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31 March 2005

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: STANDARDIZED AND OPTIMIZED REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION METHOD FOR DETECTION OF MRD IN LEUKEMIA

(57) Abstract: The invention relates to in a real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) method for minimal residual disease detection in leukemic patients through amplification of a fusion gene transcript, comprising: (i) selecting amplifiable and qualified patient samples for subsequent analysis; (ii) defining optimal conditions for performing the RT reaction; (iii) defining optimal conditions for RQ-PCR protocol; and (iv) establishing a standardized procedure for data analysis.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/004008

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

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, Sequence Search, WPI Data, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MENSINK E ET AL: "Quantitation of minimal residual disease in Philadelphia chromosome positive chronic myeloid leukaemia patients using real-time quantitative RT-PCR" BRITISH JOURNAL OF HAEMATOLOGY, OXFORD, GB, vol. 102, no. 3, August 1998 (1998-08), pages 768-774, XP002227390 ISSN: 0007-1048 the whole document ----- -/--	1,2, 15-17

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

[ ] 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
20 September 2004	05. 01. 2005
Name and mailing address of the ISA 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	Authorized officer Bradbrook, D

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMIG M ET AL: "Accurate and rapid analysis of residual disease in patients with CML using specific fluorescent hybridization probes for real time quantitative RT-PCR" LEUKEMIA, MACMILLAN PRESS LTD, US, vol. 13, no. 11, November 1999 (1999-11), pages 1825-1832, XP002249335 ISSN: 0887-6924 abstract page 1826, column 1, paragraph 5 - page 1828, column 1, paragraph 4	1-5, 15-17
X	AMABILE MARILINA ET AL: "Real-time quantification of different types of bcr-abl transcript in chronic myeloid leukemia." HAEMATOLOGICA, vol. 86, no. 3, March 2001 (2001-03), pages 252-259, XP002249336 ISSN: 0390-6078 abstract Design and Methods	1-5, 15-17
X	BOLUFER PASCUAL ET AL: "Rapid quantitative detection of BCR-ABL transcripts in chronic myeloid leukemia patients by real-time reverse transcriptase polymerase-chain reaction using fluorescently labeled probes." HAEMATOLOGICA, vol. 85, no. 12, December 2000 (2000-12), pages 1248-1254, XP002249337 ISSN: 0390-6078 the whole document	1-5, 15-17
A	DONGEN VAN J J M ET AL: "STANDARDIZED RT-PCR ANALYSIS OF FUSION GENE TRANSCRIPTS FROM CHROMOSOME ABERRATIONS IN ACUTE LEUKEMIA FOR DETECTION OF MINIMAL RESIDUAL DISEASE.REPORT OF THE BIOMED-1 CONCERTED ACTION: INVESTIGATION OF MINIMAL RESIDUAL DISEASE IN ACUTE LEUKEMIA" LEUKEMIA, MACMILLAN PRESS LTD, US, vol. 13, no. 12, December 1999 (1999-12), pages 1901-1928, XP000878700 ISSN: 0887-6924 abstract Conclusion	1-5, 15-17

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/004008

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HEID C A ET AL: "REAL TIME QUANTITATIVE PCR" GENOME RESEARCH, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 6, no. 10, 1 October 1996 (1996-10-01), pages 986-994, XP000642795 ISSN: 1088-9051 the whole document	1,17
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 16 November 2000 (2000-11-16), MITTERBAUER ADELHEID ET AL: "Quantification of minimal residual disease in patients with acute promyelocytic leukemia (APL) by real time quantitative RT-PCR with specific fluorescent hybridization probes." XP002249338 Database accession no. PREV200100312442 abstract & BLOOD, vol. 96, no. 11 Part 1, 16 November 2000 (2000-11-16), page 313a, 42nd Annual Meeting of the American Society of Hematology;San Francisco, California, USA; December 01-05, 2000 ISSN: 0006-4971	1-5, 15-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/004008

Box II Observations where certain claims were found unsearchable (Continuation of item 2 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.:
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:

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 III Observations where unity of invention is lacking (Continuation of item 3 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.:

1-4, 15-17 (in part), 5 (in full)

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

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

1. claims: 1-4,15-17 (in part) 5 (in full)

   Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the control gene is ABL, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 1-3.

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2. claims: 1-4,15-17 (in part)

   Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the control gene is B2M, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 4-6.

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3. claims: 1-4,15-17 (in part)

   Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the control gene is GUS, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 7-9.

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4. claims: 1,2,15-17 (in part), 6 (in full)

   Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is E2A-PBX1, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 10-12.

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5. claims: 1,2,15-17 (in part), 7 (in full)

   Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is MLL-AF4, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs.13-16.

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6. claims: 1,2,15-17 (in part), 8 (in full)

   Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is TEL-AML1, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 17-19.

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7. claims: 1,2,15-17 (in part), 9,10 (in full)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is BCR-ABL, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 20-22 or 23, 21 and 22 respectively.

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8. claims: 1,2,15-17 (in part), 11 (in full)

Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is SIL-TAL1, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 24-26.

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9. claims: 1,2,15-17 (in part), 12 (in full)

Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is PML-RARA, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 27-31.

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10. claims: 1,2,15-17 (in part), 13 (in full)

Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is CBFB-MYH11, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 32-36.

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11. claims: 1,2,15-17 (in part), 14 (in full)

Method for standardizing a real-time reverse transcriptase polymerase chain reaction (RQ-PCR) wherein the fusion gene is AML-ETO, and forward primer, probe and reverse primer sequences correspond to SEQ ID NOs 37-39.
