DNA-TECHNOLOGY

PRENATAL

FETAL GENDER

PRENATAL DIAGNOSTICS

NON-INVASIVE PRENATAL DIAGNOSTICS

KIT FOR DETECTING FRAGMENT OF FETAL Y-CHROMOSOME IN MOTHER'S BLOOD BY REAL-TIME PCR

Non-invasive prenatal molecular genetic diagnostics

In obstetric and gynecological practice, it is often necessary to determine the genotype of the fetus in the early stages of pregnancy. Until recently, material for such studies was obtained invasively, with the chorion, placentobiopsy, amniocentesis, and cordocentesis. The risk of spontaneous abortion, in this case, is 2-3%. The discovery of fetal DNA and RNA in maternal blood served as the basis for the development of non-invasive prenatal diagnostics, which, unlike previous methods, does not pose a threat to the course of pregnancy because the research material is the mother's blood. Fetal cells are found in the blood of a pregnant woman; their number increases with an increase in gestational age depending on the state of the placenta and the characteristics of the course of pregnancy.

Starting from 8-10 weeks of pregnancy, non-invasive prenatal molecular genetic diagnostics methods make it possible to study fetal DNA with an accuracy of 96-100%.

Detection of the fetus's gender in the first or at the beginning of the second trimester can prevent the birth of sick children in families with burdened heredity. It is possible to terminate pregnancy for medical reasons if the parents have a carrier of genes for sex-linked diseases (for example, hemophilia or progressive Duchenne / Becker muscular dystrophy in the mother).

It is also essential for the doctor to know the gender of the fetus to decide on the possibility of hormonal therapy for a pregnant woman if the patient has adrenal hyperandrogenism (congenital dysplasia of the adrenal cortex, VDKN) or other masculinizing endocrine diseases.

The primary method of prenatal determination of the gender of the unborn child today is ultrasound diagnostics; however, in the early stages of pregnancy, this method is not always correct and is often subjective.

Indications for the study:

- the presence of masculinizing endocrine diseases in pregnant women, including congenital dysfunction of the adrenal cortex (ACD), for the correction of drug therapy;
- possible carriage of hemophilia genes by women and other sex-linked diseases (mental retardation associated with the X chromosome; myodystrophy; adrenoleukodystrophy; Alport syndrome; immunodeficiency associated with the X chromosome; retinitis pigmentosa; hydrocephalus associated with the X chromosome; Lowe syndrome; X-linked ichthyosis);
- presumed violations of fetal gender determination according to the results of the ultrasound.

Kit purpose

"Fetal gender" kit for detecting fragment of fetal Y-chromosome in mother's blood by real-time PCR is intended for the detection of a multicopy segment of the fetal Y chromosome in the blood of a pregnant woman by the real-time polymerase chain reaction (PCR).

In contrast to the SRY gene, the selected DNA target is strictly specific for the Y chromosome, which is used as a target in most similar kits.

Technical characteristics and kit content

Number of tests	96 tests
Reagents format	Aliquoted (twelve 8-tube strips)
Paraffin sealed PCR-mix	20 µl per tube
Taq-polymerase solution	2 tubes – 480 µl
Mineral oil	2 tubes – 960 µl
Positive control «C+»	1 tube – 75 µl
Sample	Peripheral blood
Shelf life	12 months
Storage temperature	2°C ... 8°C

Technology:
Polymerase chain reaction with real-time detection; qualitative multiplex analysis.

Reagents for DNA extraction:
PREP-NA-FET – a set of reagents produced by DNA-Technology is explicitly designed to isolate fetal DNA from the mother's blood. It is used for DNA extraction from 50 samples (including negative controls).

Kit features:
- simultaneous detection (multiplex analysis) – several DNA targets are determined in one test tube: the presence of a DNA fragment of the Y chromosome and human genomic DNA (sample intake control, SIC);
- SIC is used to analyze the quality of extraction and allows to determine whether the amount of DNA obtained is sufficient for the research;
- since fetal DNA is in the blood of a pregnant woman in a minimal amount, the analysis of each DNA sample must be carried out in duplicate.

To carry out the analysis, the following additional consumables and equipment are required:
- rack and vortex rotor for striped plastic;
- when working with PREP-NA-FET extraction kit, a cooling rack and centrifuges for 4.5 ml (RCF not lower than 1150 g) and 1.5 ml tubes (RCF not lower than 17000 g) are required.

Equipment required for analysis:
DT devices manufactured by DNA-Technology R&P (DTlite, DTprime) (Fig. 1).

DTprime

DTlite

Fig. 1. Devices manufactured by DNA-Technology

The unique technical characteristics of the instruments can significantly reduce the overall analysis time. This substantially saves research time and ensures high laboratory throughput.

Software

DNA-Technology has developed specialized software for the “Fetal gender” kit, which allows obtaining research results in a convenient visual form (Fig. 2).

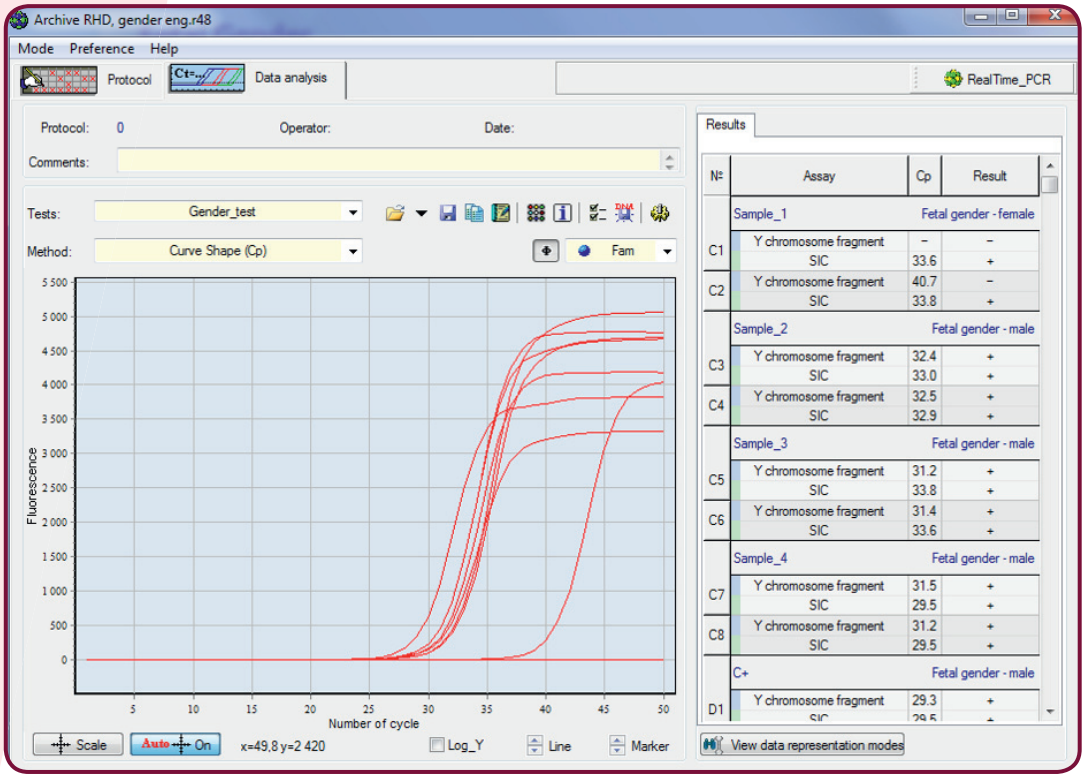

Fig. 2. Optical measurement analysis results

The test result is indicated in the upper column of the table on the right, next to the sample identifier.

The test result for each sample is determined by the software automatically taking into account the Cp values for the Fam channel (a specific fragment of the Y chromosome) and the Hex channel (SIC) in duplicates aggregate for each sample.

The result is automatically included in the answer form.

Options for research results and the type of corresponding forms
A. Fetal gender – female

	SAMPLE_2		Fetal gender – female	
C1		Y-chromosome fragment	–	–
		SIC	32,6	+
D1		Y-chromosome fragment	39,1	–
		SIC	33,1	+

Prenatal diagnostics
Fetal gender

Date:
Tube number:
Patient name:
Sex:
Age:
Organization:
Clinician name:
Comment:

Logotype

Information about laboratory

Sample ID: Sample_2

Name of research	Result	Result interpretation
Detection of Y-chromosome fragment	Not detected	Fetal gender – female

Attention: This test is performed with a gestational age of more than 8 weeks.

Study was carried out by:

Date:
Signature:

B. Fetal gender – male

	SAMPLE_14		Fetal gender – male	
C4		Y-chromosome fragment	30,3	+
		SIC	32,6	+
D4		Y-chromosome fragment	30,4	+
		SIC	32,4	+

Prenatal diagnostics
Fetal gender

Date:
Tube number:
Patient name:
Sex:
Age:
Organization:
Clinician name:
Comment:

Logotype

Information about laboratory

Sample ID: Sample_14

Name of research	Result	Result interpretation
Detection of Y-chromosome fragment	Detected	Fetal gender – male

Attention: This test is performed with a gestational age of more than 8 weeks.

Study was carried out by:

Date:
Signature:

C. Questionable or unreliable result

If questionable or unreliable results are received, (?) or (nd) will be displayed respectively.

	SAMPLE_16		Fetal gender - and	
G4		Y-chromosome fragment	-	-
		SIC	35,4	nd
H4		Y-chromosome fragment	-	-
		SIC	36,3	nd

Prenatal diagnostics
Fetal gender

Date:
Tube number:
Patient name:
Sex:
Age:
Organization:
Clinician name:
Comment:

Logotype

Information about laboratory

Sample ID: Sample_16

Name of research	Result	Result interpretation
Detection of Y-chromosome fragment	nd	It is necessary to send blood for analysis again.

Attention: This test is performed with a gestational age of more than 8 weeks.

Study was carried out by:

Date:
Signature:

When receiving questionable and unreliable results, it is necessary to redo the analysis according to the proposed algorithm (Table 1).

Table 1. Principles of determination questionable and unreliable PCR results

Fam channel result (Fam Cp)	Hex channel result (Hex Cp)	Result interpretation
Not taken into account	Cp>35 or not specified	Invalid result **
The interpretation of the results in the doubles does not match	Cp ≤35	Invalid result *
35<Cp≤37	Cp ≤35	Invalid result *

Note:
* Repeat PCR for this sample.
** Repeat PCR for this sample, either re-extract DNA and carry out PCR, or re-take clinical material from the patient (performed sequentially).

Advantages of kits for non-invasive prenatal diagnostics manufactured by DNA-Technology in comparison with analogs.

The selected DNA target is multicopy and strictly specific for the Y chromosome, in contrast to the SRY gene used as a target in most similar kits, both domestic and foreign manufacturing.

- A unified amplification program and specialized software allow combining in one protocol two types of studies ("Fetal RHD Genotyping" and "Fetal gender") while maintaining an independent automatic interpretation of the results, which significantly reduces the time required for the analysis and increases laboratory throughput.
- A small volume of a blood sample – 4.5 ml – is sufficient for carrying out a full range of studies using the "Fetal RHD Genotyping" and "Fetal gender" kits.
- Single centrifugation of blood to obtain plasma reduces the risk of sample contamination and shortens laboratory technician time.
- The aliquoted format of the kits does not require additional manipulations to prepare reaction mixtures, which reduces the time of the laboratory assistant and reduces the risk of contamination.
- The stability of the components of the reagent kits ensures long-term storage (9 months) at +2 ... +8 °C and transportation within 72 hours at temperatures from 0 to +24 ° C.
- The minimum amount of standard consumables and additional equipment for carrying out an entire cycle of PCR analysis (including no need to use columns and 5 ml tubes).
- The presence of a SIC in each tube makes it possible to assess the quality of the extraction and amplification stages and the sufficiency of the amount of isolated DNA for research, which together provides a significant reduction in the risk of obtaining incorrect results.

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