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Datasheet for the decision of 29 November 2021

Case Number: T 1608/16 - 3.3.08

Application Number: 08868000.4

Publication Number: 2231869

IPC: C12Q1/68, G01N33/48

Language of the proceedings: EN

Title of invention:
DETECTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

Patent Proprietor:
Biomerieux Sa

Opponents:
Euroimmun Medizinische Labordiagnostika AG
STRAWMAN LIMITED
James Poole Limited

Headword:
Detection of MRSA strains/BIOMERIEUX

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

Keyword:
Main Request - requirements of the EPC met (yes)

Decisions cited:

Catchword:

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Beschwerdekammern

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Case Number: T 1608/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 29 November 2021

Appellant I: (Opponent 2)	STRAWMAN LIMITED Orchard Lea Horns Lane Combe Witney, Oxfordshire OX29 8NH (GB) - appeal & opposition withdrawn -
Appellant II: (Opponent 3)	James Poole Limited One Southampton Row London WC1B 5HA (GB)
Representative:	Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)
Respondent: (Patent Proprietor)	Biomerieux Sa 100 Rodophe Street Durham, NC 27712 (US)
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Party as of right: (Opponent 1)	Euroimmun Medizinische Labordiagnostika AG Seekamp 31 23560 Lübeck (DE) - opposition withdrawn -
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 26 April 2016 concerning maintenance of the European Patent No. 2231869 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	A. Bacchin

Summary of Facts and Submissions

I. European patent No. 2 231 869 is based on European patent application No. 08868000.4 (published as International patent application WO 2009/085221) and was opposed on the grounds of Articles 100(a), (b) and (c) EPC. The opposition division considered the main request and auxiliary request 1 to contravene Article 123(2) EPC and took the view that auxiliary request 2 and the description adapted thereto complied with the requirements of the EPC.

II. The opponents 2 and 3 (appellant I and II respectively) lodged an appeal against the decision of the opposition division.

III. Together with its statement of grounds of appeal, appellant I submitted new documents D55 and D56.

IV. Appellant I withdrew both its appeal and its opposition with a letter dated 28 May 2019 and 26 May 2020 respectively. It ceased to be party to the proceedings. The appeal fee paid by Appellant I was reimbursed at 50% on 18 June 2019, in accordance with Rule 103(2) EPC as applicable at that time.

V. The party as of right (Opponent 1) withdrew its opposition with a letter dated 15 October 2020. It did not make any submissions in appeal proceedings. It ceased to be party to the proceedings.

VI. The parties were summoned to oral proceedings. The board sent a communication pursuant to Article 17(1) RPBA 2020.

VII. Appellant II informed the board with letters dated 18 September 2020 and 9 November 2020 that it would not attend the scheduled oral proceedings.

VIII. The patent proprietor (respondent) replied to the statement of grounds of appeal of appellants I and II and filed auxiliary requests 1 to 7 consisting of a new auxiliary request 1 and renumbered auxiliary requests 3 and 9-13 filed on 19 February 2016. It informed the board with letter dated 9 November 2020 that it would not attend the scheduled oral proceedings.

IX. Oral proceedings were cancelled.

X. Independent claims 1, 4, 14 and 26 of the main request read as follows:

"1. A method of detecting in a sample a methicillin-resistant Staphylococcus aureus (MRSA) having an insertion of an SCCmec cassette within Staphylococcus aureus chromosomal DNA, comprising:

performing on the sample an amplification and detection reaction utilizing:

- a) a first primer capable of specifically hybridizing in an extremity junction region of the SCCmec cassette,
- b) a second primer capable of specifically hybridizing in an extremity junction region of chromosomal Staphylococcus aureus DNA, and
- c) a probe capable of specifically hybridizing to a region of the SCCmec cassette between the region with which the first primer is capable of hybridizing and the junction,

wherein each of the first primer and the second primer is oriented such that, under amplification conditions, the junction is amplified, and

wherein if the sample contains MRSA, hybridization of the probe is detected.

4. A method of detecting in a sample a methicillin-resistant Staphylococcus aureus (MRSA) having an insertion of an SCCmec cassette within Staphylococcus aureus chromosomal DNA, the method comprising

a) performing on a sample a multiplex amplification reaction which can amplify both (1) a junction of an inserted SCCmec cassette and Staphylococcus aureus chromosomal DNA and (2) a region of mecA, wherein the amplification of the junction is performed utilizing

- 1) a first primer capable of specifically hybridizing in an extremity junction region of the SCCmec cassette,
- 2) a second primer capable of specifically hybridizing in an extremity junction region of chromosomal Staphylococcus aureus DNA, and
- 3) a first probe capable of specifically hybridizing to a region of the SCCmec cassette between the region with which the first primer is capable of specifically hybridizing and the junction,

wherein each of the first primer and the second primer is oriented such that, under amplification conditions, the junction is amplified; and

b) detecting, within the products of the amplification, the presence or absence of each of the junction and mecA,

wherein if the sample contains MRSA, the presence of both the junction and mecA in the sample is detected.

14. A kit for detection of methicillin-resistant Staphylococcus aureus (MRSA) having an insertion of an

SCCmec cassette within Staphylococcus aureus chromosomal DNA, comprising

a) a first primer capable of specifically hybridizing in a right extremity junction region of the SCCmec cassette,

b) a second primer capable of specifically hybridizing in a right extremity junction region of chromosomal Staphylococcus aureus DNA, and

c) a probe selected from the group consisting of (I) a probe capable of specifically hybridizing primarily within a region of the SCCmec cassette between the region with which the first primer is capable of hybridizing and the junction, and (2) a probe capable of specifically hybridizing fully within a region of the SCCmec cassette between the region with which the first primer is capable of hybridizing and the junction,

wherein each of the first primer and the second primer is oriented such that, under amplification conditions, the junction is amplified.

26. An oligonucleotide composition comprising:

(1) a first oligonucleotide primer having a nucleic acid sequence that specifically hybridizes to an extremity junction region of a SCCmec cassette;

(2) a second oligonucleotide primer having a nucleic acid sequence that specifically hybridizes to a Staphylococcus aureus chromosomal DNA region flanking said SCCmec cassette; and

(3) a first probe capable of specifically hybridizing to a region of the SCCmec cassette between the region with which the first primer is capable of hybridizing and the junction, wherein each of the first primer and the second primer is oriented such that, under amplification conditions, the junction is amplified."

The dependent claims define embodiments of the methods according to claims 1 and 4 or of the product according to claims 14 and 26.

XI. The following documents are cited in this decision:

D7: A. Huletsky *et al.* "New Real-Time PCR Assay for Rapid Detection of Methicillin- Resistant *Staphylococcus aureus* Directly from Specimens Containing a Mixture of Staphylococci", Journal of Clinical Microbiology, vol. 42 (5), pages 1875-84, (2004);

D8: US2007/0082340 A1 (publication date 12 April 2007);

D15: K. Oberdorfer *et al.* "Evaluation of a single-locus real-time polymerase chain reaction as a screening test for specific detection of methicillin-resistant *Staphylococcus aureus* in ICU patients." European Journal of Clinical Microbiology & Infectious Diseases, vol. 25 (10), pages 657-63 (2006);

D21: M. Holfelder *et al.* "Direct detection of methicillin-resistant Staphylococcus aureus in clinical specimens by a nucleic acid-based hybridisation assay." Clinical Microbiology and Infection, vol. 12 (12), pages 1163-7 (2006);

D24: EP0887424 A2 (publication date 28 August 1997);

D28: WO2009/018000 A1 (publication date 5 February 2009);

D32: US2005/0019893 A1 (publication date 27 January 2005);

D39: WO2007/044873 A2 (publication date 19 April 2007);

D41: M. Desjardins *et al.* "Evaluation of the IDI-MRSA Assay for Detection of Methicillin-Resistant Staphylococcus aureus from Nasal and Rectal Specimens Pooled in a Selective Broth" Journal of Clinical Microbiology, vol. 44 (4), pages 1219-23, (2006);

D54: Experimental data submitted on 19 February 2016 by former Appellant I/Opponent 2.

XII. The submissions made by Opponent 3/Appellant II, insofar as relevant to the present decision, may be summarized as follows:

Main request
Novelty

Document D8 disclosed a method of determining the MREJ sequences of MRSA strains, such as CCRI-12382 and CCRI-12383, which "harbored SCCmec type III and contained sequences specific to the yccr complex" (see paragraphs [0076] to [0078], [0076] last sentence, summarised in Figure 1, top diagram). Several elements were illustrated in a SCCmec-chromosome junction of MRSA MREJ types xiii and xiv: a first primer SEQ ID N°27 (targeting the xvccr complex sequence located in the SCCmec type III), a second primer SEQ ID N°44 (targeting the 5'end of the *orfX* gene) and a probe having the sequence of SEQ ID N°28. The primers with

SEQ ID N°27 and SEQ ID NO: 44 were capable of specifically hybridising in an extremity junction region of either the SCCmec cassette or of the chromosomal Staphylococcus aureus DNA respectively. Thus, the requirements of part (a) and (b) of claim 1 were fulfilled. These primers were capable of amplifying a mec right extremity junction "MREJ" fragment of the methicillin-resistant Staphylococcus aureus "MRSA".

The probe might be a nucleic acid probe. The use of any detection technology was also within the present invention (see paragraphs [0039] and [0042] of the patent). Since the internal sequencing primer SEQ ID N°28 was "capable of specifically hybridising to a region of the SCCmec cassette between the region with which the first primer is capable of hybridising and the junction" and allowed the determination of SEQ ID N°s: 25 and 26 of MRSA strains CCRl-12382 and CCRl-12383, said primer had to be seen as a probe according to claim 1 (see document D8 paragraph [0078]). The hybridisation of the primer with SEQ ID NO: 28 in the presence of MRSA was detected.

Document D24 described all the features of claim 1. The "mec-side" probes could be chosen from the nucleotide sequence corresponding to the mec region DNAs such as Figures 17 to 19 (see p.6, lines 5 to 7; Fig.7a). They hybridised to the cassette-side of the junction as required in claim 1 (c). The paragraphs (1), and (3) to (5) on page 5 and 6 described that primers and PCR were used to identify MRSA (paragraph (1)), that a MRSA could be identified by hybridisation with a probe (paragraph (3)), and that many primers or probes could be chosen from, e.g. on the cassette-side of the junction (paragraph (5)). The hybridisation step in

paragraph (3) clearly referred to the hybridisation of the probe to the target DNA when the target DNA was amplified.

Document D28 described a method to detect a fragment of the SCCmec cassette in a MRSA. The method comprised the use of a forward primer and a probe, in the form of a Scorpion™ primer/probe combination, that is "designed to specifically hybridize to the 3' end of sequence modified SCCmec" and may have a sequence of any of SEQ ID NOs: 3-9 and 16-24; and a reverse primer (see paragraph [0073]). The Scorpion™ probes consisted of: (i) a 3' portion comprising an oligonucleotide primer sequence, and (ii) a 5' portion comprising a hairpin structure which possessed a fluorophore/quencher pair. The 3' portion of the Scorpion™ probes was capable of "specifically hybridising to a region of the SCCmec cassette between the region with which the first primer is capable of hybridising and the junction" as required by part (c) of claim 1. The Scorpion primer/probe combined the primer and probe of part (a) and (c) of claim 1.

Document D28 disclosed furthermore a method for detecting Staphylococcus aureus in a biological sample comprising the step of contacting ... (iii) a third primer pair, one primer of which is complementary to a segment of SCCmec of the sequence modified nucleic acids and the other primer of which is complementary to a segment of the *orfx* gene of the sequence modified nucleic acids; so that an amplification products of the sequence-modified nucleic acids are produced; and (c) detecting the amplification product produced by one or more of the primer pairs (see claims 1 and 2).

Documents D8 and D24 implicitly and document D28 explicitly disclosed a kit and an oligonucleotide composition including the primers and probes recited in claim 1 (see document D28, in paragraph [0021]). Thus, they deprived at least independent claims 1, 4, 14 and 26 and of dependent claims 2, 3, 6, 8, 15, 18, 27 of novelty.

Inventive step (Article 56 EPC)

Claim 1 (at least embodiments 1 and 2)

- The first embodiment related to methods comprising the first and second primers targeting either side of a SCCmec-chromosome junction, and a probe hybridising to the cassette-side of the junction.

- The second embodiment related to methods wherein the first and second primers target either side of the SCCmec-chromosome junction, but the first primer targeted the mecA gene, and the probe hybridised to the mecA gene downstream from the first primer.

Embodiment 1:

Document D7 represented the closest prior art for embodiment 1 of claim 1.

It disclosed a real-time multiplex PCR assay for the detection of MRSA using five primers specific to different SCCmec right extremity sequences in combination with a primer and three molecular beacon probes specific for the *S. aureus* chromosomal *orfX* (cf.

abstract; p.1875, col.2, last paragraph; p.1876, col.1 paragraph 6 and col.2 paragraph 1 and 2; Table 3).

The difference between document D7 and the subject-matter of claim 1 was that the probe used for the detection of the amplification products was hybridising to the SCCmec sequence. Since no technical effect could be associated with this difference, the objective technical problem was the provision of a further method of detecting MRSA in a sample.

Document D21 used a PCR-based hybridisation assay (GenoType MRSA Direct) with a probe that hybridised to the SCCmec sequence. The PCR primers were specific to staphylococcal cassette chromosome mec (SCCmec) types I, II, Ill and IV, including the newly recognised community-acquired MRSA strain (page 1164, left column, second paragraph). The amplicons were detected using an oligonucleotide probe which targeted "the SCCmec chromosomal cassette of MRSA" (page 1165, second paragraph, "Hybridisation protocol"). All MRSA strains were tested positive, and all CoNS and the two MSSA strains were tested negative when using this assay (page 1165, "Results").

Starting from document D7 and faced with the problem of providing a further method of detecting MRSA in a sample, the skilled person would have turned to document D21 in which a probe hybridizing to the SCCmec cassette to detect MRSA was used. Thus, the subject-matter of claim 1 lacked an inventive step in view of document D7 in combination with document D21.

Embodiment 2:

Document D7 represented the closest prior art for embodiment 2 of claim 1.

The difference between document D7 and the subject-matter of embodiment 2 of claim 1 was the specific way of detecting MRSA by amplifying the junction and detecting mecA.

The technical problem was the provision of another method of detecting MRSA in a sample.

Document D8 related to "Sequences for detection and identification of MRSA" in which long range PCR reaction amplified both the junction and mecA using the primers of SEQ ID NOs: 44 and 50 (see title, paragraph [0074], Figure 1).

Starting from document D7 and faced with the problem of providing another method of detecting MRSA in a sample, the skilled person would have turned to document D8, would have used the primers to produce a large amplicon containing both the junction and mecA to detect the presence of MRSA by detecting mecA. Since the detection techniques using probe hybridisation were well-known in the art, the skilled person had no difficulties in detecting *mecA* (see document D7 probe hybridisation). Hence, the skilled person, combining the teaching of documents D7 and D8, would have arrived at embodiment 2 and accordingly at the solution of claim 1.

Claims 14 and 26

Document D7 represented the closest prior art for the kit of claim 14 and for the oligonucleotide composition of claim 26.

The difference between document D7 and the subject-matter of claims 14 or 26 was that the probe used for the detection of the amplification products was hybridising to the SCCmec sequence. Since no technical effect was associated with this difference, the technical problem had to be regarded as the provision of a further kit or oligonucleotide composition for detecting MRSA in a sample.

Starting from document D7 and faced with this problem, the skilled person would have turned to documents D21 or D8 and would have arrived at the kit or composition of claims 14 and 26.

Thus, the subject-matter of claims 1, 14 and 26 lacked an inventive step in view of D7 in combination with documents D8 or D21.

Claim 4

Documents D7 or D15 represented the closest prior art for the subject-matter of claim 4.

Starting from document D7

Two differences existed between claim 4 and document D7:

1) the junction and mecA are amplified in a multiplex amplification reaction and

2) the probe used for the detection of the junction is hybridising to the SCCmec sequence.

Since there was no technical interaction between the technique of amplifying and of detecting a target

sequence using a probe, claim 4 solved two "partial problems".

The first partial problem was regarded as the provision of an improved method wherein two markers are amplified in the same reaction container.

The multiplex amplification reaction was well-known and standard for detecting MRSA and different markers (mecA and various SCCmec types)(see document D7, paragraph spanning pages 1880 and 1881; and paragraph [0006] of the patent). Likewise, document D15 described the use of multiplex PCR for detecting *mecA* and *nuc* genes (page 659, left column, first paragraph).

The second partial problem was regarded as the provision of an alternative method of detecting MRSA in the sample.

Since, the use of a probe hybridising to the SCCmec sequence was not associated with any technical effect, the problem was the provision of a further method of detecting MRSA in a sample.

Starting from document D7 and faced with this problem, the skilled person would have, based on its common general knowledge, added the primer sets for amplifying the SCCmec junction and *mecA* in the same reaction mixture to perform a multiplex amplification reaction. It would then have turned to document D21, which used a probe that hybridised to the SCCmec cassette, and thus would have arrived at the method of claim 4.

Starting from document D15

Document D15 disclosed an "IDI-MRSA assay" which was the same assay as described in D7 (page 660, right column first sentence). The detection of the combination of the SCCmec and mecA in a multiplex approach was mentioned to be useful for reducing MRSA false-positives (page 661, right column, penultimate paragraph).

The difference between document D15 and claim 4 was that the probe was hybridising to the SCCmec junction sequence.

Since there was no technical effect associated with this difference, the technical problem vis-a-vis document D15 was regarded as the provision of a further method of detecting MRSA in a sample.

Starting from document D15 and faced with this technical problem, the skilled person would have turned to document D21 describing another method of detecting MRSA in a sample by using an oligonucleotide probe targeting "the SCCmec chromosomal cassette of MRSA". The skilled person would therefore have combined the method disclosed in documents D15 and D21 and would have arrived at the subject-matter of claim 4.

Thus, claims 1, 4, 14 and 26 lacked an inventive step.

XIII. The submissions made by former Opponent 2/Appellant I, may be summarized as follows:

Main request
Inventive step

The available data in the patent were insufficient to establish that the method of claim 1 was improved over

the method disclosed in document D7. This argument was dealt with in point 15.1.4 of the decision under appeal.

Example 1 ("approach 1 ") of the patent intended to reflect the assay carried out in document D7. Example 1 "approach 1" of the patent used one probe instead of three probes in document D7. Example 2 ("approach 2") used five probes located in the right part of the SCCmec cassette instead of three in "approach 1" (see patent Fig. 3 and paragraph [0066]).

Document D54 demonstrated that the fluorescence background generated and used to calculate the max. signal ratio for an assay using five fluorescent probes was at least 5 times higher compared to an assay using only one fluorescent probe. The signal over the noise to generate a false positive with a MSSA strain was accordingly in "approach 2" significantly higher. Hence, the reduction of falsely positive detected MRSA strains was not due to the probe specifically targeting the SCCmec chromosomal cassette but was rather due to the new signal processing. Thus, the probe localization could not substantiate a surprising technical effect. The experimental results in the patent and in document D54, the objective technical problem had to be reformulated as the provision of a further method of detecting MRSA in a sample.

XIV. The submissions made by the respondent, insofar as relevant to the present decision, may be summarized as follows:

Main request
Novelty

Document D8 failed to disclose a probe.

Document D24 described methods of identification of MRSA based on PCR 1) - Examples 8-10 used only primers; 2) methods based on LCR; 3) methods purely based on hybridization of a probe; 4) and 5) general consideration for primers and probes; 6) methods based on using reverse transcriptase and 7) methods using NASBA - explicitly used a probe that hybridizes to *intM/orfX* (see document D24 paragraph bridging pages 5 and 6 and example 12, page 21, lines 1-5). There were no methods that required a first primer, a second primer and a probe as defined in claim 1 (a) to (c). The method of item 5) on page 6 referred to Figure 7a.

Document D28 failed to at least implicitly disclose a probe capable of specifically hybridizing to a region of the SCCmec cassette between the region with which the first primer was hybridizing and the junction as defined in the present claims (see paragraph [0073]). The reference to "exemplary primer/Scorpion sequences" and that "other primers, probes and scorpions may be used" in paragraph [0073] could only confirm that primers, probes and Scorpions were not limited to the specific exemplified sequences of SEQ ID NOS: 1-9 and 14-25, nothing else. Only the probe portion of a Scorpion primer/probe had to be compared with the probe of the invention. Thus, there was no direct and unambiguous disclosure of a Scorpion primer with a probe part that bound to SCCmec.

Hence, none of documents D8, D24 and D28 anticipated the subject-matter claimed.

Inventive step

The decision under appeal was correct. Starting with document D7 or document D15 - describing essentially the same assay - in combination with document D21, the claimed subject-matter involved an inventive step.

Document D7 described a primer, not a probe, regardless of whether they hybridized to the same sequence and provided no hint that the *orfX*-binding probes should be modified.

The subject-matter of claim 1 differed from that of documents D7 or D15 in that the method used a probe hybridizing specifically to a region of the SCCmec cassette between the SCCmec primer and the junction.

The patent substantiated that the use of a probe as defined in claim 1 reduced the number of false positives compared to the prior art methods (see Tables 2 and 3 of the patent).

The technical problem had therefore to be regarded as the provision of a method for detecting MRSA with improved specificity. There was furthermore no hint in documents D7 or D15 how to solve this problem, let alone to use a probe specifically hybridising to SCCmec.

Even if, when applying approach 1 of the patent, 24% of MSSA strains tested were incorrectly reported as MRSA (see patent, page 18, line 5), approach 2 of the patent used the same number of probes to assay MRSA and MSSA strains. A number of MSSA strains detected as false positives under approach 1 were nevertheless not detected using approach 2. The improved specificity was due to the position of the probes and not to its number, the latter remained identical within the same

assay. The location of the probe within the SCCmec cassette provided therefore a clear distinction between the max. signal ratio for MSSA strains and MRSA strains within the same assay (see Table 3 of the patent).

Even if the use of five instead of one probe impacted on the fluorescence max. signal ratio, the three MSSA drop-out strains in Table 2 could nevertheless be distinguished from the other normal MSSA based on their max signal ratio of 1.9-1.7 and 1.5 for MSSA drop-out strains versus 1.3-1.2 and 1.1 for the normal MSSA strains. Thus, based on the experimental data in the patent the localization of the probe was associated with the technical effect of detecting less false positives MRSA strains.

The technical problem had to be defined as the provision of an improved MRSA detection assay compared to commercially available tests.

The solution was the method of claim 1 which used a probe that hybridizes within the amplified SCCmec cassette that was generated by a first and second primer hybridizing in an extremity junction of the SCCmec cassette and in an extremity junction of chromosomal S. aureus DNA.

Document D21 determined the location of the primers in that they were "specific to SCCmec types I, II, III and IV" (D21 page 1164, left column, paragraph 2), while the probe was "targeting the SCCmec chromosomal cassette" (page 1165, left column, paragraph 1). There was no evidence whether a junction fragment or an internal region of the SCCmec cassette was amplified. Although two drop-out MSSA strains "that carry an SCCmec cassette lacking a mecA gene", tested with a

probe targeting the SCCmec chromosomal cassette of SCCmec, generated a negative results, these results implied that the primers amplified and the probe detected a fragment including the mecA gene. A probe targeting the SCCmec region in MSSA strains including a SCCmec cassette without mecA would namely yield positive results. There was therefore no hint in document D21 to use probes hybridizing within the SCCmec cassette as defined in claim 1, to reduce the number of inappropriate detection of normal MSSA strains lacking the SCCmec cassette and thus of false positives.

Since document D21 failed to describe or suggest a probe as used in the method of claim 1, the skilled person could not "try and see" whether these probes achieved the technical effect in the method disclosed in document D7.

Since the alternative closest prior art document D15 related to the same "IDI-MRSA" assay as disclosed in document D7, the analysis of inventive step and the arguments put forward with respect to document D7 were applicable to document D15.

Claim 1 referred to "at least two embodiments which raise different inventive step considerations". Embodiment 2 was similar to embodiment 1, but involved a first primer targeting the mecA gene, and the probe hybridizing to the mecA gene, downstream from the first primer. This interpretation was based on paragraph [0026] of the patent application, where a primer and a probe was defined to be capable of specifically hybridizing to a target sequence located anywhere on the cassette-side as long as the primer can be extended across the junction. However, a first primer that

hybridized to mecA was not a primer capable of specifically binding in an extremity junction region of the SCCmec cassette as required by the claims. The mecA primer and probe were at considerable distance from the preferred right extremity junction and not in the immediate vicinity of either junction (see patent figures 2-4).

Thus, starting from document D7, based on a reduced incidence of false positives arising from choosing a probe that hybridized specifically to a region of the SCCmec cassette between the SCCmec primer and the junction, the skilled person, faced with the problem of providing a method for detecting MRSA with improved specificity, had no motivation or hint how to modify the method of document D7 to arrive at the claimed solution. The subject-matter of claim 1 involved an inventive step.

The inventive step conclusion drawn for claim 1, starting from either documents D7 or D15, also applied to claim 4, which contained the same primers and probe for detecting the junction as claim 1 and to the kit and composition of claims 14 and 26.

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

XVI. The patent proprietor (respondent) requested that the appeal be dismissed, i.e. maintenance of the European patent on the basis of the main request, corresponding to the auxiliary request 2 as maintained by the opposition division, or alternatively the maintenance of the European patent on the basis of the auxiliary requests 1-7, filed with the reply to the statement of grounds of appeal.

Reasons for the Decision

Withdrawal of the opposition by the opponents 1 and 2.

During the appeal proceedings, Opponent 1, who was not appellant, and Opponent 2, who was appellant I, withdrew their opposition and opposition and appeal respectively. They ceased to be party to the appeal proceedings in respect of the substantive issues (see decision T 789/89, OJ EPO 1994, 482). Hence the patent proprietor/respondent and Opponent 3/Appellant II were the sole remaining parties to the appeal proceedings. Thus, it remains the board's principal task to review the decision under appeal on the basis of appellant II's and respondents' requests.

Main request (Claims 1-27)

The main request is identical to the second auxiliary request of the decision under appeal.

Sufficiency of disclosure (Article 83 EPC)

1. In the decision under appeal, the opposition division found that auxiliary request 2, now the main request, did not contravene Article 83 EPC. Appellant II did not challenge this finding.

1.1 Since Appellant II did not raise an objection under Article 83 EPC and Appellant I, by withdrawing both the opposition and the appeal, ceased to be party to the proceedings, the board sees no reason to depart from the conclusion drawn by the opposition division in the decision under appeal as regards Article 83 EPC. The subject-matter of the main request complies with the

requirements of Article 83 EPC (see decision, reasons 13).

Novelty (Article 54(1)(2)(3) EPC)

2. Appellant II argued that the claimed subject-matter was anticipated by the content of documents D8, D24 and D28.

2.1 Appellant II argued that document D8 disclosed a method of determining the MREJ sequences of MRSA strains, such as CCRI-12382 and CCRI-12383, ... " and the necessary amplification primers and probe of claim 1. They were capable of amplifying a mec right extremity junction "MREJ" fragment of the methicillin-resistant Staphylococcus aureus "MRSA". Parts (a) and (b) of claim 1 were fulfilled. Since claim 1 did not specify how hybridisation of the probe had to be detected (see patent [0042]), the internal primer SEQ ID N°28 was "capable of hybridising specifically to a region of the SCCmec cassette between the region with which the first primer can hybridise and the junction", and was therefore detectable through the generated sequencing data. The primer SEQ ID N°28 was as probe within the meaning of claim 1.

2.2 The board is not convinced by Appellant II's argument, because even if a probe can be a nucleic acid which embraces an oligonucleotide whose hybridization is detectable, a probe requires to be specifically labelled in order to rapidly detect its target sequence by means of its hybridization. In other words, an oligonucleotide probe must directly enable the detection of the specific association with its target sequence due to its base-pair sequence complementarity in a sample. In contrast, an oligonucleotide primer

hybridizes to its target sequence, allowing the production of sequencing data resulting from a process requiring the presence of a primer and an extension process, but does not comply with the probe's functional requirement (see also paragraph [0005] of the patent). Thus, document D8 does not anticipate the subject matter of independent claim 1.

2.2.1 For the same reasons, claims 14 and 26 combining a set of specific primers and probe and dependent claims 6 and 8 referring back to independent claim 1 are novel.

2.3 Appellant II contended that document D24 described all the features of claim 1 and that many "mec-side" probes could be chosen from the nucleotide sequence corresponding to the mec region DNAs, as depicted in Figures 17 to 19. Finally, document D24 implicitly disclosed a kit and an oligonucleotide composition including the primers and probes recited in claim 1.

2.4 The board concurs with the opposition division that document D24 discloses a method relying on an amplification of the left and right extremity junctions using a probe hybridizing to either the *orfX* part or to the overlapping junction comprising SCCmec and *orfX* (paragraph 15 at page 6 of the decision). Document D24 does not disclose a method combining an amplification of the mec region with the detection of a probe hybridizing specifically to a region of the SCCmec cassette as required in claim 1. Even if document D24 specifies that mec-side primers or probes may be chosen from the mec region DNAs (e.g. page 6, lines 5 to 7 and Figure 17 to 19), this does not represent a direct and unambiguous disclosure of a method combining an amplification step using primers and a probe

specifically hybridizing to the amplified SCCmec cassette. Thus, independent claim 1 is novel.

2.4.1 Since document D24 does not deprive claim 1 of novelty, claims 14 and 26 and dependent claims 6 and 8 combining the same set of specific primers and probe fulfil the requirements of novelty for the same reasons as developed for claim 1.

2.5 Document D28 is prior art under Article 54(3) EPC. Appellant II contended that document D28 described a method to detect a fragment of the SCCmec cassette in a MRSA present in a biological sample. The methods could further comprise the step of detecting an amplification product produced by one or more of the primer pairs. The probe and one of the primers of the primer pair may form part of the same molecule (e.g. a Scorpion™ primer/probe). Paragraph [0073] proposed two options for the Scorpion™ primer/probe: a Scorpion primer binding to the SCCmec cassette or to the *orfX* chromosomal region.

The board does not agree that the 3' portion of any of the Scorpion ™ primer/probes is capable of "specifically hybridising to a region of the SCCmec cassette between the region with which the first primer is capable of hybridising and the junction", as the Scorpion™ primers, combining both the primer of claim 1(a) and the probe of claim 1(c), were only described as hybridizing to the *intM* or *orfX* region (see paragraphs [0073], [0074], [0100], [0105]). The board cannot accept either that Scorpion™ primers specifically binding to the SCCmec cassette were directly and unambiguously disclosed as "other" Scorpion™ primers in paragraph [0073]. Indeed, the penultimate sentence of this paragraph dealt with

exemplary primer/Scorpion™ sequences including SEQ ID N°s 1 to 9 and 14 to 24. The skilled person would have immediately understood that the "other" primers and Scorpion™ to be used had to be compared to the specific sequences recited in this penultimate sentence.

2.5.1 In view of the fact that document D28 does not anticipate the method of independent claim 1, novelty must be acknowledged for the same reasons for independent claims 4, 14 and 26 combining a set of specific primers and probe and dependent claims 2, 3, 15 and 17.

2.6 Consequently, the main request meets the requirements of Article 54 EPC.

Inventive step (Article 56 EPC)

The scope of claim 1

3. Appellant II submitted that claim 1 encompassed at least two distinct embodiments. Embodiment 1 encompassed the subject matter of "approach 2", as set out in the examples of the patent. Claim 1 did not put any limits on where the first primer hybridized. Therefore it encompassed an "Embodiment 2", similar to embodiment 1, but involving a first primer targeting the mecA gene, and a probe hybridizing to the mecA gene, downstream from the first primer.

3.1 In the board's view, this second embodiment does not fall under the scope of claim 1.

3.1.1 According to claim 1, the first primer hybridizes in an extremity junction region of the SCCmec cassette. The "extremity junction region" does not encompass the mecA

gene, which is an internal element of the SCCmec cassette, not in the immediate vicinity of either junction, and at a considerable distance from the preferred right extremity junction (see patent figures 2-4). Since both the patent and document D7 define an extremity junction of a SCCmec cassette as a region abutting on one side to the *S. aureus* genomic sequence and on the other side the *mecA* gene (see Figure 1 of the patent and document D7), a primer specifically hybridizing to a *mecA* gene cannot specifically hybridize to an extremity junction region of the SCCmec cassette as required in claim 1(a). Thus "Embodiment 2" does not fall under the scope of protection of claim 1.

4. It is common ground that document D7 or document D15 represents the closest prior art for the subject-matter of independent claims 1, 4, 14 and 26.

Starting from document D7

4.1 Document D7 is concerned with the problem of providing a real-time PCR assay for rapid detection of MRSA from samples (see title of document D7). The problem is identical to the problem to be solved by the present invention.

4.1.1 Document D7 describes a multiplex real-time assay using five primers specific to different SCCmec right extremity sequences in combination with a primer and three molecular beacon probes specific for the *S. aureus* chromosomal *orfX* sequences (see page 1875, abstract, page 1876, col.1 paragraph "primers and probes", Table 3 describing *orfX*-specific fluorescent based probes). The assay allows the distinction of both methicillin-susceptible CoNS (MSCoNS) and MSSA from

MRSA (see document D7, page 1876, left column, paragraph 1 combined with Table 5 at page 1881).

4.2 The only difference between document D7 and the method of claim 1 is that the probe used for the detection in the method claimed is located within the SCCmec chromosomal region.

4.3 Example 1 of the patent, ("approach 1") describes a MRSA detection method based on the use of five primers specific to the right extremity sequences of the SCCmec cassette in combination with one primer and one generic probe located in the *S. aureus* chromosomal *orfX* gene sequence.

Example 2 ("approach 2") differs from "approach 1" in that it uses five primers specific to the SCCmec right extremity sequence combined with one generic primer specific to the S. aureus chromosomal *orfX* gene and five specific probes located in the right part of the SCCmec cassette (see Fig.3 and paragraph [0066] of the patent).

4.4 It was argued that "approach 1" of the patent (see example 1) was not a true reproduction of the method of document D7 and differed in more than only the number of probes binding specifically to the *orfX* gene of *S. aureus*. Therefore, the experiments disclosed in the patent could not justify the definition of the technical problem as an improvement over the method disclosed in document D7.

4.4.1 The results shown in Tables 1 and 2 of document D54, reproducing approaches 1 and 2 of the patent (see Table 2 and 3 of the patent), made it apparent that when using five probes instead of one, the max. signal ratio

of MSSA strains - known to test as false positive MRSA - decreased from a mean of 11,7 to 1,7. This meant that in "approach 2" of the patent the max. signal ratio was at least 5 times lower than the one generated in "approach 1" due to the expected background noise generated by the number of fluorescent probes. Hence, the probability of generating a false positive with an MSSA strain was significantly higher for "approach 1". Thus, the reduction of false positive MRSA strains detected was not due to the "probe localization" but rather to "signal processing". The use of a probe targeting the SCCmec chromosomal cassette rather than the *S. aureus* chromosomal *orfX* region could therefore not substantiate and establish a surprising advantage.

4.5 The board agrees with the respondent that the comparison of different max. signal ratios between MRSA and MSSA within one and the same approach is permissible, while the comparison of the max signal ratio between different approaches is not (e g. Table 2 and Table 3 of the patent). Approach 1 of the patent detected 24% of MSSA strains tested incorrectly as MRSA (see patent, page 18, line 5), while approach 2 of the patent, using five specific beacon probes for the detection of MSSA and MRSA strains, detected a lower number of false positive MRSA strains (see MSSA 11, 16, 17, 18, 27). The improved specificity is assigned to the probe location and not the number of probes used, as "approach 2" uses the same number of probes for the detection of MSSA and MRSA. The location of the probes within the SCCmec cassette provides a clear distinction between the max. signal ratio for MSSA and MRSA strains within the same assay (see Table 3 of the patent).

4.5.1 Furthermore, the board considers that document D54 describes experiments which do not closely repeat the

examples of the patent. A first difference is that the NASBA uses a low temperature annealing amplification reaction, i.e. isothermal 42°C reaction, while the PCR reaction uses a more stringent annealing temperature of 59°C and an extension temperature of 72°C (see document D54). A second difference is that the location of the probe hybridizing to the SCCmec may avoid the detection of false positives due to MSSA drop-out strains (lacking mecA). A third difference is that the experiment in document D54 used a collection of three MSSA drop-out strains, still having the right part of the cassette but lacking a mecA gene, out of ten MSSA strains. Despite all these differences, the MSSA drop-out strains still show a max signal ratio of 1,9-1,7 and 1,5 while normal MSSA strains had a max signal ratio of 1,3-1,2 and 1,1 in document D54. Hence, these results cannot confirm that normal MSSA and drop-out MSSA strains are undistinguishable when using the method of claim 1.

4.6 The board considers that the reduction of detected false positive strains, attributed to the use of a probe in the method of claim 1, is supported by the data provided in the patent.

4.7 Thus, starting from document D7, the problem to be solved must be regarded as the provision of an improved MRSA detection method.

4.8 The solution to this problem is the method of claim 1.

Obviousness

5. The board accepts that document D7 discloses a primer that hybridizes to the same sequence as the probe of the invention (primer mecII574 and SEQ ID N°7 of

"approach 2" respectively). In a PCR reaction, this forward primer in combination with a reverse primer generates an amplicon (see Figure 1). As elaborated in item 2.2 above, a primer is not a probe.

5.1.1 Even though document D8 discloses primers and a probe, the probe is always hybridizing a target sequence located in the *orfX* gene of the *S. aureus* (see Example 5 and Figure 2). That "primer and probes may be selected anywhere" within the DNA sequences of the MRSA strains as indicated in paragraphs [0052] of document D8 or [0060] of document D32, does not yet specify that the probe must hybridize to the SCCmec right junction of the amplicon and not to another location in the DNA sequences of the MRSA strains (e.g. *orfX* gene).

5.2 Document D21 describes a MRSA detection method using a probe targeting the SCCmec chromosomal cassette of MRSA (see page 1164, left column, paragraph 2 combined with page 1165, left column, paragraph 1). Two drop-out MSSA strains were classified as negative when using document D21's MRSA assay, although they were detected as false positive when using the method of document D7 (see page 1164, left column, paragraph 4). This result would have motivated the skilled person to use a probe binding to the SCCmec cassette of the MRSA to reduce the false positives and therefore to improve the sensitivity and selectivity of the MRSA detection method.

5.2.1 The board observes that the probe is defined as "targeting the SCCmec chromosomal cassette" in document D21 (page 1165, left column, paragraph 1). Whether the probe is capable of hybridizing to a junction fragment or to an internal region of the SCCmec cassette remains undefined. The negative results generated with two drop-out MSSA strains using the method of document D21

suggest that the primers are either not capable of generating an amplicon, as one primer binds within the mecA gene or the probe cannot detect a fragment including the mecA gene (see page 1165, col.1, paragraph 2) - otherwise said probe targeting SCCmec would have yielded positive results for these two MSSA strains including a SCCmec cassette but lacking mecA.

5.2.2 Thus, there is no hint or incentive in documents D7, D8 or D21 that the primers/probes had to be "optimized" to a probe as defined in claim 1 nor that the probe had to be directed to the target sequence hybridizing to one specific forward primer.

5.3 Even if, for the sake of argument, the skilled person were to ignore the definition of the primer of claim 1(a) and consider embodiment 2 to be covered, he/she nonetheless had, starting from document D7, to solve the technical problem identified above in item 4.7, based on the difference and the underlying technical effect, to arrive at the subject-matter of claim 1. Even though the method of document D7 uses three molecular beacon probes specific for the *S. aureus* chromosomal *orfX* sequences, there is neither a hint nor a motivation to change the molecular beacon probes' specificity for another target DNA sequence. Thus, even under these circumstances, the subject-matter involves an inventive step.

5.3.1 Although the skilled person could under these circumstances turn to document D8, which discloses a long range PCR reaction that amplifies both the junction and *mecA* using the primers of SEQ ID NOs: 44 and 50 (see document D8 [0074] and primers of Figure 1), there is no hint or motivation in document D7 for the skilled person to select, in order to solve the

technical problem of providing an improved MRSA detection method, a primer set (primer 50 and 44 of Figure 1) capable of yielding a large amplicon extending from *mecA* gene to the *orfX* gene of *S. aureus* and at the same time use a detection probe hybridizing to an "amplified extremity junction of a SCCmec cassette" which is a region abutting on one side to the *S. aureus* genomic sequence and on the other side the *mecA* gene instead of the *orfX* gene.

5.4 In consequence, the skilled person starting from document D7, faced with the technical problem of providing a method for MRSA detection that yields improved results has no hints or motivation to use a probe hybridizing within the SCCmec cassette instead of the *orfX* gene.

5.5 Since neither the implementation nor the testing of the method of claim 1, especially its probe, is envisaged or suggested by any of the prior art documents D7, D8 and D21, the skilled person faced with the technical problem of providing an improved method was never in a "try and see" situation, which would deprive the claimed subject-matter of an inventive step.

Starting from document D15

6. Document D15 is mentioned as alternative closest prior art. It refers to an "IDI-MRSA" detection as described in document D7 (see page 658, col.1, second paragraph reference [11]). Since, the method of detection of MRSA strains in documents D7 and D15 are almost identical, the board arrives at the same inventive step conclusion.

6.1 The board concludes that the solution of claim 1 involves an inventive step.

7. The board agrees with appellant II that claim 4 differs from the methods disclosed in documents D7 and D15 in that, at least, the probe used for the detection of the junction is hybridising to the SCCmec sequence. In view of the first difference, the second difference underlying the second partial technical problem may be ignored. Thus, starting from documents D7 or D15, the skilled person faced with the partial problem of providing an improved method would have found no hint or incentive in documents D7 or D15 (see item 5.2.1 above) to modify the primers or probes to arrive in combination with document D21 at the probe used in claim 4. Thus, for the same reasons as developed for the method of claim 1, claim 4 involves an inventive step.

7.1 The kit of claim 14 and the oligonucleotide composition of claim 26 comprise all the probe used in the method of claim 1. Since the probe used for the detection of the amplification products hybridising to the SCCmec sequence is associated with a technical effect (see items 4.5 and 4.6 above), the technical problem starting from any of documents D7, D15 or D39 or D32 must therefore be regarded as the provision of an improved kit and oligonucleotide composition for detecting MRSA with improved specificity. Thus, claims 14 and 26, for the same reason as developed for the method of claim 1, involve an inventive step.

7.2 For the reasons developed above with regard to claims 1, 4, 14 and 26 the same inventive step conclusion applies to their dependent claims.

Hence, the board concludes that the main request meets the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated