

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

Paper No. 27

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte LYNN BERGMEYER, THOMAS J. CUMMINS, JOHN B. FINDLAY
and JOANNE H. KERSCHNER

Appeal No. 1996-2442
Application 08/062,021¹

ON BRIEF

Before WINTERS, ROBINSON and SCHEINER, Administrative Patent Judges.

SCHEINER, Administrative Patent Judge.

¹ Application for patent filed May 14, 1993.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 1 through 8, 10 through 13, 16 through 20, 22 through 27, 30, 31, 33 and 35 through 37, and from the rejection of claims 38 through 42, newly rejected in the Examiner's Answer. Claims 1, 22, 37 and 38 are representative of the subject matter on appeal and are reproduced as an Appendix to this opinion.

The references relied on by the examiner are:

Findlay et al. (Findlay) WO 90/08840 Aug. 9, 1990

Chamberlain et al. (Chamberlain), "Deletion Screening of the Duchenne Muscular Dystrophy Locus via Multiplex DNA Amplification," Nucleic Acids Research, Vol. 16, No. 23, pp. 11141-11155 (1988).

Gibbs et al. (Gibbs), "Multiplex DNA Deletion Detection and Exon Sequencing of the Hypoxanthine Phosphoribosyltransferase Gene in Lesch-Nyhan Families," Genomics, Vol. 7, pp. 235-244 (1990).

Brytting et al. (Brytting), "Cytomegalovirus DNA Detection of an Immediate Early Protein Gene with Nested Primer Oligonucleotides," Journal of Virological Methods, Vol. 32, pp. 127-138 (1991).

Nedjar et al. (Nedjar), "Co-amplification of Specific Sequences of HCV and HIV-1 Genomes by using the Polymerase Chain Reaction Assay: A Potential Tool for the Simultaneous Detection of HCV and HIV-1," Journal of Virological Methods, Vol. 35, pp. 297-304 (1991).

After entry of two new rejections in the Examiner's Answer, the claims stand rejected as follows:

I. Claims 1 through 8, 10 through 13, 16 through 20, 22 through 27, 30, 31, 33, 35 through 37, and 39 through 42 under 35 U.S.C. § 103 as unpatentable over Nedjar, Brytting, Gibbs and Findlay.

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II. Claims 1 through 8, 10 through 13, 16 through 20, 22 through 27, 30, 31, 33 and 35 through 38 under 35 U.S.C. § 103 as unpatentable over Nedjar, Brytting, Gibbs, Chamberlain and Findlay.

III. Claim 38 under 35 U.S.C. § 103 as unpatentable over Brytting and Gibbs.

We reverse all three rejections.

DISCUSSION

The claims on appeal are directed to methods, compositions and kits for simultaneous amplification and detection of human cytomegalovirus (hCMV) DNA and a second target DNA. Claim 1, which represents the composition in its broadest aspect, is directed to a buffered aqueous preparation containing first and second primers, differing from each other in length by no more than 5 nucleotides, specific to and hybridizable with opposing strands of hCMV; third and fourth primers, differing in length from each other by no more than 5 nucleotides, specific to and hybridizable with opposing strands of a second target DNA; and at least 10 units/100 μ l of a thermostable DNA polymerase. All four primers have melting temperatures (T_m s) within the range of about 65°C to 74°C and within about 5°C of one another; and all four primers are present in the same amount within the range of from about 0.1 to about 2 μ M. Independent claim 22, in which the composition is used, represents the method in its broadest aspect. Claim 38 is directed to oligonucleotide primers of defined sequence, corresponding to portions of hCMV DNA. Claims 39 through 42 are method and kit claims requiring specific primers and capture probes. Independent claim 37 is directed to a diagnostic element coated with a plurality of immobilized capture probes

(at least one of which is specific for hCMV) with defined hybridization and melting temperatures.

Rejections I and II

Claims 1 through 8, 10 through 13, 16 through 20, 22 through 27, 30, 31, 33, 35 through 37, and 39 through 42 stand rejected under 35 U.S.C. § 103 as unpatentable over Nedjar, Brytting, Gibbs and Findlay (Rejection I), while 1 through 8, 10 through 13, 16 through 20, 22 through 27, 30, 31, 33 and 35 through 38 stand rejected under 35 U.S.C. § 103 as unpatentable over Nedjar, Brytting, Gibbs, Chamberlain and Findlay (Rejection II). We shall address both rejections together, as they are identical but for the examiner's additional reliance on Chamberlain in Rejection II, and a perplexing difference in the claims to which they are applied.²

Gibbs discloses a premixed multiplex PCR kit containing eight sets of primer pairs for simultaneous amplification and detection of eight distinct regions of the hypoxanthine phosphoribosyltransferase gene. All sixteen primers are designed to conform as closely as possible to a general formula of 24 bases with a 50% GC content, and the concentration of each primer set in the mixture is adjusted to compensate for uneven signal strength (i.e., Gibbs' primers are not present in equal amounts, the concentration of each set is adjusted relative to the other sets to allow

² According to the examiner, Chamberlain was added as evidence that certain limitations of the claims, not taught in the other references, were known in the art. It is not clear from the examiner's discussion Chamberlain why claims 39-42 are included in Rejection I, but not Rejection II; nor is it clear why claim 38 is included in Rejection II, but not Rejection I.

approximately equal amplification of the different gene fragments). Page 238. On page 5 of the Examiner's Answer, the examiner shows that the T_m s of eight of the primers (Gibbs' first four primer pairs) range from 65.64°C to 76.55°C, based on primer length and GC content, and some of the pairs have T_m s within 5°C of each other.

Chamberlain discloses multiplex PCR using 1 μ M each of twelve primers (six primer sets), and 10 units of Taq DNA polymerase per 100 μ l of reaction mixture. There is no mention of primer T_m s, but the reference teaches that combining multiple primer sets in a single reaction requires modification of the annealing temperatures of the individual primers, among other things. Page 11146.

Nedjar discloses co-amplification and detection of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) specific sequences in the same sample, using nested primer pairs in the polymerase chain reaction (PCR). Nested PCR requires sequential rounds of amplification, rather than simultaneous, because the sequence amplified using the "inner" primer pair is embedded in the sequence amplified using the "outer" primer pair.

Brytting discloses detection of conserved sequences from the immediate early gene of human cytomegalovirus (hCMV) using nested primer pairs.

Findlay uses a "nucleic acid test article" with a specific nucleic acid probe immobilized on its surface to capture complementary nucleic acid from a sample.

On pages 5 and 6 of the Examiner's Answer, the examiner establishes that many of the individual process parameters and reagents required by the claims were known in the art at the time of the invention:

Simultaneous amplification of two distinct viral target[s] and the motivation to do so are taught by [Nedjar] . . . Primers directed to hCMV are taught by [Brytting]. One critical element of the invention is the design of primers which have the same range of melting temperature (T_m) and have T_m 's within about 2°C or about 5°C of one another to allow all the primers to function in the same amplification condition. This is taught in the multiplex method by [Gibbs]. Indeed, [Gibbs] teach[es] that all the primers are designed to conform to specific parameters regarding the melting temperature . . . [Gibbs'] primers are found in the T_m range as specified in the instant claims, i.e., between about 65°C to 74°C and within about 5°C of one another . . .

Other elements of the invention including pH, the concentrations of primers and DNA polymerase, length of primers, additional PCR reagents, capturing probes, and water-insoluble supports are all taught in the prior art.

In addition, according to the examiner, Chamberlain is newly cited to show that "the use of two-temperature PCR and 10 units of DNA polymerase is taught in the prior art" (Examiner's Answer, page 13).

Without further analysis, the examiner concludes that "[i]t would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to incorporate the teachings presented above to develop the instant method to simultaneously amplify and detect human CMV and other infectious agent[s] such as HIV" (Examiner's Answer, pages 4-5) and "[o]ne of ordinary skill in the art would have reasonably [sic] to empirically determine the specified parameters or conditions claimed . . . [i]n a time period where more diseases are found to be caused by bacterial or viral

agents, such as CMV and HIV, . . . the ordinary artisan would have been motivated to detect simultaneously more than one infectious agent (via DNA analysis) using a multiplex PCR method” (Examiner’s Answer, page 6).

We find the examiner’s reliance on “motivation” to be misplaced in its application here. While it might be fair to say that one of ordinary skill in the art would have been motivated to accomplish appellants’ outcome (i.e., efficient, simultaneous detection of more than one nucleic acid target), that does nothing to explain why one skilled in the art would have had reason to select discrete, specific teachings from the various references cited and combine them in the manner required by even the broadest composition claim, much less the broadest method and diagnostic kit claims, which require the claimed composition.³ See In re Fine, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988) (“One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention”).

Moreover, the examiner has made no attempt to address the narrower method and kit claims (e.g., claims 39 through 42), which require primers with specific sequences, or the specific limitations of the claimed diagnostic element (claim 37).

We have no doubt that the prior art could be modified in a manner consistent with appellants’ specification and claims, but the fact that the prior art could be so

³ As stated in Pro-Mold & Tool Co. v. Great Lakes Plastics, Inc., 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1629 (Fed. Cir. 1996) (citation omitted), “It is well established that before a conclusion of obviousness may be made based upon a combination of references, there must have been a reason, suggestion or motivation to lead an inventor to combine those references.”

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modified would not have made the modification obvious unless the prior art suggested the desirability of the modification. In re Gordon, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984). Here we find no reason stemming from the prior art which would have led a person having ordinary skill to the claimed invention. In our judgment, the only reason or suggestion to combine the references in the manner proposed by the examiner comes from appellants' specification.

On this record, we are constrained to reverse Rejections I and II.

Rejection III

Claim 38, directed to an oligonucleotide primer of defined sequence, stands rejected under 35 U.S.C. § 103 as unpatentable over Brytting and Gibbs. Both references are discussed above.

Again, the examiner has made no attempt to address the requirement for a primer with a specific sequence, concluding simply that “[i]t would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to arrive at the oligonucleotides directed against hCMV. [Brytting] provides the motivation for making primers directed against hCMV, . . . [Gibbs] provides the teaching and rationale to design primers which are conformed to the narrow range of melting temperature.” Examiner’s Answer, page 11.

Again, we find no reason stemming from the prior art which would have led a person having ordinary skill to the specific primers claimed. Even if the examiner had

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identified a reason for combining the teachings of these two particular references, he has not explained how those combined teachings would result in the primers specified by the claim. In our judgment, the only reason or suggestion to combine the references in the manner proposed by the examiner comes from appellants' specification. On this record, we find that the examiner has not met his burden of establishing a prima facie case of obviousness.

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Accordingly, on this record, rejection III is reversed.

REVERSED

Sherman D. Winters
Administrative Patent Judge

Douglas Robinson
Administrative Patent Judge

Toni R. Scheiner
Administrative Patent Judge

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APPENDIX

1. An aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

(a) first and second primers which are specific to and hybridizable with , respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands from 90 to 400 nucleotides,

(b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μ molar, and

(c) a thermostable DNA polymerase present at at least 10 units/100 μ l.

22. A method for the amplification and detection of human cytomegaloviral DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of hCMV DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising

first and second primers, which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing

strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μ molar, and

ii) the following additional PCR reagents: a thermostable DNA polymerase present in an amount of at least 10 units/100 μ l, a DNA polymerase cofactor and at least one dNTP, any or all of said additional PCR reagents being in the same or a different composition as defined in i),

to simultaneously amplify said opposing hCMV DNA strands and the opposing second target DNA strands wherein, in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from about 62 to about 75°C and carried out within 120 seconds,

B) capturing one of said amplified hCMV DNA strands with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of said hCMV DNA strand, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequences of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

capturing one of said amplified second target DNA strands with a second capture reagent comprising a second capture probe specific to a nucleic acid sequence of said second target DNA strand, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m 's which differ by no more than about 15°C, and

C) simultaneously detecting said captured amplified hCMV DNA strand and said captured amplified second target DNA strands as a simultaneous determination of the presence of hCMV DNA and said second target DNA.

37. A diagnostic element comprising a water-insoluble, heat or ultrasonic sealable support, having disposed thereon in distinct regions thereof, a plurality of capture reagents,

each capture reagent having a capture probe specific for and hybridizable with a distinct target DNA at a temperature from about 40 to about 55°C, each of said capture probes having from 10 to 40 nucleotides and a T_m greater than about 50°C, and the T_m 's of all capture probes differing by no more than about 15°C,

at least one of said capture probes being specific for and hybridizable with hCMV DNA.

38. An oligonucleotide having a sequence selected from the group consisting of:

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|---------------|------------|------------|-------------|
| SEQ ID NO: 3 | TGCACTGCCA | GGTGCTTCGG | CTCAT, |
| SEQ ID NO: 16 | CATTCCCCT | GACTTTCTGA | CGCACGT, |
| SEQ ID NO: 17 | TGAGGTCGTG | GAAGTTGATG | GCGT, |
| SEQ ID NO: 18 | GGTCATCGCC | GTAGTAGATG | CGTAAGGCCT, |
| SEQ ID NO: 19 | GGAATGACGC | AAGGACATAT | GGGCGT, |
| SEQ ID NO: 20 | CCCAGGTGCA | CACCAATGTG | GTGGAT, |
| SEQ ID NO: 21 | GGACTGTGCG | CGTTGTATAC | CCTGC, |
| SEQ ID NO: 22 | ACTCCCGAAG | CGAATGGCAC | GTGGA, |
| SEQ ID NO: 23 | CATAGCTTGT | GCCCGTGTGG | CACGT, |
| SEQ ID NO: 24 | CCAAGACGAG | ACCGTCAGAG | CTGGT, |
| SEQ ID NO: 25 | AAGCTGTTGC | CGCCATCAAA | TAAACG, and |
| SEQ ID NO: 26 | CTGCGTTAGA | CCGAGAACTG | TGGATAAAGG |