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**Datasheet for the decision
of 17 March 2016**

Case Number: T 0300/13 - 3.3.08

Application Number: 01917524.9

Publication Number: 1329522

IPC: C12Q1/70, C12N15/40, C12Q1/68

Language of the proceedings: EN

Title of invention:
METHOD OF DETECTING NORWALK-LIKE VIRUS (GI)

Patent Proprietor:
BML, Inc.

Opponent:
Zwicker, Jörk

Headword:
Noro Virus/BML INC

Relevant legal provisions:
EPC Art. 123(2), 56

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

Decisions cited:

Catchword:



Beschwerdekammern
Boards of Appeal
Chambres de recours

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

Case Number: T 0300/13 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 17 March 2016

Appellant: Zwicker, Jörk
(Opponent) Zwicker Schnappauf & Partner
Patentanwälte PartG mbB
Radlkoferstrasse 2
81373 München (DE)

Respondent: BML, Inc.
(Patent Proprietor) 21-3, Sendagaya 5-chome,
Shibuya-ku
Tokyo 151-0051 (JP)

Representative: Krauss, Jan
Boehmert & Boehmert
Anwaltspartnerschaft mbB
Patentanwälte Rechtsanwälte
Pettenkoferstrasse 20-22
80336 München (DE)

Decision under appeal: **Interlocutory decision of the Opposition**
Division of the European Patent Office posted on
12 December 2012 concerning maintenance of the
European Patent No. 1329522 in amended form.

Composition of the Board:

Chairman M. Wieser
Members: B. Stolz
E. Kossonakou

Summary of Facts and Submissions

- I. The appeal lies against the decision of the opposition division to maintain European patent No. 1329522 in amended form. The opposition division decided that claims 1 to 16 of the patent proprietor's (in the following respondent's) main request, filed on 18 September 2012, comply with the requirements of the EPC.
- II. In the statement setting out the grounds of appeal, the opponent (in the following appellant) argued that the request allowed by the opposition division did not comply with the requirements of Articles 123(2) and 56 EPC.
- III. In the course of the appeal proceedings, both parties made multiple submissions in writing.
- IV. The parties were summoned to oral proceedings. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), annexed to the summons, informed them of the preliminary non-binding opinion of the board on some of the issues of the appeal proceedings.
- V. Oral proceedings were held on 17 March 2016.
- VI. The subject matter of the claimed invention is a method for detecting Norwalk-like virus of genotype I (in the following Norwalk-like virus GI).
- VII. Claim 1 of the main request reads as follows:

"1. A method of detecting a virus in a specimen, whereby a Norwalk-like virus (GI) is detected by using

a nucleic acid sequence corresponding to positions 5276 to 5380 and/or a nucleic acid sequence corresponding to positions 5319 to 5446 of the nucleotide sequence of the cDNA of the prototype (standard strain) of the Norwalk-like virus (GI), whereby the prototype of the Norwalk-like virus is the strain M87661 Norwalk, and wherein the nucleic acid sequence corresponding to positions 5276 to 5380 and/or a nucleic acid sequence corresponding to positions 5319 to 5446 of the nucleotide sequence of the cDNA is a gene amplification product obtained by applying, for attaining gene amplification, gene amplification means to genes obtained from the specimen, wherein the gene amplification means comprises gene amplification primers engineered on the basis of at least two nucleic acid sequences each having consecutive 10 or more bases which are selected from the nucleic acid sequence."

Claims 2 to 11 define specific embodiments of the method of claim 1, and claims 12 to 16 define a kit and specific embodiments thereof for performing the methods of the preceding claims.

VIII. The following documents are referred to in this decision:

D3: Legeay, O. et al. (2000), "Simplified procedure for detection of enteric pathogenic viruses in shellfish by RT-PCR", J. Virology Methods 90: 1-14, available online as of September 25 2000;

D9: submitted by opponent/appellant, alignment of: complete genome sequence of NLV Genogroup I strain M87661 according to D8, complete genome sequence of NLV Genogroup I strain Southampton

according to GeneBank entry L07418, complete genome sequence of NLV Genogroup I strain KY89 according to GeneBank entry L23828, complete genome sequence of NLV Genogroup I strain Desert Shield Virus according to GeneBank entry U04469, using the alignment algorithm ClustalW2 available online at EBI-EMBL;

D13: Häfliger, D. et al. (1997), "Seminested RT-PCR systems for small round structured viruses and detection of enteric viruses in seafood", International J. of Food Microbiology 37: 27-36;

D24: Moe C.L. et al. (1994), "Application of PCR To Detect Norwalk Virus in Fecal Specimens from Outbreaks of Gastroenteritis", J. of Clinical Microbiology 32(3): 642-648.

IX. The appellant's arguments, as far as relevant for this decision, can be summarised as follows:

Article 123(2) EPC

Claim 1 required the use of a nucleic acid sequence corresponding to the indicated nucleic acid positions of the prototype cDNA. A cDNA had to be synthesized on the basis of the isolated viral genomic RNA. The Norwalk-like virus (GI) had a single stranded "plus" RNA genome (see Section [0004] of the Opposed Patent), and the "cDNA" referred to in claim 1 had a nucleic acid sequence complementary to the sequence of the single stranded RNA virus genome. According to page 14, lines 7-12 and lines 22-25, the term "corresponding to" referred to a nucleic acid sequence hybridising to the nucleic acid sequence referred to in claim 1. Thus, claim 1 required that the nucleic acid sequence used

and corresponding to positions 5276 to 5380 and/or 5319 to 5446 be complementary to the sequence of the reference cDNA. Claim 1 further required that the nucleic acid sequence used was a gene amplification product of the cDNA and as such comprised a sequence complementary to the sequence of the cDNA.

If the nucleic acid sequence used was complementary to the sequence of the cDNA, it could be used as a tool to detect the cDNA. In contrast, if the sequence of the nucleic acid sequence used was identical to the sequence of the cDNA, it could not anneal and could only be used as an index.

The patent application as filed disclosed at page 11, lines 2-21, that Norwalk-like viruses (GI) were detected "by making use of the nucleotide sequence of a nucleic acid in a highly conserved gene region". This use was further specified in the following sentence as "by use of, as an index ...". Clearly, the use referred to in the first sentence was further specified in the second sentence. Thus, the description only disclosed the use of the nucleic acid as an index but did not provide any basis for the use of the specified sequences as a tool for detecting Norwalk-like viruses (GI). Therefore, the subject-matter of claim 1 and of the dependent claims 2 to 16 did not fulfil the requirements of Art. 123(2) EPC.

Article 56 EPC

As claim 1 encompassed the use of many primers which could not provide an improved specificity, the technical problem, starting from document D13, consisted in the provision of an alternative method for the detection of Norwalk-like viruses GI.

The sensitivity of the claimed method was not 100% as alleged by the respondent. According to paragraph [0022] of the patent, 44 patient samples were analysed and 18 thereof were GI positive. Only 16 of these 18 samples were found positive by the claimed method, which equaled to a sensitivity of only 88% (16/18).

Document D9 showed that the nucleotide sequences corresponding to positions 5276 to 5380 exhibited 86% sequence identity and the nucleotide sequence corresponding to positions 5319 to 5446 exhibited 73% sequence identity. Primer SRI-2 of document D13 hybridized to the sequence corresponding to positions 5276 to 5380 and primer SRI-3 to a region which also showed 73% identity. Thus, the argument that the claimed method targeted more highly conserved regions and was therefore more sensitive was not tenable. Moreover, the relevant factor for sensitivity was the degree of sequence conservation at the site of primer binding. The primers disclosed in document D13 bound to regions which, according to document D9, exhibited 100% and 90% sequence identity. Therefore, the method disclosed in document D13 was equally sensitive and the technical problem consisted, also on this basis, in the provision of an alternative method.

Even if the underlying technical problem was defined as the provision of an improved method, the claimed solution was obvious on the basis of the teaching of document D13 in combination with that of document D24. Document D24 instructed the skilled person to look for regions with a high degree of sequence conservation. All the skilled person had to do was to look for regions of the genome with a higher degree of sequence conservation. By following the instructions of document

D24 and based on the alignment of known virus sequences as described in document D13, the skilled person would have arrived at the claimed invention in an obvious way.

- X. The respondent's arguments, as far as relevant for this decision, can be summarised as follows:

Article 123(2) EPC

There was no technical difference between a method of "*detecting by use of a nucleic acid as an index*" and a method of "*detecting by use of a nucleic acid sequence*". The omission of the term "*as an index*" did therefore not lead to a violation of the requirements of Article 123(2) EPC.

Article 56 EPC

The appellant did not provide any experimental evidence to support its allegation that the claimed method did not represent an improvement over the method disclosed in document D13. As demonstrated by paragraph [0056] of the patent, the sensitivity of the claimed method was 100%. The initial design of the method, as described in paragraph [0022], was irrelevant for the determination of the degree of sensitivity achieved by it. Based on the results described in paragraph [0056] and starting from document D13 as closest prior art, the problem to be solved consisted in providing an improved method of detecting a Norwalk-like virus GI.

Document D13 taught a semi-nested PCR method. It did not provide any incentive at all to improve the disclosed method, certainly not in the way of the patent. Document D9, which did not belong to the state

of the art but was produced by the appellant, could not fill this gap. Instead of amending the method of document D13 according to present claim 1, the skilled person, as demonstrated by document D3, could also have optimized the sample preparation in order to improve the detection of a Norwalk-like virus GI. The claimed solution was not obvious.

XI. The appellant requested that the decision under appeal be set aside and the patent be revoked.

XII. The respondent requested that the appeal be dismissed.

Reasons for the Decision

Article 123(2) EPC

1. The appellant argued that the patent application as filed only disclosed the use of the nucleic acid sequence of claim 1 "*as an index*". Due to the absence of this feature, claim 1 of the main request, read in connection with page 14 of the description, referred to the use of a nucleic acid sequence which hybridised to the strand of the cDNA which was complementary to the viral ribonucleic acid sequence. The application as filed however referred to the use of a nucleic acid sequence identical (not complementary) to the reference cDNA mentioned and could only be used as an index but not as a tool. The patent application as filed thus provided no basis for the claimed subject matter.

2. According to claim 1 (cf. item VII, above), a Norwalk-like virus GI is detected by using a nucleic acid sequence which corresponds to nucleic acids 5276 to 5380 and/or to positions 5319 to 5446 of the nucleotide sequence of the cDNA of the prototype virus. The

nucleic acid used is defined as a gene amplification product, obtained by applying primers selected from the nucleic acid sequence corresponding to positions 5276 to 5380 and/or to positions 5319 to 5446 of the cDNA of the prototype virus to genes obtained from a specimen. The board considers this language clear. The nucleic acid sequence of claim 1 is an amplification product and as such a double stranded DNA, comprising a strand matching the prototype sequence and a complementary strand. All of the afore mentioned features of claim 1 are directly and unambiguously disclosed in claims 3 and 4 of the patent application as originally filed.

3. The application as originally filed (page 11) discloses *"means for rapidly and accurately detecting NLVs (GI) by making use of the nucleotide sequence of a nucleic acid in a highly conserved gene region [...] which is determined through gene analysis [...]. Specifically, the present invention provides a detection method for Norwalk-like viruses (GI) [...] by use of, as an index, the nucleic acid fragment of a complementary nucleotide sequence or complementary nucleotide sequences [(...)] corresponding to the 5201- to 5700-positions (preferably 5276- to 5446-positions, more preferably 5276- to 5380-positions and/or 5319- to 5446-positions) of the nucleotide sequence of the cDNA of the prototype (standard strain) of NLVs (GI)."*

4. The first sentence of the cited paragraph refers to the use of highly conserved regions of the viral genome for the detection of a Norwalk-like virus GI. The following sentence states that a complementary nucleotide sequence corresponding to a highly conserved, specifically defined region of the reference cDNA is used as an index, i.e. used to indicate the presence of the virus. The second sentence does not, however, limit

in any way how the amplification product corresponding to the highly conserved region is used to indicate the presence of a Norwalk-like virus GI. Therefore, omitting the term "as an index" from claim 1 does not extend the claimed subject matter beyond the disclosure as originally filed.

5. Finally, the board does not agree with the appellant's interpretation of claim 1 in the light of page 14 of the description. The paragraph relied on by the appellant (page 14, lines 7 to 12) to arrive at its interpretation relates to the design of appropriate primers. It specifies that "*the gene region which is employed as a basis for establishing gene amplification primers must at least meet the requirement that **the resultant gene amplification product contains a complementary nucleotide sequence corresponding to a conserved region** or a highly conserved region (which will be described hereinbelow)*" (emphasis added by the board).
6. The passage emphasized above is unambiguous and cannot be interpreted as meaning that said complementary nucleotide sequence is **itself** complementary to the nucleotide sequence corresponding to the highly conserved region. The appellant's argument, that claim 1 covers subject matter which has not been disclosed in the application as filed, must therefore also fail if page 14 of the description is used to interpret the term "corresponding to".
7. The board is therefore satisfied that the subject-matter of claim 1 is directly and unambiguously derivable from the patent application as originally filed. The main request meets thus the requirements of Article 123(2) EPC.

Article 56 EPC

8. Document D13 discloses a semi-nested RT-PCR assay for the detection of small round structured viruses (SRSVs) also known as Norwalk-like viruses (cf. page 28, left column). This assay uses one primer (SRI-2) matching a sequence within the genomic region indicated in claim 1 and two primers located further downstream of the regions indicated in claim 1. The primers were designed based on an alignment of several known Norwalk-like virus (GI) RNA sequences, the identification of conserved regions within those sequences and with the help of a primer analysis software (page 29, "2.5 Computer analysis"). In order to achieve high specificity and sensitivity, a semi-nested PCR system and optimized reaction conditions were developed (page 29, "2.8 PCR"; page 31, right column, first full paragraph).
9. Starting from document D13, the respondent defined the problem to be solved as the provision of an improved method of detecting Norwalk-like virus GI.
10. As a solution to this problem, the patent proposes the method of claim 1.
11. According to paragraph [0056] of the patent, 44 patient samples were tested for the presence of Norwalk-like viruses GI. Of the 44 samples 16 tested positive whereas the remaining 28 were negative. All of the 28 GI negative samples tested positive for the presence of Norwalk-like viruses GII (genotype II). From this, the respondent concluded that the claimed method provided 100% sensitivity.

12. The appellant contested this figure on the basis of paragraph [0022] of the patent which mentioned the determination of 17 new partial and one complete new GI sequences isolated from the 44 samples. On this basis the sensitivity of the claimed method was only 88% (16/18).
13. Paragraph [0022] refers to the number of sequences used for the sequence alignment. Although it literally refers to 17 new partial sequences, the sequence numbering in brackets appears to list only 16 such sequences. Moreover, it cannot be derived from paragraph [0022] from how many of the 44 samples these new sequences were derived. Therefore, the board is not convinced by appellant's argument.
14. Although document D13 repeatedly refers to the achievement of high sensitivity and specificity, no specific figures or data are presented in this respect. Rather, high sensitivity and specificity seem to be inferred from the notion that: "*semi-nested PCR systems are among the most sensitive detection methods available today*" (page 34, right column, "Discussion"). A direct comparison of the sensitivity of the method disclosed in document D13 with that of the claimed method is therefore not possible.
15. A party to EPO proceedings who raises an objection bears the burden of proving it. In opposition proceedings, the patent proprietor is given the benefit of the doubt if a party makes contrary assertions regarding facts barring patentability which they cannot substantiate beyond reasonable doubt and which the EPO is unable to establish of its own motion (cf. Case Law of the Boards of Appeal, 7th edition, III.G.5.1.1, page 604).

16. In the present case, the patent provides experimental data upon which the respondent relied for the definition of the technical problem underlying the claimed invention. The appellant based its argument that the claimed method did not provide an improvement over the prior art, i.e. did not solve the problem defined by the respondent, on a comparison of the degree of homology of the primer sequences with several known sequences (document D9). No comparative experimental data were however provided.

17. Sensitivity and specificity of a PCR reaction depend not only on the selected primers but also on the assay conditions (apart from other factors such as sample preparation). Documents D3 and D13 describe that the sensitivity of their assays was improved by optimizing the annealing temperature and the MgCl₂ concentration (D3, page 5, "RT-PCR assays"; D13, page 31, right column, first full paragraph). Among others, these two parameters affect binding of the specific primer pairs to their target sequences. Therefore, the comparison of the homologies of primer sequences alone is not sufficient to refute the respondent's argument that the claimed method provides an improvement over the method disclosed in document D13. To do so, experimental evidence would be needed. Without comparative experimental data, the appellant's arguments do not convince the board that the technical problem as defined in point 9 above has not been solved.

18. In the light of the data provided in paragraph [0056] of the patent, the subject-matter of claim 1 credibly solves this technical problem.

19. It remains to be established whether the claimed method involves an inventive step.
20. The appellant argued that the disclosure of document D13 combined with that of document D24 rendered the claimed solution obvious.
21. The goal of document D13 was to provide a sensitive and specific PCR method for the detection of Norwalk-like viruses of genotypes I and II. The document identifies suitable regions for the design of primers (cf. point 8 above).
22. Document D24 addressed the problem of detecting Norwalk-like viruses in fecal specimens. Its authors evaluated three primer pairs, two from a presumably conserved region of the RNA-dependent RNA polymerase gene and one from a region which was expected to be more serotype specific (page 642, right column, last paragraph). The authors found that none of the isolated strains had sequences that were completely homologous with the reference Norwalk-like virus sequence and that the first open reading frame comprising the RNA polymerase gene represented the most likely region for the design of broadly reactive PCR primers (page 647, left column, first full paragraph). The authors concluded that although a primer pair targeting the polymerase gene was broadly reactive, the efficiency to detect a Norwalk-like virus might be affected by sequence diversity in a portion of the RNA polymerase region. The successful application of RT-PCR might therefore depend on the use of multiple primer pairs or primers made against regions of the genome that are more conserved (page 647; left column, last paragraph).

23. The appellant argued that the skilled person starting from document D13 would have turned to document D24 and, by designing primers to the regions with the highest degree of sequence conservation, would have arrived at the claimed solution in an obvious way.

24. In order to provide a highly sensitive assay for the detection of a Norwalk-like virus GI, the authors of document D13 used sequence alignments of known viral nucleic acid sequences and known tools for the design of optimal amplification primers. Their primers amplify a different (albeit partially overlapping) region of the viral genome than those of the opposed patent. Upon inspection of Figure 1 of the patent, it appears that the authors of document D13 already designed primers binding to regions of the viral genome with the highest degree of sequence conservation, i.e. the regions around positions 5350 and 5600 (cf. Figure 1 of the patent and Table I of document D13).

25. For the following reasons, the board is not convinced that the claimed solution was obvious. First, the board is not convinced that the skilled person would have primarily looked for regions of the genome with even higher degrees of sequence conservation. As mentioned above, the primers of document D13 already bind to regions of the viral genome with a very high degree of sequence conservation. It could also have followed the proposal in document D24 to use multiple primer pairs, i.e. it could for instance have used those disclosed in documents D13 and D24. Alternatively it could have tried to improve sample handling and preparation as also suggested in document D24 (cf. page 647, left column, 2nd full paragraph). Second, even if it would have looked for different primers binding to the regions of the viral genome with the highest degree of

sequence conservation, it would not have arrived at the claimed solution in an obvious way. While the skilled person, applying the teaching of the prior art, could have selected primers binding to different regions than those described in document D13, nothing directed it to select the specific regions mentioned in claim 1 in order to provide an improved method of detecting a Norwalk-like virus GI.

26. Therefore, the claimed method involves an inventive step and the main request meets the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:



A. Wolinski

M. Wieser

Decision electronically authenticated