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D E C I S I O N
of 18 February 2003

Case Number: T 0120/00 - 3.3.4

Application Number: 90903862.2

Publication Number: 0460041

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Probes, Kits and methods for the detection and differentiation of mycobacteria

Patentee:
Cogent Limited

Opponent:
Bio-Rad Pasteur

Headword:
Mycobacteria/COGENT

Relevant legal provisions:
EPC Art. 87, 54, 56, 114

Keyword:
"Late-filed documents - admissibility (no)"
"Priority (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
G 0002/98, T 0081/87, T 0375/91, T 0923/92, T 0548/97, T 342/98

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0120/00 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 18 February 2003

Appellant: (Opponent)
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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 1 December 1999 concerning maintenance of European patent No. 0460041 in amended form.

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: A. L. L. Marie
V. Di Cerbo

Summary of Facts and Submissions

I. European Patent EP-0 460 041, claiming priority from GB 8903968 (22 February 1989) (**GB1**) and GB 9000411 (9 January 1990) (**GB2**) was granted on the basis of a set of 16 claims, independent claims 1, 3, 5 and 10 of which read:

"1. A nucleotide probe for the diagnosis and/or epidemiological study of Mycobacterial infection, which hybridizes with Mycobacterium tuberculosis genomic DNA obtainable by screening a Mycobacterium tuberculosis genomic library with DNA of a plasmid pUS300 of Mycobacterium fortuitum which nucleotide probe in hybridisation assay is capable of distinguishing and characterising bacterial members of the Mycobacterium complex either from each other, or from other bacteria not of the complex and wherein the probe is other than said plasmid."

"3. A nucleotide probe for the diagnosis and/or epidemiological study of Mycobacterial infection, which comprises, or hybridizes with, the nucleotide sequence depicted in Fig. 2 hereof, or its complementary sequence, or which comprises, or hybridizes with a nucleotide sequence obtainable from a genomic library of an organism of the Mycobacterium tuberculosis complex by hybridization with the nucleotide sequence depicted in Fig. 2 hereof, which nucleotide probe in hybridization assay is capable of distinguishing and characterising bacterial members of the Mycobacterium tuberculosis complex, either from each other, or from other bacteria not of the complex."

"5. A nucleotide probe for the diagnosis and/or epidemiological study of Mycobacterial infection, which comprises, or hybridizes with, part or all of the nucleotide sequence shown in either Fig.2 or Fig.4 of the drawings or its complementary sequence."

"10. A nucleotide probe for the diagnosis and/or epidemiological study of Mycobacterial infection, which comprises, or hybridizes, with, part or all of an approximately 1.9kb nucleotide sequence which, in the genome of Mycobacterium tuberculosis strain 50410, occurs immediately downstream of the 3' end of the nucleotide sequence shown in Fig.2 of the drawings."

II. An opposition was filed, but despite objections raised under Articles 54 and 56 EPC, the patent in suit was maintained by the opposition division pursuant to Article 102(3) EPC on the basis of an amended set of 13 claims, in which claims 1, 2 and 4 were identical to the corresponding claims as granted, claims 5 to 9 and 11 to 13 were the same as claims 7, 9, 10 to 12 and 14 to 16 as granted. New claim 3 and amended claim 10 read:

"3. A nucleotide probe according to claim 1, wherein the probe comprises a nucleotide sequence which comprises part of said DNA obtainable by screening, and which hybridises with a repetitive insertion element in the chromosome of Mycobacterium tuberculosis strains."

"10. A nucleotide probe according to any one of claims 1 to 5 which does not show significant hybridization to nucleic acids from Mycobacterium paratuberculosis, Mycobacterium intracellulare, Mycobacterium phlei, Mycobacterium fortuitum, and Mycobacterium malmoense."

III. An appeal was lodged by the opponent (appellant) against the decision of the opposition division.

IV. The respondent (patentee) replied to the appellant's statement of grounds of appeal.

V. Further submissions were made by the appellant in his letter of 17 January 2003, to which a scientific publication, an abstract of a scientific publication, a nucleotide sequence comparison and two experimental data (**Annexes B1** and **B2**) were annexed.

VI. Oral proceedings were held on 18 February 2003.

VII. The following documents are cited in this decision:

(2) Z. Zainuddin et al., Journal of General Microbiology, 1989, Vol. 135, pages 2347 to 2355
(3) D. Thierry et al., Nucleic Acids Research, 1990, Vol. 18, No. 1, page 188
(6) WO 91/03558, with priorities **FR1** (6 September 1989) and **FR2** (2 March 1990)
(7) Letter from Mr. J.-L. Guesdon (14 April 1994)
(9) Declaration of Mme B. Gicquel-Sanzey
(13) Letter from Mme. A. Brisson-Noel (3 October 1989)
(16) Letter of Mr. J.M. Garcia Lobo (15 July 1996)
(17) Sequence comparison between IS3411 and IS6110
(18) "Current Protocols in Molecular Biology", F.M. Ausubel et al., editors, John Wiley & Sons, Inc, 1995, Vol. 1, pages 6.3.1 and 6.0.3
(18') "Short Protocols in Molecular Biology", F.M. Ausubel et al. editors, John Wiley & Sons, Inc, pages 6-1 to 6-3

(23) Declaration of Mme. F. Portaels
(24) Declaration of Mme. B. Gicquel
(30) Letter of Mr. M. Guerineau (23 September 1996)
(31) Letters from GenBank and EMBL
(32) Declaration of Mr. J. Dale
(33) Declaration of Mr. S.H. Gillespie
(34) Experimental studies from Ms. K.D. Eisenach (Annex I of respondent/patentee's letter of 16 April 1999).

VIII. The arguments submitted by the appellant in writing and during the oral proceedings can be summarized as follows:

Late-filed submissions:

- the submission of 17 January 2003, which was already announced in the statement of grounds of appeal dated 31 March 2000 (pages 8 and 9), was an answer to the objection of the opposition division that the opponent had not established on the balance of probabilities that the teaching of GB1 (the priority document of the patent in suit) did not allow the skilled person to reproduce the results therein disclosed.

Articles 87 to 89 EPC:

- plasmid pUS300, did not hybridize with the genomic DNA of *M. tuberculosis* and did not lead to the isolation of the probes A3/1 and A3/2 described in **GB1**, the first priority document of the patent in suit. Document (32) (figures A, B and C, piste 4), document (24) (figure 6), document (23) (figures 2a to 2c) showed the absence of hybridisation under high

stringency conditions. This result was confirmed by document (34), which only showed in figures 6B, 6D, 7B, 8A, 8B and 9 a non-specific hybridisation at low stringency, that the skilled person would have disregarded, since documents (18) and (18') indicated that hybridisation results were only relevant, if they were obtained under conditions of high stringency.

- the respondent's argument according to which no hybridisation was seen in document (32) (figure C, piste 4) at high stringency, because of a partial degradation of the *M. tuberculosis* DNA was meaningless, since it only was an unproven assumption and the experiment should then have been carried out again with a non-degraded DNA.

- the fact that a hybridisation was obtained with *M. tuberculosis* strain BCG in document (32) was beside the point, since **GB1** did not use this strain.

- the skilled person was hence unable to reproduce the teaching of **GB1**, the first priority document of the patent in suit, ie the isolation of a probe hybridizing with the genomic DNA of *M. tuberculosis* and pUS300. Since no deposit of the probes was made, the patent in suit was not entitled to its first priority.

Article 54(3) EPC:

- as a consequence, document (6), which was entitled to its first priority (**FR1**) (6 September 1989) was state of the art under Article 54(3) EPC and its subject-matter novelty-destroying for the claims of the patent in suit, since it described the preparation of probes

enabling the skilled person to differentiate the bacteria of the *M. tuberculosis* complex from each other and from other mycobacteria not belonging to this complex.

Article 56 EPC:

- the technical problem that the patent in suit aimed at solving was the provision of a DNA fragment originating from *M. tuberculosis* capable to distinguish the mycobacteria of *M. tuberculosis* complex from each other and from other mycobacteria not belonging to this complex. The solution proposed was a repetitive insertion element of the IS3 family. Document (9) showed that such an element, as well as its properties and its nucleotide sequence, was disclosed in several symposia in September 1989, as confirmed by documents (16) and (30). Said sequence was also published in document (3) which was to be used against claims only entitled of the second priority of **GB2**. Document (17) also showed that the skilled person was able to isolate such a DNA fragment. The skilled person was thereby motivated and guided to the solution of the patent in suit.

IX. The arguments submitted in writing and during the oral proceedings by respondent can be summarized as follows:

Late-filed submissions:

- appellant's last submission (letter of 17 January 2003) should not be allowed into the proceedings, since it was filed one month before the oral proceedings and

did not leave enough time to the respondent for a careful consideration.

Articles 87 to 89 EPC:

- **GB1** enabled the preparation of the probe for the diagnosis of *M. tuberculosis*, since, as shown in the experiments submitted (documents (32) to (34) and (2)), there was a hybridisation between the genomic DNA of *M. tuberculosis* and the plasmid pUS300.

- the use of low or medium stringency conditions for the isolation of probes was at the priority date of the patent in suit a matter of routine for the skilled person.

- **GB1** used high stringency conditions only for the study of the properties of the A3/1 and A3/2 probes obtained, but not during their isolation.

- therefore, the patent in suit was entitled to the priority date of **GB1**.

Articles 54 and 56 EPC:

- as a consequence of the valid entitlement to priority of all the claims, there could not be any objection raised under Articles 54 and 56 EPC, since none of the documents could be validly cited against the claims.

X. The appellant requested that the decision under appeal be set aside and the European patent No. 0 460 041 be revoked.

XI. The respondent requested that the appeal be dismissed.

Reasons for the Decision

Late-filed documents

1. **Annexes B1** and **B2** of the appellant's submission of 17 January 2003 are experimental data, as additional evidence in response to the judgement of the opposition division on document (23), and aim at showing that there is no hybridisation between a genomic DNA library of *M. tuberculosis* and pUS300 (**Annex B1**), even if the hybridisation conditions of **GB1** (page 4) are followed (**Annex B2**). **Annex D** is a comparison of the sequence data of pAL5000 (the nucleotide sequence of which is according to the **Annex 1** of the appellant's submission of 16 April 1999 identical to that of plasmid pUS300) with that of *M. tuberculosis* and its purpose is to show that the identical parts are too short to allow a hybridisation between the two. **Annexes E** and **C** are, respectively, an abstract of a publication and a publication, which have been published after the filing date of the patent in suit, and show that *M. bovis* BCG also contains an insertion element virtually identical to that of the strains of *M. tuberculosis* complex.

2. In the statement of the grounds of appeal (31 March 2000, pages 8 and 9), the appellant announced that an 8 month time period was necessary for the completion of the experimental data of **Annexes B1** and **B2**. However, this submission was filed four weeks prior to the oral

proceedings before the Board and thus almost three years after the filing of the grounds of appeal.

3. The facts of the present case are comparable to those in decisions T 375/91 (17 November 1995) and T 342/98 (20 November 2001). These decisions have in common that experimental data submitted about one or two months prior to the oral proceedings before the Board of appeal were not allowed into the proceedings under Article 114(2) EPC as having been late-filed. In the case of decision T 375/91, the experimental data were also introduced about two years after the last submission of the other party. The reason invoked was that the handling of such data was more cumbersome and time-consuming than that of scientific publications, since most of the time they call for counter-experiments. Therefore, to place the other party in such a situation shortly before the oral proceedings was not compatible with the principle of fair and equal treatment of the parties.

4. The other documents were not more relevant than the documents already on file and, accordingly, the Board decides under Article 114(2) EPC not to allow the documents annexed to the appellant's submission of 17 January 2003 into the proceedings.

Claims 1, 2, 4, 11 to 16/ Right to priority

*Reproducibility of priority document **GB1** (22 February 1989)*

5. The right of priority is governed by Articles 87 to 89 EPC, which require that the European patent application and the application, the priority of which is claimed

relate to the "same invention", ie to the "same subject-matter". Following the principle that a cited document must contain an enabling disclosure for it to cause lack of novelty, the priority document must also disclose the invention claimed in the subsequent application in such a way that a skilled person can carry it out. In the present case, the enabling character of the disclosure of the priority document **GB1** has been objected to by the appellant.

6. The first priority document of the patent in suit, **GB1**, discloses the probes **A3/1** and **A3/2,** their preparation process and mentions their molecular weight and their selective hybridization behaviour with bacteria of the *M. tuberculosis* complex. The preparation process involves the screening of a DNA library of a partial *Sau3AI* DNA-digest of *M. tuberculosis* ligated to *BamHI*-digested EMBL4 using pUS301, a recombinant plasmid produced by the ligation of *EcoRI*-digested pUC19 and pUS300, the latter plasmid having its origin in *M. fortuitum*.

7. The appellant argued, on the basis of experimental data (document (23) (Fig. 2A, lane 3), document (24) (Fig. 6), document (32) (Fig. C, lane 4), document (34) (Fig. 6D)) showing that under selective, high stringency conditions no hybridisation between *M. tuberculosis* and pUS300 was obtained, that the A3/1 and A3/2 probes cannot be obtained following the process described in **GB1**. The non-specific hybridisation seen under non-selective, low and medium stringency conditions (documents (23) (Fig. 2B, lane 3), (32) (Fig. A and B) and (34) (Fig. 6B and C, Fig. 7B, Fig. 9B)) would be disregarded by the skilled person,

since, as shown by documents (18) and (18'), only results obtained under discriminative, high stringency conditions are relevant.

8. The Board, however, agrees with the respondent and is convinced that the skilled person would not dogmatically stick to high stringency conditions for hybridization and disregard positive results obtained under conditions of low or intermediate stringency as non-specific, but would adapt these conditions on a case-to-case basis in order to optimize the signal obtained, thereby following the basic principle in hybridisation experiments of looking for conditions giving a signal-to-noise ratio suitable for identifying a reasonable number of positive "spots".

9. Indeed, contrary to appellant's position, neither document (18) nor document (18'), which are both only parts of scientific manuals, state *expressis verbis* that high stringency conditions have to be used for the hybridization. Document (18) only indicates on page 6.3.1 (first paragraph) that "*the probe should hybridize only to the desired clones and not to any other clones*" and mentions under the heading "*Materials*" both "*High-stringency buffer I*" and "*Low-stringency buffer I*". This being an indication that results obtained under low stringency conditions are not sought to be disregarded. Document (18') is silent on the stringency conditions which should be used for screening a DNA library.

10. The skilled person would in the present case be comforted in this attitude, because **GB1** reports the successful isolation of the A3/1 and A3/2 probes, upon

which the skilled person has *prima facie* no reason to doubt. Furthermore, **GB1** is silent on the stringency conditions used for the hybridization of pUS300 to the DNA gene library of *M. tuberculosis* (page 3, heading "Method of isolation of the probes"). The only place in **GB1** where stringency conditions are mentioned is on page 4, when considering the properties of the already isolated A3/1 and A3/2 probes. Thus, as far as the hybridisation between *M. tuberculosis* genomic DNA and pUS300 is concerned, the skilled person is neither encouraged nor restricted by the disclosure of **GB1** (and of documents (18) and (18'), cf *supra* point 9) to the use of high stringency conditions.

11. Furthermore, the experimental data submitted by the respondent (documents (34), (32) and (33)) in response to the appellant's arguments and experimental data show that pUS300 does hybridize with *M. tuberculosis* DNA at low or intermediate stringency.

12. Document (34) in Figure 9A (ethidium bromide stained agarose gel of various *Pvu*II-digested *M. tuberculosis* DNAs) and 9B (Southern blot of gel as depicted in figure 9A using pUS300 as a probe and low stringency conditions), has as an objective to "*...see if there is hybridisation between pUS300 and M. tuberculosis complex DNA and specifically DNA from M. tuberculosis strain 50410...*". It shows in lanes 3 to 5 a hybridisation between *M. tuberculosis* strain H37Rv, *bovis* and *BCG,* respectively, and pUS300. Figure 9A showing a smear suggests that a certain extent of degradation has occurred. This does not render, in the Board's view, these DNAs unsuitable for hybridisation experiments, but only reduces the strength of the

signal obtained and hence increases the risk of "false negative". In Figure 9B, a limited number of well individualised bands gives a strong hybridisation signal. This hybridisation cannot be considered as non-specific, since lane 10 corresponding to pUC19 (a negative control not supposed to hybridize with pUS300) does not give, as expected, any band.

13. Document (32) aims at reproducing the teaching of **GB1** on the hybridisation behaviour of *M. tuberculosis* and *M. tuberculosis* BCG with pUS300 at low, medium and high stringency and gives results similar to those of document (34): although the DNAs appear to be degraded to a certain extent, hybridisation can be seen with *M. tuberculosis* at low and medium stringency and for strain BCG even at high stringency. Here again a few bands give a strong signal. This satisfies the Board that a skilled person working according to GB1 would get this result.

14. Document (33) on Figures 1(a) and (b), showing the hybridisation between *Pvu*II-digested DNA of various strains of *M. tuberculosis* complex and pUS300 and pAL5000, confirms the results of documents (32) and (34) in so far as it shows a hybridization between various strains of *M. tuberculosis* and pUS300.

15. Therefore, the Board is convinced that claims 1, 2, 4, 11 to 16 are entitled to the priority right of **GB1** (22 February 1989).

Claims 3 and 5 to 10/Right to priority

*Priority document **GB2***

16. Priority document **GB2** extends the teaching of priority document **GB1** by disclosing the restriction map of "probe 5" and the nucleotide sequence of its fragments 5B and 5C in Fig. 2 and 4. Further, **GB2** indicates on page 4 (first full paragraph) that part or all of the sequences identified in fragments 5B and 5C can be used as probes. The Board is thus convinced that the claims referring to Figure 2 or Fig 4, ie claims 3 and 5 to 10 can validly claim the priority of **GB2**.

Novelty of claims 1, 2, 4 and 11 to 16

17. As a consequence of the acknowledgement of the priority right from **GB1** (22 February 1989), document (6), the first priority of which, (**FR1**)(6 September 1989), is posterior to **GB1**, is not prior art under Article 54(3) EPC against the subject-matter of claims 1, 2, 4 and 11 to 16.

Novelty of claims 3 and 5 to 10

18. The sequence called "Formula III" in document (6) is different from that given in the priority documents, **FR1** (6 September 1989) and **FR2** (2 March 1990): not only is the sequence of document (6) much longer (1684 nucleotides instead of 1152), but it also shows a different sequence of nucleotides. For instance, by reference to the numbering of document (6), positions 520, 612 and 629 show added "GC", "TAG" and "C", respectively.

19. Decision T 923/92 (OJ EPO 1996, 564) was based on a comparable technical situation: the sequence of the t-PA molecule in the first priority document differed by three amino acids from that given in the second, third priority documents and the application as filed. The priority right from the first priority document was not acknowledged, because said document was not considered as relating to the "same molecule" as in the application.

20. When applying the *ratio decidendi* of the above mentioned decision to the present situation the Board has, for the purpose of the present decision, to take the position that document (6) cannot validly claim the priority of **FR1** (6 September 1989) and **FR2** (2 March 1990), so that document (6) is no prior art under Article 54(3) EPC against claims 3 and 5 to 10 enjoying the priority right from **GB2**, which is earlier than the filing date of document (6) (6 September 1990).

21. The appellant in their statement of grounds of appeal (page 9) indicated in the first sentence under the heading *ANouveaute@* that document (3) was novelty-destroying. However, the appellant neither in writing nor during the oral proceedings substantiated this position. Document (3) was only a basis for the appellant during the written procedure for an inventive step objection under Article 56 EPC against the claims only entitled to the priority of **GB2**. The Board, hence, will not deal with document (3) in the framework of novelty.

22. The Board is thus convinced that the subject-matter of the claims of the patent in suit meet the requirements of Article 54 EPC.

Inventive step

Claims 1, 2, 4 and 11 to 16

23. The objection under Article 56 EPC was raised by the appellant in view of documents (9) and/or (3). Document (9) states that the properties of IS6110 were disclosed in three symposia in **September 1989** and its nucleotide sequence in the two last of these symposia. Document (3) also discloses said nucleotide sequence.

24. However, since claims 1, 2, 4, and 11 to 16 enjoy the priority right of **GB1** (22 February 1989), the oral disclosures made during the above mentioned symposia (document (9)) cannot, whatever their content might have been, destroy the inventive step of these claims.

Claims 3 and 5 to 10

25. As far as claims 3 and 5 to 10, referring to the sequences of Figure 2 and/or Figure 4 and enjoying the priority right from **GB2** (9 January 1990) (cf *supra* point 16), are concerned, the question is whether the teaching of documents (3) and/or (9) were made available to the public before **GB2**. The sequence depicted in Figure 4 of the patent in suit is identical to that of post-published document (3). According to document (9), it has also been shown on a diapositive at two different symposia in September 1989. In documents (16) and (30), participants at said symposia

confirm having seen said diapositive. However, this is no evidence that the nucleotide sequence shown was the same as that of document (3) and, thus, of the patent in suit. Document (13) is even an evidence to the contrary, since on 3 October 1989, ie after the two symposia, the sequence is still said "*not to be perfectly achieved*". Further, document (7) states that said sequence has been submitted to the EMBL and Genbank databanks under the accession numbers **M29899** and **X17348** on 15 November 1989. However, document (31) originating from said databanks shows that submissions **X17348** and **M29899** were made available to the public on **21 February 1990**, whereby a publication in MEDLINE occurred on **11 January 1990**. These dates are posterior to **GB2** (9 January 1990). The Board is hence convinced that the sequence of documents (3) and (9) has been made available to the public after **GB2**. Therefore, neither document (3) nor document (9) are prior art documents in the sense of Article 54(2) EPC and have not to be considered when the inventive step of the subject-matter of claims 3 and 5 to 10 is at issue.

26. Therefore, the Board considers that the claims of the patent in suit fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided that

The appeal is dismissed.

The Registrar | The Chairwoman

P. Cremona | U. Kinkeldey