First trimester screening
For better maternal and fetal care
With the cobas modular platform (cobas 4000 and 6000 analyzer series and cobas 8000 modular analyzer series) Roche has developed a platform concept based on a common architecture that delivers tailor-made solutions for diverse workload and testing requirements. The cobas modular platform is designed to reduce the complexity of laboratory operation and provide efficient and compatible solutions for network cooperation.

**Flexible and intelligent solutions**
- Multiple configurations with tailor made solutions for higher efficiency and productivity
- Consolidation of clinical chemistry and immunochemistry with more than 200 parameters for cost and workflow improvements
- Future sustainability through easy adaptation to changing throughput and parameter needs
- Consistency of interaction with hardware, software and reagents for less training and more staff flexibility
- Consistency of patient results due to a universal reagent concept

**cobas 8000 modular analyzer series**
Large volume

![cobas 8000 modular analyzer series](image1)
- 38 configurations

**cobas 6000 analyzer series**
Mid volume

![cobas 6000 analyzer series](image2)
- 7 configurations

**cobas 4000 analyzer series**
Low volume

![cobas 4000 analyzer series](image3)
- 3 configurations
Prenatal Screening for Down Syndrome

Introduction
Roughly estimated, every 800th conception resulting in a viable baby is hit by an abnormal number of chromosomes, a so-called non-gonosomal aneuploidy. The most frequent is a trisomy of chromosome 21. The affected persons will remain handicapped and prone to diseases throughout their lives. Since there is knowledge about the etiology of the condition, there has been substantial effort to develop tools for its early preterm recognition allowing to advise parents timely. This brochure is about more recent findings in this field.

History
The first report about trisomy 21 can be traced back to 1866. The author of his report, John Langdon Down, was the superintendant of an asylum for retarded children. He observed two distinct populations amongst the children in his care: As is known today, one group comprised children with a congenital hypothyroidism, the other children with a trisomy 21, a condition that is referred to today as “Down Syndrome”.

Roughly 40 years later a correlation of incidence and maternal age was reported\(^2\) (Fig. 1). In the 1930s there was increasing evidence, that chromosomal abnormalities may be the reason for the syndrome.\(^3,4\) Finally, in 1959 Lajeune\(^5,6\) and Jacobs\(^7\) identified trisomy of chromosome 21 to be one cause of it. In the 1960s more chromosomal aberrations (translocation\(^8,9\), mosaicism\(^10\)) were reported also to result in the syndrome.

Incidence and Etiology\(^11,12\)
The risk for the development of trisomy 21 correlates with maternal age and ranges from approx. 1 : 1600 to 1 : 5 or even higher. Very young mothers (15 – 18 yrs) appear to have a slightly increased risk as well. The paternal age appears to have only a small, if any influence.

Approximately 97 % of all cases are the consequence of a faulty cell division, 90 % of which would affect the maternal and 5 % the paternal genome during gametogenesis. Out of these, 2/3 are thought to be due to an impaired first meiotic disjunction, 1/3 to an incomplete second meiotic disjunction. The remaining 5 % originate in a post-zygotic, mitotic cell division error (Fig. 2).

In 95 % of all trisomy 21 cases, a complete third chromosome 21 is present in all cells of the affected person. This condition is known as “free trisomy 21”.

3 % of Down syndrome cases are due to an unbalanced translocation of parts of chromosome 21. There are 2 main types:

- 14,21-translocations, 50 % of which are inherited from balanced carriers, usually from the mothers (familial type).
- 21,21-translocations, which usually occur de novo.

A small percentage of Down syndrome cases result from so-called mosaics, which sometimes do not present very pronounced clinical manifestations and thus are often not discovered. Again there are 2 basic mechanisms that would cause a mosaic:

- Secondary somatic loss of a chromosome 21 from an initially trisomic zygote.
- Mitotic disjunction error very early during embryo development.

Today, the term “Down Syndrome” is generally accepted to describe the clinical picture of people having an aneuploidy of chromosome 21.
Manifestation\textsuperscript{12,17} The clinical picture of Down Syndrome originates from an over-expression of the genes located on chromosome 21, bringing the affected fetus out of genetic balance. The manifestation of signs and symptoms is not predictable and ranges from nearly not apparent to severely handicapping. Affected children often present a characteristic pattern of dysmorphisms, facultative malformations, growth deficiency, and mental retardation.

Down babies are very susceptible to infectious diseases. Before the availability of antibiotics 90% of them died early from infections.

**Frequent dysmorphisms in Down syndrome**
- Bradycardia
- Brushfield iris spots
- Small nose with depressed bridge\textsuperscript{18}
- Short and broad neck with excessive skin folds
- Transverse palmar crease
- Small ears with folded helix
- Frequent lid inflammations
- Fissured lips
- Wide thorax
- Klinodactyly
- Macroglossy\textsuperscript{13,14}
- Dark red tongue
- Irregularly positioned teeth\textsuperscript{15}
- Typical bowl configuration (X-ray)
- Brachymesophalangy (5\textsuperscript{th} finger), only 1 inflexion crease
- Upward slant to the eyes\textsuperscript{15}
- Epicanthal fold
- Predisposition for caries\textsuperscript{16}
- Short broad hands and fingers
- Excessive space between 1\textsuperscript{st} and 2\textsuperscript{nd} toe

Beside the dysmorphisms there are several clinical manifestations associated with the Down Syndrome.

**Some facultative clinical manifestations in Down syndrome**
- Atrioventricular septal defect
- Tetralogy of Fallot
- Duodenal stenosis or atresia
- Omphalocele
- Preaxial polydactyly
- Hypothyroidism
- Malformation of upper cervical vertebra with atlanto-occipital instability
- Esophageal and anal atresia
- Aganglionic megacolon

On the clinical side there is an increased incidence of heart defects (50%), epilepsy, hypothyroidism and celiac disease.
Invasive methods in prenatal screening

Since the discovery of the chromosomal background of Down Syndrome in the 1960s there have been continuous efforts for an early pre-term method to detect Down pregnancies. In the 1970s amniocentesis and other invasive techniques were the methods of choice. However, having a complication rate of 1 – 2 %, these examinations were restricted to the risk group (women beyond 35), so that an overall detection rate of 30 % only could be achieved.

**Amniocentesis**

Amniocentesis is used to collect amniotic fluid. A needle is inserted through the mother’s abdominal wall into the uterus, using ultrasound guidance. Approximately one ounce of fluid is taken for testing. Amniotic fluid contains fetal cells that can be examined for their chromosomes. It takes about 2 weeks to determine if the fetus has Down syndrome or not.

Amniocentesis is usually done between the 14th and 18th week of pregnancy. Side effects to the mother include cramping, bleeding, infection and leaking of amniotic fluid afterwards. There is a slight increase in the risk of miscarriage: the normal rate of miscarriage at this gestational age is 2 to 3 %, and amniocentesis increases that risk by an additional ½ to 1%. Amniocentesis is not recommended before the 14th week of pregnancy due to a higher risk of complications and miscarriage.

**Chorionic villus sampling (CVS)**

Chorionic villus sampling (CVS) is another invasive method to collect fetal cells for chromosome analysis. In this procedure, instead of amniotic fluid, a small amount of tissue is taken from the young placenta (chorionic layer). The cells can be collected transabdominally like amniotic fluid. Another method is to insert a tube into the uterus transcervically. The method depends on the mother’s anatomy and the location of the placenta.

CVS is usually done between the 10th and 12th week of pregnancy. Side effects to the mother are the same as with amniocentesis. The risk of miscarriage after CVS is around 3 – 5 %, slightly higher than with amniocentesis. In the past, after the use of CVS a number of babies were born with missing or shortened fingers or toes. However, that could be attributed to the use of CVS before the 10th week of pregnancy.

**Cordocentesis**

Cordocentesis is also known as percutaneous umbilical cord blood sampling (PUBS). It is similar to amniocentesis, but instead of sampling amniotic fluid, PUBS collects fetal blood. Under ultrasound guidance the needle is inserted through the mother’s abdomen and uterine wall into the fetal vein of the umbilical cord, where a fetal blood sample is taken. Because the fetal vein is fragile early in pregnancy, PUBS is performed no earlier than 17 weeks into pregnancy.

PUBS can not only detect chromosomal abnormalities, but also blood disorder, some metabolic disorders, infections, and other problems.

Miscarriage is the primary risk associated with PUBS; the risk is similar to CVS. Additional possible complications include blood loss at the puncture site, infection, and premature rupture of membranes.
While amniocentesis still is the “ultima ratio” today, the recent years saw the development of biochemical and imaging methods to preselect women with a clear indication for invasive techniques in order to minimize the risk of complications in probably unaffected pregnancies.

In the 1980s the so-called triple test was state of the art. Here, the initial risk based on the maternal age was refined with findings of AFP, hCG and unconjugated E3. The triple test increases the detection rate to 60 %, however, is only diagnostic during the 2nd trimester, which is relatively late.\textsuperscript{19,20,30,38,37,39,40,41,44}

Embryos in the first trimester do not yet have fully functioning excretion organs, so that they accumulate some liquid. This liquid collects in a fold at the neck. Normal embryos have very little liquid, the nuchal fold is only 1 – 2 mm thick. Embryos with a chromosomal aberration, however, may have a very pronounced edema.\textsuperscript{24}

The thickness of this neck vesicle is known as nuchal translucency (NT) and has meanwhile proven to be a very powerful marker for the prediction of Down Syndrome. But only since the availability of improved technologies in the 1990s it has been possible to measure NT by means of ultrasound. In that time, NT was used to refine the a priori age related risk to predict chromosomal aberrations and a detection rate of up to 77 % could be achieved.

Meanwhile, certain biochemical markers were identified, that have predictive potential for Down Syndrome already very early in pregnancy. Routinely used today are pregnancy associated plasma protein A (PAPP-A) and free β-hCG. Used together with NT a priori risk of Down Syndrome can be refined significantly, so that a detection around 90 % is reached. This screening algorithm is known as “First Trimester Screening”. Compared with the “old” triple test it has distinct advantages:

1. Supports early decision process: Applicable at a gestational age of 10 – 14 weeks (1st trimester), leaving ample time for further clinical investigations.
2. Higher detection rate: A detection rate of 90 % at an acceptable specificity (5 %) as compared to 60 % for the triple test.

The 1st trimester algorithm is currently state of the art for routine Down Syndrome screening.\textsuperscript{28-30,38}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{nuchal_translucency.png}
\caption{Nuchal translucency (NT)}
\end{figure}
The target of Down Syndrome screening is to identify pregnancies which are candidates for further investigations in order to avoid unnecessary invasive examinations, which carry a low, though concrete risk of complications.

There are several software solutions available that support the consolidation of the single risk factors into one total risk. The risks are usually expressed as odds, and a total value higher than 1 : 250 (1 : 300) defines a high-risk pregnancy.

The algorithm uses the risk related to the maternal age as a priori odds, which are multiplied with all positive likelihood ratios derived from the Gaussian markers (NT, PAPP-A, free β-hCG) and the static ones for other risk factors to yield the refined a posteriori odds.

While the likelihood ratios for the static risk factors are provided as a list from the software, the likelihood ratios for the Gaussian markers are calculated from mathematical approximations of the distribution curves for affected and unaffected pregnancies using the individual measured MoM-values, which are calculated as (see the graphs below):

\[
Q_{\text{posteriori}} = Q_{\text{priori}} \cdot \prod_{i=1}^{n} LR^+_i
\]

<table>
<thead>
<tr>
<th>Q</th>
<th>odds value, e.g. 1 : 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR^+</td>
<td>positive likelihood ratio</td>
</tr>
<tr>
<td>n</td>
<td>number of likelihood ratios to be considered</td>
</tr>
<tr>
<td>priori</td>
<td>before testing</td>
</tr>
<tr>
<td>posteriori</td>
<td>after testing</td>
</tr>
</tbody>
</table>

The current recommendation is that women with a risk of having a child with Down syndrome of 1 in 250 or greater should be offered amniocentesis. There is controversy over whether to use the risk at the time of screening (“risk at screening date”) or the predicted risk at the time of birth (“risk at term”). The risk at screening date is higher than the risk at term since many fetuses with Down syndrome abort spontaneously around the time of screening or afterwards (20 – 40 %). However, non-invasive screening methods support the efforts to minimize unnecessary invasive investigations with higher risk for mother and fetus.
**Nuchal translucency**

NT belongs to the so-called Gaussian markers in Down Syndrome screening. This means, that the values found for unaffected and affected fetuses follow Gaussian distribution curves. Obviously, the means (maxima) of these curves depend on the gestational age (GA). It is common practice to divide a measured NT value by the NT mean of the given gestational week to get “Multiples of the Median” (MoM), which makes the distribution independent from GA and facilitates further data processing.

The practical execution of NT measurement is difficult, because the distances to be determined are usually small (1 – 2 mm) and 1 mm more or less does make a difference in risk. If not a perfect sagittal view is achieved while sonographing the embryo, the result may be useless if not misleading. It is for this reason that some official bodies demand the sonographers, who perform such examinations, to prove their skills and be certified.

Moreover, NT has to be interpreted in context with the size of the embryo expressed as “Crown Rump Length” (CRL). For further calculations, both values have to be collected.

**PAPP-A**

PAPP-A is a high molecular weight glycoprotein (187 kDa) and belongs to the metzincin superfamily of zinc metalloproteinases. Its gene is in chromosome band 9q33.1. Amongst other sites, PAPP-A is produced by trophoblasts in the placenta and released into the maternal circulation. It is not present in amniotic fluid.

PAPP-A cleaves insulin-like growth factor binding protein-4 (IGFBP-4) and reduces dramatically the affinity of IGFBP-4 for IGF-I and IGF-II. As such it is a regulator of IGF bioactivity in several systems, including the human ovary and the cardiovascular system. Biological active PAPP-A exists as a 2 : 2 heterodimer with major basic protein (MBP).

PAPP-A increases continuously as of week 5 during normal pregnancies with a boost in the last trimester. Concentrations are lower in aneuploid pregnancies (0.5 MoM), however, its diagnostic efficiency declines after week 14. Decreased values are also seen in case of intrauterine growth restriction, premature delivery, pre-eclampsia, stillbirth.

Like NT, PAPP-A belongs to the Gaussian markers and is normalized to MoMs before further processing.
**Free β-hCG**

The well-known proteohormone hCG consists of two protein subunits, α-hCG and β-hCG, which make the holo-hormone. The holo-hormone can be cleaved into the free chains by elastase activity, however, the amount of free β-hCG in blood is usually low (1 % of total), but can increase under certain pathological conditions.

In case of aneuploid pregnancies free β-hCG is increased (2.0 MoM in the 1st trimester). The difference grows with GA.

Free β-hCG is also a Gaussian marker and is normalized to MoMs before further processing.

### Other risk factors

Besides the Gaussian markers, many more prenatal risk factors have been identified and their likelihood ratios are published. If unequivocally identified or not identified, their likelihood ratios (LR⁺) can be used to further refine the probability for the development of an aneuploid pregnancy.²¹-²⁵

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>LR⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyelectasis</td>
<td>6.77</td>
</tr>
<tr>
<td>Short femur</td>
<td>7.94</td>
</tr>
<tr>
<td>Absent nasal bone</td>
<td>26</td>
</tr>
<tr>
<td>Echogenic cardiac foci</td>
<td>6</td>
</tr>
<tr>
<td>Echogenic bowel</td>
<td>21.17</td>
</tr>
<tr>
<td>Major defect</td>
<td>32.96</td>
</tr>
<tr>
<td>Short humerus</td>
<td>22.76</td>
</tr>
</tbody>
</table>

### Summary

Down Syndrome screening is another example of how the application of state of the art diagnostic tools and algorithms improves the reliability of screening methods. Starting with a detection rate of 30 % thirty years ago, first trimester screening today achieves 90 % and more at a false positive rate of 5 %. Today, not only the sensitivity is improved, but the results are also available significantly earlier. This allows for more time for confirmation and subsequent professional counseling.
**Glossary**

**a posteriori (probability)**
The refined probability of an event to happen after further knowledge about the nature of the event was collected.

**a priori (probability)**
The estimated probability of an event to happen before further knowledge about the nature of the event has been collected.

**AFP**
Alpha-1 fetoprotein.

**Aganglionic**
Absence of normal enteric nerves.

**Amniocentesis**
Puncture of the amniotic sac through abdominal wall and uterus for the collection of amniotic fluid.

**Amniotic fluid**
The nourishing and protecting liquid contained by the amniotic sac of a pregnant woman. The amniotic sac grows and begins to fill, mainly with water, around two weeks after fertilization. After a further 10 weeks the liquid contains proteins, carbohydrates, lipids and phospholipids, urea and electrolytes, all of which aid in the growth of the fetus. In the late stages of gestation much of the amniotic fluid consists of fetal urine.

**Amniotic sac**
A two-layered membrane that surrounds the embryo or fetus in the uterus. The amniotic sac is filled with fluid in which the embryo or fetus is suspended.

**Aneuploidy**
Condition with an abnormal number of chromosomes.

**Atlanto**
Concerning the atlas.

**Atlas**
The first cervical vertebra (C1).

**Atresia**
Condition in which a body opening or passage in the body is abnormally closed or absent.

**Atrioventricular septal defect**
A hole in the wall, that separates the right from the left side of the heart (AVSD).

**Balanced (translocation)**
Translocation, that does not increase the overall number of genes in a cell.

**Brachymesophalangy**
Congenital shortening of the middle bone in a finger.

**Bradycephaly**
Smaller than usual head circumference.

**Brushfield iris spots**
Pigmentation particularity of the iris, not influencing vision.

**Chromosome**
Organized structure of DNA and proteins found in cells containing genes, regulatory elements and other nucleotide sequences.

**Celiac disease**
Autoimmune disorder of the small intestine.

**Cervical vertebrae**
The 7 vertebrae that are located directly under the skull (C1 – C7).
Chorionic layer
The fetal part of the placenta.

Chorionic villus sampling
Collection of tissue from the fetal part of the placenta. The material contains fetal cells, which are subject to e.g. chromosomal analysis.

Congenital
Influence on or damage to a developing embryo or fetus (preterm).

Cordocentesis
Collection of fetal blood from the umbilical vein. The sample is subject to subsequent examination, e.g. chromosomal analysis of the fetal cells contained herein, or the detection of infective agents.

Crown Rump Length (CRL)
The size of an embryo from the top of the head to the bottom, usually obtained sonographically.

Detection rate
The relative number of true positive results obtained from a collective of positive samples (a.k.a. sensitivity).

Disjunction
During the process of cell division the chromosomes have to duplicate at a certain stage. The initial product of this step is a pair of identical chromatids which are held together at a special position within the DNA chain (centromer) yielding the well known X-shape (metaphase). Subsequently, this metaphasic chromosome has to be cleaved in order to liberate the chromatids, which are then moved by means of the spindle apparatus to opposite poles of the dividing cell.

Down Syndrome
The clinical picture that results from a trisomy of chromosome 21.

Duodenal
Concerning the duodenum, which is the first part of the small intestine in most higher vertebrates.

Dysmorphism
An anatomical malformation.

E3
Estriol.

Embryo
The earliest stage of development of a multicellular diploid eukaryote. In humans the time from fertilization to about 8 weeks of gestation.

Epicanthal fold
A skin fold of the upper eyelid, from the nasal bone to the inferior side of the eyebrow, covering the inner corner of the eye.

Esophagus
"Entrance for eating". The main part of the connection between mouth and stomach.

Etiology
The background or cause of a clinical condition.

Expression (genes)
Normally, genes are not active. Only under distinct conditions – when the corresponding protein is needed – a gene is activated. This activation process includes a physical expression of the gene from the DNA-helix in order to make it accessible to the intra-cellular mechanisms of transcription and translation.

Facultative
Not compulsory, the opposite of obligatory.

Fetus
Gestational stage from about 9 weeks of gestation until delivery.

Gametogenesis
Process of gamete (ovums and sperms) production.
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>A piece of the genome that contains information e.g. for the production of a protein.</td>
</tr>
<tr>
<td>Genome</td>
<td>The complete genetic material of an organism.</td>
</tr>
<tr>
<td>Gestational age (GA)</td>
<td>Duration of a pregnancy since conception.</td>
</tr>
<tr>
<td>Gonosomal</td>
<td>Referring to the gonosomes.</td>
</tr>
<tr>
<td>Gonosomes</td>
<td>In humans the 23rd pair of chromosomes (gender chromosomes): XX or XY.</td>
</tr>
<tr>
<td>hCG</td>
<td>Human chorionic gonadotropin.</td>
</tr>
<tr>
<td>Helix (ear)</td>
<td>The prominent rim of the outer ear.</td>
</tr>
<tr>
<td>Heterodimer</td>
<td>A chemical or biological entity consisting of two structurally different subunits.</td>
</tr>
<tr>
<td>Holo</td>
<td>Greek prefix meaning whole, all, total.</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>Condition with a lack of thyroid hormones.</td>
</tr>
<tr>
<td>Incidence</td>
<td>Rate of occurrence of new cases per population during a given time period. Conveys information about the risk of developing a certain clinical condition.</td>
</tr>
<tr>
<td>Klinodactyly</td>
<td>Congenital sideways deviation of a finger.</td>
</tr>
<tr>
<td>Likelihood ratio</td>
<td>Statistical property defining how much more probable a true positive result is compared to a false positive (sens / (1-spec) ).</td>
</tr>
<tr>
<td>Macroglossy</td>
<td>Pathologic enlargement of the tongue.</td>
</tr>
<tr>
<td>Megacolon</td>
<td>Pathologic enlargement of the colon (last part of the digestive system).</td>
</tr>
<tr>
<td>Meiosis</td>
<td>Cell division mechanism that produces 4 haploid cells out of one parent cell, occurring for gametogenesis in ovaries and testicles. Meiosis comprises two disjunction steps (1st and 2nd meiotic disjunction).</td>
</tr>
<tr>
<td>Mitosis</td>
<td>The &quot;normal&quot; type of cell division. Mitosis produces two identical euploid cells out of one parent cell. Mitosis comprises one disjunction step.</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>The coexistence of two or more genetically distinct cell populations derived originally from a single zygote. Mosaics may arise at any stage of development, from the two-cell stage onward, or in any tissue which actively proliferates thereafter. The phenomenon may be caused by somatic mutation or chromosomal nondisjunction.</td>
</tr>
<tr>
<td>Nuchal</td>
<td>Referring to the back of the neck.</td>
</tr>
<tr>
<td>Nuchal translucency (NT)</td>
<td>Thickness of the neck fold in a fetus, obtained sonographically. NT is a powerful predictor of chromosomal aberrations.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Occipital bone</strong></td>
<td>Bone at the bottom of the skull that connects with the atlas.</td>
</tr>
<tr>
<td><strong>Omphalocele</strong></td>
<td>A type of abdominal wall defect in which the intestines, liver, and occasionally other organs remain outside of the abdomen in a sac because of a defect in the development of the muscles of the abdominal wall.</td>
</tr>
<tr>
<td><strong>Over-expression (genes)</strong></td>
<td>Too many copies of a gene are made available for transcription with the effect that the corresponding protein is overproduced which may not be in line with the organisms needs. May lead to pathological conditions.</td>
</tr>
<tr>
<td><strong>Ovum</strong></td>
<td>The female gamete (egg).</td>
</tr>
<tr>
<td><strong>Palmar</strong></td>
<td>Concerning the inner side of the hand.</td>
</tr>
<tr>
<td><strong>Physiognomy</strong></td>
<td>Characteristic facial expression.</td>
</tr>
<tr>
<td><strong>Polydactyly</strong></td>
<td>Abnormal number (&gt; 5) of fingers or toes.</td>
</tr>
<tr>
<td><strong>Preaxial polydactyly</strong></td>
<td>Polydactyly affecting the thumbs.</td>
</tr>
<tr>
<td><strong>Sagital (plane)</strong></td>
<td>An imaginary plane that travels vertically from the top to the bottom of the body, dividing it into left and right portions.</td>
</tr>
<tr>
<td><strong>Somatic (cells)</strong></td>
<td>All cells that are not gametes.</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>The relative number of true negative results obtained from a collective of negative samples.</td>
</tr>
<tr>
<td><strong>Stenosis</strong></td>
<td>Abnormal narrowing in a tubular structure.</td>
</tr>
<tr>
<td><strong>Tetralogy of Fallot</strong></td>
<td>Congenital heart defect, the most common cause for the blue baby syndrome.</td>
</tr>
<tr>
<td><strong>Transcervical</strong></td>
<td>Through the neck of the uterus.</td>
</tr>
<tr>
<td><strong>Translocation</strong></td>
<td>The rearrangement of genetic material within the same chromosome or the transfer of a segment of one chromosome to another nonhomologous one.</td>
</tr>
<tr>
<td><strong>Trisomy</strong></td>
<td>Aneuploidy in which there are three copies, instead of the normal two, of a particular chromosome.</td>
</tr>
<tr>
<td><strong>Trophoblasts</strong></td>
<td>The first cells that differentiate from the fertilized egg. They provide nutrients to the embryo and develop into a large part of the placenta.</td>
</tr>
<tr>
<td><strong>Umbilical cord</strong></td>
<td>Connection between fetus or embryo with the placenta.</td>
</tr>
<tr>
<td><strong>Unbalanced (translocation)</strong></td>
<td>Duplication of a segment of genetic material and addition of the copy to a homologous or nonhomologous chromosome.</td>
</tr>
<tr>
<td><strong>Vertebrae</strong></td>
<td>The bones that the backbone is composed of.</td>
</tr>
<tr>
<td><strong>Zygote</strong></td>
<td>The cell formed by the union of two gametes, especially a fertilized ovum before cleavage.</td>
</tr>
</tbody>
</table>
First trimester screening
For better maternal and fetal care
The aim of pregnancy examinations is the early detection of specific risks. "Risk screening" using multiple markers has achieved wide acceptance in routine antenatal care. Non-invasive screening methods in the first and second trimester for detection of fetal chromosome abnormalities have been developed over the last 15 years. Due to benefits of the earlier decision process there is a clear trend towards first trimester screening. Additionally, the highest detection rates for trisomy 21 can be achieved in the first trimester with maternal serum markers PAPP-A and free β-hCG in combination with nuchal translucency (NT) results.1,2,3

Proven Elecsys performance with highly accurate and reliable assay results*•
• High assay quality and excellent precision due to state-of-the-art electro-chemiluminescence (ECL) technology
• Assay excellence with over 20 years of development expertise leads to overachievement of FMF accreditation targets
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…for improved service to laboratory customers

FMF accreditation for certified quality supporting early decision process
• High quality results lead to effective information supporting early decision making
• Excellent detection rates for trisomy 21
• Minimize invasive investigations with higher risk for mother and fetus

…for better maternal and fetal care

Flexible solutions for consolidated testing
• Integration into routine testing with efficiency, cost and workflow improvements without compromising performance
• Flexible risk calculation software offering
• Broad system platform portfolio for every lab size with standardized reagents across the platforms

…for streamlined lab organization with efficiency and cost gains

First trimester screening with Elecsys PAPP-A and free β-hCG
For better maternal and fetal care

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References
5. Package insert Elecsys free β-hCG, Roche Diagnostics.
Elecsys® free β-hCG

Electro-chemiluminescence immunoassay (ECLIA) for the in-vitro determination of human free β-hCG in serum

Indication

Human chorionic gonadotropin (hCG) is a glycoprotein hormone (~37 kDa) composed of two non-covalently linked subunits – the α- and β-chain. During the early weeks of pregnancy, the protein is produced by trophoblast tissue and serves to maintain the corpus luteum. In addition, it also influences steroid production. Physiologically, hCG appears only in blood and urine of pregnant women. The serum of pregnant women mainly contains intact hCG. However, a minor fraction of the subunit circulates in an unbound form. The proportion of free β-hCG averages ~1% compared to intact hCG. It is now well established that the free β-hCG concentration in serum is a reliable marker for fetal aneuploidy. Free β-hCG, in combination with serum pregnancy-associated plasma protein A (PAPP-A) and the sonographic determination of nuchal translucency (NT), are markers of choice to identify women at an increased risk of carrying a fetus affected with Down syndrome during the first trimester (week 11-14) of pregnancy. Using this marker combination, detection rates of up to 70% (serum markers only) and 90% (combined with NT) have been described at a false positive rate of 5%. Median maternal serum free β-hCG levels in affected pregnancies are higher compared to the median of non affected pregnancies. Based on the maternal age, the risk for having a Down syndrome pregnancy can be calculated using an algorithm based on likelihood ratios.

Test principle: one-step sandwich assay

Elecsys technology

ECL (ElectroChemiluminescence) is Roche’s technology for immunoassay detection. Based on this technology and combined with well-designed, specific and sensitive immunoassays, Elecsys delivers reliable results. The development of ECL immunoassays is based on the use of a ruthenium-complex and tripropylamine (TPA). The chemiluminescence reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction. ECL technology can accommodate many immunoassay principles while providing superior performance.
**Free β-hCG levels in affected pregnancies:**
Median maternal serum free β-hCG levels in affected pregnancy are higher, compared to the median of non-affected pregnancies.

### Elecsys® free β-hCG test characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay time</td>
<td>18 min</td>
</tr>
<tr>
<td>Test principle</td>
<td>One-step sandwich assay</td>
</tr>
<tr>
<td>Calibration</td>
<td>2 point</td>
</tr>
<tr>
<td>Sample material</td>
<td>Serum</td>
</tr>
<tr>
<td>Sample volume</td>
<td>10 μL</td>
</tr>
<tr>
<td>Detection limit</td>
<td>&lt; 0.5 IU/L</td>
</tr>
<tr>
<td>Functional sensitivity</td>
<td>0.1 – 190 IU/L</td>
</tr>
<tr>
<td>Measuring range</td>
<td>NIBSC 75/551</td>
</tr>
<tr>
<td>Traceability</td>
<td></td>
</tr>
<tr>
<td>Total imprecision (NCCLS)</td>
<td></td>
</tr>
</tbody>
</table>

### Expected values*

- **Week 11:** median 49.9 IU/L (n = 206)
- **Week 12:** median 40.6 IU/L (n = 623)
- **Week 13:** median 33.6 IU/L (n = 1384)
- **Week 14:** median 28.8 IU/L (n = 1057)

### Certificates
- Fetal Medicine Foundation (FMF) accreditation

* Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges. For prenatal testing it is recommended that the median values should be re-evaluated periodically.

### Clinical performance data of free β-hCG and PAPP-A assays

#### A. Concordance analysis in unaffected pregnancies  
**n = 1047**

<table>
<thead>
<tr>
<th>cobas e analyzers</th>
<th>Competitive system</th>
<th>94.2%</th>
<th>92.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4 5 6 7 8 9 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cobas e analyzers</th>
<th>Competitive system</th>
<th>5.8%</th>
<th>7.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4 5 6 7 8 9 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In 1047 unaffected samples the Roche methods correctly classified 986 samples (specificity: 94.2%) in comparison to 966 (specificity: 92.3%) correctly classified by the competitor methods. Roche methods showed a false positive rate of 5.8% in comparison to 7.7% obtained with the competitor methods. **

#### B. Detection rate in confirmed trisomy 21 pregnancies, without NT  
**n = 32**

<table>
<thead>
<tr>
<th>cobas e analyzers</th>
<th>Competitive system</th>
<th>75.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
</tbody>
</table>

In 32 affected samples the Roche methods showed a detection rate of 75% (24/32) in comparison to 65.6% (21/32) obtained with the competitor methods. ** Including NT data, the detection rate can be increased up to 90 %.

### Order information

- **Elecsys® free β-hCG**
  - 100 tests  
  - 04854071 200

- **free β-hCG CalSet**
  - 4 x 1 mL  
  - 04854101

- **PC Maternal Care 1,2&3**
  - 2 x 2 mL each  
  - 04899881

** Multicenter evaluation data

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CH-6343 Rotkreuz
Switzerland

www.roche.com
Elecsys® PAPP-A
Electro-chemiluminescence immunoassay (ECLIA) for the in-vitro determination of human pregnancy associated plasma protein A (PAPP-A) in serum

**Indication**
Human pregnancy-associated plasma protein A (PAPP-A) is a large glycoprotein with a molecular weight of 200 kDa. PAPP-A was first isolated from the serum of pregnant women, where its concentration increases steadily until term. It is now well established that the PAPP-A concentration in serum is a reliable marker for fetal aneuploidy. PAPP-A, in combination with free β-hCG and the sonographic determination of nuchal translucency (NT), are the markers of choice to identify women at increased risk of carrying a fetus affected with Down syndrome during the first trimester (week 11-14) of pregnancy. Using this marker combination, detection rates of up to 70% (serum markers only) and 90% (combined with NT) have been described at a false positive rate of 5%. Median maternal serum PAPP-A levels in affected pregnancy are lower, compared to the median of non-affected pregnancies. Based on the maternal age, the risk for having a Down syndrome pregnancy can be calculated using an algorithm based on likelihood ratios.

**Test principle: one-step sandwich assay**

- **PAPP-A in the sample**
- **Streptavidin microparticle**
- **Biotinylated monoclonal antibody against human PAPP-A**
- **Ruthenylated monoclonal antibody against human PAPP-A**
- **Measurement**

**Elecsys technology**
ECL (ElectroChemiluminescence) is Roche’s technology for immunoassay detection. Based on this technology and combined with well-designed, specific and sensitive immunoassays, Elecsys delivers reliable results. The development of ECL immunoassays is based on the use of a ruthenium-complex and tripropylamine (TPA). The chemiluminescence reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction. ECL technology can accommodate many immunoassay principles while providing superior performance.
PAPP-A levels in affected pregnancies
Median maternal serum PAPP-A levels in affected pregnancy are lower, compared to the median of non-affected pregnancies.

**Elecsys® PAPP-A test characteristics**

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Test principle</td>
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</tr>
<tr>
<td>Calibration</td>
<td>2 point</td>
</tr>
<tr>
<td>Sample material</td>
<td>Serum</td>
</tr>
<tr>
<td>Sample volume</td>
<td>15 µL</td>
</tr>
<tr>
<td>Detection limit</td>
<td>4 mIU/L</td>
</tr>
<tr>
<td>Functional sensitivity</td>
<td>&lt; 20 mIU/L</td>
</tr>
<tr>
<td>Measuring range</td>
<td>4 – 10000 mIU/L</td>
</tr>
<tr>
<td>Traceability</td>
<td></td>
</tr>
<tr>
<td>Total imprecision (NCCLS)</td>
<td></td>
</tr>
</tbody>
</table>

**Expected values**

<table>
<thead>
<tr>
<th>Week</th>
<th>Median value (mIU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>1337</td>
</tr>
<tr>
<td>12</td>
<td>1919</td>
</tr>
<tr>
<td>13</td>
<td>2926</td>
</tr>
<tr>
<td>14</td>
<td>4358</td>
</tr>
</tbody>
</table>

* Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges. For prenatal testing it is recommended that the median values should be re-evaluated periodically.

**Clinical performance data of PAPP-A and free β-hCG assays**

**A. Concordance analysis in unaffected pregnancies**

<table>
<thead>
<tr>
<th>Specity (%)</th>
<th>Cobas e analyzers</th>
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**B. Detection rate in confirmed trisomy 21 pregnancies, without NT**

<table>
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<tr>
<th>Detection rate (%)</th>
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In 32 affected samples the Roche methods showed a detection rate of 75% (24/32) in comparison to 65.6% (21/32) obtained with the competitor methods.** Including NT data, the detection rate can be increased up to 90%.

**Order information**

<table>
<thead>
<tr>
<th>Test</th>
<th>Code</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys® PAPP-A</td>
<td>04854098</td>
<td>200</td>
</tr>
<tr>
<td>PAPP-A CalSet</td>
<td>04854101</td>
<td></td>
</tr>
<tr>
<td>PC Maternal Care 1,2&amp;3</td>
<td>04899881</td>
<td></td>
</tr>
</tbody>
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**Multicenter evaluation data**

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