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Published:
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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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26 February 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VIRUS DETECTION METHOD, PRIMERS THEREFOR AND SCREENING KIT

(57) Abstract: Discloses a novel detection and typing method for viruses, such as human papillomaviruses, based on real-time PCR using self-probing amplicon fluorescent primers. The method comprises: (IA) contacting the sample with a self-probing amplicon ('virus self-probing amplicon') comprising (i) a virus primer capable of hybridising to at least one target viral nucleic acid sequence and undergoing amplification thereof under primer amplification conditions to form a virus primer extension product; (ii) a virus probe comprising a nucleic acid sequence complementary to a target sequence of the virus primer extension product and capable of hybridisation thereto, provided that the self-probing amplicon is adapted to ensure that the virus probe is unresponsive to amplification under the primer amplification conditions; and (iii) a member of a virus signalling system, which system is capable of causing a detectable signal to be effected on hybridisation of the virus probe sequence to the virus primer extension product, whereby presence or absence of the target viral nucleic acid sequence in the sample is indicated by the detectable signal; (IB) amplifying the product of step (IA) under the primer amplification conditions to an extent enabling the detectable signal to be effected after step (II); and (II) separating the virus primer extension product from the target viral nucleic acid sequence; allowing the virus probe to hybridise to the target sequence of the virus primer extension product; and monitoring the signalling system. This method is quick, simple, specific, sensitive, and capable of estimating viral load per cell. The results of over 100 HPV typing reactions performed on cell lines, biopsies and cervical cytobrush samples are given which, when compared to the current reference HPV detection and typing technique, present a kappa value of 0.89. The method is also applicable to other viruses, such as SV40.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12Q1/70

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	HART KEITH W ET AL: "Novel method for detection, typing, and quantification of human papillomaviruses in clinical samples." JOURNAL OF CLINICAL MICROBIOLOGY, vol. 39, no. 9, September 2001 (2001-09), pages 3204-3212, XP002250694 ISSN: 0095-1137 the whole document -/--	1-14, 17-22, 27-34, 36-42, 44-52

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2003	13.11.03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hermann, P.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WHITCOMBE D ET AL: "Detection of PCR products using self-probing amplicons and fluorescence" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 17, no. 8, August 1999 (1999-08), pages 804-807, XP002226672 ISSN: 1087-0156 the whole document	1-14, 17-22, 27-34, 36-42, 44-52
Y	JORDENS J ZOE ET AL: "Amplification with molecular beacon primers and reverse line blotting for the detection and typing of human papillomaviruses." JOURNAL OF VIROLOGICAL METHODS, vol. 89, no. 1-2, September 2000 (2000-09), pages 29-37, XP002250695 ISSN: 0166-0934 the whole document	1-14, 17-22, 27-34, 36-42, 44-52
Y	THELWELL N ET AL: "Mode of action and application of Scorpion primers to mutation detection" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 28, no. 19, 1 October 2000 (2000-10-01), pages 3752-3761, XP002247004 ISSN: 0305-1048 the whole document	1-14, 17-22, 27-34, 36-42, 44-52
Y	US 5 639 871 A (BAUER HEIDI M ET AL) 17 June 1997 (1997-06-17) the whole document	1-14, 17-22, 27-34, 36-42, 44-52
Y	PARK JONG SUP ET AL: "Physical status and expression of HPV genes in cervical cancers." GYNECOLOGIC ONCOLOGY, vol. 65, no. 1, 1997, pages 121-129, XP002250696 ISSN: 0090-8258 cited in the application the whole document	1-14, 17-22, 27-34, 36-42, 44-52
Y	WO 93 23566 A (ISIS INNOVATION ;STICKLAND JULIA ELIZABETH (GB); RAMSHAW ANNA LOUI) 25 November 1993 (1993-11-25) the whole document	1-14, 17-22, 27-34, 36-42, 44-52

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INTERNATIONAL SEARCH REPORT

Internatic .pplication No
PCT/GB 02/02847

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with Indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 02215 A (ROYAL FREE HOSP SCHOOL MED) 4 February 1993 (1993-02-04) the whole document	1-14, 17-22, 27-34, 36-42, 44-52
A	WO 95 22626 A (STICHTING RES FONDS PATHOLOGIE ;MEIJER CHRISTOPHORUS JOANNES L (NL) 24 August 1995 (1995-08-24) the whole document	1-14, 17-22, 27-34, 36-42, 44-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/02847

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 35 and 53-55
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
see PCT/ISA/210 annex

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 35 and 53-55

The present remarks apply to the first invention, however, should further search fees be paid, similar remarks could apply to the other inventions for which the search would have to be done.

The sequence of GP5+ reverse primer is absent from the entire application therefore rendering the subject-matter of present claim 35 unclear (Article 6 PCT) to such an extend that no meaningful search for said claim could be performed.

Claim 53 lacks clarity because it relates to an oligonucleotide only defined in terms of functional features which, in the present context, are not sufficient for the skilled person to understand the actual scope of the claim. The "self-probing amplicon" is defined in claim 1 and in the description (cf p. 19 line 13-p. 27 line 1) as a VIRUS self-probing amplicon and comprises a virus-specific primer portion, a virus specific probe portion and a member of a virus signalling pathway. However, claim 53 provides neither a reference to any previous claims nor a definition of the self-probing amplicon. Moreover, the only features to which claim 53 relates are those of a tailed primer, which is consistently defined in the description as a separate entity from the virus self probing amplicon (cf p. 19 line 13-p. 27 line 1). It is thus not clear which are the features of the self probing amplicon to which this claim relates.

Moreover, in the present context, the term "degenerate" is devoid of clear technical meaning.

Therefore claim 53 lacks clarity to such an extent that no meaningful search could be performed (Article 6 PCT).

The references in claims 54 and 55 to previous claims 42 and 42 or 43 respectively are erroneous since claims 54 and 55 are product claims whereas claims 42 and 43 are method claims. Therefore said references lead to a lack of clarity of claims 54 and 55 (Article 6 PCT). Furthermore and according to the additional features set out in claims 54 and 55 read in connection to the description (cf p. 19 line 13-p. 27 line 1), it appears that the subject-matters of said claims relate to tailed primers, however none of the preceding claims are relating to tailed primers. Therefore claims 54 and 55 lack clarity to such an extent that no meaningful search with respect to their respective subject-matters could be performed.

The applicant's attention is drawn to the fact that claims relating to

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14,17-22, 27-42, 44-55 (all in part)

Invention 1:

The first invention relates to i) the detection, typing, determining viral load per cell and/or determining the integration state of specific papilloma virus type 6 in a sample suspected of comprising one or more target HPV nucleic acid sequence(s); ii) a screening method for screening an individual suspected of HPV infection with HPV type 6 by using said method; iii) a diagnostic kit for use in said method; iv) and self probing amplicon or degenerated self probing amplicon specific for HPV type 6.

Inventions 2-7:

As for invention 1 relating respectively to HPV type 11, 31, 33, 39, 51 and 56.

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2. claims: 1-14, 17-22, 23-42, 44-55 (all in part)

Invention 8:

The second invention relates to i) the detection, typing, determining viral load per cell and/or determining the integration state of specific papilloma virus type 16 in a sample suspected of comprising one or more target HPV nucleic acid sequence(s); ii) a screening method for screening an individual suspected of HPV infection with HPV type 16 by using said method; iii) a diagnostic kit for use in said method; iv) and self probing amplicon or degenerated self probing amplicon specific for HPV type 16.

Inventions 9:

As for invention 8 relating to HPV type 18.

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3. claims: 1-11, 17-22, 27-42, 44-55 (all in part)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 10:

The tenth invention relates to i) the detection, typing, determining viral load per cell and/or determining the integration state of specific papilloma virus type 40 in a sample suspected of comprising one or more target HPV nucleic acid sequence(s); ii) a screening method for screening an individual suspected of HPV infection with HPV type 40 by using said method; iii) a diagnostic kit for use in said method; iv) and self probing amplicon or degenerated self probing amplicon specific for HPV type 40.

Inventions 11-19:

As for invention 10 relating respectively to HPV types 42, 43, 44, 45, 52, 58, 59, 66 and 68.

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4. claims: 1-11, 15-16, 29, 33-34, 36-41, 43-49, 52-53 (all in part)

Invention 20:

The twentieth invention relates to i) the detection, typing, determining viral load per cell and/or determining the integration state of SV-40 in a sample suspected of comprising one or more target SV-40 nucleic acid sequence(s); ii) a screening method for screening an individual suspected of virus infection with SV-40 by using said method; iii) a diagnostic kit for use in said method; iv) and self probing amplicon or degenerated self probing amplicon specific for SV-40.

Inventions 21 and 22:

As for invention 20, relating respectively to JC and BK viruses.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatic pplication No
PCT/GB 02/02847

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		WO 9522626 A1	24-08-1995
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