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Datasheet for the decision of 20 March 2018

Case Number: T 2166/12 - 3.3.01

Application Number: 06775965.4

Publication Number: 1931998

IPC: G01N33/68

Language of the proceedings: EN

Title of invention:
A METHOD FOR QUANTIFICATION OF ALLERGENS

Patent Proprietor:
Alk-Abelló A/S

Opponent:
STALLERGENES

Headword:
Quantification of allergens/ALK-ABELLO

Relevant legal provisions:
EPC Art. 56

Keyword:
Inventive step - (no)

Decisions cited:

Catchword:

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Beschwerdekammern

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Chambres de recours

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Case Number: T 2166/12 - 3.3.01

D E C I S I O N
of Technical Board of Appeal 3.3.01
of 20 March 2018

Appellant: (Opponent)	STALLERGENES 6, rue Alexis de Tocqueville 92160 Antony (FR)
Representative:	Weiss, Wolfgang Weickmann & Weickmann Patent- und Rechtsanwälte PartmbB Richard-Strauss-Strasse 80 81679 München (DE)
Respondent: (Patent Proprietor)	Alk-Abelló A/S Bøge Allé 6-8 2970 Hørsholm (DK)
Representative:	Inspicos P/S Kogle Allé 2 2970 Hørsholm (DK)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 2 July 2012 rejecting the opposition filed against European patent No. 1931998 pursuant to Article 101(2) EPC**

Composition of the Board:

Chairman	A. Lindner
Members:	T. Sommerfeld
	L. Bühler

Summary of Facts and Submissions

I. European patent EP 1931998, based on application 06775965.4, entitled "A method for quantification of allergens" and published as international application WO 2007/031080, was granted with 18 claims.

Claim 1 as granted read as follows:

"1. A method for quantification of the absolute amount of allergen in an allergen sample where the allergen consists of more than one isoallergen(s) or homologous allergen(s) comprising the following steps:

a) providing a known amount of one or more allergen calibration standard peptide(s) having a sequence of amino acids which is identical with a sequence to be found within the allergen to be quantified by identifying a constant sequence of amino acids within the allergen to be quantified by comparing amino acid sequences of isoallergens or homologous allergens and preparing a synthetic allergen calibration standard peptide having this constant sequence and labelling said allergen calibration standard peptide(s) by introducing mass-modifying functionalities,

b) degrading the allergen sample to obtain a mixture of peptides, and optionally labelling said peptides with one or more labelling agent(s) by introducing mass-modifying functionalities, wherein if both the peptides in the degraded allergen sample and the allergen calibration standard peptide(s) are labelled, the labelling agent(s) used for labelling the allergen calibration standard peptide(s) are different from the labelling agent(s) used for labelling the peptides of the degraded allergen sample,

c) quantifying the absolute amount of allergen by correlating the amount of the allergen calibration

standard peptide(s) with the amount of the corresponding peptide(s) of the degraded allergen sample by mass spectrometry."

II. Opposition was filed against the granted patent, the opponent requesting revocation of the patent in its entirety on the grounds of lack of inventive step (Articles 56 EPC and 100(a) EPC), lack of sufficiency of disclosure (Article 100(b) EPC) and added subject-matter (Article 100(c) EPC).

III. The documents cited during the proceedings before the opposition division and the board of appeal include the following:

D1 WO 03/102220
D2 WO 03/016861
D3 WO 2004/031730
D13 van Ree R. 1997, Allergy 52: 795-805
D19 Swoboda I. et al. 1995, J. Biol. Chem. 270(6): 2607-2613
D20 Helsper J. et al. 2002, J. Allergy Clin. Immunol. 110: 131-138
D21 IUIS Allergen Nomenclature database, submission form

IV. In its decision announced at oral proceedings, the opposition division rejected the opposition under Article 101(2) EPC.

V. The opponent (appellant) filed notice of appeal against that decision. With its statement of grounds of appeal the appellant requested that the opposition division's decision be set aside and that the patent be revoked in its entirety. It also submitted new documents D19 and D20.

VI. The patent proprietor (respondent) replied with a letter dated 27 March 2013, requesting that the appeal be dismissed and that the patent be maintained as granted (main request) or alternatively according to auxiliary requests 1 to 7. New document D21 was submitted. The auxiliary requests were discussed in the letter of reply but were submitted later, with a letter dated 8 May 2013.

Claim 1 of auxiliary request 1 differs from claim 1 of the main request by inserting the following feature into step a): "... by introducing mass-modifying modalities **using isobaric or isomeric labelling reagents or using incorporated stable isotopes**".

Claim 1 of auxiliary request 2 differs from claim 1 of the main request by inserting the following features:
"a) ... one or more allergen calibration standard peptide(s) **consisting of** a sequence of amino acids, **which has 6-15 amino acids** (...),
b) **partly or fully** degrading (...) **by digestion with one or more proteolytic enzymes** (...),
c) (...)
wherein the allergen calibration standard peptide(s) has/have a sequence of amino acids, which is identical with a sequence of amino acids in a peptide obtained by degradation according to step b)".

Claim 1 of auxiliary request 3 differs from claim 1 of the main request by incorporating the amendments to both auxiliary requests 1 and 2.

Claim 1 of auxiliary request 4 differs from claim 1 of the main request by further characterising the allergen sample as follows: "... allergen sample **selected from**

the group consisting of an allergen extract, an allergen vaccine, and a food, ...".

Claim 1 of auxiliary requests 5, 6 and 7 differs from claim 1 of auxiliary request 4 by incorporating the amendments to auxiliary requests 1, 2 and 3, respectively.

VII. Observations by a third party pursuant to Article 115 EPC were filed on 24 October 2013.

VIII. Summons to oral proceedings before the board were issued. In a letter dated 1 March 2018 the respondent announced that it would not be represented at the oral proceedings.

IX. Oral proceedings before the board took place in the absence of the respondent. At the end of the oral proceedings, the chairman announced the board's decision.

X. The appellant's submissions where relevant for the present decision may be summarised as follows:

Document D13, directed to standardisation of allergen extracts, discussed the disadvantages of IgE-based techniques and proposed the use of monoclonal antibodies (mAbs) as an alternative. Page 798, right column, bottom, taught the use of mAbs specifically to detect isoforms, and referred to the limitations associated therewith. The difference compared to the patent was that the claim made use of methods based on mass spectrometry. Since there was no improvement in relation to the mAb-based methods, the technical problem could be formulated as how to provide an alternative standard method to quantify allergens. Just

as quantification of isoforms by mAb-based methods hinged on the specificity of the mAbs used (D13, page 799, left column, last paragraph), detection of isoallergens by the claimed method depended on the choice of the standard peptide. Hence D13 related to the same concept of using one common region to detect as many isoforms as possible. The claimed solution was obvious over the combination of D13 with any of D1, D2 and D3. After D13's publication in 1997, there had been rapid progress in mass spectrometry, in particular in the field of protein quantification. D1 to D3 taught internal standardisation with isotope labelling and also discussed the disadvantages of mAb-based methods for quantification: D1, page 2, line 35, to page 3, line 6; D2, page 1, paragraph 4; D3, page 1, line 12. The skilled person would thus be prompted to use the technologies disclosed in D1 to D3, which were exactly the claimed method, and would need no inventive skill to implement them. The method was known to be applicable to any samples and any proteins and to provide absolute quantification: D1, page 8, line 5, to page 9, line 12; claim 8; page 6, lines 29 to 31. Use of cell extracts as in the patent was disclosed in D1 on page 10, line 8, and the quantification of a group of proteins (splice isoforms) by using standard peptides comprising a common amino acid sequence was disclosed in claim 14, step b), on page 31, line 19, to page 32, line 5, and on page 35, lines 24 to 27; the use of further peptide standards was not excluded from the claim. As to the alleged functional difference between splice isoforms and isoallergens, this was irrelevant for the claimed method, which relied on structural similarity, as was apparent from paragraph [0040] of the patent.

Regarding auxiliary request 1, the additional feature was also disclosed in D1: page 8, line 28; page 15, line 31, to page 16, line 6. As to auxiliary request 2, degradation by proteolytic enzymes was standard in mass-spectrometry methods, as was apparent from D1 to D3, and the peptide length was also disclosed e.g. in D2 (page 14, paragraph 2 and last paragraph) and in D3 (page 36, last sentence under Example). D2 and D3 were also related to absolute quantification of protein groups: D2, page 14, last paragraph; D3, page 15, items 6 and 1 (enzymatic cleavage). Regarding auxiliary request 3, the same arguments as for auxiliary requests 1 and 2 also applied. Isobaric, isometric reagents were disclosed in D2 on page 15, paragraphs 2 and 3, and page 16, paragraph 2, and in D3, in claim 1, step 2, and claim 2. Auxiliary request 4 further defined the allergen sample as being e.g. allergen extracts, which was exactly what was used in D13 (title). As to auxiliary requests 5, 6 and 7, the same arguments as for auxiliary requests 1 to 4 also applied.

XI. The respondent's written arguments where relevant for the present decision may be summarised as follows:

It would not be obvious to use D1's technology for quantifying allergens, as was apparent from the fact that, even after mass spectrometry (MS) being known for a number of years, the standard method for quantifying allergens was still based on antibody recognition of selected allergens. It was only more than three years after D1 that quantitative MS (qMS) had been suggested as a means of allergen quantification - by the patent. The skilled person in the field of allergy vaccine preparation and in the food industry would not have taken D1 to D3, disclosing proteomics technologies, as a starting point for allergen quantification but would

rather have turned to prior art dealing with similar problems: D13. The difference compared to the patent was that D13 focused on the use of monoclonal antibodies as an improvement to methods such as ELISA for allergen quantification, while the application provided the solution of using qMS. The technical problem solved by these technical differences was how to provide a quantification method for allergens which allowed reliable and true quantification of all relevant allergens in a sample. D13 suggested the use of broadly reacting antibodies and provided no hints to use other technologies. Combination with the technologies taught in D1 to D3 would not be obvious and would also not lead to the claimed invention, because D1 to D3 taught that all unique proteins should be determined. While in fact MS had already been used to identify allergen isoforms (D19, D20), the skilled person in the proteomics field would have no interest in the quantification of proteins which were known to be allergens: no incentive was derivable from any of the cited references, D1 merely aiming at quantifying "any protein". Moreover, D1 required the use of unique calibration standard peptides (page 35, lines 27 to 29), while the patent used common calibration standard peptides.

XII. The appellant requested that the decision under appeal be set aside and that the patent be revoked. It further requested that the third-party observations filed on 24 October 2013 be admitted into the proceedings.

The respondent requested (in writing) that the appeal be dismissed and that the patent be maintained as granted (main request) or, alternatively, that the patent be maintained on the basis of auxiliary requests 1 to 7, filed with the letter dated 8 May 2013. It

further requested that the third-party observations filed on 24 October 2013 not be admitted into the proceedings.

Reasons for the Decision

1. The appeal is admissible.

2. The oral proceedings before the board took place in the absence of the patent proprietor (respondent), party as of right to the present proceedings (Article 107 EPC), who was duly summoned but decided not to attend.

According to Rule 115(2) EPC, if a party duly summoned to oral proceedings does not appear as summoned, the proceedings may continue without that party. As stipulated by Article 15(3) RPBA, the board is not obliged to delay any step in the proceedings, including its decision, by reason only of the absence at the oral proceedings of any party duly summoned who may then be treated as relying on its written case.

3. Main request - inventive step

3.1 The present patent aims at providing a sensitive method for absolute quantification of allergens.

3.2 In paragraph [0004] it states that while "[c]onventional allergen specific immunotherapy and diagnosis are currently performed by use of standardized natural allergen extracts which are further formulated to allergen vaccines[,] (...) the composition of these natural source materials (...) are [sic] known to vary considerably depending on time and

place of collection of the allergenic source materials". However, as acknowledged in paragraph [0005], "[k]nowledge of the composition of the extracts and the content of essential allergens is a prerequisite for reproducibility, safety and efficacy of the final product. A major challenge in the manufacture of allergen vaccines is standardisation, i.e. securing a constant potency from batch to batch. (...) Ideally, therefore, all components need to be controlled both qualitatively and quantitatively, but with the current technology this is not practically possible". Paragraph [0006] then teaches that: "Standardisation is currently performed in many different ways (...) by techniques such as SDS-PAGE, isoelectric focusing in addition to a variety of immunoelectrophoretic (QIE) and ELISA techniques using mono- and/or polyclonal antibodies and radio allergosorbent (RAST) or related techniques", and it goes on to remark that: "All quantitative aspects of these currently used techniques are dependent on antibodies as reagents and as such vulnerable to change over time". Paragraph [0007] states that: "Absolute quantification of specific vaccine components in complex mixtures of allergen is not straightforward and has yet not been established as a sensitive, routine high-through-put technique".

3.3 Document D13, which is directed to the same purpose as the patent, namely absolute allergen quantification (in the context of standardisation of allergen extracts), is the closest prior art. The difference compared to the claimed subject-matter is that a different method for allergen quantification is used, namely a method involving antibodies against the allergens, while the claimed method uses a mass spectrometry-based method. The shortcomings mentioned in the patent in relation to

antibody-dependent quantification methods (end of paragraph [0006]) are also acknowledged in D13 in relation to IgE-based techniques; D13 however suggests the use of mAbs as "well-defined reagents of constant quality with an unlimited availability" and teaches that they should be "selected with care" and "be neither too specific nor too cross-reactive" (page 800, left column, "Concluding remarks" section). D13 specifically elaborates on the problems linked to mAbs that are too specific and therefore fail to detect all allergen isoforms (page 798, bottom of right column) and teaches to "select mAbs that recognize the whole spectrum of isoforms" or "alternatively, a mixture of two or more different mAbs (...) to ensure complete coverage of isoforms" (page 799, left column, third paragraph). The technical problem can thus be formulated as how to provide an alternative for absolute quantification of multiple allergens, including allergen isoforms. The solution is the method as claimed, and the board is satisfied that the solution plausibly solves the problem.

3.4 The next has to be examined whether the skilled person would arrive at the claimed solution in an obvious way.

3.5 As acknowledged in the patent (paragraphs [0009] to [0014]), mass spectrometry (MS) methods were well known for use "for quantification of a variety of biomolecules from complex mixtures such as plasma, cell and tissue samples" (paragraph [0009]). Documents D1 to D3 constitute examples of disclosures of the prior art wherein mass spectrometry methods are used for quantification of proteins in samples, making use of labeled calibration standard peptides (title, abstract). The skilled person would hence be prompted by any of documents D1 to D3 to attempt mass

spectrometry as a method for quantification of allergens (protein allergens) as well. In this context, it is noted that step b) of protein degradation to obtain a mixture of peptides is part of standard mass spectrometry, as is apparent from e.g. D1, page 3, lines 8 to 14. Moreover, D1 also teaches a method for quantifying splice isoforms of polypeptides in a sample by "adding a plurality of peptide standards to said polypeptide sample, wherein said peptide standards are labeled with an isotopically distinct version of said isotope tag and wherein said plurality of peptide standards comprises at least one peptide corresponding to a common amino acid sequence of a splice isoform of a polypeptide and at least one peptide corresponding to an amino acid sequence that differs between two splice isoforms of said polypeptide" (claim 14, step b). The skilled person would thus be taught by D1 to use known amounts of calibration standard peptides "having a sequence of amino acids which is identical with a sequence of amino acids to be found within the allergen to be quantified" as in the claim. The isotope tag of D1 is a "mass-modifying functionality", and in fact the examples of the patent also use isotopes to label the internal calibration peptides: e.g. Example 7, paragraph [0131].

3.6 Accordingly, the skilled person, seeking to develop alternative methods of allergen quantification, would follow the teachings of D1 as regards protein quantification, and in particular quantification of splice isoforms, and would arrive at the subject-matter of claim 1 without the need for inventive skill.

3.7 The respondent essentially argued that D1 was not related to quantification of allergens but rather of proteins in general, that the splice isoforms mentioned

in D1 were not equivalent to the "isoallergens" of the claim, as evidenced by D21, and that D1 required the use of a plurality of unique calibration peptides, while the patent used a standard calibration peptide capable of detecting all isoallergens.

3.8 The board notes that while not all allergens are proteins, the patent itself acknowledges that "[i]t appears (...) that any protein is a potential allergen" (paragraph [0002]), and hence methods of quantification of proteins are potentially methods of quantification of allergens. In fact, the claimed method is clearly directed at quantifying allergens which are proteins, since it requires the use of calibration peptides having a common amino acid sequence with a sequence to be found in the allergen to be quantified. Moreover, methods of allergen detection making use of mass spectrometry were known in the art (paragraphs [0015] to [0017]). The documents cited therein include documents D19 and D20, filed by the appellant with the grounds of appeal. While, as stated in the patent (paragraph [0018]), said methods did not involve "the use of calibration standard peptides having a sequence of amino acids which is identical to a constant sequence of amino acids found in the group of allergens to be (..) quantified", they nevertheless allowed a relative quantification of the allergen isoforms and homologous allergens present in a sample (D19 and D20: title and abstract), albeit not absolute quantification. Mass spectrometry was also used to detect allergens in the food industry, as acknowledged in the patent in paragraph [0008], which discusses the need for "reliable detection and quantification of food allergens" and teaches that "[p]hysicochemical methods e.g. mass spectrometry, as well as immunological methods have been described".

3.9 As to the fact that "splice isoforms" are not equivalent to "isoallergens", it is noted that they nevertheless have in common that they both share portions of common amino acid sequences (D1: page 31, lines 33 to 36; patent, paragraphs [0003] and [0040]; D21, page 2, section marked by the respondent). Irrespective of any further structural or functional distinction between splice isoforms and isoallergens, it is the existence of common amino acid sequences that provides the basis for the construction of calibration peptides both according to the method as claimed and according to D1's method (e.g. page 31, line 29, to page 32, line 5). The skilled person would thus have no reason to doubt that D1's method for quantification of splice isoforms could be extrapolated for quantification of isoallergens.

3.10 Finally, D1 does indeed provide for the use not only of unique peptide standards, specific to each splice isoform, but also of peptide standards selected to assess a common portion of the splice isoforms, i.e. peptide standards according to those claimed. The use of further calibration peptides (such as the ones directed to distinct regions of the isoforms) is not excluded from the claim.

3.11 Claim 1 of the main request thus lacks inventive step. The main request is not allowable for lack of compliance with Article 56 EPC.

4. Auxiliary request 1 - inventive step

4.1 Claim 1 of this request differs from claim 1 of the main request by characterising the mass-modifying functionalities of step a) as using e.g. "incorporated

stable isotopes". This feature is also disclosed in D1 (page 8, line 28; page 15, line 31, to page 16, line 6) and hence cannot contribute to inventive step.

4.2 Auxiliary request 1 is thus also not allowable for lack of compliance with Article 56 EPC.

5. Auxiliary request 2 - inventive step

5.1 Claim 1 of this request essentially differs from claim 1 of the main request by limiting the calibration standard peptides' length to 6 to 15 amino acids and by further characterising the allergen degradation step b) as being partial or full degradation by digestion with one or more proteolytic enzymes. Sample degradation by proteolytic enzymes is standard in mass-spectrometry methods, and is disclosed in D1 (e.g. page 3, lines 8 to 14), D2 (page 20, third paragraph) and D3 (page 15, item 1). As to peptide length, this is also disclosed in D2 (page 14, second paragraph) and in D3 (page 36, last sentence under Example). Hence, these features do not contribute to inventive step either.

5.2 Auxiliary request 2 is thus also not allowable for lack of compliance with Article 56 EPC.

6. Auxiliary request 3 - inventive step

6.1 Claim 1 of auxiliary request 3 incorporates the amendments of both auxiliary requests 1 and 2. Hence for the same reasons as given above with regard to auxiliary requests 1 and 2, this claim also lacks inventive step.

6.2 Auxiliary request 3 is thus not allowable for lack of compliance with Article 56 EPC.

7. Auxiliary request 4 - inventive step

7.1 Claim 1 of this request differs from claim 1 of the main request by further restricting the allergen sample to samples selected from a group which includes, inter alia, allergen extracts. Samples consisting of allergen extracts are explicitly disclosed in document D13, which is in fact specifically directed to allergen standardisation in allergen extracts, as is apparent from its title. Hence this feature cannot contribute to inventive step either.

7.2 Auxiliary request 4 is also not allowable for lack of compliance with Article 56 EPC.

8. Auxiliary requests 5, 6 and 7 - inventive step

8.1 Claim 1 of these requests comprises the same amendment as in auxiliary request 4, in combination with the amendments of auxiliary requests 1 (for auxiliary request 5), 2 (for auxiliary request 6) and 3 (for auxiliary request 7). Hence, for the same reasons as discussed above for auxiliary requests 1 to 4, claim 1 of all these requests also lacks an inventive step.

8.2 Auxiliary requests 5, 6 and 7 are thus not allowable for lack of compliance with Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

M. Schalow A. Lindner

Decision electronically authenticated