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Datasheet for the decision of 12 May 2022

Case Number: T 0136/19 - 3.3.08

Application Number: 12729592.1

Publication Number: 2723884

IPC: C12Q1/04, C12Q1/34

Language of the proceedings: EN

Title of invention:
METHOD FOR DETECTING THE PRESENCE OF CARBAPENEMASE-PRODUCING BACTERIA IN A SAMPLE

Patent Proprietors:
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Opponent:
Strawman Limited

Headword:
Carbapenemase/INSERM

Relevant legal provisions:
EPC Art. 54, 56, 83

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

Decisions cited:
T 0009/81, T 1072/92, T 0917/94, T 0154/04

Catchword:

Europäisches Patentamt
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Beschwerdekammern

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Case Number: T 0136/19 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 12 May 2022

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Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on**

2 November 2018 concerning maintenance of the European Patent No. 2723884 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. 2 723 884 with the title "Method for detecting the presence of carbapenemase-producing bacteria in a sample" was granted from the European application No. 12729592.1 which was filed under the Patent Cooperation Treaty (PCT) and published as WO 2012/175637 A1. In the present decision, references to the "application as filed" are to the published application.

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

III. In an interlocutory decision posted on 2 November 2019, an opposition division found that, while the subject-matter of the main request filed on 26 July 2018 and of the auxiliary request 1 filed during the oral proceedings lacked an inventive step, the amended patent according to the auxiliary request 2, also filed during the oral proceedings, met the requirements of the EPC. In particular, with respect to the main request the opposition division found that the subject-matter of claim 1 was novel over documents (1) and (6) and also involved an inventive step. However, the subject-matter of claims 12 and 18 was found to lack an inventive step in view of the teachings of document (1) supplemented with the common general knowledge as exemplified by document (18).

IV. Independent claims 1, 12 and 18 of the main request read as follows:

"1. A method for detecting the presence of carbapenemase-producing bacteria in a sample, said method comprising the steps of:
a) performing cell lysis on a test sample in order to obtain an enzymatic suspension;
b) reacting a fraction of the enzymatic suspension obtained in step a) with a reagent kit, said reagent kit comprising
- a carbapenemase substrate selected from the group consisting of carbapenems and cephamycins, and
- a pH color indicator which will change color when the pH of the reaction mixture is comprised between 6.4 and 8.4,

wherein a color change after step b) indicates the presence of carbapenemase-producing bacteria in the test sample, and wherein the reaction in step b) is carried out over a period of time sufficient to observe a color change, the color change is visually observed within a time period comprised between 5 minutes and 120 minutes.

12. A kit comprising
a lysis buffer; and
a reagent kit comprising:
- a carbapenemase substrate selected from the group consisting of carbapenems and cephamycins; and
- a pH color indicator which changes color when the pH is comprised between 6.4 and 8.4.

18. A kit comprising
a lysis buffer; and
a microtiter plate comprising a well or a series of wells comprising:
- a carbapenemase substrate selected from the group consisting of carbapenems and cephamycins; and

- a pH color indicator which changes color when the pH is comprised between 6.4 and 8.4."

Dependent claims 2 to 11 and 21 relate to various embodiments of the methods of claim 1, and dependent claims 13 to 16 and 21 to embodiments of the kit of claim 12. Independent claim 17 and dependent claim 21 are directed to the use of the reagent kit of claims 12 to 16 for performing the method of claims 1 to 11. Dependent claims 19 and 21 relate to embodiments of the kit of claim 18. Independent claim 20 and dependent claim 21 are directed to the use of a microtiter plate as defined in claim 19 for performing the method of claims 1 to 11.

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

VI. Each party replied to the statement of grounds of the other party. Together with their reply, appellants I submitted additional evidence and a new set of claims as auxiliary request 3.

VII. Pursuant to their subsidiary request, the parties were summoned to oral proceedings before the board. In a communication sent in preparation of 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 raised by the appellants.

VIII. Oral proceedings were held on 12 May 2022.

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

(1): H. Knothe *et al.*, 1987, Journal of Antimicrobial Chemotherapy, Vol. 19, No. 1, pages 136 to 138;

(2): WO 2010/010083, published on 28 January 2010;

(11): D. M. Livermore, 2002, Current Opinion in Investigational Drugs, Vol. 3, No. 2, pages 218 to 224;

(12): D. M. Livermore and D. F. J. Brown, 2001, Journal of Antimicrobial Chemotherapy, Vol. 48, Suppl. S1, pages 59 to 64;

(13): J. Holt *et al.*, 1983, Antibiotics: Assessment of Antimicrobial Activity and Resistance, A. D. Russell and L. B. Quesnel, eds., pages 127 to 139;

(15): J. Escamilla, January 1976, Antimicrobial Agents and Chemotherapy, Vol. 9, No. 1, pages 196 to 198;

(16): A. Skinner and R. Wise, 1977, Journal of Clinical Pathology, Vol. 30, pages 1030 to 1032;

(17): K. Shannon *et al.*, 1985, Journal of Antimicrobial Chemotherapy, Vol. 15, Suppl. C, pages 15 to 23;

(18): WO 2009/051838, published on 23 April 2009;

(19): A. M. Queenan and K. Bush, July 2007, Clinical Microbiology Reviews, Vol. 20, No. 3, pages 440 to 458;

(21): R. Edwards *et al.*, 1997, Journal Medical Microbiology, Vol. 46, pages 807 to 809;

(23): L. Lauretti *et al.*, July 1999, Antimicrobial Agents and Chemotherapy, Vol. 43, No. 7, pages 1584 to 1590;

(24): S. Banič, 1991, Journal of Chemotherapy, Vol. 3, No. 6, pages 348 to 351;

(26): S. Shinde *et al.*, April-June 2017, Journal of Laboratory Physicians, Vol. 9, No. 2, pages 100 to 103; and

(28): P. Nordmann, 2010, Médecine/Science, Vol. 26, pages 950 to 959.

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

Main request

Article 83 EPC

The application as filed disclosed at least one way of putting the invention into practice, and this, over the whole scope of the claims. The skilled person, with a mind willing to understand the invention, was perfectly capable of reproducing the claimed invention. Thus, the requirement of Article 83 EPC was met.

Article 54 EPC

None of the cited documents disclosed a method as claimed or a kit comprising a carbapenemase substrate,

a pH color indicator and a lysis buffer. The skilled person could not derive from the passage on page 136, right-hand column, last full paragraph of document (1) a method for detecting carbapenemase-producing bacteria because the bacteria studied in document (1) were not carbapenemase producers. Hence, the claimed subject-matter was novel.

Article 56 EPC

Claim 1

Document (1) as closest state of the art

Document (1) could not be regarded as the closest state of the art because its teaching was unclear and did not relate to the very specific problematic of carbapenem-resistance resulting from carbapenemase production. In contrast, document (2) described a method having the same purpose as the claimed invention and requiring the minimum of structural and functional modifications to arrive at the present invention.

Document (2) as closest state of the art

The method of claim 1 differed from that described in document (2) in that it did not require an incubation step because the bacteria to be tested were directly lysed. Thus, the claimed method was extremely fast and specifically detected carbapenemase activity, rather than carbapenem resistance resulting from another resistance mechanism. The problem to be solved was to provide a rapid, very specific, very selective and easily reproducible method with a high medical value for detecting the presence of carbapenemase-producing bacteria in a sample.

Starting from document (2) and seeking to solve that problem, the skilled person would not have considered document (1) because this document did not teach how to identify the carbapenem resistance of the tested bacteria as resulting from carbapenemase production. Hence, the subject-matter of claim 1 involved an inventive step.

Claims 12 and 18

Document (1) was not an appropriate closest state of the art for evaluating whether the kits of claims 12 and 18 involved an inventive step. The relevant passage of document (1) (page 136, second column) did not describe a kit. Moreover, as acknowledged by the opposition division, the teaching in that paragraph was completely unclear and "out of the blue". Document (1) did not provide any reference for the Escamilla test as such, nor did it specify which modification(s) had been made "*by V. Schaefer*". The results obtained using the Escamilla test were not disclosed, let alone further discussed in document (1). All results provided and discussed were obtained using the micro-iodometric method. The skilled person would not have specifically focused on a teaching which was completely unclear, and would not have modified the method allegedly disclosed in document (1) by introducing a lysis buffer instead of using sonication, and by providing the required reagents in the form of a kit or a microtiter plate. Hence, the subject-matter of claims 12 and 18 involved an inventive step.

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

Main request

Article 83 EPC

The opposition division erred in finding that the claimed invention was sufficiently disclosed in the application as filed. Claim 1 recited the identification of carbapenemase-producing bacteria by a pH dependent color change of an indicator. According to the application as filed (see page 6, lines 4 to 8), the assay was "typically" performed in an unbuffered solution. However, claim 1 did not exclude the presence of a (relatively) high buffer concentration that would interfere with the pH dependent color change. The application did not teach at which buffer concentrations for which buffer substance the method would yield reliable results. This amounted to a fundamental lack of sufficiency of disclosure and not to a question of a "mind willing to understand" as seen by the opposition division in the decision under appeal.

Claim 16 encompassed a kit containing the carbapenemase inhibitor EDTA in the reaction mixture. As was evident from documents (23) and (21), EDTA interfered with Zn^{2+} as the carbapenemase activator. While the application mentioned concentrations for activators and inhibitors *per se*, it did not provide any teaching of how those interacted and how to select the activator and inhibitor concentrations in combination.

Article 54 EPC

The subject-matter of claims 1, 3, 7 and 8 lacked novelty over document (1). The opposition division's

view that document (1) did not directly and unambiguously disclose the feature "*wherein the color change is visually observed within a time period comprised between 5 minutes and 120 minutes*" was incorrect. The incubation time was not a technical feature because it did not change the claimed subject-matter and, therefore, could not impart novelty to known subject-matter (see decisions T 917/94 of 28 October 1999 and T 154/04, OJ EPO 2008, 46). Document (1) made direct reference to the "*rapid qualitative test of Escamilla (modified by V. Schaefer, personal communication)*" and provided the exact recipe of said test. As apparent from document (15), the Escamilla method provided results within 60 seconds, i.e. at the 5 minute time point specified in claim 1 the color change had already manifested and was visually observable. Hence, whatever the modification by V. Schaefer was, it did not change the character of the test as being a rapid test, i.e. a test clearly being within the claimed time period.

Article 56 EPC

Claim 1

Document (1) as closest state of the art

The subject-matter of claim 1 lacked an inventive step over document (1) alone or in combination with document (15). At the relevant date it had been established in the art that an acidimetric assay could be used to identify the hydrolysis of the β-lactam ring of a substrate, i.e. the mechanism on which the patent relied.

Document (1) described that resistance to the carbapenem imipenem is mediated through hydrolysis of its β-lactam (see page 136, right-hand column, last full paragraph). Document (15) confirmed that the hydrolysis was enzymatic (see page 196, left-hand column, 2nd paragraph). Hence, the skilled person took from document (1) that the resistance was by way of a carbapenem-hydrolysing enzyme. For the skilled person the term "carbapenemase" as used in claim 1 was synonymous with the expression "carbapenem-hydrolysing enzyme" explicitly taught in document (15) (see page 440, left-hand column, 1st paragraph). The mechanism underlying the claimed invention was identical to the one reported in document (1).

The opposition division had confused "slow hydrolysis" with an assay having long incubation/observation times. Even if only relatively slow hydrolysis was observed in the experiment described in document (1), it could be observed within the claimed time range of up to 120 minutes. The Escamilla assay used in document (1) was a "rapid qualitative test". Even in the slower traditional microiodometric method, hydrolysis was observable within 30 and 60 minutes (see Table II in document (1)). It was important to note that since the claimed method encompassed relatively long incubation/ observation times of up to 120 minutes it could not be characterized as a fast assay.

Whatever the difference between the disclosure of document (1) and the claimed method might be, it was certainly not associated with a surprising effect. The Escamilla assay was advertised in document (1) as being a rapid and qualitative test. Moreover, it was known already from document (15) that this test was "simpler, more rapid and more economical to perform" than other

methods including the microiodometric method (see document (15), page 196, sentence bridging the left- and right-hand columns). Hence, when improving the microiodometric method, the skilled person would have immediately turned to the Escamilla assay. Since the Escamilla method was known to be applicable to β-lactamases in general, there would also have been a reasonable expectation of success.

Document (2) as closest state of the art

The subject-matter of claim 1 was not inventive over document (2) in view of document (1). The opposition division had based the adverse decision on their misconception of the teaching of document (1) as relating to a mechanism different from carbapenemases. However, the skilled person would have taken from document (1) that it in fact related to a carbapenemase-based resistance. Hence, it provided an obvious improvement over the method of document (2) which was based on bacterial growth.

Claims 12 and 18

Independent claims 12 and 18 were product claims and hence not restricted to a specific use. The term "kit" therein was to be interpreted to mean that the different compounds referred to - i.e. the lysis buffer and the reagent components (carbapenemase substrate and pH color indicator) - represented a combination of individual components which were kept physically separate but adjacent. It was established case law that an additional functional property, such as in the present case its use in a method for detecting carbapenemase-producing bacteria, could not be inferred from the term "kit" as such (see decision T 9/81,

OJ EPO 1983, 372, points 6 and 7). The kit claims could also not be interpreted in such a way that the components of the reagent kit could not be mixed together with the lysis buffer because the enzymatic reaction would only work with a suspension of the bacterial cells. The patent explicitly encompassed the embodiment of a direct lysis protocol (see paragraph [0026]).

Document (1) was suitable as the closest state of the art for the subject-matter of claim 12 because it disclosed a method in which the carbapenemase substrate imipenem and phenol red – the components of the reagent kit as claimed – were added to a sample which had been subjected to ultrasound. The sole difference between the disclosure of document (1) and claim 12 was that the latter required a lysis buffer for lysing the bacterial cells. There was no experimental evidence that the choice of a lysis buffer was superior compared to mechanical disruption techniques like sonication.

Since no effect had been plausibly demonstrated, the technical problem to be solved would have been the provision of alternative means to determine the hydrolysis of the β-lactam ring of the carbapenemase substrate imipenem in a bacterial lysate. The skilled person starting from document (1) and faced with that technical problem would have arrived in an obvious manner to lyse the bacterial cells with a lysis buffer, not by sonication.

Document (18) was directed to methods and compositions for the detection of β-lactamases. It disclosed that "[t]*echniques for lysing bacteria are known*" and "*include the mechanical disruption techniques, freeze/ thawing techniques, and lysis buffer techniques*"; see

page 48, 1st paragraph). Likewise, paragraph [0020] disclosed that kits may contain a "*composition comprising a lysis reagent*". Thus, bearing in mind the teaching of document (18), the skilled person would have equally considered sonication or a lysis buffer to lyse bacteria. Consequently, claim 12 could not be considered to involve an inventive step.

The sole difference between claims 12 and 18 was that the latter claim defined that the components of the reagent kit were present in a microtiter plate. Starting from document (1) as the closest prior art, the technical problem to be solved was the same as for claim 12. The solution provided in claim 18 that the compositions of the reagent kit could be dried and present in the wells of a microtiter plate was disclosed in paragraph [0024] of document (18); see also paragraph [0157]. Further, Example 5.5 of document (18) - dealing with the detection of β-lactamases in the presence of lysis reagents - used the Phoenix™ panel for their enzymatic testing (see paragraph [0212]). It had been within the skilled person's common general knowledge at the relevant date that the Phoenix™ panel was a microtiter plate with wells for testing antimicrobial drugs including carbapenems (see, e.g., document (4), page 4085, abstract, as well as page 4086, left-hand column, items (ii) to (iv)). Thus, also kit claim 18 was not inventive in the light of document (1) combined with document (18).

XII. Appellants I requested that the decision under appeal be set aside and the patent be maintained on the basis of any of the sets of claims underlying the decision under appeal (main request and auxiliary requests 1 and 2) or the set of claims according to the auxiliary

request 3 filed with the reply to appellant II's statement of grounds of appeal.

XIII. Appellant II requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Main request

Sufficiency of disclosure - Article 83 EPC

1. In the decision under appeal, the requirement of sufficient disclosure of Article 83 EPC was considered to be fulfilled in connection with the auxiliary request 2 (see section 11.1 starting on page 10 of the decision). The reasons given by the opposition division for this finding, which apply equally to the present main request, were contested by appellant II.

2. As the opposition division remarked in its decision, the method of the invention is based on the technical concept that, by hydrolysing the β-lactam ring of a carbapenemase substrate, carbapenemases generate a carboxyl group which acidifies the medium. The acidity resulting from this hydrolysis is then identified by a color change of a pH color indicator present in the reaction mixture. A change in color indicates the presence of a carbapenemase (see page 2, lines 34 to 37 of the application as filed).

3. The application as filed teaches that the medium in which the reaction takes place is typically an unbuffered medium (see page 6, lines 5 and 6). The wording of claim 1 does not exclude that a buffer is

added to the reaction mixture. However, it belongs to the common general knowledge of a person skilled in the art that a buffer solution, especially at a high concentration, neutralizes added acid (or base) and thus maintains the pH of the solution relatively stable. Since the application as filed teaches that acidification of the medium is essential for the pH color indicator to change color, and thus carbapenemase activity to be detected, the skilled person would not carry out the method of claim 1 using a buffer concentration that interferes with the pH dependent color change of the pH color indicator.

4. For a skilled person at the filing date, finding out which buffer solution at which concentration interferes and is thus unsuitable for carrying out the method of the invention, was a routine task which did not amount to an undue burden or required inventive skills. Hence, the objection of lack of sufficient disclosure concerning the method of claim 1 is not justified.

5. As regards claim 16 directed to a kit comprising a carbapenemase inhibitor, appellant II alleged that the interaction between carbapenemase activator and inhibitor, in particular EDTA, and their respective concentrations in the reaction mixture were not sufficiently disclosed in the application as filed.

6. The application as filed discloses the use of divalent cations or salts thereof as carbapenemase activator in an embodiment aimed at increasing the sensitivity of the claimed method (see page 9, lines 21 to 24 of the application as filed). The preferred carbapenemase activator is Zn^{2+} (see page 9, lines 29 and 30). Typical concentrations are disclosed on page 9, lines 32 to 34 of the application.

7. An embodiment which involves the use of carbapenemase inhibitors in order to specifically identify the class of carbapenemase (Ambler class A, B or D) present in the test sample is disclosed on page 10, lines 22 to 24 of the application as filed. EDTA is mentioned as an inhibitor of a particular group of carbapenemases, namely those of Ambler class B (see page 10, lines 26 to 31). Further, the application discloses that the use of EDTA as an Ambler class B carbapenemase inhibitor is preferably associated with a depletion of $ZnSO_4$, and that "... *the activity of EDTA is enhanced by a depletion of divalent cations*" (see paragraph bridging pages 10 and 11 of the application as filed).

8. Hence, the skilled person learns from the application as filed that, when EDTA is used as an Ambler class B carbapenemase inhibitor, divalent cations should either not be added at all or added at a reduced concentration. Finding out which concentrations of activator would not interfere with the inhibitory effect of EDTA on Ambler class B carbapenemases was a routine task for the skilled person, and the necessary experimentation not unduly burdensome.

9. It follows from the above that, contrary to appellant II's view, the requirements of Article 83 EPC are met.

Novelty - Article 54 EPC

10. In the decision under appeal, the opposition division found that neither document (1) nor document (6) anticipates the subject-matter of claim 1. In appeal proceedings, only the findings concerning document (1) were contested by appellant II.

11. In particular, appellant II disagreed with the opposition division's view that document (1) does not clearly and unambiguously disclose the feature "*the color change is visually observed within a time period comprised between 5 minutes and 120 minutes*" of the method of claim 1. Appellant II did not dispute that the relevant passage of document (1) which describes a method called "*the test of Escamilla*" (see page 136, right-hand column, third full paragraph), does not explicitly disclose an incubation time, i.e. a time period until a color change can be observed. However, it alleged that the incubation time is not a technical feature.

12. The board disagrees with appellant II's view. In the test method of the present invention, which is based on the enzymatic hydrolysis of a substrate, the incubation time interacts with other technical features specified in the claim (e.g. the carbapenemase activity, the substrate and the pH color indicator) to achieve a technical effect, i.e. a color change which can be visually observed. Hence, the incubation time contributes to the technical effect underlying the method, and must therefore be regarded as a technical feature of the method which cannot be ignored when assessing novelty and inventive step.

13. Contrary to the view taken by appellant II, an incubation time as specified in claim 1 ("*between 5 minutes and 120 minutes*") is not implicit in the reference to "the test of Escamilla" on page 136, right-hand column, third full paragraph of document (1). As the opposition division held in its decision, document (1) does not provide any reference to scientific literature describing the test of

Escamilla, in particular not the required incubation time. Document (15), which describes the Escamilla test for ampicillin-resistant bacteria, was published in a specialist journal in 1987, i.e. 25 years before the filing date of the present patent. Since a person skilled in the art could only have found this document through a comprehensive search, its content does not represent the common general knowledge of the skilled person at the relevant date and, thus, cannot be taken into account in deciding on novelty over document (1). The same applies to the content of documents (16) and (17) which were cited by appellant II in this context.

14. Appellant II also pointed to Table II in document (1) which allegedly described an incubation time of 30 or 60 minutes falling under the time interval given in claim 1. However, as the opposition division found, the incubation times specified in Table II of document (1) relate to the microiodometric assay described on page 136, right-hand column, second full paragraph, rather than to the test of Escamilla. The relevant passages of this paragraph read:

> "*Hydrolysis of imipenem, ceftazidime and aztreonam by the cryde*[sic] *lysate of two of the resistant strains was measured by a microiodometric method* [...] *in non-induced as well as induced cultures (Table II).*
> [...]
> *The percentage of hydrolysis by two strains after various time intervals (corrected for control observations) is given in Table II.*"

15. Hence, the reasons given by the opposition division for its finding of novelty over document (1) are correct.

16. Moreover, the board holds that document (1) does not make available to a person skilled in the art a method for detecting the presence of carbapenemase-producing bacteria.

17. The experiments described in document (1) are aimed at investigating the mechanism underlying the imipenem- and/or ceftazidime-resistance of several bacterial strains. Carbapenemases are not specifically mentioned in document (1), and a person skilled in the art cannot unmistakably derive from this document whether the slow hydrolysis of imipenem described therein results from the presence of carbapenemases. As a matter of fact, the presence of ß-lactamases other than carbapenemases in the strains investigated in document (1) is plausible in view of the data provided in the second ("PEN-G" for benzylpenicillin) and fourth ("IMI" for imipenem) columns of Table II: lysates of both strains hydrolysed the ß-lactam ring of benzylpenicillin rapidly (100% after 30 min incubation), while hydrolysis of imipenem was very slow (24%/28% after a 8 hours incubation). Hence, contrary to the view taken by appellant II, detecting the presence of carbapenemase-producing bacteria is not the inevitable result of what is disclosed in document (1).

18. For these reasons, novelty is acknowledged.

Inventive step - Article 56 EPC

Claim 1

Document (1) as closest state of the art

19. In the decision under appeal, the opposition division regarded document (1) as the closest state of the art because this document described a method which served the same purpose as the method of claim 1. This finding was based on an interpretation of the term "carbapenemase" in claim 1 as including β-lactamase enzymes with weak carbapenemase activity. As basis for this interpretation, the opposition division referred to documents (19) and (28) (see page 4, second paragraph of section 9.2.1.1 of the decision under appeal). The board is unable to find in either document a basis for this interpretation.

20. Document (19) was filed as evidence for the common general knowledge of a person skilled in the art at the relevant date. In the passage on page 440, left-hand column, first paragraph, to which the opposition division referred, it is stated that many carbapenemases "*... recognize almost all hydrolysable β-lactams, and most are resilient against inhibition by all commercially viable β-lactamase inhibitors* [...] *Some investigators have preferred the nomenclature "carbapenem-hydrolyzing enzymes" to the term "carbapenemases," suggesting that carbapenems are but one segment of their substrate spectrum*". In the board's view, these statements do not support the opposition division's interpretation: the fact that many carbapenemases have a broad substrate spectrum does not mean that also enzymes which weakly hydrolyse carbapenems can be regarded as carbapenemases. As a

matter of fact, document (19) defines carbapenemases as "*β-lactamases with catalytic efficiencies for carbapenem hydrolysis*" (see page 441, left-hand column, lines 6 and 7). Contrary to the opposition division's view, it cannot be derived from Table 2 of document (19) that any of the listed class A carbapenemases has weak carbapenemase activity.

21. Page 951 of document (28), to which the opposition division also referred, does not provide a definition of the term "carbapenemase" in line with the opposition division's interpretation. Document (28) indicates that carbapenemases of Enterobacteria belong to classes A to D of the Ambler classification of β-lactamases (see left-hand column, lines 17 to 19 under the heading "Glossaire"), and discusses the two mechanisms responsible for carbapenem resistance, namely: (1) expression of carbapenemases, or (2) lower permeability of the bacterial membrane to carbapenems associated with overexpression of other β-lactamases, in particular cephalosporinases having weak carbapenemase activity (see left-hand column, lines 5 to 11). Hence, it is clear from these statements that the term "carbapenemase" as understood in the art at the relevant date does not include other β-lactamase enzymes with weak carbapenemase activity.

22. Document (1) describes experiments aimed at characterizing strains of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* resistant to the antibiotics imipenem and ceftazidime, or both. In particular, the authors investigated whether the imipenem and ceftazidime resistance could be transferred to other bacteria, and whether the resistant bacteria were able to hydrolyse the antibiotics. The resistance was found non-transferable (see page 137, right-hand column, last

paragraph), and only slow hydrolysis of imipenem, but no hydrolysis of ceftazidime was observed even when the crude bacterial cell lysate was incubated for up to 8 hours. The authors concluded:

> "*Whether this slow hydrolysis (Table II) can protect P. aeruginosa or K. pneumoniae cells from the rapid bactericidal effect of imipenem* [...] *remains to be shown.* ***Also*** *the resistance to ceftazidime and aztreonam* [...] *must be by a non-hydrolytic mechanism,...*" (see page 137, left-hand column, first full paragraph; emphasis added by the board)

23. Document (1) does not disclose - or even suggest - that the slow hydrolysis observed for imipenem may result from the hydrolytic activity of carbapenemase(s) produced by the resistant bacterial strains. Hence, as the opposition division concluded in its decision document (1) does not teach a person skilled in the art how to detect the presence of carbapenemase-producing bacteria in a sample, as required by claim 1.

24. At the relevant date for the assessment of inventive step, bacterial strains resistant to imipenem and other carbepenems had become a serious problem, and it belonged to the common general knowledge of a person skilled in the art that carbapenem resistance is associated with the production of carbapenemases by the resistant bacterial strains (see document (19)). However, while in document (19) various methods for detecting the presence of carbapenemase-producing bacteria (see chapter under the heading "Detection of carbapenemases" starting on page 450), including microbiological, biochemical and molecular methods are discussed, neither acidimetric methods used for other

β-lactamases in general, nor in particular the method described in document (1) (the Escamilla method modified by V. Schaefer) are mentioned as biochemical tests suitable for detecting carbapenemases (see page 452, left-hand column).

25. The question arises why at the relevant date - some 25 years after the publication of document (1) - a skilled person seeking to provide a rapid and more simple test for detecting the presence of carbapenemase-producing bacteria in a sample would resort to document (1) which, as stated above, does not describe the detection of carbapenemases. In the board's view, an analysis of inventive step starting from document (1) as the closest state of the art is already tainted with hindsight.

26. If, for the sake of argument, document (1) is nevertheless regarded as the starting point for the assessment of inventive step, the next question to be answered is why the skilled person would choose the Escamilla method among the two methods described in this document to detect hydrolysis, both methods providing unclear results as to the relevance of the observed slow hydrolysis of imipenem for the resistance to this antibiotic.

27. In appellant II's view, in the light of document (15) describing the method as a rapid (one-minute) β-lactamase test, the skilled person would prefer the Escamilla test because the test of Escamilla was known to be applicable to ß-lactamases in general. However, the evidence on which appellant II relied does not support this allegation. Document (15) relates solely to penicillinases, and the further documents on file describing an acidimetric test relate to ß-lactamases

that hydrolyse ß-lactam antibiotics of first- and second generation, in particular penicillinases and cephalosporinases (see documents (12), (13) and (24)). Document (11), which was also cited by appellant II, discusses the impact of carbapenemases on antimicrobial therapy, but does not deal with biochemical methods for detecting the presence of carbapenemase-producing bacteria.

28. Like the opposition division, the board is not persuaded that the skilled person at the relevant date, in view of the teachings in documents cited by appellant II, would seriously contemplate applying the modified method of Escamilla described in document (1) as a rapid method for detecting the presence of carbapenemase-producing bacteria in a sample.

29. Thus, appellant II's objection of lack of inventive step based on document (1) as the closest state of the art fails.

Document (2) as closest state of the art

30. Appellant II contested also the opposition division's finding that the subject-matter of claim 1 is not obvious in view of document (2) as the closest state of the art in combination with the teachings of document (1).

31. Document (2) relates generally to a method for detecting the presence of carbapenem-resistant bacteria in a sample, preferably bacteria containing a gene coding for a β-lactamase which hydrolyses carbapenems of the KPC family. In the method described in document (2), a solid culture medium (a Petri dish) comprising at least meropenem and/or ertapenem and at

least one chromogenic agent is inoculated with the sample. The chromogenic agent is a compound with a chromophore which is released after hydrolysis by a specific enzyme of a particular bacterial genus or species (see page 5, lines 19 to 21), and serves solely to distinguish between various bacterial strains growing on solid agar comprising a carbapenem, i.e. to assign carbapenem-resistant bacteria to a particular genus or species (see page 3, lines 14 to 22; page 4, lines 5 to 8, and the passage from page 5, line 19 to page 7, line 3). This is confirmed by the example on page 10 in which two different chromogenic agents are used to differentiate between carbapenem-resistant *E. coli* (red colonies) and carbapenem-resistant *Klebsiella* (metallic blue colonies) strains. Thus, the method "*... makes it possible, in a single culturing step, to specifically detect* ***and*** *differentiate carbapenem-resistant bacteria without the need for a preliminary isolation step or subsequent differentiation step*" (see page 10, lines 14 to 16). Visual analysis allows direct identification after incubation of the Petri dish for 18 to 24 hours.

32. The opposition division found that the method of claim 1 differs from that described in document (2) in that the cells in the sample are lysed prior to the reaction, and that the method is extremely rapid because it does not require colonies to grow on a medium. While this is correct, the most important difference lies in the mechanisms underlying the two methods: the method of the invention involves the use of a pH color indicator which changes color in response to the acidification of the medium when a carbapenem is hydrolysed by a carbapenemase, while in the method of document (2) the chromogenic agent, which does not change color, serves to distinguish between various

bacterial strains growing on a growth medium containing a carbapenem.

33. The board is unable to see how a person skilled in the art, starting from document (2) and seeking to improve or simply modify the method described therein, would arrive at the claimed method without applying inventive skills. The same applies if the teachings of documents (2) and (1) are combined because, as discussed above, document (1) does not teach to detect carbapenemase-producing bacteria. Hence, also appellant II's objection based on document (2) as the closest state of the art fails.

Claims 12 and 18

34. The adverse decision on inventive step for the kits of each claim 12 and claim 18 was contested by appellants I.

35. In the decision under appeal, the opposition division correctly stated that the kit claims 12 and 18 are product claims which are not restricted to a specific use. Also correct is the opposition division's finding that the kit of claim 12 differs from the teaching of the modified Escamilla test in document (1) in that: (i) the reagents employed in the test are components of a kit, and (ii) the kit includes a lysis buffer which replaces the sonication step.

36. However, the opposition division failed to apply correctly the problem-solution approach. First, the opposition division did not acknowledge that the established differences are in fact associated with a technical effect, namely the test can be carried out more efficiently and is amenable to automation. And

secondly, the technical problem to be solved starting from document (1), as formulated by the opposition division ("*the provision of a kit for detecting the presence of carbapenemase-producing bacteria in a sample*"), at least partially anticipates the solution provided by the invention.

37. Appellant II formulated the problem to be solved as the provision of improved means to perform the method described in document (1). It was not disputed that this problem is solved by the kits of each claim 12 and claim 18. Hence, the decisive questions are whether document (1) provides an incentive to introduce any changes in the method described therein and, if so, whether the specific changes were obvious to the skilled person.

38. Document (1) describes that a high hydrolysis rate for penicillin G, but only slow hydrolysis of imipenem was observed in imipenem-resistant bacterial strains when a modified Escamilla test was employed. As document (1), while describing the effect, does not provide a clear explanation why the purportedly rapid Escamilla test failed when imipenem was used as substrate, a skilled person may have a motivation to improve the method in order to obtain a higher hydrolysis rate for imipenem.

39. Modifying the reaction mixture by, e.g., adding more crude cell lysate and/or increasing or decreasing the concentration of the reagents (imipenem, phosphate buffer, phenol red and NaOH) would be obvious measures. However, contrary to the opposition division's view the skilled person would not envisage replacing the sonication step described in document (1) by a lysis buffer as required by claims 12 and 18, because it was common general knowledge that EDTA, a typical component

of a lysis buffer, may interfere in some cases with the hydrolysis of imipenem (see document (19), page 452, left-hand column, third full paragraph "*If the carbapenemase is a metalloenzyme, a brief incubation with EDTA prior to the initiation of the reaction will result in a lower* [imipenem] *hydrolysis rate*"). It should be noted that the method described in document (18), to which the opposition division referred as evidence that a lysis buffer is an obvious equivalent to sonication, is not based on imipenem hydrolysis by carbapenemases, but on nitrocefin hydrolysis by (unidentified) β-lactamases (see section 5.5 starting on page 79 of document (18)).

40. Moreover, since document (1) does not provide a reliably rapid test using imipenem as substrate, the skilled person had no motivation whatsoever to provide the test reagents used therein as components of a kit. Since in the present case the components of the kit provide a joint effect, decision T 9/81 (*supra*) cannot support appellant II's argument that the provision of the test reagents as a kit represents an obvious alternative.

41. This applies equally to the kit of claim 18 which differs from the kit of claim 12 in that the carbapenemase substrate and the pH color indicator are provided in a microtiter plate.

42. The board thus concludes that the kits of claim 12 and claim 18 are not obvious to a person skilled in the art in view of the teachings of document (1) either alone or in combination with document (18).

43. Although indicia are merely secondary considerations in the assessment of inventive step (see decision

T 1072/92 of 28 June 1994, point 3.5 of the reasons), in the present case the age of document (1), the successful commercial implementation of the claimed kit and the recognition of the inventor's merits by the scientific community, as shown in document (26), support the finding that the claimed subject-matter involves an inventive step.

Conclusion

44. The subject-matter of the claims of the main request and the invention to which they relate fulfil 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 the claims 1 to 21 of the main request filed on 26 July 2018 and a description to be adapted thereto.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated