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# D E C I S I O N
# of 6 April 2004

**Case Number:** T 1024/01 - 3.3.8

**Application Number:** 90200678.2

**Publication Number:** 0389063

**IPC:** C07H 1/08

**Language of the proceedings**: EN

**Title of invention:**
Process for isolating nucleic acid

**Patentee:**
Akzo Nobel N.V.

**Opponents:**
Eppendorf AG
Abbott Laboratories

**Headword:**
Isolation of nucleic acid/AKZO

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

**Keyword:**
"Added subject-matter (no)"
"Inventive step (yes)"

**Decisions cited:**
G 0010/91

**Catchword:**
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Beschwerdekammern | Boards of Appeal | Chambres de recours

**Case Number:** T 1024/01 - 3.3.8

# D E C I S I O N
**of the Technical Board of Appeal 3.3.8**
**of 6 April 2004**

| | |
|---|---|
| **Appellant:** (Opponent 02) | Abbott Laboratories<br>100 Abbott Park Road, Abbott Park<br>Illinois 60064-6050 (US) |
| **Representative:** | Modiano, Guido, Dr.-Ing.<br>Modiano, Josif, Pisanty & Staub<br>Baaderstrasse 3<br>D-80469 München (DE) |
| **Respondent:** (Proprietor of the patent) | Akzo Nobel N.V.<br>Velperweg 76<br>NL-6824 BM Arnhem (NL) |
| **Representative:** | Prins, Hendrik Willem<br>Arnold & Siedsma<br>Advocaten en Octrooigemachtigden<br>Sweelinckplein 1<br>NL-2517 GK Den Haag (NL) |
| **Other party:** (Opponent 01) | Eppendorf AG<br>Barkhausenweg 1<br>D-22339 Hamburg (DE) |
| **Representative:** | Emmel, Thomas, Dipl.-Biol., Dr.<br>Schaefer & Emmel<br>Gehölzweg 20<br>D-22043 Hamburg (DE) |
| **Decision under appeal:** | **Interlocutory decision of the Opposition Division of the European Patent Office posted 30 July 2001 concerning maintenance of European patent No. 0389063 in amended form.** |

**Composition of the Board:**

**Chairman:** L. Galligani
**Members:** M. R. Vega Laso
M. B. Günzel

## Summary of Facts and Submissions

I. The appeal lies from the interlocutory decision of the opposition division issued on 30 July 2001, whereby the European patent No. 0 389 063 (European patent application No. 90 200 678) with the title "Process for isolating nucleic acid" was maintained in amended form.

II. In its decision the opposition division held that, having regard to the prior art documents cited by the opponents, the subject-matter of claims 1 to 10 of the second auxiliary request filed during the oral proceedings was novel within the meaning of Article 54 EPC, and not obvious to the skilled person (Article 56 EPC). Thus, the patent was maintained on the basis of the second auxiliary request and the description amended accordingly.

III. Claim 1 of the second auxiliary request read:

"1. A process for isolating nucleic acid from a nucleic acid-containing complex biological starting material, characterized by mixing the complex biological starting material, a chaotropic substance and a nucleic acid binding solid phase comprising silica or a derivative thereof, separating the solid phase with the nucleic acid bound thereto from the liquid, whereafter thus obtained solid phase-nucleic acid complexes are washed and, if required, the nucleic acid is eluted from said complexes, wherein the starting biological material is selected from: whole blood, blood serum, buffy coat, urine, feces, liquor cerebrospinales, sperm, saliva, tissue, cell culture, foods products, vaccines, milk infected with a virus or a bacterium, vegetable

material, gram-positive bacteria yeast, mould, body fluid and biological material possibly infected with virusses or bacteria."

Claims 2 to 10 concerned different embodiments of the process according to claim 1.

IV. Opponent 01 and opponent 02 lodged an appeal against the decision of the opposition division, filed a statement of grounds of appeal and requested that the decision be set aside and the patent revoked. The respondent (patent proprietor) requested that the appeals be dismissed. Opponent 02 and the respondent requested oral proceedings in the event that the board did not intend to grant their respective requests. Opponent 01 withdrew its appeal in a letter dated 5 April 2004.

V. The parties were summoned to oral proceedings. In a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal sent with the summons, the board expressed its provisional opinion on procedural matters arising from the submissions of the parties, as well as on substantive matters in connection with Articles 123(2) and 56 EPC.

VI. Oral proceedings took place on 6 April 2004 in the absence of the appellant (opponent 02), who had informed the board that it would not attend. In response to issues relating to Article 123(2) EPC raised by the board in its communication and during the oral proceedings, the respondent filed an amended main request that replaced the main request previously on file. Claim 1 of the amended main request differed from

the corresponding claim in the set of claims allowed by the opposition division (see section III above) in that the terms "tissue, cell culture", "milk infected bacteria yeast, mould, body fluid" and "virusses" were replaced by "tissue**s and** cell culture**s**", "milk infected bacteria**,** yeast**s**, mould**s**, body fluid**s**" and "viruses", respectively.

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

(S1): US 4 483 920;

(S5): Kristensen, T. et al., Nucleic Acids Research, July 1987, Vol. 15(14), pages 5507 to 5516;

(S7): BIO 101 Inc., 1986, Instructions sheet for the Geneclean kit;

(S8): WO 87/06621;

(S9): Manser, T. and Gefter, M.L., April 1984, Proc. Natl. Acad. Sci. USA, Vol. 81, pages 2470 to 2474.

VIII. The submissions made in writing by the appellant, as far as they are relevant to this decision, may be summarised as follows:

Claim 1 insofar as it related to "vegetable material, gram-positive bacteria, yeasts and moulds" as starting materials, did not meet the requirement of industrial applicability (Article 57 EPC) and/or solve the problem of isolating nucleic acid from complex, untreated

biological materials, because such materials would require pre-treatment.

In giving weight and importance just to the examples in document (S1) while neglecting or downgrading to a "speculative part" the teaching given in the generic disclosure of (S1), the opposition division made an erroneous assessment both from a legal and a technical standpoint. The actual teaching of document (S1) focused on a direct immobilization of RNA from cells, ie from a non-purified, complex material. The examples given in (S1) were directed to demonstrate partial aspects of the general method taught in (S1). The choice in Example 4 of one of the filter materials (nitrocellulose) indicated in the generic disclosure and demonstrated in Example 1 to be suitable to immobilize RNA, did not disprove the usefulness of the other one, ie of glass fibres.

Both (S8) and (S9) disclosed the technical problem of achieving a purification of nucleic acids directly from complex materials without requiring a prior purification, and the solution thereto consisting in the taught "principle of immobilization" by using a chaotrope and a solid binding support. Document (S8), while indicating nitrocellulose as a preferred filter material, still indicated to the skilled man the existence of other possibilities, thus suggesting to him the possibility of broadening the "spectrum" of filters still usable in the disclosed method.

While no more citable against novelty, document (S5) remained a particularly relevant reference against the inventive step by teaching the usefulness of a glass

support in binding non pre-purified nucleic acid directly from mixtures thereof with further components. Also document (S7) suggested to the skilled man the possibility of using glass for isolating nucleic acids from mixtures with proteins in the presence of a chaotrope (under the Heading "Removing RNA and proteins").

The skilled man not only **could** have, but also **would** have used, or at least would have made an attempt to use, glass in the method of (S8) or (S9), as an alternative to nitrocellulose with a good expectation of success based on the disclosure given in (S1), (S5) or (S7).

IX. The respondent's submissions in writing and at oral proceedings may be summarized as follows:

Document (S1) solely disclosed the use of nitrocellulose filters in relation to the binding of mRNA directly from dissolved cells. The authors of (S1) did not contemplate the use of any other filter materials or the binding of other nucleic acids.

In document (S5) nucleic acid was isolated from a phage suspension by subjecting the suspension to a precipitation with acetic acid, and applying the precipitate to a glass fibre filter. Although the procedure was identified as simple and rapid, it was not a one-step procedure as in the present invention.

Starting from document (S8) as the closest prior art, the problem to be solved by the invention was to provide a non-selective process for isolation of

nucleic acids without pre-treatment of the biological material. By "non-selective" it was meant that essentially all kinds of nucleic acid (ssDNA, dsDNA, mRNA, tRNA and rRNA) present in the starting material could be isolated by the process according to the invention. The claimed processes did not require a previous purification or isolation treatment of the starting material by which the target nucleic acid was isolated from its accompanying components (and thereby purified). Cell permeabilization as required for some of the starting materials mentioned in claim 1 (eg vegetable material) was not considered a pre-treatment in the context of the invention.

X. The appellant requested in writing that the decision under appeal be set aside and the patent revoked.

XI. The respondent requested that the decision under appeal be set aside and the patent maintained on the basis of the main request filed during the oral proceedings or on the basis of one of auxiliary requests 1 to 3 filed on 5 March 2004.

## Reasons for the Decision

*Formal issues*

1. In the absence of the approval of the respondent, the new opposition ground of lack of industrial applicability (Article 57 EPC) raised by the appellant in its statement of grounds of appeal, cannot be taken into account (see decision of the Enlarged Board of Appeal G 10/91, OJ EPO 1993, 420).

*Main request*

2. The requirements of Article 123(2) EPC are satisfied by the amended main request filed during the oral proceedings, support for the amendments being found on page 2, lines 5 and 6 ("tissues" and "cell cultures") and line 49 ("foods products"), and on page 3, line 1 ("yeasts" and "moulds") and line 21 ("body fluids") of the application as filed (cf. published version).

3. The finding of the opposition division with respect to novelty (Article 54 EPC) has not been contested by the appellant. The board sees no reason to question novelty of the subject-matter of the amended main request, in view of any of the documents on file.

4. Thus, the sole issue that remains to be assessed is whether the subject-matter of claims 1 to 10 of the main request involves an inventive step within the meaning of Article 56 EPC, ie whether, having regard to the prior art on file, the claimed subject-matter was not obvious to a person skilled in the art.

5. The closest prior art is represented by document (S1), which discloses a process for isolating and immobilizing a nucleic acid (mRNA) from a complex biological material (eg blood, urine, sputum, lymph, etc; see column 3, lines 41 to 42 of (S1)) comprising the steps of:

(i) solubilizing cellular components with a chaotropic salt (NaI; see step (c) in column 3, lines 7 to 8 and column 4, lines 15 to 29),

(ii) filtering the mixture through filters which selectively bind message RNA, for instance a nitrocellulose or a glass fibre filter (see step (d) in column 3, lines 9 to 10 and column 4, lines 31 to 41), and

(iii) washing the filter with the mRNA bound to it (see step (e) in column 3, lines 11 to 13 and column 4, lines 43 to 52).

6. In the light of document (S1), the technical problem to be solved can be defined as being the provision of an alternative process for isolating nucleic acid from a complex biological starting material.

7. As a solution to this problem, claim 1 of the main request at issue proposes a process characterized by the steps of mixing the complex biological starting material, a chaotropic substance and a nucleic acid binding solid phase comprising silica or a derivative thereof, separating the solid phase with the nucleic

acid bound thereto from the liquid, and washing the solid phase-nucleic acid complexes thus obtained. In a final optional step, the nucleic acid can be eluted from the solid phase-nucleic acid complexes.

8. The process according to claim 1 differs from the process disclosed in document (S1) in that a filtration step is not required for binding the nucleic acid to the solid phase. According to the invention, mixing the starting material with the chaotropic substance and the silica or a derivative thereof allows solubilization of the biological material and binding of the nucleic acid in solution to the silica solid phase in only one step, without the need for filtration. Document (S1) does not give the skilled person any hint in this respect.

9. Nor are any hints provided by documents (S5), (S8) and (S9), which relate exclusively to processes for isolating nucleic acid by filtering the nucleic acid-containing material through a nitrocellulose (see documents (S8) and (S9)) or glass fibre filter (see document (S5)). Thus, contrary to the appellant's allegation, the skilled person could not have arrived at a process falling under the terms of claim 1 by combining the teaching of document (S1) with the disclosure of any of the documents (S5), (S8) or (S9).

10. Document (S7), which has also been cited by the appellant in this context, relates to a process requiring several purification steps prior to the binding of the thereafter nearly pure DNA (for instance, plasmid DNA or DNA isolated from an agarose gel) to glassmilk in the presence of a chaotropic salt. In the board's view, the skilled person seeking a simple

method to isolate nucleic acid from a complex biological material, would not have considered the method of (S7) to be suitable for replacing the filtration step in the process disclosed in document (S1) in order to simplify the process. Even if one presumes that the skilled person would have considered using glassmilk to bind nucleic acid in solution, he/she did not have, in the board's judgement, a reasonable expectation of success of isolating nucleic acid free of cellular components and proteins without prior purification and in only one step.

11. For these reasons, the board concludes that, having regard to the prior art on file, the subject-matter of claim 1 of the main request was not obvious to the skilled person at the priority date and that, therefore, it involves an inventive step within the meaning of Article 56 EPC. Since dependent claims 2 to 10 of the main request relate to particular embodiments of the process according to claim 1, an inventive step has to be acknowledged for their subject-matter.

12. Page 2a was amended to bring the description into line with the claims of the main request. The amendments do not introduce subject-matter which extends beyond the content of the application as filed. The requirements of Article 123(2) EPC are thus fulfilled.

## Order

**For these reasons it is decided that:**

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent with the following documents:

   claims of the main request and description page 2a filed during the oral proceedings before the board, description pages 2, 2b, 4, filed during oral proceedings before the opposition division, description pages 1, 3, 5 to 16 of the patent specification.

The Registrar: The Chairman:

A. Wolinski L. Galligani