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Datasheet for the decision of 8 October 2013

Case Number: T 1588/10 - 3.3.08

Application Number: 04724275.5

Publication Number: 1611254

IPC: C12Q 1/68, C12Q 1/70

Language of the proceedings: EN

Title of invention:
Compositions and methods for detecting certain flaviviruses, including members of the Japanese encephalitis virus serogroup

Applicant:
Roche Diagnostics GmbH
F.Hoffmann-La Roche AG

Headword:
Detection Japanese encephalitis viruses/ROCHE

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

Keyword:
"Main request - admissibility (yes); requirements of the EPC met (yes)"

Decisions cited:
G 0010/93

Catchword:
-

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Case Number: T 1588/10 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 8 October 2013

Appellants: (Applicant 1)
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(Applicant 2)
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Representative:
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 20 October 2009 refusing European patent application No. 04724275.5 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: M. Wieser
Members: P. Julià
J. Geschwind

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division to refuse the European patent application no. 04 724 275.5, published as International patent application WO 2004/092412 (hereinafter "*the application*").

II. The sole claim request before the examining division was filed on 2 September 2009 and consisted of sixteen claims. Claim 1 of this request read as follows:

"1. A kit for the detection of a nucleic acid of a member of the Japanese encephalitis virus serogroup, comprising:

a) a first oligonucleotide that hybridizes to a nucleic acid of SEQ ID NO: 1 or a complement thereof;

b) a second oligonucleotide that hybridizes to a nucleic acid of SEQ ID NO: 9 or a complement thereof; and

c) a detectably-labeled third oligonucleotide that hybridizes to a nucleic acid of SEQ ID NO: 16, or the complement thereof."

Claims 2 to 16 were directed to preferred embodiments of claim 1.

III. In its decision, the examining division acknowledged the claim request to fulfil the requirements of Articles 123(2) and 54 EPC but not those of Article 56 EPC.

According to the examining division, any of documents D29 and D30 (*infra*) could be the closest prior art. Document D29 disclosed the detection of the West Nile virus (WNV) and the Japanese encephalitis virus (JEV) by reverse transcription polymerase chain reaction (RT-PCR) using a pair of primers. In document D30, WNV was also detected by RT-PCR. Both documents identified the amplification product by gel electrophoresis and size determination or sequencing. The claimed subject-matter differed from these documents in: i) the reagents being provided in form of a kit, ii) the specific sequence of the oligonucleotides, and iii) the presence of a detectably labelled probe for detection. These differences failed to produce a synergistic effect going beyond the sum of their individual effects. Each difference solved only one of the three different partial problems identified, namely i) standardization of the reagents required for a routine use of the assay disclosed in the closest prior art document, ii) provision of alternative primer oligonucleotides for targeting nucleic acid of the viruses disclosed in this prior art, and iii) provision of alternative means to detect the amplification product generated.

The provision of a kit derived from a known successful laboratory method was a standard procedure for a skilled person and did not require inventive skill. The primer oligonucleotides of sequences SEQ ID NO: 1 and 9 represented a random selection among the many possible sequences available to a skilled person. The detectably labelled probe of part c) of claim 1 did not generate any technical effect that had not already been achieved by the detection methods disclosed in the closest prior

art document. No inventive merit could be attributed to any of the solutions proposed in claim 1 or in any of the dependent claims.

Moreover, there was no evidence on file showing that a kit with the primers and probe oligonucleotides of claim 1 allowed the detection of a broad variety of viruses belonging to the JEV serogroup in one single step. An alignment of nucleic acid sequences of viruses of this serogroup was only the starting point for developing primers and probes suitable for detecting a broad variety of viruses belonging to the JEV serogroup in one single step but not yet the actual technical solution thereto.

IV. The applicants (appellants) filed a notice of appeal and a statement setting out their Grounds of Appeal with new documentary evidence (documents D31 to D33, *infra*), a new Main Request and an Auxiliary Request 1 identical to the claim request considered by the examining division in the decision under appeal.

V. Summons to oral proceedings were issued on 3 May 2013. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), the board informed the appellants of its preliminary opinion on the substantive issues of the case. In particular, reference was made to Article 12(4) RPBA concerning the admissibility of the appellants' new Main Request and, with reference to decision G 10/93 (OJ EPO 1995, page 172, Headnote), several objections were raised under Article 84 EPC against claim 1 of the Auxiliary Request 1.

VI. On 9 September 2013, the appellants filed a new Main Request and Auxiliary Requests 1 and 2. The Main Request was identical to the Auxiliary Request 1 filed with their Grounds of Appeal and identical to the request considered by the examining division in the decision under appeal (cf. point II *supra*).

VII. Oral proceedings were held on 8 October 2013. At these proceedings, the appellants withdrew all their previous requests and filed a new and sole Main Request.

VIII. Claim 1 of the **Main Request** read as follows:

"1. A kit for the detection of a nucleic acid of several members of the Japanese encephalitis virus serogroup in a biological sample under stringent hybridization conditions, comprising:

a) a first primer oligonucleotide comprising SEQ ID NO:8 or a complement thereof;

b) a second primer oligonucleotide comprising SEQ ID NO:15 or a complement thereof; and

c) a detectably-labeled third probe oligonucleotide comprising SEQ ID NO:28, or the complement thereof,

wherein the detectable label in the third probe oligonucleotide is a fluorescent moiety."

Claims 2 to 11 were directed to preferred embodiments of claim 1.

IX. The following documents are cited in the present decision:

D1: M. Tanaka, J. Virol. Methods, 1993, Vol. 41, pages 311 to 322;

D29: A. Igarashi et al., Microbiol. Immunol., 1994, Vol. 38, No. 10, pages 827 to 830;

D30: K.R. Porter et al., Am. J. Trop. Med. Hyg., 1993, Vol. 48, No. 3, pages 440 to 446;

D33: K. Young, Report of Experimental Results, signed on 20 February 2010.

X. Appellants' submissions, insofar as relevant to the present decision, may be summarised as follows:

Admissibility of the Main Request

The Main Request was based on an Auxiliary Request filed in direct reply to the board's communication in which, for the first time in the proceedings, several objections were raised under Article 84 EPC. The Main Request intended to overcome the objections raised by the board under Articles 123(2) and 84 EPC at the oral proceedings.

Article 56 EPC

Document D30 amplified WNV by a RT-PCR reaction. The primers used were useful to detect WNV and Kunjin Virus (KUN) but not isolates from JEV, St. Louis encephalitis (SLE) and Yellow Fever Viruses (YFV). These primers did

not cross-react with these viruses. Document D29 showed the RT-PCR results on 24 cerebrospinal fluid specimens. In a first amplification, a flavivirus cross-reacting primer pair (YF-1 and YF-3) was used and positive samples were subjected to a second amplification using specific JEV and WNV primer pairs. In both documents, laborious and time-consuming methods were used to detect the amplified product (gel electrophoresis, sequencing, staining). Since the primers of document D30 did not amplify a broad variety of viruses from the JEV subgroup, document D29 was the closest prior art document.

Starting from document D29, the technical problem to be solved was the provision of a kit that allowed for a faster and more efficient amplification and detection of a broad variety of members of the JEV serogroup. The claimed kit with the specific amplification primer pair and the specific probe for concurrent detection of the amplified product, in a single reaction and without needing to further handle the samples for detection, solved this problem.

The specific sequences of the primers and of the detectable fluorescent label in the probe produced a synergistic effect that went beyond the sum of their individual effects. Example 1 of the application showed a WNV amplification and, during the amplification reaction, a detectable fluorescent signal was emitted and detected without needing a subsequent detection step as required in the closest prior art document. Document D33 showed that the specific primer and probe used in Example 1 allowed the combined amplification

and detection of SEV, Murray Valley Encephalitis Virus, JEV and KUN.

The primers disclosed in documents D29 and D30 did not achieve the advantageous effects shown for the claimed combination of primer and probe oligonucleotides (high cross-reactivity, two binding sites leading to higher sensitivity). Moreover, the combination of documents D29 or D30 with prior art documents disclosing fluorescent-based PCR assays would not have guided a skilled person to the specific primer and probe oligonucleotide sequences of the Main Request.

XI. The appellants (applicants) requested that the decision under appeal be set aside and that a patent be granted on the basis of the set of claims 1 to 11 of the new Main Request filed during the oral proceedings on 8 October 2013.

Reasons for the Decision

Admissibility of the Main Request

1. The filing of the Main Request at oral proceedings is an amendment of the appellants' case and thus, the Main Request may be admitted and considered only at the board's discretion (Article 13(1) RPBA).

2. The Main Request is based on an Auxiliary Request that was filed in direct reply to the board's communication pursuant to Article 15(1) RPBA. In this communication, the board raised, for the first time in the proceedings, several objections under Article 84 EPC (cf. point V

supra). At oral proceedings, the board raised further objections under Articles 123(2) and 84 EPC against this Auxiliary Request. The filing of the Main Request at oral proceedings is a reply to these objections.

3. The Main Request does not introduce new subject-matter since it results from a mere combination of the subject-matter of claim 1 of previous requests with that of dependent claims. It does not add complexity to the case or raise new objections. On the contrary, it contributes to the procedural economy of the appeal proceedings.

4. Thus, the board, in the exercise of its discretion, decides to admit the Main Request into the appeal proceedings (Article 13(1) RPBA).

Admissibility of new evidence

5. The experimental evidence of document D33 was filed with the appellants' statement of Grounds of Appeal, i.e. at the earliest stage of the appeal proceedings.

6. Although an objection under Article 56 EPC was raised by the examination division at an early stage of the examination proceedings, the filing of document D33 is a direct reply to the decision of the examining division that no evidence was on file to show that a kit with the primers and probe oligonucleotides of claim 1 allows the detection of a broad variety of viruses from the JEV serogroup in one single step (cf. point III *supra*). Since the subject-matter of the Main Request is limited to the specific oligonucleotide sequences used in the experimental evidence of document

D33, the results shown in this document are relevant for the assessment of Articles 83 and 56 EPC (*infra*).

7. Thus, the board, in the exercise of its discretion, decides to admit the experimental evidence of document D33 into the appeal proceedings (Article 12(4) RPBA).

Articles 123(2), 84, 83 and 54 EPC

8. Claim 1 of the Main Request results from a combination of the subject-matter of claims 1, 2, 4-5 and 15 of the sole request considered by the examining division in the decision under appeal with disclosures found on, *inter alia*, page 2, lines 4 to 12 (several members of the JEV serogroup), page 21, lines 3 and 4 (biological sample) and page 23, line 29 to page 24, line 18 (stringent hybridization conditions). The sole request was acknowledged by the examining division to fulfil the requirements of Articles 123(2) and 54 EPC (cf. point II *supra*). The board sees no reason to raise any objections of its own under these articles or under Article 84 EPC for the subject-matter of the Main Request.

9. In the decision under appeal, there is no reference to Article 83 EPC. In view of the subject-matter of the Main Request and of the experimental evidence in the application (Example 1) and in document D33 (both using the primers and the probe with sequences SEQ ID NOs of the Main Request), the Main Request is also considered to fulfil the requirements of this article.

Article 56 EPC

10. Document D29 discloses the detection of a nucleic acid of several members of the JEV serogroup (WNV and JEV) in a biological sample (cerebrospinal fluid) by a first RT-PCR amplification using flaviviral cross-reacting primer pairs YF-1 (19 bases) and YF-3 (25 bases). In the bibliographic reference 26 of document D29 (document D1 in the present proceedings), these universal primer pairs are characterized as corresponding to the highly conserved sequence at the 3'-noncoding or untranslated region (UTR) among flaviviruses. Specimen showing positive bands with the YF primer pairs are identified by a second RT-PCR using primer pairs specific for JEV and WNV. The amplified PCR products are visualized by ethidium bromide stained band on agarose gel electrophoresis.

11. Document D30 discloses the RT-PCR amplification and detection of a nucleic acid of several members of the JEV serogroup (WNV and KUN) in a biological sample which is obtained, as in Example 1 of the application, from a lysate of virus-infected cell culture supernatant and further RNA purification. The RT-PCR primer pairs KP7 (19 bases) and KP8I (20 bases) are derived from the WNV NS3 non-structural gene and labelled at the 5'-end with biotin molecules. The resulting double-stranded biotinylated products are attached to streptavidin-coated magnetic beads and, after denaturation and removal of the unbiotinylated strands, the nucleotide sequences of the attached strands are sequenced. Biological samples containing JEV, SLE and YFV fail to show any band when using these RT-PCR KP primers.

12. Both documents D29 and D30 disclose the detection of several members of the JEV serogroup by RT-PCR using oligonucleotide primer pairs (YF and KP) in biological samples. Since the primer pairs of document D30 and those of the Main Request are derived from the same conserved region (3' UTR of the flavivirus genomes; cf. page 30, lines 12 to 15 of the application) and in view of the fact that the RT-PCR assay of document D30 fails to detect JEV and SLE, the board agrees with the appellants that document D29 represents the closest prior art document.

13. Whereas the examining division, starting from either document D29 or D30 as closest prior art, formulated three different partial technical problems (cf. page 3 of the decision under appeal and point III *supra*), the appellants, starting from document D29, have formulated the technical problem as "*the provision of methods and kits that allow a faster and more efficient amplification and detection of any or a broad variety of members of the Japanese encephalitis virus serogroup*" (cf. page 3, first full paragraph of the appellants' Grounds of Appeal and point X *supra*).

14. In view of the actual disclosure of the closest prior art document D29 and the specific subject-matter of the Main Request, the board cannot follow the examining division and considers that hindsight knowledge of the present application would be required in order to formulate the above three partial technical problems from the disclosure of document D29. Starting from this document, the board considers that the objective technical problem to be solved is the provision of an

improved system for amplifying and detecting a nucleic acid of several members of the JEV serogroup.

15. As a solution to this problem, the patent application proposes a kit according to claim 1 of the Main Request comprising the primers and the labelled probe according to features (a)-(c). The fact that both the primer pair (25 and 16 bases) and the probe oligonucleotide (28 bases) are derived from specific conserved regions of the JEV serogroup provides, *prima facie*, a kit for a high sensitivity and efficiency PCR amplification and detection of several members of this serogroup, as shown in the experimental results of document D33. Moreover, the resulting PCR amplification products are advantageously detected by the presence of the fluorescent moiety in the probe oligonucleotide, since it allows the PCR amplification and detection to occur simultaneously. Therefore, the board is convinced that the subject-matter of claim 1 of the Main Request solves the above formulated technical problem.

16. Although the skilled person was aware of the relevance of highly conserved regions of the flaviviruses genome (such as the 3' UTR region) for synthesis of suitable (universal/JEV serogroup) PCR probes (cf. point 10 *supra*) and was certainly interested in aligning the sequences of known JEV genomes in order to look for alternative conserved sequences, there is no indication in document D29, nor in any prior art on file, that would have led the skilled person to select sequences SEQ ID NO: 8 and SEQ ID NO: 15 for a primer oligonucleotide pair, let alone to combine this primer pair with a probe of sequence SEQ ID NO: 28. Moreover, there is also no hint in document D29 that would have

led the skilled person to further modify this probe oligonucleotide with a fluorescent moiety.

17. Thus, in view of the prior art on file and the specific claimed subject-matter, the Main Request is considered to fulfil the requirements of 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 grant a patent on the basis of claims 1 to 11 of the Main Request filed during the oral proceedings on 8 October 2013 and the description to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski M. Wieser