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Datasheet for the decision of 28 July 2020

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Application Number: 09719709.9

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Language of the proceedings: EN

Title of invention:
Immuno-based botulinum toxin serotype A activity assays

Patent Proprietor:
Allergan, Inc.

Opponents:
Ipsen Pharma S.A.S.
Merz Pharma GmbH & Co. KGaA

Headword:
Botulinum toxin assays/ALLERGAN

Relevant legal provisions:
EPC Art. 56, 83, 100(a), 100(b), 100(c), 123(2)

Keyword:
Amendments - added subject-matter (no)
Sufficiency of disclosure - (yes)
Inventive step - (yes)

Decisions cited:
T 0019/90, T 0890/02

Catchword:

Europäisches Patentamt
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Beschwerdekammern

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Case Number: T 1153/17 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 28 July 2020

Appellant: (Patent Proprietor)	Allergan, Inc. 2525 Dupont Drive Irvine, CA 92612 (US)
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Appellant: (Opponent 2)	Merz Pharma GmbH & Co. KGaA Eckenheimer Landstrasse 100 60318 Frankfurt/Main (DE)
Representative:	Altmann Stößel Dick Patentanwälte PartG mbB Dudenstrasse 46 68167 Mannheim (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 14 March 2017 concerning maintenance of the European Patent No. 2271670 in amended form.**

Composition of the Board:

Chair	G. Alt
Members:	B. Rutz
	P. de Heij

Summary of Facts and Submissions

I. Appeals were lodged by the patent proprietor (appellant I) and the two opponents (appellants II and III) against the decision of the opposition division maintaining European patent No. 2 271 670 in amended form. The patent is entitled "*Immuno-based botulinum toxin serotype A activity assays*". For ease of understanding, the parties are referred to as "proprietor", "opponent 1" and "opponent 2" in the following.

II. The patent was opposed on the grounds in Article 100(a) EPC, in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC), and in Articles 100(b) and 100(c) EPC.

III. The opposition division decided that claims 1, 5 and 8 of the main request (claims as granted) and auxiliary request 1 infringed the requirements of Article 123(2) EPC and that the set of claims of auxiliary request 2 complied with the requirements of Articles 123(2) and (3), 84, 83, 54 and 56 EPC.

IV. With the statement of grounds of appeal, the proprietor maintained the set of claims of the main request (claims as granted) and filed sets of claims of auxiliary requests 1 to 10 (identical to auxiliary requests 1 and 2 underlying the decision under appeal and auxiliary requests 3 to 10 filed during opposition proceedings but not considered in the decision) and sets of claims of auxiliary requests 11 to 15. With their reply to the opponents' statements of grounds of appeal, the proprietor filed a set of claims of auxiliary request 16.

V. Independent claims 1, 5 and 8 of the main request read:

"1. A method of detecting BoNT/A activity, the method comprising the steps of:

a. treating a cell from an established cell line with a sample comprising a BoNT/A, wherein the cell from an established cell line is susceptible to BoNT/A intoxication by about 500 pM or less of a BoNT/A, and wherein said cell is selected from the group, consisting of SiMa cell line (DSMZ No. ACC 164), Neuro-2a cell line (ATCC Catalog No. CCL-131™), N18 (ECACC No. 88112301), LA1-55n (ECACC No. 06041203), PC12 (ATCC Catalog No. CRL-1721™), and SH-SY5Y (ATCC Catalog No. CRL-2266™):

b. isolating from the treated cell a SNAP-25 component comprising a SNAP-25 cleavage product having a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond;

c. contacting the SNAP-25 component with an α-SNAP-25 antibody linked to a solid-phase support, wherein the α-SNAP-25 antibody binds an epitope comprising a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product, the α-SNAP-25 antibody has an association rate constant for the intact SNAP25 of less than 1×10^{1} M^{-1} s^{-1}; and the α-SNAP-25 antibody has an equilibrium disassociation constant for the epitope of less than 0.450 nM,

d. detecting the presence of an antibody-antigen complex comprising the α-SNAP-25 antibody and the SNAP-25 cleavage product having a carboxyl-terminus glutamine from the BoNT/A cleavage site scissile bond;

wherein detection by the antibody-antigen complex is indicative of BoNT/A activity.

5. The method of any of Claims 1 to 4, wherein the sample comprises at most 100 pM of a BoNT/A.

8. A method of determining BoNT/A immunoresistance in a mammal comprising the steps of:

a. adding a BoNT/A to a test sample obtained from a mammal being tested for the presence or absence of α-BoNT/A neutralizing antibodies;
b. treating a cell from an established cell line with the test sample, wherein the cell from an established cell line is susceptible to BoNT/A intoxication and wherein said cell is selected from the group, consisting of SiMa cell line (DSMZ No. ACC 164), Neuro-2a cell line (ATCC Catalog No. CCL-131™), N18 (ECACC No. 88112301), LA1-55n (ECACC No. 06041203), PC12 (ATCC Catalog No. CRL-1721™), and SH-SY5Y (ATCC Catalog No. CRL-2266™);
c. isolating from the treated cells a SNAP-25 component comprising a SNAP-25 cleavage product having a carboxyl-terminus glutamine from the BoNT/A cleavage site scissile bond;
d. contacting the SNAP-25 component with an α-SNAP-25 antibody linked to a solid-phase support, wherein the α-SNAP-25 antibody; wherein the α-SNAP-25 antibody binds an epitope comprising a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product, the α-SNAP-25 antibody has an association rate constant for the intact SNAP25 of less than 1 x 10^{1} M^{-1} s^{-1}; and the α-SNAP-25 antibody has an

equilibrium disassociation constant for the epitope of less than 0.450 nM.

e. detecting the presence of an antibody-antigen complex comprising the α-SNAP-25 antibody and the SNAP-25 cleavage product having a carboxyl-terminus glutamine from the BoNT/A cleavage site scissile bond;

f. repeating steps b-e with a negative control sample instead of a test sample, the negative control sample comprising a BoNT/A and a serum known not to contain α-BoNT/A neutralizing antibodies; and

g. comparing the amount of antibody-antigen complex detected in step e to the amount of antibody-antigen complex detected in step f, wherein detection of a lower amount of antibody-antigen complex detected in step e relative to the amount of antibody-antigen complex detected in step f is indicative of the presence of α-BoNT/A neutralizing antibodies."

VI. The parties filed replies in response to each others' statements of grounds of appeal.

VII. The board summoned the parties to oral proceedings as requested and informed them of its preliminary opinion in a communication pursuant to Article 15(1) RPBA.

VIII. In letters dated 28 May 2020 and 9 June 2020, the opponents asked the board whether the oral proceedings scheduled for 28 July 2020 could be postponed in view of the on-going COVID-19 pandemic. In reply, the proprietor agreed to the postponement but requested that it be to 2021 in view of increased work-load due to many postponed oral proceedings.

IX. The board informed the parties that since travel restrictions had been lifted in Europe as of 15 June 2020, it considered the arguments for postponing the oral proceedings not persuasive, and the date for oral proceedings was maintained.

X. Oral proceedings before the board took place on 28 July 2020. At the end of the oral proceedings, the chair announced the board's decision.

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

D3 Jones, R. et al., "Development of improved SNAP25 endopeptidase immunoassays for botulinum type A and E toxins", 2008, Journal of Immunological Methods, 329:92-101

D5 WO 95/33850

D6 Pellett, S. et al., "A neuronal cell-based botulinum neurotoxin assay for highly sensitive and specific detection of neutralizing serum antibodies", 2007, FEBS Letters, 581:4803-4808

D11 US 2008/0064054

XII. The proprietor's arguments submitted in writing and at the oral proceedings relevant to the decision may be summarised as follows.

Main request (claims as granted)
Amendments (Article 100(c) EPC)

The claimed method comprised the treatment of undifferentiated and differentiated cell lines.

The basis for claim 1 could be found in claim 8 as filed in combination with Example II (paragraphs [0156] and [160] and Table 3) of the application as filed.

Claim 8 as filed disclosed in particular the feature that the susceptibility to Botulinum toxin A (BoNT/A) of the established cell lines was 500 pM or less. Therefore, no additional combination with even one single sub-claim or disclosure was necessary.

The concentration used in Example II was five times lower than the reference point referred to in the claim, i.e. 100 pM instead of 500 pM. It could thus not be inferred from Table 3 and paragraph [156] that only the two cell lines for which this was expressly indicated were susceptible to BoNT/A in the undifferentiated state.

Tables 3 to 6 as filed clearly singled out the specific cell lines referred to in the claim as being particularly preferable (see also paragraph [0160]).

Example II aimed to identify suitable established cell lines before suitable antibodies were created in the following examples. This was the only possible and appropriate way to carry out the experiments. It was

therefore irrelevant that it used a different assay format than the one referred to in the claims.

Hence, the subject-matter of claims 1, 5 and 8 was directly and unambiguously derivable from the application as filed.

Sufficiency of disclosure (Article 100(b) EPC)

The opponents failed to show any proof for their allegations of lack of sufficient disclosure.

The alleged lack of key features cited by the opponents was not an objection of a possible lack of sufficiency, but rather a clarity objection. Article 84 EPC was however not a ground for opposition.

The fact that the antibodies had been developed using a 13-mer SNAP 25 peptide spanning only the very C-terminal of the cleaved SNAP-25 would not have been a reason for the skilled person not to expect it to bind to the cleaved SNAP-25 in the cell or cell lysate. The interaction between antibody and antigen was the same, independent of the origin of the antigen provided and no harsh conditions had been used which could potentially have denatured the antibody or antigen.

The person skilled in the art would have found suitable differentiation conditions on the basis of the information available in the patent.

As regards claim 5, Table 3 and paragraph [156] clearly supported the susceptibility of the cell lines to detect BoNT/A at 100 pM.

The objection that claim 8 contained no reference to a BoNT/A concentration was a clarity objection. Article 84 EPC was however not a ground for opposition.

The specification provided clear and sufficient instructions for carrying out the claimed method including the description of very specific cell lines and antibodies and how these were provided. The requirements of Article 83 EPC were fulfilled.

Inventive step
(Article 100(a) in conjunction with Article 56 EPC)

Claim 1

Document D11 was the closest prior art. Claim 1 of the main request differed from the disclosure of document D11 in that it related to the use of an antibody linked to a solid-phase support instead of being in solution. Further differences were the epitope bound by the antibody and the binding characteristics of the antibody.

The problem to be solved was the provision of an improved cell-based assay for the detection of BoNT/A activity which was amenable to high-throughput automation and provided an increased specificity and sensitivity.

It would have been impossible for the person skilled in the art to provide in an obvious manner, or with any reasonable expectation of success - in particular in view of the large amount of research that had been done precisely on providing a functioning assay - the claimed method, with all the above differing features.

It would also not have been obvious for the skilled person starting from document D11 to identify antibodies with the indicated characteristics (association/dissociation constants) which were critical in enabling a detection system amenable to automation by solid-phase attachment of the antibody. These constants were not arbitrary selections but were suitable to provide the person skilled in the art with the necessary information to achieve the cell-based assay, in conjunction with the further features of the independent claims.

Claim 8

With regard to the subject-matter of claim 8, document D6 could be chosen as the closest prior art. However, by referring to very low BoNT sensitivity of continuous cell lines, it was clearly leading away from the claimed invention. Moreover, the antibody disclosed in document D6 recognised both cleaved and intact SNAP-25.

XIII. The opponents' arguments submitted in writing and at the oral proceedings relevant to the decision may be summarised as follows.

Main request (claims as granted)
Amendments (Article 100(c) EPC)

Claim 1 did not encompass that a cell from an established cell line was treated in a differentiated state. From the syntax of the claim, it was clear that the cell had to be selected from the "group, consisting of SiMa cell line [...], and SH-SY5Y [...]". Those cell lines, however, were all undifferentiated (opponent 1).

Claim 1 encompassed treating a cell from an established cell line in an undifferentiated or differentiated state (opponent 2).

However, there was no direct and unambiguous disclosure for the skilled person, using common general knowledge, in Example II of the application as filed for N18, LA1-55n, PC12 and SH-SY5Y cells exhibiting an uptake of about 500 pM or less of a BoNT/A, let alone 100 pM BoNT/A (as in claim 5), in the undifferentiated state.

The assay used in Example II was a Western blot assay, i.e. not the kind of assay referred to in the claims. Hence, in the assay according to Example II, the rabbit polyclonal anti-$SNAP_{197}$ antibody serum was not linked to a solid-phase support but was added in solution. Moreover, it was not disclosed in this example that this antibody had an association rate constant for the intact SNAP-25 of less than 1×10^{1} M^{-1} s^{-1} and an equilibrium dissociation constant for the epitope of less than 0.450 nM.

The claimed ELISA (enzyme-linked immunosorbent assay) was actually exemplified by Examples VI and VII in which, however, the established cell line was not specified and GT1b ganglioside treatment of the cells was used. The monoclonal antibody used in the ELISA was specifically 2E2A6 (see paragraphs [0200], [0201] and [0205] of the application as filed).

Claim 1 therefore represented an unallowable generalisation of the disclosure of the application as filed.

The above-mentioned arguments applied *mutatis mutandis* to claim 5 of the main request defining that the sample

of claim 1 comprised at most 100 pM of a BoNT/A and to claim 8 of the main request.

Hence, the subject-matter of claims 1, 5 and 8 was not directly and unambiguously derivable from the application as filed.

Sufficiency of disclosure (Article 100(b) EPC)

For the following reasons, the invention claimed in claim 1 could not be carried out without undue burden.

The patent did not disclose that undifferentiated cells from all the specific established cell lines referred to in claim 1 achieved the level of sensitivity to BoNT/A intoxication required by claims 1 and 5.

The patent failed to show any association rate constant or equilibrium dissociation constant for the antibodies referred to in claims 1(c) and 8(d) with regard to a SNAP-25 cleavage product isolated from a cell or cell lysate. All the binding assays disclosed in the patent had been carried out with purified peptides. A cell-based assay or immuno-histochemistry was not suited to characterise the specificity of an antibody.

How the cell line was differentiated was not defined in claim 1. Different differentiation processes affected the sensitivity of cells to BoNT/A differently.

The invention referred to in claim 5 required that a sample comprising at most 100 pM BoNT/A was used in the detection assay according to claim 1. However, claim 1 defined the susceptibility of cells as being only about 500 pM BoNT/A. Thus, even if the invention according to claim 1 was found to be sufficiently disclosed, for

this additional reason, carrying out the method of claim 5 involved an undue burden.

Method-step b) of claim 8 did not indicate for which concentration of BoNT/A the cells had to be susceptible to BoNT/A intoxication, i.e. the claim included very high sensitivities for which no appropriate cells had been disclosed.

Inventive step
(Article 100(a) in combination with Article 56 EPC)

Claim 1

Document D11 was the closest prior art document. The distinguishing features were the used assay, involving a solid support, and the specific association and disassociation constants of the antibody.

The objective technical problem to be solved was "*the provision of a method of detecting BoNT/A activity, using an alternative antibody and an alternative assay*".

Replacing a Western blot assay by ELISA techniques was routine in 2008 (the priority year). For ELISA techniques, the skilled person would have needed to generate high affinity antibodies able to selectively bind the cleaved α-SNAP-25 and not the intact α-SNAP-25. The skilled person would therefore have considered document D3, which provided a method for generating such selective antibodies. From document D3, generating antibodies which selectively bound the cleaved α-SNAP-25, and not the intact α-SNAP-2, would clearly have fallen within the ability of the skilled person.

It would have been known to the skilled person that Western blot assays as used in document D11 were not suitable for quality control validation because quantification of the obtained signals was problematic. In contrast, ELISA was used as a quantitative method in routine testing being able to reliably determine the quantitative amount of an analyte in a sample where the method could also be validated with suitable acceptance criteria and according to the requirements of the ICH Q2(R1). In addition, ELISA assays for determining Clostridial toxin activity and their benefits were well known in the art at the priority date of the contested patent, as exemplified by documents D3 and D5.

Furthermore, there was no evidence on file demonstrating that a particular technical effect was achievable with antibodies falling into the claimed range of claim 1. The anti-SNAP-25 antibodies defined in claim 1 did not exhibit any surprising or unexpected features compared with other antibodies of the prior art, such as the rabbit polyclonal anti-SNAP-25 serum pAb anti-SNAP-25_{197} #1 used in document D11. The polyclonal anti-SNAP-25_{197} antibody of document D11 was able to detect BoNT/A-cleaved SNAP-25_{197} in differentiated Neuro-2a cells incubated with 50 pM BoNT/A (see Figure 8a of D11) and did not cross-react with full-length, non-cleaved SNAP-25_{206} (see Example III, page 83, paragraph [0552] of D11), indicating that it had an association and dissociation constant, as required in claim 1.

Though no specific values were indicated for the association rate constant and equilibrium dissociation constant for the polyclonal anti-SNAP-25_{197} antibody of document D11, it had to be presumed that this antibody

was equally suitable for detecting BoNT/A activity in a cell-based assay because it was able to detect BoNT/A activity in the picomolar range. The specific features, by which the antibody of claim 1 was defined, were thus arbitrary.

Claim 8

Document D6 was the closest prior art for the subject-matter of this claim. It disclosed monitoring patients treated with BoNT/A formulations for the presence of neutralising antibodies using a cell-based assay for measuring BoNT/A activity in primary cells. Cleaved and intact SNAP-25 were detected in a Western blot assay with an antibody that detected both (see page 4805, right column, point 2.4.; Figures 2 to 4).

The differences between the method of claim 8 and the disclosure of document D6 were thus the use of established cell lines and of antibodies specifically recognising cleaved SNAP-25 substrate and having the binding characteristics defined in the claim.

The use of the antibodies having the functional features recited in the claim was not associated with a particular technical effect. The technical problem to be solved was the provision of a method for BoNT/A immunoresistance using alternative antibodies for detection and being independent of primary cells.

Knowing the drawbacks of primary cells, the skilled person would, in the light of document D11, have replaced them by cells of an established cell line, such as Neuro-2a. The skilled person would also have replaced the antibody detection system disclosed in document D6 by that disclosed in document D11. Thus,

the skilled person would have arrived at the subject-matter of claim 8.

XIV. The proprietor's requests, as far as relevant to the present decision, are that the decision under appeal be set aside and that the patent be maintained as granted.

XV. Both opponents' requests, as far as relevant to the present decision, are that the decision of the opposition division be set aside and that the patent be revoked in its entirety.

Reasons for the Decision

Main Request (claims as granted)

Claim interpretation

1. The parties interpret the expression: "treating a cell from an established cell line with a sample comprising a BoNT/A, wherein the cell from an established cell line is susceptible to BoNT/A intoxication by about 500 pM or less of a BoNT/A, and wherein said cell is selected from the group, consisting of SiMa cell line (DSMZ No. ACC 164), Neuro-2a cell line (ATCC Catalog No. CCL-131™), N18 (ECACC No. 88112301), LA1-55n (ECACC No. 06041203), PC12 (ATCC Catalog No. CRL-1721™), and SH-SY5Y (ATCC Catalog No. CRL-2266™)" recited in step (a) of claim 1 differently. The proprietor and opponent 2 are of the opinion that the claimed method includes treating undifferentiated as well as differentiated cells. In contrast, opponent 1 argues that the above expression refers only to treating undifferentiated cells because cells "from an

established cell line" as listed in claim 1 are *per definitionem* undifferentiated.

2. The board finds opponent 1's argument not persuasive. Firstly, the words alone "treating a cell from an established cell line" do not exclude that the cell taken from the established cell line is modified before it is treated with BoNT/A. Secondly, opponent 1's interpretation is also contrary to the interpretation that the skilled person, having regard to the whole of the application, would have made. The patent makes it clear that cells from an established cell line can be differentiated or undifferentiated (see patent as granted, figure legends to Figures 3, 4 and 8 on page 4; paragraphs [0090], [0091], [0103], [0104]; and Examples). In particular, the figure legends explicitly mention "*cell differentiation time for cells comprising an established cell line*" and "*differentiated SiMa cells*". Example II in paragraph [0147] reports: "[...] *besides SiMa and Neuro-2a, the cell lines N18, LA1-55n, PC12, and SH-SY5Y all exhibited an uptake of 0.1 nM BoNT/A in the differentiated state*" indicating that, in the language of the patent, cell lines (and thus their cells) can be in the differentiated and undifferentiated state.

3. In conclusion, claim 1(a) of the main request relates to treating differentiated as well as undifferentiated cells with BoNT/A.

4. This conclusion also applies to claims 5 and 8 (see section V. above). Claim 5 is dependent on claim 1 and encompasses the same method step (a) as claim 1 except for the concentration of BoNT/A being 100 pM. Claim 8 comprises method-step (a) of claim 1 as step (b).

Amendments (Article 100(c) EPC)

5. Claim 1 is identical to claim 8 as filed except that method-step (a) recites specific cell lines (i.e. "and wherein said cell ....").

6. It was established above in points 1. to 3. that step (a) of claim 1 relates to treating undifferentiated and differentiated cells.

7. Hence, the question to be asked is whether or not the skilled person, having regard to the application as a whole, would have directly and unambiguously considered that the six cell lines listed in Table 3 and mentioned in current claim 1 qualify, either in their undifferentiated or differentiated state, as an "established cell line [...] susceptible to BoNT/A intoxication by about 500 pM or less of a BoNT/A" (see claim 8 as filed).

8. While the proprietor refers to Example II, the opponents submit that it is related to an assay different from the one referred to in claim 8 as filed, thus implying that the skilled person would not read the two together.

9. The board considers that the skilled person would have recognised that Examples I and II are about determining cell lines susceptible to intoxication with BoNT/A and would therefore not have neglected the disclosure in these examples when considering the meaning of the feature "established cell line".

10. Example I in paragraph [0149] "*illustrates how to identify established cell lines susceptible to BoNT/A intoxication*". It tested 1) which cell lines have an

uptake of 1 nM BoNT/A and 2) which cell lines adhere to a substrate surface. In paragraph [0152], last sentence, a list of established/candidate cell line is given.

11. Example II "*illustrates how to determine growth conditions for established cell lines that maximize susceptible [sic] to BoNT/A intoxication or have BoNT/A uptake capacity*" (paragraph [0154]). It establishes within the list of established cell lines the ones that have a susceptibility of 0.1 nM/100 pM, i.e. five times more sensitive than required in claim 8 as filed. The cell lines that fulfil this criterion are the six listed in paragraph [0156]. Those cell lines represent a limitation of the broader list mentioned in the last sentence of paragraph [0152]. It would have been clearly and unambiguously disclosed to the skilled person that these are the cell lines that can be used in the method of claim 8 as filed, in an differentiated or undifferentiated state, depending on the selected cell line.

12. Consequently, the subject-matter of claim 1 finds a basis in the combination of claim 8 as filed and Examples I and II.

13. The same conclusion is drawn for claim 5 which contains the concentration disclosed in Example II (100 pM = 0.1 nM) and for claim 8 which contains no concentration requirement and finds basis in original claim 15 in combination with paragraph [0156] and Table 3.

14. The subject-matter of claims 1, 5 and 8 as granted does not extend beyond the content of the application as filed.

Sufficiency of disclosure (Article 100(b) EPC)

15. A successful objection of lack of sufficiency of disclosure presupposes that there are serious doubts, substantiated by verifiable facts (see e.g. decision T 19/90, OJ 1990, 476 and decision T 890/02, OJ 2005, 497 and Case Law of the Boards of Appeal of the EPO, II.C.9).

16. As outlined above (points 1. to 3.), claim 1 includes methods involving differentiated as well as undifferentiated cells and does not require that all cells are used under the same conditions. The claim lists six cell lines shown in the application to fulfil the requirements of the method as claimed (see Example II and Table 3). The patent teaches the skilled person how to select the appropriate conditions to make the invention work, e.g. to decide whether to use differentiated or undifferentiated cells. The board thus fails to see evidence ("verifiable facts", see point 15. above) that the claimed methods could not have been carried out by the skilled person having regard to the application as filed and taking into account common general knowledge.

17. Opponent 2 argues that the antibodies disclosed in the application had not been tested for their association and dissociation constants using the substrates isolated from a cell or cell lysate (i.e. full-length SNAP-25 comprising 206 amino acids or cleaved SNAP-25 comprising 197 amino acids) but instead using short synthetic peptides ($SNAP\text{-}25_{134\text{-}206}$ and $SNAP\text{-}25_{134\text{-}197}$, see paragraphs [0175] and [0176] of the application as filed), and therefore their suitability was not disclosed in the application.

18. The board does not agree with this argument because the measurements obtained with synthetic peptides comprising or lacking the epitope recognised by the antibody would have provided an assay for the skilled person to select suitable antibodies. The claim in this respect does not require the antibody to achieve those constants in a cell lysate. The board considers the values obtained with synthetic peptides to represent a reasonable approximation of the values obtainable with the full-length SNAP-25 substrate or cleavage product. Moreover, the board has seen no evidence that the antibodies disclosed and used in the examples of the patent did not fulfil the criteria of claim 1.

19. The board also sees no contradiction in the requirement of claim 5 that a sample comprising at most 100 pM BoNT/A be used in the detection assay and the requirements of claim 1 which define the cells as susceptible to 500 pM or less of BoNT/A. Claim 1 explicitly states that the cells can also be susceptible to less then 500 pM of BoNT/A. Claim 5 therefore represents a more limited embodiment in which cells and conditions have to be chosen which are capable of detecting 100 pM BoNT/A. In fact, the cells from the six cell lines listed in claim 1 have been shown in the application to be capable of detecting BoNT/A at a concentration of 100 pM under the appropriate conditions (see Table 3).

20. The board also does not agree with opponent 2's argument that the lack of a specific BoNT/A concentration in claim 8 would result in insufficient disclosure because the application contained sufficient information for the skilled person to choose an appropriate concentration (see Example II and Table 3).

21. Finally, the board finds that the person skilled in the art would have been able to choose suitable differentiation conditions on the basis of the information available in the patent. Even if different differentiation protocols might result in different BoNT/A susceptibilities with the assays and results disclosed in the patent, the skilled person would have been able to identify the relevant conditions without undue burden.

22. The invention as claimed in claims 1, 5 and 8 is disclosed in a manner sufficiently clear and complete for it to be carried out by the skilled person.

Inventive step (Article 100(a) EPC and Article 56 EPC)

Claim 1
Closest prior art and problem to be solved

23. All parties agreed on document D11 as the closest prior art. The document discloses detecting BoNT/A activity by a Western blot asssay (see for example paragraph [0258]: "[...] *western blot analysis using an antibody that specifically recognizes BoNT/A SNAP-25-cleaved product can be used to assay for uptake of BoNT/A*"). This type of assay requires that the antibody is in solution while the protein is bound to a membrane.

24. All parties agreed that the difference of the method of claim 1 over the method disclosed in document D11 was the solid-phase attachment of the antibody and the specific association and disassociation constants of the antibody.

25. Independent of the contribution of the binding characteristics, the solid-phase attachment of the

antibody has the effect that the assay delivers reliable quantitative data and can be standardised and automated (e.g. in ELISA format). This is in contrast to the Western blot detection used in document D11.

26. Opponent 2 argued that this effect was not achieved by all embodiments encompassed by claim 1 because the patent showed only examples for one specific monoclonal antibody (2E2A6) and one specific cell line (SiMa).

27. The claim lists six established cell lines to be used in the method and defines an epitope and binding characteristics for the antibody. The examples show that all listed cell lines are susceptible to BoNT/A in the required concentration range (see Example II; Table 3) and that antibodies fulfilling the binding characteristics are suitable for the differential detection of cleaved SNAP-25 (see Tables 8 and 9). Thus, the board finds that the method as outlined in claim 1 achieves the above effect.

28. The problem in view of this effect can be formulated as the provision of a quantitative cell-based assay for detecting BoNT/A activity which has high sensitivity and is amenable to a high-throughput assay format.

Obviousness

29. In the assessment of the obviousness of the claimed subject-matter, the question to be asked is whether the skilled person, faced with the problem of providing an assay with high sensitivity and amenable to high-throughput assay formats, would have replaced the Western blot assay disclosed in document D11 with an assay in which the antibody is attached to a solid phase.

30. Document D5 discloses an epitope for the polyclonal antibody used (see page 19, middle: "*The antibody is specific to either of the following cleaved sequences of SNAP-25: RIDEANQ-COOH*") identical to the epitope recognised by the polyclonal antibodies disclosed in document D11. Document D5 also mentions that the antibody can be linked to a solid support, see page 11: "*the assay of the invention can also be performed with a solid phase that comprises antibody according to the second aspect of the invention [...] combining the toxin substrate with the test compound that possibly contains toxin. This mixture is then combined with the solid-phase antibody and thereafter the presence of cleaved peptide bound to the solid-phase antibody is detected [...] solid-phase antibody for use in a toxin assay*".

31. However, the method disclosed in document D5 uses synthetic peptides brought in contact with a sample to be tested for toxin (see Example 4). This is substantially different to a cell-based assay where cells are contacted with the toxin sample, the toxin is taken up by the cell, BoNT/A is cleaved in the cell (see Figure 1B of thepatent at issue) and the cleavage product is analysed in a (crude) cell lysate. In such a situation, the antibody is in contact with a complex mixture of cellular compounds from which the cleavage product has to be detected. From the disclosure of document D5, it can therefore not be concluded that the disclosed solid-phase assay would also be capable of detecting SNAP-25 cleavage product in cell lysate.

32. Moreover, although document D5 provides a theoretical disclosure of an assay with a solid-phase antibody, it does not show any results for this format which is

reversed compared to the actual examples of document D5 which all use solid-phase substrate peptides (see Figure 1).

33. Thus, the skilled person reading document D5 would have been left in doubt whether the detection assay disclosed would be suitable for detecting SNAP-25 cleavage products resulting from a cell-based assay.

34. Document D3 equally discloses an ELISA assay for BoNT/A cleavage products but, like document D5, shows only experiments with synthetic SNAP-25 peptides immobilised to a solid phase, i.e. no cell-based assay and no solid-phase antibody.

35. Consequently the skilled person, faced with the problem of providing an assay with high sensitivity and amenable to high-throughput assay formats, would not, in the light of documents D3 or D5, have replaced the Western blot assay disclosed in document D11 with an assay in which the antibody is attached to a solid phase.

36. The subject-matter of claim 1 involves an inventive step.

Claim 8
Closest prior art and problem to be solved

37. Document D6 was cited by opponent 2 as the closest prior art for the subject-matter of claim 8. It discloses monitoring patients treated with BoNT/A formulations for the presence of neutralising antibodies, i.e. the determination of immunoresistance (see page 4803, abstract) using a cell-based assay for measuring BoNT/A activity in primary cells (i.e. not

established cell lines). Cleaved and intact SNAP-25 are detected in a Western blot assay with an antibody that recognises both forms of SNAP-25 (see page 4805, right column, point 2.4.; Figures 2 to 4).

38. The differences between the method of claim 8 and the disclosure of document D6 are thus, as put forward by opponent 2, (i) the use of particular established cell lines and (ii) the use of antibodies recognising a specific epitope on the cleaved SNAP-25 substrate and having the binding characteristics defined in the claim but, in the board's view, in addition (iii) the attachment of those antibodies to a solid phase.

39. Opponent 2 considered that the use of the antibodies having the functional features recited in the claim was not associated with a particular technical effect. The board does not agree because the differentiation between cleaved and intact SNAP-25 by specific antibodies together with the solid phase format allow high-throughput and automation of the method.

40. The objective technical problem to be solved is thus the provision of an improved method of determining BoNT/A immunoresistance in a mammal based on a quantitative cell-based assay for detecting BoNT/A activity which has high sensitivity and is amenable to a high-throughput assay format.

Obviousness

41. Opponent 2 argued that the subject-matter of claim 8 was obvious in view of a combination of the disclosures of document D6 and document D11.

42. However, first of all, the board is not convinced that the skilled person, faced with the problem of providing a quantitative cell-based assay for detecting BoNT/A activity which has high sensitivity and is amenable to a high-throughput assay format, would have consulted document D11 when starting from the teaching of document D6 because document D6 dismisses the use of established cell lines: "*Several cell-based assays have been developed, including continuous cell lines such as neuro-2a, PC12, or SK-N-SH cell* [...] *However, continuous cell lines exhibit very low BoNT sensitivities and therefore cannot be used for detection of serum antibodies*" (see page 4804, column 1).

43. Even if the skilled person had taken the disclosure of document D11 into account, this would not have led to the solution presented in claim 8. While document D11 discloses some of the established cell lines referred to in claim 8 (e.g. Neuro 2a and SH-SY5Y) and also describes antibodies specifically detecting cleaved SNAP-25 (see Example III, paragraph [0552]), it does not disclose antibodies having the particular binding characteristics required in the claim and, most importantly, it does not disclose the attachment of such antibodies to a solid phase (see also arguments with regard to inventive step of the subject-matter of claim 1 in points 23. to 29. above).

44. Hence, the subject-matter of claim 8 involves an inventive step.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside. The patent is maintained as granted.

2. The appeals of appellants II and III are dismissed.

The Registrar: The Chair:

I. Aperribay G. Alt

Decision electronically authenticated