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Datasheet for the decision of 12 September 2017

Case Number: T 2132/16 - 3.3.08

Application Number: 08776043.5

Publication Number: 2183693

IPC: G06F19/00, C12Q1/68

Language of the proceedings: EN

Title of invention:
DIAGNOSING FETAL CHROMOSOMAL ANEUPLOIDY USING GENOMIC SEQUENCING

Patent Proprietor:
The Chinese University of Hong Kong

Opponents:
OLSWANG LLP
Polz, Leo
Premaitha Health PLC

Headword:
Fetal chromosomal aneuploidy/CHINESE UNIVERSITY OF HONG KONG

Relevant legal provisions:
EPC Art. 56, 83, 84, 87, 123(2)
RPBA Art. 12(4), 13(1)

Keyword:
Late filed evidence - not admitted
Added matter - (no)
Clarity and sufficiency of disclosure - (yes)
Priority - (no)
Inventive step - (yes)

Decisions cited:
G 0003/14

Catchword:

Europäisches Patentamt
European Patent Office
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Beschwerdekammern
Boards of Appeal
Chambres de recours

European Patent Office
D-80298 MUNICH
GERMANY
Tel. +49 (0) 89 2399-0
Fax +49 (0) 89 2399-4465

Case Number: T 2132/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 12 September 2017

Appellant I: (Patent Proprietor)	The Chinese University of Hong Kong Office of Research and Knowledge Transfer Services Room 301, Pi Ch'iu Building, Shatin New Territories Hong Kong (HK)
Representative:	von Menges, Albrecht Uexküll & Stolberg Partnerschaft von Patent- und Rechtsanwälten mbB Beselerstraße 4 22607 Hamburg (DE)
Appellant II: (Opponent 2)	Polz, Leo Klebelstr. 18 85356 Freising (DE)
Representative:	Hoffmann Eitle Patent- und Rechtsanwälte PartmbB Arabellastraße 30 81925 München (DE)
Appellant III: (Opponent 3)	Premaitha Health PLC Rutherford House Manchester Science Park Manchester M15 6SZ (GB)
Representative:	Gibson, Mark Sagittarius IP Three Globeside Fieldhouse Lane Marlow, Buckinghamshire SL7 1HZ (GB)

Other party: (Opponent 1)	OLSWANG LLP 90 High Holborn London Greater London WC1V 6XX (GB)
Representative:	Stephen, Robert John CMS Cameron McKenna Nabarro Olswang LLP Cannon Place 78 Cannon Street London EC4N 6AF (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 18 July 2016 concerning maintenance of the European Patent No. 2183693 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. R. Vega Laso
	D. Rogers

Summary of Facts and Submissions

I. European patent No. 2 183 693 with the title "Diagnosing fetal chromosomal aneuploidy using genomic sequencing" was granted on the European application No. 08776043.5, which had been filed as international application under the Patent Cooperation Treaty and published as WO 2009/013496 (in the following "the application as filed") claiming the priority of the earlier US application US 60/951,438 filed on 23 July 2007.

II. The patent, which was granted with 24 claims, was opposed by two parties (opponents 01 and 02) on the grounds for opposition of Article 100(a), 100(b) and 100(c) EPC. A third party (opponent 03) intervened in accordance with Article 105 EPC.

III. In an interlocutory decision under Article 101(3)(a) and 106(2) EPC posted on 18 July 2016, an opposition division of the European Patent Office found that the ground for opposition of Article 100(c) EPC prejudiced the maintenance of the patent as granted (main request), but that, account being taken of the amendments introduced by the patent proprietor into claims 1 to 21 according to the auxiliary request 1, the patent and the invention to which it relates met the requirements of the EPC.

IV. Claim 1 according to auxiliary request 1 reads as follows:

"1. A method for performing prenatal diagnosis of a fetal chromosomal aneuploidy in a biological sample obtained from a female subject pregnant with a fetus, wherein the biological sample is maternal plasma or

serum and wherein the sample includes cell-free nucleic acid molecules from the female subject and the fetus, the method comprising:
performing a random sequencing on at least a portion of a plurality of the nucleic acid molecules contained in the biological sample to obtain a pre-determined number of sequences, wherein the sequences represent a fraction of the human genome;
aligning, with a computer system, each sequence to a human genome;
determining a first amount of sequences identified as being aligned to a first chromosome;
determining a second amount of sequences identified as being aligned to one or more second chromosomes;
determining a parameter from the first amount and the second amount; wherein the parameter represents a relative amount between the first and second amounts; and
comparing the parameter to one or more cutoff values, to determine a classification of whether a fetal chromosomal aneuploidy exists for the first chromosome."

Dependent claims 2 to 17 and 19 to 21 are directed to specific embodiments of the method of claim 1. Independent claim 18 relates to a computer program product comprising a computer readable medium for performing prenatal diagnosis of a fetal chromosomal aneuploidy.

V. The patent proprietor and the three opponents each lodged an appeal against the interlocutory decision of the opposition division. However, opponent 01 withdrew its notice of appeal.

VI. A statement setting out the grounds of appeal was submitted by each the patent proprietor (appellant I) and opponents 02 and 03 (appellant II and III, respectively). Together with its statement, appellant II filed new evidence.

VII. Both appellant III and the Swiss Federal Patent Court requested accelerated processing of the appeal. After considering the appellants' submissions on this issue, the board decided to grant the request and summoned the parties for oral proceedings.

VIII. Appellant I replied to the grounds of appeal of the other parties. It maintained its main request (claims as granted) and the auxiliary requests 1 to 17 submitted in opposition proceedings, and filed three additional sets of claims as auxiliary requests 18 to 20, as well as further evidence. By letter dated 18 April 2017, appellant I filed further evidence.

IX. Appellants II and III submitted observations on the grounds of appeal of appellant I. The other party (opponent 01) did not make any submissions.

X. In a communication in preparation of the oral proceedings, the board made observations concerning the admission of the new requests into the proceedings and expressed a provisional opinion on various issues under Articles 123(2), 83, 87 and 56 EPC.

XI. In reply to the board's communication, appellants I and III submitted additional observations and evidence.

XII. Oral proceedings were held on 12 September 2017. During the oral proceedings, appellant I withdrew its request to set aside the decision under appeal and maintain the

patent as granted, and requested dismissal of the appeals of appellants II and III.

XIII. The following documents are referred to in the present decision:

(2): Y.M. Dennis Lo et al., 7 August 2007, PNAS, Vol. 104, No. 32, pages 13116 to 13121;

(5'): H.Ch. Fan and S.R. Quake, 1 October 2007, Anal. Chem., Vol. 79, No. 19, pages 7576 to 7579;

(6): US 2005/0221341 A1, published on 6 October 2005;

(14): P.J. Campbell et al., June 2008, Nature Genetics, Vol. 40, No. 6, pages 722 to 729;

(16): A. Weise et al., 2012, Journal of Histochemistry & Cytochemistry, Vol. 60, No. 5, pages 346 to 358;

(23b): M. Margulies et al., 15 September 2005, Nature, Vol. 437, pages 376 to 380;

(28): T.J. Jensen et al., 2012, Clinical Chemistry, Vol. 58, No. 7, pages 1148 to 1151;

(29): A. Srinivasan et al., 7 February 2013, The American Journal of Human Genetics, Vol. 92, pages 167 to 176;

(69): M.A. Hultén et al., 2003, Reproduction, Vol. 126, pages 279 to 297;

(70): Extract of transcript of cross-examination of Professor William Allen Hogge in the High Court

of Justice, Chancery Division, Patents Court, dated 10 July 2017;

(74): Extract of first expert report of Dr Henry Anthony Erlich, dated 8 May 2017;

(78): Extract of transcript of cross-examination of Professor Michael Lovett in the High Court of Justice, Chancery Division, Patents Court, dated 12 July 2017;

(79): Extract of first expert report of Professor Jonathan Marchini, dated 8 May 2017.

XIV. The submissions made by appellant I concerning issues relevant to this decision, were essentially as follows:

Admission of documents (69), (70), (74), (78) and (79) into the proceedings

The documents in question had been filed at a late stage of the proceedings and were irrelevant for assessing the patentability of the claimed subject-matter. The parts of the expert statements on which appellant III relied did not contain an explanation of technical facts, but provided only an assessment of obviousness or inventive step as applied in the United Kingdom (UK), which differed from the assessment made by the Boards of Appeal. The evidence was incomplete, had been taken out of context, and provided a misleading impression of the arguments and evidence assessed in the UK litigation. If the board were to admit these documents, it was requested to admit further evidence from the UK litigation filed in reply to appellant III's submission. Otherwise, appellant I's right to be heard would be violated. Therefore, the

board should not admit the documents into the proceedings.

Article 123(2) EPC – added matter

Re: "maternal plasma or serum sample including cell-free nucleic acid molecules"

The application as filed explicitly disclosed that the methods of the invention were preferably carried out using a sample comprising cell-free nucleic acid molecules. The use of maternal plasma as a biological material represented only an example or a preferred form of a cell-free sample. It was apparent from paragraph [0054] of the application as filed that the methods could be carried out with a serum sample, which was also a cell-free sample.

Re: "a pre-determined number of sequences"

The application as filed, in particular claim 14 and paragraphs [0081], [0082], [0101], [0102] and [0110] to [0114] disclosed that the method of the invention was carried out to obtain a pre-determined number of sequences.

Re: "identified as being aligned to the first/second chromosome"

The step of aligning the sequence to a human genome was disclosed throughout the application as filed. In spite of the different wording there was no substantive difference between identifying the sequences as "*originating from a first/second chromosome*" (as in claim 1 as filed) and "*as being aligned to a first/second chromosome*" (as in amended claim 1 of the

patent). Both claims required that an amount of sequences was determined and that it was known from which chromosome the sequences originated.

Re: "the parameter represents a relative amount between the first and second amounts"

The feature had a basis in, e.g., paragraphs [0043], [0062] or [0074] of the application as filed. From paragraph [0043] it was readily apparent to one of ordinary skill that, as used in the application, a parameter could be any numerical relationship between quantitative data sets, which was in substance the same as the "relative amount" specified in claim 1.

Articles 84 and 83 EPC – clarity and sufficiency of disclosure

The term "cut-off value" was defined in paragraph [0041] of the patent and its determination was illustrated in detail in Figure 7 and paragraphs [0110] to [0112]. The patent also disclosed a pre-determined number of sequences for a desired range of accuracy.

Article 87 EPC – priority

The claimed subject-matter was entitled to the filing date of the priority application. The opposition division had misinterpreted the disclosure of the priority application on random sequencing (in particular paragraph [0192]) and erred in finding that in the priority application "random sequencing" was not used in a general context applicable to all embodiments of the invention, but only in relation to emulsion PCR. The reference to emulsion PCR in paragraph [0192] of

the earlier application characterized a method for amplifying a nucleic acid target and not a sequencing step a such. Massively parallel sequencing of samples via a preparatory emulsion PCR step represented only "another example" of carrying out the method disclosed in the earlier application (see page 52, line 30). Only two of the three example sequencing systems disclosed in paragraph [0192] of the priority application used emulsion PCR, while the third system, the Illumina Solexa system, used a non-emulsion method for amplifying the sequences.

Article 56 EPC – inventive step

Document (6), which represented the closest state of the art, described a method of karyotyping a genome of a test cell with the aim of detecting abnormalities. The ratios used for aneuploidy determination were clearly based on the analysis of a single chromosome from an isolated fetal cell, not a mixture of maternal and fetal DNA. Thus, both the starting material and the steps of the method of document (6) differed from those of the method of the invention. In the method of document (6) neither a second amount of sequences identified as being aligned to a second chromosome, nor a relative amount from a first and second amount of sequences was determined.

Starting from document (6), the problem to be solved was to provide an improved method for performing prenatal diagnosis of a fetal chromosomal aneuploidy. The problem was solved by the method defined in the claims. The solution was not obvious in view of document (6) alone. A person skilled in the art would not have combined the method of document (6) with that of document (2) because the two approaches differed

significantly in the starting material. A combination of features of the two methods could only be done applying hindsight considerations. Thus, the claimed method involved an inventive step.

XV. The submissions by appellant II, insofar as they are relevant to the present decision, may be summarised as follows:

Article 123(2) EPC – added matter

Re: "maternal plasma or serum sample including cell-free nucleic acid molecules"

While the term "cell-free" was disclosed in the application as filed in connection with plasma DNA, there was no reference to any other cell-free nucleic acids from other sources, in particular to RNA in serum. Paragraph [0054] cited in the decision under appeal did not indicate whether serum always contains cell-free nucleic acids, and whether serum and plasma always contain not only cell-free DNA, but also RNA. Since RNA was known to be less stable than DNA, there was an inextricable link between the term "cell-free" and "plasma DNA" in the application as filed.

Re: "a pre-determined number of sequences"

The introduction of this feature into claim 1 offended against Article 123(2) EPC. As apparent from Figure 2 and paragraph [0110], the method disclosed in the application as filed included a step in which the number of sequences required for the analysis was determined. This essential method step was not specified in claim 1.

Re: "identified as being aligned to the first/second chromosome"

The feature had no basis in the application as filed. The teaching conveyed by this feature differed from that of the feature "*determining a first amount of a first chromosome from sequences identified as originating from the first chromosome*" in claim 1 of the application as filed. While the latter required the actual chromosomal origin to be identified, this was not the case for the feature in claim 1 at issue. None of the passages of the application as filed referred to in the decision under appeal allowed to conclude, directly and unambiguously, that it would be unnecessary to determine the chromosomal origin of the sequences. In the case of a repetitive sequence aligning to different chromosomes, the mere "aligning" would not allow to determine whether the sequence originated from a particular chromosome.

Re: "the parameter represents a relative amount between the first and second amounts"

Paragraph [0043] of the application as filed provided no explicit mention of a "relative amount". The generic definition provided in this paragraph did not apply to the very specific context of the method of claim 1. Hence, claim 1 contravened Article 123(2) EPC.

Articles 84 and 83 EPC – clarity and sufficiency of disclosure

The patent did not provide an experimental example of an actual prenatal diagnosis of a fetal chromosomal aneuploidy or a computer programme for performing the diagnosis. In particular, no specific cut-off values

for the "parameter" were tested in the patent. Additionally, the theoretical calculations of the number of sequences required for a statistically significant diagnosis of trisomy were not based on experimental analysis of trisomy 21 samples, but on permutations of subsets of sequences obtained from a single euploid male fetus.

The accuracy of a classification of a fetal aneuploidy according to the method of the patent depended on the number of sequences analysed. However, the minimal amount of sequencing required for prenatal diagnosis was not defined in the claims.

In view of the disclosure in the application itself, it was not plausible that the claimed method was suitable for the detection of fetal aneuploidies in small chromosomal regions. The term "chromosomal aneuploidy" as used in the application did not only comprise gains or losses of whole chromosomes, but also of regions of chromosomes. The extent of a "region" was not limited in any way. Thus, also microdeletions and microduplications as small as 2 kb as disclosed in document (16) were included. It was apparent from documents (28) and (29) that, in order to detect such small deletions the required genomic coverage was much larger than the less than one-fold coverage disclosed in the application.

Article 87 EPC - priority

The opposition division was right to acknowledge that, since the features "random sequencing" and "fraction of the human genome" were not disclosed in the priority document, the priority date claimed for the patent was not valid. Additionally, the claims were not entitled

to the priority date because i) in the priority application the only parameter disclosed in connection with "random sequencing" was a "normalized frequency", rather than a relative amount as specified in claim 1; and ii) the term "random sequencing" was given a different meaning in the priority application and the opposed patent.

Article 56 EPC – inventive step

Document (2) represented the closest state of the art because it related to non-invasive methods for the diagnosis of fetal aneuploidies. The sole difference between the method described in this document and that of claim 1 was that the latter used random sequencing instead of digital PCR and that, following sequencing, the sequences were aligned to the human genome by using a computer system.

The opposition division had failed to determine the objective technical problem in view of document (2). Since the patent in suit did not include any comparative examples demonstrating enhanced accuracy of diagnosis compared to the method described in document (2), the technical problem to be solved had to be formulated as the provision of an alternative method for performing prenatal diagnosis of a fetal chromosomal aneuploidy in maternal plasma, and a computer program product for performing the method. The solution proposed in claim 1 which involved random sequencing followed by computer-based alignment was clearly obvious in view of either the common general knowledge, or document (23b).

At the effective date of the patent, it was common general knowledge that "massively parallel genomic

sequencing" as mentioned in document (2), meant random sequencing. A person skilled in the art reading document (2) would understand from the word "*genomic*" together with the reference to document (23b), which described random sequencing of a whole bacterial genome, that random massively parallel sequencing of the complete genome rather than a targeted approach was meant. At the effective date, the skilled person was aware from, e.g., document (5') (see page 7576, second column, first paragraph) that random, rather than targeted, sequencing was an alternative approach to conducting digital PCR.

It was immediately apparent to the skilled person that, when random sequencing was used, the sequences were identified not prior to, but after the sequencing, by alignment to the human genome using a computer system. The patent did not provide any additional teaching that went beyond this simple modification of the method of document (2). In view of the statement in document (2) that the suggested alternative approaches would greatly enhance the clinical applicability of the methods described therein, the skilled person would be motivated to follow them. Hence, the claimed method did not involve an inventive step.

XVI. The submissions by appellant III, insofar as they are relevant to the present decision, may be summarised as follows:

Admission of documents (69), (70), (74), (78) and (79) into the proceedings

Document (69) was highly pertinent to the discussion of the common general knowledge and inventiveness over document (6), because it demonstrated that as early as

2003 there was a clear sign-post motivating a skilled person or a team of skilled persons to apply new techniques which had been used on fetal cells to the non-invasive analysis of DNA retrieved from maternal blood samples. Since appellant III had become aware of it only during litigation in the UK, document (68) could not have been submitted earlier. Documents (70), (74), (78) and (79) related to transcripts of cross-examination given under oath and written reports signed with statements of truth from technical experts called during the litigation, the trial of which was conducted in July 2017, i.e. only few days before the documents were submitted to the board. The documents were *prima facie* relevant. Documents (69) and (70) were evidence on the common general knowledge at the priority date of the patent in suit, and documents (74), (78) and (79) provided clear guidance that the patent lacked inventive step in the light of document (6). Since appellant I had been party to the proceedings from which the documents were derived, they were known to it. Hence, it was not disadvantaged. For these reasons, the evidence should be admitted into the appeal proceedings.

Article 123(2) EPC – added matter

Re: "maternal plasma or serum sample including cell-free nucleic acid molecules"

References to "cell-free" within the application as filed were all with respect to the sample being maternal plasma, not maternal plasma or serum as required by claim 1. The term "cell-free" in claim 1 in connection with serum added subject-matter, because in the application as filed the term "cell-free" was inextricably linked to the use of maternal plasma.

Re: "a pre-determined number of sequences"

While claim 14 of the application as filed used the term "pre-determined", it did not use it in the specific context of obtaining a pre-determined number of sequences. Rather, claim 14 specified a pre-determined fraction of the human genome. The number of sequences and the fraction of the human genome could be two entirely independent values depending upon the sizes of the fragments analysed by random sequencing. Paragraphs [0081] and [0082] of the application as filed referred to the step of calculating a number of sequences to be analysed as being essential "for a desired accuracy". The isolation of the "pre-determined" feature in the absence of the specific technical context of the accuracy and fetal fraction features of paragraph [0081] of the application as filed amounted to an impermissible intermediate generalisation.

Re: "identified as being aligned to the first/second chromosome"

This feature constituted added matter as it was taken out of the specific technical context in which it was disclosed in paragraphs [0070] and [0071] of the application as filed. It represented an intermediate generalisation of the term "*originate*" used in claim 1 of the application as filed.

Re: "the parameter represents a relative amount between the first and second amounts"

There was no basis in the application as filed for the feature "relative amount" introduced into claim 1.

While it was stated in paragraph [0043] of the application as filed that a parameter as used therein meant a numerical relationship between quantitative data sets, there was no mention in claim 1 of such "quantitative data sets".

Article 87 EPC – priority

The claimed subject-matter was not entitled to priority. The skilled person would not be able to derive the subject-matter of claim 1 directly and unambiguously, using common general knowledge, from the previous application which disclosed only massively parallel sequencing using emulsion PCR. The term "massively parallel sequencing (MPS)" used in paragraph [0192] of the priority application did not refer to "random sequencing of at least a portion of a plurality of sequences" as required in claim 1. MPS was not the same as random sequencing which required that the genomic origin of the nucleic acid molecules is not known *a priori*. MPS could be conducted in both a random and targeted manner.

Article 56 EPC – inventive step

Document (6) represented the closest prior art. The sole difference between the method described in document (6) and the method of the invention was that the biological sample was maternal plasma or serum, instead of amniotic fluid. Hence, the problem to be solved was the provision of a non-invasive detection method of fetal chromosomal aneuploidy.

The solution proposed in the claims was obvious in view of document (6) combined with document (2) which provided a clear motivation to use MPS to analyse a

biological sample which included cell-free nucleic acid molecules from the female subject and the fetus. Document (2) provided explicit guidance regarding how to calculate a parameter from a comparison of a first chromosome with one or more second chromosomes. Hence, the claimed method lacked an inventive step.

XVII. Appellant I (patent proprietor) requested that the appeals of appellant II and appellant III be dismissed.

XVIII. Appellants II and III (opponents 01 and 02, respectively) requested to set aside the decision under appeal and to revoke the patent.

Reasons for the Decision

Admission of documents (69), (70), (74), (78) and (79) into the proceedings

1. In reply to the communication sent by the board in preparation of the oral proceedings, appellants I and III submitted new evidence, including various pieces of evidence originating from litigation in the United Kingdom concerning validity and infringement of *inter alia* the patent in suit. In its submissions at the oral proceedings, appellant III sought to rely in particular on documents (69), (70), (74), (78) and (79) as support for its line of argument concerning inventive step in the light of document (6) in combination with the common general knowledge.

2. Pursuant to Article 12(4) of the Rules of Procedure of the Boards of Appeal (RPBA), the board has the discretionary power to hold inadmissible facts, evidence or requests which could have been presented or

were not admitted in opposition proceedings. Moreover, according to Article 13(1) RPBA, it is at the board's discretion to admit and consider any amendment to a party's case after it has filed its grounds of appeal or reply. The discretion shall be exercised in view of *inter alia* the complexity of the new subject-matter submitted, the current state of the proceedings and the need for procedural economy.

3. Document (69) is a review paper published in 2003 discussing molecular methods being applied to aneuploidy detection using fetal cells. It was filed by appellant III at a very late stage of the appeal proceedings - roughly 5 weeks before the oral proceedings - as evidence of the common general knowledge at the filing date. The board observes that the objection of lack of inventive step based on document (6) as the closest state of the art combined with the common general knowledge had been raised already in opposition proceedings. The objection was contested by the patent proprietor (present appellant I) and considered by the opposition division in arriving at its decision (see section 5.15 of the decision under appeal). Hence, objectively, document (69) could - and should - have been filed in opposition proceedings, or, at the latest, together with appellant III's statement of grounds of appeal. Appellant III's argument that it had become aware of this document only during the litigation in the United Kingdom cannot be accepted, as it is the responsibility of a party to search for and submit relevant evidence as soon as an issue becomes a subject of dispute.

4. Since document (69) was not filed in due time, the board has the discretionary power to admit it into the proceedings or disregard it (Article 12(4) RPBA).

Contrary to appellant III's view, the board does not consider the evidence provided in document (69) to be highly relevant in the sense that it is highly likely to prejudice the maintenance of the patent as amended. Therefore, exercising its discretion, the board decides not to admit this document into the proceedings.

5. Documents (70) and (78) are extracts from a transcript of the cross-examination of two technical experts called by the claimants in the UK litigation, among which was the present appellant I. Documents (74) and (79) are written reports by two technical experts called by the present appellant III in the UK litigation. These four documents contain the opinion of different technical experts on various issues relating to inventive step, in particular the interpretation of the content of document (6), and the knowledge and ideas of a skilled team in the relevant technical fields, which in the UK litigation was considered to comprise a clinician, a molecular biologist and a biostatistician.

6. It should be noted that, while in the UK litigation procedure technical experts are regularly called by the parties to provide technical assistance to the judge, the composition of the Technical Boards of Appeal includes at least two technically qualified members who are themselves able to assess technical facts. In the present case, the board considers itself in the position to decide upon the matter without the further technical assistance provided by the experts who gave evidence in UK litigation.

7. Appellant I opposed the admission of documents (70), (74), (78) and (79) into the proceedings, and requested that, if these documents were admitted, additional

evidence from the UK litigation, which was submitted with its reply, be admitted as well. The board, when exercising the discretion conferred by Article 13(1) RPBA, has to take into account the degree of procedural complication that the admission of documents (70), (74), (78) and (79) at the very late stage of the appeal proceedings is likely to cause. Also in view of the fact that, as stated above, these documents do not include any new technical facts, but rather mere opinions on technical and legal issues which the members of the board are able to understand and decide upon without the assistance of technical experts, the board decides not to admit these documents into the proceedings.

Article 123(2) EPC – added matter

Re: "maternal plasma or serum sample including cell-free nucleic acid molecules"

8. In the decision under appeal, the opposition division found that the amendments introduced into claims 1 and 18 according to the auxiliary request 1 did not offend against Article 123(2) EPC because the feature "*maternal plasma or serum including cell-free nucleic acid molecules*" had a basis in the application as filed, specifically in claim 1 combined with claim 3, and in paragraph [0054] referring to step 110 in Figure 1, in which plasma or serum samples containing nucleic acid molecules from the fetus and the pregnant female are mentioned (see section 2.1.3 of the decision under appeal).

9. Appellants II and III did not dispute that the application as filed discloses maternal plasma or serum sample as a biological sample, but contested the

findings in the decision under appeal arguing that the feature "cell-free" was disclosed only in connection with plasma DNA.

10. In the board's view, this objection is not justified. It can be derived from paragraph [0006] of the application as filed that fetal **nucleic acids** circulate in maternal plasma predominantly in a cell-free form (see last sentence in paragraph [0006]). Undisputedly, plasma, the fluid fraction of blood which can be obtained by removing suspended material like fat globules or cells, is, as such, "cell-free". As regards serum, which is disclosed in claim 3 and paragraph [0054] of the application as filed as one of the biological samples used in the method of the invention as source of nucleic acid, the board shares the opposition division's view that, when reading the application in the light of the common general knowledge in the art, it would be immediately apparent to the notional skilled person - which according to appellant III is a team including a clinician interested in the analysis of genetic material in the blood - that any fetal and maternal nucleic acid molecules contained in a serum sample must necessarily be "cell-free" because serum, like plasma, is a "cell-free" fraction of blood.

11. The board cannot acknowledge in the disclosure in the application as filed an inextricable link between the terms "cell-free" and "plasma DNA", which in appellant II's view is suggested by the fact that RNA is known to be less stable than DNA. Moreover, the fact that the application does not indicate whether serum **always** contains cell-free nucleic acids, in particular cell-free RNA is, contrary to appellant II's view, irrelevant to the assessment of Article 123(2) EPC.

12. Hence, as regards the use of either plasma or serum containing cell-free nucleic acid molecules as a biological sample, the method for performing prenatal diagnosis of a fetal chromosomal aneuploidy according to claim 1 does not extend beyond the content of the application as filed.

Re: "a pre-determined number of sequences"

13. Appellants II and III contested the opposition division's finding that a method characterized by the feature "*... a pre-determined number of sequences, wherein the sequences represent a fraction of the human genome*" has a basis in claim 14 and paragraphs [0081], [0082], [0101], [0102], [0110] to [0114] and Figure 2 of the application as filed (see section 2.1.4 of the decision under appeal).

14. Claim 1 of the application as filed specifies the step of "*... sequencing at least a portion of a plurality of nucleic acid molecules contained in the biological sample, wherein the sequenced portion represents a fraction of the human genome*". In claim 14, which refers to claim 1, the sequenced portion is said to represent at least a pre-determined fraction of the human genome. In the board's view, it is apparent to a person skilled in the art reading claims 1 and 14 in the light of paragraphs [0081], [0082], and [0110] to [0114] of the application as filed, that sequencing a pre-determined fraction of the human genome can only be understood as obtaining - by random sequencing - a pre-determined number of sequences which corresponds to a pre-determined fraction of the human genome. This is particularly clear from paragraph [0114] of the application as filed, from which the skilled person can

derive that, for the calculation of the number of sequences required in order to sequence a pre-determined fraction of the human genome, the size of the sequences generated has to be taken into account.

15. Contrary to appellant II's view, the fact that the particular embodiment of the method of the invention illustrated in Figure 2 includes the step of determining the required number of sequences in order to sequence a pre-determined fraction of the human genome, does not necessarily mean that this step is an essential feature of the method disclosed in the application as filed. In the board's view, it is apparent from the application as filed that the determination of the number of sequences corresponding to the fraction of the human genome to be sequenced for a desired accuracy belongs to the theoretical background of the claimed invention, and is not an essential part of the method of diagnosis of aneuploidy of the invention which is intended for use in the clinical practice.

16. The same applies, *mutatis mutandis*, to the theoretical considerations relating to the determination of the number of sequences to be obtained for a desired degree of accuracy depending on the concentration of fetal nucleic acid in maternal plasma or serum (see, *inter alia*, the second sentence of paragraph [0114] of the application as filed). It cannot be derived from the disclosure in the application as filed that such theoretical considerations are necessarily an additional step of the method of the invention. Hence, the board does not consider that the fact that claim 1 does not specify an accuracy or fetal fraction feature amounts to an impermissible intermediate generalisation of the disclosure in the application as filed.

Re: "identified as being aligned to the first/second chromosome"

17. In section 2.1.5 of the decision under appeal, the opposition division found that this feature had a basis in, *inter alia*, paragraphs [0067] to [0071] of the application as filed.

18. The arguments put forward by appellants II and III against this finding fail to convince the board. For the board, the question whether or not aligning the obtained sequences to a first/second chromosome (as specified in amended claim 1) is identical to identifying the actual origin of the sequences (as specified in claim 1 of the application as filed) is not decisive for assessing compliance of the amendment with Article 123(2) EPC. Rather, the relevant question is whether or not the application as filed discloses the step of aligning the sequences obtained by random sequencing to particular chromosomes. This is certainly the case. The heading of the passage starting from paragraph [0067] of the application as filed reads "*Sequencing, **aligning**, and determining amounts*" (see page 14, line 6 of the application). Moreover, it is clearly and unambiguously derivable from the paragraphs [0070] and [0075] of the application as filed that the sequences generated in the previous sequencing step of the method are aligned to the human genome to determine the chromosomal origin, and that the amount of sequences aligned to a particular chromosome is compared to the amount of sequences aligned to other chromosome(s).

19. As regards the findings in the decision under appeal concerning repetitive sequences (see passage starting

from line 8 of section 2.1.5), the board shares the opposition division's view that the application as filed does not disclose the removal of such sequences as being essential to the method of the invention. In fact, it is stated in paragraph [0093] that, as an alternative to counting only those sequences that have been mapped to a unique location in the repeat-masked human genome reference, "*... the entire set of the sequenced data could* [...] *be used*".

Re: "the parameter represents a relative amount between the first and second amounts"

20. As basis in the application as filed for the wording "relative amount" in present claim 1, the opposition division pointed to claim 1 as originally filed ("*determining a parameter from the first and second amount*") combined with the general definition of "parameter" in paragraph [0043], which reads:

> "*The term "parameter" as used herein means [...] a numerical relationship between quantitative data sets. For example, a ratio (or function of a ratio) between a first amount of a first nucleic acid sequence and a second amount of a second nucleic acid sequence is a parameter*"

21. While it is true that a "relative amount" is not explicitly mentioned in the application as filed, it has not been disputed that the wordings "*numerical relationship between quantitative data sets*" in paragraph [0043] and "*relative amount*" in claim 1 have the same meaning. There is no doubt either that an amount of sequences aligned to a first/second chromosome - as specified in claim 1 - represents a quantitative data set.

22. Moreover, appellant II's allegation that the generic definition provided in paragraph [0043] may not apply to the specific context of the method of claim 1, cannot be accepted, because the wording "*parameter as used herein*" in the passage quoted above implies that the definition given in paragraph [0043] applies to all embodiments of the invention disclosed in the application as filed.

Re: Further issues under Article 123(2) EPC

23. The findings in sections 2.3 and 2.4 of the decision under appeal concerning the compliance of the amendments introduced into some of the dependent claims and the adapted description have not been contested in appeal proceedings. An objection to claim 21 raised for the first time at the oral proceedings before the board was not admitted into the proceedings.

24. Summarising the above: the arguments put forward in appeal proceedings fail to persuade the board that the claimed subject-matter, in particular that of claim 1 extends beyond the content of the application as filed. Hence, the claims according to auxiliary request 1 do not include any amendments that contravene Article 123(2) EPC.

Articles 84 and 83 EPC – clarity and sufficiency of disclosure

25. In the decision under appeal, the opposition division asserted that the opponents had failed to cast serious doubts substantiated by verifiable facts with respect to the disclosure of the claimed invention (see section 6.12 of the decision).

26. In its statement of grounds of appeal, appellant II maintained the objection raised in opposition proceedings based on the definition of "*chromosomal aneuploidy*" in paragraph [0043] of the patent in suit and on document (16). In the board's view, rather than the sufficiency of the disclosure in the application as filed (Article 83 EPC), what appellant II seemed to question was the clarity of the amended claim 1 (Article 84 EPC). However, neither the wording "*chromosomal aneuploidy*" nor the definition in paragraph [0043] has been amended in opposition or appeal proceedings. Hence, in the light of decision G 3/14 (OJ EPO 2015, A102; see Order) the compliance with the requirements of Article 84 EPC cannot be examined. The same applies, *mutatis mutandis*, to the wording "*fraction of the human genome*" and "*portion of the human genome*" (see paragraph [0045] of the patent), to which appellant II referred in support of a further objection under Article 83 EPC raised for the first time in its statement of grounds of appeal.

27. Appellant II further argued that the claimed invention is not sufficiently disclosed over the whole scope of the independent claims because the claims fail to define the minimal amount of sequencing that is required for the diagnosis of a fetal aneuploidy.

28. Article 83 EPC requires that the patent application **as a whole** discloses the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. It is undisputed that the application discloses a method for determining the minimal amount of nucleic acids to be sequenced for a reliable diagnosis (see page 26 under the heading "Determination of number of sequences required"). As an example, the application discloses that, if fetal DNA

is present in maternal plasma at a concentration of about 5%, at least 0.6% of the human genome has to be sequenced. Hence, there are no serious doubts substantiated by any facts that a person skilled in the art applying the technical teaching provided in the application could determine the minimal amount of sequencing required for a given - higher or lower - fetal DNA concentration, without an undue burden of experimentation or inventive skills.

29. As further arguments concerning the disclosure of the invention, appellant II first put forward an alleged failure to test specific cut-off values for the parameter specified in claim 1. Second, appellant II was critical as regards the fact that in the application as filed the number of sequences required for statistically significant diagnosis of trisomy 21 is based on data obtained from a single euploid male fetus. In the board's view, these arguments fail to support the objection of lack of sufficient disclosure. While it is true that the application as filed does not disclose specific cut-off values for the parameter specified in claim 1, it does, however, provide a clear definition of "cut-off value" (see paragraph [0044] of the application) and describes how it can be determined (see paragraphs [0064] to [0066]). There is no evidence on file that the determination of such values goes beyond the ordinary skills of an average biostatistician and represents an undue burden.

30. As regards the teaching how to determine the number of sequences required for a statistically significant diagnosis of trisomy 21, the data provided in Figure 7 and paragraphs [0110] to [0114] of the application as filed are considered to represent a theoretical numerical example indicating which parameters may

influence the amount of sequencing required. While it is true that the theoretical calculations in Figure 7 and paragraphs [0110] to [0114] are based on sequences obtained from an euploid male fetus, appellant II has not provided any evidence which may cast doubts on whether, at the relevant date, a person skilled in the art, applying the teachings derivable from the figure and the passage indicated above, was able to determine the number of sequences required for a statistically significant diagnosis of any chromosomal aneuploidy.

31. The board thus concludes that the application as filed discloses the invention as claimed according to auxiliary request 1 in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

Article 87 EPC – priority

32. The adverse findings in the decision under appeal concerning the priority of the subject-matter of claims 1 and 18 (see sections 3.8 to 3.10 of the decision) were contested by appellant I. In particular, appellant I contested the opposition division's finding that the earlier US application filed on 23 July 2010 does not disclose the method of present claim 1, but only a specific embodiment of the method in which massively parallel genomic sequencing using emulsion PCR is performed. In its line of argument, appellant I relied essentially on paragraphs [192] and [132] of the earlier US application.

33. The earlier US application is primarily concerned with the use of digital PCR for determining an imbalance between two different nucleic acid sequences. The method is used for, *inter alia*, detecting chromosomal

aneuploidies in a fetus via testing a sample of maternal blood. Paragraph [0192] under the heading "Massively parallel genomic sequencing using emulsion PCR", in particular the passage on page 53, lines 1 to 7 reads:

> "*The variant of digital PCR is the performance of massively parallel genomic sequencing using emulsion PCR in a sequencing machine such as the Roche GS20 system (http://www.454.com/about-454/partners.asp) the Applied Biosystems 'supported oligo ligation detection' (SOLiD) and the Illumina Solexa sequencing technology. The general principle of this strategy is that if one is to do random sequencing of DNA fragments that are present in the plasma of a pregnant woman, then one would obtain genomic sequences which would originally have come from either the fetus or the mother.*"

34. While random sequencing for the purpose of obtaining genomic sequences that originate from either the fetus or the mother is mentioned in this passage, the board is not persuaded that a person skilled in the art may derive, clearly and unambiguously, from the passage a method for performing random sequencing on a plurality of nucleic acids in parallel (i.e. random massively parallel genomic sequencing), other than a method using emulsion PCR.

35. The board does not share appellant I's view that the skilled person would not regard the random sequencing method disclosed in the passage quoted above to be limited to one using emulsion PCR, because among the technologies mentioned therein the Roche GS20 system and the Applied Biosystems' supported oligo ligation detection (SOLiD) in fact used emulsion PCR, but the

Illumina Solexa technology did not. In the board's view, the wording "*... the performance of massively parallel genomic sequencing using emulsion PCR in a sequencing machine such as ...*" clearly indicates that each of the three sequencing technologies mentioned uses emulsion PCR. Even if the board were to accept that the average skilled person reading the earlier US application was familiar with all three sequencing technologies mentioned in the passage quoted above, and realized that there was a discrepancy between the clear indication in both the headline and the text of paragraph [0192] ("*using emulsion PCR*") and one of the exemplary technologies mentioned, it is uncertain whether he/she would regard the reference to the Illumina Solexa sequencing technology to be erroneous, or whether he/she would derive therefrom that the suggested massively parallel genomic sequencing did not necessarily involve emulsion PCR. Under these circumstances, the board cannot acknowledge in paragraph [0192] of the earlier US application a direct and unambiguous disclosure of a random sequencing method which does not involve performing emulsion PCR.

36. Appellant I pointed also to paragraph [0132] of the earlier US application which reads:

> "*Additionally, there are now a number of alternative approaches to the manual set up of digital real-time PCR analyses as used in the current study for conducting digital PCR. These alternative approaches include microfluidics digital PCR chips (Warren, L et al. 2006 Proc Natl Acad Sci USA 103, 17807-17812; Ottesen, EA et al. 2006 Science 314, 1464-1467), emulsion PCR (Dressman, D et al. 2003 Proc Natl Acad Sci USA 100, 8817-8822), and massively parallel genomic*

sequencing (Margulies, M. et al. 2005 Nature 437,376-380), etc. With the use of these methods, digital RNA-SNP and digital RCD could be performed rapidly on a large number of samples, thus enhancing the clinical feasibility of the methods proposed here for noninvasive prenatal diagnosis."

37. It should be noted that, while emulsion PCR is not mentioned in this passage, there is also no explicit disclosure of **random** massively parallel genomic sequencing. Appellant I contended that the skilled person would derive from the reference to the publication by Margulies et al. (document (23b) in the present proceedings) that what is meant in paragraph [0132] is random massively parallel genomic sequencing. However, the board observes that, while document (23b) in fact describes random sequencing, the method also involves an **emulsion-based** clonal amplification step (see first paragraph under the heading "Methods" on page 380). There is no apparent reason why the skilled person would derive from the reference to document (23b) only the "random aspect" of the method described therein, but not the "emulsion aspect". Hence, the board concludes that, even if the skilled person reads paragraph [0132] in the light of document (23b), he/she does not derive therefrom the claimed subject-matter.

38. Finally, also appellant I's argument relying on a combination of paragraphs [0132] and [0192] fails to convince the board that the earlier US application disclosed the method claimed in the patent. First, there is no apparent link between the disclosure in the two paragraphs. And secondly, if both paragraphs were nevertheless read together, the reference to document (23b) in paragraph [0132] would corroborate

the disclosure in paragraph [0192] of MPS using emulsion PCR.

39. It is therefore concluded that, since the earlier US application does not disclose the claimed subject-matter, its priority cannot be validly claimed. Consequently, the relevant date for the purpose of determining what is comprised in the state of the art (see Articles 54 and 56 EPC) is the date of filing of the patent application.

Article 56 EPC – inventive step

Document (6) as the closest state of the art

40. In the decision under appeal, the opposition division held that the method of claim 1 was not obvious to a person skilled in the art in view of the teachings of document (6) combined with those of document (2), document (8) or the common general knowledge (see sections 5.3 to 5.15 of the decision). Appellant III contested this finding relying essentially on document (6) combined with the common general knowledge and/or document (2).

41. Document (6) describes a method for genomic analysis of a test cell, termed "Sequence-Based Karyotyping". In appellant III's view, the sole difference between the method described in document (6) and the claimed method is the use of maternal plasma or serum as the biological sample to be analysed. The board disagrees. It is apparent from the passages of document (6) on which appellant III relied (see paragraphs [0007], [0011] to [0014], [0071] to [0073] and [0265] to [0267], that the method described therein is aimed at the identification of karyotypic differences between a

test cell (e.g. a cancer cell) and a reference cell (e.g. a karyotypically "normal" cell). The method comprises generating a pool of fragments of genomic DNA by a random fragmentation method, determining the sequence of at least a part of each fragment, mapping the fragments to the genome of the organism from which the test cell originates, and comparing the distribution of the fragments relative to that in the genome **of the reference cell**. While it is stated in document (6) that the window for comparison can be restricted to one or more regions on the same chromosome, a comparison of the amount of sequences mapped to one chromosome to that mapped to a second chromosome of the same test cell is not described. Hence, the board considers that the findings in sections 5.4 to 5.7 of the decision under appeal are correct.

42. The opposition division considered the technical effect associated with the identified differences to be that no (fetal) cells and no external reference were required, and that the method could therefore be performed in a non-invasive manner and in simplified form. Consequently, it formulated the objective technical problem to be solved starting from document (6), as the provision of a simplified non-invasive method for prenatal diagnosis of a fetal chromosomal aneuploidy, and held that the problem appeared to be solved by the method of claim 1 and the computer program product of claim 18 (see sections 5.8 to 5.10 of the decision under appeal). The board observes that document (6) neither mentions nor suggests that an invasive method for obtaining amniotic fluid as the source of fetal cells may be problematic. However, it may be assumed – as the opposition division

apparently did - that this was a fact well-known in the art at the filing date.

43. While appellant III did not contest the formulation of the problem in the decision under appeal, it contended that claim 1 failed to specify features essential for solving the technical problem. In its view, in the absence of a specific enrichment step for the fetal nucleic acid, neither random sequencing nor the steps for obtaining the parameter solved the problem of high maternal nucleic acid background.

44. For the following reasons, the board cannot accept this objection. First, rather than raising an issue of inventive step appellant III seems to question whether claim 1 in fact defines the matter for which protection is sought (Article 84 EPC). Compliance with Article 84 EPC is, however, not a ground for opposition, and an objection in this respect can only be considered by the board if the deficiency arises from an amendment introduced into the claims in opposition or appeal proceedings (see decision of the Enlarged Board of Appeal G 3/14, *supra*). This is not the case in the present appeal.

45. Secondly, appellant III's contention is not supported by the passage of document (2) on which it relied (see page 13121, left-hand column, second paragraph). The passage in question concerns selective enrichment in order to achieve a fractional fetal DNA concentration of 25%, which is considered by the authors of document (2) to allow correct disease classification applying the methods described therein. However, the methods of document (2) undisputedly differ from the method of the invention (see below), the latter allowing prenatal diagnosis of fetal chromosomal

aneuploidy with a significantly lower fractional fetal DNA concentration.

46. And thirdly, although the application itself teaches the benefits of the selective enrichment of fetal DNA (see paragraphs [0077] to [0079]) as a possible embodiment of the method of the invention, this cannot be considered as cogent evidence that the method of claim 1, which does not expressly include an enrichment step, does not solve the technical problem.

47. Appellant III put much emphasis on the definition of the person skilled in the art, which in its view is a skilled team comprising a clinician, a molecular biologist and a biostatistician. This has not been contested by appellant I.

48. The issue that remains to be decided is whether this skilled team, starting from document (6), would arrive at the claimed invention in an obvious manner. Appellant III pointed to various passages of document (6) (*inter alia* paragraphs [0090], [0259] and [0253]) allegedly providing an incentive to increase the amount of sequencing and/or to use cell-free fetal DNA instead of DNA extracted from a cell. This line of argument is unconvincing because, even if it were accepted that the fact that maternal plasma contains fetal DNA was part of the common general knowledge at the filing date, it does not explain how the skilled team would deal with the problem of the maternal nucleic acid background.

49. Alternatively, appellant III relied on document (2), in particular the statements on page 13121, right-hand column, first full paragraph. Like the opposition division in the decision under appeal, the board is not

persuaded that document (2) provides an incentive to use **random** massively parallel genomic sequencing for the purpose of prenatal diagnosis. While it is true that the passage indicated by appellant III mentions massively parallel genomic sequencing among various promising approaches for conducting digital PCR, there is no clear suggestion of **random** sequencing. As appellant III asserted in the context of the discussion as to whether the claimed priority is valid, "massively parallel genomic sequencing" (MPS) is not the same as random sequencing. MPS can be conducted in either a random or a targeted manner. While the first does not require that the genomic origin of the nucleic acid molecules be known a priori, in the second – which is in fact the method described in document (2) - primers are designed to target specific desired genomic locations which must be known *a priori*.

50. For these reasons, the board concludes that, starting from document (6) and in view of either the common general knowledge or document (2), it was not obvious to a skilled team to arrive at the claimed method.

Document (2) as the closest state of the art

51. The opposition division held further that the claimed method was not obvious in view of a combination of document (2) with any of documents (14), (23b) and (6). In appeal proceedings, appellant II has contested the opposition division's findings only as regards document (2) in combination with either document (23b) or document (14).

52. The board considers document (2) to be closer to the present invention than document (6) because the methods described therein are non-invasive and based on the

detection of cell-free DNA in maternal plasma. Document (2) forms part of the state of the art for inventive step purposes as priority is not validly claimed in this case (see paragraph 39 above). However, rather than on random sequencing, the methods of document (2) are based on the amplification of defined polymorphisms ("Digital RNA SNP strategy") or genomic regions ("Digital RCD") (see Abstract and Figure 1). The implementation of these methods is said to be "*... rather labor-intensive*" (see page 13121, right-hand column, lines 3 and 4). Moreover, document (2) does not disclose the step of aligning the sequences obtained with a first and one or more second chromosomes.

53. Hence, the objective technical problem to be solved can be formulated as the provision of a simplified, possibly more reliable method for performing prenatal diagnosis of a fetal chromosomal aneuploidy in maternal plasma. This problem is solved by the method of claim 1.

54. Like the opposition division, the board is not convinced that either document (23b), to which document (2) refers, or document (14) provide a hint to the solution proposed in claim 1. While document (23b) describes random sequencing, it does not disclose aligning the sequences with a first and one or more second chromosomes. As regards document (14), which relates to the detection of somatically acquired rearrangements in cancer **cell lines**, the board shares the opposition division's view that the skilled person would not have a reasonable expectation that the method disclosed in this document could be successfully applied to a situation as in maternal plasma, where

differences in fetal DNA have to be assessed with a large background of maternal DNA.

55. Consequently, the board concludes that also in view of document (2) combined with either document (23b) or document (14), the claimed subject-matter was not obvious to a person skilled in the art at the filing date.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chairman:

N. Maslin B. Stolz

Decision electronically authenticated