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# Datasheet for the decision of 3 February 2015

**Case Number:** T 2012/10 - 3.3.08

**Application Number:** 03729220.8

**Publication Number:** 1466018

**IPC:** C12Q1/68, C12N15/10

**Language of the proceedings:** EN

**Title of invention:**
USE OF SILICA MATERIAL IN AN AMPLIFICATION REACTION

**Patent Proprietor:**
Roche Diagnostics GmbH
F.Hoffmann-La Roche AG

**Opponent:**
QIAGEN GmbH

**Headword:**
Nucleic acid purification/ROCHE DIAGNOSTICS GmbH

**Relevant legal provisions:**
EPC Art. 54, 56, 123(2)

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

**Decisions cited:**
T 0464/94

**Catchword:**

Europäisches Patentamt
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**Case Number: T 2012/10 - 3.3.08**

**D E C I S I O N**
**of Technical Board of Appeal 3.3.08**
**of 3 February 2015**

| | |
|---|---|
| **Appellant:** (Opponent) | QIAGEN GmbH<br>Qiagen Str. 1<br>40724 Hilden (DE) |
| **Representative:** | Roth, Carla<br>König-Szynka-Tilmann-von Renesse<br>Patentanwälte Partnerschaft mbB<br>Postfach 11 09 46<br>40509 Düsseldorf (DE) |
| **Respondents:** (Patent Proprietors) | Roche Diagnostics GmbH<br>Sandhofer Strasse 116<br>68305 Mannheim (DE)<br><br>F.Hoffmann-La Roche AG<br>Grenzacherstrasse 124<br>4070 Basel (CH) |
| **Representative:** | Teschemacher, Andrea<br>Isenbruck Bösl Hörschler LLP<br>Patentanwälte<br>Prinzregentenstraße 68<br>81675 München (DE) |
| **Decision under appeal:** | **Interlocutory decision of the Opposition Division of the European Patent Office posted on 4 August 2010 concerning maintenance of the European Patent No. 1466018 in amended form.** |

**Composition of the Board:**

| | |
|---|---|
| **Chairman** | M. Wieser |
| **Members:** | B. Stolz |
| | C. Heath |

## Summary of Facts and Submissions

I. The opponent (appellant) lodged an appeal against the interlocutory decision of the opposition division dated 4 August 2010, whereby the European patent No. 1 466 018 was maintained on the basis of auxiliary request A1 filed at the oral proceedings on 19 May 2010. The main request (claims as granted) was refused for lack of inventive step (Article 56 EPC).

II. The opposition was based on the grounds that the subject-matter of the claims as granted was not new (Articles 100(a) and 54 EPC), not inventive (Articles 100(a) and 56 EPC), and extended beyond the content of the application as filed (Article 100(c)) EPC).

III. With its statement setting out the grounds of appeal, the appellant filed new documents D25 to D28.

IV. The patent proprietors/respondents reply was accompanied by fourteen auxiliary requests (1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, 4C, 5A, 5B and 5C). New document D29 and several pieces of evidence to prove the entitlement to the claimed priority date, were annexed to the reply.

V. In a further submission, the appellant filed new documents D30 and D31.

VI. 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.

VII. With its response to the board's communication, the respondent submitted further evidence as documents D32 to D38 and revised auxiliary requests 2A', 3A', 4A', 5A', 2A'', 2B'', and 2C''. On 26 January 2015, it submitted corrected sets of these auxiliary requests.

VIII. Oral proceedings were held on 3 February 2015. The respondent made auxiliary request 2A' its new main request and withdrew the preceding requests.

IX. Claims 1 and 2 of the main request read as follows:

"1. A method for the purification and amplification of a target nucleic acid from a biological sample comprising said target nucleic acid and non-target nucleic acids, said method comprising the steps of:

a) adding a material comprising magnetic glass particles with an unmodified silica surface to said sample to bind said target nucleic acid and non-target nucleic acids to said material,

b) separating said material from said sample,

c) eluting said target nucleic acid and non-target nucleic acids from said material and

d) amplifying said target nucleic acid in the presence of said material,

wherein directly after step c)

(i) said target nucleic acid, non-target nucleic acids and said material are transferred to a reaction tube containing all reagents necessary for amplification or

(ii) all reagents necessary for amplification are added to said target nucleic acid, non-target nucleic acids and said material,

wherein said magnetic glass particles are manufactured by the sol-gel method comprising suspending magnetic objects in a sol, hydrolyzing the sol to cover the magnetic objects with a gel, spray-drying the magnetic objects covered with a gel in a spray-drying system, and sintering the spray-dried powder to form a glass from the gel covering the magnetic objects.

2. Method for the amplification of a target nucleic acid in a sample comprising said target nucleic acid and non-target nucleic acids, said method comprising the steps of:

(a) adding a material comprising magnetic glass particles with an unmodified silica surface to said sample,

(b) separating said material from said sample,

(c) eluting said target nucleic acid and non-target nucleic acids from said material, and

(d) amplifying said target nucleic acid in the presence of said material,

wherein directly after step c)

(i) said target nucleic acid, non-target nucleic acids and said material are transferred to a reaction tube containing all reagents necessary for amplification or

(ii) all reagents necessary for amplification are added to said target nucleic acid, non-target nucleic acids and said material,

wherein said magnetic glass particles are manufactured by the sol-gel method comprising suspending magnetic objects in a sol, hydrolyzing the sol to cover the magnetic objects with a gel, spray-drying the magnetic objects covered with a gel in a spray-drying system, and sintering the spray-dried powder to form a glass from the gel covering the magnetic objects."

Claims 3 to 12 define specific embodiments of the method according to claim 1.

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

D3: WO 96/18731

D4: WO 99/39010

D5: DE 198 01 730

D6: cDNA Synthesis Protocol von Narendra Kaushik, 1997

D7: WO 01/14590

D9: WO 96/41811

D11: Rudi et al; "Detection of Toxin-Producing Cyanobacteria by Use of Paramagnetic Beads for Cell Concentration and DNA Purification" Applied and Environmental Microbiology, January 1998, S. 34 - 37

D12: WO 03/058649

D20: WO 99/16781

D22: EP 389 063

D23: WO 95/04140

D24: WO 98/51693

XI. The arguments of the appellant, as far as relevant for this decision, can be summarized as follows:

Article 123(2) EPC

Although claim 1 specified the method by which the magnetic glass particles were manufactured, the glass particles added according to step (a) were not limited to glass particles with a glass surface. The wording of step (a), "a material comprising magnetic glass particles with an unmodified silica surface", encompassed glass beads which comprised an additional layer of unmodified, non-glass, silica. There was however no basis for the use of such beads.

As for the specified method of manufacturing the glass beads, the description only disclosed a spray drying process involving the use of a two nozzle spray drier. Omission of this feature led to an undisclosed generalisation.

As for feature (ii) of step (d), the patent application as filed only disclosed the addition of a solution comprising all reagents necessary for amplification.

Article 54 EPC

Document D20 disclosed a method according to claim 1 including a step of eluting DNA bound to glass beads followed by PCR amplification. The method disclosed in Example 4.1. did not, neither explicitly nor implicitly, comprise a step of separation of the glass beads from the eluted DNA.

Article 56 EPC

Document D1 disclosed the use of magnetic glass particles for the isolation and amplification of nucleic acids, and suggested that the step of separating the magnetic beads from the eluate was optional. Starting from document D1, the technical problem consisted in the provision of a simplified procedure for the purification and amplification of nucleic acids with less steps. The solution to this problem was obvious because the step of separating the eluate from the beads was optional and it was known from documents D3, D7 or D11, as well as from D22 or D23, that PCR could be performed in the presence of magnetic glass particles.

XII. The arguments of the respondents can be summarized as follows:

Article 123 EPC

The method of manufacturing the magnetic glass particles as specified in the claims clearly limited the use of said particles in step (a) to magnetic glass particles with an unmodified glass surface.

As for the type of spray drying system used, page 17 provided basis for a generalization of the spry drying system, as did the reference on page 16 to the methods of documents D1 and D9, both of which disclosed the use of single nozzle systems.

Basis for feature (ii) of step (d) could be found on page 10.

Article 54 EPC

The method disclosed in Example 4.1 of document D20 included a step of separating magnetic glass beads and eluted DNA. This was clear from the disclosure of the document as a whole, and the sentence in Example 4.1, describing the transfer of the eluate into a new reaction vessel.

Article 56 EPC

Document D1 represented the closest prior art. The problem to be solved by the present invention was the provision of a simplified process for the purification and amplification of nucleic acids containing less working steps than the prior art. Document D1 disclosed the step of separating the magnetic beads from the eluate as being optional depending on the intended further use. D1 did however not suggest to perform an amplification of the eluted nucleic acids in the presence of the magnetic glass beads. Documents D3, D7 and D11 taught the performance of the amplification reaction with the nucleic acids bound to the glass beads. Therefore, the claimed solution was not obvious.

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

XIV. The respondents requested that the decision under appeal be set aside and that the patent be maintained on the basis of the new main request.

## Reasons for the Decision

1. The main request, filed at the oral proceedings, is based on auxiliary request 2A' which was submitted in response to the board's communication annexed to the summons to oral proceedings. After an additional modification, which brought claim 2 in line with claim 1, auxiliary request 2A' was resubmitted on 26 January 2015.

The opposition division has decided that the main request before it complied with the requirements of Article 123(2) EPC. The amendments introduced into auxiliary request 2A' addressed issues under Article 123(2) EPC which have been raised for the first time by the board in its communication and which therefore could not not have been made at an earlier stage. The appellant did not object to the resubmission (see above) of an amended auxiliary request 2A' on 26 January 2015. The board, exercising its discretion under Article 114(2) EPC in conjunction with Article 13(1) RPBA, decides to admit the new main request into the procedure.

Article 123(2) EPC

2. Glass is an amorphous, i.e. non-crystalline, solid. The term basically encompasses any solid with these properties, be it silica based or not. A known method

of manufacturing silica based glass is the so called sol-gel method which comprises the acidification of sodium silicate solutions, the formation of polysilicic acids, the formation of colloidal silica particles, and gelling. The gel is dried to form a porous xerogel (cf. e.g. document D30). If transformation into a glass is desired, a sintering (heating) step is added.

3. The appellant submitted that step (a) of claims 1 and 2 encompassed the use of magnetic glass particles with an unmodified silica surface other than glass, e.g. glass beads covered with a xerogel, for which there was no basis.

4. Step(a) requires the addition of "*a material comprising magnetic glass particles with an unmodified silica surface*" to a sample. The board is convinced that upon proper construction of the claim wording, the unmodified silica surface is the surface of the glass particles. According to the last paragraph of claims 1 and 2, "*said magnetic glass particles*", i.e. magnetic glass particles with an unmodified silica surface, are manufactured by the sol-gel method which, as specified in the claims, comprises a step of *"sintering the spray dried powder to form a glass from the gel covering the magnetic objects"*. Thus, there can be no doubt that the unmodified silica surface of the magnetic glass particles mentioned in step (a) is an unmodified silica glass surface. The board therefore dismisses appellant's objection.

5. It was not disputed that there is disclosure of the use of magnetic glass particles with an unmodified glass surface throughout the application as filed (e.g. pages 13 and 14, original claim 8).

6. The appellant submitted, however, that the method of manufacturing the magnetic glass particles according to the last paragraph of claims 1 and 2 represented an undisclosed generalisation because the description only disclosed methods comprising the use of a two nozzle spray drier.

7. Basis for the method of manufacturing the glass beads can be found on page 16, lines 4 to 14, of the application as filed which reads as follows:

*"In a preferred embodiment of the invention, the magnetic glass particles with an unmodified glass surface are manufactured by the sol-gel method described in EP1154443 and WO 96/41811, in particular wherein the sol-gel method comprises the steps of:*
*(a) ...*
*(b) ...*
*(c) spray-drying the magnetic objects covered with a gel in a two-nozzle spray-drier, and*
*(d) ... ."*

In the subsequent paragraph the use of particular spray dryers with two nozzles and the setting of several parameters is described. At the end of the paragraph (page 17, line 13) the following is stated: *"However, he can transfer the teachings of the present invention to any other spray drying system and find out the parameters by taking the disclosures of this invention into account."*

8. Thus, there is an unambiguous disclosure that the method defined by steps (a) to (d) can be performed with any spray drying system.

9. Finally, the appellant submitted that feature (ii) of step (d) of claims 1 and 2 represented a generalisation because, in its view, the description only provided basis for the addition of solutions comprising all reagents necessary for amplification.

10. According to page 4, lines 11 to 16, the *"present invention contemplates a method for the purification and amplification of a target nucleic acid comprising binding of nucleic acids to a material comprising an unmodified silica surface, wash and elution steps followed by amplification in the presence of said material"*. Similar statements can be found on page 6, lines 1 to 4, and page 9, lines 26 to 28. Again, on page 10, lines 6 to 9, it is stated that the solution containing the purified target and non-target nucleic acids and the material with the unmodified silica surface is now ready to be used. Any further use is possible in two ways only, either by adding all the necessary reagents to the tube containing the eluate and the glass beads, or by transferring the eluate and the glass beads to a new tube comprising all the necessary reagents. Both are commonly known. Thus, there is an implicit disclosure of feature (ii) of step (d).

The disclosure in this respect is therefore not limited to the addition of a solution comprising all necessary reagents as explicitly described on page 10, lines 9 to 12.

11. In view of the above, the board dismisses the objections under Article 123(2) EPC.

Article 54 EPC

12. Based on the disclosure in document D12 and on an alleged lack of entitlement to priority, the appellant raised an objection under Article 54(3) EPC against the previous main request. Since document D12 discloses a method of isolating and amplifying nucleic acids comprising the use of magnetite particles covered by a silica xerogel, but not the use of glass particles with an unmodified glass surface, it is not relevant for the examination of novelty of the claims of the new main request. Thus, there is no need for the board to further examine the issue of entitlement to the claimed priority date.

13. The appellant based a further novelty objection on document D20 which discloses the use of magnetic glass particles, manufactured as for instance described in document WO 96/41811 (document D9 on file), for the isolation and purification of a nucleic acid (cf. page 3, line 31). Basically, a nucleic acid is adsorbed to magnetic glass beads which are then washed several times. The nucleic acid is subsequently eluted and separated from the glass beads. The eluate can be used for an amplification reaction (cf. e.g. example 3, claims 1 and 16).

14. Example 4.1 of document D20 describes a protocol for the detection of HIV-RNA, comprising the steps of binding nucleic acids to the magnetic glass particles, washing the bound nucleic acid five times and thoroughly removing the wash buffer (page 19). For the elution step, the following is disclosed:

*"Dann werden 100 µl Elutionspuffer zugegeben und die MGP resuspendiert. Nach 1 minütiger Inkubation bei 80°C*

*auf einem Eppendorf Thermomischer (13.000 RPM) werden 90 µl Eluat in ein neues Reaktionsgefäß überführt. Für die anschließende HIV-Bestimmung durch RT-PCR werden 40 µl Eluat verwendet."*

15. Since this paragraph did not explicitly refer to a step of separating the magnetic glass beads from the eluate before starting the amplification reaction, the appellant concluded that such a step had not taken place. In its view, the method of Example 4.1. comprised all the features of the methods of claims 1 and 2.

16. The board disagrees because the methods disclosed throughout document D20, with the exception of Example 4.1., explicitly include a separation step. This, on its own, is not a convincing argument. It lends however support to the argument that the paragraph quoted from example 4.1. has to be read as including a separation step because it states that 90 µl of the eluate, and not of a suspension comprising eluted nucleic acids and magnetic beads, are transferred to a new reaction tube.

17. According to established case law, if a patent is revoked, or a request is held unallowable, for lack of novelty, the board has to be certain, taking into consideration all the facts and arguments put forward during the proceedings, that its decision is justified (cf. point 16 of decision T 464/94 of 21 Mai 1997). In the present case, the board takes the view that the appellant's reading of Example 4.1. is not correct. A decision that this document is prejudicial to the novelty of the claimed subject-matter is not justifiable.

18. In view of the above, the board decides that the subject matter of claims 1 and 2 is novel (Article 54 EPC).

Article 56 EPC

19. Document D1 represents the closest prior art. It discloses the use of magnetic glass particles, preferably manufactured according to the method disclosed in document D9 (cf. [0064]), for the isolation and amplification of nucleic acids. The steps of the amplification method include the addition of the magnetic glass particles to a sample, the magnetic separation of the glass beads, wash steps, the elution of the bound nucleic acids from the glass beads, the magnetic separation of the glass beads from the eluate followed by amplification reactions (cf. [0140- 0148]).

20. Starting from document D1, the technical problem to be solved is the provision of a simplified method for the isolation and amplification of nucleic acids from a sample.

21. The patent proposes the methods of claims 1 and 2 as its solutions.

22. The patent itself does not provide an example of the claimed methods. It has however not been contested, and additional evidence on file (documents D19a and D21) supports the conclusion, that the claimed method indeed leads to the isolation and amplification of nucleic acids. Since it omits one step compared to the method of the closest prior art, it solves the underlying technical problem.

23. It remains to be established whether the claimed methods involve an inventive step.

24. The only difference between the method of document D1 and the methods of claims 1 and 2 is the separation of the magnetic beads from the eluate before the amplification reaction.

25. The appellant submitted that the omission of the separation step was obvious for several reasons. Document D1 stated that following the last wash step, *"if desired, the biological material purified in this manner can be separated from the magnetic particles"* ([0058], lines 44-45), *"the nucleic acids can be removed from the particles using an elution buffer"* ([0058], lines 47-48), or *"depending on the intended further use of the nucleic acids, the fluid can now be separated from the particles and processed further"* ([0063], lines 24-25). The appellant submitted that this wording implied that the step of separating the glass beads from the eluate was optional.

26. The board does not agree. The statement that depending on the intended use, the fluid can be separated and further processed rather suggests that, if the nucleic acids are eluted, the eluate is also separated from the magnetic particles. Moreover, the disclosure of document D1 is not limited to methods of isolating and amplifying nucleic acids. Further uses of the isolated nucleic acids such as sequencing, probe based assays or restriction digests, some of which require separation of the nucleic acids from the magnetic beads, are also contemplated ([0066]). When read in this context, the statements referred to by the appellant do not suggest a method of isolating and amplifying nucleic acids wherein the step of separating the eluted nucleic acids

from the magnetic particles is omitted. This is further supported by the methods disclosed in examples 7.1 and 7.2 which include an elution and a separation step followed by PCR.

27. Therefore, the claimed solution is not obvious, based on document D1 alone.

28. Document D3 discloses methods of separating nucleic acids from a sample with magnetic beads. Beads can be made of glass, silica, latex or a polymeric material (page 9, line 17). Following the separation step and any optional wash steps, the support carrying the nucleic acid may be transferred, e.g. resuspended or immersed into any suitable medium (page 12, lines 10-14), and depending on the support and the nature of any subsequent use, it may or may not be desirable to release the nucleic acid from the support (page 12, lines 14-18). In the case of e.g. magnetic beads the support may be used directly, for example in PCR or other amplifications, without eluting the nucleic acid from the support (page 12, lines 18 to 22). Regarding an elution step, it is stated that "*elution of the nucleic acid may be readily achieved using known means, for example by heating , e.g. to 65$^{o}$C for 5 to 10 minutes, and following which the support may be removed from the medium, leaving the nucleic acid in solution*."

29. This cannot be interpreted, as has been done by the appellant, as a suggestion to perform an elution step while omitting a separation step. Rather, the wording *"elution ... may be readily achieved ... following which the support may be removed"* suggests an optional elution step in combination with a separation step. The board concludes that document D3 does not suggest the

omission of a separation step following the elution of the bound nucleic acids from the magnetic particles.

30. Document D7 discloses methods of isolating and amplifying nucleic acids bound to silica magnetic particles (e.g. p. 12, 14). The document discloses two ways of amplifying the nucleic acids. The complex obtained after the washing steps may be processed (i.e. amplified) directly without separating the nucleic acid from the silica magnetic particles (page 22, lines 22-23). Preferably however, the DNA target material is eluted from the silica magnetic particles (page 22, line 24) and the DNA target material is separated from the silica magnetic particles (page 23, line 10).

31. The appellant referred to example 7 of document D7 which discloses the resuspension of the particles with bound nucleic acids in 20 µl of wash buffer. Tests with aliquots of the suspension were then performed to assess whether DNA bound to the particles could be used in amplification reactions (cf. page 33, line 38). The appellant submitted that the step of resuspending the glass beads in 20 µl of Tris buffer before performing the amplification reactions corresponded to an elution step. However, an elution step would require either heating of the suspension before the amplification reactions take place (e.g. 5 min at 60°C as in Example 9 of document D7), or prolonged incubation of the sample at lower temperatures (e.g. incubation overnight at 5°C as in example 10 of document D7). Such an elution step is clearly not present in Example 7 of document D7.

32. Document D7 does therefore not suggest the omission of a separation step following the elution of the bound nucleic acids from the magnetic particles.

33. Document D11 discloses the use of magnetic particles (Dynabeads) for the isolation and amplification of nucleic acids. According to the section *"Materials and Methods"*, beads were bound to the support, washed and the beads with the bound nucleic acids were dried. Before the amplification reaction, the beads were resuspended in 5 µl of water. While water could be used as an eluent, there is no indication of heating, or prolonged incubation of the resuspended beads before the addition of the amplification reagents. Thus, there was no elution step. This is in line with the statement that all of the bead-DNA complex was used in the subsequent PCR reaction (cf. abstract, line 9). Thus, there is no suggestion in document D11 to elute the DNA from the Dynabeads.

34. Example H1 of document D22 (and Example 1 of document D23) discloses a DNA purification protocol using silica particles or latex particles to which DNA was bound and washed. The washed and dried particles were resuspended in water, and an aliquot was added to a solution comprising PCR reagents. While water could be used as an eluent, there is no heating or prolonged incubation of the resuspended beads, which could be regarded as an elution step, before the addition of the amplification reagents. To the contrary, it is explicitly stated that no elution step took place (page 18, line 22). Thus, there is no suggestion pointing to an elution step without separation of the released nucleic acids from the silica particles.

35. In summary, documents D3, D7, D11, D22 and D23 taught the addition of amplification reagents to magnetic and/or silica particles with bound nucleic acids but did not suggest an elution step without subsequent separation of the eluent from the particles.

36. Documents D4 to D6, and D24, which were also referred to, disclose procedures for the isolation and purification of nucleic acids wherein amplification reactions were either started with nucleic acids bound to a solid support or with nucleic acids which have been eluted and separated from a solid support. The content of these documents does not disclose anything more relevant to the assessment of inventive step than the above mentioned documents D3, D7, D11, D22 and D23.

37. The subject matter of claims 1 and 2 could therefore not be derived in an obvious way from the teaching of document D1 in combination with any of the cited documents.

38. At the oral proceedings, the appellant submitted amended pages 3, 3a, 3b, 4, 5, 6, 8, 9 and 14 of the description to bring it in line with the main request. The board is satisfied that this has been done in agreement with the requirements of the EPC.

## Order

## For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent in the following version:

   - Claims 1-12 of auxiliary request 2A' filed with letter dated 26 January 2015 and re-filed as main request during oral proceedings;

   - Description pages 2, 7, and 10 - 13 as granted, pages 3, 3a, 3b, 4, 5, 6, 8, 9 and 14 as filed during oral proceedings

   - Figures 1 and 2 of the patent as granted.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated