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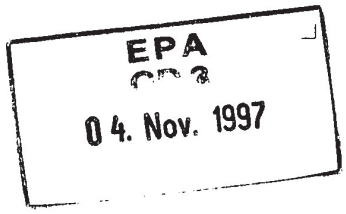

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Appeal Case T 0945/97 - 3.3.4.
ENZO BIOCHEM, INC.
Our Ref.: S 808 EP

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Uex/Ba/ALR/ne

In the following we submit the grounds in support of the formal appeal dated September 3, 1997.

In its Decision the Opposition Division has revoked the patent alleging that neither the subject matter of the claims according to the main request nor to the auxiliary requests is allowable under Art. 123(2) EPC, is sufficiently disclosed (Art. 100(b) and Art. 83 EPC), and is novel (Art. 100(a) and Art. 54 EPC).

We cannot agree thereto for the following reasons:

1. THE SUBJECT MATTER OF THE PATENT

The subject matter of the patent provides a method and an arrangement for the detection of polynucleotide sequences whereby detection is effected by fixing a single-stranded polynucleotide to a solid support which is or is contained within a system, forming an entity with a labelled polynucleotide probe

and generating and detecting the signal originating from the label, whereby the system is transparent or translucent and non-porous and the signal is a soluble signal.

2. THE SUBJECT MATTER OF THE CLAIMS SET DOES NOT GO BEYOND THE DESCRIPTION AS ORIGINALLY FILED (ART. 123(2) EPC)

2.1 The terms "transparent/translucent system" and "non-porous substrate or system"

We are of the opinion that the terms "transparent/translucent system" and "non-porous substrate or system" are disclosed in the specification as originally filed. In order to avoid unnecessary repetitions we want to refer the Board of Appeal to our reply of February 12, 1996 to the Communication pursuant to Art. 101(2) and Rule 58(1) to (4) EPC dated August 2, 1995, where we extensively discussed why, according to our opinion, the features "non-porous substrate or system" and "translucent or transparent system" are unambiguously derivable from the specification or contained within the specification as self-evident features, even if these features are not literally mentioned in the specification as originally filed.

In this context we gave a short summary of the prior art (see item 2.1.1) thereby providing a series of documents partly incorporated within the description demonstrating that the objected to features of claim 1 are self-evident features implicitly contained within the disclosure as originally filed. We further referred in item 2.1.2 the Opposition Division to the specific disclosure in the description (see pages 50-58) from which the features "non-porous substrate or system" and "transparent/translucent system" are clearly derivable as the support or the system described would not function would it be porous or non-transparent/non-translucent. It is furthermore stated that the embodiments specifically described in the application (see the reference to pages 50 to 58) referring to the later and more difficult embodiments of ELISA as they have been

developed in the prior art for e.g. antigen/antibody reactions, certainly communicate the older and better known established ELISA detection utilizing beads and other solid supports within a distinct and separate system (see the paragraph bridging pages 10 and 11).

In summary, our comments provided in the reply to the EPO demonstrate that the embodiments of claim 1 wherein the support is the system or the support is contained within a system, the system thereby being transparent or translucent and non-porous are self-evident and comprised by or derivable from the description as originally filed.

2.2 The terms "soluble signal", "non-porous system or support" and "transparent or translucent system" as objected to in the Decision Revoking the European Patent

In the following we want to specifically refer to the statements of the Opposition Division in the Decision revoking the European Patent whereby we will demonstrate that the decision and the grounds for the decision are not justified in view of the disclosure of the application as originally filed.

2.2.1 Soluble Signal

2.2.1.1 The Opposition Division first discussed a meaningful interpretation of the term "soluble signal" as this expression was seen as being unclear (which is, however, not a ground for opposition). In view of the proprietor's submission of December 28, 1994 it thereby referred to spectrophotometric and ELISA techniques involving enzyme-linked reagents which produce a color change in a substrate or precipitate and to Table II disclosing chromogens which produce an insoluble product. In view of this the feature "soluble signal" was interpreted in a broader sense as "a signal that can be detected in solution".

However, in view of the fact that radioactive signals which are detectable in solution are excluded from the disclosure of the

application as filed, the Opposition Division came to the conclusion that the expression "soluble signal" describes a novel class of signals which were not disclosed in the application as filed. Thus, the use of such an expression allegedly violates Art. 123(2) EPC.

2.2.1.2 The term "soluble signal" per se, in the context of claim 1 and in view of the description unambiguously implicates to the skilled person that a soluble signal per se is soluble in a fluid in contrast to the Opposition Division's interpretation that insoluble precipitates or fixed signalling agents generate a "soluble signal".

There are numerous locations throughout the specification indicating the generation of soluble signals being measured while being dissolved in a fluid. We want to refer the Board of Appeal to representative disclosure in the specification as e.g. on page 21, lines 9 to 26, already referred to in the statement of December 28, 1994, item 3.3.1.2, relating to spectrophotometric and ELISA techniques. The reference to spectrophotometric techniques including the passage of lines 13 to 21 referring to the measurement of an enzymatically generated product for quantitative determination and the passage on page 53, lines 1-3 mentioned in the above statement referring to an enzymatically generated product measured by spectrophotometry clearly show that by the term "soluble signal" the measurement of a signal in a solution is comprised.

We cannot share the Opposition Division's opinion that the term "soluble signal" comprises signals generated by the chromogen products of Tables I and II and also radioactive signals detectable e.g. by a scintillation counter. Tables I and II substantially relate to insoluble products which are visually evaluated while being bound to a support usually not allowing a quantitative determination as is e.g. a significant property of the soluble signals. Such precipitates do not need the detection in solution although detection is possible by e.g. submersing the support into a clear fluid. This, however, cannot be equalled to a soluble signal measured in the fluid whereas a precipitate remains an

insoluble signal. Also the arguments of the Opposition Division relating to radioactive labels cannot hold. The radioactively labelled oligo- or polynucleotide is fixed to the membrane and represents therefore an insoluble signal not comparable to signals being soluble in a fluid.

2.2.1.3 Thus, according to our opinion, the term "soluble signal" is not only unequivocally derivable from the description but is furthermore clearly delimited from other kinds of signals mentioned in the description, as these signals are insolubly precipitated or fixed signals. Such kinds of signals do not fulfil the requirements of a soluble signal. Therefore, no novel class of soluble signals is described, but the signals comprised by the term "soluble signal" are clearly derivable from the description.

2.2.2 Non-Porous System or Support

2.2.2.1 The Opposition Division is of the opinion that the term "non-porous" cannot be derived from the application as filed neither in connection with the term "support" nor with the term "system". The Opposition Division argues that despite the presence of the word "system" in items 71 and 101-108 and original claims 34 to 37 no meaningful information in connection with the term "non-porous" could be derived. Also the use of a soluble signal does not imply the use of a non-porous system in view of the different embodiments which can be represented by a system.

With respect to the term "non-porous support", the Opposition Division has acknowledged that "non-porous supports" are disclosed in the specification. However, it emphasizes that since the specification as filed does not attach any importance to this feature, a generalization as in claim 1 seems to be unjustified.

2.2.2.2 We cannot agree to the Opposition Division's arguments. The term "non-porous" is not literally mentioned in the specification. Such literal disclosure is, however, not required. We are of the opinion that the specification discloses embodiments of claim 1 which allow the conclusion that the feature "non-porous" in

association with system or substrate is comprised by the application so that claiming this feature does not result in a violation of Art. 123(2) EPC.

Suitable disclosures supporting the terms "non-porous substrate" or "non-porous system" are inter alia found in item 101 and following items and on page 50 of the invention as originally filed. Item 101 discloses a system comprising besides other components a transparent substrate to which the DNA probe to be detected is fixed. The system allows a photometrically detectable chemical reaction. From this it may be followed that a soluble signal is generated. On page 50, a transparent substrate with the DNA material bound thereto with arrays of depressions or wells allowing the photometrical detection of a soluble signal is disclosed. This means that a fluid with a soluble signal is present in the depressions or wells.

The cited locations in the description are embodiments supporting claim 1. While item 101 literally mentions the term "system", this is not the case on page 50. In this case the support is the system and it is just a matter of designation to designate the transparent substrate (see page 50, lines 10 and 11) in claim 1 as a support or a system.

In order for a feature to be allowable in a claim not violating Art. 123(2) EPC it does not have to be literally mentioned in the description as long as this feature is indirectly but clearly derivable. This is the case here. Although the term "non-porous" is not literally comprised in the description it is self-evident to the skilled person that nothing else than a non-porous support or system can be comprised by the embodiments disclosed and claimed in claim 1. A support or system (as stated above, this is just a matter of designation) to which DNA is bound, being a depression or a well and allowing the determination of the DNA whereby washing steps and substrate reactions (see page 50, lines 22 and 29-31) are performed in the support/system must be non-porous.

Furthermore, a system comprising a substrate (support) and allowing the photometric detection of a chemical reaction in a fluid also requires that the system is non-porous.

Thus, the term "non-porous" in connection with "system" or "support" is a logical consequence of the diverse embodiments comprised by claim 1 for the system or the support.

2.2.2.3 The Opposition Division alleges that the term "system" may comprise further definitions so that the attribute "non-porous" does not seem to apply. The Opposition Division mentions e.g. "a biochemical detection system". However, the meaning of a term used has to be interpreted based on the disclosure in the whole specification. We are of the opinion that there is a suitable disclosure for the term "non-porous" in connection with "support" or "system". The wording of claim 1 makes clear that the term "system" being or containing a solid support is used in a limited sense and cannot be interpreted as being any system even if further systems are contained in the specification. In the sense the term "system" in connection with the term "non-porous" is used in claim 1, it is disclosed in the specification. The addition of the attributes "transparent or translucent" (see item 2.2.3) or "non-porous" is just a logical consequence which is obvious for the person skilled in the art.

2.2.3 Transparent or Translucent System

2.2.3.1 The Opposition Division objects to the term "transparent or translucent system" as the terms "transparent" and "translucent" in connection with the term "system" have allegedly no counterpart in the specification. The Opposition Division states that the qualifiers "transparent or translucent" are never attached to the term "system".

2.2.3.2 The Opposition Division has acknowledged the term "transparent or translucent substrate" being disclosed throughout the specification. This means that at least part of claim 1 with respect to the term "transparent or translucent" should be

acknowledged as being allowable. Suitable disclosure to support the term "transparent or translucent system" may inter alia be found on page 50 disclosing a transparent glass substrate with an array of depressions or wells in which the reaction for the detection of nucleic acids is performed. As already stated above with respect to this embodiment of claim 1 it is only a matter of designation whether the transparent substrate is called a "system" or a "support" in claim 1.

The term "transparent system" is also obvious from item 101 as a further embodiment of claim 1. The determination of a photometrically detectable chemical reaction is possible only if light can fall through the system onto the solution. Therefore, the "system" should be transparent or translucent.

2.2.4. Summary

In view of our above arguments and the cited disclosure of the invention as originally filed it can be concluded that the various embodiments of claim 1 are disclosed or implicitly contained in the specification as originally filed. The features objected to are a logical consequence for the functioning of the method of claim 1 and are implicitly contained within the embodiments described in the specification. We are therefore of the opinion that the features objected to do not go beyond the description as originally filed.

3. SUFFICIENCY OF DISCLOSURE (ART. 100(b) and 83 EPC)

The Opposition Division considers claim 29 as being insufficiently disclosed within the meaning of Art. 83 EPC.

According to our opinion the Opposition Division's objections are not justified. The skilled person is able to recognize the deficiency of claim 29 and to compensate this deficiency by applying his/her general knowledge so that the method of claim 29 can be performed resulting in the detection of selected polynucleotides. Therefore, the method of claim 29 supplemented by general

knowledge, as is a prerequisite under Art. 83 EPC, can be performed.

4. NOVELTY (ART. 100(a) and 54 EPC)

The Opposition Division objects to claims 1 and 26 being allegedly anticipated by documents 04, 05 and 07 (erroneously assigned as D4, D5 and D7) and D2.

4.1 Documents 04, 05 and 07 describe the detection of nucleic acids by *in situ* hybridization. The visualization of the hybridizing polynucleotides in document 04 occurs inter alia by using rhodamine or an avidin-biotin peroxidase complex and viewing the labelled DNA through a microscope.

The system of document 05 is similar to that of 04. On page 4384, left-hand column, third paragraph, the generation of an insoluble precipitate generated by peroxidase is described.

Document 07 describes the preparation and use of modified nucleotides. This document also relates to the generation of insoluble products.

As has been analyzed above, these documents disclose the generation of insoluble products. However, as already outlined in item 2.2.1.2, above, insoluble products are according to our opinion not comprised by the term "soluble signal" generated by the signalling moiety. Therefore, the subject matter of claims 1 and 26 is not anticipated by these documents.

4.2 Furthermore, we are of the opinion that document D2 cited by the Opposition Division as anticipating claims 1 and 26 is not relevant for the evaluation of novelty as outlined below.

According to our opinion, the procedures described in D2 do not enable the skilled artisan to practice the invention of the opposed patent. Furthermore, the procedures of D2 are inoperative and would not be useful for the detection of a polynucleotide in a

soluble signalling format using a transparent or translucent, non-porous system.

D2 does not enable the practice of the invention claimed in the opposed patent because it does not teach or suggest the use of a non-porous, transparent or translucent system. D2 only describes supports on which the target polynucleotide is bound and nowhere does it describe the system in which the supports are contained. Even if the support is considered to be equivalent to the substrates claimed in the opposed patent, D2 is not anticipating.

In addition to failing to teach or suggest the system or support claimed in the opposed patent, D2 fails to teach the claimed essential feature of generating a soluble signal. D2 discloses on page 8, lines 3-15:

For example, the following reactions illustrate how a light response is elicited using three different light-emitting catalysts as the light labels, namely bacterial luciferase, firefly luciferase, and peroxidase:

$$NADH_2 + FMN + RCHO + O_2 \xrightarrow{\text{bacterial luciferase}} NAD + FMN + RCOOH + H_20 + hv$$

$$\text{luciferin} + ATP + O_2 \xrightarrow[Mg^{++}]{\text{firefly luciferase}} \text{oxyluciferin} + AMP + CO_2 + PPi + hv$$

$$H_2O_2 + \text{luminol} \xrightarrow{\text{peroxidase}} \text{oxyluminol} + H_2O + N_2 + hv$$

But the three reactions above are merely copied from another 1978 publication, Methods in Enzymology, Volume LVII, pages 410, 108 and 96, respectively. A copy of pages 410, 108 and 96 and their respective title pages are attached to this declaration as Exhibit 1 The three above-quoted equations are designated in Exhibit 1 as circled numbers, 1, 2 and 3, respectively.

Each of those reactions copied in D2 from Methods in Enzymology are presented in connection with assays for measuring protein or enzymes - but not in any case for detecting nucleic acids or polynucleotides. The first equation is cited as an oxidative chemiluminescent reaction for luminol in a short article that is titled "The Chemiluminescence of Luminol and Related Hydrazides." The other two equations concern the assay of modulator protein and the measurement of enzymes, (cyclic nucleotide phosphodiesterases), respectively. Detecting nucleic acids is not even disclosed in the three Methods in Enzymology articles (Exhibit 1). D2 fails to establish or demonstrate or even present a rationale as to how (or even why) these reactions could be usefully applied to nucleic acids and their detection.

In addition to failing to disclose or suggest claimed elements in the opposed patent, D2 is not operable and thus, the document does not constitute a disclosure that would permit the skilled artisan to carry out successfully the procedures outlined in the document. D2 lacks any disclosure or suggestion regarding the practical immobilization of a polynucleotide to a solid support -- an essential feature required to practice the procedure of D2, and, moreover, to practice the claimed invention in the opposed patent. Nowhere is it established in D2 that a polynucleotide can be immobilized on a support and retain its ability to hybridize to another polynucleotide.

The only disclosure in D2 concerning immobilization of a polynucleotide to a solid support is as follows:

> Immobilization of the sample single-stranded polynucleotides can be accomplished by any suitable method which does not inactivate a significant number of bases in the polynucleotide sequence, since a representative intact sequence must be available for base pairing with the reagent strands. The single-stranded polynucleotide segment can be attached or immobilized . . .

Later in the same paragraph, D2 alleges:

Numerous methods exist for coupling either terminal end of a polynucleotide to a given support (Weissback, A., and Poonian, M., Methods in Enzymology, Vol. XXXIV, Part B, 463-475, 1974). Also, derivatized forms of polynucleotides, for example, one carrying a terminal variety of supports (Mosbach, K., et al., Methods in Enzymology, Vol. XLIV, 859-886, 1976). Oligoribonucleotides may be immobilized on boronate derivates of various supports (Schott, H., et al., Biochemistry, 12, 932, 1973).

A. None of the three above-cited disclosures would permit, however, the immobilization of a polynucleotide to a solid support that could be used in a transparent or translucent, and non-porous system. The supports used in the three above-cited disclosures have not been shown to be at all useful in the assay procedures of D2. With respect to Weissback, the DNA material was immobilized on Sephadex G-200 beads, and the rigorous chemistry required in that procedure has not been established as compatible with the procedure in D2 or for that matter, in a format for generating a soluble signal. The carbodiimide treatment disclosed in Weissback would modify residues to the point of rendering hybridization altogether unlikely if not impossible. This consequence was vividly disclosed long before D2 or the present invention in the opposed patent. In the 1972 textbook Organic Chemistry of Nucleic Acids, Part B [Plenum Press, London and New York], the authors discuss reactions with carbodiimide on pages 331-332 (Exhibit 2):

> Single-stranded polynucleotides with no intramolecular hydrogen bonds between the bases (polyuridylic acid, for example), react smoothly with the carbodiimide CII [304, 308]; the reaction velocity in this case is somewhat lower than for uridine (Table 5.7). Virtually no reaction takes place with double-stranded complexes of polyribonucleotides and DNA [308]. . .

The authors later disclose that carbodiimide treatment for the purpose of immobilization is incompatible with subsequent hybridization reactions:

> Because the course of the reaction with the carbodiimide CII is so strongly dependent on secondary structure, and because of restriction of nuclease action after modification, the reaction with this carbodiimide can be used to identify polynucleotide segments in which separation of the double-stranded complex takes place during partial denaturation of DNA [312]. After treatment of DNA with the carbodiimide CII, followed by treatment with pancreatic DNase and phosphodiesterase from snake venom, long oligonucleotides arising from "defective" segments of the polymer can be isolated.

B. In Mosbach, the only immobilization taught is for coenzymes on a chromatographic column -- and not for polynucleotides as alleged by D2. In fact, the title of the Mosbach article is "Immobilized Coenzymes." As outlined in the beginning of Mosbach's article:

> The aspects of affinity chromatography have been covered in a recent volume of this series and are not dealt with here. In this volume the main emphasis is on their use as active coenzymes together with a brief account of their application in basic enzymology. The methodological part will be centered around the various adenine nucleotides, NAD+, NADP+, ATP, ADP, whereas work on other coenzymes will be treated only in a summary fashion. The various aspects will be treated as outlined below: (1) synthesis of a number of adenine nucleotide coenzymes, (2) coupling to matrices, (3) coenzymic activity, (4) application in enzyme technology and analysis, (5) application in enzymological and protein studies, (6) other immobilized coenzymes, (7) general discussion.

Absolutely nowhere in Mosbach's article is there any disclosure or suggestion that nucleic acid or polynucleotides could be immobilized to carry out successfully the procedure in D2.

C. In Schott, there is disclosed a borate column for distinguishing molecules containing a cis-OH group from all others. Molecules with a cis-OH group will complex with the diborate moiety in the borate column and be retained. In its uncharged state (with no amino acid attached), transfer RNA (tRNA) contains a cis-OH group and will bind to the borate column; whereas aminoacyl-tRNA

(having no cis-OH group) will not bind to the borate column. Hence, Schott et al. merely describes a chromatographic procedure for distinguishing or separating charged tRNA from uncharged tRNA. As in the case of Weissback, Schott's chemistry cannot be successfully applied in a transparent or translucent, and non-porous system for nucleic acid detection. In particular, borate columns are known to be unreliable because they have been found to be leaky. Furthermore, D2 fails to show that tRNA bound to the borate column as in Schott will hybridize to another nucleic acid, such as a probe.

Even if immobilization could be achieved to carry out the procedure in D2, no signal could be generated that would be detectable. The supports disclosed in D2 and in the underlying cited articles (Weissback, Mossbach and Schott) would immerse the target nucleic acid and any labeled probe hybridized thereto. Accordingly, generation of the signal by exposure to a light emitting reactant as required by D2 would not be possible, nor would the detection of any resulting signal be possible through the solid support in which the polynucleotides are immersed.

A telling point regarding the inoperability of D2 is evidenced by the fact that Michael J. Heller, the leading inventor named on the document, subsequently filed in 1985 a different patent application disclosure involving nucleic acid immobilization, that application having issued as U.S. Patent No. 4,824,776. To obtain that patent, Heller included additional disclosure concerning nucleic acid immobilization and he also claimed additional steps in his generic method claim. Thus, Heller's later issued U.S. patent required additional teachings on nucleic acid immobilization.

5. REQUESTS

For the reasons given above and for all the reasons brought before the Opposition Division, the patent should be maintained on the basis of the main request or the auxiliary requests. If the Board of Appeal cannot follow our argumentation with regard to the pending requests, Patentee reserves the right to submit further auxiliary requests.

A. v. Uexküll

Dr. Alexa von Uexküll
European Patent Attorney

Encl.

Exhibit 1: Roswell, D.F. and White, E.H., Matthews, J.C. and Cormier, M.J., and Fertel, R. and Weiss, B., Methods in Enzymol. Vol. LVII, Bioluminescence and Chemiluminescence, DeLuca, M.A. (Ed.)(1978), 409-410, 107-108, and 94-96, respectively

Exhibit 2: Kochetkov, N.K., et al., Organic Chemistry of Nucleic Acids, Part B, Kochetkov, N.K. and Budovskii, E.I. (Eds.)(1972), 331-332

Two copies of this letter for Opponents I and II