IV. PRENATAL DIAGNOSIS

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11. NON-INVASIVE PRENATAL DIAGNOSIS

In current obstetric practice it is often necessary to perform genetic testing of the fetus during early pregnancy. Traditionally, fetal genetic material has been obtained using invasive techniques, such as chorionic villus biopsy or placenta sampling or amnio- and cordocentesis. These procedures, however, carry a risk of miscarriage around 2-3 %. The discovery of cell-free fetal DNA and RNA in maternal blood offered a new source of fetal genetic material for non-invasive prenatal diagnosis. Since material for the research is the mother's blood, these new techniques are safe for the pregnancy compared to the previous methods. The number of fetal DNA in maternal blood increases with increasing of gestational age and depends on the placenta state and characteristics of pregnancy course. Starting from the 8-10 weeks of gestation the methods of non-invasive prenatal genetic testing allow to achieve the level of accuracy 96-100 %.

11.1. Fetal Gender Real-Time PCR Detection Kit

Early determination of fetal gender during first – beginning of the second trimester may help to prevent the birth of children with negative family history. It becomes possible to terminate the pregnancy for medical reasons if the parents have gene variants associated with sex-linked diseases (such as hemophilia or progressive Duchenne/Becker muscular dystrophy in the mother).

Fetal gender must be considered in the appointment of the hormonal therapy to the patients with adrenal hyperandrogenism (congenital adrenal hyperplasia) or other masculinizing endocrine diseases.

The main method of prenatal fetal gender determination is ultrasonography, but it cannot be done reliably in the first trimester of pregnancy because of uncompleted development of the external genitalia and in this regard, ultrasonography can be acknowledged as subjective method.

Indications for the study:

- masculinizing endocrine diseases in pregnant women, including congenital adrenal hyperplasia (CAH); correction of drug therapy.
- genetic variants in pregnant women linked to the sex-associated diseases (hemophilia, X-linked mental retardation, myodystrophy, adrenoleukodystrophy, Alport syndrome, X-linked immunodeficiency, retinitis pigmentosa, X-linked hydrocephalus, Lowe syndrome, X-linked ichthyosis)
- non reliable gender determination by means of ultrasonography.

DNA-Technology Company developed and implemented Fetal Gender REAL-TIME PCR Detection Kit which is intended for detection of cell-free fetal DNA, which derived from multi-copy fragment of Y chromosome, in the blood of pregnant women by Real-Time PCR method (table 20).

The selected target DNA fragment is strictly specific to the Y chromosome in contrast to the SRY gene, which is used as a target in the majority of similar kits of both domestic and foreign manufacturers

Table 20. Fetal Gender REAL-TIME PCR Detection Kit

Name	Detection format				Registration*
	Forez	Flash	Rt	qPCR	
Fetal Gender REAL-TIME PCR Detection Kit	–	–	–	*	RUO

*** Note:**

RU/IVD – kits for *In Vitro* Diagnostic, which are registered in Russia only

CE/IVD – kits for *In Vitro* Diagnostic, which are registered in EU

RUO – kits for Research Use Only

Method: Multiplex real-time PCR, qualitative analysis

Kit format: Strip tubes (8 pcs., 0.2 ml each).

Storage temperature: +2 to +8 °C.

Shelf life: Rt – 9 months.

DNA extraction kits:

- *PREP-NA-FET.*

Specimen for screening:

Peripheral blood.

Equipment required for analysis:

- DT devices produced by DNA-Technology (DTlite, DTprime, DT-96).

The following additional equipment is needed for analysis using strip tubes:

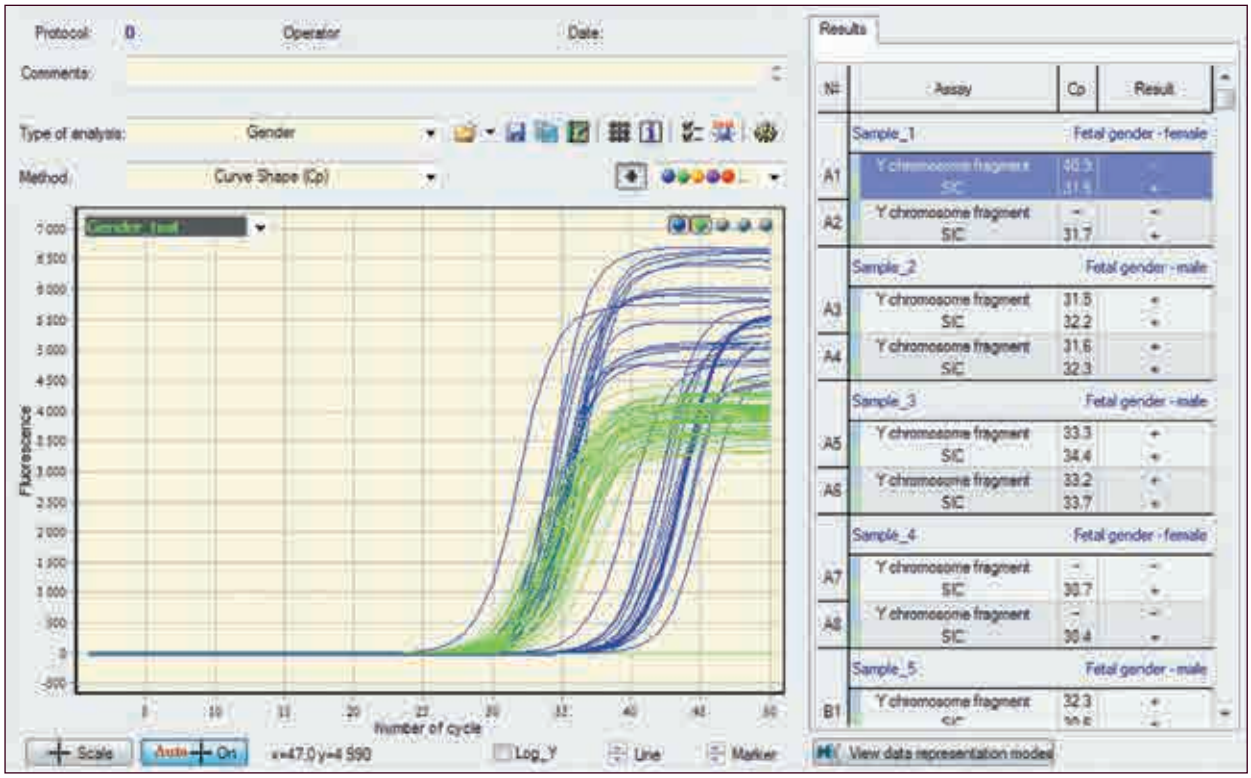
- strip plastic rack and centrifuge (vortex) rotor;
- tube rack and vortex adaptor suitable for stripped PCR tubes;
- cooling tube rack (is necessary during the DNA extraction step!)

Features of the Kit:

- Multiplex analysis – simultaneous detection of multiple targets in the one tube (Y chromosome fragment and human genomic DNA)
- SIC is intended for extraction quality assessment as well as for evaluation of sufficiency of sample for obtaining reliable result.
- Due to the small amount of fetal DNA in the blood of pregnant women analysis of each DNA sample **must be done in duplicate**. Otherwise the results will not be obtained.

Software: Reaction results are analyzed and interpreted automatically (for devices produced by DNA-Technology) (fig. 13).

A



B

	Sample_4	Fetal gender – female	
A7	Y-chromosome fragment	–	–
	SIC	30.7	+
A8	Y-chromosome fragment	–	–
	SIC	30.4	+

Prenatal diagnostics.
Fetal gender.

Date
Nomber tube
Patient name
Sex
Age
Organization
Clinician name
Comments

logotype

information about laboratory

Sample ID: Sample_4

Name of research	Result	Interpretation of result
Detection of Y-chromosome fragment	Not detected	Fetal gender – female.

Attention: Precision of test is 96-100 % at over 8 weeks gestationalage.

Study was carried out by

Data
Signature

C

	Sample_5		
B1	Y-chromosome fragment	32.3	+
	SIC	30.6	+
B2	Y-chromosome fragment	32.1	+
	SIC	30.6	+

Prenatal diagnostics.
Fetal gender.

Date
Nomber tube
Patient name
Sex
Age
Organization
Clinician name
Comments

logotype

information about laboratory

Sample ID: Sample_5

Name of research	Result	Interpretation of result
Detection of Y-chromosome fragment	Detected	Fetal gender – male.

Attention: Precision of test is 96-100 % at over 8 weeks gestationalage.

Study was carried out by

Data
Signature

Fig. 13. Analysis results for Rt optical measurements (DT devices)
A – Optical measurement analysis (Fam channel)
B – Form for presentation of results (Fetal gender – Female)
C – Form for presentation of results (Fetal gender – Male)

When doubtful or non reliable results are obtained, analysis must be repeated according to the proposed algorithm (Table 21).

Table 21. Definition of doubtful and non reliable results

Results on the FAM channel (FAM Cp)	Results on the HEX channel (HEX Cp)	Interpretation
Is not considered	Cp>35 or is not specified	Non reliable result**
Results interpretation does not match in duplicates	Cp≤35	Non reliable result*
35<Cp≤37	Cp≤35	Doubtful result*

* PCR amplification must be repeated

** PCR amplification must be repeated or DNA extraction and PCR amplification must be repeated or blood sampling, DNA extraction and PCR amplification must be repeated (performed sequantially).

11.2. Fetal RHD Genotyping Real-Time PCR Kit

There are five antigens in Rh system. The most immunogenic is D-antigen, the presence of which on erythrocyte surface defines positive Rh factor (Rh+). Portion of antigen D carriers in population reaches 86 % and rhesus negative people (Rh-) who lack antigen D is around 14 %. The cases when Rh- woman is pregnant with Rh+ fetus often complicated with development of hemolytic disease of newborn or fetal erythroblastosis, associated with transplacental passage of fetal erythrocytes to maternal blood stream. The 98 % of hemolytic disease of newborn associated with D-antigen. Upon getting into Rh- mother's blood, D-antigen causes formation of specific antibodies, which cross the placenta and destroy fetal erythrocytes, provoking the development of hemolytic disease of newborn. Early manifestation of Rh disease can cause premature birth or spontaneous abortion. The maternal sensibilization to D-antigen and risk of Rh disease development increase with every subsequent pregnancy with Rh+ fetus, independently of whether abortion was applied or successful delivery was achieved.

The standard methods of Rh incompatibility evaluation include costly and time-consuming assays:

- Evaluation of maternal specific antibodies to fetal D-antigen
- Invasive approaches based on obtaining of fetal sample, like chorionic villus biopsy or placenta sampling or amnio- and cordocentesis.
- Doppler velocimetry based evaluation of blood flow velocity in medial cerebral artery and aorta of the fetus.

All Rh- pregnant women passing dynamic control of antibodies level to fetal D-antigen. The absence of antibodies cannot guarantee that fetus is also Rh- because production of maternal antibodies can be inhibited due to integrity of placenta or suppressed immune response. Risk of spontaneous Rh disease development remains under given circumstances. Moreover, mother will be sensitized in the course of delivery.

DNA-Technology's Fetal RHD Genotyping REAL-TIME PCR Kit is intended for detection of fetal RHD gene from maternal blood by real-time PCR method and allows to evaluate Rh state of fetus at early gestational age in Rh- female. This information is helpful for early Rh disease risk detection and implementation of preventive measures. The kit can be used in framework of prenatal screening programs aimed at prevention of pregnancy complications in Rh- women.