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Datasheet for the decision of 15 June 2021

Case Number: T 0866/16 - 3.3.08

Application Number: 10013064.0

Publication Number: 2311934

IPC: C12M1/34

Language of the proceedings: EN

Title of invention:
Rapid detection of replicating cells

Patent Proprietor:
Rapid Micro Biosystems, Inc.

Opponent:
Merck Patent GmbH

Headword:
Detection of replicating cells/RAPID MICRO BIOSYSTEMS

Relevant legal provisions:
EPC Art. 54, 56, 83, 123(2), 123(3)
EPC R. 80
RPBA 2020 Art. 13(2)

Keyword:
Main request - added matter - (no)
Extension of scope of protection - (no)
Sufficiency of disclosure - (yes)
Novelty - (yes)
Inventive step - (yes)
Admission of late submissions (arguments and experimental evidence) - (no)

Decisions cited:
G 0001/93, T 0220/83, T 0305/94, T 1395/07, T 2769/17

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 0866/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 15 June 2021

Appellant: (Patent Proprietor)	Rapid Micro Biosystems, Inc. 1001 Pawtucket Blvd. West Lowell, MA 01854 (US)
Representative:	Lahrtz, Fritz Patentanwälte Isenbruck Bösl Hörschler PartG mbB Prinzregentenstraße 68 81675 München (DE)
Appellant: (Opponent)	Merck Patent GmbH Postfach 64271 Darmstadt (DE)
Representative:	Selg, Peter c/o Merck Patent GmbH Frankfurter Straße 250 64293 Darmstadt (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 28 January 2016 concerning maintenance of the European Patent No. 2311934 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. R. Vega Laso
	A. Bacchin

Summary of Facts and Submissions

I. European patent No. 2 311 934 with the title "Rapid detection of replicating cells" was granted from the European application No. 10013064.0, a divisional application of the patent application No. 09166028.2 which is in turn a divisional application of the patent application No. 02757646.1. In the present decision, references to the "the application as filed" are to the documents of the application as originally filed.

II. Claim 1 of the patent as granted reads as follows:

"1. A method for detecting living target cells in a sample, said method comprising the steps of:

(a) providing living target cells present in said sample in a detection zone comprising a detection area at a density of less than 100 target cells per mm^2 of the detection area, wherein within said detection zone said cells are randomly dispersed and immobilized;

(b) allowing the formation of one or more microcolonies of said target cells by in situ replication;

(c) labeling said one or more microcolonies with a labeling reagent; and

(d) detecting said one or more microcolonies by detecting the signal generated by said labeling reagent;

wherein the longest linear dimension of said detection area is greater than 1 mm; said one or more microcolonies have a mean measurement of less than 50 microns in at least two orthogonal dimensions; wherein said detecting does not entail magnification of more than 5x; wherein said cells in said one or more

microcolonies remain competent to replicate following said detecting; and wherein said detection method does not employ laser-scanning".

Dependent claims 2 to 15 are directed to various embodiments of the method of claim 1.

III. The patent was opposed on the grounds for opposition of Article 100(a) in conjunction with Articles 54 and 56; 100(b) and 100(c) EPC.

IV. In an interlocutory decision posted on 28 January 2016, an opposition division found that Article 100(c) EPC prejudiced the maintenance of the patent as granted (main request). The auxiliary requests 1a, 1b and 1c then on file were found to contravene Article 123(3) EPC, and the auxiliary requests 2 and 3 to offend against Articles 123(2) and 76(1) EPC. However, account being taken of the amendments introduced into the claims according to the auxiliary request 4a, the patent and the invention to which it relates were found to meet the requirements of the EPC.

V. Each the patent proprietor (appellant I) and the opponent (appellant II) filed an appeal and submitted a statement setting out the grounds of appeal.

VI. Together with its statement, appellant I submitted fifteen sets of claims as auxiliary requests 1 to 15 in appeal proceedings, the patent as granted remaining its main request.

VII. Appellant II submitted together with its statement new experimental data as document (15).

VIII. Each party replied to the statement of grounds of the other party.

IX. The parties were summoned to oral proceedings before the board.

X. In a communication sent in preparation for the oral proceedings, the board drew attention to matters which seemed to be of special significance and expressed a provisional opinion on some of the issues to be discussed.

XI. Upon request by appellant I, oral proceedings were held by videoconference on 15 June 2021. During the oral proceedings, appellant I withdrew the previous main request and auxiliary request 1, and pursued as new main request the auxiliary request 2 filed on 7 June 2016 together with the statement of grounds of appeal.

XII. Amended claim 1 of the present main request differs from claim 1 of the patent as granted in that the disclaimer "*wherein said detection method does not employ laser-scanning*" has been omitted and a new feature introduced as follows:

"[...]
wherein the longest linear dimension of said detection area is greater than 1 mm, **wherein said detection area is the area that is simultaneously analyzed by a detection device**; said one or more microcolonies have a mean measurement of less than 50 microns in at least two orthogonal dimensions; wherein said detecting does not entail magnification of more than 5x; and wherein said cells in said one or more microcolonies remain competent to replicate following said detecting." (emphasis added by the board)"

Claims 2 to 15 are identical to the corresponding claims of the patent as granted.

XIII. The following documents are referred to in this decision:

(1): US patent No. 5,766,868, published on 16 June 1998;

(2): DE 196 08 320 A1, published on 28 August 1997;

(3): M. Masuko *et al.*, 1991, FEMS Microbiology Letters, Vol. 81, pages 287 to 290;

(4) K. G. Porter and Y. S. Feig, 1980, Limnol. Oceanogr., Vol. 25, No. 5, pages 943 to 948;

(5): J. C. Gray *et al.*, January 2011, PDA Journal of Pharmaceutical Science and Technology, Vol. 65, No. 1, pages 42 to 54;

(9): Print-out of Webpage http://www.rapidmicrobio.com/blog/bid/38714/Background-lighting-and-the-genesis-..., printed on 27 October 2015;

(10): "Reproduction of the experiments disclosed in D1", neither dated nor signed;

(11): Material Safety Data Sheet for ATP Releasing Agent, 2010, Millipore Corporation;

(13): M. Zschöck and R. Böhm, 1985, Zbl. Vet. Med. B, Vol. 32, pages 157 to 168; and

(15): "Reproduction of the experiments disclosed in D1", neither dated nor signed.

XIV. The submissions made by appellant I, insofar as relevant to the present decision, were essentially as follows:

Admissibility of the appeal of appellant II

In its statement of grounds of appeal, appellant II had not put forward any particular reasons why the decision under appeal should be set aside. The statement of grounds amounted basically to nothing more than repetitions of previous or even initial allegations. It was not adequately substantiated and did not meet the requirements of Rule 99(2) EPC. Consequently, the appeal had to be rejected as inadmissible.

Article 123(3) EPC – scope of protection

The disclaimer in claim 1 of the patent as granted did not exclude any subject-matter. Thus, its omission in the amended claim 1 could not result in the scope of the claim being extended beyond the scope of protection conferred by the patent as granted.

Article 83 EPC – sufficiency of disclosure

There were no serious doubts substantiated by verifiable facts that the disclosure in the application as filed was sufficient to carry out the claimed invention without undue burden. Example 1 and Figure 6 of the application as filed disclosed a method according to the invention in which the microcolonies were labeled with reagents that allowed the cells to replicate after detection. By using antibodies to label

cell surface antigens, living cells could be detected **without** disruption of the cell membrane, and subsequent cultivation of the cells was possible. Detection using labeling reagents was clearly disclosed on page 13, lines 9 to 11 and 22 to 24; page 19, lines 3 to 13; page 22, lines 28 to 43; and page 23, lines 26 to 31 of the application as filed.

Article 54 EPC – novelty

Admittance of document (15) into the proceedings

Document (15) could and should have been filed in opposition proceedings. Thus, it should not be admitted into the appeal proceedings.

Document (1)

Document (1) did not describe the step of allowing the formation of microcolonies, let alone microcolonies of less than 50 microns. This feature was neither explicitly described in document (1) nor inherent in what this document described. As correctly found by the opposition division, the experiments in document (10) were not a direct reproduction of the Examples 3 and 4 of document (1). In particular, Experiment I in document (10) included a cultivation step to promote cell growth, whereas the method described in Examples 3 and 4 of document (1) did not comprise such a step.

The skilled person could not derive from document (1) that the bacteria immobilized to the membrane retained the capability to replicate. As described in document (2) and document (5), spraying the cells with an ATP releasing agent resulted in cell lysis. While document (5) had been submitted allegedly as evidence

that the method of document (1) was non-lethal, it had not been demonstrated that the same method was actually used in both documents. Moreover, since the latter document had been published about a decade after the priority date of the patent, a person skilled in the art at the priority date of the patent could not have been aware of the teachings of document (5). Hence, document (1) did not destroy the novelty of the claimed methods.

Document (3)

The claimed methods were novel also over document (3). Document (3) described the detection of single bacterial cells. The formation of colonies was described only for the purpose of validating the single cell detection, not for detecting the colonies themselves. Wherever the term "colony" was used in document (3), only macrocolonies were meant.

While document (3) described that luminous bacteria were able to replicate after detection, the document was completely silent on the replication capacity of **labeled** cells. Contrary to the opposition division's view, document (3) did not describe or even suggest that labeled cells needed to be viable after detection, and it was not inherent in the reference to a method for labeling the cells by enzyme immunoassay that the cells remained competent to replicate. Furthermore, no details were given in document (3) for the detection of labeled cells, e.g., the number of cells immobilized per mm^2 detection area, the dimension of the detection area, the magnification or the specific detection-illumination method.

Article 56 EPC – inventive step

Document (3) as the closest state of the art

Starting from document (3), the problem to be solved was the provision of an improved method for the detection of living bacteria that remained competent to replicate. Since document (3) taught that detecting single cells was advantageous over colony-counting methods requiring cultivation, it taught away from detecting microcolonies. The teachings of document (3) did not motivate the skilled person to include a cultivation step because this would make the method described therein slower. Moreover, the skilled person did not find in document (3) an incentive to provide **labeled** cells that remained competent to replicate after detection. The reference to staining or labeling methods which disrupted or destroyed the cells was a clear indication that it was not intended that the microorganisms remained competent to replicate after detection.

The combination of the teachings of document (3) with those of document (1) did not render the claimed method obvious. The purpose of the cultivation suggested in document (1) was to allow formation of a detectable signal. In contrast, formation of microcolonies in the method of the invention ensured that only viable microorganisms were detected. As in the method described in document (1) the cells were disrupted by the ATP releasing reagent, this document could not provide an incentive to the skilled person to ensure that the cells remained competent to replicate. Hence, the claimed subject-matter involved an inventive step over document (3), either alone or in combination with document (1).

Document (2) as the closest state of the art

The method of claim 1 differed from the method described in document (2) in that, before detection, the target cells were allowed to form microcolonies of a size of less than 50 microns by *in situ* replication; thus, only viable cells capable of replicating were detected. Moreover, while in document (2) the cells were disrupted, in the method of the invention they remained competent to replicate.

Starting from the method described in document (2), a skilled person seeking to improve it had no motivation to propagate the cells, because it was precisely that step what the method of document (2) tried to avoid by detecting single cells. The fact that the use of reagents that destroyed the cells (e.g. Na-laurylsulphate or ATP releasing agents) was suggested for labeling indicated that maintaining replication competency of the cells was of no interest for practical applications of the method described in document (2).

In both document (2) and document (1), the detection method resulted in the destruction of the cells. Neither document taught the skilled person to keep the cells alive, so that they could replicate after detection. Hence, a combination of the teachings of documents (2) and (1) did not render obvious the claimed method.

XV. The submissions made by appellant II, insofar as relevant to the present decision, may be summarized as follows:

Article 123(3) EPC – scope of protection

The omission of the disclaimer in amended claim 1 resulted in an extension of the protection conferred by the patent as granted. This was not compensated by the introduction of the new feature defining the detection area as the area that is simultaneously analysed by a detection device. The term "laser scanning" related solely to the illumination of the detection area, and did not define how the detection as such was performed. In view of the definition provided in the patent for the terms "detection area", "large area detection" and "simultaneous detection", the skilled person would understand that the detection as such was independent of the type of illumination used. Since laser scanning and simultaneous analysis of a large detection area in one step did not exclude each other, the scope of protection of the amended claim 1 was extended beyond the protection conferred by the patent as granted, contrary to Article 123(3) EPC.

Article 83 EPC – sufficiency of disclosure

The claimed invention was not disclosed in the application as filed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. The application as filed disclosed competency of the cells to replicate after detection only in connection with microcolonies which had not been labeled. In the sole example for labeled microcolonies provided in the application as filed, filters with microcolonies were fixed with formaldehyde which resulted in the cells being destroyed. The skilled person could not derive from the application as filed which labeling reagents would be suitable for carrying out the claimed invention. The inventor

himself acknowledged in document (9) that at the relevant date a non-destructive detection was only possible if the cells were not labeled.

Article 54 EPC – novelty

Admittance of document (15)

Document (10) filed in opposition proceedings provided experimental evidence which supported the objection of lack of novelty. The differences in the experimental conditions compared to Examples 3 and 4 of document (1) were indicated in document (10), but not considered to be relevant for the results. Document (15) was an exact reproduction of the relevant examples of document (1).

Document (1)

The method of claim 1 lacked novelty over document (1) which described a detection method having the same features. The method of document (1) was based on the release of ATP from cells immobilized on a membrane filter by spraying an ATP releasing reagent, followed by inducing luminescence with a luminescence-inducing reagent and detecting the luminescence. Detection of microorganisms, especially bacteria was possible either individually or as colonies formed by cultivating the bacteria after filtration.

As described in document (5), cells sprayed and detected with the Milliflex Rapid System test based on ATP bioluminescence could be re-grown for genotypic identification, thus showing that the cells were not destroyed upon application of the reagents for the ATP bioluminescence reaction. This demonstrated that the

cells detected in document (1) remained competent to replicate after detection.

Document (3)

Document (3) was novelty-destroying for the subject-matter of claims 1 to 7, 10, and 13 to 15. Document (3) described a method for counting living bacteria which emitted light or could be stimulated to emit light by various techniques (see page 290, left-hand column, lines 1 to 5 from the bottom). After a first detection of the cells labeled with a labelling reagent, the membrane filter with the immobilized cells was incubated at 20°C until colonies were formed, and the signal generated by the labeled colonies was detected. It was apparent from Figure 2 that single microcolonies could be detected employing the described method.

Article 56 EPC – inventive step

Document (3) as closest state of the art

The method of claim 1 was obvious to a person skilled in the art in view of the teachings of document (3) alone or combined with document (1). The claimed method differed from the method of document (3) solely in that microcolonies of less than 50 microns instead of single cells or macrocolonies were detected. The technical effect was that results could be obtained more rapidly, compared to the detection of macrocolonies. The problem to be solved was thus the provision of a more rapid detection method.

It was obvious to a skilled person that the method described in document (3) was suitable for the detection of colonies of any size. Hence, the claimed

method lacked inventive step in view of document (3) alone. Alternatively, the skilled person learnt from document (1) that, if the cells were cultivated for a short time period before detection, the method became more simple and rapid. Even though the term "microcolonies" was not used in document (1), cultivation of the cells for a short period resulted in such colonies being formed. Thus, in view of a combination of documents (3) and (1) the method of claim 1 did not involve an inventive step.

Document (2) as closest state of the art

In the method for detecting single cells described in document (2), the cells were disrupted prior to detection. However, a skilled person seeking to improve the method of document (2) was taught by document (1) that cultivation of the cells for a short time period increased the viable count by forming colonies and provided for a more accurate determination. Hence, allowing the formation of microcolonies prior to detection was an obvious measure which could not impart an inventive step over document (2).

XVI. Appellant I requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed as auxiliary request 2 on 7 June 2016. It further requested to dismiss the appeal of appellant II as inadmissible and to disregard document (15).

XVII. Appellant II requested that the decision under appeal be set aside and the patent be revoked. It further requested that document (15) be admitted into the proceedings.

Reasons for the Decision

Admissibility of the appeal of appellant II

1. The statement of grounds of appeal of appellant II enables the board to understand immediately why the decision under appeal is alleged to be incorrect, and on what facts appellant II bases its arguments (see, e.g. decision T 220/83, OJ 1986, 249). Even though only a few references to particular findings of the opposition division are made in the statement, this does not necessarily mean that the appeal is not substantiated as required by Article 108 EPC, in conjunction with Rules 99(2) and 101 EPC.

2. Hence, contrary to appellant I's view the appeal of appellant II is admissible.

Main request

Rule 80 EPC

3. The claims of the present main request, which was submitted as auxiliary request 2 together with appellant I's statement of grounds of appeal, are identical to those of the auxiliary request 1b underlying the decision under appeal. As stated above, amended claim 1 differs from claim 1 of the patent as granted in that: (i) the negative feature (disclaimer) "*wherein said detection method does not employ laser-scanning*" introduced during examination proceedings has been deleted, and (ii) the new feature "*wherein said detection area is the area that is simultaneously analyzed by a detection device*" introduced. Both amendments are occasioned by the ground for opposition

of Article 100(c) EPC. Thus, the requirement of Rule 80 EPC is fulfilled.

Articles 123(2) and 76(1) EPC – added matter

4. In the decision under appeal, the opposition division found that there was no basis in either the application as filed or the parent application(s) for the disclaimer "*wherein said detection method does not employ laser-scanning*" in claim 1 of the patent as granted. Consequently, the claimed subject-matter was considered to extend beyond the content of each of those applications and offend against Articles 123(2) and 76(1) EPC (see sections 14 to 17 of the decision).

5. In claim 1 of the present main request, the disclaimer has been omitted. The introduced feature defining the detection area is disclosed on page 23, lines 6 and 7 of each the application as filed and the parent applications.

6. At the oral proceedings before the board, appellant II raised an objection under Article 123(2) EPC arguing, for the first time, that the subject-matter of amended claim 1 was the result of a combination of selections from two lists. This argument represents an amendment to appellant II's appeal case, the admission of which is at the discretion of the board (see Article 13(2) RPBA 2020, which applies to the present case by virtue of Article 25(3) RPBA 2020).

7. As apparent from the minutes of the oral proceedings before the opposition division (see second sentence of the last paragraph on page 1), the opponent (present appellant II) "*... did not raise arguments under Art. 123(2),...*" against, among others, the auxiliary

request 1b which was identical to the present main request. Nor did appellant II put forward any objections under Article 123(2) EPC concerning this particular auxiliary request (now the main request) in its statement of grounds of appeal or the reply to the statement of the other appellant.

8. The board holds that the arguments brought forward by appellant II during the oral proceedings should have been submitted earlier, either already in opposition proceedings or, at the latest, with the reply to appellant I's statement of grounds of appeal in which the set of amended claims filed in opposition proceedings as auxiliary request 1b was pursued in appeal proceedings as auxiliary request 2. Appellant II did not put forward cogent reasons for the belated submission of arguments, and the board is not aware of any circumstances that, in the present case, may justify the admission of a new objection at such a late stage of the appeal proceedings. Hence, in application of Article 13(2) RPBA 2020 the board decided to exercise its discretion not to take into account appellant II's arguments under Article 123(2) EPC.

Article 123(3) EPC – scope of protection

9. In the decision under appeal, the opposition division held that the scope of the amended claim 1 of auxiliary request 1b then on file extended beyond that of claim 1 of the patent as granted and that, consequently, Article 123(3) EPC was contravened (see last paragraph of section 19.2 of the decision). The reasons given in the decision would apply equally to the identical claims of the present main request. However, the board does not share the opposition division's views on Article 123(3) EPC.

10. In decision G 1/93 (OJ EPO 1994, 541), the Enlarged Board of Appeal ruled that if, as in the present case, a claim of the patent as granted contains subject-matter which extends beyond the content of the application as filed (Article 123(2) EPC), but limits the scope of protection conferred by the patent, the patent can be maintained in amended form if the added undisclosed feature is replaced by another feature disclosed in the application as filed without breaching Article 123(3) EPC (see decision G 1/93, *supra*, Headnote).

11. In the present case, the opposition division's adverse decision on Article 123(3) EPC was based essentially on two findings. First, in connection with the main request and auxiliary request 1a then on file, the opposition division found that the disclaimer in claim 1 of the patent as granted limits the scope of the claim and, consequently, omitting it extends the protection conferred by the claim (see section 18.2 of the decision under appeal referring to section 16). This finding applies also to the auxiliary request 1b then on file, which was identical to the present main request. And second, the opposition division found that the additional feature introduced into the amended claim 1 of the auxiliary request 1b did not narrow the scope of a claim in which the disclaimer had been omitted (see section 19.2 of the decision under appeal).

12. While the board agrees in principle with the finding that the omission of the disclaimer in claim 1 extends the protection conferred by the patent as granted, it does not share the opposition division's assessment of

the extent to which the disclaimer limits the scope of protection.

13. The opposition division found that "*... in the light of the description the term "laser scanning" also encompasses embodiments relating to large area of detection (over 1 mm in the longest dimension)*" (see last sentence in section 13 of the decision under appeal). The board disagrees with this broad interpretation of the term "laser scanning".

14. According to the case law of the Boards of Appeal, if a term used in a claim has a clear technical meaning for a person skilled in the art, the description cannot be used to interpret such a term in a different way ("*in claris non fit interpretatio*"; see, e.g., decisions T 1395/07 of 7 July 2009, section 4 of the reasons; and T 2769/17 of 6 December 2019, section 1.1.1 of the reasons).

15. The term "laser scanning" imparts to the skilled person a clear and unambiguous technical teaching. As understood in the art, this technique employs a laser beam to illuminate and query a microscopic area, larger macroscopic areas being covered by scanning the area with the laser beam progressively. This understanding of the term "laser-scanning" is confirmed by the passage on page 7, lines 1 to 5 of the application as filed to which the opposition division referred in its decision (see last sentence on page 4 of the decision under appeal).

16. The opposition division referred to Figure 4 and the passage bridging pages 23 and 24 of the application as filed to support a broader interpretation of the term "laser-scanning" as encompassing also methods in which

the longest linear dimension of the detection area (i.e. the area that is simultaneously sampled by the detection device) is greater than 1 mm. Figure 4 does, however, not illustrate laser **scanning**, but only illumination of the sample with an excitation laser, and the passage bridging pages 23 and 24 discloses imaging a 2 cm x 1 cm rectangle by scanning with a linear array **detector** that has a long dimension of 1 cm as an example of large area detection. A laser is not mentioned in this context. Hence, the description does not support the opposition division's broad interpretation of the term "laser-scanning".

17. Based on its interpretation of the term "laser-scanning", the opposition division found that the disclaimer in claim 1 "*... alters the scope of the claimed subject-matter*[sic] *to exclude methods employing laser illumination and wherein the detection zone is scanned with a detector. Said scanning is not limited to a particular size of detection area that is to be simultaneously detected*" (see first full paragraph on page 7 of the decision). The board disagrees with this finding. The disclaimer in claim 1 of the patent as granted limits the scope of protection conferred by the claim only insofar as it excludes methods in which a laser beam detecting a microscopic area scans a large detection zone progressively.

18. Contrary to the opposition division's second finding (see paragraph 11 above), the new feature introduced into amended claim 1, replacing the diclaimer, limits in fact the scope of protection conferred by the claim and excludes methods employing a laser beam detecting a microscopic area to scan a large detection zone progressively. The board agrees with the opposition division in that the wording of amended claim 1 does

not preclude detecting multiple detection areas in sequence by scanning the detection zone with the detection device. However, claim 1 requires that the longest linear dimension of the area which is simultaneously analysed by the detection device (i.e. the detection area) is greater than 1 mm. Since this requirement is not met by a laser beam which generally detects simultaneously only an area of a few micrometres, the scope of protection of the amended claim 1 according to the present main request – like that of claim 1 of the patent as granted - does not include detection methods employing laser scanning. Consequently, the scope of protection of amended claim 1 does not extend beyond the scope of the patent as granted.

19. For these reasons, the board concludes that the amendments introduced into claim 1 of the present main request do not contravene Article 123(3) EPC.

Article 84 EPC – clarity and support

20. An objection under Article 84 EPC to the term "*simultaneously*" in claim 1 was raised by appellant II for the first time at the oral proceedings before the board. Since appellant II did not bring forward any cogent reasons why this objection could not have been raised in opposition proceedings or at an earlier stage of appeal proceedings, the board decided to exercise its discretion not to admit the new objection into the proceedings.

21. Hence, the requirements of Article 84 EPC are regarded as met.

Article 83 EPC – sufficiency of disclosure

22. In the decision under appeal, the opposition division held - in connection with the auxiliary request 4a then on file - that the patent provides a worked example for carrying out the invention and discloses various alternative antibody dyes. Hence, the opposition division found that the claimed invention is sufficiently disclosed in the patent.

23. This finding was contested by appellant II arguing that only detection of microcolonies competent to replicate which are not labeled for detection are disclosed in the application as filed.

24. The board does not share appellant II's view. It is apparent from various passages of the application that the use of externally applied labeling reagents for signal generation is an embodiment of the detection method of the invention. See page 13, lines 9 to 11 and 20 to 23; page 19, line 4 to page 20, line 30. On page 20, lines 14 to 30 various labelling agents that generate a chemiluminescent or fluorescent signal are disclosed. In Example 1, microcolonies grown on a filter are labeled with Syber Green I and FITC-labeled antibody before imaging. Contrary to appellant II's view, only the filters labeled with Syber Green I were fixed in formaldehyde, which killed the cells, whereas the filters used for FITC-labeling were not fixed (see page 55, lines 13 to 22 of the application as filed).

25. Document (9), on which appellant II relied to support its objection, is a print-out of the patent proprietor's website relating to the genesis of the so-called "Growth Direct technology" which exploits the natural autofluorescence of the cells for detecting

microcolonies. It is stated therein that, before the Growth Direct technology was developed, "*our technology*" was destructive because it required staining. In fact, staining mostly requires fixation and permeabilization of the cells to disrupt the cell membrane and allow entry of the stain, as exemplified with Syber Green I in Example 1 of the application. As disclosed in the application, the invention requires, however, the use of non-destructive labels which allow the cells to remain competent to replicate after detection.

26. For these reasons, the board is persuaded that the opposition division's decision on Article 83 EPC is correct.

Article 54 EPC – novelty

Admission of document (15) into the proceedings

27. Appellant II submitted document (15) together with its statement of grounds of appeal. The experiments described therein allegedly reproduce the experiments in Example 3 of document (1) and support the objection of lack of novelty over the method described in this document. However, appellant II did not put forward any cogent reasons why the experimental evidence in document (15) could not have been filed in opposition proceedings. Nor did appellant II indicate to which extent the new evidence differs from the experimental evidence in document (10) already on file and takes into account the issues raised in the decision under appeal concerning the experimental conditions in the latter document (see section 26.3 of the decision under appeal).

28. Under these circumstances, the board decided to exercise its discretion not to admit document (15) into the proceedings.

Document (1)

29. In the decision under appeal, novelty over document (1) was acknowledged on the grounds that document (1) itself did not describe that microcolonies remain competent to replicate after detection, and that documents (5) and (10) failed to plausibly show that, applying the method described in document (1), the cells in the microcolonies remained competent to replicate after detection (see sections 26.2 and 26.3 of the decision).

30. Document (1) describes a method of determining an approximate number of living microorganisms in a sample which comprises: (i) immobilising the microorganisms on an hydrophobic membrane, (ii) releasing adenosine triphosphate (ATP) from the microorganisms by spraying an ATP releasing reagent, (iii) inducing luminescence by spraying a solution of luminescence-inducing reagent, and (iv) identifying the number of luminescent spots using an ultrahigh-sensitivity TV camera as the detection device.

31. It was not contentious between the parties that steps (a), (c) and (d) of the method of claim 1 are described in document (1) and that the features of the detection device specified in claim 1 correspond to those of an ultrahigh-sensitivity TV camera.

32. However, the parties disagreed on the question whether document (1) describes a method which detects microcolonies of cells that remain competent to

replicate after the detection. In Examples 3 and 4 of document (1), a hydrophobic membrane on which *E. coli* cells had been immobilized was sprayed first with methanol as ATP releasing reagent, and then with a solution of luminescence-inducing reagent (Lucifer-LU) of five-fold concentration. Replication of the cells *in situ* to form microcolonies (step (b) of the method of claim 1) is not described in Examples 3 and 4, but it is stated in the general part of the description (see column 9, lines 23 to 33) that, preferably, the bacteria immobilized on the membrane are cultivated for a short time period (e.g. three hours) prior to detection, "*... thereby increasing the viable count by forming colonies of microbes within the same sections*".

33. Document (1) does not expressly describe that, following detection, the cells remain competent to replicate. Nor did a person skilled in the art reading document (1) in the light of the common general knowledge at the relevant date expect this to be the case, because both methanol and the further ATP releasing reagents mentioned in this document ("*... alcohols, ethers, esters, and halogenated derivatives of methane, ethane, or ethylene, as well as acetonitrile, triethylamine and others*", see column 6, lines 24 to 27) were known to disrupt the cell wall and membrane to release the ATP. According to the established case law of the Boards of Appeal (see, *inter alia*, decision T 305/94 of 20 June 1996), for the purposes of examining novelty, a document forming part of the art within the meaning of Article 54(2) EPC is to be assessed from the perspective of the skilled person on the publication date.

34. Document (5) – published in 2011, i.e. more than 12 years after the publication of document (1) – confirms this finding:

> "*The* ATP-*releasing reagent contains chemical components that lead to cell rupturing to release ATP from the microbial cells.* [...] *As all present cells were said to be ruptured after the reagent treatment, it was assumed that no intact cells could be used for further identification ...*" (see page 43, right-hand column, second paragraph)

35. The board does not share appellant II's view on the probative value of document (5) for the question whether the cells remained competent to replicate after applying the detection method described in document (1). As stated in the decision under appeal, it cannot be derived from document (5) whether the conditions for ATP release used in the experiments therein are the same as those described in document (1). As a matter of fact, the ATP releasing agent described in document (11), which is allegedly the same reagent as in the Milliflex Rapid System used in document (5), contains an (unknown) proprietary compound and (an unknown amount of) ethanol, whereas in Examples 3 and 4 of document (1) only methanol is used. The same applies to the experiments in document (10) performed with the Milliflex Rapid System.

36. Hence, the opposition division correctly acknowledged novelty of the subject-matter of claim 1 over document (1). The same applies to the dependent claims.

Document (3)

37. Document (3) describes a method for detecting and counting **single** bacterial cells, in particular of a luminous bacterium named *Photobacterium* sp. strain Ohtake 3-11, using a photon-counting TV camera to obtain a photon-counting image of a filter on which the single cells had been deposited. In order to confirm that the bright spots on the image arose in fact from the luminous bacterium, the same filter was incubated until bacterial colonies formed, and an illuminated image of the colonies was obtained. Almost all formed colonies on this image coincided with the bright spots observed in the luminous image of the single cells.

38. Document (3) suggests that the method described therein "*... can be extended to identify and count almost any kind of bacteria because there are several methods to make non-luminous bacteria to emit light. These include (1) staining them with 4',6-diamidino-2-phenylindole to cause fluorescence [9], enzyme immunoassay to selectively cause luminescence [10] and introduction of lux genes [11]*" (see page 290, left-hand column, lines 1 to 8 from the bottom). It should be noted that this suggestion is made only in connection with the detection of single cells, because the bacterial (macro)colonies formed by incubating the filter after detection of the single cells may be observed with the naked eye.

39. The board shares the opposition division's view that document (3) does not describe step (b) of the method of claim 1, namely allowing the formation of microcolonies of the target cells by *in situ* replication, prior to labeling them and detecting the signal generated by the label (steps (c) and (d) in

claim 1). Moreover, the board holds that a person skilled in the art cannot derive directly and unambiguously from document (3) a method in which, after labeling and detection, the cells remain competent to replicate.

40. Thus, the board holds that the opposition division's finding on novelty of the subject-matter of claim 1 over document (3) is correct. Also the dependent claims are novel.

Article 56 EPC – inventive step

Document (3) as the closest state of the art

41. The teachings of document (3) were summarized in paragraphs 37 and 38 above.

42. The method of claim 1 differs from the method described in document (3) in that, prior to the labeling and detection steps, the target cells are allowed to replicate *in situ* to form microcolonies having a mean measurement of less than 50 microns in at least two orthogonal dimensions, and that, after labeling and detection, the cells remain competent to replicate.

43. In the decision under appeal, the opposition division formulated the technical problem to be solved as the provision of a more sensitive/faster method of detecting living cells. In the board's view, the problem should be formulated more generally as the provision of an improved method for detecting living cells in a sample. It has not been disputed that this problem is solved by the method of the invention as claimed.

44. Having considered the arguments put forward by the parties, the board is persuaded that the claimed method involves an inventive step. In document (3) it is stated that colony-counting methods are disadvantageous, and the method described therein is praised as "*... more rapid than the colony counting method because cultivation is not needed*" (see page 287, right-hand column, lines 4 and 5 from the bottom). In the light of the successful detection of single cells employing the method described in document (3), the skilled person does not derive from this document any motivation to modify that method by cultivating the cells to form microcolonies **prior** to detection, because such a modified method would be less rapid and more cumbersome. While document (3) describes that, **after** detection of the single cells, the membrane filter was incubated to grow bacterial colonies which were then detected, a person skilled in the art understands that these steps do not form part of the described method, but are only intended for validating the results obtained for the single bacterial cells.

45. The method of document (3) is aimed at detecting and counting bacteria in a sample. There is no indication in document (3) whatsoever that the bacteria or the colonies obtained by incubating the membrane filter could be used for any other purpose. Hence, even though document (3) suggests that, for detection according to the method described therein, non-luminous bacteria could be stimulated to emit light, there is no incentive for the skilled person to ensure that, after labeling and detection, the cells remain competent to replicate. On the contrary, two of the methods referred to in document (3) to illustrate methods for inducing non-luminous bacterial cells to emit light (see page 290, left-hand column, lines 1 to 5 from the

bottom) result in the cells being destroyed (see staining with 4',6 diamidino-2 phenylindole (DAPI) in document (4), and the enzyme immunoassay to induce chemoluminiscence described in document (13)). This has not been disputed by appellant II.

46. In view of the above, the board concludes that document (3) alone does not provide any hint towards a detection method as claimed and, thus, cannot render it obvious to a person skilled in the art.

47. The same conclusion is reached if the skilled person were aware of the teaching in document (1) that a more accurate determination is achieved if the membrane filter with the entrapped cells is cultivated for a short time period. Not only would this measure run counter to the purpose of the method of document (3) which is to provide a rapid method. More importantly, the skilled person cannot derive from document (1) that, after labeling and detection, the cells remain competent to replicate. As stated above in the context of novelty, the technique used in document (1) to detect cells in a sample (ATP bioluminescence reaction) involves spraying an ATP releasing reagent to disrupt the cells. Hence, the skilled person is neither expressly taught nor expects in view of the common general knowledge that any cells competent to replicate remain after labeling and detection.

48. For these reasons, the method of claim 1 involves an inventive step over document (3) combined with document (1).

Document (2) as the closest state of the art

49. Document (2) describes a rapid method for determining both total bacterial count and the amount of *Escherichia coli* and coliform bacteria in water or an aqueous sample, the method not involving cultivation of the bacteria ("*ohne kulturelle Anzucht*", see claim 1 of document (2)). The described method requires that, after incubation of a membrane filter on which the bacteria have been immobilized with a fluorogenic substrate, and detection of the fluorescent spots, the bacteria are killed ("*alle Bakterien abgetötet werden*") before the total bacterial count on the filter is determined by measuring fluorescence or chemoluminiscence.

50. Hence, the skilled person cannot derive from document (2) a method in which the cells are allowed to form microcolonies with a size of less than 50 microns before labeling and detection, and in which the cells remain competent to replicate after detection. Like for document (3), a person skilled in the art seeking to improve the method of document (2) does not find in this document any hint towards the solution provided in claim 1.

51. Appellant II supported its objection of lack of inventive step also by a combination of the teachings of document (2) with those of document (1). However, the reasons given in paragraph 47 above starting from document (3) as the closest state of the art apply, *mutatis mutandis*, also to document (2) as the closest state of the art in combination with document (1).

52. Hence, the claimed methods involve an inventive step in view of a combination of the teachings of documents (2) and (1).

Conclusion

53. The present main request meets the requirements of the EPC.

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 on the basis of claims 1 to 15 of the main request, filed as auxiliary request 2 on 7 June 2016 and a description to be adapted.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated