

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.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. 19

**UNITED STATES PATENT AND TRADEMARK OFFICE**

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**BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES**

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Ex parte  
JONATHAN N. ROTH and WILFRED J. FERGUSON

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Appeal No. 1999-1206  
Application No. 08/394,608<sup>1</sup>

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ON BRIEF

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Before WINTERS, SCHEINER and MILLS, Administrative Patent Judges.

SCHEINER, Administrative Patent Judge.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 18, 20 through 25, and 27 through 38, the only claims remaining in the application.

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<sup>1</sup> Application for patent filed February 27, 1995. According to appellants, this application is a divisional of application serial no. 08/024,212, filed March 01, 1993, now U.S. Patent No. 5,393,662, which is a divisional of application serial no. 07/512,188, filed April 20, 1990, now U.S. Patent No. 5,210,022.

Claims 18, 22, 28, 33 and 36 are representative of the subject matter on appeal and read as follows:

18. A test medium for detecting the presence of biological material having  $\beta$ -galactosidase enzyme specificity, said test medium comprising:

a base medium for maintenance of said biological material; and

a chromogenic indolyl  $\beta$ -galactoside substrate forming an insoluble compound of a first color upon reacting with  $\beta$ -galactosidase, wherein said chromogenic  $\beta$ -galactoside substrate is selected from the group consisting of 6-chloroindolyl- $\beta$ -D-galactoside, 4,6-dichloroindolyl- $\beta$ -D-galactoside, 6, 7-dichloroindolyl- $\beta$ -D-galactoside, 4,6, 7-trichloroindolyl- $\beta$ -D-galactoside and salts thereof.

22. The test medium of claim 18, further comprising 5-bromo-4-chloro-3-indolyl- $\beta$ -glucuronide.

28. A method for testing the presence of and quantitatively identifying and differentiating microorganisms in a biological test sample, said test sample containing first microorganisms having  $\beta$ -galactosidase but not  $\beta$ -glucuronidase activity, and second microorganisms having  $\beta$ -glucuronidase activity comprising the steps of

inoculating a solid test medium or substrate capable of forming a solid with said test sample, said test medium comprising a chromogenic  $\beta$ -galactoside substrate capable of forming a first water-insoluble precipitate of a first color upon reacting with  $\beta$ -galactosidase, a chromogenic  $\beta$ -glucuronide substrate capable of forming a second water-insoluble precipitate of a second color contrasting with said first color upon reacting with  $\beta$ -glucuronidase, and a base medium, said first and second colors being visibly distinguishable in daylight,

incubating said test medium to produce colonies of said first and second microorganisms and first and second colored precipitates corresponding to said colonies,

examining said test medium for the presence of colonies having said first color, such colonies being colonies of microorganisms having  $\beta$ -galactosidase but not  $\beta$ -glucuronidase activity, and the presence of colonies having said second color, such colonies being colonies of microorganisms having  $\beta$ -glucuronidase activity, and

enumerating said colonies of microorganisms having  $\beta$ -galactosidase activity and said colonies of microorganisms having  $\beta$ -glucuronidase activity.



Sadler et al. (Sadler), "Synthesis and Absorption Spectra of the Symmetrical Chloroindigos," J. Am. Chem. Soc., Vol. 78, pp. 1251-1255 (1955)

Claims 18, 20 through 25 and 27 through 38 stand rejected under 35 U.S.C. § 103 as unpatentable over Edberg, Ley, Sadler and Watkins.

We reverse the examiner's rejection of the claims; moreover, we raise additional matters for consideration by the examiner and appellants.

### BACKGROUND

Coliform<sup>2</sup> bacteria in general, and Escherichia coli in particular, are widely used indicators of contamination in the production and/or purification of water and food,

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<sup>2</sup> "A general, ill-defined term used to denote Gram-negative, fermentative rods that inhabit the intestinal tract of man and other animals. Sometimes used to refer to all enteric bacteria, or used to refer only to lactose-fermenting enteric bacteria." Stedman's Medical Dictionary, Illustrated, 24th Edition, Williams & Wilkins, Baltimore/London, page 297 (1982). According to the specification (page 7), "[t]he genera Citrobacter, Enterobacter, Klebsiella, and Escherichia are the generally listed members of the coliform group."

especially dairy products. According to the specification, “[n]umerous methods for determining, identifying and enumerating coliforms and E. coli [are known],” but none can be used to both quantify, and distinguish between, general coliforms and E. coli, in a single test on a single sample. Specification, page 2. For example (Id., pages 3-4)

The Presence/Absence (P/A) test . . . which involves the reagents O-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG), a  $\beta$ -galactosidase substrate and 4-methyl-umbelliferyl- $\beta$ -D-glucuronide (MUG), a  $\beta$ -D-glucuronidase substrate, results in the determination of the presence or absence of general coliforms and E. coli. The test relies on the fact that generally all coliforms produce  $\beta$ -galactosidase, but only E. coli strains produce  $\beta$ -glucuronidase. If any coliforms are present, the broth medium turns a yellow color due to the activity of galactosidase enzyme on the ONPG material causing the release of a diffusible yellow pigment. If E. coli is present, the broth medium will demonstrate a blue fluorescence when irradiated with ultraviolet rays due to the breakdown of the MUG reagent with the release of the fluorogenic dye caused by . . . glucuronidase . . . . These reactions are very specific and allow both general coliforms and E. coli to be identified in a single test in a single sample. But since both reagents produce diffusible pigments, the test has the disadvantage of not being directly quantitative for either bacterial type.

[t]he reagent 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-gal) is a known test compound for identifying coliforms. When acted on by the  $\beta$ -galactosidase enzyme produced by coliforms, X-gal forms an insoluble indigo blue precipitate. X-gal can be incorporated into a nutrient medium such as an agar plate, and if a sample containing coliforms is present, the coliforms will grow as indigo blue colonies. X-gal has the advantage over . . . ONPG . . . in that it forms an insoluble precipitate, rather than a diffusible compound, thereby allowing the quantitative determination of coliforms.

[a] similar compound, 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide (X-gluc) has been developed for the identification of E. coli. When acted on by the  $\beta$ -glucuronidase enzyme produced by E. coli, X-gluc forms an insoluble indigo blue precipitate. X-gluc has the advantages over . . . MUG . . . in that it forms an insoluble precipitate, rather than a diffusible compound, thereby allowing the quantitative determination of E. coli [and] it does not require the use of ultraviolet light.

X-gal and X-gluc have the disadvantage that they each contain the exact same chromogen and therefore cannot be used together to identify and

distinguish between both E. coli and general coliforms in a single test with a single sample. Both X-gal and X-gluc cause the formation of identically hued indigo blue colonies. A person using both reagents together would be able to quantitatively identify the total number of coliforms, the same as if X-gal were used alone, but would not be able to tell which of the colonies were E. coli and which were other coliforms . . .

The present specification describes a number of chromogenic substrates of  $\beta$ -galactosidase<sup>3</sup> and  $\beta$ -gucuronidase,<sup>4</sup> each of which forms an insoluble magenta precipitate upon enzymatic hydrolysis, readily distinguishable from the insoluble indigo blue precipitate produced upon enzymatic hydrolysis of X-gal or X-gluc. Pages 6 and 10-16. Thus, colonies of E. coli (indigo blue) are easily differentiated from colonies of general coliforms (magenta or purple) when both are grown on solidified culture medium containing one of the magenta precipitate-forming  $\beta$ -galactosidase substrates, e.g., 6-chloroindolyl- $\beta$ -D-galactoside, and an indigo blue precipitate-forming  $\beta$ -gucuronidase substrate, e.g., X-gluc. Similarly, colonies of E. coli will be magenta, while colonies of general coliforms will be indigo blue, when grown on a medium containing one of the magenta precipitate-forming  $\beta$ -glucuronidase substrates, e.g., 6-chloroindolyl- $\beta$ -D-glucuronide, and an indigo blue precipitate-forming  $\beta$ -galactosidase

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<sup>3</sup> E.g., 6-chloroindolyl- $\beta$ -D-galactoside, 4,6-dichloroindolyl- $\beta$ -D-galactoside, 6,7-dichloroindolyl- $\beta$ -D-galactoside and 4,6,7-trichloroindolyl- $\beta$ -D-galactoside.

<sup>4</sup> E.g., 6-chloroindolyl- $\beta$ -D-glucuronide, 4,6-dichloroindolyl- $\beta$ -D-glucuronide, 6,7-dichloroindolyl- $\beta$ -D-glucuronide and 4,6,7-trichloroindolyl- $\beta$ -D-glucuronide.

substrate, e.g., X-gal. In addition, the method is directly quantitative because each blue or magenta spot on the solidified medium represents a colony-forming unit. See the Specification, page 5.

### DISCUSSION

As even a cursory review of the representative claims reveals, there are substantial differences in scope between the claims on appeal. Despite these differences, the examiner has rejected all of the claims together, on the premise that “Edberg discloses a medium for differential detection of E. coli from other enterobacteria” which “couples both glucuronides and galactosides as chromogenic substrates each having different visual endpoints,” while Lay, Sadler and Watkins “appear[] to disclose all of the dye-galactoside substrates designated in the instant claims,” thus “[i]t would have been obvious . . . to substitute the known galactosidase and glucuronidase substrates taught by Levy [sic, Ley], Sadler and Watkins for the substrates taught by Edberg because they are similar in structure, function and form and taught by Levy [sic, Ley] to be superior.” Examiner’s Answer, pages 4 and 5.

As so often happens, in taking this “shotgun” approach to the claims, the examiner has managed to miss the mark in every instance. Frankly, we are at a loss as to how the examiner’s rejection relates to any one of the claims in particular.

Each of claims 18, 20 through 24, 29, 30, 34 and 35 requires, at a minimum, a medium containing a magenta precipitate-forming  $\beta$ -galactosidase substrate: 6-chloroindolyl- $\beta$ -D-galactoside, 4,6-dichloroindolyl- $\beta$ -D-galactoside, 6,7-dichloroindolyl- $\beta$ -D-galactoside, 4,6,7-trichloroindolyl- $\beta$ -D-galactoside, or a salt thereof. Similarly, claims

25, 27, 31 and 32 require, at a minimum, a magenta precipitate-forming  $\beta$ -glucuronidase substrate: 6-chloroindolyl- $\beta$ -D-glucuronide, 4,6-dichloroindolyl- $\beta$ -D-glucuronide, 6,7-dichloroindolyl- $\beta$ -D-glucuronide, 4,6,7-trichloroindolyl- $\beta$ -D-glucuronide, or a salt thereof. The examiner has not pointed to a description of any of the required magenta precipitate-forming substrates in the prior art. Rather, Ley, Sadler and Watkins, relied on by the examiner as describing “all of the dye-galactoside substrates designated in the instant claims,” appear to describe chromogenic substrates that form blue precipitates (e.g., 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide (X-gluc)). Blue precipitate-forming substrates like X-gluc or indoxyl- $\beta$ -D-glucuronide are required by some of the claims on appeal, but only in addition to magenta precipitate-forming substrates.

In our judgment, the combined disclosures of the cited references are clearly insufficient to support a conclusion of obviousness of claims containing the limitations discussed above. 35 U.S.C. § 103 requires that obviousness be determined based on the claimed subject matter as a whole. Where, as here, the determination of obviousness was based on less than the entire claimed subject matter, the examiner’s conclusion of obviousness for claims with these limitations is legally unsound and cannot stand.

Claim 28, directed to a generic method of “quantitatively identifying and differentiating” organisms having  $\beta$ -galactosidase - but not  $\beta$ -glucuronidase activity - and organisms with  $\beta$ -glucuronidase activity, in a single test with a single sample, is quite a

bit broader than the claims discussed above. The claimed method requires growing the sample on or in a solid culture medium containing chromogenic  $\beta$ -glucuronidase and  $\beta$ -galactosidase substrates capable of forming insoluble precipitates of two different colors, "visibly distinguishable in daylight," and quantifying and identifying the two types of organisms on the basis of colony color. Claim 33 is directed to a method similar to the method of claim 28, but is even broader in that it is not limited to identifying organisms with any particular type of enzymatic activity.

The examiner's proposed reason or motivation for substituting the chromogens described by Ley, Sadler and Watkins for Edberg's indicators in Edberg's method does not withstand scrutiny for a number of reasons. Edberg combines various  $\beta$ -glucuronidase and  $\beta$ -galactosidase substrates in a single vessel, but always in solution.

The color of the solution and its fluorescence at a given wavelength are determined by autoanalyzer, and the method only works if the reaction products are diffusible. The examiner does not explain how or why one skilled in the art would substitute substrates that form insoluble precipitates for Edberg's substrates that form diffusible products. On the other hand, the examiner has not explained why one would convert Edberg's format to one requiring solid media. Moreover, even if some of the chromogens described by Ley, Sadler and Watkins are capable of forming insoluble precipitates upon enzymatic hydrolysis, the examiner has not identified any two that form precipitates "visibly distinguishable [from each other] in daylight," as required by the claims.

Claim 36, directed to a test medium containing two chromogens capable of forming insoluble compounds of two different colors, is broader still. Yet the examiner

has not directly addressed the limitations of even this claim.

As explained in In re Kotzab, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316 (Fed. Cir. 2000):

Most if not all inventions arise from a combination of old elements. [] Thus, every element of a claimed invention may often be found in the prior art. [] However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. [] Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant. [citations omitted]

In other words, “[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.” In re Fine, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988). “[T]here still must be evidence that ‘a skilled artisan, . . . with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.’” Ecolchem Inc. v. Southern California Edison, 227 F.3d 1361, 1375, 56 USPQ2d 1065, 1075-76 (Fed. Cir. 2000). In our view, that evidence is lacking in the examiner’s rejection and in the art cited.

The initial burden of presenting a prima facie case of obviousness rests on the examiner. In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Thus, the examiner is charged with addressing every limitation of a claimed invention. In our judgment, the examiner has failed to establish a prima facie case of obviousness within the meaning of 35 U.S.C. § 103. On this record, we are constrained to reverse the examiner’s rejection of claims 18, 20 through 25 and 27 through 37.

ADDITIONAL MATTERS

In the first office action (October 2, 1995, paper no. 5), the examiner rejected claims 18 through 38 under the judicially-created doctrine of obvious-type double patenting, as unpatentable over “claims 1-13 of copending application Serial No. 08/457,876. Appellants pointed out in their response to the first office action (paper no. 6, January 2, 1996), that “the serial number [was] unknown” to them, and its citation was apparently a clerical error. Evidently, the examiner agreed, and no further mention was made of the rejection. Nevertheless, we believe appellants and the examiner should revisit this matter to determine whether the present claims and the claims of parent application serial no. 07/512,188 (now patent no. 5,210,022) are patentably distinct. At least some of the claims in each appear to be directed to very similar methods. We note that there was a restriction requirement in the parent application between methods, compounds and compositions, but that would not seem to preclude an obviousness type double patenting rejection between the methods of the present application and those of the patent.

Finally, we wish to express our dismay at the seemingly indifferent examination of this application. It is axiomatic that broader claims are more vulnerable to prior art than narrower claims, but it is not apparent from the record that the examiner appreciated the breadth of certain of the claims (claim 36 leaps to mind) during the prosecution of this case.

Moreover, we are troubled by the complete lack of a meaningful response to appellants' arguments. Throughout the prosecution of this case, appellants have maintained, among other things, that none of the references cited by the examiner

describes or suggests the magenta precipitate-forming substrates required by many of the claims. Rather than actually addressing appellants' very relevant criticism of the rejection, the examiner's response (Examiner's Answer, page 4) is nothing more than boilerplate:

Appellant's arguments have been fully considered but they are not deemed persuasive. The rejection is maintained for the reasons set forth in the previous office action. A prima facie case of obviousness has been set forth as it appears that the substartes [sic] used are if [sic] fact not novel or unobvious in view of the cited references.

Turning to the previous office action (final rejection, paper no. 7), for the "reasons," we find only the same boilerplate paragraph. Needless to say, this treatment of appellants' arguments is manifestly improper.

REVERSED

Sherman D. Winters )  
Administrative Patent Judge )  
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