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Datasheet for the decision of 9 February 2017

Case Number: T 1818/12 - 3.3.04

Application Number: 05803527.0

Publication Number: 1814912

IPC: C07K16/00, C12N1/00, B01D15/00, C12P21/00

Language of the proceedings: EN

Title of invention:
Process for obtaining antibodies

Patent Proprietor:
UCB Pharma, S.A.

Opponent:
Zimmer, Franz-Josef

Headword:
Production of antibodies/UCB PHARMA

Relevant legal provisions:
EPC Art. 56, 83

Keyword:
Sufficiency of disclosure - (yes)
Inventive step - main request (yes)

Decisions cited:

Catchword:

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Case Number: T 1818/12 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 9 February 2017

Appellant: (Opponent)	Zimmer, Franz-Josef Savitsstr. 18 81929 München (DE)
Representative:	Grünecker Patent- und Rechtsanwälte PartG mbB Leopoldstraße 4 80802 München (DE)
Respondent: (Patent Proprietor)	UCB Pharma, S.A. Allée de la Recherche 60 1070 Bruxelles (BE)
Representative:	Campbell, Patrick John Henry J A Kemp 14 South Square Gray's Inn London WC1R 5JJ (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 19 June 2012 concerning maintenance of the European Patent No. 1814912 in amended form.**

Composition of the Board:

Chairwoman	G. Alt
Members:	A. Chakravarty
	P. de Heij

Summary of Facts and Submissions

I. An appeal was lodged by the opponent (appellant) against the interlocutory decision of the opposition division that European patent No. EP-B-1 814 912, entitled "*Process for obtaining antibodies*", in amended form, met the requirements of the EPC (Article 101(3)(a) EPC). The patent proprietor is party as of right to the appeal proceedings (respondent - Article 107 EPC).

II. An opposition was filed against the patent as a whole based on Article 100(a) EPC (lack of novelty, Article 54 EPC, and lack of inventive step, Article 56 EPC), Article 100(b) EPC (disclosure of the invention), and Article 100(c) EPC (added subject-matter). The opposition division held that the latter ground had not been substantiated and that the subject-matter of the main request was novel, involved an inventive step and that the patent disclosed the claimed invention sufficiently clearly and completely for it to be carried out by a person skilled in the art.

III. The following documents are cited in the present decision:

D3: US 5 665 866

D5: Middelberg A., Biotechnol. Adv.. 1995, 13(3), 491-551.

D6: Middelberg A. et al., 1991, Biotechnol. Bioeng., 38, 363-370.

D14: Ramanan R.N. et al., 2009, Am. J. Biochem. Biotechnol., 5(1), 21-29.

Declaration of Dr David Humphreys, dated 21 March 2013

Annex 1 filed before the opposition division

Annex A filed with the statement of grounds of appeal.

IV. With the statement of grounds of appeal, the appellant maintained objections of lack of an inventive step (Article 56 EPC) and lack of sufficient disclosure (Article 83 EPC) against the subject-matter of the claims considered allowable in the decision under appeal. Moreover, they submitted Annex A containing "*further examples [...] which confirm the results of Annex 1* [filed before the opposition division]".

V. The respondent replied to the appellant's statement of grounds of appeal and re-submitted the set of claims considered allowable in the decision under appeal. They also filed a declaration of Dr David Humphreys.

VI. Claim 1 of the set of claims found allowable in the decision under appeal (main request) reads as follows:

"1. A method for the manufacture of recombinant antibody molecules comprising culturing an *E. coli* host cell sample transformed with an expression vector encoding a recombinant antibody molecule that is expressed in the periplasm of the host cell and subjecting said host cell sample to a heat treatment step, characterised in that said sample is subjected to a non-lysing pressure treatment step between 1000 psi

(68.9 bar) and 4000 psi (275.8 bar) before being subjected to an increase in temperature within the range of 30°C to 70°C for a period of up to 24 hours".

Claims 2 to 5 depend on claim 1 and relate to embodiments thereof.

VII. The board issued a communication pursuant to Article 15(1) RPBA, setting out its preliminary appreciation of substantive and legal matters concerning the appeal.

VIII. Both parties replied to the board's communication. The respondent submitted document D14 with their reply. In a further letter, the appellant informed the board that they would not attend the scheduled oral proceedings.

IX. Oral proceedings before the board were held in the absence of the appellant on 9 February 2017. At the end of the proceedings the Chairwoman announced the decision of the board.

X. The appellant's arguments relevant to the decision can be summarised as follows.

Main Request - Claims 1 to 5

Disclosure of the invention - Article 100(b) and Article 83 EPC

The opposition division had been wrong to accept that the patent met the requirements of Article 83 EPC.

The non-lysing pressure step included in claim 1 was critical to the effect of improved yield, allegedly achieved. However, there was evidence in Annexes 1 and

A, in the patent itself and in document D6, that many bacterial strains lysed under the pressures mentioned in the claim. For instance, document D6 disclosed non-working embodiments including strain JM-109, in which the cells lysed under the pressure conditions set out in the claim. In fact, apart from the single example in the patent, there was no known cell system available which would not lyse under the conditions of the claim. The data both in Annex 1 and in newly filed Annex A showed that there was no correlation at all between pressure treatment and cell lysis in the range between 1000 to 4000 psi.

The opposition division cited decision T 292/85 to support the view that "*inoperable components*" were immaterial for the assessment of enablement as long as there are suitable variants known to the skilled person. However, this decision did not reflect the case law that held that the disclosure of a patent must enable the skilled person to carry out the invention over the entire range claimed, see decisions T 409/91, T 435/91, T694/92 and T 187/93.

In view of the lack of known suitable cell types that did not lyse under the pressures mentioned in the claim, the skilled person trying to carry out the invention as claimed would have had to carry out trial and error experiments to determine which pressure to use for any particular strain to avoid cell lysis, amounting to an undue burden for the skilled person.

Inventive step - Article 56 EPC

The claim related to a method for the production and isolation of functional recombinant antibodies employing the particular combination of pressure and

heat treatment set out in claim 1, which was alleged to result in increased yields as compared to the methods known in the prior art. In particular, the antibodies were expressed in *E. coli* and produced in sufficient quality and quantities needed for therapy. Figures 4a and 4b of the patent showed that the yield of an Fab fragment produced by a method as claimed, was slightly increased between 1000 psi and 4000 psi but at higher pressures the yield slightly decreased again. However, the available evidence showed that increased yield could in fact only be obtained using the unique system used in the Examples of the patent and not over the entire scope claimed.

As mentioned in the context of disclosure of the invention, document D6 provided evidence of *E. coli* cell lysis under the pressure and temperature conditions of the claim. Similarly, Annexes 1 and A were submitted as additional evidence to show, *inter alia,* that due to cell lysis under conditions of the claim, the skilled person would not be able to carry out the invention other than by using the strain exemplified in the patent.

Annex 1 showed that a non-lysing pressure treatment did not lead to an improved antibody yield over the entire scope claimed. This evidence was now confirmed by Annex A.

Annex A reported the results of experiments done to repeat the work shown in Figure 4 and Table 1 of the patent using an inducible expression system, similar to that used in the patent. In addition, the claimed method was tested using an auto-induced expression system. The results from these experiments showed that an increased yield of functional antibodies could be

obtained for the specific expression system used in the patent at temperatures of 50° and 60° Celsius. However, it was not plausible that the same was true at lower temperatures within the claimed range or in an auto-induced expression system. Annex A (Figures 2a to 2c) showed that there was no yield increase with pressure in the claimed range at any temperature in an auto-induced system, where an increase in pressure and temperature was expected to lead to a reduction in the yield.

Obviousness

The claimed subject-matter was obvious. The opposition division's choice of closest prior art was document D3 and their formulation of the problem to be solved was the provision of a method to increase the selective extraction of antibodies expressed in the periplasm of *E. coli*. The opposition division had concluded that the application of a non-lysing pressure of 1000 to 4000 psi prior to the heat treatment lead to an improved selective extraction of antibodies.

However, as noted above, this effect was not achievable over the whole scope claimed. There was in fact no correlation between the degree of cell lysis and yield, which, in the auto-inducible system (see Annex A), were almost anti-correlated.

Document D5 was a review of effective techniques for performing cell disruptions. It disclosed the use of heat and gentle shaking to extract proteins secreted to the periplasm of *E. coli*. It also disclosed the use of various pressure techniques such as bead milling, homogenizing or using a microfluidiser, and the combination of these with a heat treatment step.

The combination of pressure treatment with the heat treatment method of document D3 was derivable from the disclosure of document D5, a combination of the disclosure of document D3 with that of document D5 leading directly to the claimed method.

Experimental data

The experimental data in Annex A had been filed to support arguments relating to sufficiency of disclosure and inventive step. It was reliable for the following reasons:

- the experiments were performed under conditions as close as possible to those described in the patent, for instance they employed a derivative of the *E. coli* strain W3110 used in the patent,

- the experiments employed conventional conditions and were scientifically reasonable. Final biomass concentrations were in comparable ranges to those reported in the examples of the patent,

- the experiments included a heat treatment step very similar to that described the patent.

- the respondent had confirmed that the system tested in Annex A involved periplasmic expression, by stating that "*Annex A contains data that supports the claims of the patent*".

- the experiments used pressures within the claimed range. Non-lysing conditions were defined in the patent to be between 1000 and 4000 psi and thus were an inherent feature of any method carried out at these pressures.

XI. The respondent's arguments relevant to the decision can be summarised as follows:

Main Request- Claims 1 to 5

Disclosure of the invention - Article 100(b) and Article 83 EPC

There were three physical steps in claim 1, all of which could easily be performed by a skilled person. There were also two functional limitations, i) that antibody molecules are produced and ii) that the pressure treatment is non-lysing. The question on sufficiency was therefore whether or not the skilled person could have readily performed the three physical steps of the claim in a manner that resulted in antibody being produced and did not lead to cell lysis. The answer to this question was 'yes', as the matter of identifying those embodiments that worked was a matter of routine experimentation.

The data in the patent, as shown in Figures 2, 3a, 3b, 4a, 4b and 5 taken as a whole, showed that, at pressures in the range between 1000 and 4000 psi, there was, surprisingly, a significant improvement in the yield of antibody without any significant increase in the release of undesirable non-specific protein.

The appellant's arguments on lack of sufficient disclosure due to the presence of non-working embodiments failed because there was no evidence to back them up. Document D6 did not provide evidence of non-working embodiments because it concerned the production of antibodies in "*inclusion bodies*", i.e. intracellularly and not in the periplasm as required by the present invention. The evidence provided by Annexes

1 and A was unreliable - see below under "*Experimental data*" - and could not therefore be taken into account.

Inventive step - Article 56 EPC

The closest prior art was represented by document D3 which focused on a process comprising a heat treatment step to facilitate the isolation of correctly folded and assembled antibody molecules, substantially free of host cell protein and also free of partially degraded or incorrectly folded antibody molecules.

The claimed process constituted an improvement over the closest prior art in terms of the yield of high purity antibodies obtained. Evidence for this was to be found in the patent, for instance in Figures 2, 3 and 4a and in Table 1. The improvement was due to the addition of a non-lysing pressure treatment at between 1000 and 4000 psi.

The problem to be solved by the claimed invention was therefore to provide an improved process for manufacturing antibodies.

Document D3 did not disclose either pressure treatment of between 1000 psi and 4000 psi or the treatment of cells under non-lysing conditions.

Document D5, on the other hand, was a general review and disclosed an array of different techniques for disrupting microorganisms (see e.g. page 498). The pressure treatments disclosed were all of a kind that disrupted the entire cell.
There was nothing in D5 that suggested that pressure could be used in a gentle, specific manner, as claimed, nor that a mild non-lysing pressure of 1000 to 4000 psi

could be used to extract antibodies from the periplasm without also extracting intracellular material. Pressure was seen in the prior art simply as a way of crudely breaking open cells, not of selectively stripping antibody out of the periplasm.

Starting from document D3 there was also nothing in this document that would have suggested the use of pressure implemented in the claimed method. Thus, the claimed method involved an inventive step.

Experimental data

The appellant had filed Annexes 1 and A in support of arguments relating to disclosure of the invention and inventive step. However, the experiments disclosed therein were not reliable or convincing and should be disregarded by the board in its considerations under Article 100(b)/Article 83 EPC and Article 56 EPC, for the reasons set out in the declaration of Dr David Humphreys. They were *inter alia*:

- the lack of an identifiable author - both annexes contained the results of experiments but gave no information as to who carried out the experiments.

- there was no indication that the experiments were done according to the method claimed. In particular, there was no evidence that the expression of the antibody was periplasmic or that the pressure conditions used were non-lysing.

- there were major inconsistencies between the results of Annex 1 and Annex A, which cast a doubt on the fundamental reliability of methods used and thus on the information presented in both documents.

XII. The appellant requested that the decision of the opposition division be set aside and the patent be revoked in its entirety.

XIII. The respondent requested that the appeal be dismissed.

Reasons for the Decision

1. The oral proceedings were held in the absence of the appellant, in accordance with Rule 115(2) EPC and Article 15(3) RPBA. Accordingly, the appellant is treated as relying on its written case.

Main Request - Claims 1 to 5

Disclosure of the invention - Article 100(b) and Article 83 EPC

2. The ground for opposition Article 100(b) EPC requires that the European patent discloses the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. According to the case law of the boards of appeal, the subject-matter of a patent is sufficiently disclosed if the skilled person is able to obtain substantially all embodiments falling within the ambit of the claims. Moreover, it must be possible to reproduce the invention on the basis of the patent without any inventive effort and undue burden (see Case Law of the Boards of Appeal of the European Patent Office, 8th edition, II.C. 4.1, 4.4 and 5.6)

3. The appellant argued that finding *E. coli* strains suitable for use in the claimed method, other than the strain W3110, was an undue burden for the skilled person. Document D6, provided evidence of cell lysis under the pressure and temperature conditions of the

claim. Similarly, Annexes 1 and A were submitted as additional evidence to show, *inter alia*, that due to cell lysis under conditions of the claim, the skilled person would not be able to carry out the invention other than by using the strain exemplified in the patent.

4. Document D6 concerns "*the high-pressure homogenization of Escherichia coli, strain JM101, containing inclusion bodies of recombinant porcine somatotropin*" (see abstract). None of the findings on the lysis of *E. coli* strains under homogeniser pressure (see page 368, right column, penultimate paragraph and Figure 7) relate to periplasmic expression of a protein. It was concluded that "*the overexpression of a foreign protein leads to weakening of the cell wall*" (*Id.*, paragraph 1), while "*at higher pressures E. coli which did not contain recombinant inclusion bodies (batch 5) disrupt less easily than the equivalent induced cells*" (*Id.*, left column, final paragraph).

5. There is therefore no evidence in document D6 that *E. coli* having periplasmic expression also have weakened cell walls. Thus, the lysis of *E. coli* cells expressing a foreign protein to inclusion bodies, reported in document D6, is not evidence that *E. coli* cells expressing antibodies to the periplasm would lyse under the same conditions. In view of this, the board considers that there is no evidence in document D6 that the skilled person would encounter any difficultly in identifying strains of *E. coli* periplasmically expressing recombinant proteins that do not lyse under conditions of the claim.

5.1 Thus, document D6 does not provide evidence that the skilled person cannot carry out the invention as claimed without undue burden.

6. In relation to the evidence provided in Annexes 1 and A, the established case law of the boards is that there are no firm rules according to which types of evidence are, or are not, convincing. Each piece of evidence is given an appropriate weighting according to its probative value on a case-by-case basis (see Case Law of the Boards of Appeal of the European Patent Office, 8th edition, III.G.4.2).

7. In the case at hand, the board has noted a number of issues that arise when considering the Annexes. These include the following:

7.1 Neither Annex 1 nor Annex A has an identifiable author. In the case law of the boards "*an unsigned statement by an unknown and unnamed person should in principle be given minimal weight*" (see *Id.*, 4.2.1). Moreover, the level of skill of the person who carried out the experiments is unknown.

7.2 It is not clear if, in the experiments reported in the Annexes, the expression of the antibody was to the periplasm. The board notes that expression of a protein to the periplasm in *E. coli* is generally governed by the presence of an appropriate signal sequence. Neither Annex nor any of the appellant's written submissions provide any details about the expression vector used.

7.3 The information presented in Annex 1 is, in part, inconsistent with that presented in Annex A. For instance Annex 1 (Abb. 4) and Annex A (Fig. 1b) relate to the same experiment and both show the amount of

product (antibody) recovered in mass per unit volume. The yield obtained for induced and auto-induced systems at 70 and 200 bar shown in Abb. 4 of Annex 1 and Fig. 1b of Annex A is shown below;

	Annex 1 (Abb.4)	Annex A (Fig. 1b)
70 bar		
Auto-induced	ca. 50 mg/l	0 mg/l
Induced	ca. 250 mg/l	1050 mg/l
200 bar		
Auto-induced	ca. 230 mg/l	0 mg/l
Induced	ca. 420 mg/l	1200 mg/l

7.4 The fact that there is no correspondence between the yield achieved for the induced and the auto-induced systems between the two sets of results, although these relate to the same experiment, leads to the conclusion the there must be an error, either in the reporting of the results or in the experiments themselves.

8. All of the above factors lead the board to the conclusion that the evidence in both Annex 1 and A is of a nature that it cannot convincingly demonstrate that the skilled person would face an undue burden in carrying out the invention as claimed.

9. The board is therefore satisfied that the requirements of Article 83 EPC are met for the subject-matter of the claims.

Inventive step - Article 56 EPC

10. To assess whether or not a claimed invention meets the requirements of Article 56 EPC, the board applies the

"*problem and solution*" approach, long established in the case law of the boards of appeal (see Case Law of the Boards of Appeal of the European Patent Office, 8th edition, I.D.2).

The closest prior art

11. Document D3 discloses, *inter alia,* methods for the manufacture of recombinant antibodies from the periplasm of *E. coli* strain W3110 and retrieval of folded and soluble material by applying heat treatment (see column 13, line 64 to column 14, lines 1 to 19). This document was seen as representing the most relevant state of the art for assessing inventive step by the opposition division and both parties. The board has no reasons to depart from this assessment.

The technical problem and its solution

12. The method of claim 1 differs from that disclosed in document D3 in the inclusion of a step of subjecting the *E. coli* host cell sample to a non-lysing pressure treatment. According to the patent, the technical effect of this is "*that non-lysing treatment in combination with heat treatment, brings an increase in the yield of functional antibody at the primary extraction stage of up to 50%; i.e. the yield of functional antibody is increased above that of heat treatment alone*" (see paragraph [007]).

13. The appellant, on the basis of evidence in the patent itself, in Annexes 1 and A and in document D6, disputes that this effect is achievable over the entire scope of the claim.

14. It is established case law of the boards of appeal that "*If the inventive step of a claimed invention is based on a given technical effect,* [it] *should, in principle, be achievable over the whole area claimed*" (see Case Law of the Boards of Appeal of the European Patent Office, 8th edition 2016, ID. 9.8.3).

15. The data provided in the patent in Figures 2, 3b, 4a and Table 1 shows that the yield of antibody obtainable by carrying out the method as claimed is improved in comparison to the yield obtainable without the non-lysing pressure conditions, i.e. in comparison with a method representing the closest prior art. Figure 3(a) of the patent (a histogram showing the effect of pressure treatment on the yield of functional antibody at 60°C) shows that the yield of functional antibody at 1000 psi (319 mg/l) was slightly less than the control (atmospheric pressure; 343 mg/l). However, in the same experiment, the yield at pressures of 2000 and 4000 psi was greater than the control (460 and 921 mg/l, respectively).

16. Thus, while the patent discloses that a single set of conditions, falling within the ambit of claim 1, did not result in an improved yield, it also discloses several other working embodiments of the claimed method which did result in an improved yield. For the board, the overall evidence in the patent convincingly demonstrates that using the claimed method leads to an improved yield of high purity antibodies, achievable over substantially the whole area claimed.

17. As far as document D6 and Annexes 1 and A are concerned, the board considers that none of them provides evidence that the technical effect of improved yield of high purity antibodies cannot be achieved over

the whole area claimed for the reasons given in points 4. to 8. above.

18. Hence, the technical problem can be seen as to improve the yield of a method for the production of high purity recombinant antibodies.

Obviousness

19. The question to be answered in considering obviousness is whether the person skilled in the art, seeking a solution to the above formulated problem, and starting from the closest prior art as represented by document D3, would have considered that subjecting an *E. coli* sample, periplasmically expressing recombinant antibody, to a non-lysing pressure treatment step at between 1000 psi and 4000 psi, before subjecting it to an increase in temperature, was obvious.

20. The appellant has argued that the solution as presented in claim 1 was obvious because it combines heat with pressure treatment which combination was derivable from the disclosure of document D3 combined with that of document D5.

21. Document D5 is a review of process-scale techniques used to disrupt host cells for the large-scale manufacture of biological products. In relation to the release of proteins from the periplasmic proteins it states "[...] *chemical attack of the outer membrane allows periplasmic proteins to be released. Enzymatic methods generally involve enzymatic attack of the peptidoglycan layer in gram-negative bacteria, and of the mannoprotein and glucan components of the yeast wall*" (page 499, first paragraph). More specifically it states "*EDTA is clearly effective at disrupting the*

outer membrane, and may therefore be employed to recover periplasmic proteins" (page 501, final paragraph).

22. On the other hand, pressure treatment is mentioned on page 498 as follows: "*Complete destruction of the wall in a non-specific manner is usually achieved by mechanical means. Laboratory-scale methods [...] include the French press, shaking with glass beads and sonication. At process scale, mechanical methods are restricted primarily to bead milling, high-pressure homogenization, and microfluidization...*".

23. In summary, document D5 suggests chemical means for the specific release of proteins from the periplasm and discloses pressure treatments as a means for the complete destruction of the cell wall and the release of the entire intracellular content.

24. From this, the board concludes that the skilled person starting from document D3 and seeking to improve the yield of high purity antibody would not have considered including an additional pressure step before the heat treatment step, since such steps were seen as a means of totally destroying the cell wall.

25. It follows that the board holds that the subject-matter of claim 1 was not obvious to the person skilled in the art at the effective date of the patent. Dependent claims 2 to 5 relate to embodiments of claim 1. The conclusions on inventive step reached for the subject-matter of claim 1 therefore apply equally to the subject-matter of claims 2 to 5.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

P. Cremona G. Alt

Decision electronically authenticated