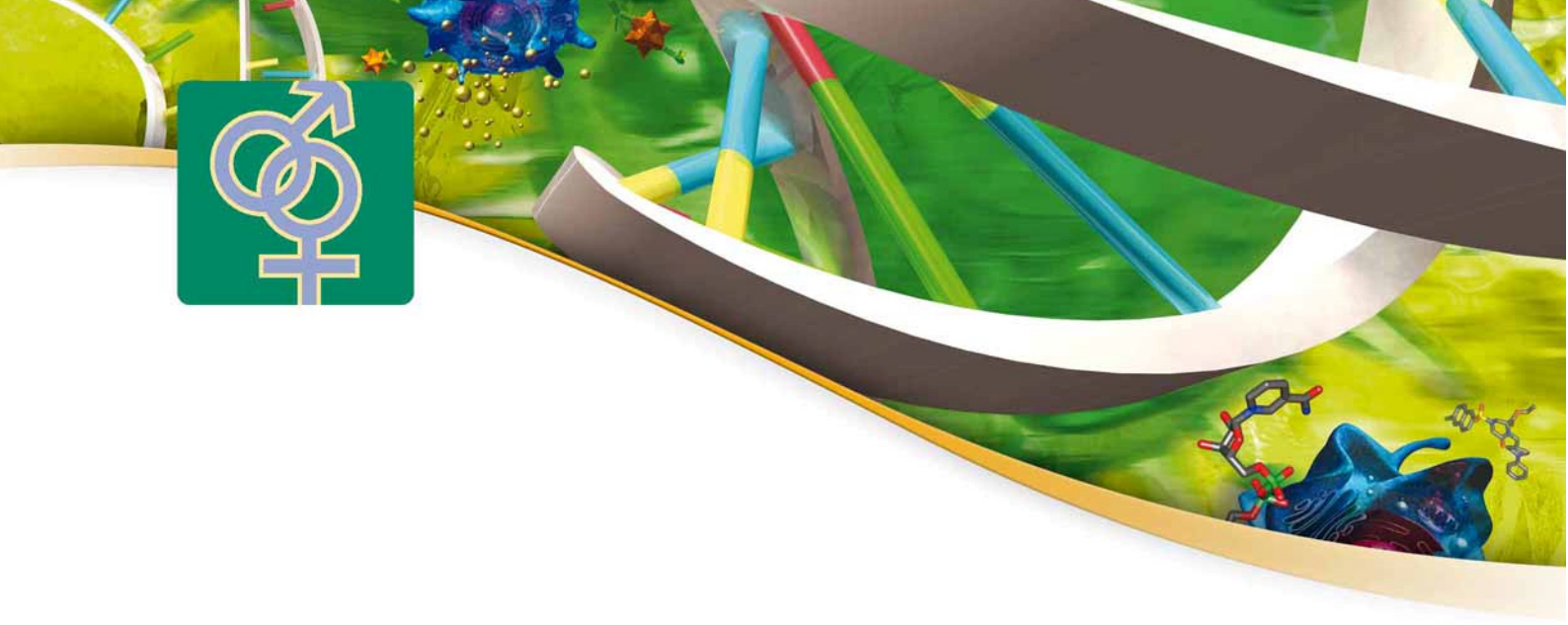
BECKMAN COULTER

Prenatal screening
Clinical aspects



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PRENATAL SCREENING

Aim of prenatal screening

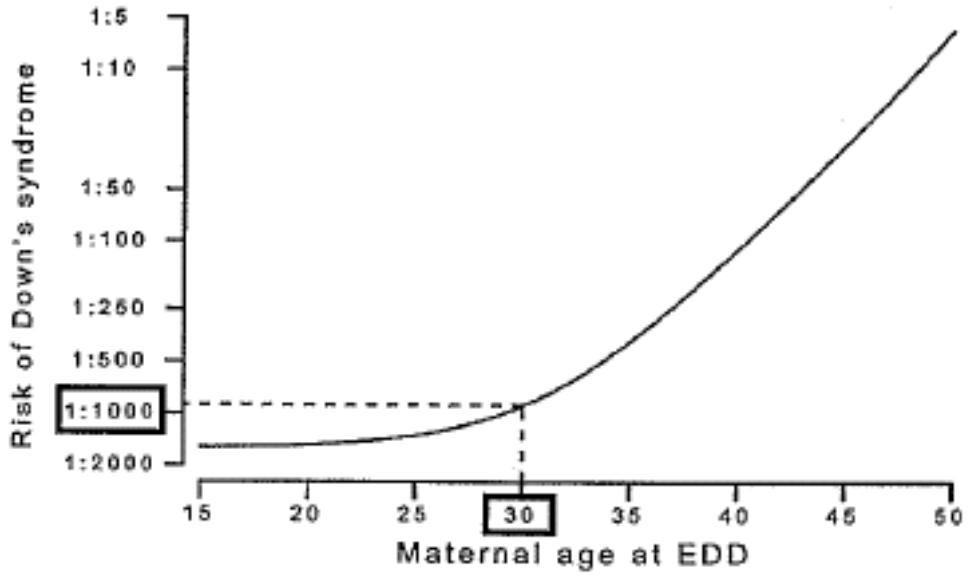
The goal of medical screening is to identify the individuals with significant risk of certain disorder before its clinical manifestation. The positive results of screening examinations obtained by a simple, accessible, and inexpensive screening method are followed by a series of more specific and more complex examinations.

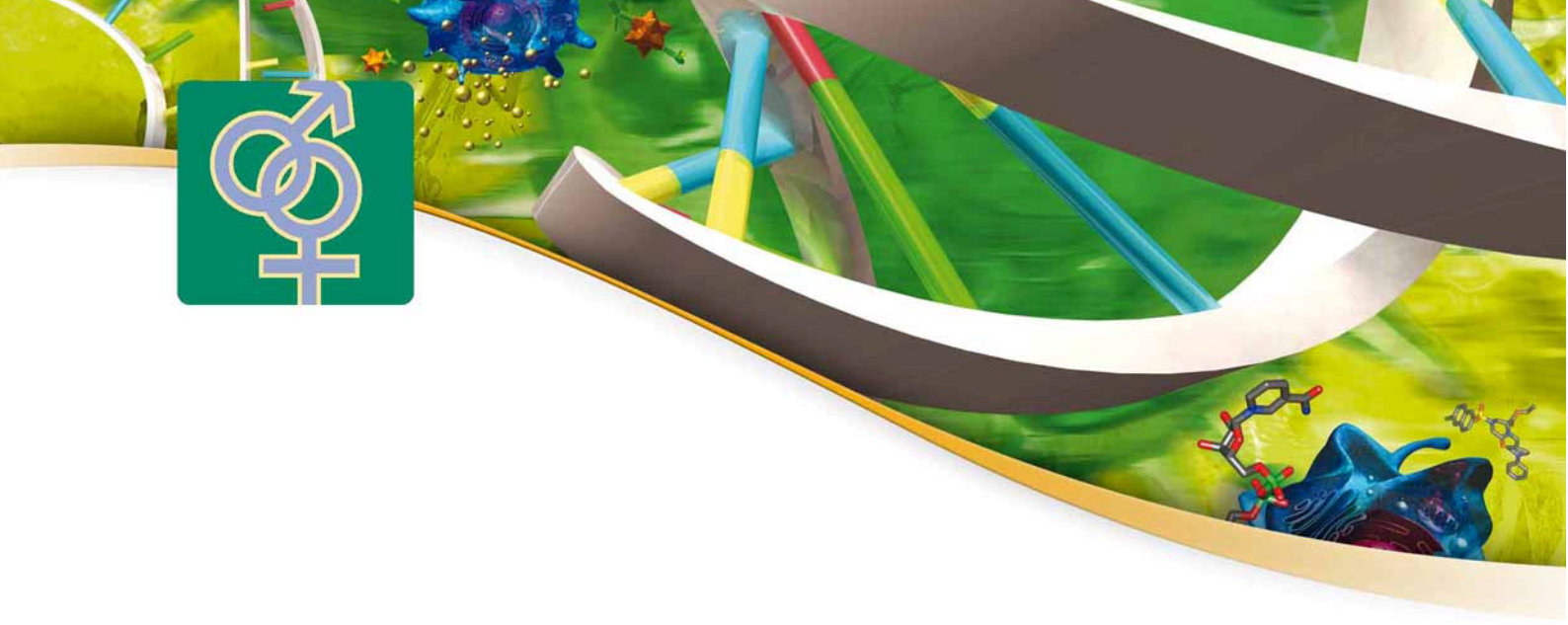
Prenatal screening should prevent from the birth of child affected by one of congenital malformances, particularly neural tube defects or chromosomal malformances. It may also help in finding pregnancies with increased risk of pregnancy complications like spontaneous abortion or perinatal death.
The term "prenatal screening" is sometimes reduced to the screening for the pregnancies affected by Down's syndrome (DS). Although there is no doubt about seriousness of this condition, screening contribution to the detection of other malformances should not be neglected.

Chromosomal malformances

Trisomy 21 (Down's syndrome, DS)

It is responsible for more than 50 % of all chromosomal malformances.
Probability of Down's syndrome affected pregnancy is approx. 1 : 800 in total population.
Risk significantly increases with the age of mother, see figure below





Gradual increase in maternal age may be seen in many countries in the course of last decades. Consequently, it also means increase in frequency of Down's syndrome affected pregnancies. In spite of the changes in maternal age, the vast majority of babies (95 %) are still born to women under 35 years of age. Therefore, majority of DS affected pregnancies (~80 %) is still born to women under 35 years.
It is apparent that maternal age itself, though it is significant risk factor for DS, does not have sufficient statistical power to discriminate (at satisfactory probability level) between DS affected and not affected pregnancies. Other factors have to be considered to enable better discrimination between high risk and low risk pregnancies.

Sexual chromosome aberrations

Responsible for about 25 % of chromosomal malformances.
The most frequent are Klinefelter's syndrome (XXY) and Turner's syndrome (X0).

Other findings

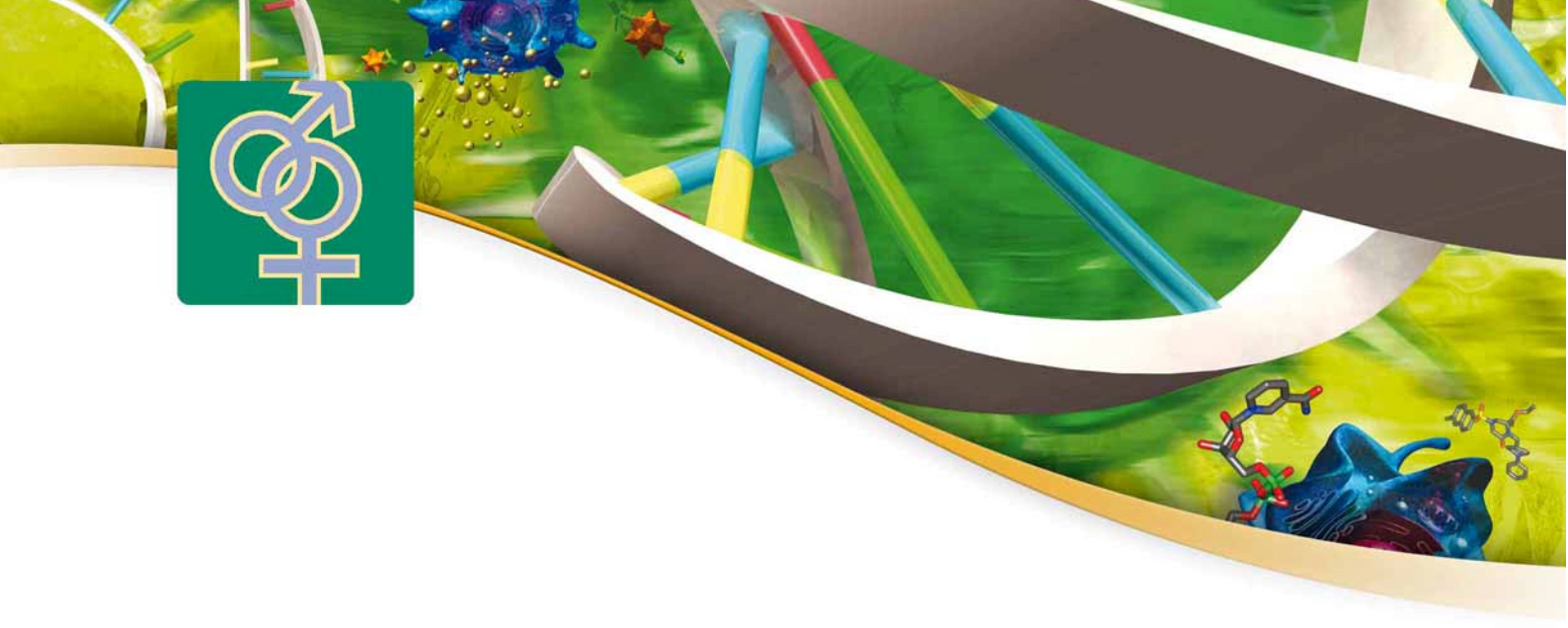
There are other less common findings like trisomy 13 (Patau's syndrome) or trisomy 18 (Edward's syndrome).

Neural tube defects

Neural tube defects (NTD) belong to the most frequent morphological impairments with the incidence of 0.3 - 3 per 1000 live births.
They cover:

- Anencephaly
- Rachischisis (spina bifida)
- Spinal column closure defects.

The NTD etiology is multifactorial. The repeated incidence of NTD risk among relatives of the first degree is estimated to be 3.5 %. The highest NTD risk is found among mothers of the youngest and the oldest age group and among those of the lower social-economic level. In 95 % of cases, the affected fetus is born as the first in the order. The recently reported decrease of NTD incidence is a result of the biochemical and ultrasonographic screening and likely of the nutrition quality improvement as well. A long-term pre-conceptional nutrition complementation by low doses (0.4 - 4 mg per day) of folic acid decreases the NTD occurrence in the population with genetic predisposition.



Risk of complications in the 3rd trimester

Abnormal prenatal screening results may also refer to increased risk of some complications in the 3rd trimester of pregnancy, including:

- Low birth weight
- Intrauterine growth retardation
- Perinatal fetal death

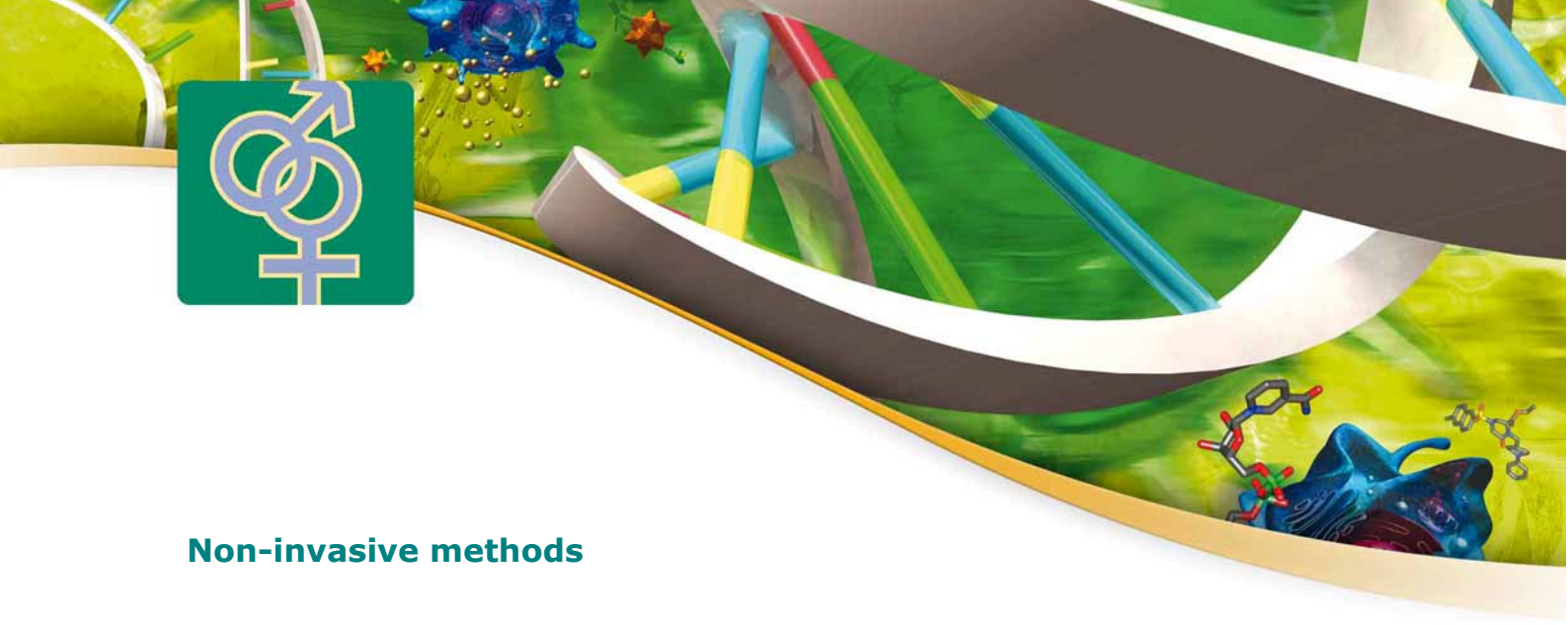
Diagnostic methods

As already mentioned, screening method should be simple, accessible, and inexpensive. Also, it should not bring any additional health risk for the screened person. These requirements are fulfilled by non-invasive methods as determination of biomarkers in maternal blood and ultrasonography examination of the fetus. These methods do not give definite reply concerning fetal status and development, but they help to identify pregnancies with higher probability of affected fetus.

The result is considered as screening positive usually when the risk is higher than 1 : 270 (this "cut off" of risk is laboratory/hospital dependent and ranges between 1 : 250 and 1 : 300). This risk cut off ratio between positivity and negativity is usually set up so as it corresponds given false positivity rate, usually 5 %.

Genetic counseling and further examinations are indicated in high risk (screening positive) pregnancies in order to definitely exclude or confirm the suspected malformances. These examinations are based on invasive method of biological material collection, and therefore more risky for the pregnancy.

It is desirable to adopt such screening program that would enable high rate of affected pregnancy detection and, in parallel, the lowest possible amount of unnecessary invasive examinations.



Non-invasive methods

Biochemical markers

It has been found that affected pregnancies are accompanied by increased or decreased levels of various substances in maternal blood, when compared with typical healthy pregnancy. The first such identified marker was AFP. If it is determined in mother blood in the second trimester of pregnancy, increased levels were found to be connected with neural tube defects and, on the contrary, decreased values with Down's syndrome. Since then, other markes has been identified. Combination of several such markers improves significantly the discriminating ability. The most beneficial markers used in the second trimester are particularly AFP and hCG, but also uncojugated Estriol and Inhibin A.

Screening in the first trimester has been introduced in countries with high level of prenatal healthcare, bringing the new biochemical parameter PAPP-A. This determination may be supplemented by the determination of hCG or Free-beta hCG subunit.

Ultrasonography

Ultrasound may be employed several times during fetal development, looking for specific information in dependence on gestational age:

- From 7th week of pregnancy, so called dating scan is done to look for multiple pregnancy and to confirm the gestational age. Exact determination of gestational age is crucial for successful screening results. The levels of markers significantly change with time, so it is necessary to link their values with as precisely determined gestation age as possible.
- Around 11-13th weeks, nuchal translucency (NT) may be measured and presence of nasal bone evaluated as part of Down's syndrome screening
- From 18th week, morphology scan may check for any abnormal development like e.g. cardiac or renal tract abnormalities.

Invasive methods

Invasive methods are used for confirmation/exclusion of the malformances in high risk pregnancies (usually when risk is higher than 1 : 270). Although invasive methods are considered to be very accurate, even they are not absolutely perfect with reported error rate approx. 0.2 % (e.g. mosaic Down's syndrome in which only some cells carries the genetic abnormality).

Amniocentesis

The most frequent method used for exclusion/confirmation of chromosomal aberrations is amniocentesis. It is a procedure of amniotic fluid sampling and testing fetal DNA. This can be done once enough amniotic fluid has developed to enable sampling (approx. from 14th week). Cells from the fetus are present in this fluid, and can be separated and tested. Miscarriage risk of amniocentesis is commonly quoted as 0.5%, but may be even higher.

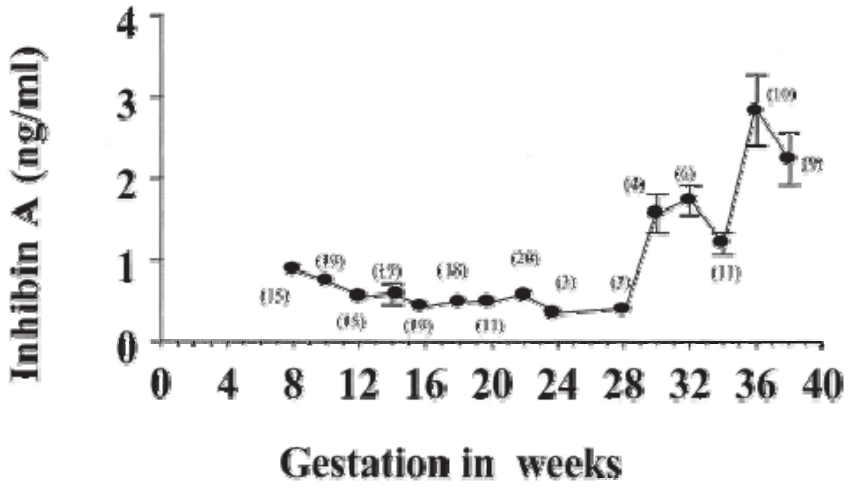
Chorionic villus sampling

Chorionic villus sampling is based on testing the sample of placental tissue called chorionic villus. Significant advantage is that it may be performed earlier than amniocentesis (between 9.5 and 12.5 weeks of gestation). On the other hand, the risk of miscarriage is higher, at about 1 %.

Screening methodology

Screening is based on combination of several "risk" parameters, including biochemical marker values, ultrasound marker values, but also maternal age, history of previous pregnancies etc. Other factors necessary for correct interpretation of these parameters are gestational age, maternal weight, multiplicity of the pregnancy. All these data are statistically evaluated and results are reported as the final risks of having affected pregnancy by the individual malformances.

The concentrations of biochemical markers and NT results change significantly with gestational age – see example of Inhibin A below.



As a consequence of this variability, the results of biochemical markers and NT are not therefore expressed in units, but in so called MoMs (multiple of medians).

MoM (multiple of medians) = obtained result/median value corresponding to the gestational age

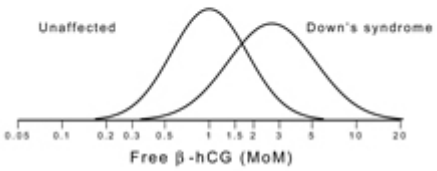
As MoM are already recalculated to gestational age-dependent median values, they are independent of this age and may be directly used for calculation of risk. Figures below show the distribution of prenatal screening marker values differs between normal pregnancies and Down's syndrome pregnancies.

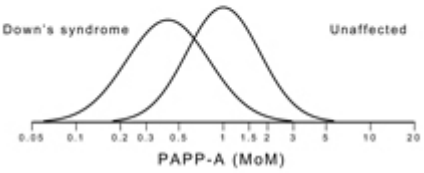
Medians correspond to median values obtained on healthy population. Medians are calculated using specific software and are specific for each laboratory, as they are based on laboratory specific collection of data.
Medians are dependent on:

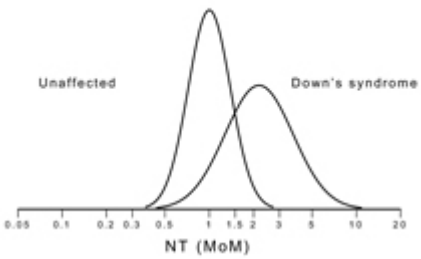
- Gestational age
- Assay method
- Tested population

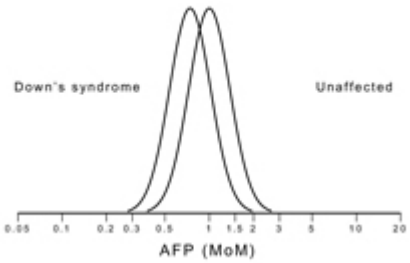
Individual results (expressed in MoMs) may need to be adjusted for:

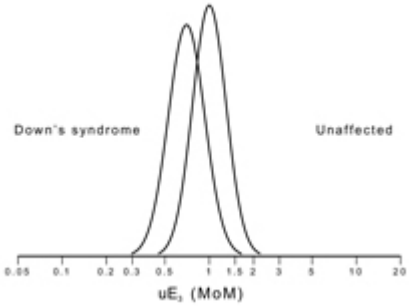
- weight
- ethnic group
- other conditions e.g. diabetes
- multiple pregnancies

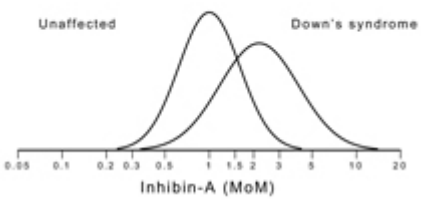












Screening programs

New approaches for the screening programs has been revealed with finding the possibility to move screening to earlier stage of pregnancy thanks to ultrasound marker NT and biochemical marker PAPP-A.
Consequently, the need of objective evaluation of possible benefits and disadvantages of the movement the screening towards the first trimester arose. It became important to evaluate what approach would be the most beneficial and cost-effective and therefore brings the best outcome. Two such big studies have been performed recently.

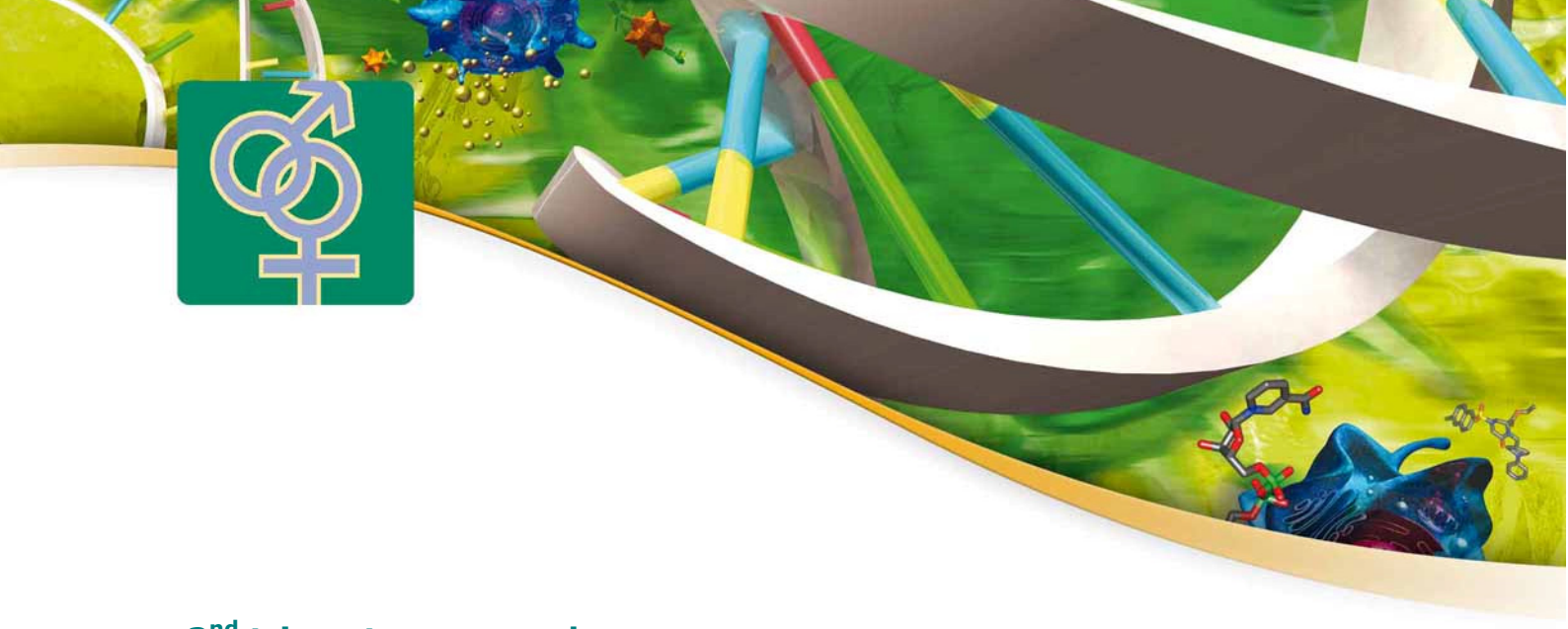
SURUSS and FASTER studies

The first one - SURUSS (Serum, Urine, and Ultrasound Screening Study) - was performed in UK on more than 48 000 pregnant women. Their results were presented in 2003.
The second study - FASTER (First and Second trimester Evaluation of Risk) – was performed in US on more than 38 000 pregnant women. Results were published in 2005.
The results of both studies are in a good agreement.

Table 1: False positive rates (FPR) at given detection rate (DR) level - 85 %. Results are compared for various combinations of markers as found by SURUSS study.

Test	Markers combination	FPR for 85% DR (%)
Integrated test with Inhibin A	NT and PAPP-A (1st tr.) AFP, hCG, uE3, Inhibin A (2nd tr.)	1.3
Integrated test without Inhibicn A	NT and PAPP-A (1st tr.) and AFP, hCG, uE3 (2nd tr.)	2.0
Serum integrated test with Inhibin A	PAPP-A (1st tr.) and AFP, hCG, uE3 and Inhibin A (2nd tr.)	3.0
Serum integrated test without Inhibin A	PAPP-A (1st tr.) and AFP, hCG, uE3 (2nd tr.)	4.8
Combined test	NT, PAPP-A and free-beta hCG (1st tr.)	6.1
Quadruple test	AFP, hCG, uE3 and inhibin A	7.1
Triple test	AFP, hCG, uE3	10.9
Double test	AFP, hCG	16

Consequences and possible approaches of the studies are presented and discussed below.



2nd trimester approach

Testing in the second trimester of pregnancy still remains the most frequently used method. It employs biochemical markers only. Samples of maternal blood are collected between 14th and 18th weeks (up to 20th week maximally). In dependence on the number of markers used, we recognize so called double (AFP and hCG), triple (AFP, hCG or free-beta hCG, and unconjugated estriol), or quadruple test (AFP, hCG, unconjugated estriol and inhibin A).
Quadruple test proved significantly better detection rates than triple test, or double test, as shown in tables below (SURRUS study).

Table 2: Screening in 2nd trimester; comparison of detection rates (DR) at given false positivity rates (FPR) for various marker combinations. Data from SURUSS study.

FPR (%)	1	3	5
	DR (%)		
Double test	40	57	66
Triple test	51	67	74
Quadruple test	62	75	81

Table 3: Screening in 2nd trimester; comparison of false positivity rates (FPR) at given detection rates (DR) for various marker combinations. Data from SURUSS study.

DR (%)	70	80	90	95
	FPR (%)			
Double test	6.4	11.7	23	37
Triple test	3.7	7.4	17	28
Quadruple test	2.0	4.5	11.7	22

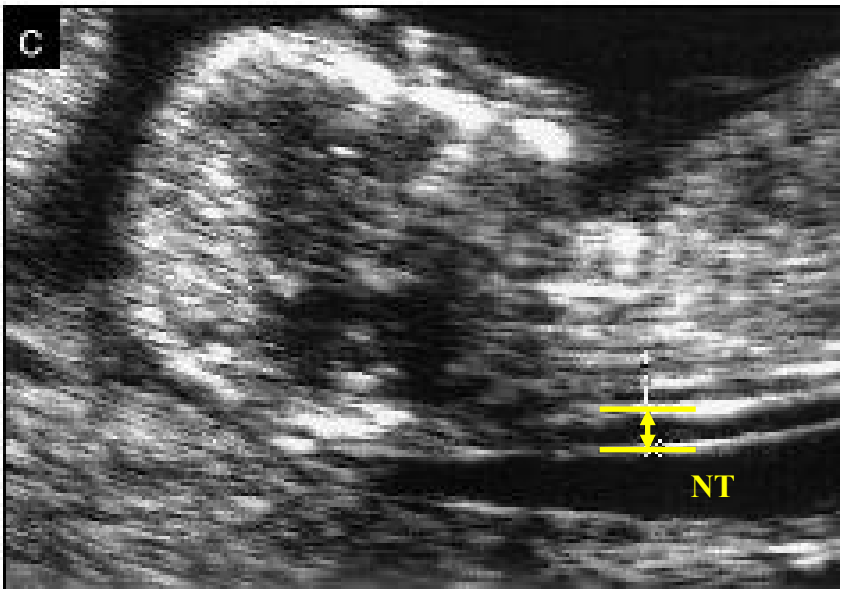
1st trimester approach (so called combined screening)

First trimester testing was introduced several years ago, and it has become very popular since then. It combines ultrasound examination and biochemical markers.

Ultrasound examinations are performed between weeks 11 and 13, and they are focused mainly on the measurement of nuchal translucency thickness (NT), corresponding the amount of fluid at the back of fetal neck, see fig. 1. Another ultrasound marker that may be used is the presence/absence of nasal bone (may or may not be absent in Down's syndrome).

Fig. 1

Ultrasound measurement of nuchal translucency



The most important biochemical marker is PAPP-A (pregnancy associated plasma protein A). Another parameter that may be used in combination with PAPP-A is free-beta hCG or hCG. The optimal gestational age for the determination of biochemical markers is between weeks 10th and 13th. Nevertheless, as shown in table 4, the most significant benefit of PAPP-A determination is in 10th week (when the benefit for hCG and free-beta hCG is rather low). On the contrary, the benefit of PAPP-A is lower in 13th week which is ideal for hCG and free-beta hCG (what is not so surprising in the markers connected rather with second trimester).

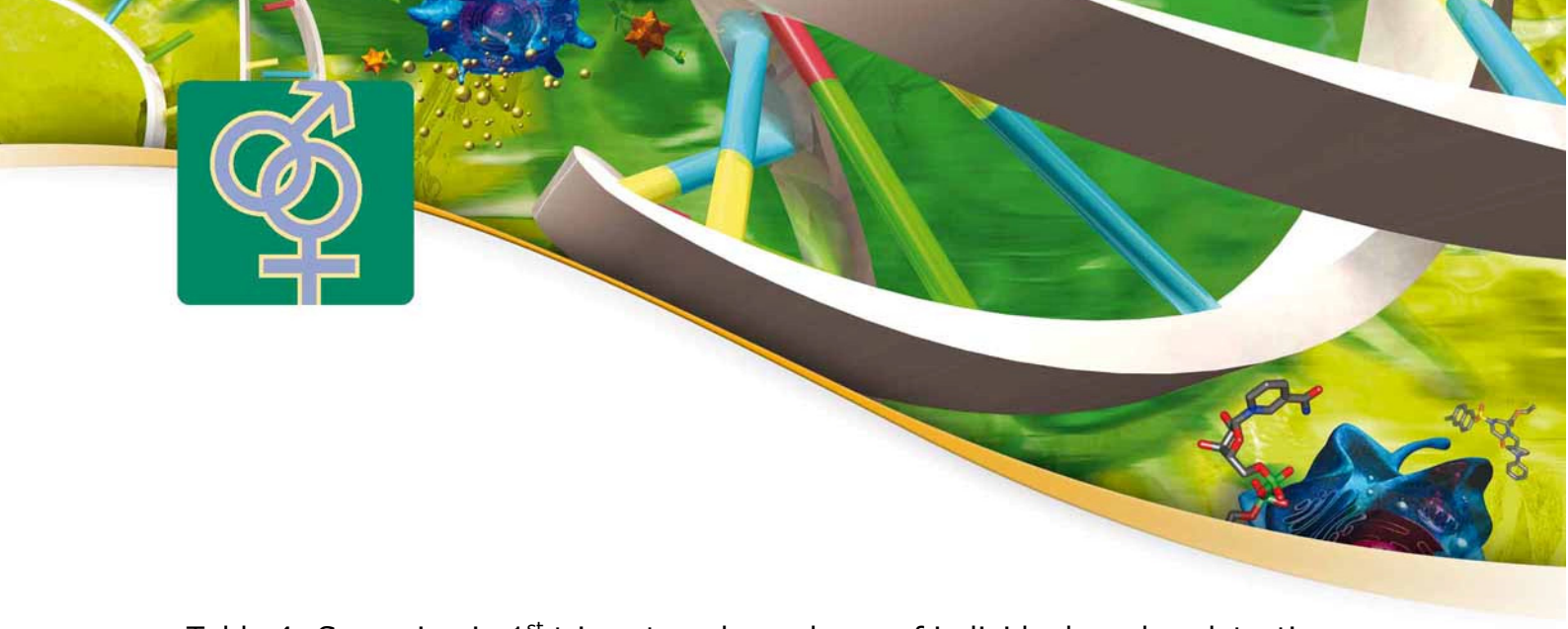


Table 4: Screening in 1st trimester; dependence of individual marker detection rates on the lengths of gestational age (results reported for given 5% false positivity rate). Data from SURUSS study.

GA (weeks)	10	11	12	13
	DR (%) in FPR 5%			
NT (US)	51	59	62	62
PAPP-A	58	45	35	27
Free β-hCG	19	28	35	44
Total hCG	5	15	26	41
uE3	13	21	29	37
Inhibin A	5	16	30	48

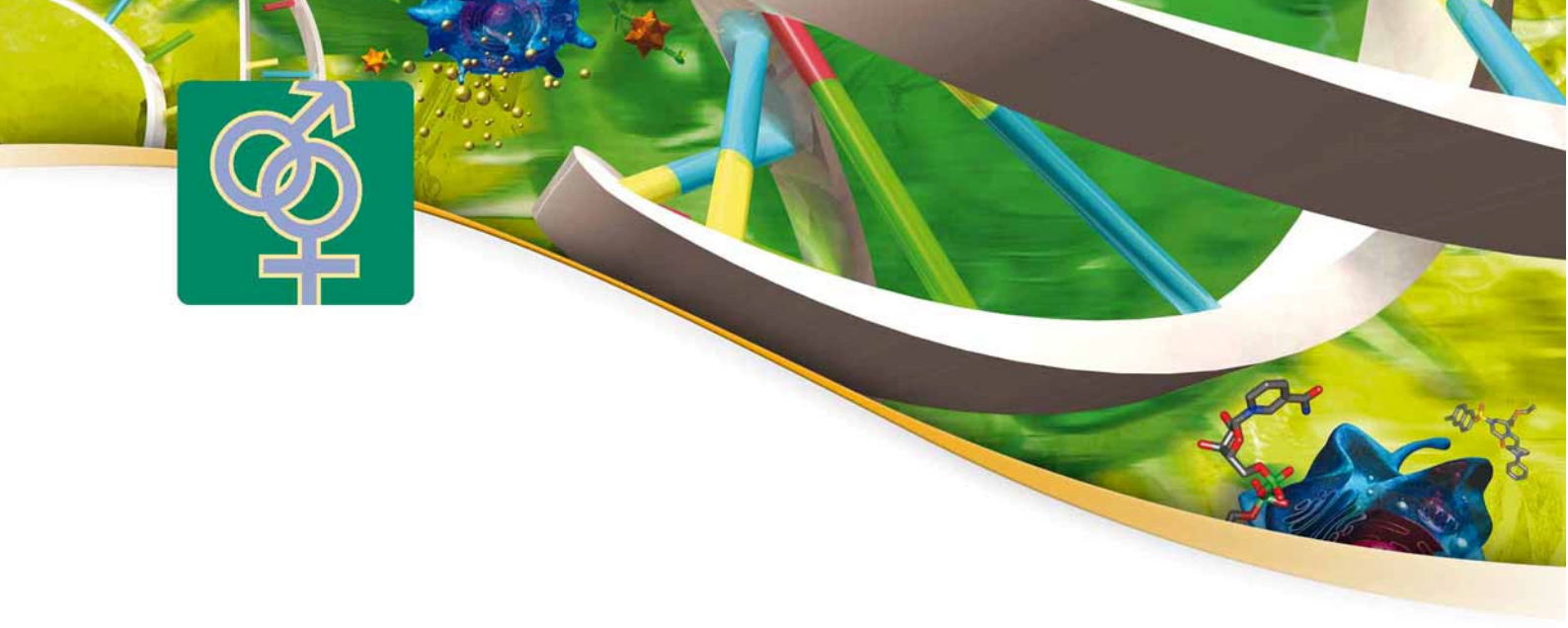
FPR – false positivity rate
DR – detection rate

GA – gestational age
NT (US) – nuchal translucency (ultrasound)

Evaluation of these data may provide an estimation of the risk of chromosomal aberrations, especially the Down's syndrome, but also the risk of Edward's and Turner's syndrome. In any case, the results of the first trimester screening are not useful for the detection of neural tube defects.

Clear benefit of first trimester screening is the prospect of early prenatal diagnosis.

On the other hand, it may detect also those affected fetuses which would be lost spontaneously, bringing thus additional stress to women. Also, as already mentioned, combined screening do not contribute to detection of neural tube defects. If second trimester testing is omitted, morphology ultrasound becomes necessary to reduce risk of NTD.



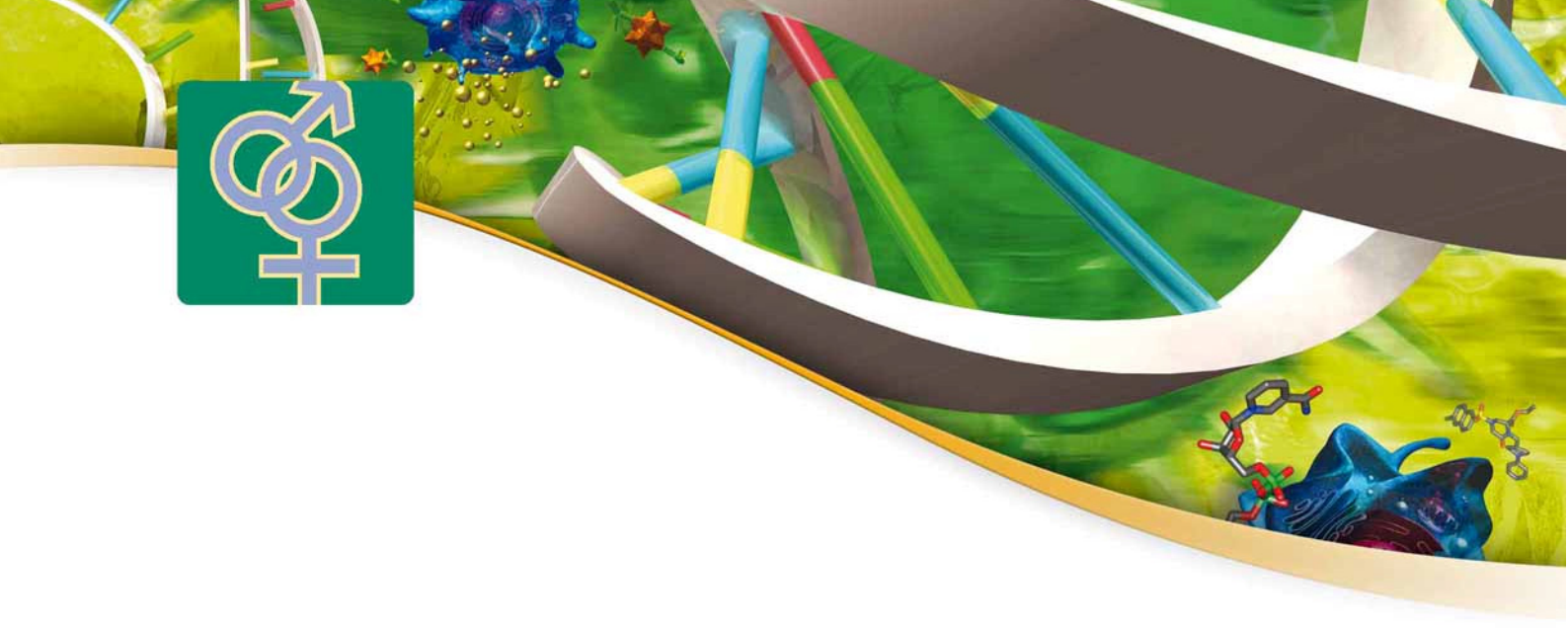
Another aspect is the necessity of reliable NT results, which may be provided only by well-trained and experienced ultrasonographist working on enhanced type of instrument. Otherwise the NT results may cause failure of the whole screening.

Table 5: Screening in 1st trimester; comparison of detection rates (DR) at given false positivity rates (FPR) for various marker combinations. Data from SURUSS study.

FPR (%)	1	3	5
	DR (%)		
Combined test NT, PAPP-A	59	73	79
Combined test NT, PAPP-A, hCG	60	74	80
Combined test – NT, PAPP-A, Free beta hCG	66	78	83

Table 6: Screening in 1st trimester; comparison of false positivity rates (FPR) at given detection rates (DR) for various marker combinations. Data from SURUSS study.

DR (%)	70	80	90	95
	FPR (%)			
Combined test NT, PAPP-A	2.3	5.3	13.9	26
Combined test NT, PAPP-A, hCG	2.2	5.1	13.4	26
Combined test – NT, PAPP-A, Free beta hCG	1.5	3.7	10.8	22



Integrated screening approach

Integrated screening program combines results of the first trimester (usually PAPP-A and NT) with second trimester data (AFP and hCG, and possibly also of unconjugated Estriol and/or Inhibin A). All the data are evaluated together in the second trimester. Such integrated screening has the best reported outcome, with 95 % Down's syndrome detection rate (at 5 % false positivity level).

Another option, suitable if NT measurement is not available, is so called serum integrated screening (PAPP-A and second trimester markers).

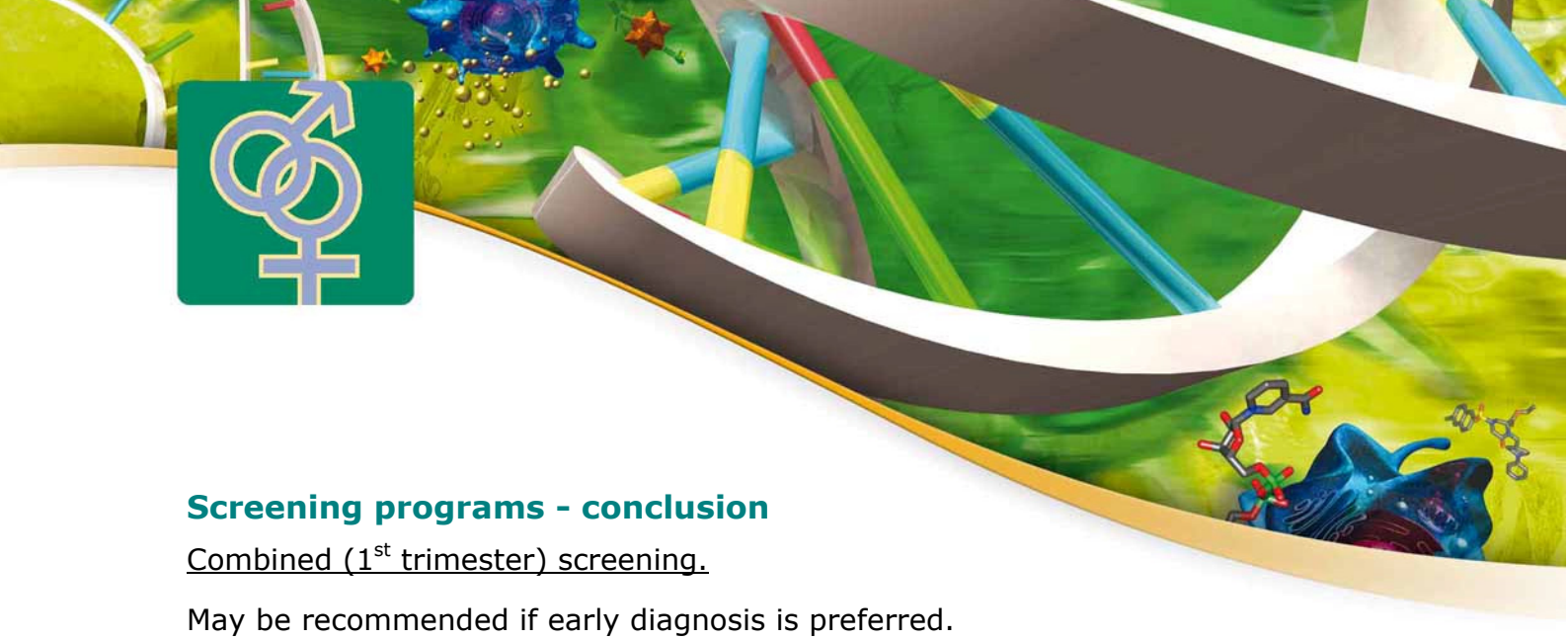
The table below shows apparent superiority of integrated screening comparing to both above mentioned approaches (second trimester or combined screening).

Table 7: Integrated screening; comparison of detection rates (DR) at given false positivity rates (FPR) for various marker combinations. Data from SURUSS study.

FPR (%)	1	3	5
	DR (%)		
Integrated test NT, PAPP-A, AFP, hCG, uE3	79	88	92
Integrated test NT, PAPP-A, AFP, hCG, uE3, Inhibin A	83	90	93
Serum integrated test PAPP-A, AFP, hCG, uE3	69	80	85
Serum integrated test PAPP-A, AFP, hCG, uE3, Inhibin A	75	85	89

Table 8: Integrated screening; comparison of false positivity rates (FPR) at given detection rates (DR) for various marker combinations. Data from SURUSS study.

DR (%)	70	80	90	95
	FPR (%)			
Integrated test NT, PAPP-A, AFP, hCG, uE3	0.4	1.1	3.9	9.4
Integrated test NT, PAPP-A, AFP, hCG, uE3, Inhibin A	0.2	0.7	2.8	7.4
Serum integrated test – NT, PAPP-A, AFP, hCG, uE3	1.1	2.8	8.5	17
Serum integrated test – NT, PAPP-A, AFP, hCG, uE3, Inhibin A	0.6	1.7	5.8	13.2



Screening programs - conclusion

Combined (1st trimester) screening.

May be recommended if early diagnosis is preferred.

NT (weeks 11-13) and PAPP-A (week 10)
hCG or free-beta hCG may be determined as well. Nevertheless, the benefit of such determination would be very low if it is performed together with PAPP-A in 10th week. It seems to be better to take another sample in week 12-13 for this determination (see table 4; showing the performance of individual markers in different gestational age).

Advantages	- early diagnosis
Disadvantages	- lower sensitivity, higher risk of invasive procedure
	- does not contribute to the detection of neural tube defects
	- unnecessary detection of pregnancies which would terminate naturally

Second trimester screening

May be recommended for women who come too late for the first trimester testing.

Preferably AFP, hCG, uE3 and inhibin A

Advantages	- no special ultrasound equipment and experience necessary
	- detection of NTD
Disadvantages	- low sensitivity, higher risk of invasive procedure. especially in case of double and triple test

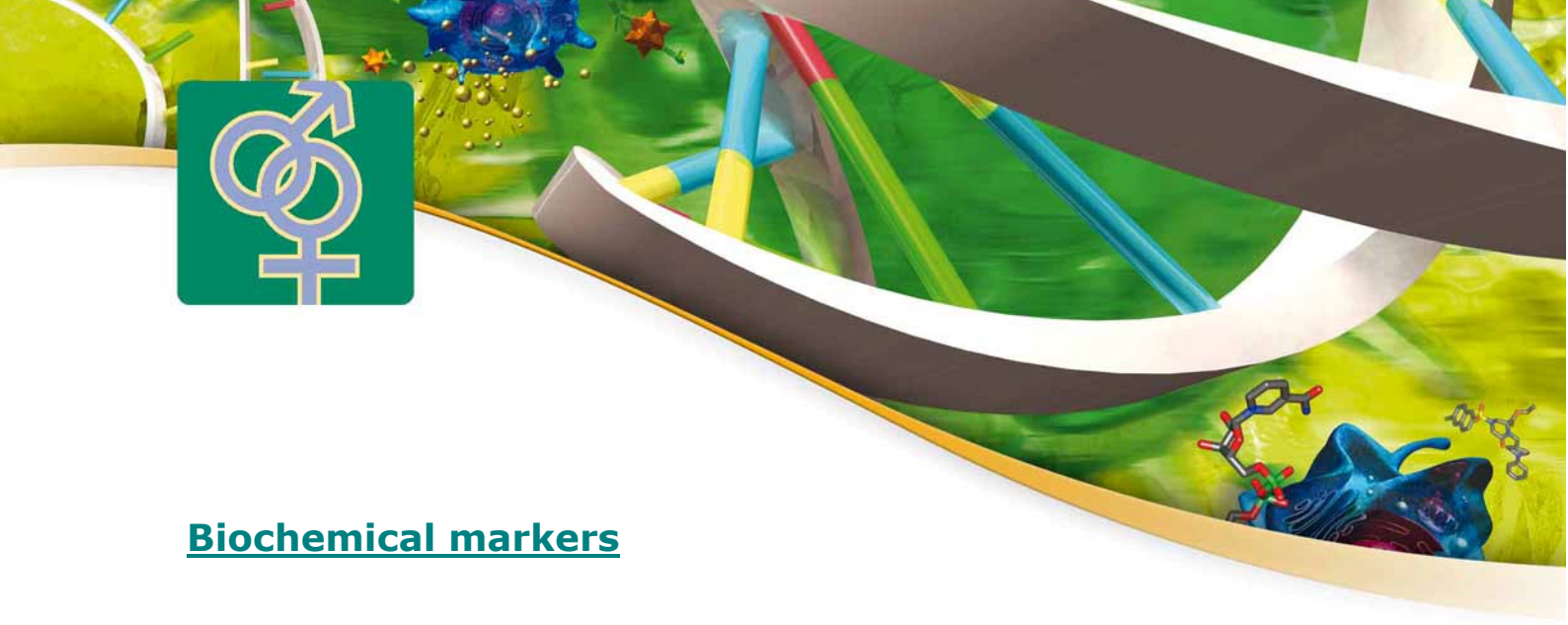
Integrated screening

The most reliable outcome.

PAPP-A and NT in 1st trimester, hCG and AFP (possibly also uE3 and Inhibin A) in 2nd trimester
If NT measurement not available, possibility of integrated serum screening only.

Advantages	- very good sensitivity, very low risk of invasive procedure
	- detection of NTD
Disadvantages	- long time between the first collection and information about the risk (stress for mother)

Note: Omission of NT would decrease sensitivity of screening to the level comparable to the combined (1st trimester) screening.



Biochemical markers

PAPP-A (Pregnancy Associated Plasma Protein, synonym: IGFBP-4 protease)

PAPP-A is a high-molecular weight (720 -820 kDa) tetramer, enzyme of the class of metalloendopeptidases.

Four polypeptide chains are paired by disulphide bridges to form two dimers that are bound non-covalently. Each dimer contains one PAPP-A subunit and one subunit of glycosylated pro-form of the main basic eosinophilic protein (proMBP).

PAPP-A is not specific to pregnancy only, measurable levels can be found also in non-pregnant women and in men. Its concentration increases e.g. if unstable atherosclerotic plaques are present, making PAPP-A very promising marker in acute coronary syndrome events.

Diagnostic significance of PAPP-A in pregnancy

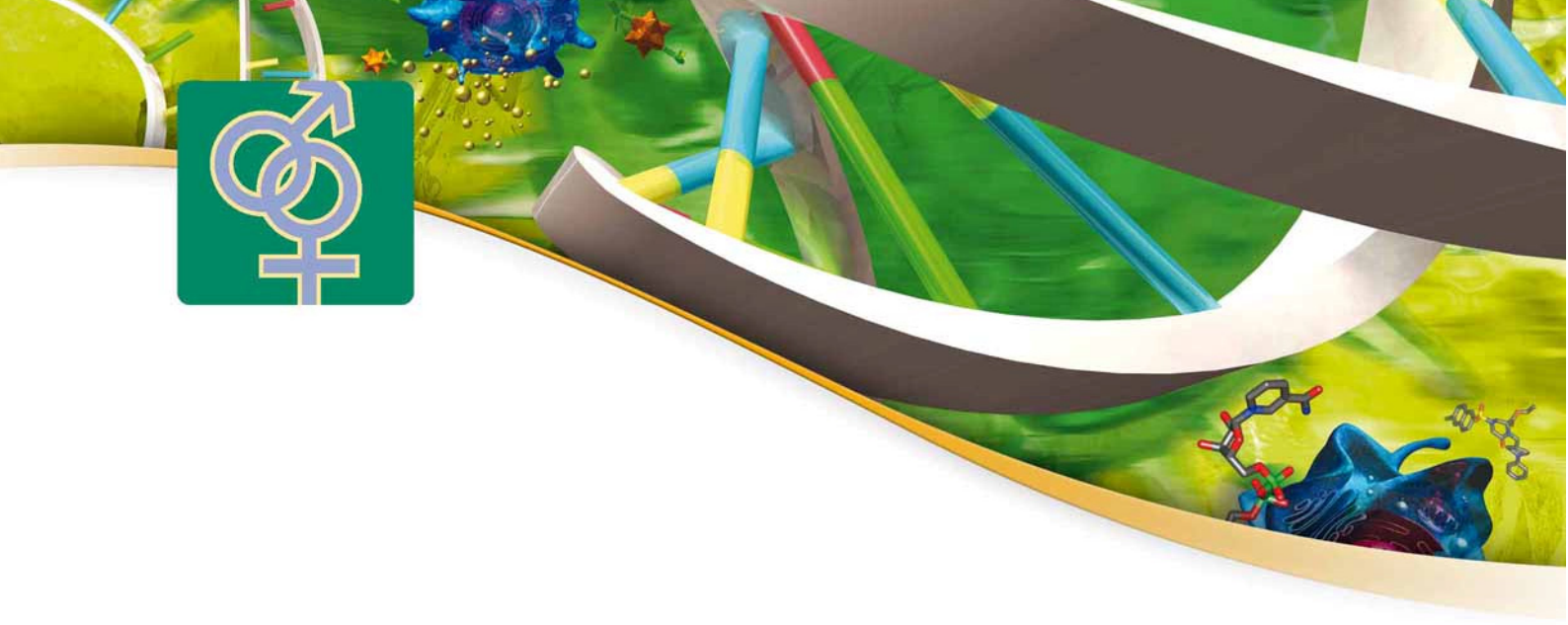
The PAPP-A level starts to increase significantly from the 7th week of normal pregnancy. The growth is nearly exponential in the beginning and continues during the whole pregnancy. After delivery the levels slowly decrease. Numerous studies have shown that in fetuses with chromosome 21 or 18 trisomy (Down's syndrome, Edward's syndrome) as well as at some other congenital malformations the PAPP-A levels are usually significantly reduced, particularly in the 1st trimester. After the 12th week PAPP-A serum levels gradually decrease towards the normal range.

Correct interpretation of the test is conditioned by exact information about the gestational age as the PAPP-A levels increase very rapidly in the 1st trimester.

Table 10: Examples of PAPP-A medians for congenital malformation screening in the 1st trimester

Note: Each laboratory must generate its own range of median values and keep it updated. Values shown in the table below were obtained during the clinical testing and are informative only.

Gestation week	n	Median (IU/L)
9	63	0.58
10	292	0.93
11	245	1.49
12	113	2.50
13	164	3.91
14	60	5.43



Chorionic gonadotropin (hCG), free-beta subunit of hCG (free beta-hCG)

Chorionic gonadotropin is the major gestational hormone. It is generated in the syncitiotrophoblast cells of the placenta and in certain tumours, insignificant quantities are produced by the pituitary gland as well.

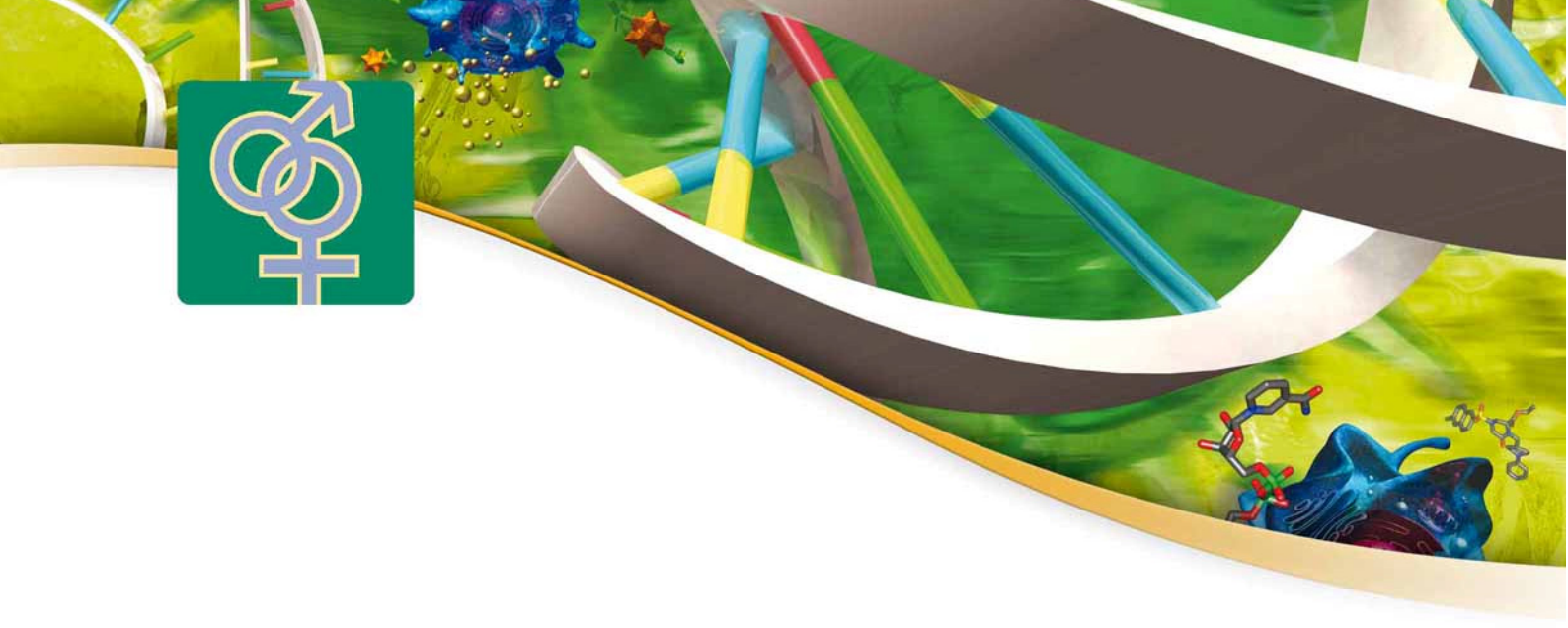
Like pituitary gonadotropins, hCG is a glycoprotein (molecular weight about 40 kDa) consisting of two subunits - alpha and beta. As to amino acid sequences, the hCG β-subunit contains the substantial part identical to LH β-subunit and moreover it contains 30 further amino-acids on the carboxyl terminal of the molecule. As a result of this high homology, the immunochemical discrimination between hCG and LH is dependent on the ability of an antibody to recognise just this tiny different part of the hCG molecule.

Besides the complete, undissociated hCG molecules, smaller amounts of free α- and β-subunits, or other forms, so-called "nicked beta-hCG" and "beta-core fragment", may circulate in the peripheral blood. Levels of any subunit may be elevated at certain pathological conditions.

Some available tests designated as "hCG" measure the levels of intact, undissociated hCG molecule. This type of tests does not measure the levels of free α-subunit, free β-subunit or its other forms. On the contrary, tests designated as "hCG Total" or "Total β-hCG" measure not only the level of undissociated molecules but also the levels of free β-hCG subunit and its other forms. Values obtained by these tests are higher than those measured using the "hCG" test. Tests designated as "Free β-hCG" are then intended for determination of free β-hCG subunit levels only.

The diagnostic significance of hCG and free β-hCG in pregnancy.

The measurable hCG levels appear in the maternal blood very soon, already in the day 8 or 9 after conception. During the 1st trimester, the hCG concentration increases very rapidly. In the course of normally proceeding pregnancy, the doubled hCG concentration value may be expected every 2 to 3 days. The hCG concentration reaches its peak by the week 8th – 10th, then decreases, and in the second half of the pregnancy exhibits a more or less stable level. The rapid hCG concentration increase both in the peripheral blood and in urine, what predetermines hCG to be an ideal early pregnancy recognition test.



In general, it is supposed that the physiological role of hCG in an early pregnancy is to stimulate the progesterone synthesis in the corpus luteum. In addition, it is supposed that hCG stimulates the testosterone production in the male fetus gonads and also affects the fetal adrenal cortex.

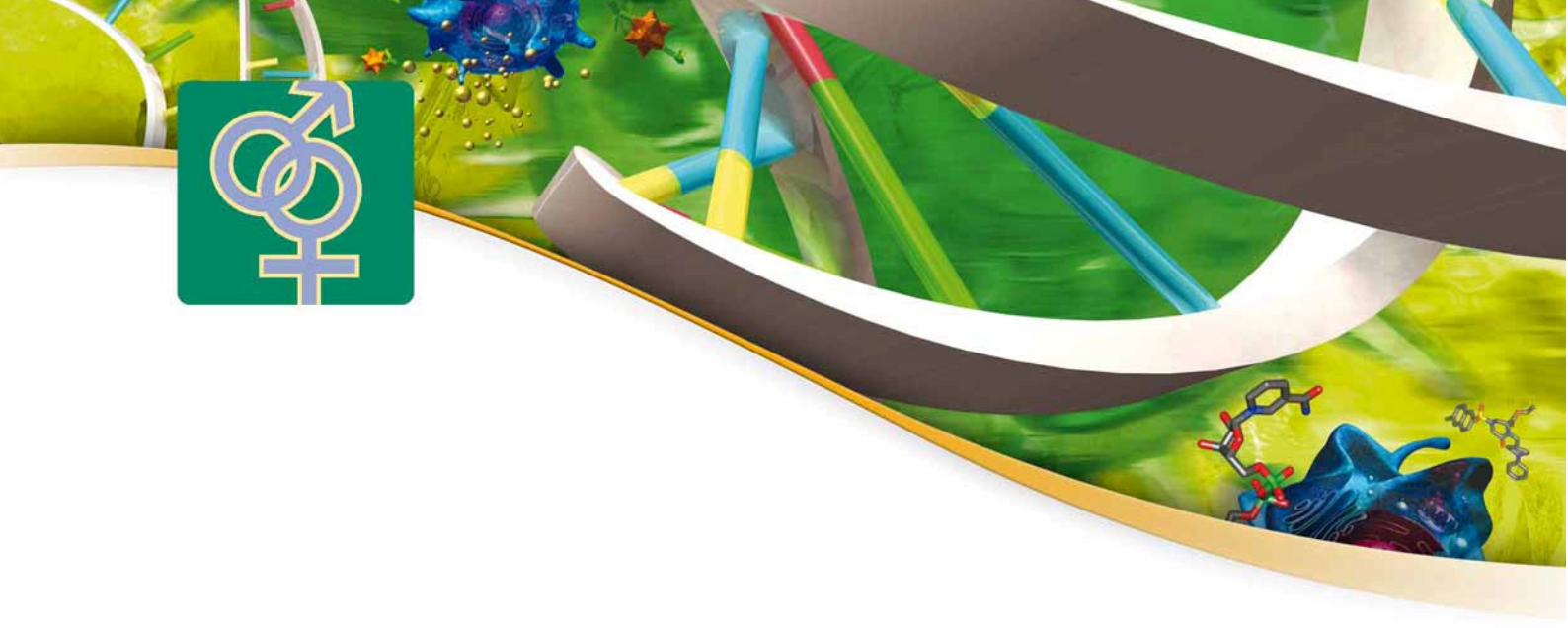
Various degradation products of hCG molecule, including free β-subunit, co-exist in peripheral blood. Concentrations of β-hCG in maternal serum are, of course, considerably lower than the concentrations of intact molecules of the hormone. In the 1st trimester the ratio of free β-hCG to hCG is about 1-4%, and in the 2nd and 3rd trimester this percentage drops below 1%.

The hCG molecule is relatively stable in collected material - serum as well as plasma. When, however, samples are left for a longer period at room temperature, the molecules dissociate into free subunits and their concentration in the sample increases. For exact determination of β-hCG, a standard procedure of sample collection and storage should be adhered to. The samples should preferably be stored frozen.

Table 9: Examples of hCG medians for congenital malformation screening in the 2nd trimester

Note: Each laboratory must generate its own range of median values and keep it updated. Values shown in the table below were obtained during the clinical testing and are informative only.

Gestation week	n	Median (kIU/L)
15	172	48.9
16	268	38.1
17	193	32.5
18	49	25.7
19	18	25.1



Alphafetoprotein (AFP)

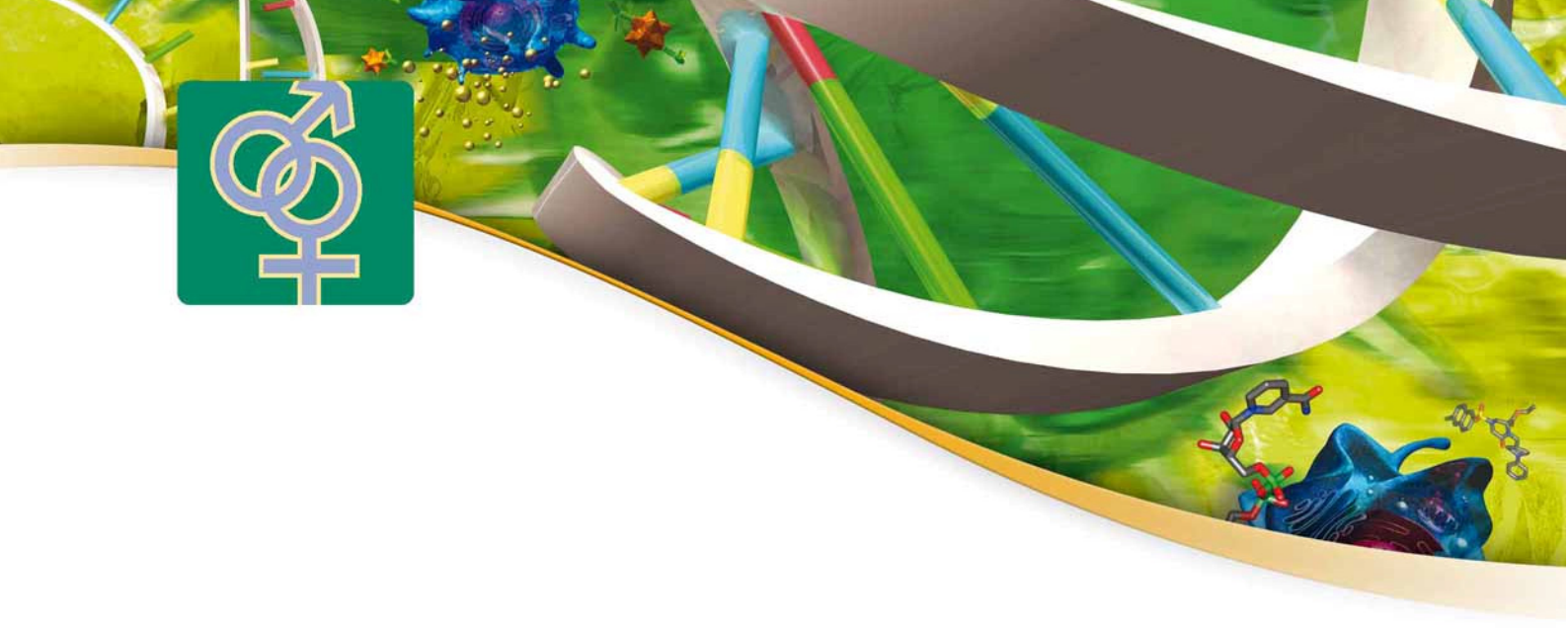
AFP is a specific fetal alpha-globulin of molecular weight 65 – 70 kDa. AFP is primarily produced by the yolk sac. By the 13th week, its synthesis is taken over by fetal liver and declines till the delivery. During the first year of life, the AFP level decreases to a very low level, normally found in adults.

The diagnostic significance of AFP in pregnancy

During a physiological pregnancy, the AFP level decreases in amniotic fluid and, on the contrary, increases in the maternal serum. The elevation above physiological values always signalizes a serious pathological state. The AFP level determination in amniotic fluid obtained by means of amniocentesis between week 15 and 20 has been utilized since the seventieth for the prenatal diagnosis of neural tube closure defects which are not covered by skin. The determination of maternal serum AFP is used as a screening test for the NTD since 1977. The AFP MoM increase to values above 2.5 represents significant evidence of the congenital malformation risk, or imminent abortion or of a dead fetus.

Table 11: Review of MS AFP level increase and decrease causes during pregnancy.

Elevation causes	Decrease causes
Fetus	
Anencephaly Rachischisis Omphalocele Gastroschisis Thoracoabdominal defect Renal agenesis Teratoma Hydrocephaly Multiple malformations Hygroma colli cysticum Oligohydramnion Meckel's syndrome Encephalocele	Down's syndrome and other chromosomal aberrations

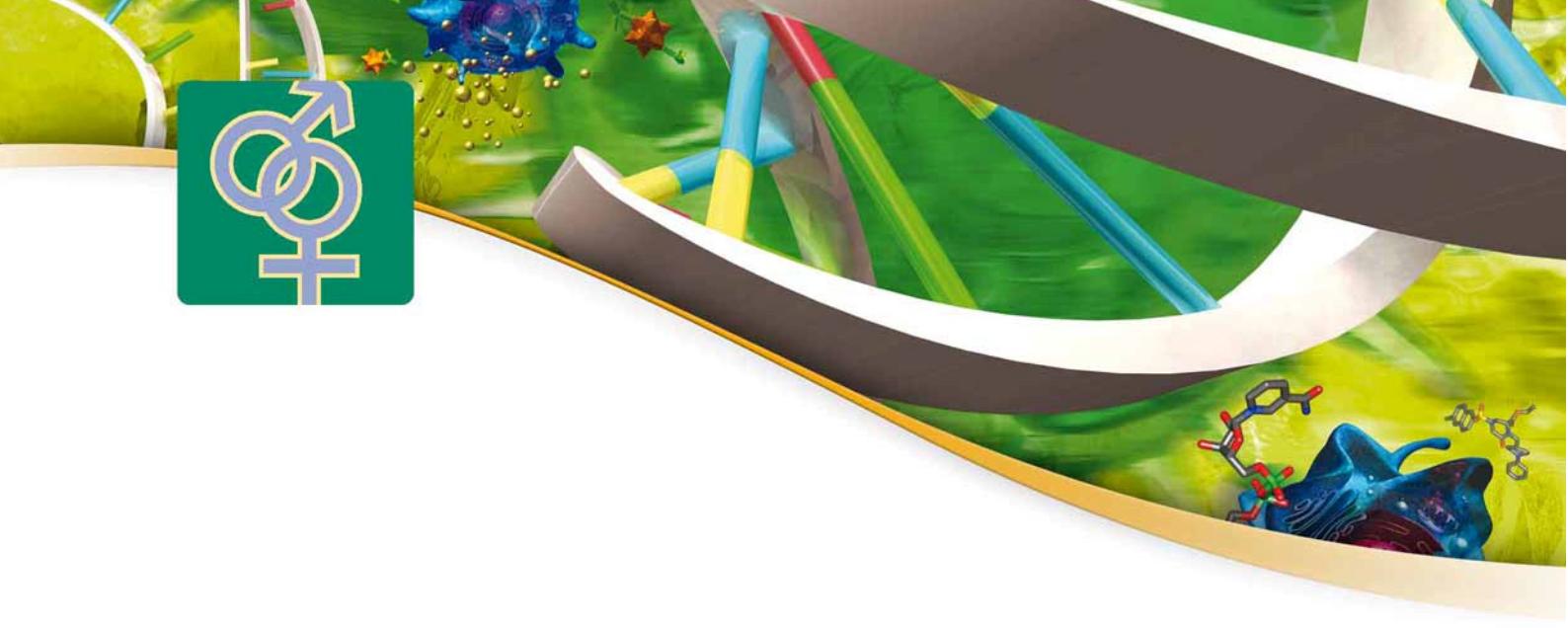


Elevation causes	Decrease causes
Mother	
Extra uterine pregnancy Preeclampsia Placental shape deviations Viral hepatitis Primary liver carcinoma Malign gastrointestinal tumours Tumours of germ cell origins	Diabetes mellitus type 1 Mola hydatidosa Choriocarcinoma
Pregnancy disorders	
Fetal death Placental dysfunction Rh incompatibility	

Table 12: Examples of AFP medians for congenital malformation screening in the 2nd trimester

Note: Each laboratory must generate its own range of median values and keep it updated. Values shown in the table below were obtained during the clinical testing and are informative only.

Gestation week	n	Median (IU/mL)
15	48	27.2
16	65	35.4
17	74	40.3
18	68	46.2
19	59	57.5
20	46	65.5



Unconjugated estriol (uE3)

Estriol is the major steroid hormone synthesised by the placenta. The first synthesis stage proceeds in the fetus, where cholesterol, either generated de novo or brought from maternal circulation, changes to pregnenolon, is sulphated in the fetal adrenal cortex and converted to DHEAS Dehydroepiandrosterone sulfate). This compound is 16-alpha hydroxylated in fetal livers and finally transported into the placenta. The sulphate is cleaved by placental sulphatase and the free steroid by-product is converted into estriol. For the estriol generation, the co-operation of the fetus and the placenta is necessary. Therefore, the estriol excretion is an ideal marker for the monitoring of the whole feto-placental unit function.

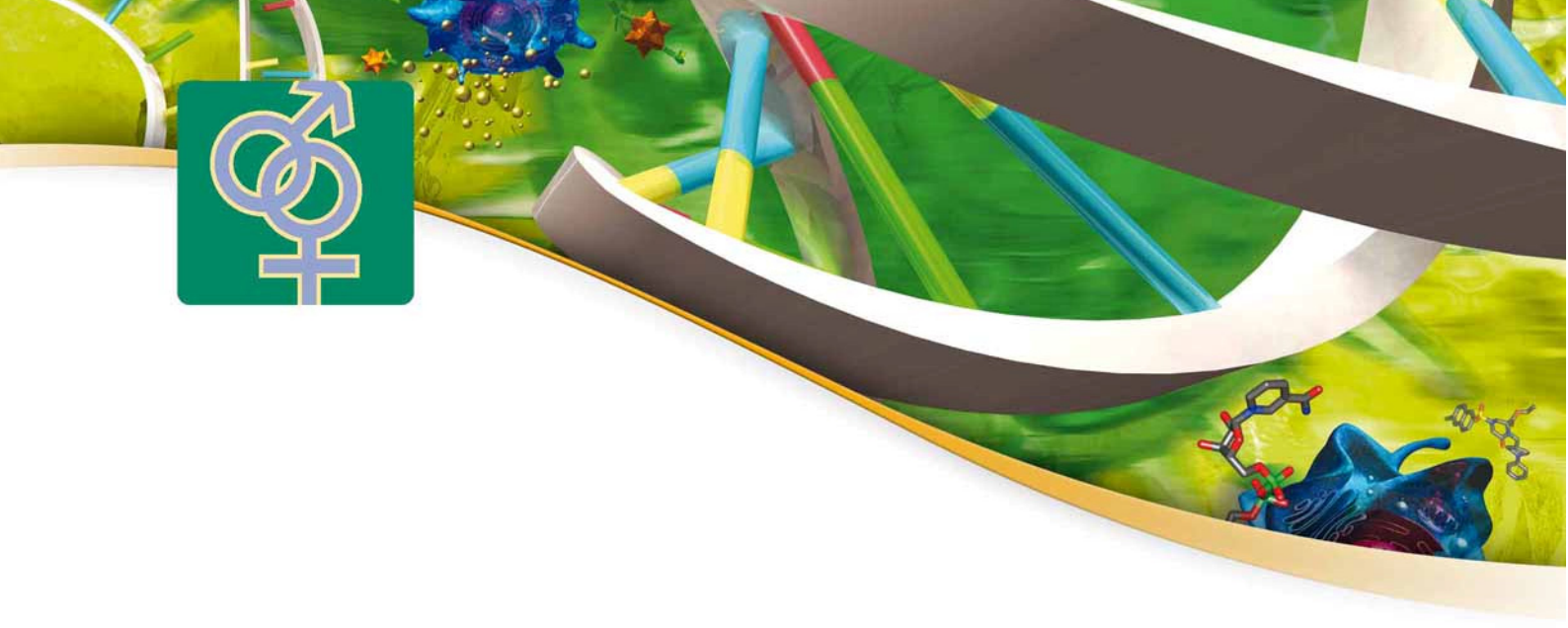
The diagnostic significance of estriol in pregnancy

Estriol is present in the maternal circulation in small amounts as an unconjugated steroid and, in particular, as glucuronide and sulphate. In the physiological pregnancy, the estriol level increases continuously by the 40th week. The decreased estriol values or its rapid drop signalize an intrauterine fetal distress. The free or the total estriol levels are used for monitoring. Unconjugated estriol may reflect the undesirable change of the fetoplacental function more rapidly.

Table 13: Examples of unconjugated estriol medians for congenital malformation screening in the 2nd trimester

Note: Each laboratory must generate its own range of median values and keep it updated. Values shown in the table below were obtained during the clinical testing and are informative only.

Gestation week	n	Median (ng/mL)
14	33	0.61
15	202	0.89
16	181	1.16
17	68	1.37
18	11	1.78



Inhibin A

Inhibins are heterodimeric proteins that suppress the secretion of FSH (follicle stimulating hormone) from the pituitary. Inhibin consists of two distinct chains, or subunits (alpha and beta), linked together. Inhibin A consists of the alpha-subunit and β_A-subunit. Only the dimeric forms of the molecule, containing both the alpha and beta subunits, are bioactive. The free subunit forms exist in circulation as well.

Inhibin A is secreted by ovarian granulosa cells. At the onset of menstruation during the early follicular phase, very low levels of inhibin A are found. Levels increase dramatically in the late follicular phase and maximize in the mid-luteal phase. During the menstrual cycle and very early pregnancy, inhibin A is produced by the corpus luteum.

The diagnostic significance of Inhibin A in pregnancy

The feto-placental unit appears to be the major source of increased circulating concentrations of inhibin A in early pregnancy. Production occurs at a number of sites, including the fetus and placental and fetal membranes. Maternal serum levels of inhibin A increase during the first trimester and decline after about 10 weeks. Levels remain stable at 15 to 25 weeks and then increase, reaching peak at term.

Inhibin A has become integral part of prenatal screening in the second trimester of pregnancy. Down's syndrome pregnancies show increased values of this marker.

Inhibin A may be also used for the detection of pregnancy in case of IVF (in vitro fertilization), when hCG levels are less reliable due to intake of extragenous hCG.

Inhibin A may be also used as an early marker of pre-eclampsia. Measurements of inhibin A at 15-20th weeks of pregnancy showed that the increase of serum level can be predictive of later onset of pre-eclampsia.

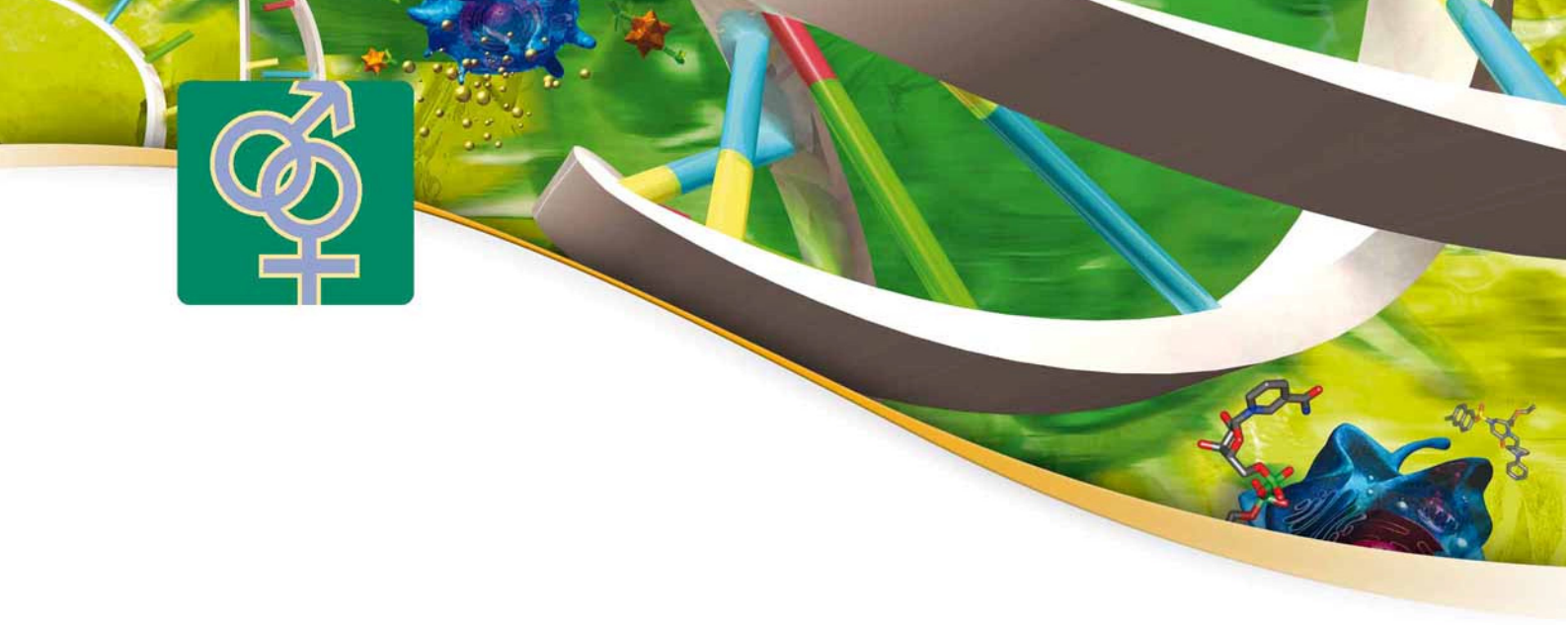
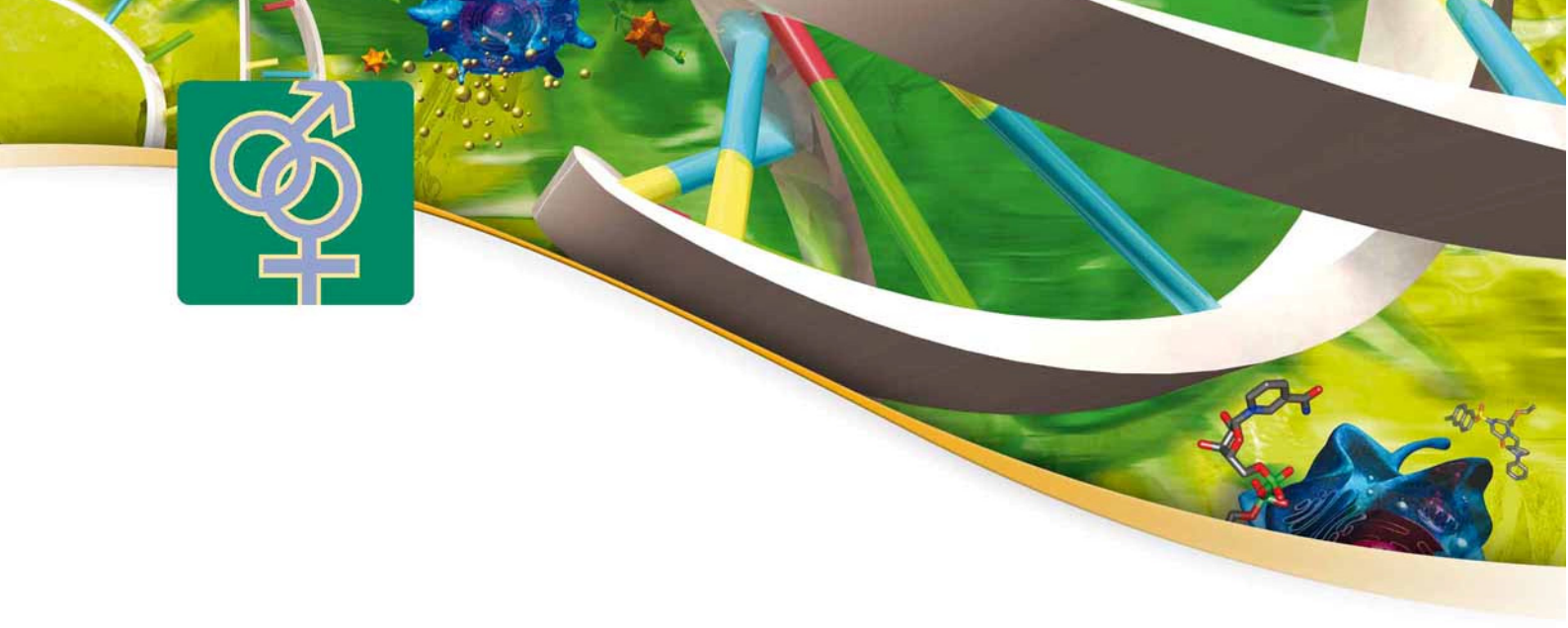


Table 14: Examples of Inhibin A medians for congenital malformation screening in the 2nd trimester

Note: Each laboratory must generate its own range of median values and keep it updated. Values shown in the table below were obtained during the clinical testing and are informative only.

Gestation week	n	Median (pg/mL)
15	30	157.6
16	124	153.3
17	74	151.2
17	45	155.1
18	20	165.2
19	16	185.8



Conclusion

Prenatal screening becomes part of routine diagnostic procedures in pregnancy in more and more countries. Big effort has been made to find screening program enabling further reduction of false positivity and simultaneous increase of detection rate. The best results has been found for integrated approach, consisting of PAPP-A and NT determination in the first trimester, and AFP, hCG, uE3 and Inhibin A determination in the second trimester.
Nevertheless, even this approach has certain limitations, mainly:

- the need of well trained ultrasonographist and advanced instrument
- the need of visit of clinician early in the course of pregnancy (ideal time for PAPP-A determination is in 10th week)

Short summary of screening programs is in the table below:

Table 15: Comparison of screening programs

Screening program	**Convenient at condition**	**Disadvantage**
Combined screening (1st trimester)	Early information is required	Lower sensitivity Higher risk of invasive examination Equipment for NT necessary
Second trimester screening	Mother visits clinician too late	Low sensitivity Higher risk of invasive examination
Integrated screening	The most reliable information is required	Long time between first determination and the results Equipment for NT necessary
Serum integrated screening	Equipment for NT is not available	Long time between first determination and the results Lower sensitivity Higher risk of invasive examination