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D E C I S I O N
of 21 September 1999

Case Number: T 0744/97 - 3.3.4

Application Number: 86117754.1

Publication Number: 0254771

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Language of the proceedings: EN

Title of invention:
Detection of microbes in a sample

Patentee:
Edberg, Stephen C.

Opponent:
-

Headword:
Detection of microbes/EDBERG, S.

Relevant legal provisions:
EPC Art. 54

Keyword:
"Main request - novelty - yes"

Decisions cited:
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Case Number: T 0744/97 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 21 September 1999

Appellant: (Proprietor of the patent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 9 May 1997 concerning maintenance of the European patent No. 0 254 771 in amended form pursuant to Article 102(3) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
W. Moser

Summary of Facts and Submissions

I. European patent No. 0 254 771 with the title "Detection of microbes in a sample" was granted on the basis of European application No. 86 117 754.1 with 17 claims.

Granted claims 1 and 9 read as follows:

"1. A specific medium for combination with a specimen sample to determine the presence or absence of a target microbe in said specimen sample, said medium comprising:

(a) ingredients for supporting growth of said target microbe;

(b) a nutrient-indicator including a moiety metabolizable by said target microbe and a sample-altering moiety which is released only when said nutrient-indicator is metabolized by said target microbe and provides alteration of a sensible characteristic of said specimen sample;

(c) said ingredients and said nutrient-indicator being selected such that significant reproductive growth of only said target microbe takes place, said nutrient-indicator being a nutrient which is preferred by said target microbe and cannot significantly be metabolized by other viable microbes in said specimen sample."

"9. The medium of claim 8 wherein said chromogenic substrate for B-glucuronidase is a glucuronide selected from the group consisting of orthonitrophenyl-B-D-

glucuronide, B-naphtalamide-B-D-glucuronide, alpha-naphtol-**B-C**-glucuronide, methylumbilliferyl-B-D-glucuronide, and bromo-chlor-indole-B-D-glucuronide, or mixtures thereof."(emphasis added by the board)

Dependent claims 2 to 8, 10 to 14 related to further features of the medium of claim 1. Claim 15 related to a method of testing a specimen to determine the presence or absence of a target microbe making use of the medium of claims 1 to 14. Dependent claims 16 and 17 related to further features of the method of claim 15.

II. An opposition was filed requesting the revocation of the patent under Article 100(a) to (c) EPC.

III. The Opposition Division decided that the granted claim request fulfilled the requirements of Article 123(2) EPC since a basis for claim 1, part (c), second half of the sentence could be found in the application as filed on page 4, lines 19 and 20. In the same manner, a basis for claim 4 could be found in said application on page 3, lines 36 and 37 and on page 6, lines 43, 50 and 51 whereas a basis for claim 7 could be found on page 5 and page 6, line 25. They, however, refused the maintenance of the patent as granted for the reason that claim 1 lacked novelty over the disclosures of either of documents (MP-1), (MP-5) and (MP-8).

They maintained the patent in amended form on the basis of an auxiliary request filed at oral proceedings. In this request, the subject-matter of granted claim 4 was inserted into claim 1. Claims 2, 3 and 4 to 16 were identical to claims 2, 3 and 5 to 17 as granted. It was decided that the amendment introduced into claim 1 conferred novelty to its subject-matter. Inventive step was acknowledged for the reason that none of documents MP-1, MP-5 or MP-8 alone or in combination made it

obvious to use the nutrient-indicator as a means for selection of the target microbe. Furthermore, many examples were provided in the patent specification for the specific detection of different microbes and, thus, the requirement of sufficiency of disclosure was fulfilled.

IV. The Appellant (Patentee) lodged an appeal, paid the appeal fee and submitted a statement of grounds for the appeal together with eight auxiliary requests. His main request was that the patent be maintained on the basis of the claims as granted.

V. The Board of Appeal sent a communication requiring that the content of the auxiliary requests be clarified and providing their provisional non-binding opinion with regard to novelty of the claims of the main request.

VI. On 24 February 1999, the Appellant filed a further submission together with four new auxiliary requests in replacement of the previous eight auxiliary requests, and three further documents.

VII. The Respondent (Opponent) withdrew the opposition.

VIII. The Board of Appeal sent a further communication to draw the Appellant's attention to the fact that claim 9 of the main request (claim 9 as granted; see section I supra) contained subject-matter which was not present in the application as filed insofar as it mentioned alpha-naphtol-B-**C**-glucuronide (see section I supra) rather than alpha-B-**D**-glucuronide as chromogenic substrate.

IX. With a letter received by the EPO on 25 August 1999, the Appellant submitted a new main request in replacement of the previous main request, whereby claim 9 read as follows:

"9. The medium of claim 8 wherein said chromogenic substrate for B-glucuronidase is a glucuronide selected from the group consisting of orthonitrophenyl-B-D-glucuronide, B-naphtalamide-B-D-glucuronide, alpha-naphtol-**B-D**-glucuronide, methylumbilliferyl-B-D-glucuronide, and bromo-chlor-indole-B-D-glucuronide, or mixtures thereof."(emphasis added by the board)

All other claims remained as granted.

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

(MP-1) : Kilian, M. and P. Bülow, Acta Path. Microbiol.Scand, Sect.B., Vol. 87, pages 271 to 276, 1979,

(MP-5) : Petzel, J. and P. A. Hartman, Applied and Envir. Microbiol., Vol. 49, pages 925 to 933, 1985,

(MP-8) : US 4 591 554 issued on 27 May 1986,

(E-10) : Porter, J. R. in "Bacterial Chemistry and Physiology", edited by John Wiley and Sons, page 820, 1950.

(E-17) : Difco Manual, "Dehydrated Culture media and Reagents for Microbiology", pages 453 to 454, 1984,

(E-21) : Difco Manual, "Dehydrated Culture media and Reagents for Microbiology", page 680, 1984,

(E-22) : "Food Chemistry", edited by O. Fennema, Marcel Dekker, Inc., pages 742 and 751, 1985.

XI. The arguments by the Appellant with regard to the main request were essentially as follows:

Novelty

Documents (MP-1) and (MP-5) described media which contained in addition to a galactoside-indicator, significant amounts of free galactose or 1 g/l of free glucose, respectively. The assertion by the Opposition Division that the free galactose or glucose would be less available in the gelified agar medium than the galactoside comprised in the indicator was without scientific basis and wrong. In fact, the free galactose and glucose being monosaccharides would be used by micro-organisms in preference to the galactoside present in the galactoside-indicator. Accordingly, neither document (MP-1), nor document (MP-5) could be novelty destroying for the subject-matter of granted claim 1.

Document (MP-8), Example 1 described a method for determining the number of micro-organisms in a suspension, which involved growing them in the presence of 4-methyl umbelliferone derivatives and measuring the fluorescence of the umbelliferone released in the medium following cleavage of said derivatives by said micro-organisms. Micro-organisms comprising coliforms, gram-positive bacteria, yeast and fungi were shown to grow to a cell density in the range from 10^6 to 10^9 /ml in a medium described as "a medium of pH: 7.0 contain[*ing*] 0.5% peptone and 10^{-3} 4-methyl umbelliferone derivatives". This information on the growth medium was obviously incomplete: at least the yeast and fungal strains which were not able to

hydrolyse the umbelliferone derivatives (Table 2) would not be expected to grow to the disclosed cell density in the presence of 0.5% peptone. Example 1 was not a clear and unmistakable disclosure of the subject-matter of granted claim 1.

Document (MP-8), Example 7 disclosed growing the micro-organisms to be numbered in heart infusion medium. This medium was derived from heart muscle which contained simple sugars as shown not only in document (E-22) but also in Example 7 itself where the significant gaz production observed was proof of sugar utilisation. The simple sugars would be used by the micro-organisms in preference to the nutrient-indicator. This example like Example 1 was not relevant to novelty.

Inventive step

The underlying problem addressed by the present invention was to provide a medium for an improved method of detecting a small number of microbes in a sample. In contrast to the prior art, the claimed medium used an active selector for the target microbe rather than passive reactors. This approach to the problem was elegant, simple and unexpected.

Sufficiency of disclosure

Many examples were provided in the patent specification of how to perform the invention with samples containing different types of bacteria. Following these examples, the skilled person would only have to adapt the parameters of the medium such as the pH or the concentration in antibiotics to the kind of bacteria, the presence of which in a sample was to be investigated. This could be achieved without undue burden.

XII. The Respondent did not put forward any submissions or requests.

XIII. The Appellant requested that the decision under appeal be set aside and that the patent be maintained on the basis of the following documents:

(a) claims 1 to 8, 10 to 17 as granted and claim 9 filed on 25 August 1999 as main request; or

(b) claims of either of the first to fourth auxiliary requests filed on 24 February 1999 and

(c) description, pages 2 to 8 and page 9 lines 1 to 34, as granted.

Oral proceedings were requested in case the Board would not allow the main request.

Reasons for the Decision

1. The appeal is admissible.

Main request

Article 123(2) EPC, added subject-matter:

2. The passages in the application as filed which serve as a basis for claim 1, part (c), second half sentence, and for claim 7 are the last paragraph on page 8 and page 11 respectively. The passages which serve as a basis for claim 4 are the second paragraph on page 6 and the first paragraph on page 14. Furthermore, the

amendment brought to claim 9 ensures that all claimed specific chromogenic substrates were originally disclosed. From the above, it follows that the main request fulfills the requirements of Article 123(2) EPC.

Article 54 EPC; novelty:

3. Document (MP-1) discloses a rapid method for the identification of enterobacteriacae. Bacterial samples are grown on PGUA agar, a medium which contains amongst other ingredients an indicator: 4-nitrophenyl-β-D glucopyranosiduronic acid. This compound is cleaved by the enterobacteriacae exclusively, as only they synthesize the enzyme β-glucuronidase. Furthermore, gram-positive bacteria and yeasts do not grow on PGUA agar (Table 1). Accordingly, document (MP-1) discloses a medium having the characteristics (a), (b) and also (c) of the medium of claim 1, insofar as:

- the viable microbes other than the target microbe do not metabolize the indicator and,

- only the target microbe is capable of significant reproductive growth.

4. The question which remains to be answered in relation to novelty is whether, as required in part (c) of claim 1, the indicator, 4-nitrophenyl-β-D glucopyranosiduronic acid, is also the nutrient **preferred** by the target microbe. In this respect, it is important that PGUA agar contains Japan agar which comprises significant amounts of free galactose (document (E-10)) which evidently will be available to

the micro-organisms. As it is a monosaccharide, it will be used as a carbon and energy source in preference to any other nutrients of higher molecular complexity such as the 4-nitrophenyl-β-D glucopyranosiduronic acid. Accordingly, the nutrient-indicator present in the medium described in document (MP-1) is not the **preferred** nutrient of the target microbe. Thus, the teaching of this document does not affect novelty.

5. Document (MP-5) discloses a medium for determination of total gram-negative bacteria and E.coli. It contains in addition to the indicator 4-methylumbelliferyl-β-D-glucuronide, 23.3 g/l of plate count agar (page 926, left hand column). This agar comprises 1 g/l of free glucose as monosaccharide (document (E-21)). Thus, document (MP-5) does not affect novelty for the same reasons as given with regard to document (MP-1).

6. Document (MP-8) discloses media for determining the number of micro-organisms present in a sample on the basis of their ability to hydrolyse non fluorescent umbelliferone derivatives (for Example 4 methyl-umbelliferyl-galactoside) to fluorescent umbelliferone or 4-methyl-umbelliferone.

7. In Example 1, column 5, one such medium is described which allows coliform organisms, other gram-negative bacteria, gram-positive bacteria, yeast and fungi to grow from 100 microbial cells to 10^6 to 10^9 cells/ml in 24 hours. This growth occurs independently from whether or not the micro-organims are able to hydrolyse the non fluorescent umbelliferone derivatives, i.e independently from whether or not they have access to the nutrient (for example galactoside) these derivatives contain. Example 1, thus, discloses a medium, the ingredients of which allow significant growth of all microbes rather than significant growth of **only** a target microbe. This medium lacks the

property corresponding to feature (c) of claim 1 that the claimed ingredients are being "selected such that significant reproductive growth of only said target microbe takes place...".

8. In Example 7, HI medium is used which contains in particular a heart infusion, sodium desoxycholate and umbelliferyl-β-D-galactoside (U-β-Gal) as the indicator. The presence of desoxycholate ensures that only coliform bacteria will grow (column 4). U-β-Gal can only be hydrolysed by coliform bacteria to give the fluorescent compound and the galactoside. This medium, thus, possesses the features (a) and (b) of the medium of claim 1 and also the feature (c), insofar as the ingredients and indicator are selected such that significant growth of only the target microbe takes place.

9. The question which remains to be answered in relation to novelty over document (MP-8) is whether U-β-Gal is the nutrient preferred by the coliform bacteria. Heart infusion is made from beef hearts and beef heart muscle contains glucose (document (E-22), page 751). It also contains glycogen which, according to the Appellant, would be degraded into glucose while the infusion is being produced. Thus, and in absence of any evidence to the contrary, the Board is prepared to accept that glucose is indeed present in the HI medium. Accordingly, and for the same reasons as given under point 4 above, it is concluded that U-β-Gal is not the **preferred** nutrient in the medium and that, therefore, said medium lacks the property corresponding to feature (c) of claim 1 according to which "said nutrient indicator being a nutrient which is preferred by said target microbe...".

10. The teachings of document (MP-8) are not detrimental to novelty.

11. No other documents on file disclose a medium with the features of claim 1 or of dependent claims thereof. Method claim 15 makes use of the media of claims 1 to 14 and, thus, is also novel.

12. The requirements of Article 54 EPC are fulfilled.

Inventive step

13. Document (MP-8) is considered to be the closest prior art. It discloses a medium for the detection of a small number of micro-organisms in a sample, which comprises an indicator in addition to a nutrient metabolizable by all micro-organisms. In relation to the specific detection of coliform bacteria, it is stated in column 4: "...it is preferable to add a bile acid or desoxycholic acid which inhibits growth of microorganisms other than coliform bacteria to the nutrient culture medium."

14. In the light of this prior art, the technical problem to be solved can be defined as the provision of a medium for the detection of specific microorganisms in a sample.

15. As a solution thereto a medium is proposed, one constituent of which simultaneously acts as a nutrient to be metabolized **only** by the micro-organisms to be detected and as an indicator of the presence of said micro-organisms in the sample.

16. The examples given in the patent show that this medium solves the technical problem.

17. The difference between the claimed medium and the medium disclosed in document (MP-8) is that one of its constituents: the nutrient-indicator, fulfills the tasks of three of the constituents of the latter medium: the nutrient, the indicator and the inhibitor of microbial growth other than that which is to be detected. No suggestions can be found in document (MP-8) taken either alone or in combination with the other documents of the state of the art, of such a simple and straightforward solution to detecting the presence of specific micro-organisms in a sample. Inventive step is acknowledged.

Sufficiency of disclosure

18. Numerous examples are provided of media suited for detecting different types of micro-organisms. In the Board's judgment, the skilled person could, thus, reproduce the claimed invention without undue burden.

19. Since the main request is allowable, there was no need for oral proceedings. Neither had the auxiliary requests to be considered.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the following documents:

   (a) claims 1 to 8, 10 to 17 as granted and claim 9 filed on 25 August 1999, and

   (b) description, pages 2 to 8 and page 9, lines 1 to 34, as granted.

The Registrar: The Chairwoman:

U. Bultmann U. Kinkeldey