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Datasheet for the decision of 28 August 2007

Case Number: T 0099/04 - 3.3.04

Application Number: 90913621.0

Publication Number: 0497784

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Quantitation of nucleic acids using the polymerase chain reaction

Patentee:
F. HOFFMANN-LA ROCHE AG

Opponent:
Becton Dickinson and Company

Headword:
Quantitation/HOFFMANN-LA ROCHE

Relevant legal provisions (EPC 1973):
EPC Art. 54, 56, 83, 123(2), 123(3)
EPC R. 29(2), 57a, 61a

Keyword:
"Prohibition of reformatio in peius and extent of examination"
"Amendments prohibited by Rule 57a or Rule 29(2) EPC (no)"
"Added matter (no)"
"Sufficiency of disclosure, novelty, inventive step (yes)"

Decisions cited:
G 0009/92, G 0004/93, G 0001/99, T 0223/97, T 0937/00, T 0149/02, T 0181/02, T 0991/02, T 0498/03, T 0168/04

Catchword:
See points 3 to 15

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Case Number: T 0099/04 **-** 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 28 August 2007

Appellant: (Patent Proprietor)
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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 6 November 2003 concerning maintenance of European patent No. 0497784 in amended form.

Composition of the Board:

Chairman: R. Moufang
Members: B. Claes
R. Gramaglia

Summary of Facts and Submissions

I. European patent no. 0 497 784 was granted with 39 claims on the basis of European patent application 90913621.0 (published as WO 91/02817, referred to in this decision as "the application as filed") and was opposed on the grounds of Article 100(a) EPC, for lack of novelty and inventive step (Articles 54 and 56 EPC), Article 100(b) EPC and Article 100(c) EPC.

II. Independent claims 1, 12 and 30 as granted read:

"1. Use of an internal standard for the quantitation of at least one target nucleic acid segment contained within a sample in an amplification method, said internal standard comprising on one strand a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment, wherein said internal standard nucleic acid segment and said target nucleic acid segment are co-amplified using the same set of primers and wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid segment can be distinguished."

"12. A kit for the quantitation of a target nucleic acid segment in a biological sample comprising individual containers which provide:

(a) a predetermined initial amount of an internal standard comprising a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid segment can be distinguished;
(b) at least one oligonucleotide primer pair for the co-amplification of said internal standard and said target nucleic acid segment."

"30. A method for quantifying a target nucleic acid segment in a sample, which method comprises the steps of:

(a) adding to said sample a predetermined initial amount of an internal standard as characterized in any one of claims 1 to 11, wherein said internal standard comprises a nucleic acid segment that binds to the same primers as are bound by said target nucleic acid segment;
(b) treating said sample under conditions suitable for carrying out a polymerase chain reaction, wherein said nucleic acids are rendered single-stranded and exposed to an agent for polymerization, deoxynucleotide 5' triphosphates, and a pair of oligonucleotide primers, wherein said primer pair can hybridize to both the target and standard nucleic acid segments, such that each primer can serve to initiate synthesis of an extension product on a DNA strand of each of the target

and standard nucleic acid segments, such that the extension production of one primer, when it is separated from the template strand, serves as a template for the synthesis of the extension production of the other primer of said pair wherein said amplified target and standard nucleic acid segments are distinguishable;
(c) separating the primer extension products from the template on which they were synthesized to provide single-stranded molecules;
(d) repeating steps (b) and (c) on the single stranded molecules produced in step (c) at least once, whereby each repeat of steps (b) and (c) is one amplification cycle;
(e) measuring the amounts of the amplified target and standard segments produced in step (d); and
(f) calculating from the amplified target and standard segments produced in step (d) the amount of said target nucleic acid segment present in the sample before amplification."

III. The opposition division decided that the patent as amended on the basis of a third auxiliary request, consisting of 70 claims and filed by the proprietor during the oral proceedings on 21 May 2003, fulfilled the requirements of the EPC.

Independent claims 1, 11 and 26 of this third auxiliary request read:

"1. Use of an internal standard for the quantitation of **one** target nucleic acid segment contained within a sample in **a polymerase chain reaction** amplification method, said internal standard comprising on one strand

a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment, wherein said internal standard nucleic acid segment and said target nucleic acid segment are co-amplified using the same set of primers and wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid segment **are distinguished by segment specific probes which are differentially labeled**." (emphasis added by the board)

"11. A kit for the quantitation of a target nucleic acid segment in a biological sample **in a polymerase chain reaction amplification method** comprising individual containers which provide:

(a) a predetermined initial amount of an internal standard comprising a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid segment can be distinguished **by segment specific probes**;

(b) at least one oligonucleotide primer pair for the co-amplification of said internal standard and said target nucleic acid segment.
(c) segment specific probes which are differentially labeled for detecting said internal standard nucleic acid segment and said target nucleic acid segment."
(emphasis added by the board)

"26. A method for quantifying a target nucleic acid segment in a sample **in a polymerase chain reaction amplification method**, which method comprises the steps of:

(a) adding to said sample a predetermined initial amount of an internal standard as characterized in any one of claims 1 to 10, wherein said internal standard comprises a nucleic acid segment that binds to the same primers as are bound by said target nucleic acid segment;
(b) treating said sample under conditions suitable for carrying out a polymerase chain reaction, wherein said nucleic acids are rendered single-stranded and exposed to an agent for polymerization, deoxynucleotide 5' triphosphates, and a pair of oligonucleotide primers, wherein said primer pair can hybridize to both the target and standard nucleic acid segments, such that each primer can serve to initiate synthesis of an extension product on a DNA strand of each of the target and standard nucleic acid segments, such that the extension production of one primer, when it is separated from the template strand, serves as a template for the synthesis of the extension production of the other primer of said pair wherein said amplified

target and standard nucleic acid segments are distinguishable **by segment specific probes**;
(c) separating the primer extension products from the template on which they were synthesized to provide single-stranded molecules;
(d) repeating steps (b) and (c) on the single stranded molecules produced in step (c) at least once, whereby each repeat of steps (b) and (c) is one amplification cycle;
(e) measuring the amounts of the amplified target and standard segments produced in step (d) **with segment specific probes which are differentially labeled**; and
(f) calculating from the amplified target and standard segments produced in step (d) the amount of said target nucleic acid segment present in the sample before amplification."

Independent claims 36, 45 and 61 of this third auxiliary request read:

"36. Use of an internal standard for the quantitation of at least one target nucleic acid segment contained within a sample in **a polymerase chain reaction** amplification method, said internal standard comprising on one strand a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment, wherein said internal standard nucleic acid segment and said target nucleic acid segment are co-amplified using the same set of primers and wherein upon amplification said

internal standard nucleic acid segment and said target nucleic acid segment can be distinguished**, wherein said internal standard is suitable for the quantitation of between 2 and 32 target nucleic acid segments**."
(emphasis added by the board)

"45. A kit for the quantitation of a target nucleic acid segment in a biological sample **in a polymerase chain reaction amplification method** comprising individual containers which provide:

(a) a predetermined initial amount of an internal standard comprising a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid segment can be distinguished **by segment specific probes, wherein said internal standard is suitable for the quantitation of between 2 and 32 target nucleic acid seqments**;
(b) at least one oligonucleotide primer pair for the co-amplification of said internal standard and said target nucleic acid segment." (emphasis added by the board)

"61. A method for quantifying a target nucleic acid segment in a sample **in a polymerase chain reaction amplification method**, which method comprises the steps of:

(a) adding to said sample a predetermined initial amount of an internal standard as characterized in any one of claims 36 to 44, wherein said internal standard comprises a nucleic acid segment that binds to the same primers as are bound by said target nucleic acid segment;
(b) treating said sample under conditions suitable for carrying out a polymerase chain reaction, wherein said nucleic acids are rendered single-stranded and exposed to an agent for polymerization, deoxynucleotide 5' triphosphates, and a pair of oligonucleotide primers, wherein said primer pair can hybridize to both the target and standard nucleic acid segments, such that each primer can serve to initiate synthesis of an extension product on a DNA strand of each of the target and standard nucleic acid segments, such that the extension production of one primer, when it is separated from the template strand, serves as a template for the synthesis of the extension production of the other primer of said pair wherein said amplified target and standard nucleic acid segments are distinguishable;
(c) separating the primer extension products from the template on which they were synthesized to provide single-stranded molecules;
(d) repeating steps (b) and (c) on the single stranded molecules produced in step (c) at least once, whereby each repeat of steps (b) and (c) is one amplification cycle;
(e) measuring the amounts of the amplified target and standard segments produced in step (d); and
(f) calculating from the amplified target and standard segments produced in step (d) the amount of said target

nucleic acid segment present in the sample before amplification." (emphasis added by the board)

IV. The interlocutory decision of the opposition division was appealed by the patent proprietor (appellant).

V. The sole remaining opponent, who is the respondent in the present case, replied to the appellant's statement of grounds of appeal.

VI. With letter dated 23 January 2006 the appellant made further submissions concerning its appeal.

VII. In reaction to the summons to oral proceedings, the respondent informed the board of its intention not to attend the oral proceedings.

VIII. Oral proceedings took place on 28 August 2007 in the absence of the respondent. The appellant filed a new main request consisting of 88 claims.

Independent claim 1 of this new main request corresponded to claim 1 of the third auxiliary request before the opposition division which however was amended so as to now have as subject-matter the "Use of an internal standard for the quantitation of **a** target nucleic acid segment ..." (emphasis added) as opposed to "Use of an internal standard for the quantitation of **one** target nucleic acid segment ..." (emphasis added).

Independent claim 10 of the new main request was identical to claim 11 of the third auxiliary request before the opposition division whereas independent claim 24 corresponded to claim 26 of the third

auxiliary request before the opposition division having the back-reference in item (a) amended to claims "1 to 9".

Dependent claims 2 to 9, 11 to 23 and 25 to 32 were identical to claims 2 to 7, 9, 10, 12 to 17, 19 to 25, 27 to 29 and 31 to 35 of the third auxiliary request before the opposition division and corresponded to claims 3 to 8, 10, 11, 14 to 19, 21 to 24, 27 to 29, 31 to 33 and 35 to 39 as granted, albeit having their dependencies adapted.

Independent claims 33, 40 and 50 had no counterpart in the third auxiliary request before the opposition division and read:

"33. Use of a **cRNA** internal standard for the quantitation of at least one **RNA** target nucleic acid segment contained within a sample **in a polymerase chain reaction amplification method**, said internal standard comprising on one strand a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment, wherein said internal standard nucleic acid segment and said target nucleic acid segment are co-amplified using the same set of primers**, wherein the reverse transcriptase reaction of the standard cRNA and target RNA are carried out in the same reaction** and wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid

segment can be distinguished." (emphasis added by the board)

"40. A kit for the quantitation of **an RNA** target nucleic acid segment in a biological sample **in a polymerase chain reaction amplification method** comprising individual containers which provide:

(a) a predetermined initial amount of **a cRNA** internal standard comprising a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid segment can be distinguished;
(b) at least one oligonucleotide primer pair for the co-amplification of said internal standard and said target nucleic acid segment;
(c) a thermostable polymerase; and
(d) optionally appropriate buffers for a polymerase chain reaction and nucleoside triphospates."

"50. A method for quantifying **an RNA** target nucleic acid segment in a sample **in a polymerase chain reaction amplification method**, which method comprises the steps of:

(a) adding to said sample a predetermined initial amount of **a cRNA** internal standard as characterized in any one of **claims 33 to 39**, wherein said internal

standard comprises a nucleic acid segment that binds to the same primers as are bound by said target nucleic acid segment and wherein said internal standard nucleic acid segment and said target nucleic acid segment are co-amplified using the same set of primers;

(b) reverse transcribing of said target nucleic acid segment and said internal control nucleic acid segment into cDNA molecules;

(c) treating said sample under conditions suitable for carrying out a polymerase chain reaction, wherein said **cDNA molecules** are rendered single-stranded and exposed to an agent for polymerization, deoxynucleotide 5' triphosphates, and a pair of oligonucleotide primers, wherein said primer pair can hybridize to both the target and standard nucleic acid segments, such that each primer can serve to initiate synthesis of an extension product on a DNA strand of each of the target and standard nucleic acid segments, such that the extension production of one primer, when it is separated from the template strand, serves as a template for the synthesis of the extension production of the other primer of said pair wherein said amplified target and standard nucleic acid segments are distinguishable;

(d) separating the primer extension products from the template on which they were synthesized to provide single-stranded molecules;

(e) repeating steps **(c)** and **(d)** on the single stranded molecules produced in step **(d)** at least once, whereby each repeat of steps **(c)** and **(d)** is one amplification cycle;

(f) measuring the amounts of the amplified target and standard segments produced in step **(e)**; and

(g) calculating from the amplified target and standard segments produced in step **(e)** the amount of said target nucleic acid segment present in the sample before amplification." (emphasis added by the board)

Dependent claims 34 to 36, 38, 39, 41 to 49 and 51 to 56 were identical or corresponded to claims 3 to 5, 10, 11, 14 to 16, 22, 23, 25 to 27, 29, 32, 33, 35 to 37 and 39 as granted, having their dependencies adapted. New dependent claim 37 is based on claim 8 as granted and read:

"37. The use of any one of claims 33 to 36, wherein said internal standard cRNA molecule is synthesized using a plasmid as a template".

Independent claims 57, 65 and 80 and the claims depending thereon of the new main request were, apart from a renumbering of back-references, identical to claims 36, 45 and 61 and claims depending thereon of the third auxiliary request before the opposition division.

In addition to the claims of the main request, the appellant filed an amended page 13 of the patent description.

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

(E1) Murakawa *et al.* (1988), DNA, Vol.7, pages 287-295.

(E2) Zaia & Rossi (1989), Transfusion Medicine Reviews, Vol.3, Pages 27-30.

(E3) Gilliland *et al.* (1989), J. Cell. Biochem., Suppl. 13E, page 270, abstract.

X. The appellant's arguments which are relevant for the present decision are summarised as follows:

Reformatio in peius

– Decisions G 9/92 and G 4/93 (OJ EPO 1994, 875) and G 1/99 (OJ EPO 2001, 381) established the principle of the prohibition of *reformatio in peius* stipulating that a decision may not be reached which would put an appellant in a worse position than it was in under the decision which is the subject of the appeal. When applied to the present case, the principle meant that the board had no competence to examine the subject-matter of claims 57 to 88 to its merits, seeing that the opposition division had considered the subject-matter of these claims, contained in claims 36 to 70 of the third auxiliary request before the opposition division, to comply with the requirements of the EPC.

Rule 29(2) EPC

– Article 2 of the Decision of the Administrative Council of the European Patent Organisation of 13 December 2001 amending Rule 29(2) EPC (OJ EPO 2002, 2) stipulated expressly that this new Rule shall enter into force on 2 January 2002 and shall apply to all European patent applications in respect of which a communication under Rule 51(4)

EPC had not yet been despatched by that date. In the present case the communication under Rule 51(4) EPC was issued on 30 April 1998. The newly formulated Rule 29(2) EPC was therefore not applicable to the present case.

– Amended Rule 29(2) EPC was relevant only during the examination procedure wherein, contrary to the opposition procedure, it was possible to file divisional patent applications.

Added subject-matter

– The restriction of the independent claims to subject-matter in which the referred to "amplification method" is specified as "a **polymerase chain reaction** amplification method" found plenty of support in the application as filed.

– The amendment to "the quantitation of **a** target nucleic acid sequence" of claim 1 found support *inter alia* in claim 1 of the application as filed.

Sufficiency of disclosure

– The patent disclosed the subject-matter of the claims of the main request in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

Novelty

– None of the cited prior art disclosed the subject-matter of claims 1 to 32 or the subject-matter of claims 33 to 56. In particular neither of documents (E1) to (E3) disclosed segment specific probes which were differentially labelled or a cRNA internal standard in the quantitation of RNA target nucleic acid segments wherein the reverse transcriptase reaction of the standard cRNA and target RNA were carried out in the same reaction.

Inventive step

– Closest prior art for the subject-matter of both groups of claims 1 to 32 and 33 to 56 was document (E3).

– The problem to be solved by the invention in claims 1 to 32 was the provision of an improved PCR method for simple, reliable and accurate quantitation. The solution to this problem, the use of segment specific and differentially labelled probes in order to distinguish two amplificates was not suggested in any other cited prior art document. In particular, document (E1) and (E2) solely disclosed the use of a single radio-labelled probe in Southern gel electrophoresis (document (E2), page 28, right-hand column, lines 16 to 38) and alternatively the use of radio-labelled nucleotides in preference to probes (document (E2), page 30, left-hand column, lines 7 to 9). For these reasons the solution in claims 1 to 32 was not rendered obvious by the prior art to the skilled person.

– The technical problem to be solved by the subject-matter of claims 33 to 56 was the provision of a source of an internal standard alternative to the cDNA used in the document. The solution as claimed was the use of RNA molecules as internal standard. This not only allowed for the co-amplification of the cDNA amplificates of the target and the standard but also for the simultaneous reverse transcription of the target mRNAs and the control RNA. Accordingly, this method provided for the accurate measurement of the starting mRNA instead of the measurement of the initial concentration of the target cDNA. Since documents (E1) and (E2) merely suggested the use of control RNA in the context of quality of the polymerase chain reaction on target mRNA, the document could not teach the skilled person to implement the same control RNAs in a quantitative context.

XI. The respondent's arguments which are relevant for the present decision are summarised as follows:

Rule 29(2) EPC

– The number of independent claims formulated by the appellant was contrary to Article 84 EPC in combination with Rule 29(2) EPC.

Novelty

– Document (E2), which referred to the methods disclosed in (E1), mentioned the use of an RNA internal standard in respect of quantitation (see page 29, sole full paragraph in the right-hand

column) and therefore anticipated the subject-matter of claims 33 to 56.

Inventive step

– Closest prior art for the subject-matter of claims 1 to 32 was document (E3). The problem to be solved was the provision of an alternative method of distinguishing the amplificates of the target and standard segments. The skilled person would know that such probes must be segment specific and differentially labelled in order to distinguish target and standard.

– Closest prior art for the subject matter of claims 33 to 56 was document (E2). The technical problem to be solved was the provision of an alternative source of RNA molecules as internal standard. The skilled person would know from document (E2) that quantitation was possible and would therefore design further alternative RNA molecules as internal standard.

XII. The appellant requested that the decision under appeal be set aside and that the patent be maintained in amended form on the basis of claims 1 to 88 of the main request filed at the oral proceedings on 28 August 2007 before the board and the description as amended at the oral proceedings before the opposition division on 21 May 2003, with the exception of page 13 which is to be replaced by the new page 13 filed at the oral proceedings before the board. The respondent, with letter of 27 July 2007, requested that the appeal be dismissed.

XIII. At the end of the oral proceedings, the chairman announced the board's decision.

Reasons for the Decision

1. The appeal is admissible as it complies with the requirements of Articles 106 to 108 and Rule 64 EPC.

The claimed subject-matter

2. The main request concerns three groups of claims which are related to the following subject-matter:

The first group of claims (claims 1 to 32) is related to uses, kits and methods for the quantitation of a target nucleic acid segment in a biological sample in a polymerase chain reaction amplification method involving an internal standard and **segment specific probes which are differentially labelled**.

The second group of claims (claims 33 to 56) is related to uses, kits and methods for the quantitation of a target nucleic acid segment in a biological sample in a polymerase chain reaction amplification method involving a **cRNA internal standard**.

The third group of claims (claims 57 to 88) is related to uses, kits and methods for the quantitation of a target nucleic acid segment in a biological sample in a polymerase chain reaction amplification method involving an internal standard suitable for the

quantitation of **between 2 and 32 target nucleic acid segments**.

Prohibition of reformatio in peius and extent of examination by the board

3. In the present case, the proprietor is the sole appellant against the interlocutory decision of the opposition division according to which the patent could be maintained in amended form on the basis of the then third auxiliary request. Thus neither the board of appeal nor the non-appealing opponent may challenge the maintenance of the patent in the amended form (G 9/92 and G 4/93, OJ EPO 1994, 875).

4. The board notes that, apart from a renumbering of claims and consequential amendments of back-references, the third group of claims (claims 57 to 88) is identical to claims 36 to 41, 43 to 50, 52 to 64 and 66 to 70 of the third auxiliary request before the opposition division which was considered to fulfil the requirements of the EPC. Thus the question arises whether the above-mentioned principle of prohibition of *reformatio in peius* limits the power of the board to reconsider the allowability of these claims.

5. The board is aware of decision T 498/03 of 28 November 2006 (point 1.1) where it was found that an independent method claim held allowable by the opposition division in its interlocutory decision which was appealed solely by the proprietor could not be considered any more at the appeal stage (see also T 149/02 of 25 July 2003, point 2, and T 168/04 of 8 September 2005, point 2). Although it may be doubted whether the prohibition of

reformatio in peius should generally be extended so far, in the present case the respondent has not brought forward any substantive arguments during the whole appeal proceedings as to why, contrary to the conclusions of the opposition division, the third group of claims would not comply with the requirements of the EPC. Under these circumstances the board does not consider it appropriate to reconsider the allowability of this group of claims on its own motion.

6. The first group of claims (claims 1 to 32) also corresponds to a large extent to claims which have been considered allowable by the opposition division. However, since the wording of independent claims of this group has been modified, albeit only slightly, their formal and substantive allowability has to be fully considered by the board. The same holds true for the second group of claims (claims 33 to 56) which does not find a counterpart in claims of the request considered to be allowable by the opposition division.

Rule 29(2) EPC

7. The patent as granted contained three independent claims falling within different categories (use, product and method). The appellant's main request contains nine independent claims in the same three categories, i.e. three independent use claims, three independent product claims and three independent method claims.

8. According to Rule 29(2) EPC in its current version, a European patent application may not contain more than one independent claim in the same category unless certain specific conditions set out in paragraphs (a) to (c) are fulfilled. However, this requirement does not apply to the present case in view of the transitional provisions stipulated by the legislator when Rule 29(2) EPC was amended in 2001; pursuant to Article 2 of the Decision of the Administrative Council of the European Patent Organisation of 13 December 2001 (OJ EPO 2002, 2), the amended Rule 29(2) EPC "shall enter into force on 2 January 2002 and shall apply to all European patent applications in respect of which a communication under Rule 51(4) EPC has not yet been despatched by that date".

9. In the present case the communication under Rule 51(4) EPC was already issued on 30 April 1998. Thus, the current version of Rule 29(2) EPC cannot apply. In view of this conclusion which follows the legal analysis underlying the decision T 991/02 of 26 September 2003 (point 2), the board does not need to consider whether the opposition division was correct in holding that, notwithstanding Rule 61a EPC, Rule 29(2) EPC in its current version does not apply in opposition proceedings.

10. According to Rule 29(2) EPC in the version prior to the above amendment, a European patent application may contain two or more independent claims in the same category where it is not appropriate, having regard to the subject-matter of the application, to cover this subject-matter by a single claim. In the present case a set of claims which only contained one independent use

claim, one independent product claim and one independent method claim would either be likely to be unallowable in view of the relevant prior art or would give the appellant considerably less protection than the claims of the main request. The board therefore does not consider the relatively high number of independent claims in the present case as inappropriate. Thus, Rule 29(2) EPC - in its former version - is complied with.

Rule 57a EPC

11. Rule 57a EPC lays down the principle that the description, claims and drawings of an opposed European patent may be amended, provided that the amendments are occasioned by grounds for opposition. In the present case, the number of the claims of the appellant's main request (eighty-eight) considerably exceeds the number of the granted claims (thirty-nine). The same holds true for the number of the independent claims (nine *vs*. three). Whether such a proliferation of claims can fairly be said to be occasioned by grounds of opposition, needs closer scrutiny.

12. The respondent had raised several objections under Article 100 EPC against the claims of the patent as granted. In response to this challenge, the appellant chose not to defend the claims unamended but to submit a main request which contains three groups of claims, each group having three independent claims. The first group is characterised by the additional limiting feature "segment specific probes differentially labelled", the second group by the additional limiting feature "cRNA internal standard" and the third group by

the additional limiting feature "suitable for quantitation of between 2 and 32 target nucleic acid segments". Each group furthermore contains dependent claims which are based on dependent claims of the patent as granted.

13. In the board's view, the condition stipulated in Rule 57a EPC aims at preventing the proprietor from using the opposition procedure as a mere extension of the examination procedure. Its purpose is not to prevent the proprietor from formulating a request which preserves as much of the scope of protection of the granted patent as possible in the light of the grounds of opposition. It appears to be a legitimate reaction for a proprietor who sees no basis for defending a granted independent claim against an opposition to replace this claim by two or more independent claims each of which contains a different limiting feature. This reaction may furthermore cause a proliferation of dependent claims if, as in the present case, many of the dependent claims as granted are then made dependent on the new independent claims. Amendments of this type can therefore fairly be said to be occasioned by grounds of opposition.

14. This conclusion finds support in the relevant appeal case law. In decision T 223/97 of 3 November 1998 (point 2.2) it was held that the replacement of one independent claim as granted by two independent claims each directed to a respective specific embodiment covered by the independent claim as granted was admissible in principle. In decision T 937/00 of 12 June 2003 (point 2.1), the competent board did not see any objection in principle to a patentee amending

its claims in response to an opposition so that they comprise several independent claims directed to different objects originally covered by a single generic claim of a given category, when such claim could not be maintained. Although decision T 181/02 of 13 October 2003 (point 3.2) considered the replacement of a granted single independent claim by two or more independent claims to be occasioned by grounds of opposition "only in exceptional cases", it cited the decision T 223/97 (*supra*) with approval and went on to observe that, where two granted dependent claims (e.g. claims 2 and 3) are linked in parallel to a single independent claim (e.g. claim 1), the filing of two independent claims (e.g. including the features of claims 1 and 2, and 1 and 3) might "of course" be possible.

15. For the above reasons the board considers the claims of the main request to comply with the requirements of Rule 57a EPC.

Added subject-matter

16. The respondent has argued that the claims as granted contained added subject-matter since they did not specify the referred to amplification method as "a **polymerase chain reaction** amplification method". The board notes that all the independent claims of the new main request now refer to this specific amplification method. These amendments comply with Article 123(2) EPC, since numerous references to PCR amplification methods can be found throughout the application.

17. Independent claim 1, as compared to claim 1 as granted, is characterised by the feature that upon amplification the internal standard nucleic acid segment and the target nucleic acid segment "**are distinguished by segment specific probes which are differentially labeled**". Corresponding amendments are contained in the other independent claims of the first group of claims, i.e. claims 10 and 24. The amendments find support in the application as filed at page 15, lines 15 to 19 where it states that "[t]*he present invention does not require that the amplified product be of different sizes, however, for other methods can be utilized to distinguish one amplified segment from another. For instance, the internal probe specific for one segment can be labeled differently than the internal probe specific for the other segment."*

18. Claim 1 of the main request now refers to the use of an internal standard "for the quantitation of **a** target nucleic acid sequence" and no longer contains the feature that the internal standard be used "for the quantitation of **at least one** target nucleic acid sequence". Literal support for this amendment can be found in the first line of claim 1 as originally filed. Accordingly, the objection raised by the respondent in this context under Article 123(2) EPC against an earlier version of the claim has been overcome.

19. Independent claims 33, 40 and 50 (i.e. the second group of claims) are related to uses, kits and methods for the quantitation of a target nucleic acid segment in a biological sample in a polymerase chain reaction amplification method involving a **cRNA internal standard**.

The amendments find support in the application as filed on page 5, lines 10 to 15 and 22 to 27.

20. Independent claim 33 contains the further feature that "the reverse transcriptase reaction of the standard cRNA and target RNA are carried out in the same reaction". A basis for this amendment is contained in the application as originally filed on page 11, line 23.

21. Dependent claim 37, corresponding to claim 8 as granted, has been amended to stipulate that "wherein said internal standard cRNA molecule is synthesized using a plasmid as a template". A basis for this amendment can be found in the application as filed on page 5, lines 10 to 15.

22. The respondent has not formulated any further objections under Article 123(2) EPC against the claims of the main request and the board also sees no reason for such an objection.

23. In view of the above considerations the board is satisfied that claims 1, 10, 24, 33, 40 and 50 as well as the claims dependent thereon (i.e. the first and second group of claims) comply with the requirements of Article 123(2) EPC.

24. The subject-matter of the independent claims of all three groups of claims has been restricted as compared to that of the independent claims as granted. Accordingly, the claims comply with the requirements of Article 123(3) EPC.

Sufficiency of disclosure

25. With respect to auxiliary request III then before it, the opposition division came to the conclusion that the subject-matter of the claims complied with the requirements of Article 83 EPC. During the appeal procedure, the respondent has not formulated any objections relating to sufficiency of disclosure. Also the board sees no reason for questioning the sufficiency of disclosure of the subject-matter of the first and second group of claims of the new main request.

Novelty

26. Document (E1) discloses modification of the polymerase chain reaction method for the amplification of HIV-1 RNA templates allowing the direct detection of HIV-1 infection. The amplification of an HIV-1 RNA template enhanced the polymerase chain reaction as compared to the amplification of HIV DNA. The document provides for a negative control system for the polymerase chain reaction (page 293, right hand column, lines 15 to 17). In order to be able to distinguish a negative result indicative of the absence of HIV-1 in the sample (i.e. a genuine negative result indicating the HIV negative status of a patient) from a negative result due to an abortive PCR reaction (i.e. a so-called false negative), the document teaches the inclusion of a control RNA template in the polymerase chain reaction which can be amplified with the same primers as the target RNA. The amplificate of the control template was 21 bases longer than the amplificate of the template which was 151 nucleotides in size. This allows for distinguishing

both amplificates (page 292, right-hand column, lines 13 to 21; page 293, lines 15 to 20, Figures 1, 7 and 8). The document states that when equimolar amounts of RNA from both the HIV template and the control template are added both templates are simultaneously amplified with approximately equivalent efficiencies (page 292, right-hand column, lines 18 to 21).

27. Document (E2) reviews different approaches for confirming an HIV infection, e.g. Western blot, gene amplification in general and the so-called "Rossi" method (see part of the article bridging pages 28 and 29; subtitle in line 3 of the right-hand column on page 28). The latter corresponds to the method as disclosed in document (E1) (page 28, right-hand column, line 3 to page 29, right-hand column, line 15). After having explained how polymerase chain reaction amplificates of HIV-1 RNA can be detected by Southern gel electrophoresis, the document states on page 28, right-hand column, line 45 to page 29, left-hand column, line 8: "*The RNA can be quantitated in the blood by extracting HIV-1-specific RNA directly from WBCs. When 1 ng is added to the reaction as a positive control, the size of the spot on the gel becomes the internal control for 1 ng of RNA. When this technique is used to test recipients of HIV-1-infected blood who have developed AIDS, discernable blots are seen. In experiments using either HIV-1-positive RNA or RNA extracted from infected cells or uninfected cells, as few as 1 x 10^{-7} pmol of RNA from the plasmid were detected*[3]*.*" ("3" being a reference to the document designated (E1) in the present proceedings). In a next part of the document headed by the subtitle "Quantitation" on page 29, right-hand column, lines 1

to 15, document (E2) states: "*Because a specific-sized region of amplification is constructed, a positive test determines a band of known size. Through the construction of an extra insert of 21 bases into the HIV-1 sequence to be amplified, the extra 21 bases will make the amplified DNA larger, so that it will migrate more slowly on the gel ... Testing a standard amount of the extra-long fragment with an unknown permits quantitation by comparing the unknown with the known. This has been confirmed using a specimen from an AIDS patient*[3]".

28. Document (E3) is an abstract which describes a method for the quantitation of specific mRNA species involving co-amplification of a competitive template which uses the same primers as a target cDNA, but can be distinguished from target cDNA following amplification (lines 13 to 17). The target cDNA is co-amplified with a dilution series of competitive template of known concentration (lines 19 to 21). Radiolabelled dNTP is used to quantitate the amount of competitive template and target cDNA after amplification (lines 23 to 25). The method is reported to give accurate quantitation of less than 1 pg of target cDNA from 1 ng of total starting mRNA and to be able to distinguish two-fold differences in mRNA concentration (lines 27 to 29).

29. The board notes that none of documents (E1) to (E3) discloses kits for use in the methods as disclosed so that they do not anticipate the subject-matter of independent claims 10 and 40. Furthermore, none of the above documents nor any other cited prior art document discloses a polymerase chain reaction amplification method involving segment specific probes which are

differentially labelled so that the subject-matter of independent claims 1 and 24 is not anticipated either. In addition, document (E3) does not disclose a cRNA template for use as an internal standard for the measurement of the initial mRNA amount but applies a competitive cDNA template and can therefore not destroy the novelty of independent claims 33 and 50.

30. In view of the above, it remains to be decided whether the disclosure in documents (E1) and (E2), as argued by the respondent, anticipates the subject-matter of independent claims 33 and 50.

In this context the respondent has in particular referred to the passage on page 29, right-hand column, lines 1 to 15, in document (E2) which contains the statement that "[t]*esting a standard amount of the extra-long fragment with an unknown permits quantitation by comparing the unknown with the known*". The board notes however that document (E2) refers immediately after this sentence to experimental details disclosed only in document (E1), by stating that "[t]*his has been confirmed using a specimen from an AIDS patient*[3]". Therefore, when determining the meaning of the passage relied on by the respondent on page 29 in document (E2), the skilled person would necessarily consult document (E1).

The only experiment in document (E1) to which the above statements in document (E2) can relate is the one shown in Figure 8 (Southern blot X-ray photo of polymerase chain reaction amplificates, including an amplificate of mRNA from a blood sample of an HIV positive patient) and the text passages relating to it. However, neither

the Figure nor these text passages disclose or describe how a result obtained by the experiment can be quantitated. Indeed, as noted in point 26 above, document (E1) does not relate to the quantitation of mRNA but discloses the use of a mRNA internal standard for the qualitative control of the polymerase chain reaction. The document is silent on the determination of the initial amount of HIV-1 mRNA in patients' blood samples. The board considers that even when reading document (E2) in combination with document (E1), the skilled person would not obtain any instructions concerning concrete method steps for implementing the mentioned quantitation. For these reasons the subject-matter of claims 33 and 50 is not made available to the public.

31. In view of the above considerations the board is satisfied that the subject-matter of claims 1, 10, 24, 33, 40 and 50 as well as the claims dependent thereon is novel over the cited prior art.

Inventive step

32. For assessing whether or not a claimed invention meets the requirements of Article 56 EPC, the boards of appeal apply the "problem and solution" approach, which requires as a first step the identification of the closest prior art. In accordance with established case law of the boards of appeal, the closest prior art is a teaching in a document conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common, i.e. requiring the minimum of

structural modifications to arrive at the claimed invention.

Closest prior art

33. The present invention as subject-matter of both the first and the second group of claims aims at the quantitative determination of a particular nucleic acid segment in a sample by means of an internal standard in a polymerase chain reaction method. Document (E3) explicitly aims at the same objective as the invention, namely the quantitative amplification of mRNA using an internal standard in a polymerase chain reaction (see title and lines 27 to 32). In particular, the strategy involves co-amplification of a competitive template which uses the same primers as the target cDNA, but can be distinguished from target cDNA following amplification (see lines 14 to 17). Documents (E1) and (E2) on the other hand, both relate to the qualitative control of the polymerase chain reaction. Although document (E2) contains a short passage concerning a possible use of the described method as a means for quantitation, this passage leaves the skilled reader without any concrete instructions for implementing the mentioned quantitation. Furthermore, as emphasised in point 30 above, this lack of concrete instruction would not be remedied even if the skilled person were to consult document (E1) as referenced in that passage of document (E2).

For the above reasons, the board considers document (E3) to qualify as representing the closest prior art for the inventions in both the first and the second group of claims.

First group of claims

34. Starting from the closest prior art as identified above, the problem to be solved by the invention as subject-matter of the independent claims 1, 10 and 24 of the first group of claims can be formulated as the provision of alternative means for distinguishing between an amplified internal standard and an amplified target nucleic acid segment. The board has no reason to doubt that the subject-matter of these claims solves this problem.

35. The competitive template in document (E3) is either a mutant cDNA containing a new restriction site, or, if the primers are in separate exons and flank a small intron (100-200 bp), genomic plasmid DNA (lines 17 to 19). The document describes the use of radiolabelled dNTP to quantitate the amount of competitive template and target cDNA after amplification (lines 23 to 25).

36. It therefore needs to be established whether or not the skilled person, in order to solve the above technical problem, would adapt the teaching of document (E3) in an obvious manner and arrive at the subject-matter of the independent claims 1, 10 and 24, by using segment specific probes which are differentially labelled.

37. Neither document (E3) itself nor any of the other cited prior art documents including documents (E1) and (E2) suggest, for distinguishing between two amplificates, the use of segment specific probes which are differentially labelled. In fact, document (E2) discloses for distinguishing between the differently

sized or restriction nuclease digested amplificates of standard and target, the use of Southern blotting with radiolabeled nucleotides (page 28, right-hand column, lines 27 to 38) and states that this method eliminates the need for probes (page 30, left-hand column, lines 7 to 11). Document (E1) discloses differently sized target and standard (see e.g. Figure 7).

38. In view of the above considerations the board judges that the subject-matter of the independent claims 1, 10 and 24 of the first group of claims was not rendered obvious to the skilled person by the prior art. The same holds true for the claims dependent thereon.

Second group of claims

39. Starting from document (E3), identified above as the closest prior art, the problem to be solved by the invention as subject-matter of the independent claims 33, 40 and 50 of the second group of claims can be formulated as the provision of more accurate means for the quantitative determination of the amount of a target RNA nucleic acid segment contained within a sample in a polymerase chain reaction.

40. The subject-matter of these claims solves this problem by providing for a cRNA standard which, when added to the reaction mixture, allows simultaneous reverse transcription of the target and internal standard RNA in the same reaction. Although only independent claim 33 explicitly requires that the reverse transcription reaction is to be carried out in the same reaction the board considers that the same applies to independent claims 40 and 50. In particular, the

skilled person, when interpreting claims 40 and 50, would immediately recognise that cRNA molecules can only function as quantitative internal standards if the reverse transcription of the standard and the target is not performed in separate reactions, since otherwise the different reaction environment of the reverse transcription of the cRNA and the target may lead to not fully correlatable and therefore inaccurate start amounts for the ensuing PCR reaction. The examples of the patent show that the subject-matter of independent claims 33, 40 and 50 indeed solves the above problem.

41. It therefore needs to be determined whether the skilled person, in order to find a solution for the above technical problem, would modify the disclosure of document (E3) in an obvious manner and therefore arrive at the subject-matter claimed.

Document (E3) itself does not provide any suggestions how to further improve the disclosed quantitation. Furthermore, the respondent has not brought forward arguments why the skilled person starting from the disclosure in document (E3) and using common general knowledge would arrive at the subject-matter of the independent claims of the second group of claims.

Accordingly, it needs to be established whether or not the remaining cited prior art renders the claimed invention obvious to a skilled person.

42. As established in point 30 above, documents (E1) and (E2) do not disclose or teach how to quantitate the amount of RNA, such as mRNA, initially present in a sample, by using co-amplification of an internal

standard and the target nucleic acid. The documents rather focus on the use of an internal standard to qualitatively control the functioning of the polymerase chain reaction conducted on target mRNA. In view of this focus, the board doubts that the skilled person, when trying to improve the teaching of document (E3), would seek assistance from documents (E1) or (E2).

43. The board furthermore considers that even if the skilled person were to consult document (E1) or (E2), he would not arrive at the invention as subject-matter of the independent claims of the second group of claims:

As already emphasised in points 30 and 33 above, the short passage in document (E2) concerning a possible use of the described method as a means for quantitation leaves the skilled reader without any concrete instructions for its implementation. Furthermore, if the skilled person were to consult document (E1) as referenced in that passage of document (E2), he would, as already concluded above (point 30), necessarily focus upon the experiments relating to Figure 8. The legend of that figure states:

"*PCR and transcriptional amplification of RNA from a patient blood sample. One microgram of patient RNA prepared from peripheral blood lymphocytes was amplified for 15 rounds with the HTLVAT7 and HTLVB oligonucleotide primers. From this reaction, 1/20th of the sample was withdrawn and mixed with about 5.0 ng of PGM92+21 RNA, and the two samples were amplified with AMV reverse transcriptase and then DNA polymerase I (Klenow) for and* [sic] *additional 10 rounds with the same oligonucleotide primers. One-twentieth of the*

mixture was phenol-extracted, ethanol-precipitated, and then included in a transcription reaction. The exposure was for 12 hr with an intensifying screen at -70°C.".

Accordingly, the patient's RNA prepared from the blood was amplified separately from the control mRNA. It was only from this reaction that 1/20th of the sample was withdrawn and then mixed with a particular amount of RNA standard, i.e. pGM92+21 RNA for co-amplification. In view of these experimental conditions the skilled person would not consider the experiments disclosed in Figure 8 as suitable for adaptation so as to provide for the quantitation of the initial amount of target mRNA.

The board notes that document (E1) additionally discloses the possibility of simultaneous reverse transcription of a target and standard RNA and subsequent co-amplification in one reaction (see Figure 7). However, these experiments are conducted under controlled conditions, i.e. applying predetermined and equal amounts of target and standard without providing or suggesting that this could be used for the quantitation of an unknown amount of target mRNA.

44. In view of the above considerations the board judges that the subject-matter of claims 33, 40 and 50 and the claims dependent thereon was not rendered obvious to the skilled person.

45. For the above reasons the subject-matter of claims 1 to 56 involves an inventive step (Article 56 EPC).

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 in amended form on the basis of the following documents:

   – Claims: 1 to 88 filed at the oral proceedings before the board on 28 August 2007.
   – Description: pages 2 and 3 filed at the oral proceedings before the opposition division on 21 May 2003; pages 4 to 12 and 14 to 15 of the patent specification; page 13 filed at the oral proceedings before the board on 28 August 2007.
   – Drawings: figures 1 to 4 of the patent specification.

The Registrar: The Chairman:

P. Cremona R. Moufang