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Datasheet for the decision of 14 February 2014

Case Number: T 0307/10 - 3.3.08

Application Number: 02765125.6

Publication Number: 1436420

IPC: C12Q1/68

Language of the proceedings: EN

Title of invention:
Test Strip Assay System and Method for the Detection of Specific Nucleic Acid Sequences

Patent Proprietor:
Soufla, Giannoula

Opponent:
Medicon Hellas S.A.

Headword:
Assay system/SOUFLA

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

Keyword:
Main request
Novelty - (yes)
Inventive step - (yes)

Decisions cited:

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

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Case Number: T 0307/10 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 14 February 2014

Appellant: (Opponent)	Medicon Hellas S.A. 5-7 Melitona 153 44 Gerakas/athens (GR)
Representative:	Rasmussen, Torben Ravn Awapatent A/S Rigensgade 11 1316 Copenhagen K (DK)
Respondent: (Patent Proprietor)	Soufla, Giannoula 84 Anakreontos str 15771 Zografou Attikis, Athens (GR)
Representative:	Friede, Thomas Wächtershäuser & Hartz Patentanwaltspartnerschaft mbB Ottostrasse 4 80333 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 7 December 2009 rejecting the opposition filed against European patent No. 1436420 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman: M. Wieser
Members: T.J.H. Mennessier
D. Rogers

Summary of Facts and Submissions

I. The opponent (appellant) lodged an appeal against the decision of the opposition division dated 7 December 2009, whereby the opposition filed against European patent No. 1 436 420, which had been granted on European patent application No. 02765125.6, published as the international application WO 03/33735, was rejected.

II. The opposition was filed on the grounds of Article 100(a) (lack of novelty and inventive step, Articles 54 and 56 EPC) and 100(b) EPC. At the oral proceedings before the opposition division the appellant withdrew its objection under Article 100(b) EPC.

III. The statement setting out the grounds of appeal was accompanied by two new documents, hereinafter referred to as documents D16 and D17. Additionally, the appellant requested that document D14, not admitted into the opposition proceedings, be admitted into the appeal proceedings. The patent proprietor (respondent) replied on 18 October 2010 by filing submissions together with three auxiliary requests. Oral proceedings were requested by both parties.

IV. The board issued, as an annex to the summons to oral proceedings, a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), expressing its preliminary and non-binding views and indicating that it was inclined to dismiss the appeal.

V. The appellant withdrew its request for oral proceedings.

VI. The board informed the parties that the scheduled oral proceedings were cancelled.

VII. The set of claims as granted (main request) consists of 16 claims of which claim 1 reads as follows:

"1. A dipping test strip assay system for the qualitative and/or quantitative one step determination of a specific nucleic acid sequence (16, 19) in a liquid sample, comprising:

A) a test strip (1), said test strip (1) having four distinct areas:

a) a first area (2), which contacts said sample or assay-development solution;
b) a second area (3) onto which a visible signal producing substance is dried;
c) a third area (6) for signal development and assay completion control, comprising a first zone (8) containing immobilized streptavidin and a second zone (9) containing an immobilized oligonucleotide; and
(d) a fourth area (7) to withhold the excess of said liquid sample;

characterized in that

said visible signal producing substance consists of colloidal gold particles (14) conjugated with an oligonucleotide (15) of a certain length and having a defined nucleotide sequence and can be solubilized by the liquid sample or assay-development solution; said immobilized oligonucleotide (12) in the second zone (9) of said third area (6) of the test strip (1) has a nucleotide sequence complementary to the nucleotide

sequence of said oligonucleotide (15) conjugated with said colloidal gold particles (14);

and in that

said dipping test strip assay comprises further:

B) a distinct reagent (17; 20, 21) consisting of one or more oligonucleotides that hybridize specifically with said hybridized nucleic acid and that confer to said hybridized nucleic acid the ability to simultaneously hybridize to said oligonucleotide (15) that is conjugated with said colloidal gold particles (14) and to bind to said immobilized streptavidin of the first zone (8) of said third area (6)."

Claims 2 to 9 are dependent on claim 1.

Claim 10 is directed to a method for the qualitative and/or quantitative one-step determination of a specific nucleic acid sequence in a liquid sample using a dipping test strip assay system according to any one of claims 1 to 9.

Claims 11 to 13 are dependent on claim 10.

Claim 14 is directed to the use of the dipping test strip assay according to any one of claims 1 to 9 or of the method of any one of claims 10 to 13 for the detection and/or determination of specific nucleic acid sequences in a sample, which are indicative of a disease.

Claims 15 and 16 are dependent on claim 14.

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

(D5) English translation of document D5' (see below) comprising a front page entitled "*Detailed Form for the Registration of Intellectual Property*" regarding two studies on dry reagents for the detection of DNA by the inventor of the patent at issue and, attached thereto, a technical report of 15 pages numbered 1/414 to 15/428 with page 1/414 carrying the reference "No. 3124/31-7-2001, and entitled "*Dipping test strip assay system and assay method for the detection and/or determination of specific nucleic acid sequences*"

(D5') Document in Greek language dated 31 July 2001

(D6) J. Klepp, Biochemica (Roche Molecular Biochemicals), No. 2, 2000, 3 pages entitled "DNA Detection Test Strip for the Rapid Detection of Digoxigenin- or Biotin-labeled PCR products"

(D6a) Instruction manual of Roche for the 'Test strips for the rapid detection of digoxigenin-or biotin-labeled PCR products', Version 2, March 2000

(D10) Patent application CA 2 223 705 A1 (published on 25 August 1999)

(D11) Letter of the National Library of Greece to the inventor/respondent dated 13 February 2008 (in Greek language)

(D11a) English translation of document D11

(D14) 'Affinity Chromatography, Methods and Protocols' in 'Methods in Molecular Biology', Edited by P. Bailon et al., Humana Press, Totowa, New Jersey, 2000, pages 1 to 5 (Chapter 1) and 141 to 153 (Chapter 14)

(D15) One page document in Greek language from the National Library of Greece dated 5 October 2009

(D15a) English translation of document D15

(D16) Patent application CA 2 256 943 A1 (published on 24 June 1999)

(D17) J. Chandler et al., IVD Technology, March 2001, as retrieved from an internet website, 10 pages

(D18) English translation of Greek law 2121/1993 regarding copyright, related rights and cultural matters, as retrieved from the WIPO's internet site

IX. The submissions made by the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

Admissibility of documents D14, D16 and D17

The content of document D14 represented common general knowledge in all technical fields concerned with the use of affinity binding partner pairs. Although D14 focused on affinity chromatography, Table 2 on page 3 listed various techniques stemming from affinity chromatography. A skilled person would have known from D14 that all the affinity binding pairs mentioned therein, in particular the oligonucleotides described

in Chapter 14, could replace any other affinity binding partner pairs commonly employed in any technology involving specific and selective binding of an entity fitted with one binding partner to another entity fitted with a complementary binding partner. In particular this was the case for a dipstick assay, independent of whether the assay was based on a chromatographic technique or not. Therefore, document D14 was relevant for the assessment of inventive step and should have been admitted into the opposition proceedings.

Document D16, co-authored by one of the inventors, related to the same dipstick assay as the one described in documents D6 and D6a. Therefore, D16 was relevant for the assessment of novelty.

Document D17 dealt with the problem of false signals in gold-based rapid tests. Although it did not discuss oligonucleotides in the context of dipstick assays, it dealt with stability of protein-gold conjugates. Considering that oligonucleotides and proteins interacted with gold surfaces via the same type of interactions, the information provided by document D17 could be applied to evaluate the stability of oligonucleotide-gold conjugates. Document D17 was therefore relevant for the assessment of inventive step.

Main request

Novelty (Article 54 EPC)

Although document D11, which referred to Articles 2 to 4 of the Greek law 2121/1993, certified that the technical report of document D5 has not been made

available to the public for "reading-studying-researching", it could not be derived therefrom that document D5 had not been made publicly available. In particular, Article 4(1) provided that the author could decide on "the time, place and manner in which the work shall be made accessible to the public". Therefore, the claimed invention was not new over document D5.

Inventive step (Article 56 EPC)

Document D6 represented the closest state of the art. The technical problem was the provision of an alternative dipping test strip assay system. The only difference between the dipstick according to claim 1 and that of document D6 resided in the binding pairs used in the detection and in the control regions of the strip. Document D10 related to a dipstick assay system for detecting an end product in a PCR reaction. This system employed gold nanoparticles to form one or two visible bands in the test strip.

If the skilled person had replaced the monoclonal anti-DIG antibody on the gold nanoparticles in the strip of document D6 with an oligonucleotide as suggested in document D10, it would have been immediately evident that a pair of complementary oligonucleotides had to be used as the other binding partner pair.

The alleged advantages of the test strip of the assay system of claim 1, namely facilitated synthesis and improved stability, were not documented in the patent at issue.

Therefore, following the suggestion of document D10 to use an oligonucleotide in place of an antibody, the skilled person, starting from the dipstick of document

D6, would have arrived at the assay system of the main request in an obvious way.

X. The submissions made by the respondent, insofar as they are relevant to the present decision, may be summarised as follows:

Admissibility of documents D14, D16 and D17

Late filed document D14 was a textbook relating to affinity chromatography , i.e. to a purification technique which at its face value was irrelevant for the purpose of detecting a nucleic acid in the format of a dipstick assay. The opposition division had correctly decided not to admit D14 into the opposition proceedings.

Document D16, filed only in appeal proceedings, did not disclose any nucleic acid specific hybridization with an oligonucleotide conjugated to colloidal gold particles and a target DNA. Rather it disclosed colloidal gold particles only which were bound to antibodies and was irrelevant for the question of novelty.

Document D17 was silent with regard to oligonucleotides and, therefore, could not provide any information on the stability of gold oligonucleotide conjugates compared to gold antibody conjugates. It did not support the appellant's position that antibodies conjugated with gold particles were equally abundant, stable and reliable as oligonucleotides. Therefore, document D17 was irrelevant for the assessment of inventive step.

Main request

Novelty (Article 54 EPC)

Document D5' did not belong to the state of the art. The burden of proof to demonstrate the public availability of the intellectual property documents deposited with the National Library of Greece lay with the appellant. No evidence had been presented to this effect. Documents D11/D11a and D15/D15a showed that, according to the provisions of the Greek copyright law, the deposit of a document with the National Library did not render it available to the public. No actual release of a copy of the technical report of document D5' occurred before the priority date of the patent at issue.

Inventive step (Article 56 EPC)

Document D6 represented the closest state of the art. It described a DNA detection test strip for the rapid detection of digoxigenin- or biotin-labeled PCR products. The technical problem was the provision of an improved dipping test assay for qualitative and/or quantitative one-step determination of a specific nucleic acid sequence. The claimed dipping test assay strip was associated with a number of advantages pointing to the presence of an inventive step, namely lower production costs, facilitated synthesis, improved stability, conjugation of oligonucleotides at a higher molecular ratio than for antibodies, increased sensitivity and easy solubility.

Document D10 described a further one-step assay method for detecting the end product of a nucleotide amplification process, involving the use of a solution

comprising colloidal gold particles conjugated with a molecule/ligand containing a segment of DNA complementary to the DNA to be detected. Thus, in contrast to the test strip according to claim 1, these colloidal gold particle conjugates were not contained in the test strip. Furthermore, in the test strip of document D10, the ligand was not the probe as such. Document D10 was also silent on a distinct reagent consisting of one or more oligonucleotides being capable at the same time to hybridize to the oligonucleotide gold particle conjugates and to bind to the immobilised streptavidin in the third area of the test strip. Document D10 also failed to describe a reagent such as the distinct reagent of part B of claim 1, which allowed the design of an universal dip stick assay that, nevertheless, was specific for a nucleic acid to be analysed. Moreover, if a skilled person had considered to replace any one of the antibodies of the dipstick of document D6 with an oligonucleotide, he/she would not have known that the complementary binding partner of the replaced antibody would also need to be replaced.

Therefore, a skilled person, using the teaching of document D10 to modify the test strip of document D6, would not have arrived at the assay system of claim 1 of the main request.

XI. The appellant requests that the decision under appeal be set aside and the patent be revoked. The respondent requests that the appeal be dismissed or the patent be maintained on the basis of one of the three auxiliary requests filed with the letter of 18 October 2010.

Reasons for the Decision

Review of the decision of the opposition division not to admit document D14 into the opposition proceedings

1. Document D14 was submitted by the appellant (then opponent) with its letter of 14 September 2009 two months in advance of the oral proceedings before the opposition division. At the oral proceedings the opposition division found that D14 was not *prima facie* relevant and the document was therefore not admitted into the opposition proceedings.

2. Document D14 consists of extracts (Chapters 1 and 14) selected from a textbook dealing with methods and protocols for affinity chromatography. Chapter 1 provides a very brief overview of the technique, while Chapter 14 concerns the use of DNA affinity chromatography for purification of polynucleotides and polynucleotide-binding proteins. Specific procedures used for the synthesis of (dT)18-silica and -Sepharose and for enzymatic primer extension using DNA and RNA templates are described. Document D14, *prima facie*, does not contain any disclosure generally useful for the design of a complete dipping test strip assay system, let alone one according to claim 1 as granted.

3. The board concludes that the opposition division has properly exercised its discretion when deciding not to admit document D14 into the opposition proceedings and has therefore no reason to set this decision aside.

Admissibility of documents D16 and D17 into the appeal proceedings

4. Documents D16 and D17 were submitted by the appellant together with its statement of grounds of appeal.

5. Document D16 is a Canadian patent application published on 24 June 1999, i.e. some months before documents D6 and D6a. The author of document D6 is named as one of the inventors in document D16. The disclosure of documents D6 and D6a is found within document D16 (see Example 1).

6. Document D16 does not disclose colloidal gold particles conjugated with an oligonucleotide. Thus, an essential technical feature of a dipping test strip assay system according to claim 1 of the main request is not disclosed in document D16. The disclosure in document D16 is therefore no more relevant than the disclosure in documents D6 and D6a. Thus, document D16 is not admitted into the procedure.

7. Document D17 deals exclusively with the handling of false signals in membrane-based lateral-flow immunoassay tests involving gold particles conjugated to antibodies. It is totally silent about interactions between gold particles and oligonucleotides and does not contain any pointer allowing an extrapolation of its teaching to the design of assay systems based on the use of gold particles conjugated to oligonucleotides. Therefore, document D17 is considered to be *prima facie* irrelevant for the assessment of inventive step and is not admitted into the procedure.

Main request

Article 54 EPC (novelty)

8. The appellant argues that the main request lacks novelty over document D5'.

9. The front page of document D5 (English translation of document D5') is a form filed for the registration of an intellectual property right, dated 31 July 2001. This form refers to an intellectual creation to be registered in the name of the author who is the respondent. Said intellectual creation consists of two studies both entitled "*Study on dry reagents for the detection of DNA*".

10. Document D11a (English translation of document D11) is a letter, dated 13 February 2008, from the National Library of Greece to the respondent. It clarifies that the registration referred to in document D5' was made under Greek law 2121/1993 regarding copyright, related rights and cultural matters (see document D18). In document D11a, it is certified that the documents deposited for the registration were not made available to the public.

11. Document D15a (English translation of document D15) is a certificate, dated 5 January 2009, delivered by the National Library of Greece. It certifies that no request to obtain certified copies of the deposited material referred to in document D5' was submitted to the National Library of Greece during the period beginning on 31 July 2001 and ending on 16 October 2001 (included), the priority date claimed for the patent at issue.

12. Attached to the registration form of document D5' is a set of 15 pages, numbered '414' to '428'. According to the English translation provided by document D5, page '414' relates to a dipping test strip assay system and an assay method for the detection and/or determination of specific nucleic acid sequences. The same reference, namely "No. 3124/31-7-201" appears on both the registration form and page 414.. Therefore, pages 414 to 428 are considered to constitute the second 'intellectual work' mentioned on the registration form of document D5'. Their content is almost identical to the disclosure contained in the description of the patent at issue.

13. Document D5' proves that, on 31 July 2001, the inventor/respondent applied for protection under the provisions of Greek law 2121/1993 regarding copyright, related rights and cultural matters. There is no evidence on file that said application had the effect that the content of pages 414 to 428 (see above) was made available to the public without the consent of the inventor.

14. The appellant argues in its statement of grounds of appeal, that "*[H]owever, from these articles [articles 2, 3 and 4 of the Greek law 21212/1993; added by the board] it cannot be derived that D5 was not publicly available*" (see first paragraph on page 8 of the statement of grounds). This mere statement cannot discharge the appellant from its burden of proof. The appellant has to provide evidence showing that the technical report of pages 414 to 428 attached to the registration form of document D5' was indeed made available to the public before the priority date.

15. As this evidence has not been provided, document D5' does not form part of the state of the art for the purpose of considering the novelty of the main request.

16. The dipping test strip system of document D16, the second document referred to by the appellant in this respect, lacks at least one essential feature of the assay system according to claim 1 (see point 6 supra).

17. Accordingly, the subject-matter of claim 1 of the main request is novel and meets the requirements of Article 54 EPC.The same applies to the subject-matter of claims 2 to 16.

Article 56 EPC (inventive step)

18. The assessment of inventive step is performed following the problem-and-solution approach. In a first step, the closest prior art is determined. In this respect, the appellant has referred to two documents, documents D6 and D14. As document D14 is not admitted into the proceedings (see points (1) to (3) above), document D6 is considered to represent the closest prior art document.

19. Document D6 describes a DNA test strip which allows to routinely check the success of a PCR amplification of digoxigenin- or biotin-labeled products. The test strip contains: (i) 0,2 µl anti-digoxigenin (DIG) mouse monoclonal antibody conjugated to gold particles (anti-DIG-gold) and impregnated in a conjugate pad, (ii) 1,3 µg streptavidin immobilised as a line on a nitrocellulose membrane, and (iii) 0,1 µg anti-mouse polyclonal antibody - to serve as an internal control - immobilised as a second line at a higher position on the nitrocellulose membrane. 5 µl of the hybridization

mixture of labeled PCR product and respective labeled hybridization probe are pipetted onto the sample application pad of the test strip. The strip's bottom tip is then dipped for a few seconds into a chromatographic buffer. The buffer moves along the test strip and resolubilizes the monoclonal anti-digoxigenin antibody that is conjugated to gold particles. As the buffer and antibody pass through the sample, the labeled PCR product is bound by the anti-DIG-gold conjugate and carried further along the test strip. As the DNA-anti-DIG-gold complex migrates across the streptavidin that is fixed to the nitrocellulose membrane, the biotin label within the complex is captured. Thereby, the conjugated gold particles from the anti-DIG-gold complex are concentrated to form a visible red line.

20. The technical problem to be solved in the light of the content of document D6 is seen as the provision of an improved assay system. As a solution to this problem, the application provides the assay system according to claim 1, in which the use of antibodies is avoided. Colloidal gold particles are conjugated with a poly-A oligonucleotide and the antibodies in the control area are replaced by poly-T oligonucleotides. Furthermore, the hybridization of the PCR product to the probe takes place on the test strip. In view of the disclosure contained in the patent at issue, in particular in the examples, the claimed subject-matter solves the technical problem.

21. In support of its objection of lack of inventive step, the appellant has referred to a combination of documents D6 and D10.

22. Document D10 very briefly and generally describes an assay test strip and a method of using it. Due to its unclear wording and in view of the absence of any experimental illustration, the enablement of the disclosure of D10 is highly questionable. According to the disclosure of document D10, when performing the assay test strip the PCR product is reacted with the probe attached to colloidal gold particles and the reaction mixture is applied to the test strip. Furthermore, anti-colloidal gold antibodies are immobilised in the control area. Therefore, the skilled person, facing the technical problem underlying the patent in suit, will not find any incentive in document D10 to modify the assay system disclosed in document D6. Nonetheless, even if a skilled person would disregard the scanty disclosure of document D10 and would consider to combine the teaching of the two documents, this would lead to a test strip assay system having antibodies immobilised in the control area, i.e. a test strip assay system which is different from that of claim 1.

23. Therefore, the subject-matter of claims 1 to 16 involves an inventive step and 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