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Case Number: T 1147/02 - 3.3.04

Application Number: 93909875.2

Publication Number: 0639229

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Language of the proceedings: EN

Title of invention:
Rapid detection of antibiotic resistance in Mycobacterium tuberculosis

Patentee:
INSTITUT PASTEUR, et al

Opponent:
Innogenetics N.V.

Headword:
Antibiotic resistance/PASTEUR

Relevant legal provisions:
EPC Art. 54, 56, 83, 123(2),(3)
PCT Art. 8(2)(a)
PCT R. 17.1
Paris Convention Art. 4A(1), 4C(1)

Keyword:
"Added matter (no)"
"Sufficiency, novelty, inventive step (yes)"
"Admission of late filed documents (no)"
"Validity of priority claim based on third priority document (yes)"

Decisions cited:
T 0204/83, T 0677/91, T 0776/96, T 0838/97

Catchword:
see points 12 to 18

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1147/02 **-** 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 31 May 2006

Appellant I: (Patent Proprietor)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 30 September 2002 concerning maintenance of the European Patent No. 0639229 in amended form.**

Composition of the Board:

Chair: U. Kinkeldey
Members: B. Claes
G. Weiss

Summary of Facts and Submissions

I. The European application with the title "Rapid detection of antibiotic resistance in Mycobacterium tuberculosis" was filed as international application PCT/EP93/01063, claiming priorities from US 875,940 (P1; filed on 30 April 1992), US 929,206 (P2; filed on 14 August 1992), FR 92/11098 (P3; filed on 17 September 1992) and FR 93/04545 (fourth priority document; filed on 16 April 1993).

Claims 1 and 16 of the application as originally filed read:

"1. A process for the detection of a resistance to an antibiotic in a mycobacterium which comprises detecting a mutation in a gene selected from the katG gene or fragment thereof, the rpoB gene or fragment thereof and rpsL gene or fragment thereof."

"16. The process of claim 1 for the detection of resistance to the selected antibiotic which comprises:
- fragmenting the relevant gene or part thereof likely to carry the mutation into a plurality of fragments, such as by digestion of said relevant gene by selected restriction enzymes,
- hybridizing these fragments to complementary oligonucleotide probes, preferably a series of labelled probes recognizing under stringent conditions, all of the parts of the relevant gene of a corresponding control DNA of a strain non-resistant to the corresponding antibiotic,
- and relating the absence of hybridization of at least one of said oligonucleotide probes to any of the DNA

fragments of the relevant gene of the mycobacterium under study as evidence of the presence of a mutation and, possibly, of a resistance to the corresponding antibiotic, particularly as compared to results obtained upon running the test under the same conditions with the same oligonucleotides on the relevant gene(s) obtained from a strain (strains) not resistant to said antibiotic, wherein said relevant gene is either the katG gene or a fragment thereof, the rboB gene or a fragment thereof, the rpsL gene or a fragment thereof."

II. The patent was granted with 32 claims and contained *inter alia* the following claims:

"1. A process for the detection of a resistance to an antibiotic in a mycobacterium which comprises detecting a mutation in a gene selected from the group comprising

the katG gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56,

the rpoB gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the sequence shown in Figure 13, and

the rpSL gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the Mycobacterium tuberculosis sequence shown in Figure 14."

"2. A process of claim 1 for detecting in vitro the presence of nucleic acids of Mycobacterium tuberculosis

resistant to isoniazid, wherein the process comprises the steps of:

- contacting said nucleic acids previously made accessible to a probe if required under conditions permitting hybridization;
- detecting any probe that had hybridized to said nucleic acids;

wherein said probe comprises a nucleic acid sequence, which is 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56 or of part thereof, and wherein said fragment contains a BamHI cleavage site, wherein said part is nonetheless sufficiently long to provide for the selectivity of the in vitro detection of a Mycobacterium tuberculosis resistant to isoniazid."

"10. A nucleic acid probe for detecting Mycobacterium tuberculosis resistant to isoniazid, wherein said probe consists of a 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56, wherein said fragment contains a BamHI cleavage site, or of a part of said fragment nonetheless sufficiently long to provide for the selectivity of the in vitro detection of a Mycobacterium tuberculosis resistant to isoniazid."

"11. A hybrid duplex molecule consisting essentially of the probe of claim 10 hydrogen bonded to a nucleotide sequence of complementary base sequence."

"12. A process for selecting a nucleotide sequence of a Mycobacterium tuberculosis resistant to isoniazid from a group of nucleotide sequences, comprising the step of

determining which of said nucleotide sequences hybridizes to a probe as claimed in claim 10 or 11."

"14. The process of claim 1 for the detection of resistance to the selected antibiotic which comprises:

- fragmenting the relevant gene or part thereof likely to carry the mutation into a plurality of fragments,
- hybridizing these fragments to a series of labelled oligonucleotide probes recognizing under stringent conditions, all of the parts of the relevant gene of a corresponding control DNA of a strain non-resistant to the corresponding antibiotic,
- and relating the absence of hybridization of at least one of said oligonucleotide probes to any of the DNA fragments of the relevant gene of the mycobacterium under study as evidence of the presence of a mutation and, possibly, of a resistance to the corresponding antibiotic, particularly as compared to results obtained upon running the test under the same conditions with the same oligonucleotides on the relevant gene(s) obtained from a strain (strains) not resistant to said antibiotic, wherein said relevant gene is either the katG gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with a 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56, the rpoB gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the sequence shown in Figure 13, the rpsL gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the Mycobacterium tuberculosis sequence shown in Figure 14."

"16. The process of claim 1 which comprises:

- digesting the DNA to be analyzed,
- amplifying the fragments obtained,
- recovering the amplified fragments, and
- separating them from one another according to sizes by causing them to migrate,
- comparing the sizes of the different fragments with those obtained from the DNA(s) of one or several control strains not resistant to the antibiotic, which had been subjected to a similar assay, and
- relating the polymorphism possibly detected to the existence of a mutation in the relevant gene, accordingly to a possible resistance to the corresponding antibiotic of the strain from which the DNA under study had been obtained, wherein said relevant gene is either the katG gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with a 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56, the rpoB gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the sequence shown in Figure 13, the rpsL gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the Mycobacterium tuberculosis sequence shown in Figure 14."

"18. A kit for the in vitro diagnostic of the resistance of a bacteria of a mycobacterium genus to isoniazid, characterized in that it comprises:

- means for carrying out for a genic amplification of the DNA of the katG gene or of a fragment thereof,

which gene or fragment thereof is capable of hybridizing with a 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56,

- means to bring into evidence one or several mutations on the amplification products so obtained, and
- a preparation of control DNA of a katG gene of a strain of said bacteria sensitive to isoniazid or of a fragment thereof."

"20. A kit for the in vitro diagnostic of the resistance of a bacteria of a mycobacterium genus to rifampicin or its analogues, characterized in that it comprises:

- means for carrying out for a genic amplification of the DNA of the rpoB gene or of the ß-sub-unit of the RNA polymerase of said mycobacteria, or of a fragment thereof, which gene or fragment thereof is capable of hybridizing with the sequence shown in Figure 13,
- means to bring into evidence one or several mutations on the amplification products so obtained, and
- a preparation of control DNA of a rpoB gene coding for the ß-sub-unit of the RNA polymerase of a strain of said bacteria sensitive to rifampicin or of a fragment thereof."

"22. A kit for the in vitro diagnostics of the resistance of the M. tuberculosis to streptomycin, characterized in that it includes:

- means for carrying out a genic amplification of the rpsL gene coding for the S12 protein of the small ribosome sub-unit, or fragment thereof, which gene or fragment thereof is capable of hybridizing with the Mycobacterium tuberculosis sequence shown in Figure 14,
- means which enable the bringing to evidence of one or several mutations on the amplification products obtained, and
- a control preparation of a DNA sequence of the rpsL gene coding for the S12 protein of the small sub-unit of the ribosome of a M.tuberculosis strain sensitive to streptomycin."

"24. A nucleotide sequence comprising the 263 base sequence or a portion thereof as described in Figure 15."

"26. A nucleotide sequence comprising the 3447 base sequence or a portion thereof as described in Figure 12."

"27. A nucleotide sequence comprising the 432 base sequence or a portion thereof as described in Figure 13."

"30. Nucleic acid sequence comprising a 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56 or a nucleic acid sequence capable of hybridizing with said fragment."

"31. Nucleic acid sequence according to claim 30 comprising a 4.5 kb KpnI fragment of plasmid pYZ56, wherein said fragment contains a BamHI cleavage site,

or a nucleic acid sequence capable of hybridizing with said fragment."

"32. Use of a nucleic acid sequence comprising the sequence of any of claims 24 to 31 for the detection of an antibiotic resistance in Mycobacteria."

III. The patent was opposed as a whole on the basis of the grounds of opposition in Article 100(a) EPC concerning novelty and inventive step, Article 100(b) EPC and Article 100(c) EPC. The opposition division decided that they could maintain the patent in amended form (Article 102(3) EPC) on the basis of the claims of an auxiliary request 4 filed during oral proceedings before them.

IV. Besides the priority documents P1, P2 as well as the fourth priority document (see section I above), the following documents are cited in the present decision:

Certified copy (copie officielle) dated 9 April 2001 of an application for a industrial property right filed with the Institut national de la proprieté industrielle (French Patent Office; hereafter INPI) which was filed on 17 September 1992 and has the number FR 92/11098 (P3).

Form PCT/IB/304 mailed on 14 July 1993 relating to international patent application No. PCT/EP93/01063.

Annex 3: Evidence that priority document P3 as transmitted by the International Bureau (hereafter "IB") and contained in the EPO dossier pertaining to the patent in suit does not contain the figures

mentioned on pages 6 and 7. This document was submitted by appellant II (see further section V) as Annex B to the notice of opposition dated 10 December 1999.

Annex 6: sequence alignment of the sequence of Figure 14 with the M smegmatis rpsL gene sequence.

Annex 10: Certification issued by the IB and dated 10 September 2003 that an attached copy is a true copy of the certified copy of P3 as established by the INPI and transmitted to the IB under Rule 17.1 PCT.

Annex 11: Fax addressed to appellant II (see further section V) concerning the corresponding file at the USPTO.

Annex 12: Fax addressed to appellant II (see further section V) concerning the corresponding file at the JPO.

Annex 13: Copy of the USPTO file wrapper of patent US 5,851,763 based on international application PCT/EP93/01063

(D1): Zhang *et al.* (1992), Nature, Vol. 358, p.591-593.

(D5): Winder (1982), In "The Biology of Microbacteria" Ratledge & Stanford (Eds.), Academic Press, London, p.353-438.

(D6): Devi *et al* (1975), Biochem. J. Vol. 149, No. 1, p.187-197.

(D9): Jin & Gross (1988), J. Mol. Biol., Vol. 202, p.45-58.

(Da2): Sweetser *et al.* (1987), Proc. Nat. Acad. Sci. USA, Vol. 84, p.1192-1196.

(P4): Sreevatsan *et al.* (1997), Proc. Nat. Acad. Sci. USA, Vol. 94, p.9869-9874.

V. The patent proprietors (appellant I) and the opponent (appellant II) have lodged appeals against the interlocutory decision of the opposition division posted on 30 September 2002.

VI. When summoning to oral proceedings, the board issued a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal, setting outs its preliminary opinion on a number of issues. The final date for making written submissions was set to one month before the date of the oral proceedings.

VII. With a letter dated 2 May 2006, appellant II filed documents (Da2) and annex 13.

VIII. Oral proceedings took place on 30 and 31 May 2006, during which appellant I filed a new main request. The claims of the new main request relevant for the present decision are the following:

Claim 1 corresponded to claim 1 as granted, having the alternative feature "the katG gene or fragment thereof, which gene or fragment thereof is capable of

hybridizing with 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56" deleted. This resulted in the following claim:

"1. A process for the detection of a resistance to an antibiotic in a mycobacterium which comprises detecting a mutation in a gene selected from the group comprising

the rpoB gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the sequence shown in Figure 13, and

the rpSL gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the Mycobacterium tuberculosis sequence shown in Figure 14."

Claim 2 corresponded to claim 2 as granted, but was now formulated as an independent claim reading:

"2. A process for detecting in vitro the presence of nucleic acids of Mycobacterium tuberculosis resistant to isoniazid, wherein the process comprises the steps of:

- contacting said nucleic acids previously made accessible to a probe if required under conditions permitting hybridization;
- detecting any probe that had hybridized to said nucleic acids;

wherein said probe comprises a nucleic acid sequence, which is 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56, and wherein said fragment contains a BamHI cleavage site, wherein isoniazid-resistant

Mycobacterium tuberculosis do not contain DNA which hybridises with this fragment."

Claims 3 to 7 were identical to granted claims 3 to 7.

Claim 8 was an amended version of claim 10 as granted and read:

"8. A nucleic acid probe for detecting Mycobacterium tuberculosis resistant to isoniazid, wherein said probe consists of a 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56, wherein said fragment contains a BamHI cleavage site, or of a part of said fragment which encodes a polypeptide of the formula APLNSWPDNASLDKARRLLWPSKKKYGK KLSWADLIV."

Claims 9 and 10 corresponded to claims 10 and 11 as granted having the reference to claim 10 and claims 10 or 11, respectively, amended to claim 8 and claims 8 and 9, respectively.

Claim 11 was an amended version of claim 14 as granted. The last feature now read:

– "and relating the absence of hybridization of at least one of said oligonucleotide probes to any of the DNA fragments of the relevant gene of the mycobacterium under study as evidence of the presence of a mutation and, possibly, of resistance to the corresponding antibiotic, particularly as compared to results obtained upon running the test under the same conditions with the same oligonucleotides on the relevant gene(s) obtained from a strain (strains) not resistant to said

antibiotic, wherein said relevant gene is either the rpoB gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the sequence shown in Figure 13, or the rpsL gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the Mycobacterium tuberculosis sequence shown in Figure 14."

Claim 12 corresponded to claim 15 as granted being now dependent on claim 11.

Claim 13 was an amended version of claim 16 as granted. The last feature now read:

– "comparing the sizes of the different fragments with those obtained from the DNA(s) of one or several control strains not resistant to the antibiotic, which had been subjected to a similar assay, and relating the polymorphism possibly detected to the existence of a mutation in the relevant gene, accordingly to a possible resistance to the corresponding antibiotic of the strain from which the DNA under study had been obtained, wherein said relevant gene is either the rpoB gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the sequence shown in Figure 13, or the rpsL gene or fragment thereof, which gene or fragment thereof is capable of hybridizing with the Mycobacterium tuberculosis sequence shown in Figure 14."

Dependent claim 14 corresponded to granted claim 17 having the dependency adapted to claim 13.

Claims 15, 17 and 19 were identical to claims 18, 20 and 22 as granted. Dependent claims 16, 18 and 20 corresponded to claims 19, 21 and 23 as granted, their dependencies being adapted.

Claim 21 was an amended version of claim 24 as granted and read:

"21. A nucleotide sequence which is the 263 base sequence as described in Figure 15."

Dependent claim 22 corresponded to granted claim 25, being now dependent on claim 21.

Claim 23 corresponded to claim 26 as granted, amended as follows:

"23. A nucleotide sequence which is the 3447 base sequence as described in Figure 12, or a portion thereof, said portion being

- the sequence illustrated in Figure 11A
- the 710 base pair fragment obtainable by amplification of the sequence illustrated in Figure 12, with the primers CAGGACGTCGAGGCGATCAC and AACGACGACGTGGCCAGCGT;
- the nucleotide sequence extending from nucleotides 1195 to 1293 in Figure 12, encoding the amino acid sequence illustrated in Figure 11B."

Claim 24 corresponded to claim 27 as granted amended as follows:

"24. A nucleotide sequence which is the 432 base sequence described in Figure 13, or a portion thereof said portion being:

- a sequence including the codons 400-450 of the rpoB gene;
- the nucleotide sequence extending from nucleotides 169 to 267 in Figure 13, encoding the amino acid sequence illustrated in Figure 11B."

Dependent claims 25 and 26, corresponding to claims 28 and 29, were now dependent on claims 23 or 24 and claim 25, respectively.

Claim 27 corresponded to claim 30 as granted, amended as follows:

"27. Nucleic acid sequence comprising a 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56."

Dependent claim 28, corresponding to claim 31 as granted, now depended on claim 27 and read:

"28. Nucleic acid sequence according to claim 27 comprising a 4.5 kb KpnI fragment of plasmid pYZ56, wherein said fragment contains a BamHI cleavage site."

Claim 29 corresponded to claim 32 as granted now referring to the sequences of any of claims 21 to 28.

IX. The arguments of appellant I may be summarised as follows:

Late filed documents

– Both documents (Da2) and annex 13 should not be allowed into the proceedings as they were not more relevant than the documents cited.

Added subject-matter

– Support for the missing wording "complementary" in claim 11 as compared to claim 16 as originally filed found support in the passages at page 19, lines 7 to 11 and page 51, line 30 to page 52, line 3 of the description as originally filed.

Sufficiency of disclosure

– As could be taken from post-published document (P4), which reported the results of studies investigating the nature of nucleotide substitutions in 26 different structural genes in Mycobacterium tuberculosis and three other members of the M. tuberculosis complex, it was widely recognised in the scientific community that mutations in Mycobacterium are almost invariably associated with antibiotic resistance. In fact greater than 95% of nucleotide substitutions caused amino acid replacements or other mutations in gene regions linked to antibiotic resistance (see (P4), page 9870, last 5 lines to page 5871, line 1) which was a "*striking lack of silent substitutions in M. tuberculosis complex members from global sources*"

(page 5871, line 10 to 12). With regard to the rpsL gene, document (P4) taught that of 178 strains analysed from diverse geographical localities, only *one* silent polymorphic site was identified (see Table 1). Accordingly, the detection of a mutation in the rpoB and rpsL genes of *Mycobacterium* as taught in the patent provided an indication of antibiotic resistance with greater than 95% certainty. On the basis of the teaching of the patent the skilled person can thus detect mutations, and consequently resistance to antibiotics, without undue effort.

– the skilled person was in a position to identify "fragments" of the genes as defined in claims 1, 17 and 19 and knew that they had to be large enough to hybridise to the indicated sequences.

– The nucleotide sequence of claim 25 was required to be "according to" either claim 23 or claim 24. There could therefore be no doubt about the nature of the claimed sequence.

Right to priority

based on document P1 and the fourth priority document

– The claimed embodiments which relate to the katG gene, except the aspects in claims 15 and 16 relating to the defined "fragments" of the katG gene, can enjoy as relevant date that of the first priority document (P1), whereas the relevant date for the rpsL embodiments was the filing date of the fourth priority document.

based on priority document P3

– There was no legal basis for a requirement that the extent of the priority right is determined by the content of a certified copy of an earlier application. The validity of a priority claim should either be acknowledged and the extent of the priority right determined on the basis of the prior application as filed. The priority claim should not be acknowledged if no certified copy of the prior application is filed. If the figures corresponding to figures 12 and 13 of the patent were indeed missing from the copy of P3 sent by INPI to the International Bureau, then it cannot be considered that the certified copy had been transmitted seeing that such would, by definition, have contained these figures, as does the certified copy received from INPI by appellant I and which was dated 9 April 2001. If this copy were incomplete and would be considered as constituting the required certified copy, then appellant I should have been given the opportunity to correct this deficiency. The EPO had, however, never issued a notification inviting appellant I to file a complete certified copy. On the basis of the available evidence, appellant I has thus fulfilled the requirements for a valid priority claim. The extent of the right to priority is therefore to be determined on the content of priority application P3 as filed.

– Seeing that priority application P3 covers all the claimed embodiments relating to the rpoB gene, except for claim 22, these remaining claims are

entitled to the filing date of priority application P3.

Novelty

None of the prior art documents disclosed any of the different claimed embodiments.

Inventive step

– If the closest prior art for the assessment of inventive step of claim 1, i.e. the rpoB and the rpsL embodiments, was in both aspects document (D1), then the problem to be solved by the invention was the provision of a detection method for rifampicin or streptomycin. The solution in the patent was then a detection method of mutations in specific mycobacterial genes which had been unidentified in the prior art. Nothing in the cited prior art rendered this subject-matter obvious.

– Closest prior art for the assessment of inventive step of claim 2, relating to the katG embodiment, was document (D6). Neither the disclosure of document (D6) taken alone, not combined with that of any of the cited prior art, rendered the claimed invention obvious when solving the problem of providing a method for the detection of isoniazid resistance in M. tuberculosis.

– Closest prior art for the subject-matter of claim 15 was document (D1) leading to the problem to be solved to provide means detect resistance to isoniazid irrespective whether the catalase activity

is present or not. The claimed solution was not rendered obvious to the skilled person by the cited prior art.

X. The arguments of appellant II may be summarised as follows:

Late filed documents

– Document (Da2) was not more relevant than e.g. document (D5) or (D9).

Added subject-matter

– The deletion of the term "complementary" from the wording of claim 16 as originally filed resulting in the corresponding wording of claim 11 of the new main request went beyond the content of the application as originally filed (Article 100(c) EPC). The original wording "complementary oligonucleotide probes" referred to probes containing the exact complementary sequence of the target DNA to which they hybridize, i.e. they require an exact match with the target DNA sequence. The term was more limiting than the term "oligonucleotide probes recognizing (the target DNA) under stringent conditions" as now present in claim 11 of the new main request. Furthermore, the passages in the description referred to by the opposition division for accepting the amendment (i.e. page 17, last paragraph and page 19, second paragraph of the application as originally filed) merely referred to the "isoniazid resistance" embodiments and not to other resistances.

Sufficiency of disclosure

– The subject matter of claims 1, 17 and 19 related to a method or kit for the detection of mutations in the rpoB and rpsL genes which are not characterised by their exact sequence, but by their capability of hybridizing to gene sequences as depicted in figures 13 or 14, or fragments thereof. The sequences provided for in annexes 1 to 5 demonstrated that there exists sequence variability in the mycobacterial rpoB and rpsL genes which does not cause antibiotic drug resistance. The patent therefore did not teach the skilled person in a sufficient manner how to distinguish between mutations in rpsL genes which confer resistance to the respective antibiotics and those which do not and cause merely sequence variability, a knowledge which is indispensable for carrying out the method of claim 1 or prepare the kits of claims 17 and 19.

– it was an undue burden for the skilled person to identify "fragments" of the genes as defined in claims 1, 17 and 19 which would fulfil the function as required in the claims, i.e. "*capable of hybridizing with the sequence shown in Figure*" 13 or 14.

– Dependent claim 25, relating to a sequence comprising a mutation in the region 400-450 refers to both independent claims 23 and 24, which however refer to the sequence of figure 12 or 13, respectively. It was undue burden for the skilled

person to determine to which figure the region indicated in claim 25 referred to.

Right to priority

based on priority document P1 and the fourth priority document

– The embodiments claimed which relate to the katG gene, except the aspects in claims 15 and 16 relating to the defined "fragments" of the katG gene as defined, could enjoy the filing date of priority document P1, whereas the relevant date for the rpsL embodiments was the filing date of the fourth priority document.

based on priority document P3

– From the evidence presented in annexes 10 to 12 it was clear that priority document P3 as filed with the IB was not complete as it lacked figures 3 and 4 corresponding to figures 12 and 13 contained in the patent. Accordingly, any presently claimed subject-matter defined by reference to these figures cannot enjoy as relevant date the filing date of document P3. Consequently documents (D2) to (D4), all published before the filing date for the international application but after the filing date for priority application P3 are contained in the prior art relevant for such subject-matter.

Novelty

– During oral proceedings, appellant II has merely maintained the objection that the subject-matter of claim 1 relating to the **rpsL** embodiment was not novel over the disclosure in document (D5) on page 390, lines 18 to 20 where it was disclosed that in M. smegmatis high level resistance to streptomycin arose by a single mutation in a gene *str* (a gene which corresponded to the rpsL gene). This implied that streptomycin resistance could be detected by mutation in this gene and based on this information the skilled person would know numerous methods for detection of a mutation in this rpsL gene. As M. smegmatis was closely related to M. tuberculosis, the rpsL gene of M. smegmatis would, as evidenced by annex 6 (sequence alignment of the sequence of figure 14 with the M. smegmatis rpsL gene sequence) evidently hybridise with the sequence of figure 14 as required in claim 1.

Inventive step

– For both embodiments of claim 1, document (D5) represented the closest prior art, which described the mode of action of antimyobacterial agents, including rifampicin and strepromycin, and associated aspects of the molecular biology of the mycobacteria (see title). The problem to be solved was then the provision of the respective mycobacterium rpoB and rpsL genes. Based on routine experimentation and the similarities indicated in document (D5) such isolation did not involve an inventive step.

– Closest prior art for the subject-matter of the claims relating to the katG embodiment was document (D6). The mere identification of a particular fragment of the katG gene for formulating the claimed solution could not contribute to an inventive step as it was obvious to the skilled person to use the E. coli or Bacillus gene for identifying the mycobacterial gene.

– The relevant date for the subject-matter of claim 15 was the filing date of the international application. Document (D1) represented therefore the closest prior art. The formulation of a kit for the implementation of the method as disclosed in document (D1) did not involve an inventive step.

XI. Appellant I requested that the decision under appeal be set aside and that the patent be maintained in amended form on the basis of claims 1 to 29 of the new main request filed at the oral proceedings and a description yet to be adapted thereto.

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

Reasons for the Decision

Admission into the proceedings of late filed document (Da2) and annex 13

1. Both documents (Da2) and annex 13 were filed by appellant II with a letter dated 2 May 2006, i.e. after

the time limit for submitting written submissions set in the board's communication accompanying the summons to oral proceedings (see sections VI to VII).

The documents are thus considered as having been late filed. Accordingly, the criteria established in the jurisprudence of the Boards of Appeal for late filed documents (cf. "Case Law of the Boards of Appeal of the European Patent Office", 4th edition 2001, chapter VI.F.3.1) apply. No exceptional circumstances that could justify the late filing of these documents have been advanced by appellant II. Furthermore, during the oral proceedings, appellant II agreed that the content of document (Da2) was not more relevant than the content of e.g. document (D5) or (D9). The board considers also late filed annex 13 not to be more relevant for the present decision than e.g. annex 11, i.e. a fax addressed to appellant II concerning the corresponding file at the USPTO and stating that figures corresponding to figures 12 and 13 of the patent were absent from the priority document P3 contained in this file. From the above the board concludes that late filed documents (Da2) and annex 13 do not add any further elements which might convince the board to adopt a different position as regards the issues being judged, and ultimately change the outcome of the decision. The board does therefore not admit these documents in the proceedings.

Added subject-matter

2. Concerning the issue of added subject-matter relating to the ground of opposition under Article 100(c) EPC, appellant II has only maintained its objection as to

the deletion of the term "complementary" from the wording of the second method step of claim 16 as originally filed, which qualified the oligonucleotide probes used for hybridizing to the fragments as obtained in the first step of the process, thereby resulting in the wording of claim 11 of the new main request. Appellant II argued in particular that the original wording required the oligonucleotide probes used to exactly match the sequence of the target DNA, whereas the amended wording did no longer require such an exact match with the target DNA sequence.

2.1 The description of the application as originally filed contains at page 19, lines 7 to 11 the following passage: "*Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for hybridisation between the probe and the polynucleotide for duplex formation.*" and on page 51, line 30 to page 52, line 3 the following passage: "*The invention relates also to the "mutated" DNA fragments. They can in turn be used as hybridisation probes for use for the detection in suitable hybridization procedures and for the detection of similar mutation in DNA extracted from a M.tuberculosis strain suspected to include resistance to any one of the above illustrated antibiotics.*".

2.2 This passage does not discriminate between the various antibiotic resistance embodiments contained in the application and forms a basis for the use of oligonucleotide probes which do not require an exact match with the target DNA. Accordingly, the amendment

does not introduce subject-matter which extends beyond the content of the application as filed.

3. Appellant II has not objected to any of the further amendments introduced in the claims of the new main request as compared to the claims of the patent as granted. The board is satisfied that also these amendments comply with the requirements of Article 123(2) EPC.

3.1 The amendments to claims 1, 2, 10, 14, 16, 24, 30 and 31 as granted, resulting in claims 1, 2, 8, 11, 13, 21, 27 and 28 of the main request, respectively, amounting to the **deletion of alternative embodiments** from the claimed subject-matter do not infringe the requirements of Article 123(2) EPC. The board is furthermore satisfied that the amendments in claims 9, 10, 12, 14, 16, 18, 19, 21, 25, 26, 28 and 29 of the main request concerning adaptations of the claim references are also conform Article 123(2) EPC.

3.2 The amendment to claim 2 of the new main request amounting to the **deletion of the dependency on claim 1** finds a basis in the application as filed on page 4, lines 4 to 16. The amendment to the claim qualifying the probe used in the process of detection, i.e. "wherein isoniazid-resistant Mycobacterium tuberculosis do not contain DNA which hybridises with this fragment" finds support on page 4, last line to page 5, line 9 of the application as filed.

3.3 The amendment to claim 8 of the main request relating to the wording "which encodes a polypeptide of the formula APLNSWPDNASLDKARRLLWPSKKKYGKKLSWADLIV",

qualifying the part of the fragment finds support in claim 4 in combination with page 15, lines 5 to 13 of the application as filed.

3.4 The amendments to claim 23 of the new main request finds a basis in figure 11A; page 50, line 2 in combination with figure 10, page 47, lines 31 to 32 and page 49, table; and figure 11B, in combination with the legend of figure 11 at page 47, lines 24 to 31, and page 50, lines 15 to 18.

3.5 The amendments to claim 24 of the new main request finds a basis in page 50, lines 22 to 24, in combination with page 48, lines 1 and 2, page 45, lines 19 to 23; and figure 11B, in combination with the legend of figure 11 at page 47, lines 24 to 31, and page 50, lines 15 to 24.

3.6 In view of the above, the board concludes that the claims of the main request do not violate the requirements of Article 123(2) EPC.

Article 123(3) EPC

4. Appellant II has not objected to the claims of the main request under Article 123(3) EPC. The board is satisfied that in particular the amendment of dependent claim 2 as granted resulting in independent claim 2 of the main request is in conformity with the requirements of Article 123(3) EPC seeing that claim 2 now specifies that the probe is the 2.5 kb EcoRV-KpnI fragment of plasmid pYZ56. This probe can only hybridise with the katG gene as originally specified in claim 1. Claim 2 of the main request therefore does not extend the

protection conferred by claim 1 of the patent as granted.

The remaining amendments amount to deletions of alternative embodiments from or to limitations of the claimed subject-matter as granted and hence do not infringe the requirements of Article 123(3) EPC.

The board therefore concludes that the claims of the main-request complies with the requirements of Article 123(3) EPC.

Sufficiency of disclosure

5. The board considers that the patent discloses a straightforward correlation of mutations in the mycobacterium katG, rpoB and rpsL genes with resistance phenotypes against isoniazid, rifampicin and streptomycin, respectively, processes for the detection of such resistances by the detection of mutations in those genes as well as the appropriate tools for implementing such detection processes based on particular sequences which sufficiently disclose the claimed invention.

6. Appellant II has pursued three objections in relation to the subject-matter of the claims of the main request under the heading of the ground for opposition defined in Article 100(b) EPC. Although the board is of the opinion that all three arguments rather relate to the clarity of the claims than to sufficiency of disclosure of the claimed invention, the board will nevertheless answer these objections.

6.1 Firstly, appellant II has argued that since there exists sequence variability in the rpoB and rpsL genes of mycobacteria which does not give rise to antibiotic resistance, the patent fails to teach the skilled person in a sufficiently clear and complete manner how to distinguish between mutations in rpoB and rpsL genes which confer resistance to the respective antibiotics and those which do not and cause merely sequence variability. Without this knowledge the skilled person was not in a position to carry out the method of claim 1 or prepare the kits of claims 17 and 19.

6.2 The board notes that claims 1, 17 and 19 of the new main request relate to a **process for the detection of a resistance to an antibiotic in a mycobacterium which comprises** bringing into evidence mutations in a rpoB or rpsL gene, respectively, and to **kits comprising certain compounds for the in vitro diagnostic of the resistance of a mycobacterium to rifampicin or streptomycin, respectively,** based on similar principles, whereby all three claims define the relevant reference genes by their capability of hybridizing with the sequence shown in either figure 13 or 14, containing the DNA sequences from the rpoB and rpsL genes of rifampicin and streptomycin sensitive Mycobacterium tuberculosis, respectively.

6.3 The argument of appellant II aims at the fact that the patent does not teach a 100% correlation of a detection of a mutation in the respective gene with the occurrence of resistance to the respective antibiotic. The board considers however that the detection of the mutation in the respective gene is a first step in the process of claim 1, which step however, on its own

already, not necessarily would lead to the detection of the resistance. This understanding is in line with the formulation of process claim 11, which is directly dependent on claim 1. Claim 11 states that a crucial step of the process consists in relating the absence of hybridization of at least one of oligonucleotide probes, which recognise under stringent conditions all of the parts of the relevant gene of the mycobacterium under study to any of the DNA fragments of the relevant gene of the mycobacterium under study as evidence of the presence of a mutation **and, possibly, of a resistance to the corresponding antibiotic,** particularly as compared to results obtained upon running the test under the same conditions with the same oligonucleotides on the relevant gene(s) obtained from a strain (strains) not resistant to said antibiotic. The board considers that the further step in the process of claim 1, i.e. correlating the detected mutation in a particular gene with a particular antibiotic resistance, does not constitute an undue burden for the skilled person in view of the fact that the patent teaches that such correlations can be determined. Therefore, the fact that possibly not all detected mutations lead to the presence of a resistance does not impair the reproducibility of the invention.

6.4 Accordingly, a 100% correlation of the detection of a mutation in the respective gene with the occurrence of resistance to the respective antibiotic is not required to carry out the invention so that the argument of appellant II must fail.

6.5 It was furthermore not contested by appellant II that it is now widely recognised in the scientific community

that mutations in Mycobacterium are almost invariably associated with antibiotic resistance. Indeed, it can be taken from post-published document (P4) in the paragraph bridging pages 9870 and 9871, that greater than 95% of all nucleotide substitutions in 26 tested genes of mycobacteria (including rpoB and rpsL) caused amino acid replacements or other mutations in gene regions linked to antibiotic resistance and were driven to high frequency by direct drug slection. Accordingly, post-published evidence shows that the detection of a mutation in the rpoB and rpsL genes of *Mycobacterium* provides an indication of antibiotic resistance with more than 95% certainty. Hence, based on the teaching of the patent the skilled person can thus detect mutations, and consequently resistance to antibiotics, without undue effort.

6.6 The board considers that the above considerations also apply to the subject-matter of claims 17 and 19. Indeed, these claims relate to kits which are suitable for the in vitro diagnostic of a mycobacterium to either rifampicin or streptomycin which also involve the bringing into evidence of mutations in the respective genes.

7. Appellant II has furthermore argued that it was undue burden for the skilled person to identify "fragments" of the genes as defined in claims 1, 17 and 19 which would fulfil the function as required in the claims, i.e. "*capable of hybridizing with the sequence shown in Figure*" 13 or 14 and be determinant for the detection of mutations leading to resistance. The board however considers, in agreement with appellant I, that the skilled person at the relevant date would have been in

a position to identify such fragments of the sequences in figures 13 and 14 as they merely must be large enough to hybridise to the indicated sequences which then easily can be detected.

8. Finally, appellant II has put forward that dependent claim 25, relating to a sequence comprising a mutation in the region 400-450 refers to both independent claims 23 and 24, which however refer to the sequence of figure 12 or 13, respectively. It was undue burden for the skilled person to determine to which figure the region indicated in claim 25 referred to.

9. The board can however not concur with this argument under the heading of the ground of opposition under Article 100(b) EPC. In fact, the DNA of claim 25, as it stands, is by reference required to be "according to" the nucleic acid sequences of claims 23 or 24 and to comprise a mutation localised in the region 400-450. Appellant II has not submitted any verifiable facts why the skilled person in the art was, at the relevant date, not in a position to produce and identify such nucleotide sequences which comply with the requirements of claim 25.

10. In view of the above considerations the board concludes that no case has been made out which should lead to the conclusion that the claimed subject-matter is not sufficiently disclosed in the patent, which therefore fulfils the requirements of Article 83 EPC.

Right to priority

based on priority documents P1 or the fourth priority document

11. During the oral proceedings, the parties were in agreement that the embodiments claimed in the new main request which relate to the katG gene, except the aspects in claims 15 and 16 relating to the defined "fragments" of the katG gene as defined, can enjoy as relevant date for determining the prior art in accordance with Articles 54(2) and 89 EPC the filing date of priority document P1, whereas the relevant date for the rpsL embodiments was the filing date of the fourth priority document. In view of the fact that these findings were undisputed among the parties the board sees no necessity to decide on these point.

based on priority document P3

12. Appellant II has challenged the validity of the priority claim based on priority document P3 for the claimed embodiments relating to the rpoB gene based on the argument that priority document P3 (FR92/11098), as transmitted by the IB and contained in the EPO file pertaining to the present case, did not include figures corresponding to the figures 12 and 13 of the patent as granted. Therefore, the relevant date for any claims referring to these figures 12 and 13 and in particular independent claims 1, 11, 17, 19, 23 and 24 of the main request relating to the rpoB gene embodiments could not be the filing date of P3 but had to be the filing date of the international application.

13. Pursuant to Article 8(2)(a) PCT in conjunction with Article 4A(1) and 4C(1) Paris Convention a person who has duly filed an application for a patent, shall enjoy, for the purpose of filing an international patent application in respect of the **same invention**, a right of priority during a period of twelve months from the date of filing of the first application. Accordingly, it needs to be established whether or not the French patent application FR 92/11098, which was filed on 17 September 1992, contained figures corresponding to the figures 12 and 13 of the patent.

Appellant I has filed a certified copy (copie officielle) issued by the INPI which is dated 9 April 2001 of an application for a industrial property right filed with INPI which has the number FR 92/11098 and was filed on 17 September 1992, i.e. the priority application P3. This certified copy contains figures 3 and 4 the contents of which is identical to figures 12 and 13 contained in the patent.

14. Appellant II has filed two documents which are of relevance for determining the validity of the priority claim of subject-matter related to the subject-matter as contained in figures 12 and 13 of the patent based on priority document P3. A first document is designated annex 3 and was filed with the ground of opposition and contains confirmation from the EPO that priority document P3 as transmitted by the International Bureau and contained in the EPO dossier does not contain figures corresponding to figures 12 and 13 of the patent. A second document is annex 10 which constitutes a certified copy established by the IB and dated 10 September 2003 of the certified copy of P3 as

established by the INPI and transmitted to the IB under Rule 17.1 PCT.

15. From the document filed by appellant I the board takes it that, upon request, INPI in 2001 was issuing a certified copy of its application FR 92/11098 which contained figures corresponding to the figures 12 and 13 of the patent. From the annex 3 it can be taken that the priority document contained in the EPO file, and transmitted to the EPO by the International Bureau, is devoid of these same figures. The same is apparently true for the corresponding dossiers relating to the American and Japanese counterparts (see annexes 11 and 12). Thirdly, from annex 10, also submitted by appellant II, it can be taken that the "certified copy" of 10 September 2003 as established by the International Bureau of the certified copy of application FR 92/11098 as established by INPI on 7 June 1993 and transmitted to the International Bureau under Rule 17.1 PCT was equally devoid of these figures.

16. The board considers that from the above evidence there is a *prima facie* assumption that the figures in question were contained in the application filed with INPI. This, however, is put into question by the documents filed as priority documents. The board is therefore confronted with contradicting evidence each of which as such has to be taken as a reliable source of facts. It can hence not straightforwardly be taken whether or not figures 3 and 4 corresponding to figures 12 and 13 of the patent were contained in the French patent application FR 92/11098 as filed at INPI on 17 September 1992.

17. The question of the validity of the priority based on P3 therefore needs to be resolved by free evaluation of the facts described above and by taking into account considerations as to which party in these opposition/appeal proceedings has the burden of proving the exact content of priority document P3.

18. Appellant II has challenged the priority date of the subject-matter related the rpoB embodiments claimed, in particular such subject-matter defined by reference to figures 12 and 13 of the patent. This date is necessary for establishing the prior art available on file for examining novelty and inventive step of this subject-matter. Accordingly, following the principles as established in the case law, appellant II also bears the burden of proving by convincing evidence that the relevant date for this subject-matter is not the filing date of priority document P3. The board accepts that the weight of a priority document is considerable and could provide the desired certainty. Here however, appellant II, in the light of the documents showing that in the original application filed with INPI the figures were contained, bears the burden of proof to convince the board that this was not so. Such proof has not be brought by appellant II. It is thus not proven that figures 3 and 4 corresponding to figures 12 and 13 of the patent were absent from the documents filed at the INPI on 17 September 1992 and leading to French patent application FR 92/11098. Accordingly the board decides that the relevant date for establishing the state of the art for independent claims 1, 11, 17, 19, 23 and 24 of the new main request relating to the rpoB gene embodiments is the filing date of document P3.

19. To summarise,

- the katG embodiments as contained claims 2 to 10 and 27 to 29 of the main request can enjoy the first priority date, whereas the embodiments in claims 15 and 16 have as relevant date the filing date of the international application. Consequently, document (D1) is no prior art for the subject-matter of claims 2 to 10 and 27 to 29 of the main request.

- The rpoB embodiments as contained in claims claims 1, 11, 17, 19, 23 and 24 can enjoy as the relevant date the filing date of third priority document. Consequently, documents (D2) to (D4) do not constitute prior art for these embodiments. Claim 22 enjoys as relevant date the filing date of the fourth priority document.

- The rpsL embodiments have the filing date of the fourth priority document as relevant date.

Novelty

20. The board has now to examine whether the disclosure in the cited documents contained in the prior art relevant for the three different claimed embodiments is prejudicial to the novelty of the claimed subject-matter of the new main request.

21. Appellant II has argued that the subject-matter of claim 1 relating to the rpsL embodiment is not novel over the disclosure in document (D5) on page 390, lines 18 to 20. In particular, document (D5) disclosed that in M. smegmatis high level resistance to

streptomycin arose by a single mutation in a gene *str* (a gene which corresponded to the rpsL gene). This implied that streptomycin resistance could be detected by mutation in this gene. Based on this information the skilled person would know numerous methods for detection of a mutation in this rpsL gene. As M. smegmatis was closely related to M. tuberculosis, the rpsL gene of M. smegmatis would, as evidenced by annex 6 (sequence alignment of the sequence of figure 14 with the M. smegmatis rpsL gene sequence) evidently hybridise with the sequence of figure 14 as required in claim 1. Therefore document (D5) implicitly disclosed the process of detection of a mutation in the rpsL gene of mycobacteria as claimed in claim 1.

22. Appellant II has not argued that the process of claim 1 relating to the rpsL gene is as such disclosed in document (D5) or document (D3), but rather that, in document (D5), the scientific presentation of the fact of resistances or the designation of S12 as a potential target of streptomycin implicitly disclosed the detection method of claim 1. The board, in accordance with established principles of case law of the boards of appeal, considers however that in order to be novelty-destroying, a prior art document has to contain a clear, unambiguous and unmistakable disclosure for the skilled person of the subject-matter of a claim in question (cf. e.g. T 204/83 OJ EPO 1985, 310; T 776/96 of 23 September 1997, T 677/91 of 3 November 1992 or T 838/97 of 14 November 2000). There must be no doubt that the prior disclosure, as read by the skilled person, unambiguously corresponds in all its technical features to the subject-matter as claimed. Such is not the case here as document (D5) does not disclose a

method of detection of a resistance in a mycobacterium rpsL gene, which is capable of hybridising to a specific sequence. In particular the document does not disclose any such specific mycobacterium rpsL gene sequence.

23. Therefore, in accordance with the above referred to principles the argument of appellant I must fail.

Inventive step

24. The claims of the new main request comprise three main embodiments, i.e. those relating to the rpoB gene and rpsL gene and those relating to the katG gene, respectively. The three embodiments and the claims which relate to them enjoy different priority dates (see point 20, above), will be assessed separately. Moreover, as the reasoning in favour of inventive step for the embodiments relating to the rpoB gene and rpsL gene embodiments is very similar, these two embodiments will be dealt with simultaneously.

25. For assessing whether or not a claimed invention meets the requirements of Article 56 EPC, the boards of appeal normally apply the "problem and solution" approach, which requires as a first step the identification of the closest prior art. In accordance with established case law of the boards of appeal the closest prior art is a teaching in a document conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common, i.e. requiring the minimum of structural modifications to arrive at the claimed invention.

The rpoB and rpsL embodiments

26. The relevant claims in the new main request for the rpoB embodiments are claims 1, 11 to 14, 17, 18, 23 to 26 and 29 whereas the relevant claims for the rpsL embodiments are claims 1, 11 to 14, 19 to 22 and 29.

27. For both embodiments of claim 1, appellant II has considered document (D5) to represent the closest prior art which describes the mode of action of antimyobacterial agents, including rifampicin and strepromycin, and associated aspects of the molecular biology of the mycobacteria (see title). In section 3 of document (D5), dealing with the action of rifampicin in mycobacteria (page 368, line 34 ff.), it is *inter alia* stated that the action of rifampicin is similar in mycobacteria and E. coli, i.e. it acts as an inhibitor of RNA synthesis (see page 369, lines 16, 17 and 31 to 34) and that mutation to resistance to high concentrations of rifampicin can occur in mycobacteria, as in E. coli, in a single step (see page 371, lines 6 to 9). The document is silent on the particular gene involved in the mutational development of resistance to rifampicin in mycobacteria. Similarly, as far as the claimed subject-matter related to the rpsL gene and streptomycin resistance is concerned, document (D5) discloses in the section dealing with the action of streptomycin in mycobacteria (page 389, line 7 ff.) that all the indications are that the mechanism of action of streptopmycin in mycobacteria is similar to that in E. coli (see page 389, lines 29 to 30) and that in M. smegmatis high-level resistance to steptomycin arises by a single mutation change in a gene *str*,

specifying a component of the 30S subunit (see page 390, lines 18 to 20).

28. The subject-matter of claim 1 is a process for the detection of a resistance to an antibiotic in mycobacterium, which comprises detecting a mutation in a gene. Therefore, and in accordance with the principles of case law referred to above, the board considers, rather than document (D5), document (D1) to represent the closest prior art for both embodiments in claim 1. Indeed, document (D1) discloses the use of mycobacterial genetics to study the molecular basis of INH resistance. A single M. tuberculosis gene, katG, encoding a catalase, restored sensitivity to INH in a resident mutant of M. smegmatis and conferred INH susceptibility in some strains of E. coli (see Abstract lines 11 to 16). The authors of document (D1) note that in many INH resistant isolates of M. tuberculosis a decreased catalase activity is observed (see p.592, left hand column, lines 11 to 13) and could conclude that in a subset of the tested INH resistant strains the loss of catalase activity is due to deletion of the catalase gene (see page 592, right hand column, lines 14 to 16). Accordingly, document (D1) is concerned with the detection of mutations in particular genes correlating with the occurrence of antibiotic resistance in M. tuberculosis.

29. The problem to be solved by the invention as subject-matter of claim 1 can therefore be considered as the provision of a detection method for resistance of mycobacteria to either rifampicin or streptomycin.

30. The board is satisfied that the subject-matter of claim 1, i.e. the method involving the detection of a mutation in the rpoB or rpsL gene of the mycobacterium, solves this problem and appellant II has not contested this finding.

31. Document (D1) itself does not provide a solution to the above technical problem as it does not address the molecular biology or the action of rifampicin and streptomycin in mycobacteria. The only document contained in the prior art relevant for the embodiments in claim 1 which addresses the mode of action of rifampicin and streptomycin in mycobacteria and the development of resistance thereto is document (D5). It therefore needs to be established, whether or not document (D5) when combined with the teachings of document (D1) renders the detection of mutations in the rpoB or rpsL genes of mycobacteria obvious when addressing the above formulated problem.

32. Document (D5) neither in the section dealing with rifampicin resistance in mycobacteria (page 368, line 34 ff.) nor in relation to streptomycin resistance of mycobacteria (page 389, line 7) identifies either the mycobacterial rpoB gene or the mycobacterial rpsL gene or gene product as being instrumental for the development of resistances to these antibiotics. In both the claimed processes as subject-matter of claim 1, however, the detection of mutations in these specific genes is part of the claimed solution to the above formulated problem.

33. The board therefore concludes that also the combination of the teaching of document (D1) with that of document

(D5) does not render the subject-matter of claim 1 obvious to the skilled person. Accordingly, the subject-matter of claim 1, and dependent claims 11 to 14, involves an inventive step. Furthermore, claims 17 to 20 are directed to kits for implementing the method of claim 1 and relate explicitly to the mycobacterial rpoB and rpsL genes, whereas claims 17 to 26 and 19 are directed to specific sequences and mutations of these genes. In accordance with the reasoning for the subject matter of these claims is also considered inventive.

The katG embodiments

34. The relevant claims for these embodiments are claims 2 to 10, 15, 16, 27 to 29. The relevant prior art for the assessment of the involvement of inventive step of the subject matter of claim 2 are documents (D5) to (D11).

35. The board agrees with the parties that document (D6) represents the closest prior art for the assessment of inventive step of the subject-matter of claim 2. It discloses that the catalase and peroxidase activities of mycobacteria are involved on the mechanism of isoniazid action (see page 196, left hand column, lines 12 to 15) and that in M. tuberculosis H37Rv the two activities are catalysed by a single protein (see page 196, left hand column, lines 27 to 29). The document furthermore describes the purification of this protein (see page 190, left hand column, line 7 ff.). On page 197, left hand column, lines 13 to 18 the authors of document (D6) additionally disclose that it is clear that a single mutation from isoniazid sensitivity to resistance in M. tuberculosis leads to

the loss of isoniazid uptake and a loss of catalase, peroxidase and Y-enzyme activities.

36. The board considers that, in view of the disclosure in document (D6) and in accordance with the problem as defined in the patent on paragraph [0041] the problem to be solved by the invention as subject-matter of claim 2 is the provision of a method for the detection of isoniazid resistance in M. tuberculosis.

37. The board is satisfied that the subject-matter of claim 2, i.e. the detection of the capability of hybridisation of the test genome to a particular genomic 2,5 kb DNA fragment isolated from a isoniazid sensitive strain and coding for the catalase/peroxidase enzyme, solves the above problem in view of the data presented in paragraph [0068] of the patent.

38. Document (D6) itself does not render the genetic detection method of claim 2 obvious to a skilled person as it does not go beyond the mere detection of the enzymatic activity associated with the resistance. While the board accepts that this is a necessary step for the development of a method as claimed, it does not constitute a hint to the genetic detection methods as claimed.

39. The relevant question is thus whether any other cited document contained in the prior art renders it obvious that the **deletion** of a particular and defined part of the M. tuberculosis, i.e. the gene coding for the catalase/peroxidase activity described in the prior art as represented by the specific 2,5 kb fragment defined

in the claim, provides isoniazid resistance in M. tuberculosis.

40. The only other cited prior art document dealing with isoniazid resistance in mycobacteria is again document (D5), a review document (see above point 27) which endorses the findings in earlier document (6) by stating that resistance to high concentrations of isoniazid in M. tuberculosis arises almost always by a single mutational step which involves also the loss of catalase and peroxidase activity and concluding that the catalase and peroxidase activity are due to the same enzyme (see page 403, lines 2 to 11, lines 33 to 35). The document is however silent on the nature of the gene coding for this catalase/peroxidase activity in M. tuberculosis, let alone the nature of the deletion in the gene which provides resistance. The board therefore considers that none of these the prior art documents, either taken alone or combined with each other render it obvious to the skilled person that detection of the absence of specific genomic sequences coding for the catalase/peroxidase gene correlates with the occurrence of isoniazid resistance.

41. In view of the above considerations the board considers the subject-matter of claim 2 to 10 to involve an inventive step. Furthermore, claims 27 to 29 relate explicitly to the mycobacterial katG gene. In accordance with the reasoning for the subject matter of claim 2 these claims is also considered inventive.

42. Since the priority date of the subject-matter of claims 15 and 16 is later than the publication date of document (D1), this document is contained in the prior

art. The board agrees with the parties that this document represents the closest prior art for the assessment of inventive step of the subject-matter of claim 15.

43. As already mentioned in point 28 above, document (D1) discloses that a single M. tuberculosis gene, i.e. the katG gene encoding a catalase/peroxidase, was able to restore sensitivity to INH in a resident mutant of M. smegmatis and conferred INH susceptibility in some strains of E. coli (see Abstract lines 11 to 16). Furthermore, in many INH resistant isolates of M. tuberculosis a decreased catalase activity was observed (see p.592, left hand column, lines 11 to 13) and in a subset of the tested INH resistant strains the loss of catalase activity was due to deletion of the catalase gene (see page 592, right hand column, lines 14 to 16). The authors report that *"[g]ene deletion represents an unexpected and unusual mechanism for the development of drug resistance. Effective drugs must be active against cell components that are essential for bacterial viability, and resistance is generally conferred either by an altered structure of the drug target, or by acquisition of an effective drug degradation system or permeability barrier. It is likely that in M. tuberculosis other forms of INH resistance may also occur. Inactivation of the catalase-peroxidase gene by movement of an insertion element, or point mutations, are attractive theoretical possibilities and screening of extended panels of INH-resistant isolates of M. tuberculosis will be required to assess the relative frequency of gene deletion compared with other potential mechanisms of INH resistance. The multiple-drug-resistant strains in*

which there is a correlation between INH resistance and decreased catalase activity are particularly important because, owing to the contagiousness of tuberculosis, these strains pose a public health threat to both HIV-infected and healthy individuals. An improved understanding of the mechanisms of drug resistance will enable rapid tests for drug-resistance isolates to be developed and should facilitate the design of antituberculosis drugs." (see page 592, right hand column, line 36 to page 593, right hand column, line 4).

44. Claim 15 is directed to a kit for the *in vitro* diagnostic of the resistance of a mycobacterium to isoniazid based on the identification of mutations in sequences amplified from the katG gene. The subject-matter of claim 15 differs from the teaching in document (D1), that it is concretely directed to a kit for the *in vitro* diagnostic and that it relates to the INH resistance phenotype of the strains to be tested to mutations in the katG gene. It has been argued by appellant I, and the board agrees therewith, that this kit enables the implementation of a method for the detection of INH resistance independent of the presence of catalase activity in a clinical isolate.

45. Starting from the disclosure in document (D1), the board considers that the problem to be solved by the invention as defined in claim 15 of the new main request is therefore the provision of means for the diagnostic in mycobacteria of resistance to isoniazid and this irrespective of the catalase activity of the isolate. The board is satisfied on the experiments disclosed in example 1 the application solves this problem. Appellant II has not contested this finding.

46. The board notes that neither document (D1) itself, nor any of the other documents contained in the prior art, renders a kit serving such purpose based on a method i) which requires the presence of katG gene sequences and ii) requires the identification of mutations in such sequences obvious to the skilled person.

47. For the above reasons the board considers the subject matter of claims 15 and 16 of the new main request to involve an inventive step.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent on the basis of claims 1 to 29 of the new main request filed at the oral proceedings and a description yet to be adapted thereto.

Registrar Chair

P. Cremona U. Kinkeldey