Internal distribution code:

(A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 13 October 2021

Case Number: T 1688/16 - 3.3.08

Application Number: 10755006.3

Publication Number: 2606145

IPC: C12Q1/02, G01N33/569, G01N33/68

Language of the proceedings: EN

Title of invention:
Methods for determining antibiotic resistance in microorganisms

Applicant:
Erasmus University Medical Center Rotterdam

Headword:
MS-based determination of microbial resistance/ERASMUS UNIVERSITY MEDICAL CENTER

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 123(2)

Keyword:
New main request - requirements of the EPC met - (yes)

Decisions cited:

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1688/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 13 October 2021

Appellant: (Applicant)	Erasmus University Medical Center Rotterdam Dr. Molewaterplein 50 3015 GE Rotterdam (NL)
Representative:	Dr. Albrecht Bähring Kohler Schmid Möbus Patentanwälte Partnerschaftsgesellschaft mbB Gropiusplatz 10 70563 Stuttgart (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 10 February 2016 refusing European patent application No. 10755006.3 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. Montrone
	C. Almberg

Summary of Facts and Submissions

I. This appeal is against the decision of an examining division to refuse the European patent application No. 10 755 006.3, which was filed under the PCT and published as international patent application WO 2012/023845 ("patent application").

II. The examining division held in the decision under appeal that the subject-matter of claims 1, 15 and 16 of the main request and auxiliary request 1, and of claim 1 of auxiliary request 2 lacked an inventive step (Article 56 EPC), and that the subject-matter of claims 15 and 16 of the main request, and of claim 1 of auxiliary request 1 lacked clarity (Article 84 EPC).

III. With their statement of grounds of appeal, the applicant ("appellant") submitted a main request and five auxiliary requests.

IV. In a communication pursuant to Article 15(1) RPBA 2020, the appellant was informed of the board's provisional, non-binding opinion. The board *inter alia* raised new objections under Articles 54, 83 and 84 EPC and introduced three new documents into the proceedings.

V. In reply, the appellant submitted a main request and six auxiliary requests which were all new to the proceedings.

VI. Oral proceedings before the board were held on 13 October 2021. During the oral proceedings, the appellant submitted a new main request that replaced the previous main request.

VII. Claim 1 of the main request filed during oral proceedings reads as follows:

"1. A method for establishing whether a microorganism is potentially resistant to an antimicrobial compound, said method comprising the steps of:

(a) providing a reference mass spectrum of the antimicrobial compound or its enzymatic modification product, wherein said modification comprises enzymatic inactivation or enzymatic degradation of said antimicrobial compound;

(b) exposing a microorganism, a cell lysate thereof, or a growth medium supernatant thereof, to said antimicrobial compound in aqueous liquid to thereby provide an exposed sample;

(c) acquiring a mass spectrum of the exposed sample;

(d) comparing the mass spectrum acquired in step (c) with the reference mass spectrum of step (a), and

(e) determining from said comparison of mass spectra in step (d) whether modification of said antimicrobial compound has occurred following said exposure, and

(f) establishing from said determination in step (e) that said microorganism is potentially resistant to said antimicrobial compound when said modification is observed in step (e)."

Dependent claims 2 to 11 are directed to various embodiments of the method of claim 1.

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

D1: Yazawa K, *et al.*, Microbiology and Immunology, 1991, Vol. 35(1), 39-48;

D2: Mosher R H, *et al.*, Journal of Biological Chemistry, 1995, Vol. 270(45), 27000-27006;

D3: GB-A-2438066 (published 14 November 2007).

IX. The appellant's submissions, insofar as relevant to the present decision, may be summarised as follows:

Inventive step

Documents D1 and D2 assessed the resistance mechanisms towards kanamycin A ("kan A") or chloramphenicol ("Cm"), respectively, in different pathogenic bacterial strains. While mass spectrometry ("MS") was used to determine the enzyme-mediated structural modifications of these two antibiotics, microbial resistance itself was assessed by physiological growth tests only. Since documents D1 and D2 thus described the use of MS for structure analysis but not for establishing microbial resistance, MS was used in these documents for a different purpose compared to the claimed method.

The claimed method differed from those of documents D1 and D2 in that a MS-based structural comparison of degraded or inactivated antibiotics with unmodified antibiotics was used as marker for determining microbial resistance. This allowed a faster detection of resistant strains in samples, since less process steps were required.

Document D3 disclosed the use of MS for the same purpose as in claim 1, i.e. the determination of microbial resistance. This was achieved by comparing as biomarkers mass spectra of microbial proteins obtained from bacteria exposed and non-exposed to antibiotics.

The claimed method differed from the method in document D3 in using instead as markers the mass spectra of enzymatically inactivated/degraded antibiotics following a microbial exposure which indicated a potential microbial resistance when compared with reference spectra of unmodified antibiotics. This allowed a faster determination of resistant strains, because antibiotics were enzymatically modified by resistant strains within minutes. An antibiotic-mediated change of a microbial protein profile as detected in document D3 required more time due to the need for growing cells.

The technical problem underlying the claimed method compared to the methods of documents D1 to D3 was thus the provision of an improved method for establishing a potential microbial resistance.

The method of claim 1 as solution to this problem was inventive. Documents D1 to D3 provided no hints or suggestions that microbial resistance could be determined by comparing MS-spectra of unmodified antibiotics and enzymatically inactivated/degraded antibiotics following a microbial exposure.

X. The appellant requests that the decision under appeal be set aside and that a patent be granted on the basis of the main request filed on 13 October 2021 during the oral proceedings.

Reasons for the Decision

Main request

Added subject-matter

1. A method as defined in claim 1 is directly and unambiguously derivable from claims 1 and 2 as filed, including the passages disclosed on page 3, line 18 to page 4, line 8 of the patent application.

1.1 The subject-matter of dependent claims 2 to 4 has a basis in claims 6, 7 and 10 as filed, respectively.

1.2 The subject-matter of dependent claim 5 has a basis in claim 11 as filed in conjunction with the passage on page 20, lines 3 to 6 of the patent application.

1.3 The subject-matter of dependent claims 6 and 7 has a basis in claims 12 and 13 as filed, respectively.

1.4 The subject-matter of dependent claim 8 is derivable from page 16, lines 16 to 19 of the patent application.

1.5 The subject-matter of dependent claim 9 is disclosed in claim 7 as filed.

1.6 The subject-matter of dependent claims 10 and 11, respectively, is derivable from page 20, lines 23 to 26, and lines 18 to 22 of the patent application in conjunction with the disclosure on page 8, lines 3 and 4 of the patent application.

2. Consequently, the claimed subject-matter complies with the requirements of Article 123(2) EPC.

Clarity and support

3. In the decision under appeal, the examining division was of the view that in the main request before them, independent claims 15 and 16 contained terms with unclear functional definitions (see point 4.1 of the decision under appeal). These claims have been deleted from the main request.

4. The board raised in its communication corresponding objections of lack of clarity against the subject-matter of claim 1. Amended claim 1 of this main request remedies these deficiencies.

5. Hence, the main request complies with Article 84 EPC.

Sufficiency of disclosure

6. The amendment in step (a) of claim 1 concerning the provision of reference mass spectra of enzymatically inactivated or degraded antimicrobial compounds overcomes the deficiency under Article 83 EPC set out in the board's communication.

7. The patent application describes in general the use of mass spectrometry ("MS") for obtaining mass spectra of native (reference) and inactivated or degraded antibiotics exposed to microorganisms. The patent application provides no experimental data demonstrating the suitability of the claimed method for establishing a potential microbial resistance based on a comparison of these spectra. Since, however, MS was an established analytic method at the priority date of the patent application, the board has no serious doubts substantiated by verifiable facts that the claimed

method is suitable for the claimed purpose, and can be carried out by a skilled person in the art.

8. The main request, hence, complies with Article 83 EPC.

Novelty

9. None of the available prior art documents discloses a method as defined in claim 1.

10. Thus the main request complies with Article 54 EPC.

Inventive step

11. In the decision under appeal (see points 3.1 to 3.7, 4.2 and 4.3), the examining division held that in view of the teaching of documents D1, D2 or D3, the method of claim 1 and the kit or system according to claims 15 and 16, respectively, in the main request before them did not involve an inventive step within the meaning of Article 56 EPC. Claims 15 and 16 have been deleted from the main request.

12. The appellant accepted that either document D1 or D2 represented the closest prior art for the method of claim 1.

13. Document D1 discloses studies in pathogenic *Nocardia* strains on resistance mechanisms, which includes the structural determination of an inactivated substance from kanamycin A ("kan A") (see abstract, page 40, second paragraph). According to an earlier study, kan A resistance was primarily caused by inactivating enzymes, such as amino glycoside phosphotransferases (see page 39, third paragraph).

13.1 Document D1 further reports that MS and nuclear magnetic resonance ("NMR") are used to structurally determine the enzymatic inactivation sites on kan A (see page 41, fifth and sixth paragraphs, Figure 3 and page 44, second paragraph).

13.2 The production of inactivated kan A (kan A-3'-phosphate) in *Nocardia* strains is then monitored by thin layer chromatography (see Figure 4). Producer strains are further tested for kan A resistance by determining the minimum inhibitory concentration of kan A in an agar dilution sensitivity test (see paragraph bridging pages 44 and 45, and Table 1).

13.3 The authors of document D1 conclude from this data that "*Resistance of Nocardia to kanamycin A thus appears to be aligned with the production of kanamycin A 3'-phosphate, and the high sensitivity of N. otitidiscaviarum to kanamycin A, therefore, seems attributable to the lack of an inactivation enzyme APH (3')*", and that "*Our data clearly indicate that inactivation enzymes played a major role in our drug sensitivity pattern test results. We have been applying this identification system (11) to clinical isolates and, to date, have found good correlation between the standard identification method and the present drug sensitivity pattern test used*" (see page 46, first and third paragraph).

13.4 In other words, the drug sensitivity pattern test disclosed in document D1 compares the antibiotic susceptibility of *Nocardia* strains as determined in a physiological growth assay with the production of inactivated kan A as determined by a chromatographic assay.

14. Document D2 discloses a study on the inactivation of chloramphenicol ("Cm") by 3'-O-phosphorylation in a *Streptomyces lividans* strain transformed with a gene encoding a Cm 3'-O-phosphotransferase (see abstract, page 27005, column 2, first paragraph). The aim of the study is to isolate and characterise the major product of Cm metabolism (3'-phosphoryl ester of Cm) using MS and NMR for structure determination (see abstract, page 27000, column 2, third paragraph, page 27002, column 2, first paragraph to page 27003, column 1, first paragraph, Figure 3, and page 27005, column 2, first paragraph). Modified 3'-phospho-Cm and unmodified Cm are tested for their antibiotic activity in a disk diffusion assay on a sensitive test bacterium. The data demonstrate that Cm is inactivated by an enzyme-mediated phosphorylation (see page 27003, column 1, second paragraph, page 27005, column 2, second paragraph).

15. The appellant contested the examining division's finding that the method of claim 1 differed from the disclosure in documents D1 and D2 solely by the use of a reference spectrum for the inactivated/degraded antibiotics. Furthermore, these documents used MS for a different purpose, i.e. the structural determination of inactivated antibiotics in strains known to be resistant. Microbial resistance itself was determined by physiological growth tests, and not by an MS-based structural comparison of antibiotics before and after their microbial exposure.

16. It is established case law that in a process claim directed to a purpose for carrying out the process, wherein the purpose is not directed at the production of a product but rather defines one of the steps in the claimed process, that purpose is a functional technical

feature limiting the claim (see Case Law of the Boards of Appeal of the EPO, 9th edition 2019, ("Case Law"), I.C.8.1.3.c)).

17. As set out above, documents D1 and D2 use *inter alia* MS to determine the structure of enzymatically inactivated kan A and Cm. The determination of whether or not the microorganisms are resistant against these antibiotics is carried out, however, by growth tests, an agar dilution sensitivity test, and a disk diffusion assay.

18. Therefore, and contrary to the examining division's finding (see point 3.5 of the decision under appeal), the board is not convinced that documents D1 and D2 disclose the use of MS as one of the techniques for determining microbial resistance. Hence, step (f) of claim 1 is not disclosed in documents D1 and D2 too.

19. Although claim 1, due to the use of the comprising language does not exclude the presence of further process steps, the claim defines the minimum number of steps to achieve the claimed purpose, i.e. the determination of a potential microbial resistance. The steps of claim 1 require *inter alia* that mass spectra are obtained from antibiotics exposed to microorganisms/microbial cell lysates/medium supernatants and compared to mass spectra of non-exposed antibiotics, i.e. reference spectra. Claim 1 requires too that based on this comparison of mass spectra, a potential microbial resistance is determined. Since MS is the sole technique for obtaining mass spectra, its use is necessarily implied in claim 1 for achieving this purpose.

20. In the board's opinion the question can be left unanswered in this case whether an advantageous

technical effect can be ascribed to the features distinguishing claim 1 from the methods disclosed in documents D1 and D2. This is so, because even if the technical problem is defined as the provision of a further/alternative method for determining microbial resistance, the solution provided by the method of claim 1 is based on an inventive step.

21. It is established case law that for assessing whether a claimed invention involves an inventive step, the issue to be decided is whether the skilled person seeking a solution to the objective technical problem starting from the closest prior art would have solved the problem by modifying the closest prior art to arrive at the claimed subject-matter. In this context a teaching has to be available either from the closest prior art or in combination with another document that would have prompted the skilled person to act in one way or another (see Case Law, I.D.5).

22. As set out above, while documents D1 and D2 use MS for characterising the structural modifications in kan A and Cm that caused their inactivation, these documents disclose growth tests only for determining microbial resistance. Although based on the teaching of documents D1 and D2, MS could have certainly been used for determining microbial resistance too, both documents are silent on hints or suggestions that would have prompted the skilled person to use MS for this purpose. In this situation, in the board's opinion, the skilled person would rather have looked for other, alternative growth assays to provide a further/alternative method for determining microbial resistance.

23. During the oral proceedings, the appellant was also heard on the disclosure of document D3, and its relevance in the assessment of inventive step.

23.1 Document D3 discloses the use of MS for determining microbial resistance. Hence, its disclosure is directed to the same purpose as the claimed method. Document D3 differs from the claimed method in that mass spectra of protein profiles are compared which are obtained from microorganisms that were exposed and non-exposed to antibiotics (see abstract).

23.2 Document D3, however, neither suggests nor points at using instead a comparison of mass spectra of antibiotics obtained from samples exposed and non-exposed to microorganisms. In the absence of hints that would have prompted the skilled person to use these mass spectra as an alternative marker for determining microbial resistance, the claimed method is not obvious in light of the teaching of document D3 too.

24. The method of claim 1, and hence the main request complies with Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent on the basis of claims 1 to 11 of the new main request filed during the oral proceedings, and a description to be adapted thereto.

The Registrar: The Chair:

L. Malécot-Grob B. Stolz

Decision electronically authenticated