Internal distribution code:

(A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 28 August 2014

Case Number: T 2111/09 - 3.3.08

Application Number: 98955810.1

Publication Number: 1044283

IPC: C12Q1/68

Language of the proceedings: EN

Title of invention:
Fluorometric method for monitoring and detecting nucleic acid amplification

Patent Proprietor:
The Secretary of State for Defence

Opponent:
Becton, Dickinson and Company

Headword:
Fluorometric method/THE SECRETARY OF STATE FOR DEFENCE

Relevant legal provisions:
EPC Art. 54(3), 54(4), 56
RPBA Art. 12(4), 13(1), 13(3)

Keyword:
Admission of new ground for opposition - (no)
Admission of new evidence - (yes)
Admission of new main request - (yes)
Novelty - (yes)
Inventive step - (yes)

Decisions cited:
T 0305/87

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern
Boards of Appeal
Chambres de recours

European Patent Office
D-80298 MUNICH
GERMANY
Tel. +49 (0) 89 2399-0
Fax +49 (0) 89 2399-4465

Case Number: T 2111/09 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 28 August 2014

Appellant: (Opponent)	Becton, Dickinson and Company One Becton Drive Franklin Lakes, New Jersey 07417-1880 (US)
Representative:	Dörries, Hans Ulrich df-mp Dörries Frank-Molnia & Pohlman Patentanwälte Rechtsanwälte PartG mbB Theatinerstrasse 16 80333 München (DE)
Respondent: (Patent Proprietor)	THE SECRETARY OF STATE FOR DEFENCE DSTL Porton Down Salisbury, Wiltshire SP4 0JQ (GB)
Representative:	Haseley, Simon Defence Intelectual Property Rights Military of Defence Formalities Section, Poplar 2 #2214 Abbey Wood South Bristol BS34 8JH (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 18 August 2009 rejecting the opposition filed against European patent No. 1044283 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	M. Wieser
Members:	M. R. Vega Laso
	C. Heath

Summary of Facts and Submissions

I. European patent No. 1 044 283 with the title "Fluorometric method for monitoring and detecting nucleic acid amplification" was granted on European patent application No. 98955810.1, which was filed as international application PCT/GB98/03563 on 27 November 1998 claiming the priority of an earlier application filed in the United Kingdom on 29 November 1997. The application was published as WO 99/28501.

II. The patent was granted with 29 claims. Independent claims 1 and 28 read as follows:

"1. A method for detecting nucleic acid amplification in a sample, comprising performing nucleic acid amplification on a target polynucleotide in the presence of (a) a nucleic acid polymerase, (b) a primer capable of hybridising to said target polynucleotide, and (c) an oligonucleotide probe which is capable of hybridising to said target oligonucleotide and which comprises a fluorescence donor and a fluorescence acceptor molecule spaced along the length at a distance at which fluorescence from the donor is reduced by the acceptor, and a single stranded sequence recognised by a restriction enzyme which cuts at said sequence when in double stranded form, said sequence being located intermediate said donor and said acceptor molecule; applying to the amplification product said restriction enzyme such that double stranded amplification product is cleaved so as to liberate acceptor molecule from donor molecule, and detecting a fluorescent signal from the sample, wherein said oligonucleotide probe is capable of hybridising to said target polynucleotide in the region of the 3' end of said target polynucleotide such that the 3' end of the polynucleotide can extend

during amplification to form a complementary region of the probe.

28. A kit for carrying out a method according to any one of claims 1 to 18, said kit comprising an oligonucleotide probe which is capable of hybridising to a target polynucleotide and which comprises a fluorescence donor molecule a fluorescence acceptor molecule and a single stranded sequence recognised by a restriction enzyme which cuts at said sequence when in double stranded form, said sequence being located intermediate said donor and said acceptor molecule, a restriction enzyme which is able to cut said probe intermediate the acceptor and donor molecules and a modified nucleotide which is able to generate a nucleotide chain which is resistant to restriction enzymes."

Dependent claims 2 to 18 specified variants of the method according to claim 1. Claims 19 and 20 related to a method of assessing the activity of a restriction enzyme, and claims 21 to 27 to the use of a defined oligonucleotide probe in a method according to any of the preceding claims. Dependent claim 29 was directed to a particular embodiment of the kit of claim 28.

III. An opposition to the grant of the patent was filed. The opposition was based on the grounds for opposition under Article 100(a) EPC in connection with Articles 54(3)(4) and 56 EPC 1973, in particular on the grounds that the subject-matter of claims 1 to 18 and claims 21 to 28 - as far as they referred to claims 1 to 18 - of the patent as granted lacked novelty, and that the subject-matter of claim 19 and claims 20 to 27 - as far as they depended on or referred to claim 19 - did not involve an inventive step.

IV. In a decision under Article 101(2) EPC posted on 18 August 2009, an opposition division of the European Patent Office rejected the opposition because it found that the subject-matter of claims 1 and 28 was novel over document (1) (see section XVII below), and that claims 19 and 20 involved an inventive step within the meaning of Article 56 EPC.

V. The opponent (appellant) lodged an appeal against the decision of the opposition division. In its statement of grounds of appeal, the appellant contested the findings of the opposition division on novelty and inventive step. In support of the objection of lack of novelty, the appellant filed additional evidence (documents (7) and (8); see section XVII below).

VI. The respondent (patent proprietor) replied to the statement of grounds of appeal.

VII. Both parties requested oral proceedings as a subsidiary request.

VIII. On 19 October 2011, the appellant submitted further arguments and evidence (document (9); see section XVII below).

IX. The respondent replied to the appellant's arguments by filing a set of amended claims as its "first auxiliary request".

X. By letter of 19 May 2014, the respondent requested acceleration of the proceedings on the grounds that it had become aware of an apparent infringement of the patent, and that it was considering initiating infringement proceedings.

XI. The parties were summoned to oral proceedings. In a communication under Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) attached to the summons, the board expressed a provisional opinion on the issues of novelty and inventive step, and made some observations with regard to the new evidence filed in appeal proceedings.

XII. By letter dated 16 June 2014, the appellant opposed to the acceleration of the proceedings.

XIII. By a further letter in reply to the board's communication, the appellant put forward further arguments on the issues of novelty and inventive step.

XIV. The respondent submitted some arguments with respect to the claims of the "first auxiliary request".

XV. During the oral proceedings, which were held on 28 August 2014, the respondent filed a set of amended claims and an amended page 4 of the patent specification as its new main request.

XVI. The set of claims according to the **new main request** differs from the claims of the patent as granted in that claims 19, 20 and 22 have been deleted, and claims 21 and 23 to 29 have been renumbered as claims 19 to 26 and their dependencies amended accordingly.

XVII. The following documents are referred to in the present decision:

(1): EP 0 878 554 A2, published on 18 November 1998;

(7): EP 0 684 315 A1, published on 29 November 1995;

(8): Diagnostic Molecular Microbiology, 1994, ed. D. H. Persing et al., pages 65 to 67;

(9): Interlocutory decision dated 2 November 2010 issued in the opposition proceedings against EP 0 878 554.

XVIII. The submissions made by the appellant concerning issues relevant to this decision, were essentially as follows:

New ground for opposition under Article 100(b) EPC

Claim 1 was not supported over the whole of its breath and did not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

Admission of new evidence into the proceedings

Documents (7) and (8) were filed in order to better illustrate the disclosure content the skilled person would derive from document (1), which had been misunderstood by the opposition division. Prior to the oral proceedings before the opposition division, a need for additional evidence concerning the technical content of document (1) had not been apparent, because the opposition division's provisional opinion on the issue of novelty over document (1) which had been expressed in the summons to oral proceedings coincided with the opponent's own position. Since it had been for the first time at the oral proceedings that the opposition division adopted a different view as regards the content of document (1), which resulted in a positive decision on the issue of novelty, the opponent

could not have filed additional evidence earlier in the proceedings.

New main request - Admission into the proceedings

The set of claims of the new main request should not be admitted into the proceedings. The claims were filed at a late stage of the proceedings and, contrary to Rule 80 EPC, the deletion of claim 22 was not occasioned by a ground for opposition.

Article 54(3)(4) EPC 1973 - Novelty over document (1)

Claim 1

The opposition division had erred in its conclusions on novelty because it had not understood the disclosure of the second embodiment in document (1) as a person skilled in the art would have done it. The relevant paragraphs in document (1) were those on page 3, lines 48 to 53; page 4, lines 13 to 17; page 4, line 51 to page 5, line 2; page 6, lines 37 to 51; and page 9, lines 27 to 51.

In the passages on pages 3, 4 and 6, two alternative embodiments were described, one of which was characterized as being "*employed in an amplification reaction*" and the other as an "*embodiment for non-amplification based detection of target sequences*". However, it was clear from the document as a whole that the two embodiments were not mutually exclusive, and that the features of the "non-amplification" embodiment could be used in the "amplification" embodiment. The skilled person would have understood that the first embodiment **required** amplification in order for it to function, whereas the second embodiment did **not require**

amplification, but could equally function in an amplification reaction. This was made clear in the passage on page 6, lines 48 and 49.

It was apparent from the passage stretching from page 9, line 27 to page 10, line 10 that document (1) describes the use of both embodiments in PCR and other amplification reactions, in particular SDA, 3SR, NASBA and TMA. There was no suggestion in the whole document that the statements in this passage applied only to the first embodiment and not to the second – they covered both "*methods of the invention*". Moreover, there was no indication in the application that the use of the signal primers described in the passage from page 4, last line to page 5, line 2 in an embodiment involving amplification was prohibited.

The oligonucleotide probe specified in claim 1 was not distinguished over the labelled amplification primer described in the passage on page 9, lines 54 and 55 of document (1). Since the methods described in this document used labelled amplification primers in connection with nucleic acid amplification, in particular SDA, which as shown in document (7) required two primers on either strand, the subject-matter of claim 1 lacked novelty.

Claim 25

Claim 25 defined a kit comprising an oligonucleotide probe, a restriction enzyme and a modified nucleotide. All these components could be directly derived from the examples in document (1): there was a signal primer equivalent to the "oligonucleotide probe" specified in claim 25, a restriction enzyme (*AvaI* in Example 1 and *BsoBI* in Example 2) and "a modified nucleotide"

(designated as dCTPαS in Example 1 and α-thio dCTP in Example 2).

Claim 25 did not specify that the reagents must be in a single container. Thus, the order in which the reagents were combined in Example 2 was irrelevant. In contrast to the opposition division's understanding, the reaction mixture in either Example 1 or 2 of document (1) did not contain all the components required for reaction, because the polymerase and the restriction enzyme were not added at first and were kept in separate compartments. Nor was the target, which the opposition division wrongly considered to be present in all reaction mixtures described in document (1). However, the control reactions did not contain any target.

Contrary to the opposition division's view, it was not apparent why the kit described in document (1) could not be shipped and stored.

XIX. The submissions made by the respondent, as far as they relate to the decisive issues, may be summarized as follows:

Admission of new evidence into the proceedings

Document (7) should not be admitted into the appeal proceedings because it could have been filed already in opposition proceedings.

New main request - Admission into the proceedings

The amendments introduced into the claims of the new main request were straightforward, did not increase the complexity of the case and met the objection of lack of

inventive step. Therefore, the set of claims should be admitted into the proceedings.

Article 54(3)(4) EPC 1973 – Novelty over document (1)

Claim 1

The invention described in document (1) employed hybridization and extension of a signal primer for detection of a nucleic acid target sequence by fluorescence quenching mechanisms (see page 3, lines 37 and 38). In one embodiment, the signal primer was employed in an amplification reaction for detection of target sequence amplification. In an alternative embodiment, the signal primer was used for non-amplification based detection of target sequences. While the signal primer described in the passage on page 4, lines 51 to 55 was used in both embodiments, the way in which the signal primer functions was different depending on which embodiment was being considered. In the amplification embodiment, "*the signal primer hybridises to the target sequence downstream of an amplification primer*" (see page 5, lines 15 and 16). In the alternative non-amplification embodiment "*the target binding sequence of the signal primer hybridizes to the 3' end of the target oligonucleotide such that the RERS form a 5' overhang*" (see page 6, lines 38 to 40), such that it is cut by a restriction enzyme when the target is extended. Clearly, two separate embodiments were described in document (1), but there was no specific suggestion to combine separate items from each of the embodiments. Thus, document (1) did not anticipate the subject-matter of claim 1.

Claim 28

Neither Example 1 nor Example 2 in document (1) described a kit as claimed in claim 25. The kit of claim 25 had to be suitable for carrying out the methods of claims 1 to 18, which required nucleic acid amplification. The experiment described in Example 2 was conducted, however, "*in the absence of target amplification*" (see page 11, lines 4 and 5). Similarly, claims 1 to 18 required that the probe hybridises to the target polynucleotide in the region of the 3' end so as to form an overhang. Example 1 described a "*SDA performed generally as described in EP 0 684 315*", which corresponded to document (7). This document did not describe a signal primer which hybridises in the region of the 3' end so as to form an overhang.

XX. The appellant (opponent) requested that the decision under appeal be set aside and the European patent be revoked.

XXI. The respondent (patent proprietor) requested that the decision under appeal be set aside and the patent be maintained based on the set of claims of the new main request and the adapted description.

Reasons for the Decision

New ground for opposition under Article 100(b) EPC

1. Pursuant to decision G 1/95 of the Enlarged Board of Appeal (OJ EPO 1996, 615), a fresh ground for opposition, i.e. a ground for opposition which was neither raised and substantiated in the notice of opposition, nor introduced into the proceedings by the

opposition division, can only be introduced into the appeal proceedings with the agreement of the patent proprietor.

2. In the present case, the respondent (patent proprietor) did not agree to the introduction of the fresh ground for opposition under Article 100(b) EPC raised by the appellant in its statement of grounds of appeal. Consequently, the appellant's objection that the invention claimed in claim 1 is not sufficiently disclosed, cannot be considered by the board.

Admission of new evidence into the proceedings (Article 12(4) EPC)

3. Documents (7) and (8), which were filed by the appellant together with its statement of grounds of appeal, relate to nucleic acid amplification techniques, in particular strand displacement amplification (SDA) and self-sustaining sequence replication (3SR), respectively. The respondent opposed to the introduction of document (7) into the appeal proceedings, but it did not dispute that the technical content of document (8) formed part of the common general knowledge of a person skilled in the art at the priority date.

4. Having regard to Article 12(4) of the Rules of Procedure of the Boards of Appeal (RPBA) which empowers the board to hold inadmissible facts, evidence or requests which could have been presented in the proceedings before the opposition division of the European Patent Office, the issue to be decided is whether or not document (7) could have been filed during the opposition proceedings.

5. Even though it is true that document (7) could, in principle, have been filed in opposition proceedings because the document was available at the time, the board judges that, under the circumstances of the present case, it could not have been apparent to the opponent (the present appellant) that additional evidence was required. As the opposition division's opinion on the content of document (1) changed during the oral proceedings, the opponent, caught by surprise, was not able to react immediately by filing additional evidence. Its first opportunity to submit the evidence was the statement of grounds of appeal.

6. Since document (7) was submitted as early as possible in response to the opposition division's changed position, the document must be considered to have been submitted in due time. Hence, document (7) was admitted into the proceedings. As the respondent did not oppose to the admission of document (8), also this document was admitted into the proceedings.

7. Document (9) was filed as evidence for a view expressed by an opposition division in different opposition proceedings concerning the content of document (1). Since the purpose of the present appeal proceedings is to give a judicial decision upon the correctness of the opposition division's decision in the present case, and the board has both the legal and technical competence to assess the content of document (1) on its own, this document was not admitted into the proceedings.

New main request - Admission into the proceedings

8. The appellant objected to the admission into the proceedings of the set of claims according to the new

main request (see section XVI above) filed during the oral proceedings before the board.

9. Pursuant to Article 13(1) RPBA, any amendments to a party's case after it has filed its grounds of appeal or reply may be admitted and considered at the board's discretion. When exercising its discretion, the board must take into account, *inter alia*, the complexity of the new subject-matter submitted, the current state of the proceedings and the need for procedural economy.

10. The set of amended claims according to the new main request was filed at a very late stage of the appeal proceedings. In principle, the amended claims could have been presented together with the respondent's reply to the statement of grounds of appeal.

11. However, the board, exercising the discretion conferred by Article 13(1) RPBA and taking into account that the complexity of the claimed subject-matter has not been increased, but rather reduced, and that the amendments introduced into the claims meet the objections under Article 56 EPC raised by the appellant, decided to admit and consider the new main request. It should be noted that, as required by Article 13(3) RPBA, the amendments did not give rise to any issues which the board or the other party could not reasonably be expected to deal with without adjournment of the oral proceedings.

Article 54(3)(4) EPC 1973 – Novelty over document (1)

Claim 1

12. In the decision under appeal, the opposition division found that the content of document (1) does not

anticipate the method according to claim 1. In the view of the opposition division, document (1) discloses two alternative embodiments, a first embodiment in which a signal primer is employed in an amplification reaction for detection of target sequence amplification, and an alternative embodiment for non-amplification based detection of target sequence. The opposition division held that it was not permissible to combine features of these two embodiments, especially in view of the fact that they were described as alternative embodiments (see section 4.3 of the decision under appeal).

13. In appeal proceedings, the appellant contested these findings relying on different passages of document (1), particularly on the passage on page 9, lines 54 and 55. The arguments put forward by the appellant fail, however, to convince the board that the content of document (1) destroys the novelty of the subject-matter of independent claim 1.

14. Claim 1 is directed to a method for detecting nucleic acid amplification of a target polynucleotide comprising, as a first step, the amplification of the target sequence in the presence of a nucleic acid polymerase, a primer and an oligonucleotide probe.

15. Structurally, the oligonucleotide probe is characterized by comprising a single stranded sequence located between a fluorescence donor and a fluorescence acceptor molecule. These two molecules are spaced along the length of the probe at a distance at which the fluorescence from the donor is reduced by the acceptor. The single stranded sequence is characterised as being recognised by a restriction enzyme which cuts at said sequence when in double stranded form.

16. According to claim 1, the oligonucleotide probe is capable of hybridising to the target polynucleotide in the region of the 3' end of the polynucleotide, such that the 3' end of the polynucleotide can extend during amplification to form a complementary region of the probe. Since the restriction site is located precisely in this region, when the restriction enzyme is applied to the double stranded amplification product, the amplification product is cleaved, and the acceptor molecule is liberated from the donor molecule. Since the fluorescence from the donor is no longer quenched, the fluorescence signal from the sample can be detected and serves to monitor the amplification of the target sequence. This is illustrated in Figures 1D to 1F of the patent as granted:

Fig.1D.

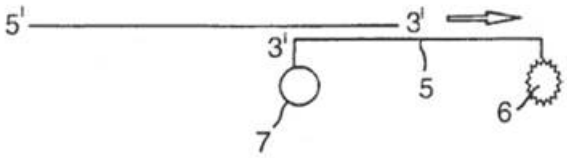


Fig.1E.

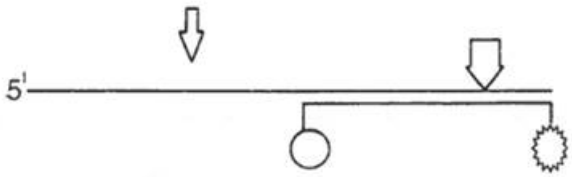
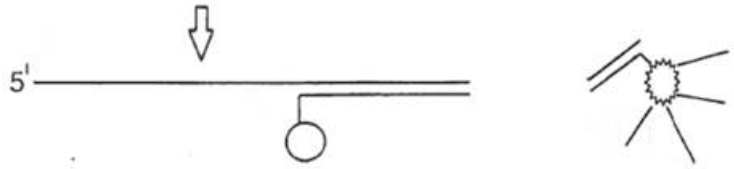


Fig.1F.



17. It is undisputed that document (1) describes a method in which a signal primer is used in hybridization and extension reactions to produce double-stranded products

which contain a donor/acceptor fluorescence dye pair. The signal primer includes a single-stranded restriction endonuclease cleavage site that, when converted to the double-stranded form, renders the cleavage site cleavable or nickable by the restriction endonuclease, the cleavage resulting in decreased quenching of the donor fluorescence and an increase in donor fluorescence intensity (see page 4, lines 8 to 17).

18. Document (1) describes two embodiments of the method for detecting nucleic acid target sequences employing fluorescence quenching, a first embodiment in which "*... the signal primer of the invention is employed in an amplification reaction for detection of target sequence amplification*", and "*... an alternative embodiment* ***for non-amplification based detection of target sequences,*** [in which] *the signal primer is hybridized at the 3' end of the target oligonucleotide such that the restriction endonuclease recognition site forms a 5' overhang. Extension of the target sequence on the signal primer using polymerase produces a fully double-stranded restriction site which is cleaved or nicked to separate the dyes. This results in a change in fluorescence which indicates* ***the presence*** *of the target sequence.*" (see page 3, lines 48 to 53; emphasis added by the board).

19. In document (1), there is no explicit mention of a 5' overhang in the context of an amplification embodiment. Nor is a combination of features belonging to the two embodiments described in document (1) suggested in this document. Rather, the two embodiments are clearly described as **alternative** embodiments. In line with decision T 305/87 (OJ EPO 1991, 429; see point 5.3 of the Reasons), the board is of the view

that, in the framework of determining the content of a prior art document for the assessment of novelty, it is not permissible to draw features pertaining to different embodiments in order to create artificially a particular embodiment which destroys the novelty of the claimed subject-matter, unless the document itself suggests that specific combination of features. On this account, the board cannot accept appellant's argument that a combination of the two embodiments described in document (1) is not only not precluded, but would be clearly obvious to a person skilled in the art. In the board's view, if such an argument were accepted, the boundary between the concepts of novelty and inventive step would become blurred.

20. As regards the passage on page 9, lines 54 and 55 of document (1) ("*However, it will be apparent that the amplification primers known for use in the various nucleic acid amplification reactions may also be labeled and modified as described for signal primers*"), which according to the appellant allegedly suggests to use a signal primer with a 5' overhang in the amplification embodiment, the board considers that this passage refers to labels and modifications of oligonucleotide primers in general, rather than to the specific **design** of a primer that hybridizes at the 3' end of the target such that the sequence having a restriction endonuclease recognition site forms a 5' overhang. Thus, this passage cannot be interpreted as a suggestion to combine the two alternative embodiments described in document (1). Nor does any of the further passages cited by the appellant provide such a suggestion.

21. For these reasons, the board is convinced that document (1) does not describe a method with the

features specified in claim 1. Thus, the objection of lack of novelty fails.

Claim 25

22. The findings of the opposition division on the novelty of a kit according to claim 28 have been contested by the appellant. Like the opposition division, the board cannot accept the appellant's argument that Example 2 in document (1) describes a kit. A kit can be defined as a set of separate reagents required for carrying out a method, which is packed and sold as a unit. No such set of reagents is described in Example 2. As regards Example 1, neither document (1) nor document (7), which is cited therein, describe a signal primer which hybridises in the region of the 3' end so as to form a 5' overhang. Thus, the subject-matter of claim 25 is not anticipated by document (1).

Article 56 EPC

23. The appellant has not raised an objection of lack of inventive step with regard to any of the claims of the new main request, and the board sees no reason to raise any of its own.

Conclusion

24. Since the claims according to the new main request and the invention to which they relate fulfil the requirements of the EPC, the patent can be maintained in amended form.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent based on the following documents:

   - claims 1 to 26 of the new main request filed during oral proceedings;
   - description pages 2, 3, 5 - 8 of the patent as granted;
   - description page 4 filed during oral proceedings;
   - figures of the patent as granted.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated