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# D E C I S I O N
## of 22 November 1999

**Case Number:** T 0013/97 - 3.4.2

**Application Number:** 88107089.0

**Publication Number:** 0301182

**IPC:** G01N 1/30, C12Q 1/06, G01N 15/14, G01N 33/49

**Language of the proceedings:** EN

**Title of invention:**
Reagent for reticulocyte counting by flow cytometry

**Patentee:**
Toa Medical Electronics Co., Ltd.

**Opponent:**
Bayer Corp., One Mellon Center

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

**Keyword:**
"Inventive step (main request: no)"
"Amendments - insertion of a disclaimer (auxiliary request - not allowable)"

**Decisions cited:**
G 0002/88, T 0645/95, T 0863/96, T 0313/86, T 0170/87, T 0623/91

**Catchword:**

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Beschwerdekammern | Boards of Appeal | Chambres de recours

**Case Number:** T 0013/97 - 3.4.2

**D E C I S I O N**
**of the Technical Board of Appeal 3.4.2**
**of 22 November 1999**

**Appellant:** (Proprietor of the patent)
Toa Medical Electronics Co., Ltd.
2-1, Minatojimanakamachi 7-chome
Chuo-ku
Kobe-shi
Hyogo-ken (JP)

**Representative:**
Hansen, Bernd, Dr. Dipl.-Chem.
Hoffmann Eitle
Patent- und Rechtsanwälte
Postfach 81 04 20
81904 München (DE)

**Respondent:** (Opponent)
Bayer Corp., One Mellon Center
500 Grant Street
Pittsburgh
PA 15219-2502 (US)

**Representative:**
Burkert, Frank
Bayer AG
Konzernbereich RP
Patente und Lizenzen
51368 Leverkusen (DE)

**Decision under appeal:** **Decision of the Opposition Division of the European Patent Office posted 31 October 1996 revoking European patent No. 0 301 182 pursuant to Article 102(1) EPC.**

**Composition of the Board:**

**Chairman:** E. Turrini
**Members:** R. Zottmann
B. J. Schachenmann

## Summary of Facts and Submissions

I. The Appellant (Patentee) lodged an appeal against the decision of the Opposition Division revoking European patent No. 0 301 182 with the application No. 88 107 089.0.

The opposition was based on the ground of opposition laid down in Article 100(a) EPC that all claims were not novel with respect to document

E1: Cytometry 5:589-600 (1984), Alan R. Liss Inc.

The reason for said decision was that the subject-matter of amended independent claims 1 according to main and auxiliary requests did not involve an inventive step with respect to document E1.

II. Among other documents the following further documents were cited during the appeal proceedings:

E2: Cytometry 7:508-517 (1986), Alan R. Liss Inc.

E4': English translation of "Hematology of Experimental Animals", Soft Science Publishing, 1981, pages 195-196, submitted by the Appellant

E5: LABMEDICA, 6 (3), 1989, pages 27-32

E6: Clin. lab. Haemat. 1991, 13, pages 177-188

E8: Documenta Geigy, Wissenschaftliche Tabellen, 1962, page 545

III. Oral proceedings were held before the Board of Appeal at the end of which the decision was announced.

IV. The Appellant requested that the decision under appeal be set aside and that the patent be maintained in amended form on the basis of claims 1 to 3 filed during the oral proceedings as main request, or, as auxiliary request, on the basis of the claims 1 to 3 filed as auxiliary request with the letter dated 22 October 1999.

The Respondent (Opponent) requested that the appeal be dismissed.

V. The claims of the main request read as follows:

"1. Use of a reagent comprising an aqueous solution of a dye, a buffer and a carbonate salt, for human reticulocyte counting by flow cytometry.

2. Use according to claim 1 in which the carbonate salt concentration is in the range of 1-300 mM.

3. Use according to claim 1 in which the carbonate salt is $NaHCO_3$ or $Na_2CO_3$."

The claims of the auxiliary request read as follows:

"1. Use of a reagent comprising an aqueous solution of a dye, a buffer and a carbonate salt, for reticulocyte counting by flow cytometry, wherein the reticulocytes are within a concentration of 0.7 to 2.2 % of the total count of erythrocytes and wherein the dye 3,3'-dimethyloxacarbocyanine

is excluded."

2. The use according to claim 1 in which the carbonate salt concentration is in the range of 1-300 mM.

3. The use according to claim 1 in which the carbonate salt is $NaHCO_3$ or $Na_2CO_3$."

VI. The arguments of the Appellant are summarized as follows:

Reference to "patients" or "person" in the application is a sufficient basis for a restriction of claim 1 (main request) to "human reticulocyte counting".

Documents E2, E5 and E6 show that human reticulocyte counting with 3,3'-dimethyloxacarbocyanine (hereinafter called "$DiOC_1$[3]") does not work, is in particular not usable for the manual method and has no clinical utility. Therefore, a reagent containing said dye does not solve the problem underlying the patent and a corresponding disclaimer should be allowable (see claim 1 of the auxiliary request); this is established jurisprudence of the EPO.

The last two of the five dye solutions (hereinafter called solutions A to E) used in the method of E1 listed on page 590 in the middle of the left-hand column do not contain $NaHCO_3$. Otherwise, the carbonate would be mentioned there. According to Figure 7 and the corresponding description on page 595 right-hand column second paragraph, only with the solutions which do not contain Mg satisfying results can be obtained. Of said

solutions, only solution E is free of Mg ions; however, said solution does not contain $NaHCO_3$. Nowhere in E1 can be found a hint that carbonate salt is important to improve the results.

The method described in E1 is only applicable for blood of mice with a much higher ratio of concentrations of reticulocytes and (mature) erythrocytes. When the concentrations are lower, a very accurate method is needed which is not provided by the method of E1.

E2 is more relevant than E1, since the method according to the latter document is carried out with human blood. In the methods described in E2 published two years after E1 no carbonate salt is contained in the dye solutions.

Therefore, a combination of E1 and E2 would not lead to the subject-matter of the independent claims.

VII. The arguments of the Respondent are summarized as follows:

There is no basis in the application as filed for a restriction to human reticulocyte counting of claim 1 of the main request or to the concentration range of claim 1 of the auxiliary request. Said range of claim 1 of the auxiliary request violates Articles 84 and 123 EPC. According to the patent, only healthy people have such reticulocyte percentages and the values in said ranges were not determined by flow cytometry.

As to the applicability of the dyestuffs, it is important that they bind to the nuclear acid of the

reticulocytes. If a dyestuff, e.g. $DiOC_1$[3], does not work well, the skilled person would replace it by one of the numerous other dyestuffs known to the skilled person in this connection.

All five solutions A to E of E1 do contain $NaHCO_3$. Since E1 is cited in E2, these two documents have to be seen as one document. E2 describes testing of human blood using several different dyes. Therefore, the claims of both requests lack novelty. In case of not acceptance of the argumentation that E1 and E2 should be considered as one document a skilled artisan would be clearly guided to combine them. Therefore, the claims would at least lack an inventive step.

## Reasons for the Decision

1. The appeal complies with the requirements of Articles 106 to 108 and Rule 64 EPC and is, therefore, admissible.

2. *Amendments*

2.1 The granted claims are directed to a reagent for reticulocyte counting whereas the present claims of the main and auxiliary requests have been reworded as use claims. In view of decision G 2/88 of the Enlarged Board of Appeal, such an amendment is not open to objection under Article 123(3) EPC. This has not even been disputed by the Respondent.

2.2 Further, the Board is satisfied that mention of "person" in the general description of the invention in

the application as originally filed (column 5, lines 41 to 45: "... the amount of $CO_2$ in erythrocytes differs from person to person ...") is a sufficient basis for a restriction of claim 1 of the main request to "human reticulocyte counting" (requirement of Article 123(2) EPC.

2.3 When compared with the claims as granted, claim 1 of the auxiliary request contains a supplement in the form of an exclusion based on the teachings of document E1, namely clause (a): "with the exception of 3,3'-dimethyloxa-carbocyanine".

Apparently, a feature corresponding to said supplement is not disclosed in the original application documents as required by Article 123(2) EPC.

Though an insertion of an exclusion in claims could be acceptable in form of a so-called **disclaimer**, this is an exceptional case. First, it would be allowable if such a **disclaimer** is an accidental novelty-destroying disclosure, that is if the cited document containing said disclosure has no relevance for any further examination of the claimed invention, in particular of inventive step, and if it thus disappears from the prior art field to be taken into consideration (see e.g. decisions T 645/95 and T 863/96, both unpublished). Second, it would be allowable if the excluded subject-matter does not contribute to the solution of the problem (see e.g. decisions T 313/86 unpublished, T 170/87 EPO OJ 1989,441, and T 623/91 unpublished).

In the present case, E1 belongs to the same technical

field as that of the claimed invention. In particular, E1 does not fulfill the condition that after insertion of clause (a) the prior art of E1 remains insignificant for the further examination of the patent, in particular of inventive step (see also section 4 below).

Moreover, the Board is not convinced that reagents comprising the disclaimed $DiOC_1[3]$ dye cannot be used for reticulocyte counting by flow cytometry when the reticulocytes are within a concentration of 0.7 to 2.2%. Though Auramine O is used for the Example, the patent does not exclude or prefer a certain dye. It is true that according to E2 reticulocyte analysis of human blood with thiazole orange dye offers advantages, however also a potential disadvantage is mentioned (see e.g. the second paragraph of page 517) and it is not alleged there that reticulocyte counting by flow cytometry with other dyes - including $DiOC_1[3]$ - does not work. According to E5, dye solutions comprising $DiOC_1[3]$ show advantages as well as disadvantages over solutions with other dyes (see Table 1 of E5). E6 states (on page 178 second paragraph) only that $DiOC_1[3]$ suffers from drawbacks limiting its clinical utility. However, claim 1 is not restricted to clinical use. Moreover, none of the methods described in E2, E5 and E6 uses a dye solution comprising a carbonate salt.

Under these circumstances the insertion of clause (a) disclosed in E1 as a disclaimer is not allowable within the framework of Article 123(2) EPC.

2.4 Moreover, the Board considers that the insertion of the range "0.7 to 2.2%" for the reticulocyte concentration

into claim 1 of the auxiliary request renders the claim unclear. In column 1, second paragraph of EP-A-0 301 182 it is stated: "Immature erythrocytes in the blood are called reticulocytes, and normally account for 0.7 to 2.2% of the total count of erythrocytes. Determination of reticulocyte count helps ... diagnosis of such diseases ..." (emphasis added by the Board). This range does not even cover the range of healthy adult persons - let alone that of sick persons -, of persons with anomalous blood or of new-born. For example, according to E8, the **mean** value of the reticulocyte concentration for men is 0.6% and for babies during the first 24 hours is 3.92%; according to E2, reticulocyte concentrations of healthy volunteers of up to 3.2% are obtained with the best of the tested dyes (see Tables 2 and 3); according to E4', said range is 0.2 to 2.3 for human blood; and according to E6, at least the upper limit of reticulocyte normal range is much higher, namely at least 2.8, for female up to 3.1% (see Table 1). From the term "normally" in the above cited passage of the description follows that said range is at least valid for the blood of the average and healthy patient. Since reticulocyte counting is just important for determination of diseases with anomalous reticulocyte concentrations - corresponding to the second sentence of said passage of the patent in suit - restriction of the use to said range is not supported by the description.

Therefore, claim 1 of the auxiliary request infringes Articles 84 EPC, too.

2.5 Thus, the amendments of claim 1 of the main request are acceptable, whereas those of claim 1 of the auxiliary

request are not allowable under Article 123(2) and 84 EPC.

3. *Novelty of claim 1 of the main request*

Document E1 is only cited together with four other documents in the introductory part of E2 describing currently available methods for reticulocyte counting in a general manner and there is no hint that the method of E2 should be preferred or the like. Therefore, E1 and E2 cannot be seen as one single reference. None of documents E1 and E2 and of the remaining prior-published documents cited during the appeal proceedings discloses the use of a reagent according to claim 1. Therefore, said claim is novel in the sense of Articles 54 EPC.

4. *Inventive Step of claim 1 of the main request*

4.1 Document E1 describes the use of a reagent comprising an aqueous solution of a dye ($DiOC_1$[3]), a buffer and a carbonate salt ($NaHCO_3$), for reticulocyte counting by flow cytometry (see the abstract and the chapter "Buffered Salts and Media" on page 590).

The method was carried out with blood of mice which normally has reticulocyte concentrations between 1 and 5% (see E1, page 591, left-hand column at the bottom); concentrations down to less than 0.01% were measured (see page 591, left-hand column at the bottom). Thus said range overlaps with that of human blood (see section 2.4 above).

It is undisputed that solutions A to C listed in E1

(see point V1, above) contain 20 mM carbonate salt. Each of the solutions B to E contains the preceding solution with possible changes. $NaHCO_3$ is only mentioned as a component of solution A. Solution D (HeSlM-1) is solution C with changes of five components. Solution E is solution D with changes of five components whereby $NaHCO_3$ is not mentioned, too; however, in the list of the solutions A to E missing components with respect to the preceding solution are explicitly cited: "0 $MgCl_2$, 0 $CaCl_2$". Hence, all five dye solutions A to E used in the flow cytometric analysis of E1 must be considered to contain the same concentration (20 mM) of carbonate salt. In any case the skilled person would have to assume that when reading the article E1.

Therefore, the subject-matter of claim 1 differs from the prior art E1 only in that the reagent is used for **human** reticulocyte counting.

The method described in E2 is used for human blood, but the dye reagent does not contain a carbonate salt. Since the reticulocyte concentration ranges of the tested blood of E1 and that of human blood overlap, the prior art of E2 at least does not come nearer to the subject-matter of claim 1 than the prior art of E1. The other cited documents are still less relevant.

4.2 The objective problem underlying claim 1 of the main request is, therefore, to find a further application of the method described in E1.

4.3 Use of the reagents of E1 for analysing human blood is suggested by E1 itself. In the abstract, it is stated that $DiOC_1$[3] is taken up by all cells in mammalian

blood. Mammalian blood encompasses human blood. In the introductory part, it is stated that there is no reason to suspect that this analysis will not work for any mammalian erythrocyte population and that a report on the use of a homologue of $DiOC_1[3]$, namely $DiOC_6[3]$, supports this optimistic view.

Documents E5 and E6 are post-published. In the method of E2, human blood was used and it is stated that use of $DiOC_1[3]$ or other dyes have disadvantages with respect to thiazole orange (see page 513 last paragraph), but it is nowhere stated that it does not work with $DiOC_1[3]$. The reagent of E2 does not contain a carbonate salt such that the results of E2 and E1 are not directly comparable. Therefore, documents referred to above could not establish a prejudice against the use of the known reagent of E1 also for analysing the reticulocyte concentration of human blood and thus could not refrain the skilled person from such a use. Moreover, the skilled person would, as suggested by E1 itself (see the last sentence of the abstract, the last paragraph of the introductory part, the last sentence of the last but one paragraph of page 591 and page 599, right-hand column, first paragraph), also test other dyes than $DiOC_1[3]$, all the more so as in E1 also disadvantages of said dye are mentioned (see the sentence bridging pages 595 and 596, the last five lines of page 596 and page 599, right-hand column, first paragraph).

In addition, tests of new methods with samples taken from animals are usually a preparatory step for the application to human samples.

Paper E1 comes to the conclusion that $DiOC_1[3]$ may be useful in clinical hematology, but it seems to be unlikely to be suited for the **routine** determination of reticulocyte frequencies as they are now performed in the clinic (see page 599, right-hand column, first paragraph). However, this is of no importance in the present context, since claim 1 of the main request is not restricted to such a kind of determination.

Therefore, claim 1 of the main request does not involve an inventive step in the sense of Articles 56 EPC.

5. Since neither claim 1 of the main request nor that of the auxiliary request are allowable, none of the requests is allowable and it is not necessary to examine the remaining claims.

## Order

## For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

P. Martorana E. Turrini