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D E C I S I O N
of 22 November 2005

Case Number: T 0971/04 - 3.3.08

Application Number: 98921592.6

Publication Number: 0988307

IPC: C07H

Language of the proceedings: EN

Title of invention:
Solid-phase Nucleic Acid Isolation

Patentee:
Genpoint AS

Opponent:
Merck Patent GmbH

Headword:
Nucleic acid isolation/GENPOINT

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main request - added subject-matter (no) "
"Extension of protection (no) "
"Novelty and inventive step (yes) "

Decisions cited:
G 0009/91, T 0420/00

Catchword:
-



Case Number: T 0971/04 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 22 November 2005

Appellant:
(Proprietor of the patent)

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Decision under appeal:

Decision of the Opposition Division of the
European Patent Office posted 18 May 2004
revoking European patent No. 0988307 pursuant
to Article 102(1) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
T. Bokor

Summary of Facts and Submissions

I. European patent No. 0 988 307 with the title "Solid-phase nucleic acid isolation" was granted with 20 claims on the basis of European patent application No. 98 921 592.6, which originated from an international application published as WO 98/51693, to be referred to in the present decision as the application as filed.

Claim 1 as granted read as follows:

"1. A method of isolating nucleic acid from a sample of cells, said method comprising:

- (a) binding cells in said sample to a solid support to isolate cells from the sample;
- (b) lysing the isolated cells; and
- (c) binding nucleic acid released from said lysed cells to said same solid support."

Claims 2 to 16 were further embodiments of claim 1, wherein claim 7 defined the solid support as being particulate. Claim 17 related to the use of the method of any one of claims 1 to 16 in the preparation of nucleic acid for use in a nucleic acid-based target cell detection method. Claims 18 and 19 related to a method for detecting the presence or absence of a target cell in a sample, said method comprising the steps of claim 1 and an additional step for detecting the presence or absence of nucleic acid characteristic of said target cells within the bound nucleic acid (claim 18), and wherein said detection step comprised *in situ* hybridisation and/or *in vitro* amplification

- and/or nucleic acid sequencing (claim 19). Claim 20 related to a kit for isolating nucleic acid from a sample comprising a solid support and several means for carrying out the method of claim 1.
- II. On 24 July 2002 an opposition was filed on the grounds of Articles 100(a) EPC (lack of novelty and lack of inventive step, Articles 54 and 56 EPC) and 100(b) EPC (insufficiency of disclosure, Article 83 EPC).
- III. On 18 May 2004 the opposition division issued a decision pursuant to Article 102(1) EPC revoking the patent since neither the main request nor the auxiliary requests filed at the oral proceedings before the opposition division were considered to fulfil the requirements of the EPC: the main request and the first auxiliary request were held to lack an inventive step (Article 56 EPC) and the second auxiliary request to lack clarity (Article 84 EPC).
- IV. The patentee (appellant) lodged an appeal against the decision of the opposition division. A main request and auxiliary requests A, B and C were filed on 28 September 2004 with the statement of grounds of appeal.
- V. With letter dated 3 February 2005, the opponent (respondent) replied to the statement of grounds of appeal. Objections were raised under Articles 123(2), 54 and 56 EPC against the new requests.

- VI. Pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal, the board sent a communication to the parties on 29 July 2005 and informed them of its preliminary, non-binding opinion.
- VII. In reply to the board's communication, both appellant and respondent filed further observations with letters of 24 and 19 October 2005, respectively. The appellant also filed a new main request and auxiliary requests B, D and E and maintained the auxiliary requests A and C filed with the statement of grounds of appeal.
- VIII. Oral proceedings took place on 22 November 2005. At the oral proceedings the appellant made the auxiliary request B its main request and withdrew the previous main request and auxiliary request A. The appellant further filed a description adapted to this new main request.

Claim 1 of the **main request** read as follows:

"1. A method for detecting the presence or absence of a target eukaryotic or prokaryotic cell in a sample, said method comprising:

(a) providing a particulate solid support and mixing it with the sample and allowing binding of cells in said sample to the solid support to isolate cells from the sample;

(b) lysing the isolated cells;

(c) binding nucleic acid released from said lysed cells to said same solid support; and

(d) detecting the presence or absence of nucleic acid characteristic of said target cells within said bound nucleic acid."

Claims 2 to 15 were further embodiments of claim 1 and corresponded to claims 2 to 6, 8 to 13, 15 to 16 and claim 19 as granted.

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

D1: A. Metzler et al., Mitt. Gebiete Lebensm. Hyg., 1996, Vol. 87, pages 55 to 72;

D2: WO-A-92/07863 (publication date: 14 May 1992);

D5: WO-A-91/12079 (publication date: 22 August 1991);

D29: DE-A-195 20 398 (publication date: 12 December 1996);

D36: D.W. Johnson et al., Appl. Environ. Microbiol., 1995, Vol. 61, pages 3849 to 3855.

X. The appellant's arguments in writing and during oral proceedings, insofar as they are relevant to the present decision, may be summarised as follows:

Main request

Article 123(2) EPC (Added subject-matter)

There was a formal basis in the application as filed for particulate solid supports. Claim 7 in the application as filed defined the support used in the method of isolating nucleic acid of claim 1 as being a particulate solid support. References were also found in the description as filed to particulate solid

supports for use in the methods disclosed therein. In fact, these particulate supports were referred to as preferred material due to their greater binding capacity and other advantageous properties. Thus, the use of these particulate solid supports was not an arbitrary selection but had a formal basis in the application as filed.

The restriction to the use of the claimed method for detecting target eukaryotic or prokaryotic cells in a sample was an appropriate limitation to an embodiment found in the application as filed. The general context of the application as filed and the type of samples used in the single example disclosed therein clearly showed that this embodiment was in fact a preferred embodiment. The limitation to this subject-matter did not result in a different teaching and it did not create an aliud.

Article 84 EPC (Clarity)

Step (d) of claim 1 only stated that the nucleic acid of interest was the one which remained bound to the particulate solid support in step (c) of that claim. The meaning and intention of step (d) was clear and it did not imply anything more.

Article 54 EPC (Novelty)

Document D2 disclosed a method of isolating nucleic acids from prokaryotic or eukaryotic cells for detecting the presence or absence of these cells in a sample. The method used a fixed solid support (silica particles embedded in an inert matrix) that could not

be mixed with the sample (it only passed through the fixed support). The claimed method, however, required both the sample and the particulate solid support to be mixed, i.e. both were to be mobile. Moreover, whereas the nucleic acid released by cell lysis was bound to the silica particles embedded in the inert matrix, the cells present in the sample were not bound to these particles nor to the fixed solid support, they were only retained by said support. The claimed method, however, required both the cells and the released nucleic acid to bind to the same particulate solid support. Thus, the teachings of document D2 were different from the ones of the opposed patent.

Article 56 EPC (Inventive step)

Document D29 represented the closest prior art since it had the same purpose as the claimed invention, namely a method of isolating nucleic acids for the detection of prokaryotic or eukaryotic cells in a sample using a particulate solid support as well. The method comprised a first step wherein, after mixing a sample and a solid support, the cells of the sample remained bound to the support, and a second step wherein, after lysis of the bound cells, the released nucleic acid was bound to a particulate solid support. However, the solid support used in the first step was adapted to bind the specific cells of the sample (in the presence of antibodies on the surface of the support), whereas this adaptation was absent in the particulate solid support of the second step. Starting from this closest prior art, the problem to be solved was therefore the improvement of this method. Although it was obvious from document D29 itself that the first solid support was not appropriate

for binding the released nucleic acid of the second step, there was no indication suggesting that this was disadvantageous nor that the use of two different solid supports resulted in some technical drawbacks. Nor was such a suggestion found in the prior art on file, which thus did not provide the skilled person with any motivation for replacing the solid supports disclosed in document D29.

In particular, the combination of document D29 with document D5 could only be done with hindsight since document D5 was concerned only with the isolation of biopolymers (nucleic acids) and not with the specific detection of eukaryotic or prokaryotic cells in a sample. The starting material, namely nucleic acid molecules in solution, viruses or bacterial cells, was clearly differentiated in document D5 and this was reflected in the examples as well. Examples 1-2 were concerned with nucleic acid material, examples 3-5 with bacteriophages and examples 6-7 were concerned with the isolation of nucleic acids from prokaryotic (bacteria) cells. Thus, the method and the experimental conditions referred to in example 5 would be considered not to be appropriate for the isolation of nucleic acids from a (starting) material other than viruses. For prokaryotic cells only examples 6-7 would come into consideration. Example 7 disclosed a method which used two sets of magnetic beads, a first set for binding the prokaryotic cells (and lysing the cells bound thereto) and a second set for binding the released plasmid DNA. Although both sets of beads were of the same type, they were not the same beads in the sense of the opposed patent. In fact, they were used in a manner identical to that known from document D29. The reference in document D5 to the

convenient use of the same beads for a sequence of manipulations had to be understood in this context too. This interpretation was further supported by the reference to section IIIc (examples 6-7) when the possibility to carry out cell lysis directly on the beads was discussed. Nowhere in document D5 was suggested to combine the methods applied to one particular starting material (viruses) with those applied to another very different one (bacterial cells). It was only with the benefit of hindsight that a general disclosure of document D5 could be taken out of its context and applied to a very specific situation for which no suggestion, let alone an example, was found in that document.

Document D1 related to the detection of viruses and eukaryotic cells in a sample and thus, it had the same purpose as the opposed patent. The specific methods for detecting eukaryotic cells were clearly differentiated from the ones used for detecting viruses and both methods were exemplified in separate sections. The deficiencies of several methods used for detecting eukaryotic cells were also explicitly mentioned. Starting from document D1 as the closest prior art, the problem to be solved was to improve these methods for the detection of eukaryotic cells. Document D1 itself hinted at a very promising method based on molecular biology techniques (PCR). This method - disclosed in the bibliographic reference (30) and corresponding to document D36 in the present appeal proceedings - would have been the first choice of the skilled person. However, Figure 2 of document D36 showed that this method comprised only a cell-binding step which used magnetic beads with antibodies bound on their surface.

This step was similar to the first step of document D29. Thus, both documents D1 and D36 did not go beyond the teachings of document D29. The authors of document D1, being well aware of the disclosed method for detecting viruses, failed to recognize the possible application of this method for detecting eukaryotic cells as well. There was no indication that could readily lead a skilled person to such application. References were made to the "could-would approach" and the ex-post-facto analysis established in the case law of the Boards of Appeal.

XI. The respondent's arguments in writing and during oral proceedings, insofar as they are relevant to the present decision, may be summarised as follows:

Main request

Article 123(2) EPC (Added subject-matter)

The limitation of the solid support in claim 1 to a particulate solid support represented an arbitrary selection among all possible supports disclosed in the application as filed. The selection of this support, which was not singled out in the application as filed, was not directly derivable from the application as filed.

The application as filed defined "cell" as comprising *inter alia* eukaryotic and prokaryotic cells as well as viruses. The methods disclosed therein were to be applied to all these cells without further modifications or specific adaptations to the particular type of cell chosen. If the limitation to eukaryotic and prokaryotic cells were to imply an (advantageous)

technical effect over other cells (viruses), then neither the effect nor these advantages were directly derivable from the application as filed and the selection of these eukaryotic or prokaryotic cells resulted in an aliud, i.e. a new (selection of) subject-matter based on an (advantageous) technical effect not disclosed in the application as filed.

Article 84 EPC (Clarity)

From the wording of step (d) in claim 1 ("within said bound nucleic acid"), it was not clear whether the detection of the nucleic acid had to be carried out on the nucleic acid once eluted from the support or else on nucleic acid still bound to the solid support. Although this wording was already present in the claims as granted, it was only by its introduction in independent claim 1 that the unclarity become evident. Thus, the objection for lack of clarity resulted from the amendments introduced into the claims.

Article 54 EPC (Novelty)

The term "mixing" was broadly defined in the description as filed, wherein both an active stirring and a passive pass through a filter, membrane, etc. were considered appropriate mixing means. It was acknowledged, however, that the combination of the terms "mixing" and "a particulate solid support" differentiated the claimed subject-matter from the disclosure of document D2. Thus, novelty objections were no longer maintained for the claimed subject-matter of this request.

Article 56 EPC (Inventive step)

Three different approaches to inventive step were made, each one having a different starting point (closest prior art), namely documents D29, D5 and document D1.

Document D29 disclosed a method of isolating nucleic acids from eukaryotic and prokaryotic cells for the detection of those cells in a sample. The method comprised a first cell-binding step and a second nucleic acid binding step, wherein both steps used a particulate solid support. In the first step the solid support carried on its surface antibodies specific for the cells to be bound. Therefore, it was immediately evident to the skilled person that this method was disadvantageous since it required the production of specific antibodies which were expensive and of limited use (only for a particular type of cells). Moreover, document D29 emphasized the importance of carrying out the complete method in a single tube and explicitly referred to the advantages associated to this single-tube-method. Thus, it was evident to the skilled person that the use of a second set of beads in the second nucleic acid binding step was disadvantageous and that the method could be, therefore, improved. Starting from this document as the closest prior art, the problem to be solved was then the provision of a solid support for use in both the cell-binding and the nucleic acid binding steps and the determination of appropriate conditions therefor. For solving this problem, there was a clear teaching in document D5 which disclosed that for a stepwise method the same solid support could be used in all steps (cell binding, cell lysis and nucleic acid binding). This teaching was

demonstrated in Example 5. Although the starting material in this example were viruses and not eukaryotic or prokaryotic cells, this was irrelevant since a skilled person concerned with the isolation of nucleic acids would not have made any differences between these materials. This was also shown by the patent as granted which made no differences between these materials. The combination of documents D29 and D5 was therefore detrimental for the inventive step of the claimed subject-matter.

In fact, document D5 per se could also be considered as the closest prior art since it disclosed a method for isolating biological material using a particulate solid support (magnetic beads). The method comprised a first step in which the sample was mixed with the solid support, followed by the precipitation of the biological material on the support and the isolation of the support with the biological material bound thereto. The document disclosed the isolation of nucleic acids as well as the precipitation of bacteria (*inter alia* Examples 6 and 7, Figure 3). Thus, it provided instructions to enable the skilled person to carry out steps (a) and (c) of the method of claim 1. Starting from this closest prior art, the problem to be solved was to improve the methods therein disclosed. Document D5 itself indicated that for a sequence of manipulations (stepwise method) the same beads could be used and that, when bacteria were to be lysed, this cell lysis could be carried out directly on the solid support (i.e. step (b) of claim 1). Thus, it was obvious to combine the different teachings of document D5 in the manner done in the opposed patent and it did not require any inventive contribution to follow these

instructions so as to arrive at the claimed method (steps (a) to (c)). Example 5 showed the application of these teachings (use of the same solid support) in the isolation of nucleic acids from a bacteriophage. Thus, the skilled person had also a reasonable expectation of success. Although document D5 only indicated that the isolated nucleic acid was kept for further uses, these (clinical, diagnostic) uses were clearly known from the prior art on file (*inter alia* documents D1, D2 and D29). Thus, the claimed subject-matter did not fulfil the requirements of Article 56 EPC.

Document D1, which concerned methods for detecting the presence of microorganisms in drinking water, could also be considered the closest prior art since it disclosed a method for detecting viruses comprising the steps: a) mixing the water sample with a particulate solid support and binding of the viruses thereto, b) lysis of the viruses with an appropriate buffer, c) binding of the released nucleic acid into the same solid support and elution of contaminants and debris and d) elution and (PCR) detection of the nucleic acid. This method was identical to the claimed one, except that viruses were the targeted microorganisms instead of eukaryotic or prokaryotic cells. In fact, document D1 itself referred to several known methods for the detection of these cells and outlined the shortcomings of these methods. Thus, starting from this document, the problem to be solved was to improve these methods. The use of the method disclosed in document D1 in respect of viruses for detecting eukaryotic or prokaryotic cells was obvious to the skilled person. As shown by the opposed patent (which made no difference between these cells and viruses), no particular

modification (let alone an inventive one) was required, thus no inventive contribution could be acknowledged.

XII. The appellant (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request, filed on 24 October 2005 (at that time as auxiliary request B), or auxiliary request C filed on 28 September 2004, or auxiliary request D filed on 24 October 2005 or auxiliary request E on 24 October 2005.

XIII. The respondent (opponent) requested that the appeal be dismissed.

Reasons for the Decision

Main request

Rule 57a EPC, Articles 123(2), (3) EPC and Article 84 EPC

1. According to Rule 57a EPC, the description, claims and drawings may be amended provided that the amendments are occasioned by grounds for opposition specified in Article 100 EPC. These amendments are to be examined for compliance with the requirements of the EPC, in particular with Articles 123(2), (3) EPC as well as Article 84 EPC (cf. G 9/91, OJ EPO 1993, 408, point 19 of the Reasons and "Case Law of the Boards of Appeal of the EPO", 4th edition 2001, VII.C.10.2, page 488).
2. The scope of present claim 1 in comparison to that of granted claim 18 has been restricted by limiting the target cells to eukaryotic or prokaryotic cells and the solid support to a particulate solid support. These

amendments have been introduced in order to overcome a ground of opposition - lack of novelty over *inter alia* document D2 (Article 100(a) EPC) - as required by Rule 57a EPC.

3. The subject-matter of claim 1 is a combination of granted claims 1 (method of isolating nucleic acid from a sample of cells), 7 (particulate solid support), 16 (mixing the sample with particulate support) and 17 (use of the method in the preparation of nucleic acid for use in a nucleic acid-based target cell detection method), wherein the target cells have been restricted to eukaryotic or prokaryotic cells. Alternatively, the subject-matter of claim 1 corresponds to granted claim 18 (method for detecting a target cell in a sample) with the limitations referred to in point 2 above. Both the combination or the restriction of the granted claims constitute clear limitations of the protection conferred and thus, Article 123(3) EPC is fulfilled.

4. There is a formal basis in the application as filed for claim 1 resulting from the combination of claims 1 (method of isolating nucleic acid from a sample of cells), 7 (particulate solid support) and 15 (use of the method in the preparation of nucleic acid for use in a nucleic acid-based target cell detection method) as well as claim 16 (method for detecting a target cell in a sample), all claims as filed. Moreover, the application as filed also refers to the mixing of the various components (sample and solid support), to leaving the mixture to stand for a suitable interval of time so as to allow the cells to bind to the support (cf. page 8, first paragraph of application as filed)

and to prokaryotic and eukaryotic cells as targeted cells (cf. page 4, lines 28 to 34). Particulate materials and prokaryotic cells are disclosed as preferred embodiments (cf. page 9, lines 23 to 25 and page 20, example 1). Thus, Article 123(2) EPC is also fulfilled.

5. It is established case law of the Boards of Appeal that clarity objections arising from amendments that introduce subject-matter of dependent claims into an independent claim may be considered in some cases (cf. *inter alia* T 420/00 of 21 January 2003, point 3.6 of the Reasons). However, in the present case the wording of step (d) of present claim 1 was already found in granted claim 18, which was one of the two independent claims granted. Moreover, both present claim 1 and granted claim 18 relate to the same subject-matter, namely a method for detecting target cells in a sample. The board is therefore unable to follow the respondent's argument that the wording of step (d) was hidden in the granted claims and that it has become apparent only by its introduction into an independent claim. Thus, the objection under Article 84 EPC does not arise out of the amendments made and the board is not empowered to consider the issue (cf. "Case Law", *supra*, VII.C.10.1.2, 484).

Articles 83 and 54 EPC

6. The ground of insufficiency of disclosure (Article 100(b) EPC) has not been further pursued during appeal proceedings. The respondent has also raised no novelty objections for the subject-matter of this request (cf. point XI *supra*). Nor does the board,

in the light of appellant's arguments and the documents on file (cf. point X *supra*), see any basis for such an objection. Thus, the main request is considered to be novel and sufficiently disclosed (Articles 54 and 83 EPC).

Article 56 EPC

7. According to the established case law of the Boards of Appeal, the closest prior art is normally a document disclosing subject-matter conceived for the same purpose as the claimed invention (cf. "Case Law", *supra*, I.D.3, 102). The subject-matter of the present request relates to a method for detecting the presence or absence of target eukaryotic or prokaryotic cells in a sample, which comprises three steps (steps (a), (b), (c)) for isolating the nucleic acid from those cells and a fourth step (step (d)) for detecting the nucleic acid characteristic of those target cells (cf. point VIII *supra*).

8. Three documents have been referred to as possible closest prior art, namely documents D1, D5 and D29 (cf. points X and XI *supra*). Documents D1 and D29 disclose methods for identifying eukaryotic or prokaryotic cells based on the isolation and detection of their nucleic acids. However, document D5 refers only to keeping the isolated nucleic acids "for further uses" but without specifying which uses are meant (cf. page 1, lines 11 to 23). Thus, the appropriate closest prior art is considered to be represented by either document D1 or D29.

9. It is also established case law of the Boards of Appeal that for assessing inventive step it must be avoided to interpret the prior art as influenced by the problem solved by the invention if this problem is neither mentioned nor even suggested in this prior art (ex post facto analysis). Starting from the closest prior art the relevant question is not whether the skilled person **could** have carried out the invention, but whether it **would** have done so in the hope of solving a technical problem or in the expectation of some improvement ("could-would" approach) (cf. "Case Law", *supra*, I.D.6.1, 116).
10. Document D29 discloses the production of magnetic particles (with an external glass surface) which are used for isolating and detecting biological material, in particular nucleic acids, in a sample (cf. page 2, lines 44 to 46, page 4, lines 31 to 34, page 5, line 61 to page 6, line 3). These particles are particularly advantageous for isolating nucleic acids (cf. *inter alia* page 3, lines 64 to 66). Eukaryotic and prokaryotic cells are also explicitly mentioned (cf. page 4, lines 27 to 28). For separation of these cells, document D29 refers to the use of (magnetic or other commercially available) particles with antibodies on their surface. Cells bound to these particles coated with antibodies are lysed and their nucleic acid released (cf. page 5, lines 51 to 60). The released nucleic acid is then isolated by further addition of the magnetic particles disclosed in the document. The combination of these two isolation steps results in an advantageous "single-tube-method" for purifying nucleic acids (high sensitivity, easy automation, etc.) (cf.

page 5, lines 61 to 65, page 6, lines 11 to 39 and Figure 1).

11. Starting from this closest prior art, the problem to be solved is considered to be the improvement of this "single-tube-method". The combination of features defined in claim 1 of the present main request solves this problem by using the very same particulate solid support (magnetic particles) in the two separation steps, i.e. for binding the eukaryotic or prokaryotic cells (first step) and for binding the released nucleic acids (second step).

12. Document D29 itself refers to possible improvements, such as in the selection of optimal binding conditions or in the modification of the particles for achieving an optimal binding of the biological material to these particles (cf. page 4, lines 60 to 65). Thus, for the separation of nucleic acids reference is made to the use of a biotin/streptavidin binding, although a direct binding to the glass surface of the magnetic particles is preferred (cf. page 4, line 67 to page 5, line 8). Notwithstanding the known problems encountered when using antibodies (cf. point X *supra*), document D29 refers only to the use of antibodies (on the surface of the support) for separating eukaryotic or prokaryotic cells and it emphasizes the importance of selecting appropriate (specific) antibodies and suitable conditions for obtaining antigen/antibody complexes as well (cf. page 4, lines 65 to 67, page 6, lines 15 to 18). There is no suggestion, however, to modify the particles and/or to select appropriate conditions in such a manner as to achieve a binding of two different biological materials (cells, nucleic acids) to the same

- (type of) particles, let alone to use the very same particles in two different isolation steps. The addition of a second set of particles for the isolation of the second biological material (nucleic acid) is always contemplated in document D29.
13. The teachings of document D5, referred to in document D29 (cf. page 2, lines 30 to 37), do not go much beyond the ones of document D29. Although document D5 refers to the use of "*the same beads*" for a sequence of manipulations (cf. page 6, lines 13 to 14) and to the lysis of bacteria "*directly on the beads*" (cf. page 16, lines 8 to 10), when these teachings are exemplified in section IIIc two sets of beads are used, a first one for binding bacteria and a second one for binding the plasmid DNA released after lysis of the bacteria (cf. pages 20 and 21). Similarly for the isolation of nucleic acids from (bacteriophages) viruses, a first isolation step for precipitating bacterial DNA, membranes and proteins from bacterial lysates is followed by a second isolation step of the (viral) nucleic acid. Although both steps use the same (type of) beads, they are not the very same beads but two different sets (cf. page 13, line 23 to page 14, line 27 and Figure 3).
14. As correctly described in the decision under appeal, in the first isolation step of the said procedure the bacterial nucleic acid remains bound to the first set of beads. It might well be possible - as further argued in the said decision - to find appropriate (washing) conditions for eliminating membranes and proteins from the bacterial lysate and to find out suitable conditions for later elution of the bacterial nucleic

- acid (if required) and its further detection. However, this is a mere speculation based on hindsight, since there is nothing in document D5 that could lead the skilled person to follow this approach, let alone to expect any success (see, for instance, the reference to the destruction of long nucleic acids by immobilisation on particles; page 3, lines 64 to 66 of document D29). (cf. point 9 *supra*).
15. Reference has also been made to Example 5 of document D5, which discloses a method for isolating the nucleic acid from bacteriophages. The method comprises the binding of these viruses to magnetic beads (after pre-cleared of bacteria by centrifugation), lysis of the viruses and binding of the released viral nucleic acid to the very same magnetic beads (cf. page 19, line 26 to page 20, line 8). However, nothing in document D5 suggests using this method for the isolation of nucleic acids derived from eukaryotic or prokaryotic cells. On the contrary, whenever lysis of those cells is envisaged, the use of a second set of magnetic beads is always contemplated (cf. Examples 6 and 7 and point 13 *supra*). Therefore, in the absence of such a suggestion, the board considers that it would only be with the benefit of hindsight that the skilled person would follow this approach. In fact, this method of Example 5 in document D5 is identical to the one disclosed in document D1 for the isolation of viral nucleic acids in samples of drinking water and detection by PCR (cf. page 66).
16. Document D1, which might also be considered an appropriate starting point (cf. point 8 *supra*), discloses methods for detecting the presence of viruses

and of eukaryotic (protozoan) cells in samples of drinking water. With respect to viruses a method is disclosed that comprises a series of steps identical to steps (a) to (d) of the present case. However, this method is not disclosed for detecting eukaryotic cells. For these eukaryotic cells, several prior art methods as well as their shortcomings and drawbacks are reviewed (cf. *inter alia* paragraph bridging pages 61 to 62). Starting from this document, the technical problem to be solved is seen in the improvement of these known methods for detecting eukaryotic cells. Document D1 itself refers to a very promising method based on the isolation of the nucleic acid of those cells and their detection by PCR (cf. page 62, lines 8 to 9). This method is disclosed in bibliographic reference (30) which corresponds to document D36 in the present proceedings. Figure 2 of document D36 shows that the method relies on a separation step based on the binding of eukaryotic cells to magnetic beads with antibodies on their surface, lysis of these cells and recovery of the released nucleic acid for further detection (cf. page 3851), which is thus identical to the first separation step of the method disclosed in document D29 (cf. point 12 *supra*). There is, however, no reference in document D36 to a second separation step, let alone to a possible use of the same magnetic beads for binding the released nucleic acid. Therefore, neither the teachings of document D1 nor the ones of the cross-reference document D36 go much beyond the ones of document D29 and/or document D5, which (when lysis of eukaryotic or prokaryotic cells is envisaged) always comprise two separation steps with two different sets of magnetic beads. There is absolutely no suggestion or hint in document D1 to try for the detection of

eukaryotic cells the method disclosed for the detection of viruses. Under these circumstances, the board considers that only with the benefit of hindsight such an approach would be obvious to a skilled person (cf. point 15 *supra*).

17. Thus, the subject-matter of claim 1 and all the dependent claims are considered to fulfil the requirements of Article 56 EPC.

Adaptation of the description

18. The appellant has proposed amendments to the description. No objections have been raised by the respondent to the amended description. The board considers that these amendments result in an appropriate adaptation of the description to the claims of the present request and that they are in compliance with the requirements of Article 123(2) EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance, with the order to maintain the patent on the basis of:
 - Claims 1-15 of the main request, filed on 24 October 2005 (at that time as auxiliary request B),

 - Description pages 2-8, 3a filed during oral proceedings,

 - Figure as granted.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani