestion reaction in step two comprises the following system: 30.0 mu l of pBSAs overall-length recombinant plasmid EcoRT22I digestion recycled products, 2.5 mu l of SmaI, 5 mu l of 10xT Buffer, 5 mu l of bovine serum albumin (BSA), and 7.5 mu l of sterile water. The system of the double digestion reaction in step two comprises the following system: 30.0 mu l of blended products, 2.5 mu l of EcoRt22I, 5 mu l of 10xH Buffer, and 12.5 mu l of sterile water. The system of the double digestion reaction in step two comprises the following system: 30.0 mu l of blended product EcoRT22I double digestion reaction recycled products, 2.5 mu l of SmaI, 5 mu l of BSA, and 7.5 mu l of sterile water. Connecting system in (b) is as follows: 1.0 mu l of 10xT4 DNA connecting enzyme buffer solution, 6.0 mu l of blended products after double digestion, 2.0 mu l of pBSAs overall-length recombinant plasmid after double digestion, and 1.0 mu l of T4 DNA connecting enzyme.

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PN	US2007065814A1 2007-03-22 DW200731 EP1767657A1 2007-03-28 DW200731 WO2007038117A2 2007-04-05 DW200731 WO2007038117A3 2007-08-02 DW201229
TI	New composition comprises mixture comprising primer pairs capable of amplifying a sequence present in a foot-and-mouth disease virus, useful for determining whether or not an animal contains a foot-and-mouth disease virus
PA	(ENGE-I) ENGELHARD E K(FAIR-N) FAIR ISAAC CORP
ICAI	A61K39/125; C12M1/34; C12Q1/68; C12Q1/70;
AB	- NOVELTY : A composition comprising a mixture, where the mixture comprises at least one primer pair selected from primer pair numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, where the primer pair is capable of amplifying a sequence present in a foot-and-mouth disease virus, is new. - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are: (1) an article of manufacture comprising (a) a substrate comprising a microfluidic chamber, and (b) a mixture comprising at least one primer pair selected from primer pair numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, where the mixture is within the chamber, where the primer pair is capable of amplifying, within the chamber, a sequence present in a foot-and-mouth disease virus; (2) a diagnostic card for determining whether or not a cow contains a foot-and-mouth disease virus, where the card comprises microfluidic chambers, where at least one of the microfluidic chambers comprises at least one primer pair selected from primer pair number 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, which are capable of amplifying, within the chamber, a sequence present in a foot-and-mouth disease virus; (3) a method for determining whether or not a cloven-hooved animal contains a foot-and-mouth disease virus; and (4) a method for making an article of manufacture for determining whether or not a cloven-hooved animal contains a foot-and-mouth disease virus. - USE : The composition and methods are useful for determining whether or not an animal contains a foot-and-mouth disease virus. - BIOTECHNOLOGY : Preferred Composition: In the composition above, the mixture is a solid or a liquid. Preferred Method: Determining whether or not a cloven-hooved animal contains a foot-and-mouth disease virus comprises performing an amplification reaction with at least one primer pair selected from primer pair numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 to determine whether or not a sample from the animal contains nucleic acid capable of being amplified with the primer pair, where the presence of the nucleic acid indicates that the animal contains a foot-and-mouth disease virus. The animal is a cow, and the sample is a blood sample. Making an article of manufacture for determining whether or not a cloven-hooved animal contains a foot-and-mouth disease virus comprises providing a substrate defining a microfluidic chamber, and (b) placing a mixture into the chamber to form the article of manufacture, where the mixture comprises at least one primer pair selected from primer pair numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, where the mixture is within the chamber, where the primer pair is capable of amplifying, within the chamber, a sequence present in a foot-and-mouth disease virus.SPECIFIC SEQUENCES : The primer pair numbers 1-10 comprises: Primer pair number 1: comprising 27 and 25 bp (SEQ ID NO. 1 and 2), Primer pair number 2: comprising 25 and 26 bp (SEQ ID NO. 3 and 4), Primer pair number 3: comprising 25 bp (SEQ ID NO. 3 and 5), Primer pair number 4: comprising 26 and 25 bp (SEQ ID NO. 6 and 7), Primer pair number 5: comprising 25 bp (SEQ ID NO. 8 and 7), Primer pair number 6: comprising 25 bp (SEQ ID NO. 9 and 7), Primer pair number 7: comprising 25 and 24 bp (SEQ ID NO. 10 and 11), Primer pair number 8: comprising 24 and 27 bp (SEQ ID NO. 12 and 13), Primer pair number 9: comprising 24 and 27 bp (SEQ ID NO. 12 and 14), Primer pair number 10:

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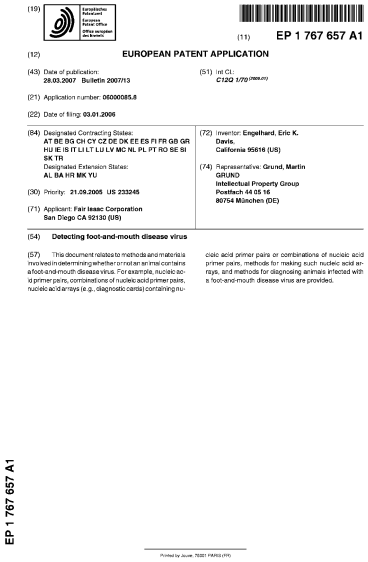
comprising 24and 26 bp (SEQ ID NO. 12 and 15) (disclosed).EXAMPLE :
No example given.



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 767 657 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
28.03.2007 Bulletin 2007/13

(51) Int Cl.:
C12Q 1/70 (2006.01)

(21) Application number: 06000085.8

(22) Date of filing: 03.01.2006

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR
Designated Extension States:
AL BA HR MK YU

(30) Priority: 21.09.2005 US 233245

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(54) Detecting foot-and-mouth disease virus

(57) This document relates to methods and materials involved in determining whether or not an animal contains a foot-and-mouth disease virus. For example, nucleic acid primer pairs, combinations of nucleic acid primer pairs, nucleic acid arrays (e.g., diagnostic cards) containing nucleic acid primer pairs or combinations of nucleic acid primer pairs, methods for making such nucleic acid arrays, and methods for diagnosing animals infected with a foot-and-mouth disease virus are provided.

EP 1 767 657 A1

Printed by Jouve, 75001 PARIS (FR)

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PN	WO2006024542A1 2006-03-09 DW200622 EP1650308A1 2006-04-26 DW200628 US2006165723A1 2006-07-27 DW200650 EP1789566A1 2007-05-30 DW200735 AU2005279303A1 2007-03-15 DW200759 BRPI0514911A 2008-06-24 DW200845 AU2005279303B2 2011-10-27 DW201173 AU2011236056A1 2011-11-03 DW201175
TI	Novel nucleic acid comprising sequences from a replication competent transmissible gastroenteritis virus, and sequences encoding proteins of different virus, useful for preparing vaccine protecting human against disease caused by virus
PA	(CNSJ) CONSEJO SUPERIOR INVESTIGACIONES CIENTIF(FORT-N) FORT DODGE VETERINARIA SA
ICAI	A61K39/15; A61K39/395; C07H21/04; C07K14/005; C07K14/14; C07K14/165; C07K14/17; C12N15/86; C12P21/06; C12Q1/70;
AB	- NOVELTY : A nucleic acid (I) comprising sequences of a replication competent transmissible gastroenteritis virus (TGEV), and sequences encoding one or more proteins of a different virus, where the one or more proteins are capable of associating into a virus-like particle (VLP) that does not contain any infectious nucleic acid, is new. - DETAILED DESCRIPTION : A nucleic acid (I) comprising: (a) sequences of a replication competent transmissible gastroenteritis virus (TGEV), where sequences encode a TGEV replicase under the control of expression regulatory sequences such that expression of the replicase in a cell containing the nucleic acid will initiate replication of the nucleic acid, and thus increase the number of nucleic acids in the cell; and (b) sequences encoding one or more proteins of a different virus, where the one or more proteins are capable of associating into a virus-like particle (VLP) that does not contain any infectious nucleic acid. (I) preferably comprises: (i) sequences of a replication competent but non-infectious TGEV, or sequences of a replication competent and infectious TGEV, which encode a TGEV replicase under the control of expression regulatory sequences such that expression of the replicase in a cell containing the nucleic acid will initiate replication of the nucleic acid, and thus increase the number of nucleic acids in the cell and a sequence encoding the TGEV N protein;

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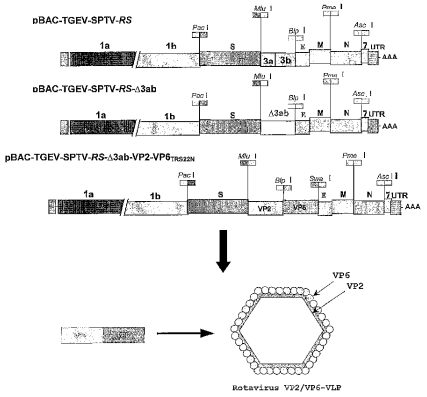
and at least two of the rotavirus sequences including VP2, VP4, VP6 and VP7, having 4 fully defined 2643, 2328, 1194 and 894 nucleotide (SEQ ID No. 1-4) sequences given in the specification, respectively, or sequences having a homology of at least 60% to SEQ ID No. 1-4; or
(ii) sequences of a replication competent but non-infectious TGEV, or sequences of a replication competent and infectious TGEV, which encode a TGEV replicase under the control of expression regulatory sequences such that expression of the replicase in a cell containing the nucleic acid will initiate replication of the nucleic acid, and thus increase the number of nucleic acids in the cell, and a sequence encoding the TGEV N protein; and two or all of the severe acute respiratory syndrome-coronavirus (SARS-CoV) sequences including S, M and E, having 3 fully defined 3768, 666 and 231 nucleotide (SEQ ID No. 17-19) sequences given in the specification, respectively, or sequences having a homology of at least 60% to SEQ ID No. 17-19.
INDEPENDENT CLAIMS are also included for:
(1) recombinant RNA (II) encoded by (I);
(2) vector (V1) comprising (I) or (II);
(3) a host cell (III) comprising V1;
(4) virus particle (IV) comprising (I) and at least one TGEV coat protein, where (IV) is preferably the virus particle deposited under CNCM I-3289;
(5) preparing virus-like particles that do not contain any infectious nucleic acid, involves expressing (I) in a host cell in cell culture, and isolating the virus-like particles from the medium and/or from the host cells;
(6) a pharmaceutical preparation (C1) comprising (I)-(IV) or V1;
(7) vaccine (V) capable of protecting an animal or a human against a disease caused by an infectious virus, comprising (I)-(IV) or V1;
(8) vaccine (VI) capable of protecting a human against a disease caused by rotavirus or SARS-CoV infection, comprising a virus particle, which comprises (I) and at least one or all of the TGEV coat proteins;
(9) a vaccine (VII) capable of protecting an animal against foot and mouth disease, comprising a virus particle, which comprises (I) and at least one or all of the TGEV coat proteins; and
(10) diagnosing whether an animal or a human is infected with a virus or has been vaccinated using (V)-(VII), involves utilizing antibodies specific for proteins of the wild-type virus but not expressed by the vaccine strain.ACTIVITY :
Virucide.
No biological data given.MECHANISM OF ACTION :
Vaccine (claimed).
- USE :
(I) is useful for preparing a vaccine capable of protecting an animal or a human against a disease caused by an infectious virus.
(V) is useful for protecting an animal or a human against a disease caused by an infectious virus, and is suitable for vaccinating an animal such as human, ruminant, swine or bird.
(V)-(VII) is useful for inducing both a systemic immune response and a mucosal immune response against infectious viral agents. The infectious agent is rotavirus, PCV, SARS virus, FMDV or TGEV.
(V)-(VII) is useful for protecting animals against viral infection (claimed).
- BIOTECHNOLOGY :
Preparation: (I) is obtained by standard recombinant methods.
Preferred Nucleic Acid: (I) encodes a TGEV replicase and a sequence encoding the TGEV N protein. (I) further includes a fully defined 534 nucleotide (SEQ ID No. 16) sequence given in the specification, or a sequence having a homology of at least 60% to SEQ ID No. 16. The replication competent TGEV vector is not infectious, or is infectious.
(I) further comprises one or more of TGEV genes including S, E, M and/or N or sequences having a homology of at least 60% to the given sequence. The TGEV infectious viral particles obtainable from the association of TGEV proteins and the nucleic

(Volver al Sumario)



acid sequences are attenuated viral particles. The nucleic acid sequences as mentioned in (b) are derived from any virus, which is not foot and mouth disease virus (FMDV). The VLPs are VLPs of rotavirus, SARS virus, porcine circovirus (PCV), FMDV or parvovirus that are capable of generating an immune response in a mammal. The nucleic acid sequences encoding rotavirus proteins are of human and/or animal origin and comprises sequences encoding at least two of the proteins including VP2, VP4, VP6 and VP7, having SEQ ID No. 1-4, or sequences having a homology of at least 60% to the SEQ ID No. 1-4. The nucleic acid sequences encoding rotavirus proteins further comprise sequences encoding fusion proteins. The nucleic acid sequence encoding a rotavirus VP8-VP2 fusion protein has a fully defined 3105 nucleotide (SEQ ID No. 5) sequence given in the specification or a sequence having a homology of at least 60% to SEQ ID No. 5. The nucleic acid sequences encode FMDV proteins comprising sequences encoding at least two of the proteins including VP1, VP2, VP3, VP4 or 3C, or sequences having a homology of at least 60% to the sequences.

(I) further comprises sequences encoding FMDV protein 3D or sequences having a homology of at least 60% to the sequence. The nucleotide sequence encoding the FMDV polymerase 3D gene is truncated. The nucleotide sequence encoding the FMDV polymerase 3D is truncated at the 5' end. The nucleic acid sequences encoding FMDV proteins comprise sequences encoding the FMDV polyprotein P1 (VP4, VP3, VP2 and VP1) and the 3C protein, or sequences having a homology of at least 60% to the sequences. The nucleic acid sequences encoding FMDV proteins VP1, VP2, VP3 and VP4 are expressed in the form of a polyprotein, which optionally further comprises protein 3D. The proteins VP1, VP2, VP3 and VP4 and optionally protein 3D are expressed in the form of a polyprotein under the control of a strong promoter, preferably a promoter comprising the natural promoter of the FMDV 3a gene which comprises a fully defined 109 nucleotide (SEQ ID No. 20) sequence given in the specification. The sequence encoding FMDV protease 3C is expressed under the control of a weak promoter, preferably under the control of the synthetic 22N promoter comprising a fully defined 31 nucleotide (SEQ ID No. 21) sequence given in the specification. The sequences encoding FMDV proteins are sequences derived from FMDV serotypes O (isolate O PanAsia) or C (isolate C-Sta.Pau/Sp70). The nucleotide sequence encoding FMDV serotype C capsid protein VP1 is modified to obtain proteins with modified amino acid residues at position 140-160. (I) is DNA or RNA.

Preferred Vector: V1 is cDNA vector or is BAC-TGEV <FL> vector. V1 is capable of replicating (I) or (II) within a host cell.

Preferred Host Cell: (III) is a bacterial cell, yeast cell, insect cell, animal cell or human cell. (III) is a porcine swine testis cell line, such as the cell line deposited under ATCC CRL-1746.

Preferred Virus Particle: (IV) comprises all TGEV coat proteins of the native TGEV virus particle.

Preferred Pharmaceutical: C1 or (V) further comprises a carrier, excipient and/or adjuvants.ADMINISTRATION :

(V)-(VII) is administered by intramuscular, intravenous or oronasal route (claimed).

No specific dosage details given.EXAMPLE :

No relevant examples given.

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PN RU2242513C1 2004-12-20 DW200511

(Volver al Sumario)

TI	Foot and mouth disease virus strain A (Georgia) 1999/ 1721 type a for preparing diagnostic and vaccine preparations
PA	(ANIM-R) ANIMAL HEALTH CONSERVATION CENTRE
ICAI	A61K39/135; C12N7/00;
AB	- NOVELTY : The parent virus for preparing the strain A (Georgia) 1999/N1721 is isolated in 1999 year from sick cows in private sector of village Ude in Adigensky district, Georgia Republic. The industrial strain A (Georgia) 1999/N1721 is prepared by successive passages in sensitive hetero- and homological cell cultures. - DETAILED DESCRIPTION : The strain is deposited in collection of microorganisms VGNKI at registration number A (Georgia) 1999/N1721. Virus of the strain A (Georgia) 1999/N1721-DEP is reproduced in sensitive cell cultures with accumulation up to 6.0-8.0 lg TCD50/ml for 18-24 h of incubation. At mass infection virus induces cytopathogenic effect (CPE) in 4 h and retains the parent indices in passage in cell cultures for 10 passages. The strain A (Georgia) 1999/N1721-DEP elicits high biological, antigenic and immunogenic activity and can be used for preparing agents for specific prophylaxis and diagnosis of foot and mouth disease virus type A that shows homological properties with epizootic virus distributing in Transcaucasian and Near East countries. - USE : Biotechnology, virology, veterinary science. - ADVANTAGE : Valuable properties of strain. 7 tbl, 3 dwg, 5 ex

7/29	@ WPI / Thomson
PN	WO2004058300A1 2004-07-15 DW200451 AU2003300192A1 2004-07-22 DW200476 EP1583556A1 2005-10-12 DW200567 BR0316917A 2005-10-18 DW200571 US2008032285A1 2008-02-07 DW200812 EP1583556B1 2008-08-20 DW200857 DE60323153E 2008-10-02 DW200866 US7790876B2 2010-09-07 DW201058
TI	Detecting the presence of foot and mouth disease virus in a sample comprises performing RT-PCR amplification of the sample using primer pairs and examining the amplification result to detect amplification product of the primer pairs
PA	(DUPO) DU PONT DE NEMOURS&CO E I(DUPO) DU PONT DE NEMOURS & CO E I(DECA-I) DECAROLIS L J(EBER-I) EBERSOLE R C(JACK-I) JACKSON R E
ICAI	A61K39/125; A61K39/135; C07H21/00; C07H21/04; C12Q1/68; C12Q1/70;
AB	- NOVELTY : Detecting the presence of foot and mouth disease virus (FMDV) in a sample comprises performing RT-PCR amplification of the sample using at least one primer pair given in the specification to produce an RT-PCR amplification result, and examining the RT-PCR amplification result to detect for an amplification product of the primer pair, where a positive detection of the amplification product indicates the presence of FMDV in the sample. - DETAILED DESCRIPTION : Detecting the presence of foot and mouth disease virus (FMDV) in a sample comprises performing RT-PCR amplification of the sample using at least one primer pair given in the specification to produce an RT-PCR amplification result, and examining the RT-PCR amplification result to detect for an amplification product of the primer pair, where a positive detection of the amplification product indicates the presence of FMDV in the sample. The primer pair comprises a sequence of 27 bp (SEQ ID NO: 16) fully defined in the specification together with any of the sequences having 23, 29, 30 or 25 bp given in the specification (SEQ ID NOS: 17, 18, 19 and 20, respectively). INDEPENDENT CLAIMS are also included for:

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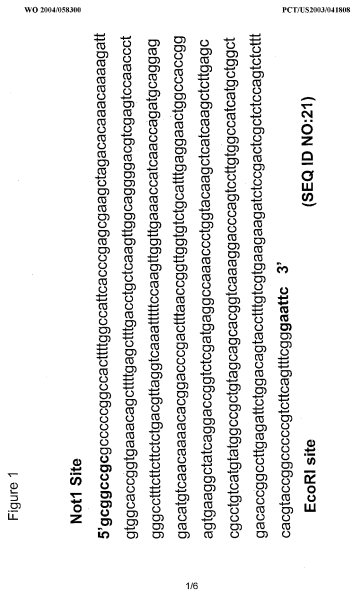
(1) an isolated polynucleotide for detecting FMDV comprising any of SEQ ID NOS: 16, 17, 18, 19 and 20;
(2) a replication composition for use in performing RT-PCR, comprising at least one primer pair cited above, reverse transcriptase, and thermostable DNA polymerase;
(3) a tablet comprising the replication composition cited above; and
(4) a kit for detecting FMDV in a sample, comprising the tablet cited above or at least one of the above primer pairs, reverse transcriptase, and thermostable DNA polymerase.
- USE :
The composition and method are useful for detecting or diagnosing foot and mouth disease in a rapid, accurate and easy manner.
- BIOTECHNOLOGY :
Preferred Method: In the examining step of the above method of detecting the presence of FMDV in a sample, a melting curve analysis is used to detect for an amplification product. The method further comprises a step of extracting RNA from the sample prior to the performing step.


WO 2004/058300 PCT/US2003/041808
Figure 1
Not1 Site
5'gcggccgcggccccggccacttttggccattcaccggagcgaagctagacacaacaaaagatt
gtggcaccggtgaaacagctttgagctgctcaagttggcgaggggacgtcgagtccaacct
gggccttctttctctgacgttaggtcaaatttttccaagttggttgaaaccatcaaccagatgcaggag
gacatgtcaacaaaacacggacccgacttaaccgttggtgtctgcatttgaggaactggccacggg
agtgaaggctatcaggaccggtctcgatgaggccaaaccctggtacaagctcatcaagctcttgagc
cgcctgtcatgtatggccgctgtagcagcacggtcaaaggacccagtccttgtggccatcatgctggct
gacaccggccttgagattctggacagtacctttgctgtgaaagaatctccgactcgctctccagtctctt
cacgtaccggccccgtctccagtttcgtggaattc 3'
(SEQ ID NO:21)
EcoRI site
1/6

8/29	@ WPI / Thomson
PN	WO02095074A1 2002-11-28 DW200312 US2003149259A1 2003-08-07 DW200358 AU2002339780A1 2002-12-03 DW200452
TI	Novel isolated nucleic acid useful for detecting foot and mouth disease virus infection in patient, and for detecting infection caused by serotypes of the virus
PA	(CALL-I) CALLAHAN J D(MANG-I) MANGOLD B L(NELS-I) NELSON W M(TETR-N) TETRACORE INC
ICAI	C12Q1/70; G01N33/569;
AB	- NOVELTY : An isolated nucleic acid (I) comprising a sequence consisting of any of at least 10 contiguous nucleotides of a portion of the 3D coding region of a foot and mouth disease virus (FMDV) genome, where the portion comprises the 3'-terminal third of the coding region or its complement, or that hybridizes under stringent hybridization conditions to the portion or its complement, is new. - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are also included for the following: (1) a pair (II) of two different nucleic acids, each of which comprises (I), and capable of priming a polymerase chain reaction (PCR) that amplifies a region of the nucleic acid with the portion; (2) a vector (III) comprising (I); (3) a cell containing (III); (4) a pair of two different nucleic acids, each having a sequence (S1) of 5'-CTgggTTTTATAACCTgTgATg-3', 5'-ACTgggTTTTACAAACCTgTgA-3', 5'-CTgggTTTTATAAACCTgTgAT-3', 5'-ACTgggTTTTACAAACCTg-3', 5'-TCCTTTgCaCgCCgTgggAC-3', 5'-CTTTgCACgCCgTgggaCCAT-3', 5'-gCgAgTCCTgCCACggA-3', 5'-AgTCCTgCCACggA-3', 5'-TCCgTggCAggACTCgC-3', 5'-CCgTggCAggACTCgC-3', 5'-CACACggCgTTCACCCA-3', 5'-aCaCggCgTTCAC-3', 5'-CTACAgATCACTTTACCTgCg-3', or 5'-AgCTACAgATCACTTTACCTg-3'; (5) an antibody (IV) that specifically binds to a polypeptide expressed from a 3D coding region of a FMDV genome; (6) a kit (V) for detecting FMDV infection in a patient, comprising (II), or (IV); and

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(7) a kit (VI) for detecting an infection caused by any of the several serotypes of FMDV, comprising a pair of primers for PCR amplification of at least a portion of 3D coding region of a FMDV genome.
- USE :
(I) is useful for detecting a FMDV infection in a patient or for detecting an infection caused by any of several serotypes of FMDV, by amplifying a portion of the nucleic acid of a biological sample obtained from the patient by PCR amplification to produce an amplification product which contains a sequence derived from the 3D coding region of a FMDV genome, and detecting the FMDV infection in the patient by the presence of the amplification product. The FMDV infection caused by any of the FMDV serotypes such as Asia, A, C, O, Sat 1, Sat 2 or Sat 3, in a patient, especially in cattle, horses, pigs, sheep, camels or goats. The amplification involves PCR, RT-PCR, or a probe hydrolysis RT-PCR amplification. The biological sample is sample of tissue, fluid or their combination obtained from the patient. The sample comprises materials collected from a vesicle or lesion of the patient. The nucleic acid is isolated from the sample prior to amplification, and the amplification product contains a sequence derived from a 3'-terminal portion of the 3D coding region of FMDV. The method distinguishes a FMDV-infected patient from a patient infected with one or more of the viruses such as swine vesicular disease virus, vesicular stomatitis virus, vesicular exanthema of swine virus, and distinguish a FMDV-vaccinated patient from a FMDV-infected patient. (IV) is useful for detecting an infection of a patient caused by any of several serotypes of FMDV, by contacting a biological sample obtained from the patient with (IV) directed against a 3D coding region of an expressed portion of FMDV genome to form antibody/antigen complexes, and detecting the FMDV infection in the patient by the presence of antibody/antigen complexes (all claimed).
- DESCRIPTION OF DRAWINGS :
The figure shows the polypeptide structure of FMDV.
- BIOTECHNOLOGY :
Preferred Nucleic Acid: At least 10 contiguous nucleotides comprises at least 15, 20, 30, 35 or 50 contiguous nucleotides. (I) comprises DNA or peptide nucleic acid (PNA). The 3'-terminal portion comprises a sequence chosen from positions of 6685-6996 of an O serotype, isolate 01 Campos, positions 7769-8076 of an O serotype, positions 7401-7712 of an O serotype, isolate 01K, positions 7400-7707 of an O serotype, isolate O/SKR/200, positions 7319-7626 of an O serotype, isolate Chu-Pei strain, positions 7336-7643 of an O serotype, isolate Tau-Yuan TW9 strain, positions 7711-8018 of an O serotype, strains rp146, rp99 and c-s8cl, and positions 7371-7678 of an Sat2 serotype. The 3'-terminal portion comprises a sequence chosen from the positions 1102-1401 of the 3D coding region of Asia 1 serotype, and positions 1281-1582 of the 3D coding region of a C-1-Santa Pau (C-s8) replicase genotype. The foot and mouth disease virus genome is genomes of serotype A, C, O, Asia 1, Sat 1, Sat 2, or Sat 3.
Preferred Kit: (V) is capable of detecting several serotypes of FMDV, and the antibody is labeled with a detectable label, especially a fluorescent. The kit comprises several greater than 3,4,5,or 6 different serotypes of FMDV. In (VI), the method is performed and FMDV infection is detected within 2 hours. (VI) further comprises dried reagents for reverse transcription (RT)-PCR analysis of the biological sample plus trehalose. (VI) can be stored at room temperatures for at least one year. The kit comprises several 3,4,5,6 or 7 different serotypes of FMDV. Each primer comprises a sequence selected from S1.
Preferred Antibody: (IV) is monoclonal antibody or polycolonal antibody or their fragments.EXAMPLE :
Foot and mouth disease virus (FMDV) isolates that represented all 7 serotypes were grown in monolayers of a continuous bovine kidney cell line (LF-BK). Viral infectivity was measured. Viruses that cause similar clinical signs, namely swine vesicular disease virus (SVDV)-UK and It-1/66, vesicular stomatitis virus (VSV)-Indiana, and vesicular exanthema of swine virus (VESV) A-48 were grown in monolayers of pig kidney cells (IBRS2), baby hamster kidney cells (BHK-21) and LF-BK cells. Viral RNA was extracted from cell culture supernatants or plasma, whole blood or tissue. Clinical samples from a

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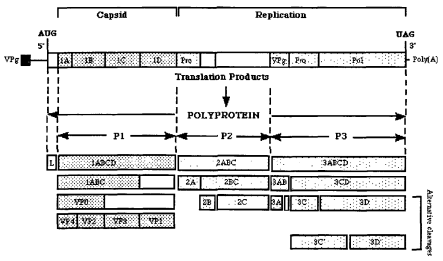

foreign animal disease which included epithelial tissue and saliva samples obtained from steers, sheep and pigs seven days after contact exposure to a pig that was infected by ID inoculation of 10<5> tissue culture infective doses (TCID)50 of a FMDV (type O), were examined. Additional clinical samples included blood samples obtained from pigs 5 days after ID inoculation of 10<5> TCID50 of A type a virus. The blood samples were divided into two aliquots, and the other of which was untreated. Both aliquots underwent RNA extraction and were tested in parallel for viral infectivity and viral RNA. A group of experimentally infected animals were evaluated. Prior to exposure of the animals to FMDV, control samples were collected and included plasma, oral and nasal swab specimens, and oral-pharyngeal fluids. 24 hours later the animals were placed in separate rooms that contained four FMDV-free, healthy animals of the same species. Oral and nasal swab specimens, oral-pharyngeal fluid specimens, and blood samples were obtained at 0 (before exposure), 4,8,12,16,24, 48,72,96 or 120 and 144 hours. The animals were observed for the onset of fever and clinical signs of FMDV. Samples were collected All tests were performed in a masked fashion in parallel with viral isolation. The FMDV nucleotide sequences were retrieved from GenBank and aligned by use of sequence alignment software. Specific oligonucleotide primers and a fluorogenic probe were designed to target a highly conserved region within the FMDV RNA polymerase (3D) gene sequence alignment. The probe was labeled with 5'-receptor dye, 6-carboxyfluorescein and a 3'-quencher, tetramethylrhodamine. A blast search analysis of the primer and probe sequences confirmed 100% homology with 5 of 7 serotypes of FMDV, with the exception of a single base mismatch located within the forward primer binding area of SAT2. Sequence information for the 3D genomic region of serotypes SAT1 and SAT3 was presently not available.

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PN	WO02090572A2 2002-11-14 DW200311 US2003152942A1 2003-08-14 DW200355 AU2002311897A1 2002-11-18 DW200452 AU2002311897A8 2005-10-13 DW200619 US2008015112A1 2008-01-17 DW200807 WO02090572A3 2003-07-03 DW201208
TI	Use of an INVADER detection assay for testing nucleic acids in pooled samples without prior amplification, e.g. for detecting rare mutations, or testing large numbers of blood or plasma donations to eliminate contaminated units
PA	(BROW-I) BROW M A D(DBRO-I) D BROW M A(FORS-I) FORS L(INDI-I) INDIG M D A(NERI-I) NERI B P(ROEV-I) ROEVEN R(THIR-N) THIRD WAVE TECHNOLOGIES INC
ICAI	C12Q1/68; C12Q1/70; C40B20/04; G06Q10/00; G06Q30/00;
AB	- NOVELTY : Performing nucleic acid testing on a pooled sample comprising employing an INVADER detection assay, is new. - DETAILED DESCRIPTION : Performing nucleic acid testing comprises: (a) providing: (i) several individual biological material samples, or a pooled sample comprising biological material combined from several individual samples; and (ii) INVADER assay reagents configured to detect the presence or absence, or measure the quantity, of a target nucleic acid sequence; and (b) contacting the pooled sample with the INVADER assay reagents so that the presence or absence of the target nucleic acid sequence in the pooled sample is determined. INDEPENDENT CLAIMS are also included for the following:

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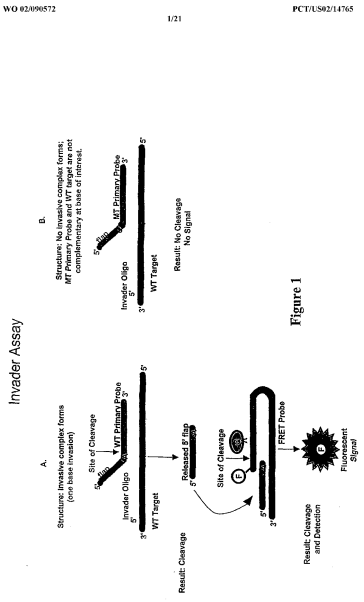
(1) detecting an allele frequency of a polymorphism comprising:
(i) providing:
(a) a pooled sample comprising a target nucleic acid sequence from several individuals; and
(b) INVADER assay reagents configured to detect the presence or absence of a polymorphism, or configured to generate distinct signals for each allele of a polymorphic locus in the target nucleic acid sequence;
(ii) contacting the pooled sample with the INVADER assay reagents to generate a detectable signal or at least one distinct signal; and
(iii) measuring the detectable signal to determine a number of target nucleic acid sequences that contain the polymorphism, or measuring each of the distinct signal to determine a proportion of each allele in the polymorphic locus within the pooled sample; and
(2) detecting a rare mutation comprising:
(i) providing:
(a) a sample from a single subject, where the sample comprises at least 10000 target nucleic acid sequences; and
(b) a detection assay capable of detecting a mutation in a population of target nucleic acid sequences that is present at an allele frequency of 1:1000 or less compared to wild type alleles; and
(ii) assaying the sample with the detection assay so that the presence or absence of a rare mutation is detected, or so that an allele frequency in the sample of a rare mutation is determined.
- USE :
The method is useful for detecting target nucleic acid sequences in pooled samples without prior amplification of the target. The method is particularly useful for detecting an allele frequency of a polymorphism, detecting a rare mutation, or testing large numbers of blood or plasma donations to eliminate units having pathogenic (e.g. viral) contamination.
- ADVANTAGE :
Polymerase chain reaction testing is very expensive and since the general donor population includes a relatively small number of positive donors, individual testing of each donation is not cost effective or economically feasible. The present method is an efficient and cost-effective method of testing large numbers of blood or plasma donations to eliminate units having pathogenic (e.g. viral) contamination.
- BIOTECHNOLOGY :
Preferred Method: The biological material comprises blood or blood plasma. The target nucleic acid sequence is RNA or DNA, and is a sequence from a microorganism or virus. In particular, the target nucleic acid sequence is from a pathogen (e.g. HIV-1, HIV-2, HCV, HBV, HTLVI, HTLV2 or HCMV). The target nucleic acid comprises a first and second non-contiguous single-stranded regions separated by an intervening region comprising a double stranded region. The INVADER assay reagents comprise:
(a) a bridging oligonucleotide capable of binding to the first and second non-contiguous single-stranded regions;
(b) a second oligonucleotide capable of binding to a portion of the first non-contiguous single-stranded region; and
(c) a cleavage means.
The step of contacting causes either the second oligonucleotide or the bridging oligonucleotide to be cleaved. The individual samples comprise at least 5-16 individual sample. The method further comprises, prior to step (b), the step of performing polymerase chain reaction on the pooled sample such that the target nucleic acid sequence is amplified if present in the pooled sample. The contacting step is performed under conditions where the target nucleic acid is not amplified before the presence or the absence of the target nucleic acid sequence is determined. The method may also comprise:
(a) providing:

(Volver al Sumario)

(i) several individual biological samples; and
(ii) INVADER assay reagents configured to detect the presence or absence of a target nucleic acid sequence; and
(b) forming a sub-pool by combining a portion of each of the individual biological samples; and
(c) contacting the sub-pool with the INVADER assay reagents so that the presence or absence of the target nucleic acid sequence in the sub-pool is determined.
Where the contacting indicates that the target nucleic acid sequence is absent from the sub-pool, the method further involves combining the individual biological samples into a primary pool. If contacting indicates that the target nucleic acid sequence is present in the sub-pool, the method further involves screening each of the individual biological samples for the presence or absence of the target nucleic acid sequence. In method (1), the group of individuals comprises at least 10-1000 individuals. The measuring comprises detection of fluorescence. At least two distinct signals are generated in step (ii), where the measuring comprises comparing at least two distinct signals. The comparing comprises applying a correction factor to a measurement of at least one distinct signal.



10/29	@ WPI / Thomson
PN	WO03038119A1 2003-05-08 DW200340 EP1308521A1 2003-05-07 DW200340 AU2002339290A1 2003-05-12 DW200464 EP1308521B1 2009-04-08 DW200925 DE60138286E 2009-05-20 DW200934
TI	Detecting foot-and-mouth disease virus (FMDV) in a biological sample by isolating the RNA molecules of the FMDV from the biological sample, subjecting the RNA molecules to amplification and detecting the amplified RNA product
PA	(HKDN-N) HONG KONG DNA CHIPS LTD
ICAI	C12N7/01; C12Q1/68; C12Q1/70;
AB	- NOVELTY : Detecting foot-and-mouth disease virus (FMDV) in a biological sample comprises: (a) isolating the RNA molecules of the FMDV from the biological sample; (b) subjecting the RNA molecules to amplification to obtain an amplified product containing a nucleic acid sequence complementary to at least a portion of the RNA sequence of FMDV; and (c) detecting the amplified RNA product. - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are also included for the following: (1) a kit for detecting FMDV in a biological sample; and (2) a DNA sequence. - USE : The method is useful for detecting FMDV in a biological sample (claimed). - ADVANTAGE : The method increases the detection sensitivity and decreases the cost and time for detection of FMDV. - BIOTECHNOLOGY : Preferred Method: In the method of detecting FMDV in a biological sample, the amplification of the RNA is based on NASBA method comprises: (1) annealing primer A to RNA molecules;

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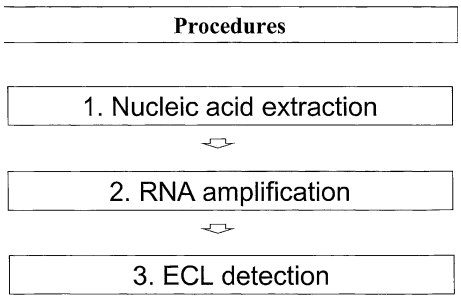
(2) allowing the primer A to extend under the presence of a reverse transcriptase to obtain an RNA-DNA hybrid;
(3) digesting the RNA sequence in the RNA-DNA hybrid with an RNase to obtain a single strand DNA molecule;
(4) annealing the primer B to the single strand DNA;
(5) subjecting the double-strand DNA to amplification in the presence of an RNA polymerase to obtain an amplified RNA product.

Fig. 1



Primer A comprises a sequence having 15-160 bp. Primer B comprises a sequence having 19-159 bp. The RNA polymerase is T7 RNA polymerase. The RNase is RNase H. The reverse transcriptase is an avian myeloblastosis virus reverse transcriptase. The second DNA sequence of primer A comprises 31 bp. The detection of amplified RNA products allowing a detection molecule to bind to the amplified RNA product. The signal generator is an electrochemiluminescent or enzymatic molecule. The capture probe comprises 25-bp sequence. The FMDV is type O strain.
Preferred Kit: The kit comprises:
(1) an isolating agent for isolating the RNA molecules of FMDV from the biological sample;
(2) an amplifying agent for amplifying the isolated RNA molecules; and
(3) a detecting agent for detecting the amplified RNA product.
Preferred DNA: The DNA sequence comprises 15-160 or 19-159 bp.EXAMPLE :
10 Microl of the FMDV RNA-specific amplification solution was added into the test tube. The amplification solution contained primers A and B comprising 19-160-bp sequence. The results showed positive FMDV detection.

11/29	@ WPI / Thomson
PN	GB2377017A 2002-12-31 DW200319 WO03002766A2 2003-01-09 DW200319 AU2002311477A1 2003-03-03 DW200452 AU2002311477A8 2005-10-13 DW200611 WO03002766A3 2003-08-28 DW201208
TI	Novel polynucleotide sequences that are useful as hybridization probes and universal primers in the detection of Foot and Mouth disease virus in a sample
PA	(ANIM-N) INST ANIMAL HEALTH
ICAI	C12Q1/70;
AB	- NOVELTY : A polynucleotide (Ia) which is a hybridization probe comprising a sequence (S1) or its fragment capable of binding specifically to the complementary sequence of S1, or universal primers (Ib) comprising sequence (S2) and (S3), respectively, are new. - DETAILED DESCRIPTION : A polynucleotide (Ia) which is a hybridization probe comprising a sequence (S1) or its fragment capable of binding specifically to the complementary sequence of S1, or universal primers (Ib) comprising sequence (S2) and (S3), respectively, are new. (S1) CCTCGGGGTACCTGAAGGGCATCC; (S2) CAC{T/C}T{T/C}AAG{G/A}TGACA{T/C}TG{G/A}TACTGGTAC; (S3) CAGAT{C/T}CC{G/A}AGTG{T/A}C{I}C{I}TGTTA. INDEPENDENT CLAIMS are also included for the following: (1) determining whether an organism is infected with Foot and Mouth disease virus (FMDV), by determining whether a sample from the organism contains FMDV using (I); (2) vaccinating an organism against FMDV, by determining whether a sample from an organism contains FMDV by the method of (1), and, if FMDV is not detected in the

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sample, administering an FMDV vaccine to the organism;
(3) treating an organism infected with FMDV, by determining whether a sample from an organism contains FMDV by the method of (1), and, if FMDV is detected in the sample, administering a therapeutic agent to the organism, which agent is effective in combating the FMDV virus;
(4) combating the spread of FMDV between organisms, by determining whether a sample from an organism contains FMDV by the method of (1), and if the organism is infected with FMDV, sacrificing the organism;
(5) determining whether a test vaccine is capable of preventing FMDV infection, by administering the test vaccine to an organism, inoculating the organism with FMDV and determining whether a sample from the organism contains FMDV by the method of (1);
(6) determining whether a test agent is capable of combating FMDV, administering the test agent to an organism infected with FMDV and determining whether the test agent is capable of combating FMDV by the method of (6);
(7) a system (II) for detecting FMDV, comprising (Ia), where the specific binding of (Ia) to a target polynucleotide of a sample is indicative of the presence of FMDV in the sample; and
(8) a kit of parts comprising (I), and optionally (Ib).ACTIVITY :
Antiviral.
No biological data is given.MECHANISM OF ACTION :
Vaccine.
- USE :
(Ia) is useful for determining whether a sample contains FMDV, by contacting the sample with a probe comprising (Ia) in vitrounder conditions that allow the probe to bind specifically to a target polynucleotide, and determining whether the probe has bound to a target polynucleotide, thus to determine whether the sample comprises a target polynucleotide, which indicates the presence of FMDV. A region of a target polynucleotide comprising the binding site of the probe is amplified using (Ib). Binding of probe to the target polynucleotide is determined by determining the fluorescence emission of the probe. The sample is a mammalian sample e.g. pig, wild or domesticated ruminant such as cattle, buffalo, sheep, goat or deer. (All claimed.)
- ADVANTAGE :
(Ia) is capable of detecting all FMDV serotypes. The method is rapid, highly sensitive, specific and reliable.
- BIOTECHNOLOGY :
Preferred Polynucleotide: (I) comprises one or two labeling moieties. The moieties are bound to the polynucleotide at positions that allow one moiety to quench the fluorescence emission of the other moiety.EXAMPLE :
A TaqMan(RTM) probe of 24 nucleotides was designed using Primer Express Software from internal ribosomal entry site (IRES) region sequences. The position of the sequence (genomic location 292-269R) was totally conserved in Foot and Mouth disease (FMD) viruses, but not so in other picornaviruses, making this genomic region highly suitable for the detection of all seven serotypes of FMD virus. This position was also highly homologous to other genes in the public databases, especially to sequences involved in RNA interactions such as promoter regions, RNA polymerase or 16S ribosomal RNA which places a functional constraint upon this sequence of the genome. However, the maximum homology between the probe sequence region and the other sequences was a stretch of 18 nucleotides in a row or 19 out of 20 nucleotides in one case. Redundant primers on each side of the conserved probe were designed (genomic locations 315-293R and 219-246F respectively) were designed using the Primer Express Software from public databases to enable the assay to detect all seven serotypes of FMD virus. Amplicon size was short (c120 nt) which enhanced the polymerase chain reaction (PCR) efficiency on the one hand and lessened the risk of RNA degradation which could lower the PCR efficiency.

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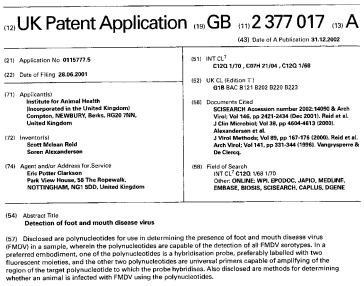

(12) UK Patent Application (19) GB (11) 2 377 017 (13) A

(43) Date of A Publication 31.12.2002

(21) Application No 0115777.5

(22) Date of Filing 28.06.2001

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(51) INT CL7
C12Q 1/70 , C07H 21/04 , C12Q 1/68

(52) UK CL (Edition 7)
G1B BAC B121 B200 B220 B223

(56) Documents Cited
SCISEARCH Accession number 2002:14090 & Arch Virol; Vol 146, pp 2421-2434 (Dec 2001). Reid et al.
J Clin Microbiol; Vol 38, pp 4604-4613 (2000). Alexandersen et al.
J Virol Methods; Vol 89, pp 167-176 (2000). Reid et al.
Arch Virol; Vol 141, pp 331-344 (1996). Vangrysperre & De Clercq.

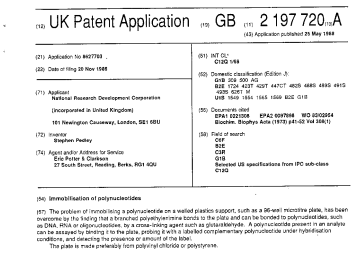
(58) Field of Search
INT CL7 C12Q 1/68 1/70
Other: ONLINE: WPI, EPODOC, JAPIO, MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS, DGENE

(54) Abstract Title
Detection of foot and mouth disease virus

(57) Disclosed are polynucleotides for use in determining the presence of foot and mouth disease virus (FMDV) in a sample, wherein the polynucleotides are capable of the detection of all FMDV serotypes. In a preferred embodiment, one of the polynucleotides is a hybridisation probe, preferably labelled with two fluorescent moieties, and the other two polynucleotides are universal primers capable of amplifying of the region of the target polynucleotide to which the probe hybridises. Also disclosed are methods for determining whether an animal is infected with FMDV using the polynucleotides.

GB 2 377 017 A

12/29	@ WPI / Thomson
PN	GB2197720A 1988-05-25 DW198821
TI	Rigid plastic support having bound polyethyleneimine - used for immobilising a polynucleotide for hybridisation assays
PA	(NATR) NAT RES DEV CORP
ICAI	C12Q1/68; G01N33/543;
AB	A novel rigid plastic support has bound to it a branched polyethylenimine (PEI) having (1) primary amino or crosslinkable secondary amino and (2) protonated amino gps.. Pref. the support is formed from PVC or polystyrene. - USE/ - ADVANTAGE : The support can be used for immobilising a polynucleotide by incubating the support with a polynucleotide and a cross linking agent to link the primary amine gps. of the PEI to reactive gps. on the polynucleotide. The PEI bonds well to ordinary plastic surfaces and esp. welled plastics plates and the bonding is sufficiently stable to allow hybridisations to be carried out in the plates between the bound polynucleotide and its complementary partner.



(12) UK Patent Application (19) GB (11) 2 197 720 (13) A

(43) Application published 25 May 1988

(21) Application No 8627703

(22) Date of filing 28 Nov 1986

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(51) INT CL4
C12Q 1/68

(52) Domestic classification (Edition J)
G1B 306 309 AG
B2E 172A 422T 422T 447CT 482S 486S 489S 491S 492S 624T M
U1S 1509 1554 1565 1569 B2E G1B

(55) Documents cited
EP A1 0223256 EP A2 0097058 WO A2 8402954
Biochim. Biophys Acta (1973) p41-52 Vol 308(1)

(58) Field of search
C4F
B2E
G1B
Selected US specifications from IPC sub-class C12Q

(54) Immobilisation of polynucleotides

(57) The problem of immobilising a polynucleotide on a welled plastics support, such as a 96-well microtitre plate, has been overcome by the finding that a branched polyethylenimine bonds to the plate and can be bonded to polynucleotides, such as DNA, RNA or oligonucleotides, by a cross-linking agent such as glutaraldehyde. A polynucleotide present in an analyte can be assayed by binding it to the plate, probing it with a labelled complementary polynucleotide under hybridisation conditions, and detecting the presence or amount of the label.
The plate is made preferably from polyvinyl chloride or polystyrene.

GB 2 197 720 A

13/29	@ WPI / Thomson
PN	CN101659999A 2010-03-03 DW201023 CN101659999B 2011-12-21 DW201208
TI	Detecting loop-mediated isothermal amplification (LAMP) product comprises amplifying the DNA in the sample by LAMP, and hybridizing the gold nanoparticle marked with molecular probe with the DNA of the product
PA	(TIAN-N) TIANJIN CHAOHAI TECHNOLOGY CO LTD
ICAI	C12Q1/68; C12Q1/70;
AB	- NOVELTY : Detecting loop-mediated isothermal amplification (LAMP) product comprises: (A) preparing gold nanoparticle marked with molecular probe; (B) amplifying the DNA in the sample by LAMP or after reverse transcription process,

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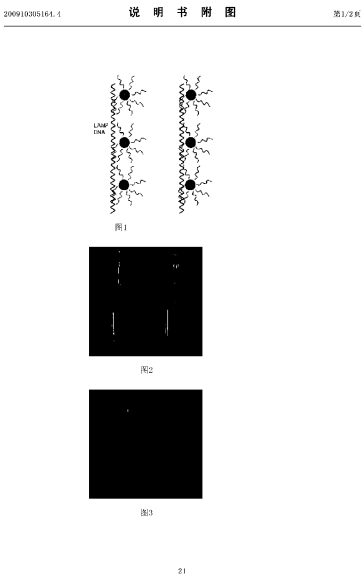
and amplifying the RNA in the sample by LAMP;
(C) hybridizing the gold nanoparticle marked with molecular probe with the DNA of the product produced by LAMP; and
(D) hybridizing to initiate the aggregation of the gold nanoparticle to make the color in the aggregation part change to analyze whether the sample contains DNA or RNA or not.
- USE :
The method is useful for detecting LAMP product. The method can be used for detecting HIV, hepatitis B, hepatitis C, viral pathogen of the viral pathogen virosis such as flu comprising H1N1 flu A, hand-foot-mouth disease, meningitis, and Escherichia coli, Staphylococcus, pathogen bacterium of bacterial disease, pathogen epiphyte of epiphyte disease such as dermatophytosis and ringworm of the nails, Chlamydia psittacosis, Chlamydia trachomatis, and Chlamydia pneumoniae. The method also can be used for diagnosing tumor, comprising liver cancer, lung cancer, cerebral cancer, esophagus cancer, prostatic cancer, uterine cancer, bone cancer and leukemia, and also can be used for identifying the grade malignancy of the tumor. The method can be used for diagnosing human disease, diagnosing animal disease, diagnosing plant disease, monitoring the food sanitation, identifying the sex of the fetus, identifying the genovariation, detecting single nucleotide polymorphism and detecting the gene change in the cell (all claimed).
- ADVANTAGE :
The method has high sensitivity and specificity, simple operation, and gives rapid detecting result without the use of professional equipment.
- BIOTECHNOLOGY :
Preferred Method: In the method above, the amplifying target can be DNA, RNA or the mixture of DNA and RNA. The gold nanoparticle probe comprises two parts, one is gold nanoparticle, the other is molecular probe fixed on the surface of the gold nanoparticle. Hybridizing the gold nanoparticle marked with molecular probe with the DNA of the product produced by LAMP and amplifying reaction by LAMP can be carried out in the same reacting condition and same reacting vessel at same time. Hybridizing the gold nanoparticle marked with molecular probe with the DNA of the product produced by LAMP can be carried out in solution or the surface of solid. Hybridizing the gold nanoparticle marked with molecular probe with the DNA of the product produced by LAMP can initiate the color change of the solution or the solid surface, the change can be seen by the naked eye, and also can use the optical instrument such as spectrophotometer to detect. The molecular probe is the functional molecule which can distinguish the DNA of the product produced by LAMP. The same condition is not limited in but comprises solution constituent, concentration, pH value, temperature and reacting time and so on.



14/29	@ WPI / Thomson
PN	WO2009150429A2 2009-12-17 DW201006 WO2009150429A3 2010-03-11 DW201019 US2011311568A1 2011-12-22 DW201201 EP2419124A2 2012-02-22 DW201214
TI	Foot and mouth disease (FMD) vaccine for preventing and/or treating FMD in a subject comprises a vaccinating entity comprising or capable of expressing a foot and mouth disease virus (FMDV) viral particle (VP)-1 polypeptide
PA	(BARN-I) BARNETT P(FOWL-I) FOWLER V(ANIM-N) INST ANIMAL HEALTH(KNOW-

(Volver al Sumario)

	I) KNOWLES N
ICAI	A61K39/00; A61K39/135; A61P31/14; A61P37/04; C12Q1/70; C40B30/04;
AB	- NOVELTY : A foot and mouth disease (FMD) vaccine comprises a vaccinating entity comprising or capable of expressing a foot and mouth disease virus (FMDV) viral particle (VP)-1 polypeptide having a deletion of at least seven amino acids in the G-H loop so that the VP-1 polypeptide lacks an RGD motif, where the vaccine is substantially free from a vaccinating entity comprising or capable of expressing an FMDV VP-1 polypeptide having a complete G-H loop. - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are: (1) a method for distinguishing between: (a) FMD infected, sub-clinically infected, or previously infected subjects; and (b) subjects vaccinated with the vaccine comprising investigating the presence of a VP-1 polypeptide comprising a G-H loop, or evidence of an immune response against the VP-1 G-H loop in the subject; the presence of a VP-1 polypeptide comprising a G-H loop, or evidence of an immune response against the VP-1 G-H loop in the subject, indicating that the subject is or has been infected with FMDV; (2) a differentiation assay to distinguish between: (a) FMD infected, sub-clinically infected, or previously infected subjects; and (b) subjects vaccinated with the vaccine, which comprises a detection system for VP-1 G-H loops or for anti-G-H loop antibodies; and (3) a kit which comprises the vaccine together with the differentiation assay.ACTIVITY : Virucide. Test details are described but no results given.MECHANISM OF ACTION : Vaccine. - USE : The vaccine is useful for preventing and/or treating FMD in a subject. It can also be used in the manufacture of a medicament for the prevention of FMD. The vaccine is also useful as a marker vaccine (all claimed). - ADVANTAGE : The vaccine provides a more definitive distinction between vaccinated and infected individuals when coupled with an appropriate diagnostic assay. - BIOTECHNOLOGY : Preferred Method: Distinguishing between: (a) FMD infected, sub-clinically infected, or previously infected subjects; and (b) subjects vaccinated with the vaccine, comprises investigating the presence of antibodies to the G-H loop in the subject, which involves: (a) removal of non-G-H loop antibodies in a sample from the subject; and (b) detecting the presence of antibodies after the removal step the presence of antibodies after the removal step indicating the presence of G-H loop antibodies in the subject. It also comprises investigating the presence of a VP-1 polypeptide comprising a G-H loop using an alpha vbeta 6 integrin. Preferred Assay: The detection system detects anti-G-H loop antibodies, which includes a vaccinating entity for pre-absorption of non-G-H loop antibodies. It also comprises an alpha vbeta 6 integrin for detection of VP-1 G-H loops.PHARMACEUTICALS : Preferred Vaccine: The vaccine comprises the vaccinating entity comprising or capable of expressing a foot and mouth disease virus (FMDV) VP-1 polypeptide having a deletion in the G-H loop. The vaccinating entity is an inactivated FMD virus or an attenuated form of FMDV. The vaccinating entity is also nucleotide sequence capable of encoding the FMD proteins needed to produce an FMDV empty capsid particle. The vaccinating entity comprises or is capable of expressing a serotype A VP-1 polypeptide having a deletion of residues 142-154 in the G-H loop.EXAMPLE : No suitable example given.

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A+	A-	Position
N	N	17
D	D	37
L	L	53
A	V	138
D	A	141
S	DELETED	142
R	DELETED	143
G	DELETED	144
D	DELETED	145
L	DELETED	146
G	DELETED	147
P	DELETED	148
L	DELETED	149
A	DELETED	150
A	DELETED	151
R	DELETED	152
V	DELETED	153
A	DELETED	154
A	K	155
K	M	203

FIG. 1

15/29	@ WPI / Thomson
PN	US2009105092A1 2009-04-23 DW200937 JP2009131242A 2009-06-18 DW200943
TI	New oligonucleotide set comprises nucleic acid sequences that are reverse translated from different amino acid sequences comprising a motif conserved in different virus family, genus, or species, useful for detecting vertebrate viruses
PA	(UYCO) UNIV COLUMBIA NEW YORK
ICAI	C12M1/00; C12N15/09; C12Q1/68; C12Q1/70; C40B40/06; G06F17/30;
AB	- NOVELTY : A set of oligonucleotides for detecting vertebrate viruses, the set of oligonucleotides comprising nucleic acid sequences that are reverse translated from 10,000-50,000 different amino acid sequences, each amino acid sequence comprising a motif conserved in a different virus family, genus, or species, is new. - DETAILED DESCRIPTION : A set of oligonucleotides for detecting vertebrate viruses, the set of oligonucleotides comprising: (a) nucleic acid sequences that are reverse translated from 10,000-50,000 different amino acid sequences, each amino acid sequence comprising a motif conserved in a different virus family, genus, or species; (b) nucleic acid sequences that are reverse translated from no more than one thousand different amino acid sequences, each amino acid sequence comprising a motif conserved in either: (i) a virus family e.g. Asfarviridae, Poxyiridae, Iridoviridae, Herpesviridae, Polydnaviridae, Papovaviridae, Adenoviridae, Circoviridae, Reoviridae, Birnaviridae, Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Bornaviridae, Filoviridae, Arenaviridae, Retroviridae, Bunyaviridae, Caliciviridae, Picornaviridae, Astroviridae, Flaviviridae, Nodaviridae, Coronaviridae, Togaviridae, and Arteriviridae; (ii) a virus genus e.g. Asfivirus, Orthopoxvirus, Parapoxvirus, Avipoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus, Yatapoxvirus, Entomopoxvirus A, Entomopoxvirus B, Entomopoxvirus C, Iridovirus, Chloriridovirus, Ranavirus, Lymphocystivirus, Simplexvirus, Varicellovirus, Cytomegalovirus, Muromegalovirus, Roseolovirus, Lymphocryptovirus, Rhadinovirus, Ichnovirus, Bracovirus, Polyomavirus, Papillomavirus, Mastadenovirus, Aviadenovirus, Orthoreovirus, Orbivirus, Rotavirus, Coltivirus, Aquareovirus, Cypovirus, Fijivirus, Phytoreovirus, Oryzavirus, Aquabirnavirus, Avibirnavirus, Entomobirnavirus, Influenzavirus A, Influenzavirus B, Influenzavirus C, Influenzavirus D, Paramyxovirus,

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Morbillivirus, Rubulavirus, Pneumovirus, Bornavirus, Marburgvirus, Ebolavirus, Arenavirus, Alpharetrovirus, Betaretrovirus, Gammaretrovirus, Type D Retrovirus group, Deltaretrovirus, Epsilonretrovirus, Lentivirus, Spumavirus, Bunyavirus, Hantavirus, Nairovirus, Phlebovirus, Tospovirus, Calicivirus, Enterovirus, Rhinovirus, Hepatovirus, Cardiovirus, Aphthovirus, Astrovirus, Flavivirus, Pestivirus, Hepacivirus, Alphanodavirus, Coronavirus, Torovirus, Alphavirus, Arterivirus, and Deltavirus; and/or (iii) a virus species from the virus family in (a) or the virus genus in (ii); where the set of oligonucleotides as a whole can detect any virus that infects vertebrates; (c) less than 10,000 different oligonucleotide sequences, where the set of oligonucleotides hybridizes to nucleic acid sequences from at least 10 viral species, where each oligonucleotide of the set comprises a nucleotide sequence reverse translated from an amino acid sequence listed in the CD-ROM Appendix Table; (d) less than 10,000 different oligonucleotide sequences, where the set comprises a nucleotide sequence listed in the CD-ROM Appendix Table; and (e) less than 10,000 different oligonucleotide sequences, where the set comprises a nucleotide sequence complementary to a nucleotide sequence listed in the CD-ROM Appendix Table, is new. INDEPENDENT CLAIMS are:
(1) a method for designing an oligonucleotide for viral screening;
(2) a microarray comprising any one of the oligonucleotides above;
(3) a method for identifying a virus from a environmental or clinical sample;
(4) a computer program product residing on a computer readable medium;
(5) a method for designing one or more primers;
(6) a method of designing a database of coding viral oligonucleotides;
(7) a method of designing a database of degenerate coding viral oligonucleotides;
(8) a method of designing a database of non-coding viral oligonucleotides;
(9) a method for identifying sequence patterns that are conserved across viral taxa or within a viral taxon;
(10) a method for generating conserved peptides that can be used as immunogens for the generation of an antibody against a virus;
(11) a computer-readable medium containing computer-executable instructions that, when executed by a processor, cause the processor to perform a method for designing an oligonucleotide for viral screening, where the method comprises the method of (1), (6), (7), or (8);
(12) a computer-readable medium for storing data for access by an application;
(13) a system for mapping a viral nucleic acid sequence to a tree structure;
(14) a system for at least one of diagnosis, surveillance, or discovery of infection or disease;
(15) a method for updating a database of genetic information; and
(16) a viral detection kit comprising the microarray of (2).
- USE :
The set of oligonucleotides is useful for detecting vertebrate viruses; designing an oligonucleotide for viral screening; identifying a virus from a environmental or clinical sample; designing one or more primers; designing a database of coding viral oligonucleotides; designing a database of degenerate coding viral oligonucleotides; designing a database of non-coding viral oligonucleotides; identifying sequence patterns that are conserved across viral taxa or within a viral taxon; generating conserved peptides that can be used as immunogens for the generation of an antibody against a virus; updating a database of genetic information; and for an oligonucleotide-related application comprising microarray screening, PCR, and RNA interference (RNAi) analysis (all claimed). It is also useful for detecting/identifying any unknown or known virus of a particular taxon.
- BIOTECHNOLOGY :
Preferred Oligonucleotides: The set of oligonucleotides for detecting vertebrate viruses, the set of oligonucleotides comprises: (a) nucleic acid sequences that are reverse translated from 10,000-50,000 different amino acid sequences, each amino acid sequence comprising a motif conserved in a different virus family, genus, or species, where the virus family is Asfarviridae, Poxyiridae, Iridoviridae, Herpesviridae,

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Polydnaviridae, Papovaviridae, Adenoviridae, Circoviridae, Reoviridae, Birnaviridae, Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Bornaviridae, Filoviridae, Arenaviridae, Retroviridae, Bunyaviridae, Caliciviridae, Picornaviridae, Astroviridae, Flaviviridae, Nodaviridae, Coronaviridae, Togaviridae, and Arteriviridae; where the virus genus is Asfivirus, Orthopoxvirus, Parapoxvirus, Avipoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus, Yatapoxvirus, Entomopoxvirus A, Entomopoxvirus B, Entomopoxvirus C, Iridovirus, Chloriridovirus, Ranavirus, Lymphocystivirus, Simplexvirus, Varicellovirus, Cytomegalovirus, Muromegalovirus, Roseolovirus, Lymphocryptovirus, Rhadinovirus, Ichnovirus, Bracovirus, Polyomavirus, Papillomavirus, Mastadenovirus, Aviadenovirus, Orthoreovirus, Orbivirus, Rotavirus, Coltivirus, Aquareovirus, Cypovirus, Fijivirus, Phytoreovirus, Oryzavirus, Aquabirnavirus, Avibirnavirus, Entomobirnavirus, Influenzavirus A, Influenzavirus B, Influenzavirus C, Influenzavirus D, Paramyxovirus, Morbillivirus, Rubulavirus, Pneumovirus, Bornavirus, Marburgvirus, Ebolavirus, Arenavirus, Alpharetrovirus, Betaretrovirus, Gammaretrovirus, Type D Retrovirus group, Deltaretrovirus, Epsilonretrovirus, Lentivirus, Spumavirus, Bunyavirus, Hantavirus, Nairovirus, Phlebovirus, Tospovirus, Calicivirus, Enterovirus, Rhinovirus, Hepatovirus, Cardiovirus, Aphthovirus, Astrovirus, Flavivirus, Pestivirus, Hepacivirus, Alphanodavirus, Coronavirus, Torovirus, Alphavirus, Arterivirus, and Deltavirus; (b) nucleic acid sequences that are reverse translated from no more than one thousand different amino acid sequences, each amino acid sequence comprising a motif conserved in either: (i) a virus family e.g. Asfarviridae, Poxyiridae, Iridoviridae, Herpesviridae, Polydnaviridae, Papovaviridae, Adenoviridae, Circoviridae, Reoviridae, Birnaviridae, Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Bornaviridae, Filoviridae, Arenaviridae, Retroviridae, Bunyaviridae, Caliciviridae, Picornaviridae, Astroviridae, Flaviviridae, Nodaviridae, Coronaviridae, Togaviridae, and Arteriviridae; (ii) a virus genus e.g. Asfivirus, Orthopoxvirus, Parapoxvirus, Avipoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus, Yatapoxvirus, Entomopoxvirus A, Entomopoxvirus B, Entomopoxvirus C, Iridovirus, Chloriridovirus, Ranavirus, Lymphocystivirus, Simplexvirus, Varicellovirus, Cytomegalovirus, Muromegalovirus, Roseolovirus, Lymphocryptovirus, Rhadinovirus, Ichnovirus, Bracovirus, Polyomavirus, Papillomavirus, Mastadenovirus, Aviadenovirus, Orthoreovirus, Orbivirus, Rotavirus, Coltivirus, Aquareovirus, Cypovirus, Fijivirus, Phytoreovirus, Oryzavirus, Aquabirnavirus, Avibirnavirus, Entomobirnavirus, Influenzavirus A, Influenzavirus B, Influenzavirus C, Influenzavirus D, Paramyxovirus, Morbillivirus, Rubulavirus, Pneumovirus, Bornavirus, Marburgvirus, Ebolavirus, Arenavirus, Alpharetrovirus, Betaretrovirus, Gammaretrovirus, Type D Retrovirus group, Deltaretrovirus, Epsilonretrovirus, Lentivirus, Spumavirus, Bunyavirus, Hantavirus, Nairovirus, Phlebovirus, Tospovirus, Calicivirus, Enterovirus, Rhinovirus, Hepatovirus, Cardiovirus, Aphthovirus, Astrovirus, Flavivirus, Pestivirus, Hepacivirus, Alphanodavirus, Coronavirus, Torovirus, Alphavirus, Arterivirus, and Deltavirus; and/or (iii) a virus species from the virus family in (a) or the virus genus in (ii); where the set of oligonucleotides as a whole can detect any virus that infects vertebrates; (c) less than 10,000 different oligonucleotide sequences, where the set of oligonucleotides hybridizes to nucleic acid sequences from at least 10 viral species, where each oligonucleotide of the set comprises a nucleotide sequence reverse translated from an amino acid sequence listed in the CD-ROM Appendix Table; (d) less than 10,000 different oligonucleotide sequences, where the set comprises a nucleotide sequence listed in the CD-ROM Appendix Table; and (e) less than 10,000 different oligonucleotide sequences, where the set comprises a nucleotide sequence complementary to a nucleotide sequence listed in the CD-ROM Appendix Table, is new. The amino acid sequences are listed in the CD-ROM Table Appendix or amino acid sequences that are at least 10 residues in length and 90% identical to the amino acid sequences listed in the CD-ROM Table Appendix. Each oligonucleotide in the set of oligonucleotides comprises a nucleotide sequence that is at least 20 nucleotides in length and has 90-99% sequence identity to sequences listed in the CD-ROM Table Appendix. The motifs comprise an amino acid sequence from a viral polymerase or from a viral capsid. The set further

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comprises oligonucleotides comprising a nucleotide sequence from a non-coding region of a genome of a vertebrate virus that is conserved in a vertebrate family, genus, or species. Preferred Method: Designing an oligonucleotide for viral screening comprises: (a) compiling a database of viral sequences, where the database of viral sequences comprises nucleotide sequences and amino acid sequences representative of at least 10 different species of virus; (b) classifying each nucleotide sequence and amino acid sequence into a viral order, family, genus, and species; (c) identifying from the database of viral sequences a set of amino acid sequences where each amino acid sequence of the set comprises a protein domain or motif; (d) identifying from the set of amino acid sequences of (c) a subset of amino acid sequence motifs that are conserved throughout a viral family, genus, and/or species; (e) determining the nucleotide sequences coding for the subset of amino acid sequence motifs of (d), where the nucleotide sequences are obtained from the database of viral sequences; and (f) designing a group of oligonucleotides comprising nucleotide sequences selected from the nucleotide sequences coding for the subset of amino acid sequence motifs. In the method, the designing of (f) comprises using a set covering algorithm to determine a minimum number of sequences that needs to be selected from the nucleotide sequences coding for the subset of amino acid sequence motifs in order to represent every viral species in the viral database. In step (f), the oligonucleotides comprise nucleotide sequences selected from nucleotide sequences that code for amino acid sequence motifs conserved in a single viral family or genus. The viral sequence database consists essentially of sequences classified to be from vertebrate viruses. The viral sequence database does not comprise sequences from viruses that infect plants or bacteria. The compiling step comprises obtaining a nucleotide sequence or amino acid sequence identified to be viral from one or more public sequence collections, where the public sequence databases comprise GenBank; DNA DataBank of Japan (DDBJ); the European Molecular Biology Laboratory (EMBL); Reference Sequence (RefSeq) collection; translated coding regions from DNA sequences in GenBank, EMBL, and DDBJ; Protein Information Resource (PIR); SWISS-PROT; Protein Research Foundation (PRF); and Protein Data Bank (PDB); and any successor entity. The viral database comprises sequences from at least 10 species of vertebrate viruses or sequences for partial genomes of a viral species or for partial coding sequences for a viral protein. The nucleotide sequences for a viral species comprise sequences from more than one representative genome of the virus species. In the method, the classifying step further comprises classifying each nucleotide sequence and amino acid sequence into a viral subfamily, serogroup, subspecies, and/or isolate. The classifying step is based on viral taxonomic tree structure criteria from the International Committee on the Taxonomy of Viruses. The identifying in (c) comprises using Hidden Markov Models (HMM). Step (d) comprises using a probabilistic model for identifying from the set of amino acid sequences of (c) the subset of amino acid sequence motifs that are conserved throughout a viral family, genus, and/or species. The probabilistic model is a multiple EM for motif estimation (MEME) algorithm. Identifying a virus from a environmental or clinical sample, the method comprises: (a) isolating nucleic acids from a sample containing the virus; (b) labeling the nucleic acids with a label; (c) hybridizing the labeled nucleic acids to a set of oligonucleotides above; and (d) identifying the nucleic acids from the set of nucleic acids above that hybridized to the labeled nucleic acids. Designing one or more primers comprises: (a) generating a similarity matrix of multiple nucleic acid sequence sub-alignments within a nucleic acid sequence alignment by pairwise comparison with a tree structure building; (b) generating a phylogenetic tree of nodes from the similarity matrix of (a) by hierarchical clustering, where each node comprises a one or more nucleic acid sequences in a sub-alignment; (c) identifying one or more nucleic acid sequences in each node of (b) by scoring on the basis of one or more parameters; (d) determining a minimum number of nucleic acid sequences identified in (c) capable of amplifying the nucleic acid sequence in the subalignment of (b) with a set covering algorithm; and (e) identifying nucleic acid sequences that are capable of forming primer pairs on the basis of one or more

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parameters. The tree structure building algorithm comprises: (a) a method of extracting sub-alignments from an entire alignment; (b) a method of filtering sub-alignments for uniqueness; and (c) a method of performing a pairwise comparison of sub-alignments. The hierarchical clustering algorithm is based on Euclidean distance. The parameters measured by the scoring function comprises: melting temperature, guanine-cytosine (GC) content, homopolymeric runs, hairpin/primer dimer formation, degeneracy, ability to hybridize to a template, total mismatches to a template. The set covering algorithm is a greedy algorithm. In the method, the parameters used to identify nucleic acid sequences capable of forming primer pairs comprise the length of an amplicon or melting temperature differences between nucleic acids. The pairs can encode a viral amino acid sequence. Designing a database of coding viral oligonucleotides comprises: (a) compiling a database of viral nucleic acid sequences; (b) compiling a database of viral protein sequences; (c) identifying a subset of viral nucleic acid sequences in the database of (a) capable of encoding one or more amino acid sequences having at least 90% sequence identity to any viral protein sequence in the viral protein database of (b), where nucleic acid sequences in the subset of viral nucleic acid sequences comprise oligonucleotides having a length of 10-250 nucleotides, preferably 25-60 nucleotides; (d) translating the oligonucleotides of (c) to generate a database of back-translated viral protein sequences; (e) identifying amino acid sequences in the back translated viral protein sequences of (d) that share at least 60% identity with conserved eukaryotic, viral, and bacterial protein domains, where the identification is made with a Hidden Markov model algorithm with an algorithm for pairwise comparison of homologous clusters; (f) identifying the viral nucleic acid sequences in the database of (a) that are capable of encoding amino acid sequences identified in (e); (g) identifying nucleic acid sequences that are statistically overrepresented in the viral nucleic acid sequences of (f), where the identification is made with a probabilistic model algorithm; (h) identifying oligonucleotides from nucleic acid sequences in (g) that are suitable for hybridization; and (i) compiling the oligonucleotides identified in (h) into a database of viral oligonucleotides, where the database is a database of coding viral oligonucleotides. Designing a database of degenerate coding viral oligonucleotides comprises: (a) compiling a database of viral nucleic acid sequences; (b) compiling a database of viral protein sequences; (c) identifying a subset of viral nucleic acid sequences in the database of (a) capable of encoding one or more amino acid sequences having at least 90% sequence identity to any viral protein sequence in the viral protein database of (b), where nucleic acid sequences in the subset of viral nucleic acid sequences comprise oligonucleotides having a length of 10-250 nucleotides, preferably 25-60 nucleotides; (d) translating the oligonucleotides of (c) to generate back-translated viral protein sequences; (e) identifying amino acid sequences in the back translated viral protein sequences of (d) that share at least 60% identity with conserved eukaryotic, viral and bacterial protein domains, where the identification is made with a Hidden Markov model algorithm with an algorithm for pairwise comparison of homologous clusters; (f) identifying a minimum set of degenerate nucleotides sequences that are capable of encoding the amino acid sequences identified in (e); (g) identifying nucleic acid sequences that are statistically overrepresented in the viral nucleic acid sequences of (f), where the identification is made with a probabilistic model algorithm; (h) identifying oligonucleotides from nucleic acid sequences in (g) that are suitable for hybridization; and (i) compiling the oligonucleotides identified in (h) into a database of viral oligonucleotides, where the database is a database of degenerate coding viral oligonucleotides. Designing a database of non-coding viral oligonucleotides comprises: (a) compiling a database of viral nucleic acid sequences; (b) compiling a database of viral protein sequences; (c) identifying a subset of viral nucleic acid sequences in the database of (a) that are not capable of encoding one or more proteins having at least 80% sequence identity any viral protein sequence inthe viral protein database of (b), where nucleic acids sequences in the subset of viral nucleic acid sequences compriseoligonucleotides having a length of 10-250 nucleotides, preferably 25-60 nucleotides; (d) identifying nucleic acid sequences that are statistically overrepresented

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in the viral nucleic acid sequences of (f), where the identification is made with a probabilistic model algorithm; (e) identifying oligonucleotides from nucleic acid sequences in (g) that are suitable for hybridization; (f) compiling the oligonucleotides identified in (h) into a database of viral oligonucleotides, where the database is a database of non-coding viral oligonucleotides. Identifying sequence patterns that are conserved across viral taxa or within a viral taxon comprises steps (a)-(g) of (6). Identifying sequence patterns that are conserved across viral taxa or within a viral taxon comprises steps (a)-(g) of (7). Identifying sequence patterns that are conserved across viral taxa or within a viral taxon comprises steps (a)-(d) of (8). The sequence patterns are nucleic acid motifs, amino acid motifs, or protein domains. Generating conserved peptides that can be used as immunogens for the generation of an antibody against a virus, where one or more oligonucleotides above are translated to produce immunogens for the generation of antibodies. Updating a database of genetic information comprises: (a) obtaining one or more sequences from at least one source of information at an interval; (b) reconciling differences among the one or more sequences obtained in (a) and sequences in a database above; (c) determining if the one or more sequences should be added to the database; and (d) adding the one or more sequences in the database, where the genetic information in the database is updated. The determining comprises determining if the sequence is covered, within a programmable difference of nucleotide mismatches, by a sequence already in the database. Preferred Product: The computer program product residing on a computer readable medium comprises instructions for causing a computer to: (a) compile a database of viral sequences, where the database of viral sequences comprises nucleotide sequences and amino acid sequences representative of at least 10 different species of virus; (b) classify each nucleotide sequence and amino acid sequence into a viral order, family, genus, and species; (c) identify from the database of viral sequences a set of amino acid sequences where each amino acid sequence of the set comprises a protein domain; (d) identify from the set of amino acid sequences of (c) a subset of amino acid sequence motifs that are conserved throughout a viral family, genus, and/or species; (e) determine the nucleotide sequences coding for the subset of amino acid sequence motifs of (d), where the nucleotide sequences are obtained from the database of viral sequences; and (f) design a group of oligonucleotides comprising nucleotide sequences selected from the nucleotide sequences coding for the subset of amino acid sequence motifs. Preferred Medium: The computer-readable medium containing computer-executable instructions that, when executed by a processor, cause the processor to perform a method for designing an oligonucleotide for viral screening, where the method comprises the method of (1), (6), (7), or (8). The computer-readable medium for storing data for access by an application comprises a tree structure stored in the computer-readable medium, where the tree structure comprises nodes connected by edges, where the nodes is a top node describing a viral nucleic acid sequence, and where the top node has at least two child nodes describing a viral nucleic acid sequence, where the at least two child nodes are generated by the method of (5). The nodes in the tree structure correspond to a viral family, genus or species. Preferred System: The system for mapping a viral nucleic acid sequence to a tree structure comprises: an interface; a memory containing the tree structure of claim 50; and a processor in communication with the memory and the interface, where the processor: (a) receives nucleic acid hybridization information from the interface; (b) receives instructions from the memory, where the instructions from the memory comprise instructions that when executed by the processor cause the processor to map the nucleic acid hybridization information of (a) to a node of tree structure of the computer-readable medium and generate an output; (c) sends the output the interface. The interface is in communication with a network. In the system, the nucleic acid hybridization information is from a micro array. The nucleic acid hybridization information comprises a pattern of positive signals. The processor receives instructions from the memory to: (a) analyze a pattern of positive signals in the hybridization information; (b) eliminate signals from internal controls and position makers; and (c)

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calculate a probability that the pattern of positive signals matches a viral family, genus, or species. The system for the diagnosis, surveillance, or discovery of infection or disease comprises: (a) a processor; (b) a storage device coupled to the processor; (c) a database above residing on the storage device, where the processor checks a database for new genetic information and updates the database with the genetic information; and (d) an input device coupled to the processor which inputs a genetic sequence and hybridization results of the genetic sequence, where the processor analyzes the hybridization results of the genetic sequence and generates information regarding the placement of the genetic sequence in the database.EXAMPLE :
No suitable example given.

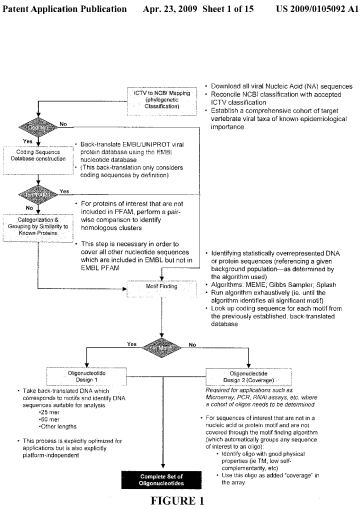

FIGURE 1

16/29	@ WPI / Thomson
PN	CN101423874A 2009-05-06 DW200936 CN101423874B 2011-08-31 DW201177
TI	Foot and mouth disease virus multiplex reverse transcriptase (RT)-PCR testing kit useful in field of testing animal epidemics, comprises buffer solution, deoxynucleotide triphosphate mixture, magnesium chloride, and inverse transcriptase
PA	(LANZ-N) LANZHOU VETERINARY RES INST ACAD AGRIC SCI(LANZ-N) LANZHOU VETERINARY RES INST CHINA AGRIC
ICAI	C12Q1/68; C12Q1/70;
AB	- NOVELTY : A foot and mouth disease virus multiplex reverse transcriptase (RT)-PCR testing kit comprising singly packed ingredients whose concentrations are not lower than the concentration of one RT-PCR reaction, RT-PCR reaction buffer solution, deoxynucleotide triphosphate (dNTP) mixture, 25 mmol/L of magnesium chloride (MgCl 2), inverse transcriptase, RNase inhibitory factor, Taq enzyme, double distilled water without RNA enzyme, positive strand amplification primer and negative strand amplification primer, is new. - DETAILED DESCRIPTION : A foot and mouth disease virus multiplex RT-PCR testing kit comprising singly packed ingredients whose concentrations are not lower than the concentration of one reverse transcriptase (RT)-PCR reaction, RT-PCR reaction buffer solution, deoxynucleotide triphosphate (dNTP) mixture, 25 mmol/L of magnesium chloride (MgCl 2), inverse transcriptase, RNase inhibitory factor, Taq enzyme, double distilled water without RNA enzyme, positive strand amplification primer and negative strand amplification primer, where positive strand amplification primer comprises primer 1: gactcg/aacgtctccc/tgccaact (SEQ ID NO.: not defined); primer 2: acgacg/cggggcttttgctttcac (SEQ ID NO.: not defined); primer 3: cg/tggaaacgcac/tgagcagtatc (SEQ ID NO.: not defined); and the negative strand amplification primer comprises primer 4: tgcgg/tacggccacc/gtactacttc (SEQ ID NO.: not defined); primer 5: agctccacgaa/gaaa/ggtgtcgag (SEQ ID NO.: not defined); and primer 6: cgtgatgtg/agcg/aagaatgaagaa (SEQ ID NO.: not defined), is new. - USE : The foot and mouth disease virus multiplex RT-PCR testing kit is useful in the field of testing animal epidemics. - ADVANTAGE : The multiplex RT-PCR testing kit has high sensibility which is 10-100 higher than that of the conventional RT-PCR testing kit, moreover it has good particularity; even if the clinical sample is not stored unduly or not fresh in the collecting process; the foot and

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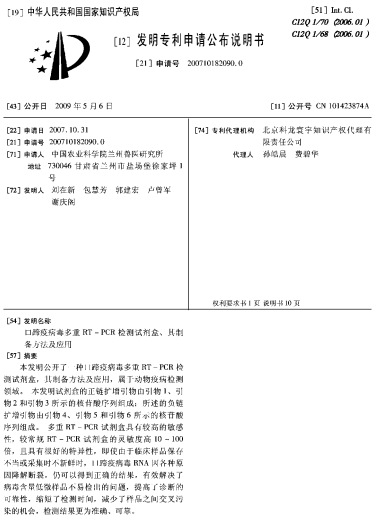
mouth disease virus RNA can be de-gradated and ruptured for different reasons, and the right result can also be obtained; the problem that the sample including low virus content is not tested can be solved; thus, the diagnosis dependability can be increased; the testing time can be shortened; the chances of cross contamination among the samples are reduced; furthermore, the testing result can be precise and dependable.

- BIOTECHNOLOGY :

Preferred Kit: In the kit above, the positive strand amplification primer comprises nucleotide sequences shown in primer 1, primer 2 and primer 3 according to their mass ratio of 1:1:2, respectively; and the negative strand amplification primer comprises nucleotide sequences shown in primer 4, primer 5 and primer 6 according to their mass ratio of 1:1:2, respectively. The positive strand amplification primer is prepared by using automatic DNA combiner to combine the primer 1, primer 2 and primer 3; diluting them to the solution whose viscosity is 12.5 mol/micron; and mixing them according to their mass ratio or volume ratio of 1:1:2; and the negative strand amplification primer is prepared by the following method: respectively using automatic DNA combiner to combine the primer 4, primer 5 and primer 6; diluting them to the solution whose viscosity is 12.5 mol/ micron; and mixing them according to their mass ratio or volume ratio of 1:1:2. The inverse transcriptase is avian myeloblastosis virus (AMV) RT XL; and the Taq enzyme is AMV-Optimized Taq. The testing kit includes a blank control or/and a positive control.



[19] 中华人民共和国国家知识产权局

[12] 发明专利申请公布说明书

[21] 申请号 200710182090.0

[51] Int. Cl.
C12Q 1/70 (2006.01)
C12Q 1/68 (2006.01)

[43] 公开日 2009 年 5 月 6 日

[11] 公开号 CN 101423874A

[22] 申请日 2007.10.31

[21] 申请号 200710182090.0

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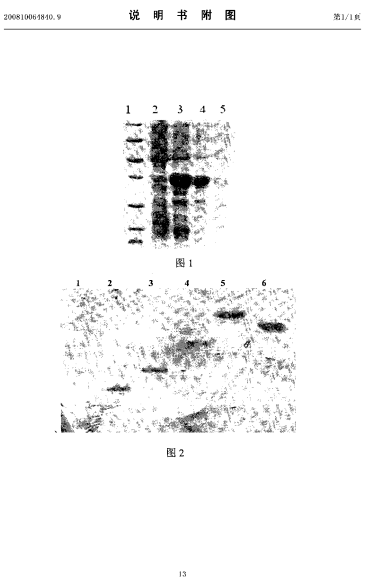
[54] 发明名称

口蹄疫病毒多重 RT－PCR 检测试剂盒、其制备方法及应用

[57] 摘要

本发明公开了一种口蹄疫病毒多重 RT－PCR 检测试剂盒，其制备方法及应用，属于动物疫病检测领域。本发明试剂盒的正链扩增引物由引物 1、引物 2 和引物 3 所示的核苷酸序列组成，所述的负链扩增引物由引物 4、引物 5 和引物 6 所示的核苷酸序列组成。多重 RT－PCR 试剂盒具有较高的敏感性，较常规 RT－PCR 试剂盒的灵敏度高 10－100 倍，且具有很好的特异性，即使由于临床样品保存不当或采集时不新鲜时，口蹄疫病毒 RNA 因各种原因降解酶，仍可以得到正确的结果，有效解决了病毒含量低致样品不易检出的问题，提高了诊断的可靠性，缩短了检测时间，减少了样品之间交叉污染的机会，检测结果更为准确、可靠。

17/29	@ WPI / Thomson
PN	CN101302512A 2008-11-12 DW200910
TI	New gene order of epitope antigen, useful for differential diagnosing foot-and-mouth disease
PA	(UYDO-N) UNIV DONGBEI AGRIC
ICAI	C12N15/11; G01N33/68;
AB	- NOVELTY : A gene order of epitope antigen for differential diagnosing foot-and-mouth disease comprising fully defined 72-699 bp (SEQ ID NOS: not defined) given in the specification, is new. - USE : The gene order of epitope antigen is useful for differential diagnosing foot-and-mouth disease (claimed). - ADVANTAGE : The invention solves the problem that prior differential antigen of foot-and-mouth disease will generate non-specific reaction and will cause false positive after detection. Additionally, the protein expressed by the gene order of the invention conquers the problem that high molecular weight recombinant protein generates non-specific reaction and false positive after detection is caused.



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1 2 3 4 5

图 1

1 2 3 4 5 6

图 2

13

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PN	US2008280296A1 2008-11-13 DW200901 TW200844441A 2008-11-16 DW200943 TWI329742B 2010-09-01 DW201176
TI	Detecting foot-and-mouth disease virus (FMDV) with chromatographic strip test, where primers are designed by the nuclei acid sequence of non-structure proteins (NSPs) of FMDV
PA	(ANIM-N) ANIMAL HEALTH RES INST COUNCIL AGRIC EXE(CHEN-I) CHEN T(JONG-I) JONG M(PANC-I) PAN C
ICAI	C12Q1/68; G01N33/569;
AB	- NOVELTY : Detecting foot-and-mouth disease virus (FMDV) with chromatographic strip test, where primers are designed by the nuclei acid sequence of non-structure proteins (NSPs) of FMDV. - DETAILED DESCRIPTION : Detecting FMDV with chromatographic strip test, where primers are designed by the nuclei acid sequence of NSPs of FMDV, a reverse transcriptase polymerase chain reaction (RT-PCR) method is utilized to amplify the nuclei acid of virus, recombinant vectors are constructed and performed through a prokaryotic system to transform and express recombinant proteins, and the purified recombinant proteins are mass produced; a chromatographic test strip (pen-side strip) is made following the above process, only one drop of body fluid is required to the test strip for completing qualitative test, and the test strip is operated with a portable POCT (Point of care testing) detector for completing quantitative test comprises: (a) searching from nuclei acid database in a GenBank, an immunity determinant gene of non-structure protein nuclei acid sequence of FMDV O/TAW/97 and O/TAW/99 was retrieved as a main target gene for detection; (b) the nuclei acid sequence of FMDV NSPs is designed by the RT-PCR method to be specific primers which specifically amplify the FMDV non-structure protein gene regions of cDNA templates, for synthesizing DNA products; (c) DNA sequence fragments of the target gene are respectively ligated into prokaryotic expressing vectors to complete the construction of recombinant plasmids; (d) inserting tests of sequencing and alignment to confirm cutting sites and size of inserted fragments of the designed DNA fragments; (e) transformation of the confirmed DNA plasmids is performed in a prokaryotic expressing system and Isopropylthiogalactoside (IPTG) of final concentration 1 mM is added to perform induced expression. SDS-PAGE assay was conducted to confirm the expected molecular weight, and then mass producing and purifying the recombinant proteins by affinity chromatography column (HisTrap HP). Completing the production of chromatographic test strip and applying the test strip to detect the body fluid antibodies; and (f) recombinant proteins were confirmed by utilizing a western blot assay to prove that about 20-40 KDa functional proteins react with the antibody of the FMDV O/TAW/97 and O/TAW/99 antiserum in signal recognition.ACTIVITY : Virucide. No biological data given.MECHANISM OF ACTION : Vaccine. - USE : The method is useful for detecting FMDV (claimed). - ADVANTAGE : The invention is easy and simple to handle, no need of elaborate equipment, only one drop of body fluid is required to quickly complete the qualitative test in 10-20 minutes, and operating with a portable POCT instrument to complete the quantitative detection within 40-50 minutes. - BIOTECHNOLOGY : Preferred Method: In detecting FMDV, principles of the design are based on immunoassay and chromatographic analysis. The specific primers are forward primers FMDV-3ABC-F and FMDV3BC-F. The FMDV3ABC-F comprises fully defined is 38 bp (SEQ ID NO. 1), and the FMDV3BC-F comprises fully defined 24 bp (SEQ ID NO. 3) given in the specification. The specific primers are reverse primers FMDV-3ABC-R and FMDV3BC-R. The FMDV3ABC-R comprises fully defined 36 bp (SEQ ID NO. 2) and

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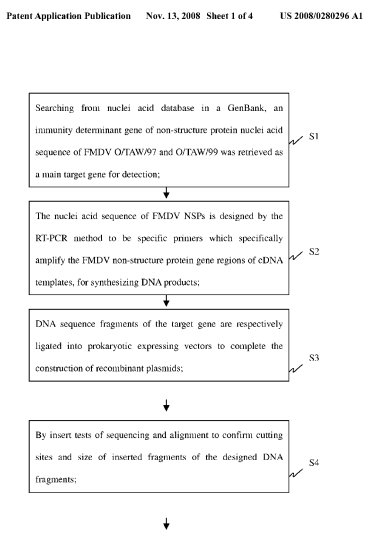

the FMDV-3BC-R comprises 27 bp (SEQ ID NO. 4) given in the specification. The structure and non-structure proteins of FMDV comprise VP1, VP2, VP3, VP4, Lb, 2B, 2C, 3A, 3D, 3AB, 3BC or 3ABC. The non-structure proteins are protein G and/or protein A. The FMDV antibodies particularly use the FMDV non-structure proteins comprising Lb, 2B, 2C, 3A, 3AB, 3BC, 3ABC or 3D. The chromatographic test strip simultaneously detect antibodies to the non-structure proteins of four serotypes of FMDV O, A, C and Asia 1. The body fluid is a whole blood or serum. The chromatographic test strip completes the qualitative test within 10-20 minutes. Portable POCT detector completes the quantitative test within 40-50 minutes. 5'-accggatcctgtggaccctacacc-3' (SEQ ID NO. 3); 5'-cccgaattcgcacgtcttcccgtcgag-3' (SEQ ID NO. 4).SPECIFIC SEQUENCES :
Specifically claimed are NSPs comprising 24-38 bp (SEQ ID NO. 1-4). All sequences are fully defined in the specification. 5'-accggatcctgtggaccctacacc-3' (SEQ ID NO. 3); 5'-cccgaattcgcacgtcttcccgtcgag-3' (SEQ ID NO. 4).EXAMPLE :
No example given.

19/29	@ WPI / Thomson
PN	CN101270155A 2008-09-24 DW200878 CN101270155B 2011-04-27 DW201168
TI	New empty capsid protein, which is a mutant protein obtained by performing the acid resistant alteration of P12A of aphthovirus, useful for preparing the aphthovirus vaccine or aphthovirus diagnostic reagent
PA	(LANZ-N) LANZHOU VETERINARY INST CHINESE ACAD AGR(LANZ-N) LANZHOU VETERINARY RES INST CHINA AGRIC
ICAI	A61K39/135; A61P31/14; C07K14/09; C12N15/42; C12N15/63; C12N15/866; C12N5/10; G01N33/569;
AB	- NOVELTY : An empty capsid protein, which is a mutant protein obtained by performing the acid resistant alteration of P12A of aphthovirus, is new. - DETAILED DESCRIPTION : An empty capsid protein, which is a mutant protein obtained by performing the acid resistant alteration of P12A of aphthovirus, where acid resistant alteration comprises mutating the histidine residue in the 140th-145th sites from the amino terminal of viral particle (VP)3 in P12A of aphthovirus to amino acid without positive charge in acidic environment; the amino acid without positive charge is asparagine, cysteine, glutamic acid, glutamine, proline, serine, threonine, tyrosine, tryptophan, leucine, isoleucine, valine, lactamine, aspartic acid, glycine, methionine, or phenylalanine, preferably leucine, isoleucine, or valine; the VP4, VP2, VP3, VP1, and 2A proteins of the P12A are from a same strain of aphthovirus or from different strains of aphthovirus respectively, is new. INDEPENDENT CLAIMS are: (1) a gene encoding the protein; (2) a recombinant carrier and transgenic cell lines containing the gene; and (3) a method of expressing aphthovirus empty capsid in insect cell comprising: (a) leading the modified non-structural protein gene 3C of P12A gene and aphthovirus into bacterium through rhabdovirus carrier for performing recombination to obtain the recombinant rhabdovirus A; and (b) using the DNA of recombinant rhabdovirus A to transfect the insect cells to obtain the aphthovirus empty capsid.MECHANISM OF ACTION :

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Vaccine.
- USE :
The aphthovirus empty capsid is useful for preparing the aphthovirus vaccine or aphthovirus diagnostic reagent (claimed).
- BIOTECHNOLOGY :
Preferred Capsid Protein: The empty capsid is one of following seven proteins: (a) the amino acid sequence of SEQ ID NO. 2; (b) the mutant protein obtained by performing the acid resistant alteration for P12A protein of O type FMDV in the 217th-953rd sites of amino terminal with amino acid sequence from GenBank Accession Number AF511039, the acid resistant alteration means that that the histidine residue in the 141st-144th sites from the amino terminal of VP3 in P12A of aphthovirus is mutated to amino acid without positive charge in acidic environment; the amino acid without positive charge in acidic environment is asparagines, cysteine, glutamic acid, glutamine, proline, serine, threonine, tyrosine, tryptophan, leucine, isoleucine, valine, lactamine, aspartic acid, glycine, methionine or phenylalanine; (c) the mutant protein obtained by performing the acid resistant alteration for P12A protein of A type FMDV in the 219th-956th sites of amino terminal with amino acid sequence from GenBank Accession Number EF494487, the acid resistant alteration means that that the histidine residue in the 142nd-145th sites from the amino terminal of VP3 in P12A of aphthovirus is mutated to amino acid without positive charge in acidic environment; the amino acid without positive charge in acidic environment is asparagines, cysteine, glutamic acid, glutamine, proline, serine, threonine, tyrosine, tryptophan, leucine, isoleucine, valine, lactamine, aspartic acid, glycine, methionine or phenylalanine; (d) the mutant protein obtained by performing the acid resistant alteration for P12A protein of C type FMDV in the 217th-948th sites of amino terminal with amino acid sequence from GenBank Accession Number AM409325, the acid resistant alteration means that that the histidine residue in the 140th-143rd sites from the amino terminal of VP3 in P12A of aphthovirus is mutated to amino acid without positive charge in acidic environment; the amino acid without positive charge in acidic environment is asparagines, cysteine, glutamic acid, glutamine, proline, serine, threonine, tyrosine, tryptophan, leucine, isoleucine, valine, lactamine, aspartic acid, glycine, methionine or phenylalanine; (e) the mutant protein obtained by performing the acid resistant alteration for P12A protein of SAT1 type FMDV in the 215th-960th sites of amino terminal with amino acid sequence from GenBank Accession Number NC011451, the acid resistant alteration means that that the histidine residue in the 142nd-145th sites from the amino terminal of VP3 in P12A of aphthovirus is mutated to amino acid without positive charge in acidic environment; the amino acid without positive charge in acidic environment is asparagines, cysteine, glutamic acid, glutamine, proline, serine, threonine, tyrosine, tryptophan, leucine, isoleucine, valine, lactamine, aspartic acid, glycine, methionine or phenylalanine; (f) the mutant protein obtained by performing the acid resistant alteration for P12A protein of SAT2 type FMDV in the 215th-956th sites of amino terminal with amino acid sequence from GenBank Accession Number NC003992, the acid resistant alteration means that that the histidine residue in the 142nd-145th sites from the amino terminal of VP3 in P12A of aphthovirus is mutated to amino acid without positive charge in acidic environment; the amino acid without positive charge in acidic environment is asparagines, cysteine, glutamic acid, glutamine, proline, serine, threonine, tyrosine, tryptophan, leucine, isoleucine, valine, lactamine, aspartic acid, glycine, methionine or phenylalanine; (g) the mutant protein obtained by performing the acid resistant alteration for P12A protein of SAT3 type FMDV in the 215th-956th sites of amino terminal with amino acid sequence from GenBank Accession Number AY593852, the acid resistant alteration means that that the histidine residue in the 142nd-145th sites from the amino terminal of VP3 in P12A of aphthovirus is mutated to amino acid without positive charge in acidic environment; the amino acid without positive charge in acidic environment is asparagines, cysteine, glutamic acid, glutamine, proline, serine, threonine, tyrosine, tryptophan, leucine, isoleucine, valine, lactamine, aspartic acid, glycine, methionine or phenylalanine. Preferred Method: Expressing aphthovirus empty capsid in insect cell

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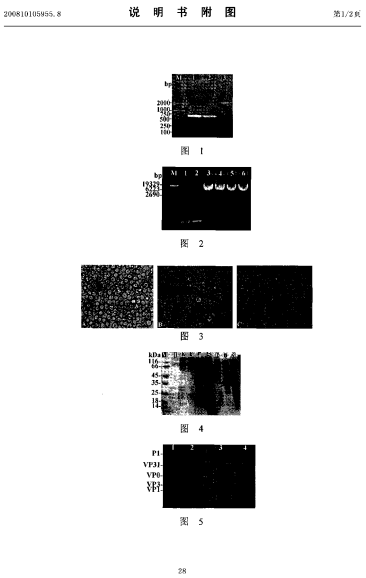
further comprises using the recombinant rhabdovirus obtained by using the DNA of recombinant rhabdovirus A to transfect the insect cells to infect the insect cells again to obtain the aphthovirus empty capsid. The insect cells are Sf9 cell lines, sf21 cell lines, or High Five cell lines. The gene and non-structural protein gene 3C of aphthovirus are transfected into bacterium by a same rhabdovirus carrier containing double promoters, the gene is inserted into the downstream of one promoter of the double-promoter rhabdovirus carrier, the non-structural protein gene 3C of aphthovirus is inserted into another promoter of the double-promoter rhabdovirus carrier; and the bacterium is Escherichia coli. The double-promoter rhabdovirus carrier is the carrier capable of generating transposable recombination in bacterium. Preferably, the double-promoter rhabdovirus carrier is pFastBacDual.



20/29	@ WPI / Thomson
PN	WO2007093816A1 2007-08-23 DW200867 EP1996719A1 2008-12-03 DW200882 US2009068672A1 2009-03-12 DW200920 JP2009526542A 2009-07-23 DW200948
TI	Use of red nucleic acid stain in the detection of nucleic acids in real-time polymerase chain reaction
PA	(ENIG-N) ENIGMA DIAGNOSTICS LTD
ICAI	C12N15/09; C12Q1/68;
AB	- NOVELTY : A red nucleic acid stain is used in the detection of nucleic acids in a polymerase chain reaction (PCR). - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are also included for the following: (1) a method for detecting a nucleic acid sequence in a biological sample during amplification comprising adding a thermostable polymerase and primers for amplification of the target nucleic acid sequence to the biological sample; amplifying the target nucleic acid sequence by the polymerase chain reaction in the presence of a nucleic acid stain and optionally additional signaling fluorophores; illuminating the biological sample with light at a wavelength absorbed by the nucleic acid stain or the optional additional fluorophore; and detecting a fluorescent emission from the sample related to the presence or amount of amplified target nucleic acid sequence in the sample; (2) a method for determining a characteristic of a sequence, comprising adding to a sample suspected of containing the sequence, a fluorescently labeled probe specific for the target sequence and a DNA duplex binding agent able to absorb fluorescence from a fluorescent label on the probe, where one of the label on the probe or the DNA duplex binding agent is a nucleic acid stain; subjecting the sample to a variable reaction condition, during which the probe hybridizes to the target sequence; monitoring fluorescence from the sample and determining a particular reaction condition, characteristic of the sequence, at which fluorescence changes as a result of the hybridization of the probe to the sample or destabilization of the duplex formed between the probe and the target nucleic acid sequence; and (3) a kit for the detection of a nucleic acid, the detection of the progress of nucleic acid amplification or for determining a characteristic of a nucleic acid sequence, comprising

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a red nucleic acid stain.
- USE :
For the detection of nucleic acids in PCR.
- ADVANTAGE :
The use of nucleic acid stains provides a Universal Acceptor arrangement where multiple light sources can transfer energy to a single DNA binding dye. This may work on many platforms without requiring the monitoring of the acceptor dye.
- BIOTECHNOLOGY :
Preferred Structure: The nucleic acid stain is a red stain which is of formula (IIA). The compound (IIA) is a compound of formula (III).
n : 0-2;
R <30>-R <32>H, 1-6C alkyl, 3-10C cycloalkyl, or (hetero)aryl;
R <2>H, 1-6C alkyl that is optionally substituted by sulfonate, carboxy or amino;
R <3>, R <4>H; 1-6C alkyl that is optionally saturated; or halo, or cyclic group (from (hetero)aryl, or 3-10C cycloalkyl which may be optionally substituted by halo, amino, alkyl, perfluoroalkyl, (di)alkylamino, alkoxy or carboxyalkyl, where each alkyl group has 1-6C; or by TAIL moiety); or -OR <8>, -SR <8>, -(NR <8>R <9>); or TAIL;
R <8>, R <9>1-6C alkyl; or 1-2 alicyclic or aromatic rings; or in combination may be -(CH 2) 2-V-(CH 2) 2;
V : single bond, -O-, -CH 2-, or -NR <10>-;
R <10>H or 1-6C alkyl;
R <5>heteroaryl group;
R <30>-R <32>H, 1-6C alkyl, 3-10C cycloalkyl, or (hetero)aryl;
L, BRIDGE : single covalent bond or covalent linkage that is optionally saturated having 1-16 nonhydrogen atoms from C, N, P, O or S, such that the linkage contains ether, thioether, amine, ester, and/or amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds;
TAIL : heteroatom-containing moiety from group of LINK-SPACER-CAP or groups of sub-formulae (1-3);
LINK : NR <20>;
R <20>H, 1-5C alkyl or group -SPACER'-CAP';
SPACER : 1-6C in saturated chain;
CAP : group OR <21>, -SR <21>, -NR <21>R <22>, or -N <+>R <21>R <22>R <23.>PSI <->;
R <21>-R <23>H or optionally substituted (cyclo)alkyl group having 1-8C; and
PSI <->counterion.
[Image]
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[Image]ORGANIC CHEMISTRY :
Preferred Component: The dye is SYTO(RTM: red fluorescent nucleic acid stain) including SYTO 17(RTM: red fluorescent nucleic acid stain), SYTO 59(RTM: red fluorescent nucleic acid stain), SYTO 60(RTM: red fluorescent nucleic acid stain), SYTO 61(RTM: red fluorescent nucleic acid stain), SYTO 62(RTM: red fluorescent nucleic acid stain), SYTO 63(RTM: red fluorescent nucleic acid stain), or SYTO 64(RTM: red fluorescent nucleic acid stain).EXAMPLE :
An aqueous polymerase chain reaction mix formulation was prepared from Tris (50 mM), Bovine Serum Albumin (250 ng/mu l), magnesium chloride (3mM), dUTP Nucleotides (200 mu M), Taq Polymerase at 0.04 units/mu l, antiTaq antibody (0.04 units/mu l), forward primer (1 mu M), reverse primer (1mu M), SYTO(RTM: red fluorescent nucleic acid stain) (5 mu M), and fluorescein labeled probe (0.2 mu M).

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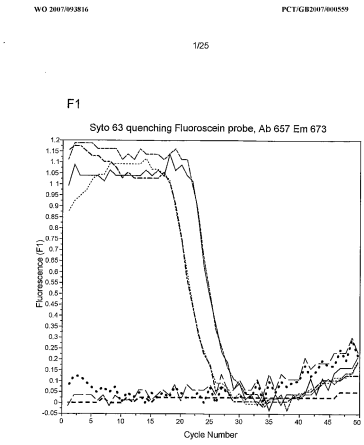

Fig. 1a

SUBSTITUTE SHEET (RULE 26)

21/29	@ WPI / Thomson
PN	WO2007046825A2 2007-04-26 DW200746 EP1831404A2 2007-09-12 DW200761 AU2005337484A1 2007-06-07 DW200765 CN101111605A 2008-01-23 DW200833 JP2008522195A 2008-06-26 DW200844 EP1831404B1 2010-04-28 DW201029 AU2005337484B2 2010-04-29 DW201031 DE602005020978E 2010-06-10 DW201038 WO2007046825A3 2007-07-26 DW201229 CN101111605B 2013-04-03 DW201363 JP5523673B2 2014-06-18 DW201440
TI	Detecting a nucleic acid sequence in a sample by combining the sample with an antigen-conjugated oligonucleotide, adding an emittor cell and measuring photon emission from the emittor cell
PA	(MASI) MASSACHUSETTS INST TECHNOLOGY
ICAI	C12M1/00; C12M1/34; C12N15/09; C12Q1/68; G01N21/76; G01N21/78; G01N33/53; G01N33/536;
AB	- NOVELTY : Detecting a nucleic acid sequence in a sample comprises: (1) combining the sample with at least one antigen-conjugated oligonucleotide under conditions suitable for hybridization of the antigen-conjugated oligonucleotide to the nucleic acid sequence; (2) adding an emittor cell; and (3) measuring photon emission from the emittor cell. - DETAILED DESCRIPTION : Detecting a nucleic acid sequence in a sample comprises: (1) combining the sample with at least one antigen-conjugated oligonucleotide under conditions suitable for hybridization of the antigen-conjugated oligonucleotide to the nucleic acid sequence; (2) adding an emittor cell comprising one or more receptors which bind to the antigen of the antigen-conjugated oligonucleotides, where binding of the one or more receptors to the antigen results in an increase in intracellular calcium, and an emittor molecule that emits a photon in response to the increase in intracellular calcium; and (3) measuring photon emission from the emittor cell. INDEPENDENT CLAIMS are:

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(1) a method of detecting a target particle in a sample;
(2) a method of detecting a soluble antigen in a sample;
(3) an optoelectronic sensor device for detecting a target particle in samples using a photon detector or for detecting a nucleic acid sequence in one or more samples using a photon detector; and
(4) a method of detecting a chemical in a sample.
- USE :
The method is useful in detecting a nucleic acid sequence in a sample (claimed).
- BIOTECHNOLOGY :
Preferred Method: Detecting a nucleic acid sequence in a sample comprises:
(1) combining the sample with at least one antigen-conjugated oligonucleotide under conditions suitable for hybridization of the antigen-conjugated oligonucleotide to the nucleic acid sequence;
(2) adding an emittor cell comprising one or more receptors which bind to the antigen of the antigen-conjugated oligonucleotides, where binding of the one or more receptors to the antigen results in an increase in intracellular calcium, and an emittor molecule that emits a photon in response to the increase in intracellular calcium; and
(3) measuring photon emission from the emittor cell.
The method comprises:
(1) combining the sample with antigen-conjugated oligonucleotides under conditions suitable for hybridization of the antigen-conjugated oligonucleotides to the nucleic acid sequence;
(2) adding an emittor cell comprising one or more receptors which binds to the antigen of the antigen-conjugated oligonucleotides, where binding of the one or more receptors to the antigen results in an increase in intracellular calcium, and an emitter molecule that emits a photon in response to the increase in intracellular calcium; and
(3) measuring photon emission from the emittor cell.
The method comprises:
(1) combining the sample, at least one antigen-conjugated oligonucleotide that is complementary to the nucleic acid sequence, and a solid substrate comprising at least one oligonucleotide complementary to the nucleic acid sequence bound to the solid substrate, under conditions suitable for hybridization of the antigen-conjugated oligonucleotide and the oligonucleotide bound to the solid substrate for hybridizing to the nucleic acid sequence, thereby producing a solid substrate comprising the nucleic acid sequence having at least one hybridized antigen-conjugated oligonucleotide;
(2) adding to the solid substrate comprising the nucleic acid sequence having at least one hybridized antigen-conjugated oligonucleotide an emittor cell comprising one or more receptors which binds to the antigen of the antigen-conjugated oligonucleotide, where binding of the one or more receptors to the antigen results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and
(3) measuring photon emission from the emittor cell.
The method comprises:
(1) combining the sample with at least one antigen-conjugated oligonucleotide under conditions suitable for hybridization of the antigen-conjugated oligonucleotide to the nucleic acid sequence, thereby producing an antigen-conjugated hybridization complex;
(2) adding one or more antibodies specific for the antigen of the antigen-conjugated hybridization complex;
(3) adding an emittor cell comprising an Fc receptor, where binding of the Fc receptor to the one or more antibodies results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and
(4) measuring photon emission from the emittor cell.
Detecting a target particle in a sample comprises combining the sample with an antibody specific for the target particle, and an emittor cell comprising an Fc receptor, where binding of the Fc receptor to the antibody results in an increase in intracellular

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calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and measuring photon emission from the emittor cell, thereby detecting a target particle in a sample. Detecting a soluble antigen in a sample comprises combining the sample with one or more antibodies that bind to two different epitopes on the soluble antigen, and an emittor cell comprising an Fc receptor, where binding of the Fc receptor to the one or more antibodies results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and measuring photon emission from the emittor cell. The method comprises combining the sample with an emittor cell comprising one or more antibodies that bind to two different epitopes on the soluble antigen, where binding of the one or more antibodies to the soluble antigen results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and measuring photon emission from the emittor cell, thereby detecting a soluble antigen in a sample. The soluble antigen is a protein. The soluble antigen is a chemical. The method comprises: (a) crosslinking the soluble antigen, thereby producing a crosslinked antigen; (b) combining with the crosslinked antigen with an emittor cell comprising an antibody that binds to the crosslinked antigen, where binding of the antibody to the crosslinked antigen results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and (c) measuring photon emission from the emittor cell. The method comprises: (a) crosslinking the soluble antigen to a solid substrate, thereby producing a crosslinked soluble antigen bound to a solid substrate; (b) adding an emittor cell to the crosslinked soluble antigen bound to the solid substrate, where the emittor cell comprises an antibody that binds an epitope on the soluble antigen, where binding of the antibody to the crosslinked soluble antigen bound to the solid support results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and (c) measuring photon emission from the emittor cell, thereby detecting a soluble antigen in a sample. The method comprises: (a) combining the sample with a solid substrate comprising a first antibody that binds a first epitope on the soluble antigen, thereby producing a crosslinked soluble antigen bound to a solid substrate; (b) adding an emittor cell to the crosslinked soluble antigen bound to the solid substrate, where the emittor cell comprises a second antibody that binds a second epitope on the soluble antigen, where binding of the second antibody to the crosslinked soluble antigen bound to the solid support results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and (c) measuring photon emission from the emittor cell, thereby detecting a soluble antigen in a sample. Detecting a chemical in a sample comprises:

(1) combining the chemical with a peptide, thereby producing a chemical-peptide complex; and

(2) adding an emittor cell comprising one or more antibodies that bind to two different epitopes on the chemical-peptide complex, where binding of the one or more antibodies to the chemical-peptide complex results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and

(3) measuring photon emission from the emittor cell, thereby detecting a chemical in a sample.

The method comprises:

(1) combining the chemical with a peptide, thereby producing a chemical-peptide complex;

(2) crosslinking the chemical-peptide complex, thereby producing a crosslinked chemical-peptide complex;

(3) combining with the crosslinked chemical-peptide complex with an emittor cell

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comprising an antibody that binds to the crosslinked chemical-peptide complex, where binding of the antibody to the crosslinked chemical-peptide complex results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and
(4) measuring photon emission from the emittor cell.
The method comprises:
(1) combining the chemical with an antigen-conjugated peptide, thereby producing a chemical-antigen-conjugated peptide complex;
(2) adding an emittor cell comprising one or more antibodies that bind to two different epitopes on the chemical-antigen-conjugated peptide complex, where binding of the one or more antibodies to the chemical-antigen-conjugated peptide complex results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and
(3) measuring photon emission from the emittor cell, thereby detecting a chemical in a sample.
The method comprises:
(1) combining the chemical with an antigen-conjugated peptide, thereby producing a chemical-antigen-conjugated peptide complex;
(2) crosslinking the chemical-antigen-conjugated peptide complex, thereby producing a crosslinked chemical-antigen-conjugated peptide complex;
(3) adding an emittor cell comprising an antibody that binds to the crosslinked chemical-antigen-conjugated peptide complex, where binding of the antibody to the chemical-antigen-conjugated peptide complex results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and
(4) measuring photon emission from the emittor cell, thereby detecting a soluble antigen in a sample.
Preferred Device: The optoelectronic sensor device for detecting a target particle in samples using a photon detector comprises: (a) a rotor comprising positions to hold samples; (b) one or more samples comprising a mixture of a sample to be tested for the target particle, and a cell comprising a receptor for the target particle, where binding of the receptor to the target particle results in an increase in intracellular calcium, and where the cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and (c) a photon detector located at a position to detect photons emitted from one or more samples upon rotation of the rotor. The rotor comprises sixteen positions to hold sixteen samples. The device comprises:
(1) a rotor comprising positions to hold samples;
(2) one or more samples comprising a mixture of a sample to be tested for the target particle, an antibody specific for the target particle, and an emittor cell, where the emittor cell comprises an Fc receptor, where binding of the Fc receptor to the antibody results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and
(3) a photon detector located at a position to detect photons emitted from one or more samples upon rotation of the rotor.
The device for detecting a nucleic acid sequence in one or more samples using a photon detector comprises:
(1) a rotor comprising positions to hold samples;
(2) one or more samples comprising a mixture of a sample to be tested for the nucleic acid sequence, at least one antigen-conjugated oligonucleotide hybridized to the nucleic acid sequence, and an emittor cell comprising one or more receptors winch binds to the antigen of the antigen-conjugated oligonucleotides hybridized to the nucleic acid sequence, where binding of the one or more receptors to the antigen results in an increase in intracellular calcium, and where the emittor cell further comprises an emittor molecule that emits a photon in response to the increase in intracellular calcium; and

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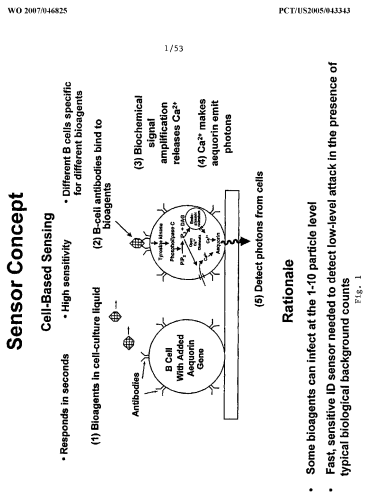
(3) a photon detector located at a position to detect photons emitted from one or more samples upon rotation of the rotor.EXAMPLE :
No suitable example given.



22/29	@ WPI / Thomson
PN	CN102220436A 2011-10-19 DW201228
TI	New specific primer used for real-time detection of foot-and-mouth disease virus group, foot-and-mouth disease virus type O, and foot-and-mouth disease virus type Asia I by fluorescent quantitative real-time-PCR
PA	(TEZH-N) TECH CENT ZHUHAI INSPECTION&QUARANTINE
ICAI	C12Q1/68; C12Q1/70;
AB	- NOVELTY : Specific primer comprising a sequence of 21 bp (SEQ ID NO: 1) and a sequence of 22 bp (SEQ ID NO: 2) for foot-and-mouth disease virus (FMDV) group; a sequence of 20 bp (SEQ ID NO: 4) and a sequence of 22 bp (SEQ ID NO: 5) for FMDV type O; and a sequence of 20 bp (SEQ ID NO: 7) and a sequence of 18 bp (SEQ ID NO: 8) for FMDV type Asia I, is new. - DETAILED DESCRIPTION : Specific primer comprising 21 bp (SEQ ID NO: 1) and 22 bp (SEQ ID NO: 2) for foot-and-mouth disease virus (FMDV) group; 20 bp (SEQ ID NO: 4) and 22 bp (SEQ ID NO: 5) for FMDV type O; and 20 bp (SEQ ID NO: 7) and 18 bp (SEQ ID NO: 8) for FMDV type Asia I, is new. tgggaccatacaggagaagtt (SEQ ID NO: 1) ccaacgcaggtaaagtgatctg (SEQ ID NO: 2) aacccmcggacgaacatgac (SEQ ID NO: 4) cgatgttggcatacaccttgat (SEQ ID NO: 5) tggaggagccgttcttgcta (SEQ ID NO: 7) gccacctgcggagtgagt (SEQ ID NO: 8) INDEPENDENT CLAIMS are included for: (1) specific probe for real-time (RT) detection of FMDV group, FMDV type O, and FMDV type Asia I, where FMDV group specific probe comprises 20 bp (SEQ ID NO: 3), FMDV type O specific probe comprises 23 bp (SEQ ID NO: 6), and FMDV type Asia I specific probe comprises 24 bp (SEQ ID NO: 9); and (2) RT detection of FMDV group, FMDV type O, and FMDV type Asia I by fluorescent quantitative RT-PCR using specific primer and specific probe comprising preprocessing sample; numbering the collected sample; respectively selecting a small amount of sample mixed green streptomycin double antibody solution; homogenizing; taking supernatant; standing overnight at 4[deg] C; extracting RNA from the collected sample; FMDV fluorescent quantitative RT-PCR augmenting; taking the extracted RNA as template; respectively applying FMDV group, FMDV type O, and FMDV type Asia I specific primer or specific probe to carry out PCR amplification; and according to fluorescent quantitative RT-PCR result, identifying or diagnosing whether FMDV is contained and judging whether it is FMDV type O or FMDV type Asia I. gtccactccggacctgagag (SEQ ID NO: 3) tacgaccagtacaaggtwcacaa (SEQ ID NO: 6) acggagccgagactttcatcgtcg (SEQ ID NO: 9) - USE :

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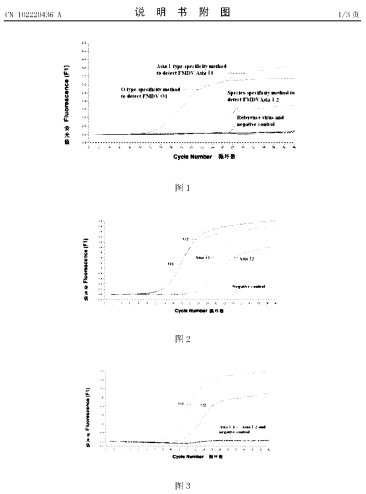

Specific primer for RT detection of FMDV group, FMDV type O, and FMDV type Asia I by fluorescent quantitative RT-PCR (claimed).
- ADVANTAGE :
Even if FMDV is mixed with other susceptible virus, the detection method by fluorescent quantitative RT-PCR using the specific primer or specific probe has sensitivity that reaches 10 copies of template. The whole reaction process of the detection method is a closed tube operation, which reduces the pollution of the system at the utmost extent. The whole process of obtaining the sample to be tested, extracting the nucleic acid, preparing fluorescent quantitative RT-PCR reaction system, and getting the reaction result can be finished in 2.5 hours, greatly shortening the detection time on the basis of the detection result.
- BIOTECHNOLOGY :
Preferred Component: The 5' ends of the FMDV group specific probe, FMDV type O specific probe, and FMDV type Asia I specific probe are marked with the light emitting radical FAM, and the 3' ends are marked with the vanish radical TAMRA. Preferred Method: The fluorescent quantitative RT-PCR system is at 25 mu l and comprises 2x 1 Step Buffer (12. 5 mu l), 10 mu mol/L upstream and downstream specific primer and specific probe (each 1. 0 mu l), PrimeScript(RTM: 1 step enzyme mix) (1.0 mu l), RNA template (2.5 mu l), and ribonuclease-free water (6.0 mu l). The fluorescent quantitative RT-PCR is conducted at 42[deg] C for 30 minutes, 92[deg] C for 2 minutes, 92[deg] C for 10 seconds, and 55[deg] C for 30 seconds for 40 repetitions.

23/29	@ WPI / Thomson
PN	WO2012033875A2 2012-03-15 DW201222 WO2012033875A3 2012-06-14 DW201240 EP2614157A2 2013-07-17 DW201347 US2013203626A1 2013-08-08 DW201352
TI	Identifying presence of related target sequences in sample involves contacting sample comprising target sequence, with pair of primers for amplifying the targets; performing amplification reaction; and then detecting amplification product
PA	(UYBR-N) UNIV BRANDEIS
ICAI	C12N15/11; C12Q1/68; C12Q1/70;
AB	- NOVELTY : Identifying (M1) presence of any of several related target sequences in sample involves: a) contacting sample comprising target sequence that is one of several related target sequences, where target sequences differ by nucleotide, with a pair of primers for amplifying the targets, where the primers are complementary or partially complementary to positions of the targets or their complements that differ by nucleotide in the position; b) performing an amplification reaction under conditions such that target sequence is amplified in single reaction; and c) detecting amplification product. - DETAILED DESCRIPTION : Identifying (M1) presence of any of several related target sequences in a sample, involves: a) contacting a sample comprising a target sequence that is one of several related target sequences, where the target sequences differ by at least one nucleotide, with a pair of primers for amplifying the targets, where the primers are complementary or partially complementary to positions of the targets or their complements that differ by at least one nucleotide in the position, and where the primers is a thermodynamic consensus primer, and where the primers is used at a concentration at least 5 times that of other primers in the primers; b) performing an amplification reaction under

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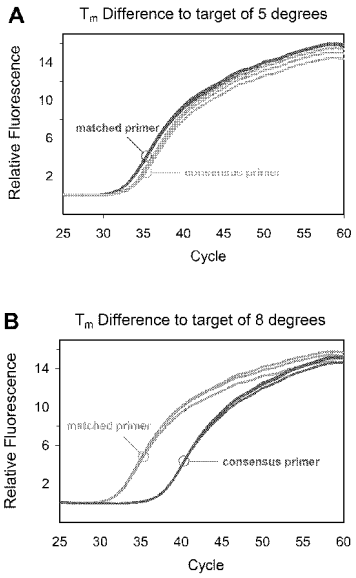
conditions such that the target sequence is amplified in a single reaction; and c) detecting an amplification product. INDEPENDENT CLAIMS are included for the following:
(1) a system comprising a processor configured to generate melting curves of at least two different amplification products generated by the method (M1); and
(2) an oligonucleotide set (S1) comprising a first primer, second primer, and optionally third primer, where the second primer is 50-98% homologous to the first primer, and where the concentration of the first primer is at least 5 times higher than the concentration of the second primer, and where the third primer is paired with the first primer for the amplification of any of several related target sequences.
- USE :
For identifying presence of related target sequences (including variants of genes, homologs of genes, orthologs of genes, and subtypes of genes, where the genes are from organism selected from pathogenic agents, animals (preferably humans, livestock and companion animals), and other organisms of interest, and where the pathogens are selected from viruses, bacteria and fungi) in a sample (all claimed). Also useful in the detection of multiple strains, variants and subtypes of the same microorganism or homologs or orthologs of prokaryotic or eukaryotic organisms; and in a variety of research, diagnostic, and screening applications (such as for identification of unknown strains of microorganisms or homologs/orthologs of genes, drug screening (e.g. screening for growth of microooroganism or expression of particular genes), and epidemiological applications (e.g. monitoring emergence of new strains of pathogens and monitoring spread of disease)).
- ADVANTAGE :
The method utilizes multiple primers with similar sequences at different concentrations in a single amplification reaction in order to amplify the targets from a wide range of related nucleic acid targets (e.g. from different strains of a bacteria or virus, or highly conserved genes from a variety of organisms) or to identify an unknown member of a known class or microorganism or gene. The method overcomes the shortcomings from the prior art method (e.g. narrow range of sequence variation which results in low efficiency or failure due to the very small concentrations of each particular primer that is included in the mix).
- BIOTECHNOLOGY :
Preferred Method: In the method (M1), the related targets vary at >= 4 nucleotide positions in the region complementary to the thermodynamic consensus primer. The concentration of the primers is at a ratio of at least 5 to 1 and the amplification method is asymmetric polymerase chain reaction (PCR) or symmetric PCR. In the method (M1), one of the pair of primer is a limiting primer that is at least 5-fold lower in concentration than a paired excess primer, and one of the pair of primer is an excess primer that is at least 5-fold higher in concentration than a paired limiting primer, and where the initial melting temperature of the limiting primer with respect to its fully complementary target is higher than or equal to the initial melting temperature of the excess primer with respect its fully complementary target. In the method (M1), the sample comprises at least two of the related target sequences. Preferred Set: In the oligonucleotide set (S1), the third primer is at a concentration at least 5 times higher than the concentration of the first primer.

FIG. 1



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24/29	@ WPI / Thomson
PN	WO2011150115A2 2011-12-01 DW201182 US2011294112A1 2011-12-01 DW201182 US2011294199A1 2011-12-01 DW201182 WO2011150115A3 2012-04-05 DW201224 EP2576833A2 2013-04-10 DW201325 US9222126B2 2015-12-29 DW201603
TI	Detecting target nucleic acid in sample, by transferring sample to nucleic acid binding element, amplifying target nucleic acid, and detecting amplification of target nucleic acid
PA	(USGO) LAWRENCE LIVERMORE NAT SECURITY LLC(BEAR-I) BEARINGER J P(DUGA-I) DUGAN L C
ICAI	B01L3/00; B01L7/00; C12M1/34; C12M1/38; C12P19/34; C12Q1/68; C12Q1/70; G01N33/50; G01N35/00;
AB	- NOVELTY : Method (M1) for detecting a target nucleic acid in a sample, involves sealing sample within a containment vessel (501), transferring the sample to a nucleic acid binding element positioned in the vessel, amplifying the target nucleic acid in the vessel, and detecting amplification of the target nucleic acid in the vessel. - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are included for the following: (1) method (M2) for diagnosing a condition in an individual, involves performing the method (M1), and diagnosing the condition, where the condition is associated with presence of a target nucleic acid in the individual, and the target nucleic acid is produced by a pathogen; and (2) apparatus comprising a containment vessel, a sample collection element (502) configured for removable coupling to the containment vessel, a nucleic acid binding element positioned within the containment vessel, where the sample collection element is configured to collect the sample and to transfer the sample to the nucleic acid binding element when the sample collection element is removably coupled to the containment vessel, several reagents (530) configured for placement in fluid communication with the nucleic acid binding element, where several reagents comprises nucleic acid purification reagents and nucleic acid amplification reagents, and a heater (506) configured to heat the nucleic acid amplification reagents in fluid communication with the nucleic acid binding element. - USE : The method (M1) and apparatus are useful for detecting a target nucleic acid in a sample. The method (M2) is useful for diagnosing a condition in an individual caused by pathogen. The pathogen is chosen from virus, bacteria, fungi and their combinations. The condition is an infectious disease chosen from foot and mouth disease, flu, swine flu, avian flu, methicillin-resistant Staphylococcus aureus(MRSA) infection, anthrax sexually transmitted disease (STDs), AIDS, Chlamydia trachomatis/ Neisseria gonorrhoeae(CT/NG) infection, human papillomavirus (HPV), hepatitis C virus (HCV), Clostridium difficileinfection, Strep A, influenza and their combinations (all claimed). - ADVANTAGE : The method is rapid and cost-effective, as it does not require large, fragile and expensive equipments. The test subjects can conduct the method for self-testing. - DESCRIPTION OF DRAWINGS : The drawing shows schematic view of disposable device for detecting target nucleic. 501 : Containment vessel 502 : Sample collection element 506 : Heater 507 : Waste collection unit 510 : Lumen 515 : Reagent cartridges 516 : Capsule 517 : Plug 530 : Several reagents - BIOTECHNOLOGY : Preferred Method: Preferred Method: The method (M1) further involves collecting the sample, preferably bodily sample from an individual before

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sealing. The collecting step involves conducting nasal swab, collecting blood sample, collecting saliva sample, collecting nasal mucus sample, collecting urine sample, collecting buccal cell sample or collecting fecal sample. The collecting step further involves collecting the sample with sample collection element. The sealing step further involves removably coupling the sample collection element to the containment vessel. The sample collecting element comprises lumen (510). The transferring step further involves lavaging one or more reagent through the lumen of the sample collection element when the sample collection element is removably coupled to the containment vessel. The method (M1) further involves purifying nucleic acid from the sample in the containment vessel after the transferring step. The purifying step involves introducing nucleic acid purification reagents into fluid communication with the nucleic acid binding element. The method (M1) further involves introducing the nucleic acid purification reagents from several reagent cartridges (515) into fluid communication with the nucleic acid binding element, where the several reagent cartridges are connected to the containment vessel. The purifying step further involves collecting a waste into a waste collection unit (507) connected to the containment vessel. The amplifying step further involves introducing nucleic acid amplification reagents in fluid communication with the nucleic acid binding element, and heating the nucleic acid amplification reagents. The nucleic acid amplification reagents are sequestered within separate polymer shells, where the polymer shells are comprised within the containment vessel, and configured to melt and release the nucleic acid amplification reagents by heating, and heating the polymer shells. The polymer shells are polycaprolactone (PCL) shells. The heating step further involves isothermally heating the nucleic acid amplification reagents in fluid communication with the nucleic acid binding element at 60-65° c for 1 hour. The heating is performed by exothermic chemical reaction, oxidative chemical reaction or catalytic chemical reaction. The heating step further involves stabilizing heating temperature using a phase change material. The heating step is performed using a disposable heater. The detecting step involves detecting a color shift in a colorimetric dye in fluid communication with the nucleic acid amplification reagents, and detecting fluorescence of a fluorescent dye in fluid communication with the nucleic acid amplification reagents. The method (M1) further involves disposing the sample, the containment vessel, the nucleic acid binding element and the nucleic acid amplification reagents after detection. The isothermal amplification is chosen from loop-mediated isothermal amplification, helicase-dependent isothermal amplification and recombinase polymerase amplification. Preferred Apparatus: The containment vessel comprises a loading port, and where several reagents are configured for loading or extracting through the loading port for placement in or removal from fluid communication with the nucleic acid binding element. The containment vessel, sample collection element, nucleic acid binding element, and several reagents are configured for a single use. The heater is configured for a single use. The apparatus further comprises a waste collection unit. The waste collection unit is coupled to sample collection element, and comprises chamber comprising first gap, plate coupled with the chamber, and second gap, where the chamber and plate surround the sample collection element and at least one of the chamber and plate is configured to-be-rotatable, and when the first gap overlaps with the second gap, content of the containment vessel is adapted to flow into the chamber of the waste collection unit. The content of the containment vessel adapted to flow into the chamber of the waste collection unit is a waste. Several reagents are enveloped within several reagent cartridges, where the reagent cartridges comprises capsule (516) and plug (517), the capsule is connected to the lumen through an opening, and plug sealing opening. The nucleic acid binding element comprises a FTA card. The heater comprises an electrical power source or fuel cell. The heater is configured to generate heat by an exothermal chemical reaction, metal oxidation, or supersaturated salt solution. The heater comprises a catalytic burner or phase change material. The apparatus further comprises a detection element configured to detect amplification of the target nucleic acid. The detection element comprises a colorimetric dye in fluid communication with the nucleic acid amplification reagents, where the colorimetric dye

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is configured to undergo a color shift when the target nucleic acid is amplified. The colorimetric dye is hydroxynaphthol blue (HNB).EXAMPLE : No suitable example given.

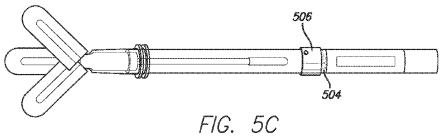

FIG. 5C

25/29	@ WPI / Thomson
PN	WO2011109440A1 2011-09-09 DW201164 AU2011223789A1 2012-09-20 DW201270 CA2791905A1 2011-09-09 DW201274 EP2542696A1 2013-01-09 DW201304 JP2013521502A 2013-06-10 DW201338 KR20130056855A 2013-05-30 DW201338 US2013287772A1 2013-10-31 DW201371 CN103237901A 2013-08-07 DW201374 IL221719A 2012-10-31 DW201518 US9128101B2 2015-09-08 DW201559 JP5808349B2 2015-11-10 DW201578 IN7533DELNP2012A 2016-01-01 DW201613 JP2016028584A 2016-03-03 DW201619
TI	Theranosing disease e.g. cancer, immune disease involves identifying biosignature comprising cell-specific/disease-specific/general vesicle biomarker, of vesicle population in sample; and comparing the biosignature to reference
PA	(CARI-N) CARIS LIFE SCI LUXEMBOURG HOLDINGS(CARI-N) CARIS LIFE SCI SWITZERLAND HOLDINGS SARL(CARI-N) CARIS MPI INC
ICAI	A61K45/00; A61P1/16; A61P15/04; A61P17/00; A61P17/06; A61P19/02; A61P21/02; A61P25/00; A61P25/14; A61P25/16; A61P25/18; A61P25/24; A61P25/28; A61P29/00; A61P3/10; A61P31/04; A61P31/06; A61P31/12; A61P31/14; A61P31/16; A61P31/18; A61P33/06; A61P35/00; A61P35/02; A61P37/06; A61P9/00; A61P9/04; A61P9/08; A61P9/10; A61P9/12; C12Q1/68; C40B30/00; G01N33/48; G01N33/50; G01N33/53; G01N33/574; G01N33/68;
AB	- NOVELTY : Theranosing a disease or disorder in a subject involves identifying a biosignature of a vesicle population in a sample from the subject, where the biosignature comprises a presence or level of at least one cell-specific biomarker and/or a presence or level of at least one disease-specific biomarker, and a presence or level of at least one general vesicle biomarker; and comparing the biosignature to a reference, where the comparison is indicative of whether the subject is a responder or non-responder to a therapeutic agent, thereby theranosing the disease or disorder. - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are included for the following: (1) use of a reagent to carry out the method of theranosing a disease; and (2) a kit comprising a reagent to carry out the method of theranosing a disease. - USE : For theranosing cancer selected from prostate cancer, colorectal cancer, lung cancer, breast cancer, ovarian cancer, melanoma, prostate cancer, hyperplastic polyp, adenoma, colorectal cancer, high grade dysplasia, low grade dysplasia, prostatic hyperplasia, prostate cancer, melanoma, pancreatic cancer, brain cancer, a glioblastoma, hepatocellular carcinoma, cervical cancer, endometrial cancer, head and neck cancer, esophageal cancer, gastrointestinal stromal tumor (GIST), renal cell carcinoma (RCC), gastric cancer, colorectal cancer (CRC), CRC Dukes B, CRC Dukes C-D, a hematological malignancy, B-cell chronic lymphocytic leukemia, B-cell lymphoma-DLBCL, B-cell lymphoma-DLBCL-germinal center-like, Burkitt's lymphoma, lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, anal cancer, appendix cancer, astrocytomas, atypical teratoid/rhabdoid tumor, basal cell carcinoma, bladder cancer, brain stem glioma, brain tumor (including brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal

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parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma), breast cancer, bronchial tumors, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, cervical cancer, childhood cancers, chordoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, endocrine pancreas islet cell tumors, endometrial cancer, ependymoblastoma, ependymoma, esophageal cancer, esthesioneuroblastoma, Ewing sarcoma, extracranial germ cell tumor, extrahepatic bile duct cancer, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal cell tumor, gestational trophoblastic tumor, glioma, hairy cell leukemia, head and neck cancer, heart cancer, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell tumors, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, lip cancer, malignant fibrous histiocytoma bone cancer, medulloblastoma, medulloepithelioma, Merkel cell carcinoma, Merkel cell skin carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndromes, multiple myeloma, plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myeloproliferative neoplasms, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, Non-Hodgkin lymphoma, nonmelanoma skin cancer, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma, other brain and spinal cord tumors, ovarian cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, papillomatosis, paranasal sinus cancer, parathyroid cancer, pelvic cancer, penile cancer, pharyngeal cancer, pineal parenchymal tumors of intermediate differentiation, pineoblastoma, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system (CNS) lymphoma, primary hepatocellular liver cancer, prostate cancer, rectal cancer, renal cancer, renal cell cancer, respiratory tract cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, Sezary syndrome, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach (gastric) cancer, supratentorial primitive neuroectodermal tumors, T-cell lymphoma, testicular cancer, throat cancer, thymic carcinoma, thymoma, thyroid cancer, transitional cell cancer, trophoblastic tumor, ureter cancer, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, or Wilm's tumor; premalignant condition selected from actinic keratosis, atrophic gastritis, leukoplakia, erythroplasia, Lymphomatoid Granulomatosis, preleukemia, fibrosis, cervical dysplasia, uterine cervical dysplasia, xeroderma pigmentosum, Barrett's Esophagus, colorectal polyp, a transformative viral infection, HIV, human papilloma virus; autoimmune disease or disorder selected from inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), pelvic inflammation, vasculitis, psoriasis, diabetes, autoimmune hepatitis, multiple sclerosis, myasthenia gravis, Type I diabetes, rheumatoid arthritis, psoriasis, systemic lupus erythematosis (SLE), Hashimoto's Thyroiditis, Grave's disease, Ankylosing Spondylitis Sjogrens Disease, CREST syndrome, Scleroderma, Rheumatic Disease, organ rejection, Primary Sclerosing Cholangitis, or sepsis; cardiovascular disease or disorder selected from atherosclerosis, congestive heart failure, vulnerable plaque, stroke, ischemia, high blood pressure, stenosis, vessel occlusion, thrombotic event; neurological disease or disorder selected from Multiple Sclerosis (MS), Parkinson's Disease (PD), Alzheimer's Disease (AD), schizophrenia, bipolar disorder, depression, autism, Prion Disease, Pick's disease, dementia, Huntington disease (HD), Down's syndrome, cerebrovascular disease, Rasmussen's encephalitis, viral meningitis, neurospsychiatric systemic lupus erythematosus (NPSLE), amyotrophic lateral sclerosis, Creutzfeldt- Jacob disease, Gerstmann-Straussler-Scheinker disease, transmissible spongiform encephalopathy, ischemic reperfusion damage (including stroke), brain trauma, microbial infection, or chronic fatigue syndrome; infectious disease selected from bacterial infection, viral infection, yeast infection, Whipple's Disease, Prion Disease, cirrhosis, methicillin-resistant staphylococcus aureus, HIV,

(Volver al Sumario)

HCV, hepatitis, syphilis, meningitis, malaria, tuberculosis, influenza; pain selected from fibromyalgia, chronic neuropathic pain, or peripheral neuropathic pain; inflammatory disease; and immune disease (claimed).
- ADVANTAGE : The method reduces unnecessary treatment options, avoids possible side effects from ineffective therapeutics, and improves the subject's symptoms and/or condition.BIOLOGY : Preferred Components: The sample comprises a bodily fluid. The bodily fluid comprises peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, or umbilical cord blood (preferably serum or plasma). The vesicle population comprises vesicles with a diameter of 20-800 (preferably 20-200) nm. The biosignature comprises DR3, STEAP, Epha2, TMEM211, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, TETS, CD9, Epidermal growth factor receptor (EGFR), CD63, MUC1, TGM2, CD81, tissue inhibitor of metalloproteinases (TIMP), EPHA2, TMEM211, UNC93A, CD66e, CD24, Ferritin, EpCAM, neutrophil gelatinase-associated lipocalin (NGAL), GPR30, p53, NCAM, B7H3, PSMA, PCSA, CD63, STEAP, STEAP1, ICAM1 (CD54), A33, DR3, CD66e, MFG-e8, Hepsin, TROP-2, Mammoglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NK-2, EpCam, NK-1R, 5T4, PAI-1, CD45, SPB, SPC, NSE, PGP9.5, P2RX7, NDUFB7, NSE, Gal3, Osteopontin, CHI3L1, iC3b, Mesothelin, SPA, TPA, PCSA, CD63, AQP5, DLL4, DR3, PSMA, GPCR 110 (GPR110), EPHA2, antigen related cell adhesion molecule (CEACAM), protein tyrosine phosphatase (PTP), CABYR, TMEM211, ADAM28, UNC93a, A33, CD24, CD10, PRO GRP, CYTO 18, FTH1, centromere protein H (CENPH), ANNEXIN I, ANNEXIN V, CRP, vascular endothelial growth factor (VEGF), CYTO 19, CCL2, Osteopontin (OST19), Osteopontin (OST22), BTUB, CD45, NACC1, MMP9, BRCAl, P27, NSE, M2PK, HCG, MUCl, CEA, CEACAM, CYTO 7, MS4A1, MUC2, PGP9, SFTPC, UNCR2, neuron specific enolase (NSE), INGA3, INTO b4, MMP1, PNT, RACK1, NAP2, HLA, BMP2, PTH1R, CD151, CKS1, follicle stimulating hormone receptor (FSHR), HIF, KRAS, LAMP2, SNAIL, TRIM29, TSPAN1, TWIST1, ASPH, AURKB, ABCC1, ABCG2, ACE2, ADA, ADH1C, ADH4, AGT, AR, AREG, ASNS, BCL2, breast cancer resistance protein (BCRP), BDCA1, beta III tubulin, BIRC5, B-RAF, BRCA1, BRCA2, CA2, caveolin, CD20, CD25, CD33, CD52, CDA, CDKN2A, CDKN1A, CDKN1B, CDK2, CDW52, CES2, CK 14, CK 17, CK 5/6, c-KIT, c-Met, c-Myc, COX-2, Cyclin D1, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, E-Cadherin, ECGF1, EML4-ALK fusion, EPHA2, Epiregulin, ER, ERBR2, ERCC1, ERCC3, High epiregulin (EREG), ESR1, FLT1, folate receptor, FOLR1, FOLR2, FSHB, FSHPRH1, FSHR, FYN, glycinamide ribonucleotide transformylase (GART), gonadotrophin-releasing hormone (GnRH) -1, GNRHR1, GSTP1, HCK, Histone Deacetylase (HDAC) -1, hENT-1, Her2/Neu, hepatocyte growth factor (HGF), HIF1A, HIG1, HSP90, HSP90AA1, HSPCA, IGF-1R, insulin-like growth factor binding protein (IGFBP), IGFRBP3, IGFRBP4, IGFRBP5, IL13RA1, IL2RA, KDR, Ki67, KIT, K-RAS, LCK, LTB, Lymphotoxin Beta Receptor, LYN, MET, alkyltransferase (MGMT), MLH1, MMR, MRP1, MS4A1, MSH2, MSH5, Myc, NFKBl, NFKB2, NFKBIA, ODC1, OGFR, pl6, p21, p27, p95, PARP-1, PDGFC, PDGFR, PDGFRA, PDGFRB, PGP, PGR, PI3K, POLA, POLA1, Peroxisome proliferator-activated receptor gamma (PPAR), PPARGC1, PR, Phosphatase and tensin homolog (PTEN), PTGS2, RAF1, RARA, RRM1, RRM2, RRM2B, retinoid receptor beta (RXRB), RXRG, SPARC, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, Survivin, TK1, TLE3, Tumour necrosis factor (TNF), TOPI, TOP2A, TOP2B, TS, TXN, TXNRD1, thymidylate synthase (TYMS), vitamin D receptor (VDR), vascular endothelial growth factor (VEGF), VEGFC, von Hippel-Lindau (VHL), YES1, and ZAP70.
- BIOTECHNOLOGY : Preferred Components: The binding agent comprises an antigen, DNA molecule, RNA molecule, antibody, antibody fragment, aptamer, peptoid, zDNA,

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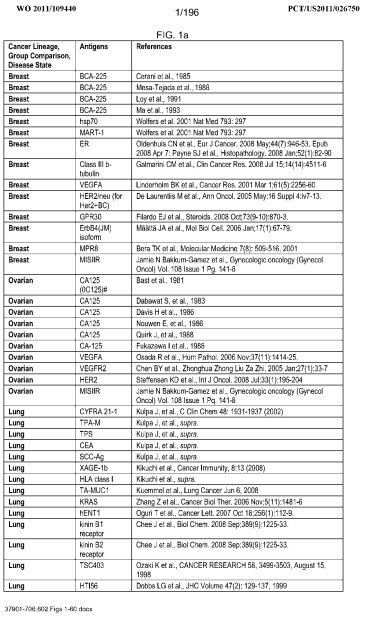
peptide nucleic acid (PNA), locked nucleic acids (LNA), lectin, peptide, dendrimer, membrane protein labeling agent, or chemical compound. The nucleic acid comprises DNA, mRNA, microRNA, snoRNA, snRNA, rRNAs, tRNAs, siRNA, hnRNA, or shRNA. The nucleic acid comprises miR-21, miR-205, miR-92, miR-147 miR-141 or miR-574. The nucleic acid comprises microRNA as given in FIGs. 3-6, 19-24, 26-30, 32, 33, 36, 40-42, 47, 51, 53-57, and/or 60 of the specification (preferably miR-21, miR-205, miR-92, miR-147 or miR-574). The general vesicle biomarker comprises tetraspanin.PHARMACEUTICALS : Preferred Method: In the method, the subject has not been exposed to the therapeutic agent previously; the theranosis involving determining a treatment efficacy; the identifying is performed in a single assay; the vesicle population is subjected to size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, affinity capture, immunoassay, and/or microfluidic separation; identifying the biosignature by contacting the sample with at least three binding agents specific for three different analytes; identifying the biosignature involves assessment of at least one nucleic acid, peptide, protein, lipid, antigen, carbohydrate, and/or proteoglycan; the subject is not currently being treated for the disease or disorder; the subject is on an existing treatment for the disease or disorder; identifying the subject as a non-responder or responder to the therapeutic agent involves correlating the vesicle biosignature of the subject against a set of vesicle biosignatures from previously identified responders and non-re sponders to the therapeutic agent; the subject is identified as a responder if the subject's vesicle biosignature correlates more closely with the set of vesicle biosignatures from previously identified responders than with the set of vesicle biosignatures from previously identified non-responders; the subject is identified as a non-responder if the subject's vesicle biosignature correlates more closely with the set of vesicle biosignatures from previously identified non-responders than with the set of vesicle biosignatures from previously identified responders; identifying the subject as a non-responder or responder to the therapeutic agent involves classifying the vesicle biosignature of the subject against a classifier trained using previously identified responders and non-responders; overexpression, underexpression or mutation of the marker as compared to a reference is used to select the therapeutic agent; the marker comprises K-ras oncogene protein (KRAS) and a mutation in KRAS as compared to a wild type reference is used to select the therapeutic agent; and the mutation is KRAS is determined by sequencing KRAS mRNA; the KRAS mRNA is payload within the vesicle population. The method further involves administering the therapeutic agent to the subject. The theranosing a disease or disorder in a subject involves identifying a biosignature of a vesicle population in a sample from the subject, where the biosignature comprises a mutation of KRAS, BRAF, PIK3CA, and/or c-kit; and comparing the biosignature to a reference to identify the presence of a mutation in the KRAS, BRAF, PIK3CA, and/or c-kit, thereby theranosing the disease or disorder, where the mutation is detected in mRNA isolated from the vesicle population, the biosignature comprises a mutation in KRAS, and the method is performed in vitro. Preferred Components: The cell-specific biomarker, disease-specific biomarker, and vesicle biomarker comprise proteins. The disease-specific biomarker comprises Epithelial cell adhesion molecule (EpCAM), B7H3, CD24, and/or Tissue Factor. The general vesicle biomarker comprises CD63, CD9, CD81, CD82, CD37, CD53, Rab-5b, MFG-E8, and/or Annexin V. The biosignature comprises analysis using a binding agent. The general vesicle biomarker comprises at least one marker listed in Table 3 of the specification.EXAMPLE : No suitable example given.

(Volver al Sumario)



WO 2011/109440 1/196 PCT/US2011/026750

FIG. 1a

Cancer Lineage, Group Comparison, Disease State	Antigens	References
Breast	BCA-225	Ceriani et al., 1985
Breast	BCA-225	Mesa-Tejada et al., 1988
Breast	BCA-225	Loy et al., 1991
Breast	BCA-225	Ma et al., 1993
Breast	hsp70	Wolfers et al. 2001 Nat Med 793: 297
Breast	MART-1	Wolfers et al. 2001 Nat Med 793: 297
Breast	ER	Oldenhuis CN et al., Eur J Cancer. 2008 May;44(7):946-53. Epub 2008 Apr 7. Payne SJ et al., Histopathology. 2008 Jan;52(1):82-90
Breast	Class III b-tubulin	Galmarini CM et al., Clin Cancer Res. 2008 Jul 15;14(14):4511-6
Breast	VEGFA	Lindenholm BK et al., Cancer Res. 2001 Mar 1;61(5):2256-60
Breast	HER2/neu (for Her2+BC)	De Laurentiis M et al., Ann Oncol. 2005 May;16 Suppl 4:iv7-13.
Breast	GPR30	Filardo EJ et al., Steroids. 2008 Oct;73(9-10):870-3.
Breast	ErbB4/JM isoform	Määttä JA et al., Mol Biol Cell. 2006 Jan;17(1):67-79.
Breast	MPR8	Bera TK et al., Molecular Medicine 7(8): 505-516, 2001
Breast	MISIIR	Jamie N Bakkum-Gamez et al., Gynecologic oncology (Gynecol Oncol) Vol. 108 Issue 1 Pg. 141-8
Ovarian	CA125 (0C125)#	Bast et al., 1981
Ovarian	CA125	Dabawat S, et al., 1983
Ovarian	CA125	Davis H et al., 1986
Ovarian	CA125	Nouwen E. et al., 1986
Ovarian	CA125	Quirk J, et al., 1988
Ovarian	CA-125	Fukazawa I et al., 1988
Ovarian	VEGFA	Osada R et al., Hum Pathol. 2006 Nov;37(11):1414-25.
Ovarian	VEGFR2	Chen BY et al., Zhonghua Zhong Liu Za Zhi. 2005 Jan;27(1):33-7
Ovarian	HER2	Stiefensen KO et al., Int J Oncol. 2008 Jul;33(1):195-204
Ovarian	MISIIR	Jamie N Bakkum-Gamez et al., Gynecologic oncology (Gynecol Oncol) Vol. 108 Issue 1 Pg. 141-8
Lung	CYFRA 21-1	Kulpa J, et al., C Clin Chem 48: 1631-1937 (2002)
Lung	TPA-M	Kulpa J, et al., supra.
Lung	TPS	Kulpa J, et al., supra.
Lung	CEA	Kulpa J, et al., supra.
Lung	SCC-Ag	Kulpa J, et al., supra.
Lung	XAGE-1b	Kikuchi et al., Cancer Immunity, 8:13 (2008)
Lung	HLA class I	Kikuchi et al., supra.
Lung	TA-MUC1	Kuzmind et al., Lung Cancer Jun 6, 2008
Lung	KRAS	Zhang Z et al., Cancer Biol Ther. 2006 Nov;5(11):1481-6
Lung	hENT1	Oguri T et al., Cancer Lett. 2007 Oct 18;256(1):112-9.
Lung	kinin B1 receptor	Chee J et al., Biol Chem. 2008 Sep;389(9):1225-33.
Lung	kinin B2 receptor	Chee J et al., Biol Chem. 2008 Sep;389(9):1225-33.
Lung	TSC403	Ozaki K et al., CANCER RESEARCH 58, 3496-3503, August 15, 1998
Lung	H*156	Dobbs LG et al., JHC Volume 47(2): 129-137, 1999

37901-706.602 Figs 1-90.docx

26/29	@ WPI / Thomson
PN	WO2011098775A1 2011-08-18 DW201156
TI	Use of modified virus such that cell to cell movement of virus is compromised, for producing heterologous reference nucleic acid standard in nucleic acid-based assay, where reference nucleic acid is encapsidated in the viral particle
PA	(PBIO) PLANT BIOSCIENCE LTD
ICAI	C12N15/82; C12Q1/68;
AB	- NOVELTY : In the production of a heterologous reference nucleic acid standard in nucleic acid-based assay, where reference nucleic acid is encapsidated in the viral particle, a modified virus such that cell to cell movement of the virus is compromised, is used. - DETAILED DESCRIPTION : INDEPENDENT CLAIMS are included for the following: (1) preparing (P1) reference nucleic acid in a nucleic acid based assay involving providing all or part of a viral genome which is modified such that cell to cell movement of the encoded virus is compromised and into which a reference nucleic acid sequence is introduced, and introducing genome into a population of cells such that compromised virus comprising nucleic having reference sequence is produced, where the reference nucleic acid is encapsidated in the viral particle; (2) preparing (P2) reference RNA in a nucleic acid based assay involving providing DNA encoding a viral RNA genome which is modified such that cell to cell movement of the encoded virus is compromised and into which RNA-reference-encoding DNA sequence is introduced, introducing DNA into a population of cells such that compromised virus comprising reference RNA is produced, where the reference RNA acid is encapsidated in the compromised; (3) making a reference standard in a nucleic acid based assay involving preparing a movement protein-deleted virus into which a target sequence of interest is inserted such that the reference standard nucleic acid is encapsidated in the virus; (4) a modified virus or reference nucleic acid standard obtained by the process (P1) or (P2); (5) a kit (K1) for performing a nucleic-acid based assay, comprising DNA encoding a modified viral genome is modified such that cell to cell movement of the encoded virus is compromised plus optionally at least one further components for performing the assay, or a modified virus which is modified such that cell to cell movement of the virus

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is compromised and into which RNA-reference sequence is introduced plus optionally at least one further components for performing the assay.
- USE :
For producing a heterologous reference nucleic acid standard used in nucleic acid-based assay such as reverse-transcription-polymerase chain reaction (claimed); and as an internal control for diagnostic testing for pathogenic human viruses such as HIV.
- ADVANTAGE :
The modified viruses provide good internal standard; and are not able to spread infection such as Cowpea mosaic virus (CPMV) infection.
- BIOTECHNOLOGY :
Preferred Process: The process (P1) involves providing a sample to be assayed, providing modified virus which includes reference nucleic acid, mixing the sample with the viral particle, isolating test nucleic acid from the admixture, and assaying for the presence of the reference nucleic acid in the test mixture; providing all or part of a viral genome which is modified such that cell to cell movement of the encoded virus is compromised, introducing a reference sequence into genome, and introducing genome into a population of cells such that compromised virus comprising nucleic having reference sequence is produced; and providing all or part of a viral genome, modifying the viral genome such that cell to cell movement of the encoded virus is compromised, introducing a reference sequence into modified genome, and introducing modified genome into a population of cells such that compromised virus comprising nucleic having reference sequence is produced. In the process (P1), the modified virus is a Comovirus, which is optionally Cowpea mosaic virus (CPMV). The reference nucleic acid is non-translatable. The modified virus includes a heterologous reference nucleic acid sequence, which is derived from a virus selected from avian influenza virus, bovine diarrhea virus, rabies virus, foot and mouth disease virus, classical swine fever virus, H1N1 influenza, H5 influenza, H7 influenza, N1 influenza, swine vesicular disease virus, HIV-1, HIV-2, hepatitis C virus, human T-cell lymphotrophic virus-1 (HTLV-1), HTLV-2, hepatitis G, and an enterovirus. The virus has RNA genome and is used to prepare reference RNA. The process (P2) involves providing DNA encoding a viral RNA genome which is modified such that cell to cell movement of the encoded virus is compromised, introducing RNA-reference-encoding DNA sequence into genome-encoding DNA sequence, and introducing resulting DNA into a population of cells such that compromised virus comprising reference RNA is produced; and providing DNA encoding a viral RNA genome, modifying the DNA encoding the viral RNA genome such that cell to cell movement of the encoded virus is compromised, introducing RNA-reference-encoding DNA sequence into genome-encoding DNA sequence, introducing resulting DNA into a population of cells such that compromised virus comprising reference RNA is produced. In the process (P2), the virus is produced by introducing the DNA into the cells via at least one vectors, where at least one vector encode a modified viral RNA2 segment and RNA1 segment, and the RNA2 segment is modified such that cell to cell movement of the encoded virus is compromised and which incorporates the RNA-reference-encoding sequence; and permitting expression of segments such that the RNA2 and RNA1 are produced in the host cells in viral-encapsulated form. In the process (P1) and (P2), the reference nucleic acid, in encapsulated form, is used in the nucleic acid based assay. The modification which compromises cell to cell movement of the encoded virus is within the movement protein encoded by the viral genome. The modification is deletion of all or part of the movement protein to render it non-functional. The virus is a bipartite RNA virus and the reference sequence is introduced into RNA2 following a stop codon. Preferred Kit: In the kit (K1), the modified virus is obtained by the process (P1) or (P2). The modification involves cell to cell movement of the encoded virus is within the movement protein encoded by the viral genome. The modification is deletion of all or part of the movement protein to render it non-functional. The kit is for assaying for a virus including a target nucleic acid sequence, and the modified viral genome includes a reference nucleic acid sequence, and the target nucleic acid and the reference nucleic acid include corresponding primer

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binding sites such that an amplification product can be generated from the reference nucleic acid and from the target nucleic acid using a set of primers, where the amplification product of the reference nucleic acid and the amplification product of the target nucleic acid are distinguishable. The kit (K1) further involves set of primers, units for distinguishing the amplification product of the reference nucleic acid and the amplification product of the target nucleic acid; and at least one nucleic acid probes specific for one of the amplification products.

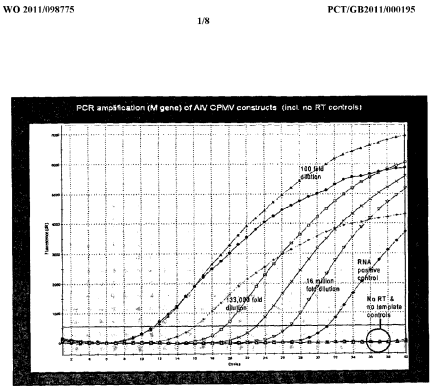

FIGURE 1

27/29	@ WPI / Thomson
PN	WO2011054011A2 2011-05-05 DW201133 WO2011054011A3 2011-08-04 DW201151 WO2011054011A4 2011-09-22 DW201162 CA2777705A1 2011-05-05 DW201241 AU2010313132A1 2012-05-17 DW201253 EP2498814A2 2012-09-19 DW201261 KR20120114217A 2012-10-16 DW201270 US2012282217A1 2012-11-08 DW201274 CN102712931A 2012-10-03 DW201304 MX2012004967A1 2012-11-30 DW201307 JP2013509203A 2013-03-14 DW201319 AU2010313132B2 2015-01-15 DW201508 AU2014271312A1 2015-01-15 DW201512 US9109014B2 2015-08-18 DW201555 EP2498814B1 2015-09-09 DW201559 IN3928DELNP2012A 2015-09-04 DW201561 JP2015166353A 2015-09-24 DW201564 MX333490B 2015-09-25 DW201603 US2016039886A1 2016-02-11 DW201612
TI	New isolated nucleic acid comprising consensus amino acid sequence of foot and mouth disease virus protein useful as vaccine for eliciting immune response against foot and mouth disease virus subtypes in mammal
PA	(UPEN) UNIV PENNSYLVANIA(UPEN) UNIV PENNSYLVANIA TRUSTEE(BOWL-I) BOWLING R A(BROW-I) BROWN P A(FERR-I) FERRARO B(INNO-N) INNOVIA PHARM INC(INOV-N) INOVIO PHARM INC(KERN-I) KERN D R(MUTH-I) MUTHUMANI K(RAMA-I) RAMANATHAN M P(SARD-I) SARDESAI N Y(WEIN-I) WEINER D B(YANJ-I) YAN J
ICAI	A61K38/20; A61K39/00; A61K39/12; A61K39/125; A61K39/135; A61K39/39; A61K48/00; A61M37/00; A61N1/30; A61N1/32; A61P31/14; A61P37/04; C07K14/005; C07K14/09; C07K19/00; C12N15/09; C12N15/12; C12N15/42; C12N15/57; C12N15/62; C12N15/63; C12N7/00; C12N9/50; C12Q1/70; G01N33/564; G01N33/569; G01N33/68;
AB	- NOVELTY : An isolated nucleic acid comprising consensus amino acid sequence of foot and mouth disease virus protein is new. - DETAILED DESCRIPTION : An isolated nucleic acid (A1) comprising a sequence encoding a protein (A2) having at least one sequence selected from at least one of (SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 or 42), not given in specification, with or without a leader sequence; variants with ≥ 80% homology to (SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 or 42), not given in specification; or their immunogenic fragments comprising

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at least 20 amino acids, or complements, is new. INDEPENDENT CLAIMS are included for the following:
(1) a vaccine (A3) comprising the nucleic acid (A1) and/or at least one protein (A2);
(2) a composition comprising at least one protein (A2);
(3) eliciting an immune response against at least one foot and mouth disease virus (FMDV) subtype in a mammal involving administering the vaccine (A3); and
(4) diagnosing a mammals infected with FMDV in mammal vaccinated with the vaccine (A3) involving a1) isolating a fluid sample from the mammal; and b1) detecting the presence of FMDV proteins not included in the vaccine and/or antibodies against FMDV proteins not included in the vaccine, where the presence of FMDV proteins not included in the vaccine and/or antibodies against FMDV proteins not included in the vaccine indicates the mammal has been infected with FMDV.ACTIVITY : Virucide; Immunostimulant.MECHANISM OF ACTION : Vaccine; Gene therapy. Test details are described, but no result given.
- USE : As vaccine for eliciting an immune response against at least one foot and mouth disease virus (FMDV) subtype in a mammal (where the mammal has not been infected with FMDV and the immune response is a protective immune response, or the mammal has been infected with FMDV and the immune response is a therapeutic immune response); for diagnosing a mammals infected with FMDV (claimed).
- ADVANTAGE : The vaccine directed against the consensus amino acid sequences of viral capsid proteins VP1, VP2, VP3, and/or VP4 for at least one subtype of FMDV presents a large repertoire of epitopes that are effective in eliciting an effective immune response (either humoral, cellular or both) against a majority of the species within each subtype of FMDV. The vaccine provides protection against several epitopes of FMDV across the various subtypes of FDMV.
- BIOTECHNOLOGY : Preparation (disclosed): The DNA plasmid is prepared by standard recombinant method. Preferred Nucleic acid: In (A1), the sequence is selected from (SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41), not given in specification, with or without coding sequence for a leader sequence; or nucleic acid molecules 80% homologous to (SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41), not given in specification; or their fragments encoding at least 20 amino acids or complements. The nucleic acid (A1) preferably comprises a sequence encoding a protein selected from (SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 or 42), not given in specification, with or without a leader sequence; or a sequence selected from (SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41), not given in specification. The nucleic acid is a plasmid (preferably expression vector). Preferred Vaccine: The vaccine further comprises an adjuvant selected from interleukin-12 (IL-12) and/or IL-15 or nucleic acid sequence encoding IL-12 and/or IL-15. Preferred Method: The method of eliciting an immune response against at least one FMDV virus subtype in a mammal involves a) administering the nucleic acid molecule to the tissue of the mammal; and b) electroporating cells of the tissue with a pulse of energy at a constant current effective to permit entry of the DNA plasmid into the cells. The current is preset for delivering to the tissue and pulse of energy is at a constant current that equals the preset current. The electroporating step further involves measuring the impedence in the electroporated cells; and adjusting the energy level of the pulse of energy relative to the measured impedance to maintain a constant current in the electroporated cells, where the measuring and adjusting steps occur within a lifetime of the pulse of energy. The electroporation step involves delivering the pulse of energy to several electrodes according to a pulse sequence pattern that delivers the pulse of energy in a decentralized pattern. Preferred Components: The leader sequence is an immunoglobulin E (IgE) leader sequence.ADMINISTRATION : The DNA plasmid vaccine is administered to the mammal by intradermic, subcutaneous, or muscle tissue injection (claimed). The vaccine is also administered orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, or by intravenous, intraarterial, intraperitoneal,

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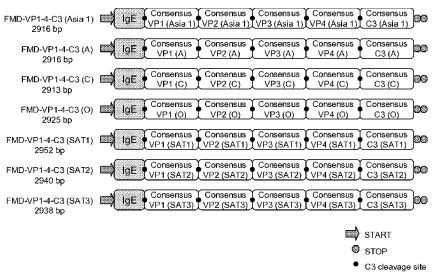
intramuscular, intranasal, intrathecal or intraarticular route. The dosage of DNA is 1 nanogram to 10 mg.EXAMPLE : No suitable example given.



28/29	@ WPI / Thomson
PN	US2011070586A1 2011-03-24 DW201126 US8354514B2 2013-01-15 DW201306
TI	Kit for determining the presence or absence of agricultural pathogens, comprises nucleic acid reagents for detection of nucleic acid signature sequence from each of the pathogens
PA	(USGO) LAWRENCE LIVERMORE NAT SECURITY LLC
ICAI	C07H21/02; C12Q1/68;
AB	- NOVELTY : A kit for determining the presence or absence of at least two porcine or bovine pathogens in a sample, comprises nucleic acid reagents for detection of at least one nucleic acid signature sequence from each of the at least two pathogens. - DETAILED DESCRIPTION : A kit for determining the presence or absence of at least two porcine pathogens in a sample, the pathogens selected from foot and mouth disease virus (FMDV), porcine reproductive and respiratory syndrome (PRRS), Swine Vesicular Disease (SVD), Vesicular Exanthema of Swine Virus (VESV), and vesicular stomatitis virus (VSV), the kit comprises nucleic acid reagents for detection of at least one nucleic acid signature sequence from each of the at least two pathogens, where the following nucleic acid signature sequences are detected: for pathogen FMDV, signature sequences consisting of SEQ ID NO: 129 or 133; for pathogen PRRS, signature sequences consisting of SEQ ID NO: 137, 141, 145, 149 or 153; for pathogen SVD, signature sequences consisting of SEQ ID NO: 157, 161 or 165; for pathogen VESV, signature sequences consisting of SEQ ID NO: 169, 173 or 177; and for pathogen VSV, signature sequences consisting of SEQ ID NO: 181, 185, 189, 193, 197 or 201. A kit for determining the presence or absence of at least two bovine pathogens in a sample, the pathogens selected from Ovine herpesvirus-2 (OvHV-2)/AHV1, Bovine Herpes Virus (BHV), Parapox (PPDX), FMDV, Bovine Viral Diarrhea (BVD), Bluetongue Virus (BTV), VSV, and Rinderpest Virus (RPV), the kit comprises nucleic acid reagents for detection of at least one nucleic acid signature sequence from each of the at least two pathogens, where the following nucleic acid signature sequences are detected: for pathogen OvHV-2/AHV1, signature sequences consisting of SEQ ID NO: 211, 215 or 219; for pathogen BHV, signature sequences consisting of SEQ ID NO: 223 or 227; for pathogen PPDX, signature sequences consisting of SEQ ID NO: 231, 235 or 239; for pathogen FMDV, signature sequences consisting of SEQ ID NO: 243 or 247; for pathogen BVD, signature sequences consisting of SEQ ID NO: 251, 255, 259, 263, 267 or 271; for pathogen VSV, signature sequences consisting of SEQ ID NO: 275, 279, 283 or 287, and for pathogen RPV, signature sequences consisting of SEQ ID NO: 291, 295 or 299. An INDEPENDENT CLAIM is a method for determining the presence or absence of at least two pathogens selected from FMDV, PRRS, SVD, VESV, VSV, OvHV-2/AHV1, BHV, PPDX, FMDV, BVD, BTV, VSV, and RPV in a sample, by using the kit above. - USE : The kit and method are used for determining the presence or absence of at least two porcine or bovine pathogens in a sample (claimed). Sequences are available in electronic form from the USPTO web site http://seqdata.uspto.gov/sequence.html;

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Document ID: US20110070586A1.

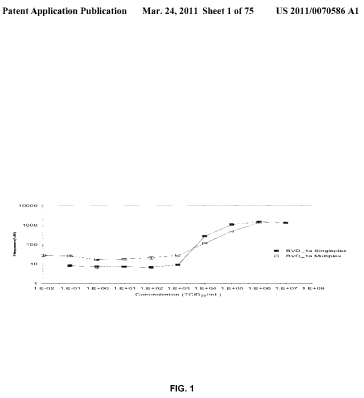

FIG. 1

- BIOTECHNOLOGY :
Preferred Kit: In the kit above, the reagents comprise a set of oligonucleotides for each signature sequence to be detected, the set comprising PCR primers and hybridization probes for each signature sequence. The reagents comprise at least two sets selected from PCR primers and hybridization probes disclosed in Table 11, given in the specification. The reagents comprise all of PCR primers and hybridization probes disclosed in Table 11. The kit further comprises reagents for detection of control sequences disclosed in Table 11. A kit for determining the presence or absence of at least two pathogens in a sample, the pathogens selected from FMDV, PRRS, SVD, VESV, VSV, OvHV2, AHV1, BHV, PPDX, BVD, BTV, and RPV, the kit comprises oligonucleotides for detection of at least one nucleic acid sequence from each of the at least two pathogens, where the following oligonucleotide probes are used: for pathogen FMDV, oligonucleotide probes consisting of SEQ ID NO: 306-8574; for pathogen PRRS, oligonucleotide probes consisting of SEQ ID NO: 8575-9300; for pathogen SVD, oligonucleotide probes consisting of SEQ ID NO: 9301-10720; for pathogen VESV, oligonucleotide probes of SEQ ID NO: 10721-11518; for pathogen VSV, oligonucleotide probes consisting of SEQ ID NO: 11519-12572; for pathogen OvHV-2, oligonucleotide probes consisting of SEQ ID NO: 12573-12701; for pathogen AHV1, oligonucleotide probes consisting of SEQ ID NO: 12702-12775; for pathogen BHV, oligonucleotide probes consisting of SEQ ID NO: 12776-13173; for pathogen PPDX, oligonucleotide probes consisting of SEQ ID NO: 13174-14235; pathogen BVD, oligonucleotide probes consisting of SEQ NO: 14236-16339; for pathogen BTV, oligonucleotide probes consisting of SEQ ID NO: 16340-22724; and for pathogen RPV, oligonucleotide probes consisting of SEQ ID NO: 22725-23046. The oligonucleotide probes are affixed to a solid support. A kit for determining the presence or absence of agricultural pathogens FMDV and VSV in a sample, the kit comprises (a) nucleic acid reagents for detection of at least one nucleic acid signature sequence from each pathogen or (b) oligonucleotide probes for detection of each pathogen or both (a) and (b); and the nucleic acid reagents and oligonucleotide probes are selected from the following: nucleic acid reagents for detection of the following FMDV nucleic acid signature sequences: SEQ ID NO: 129, 133, 243 or 247; nucleic acid reagents for detection of the following VSV nucleic acid signature sequences: SEQ ID NO: 181, 185, 189, 193, 197, 201, 275, 279, 283, or 287; oligonucleotide probes for FMDV: SEQ ID NO: 306-8574; and oligonucleotide probes for VSV: SEQ ID NO: 11519-12572. Preferred Method: The method for determining the presence or absence of pathogens includes hybridization of each oligonucleotide to the sample.EXAMPLE :
No suitable example given.

29/29	@ WPI / Thomson
PN	JP2012143185A 2012-08-02 DW201253
TI	New primer set, useful for rapidly amplifying a specific base sequence of the foot and mouth disease virus with high specificity
PA	(UYMI-N) UNIV MIYAZAKI
ICAI	C12N15/09; C12Q1/68; G01N33/53;
AB	- NOVELTY : A primer set for amplifying a specific base sequence of the foot and mouth disease (FMD) virus, is new. - DETAILED DESCRIPTION : A primer set for amplifying a specific base sequence of the FMD virus, comprises Primer set 1: 5'-caaccacaagaattgccttc-3' (SEQ ID NO: 6), 5'-gtggagtcaagcacagtaca-3'

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(SEQ ID NO: 9); 5'-acaagtcctcctcaagcgacgattcgcctagttttgcccgt-3' (SEQ ID NO: 10), 5'-gtcccatttctcctgaaaacgggatgaaccttcacccggaag-3' (SEQ ID NO: 11), 5'-caaccacaagaattgccttc-3' (SEQ ID NO: 6), 5'-gacttgtacaaacacgatctactcaggcacccctctagacctggaa-3' (SEQ ID NO: 12), 5'-tttcgtgcgcagacgtccc-3' (SEQ ID NO: 13), 5'-actaggcgaatttgccgtttta-3' (SEQ ID NO: 14), 5'-gcaacttgaaactccgcct-3' (SEQ ID NO: 15), and 5'-ccgacacaaaccgtgcaa-3' (SEQ ID NO: 16); Primer set 2: 5'-gtgatggcctcaaagacc-3' (SEQ ID NO: 20), 5'-attatgcgtcaccacacac-3' (SEQ ID NO: 23), 5'-gccacggagatcaacttctcctcttgaggctatcctctcctt-3' (SEQ ID NO: 24), 5'-gacgagtaccggcgtctcttcgcaggtaaagtgatctgt-3' (SEQ ID NO: 25), 5'-ccacggcgtgcaaagga-3' (SEQ ID NO: 26), 5'-gagcccttccagggcctctt-3' (SEQ ID NO: 27), and 5'-gcctctttgagatcccaagct-3' (SEQ ID NO: 28); Primer set 3: 5'-attctgttggccgggttg-3' (SEQ ID NO: 32), 5'-ggtctgtcatggtgacaaac-3' (SEQ ID NO: 35), 5'-gtcacgtgctttgagttgtttctccgcctcgagtttcttccg-3' (SEQ ID NO: 36), 5'-tcaagaacggcgagtggcttctcttctgaggcgatcca-3' (SEQ ID NO: 37), 5'-gctctctcaaggtcttcggg-3' (SEQ ID NO: 38), and 5'-cttgccatccgcgactgga-3' (SEQ ID NO: 39); and Primer set 4: 5'-tgacaaaagcgacaaaggtt-3' (SEQ ID NO: 43), 5'-tggactgcgagtcctgc-3' (SEQ ID NO: 46), 5'-acaggtttgtaaaacccagttccacactccattgccgatgtcac-3' (SEQ ID NO: 47), 5'-atggcttcgaagaccctcgaacggagatcaacttctcctgta-3' (SEQ ID NO: 48), 5'-ggaagtgtcttttgaggaa-3' (SEQ ID NO: 49), and 5'-tttgcacgccgtgggac-3' (SEQ ID NO: 50). All sequences are given in the specification. INDEPENDENT CLAIMS are:

(1) and oligonucleotide primer for amplifying the base sequence of FMD virus gene comprising (i) a sequence comprising nucleotides 121-351 of a defined nucleotide sequence of 7822 bp (SEQ ID NO: 1), (ii) a sequence comprising 7883-8090 of a defined nucleotide sequence of 8201 bp (SEQ ID NO: 17), (iii) a sequence comprising nucleotides 4326-4552 of a defined nucleotide sequence of 8191 bp (SEQ ID NO: 29), and (iv) a sequence comprising nucleotides 7767-7964 of a defined nucleotide sequence of 8186 bp (SEQ ID NO: 40), where sequences are given in the specification;

(2) a method for detecting FMD virus by amplifying the target gene of the FMD virus by using the primer set or oligonucleotide primer above;

(3) a method for diagnosing FMD virus infection by amplifying the target gene of the FMD virus by using the primer set or oligonucleotide primer above; and

(4) a kit for a FMD virus detection or a FMD diagnosis, comprising the primer set or oligonucleotide primer above.

- USE :

The primer set and oligonucleotide primers are used for amplifying the base sequence of FMD virus gene. The primer set, oligonucleotide primer, and kit are used for detecting FMD virus, and for diagnosing FMD virus infection (all claimed).

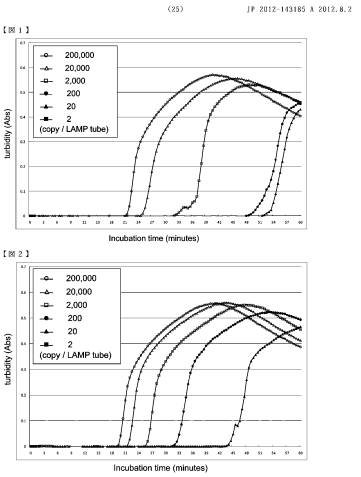
- ADVANTAGE :

The primer set, oligonucleotide primer, and kit are used for rapid FMD virus detection with high sensitivity.

- BIOTECHNOLOGY :

Preferred Oligonucleotide Primer: The oligonucleotide primer contains continuous 15 bp of any of the 11 defined nucleotide sequences selected from cgtcgcttgaggaggacttgt, attcgcctagttttgcccgt, ccgttttcaggagaaatgggac, gatgaaccttcacccggaag, caaccacaagaattgccttc, gacttgtacaaacacgatctactcagg, ttccaggtctagaggggtg, gtggagtcaagcacagtaca, acaagtcctcctcaagcgacgattcgcctagttttgcccgt, gtcccatttctcctgaaaacgggatgaaccttcacccggaag, and gacttgtacaaacacgatctactcaggcacccctctagacctggaa (SEQ ID NO: 2-12), given in the specification. The oligonucleotide primer contains continuous 15 bp of any of the 8 defined sequences selected from aggagaagttgatctccgtggc, cttgaggctatcctctcctt, gtgatggcctcaaagacc, gacgagtaccggcgtctctt, and acagatcactttacctgcg (SEQ ID NO: 18 - 25), given in the specification. The oligonucleotide primer contains continuous 15 bp of any of the 8 defined nucleotide sequences selected from gagaaacaactcaaagcacgtgac, cgcctcgagtttcttccg, attctgttggccgggttg, tcaagaacggcgagtggct, tggatcgcctcagaagaga, ggtctgtcatggtgacaaac, gtcacgtgctttgagttgtttctccgcctc, gagtttcttccg, and tcaagaacggcgagtggcttctcttctgaggcgatcca (SEQ ID NO: 30-37), given in the

(Volver al Sumario)



specification. The oligonucleotide primer contains continuous 15 bp of any of the 8 defined nucleotide sequences selected from tggaactgggttttacaaacctgt, cactccattgccgatgtcac, tgacaaaagcgacaaaggtt, atggcttcgaagaccctcga, tacaggagaagttgatctccgt, tggactgcgagtcctgc, acaggtttgtaaaacccagttccacactccattgccgatgtcac, and atggcttcgaagaccctcgaacggagatcaacttctcctgta (SEQ ID NO: 41-48), given in the specification. The oligonucleotide primer comprises (a) a base sequence has F2 region of a target nucleic acid in 3' terminal side, and has an F1c region of a target nucleic acid in 5' terminal side, (b) a base sequence has F3 region of a target nucleic acid, (c) a base sequence has B2 region of a target nucleic acid in 3' terminal side, and has B1c region of a target nucleic acid in 5' terminal side, and (d) a base sequence which has B3 region of a target nucleic acid. The oligonucleotide primer comprises (i) 5'-(base sequence complementary to cgtcgcttgaggaggacttgt (SEQ ID NO: 2))-(arbitrary base sequences of base number 0-50)-(attcgcctagttttgcccgt (SEQ ID NO: 3))-3', (ii) 5'-(base sequence complementary to ccgttttcaggagaaatgggac (SEQ ID NO: 4))-(arbitrary base sequences of base number 0-50)-(gatgaaccttcacccggaag (SEQ ID NO: 5))-3', (iii) 5'-(gacttgtacaaacacgatctactcagg (SEQ ID NO: 7))-(arbitrary base sequences of base number 0-50)-(base sequence complementary to ttccaggtctagaggggtg (SEQ ID NO: 8))-3', (iv) 5'-(base sequence complementary to aggagaagttgatctccgtggc (SEQ ID NO: 18))-(arbitrary base sequences of base number 0-50)-(cttgaggctatcctctcctt (SEQ ID NO: 19))-3', (v) 5'-(gacgagtaccggcgtctctt (SEQ ID NO: 21))-(arbitrary base sequences of base number 0-50)-(a base sequence complementary to acagatcactttacctgcg (SEQ ID NO: 22))-3', (vi) 5'-(base sequence complementary to gagaaacaactcaaagcacgtgac (SEQ ID NO: 30))-(arbitrary base sequences of base number 0-50)-(cgcctcgagtttcttccg (SEQ ID NO: 31))-3', (vii) 5'-(tcaagaacggcgagtggct (SEQ ID NO: 33))-(arbitrary base sequences of base number 0-50)-(a base sequence complementary to tggatcgcctcagaagaga (SEQ ID NO: 34))-3', (viii) 5'-(base sequence complementary to tggaactgggttttacaaacctgt (SEQ ID NO: 41))-(arbitrary base sequences of base number 0-50)-(cactccattgccgatgtcac (SEQ ID NO: 42))-3', and (ix) 5'-(atggcttcgaagaccctcga (SEQ ID NO: 44))-(arbitrary base sequences of base number 0-50)-(a base sequence complementary to tacaggagaagttgatctccgt (SEQ ID NO: 45))-3', where all sequences are given in the specification. Preferred Method: In the detection and diagnostic methods above, 1-3 reaction tubes are used, where the tubes contains the oligonucleotide primer. The method comprises performing amplification on the target nucleic acid region of any of the 4 types of FMD virus gene. Amplification reaction is by loop mediated isothermal amplification (LAMP) method.EXAMPLE :
No suitable example given.

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Literatura No Patente

1/21	@ BIOSIS / BIOSIS
AN	PREV201100435006
PD	2011-06-00
TI	Pan-serotypic detection of foot-and-mouth disease virus using a minor groove binder probe reverse transcription polymerase chain reaction assay
AU	McKillen John; McMenamy Michael; Reid Scott M; Duffy Catherine; Hjertner Bernt; King Donald P; Belak Sandor; Welsh Michael; Allan Gordon
AUAF	Agri Food and Biosci Inst, Vet Sci Div, Belfast BT4 3SD, Antrim, UK; john.mckillen@afbini.gov.uk
PUB	Journal of Virological MethodsJUN 2011
LNKD	doi:10.1016/j.jviromet.2011.03.008
IRN	ISSN 0166-0934
VOL	174
NR	1-2
PG	117-119
AB	A novel assay for the pan-serotypic detection of foot-and-mouth disease virus (FMDV) was designed using a 5' conjugated minor groove binder (MGB) probe real-time RT-PCR system. This assay targets the 3D region of the FMDV genome and is capable of detecting 20 copies of a transcribed RNA standard. The linear range of the test was eight logs from 2 x 10(1) to 2 x 10(8) copies and amplification time was approximately 2 h. Using a panel of 83 RNA samples from representative FMDV isolates, the diagnostic sensitivity of this test was shown to be equivalent to a TaqMan real-time RT-PCR that targets the 5' untranslated region of FMDV. Furthermore, the assay does not detect viruses causing similar clinical diseases in pigs such as swine vesicular disease virus and vesicular stomatitis virus, nor does it detect marine caliciviruses causing vesicular exanthema. The development of this assay provides a useful tool for the differential diagnosis of FMD, potentially for use in statutory or emergency testing programmes, or for detection of FMDV RNA in research applications. (C) 2011 Elsevier B.V. All rights reserved.

2/21	@ BIOSIS / BIOSIS
AN	PREV201000561116
PD	2010-10-00
TI	Molecular Characterization of Foot-and-Mouth Disease Viruses Collected from Sudan
AU	Habiela M; Ferris N P; Hutchings G H; Wadsworth J; Reid S M; Madi M; Ebert K; Sumption K J; Knowles N J; King D P; Paton D J
AUAF	Inst Anim Hlth, Ash Rd, Pirbright GU24 0NF, Surrey, UK; donald.king@bbsrc.ac.uk
PUB	Transboundary and Emerging DiseasesOCT 2010
LNKD	doi:10.1111/j.1865-1682.2010.01151.x
IRN	ISSN 1865-1674
VOL	57
NR	5
PG	305-314
AB	The aim of this study was to characterize foot-and-mouth disease (FMD) viruses collected between 2004 and 2008 from Sudan, a country where FMD is endemic. Using virus isolation and antigen ELISA, three FMD virus serotypes (O, A and SAT2) were detected in 24 samples that were submitted to the FAO World Reference Laboratory for FMD. Pan-serotypic real-time RT-PCR assays targeting the 5' untranslated region (5'UTR) and 3D genes of FMD virus were also used to contribute to the laboratory diagnosis of these cases. The lack of concordant results between the real-time RT-PCR assays for three serotype O viruses was attributed to four nucleotide mismatches

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	in the 5'UTR PCR primer and probe sites (three substitutions for the sense-primer and one in the TaqMan (R) probe region). Taken together, the laboratory results showed that recent FMD outbreaks that occurred during 2008 in northern and central Sudan were caused by serotypes O and SAT2, while serotype A was last detected in 2006. Phylogenetic analyses of VP1 sequences from these viruses were used to determine the relationships with 23 older viruses from Sudan and other viruses from West and East Africa. For serotype O, closest genetic identities were between concurrent and historical Sudanese isolates, indicating that within-country circulation is an important mechanism by which FMD is maintained year-on-year in Sudan. A similar pattern was also evident for serotype A and SAT2 viruses; however, these lineages also contained recent representative FMD viral isolates from other countries in the region suggesting that long-distance animal movement can also contribute to FMD dispersal across sub-Saharan Africa. These findings provide the first molecular description of FMD viruses that are circulating in Sudan, and highlight that further sampling of representative viruses from the region is required before the complex epidemiology of FMD in sub-Saharan Africa can be fully understood.

3/21	@ BIOSIS / BIOSIS
AN	PREV201000559135
PD	2010-10-00
TI	Pan-serotypic detection of foot-and-mouth disease virus by RT linear-after-the-exponential PCR
AU	Reid Scott M; Pierce Kenneth E; Mistry Rohit; Bharya Sukvinder; Dukes Juliet P; Volpe Carmelo; Wangh Lawrence J; King Donald R
AUAF	Inst Anim Hlth, Ash Rd, Woking GU24 0NF, Surrey, UK; donald.king@bbsrc.ac.uk
PUB	Molecular and Cellular ProbesOCT 2010
LNKD	doi:10.1016/j.mcp.2010.04.004
IRN	ISSN 0890-8508
VOL	24
NR	5
PG	250-255
AB	A reverse transcription Linear-After-The-Exponential polymerase chain reaction (RT LATE-PCR) assay was evaluated for detection of foot-and-mouth disease virus (FMDV). This pan-serotypic assay targets highly conserved sequences within the 3D (RNA polymerase) region of the FMDV genome, and uses end-point hybridisation analysis of a single mismatch-tolerant low temperature probe to confirm the identity of the amplicons. An Armored RNA (R) served as an internal control to validate virus negative results. The ability of the assay to identify FMDV was directly compared to a real-time RT-PCR assay routinely used by reference laboratories. The analytical sensitivity of the RT LATE-PCR assay was 10 genomic copies and the dynamic range of the test was identical to real-time RT-PCR based on decimal dilutions of an FMDV-positive sample. This pan-serotypic assay was able to detect FMDV in a broad range of clinical samples collected from field cases of FMD (n = 121), while samples of other viruses causing vesicular disease in livestock and genetic relatives of FMDV were negative. In addition to the laboratory-based utility of this diagnostic test, the RT LATE-PCR assay format has potential application for use in a portable ("point-of-care") device designed to achieve rapid detection of FMDV in the field. (C) 2010 Elsevier Ltd. All rights reserved.

4/21	@ BIOSIS / BIOSIS
AN	PREV201000398268
PD	2010-07-00
TI	Design and optimization of a novel reverse transcription linear-after-the-exponential PCR for the detection of foot-and-mouth disease virus
AU	Pierce K E; Mistry R; Reid S M; Bharya S; Dukes J P; Hartshorn C; King D P; Wangh L J

(Volver al Sumario)

AUAF	Brandeis Univ, Dept Biol, MS 008, Waltham, MA 02454 USA; pierce@brandeis.edu
PUB	Journal of Applied MicrobiologyJUL 2010
LNKD	doi:10.1111/j.1365-2672.2009.04640.x
IRN	ISSN 1364-5072
VOL	109
NR	1
PG	180-189
AB	Aims:A novel molecular assay for the detection of foot-and-mouth disease virus (FMDV) was developed using linear-after-the-exponential polymerase chain reaction (LATE-PCR).Methods and Results:Pilot experiments using synthetic DNA targets demonstrated the ability of LATE-PCR to quantify initial target concentration through endpoint detection. A two-step protocol involving reverse transcription (RT) followed by LATE-PCR was then used to confirm the ability of the assay to detect FMDV RNA. Finally, RT and LATE-PCR were combined in a one-step duplex assay for co-amplification of an FMDV RNA segment and an internal control comprised of an Armored RNA (R). In that form, each of the excess primers in the reaction mixture hybridize to their respective RNA targets during a short pre-incubation, then generate cDNA strands during a 3-min RT step at 60 degrees C, and the resulting cDNA is amplified by LATE-PCR without intervening sample processing.Conclusions:The RT-LATE-PCR assay generates fluorescent signals at endpoint that are proportional to the starting number of RNA targets and can detect a range of sequence variants using a single mismatch-tolerant probe.Significance and Impact of the Study:In addition to offering improvements over current laboratory-based molecular diagnostic assays for FMDV, this new assay is compatible with a novel portable ('point-of-care') device, the BioSeeq (R) II, designed for the rapid diagnosis of FMD in the field.

5/21	@ BIOSIS / BIOSIS
AN	PREV201000027619
PD	2009-10-00
TI	A review of RT-PCR technologies used in veterinary virology and disease control: Sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health
AU	Hoffmann Bernd; Beer Martin; Reid Scott M; Mertens Peter; Oura Chris A L; van Rijn Piet A; Slomka Marek J; Banks Jill; Brown Ian H; Alexander Dennis J; King Donald P
AUAF	Friedrich Loeffler Inst, Inst Diagnost Virol, Sudufer 10, D-17493 Greifswald, Riems, Germany; martin.beer@fli.bund.de
PUB	Veterinary MicrobiologyOCT 20 2009
LNKD	doi:10.1016/j.vetmic.2009.04.034
IRN	ISSN 0378-1135
VOL	139
NR	1-2
PG	1-23
AB	Real-time, reverse transcription polymerase chain reaction (rRT-PCR) has become one of the most widely used methods in the field of molecular diagnostics and research. The potential of this format to provide sensitive, specific and swift detection and quantification of viral RNAs has made it an indispensable tool for state-of-the-art diagnostics of important human and animal viral pathogens. Integration of these assays into automated liquid handling platforms for nucleic acid extraction increases the rate and standardisation of sample throughput and decreases the potential for cross-contamination. The reliability of these assays can be further enhanced by using internal controls to validate test results. Based on these advantageous characteristics, numerous robust rRT-PCRs systems have been developed and validated for important epizootic diseases of livestock. Here, we review the rRT-PCR assays that have been developed for the detection of five RNA viruses that cause diseases that are notifiable to the World Organisation for Animal Health (OIE), namely: foot-and-mouth disease, classical swine fever, bluetongue disease, avian influenza and Newcastle disease. The performance of these tests for viral diagnostics and disease control and prospects for

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	improved strategies in the future are discussed. (C) 2009 Elsevier B.V. All rights reserved.

6/21	@ BIOSIS / BIOSIS
AN	PREV200900513558
PD	2009-03-00
TI	Establishment of RT-LAMP for Rapid Detection of Foot-and-Mouth Disease Virus
AU	Li Jian; Chen Qin; Xiong Wei; Fang Xue-en
AUAF	Shanghai Univ, Sch Life Sci, Shanghai 200444, Peoples R China; lijiancq@yahoo.com.cn, chenqincc@yahoo.com.cn
PUB	Chinese Journal of VirologyMAR 2009
IRN	ISSN 1000-8721
VOL	25
NR	2
PG	137-142
AB	A rapid detection of foot-and-mouth disease virus (FMDV) was established by using reverse transcription loop-mediated isothermal amplification (RT-LAMP) method, meanwhile its specificity and sensitivity were assessed. The results showed that the FMDV RNA could be amplified by incubation at 65 degrees C for only 1h using six primers designed based on FMDV polyprotein gene and the amplification products could be detected easily by naked-eye. There is no cross reaction with other virus such as SVDV, SFV and PPV by detecting their RNA samples. The detection limit of this method was found to be 10(-5) dilution of RNA sample which was 100-fold higher than that of PCR and 10-fold higher than real-time PCR.

7/21	@ BIOSIS / BIOSIS
AN	PREV200900117885
PD	2009-01-00
TI	Development and laboratory validation of a lateral flow device for the detection of foot-and-mouth disease virus in clinical samples
AU	Ferris Nigel P; Nordengrahn Ann; Hutchings Geoffrey H; Reid Scott M; King Donald P; Ebert Katja; Paton David J; Kristersson Therese; Brocchi Emiliana; Grazioli Santina; Merza Malik
AUAF	Inst Anim Hlth, Pirbright Lab, Ash Rd, Woking GU24 0NF, Surrey, UK; nigel.ferris@bbsrc.ac.uk
PUB	Journal of Virological MethodsJAN 2009
LNKD	doi:10.1016/j.jviromet.2008.09.009
IRN	ISSN 0166-0934
VOL	155
NR	1
PG	10-17
AB	A lateral flow device (LFD) for the detection of all seven serotypes of foot-and-mouth disease virus (FMDV) was developed Using a monoclonal antibody (Mab 1F10) shown to be pan-reactive to FMDV strains of each serotype by ELISA. The performance of the LFD was evaluated in the laboratory on suspensions of vesicular epithelia (304 positive and 1003 negative samples) from suspected cases of vesicular disease collected from 86 countries between 1965 and 2008 and negative samples collected from healthy animals. The diagnostic sensitivity of the LFD for FMDV was similar at 84% compared to 85% obtained by the reference method of antigen ELISA, and the diagnostic specificity of the LFD was approximately 99% compared to 99.9% for the ELISA. The device recognized FMDV strains of wide diversity of all seven serotypes but weaker reactions were often evident with those of type SAT 2, several viruses of which were not detected. Reactions with the viruses of swine vesicular disease and vesicular stomatitis that produce clinically indistinguishable syndromes in pigs and cattle, did not occur. The test procedure was simple and rapid, and typically provided a result within 1-10 min of sample addition. Simple homogenizers that could be used in field

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	conditions for preparing epithelial suspensions were demonstrated to be effective for LFD application. These data illustrate the potential for the LFD to be used next to the animal in the pen-side diagnosis of FMD and for providing rapid and objective support to veterinarians in their clinical judgment of the disease. (c) 2008 Elsevier B.V. All rights reserved.

8/21	@ BIOSIS / BIOSIS
AN	PREV200800191075
PD	2007-00-00
TI	LATE-PCR detection of Foot and Mouth Disease Virus (FMDV)
AU	Pierce K E; Dukes J R; Reid S M; King D P; Waugh L J
AUAF	Brandeis Univ, Waltham, MA 02254 USA
PUB	Abstracts of the General Meeting of the American Society for Microbiology2007
IRN	ISSN 1060-2011
VOL	107
PG	729-730
CONF	107th General Meeting of the American-Society-for-Microbiology; Toronto, CANADA; 2007,
AB	No hay resumen disponible

9/21	@ BIOSIS / BIOSIS
AN	PREV200800108072
PD	2008-01-00
TI	Detection of foot-and-mouth disease virus by nucleic acid sequence-based amplification (NASBA)
AU	Lau Lok-Ting; Reid Scott M; King Donald P; Lau Anson Ming-Fung; Shaw Andrew E; Ferris Nigel P; Yu Albert Cheung-Hoi
AUAF	8 F Hang Tung Resources Ctr, Hai Kang Life Corp Ltd, 18 A Kung Ngam Village Rd, Hong Kong, Hong Kong, Peoples R China; achy@hsc.pku.edu.cn
PUB	Veterinary MicrobiologyJAN 1 2008
LNKD	doi:10.1016/j.vetmic.2007.07.008
IRN	ISSN 0378-1135
VOL	126
NR	1-3
PG	101-110
AB	A study was conducted to evaluate the performance of a nucleic acid sequence-based amplification (NASBA) assay for the detection of foot-and-mouth disease virus (FMDV). Two detection methods: NASBA-electrochemiluminescence (NASBA-ECL) and a newly developed NASBA-enzyme-linked oligonucleotide capture (NASBA-EOC) were evaluated. The diagnostic sensitivity of these assays was compared with other laboratory-based methods using 200 clinical samples collected from different regions of the world. Assay specificity was also assessed using samples (n = 43) of other viruses that cause vesicular disease in livestock and genetic relatives of FMDV Concordant results were generated for 174/200 (87.0%) of suspect FMD samples between NASBA-ECL and real-time RT-PCR. In comparison with the virus isolation (VI) data, the sensitivity of the NASBA-ECL assay was 92.9%, which was almost identical to that of the real-time RT-PCR (92.4%) for the same set of samples. There was broad agreement between the results of the NASBA-ECL and the simpler NASBA-EOC detection method for 97.1 % of samples. In conclusion, this study provides further data to support the use of NASBA as a rapid and sensitive diagnostic method for the detection and surveillance of FMDV. (C) 2007 Elsevier B.V. All rights reserved.

10/21	@ BIOSIS / BIOSIS
AN	PREV200800103958
PD	2007-12-00
TI	Development of a novel recombinant encapsidated RNA particle: Evaluation as an

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	internal control for diagnostic RT-PCR
AU	King Donald P; Montague Nick; Ebert Katja; Reid Scott M; Dukes Juliet P; Schaedlich Lysann; Belsham Graham J; Lomonossoff George P
AUAF	Inst Anim Hlth, Ash Rd, Pirbright GU24 0NF, Surrey, UK; donald.king@bbsrc.ac.uk
PUB	Journal of Virological MethodsDEC 2007
LNKD	doi:10.1016/j.jviromet.2007.07.002
IRN	ISSN 0166-0934
VOL	146
NR	1-2
PG	218-225
AB	This report describes the generation of novel encapsidated RNA particles and their evaluation as in-tube internal controls in diagnostic real-time reverse-transcription PCR (rRT-PCR) assays for the detection of RNA viruses. A cassette containing sequences of 2 diagnostic primer sets for foot-and-mouth disease virus (FMDV) and a set for swine vesicular disease virus (SVDV) was engineered into a full-length cDNA clone containing the RNA-2 segment of Cowpea Mosaic Virus (CPMV). After co-inoculation with a plasmid that expressed CPMV RNA-1, recombinant virus particles were rescued from cowpea plants (Vigna unguiculata). RNA contained in these particles was amplified in diagnostic rRT-PCR assays used for detection of FMDV and SVDV. Amplification of these internal controls was used to confirm that rRT-PCR inhibitors were absent from clinical samples, thereby verifying negative assay results. The recombinant CPMVs did not reduce the analytical sensitivity of the rRT-PCRs when amplification of the insert was performed in the same tube as the diagnostic target. This system provides an attractive solution to the production of internal controls for rRT-PCR assays since CPMV grows to high yields in plants, the particles are thermostable, RNase resistant and simple purification of RNA-2 containing capsids yields a preparation which is non-infectious. (c) 2007 Elsevier B.V. All rights reserved.

11/21	@ BIOSIS / BIOSIS
AN	PREV200700463202
PD	2007-06-00
TI	Development of an epitope-blocking-enzyme-linked immunosorbent assay to differentiate between animals infected with and vaccinated against foot-and-mouth disease virus
AU	Oem Jae Ku; Chang Byung Sik; Joo Hoo Don; Yang Mi Young; Kim Gwang Jae; Park Jee Yong; Ko Young Joon; Kim Yong Ju; Park Jong Hyeon; Joo Yi Seok
AUAF	Jenobiotech Inc, Dept Res Lab, Chucheon, South Korea; jku0622@nvrqs.go.kr, lab-chief@jenobiotech.com, jenobio@jenobiotech.com, heimlich@jenobiotech.com, gjkim@jenobiotech.com, parkjy@nvrqs.go.kr, koyj@nvrqs.go.kr, kimyj@nvrqs.go.kr, parkjh@nvrqs.go.kr, jooys@nvrqs.go.kr
PUB	Journal of Virological MethodsJUN 2007
LNKD	doi:10.1016/j.jviromet.2007.01.025
IRN	ISSN 0166-0934
VOL	142
NR	1-2
PG	174-181
AB	An epitope-blocking ELISA (EB-ELISA) was developed to distinguish animals infected with foot-and-mouth-disease (FMDV) from those immunized with commercial vaccines. The assay used monoclonal antibodies to target the 313 core repeat motif (QKPLK) and purified recombinant 3AB proteins from the major B cell line epitopes of FMDV. Sera from uninfected and regularly vaccinated cattle, pigs, goats, and sheep (raised in FMDV free areas) were screened to evaluate the specificity of the EB-ELISA. The specificity scores of the assays were 99.8-100% and 100%, respectively. Reference sera from cattle, pigs, goats, and sheep experimentally infected with FMDV tested positive, with only a single exception. Antibodies formed in response to FMDV 3B appeared 1 week after infection and persisted at high levels for more than 8 weeks within the sera collected from serial bleeding of animals infected with FMDV

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	O/SKR/2000. The EB-ELISA was used to differentiate between farms vaccinated against and those infected with FMDV (FMDV Asia serotype) during the 2005 epidemic in Mongolia by detecting antibodies against the FMDV Asia serotype in outbreak farms. This EB-ELISA method shows promise as an effective tool for FMDV control and eradication. (c) 2007 Elsevier B.V. All rights reserved.

12/21	@ BIOSIS / BIOSIS
AN	PREV200700452628
PD	2007-07-00
TI	Implementation of a one-step real-time RT-PCR protocol for diagnosis of foot-and-mouth disease
AU	Shaw Andrew E; Reid Scott M; Ebert Katja; Hutchings Geoffrey H; Ferris Nigel P; King Donald P
AUAF	Pirbright Lab, Inst Anim Hlth, Woking GU24 0NF, Surrey, UK; donald.king@bbsrc.ac.uk
PUB	Journal of Virological MethodsJUL 2007
LNKD	doi:10.1016/j.jviromet.2007.02.009
IRN	ISSN 0166-0934
VOL	143
NR	1
PG	81-85
AB	An automated one-step real-time reverse transcription polymerase chain reaction (rRT-PCR) protocol was optimised and evaluated for the routine diagnosis of foot-and-mouth disease (FMD). Parallel testing of RNA samples (n = 257) indicated that this assay has a diagnostic sensitivity at least equivalent to the automated two-step rRT-PCR protocol previously used for the laboratory detection of FMD virus (FMDV). This more rapid and economical one-step protocol will play a key role in contingency planning for any future outbreaks of FMD in the United Kingdom (UK). (C) 2007 Elsevier B.V. All rights reserved.

13/21	@ BIOSIS / BIOSIS
AN	PREV200600537870
PD	2006-05-00
TI	Microarray-based identification of antigenic variants of foot-and-mouth disease virus: a bioinformatics quality assessment
AU	Martin Veromica; Perales Celia; Abia David; Ortiz Angel R; Domingo Esteban; Briones Carlos
AUAF	Univ Autonoma Madrid, Ctr Biol Mol Severo Ochoa, Bioinformat Unit, E-28049 Madrid, Spain; vmartin@cbm.uam.es, cperales@cbm.uam.es, dabia@cbm.uam.es, aro@cbm.uam.es, edomingo@cbm.uam.es, brioneslc@inta.es
PUB	BMC GenomicsMAY 18 2006
LNKD	doi:10.1186/1471-2164/7/117
IRN	ISSN 1471-2164
VOL	7
PG	Article No.: 117
AB	Background: The evolution of viral quasispecies can influence viral pathogenesis and the response to antiviral treatments. Mutant clouds in infected organisms represent the first stage in the genetic and antigenic diversification of RNA viruses, such as foot and mouth disease virus (FMDV), an important animal pathogen. Antigenic variants of FMDV have been classically diagnosed by immunological or RT-PCR-based methods. DNA microarrays are becoming increasingly useful for the analysis of gene expression and single nucleotide polymorphisms (SNPs). Recently, a FMDV microarray was described to detect simultaneously the seven FMDV serotypes. These results encourage the development of new oligonucleotide microarrays to probe the fine genetic and antigenic composition of FMDV for diagnosis, vaccine design, and to gain insight into the molecular epidemiology of this pathogen.Results: A FMDV microarray was designed and optimized to detect SNPs at a major antigenic site of the virus. A

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screening of point mutants of the genomic region encoding antigenic site A of FMDV C-S8c1 was achieved. The hybridization pattern of a mutant includes specific positive and negative signals as well as crosshybridization signals, which are of different intensity depending on the thermodynamic stability of each probe-target pair. Moreover, an array bioinformatic classification method was developed to evaluate the hybridization signals. This statistical analysis shows that the procedure allows a very accurate classification per variant genome.Conclusion: A specific approach based on a microarray platform aimed at distinguishing point mutants within an important determinant of antigenicity and host cell tropism, namely the G-H loop of capsid protein VPI, was developed. The procedure is of general applicability as a test for specificity and discriminatory power of microarray-based diagnostic procedures using multiple oligonucleotide probes.

14/21	@ BIOSIS / BIOSIS
AN	PREV200600128635
PD	2006-01-00
TI	Utility of automated real-time RT-PCR for the detection of foot-and-mouth disease virus excreted in milk
AU	Reid Scott M; Parida Satya; King Donald P; Hutchings Geoffrey H; Shaw Andrew E; Ferris Nigel P; Zhang Zhidong; Hillerton J Eric; Paton David J
AUAF	Inst Anim Hlth, Pirbright Lab, Ash Rd, Surrey GU24 0NF, UK; scott.reid@bbsrc.ac.uk
PUB	Veterinary Research (Les Ulis)JAN-FEB 2006
IRN	ISSN 0928-4249
VOL	37
NR	1
PG	121-132
AB	Foot-and-mouth disease virus (FMDV) can be excreted in milk and thereby spread infection to susceptible animals in other holdings. The feasibility of using real-time reverse transcription polymerase chain reaction (rRT-PCR) as a diagnostic tool for detection of FMDV in milk was assessed by studying the excretion of virus from experimentally-infected cattle. Fore- and machine milk samples were collected over a 4-week period from two dairy cows infected with FMDV and from two in-contact cows held in the same pen. The whole, skim, cream and cellular debris components of the milks were tested by automated rRT-PCR and results compared to virus isolation (VI) in cell culture. The onset of clinical signs of FMD in all four cows correlated with viraemia, and the presence of FMDV in other clinical samples. rRT-PCR results matched closely with VI in detecting FMDV in all milk components and generally coincided with, but did not consistently precede, the onset of clinical signs. rRT-PCR detected FMDV in milk up to 23 days post inoculation which was longer than VI. Furthermore, the detection limit of FMDV in milk was greater by rRT-PCR than VI and, in contrast to VI, rRT-PCR detected virus genome following heat treatment that simulated pasteurisation. rRT-PCR was also able to detect FMDV in preservative-treated milk. In conclusion, this study showed that automated rRT-PCR is quicker and more sensitive than VI and can be used to detect FMDV in whole milk as well as milk fractions from infected animals.

15/21	@ BIOSIS / BIOSIS
AN	PREV200600042293
PD	2005-10-00
TI	The application of new techniques to the improved detection of persistently infected cattle after vaccination and contact exposure to foot-and-mouth disease
AU	Parida S; Cox S J; Reid S M; Hamblin P; Barnett P V; Inoue T; Anderson J; Paton D J
AUAF	Inst Anim Hlth, Pirbright Lab, Ash Rd, Surrey GU24 0NF, UK; david.paton@bbsrc.ac.uk
PUB	VaccineOCT 25 2005
IRN	ISSN 0264-410X
VOL	23
NR	44

(Volver al Sumario)

PG	5186-5195
AB	Detection of antibodies to the non-structural proteins (NSP) of foot-and-mouth disease virus (FMDV) was compared with conventional serological and virological methods and with RT-PCR for the identification of FMDV carrier animals obtained after experimental contact challenge of vaccinated cattle. Transmission from carriers to sentinels was also monitored. Twenty FMDV vaccinated and five unvaccinated cattle were challenged by direct contact with five donor cattle excreting FMDV and monitored until 28 days post challenge-exposure [1]. Twelve vaccinated and three unvaccinated animals were retained up to 24 weeks post exposure to FMDV in order to monitor viral persistence, transmission and antibody responses. In nine vaccinated animals, infection persisted beyond 28 days post exposure, virus being detected more frequently and for longer in oesophagopharyngeal samples from these animals when examined by RT-PCR rather than by virus isolation. Although recovery of FMDV RNA became increasingly sporadic over time, the number of RNA copies detected in positive samples declined only slowly. Two naive sentinel cattle housed with the persistently infected animals between 93 and 168 days after the latter had been challenge-exposed to FMDV did not become infected. There were differences in the ability of commercially available serological tests to detect antibodies to FMDV non-structural proteins (NSP) in vaccinated and subsequently challenged cattle. Although no single test could identify all of the vaccinated cattle that became persistently infected, the most poorly recognised animals were those with the least evidence of virus replication based on other tests. The potential of the detection of antibodies to the 2B NSP of FMDV for diagnosing persistent FMDV infection was demonstrated. (c) 2005 Elsevier Ltd. All rights reserved.

16/21	@ BIOSIS / BIOSIS
AN	PREV200400187804
PD	2004-03-15
TI	Evaluation of real-time reverse transcription polymerase chain reaction assays for the detection of swine vesicular disease virus.
AU	Reid Scott M; Ferris Nigel P; Hutchings Geoffrey H; King Donald P; Alexandersen Soren
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PUB	Journal of Virological Methods15 March, 2004
IRN	ISSN 0166-0934
VOL	116
NR	2
PG	169-176
AB	Differential detection of swine vesicular disease virus (SVDV) from the other vesicular disease viruses of foot-and-mouth disease (FMD), vesicular stomatitis (VS) and vesivirus is important as the vesicular lesions produced by these viruses are indistinguishable in pigs. Two independent sets of primers and probe, designed from nucleotide sequences within the 5' untranslated region (UTR) of the SVDV, genome, were evaluated in a real-time (5' nuclease probe-based or fluorogenic) PCR format. Although both primers/probe sets failed to detect one isolate, the assays successfully amplified RNA extracted from epithelial suspensions (ES) and cell culture grown virus preparations from clinical samples representing all currently designated phylogenetic groups of SVDV. Furthermore, no cross-reactivity was demonstrated when these primer/probe sets were tested with RNA prepared from all seven serotypes of FMD virus (FMDV) and from selected isolates of VS virus (VSV), vesivirus and teschoviruses. These assays provide sensitive and rapid alternatives to supplement the routine procedures of ELISA and virus isolation for SVDV diagnosis. The two independent sets of primers/probe can be used routinely while only one of the primers/probe sets would typically be used in SVDV diagnosis during an outbreak.

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AN	PREV200400036184
PD	2003-10-00
TI	Development of a novel quantitative real-time RT-PCR assay for the simultaneous detection of all serotypes of Foot-and-mouth disease virus.
AU	Rasmussen T B; Uttenthal A; de Stricker K; Belak S; Storgaard T
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PUB	Archives of VirologyOctober 2003
IRN	ISSN 0304-8608
VOL	148
NR	10
PG	2005-2021
AB	Foot-and-mouth disease virus (FMDV) spreads extremely fast and the need for rapid and robust diagnostic virus detection systems was obvious during the recent European epidemic. Using a novel real-time RT-PCR system based on primer-probe energy transfer (PriProET) we present here an assay targeting the 3D gene of FMDV. The assay was validated for the efficacy to detect all known FMDV serotypes. The test method was linear over a range of at least 7 orders of magnitude and the detection limit was below the equivalent of 10 genomic copies. Analysing recent African probang samples the method was able to detect FMDV in materials from both cattle and buffalo. When compared to traditional virus cultivation the virus detection sensitivity was similar but the RT-PCR method can provide a laboratory result much faster than virus cultivation. The real-time PCR method confirms the identity of the amplicon by melting point analysis for added specificity and at the same time allows the detection of mutations in the probe region. As such, the described new method is suitable for the robust real-time detection of index cases caused by any serotype of FMDV.

18/21	@ BIOSIS / BIOSIS
AN	PREV200300568407
PD	2003-10-00
TI	Validation of a LightCycler-based reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus.
AU	Moonen Peter; Boonstra Jan; Hakze-van der Honing Renate; Boonstra-Leendertse Christine; Jacobs Liesbeth; Dekker Aldo
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PUB	Journal of Virological MethodsOctober 2003
IRN	ISSN 0166-0934
VOL	113
NR	1
PG	35-41
AB	A specific reverse transcription polymerase chain reaction (RT-PCR) for the detection of the polymerase gene (3D) of foot-and-mouth disease virus (FMDV) was developed and validated with an analytical sensitivity of equal to, to 1000 times higher than that of a single passage virus isolation. The performance of the RT-PCR was determined in 180 runs. After implementation, 5.3% of the tests had to be rejected due to invalid controls (e.g. cross-contamination of negative controls). The diagnostic sensitivity, determined using 124 samples from experimentally infected animals, was 91.9% for RT-PCR and 84.7% for virus isolation. Diagnostic specificity, determined by testing 258 samples from uninfected animals, was 100% by both tests. Of the 627 samples tested by RT-PCR and virus isolation, 85 reacted positively in both tests (13.5%) and 447 negatively in both tests (71.3%). One sample was positive by virus isolation and negative by RT-PCR (0.2%), 94 samples were positive by RT-PCR and negative by virus isolation (15%). The majority (84 of 94) of the 15% RT-PCR positive and virus isolation negative samples were among other samples from farms that reacted positively by both tests. The new RT-PCR is a robust, reliable and sensitive test, provided that adequate measures are taken to prevent cross-contamination. A possible

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preventive measure is to exclude ELISA positive samples from the RT-PCR testing.

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AN	PREV200200420967
PD	2002-06-01
TI	Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus
AU	Callahan Johnny D; Brown Fred; Osorio Fernando A; Sur Jung H; Kramer Ed; Long Gary W; Lubroth Juan; Ellis Stefanie J; Shoulars Katina S; Gaffney Kristin L; Rock Daniel L; Nelson William M
AUAF	Tetracore Inc, 11 Firstfield Rd, Gaithersburg, MD, 20878, USA
PUB	Journal of the American Veterinary Medical AssociationJune 1, 2002
IRN	ISSN 0003-1488
VOL	220
NR	11
PG	1636-1642
AB	Objective: To evaluate a portable real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay designed to detect all 7 viral serotypes of foot-and-mouth disease virus (FMDV). Design: Laboratory and animal studies. Study Population: Viruses grown in tissue culture and animals experimentally infected with FMDV. Procedure: 1 steer, pig, and sheep were infected with serotype O FMDV. Twenty-four hours later, animals were placed in separate rooms that contained 4 FMDV-free, healthy animals of the same species. Oral and nasal swab specimens, oropharyngeal specimens obtained with a probang, and blood samples were obtained at frequent intervals, and animals were observed for fever and clinical signs of foot-and-mouth disease (FMD). Samples from animals and tissue cultures were assayed for infectious virus and viral RNA. Results: The assay detected viral RNA representing all 7 FMDV serotypes grown in tissue culture but did not amplify a panel of selected viruses that included those that cause vesicular diseases similar to FMD; thus, the assay had a specificity of 100%, depending on the panel selected. The assay also met or exceeded sensitivity of viral culture on samples from experimentally infected animals. In many instances, the assay detected viral RNA in the mouth and nose 24 to 96 hours before the onset of clinical disease. Conclusions and Clinical Relevance: The assay reagents are produced in a vitrified form, which permits storage and transportation at ambient temperatures. The test can be performed in 2 hours or less on a portable instrument, thus providing a rapid, portable, sensitive, and specific method for detection of FMDV.

20/21	@ BIOSIS / BIOSIS
AN	PREV200200230868
PD	2001-12-00
TI	Diagnosis of foot-and-mouth disease by RT-PCR: Use of phylogenetic data to evaluate primers for the typing of viral RNA in clinical samples
AU	Reid S M; Ferris N P; Hutchings G H; De Clercq K; Newman B J; Knowles N J; Samuel A R
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PUB	Archives of VirologyDecember, 2001
IRN	ISSN 0304-8608
VOL	146
NR	12
PG	2421-2434
AB	The results of type-specific RT-PCR diagnostic assays on foot-and-mouth disease (FMD) viruses in clinical samples were mapped onto serotype-specific dendrograms representing the degree of nucleotide sequence variation between the FMD virus isolates. This novel approach assisted the selection of suitable PCR primer sets for the diagnosis of FMD virus isolates belonging to different topotypes within each serotype.

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These interpretations were qualified by using a universal (FMD virus group) specific primer to confirm that FMD virus RNA had been extracted from the samples under investigation. The analyses showed that the design of primer sets for the detection of FMD virus serotypes O, A, Asia 1, SAT 1 and SAT 3 were generally satisfactory, as most virus isolates within the major virus sub-groupings were successfully detected. However, the FMD virus serotype C and SAT 2 specific primers were less efficient as certain virus sub-groups were not detected. This identified the need for additional or alternative primers to improve RT-PCR procedures for more comprehensive detection of divergent virus strains within these serotypes. There were some examples where not all virus isolates from the same outbreak reacted with particular type-specific primers which suggested that either further minor refinements may be necessary in the primer design or that there were shortcomings in the RT-PCR methodology.

21/21	@ BIOSIS / BIOSIS
AN	PREV200100570215
PD	2001-12-00
TI	Development of reverse transcription-PCR (oligonucleotide probing) enzyme-linked immunosorbent assays for diagnosis and preliminary typing of foot-and-mouth disease: A new system using simple and aqueous-phase hybridization
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PUB	Journal of Clinical MicrobiologyDecember, 2001
IRN	ISSN 0095-1137
VOL	38
NR	12
PG	4604-4613
AB	A reverse transcription-PCR (RT-PCR)-enzyme-linked immunosorbent assay system that detects a relatively conserved region within the RNA genome of all seven serotypes of foot-and-mouth disease virus (FMDV) has been developed. The high specificity of the assay is achieved by including a rapid hybridization step with a biotin-labeled internal oligonucleotide. The assay is highly sensitive, fast, and easy to perform. A similar assay, based on a highly variable region of the FMDV genome and employing a single asymmetric RT-PCR and multiple hybridization oligonucleotides, was developed to demonstrate the method's ability to type FMDV. Based on our theoretical and practical knowledge of the methodology, we predict that similar assays are applicable to diagnosis and strain differentiation in any system amenable to PCR amplification.

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