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D E C I S I O N
of 5 June 2002

Case Number: T 0295/99 - 3.3.4

Application Number: 91903835.6

Publication Number: 0469154

IPC: C12Q 1/32

Language of the proceedings: EN

Title of invention:
Method of determining glucose-6-phosphate and composition therefor

Patentee:
IATRON LABORATORIES, INC., et al

Opponent:
Roche Diagnostics GmbH

Headword:
Glucose-6-phosphate/IATRON

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
"Novelty (yes)"
"Inventive step (no)"

Decisions cited:
T 0472/88; T 0711/90; T 0522/91

Catchword:
-

Case Number: T 0295/99 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 5 June 2002

Appellant: (Proprietor of the patent)
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Respondent: (Opponent)
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Representative: -

Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 13 January 1999 revoking European patent No. 0 469 154 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: L. Galligani
S. C. Perryman

Summary of Facts and Submissions

I. The appeal was lodged by the patent proprietors against the decision of the opposition division dated 13 January 1999 by which the European Patent No. 0 469 154 entitled "Method of determining glucose-6-phosphate and composition therefor", which had been opposed by one party on grounds of lack of novelty and lack of inventive step, was revoked. Basis of the decision were a main request and two auxiliary requests. The opposition division considered that the subject-matter of all these requests lacked an inventive step having regard to the following documents:

(1) Vormbrock R. and Helger R., ENZYME, Vol. 38, Suppl. 1, September 1987, Abstract A/12, pages 20/21, to be read together with document (1a) to which explicit reference was made: Scand. J. Clin. Lab. Invest. Vol. 39, 1979, pages 1 to 5;

(2) Scopes R.K., FEBS Letters, Vol. 193, No. 2, December 1985, pages 185 to 188.

II. With the statement of grounds of appeal, the appellants filed, as a new main request, amended claims 1 to 3. They also submitted the declaration of Ms Yoko Endo.

III. The respondents filed their observations to the statement of grounds of appeal.

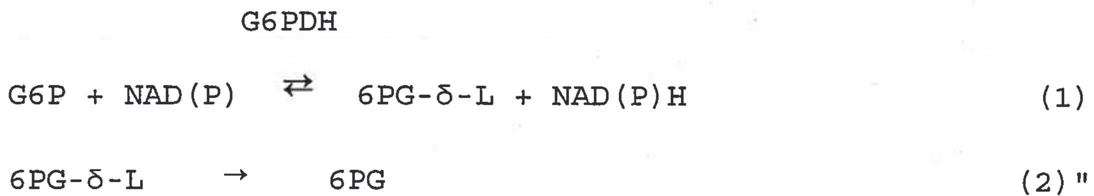
IV. On 24 January 2002, the board issued a communication with a preliminary view on some of the issues to be discussed, raising in particular objections under Article 123(2) and (3) EPC to the amended claims.

V. In reply to the board's communication, the appellants filed a new main request and four auxiliary requests. They also submitted new documents.

VI. During oral proceedings, which took place on 5 June 2002, the appellants filed a new main request and four auxiliary requests.

Claims 1 and 2 of the **main request** (consisting of six claims) and of the **first auxiliary request** (consisting of four claims) were identical and read as follows:

"1. A method for determining glucose-6-phosphate, including the step of dehydrogenating glucose-6-phosphate and NAD or NADP in the presence of glucose-6-phosphate dehydrogenase to produce 6-phosphogluconate and NADH or NADPH, characterized in that the reaction which leads to the product to be determined is carried out in the presence of 6-phosphogluconolactonase and consists essentially of the reaction according to the following reaction scheme:



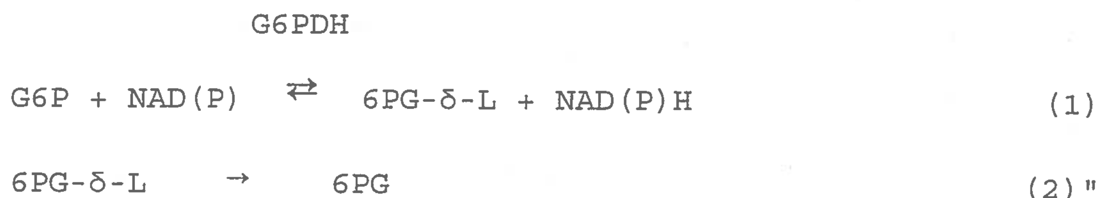
$$G6P + NAD(P) \overset{G6PDH}{\rightleftarrows} 6PG\text{-}\delta\text{-}L + NAD(P)H \quad (1)$$

$$6PG\text{-}\delta\text{-}L \rightarrow 6PG \quad (2)"$$

"2. A method for determining creatine kinase, including the step of bringing a sample into contact with creatine phosphate, glucose, hexokinase, ADP, NAD or NADP, and glucose-6-phosphate dehydrogenase, characterized in that the reaction which leads to the product to be determined is carried out in the presence of 6-phosphogluconolactonase and consists essentially of the reaction according to the reaction scheme of claim 1".

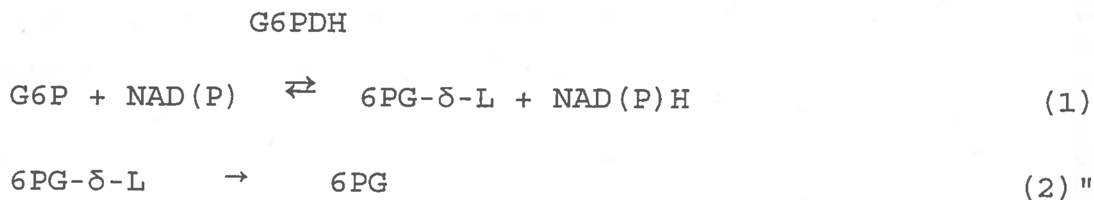
Claim 1 of the **second auxiliary request** (consisting of two claims) was as claim 2 of the preceding request except that the reaction scheme was recited as follows:

"... according to the reaction scheme:



Claims 1 and 2 of the **third auxiliary request** (claims 1 to 4) read as follows:

"1. A method for determining glucose-6-phosphate, including the step of dehydrogenating glucose-6-phosphate and NAD or NADP in the presence of glucose-6-phosphate dehydrogenase to produce 6-phosphogluconate and NADH or NADPH, characterized in that the reaction which leads to the product to be determined is carried out in the presence of 0.01 to 50 U/ml of 6-phosphogluconolactonase, 0.5 to 20 U/ml of glucose-6-phosphat[e] dehydrogenase, 0.5 to 5 mM NAD or NADP and consists essentially of the reaction according to the following reaction scheme:



"2. A method for determining creatine kinase, including the step of bringing a sample into contact with creatine phosphate, glucose, hexokinase, ADP, NAD or NADP, and glucose-6-phosphate dehydrogenase, characterized in that the reaction which leads to the

product to be determined is carried out in the presence of 0.01 to 50 U/ml of 6-phosphogluconolactonase, 0.5 to 20 U/ml of glucose-6-phosphat[e] dehydrogenase, 0.5 to 5 mM NAD or NADP and consists essentially of the reaction according to the reaction scheme of claim 1".

The sole claim of the **fourth auxiliary request** read as follows:

"A method for determining creatine kinase up to a concentration of creatine kinase of 5000 U/L, including the step of bringing a sample into contact with creatine phosphate, glucose, hexokinase, ADP, NAD or NADP, and glucose-6-phosphate dehydrogenase, characterized in that the reaction which leads to the product to be determined is carried out in the presence of 6-phosphogluconolactonase and consists essentially of the reaction according to the following reaction scheme:

$$G6P + NAD(P) \overset{G6PDH}{\rightleftarrows} 6PG\text{-}\delta\text{-}L + NAD(P)H \quad (1)$$

$$6PG\text{-}\delta\text{-}L \rightarrow 6PG \quad (2)$$

wherein 6-phosphogluconolactonase is present in 0.01 to 50 U/ml, glucose-6-phosphate dehydrogenase is present in 0.5 to 5 U/ml and NAD or NADP is present in 0.5 to 20 mM."

VII. The following abbreviations are used:

G6PDH: glucose-6-phosphate dehydrogenase

G6P: glucose-6-phosphate

6PG-δ-L: 6-phospho-D-glucono-δ-lactone

6PG: 6-phosphogluconate

6PGL: 6-phosphogluconolactonase

6PGDH: 6-phosphogluconate dehydrogenase

CK: creatine kinase

VIII. The appellants argued essentially that the feature "consists essentially of the reaction according to the following reaction scheme ..." was adequate to establish novelty over document (1) which relied on an additional reaction, namely the dehydrogenation of 6PG. The latter document represented the closest prior art. It dealt with the determination of CK-MB (creatine kinase isozyme MB) which was known to be present in only very small amounts and thus posed the problem of having to increase the signal strength and, thus, the sensitivity. In order to achieve this, document (1) proposed using in the known CK assay (cf document (1a)) two additional enzymes, namely 6PGL and 6PGDH, which resulted in the production of a double amount of NAD(P)H, due to the two reactions. However, this approach was inadequate to solve the problem underlying the patent in suit which was finding a method for determining high CK quantities (far beyond the amount of 800 U/l referred to in document (1)) in a linear manner. The use of 6PGL as the sole additional enzyme, as proposed by the claims, provided a valid solution as demonstrated by the test results and by the declaration of Ms Yoko Endo. If the amount of NAD(P)H produced in the **absence** of 6PGL was denoted by "A", the merit of the invention was to have found that in the **presence** of 6PGL the amount "A+α" was produced at creatine kinase concentrations higher than 1000 U/L (cf Example 2 and Figure 2). The method of document (1) by relying on two enzymes (6PGL and 6PGDH) resulted in the production of

2x the amount "A" of NAD(P)H. Document (1) did not disclose or suggest the existence of the amount "α". The statement in the document that, if 6PGL was omitted, the resulting CK activity was only 70%, was not a suggestion to use only 6PGL and thus benefit of the amount "α". The said statement meant rather that, when omitting 6PGL, 70% of the amount 2x "A" was obtained in the system.

As for document (2), it related to the determination of 6PGL and did not describe the determination of CK in a clinical sample. The statement at the end of the document about the possible usefulness of 6PGL in enabling a rapid quantitative conversion of G6P to 6PG was mere speculation which had no experimental basis in document (2) which, differently from the present invention, related to an **end-point** assay, where G6P was added at once, **not** to a **rate** assay. Moreover, the statement on page 187 of the document that addition of the lactonase resulted in the rate of NADH produced being considerably less than the rate of 6PG-δ-L hydrolysis would not have induced the skilled person to expect any desirable effect in the use of the lactonase in the determination of CK.

IX. The respondents submitted that the expression "consists essentially of .." used in the claims, as opposed to the expression "consists of" , did not exclude the presence of other compounds (cf decisions T 522/91 of 18 November 1993 and T 711/90 of 15 September 1993). In this respect, they observed that on page 7 of the description of the patent specification reference was made to the presence of a third component (solution F: 6PGDH). Thus, in their view, the subject-matter of some of the claims of the requests on filed (eg claims 1 and 2 of the main request) lacked novelty having regard to document (1).

As regards the issue of inventive step, they argued that the claims were not limited to the determination only of high concentrations of CK. Document (1) provided a clear demonstration of the usefulness of 6PGL in the determination of CK activity. Document (2) also pointed to the usefulness of this enzyme in combination with 6GPDH in analytical assays. Thus, none of the claims of the different requests involved an inventive step.

X. The appellants request that the decision under appeal be set aside and that a patent be maintained on the basis of the main request or one of the first to fourth auxiliary requests, all submitted at the oral proceedings on 5 June 2002.

The respondents request that the appeal be dismissed.

Reason for the Decision

Formal objections

1. As regards the formal requirements, in view of the board's finding on inventive step (cf points 5 to 13 infra), the question whether all the selected ranges which characterize the only claim of the fourth auxiliary request are in line with the requirements of Article 123(2) EPC needed not be decided.

Novelty

2. The respondents consider that, in view of the expression "consists essentially of..." used in the claims, the assay system does not exclude the presence of further components, in particular 6PGDH. They thus conclude that the amendments did not overcome the novelty objection vis-à-vis document (1) for at least

some of the claims, eg claim 1 and 2 of the main request.

3. In the board's view, while it is true that the expression "consisting essentially of ..." does not in principle exclude the presence, in addition to the mandatory components recited in the claims, of other components, the latter can only be minor components the presence of which does not affect the essential nature of the method as claimed (cf mutatis mutandis point 3 of decision T 472/88 of 10 October 1990). Accordingly, in the board's judgement, the presence of 6PGDH, as a further component, is excluded by the wording of the claims at issue. In fact, this enzyme, if present, would alter the reaction scheme by providing an extra step and further reaction products. This would mean an alteration of the essential nature of the claimed method. The passage on page 7 of the patent in suit referred to by the respondents relates to a reference example which is not within the scope of the present claims.

4. For these reasons, the board considers that the requirement of novelty is met by all claims of all requests vis-à-vis document (1).

Inventive step: all requests.

5. The subject-matter of all the claims is centred on the determination of G6P which is formed during the assay of a number of clinically relevant enzymes, eg CK, said determination being based on the conversion of G6P + NAD(P) into 6PG-δ-L + NAD(P)H. The essential proposal in all claims is to **carry out such determination in the presence of 6PGL** so that 6PG-δ-L is further converted into 6PG. In particular, focus is on a method for determining CK, a claim directed thereto being present

in all requests. Therefore, inventive step is hereinafter discussed in relation to such a method.

6. The closest prior art is represented by document (1), which is to be read together with document (1a) to which explicit reference is made. Document (1a) is concerned with the recommended method for the determination of CK. Against the background of this document, document (1) discloses a method for determining CK-MB activity (an isozyme of CK) in the presence of the additional enzymes 6PGL and 6PGDH. The first enzyme converts 6PG-δ-L into 6PG, while the second enzyme converts 6PG into D-ribulose 5-phosphate with further NAD(P)H formation. The document states:"*We demonstrate that 6-phosphogluconolactonase is essential to obtain a photometric signal that is precisely twice the signal of the recommended CK tests. If the enzyme 6-phosphogluconolactonase is omitted the resulting CK-MB activity is only 70%*". At the end of the document it is stated: "*The linear range of the new test extends up to 800 U/l CK-MB*".

7. Having regard to this prior art, the technical problem to be solved is objectively defined as being the provision of a further (improved) CK assay.

8. The solution proposed is to carry out the CK assay in the presence of 6PGL as the only additional enzyme.

9. The appellants submit essentially that it was not obvious for the skilled person to apply the approach described in document (1), let alone with only 6PGL, for the determination of high CK quantities. Moreover, they argue that the skilled person would not have expected to achieve linearity at CK concentrations higher than 1000 U/L.

10. As observed also by the respondents, the claims at

issue of all requests are in no way limited to CK determination in any given concentration range. Only claim 1 of the fourth auxiliary request makes reference to the upper limit (up to 5000 U/L). This, however, does not exclude a method applied to lower concentration ranges, eg ranges as those referred to in document (1). Thus, the arguments put forward by the appellants in relation to the CK concentration and the linearity of the assay cannot influence the decision on claims with a much broader scope such as those at issue. Here, the relevant question in respect of inventive step is only whether or not the skilled person would have been led by the prior art to carry out the CK determinations, according to the recommended method, in the presence of 6PGL.

11. In the board's judgement, the answer to the question is affirmative. This is because not only document (1) provided a strong enough incentive to use 6PGL, but also prior art document (2), which was concerned with the isolation of 6PGL from *Zymomonas mobilis*, had concluded that the enzyme "*should be a useful analytical enzyme in combination with glucose-6-phosphate dehydrogenase in enabling a rapid quantitative conversion of glucose-6-phosphate to 6-phosphogluconate even at pH values below 7, and without a large excess of NAD(P)$^+$ present*". Nothing in the quoted prior art documents indicated to the skilled person either that it was mandatory to use it in combination with 6PGDH, or that there were any kinds of disadvantages or drawbacks in using it. The statement on page 187 of document (2) relied upon by the appellants is not dissuasive in this respect. The fact that the document itself is not directly concerned with a rate assay is immaterial as the final statement constitutes an express incentive to use 6PGL in analytical assays in combination with G6PDH.

12. The reference to specific concentration ranges of the reagents which characterise the third and fourth auxiliary request does not introduce any elements that could confer an inventive step to an otherwise obvious method as the quoted concentration ranges are those usually taken into consideration by the skilled person when carrying out a CK assay: the 6PGL concentration suggested by document (1) is 110 U/L = 0.11 U/ml; document (1a) indicates a G6PDH concentration of 2000 U/L = 2 U/ml and a NADP concentration of 2 mmol.

13. For these reasons, none of the claim requests at issue satisfies the requirements of Article 56 EPC.

Order

For these reasons it is decided:

The appeal is dismissed.

The Registrar: The Chairperson:

pp. P. Cremona U. Kinkeldey