

CLAIMS

1. A method for detecting a chromosomal aneuploidy in a foetus carried by a pregnant female, said method comprising the steps:

(a) providing a sample taken from said pregnant female, which sample comprises DNA that originates from cells of a foetus and/or the placenta of a foetus in admixture with differently methylated DNA of maternal origin;

(b) treating the DNA present in said sample with a reagent that differentially modifies methylated and non-methylated DNA;

(c) determining an amount of a first target species of DNA, being the chromosome relevant to the chromosomal aneuploidy, in said sample by detecting in said sample the presence of methylation at two or more first target differentially methylated regions (DMRs) located on said chromosome, said first target DMRs differently methylated between DNA that originates from cells of a foetus and/or the placenta of a foetus and DNA of maternal origin, the modification of DNA of the first target DMRs by said reagent is sensitive to methylation of DNA, wherein a detected amount of methylated DNA at one or more of said first target DMRs indicates said amount of first target species of DNA in said sample;

(d) determining an amount of reference species of DNA, being one or more reference chromosomes, in said sample by detecting in said sample the presence of methylation at two or more reference DMRs located on said reference chromosome(s), said reference DMRs differently methylated between DNA that originates from cells of a foetus and/or the placenta of a foetus and DNA of maternal origin, the modification of DNA of such reference DMRs by said reagent is sensitive to methylation of DNA, wherein a detected amount of methylated DNA at one or more of said reference DMRs indicates said amount of reference species of DNA in said sample; and

(e) determining relative amount(s), preferable ratio(s), of an amount determined from step (c) and an amount determined from step (d), wherein one or more of said relative amount(s) indicates the presence or absence of the chromosomal aneuploidy in the foetus,

wherein, said detections in step (c) and step (d) are made using the same aliquot of DNA of said sample, and in the same vessel, and effectively simultaneously for said first target DMRs and said reference DMRs, and using: (x) the same detectable labels(s) for at least two of said reference DMRs; and (y) a different detectable label(s) for at least two of said first target DMRs; and *optionally*,

wherein in step (e) said relative amount(s) or ratio(s) are compared with threshold(s) and/or reference distribution(s), wherein one or more of said relative amount(s) or ratio(s) higher or lower than said threshold(s) and/or reference distribution(s) indicates the presence of the chromosomal aneuploidy in the foetus.

2. The method of claim 1, wherein two or more of said reference DMRs are located on different reference chromosomes.

3. The method of claim 1 or 2 further comprising the step:

(f) determining an amount of total DNA in said sample by detecting at least one other region (OR) that is not differently methylated between DNA that originates from cells of a foetus and/or the placenta of a foetus and DNA of maternal origin, the modification of which OR(s) by said reagent is insensitive to methylation of DNA,

wherein, said detections in step (c) and step (d) and step (f) are made using the same aliquot of DNA of said sample, and in the same vessel, and effectively simultaneously for said first target DMRs and said reference

DMRs and said other region(s), and using: (x) the same detectable labels(s) for at least two of said reference DMRs; and (y) a different detectable label(s) for at least two of said first target DMRs and for at least one of said OR(s); and *optionally*,

(A) wherein said OR(s) is(are) located on one or more reference chromosome(s); and/or

(B) wherein said OR is located between about 20bp and 20kb upstream or downstream of, and/or within the same gene as, at least one of said reference DMRs.

4. The method of claim 3, wherein said detection in step (f) comprises using at least two of said ORs; *preferably wherein*, the number of said ORs is the same as the number of reference DMRs used in step (d); *more preferably wherein*, one of said ORs is located on the same chromosome as, such as between about 20bp and about 20kb upstream or downstream of, a reference DMR used in step (d) and each other of the said ORs is located on the same chromosome as, such as between about 20bp and about 20kb upstream or downstream of, another of said reference DMRs; and *optionally*, wherein said detection in step (f) is made using: (x) the same detectable label(s) for each of said ORs.

5. The method of any one of claims 1 to 4, wherein said detection step (c) and step (d) and optional step (f), are made using the same aliquot of DNA of said sample, and in the same reaction/detection vessel, and effectively simultaneously with each other, and by multiplex real-time quantitative probe-based PCR using at least one labelled probe specific for each of the said DMRs and optional OR(s).

6. The method of any one of claims 1 to 5, wherein said species of DNA that originate from cells of a foetus and/or the placenta of a foetus is circulating cell-free DNA and said sample is a blood fraction such as plasma or serum.

7. The method of any one of claims 1 to 6,
(A) wherein said reagent that differentially modifies methylated and non-methylated DNA comprises bisulphite; or
(B) wherein said reagent that differentially modifies methylated and non-methylated DNA comprises an agent that selectively digests unmethylated over methylated DNA, *preferably wherein*, said agent comprises:

- at least one methylation sensitive enzyme;
- at least one methylation sensitive restriction enzyme; and/or

an agent selected from the group consisting of: AatII, AciI, AclI, AfeI, AgeI, AgeI-HF, AscI, AsiSI, AvaI, BceAI, BmgBI, BsaAI, BsaHI, BsiEI, BsiWI, BsmBI, BspDI, BsrFI, BssHII, BstBI, BstUI, ClaI, EagI, FauI, FseI, FspI, HaeII, HgaI, HhaI, HinP1I, HpaII, Hpy99I, HpyCH4IV, KasI, MluI, NaeI, NarI, NgoMIV, NotI, NotI-HF, NruI, Nt.BsmAI, Nt.CviPII, PaeR7I, PluTI, PmlI, PvuI, PvuI-HF, RsrII, SacII, SalI, SalI-HF, SfoI, SgrAI, SmaI, SnaBI, TspMI and ZraI.

8. The method of any one of claims 1 to 7,
(A) wherein one reference DMR is located on human chromosome 5 another reference DMR is located on human chromosome 12; and *optionally*, wherein each of said first target DMRs is located on human chromosome 21, on human chromosome 18 or on human chromosome 13, preferably on human chromosome 21; and/or
(B) wherein said DMRs are hypermethylated in foetal DNA and hypo methylated in maternal DNA; and/or
(C) wherein said DMRs comprise at least one, preferably at least two, methylation site(s) specific for said

reagent.

9. The method of any one of claims 1 to 8,

(A) wherein said first target DMRs are each located in a region and/or gene independently selected from:

- one disclosed in WO 2011/092592, including on selected from the list consisting of: EP1, EP2, EP3, EP4, EP5, EP6, EP7, EP8, EP9, EP10, EP11 and EP12 [SEQ ID NOs 33-44] of WO 2011/092592; or
- the list consisting of: AIRE, SIM2, and ERG or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene; or
- the list consisting of: PDE9A, PPP1R2P2, , CBR1, DSCAM, C21orf29 and HLCS or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene; or
- C21orf57 or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene; or
- the list consisting of: SEQ ID No NOs 33, 34, 35, 36, 37, 38, 39, 176, 179, 180, 184, 188, 189, 190, 191, 193, 195, 198, 199, 200, 201, 202, 203, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 221, 223, 225, 226, 231, 232, 233, 235, 239, 241, 257, 258, 259, and 261 of WO 2011/034631; and/or

(B) wherein said first target DMRs are each located in a region and/or gene independently selected from:

- VAPA-APCDDI or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene; or
- maspin or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene
- the list consisting of: SEQ ID No NOs 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32 of WO 2011/034631; and/or

(C) wherein said first target DMRs are each located in a region and/or gene independently selected from:

- the list consisting of: SEQ ID No NOs 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 of WO 2011/034631.

10. The method of any one of claims 1 to 9, wherein said reference DMRs are each located in a region and/or gene independently selected from:

- the list consisting of: RASSF1A, TBX3, ZFY, CDC42EP1, PCDHGA1, SOX14 and SPN or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene; or
- the list consisting of: SEQ ID NOs: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 and 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162 and 163 of WO 2011/034631.

11. The method of any one of claims 1 to 10, wherein at least one of said first target DMRs is located in a portion of the genome and/or a gene that is DSCAM and/or C21orf57, or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene.

12. The method of any one of claims 1 to 11, wherein (A) one of said first target DMRs is located in DSCAM and another of said first target DMRs is located in C21orf57 or a DNA sequence of no more than 10kbp, 5kbp, 1kbp,

500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene; and (B) one of said reference DMRs is located in TBX3 and another of said reference DMRs is located in PCDHGA1 or a DNA sequence of no more than 10kbp, 5kbp, 1kbp, 500bp, 250bp, 150bp, 100bp or 50bp upstream and/or downstream from such region and/or gene; *preferably* wherein: (i) one of said first target DMRs comprises SEQ ID NO.: 201 and another of said first target DMRs comprises SEQ ID NO.: 219; and (ii) one of said reference DMRs comprises SEQ ID NO.: 203 and another of said reference DMRs comprises SEQ ID NO.: 220.

13. The method of any one of claims 1 to 12, wherein a plurality of sets of determinations are made for step (c) and step (d) and optional step (f), with each set of said determinations made using a different aliquot of DNA of said sample, in a different vessel and effectively simultaneously with each other member of the plurality of sets of determinations; and *optionally*, wherein the relative amount(s) or ratio(s) determined in step (e) is determined from said plurality of sets of determinations made for step (c) and step (d) and optional step (f), preferably by using an average amount of DNA determined for each set, such as a mean, median or mode amount of DNA determined for each set, preferably a mean amount of DNA determined for each set.

14. The method of any one of claims 1 to 13,
 (A) wherein an amount of the first target species of DNA is determined from the detected amount of methylation at one of the first target DMRs and an amount of the first target species of DNA is determined from the detected amount of methylation at another of the first target DMRs; and
 (B) wherein two or more relative amount(s), preferably ratio(s), are determined in step (e) for two or more amounts of first target species of DNA, each in respect of one or more of said first target DMRs; and
 (C) wherein two or more of said relative amount(s) or ratios indicate the presence or absence of the chromosomal aneuploidy in the foetus; and *optionally*, wherein in step (e) two or more of said relative amount(s) or ratio(s) are compared with threshold(s) and/or reference distribution(s), wherein two or more of said relative amount(s) or ratio(s) higher or lower than said threshold(s) and/or reference distribution(s) indicates the presence of the chromosomal aneuploidy in the foetus.

15. The method of any one of claims 1 to 14, wherein a threshold and/or reference distribution is determined from a plurality of samples, each sample taken from a different pregnant female, such as by practicing the method as set forth in any one of claims 1 to 12 on such samples, wherein said method is conducted on a plurality of samples each taken from a different pregnant female, each method conducted in a separate vessel, and effectively simultaneously with each other member of the plurality of samples.

16. A method for detecting an increased risk of a pregnant female suffering from or developing a pregnancy-associated medical condition; said method comprising the steps:
 (i) conducting the method of any one of claims 1 to 15 when dependent on claim 3;
 (ii) determining at least one amount, such as an absolute or relative amount, of foetal DNA present in the sample; and
 (iii) comparing the amount of foetal DNA determined with a threshold and/or reference distribution, wherein an increase in, or outlying of, the amount of said foetal of DNA from said threshold and/or reference distribution indicates an increased risk of the pregnant female suffering from or developing said pregnancy-associated medical condition.

17. A composition comprising:

- two or more labelled probes each of which specific for one of the said target DMRs as set forth in any one of claims 1 to 3, each probe for detecting one of said two or more first target DMRs.

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18. The composition of claim 17 further comprising:

- two or more pairs of PCR primers, each pair for amplifying one of said two or more first target DMRs as set forth in any of claims 1 to 15; and/or
- two or more labelled probes each of which specific for one of said reference DMRs as set forth in any one of claims 1 to 3, each probe for detecting one of said two or more reference DMRs; and/or
- two or more pairs of PCR primers, each pair for amplifying one of said two or more reference DMRs as set forth in any of claims 1 to 15.

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19. A kit, preferably for detecting a chromosomal aneuploidy in a foetus carried by a pregnant female and/or for detecting an increased risk of a pregnant female suffering from or developing a medical condition, said kit comprising:

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- a combination of primers and/or probes wherein said combination is as set forth in claim 17 or 18; and
- *optionally*, further comprising:
 - (i) a printed manual or computer readable memory comprising instructions to use said primers and probes to practice a method of any one of claims 1 to 15 and/or to produce or use the composition of claim 17 or 18; and/or
 - (ii) one or more other claim, component or reagent useful for the practice of a method of any one of claims 1 to 15 and/or the production or use of the composition of claim 17 or 18, including any such claim, component or reagent disclosed herein useful for such practice or production.

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20. A computer program product comprising: a computer readable medium encoded with a plurality of instructions for controlling a computing system to perform and/or manage an operation for detecting a chromosomal aneuploidy in a foetus carried by a pregnant female from a sample taken from said pregnant female, which sample comprises DNA that originates from cells of a foetus and/or the placenta of a foetus in admixture with differently methylated DNA of maternal origin, the DNA present in said sample being treated with a reagent that differentially modifies methylated and non-methylated DNA as set forth in any one of claims 1 to 15; said operation comprising the steps of:

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- receiving: (i) two signals, each representing an amount of the first target species of DNA, being the chromosome relevant to the chromosomal aneuploidy, present in said sample as set forth in step (c) of any one of claims 1 to 15; and (ii) one signal representing an amount of reference species of DNA, being one or more reference chromosomes, present in said sample as set forth in step (d) of any one of claims 1 to 15;
- determining a classification of whether a chromosomal aneuploidy is present in the foetus carried by said pregnant female based on at least one relative amount(s) of the chromosome relevant to the chromosomal aneuploidy represented by at least one signal received in (i) compared to an amount of reference chromosomes represented by the signal received in (ii), wherein at least one indicates the presence or absence of the chromosomal aneuploidy in the foetus; and *optionally*,

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(A) wherein the signals representing said amounts of DNA determined in step (c) and step (d) are generated using the same aliquot of DNA of said sample, and in the same vessel, and effectively simultaneously for said

first target DMRs and said reference DMRs; optionally using: (x) the same detectable labels(s) for at least two of said reference DMRs; and (y) a different detectable label(s) for at least two of said first target DMRs; and/or (B) wherein said operation conducts such classification by making comparison to threshold(s) and/or reference distribution(s), wherein one or more of said relative amount(s) or ratio(s) higher or lower than said threshold(s) and/or reference distribution(s) indicates the presence of the chromosomal aneuploidy in the foetus.