

nuclear genome. The interior of the nucleus contains a number of subnuclear bodies including the RNA-comprising nucleolus, which is mainly involved in the assembly of RNA-comprising ribosomes. After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they translate
5 mRNA.

An anucleated blood cell-extracted nucleic acid fraction preferably refers to a fraction comprising chromosomal DNA, ribosomal RNA, nucleolus RNA, and/or messenger RNA.

The term "gene" as used herein, and in particular in the phrasing
10 "mutation in a gene of a nucleated cell" is meant to refer to any nucleic acid sequence, both chromosomal and extra-chromosomal, of a nucleated cell, preferably a nuclear nucleic acid sequence, and may include transcribed and non-transcribed sequences as well as ribosomal RNA sequences, most preferably chromosomal sequences that are transcribed into RNA.

15 ~~In an preferred embodiment~~example of a method of the invention~~disclosure~~ said nucleic acid of a foetus is chromosomal or genomic nucleic acid of said foetus, in particular a nucleic acid carrying a mutation that is indicative of a (predisposition to develop) foetal disease or condition in said foetus.

~~In another preferred embodiment~~example, said foetal disease or condition
20 marker is an expression profile of genes of said foetus, wherein said expression profile is indicative of a (predisposition to develop) foetal disease or condition in said foetus, i.e. a foetal disease or condition-specific expression profile.

~~In some preferred embodiments~~examples of aspects of the present invention,
said expression profile of genes is not an expression profile of genes from an
25 anucleated blood cell of said foetus, and is also not an expression profile of genes from a nucleated cell of said subject.

~~In an preferred embodiment~~example of a method of the invention~~disclosure~~ said foetal disease or condition-specific expression profile is the expression profile of chromosomal genes in (a cell of) said foetus, the expression of which is altered
30 do to the presence of a foetal disease or condition or disorder. Preferably, the

profile is the expression profile of chromosomal genes from a nucleated cell of said foetus the mRNA of which is present in anucleated blood cells of the subject carrying the said foetus.

In another ~~preferred embodiment~~ example of a method of the ~~invention~~ disclosure the foetal nucleic acid is ribonucleic acid (RNA), more preferably messenger ribonucleic acid (mRNA) or miRNA.

In a ~~preferred embodiment~~ an example of a method of the ~~invention~~ disclosure said nucleic acid is not mitochondrial DNA or mitochondrial RNA. Hence, mitochondrial nucleic acid is preferably not an aspect of the present ~~invention~~ disclosure.

In another aspect, there is provided a method for determining the stage of foetal disease or condition or the efficacy of a foetal disease or condition treatment in a subject comprising the steps of:

- analysing a blood sample of a subject for the presence of a foetal disease or condition marker using the method as defined herein at a first
time point to thereby provide a first value for the level of said foetal disease or condition marker in said subject,
- analysing a blood sample of said subject for the presence of a foetal disease or condition marker using the method as defined herein at a
second time point to thereby provide a second value for the level of said foetal disease or condition marker in said subject, wherein said subject or said foetus has been subjected to a foetal disease or condition treatment between said first and second time point, and
- comparing said first and second value to determine the efficacy of said foetal disease or condition treatment in said subject.

In yet another aspect, there is provided a method for determining the stage of a foetal disease or condition in a subject, comprising the steps of:

- analysing a blood sample of a subject carrying a foetus for the presence of a foetal disease or condition marker using the method

as defined herein to thereby provide a test value for the level of said foetal disease or condition marker in said subject,

- providing a reference value for the level of said foetal disease or condition marker wherein said reference value is correlated to a particular stage of foetal disease or condition, and

- comparing said test and reference value to determine the stage of foetal disease or condition in said subject.

In another ~~preferred embodiment~~example of a method of analysing a blood sample according to the invention said step b) of analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a foetal disease or condition marker comprises the selective amplification of

i) at least a part of said nucleic acid of a foetal disease or condition agent by (reverse transcriptase) polymerase chain reaction amplification using at least one foetal disease or condition agent-specific amplification primer or probe, or
ii) a plurality of mRNAs (or at least a part of the mRNAs) by reverse transcriptase polymerase chain reaction amplification to determine the expression level of the chromosomal genes encoding said mRNAs to thereby provide an expression profile for said genes and comparing said expression profile to a reference profile.

In an ~~preferred embodiment of aspects of the invention~~example, the foetal disease or condition is selected from the foetal disease or conditions listed in Table 1.

Table 1. Foetal diseases or conditions subject of embodiments of the present invention.

- Achondroplasia (abnormality of the skeleton causing dwarfism)
- Achromatopsia (inability to see color)
- Adrenal Hypoplasia Congenita (reduction in adrenal gland function)

CLAIMS

~~P95686PC00~~

~~Amended Claims filed in response to the second written opinion~~

1. A method of analysing a blood sample of a subject carrying a foetus for the presence of a foetal disease or condition marker, said method comprising the steps of

5 a) extracting nucleic acid from anucleated blood cells, preferably thrombocytes, in said blood sample to provide an anucleated blood cell-extracted nucleic acid fraction, and

b) analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a foetal disease or condition marker, wherein said foetal disease or condition marker is a foetal disease or condition-specific
10 nucleic acid sequence or mutation in the nucleic acid of said foetus, or wherein said foetal disease or condition marker is a foetal disease or condition-specific expression profile of genes of a cell of said foetus.

2. The method of claim 1, wherein said anucleated blood cells are
15 thrombocytes or erythrocytes, preferably thrombocytes.

3. The method of claim 1 or 2, wherein said foetal disease or condition is selected from the group consisting of congenital and genetic disorders.

20 4. The method of claim 3 wherein said genetic disorder is gene mutation of trisomy or wherein said foetal disease or condition is the foetal gender.

5. The method of any one of the preceding claims, wherein said foetal
25 disease or condition marker is a nucleic acid of a foetus.

6. The method of any one of the preceding claims, wherein said expression profile is based on ribonucleic acid (RNA), preferably mRNA in said anucleated blood cell.

5 7. The method of any one of the preceding claims, wherein said step b) of analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a foetal disease or condition marker comprises the selective amplification of

i) at least a part of said nucleic acid by (reverse transcriptase) polymerase
10 chain reaction amplification using at least one foetal disease or condition marker-specific amplification primer or probe, or
ii) a plurality of mRNAs (or at least a part of the mRNAs) by reverse transcriptase polymerase chain reaction amplification to determine the expression level of the chromosomal genes encoding said mRNAs to thereby
15 provide an expression profile for said genes and comparing said expression profile to a reference profile.

8. The method of any one of the preceding claims, wherein said method is part of a method of diagnosing said foetal disease or condition in a subject,
20 and wherein the presence of said foetal disease or condition marker in said anucleated blood cell-extracted nucleic acid fraction is indicative of said foetus suffering from or at risk of suffering from said foetal disease or condition.

9. A method for determining the stage of foetal disease or condition or
25 the efficacy of a foetal disease or condition treatment in a subject, comprising the steps of:
- analysing a blood sample of a subject for the presence of a foetal disease or condition marker using the method according to any one of claims 1-8 at a first time point to thereby provide a first value for the level of said foetal disease or
30 condition marker in said subject,
- analysing a blood sample of said subject for the presence of a foetal disease or condition marker using the method according to any one of claims 1-8 at a

second time point to thereby provide a second value for the level of said foetal disease or condition marker in said subject, wherein said subject or said foetus has been subjected to a foetal disease or condition treatment between said first and second time point, and

- 5 - comparing said first and second value to determine the efficacy of said foetal disease or condition treatment in said subject.

10. A method for determining the stage of a foetal disease or condition in a subject, comprising the steps of:

- 10 - analysing a blood sample of a subject carrying a foetus for the presence of a foetal disease or condition marker using the method according to any one of claims 1-8 to thereby provide a test value for the level of said foetal disease or condition marker in said subject,
- providing a reference value for the level of said foetal disease or condition
- 15 marker wherein said reference value is correlated to a particular stage of foetal disease or condition, and
- comparing said test and reference value to determine the stage of foetal disease or condition in said subject.