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**Datasheet for the decision
of 2 September 2014**

Case Number: T 2288/11 - 3.3.08

Application Number: 05709892.3

Publication Number: 1721991

IPC: C12Q1/68, C12N15/10, G01N33/50

Language of the proceedings: EN

Title of invention:
METHOD OF DETECTING NUCLEIC ACID AND UTILIZATION THEREOF

Applicant:
FUSO PHARMACEUTICAL INDUSTRIES LTD.

Headword:
Nucleic acid detection air-dried whole blood sample direct PCR/
FUSO

Relevant legal provisions:
EPC Art. 111

Keyword:
Main and Auxiliary Requests: remittal to the department of
first instance (yes)

Decisions cited:
G 0010/93

Catchword:



**Beschwerdekammern
Boards of Appeal
Chambres de recours**

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Case Number: T 2288/11 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 2 September 2014

Appellant: FUSO PHARMACEUTICAL INDUSTRIES LTD.
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Representative: Graf von Stosch, Andreas
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Decision under appeal: **Decision of the Examining Division of the
European Patent Office posted on 6 June 2011
refusing European patent application No.
05709892.3 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman M. Wieser
Members: P. Julià
J. Geschwind

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division to refuse the European patent application no. 05 709 892.3. The examining division considered the set of claims filed on 12 April 2011 not to fulfil the requirements of Article 56 EPC. Claim 1 of this set of claims read as follows:

"1. A nucleic acid detection method comprising the steps of:

providing a sample support divided into a plurality of compartments;

fixing a cell-containing sample on the surface of the support in the plurality of compartments according to the following sub-steps (i) through (iv):

- (i) smearing the sample in the divided compartments,
- (ii) air-drying the smeared samples,
- (iii) adding 75% ethanol to each compartment, and
- (iv) air-drying the samples with a thermal cycler after removing the 75% ethanol;

exposing nucleic acids contained in the sample fixed on the support in the plurality of compartments;

amplifying nucleic acids exposed from the sample fixed in the plurality of compartments on the support by placing a PCR mixture containing primers for amplifying a target nucleic acid into the plurality of compartments; and

determining whether the amplified nucleic acids in a PCR solution existing outside the fixed cell sample in

the plurality of compartments contains a target nucleic acid."

Claims 2 to 10 were directed to preferred embodiments of the nucleic acid detection method of claim 1.

II. The applicant (appellant) filed a notice of appeal and a statement setting out the Grounds of Appeal. With the Grounds of Appeal, the appellant maintained the set of claims underlying the decision under appeal and filed, as an Annex, new experimental evidence ("*Comparison of Claimed Invention with Direct PCR*"). The appellant requested the board to set aside the decision under appeal and to grant a patent on the basis of the set of claims filed with its Grounds of Appeal. Oral proceedings were requested as an auxiliary measure.

III. On 28 May 2014, the board summoned the appellant to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, the appellant was informed of the board's preliminary, non-binding opinion on the substantive issues of the case.

In particular and with reference to decision G 10/93 (OJ EPO 1995, page 172, Headnote) wherein the Enlarged Board of Appeal stated that in an appeal proceedings from a decision of an examining division, the board had the power to examine whether the application meet all the requirements of the EPC, the board raised several objections under Article 84 EPC which were new in the proceedings. The board introduced two new documents into the proceedings, namely G.S. Makowski et al., *Clinical Chemistry*, Vol. 41, No. 3, 1995, pages 477-479 (document D22) and A. Ulvik and P.M. Ueland, *Clinical Chemistry*, Vol. 47, No. 11, 2001, pages 2050-2054

(document D23) and made several remarks on inventive step (Article 56 EPC).

IV. On 15 July 2014, the appellant replied to the communication of the board and filed a new Main Request and a new Auxiliary Request. Substantive submissions concerning Articles 123(2), 84 and 56 EPC were provided.

Claim 1 of the new **Main Request** read as follows:

"1. A nucleic acid detection method comprising the steps of:

providing a sample support divided into a plurality of compartments;

fixing a cell-containing sample, said sample being white blood cells collected from 5 ml of blood collected from human patients suspected to have infection-causing microbes selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*, whereby the white blood cells are suspended in 150 μ l PBS, on the surface of the support in the plurality of compartments according to the following sub-steps (i) through (iv):

- (i) smearing 5 μ l of the sample in the divided compartments,
- (ii) air-drying the smeared samples,
- (iii) adding 75% ethanol to each compartment, and
- (iv) air-drying the samples with a thermal cycler after removing the 75% ethanol;

exposing nucleic acids contained in the sample fixed on the support in the plurality of compartments by treatment with an enzyme reagent containing N-acetylmuramidase, lysozyme and lysostaphin for 10 minutes at 37°C, followed by 10 minutes at 95°C;

amplifying nucleic acids exposed from the sample fixed in the plurality of compartments on the support by nested PCR, whereby in the first run a PCR mixture containing 0,16 µM of each of the primers according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 18, is placed into the plurality of compartments, and whereby in the second run 0,16 µM of each of the primers according to SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30 are used; and

determining whether the amplified nucleic acids in a PCR solution existing outside the fixed cell sample in the plurality of compartments contains a target nucleic acid."

Claims 2 to 7 were directed to preferred embodiments of the acid nucleic detection method of claim 1 and read as claims 3-6 and 9-10 of the appellant's former Main Request.

Claim 1 of the **Auxiliary Request** read as follows:

"1. A nucleic acid detection method comprising the steps of:

providing a sample support divided into a plurality of compartments, whereby the sample support is shaped to fit a gene amplifier for PCR (thermal cycler);

fixing a cell-containing sample, said sample being white blood cells collected from 5 ml of blood collected from human patients suspected to have infection-causing microbes selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*, whereby the white blood cells are suspended in 150 μ l PBS, on the surface of the support in the plurality of compartments according to the following sub-steps (i) through (iv):

- (i) smearing 5 μ l of the sample in the divided compartments,
- (ii) air-drying the smeared samples,
- (iii) adding 75% ethanol to each compartment, and
- (iv) air-drying the samples with a thermal cycler after removing the 75% ethanol;

exposing nucleic acids contained in the sample fixed on the support in the plurality of compartments by treatment with an enzyme reagent containing N-acetylmuramidase, lysozyme and lysostaphin for 10 minutes at 37°C, followed by 10 minutes at 95°C;

amplifying nucleic acids exposed from the sample fixed in the plurality of compartments on the support by nested PCR according to the following sub-steps (1) through (4):

- (1) placing a PCR mixture containing 0,2 μ l TaKaRa LA Taq, 2 μ l 10x LA Taq buffer, 2 μ l 25 mM MgCl₂, 3,2 μ l dNTP mixture (2,5 mM each), 0,16 μ M of each of the

primers for amplifying a target nucleic acid, whereby the primers according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 17 and SEQ ID NO: 18 are used, and whereby the PCR mixture is adjusted to 20 µl with sterilized pure water, into the plurality of compartments,

(2) performing a first PCR run with the following cycling parameters: retention at 94°C for 1 min, 30 cycles consisting of 98°C for 20 s and 68°C for 3 min, and retention at 72°C for 5 min,

(3) placing 1 µl solution of the first run in a PCR tube supplemented with the foregoing composition, whereby the primers according to SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30 are used, and

(4) performing a second PCR run with the following cycling parameters: retention at 94°C for 1 min, 30 cycles consisting of 98°C for 20 s and 68°C for 1 min, and retention at 72°C for 5 min; and

determining whether the amplified nucleic acids in a PCR solution existing outside the fixed cell sample in the plurality of compartments contains a target nucleic acid."

Claims 2 to 6 were directed to preferred embodiments of the acid nucleic detection method of claim 1 and read as claims 3 to 6 and 10 of the appellant's former Main Request.

- V. In a communication of 28 July 2014, the board acknowledged the amendments made in the appellant's new requests to be a *bona fide* attempt to overcome the objections raised under Articles 84 and 56 EPC by the board in its communication pursuant to Article 15(1) RPBA. The board also noted that, in view of the number and character of these amendments and of the procedural history of the case, the board intended to remit the case to the department of first instance for further prosecution. The appellant was informed that the scheduled oral proceedings will be cancelled if it agreed with the board and formally requested such a remittal (cf. point 1 *infra*).
- VI. On 1 August 2014, the appellant requested the board to remit the case to the department of first instance for further prosecution on the basis of its Main Request and Auxiliary Request filed on 15 July 2014. The appellant further requested to cancel the scheduled oral proceedings.
- VII. On 8 July 2014, the board cancelled the oral proceedings scheduled for the 7 October 2014.

Reasons for the Decision

The amendments introduced in the new Main and Auxiliary Request have been carried out in direct response to objections raised for the first time in the board's communication. As there was no need or possibility for the appellant to file such amended requests earlier in time, they are admitted into the procedure.

Article 111 EPC

1. In its communication of 28 July 2014 (cf. point V *supra*), the board noted that:

"The appellant's Main Request and Auxiliary Request have been amended by deletion of several dependent claims, introduction of the subject-matter of dependent claims into independent claim 1, and introduction of several features and SEQ ID NOs from the description and the Sequence Listing of the application as filed. These amendments are a *bona fide* attempt to overcome the objections raised under Articles 84 and 56 EPC by the board in its communication pursuant to Article 15(1) RPBA.

In view of the number and character of these amendments, it is the board's opinion that for each and every feature introduced into claim 1 and for their combination in the particular context of this claim a detailed examination for a formal basis in the application as filed is required (Article 123(2) EPC). Likewise, it is also necessary to assess whether the new requests fulfil the requirements of Article 84 EPC. Moreover, the requirements of Article 56 EPC have to be assessed in the light of the prior art cited by the board in its communication pursuant to Article 15(1) RPBA and, if necessary, of additional prior art that takes into account the features newly introduced into the claims.

As it is apparent from the file, the decision under appeal was concerned only with Article 56 EPC and the board, in its communication pursuant to Article 15(1) RPBA, has raised new objections under Article 84 EPC for the first time in the proceedings and has also introduced two new prior art documents (D22-D23)

that were considered to be of relevance for assessing the requirements of Article 56 EPC.

In view of the procedural history of the present case and, in order to give the appellant the benefit of two instances, the board intends to remit the case to the department of first instance for further prosecution."

2. In reply to the board's communication (cf. point VI *supra*), the appellant itself modified its requests and asked the board to remit the case to the department of first instance for further prosecution on the basis of its Main Request and Auxiliary Request filed on 15 July 2014. The board notes that the requirements of patentability for the very specific subject-matter of claims 1 to 7 of the Main Request and of claims 1 to 6 of the Auxiliary Request have never been examined by the first instance department. Accordingly, no comments or reasons concerning it can be found in the decision under appeal.
3. In view of all these facts, the board decides that a remittal to the department of first instance is the most appropriate course of action in the present case.

Order

For these reasons it is decided that:

The decision under appeal is set aside.

The case is remitted to the department of first instance for further prosecution on the basis of the Main Request (claims 1 to 7) and the Auxiliary Request (claims 1 to 6), both filed on 15 July 2014.

The Registrar:

The Chairman:



A. Wolinski

M. Wieser

Decision electronically authenticated