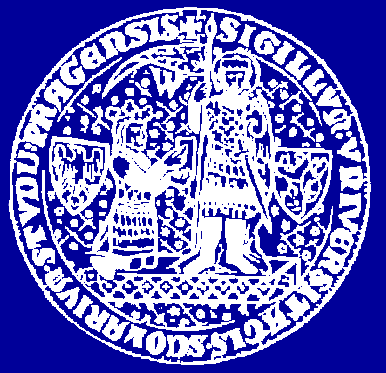
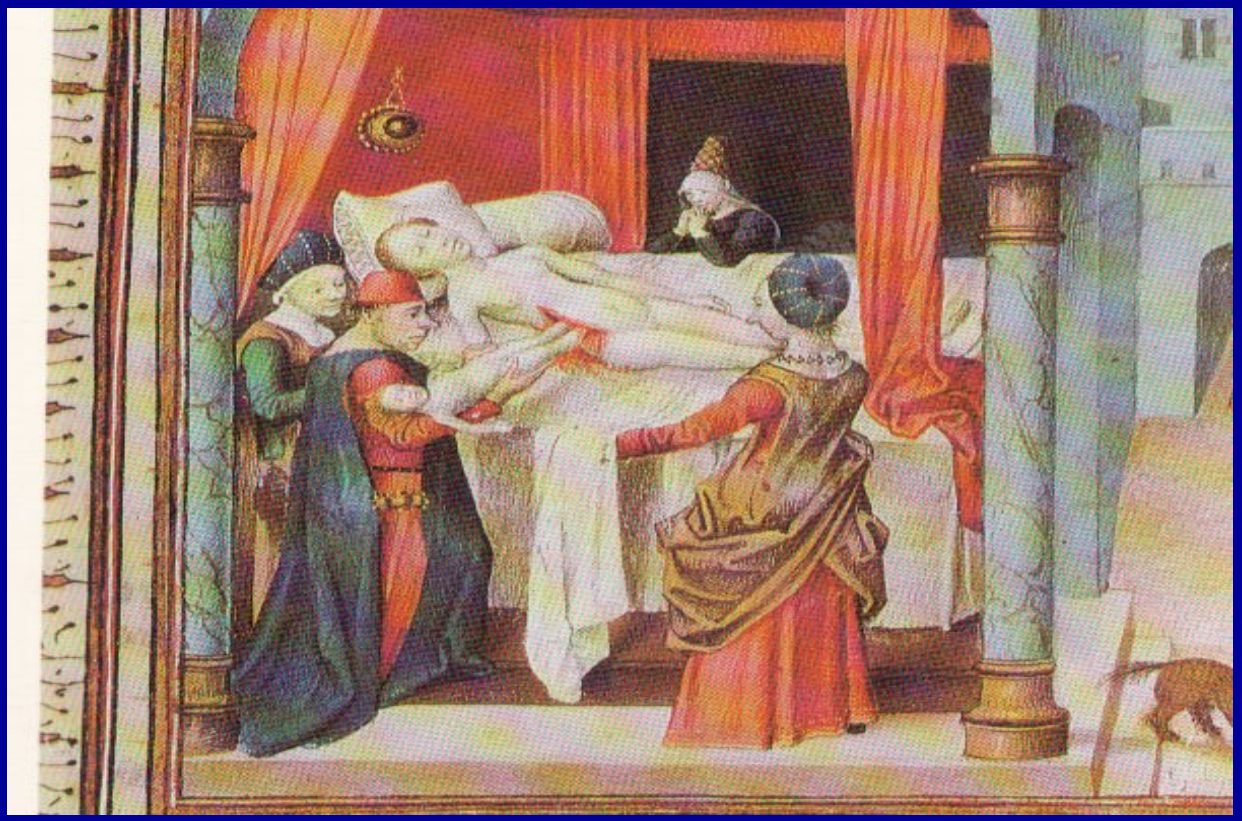
Prenatal screening of Down‘s syndrom in the first and the second trimester of pregnancy



Drahomira Springer
ULBLD and 1.LF UK
Praha

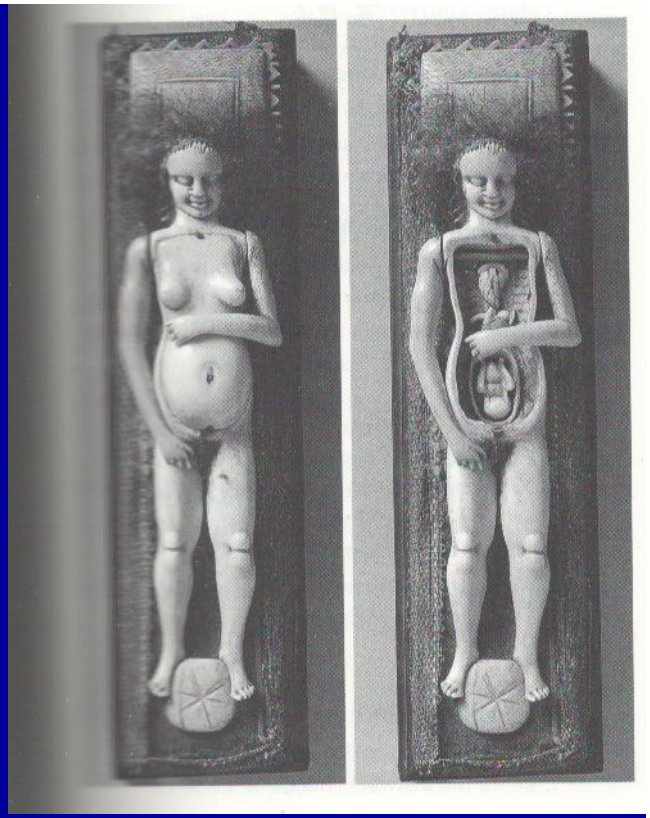
Aim of maternal-foetal care

- the uncomplicated birth of a healthy baby to a healthy mother at term



Paulus Orosius, Histoire du monde, 1460 ?

Screening tests in pregnancy



Risk for mother and foetus

- curable
  - gestational diabetes
  - infection (HIV, hepatitis and syphilis)
  - rhesus incompatibility
  - thyroid dysfunction

Screening of congenital development defects

Screening of DS and NTD in the 2nd trimester

- hCG, AFP, uE3, inhibin

Screening of DS in the 1st trimester

- Free β hCG, PAPP-A
- Nuchal translucency – NT, other US markers

Prenatal screening history

- 1866 : First description of Down Syndrom (John Langdon Down)
- 1930 - Down syndrome – maternal age association
- 1966 : First karyotype on amniotic cells culture
- 1974 : First foetal ultrasound scan in France
- 1980 - 2nd trimester AFP (w*ith maternal age)*
  - 2nd trimester Multiple markers (double, triple, quad)
- 1990 - 1st trimester nuchal translucency (NT)
  - 1st trimester NT + PAPP-A + free βhCG
- 2000 -Integrated 1st and 2nd trimester
  - Sequential 1st and 2nd trimester
- 2012 - Foetal nucleic acids in maternal plasma?

Jacob Canick, PhD, Brown University, Women and Infants Hospital, Providence, Rhode Island, USA

Prenatal screening in the Czech Republic

- In 80. started investigation with AFP in combination with age – for women over 35 was automatically offered AMC
- In 90. was prenatal testing in the second trimester obligatory for all pregnant women
- After 2000 started first trimester screening and the care shifted from biochemistry to gynaecology
- More than 95% women with prenatal diagnosed DS choose termination of pregnancy

Down's Syndrome

- **Down's syndrome (DS) is a congenital disorder, caused by a trisomy of chromosome 21**
- **First described 1866 JLH Down**
- **~1 in 900 births in Czech Republic**
- **risk increases with the mother's age**

Downs Syndrome - Trisomy 21

- **Clinical Features**
  - Average life expectancy 30 years
  - Characteristic phenotype
  - Learning disability (IQ 20-60)
  - Developmental delay / Hypotonia
  - Delayed puberty / Early menopause
- **Major Causes of Morbidity & Mortality**
  - 96% portal tract anomalies / Duodenal atresia
  - 50% congenital cardiac lesions
  - 60% Pre-senile dementia

Incidence of Down's Syndrome

- majority of babies (95%) are born to women under 35 years of age
- *majority of DS babies (80%) are born to women under 35 years of age*
- need mass screening programme for low-risk group

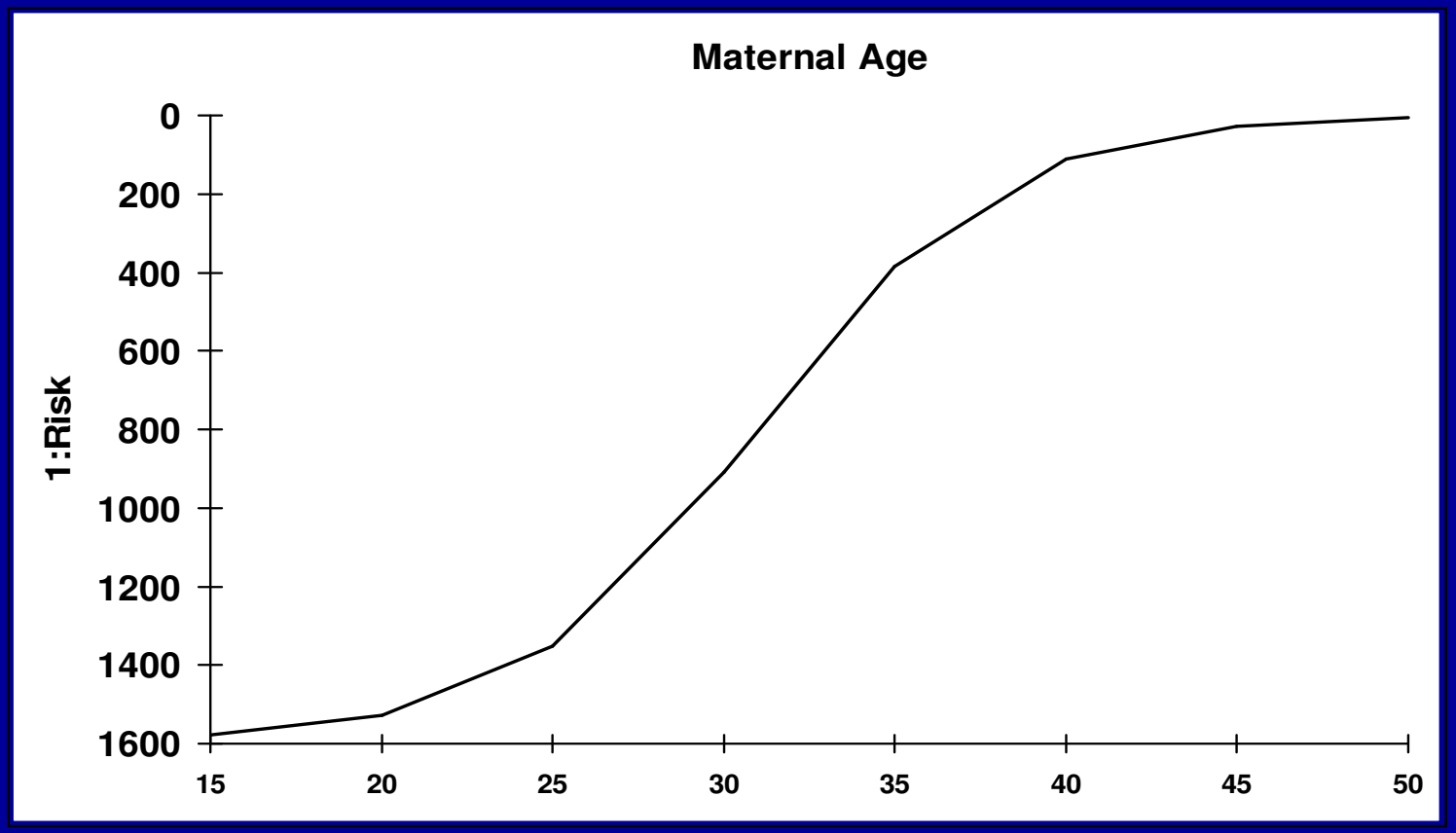
Testing for Downs Syndrome

- no screening test capable of detecting parental predisposition for a Down's syndrome birth
- earlier methods used direct foetal testing, by invasive tests (e.g.amniocentesis)
- amniocentesis not suitable for mass screening programmes
- amniocentesis can cause foetal abort

Down's Syndrome and maternal age

Maternal age at birth	Risk of Down's syndrome
24	1 in 950
30	1 in 680
36	1 in 210
42	1 in 40

Age Distribution of Risk



Aim of Antenatal Screening for Down's Syndrome

- To identify a group of women at sufficiently high risk of having an affected child to justify the offer of a diagnostic test (chorionic villus sampling or amniocentesis).

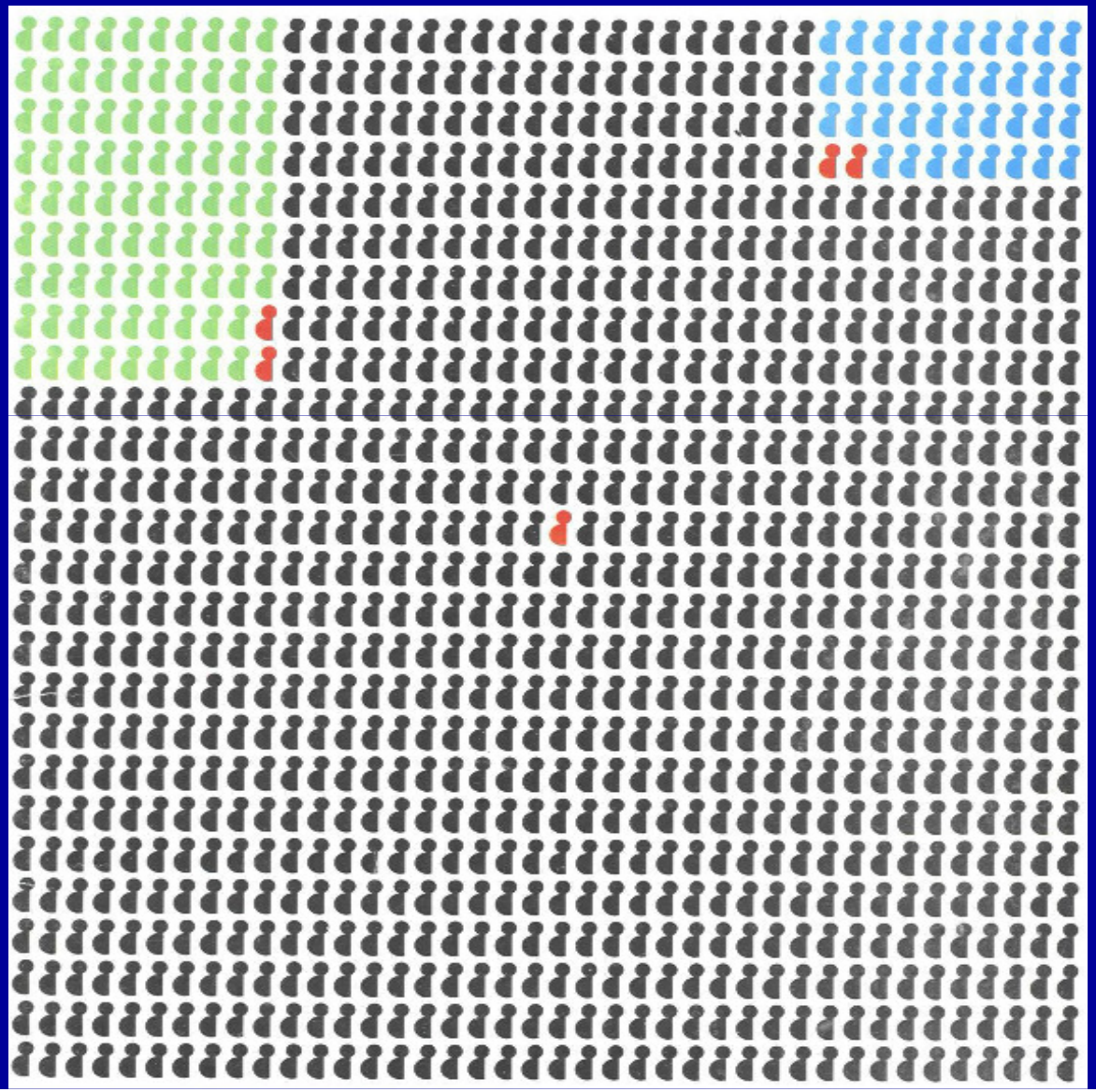
Testing for Downs Syndrome

- indirect foetal testing, by biochemical maternal serum screening
- maternal serum screening does not detect specific marker
- multiple biochemical markers used to calculate risk
- software packages available to calculate risk
- can screen in first or second trimester

Second trimester risk factors

- maternal age
- serum AFP
- serum total hCG
- unconjugated oestriol (uE3)

Scheme of distribution positivity in 2nd trimester



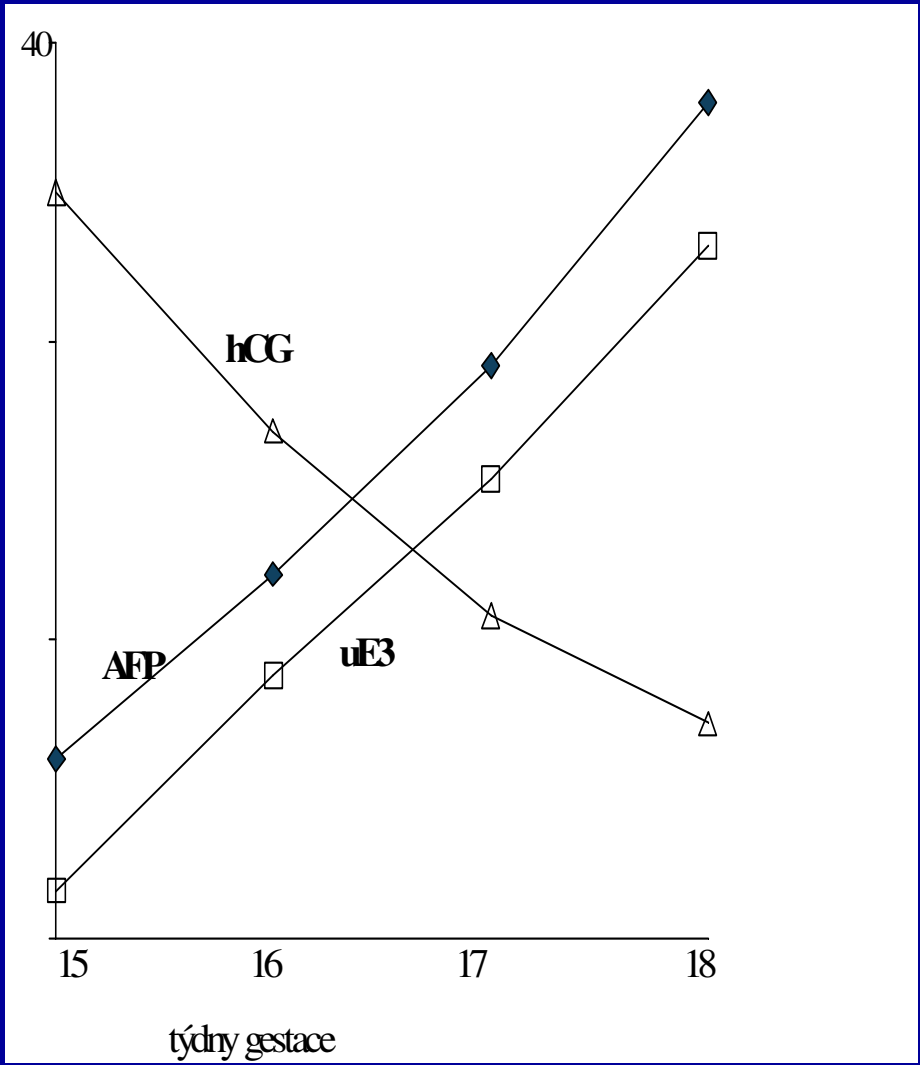
Positivity of DS

Positivity of NTD

Truly affected pregnancy

Negative results of screening

Medians for biochemical markers



- Decreasing levels of hCG
- Increasing levels of AFP and uE3
- Pregnancy with DS are delated
  - high hCG
  - low AFP and uE3

MoM (multiple of median)

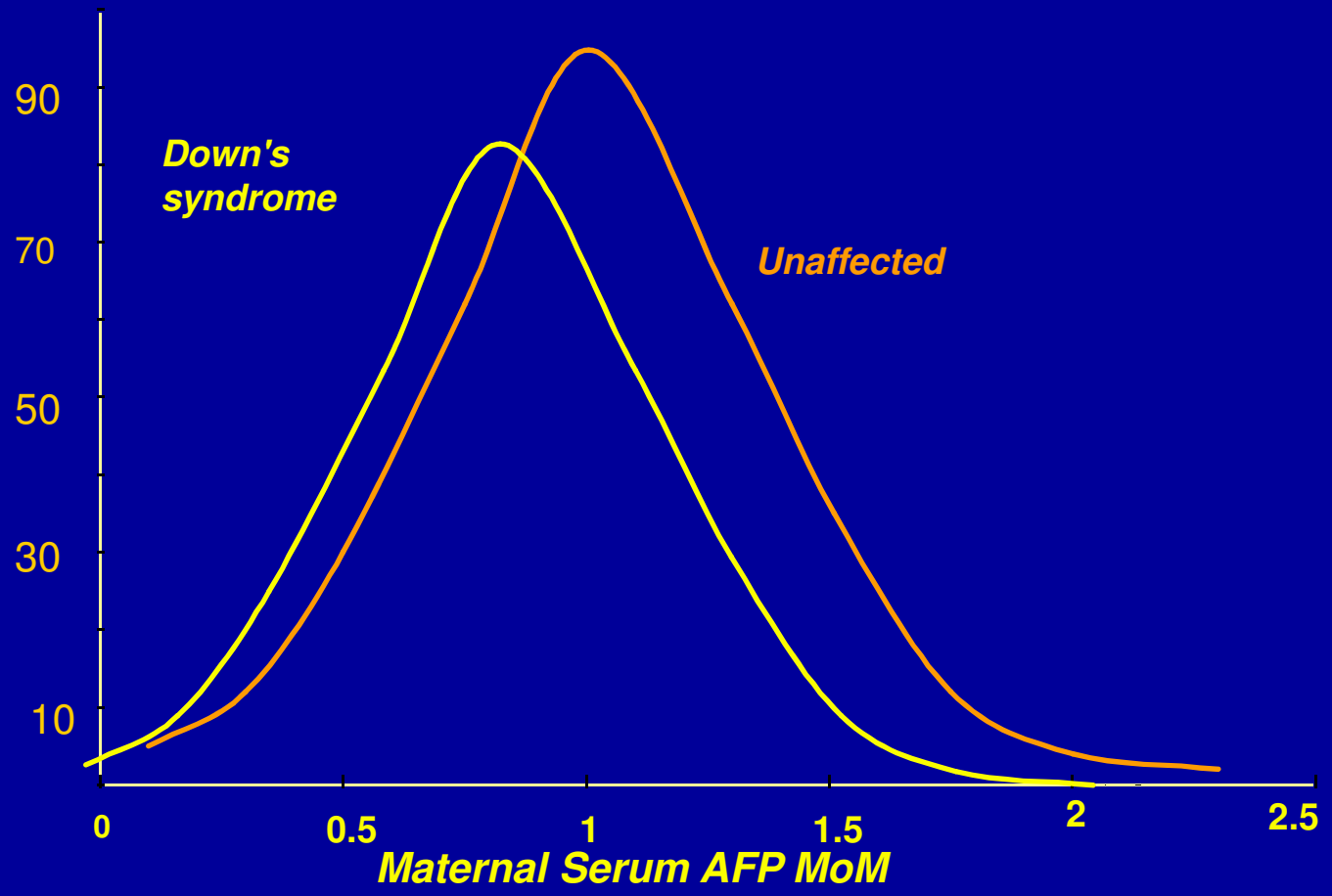
- ***result reported strictly as multiple of median***

- MoM's vary with gestational age
- MoM's vary with assay method
- MoM's vary with population tested
- May need adjustment for:
  - **weight**
  - ethnic group
  - other conditions e.g. diabetes
  - twin pregnancies

Maternal serum Alpha-fetoprotein (AFP)

- Glycoprotein of foetal origin.
- Synthesized initially in embryonic yolk sac & then by foetal liver
- Maternal serum concentration maximal at 30 weeks gestation
- Maternal serum AFP is ***lower*** in DS pregnancies
- Geometric mean MoM is 0.74
- also useful marker of neural tube defects (NTD)
- Maternal serum AFP is ***elevated*** in NTD pregnancies

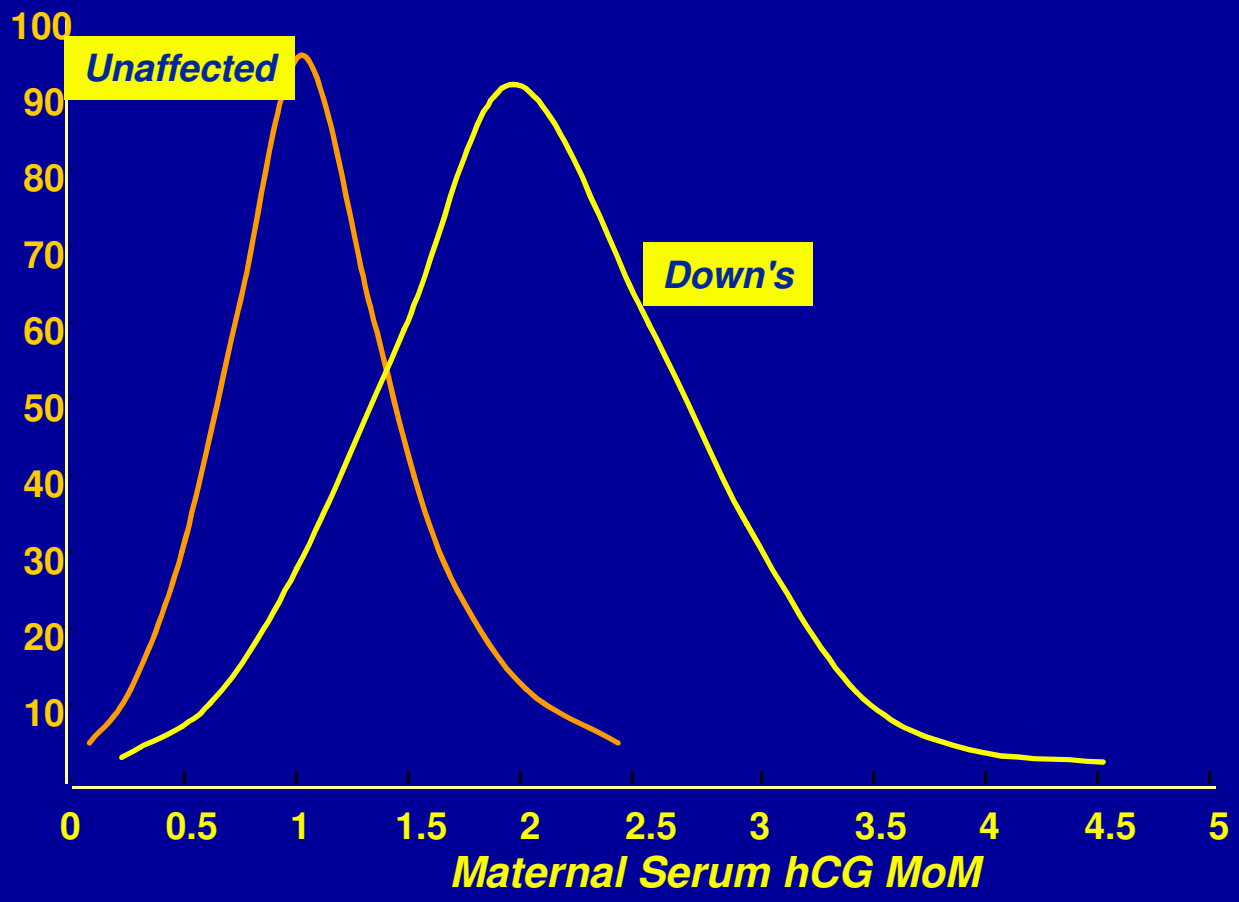
AFP Distribution curves



Serum human chorionic gonadotrophin (hCG)

- Dimeric glycoprotein hormone (α & ß subunits) secreted by the fertilised ovum and later by placental tissue.
- Primary function is to maintain the corpus luteum, later produces Prog & Oest to maintain early pregnancy
- Maternal serum hCG maximal during first trimester, then declines during second trimester
- Maternal serum hCG is elevated in DS pregnancies

hCG Distribution curves



Unconjugated Oestriol (uE3)

- Derived from foetal adrenal DHEAS. Latter hydroxylated in foetal liver & cleaved by steroid sulphatase in placenta where the unconjugated fraction converted to uE3
- Low levels uE3 + hCG can detect Edward's Syndrome
- Low levels seen in maternal serum from Down's syndrome
- circadian rhythm; levels 15% lower in the morning
- no advantage over double test (AFP/hCG)

Double or triple test?

- uE3 – very unstable
- interfere of lipaemia
- different quality of diagnostics sets
- growth of positivity don‘t comport with recovery
- long-term comparision showed irresponsibility results of triple test in our conditions

Inhibin A

- Dimeric inhibin-A (DIA) is a fourth biochemical marker for Down syndrome screening,
- It is a glycoprotein of placental origin similar to hCG. Levels in maternal serum remain relatively constant through the 15th-18th weeks of pregnancy.
- Maternal serum levels of DIA are twice as high in pregnancies affected by Down syndrome as in unaffected pregnancies.

Importance of gestational dating

- exact gestational dating is essential to calculate risk of DS
- measured value standardised against expected median value for a normal population at same stage of gestation
- DS foetuses are relatively retarded compared to normal
- results in alteration of maternal serum concentration of foetal products e.g AFP, hCG
- serum concentrations related to gestational age

Complex Data Required

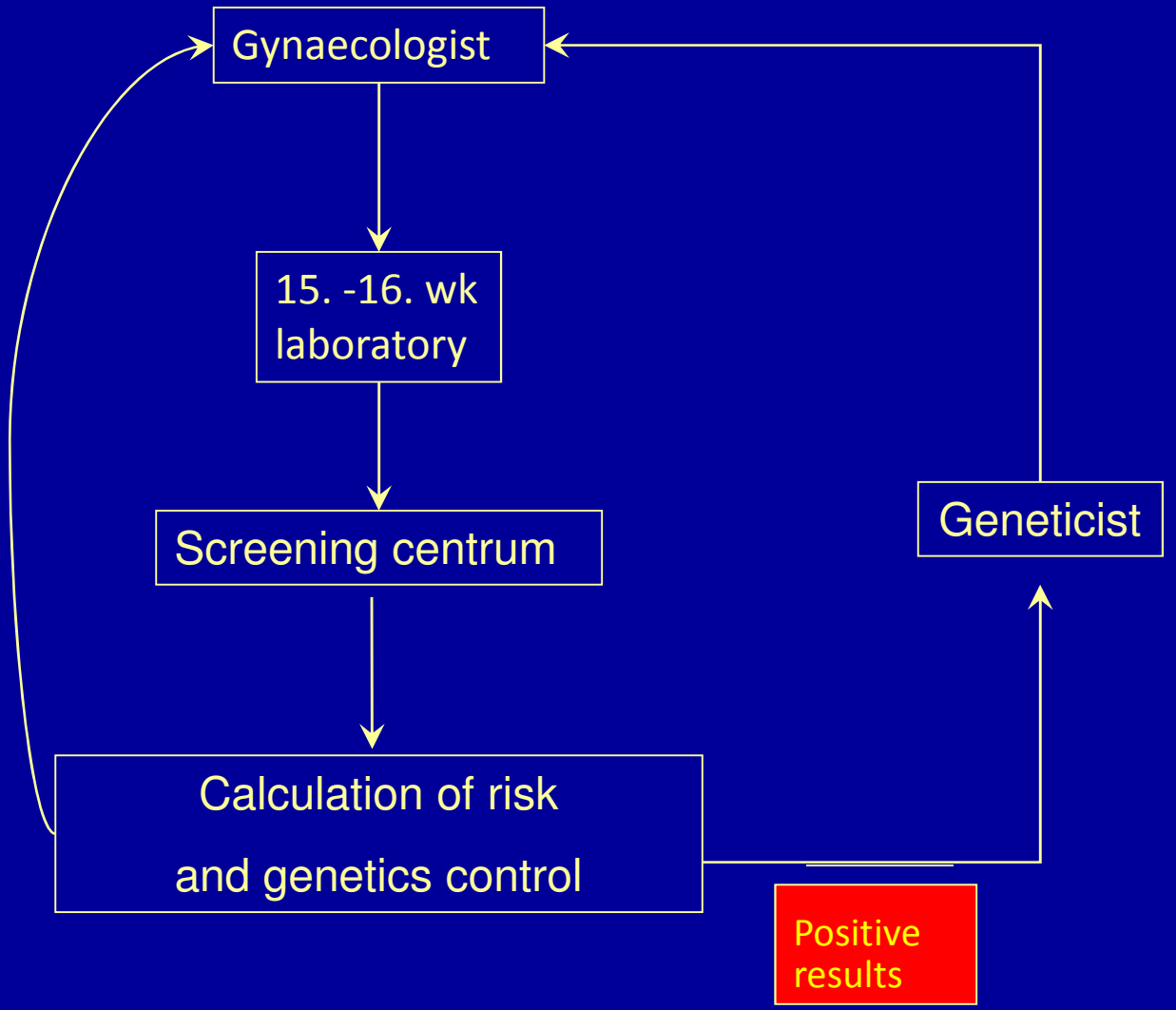
- Accurate measure of gestational age - US
- Accurate demographic details
  - maternal age
  - specimen date
  - details of other conditions
  - foetal numbers

Sophisticated software essential

NTD

- NTD (Neural tube defects) can affect 1in 500 infants
- Commonest forms of NTD known as anencephaly or spina bifida
- Neural tube beneath the backbone fails to develop
- definitive diagnosis relies on amniocentesis
- high levels of AFP (Alphafetoprotein) seen in NTD
- Amniocentesis is costly and time-consuming
- miscarriage rate of 1:100

Scheme of 2nd trimester screening



First Trimester Screening

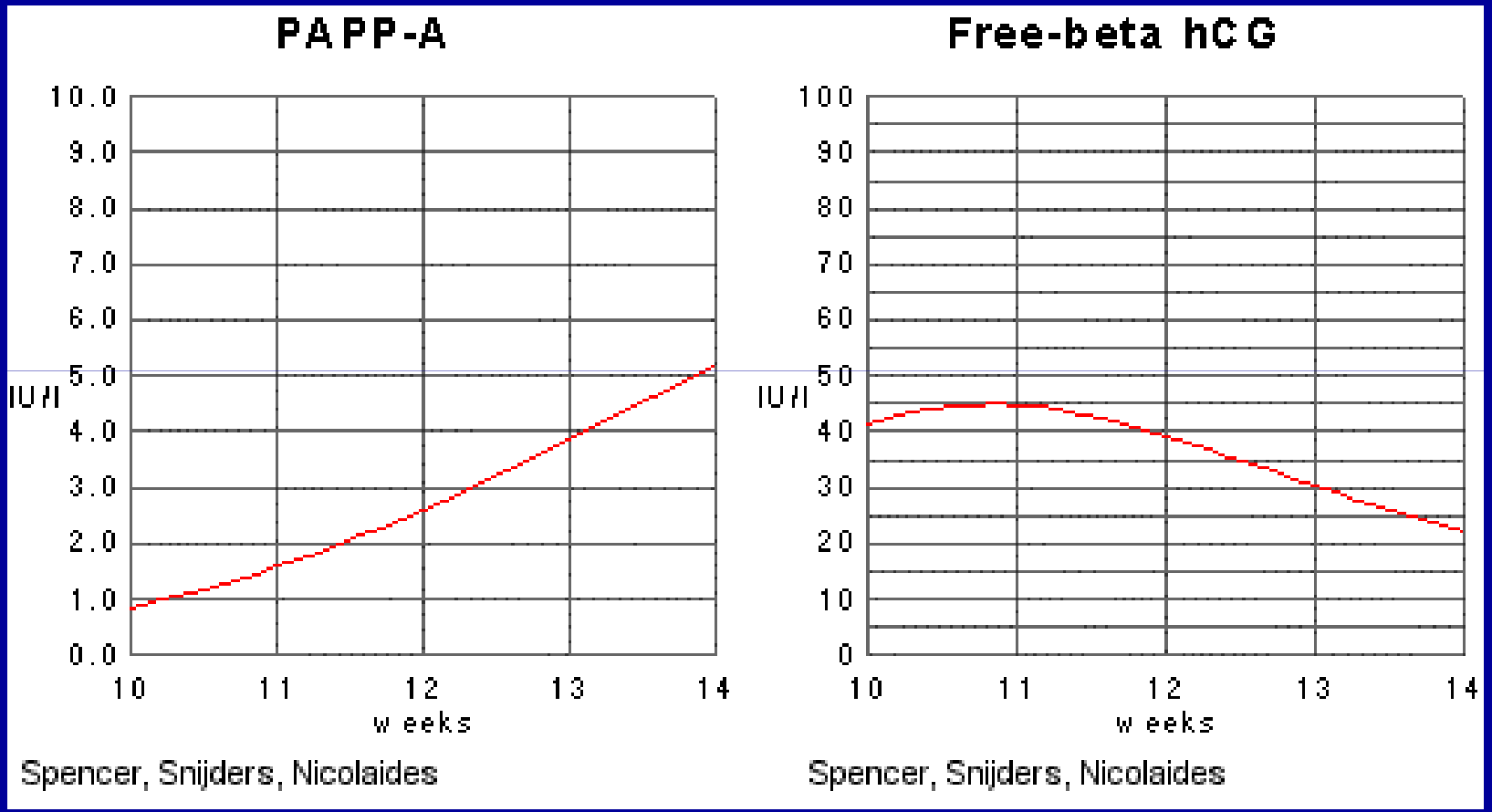
- Determining the overall risk of Down's syndrome in the unborn.
- First Trimester Screening determines how specific quantities of Free β HCG and PAPP-A in one specimen compare to the mediums of a population database.
- Measured at 10-13 completed weeks (70-97 days)
- Calculation of the Multiple of Median (MoM).

Biochemical Markers – 1st Trimester

- Pregnancy Associated Plasma Protein A (PAPP A)
- Free ßhCG
- Used with the ultrasound marker - Nuchal Translucency (NT)

- **Gold Standard test for Trisomy is karyotyping of foetal cells**

PAPP-A and Free β-hCG

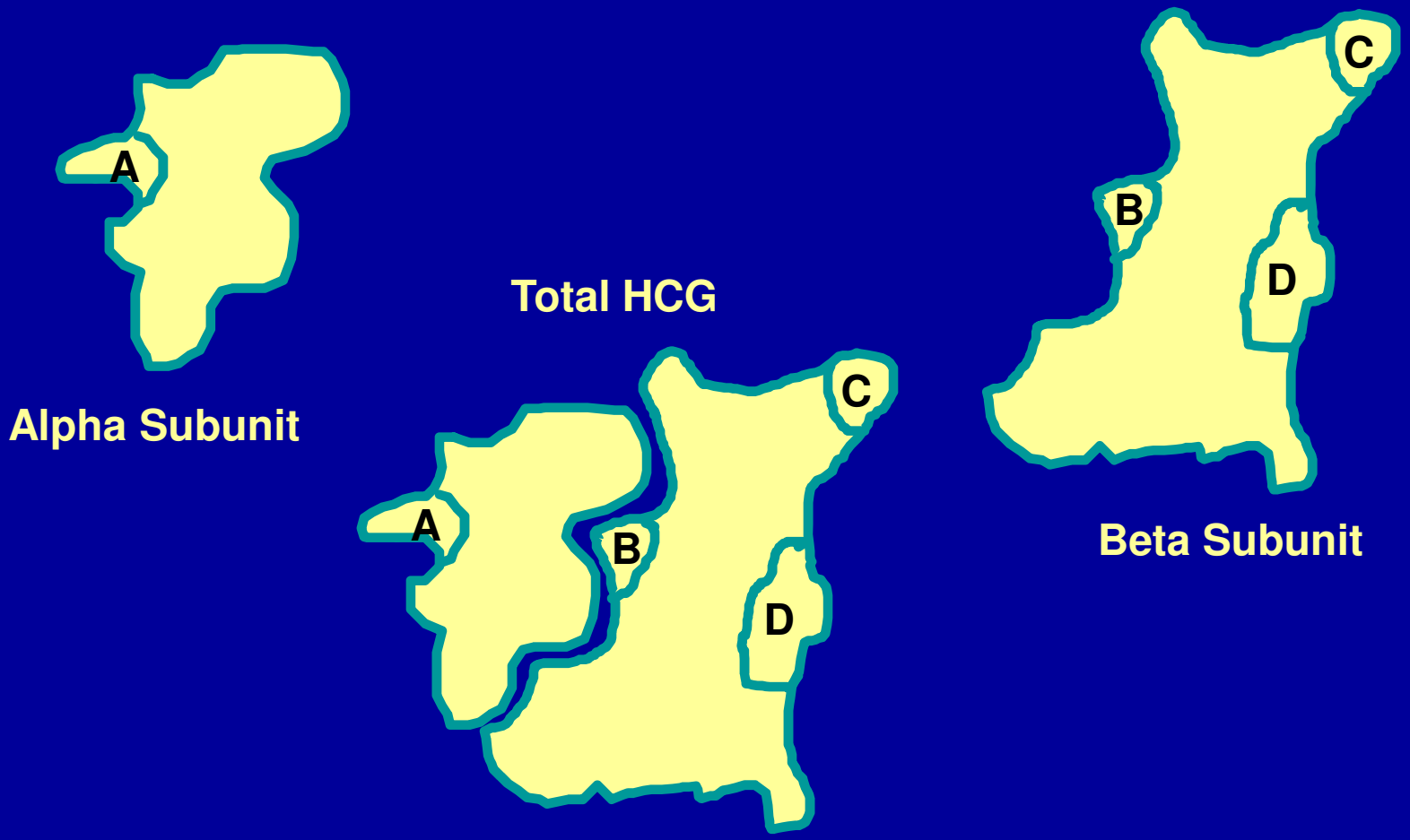


On average, baby with trisomy 21 will have 2.0 MoM for β-hCG and 0.4 MoM PAPP-A

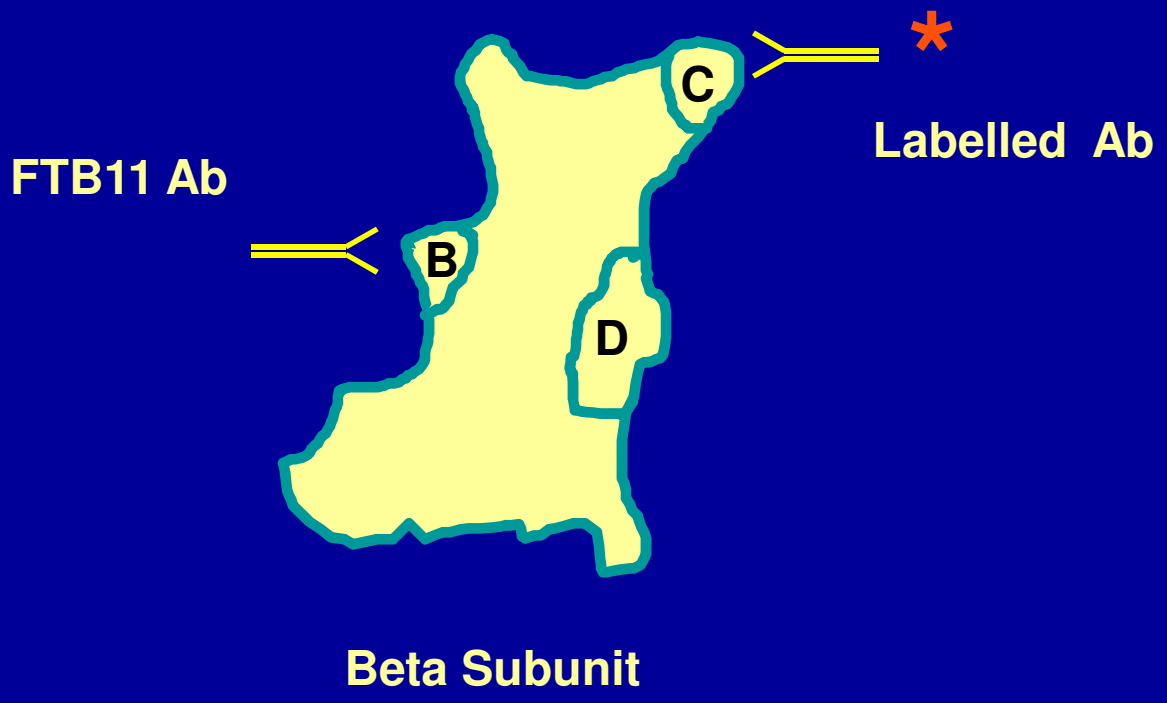
Free β hCG

- Free β chain of Human Chorionic Gonadotropin. Very high in the early stages of the first trimester, declines in the late first trimester
- Free ßhCG higher in Down's Pregnancies during 1st trimester screening period.
- free β hCG is not stable in blood samples. ***This is a serious disadvantage***, as blood samples sent to screening centres may be unseparated for 24 hours or more

Free Beta-hCG



Free Beta hCG Assay Specificity



Pregnancy Associated Plasma Protein A (PAPP A)

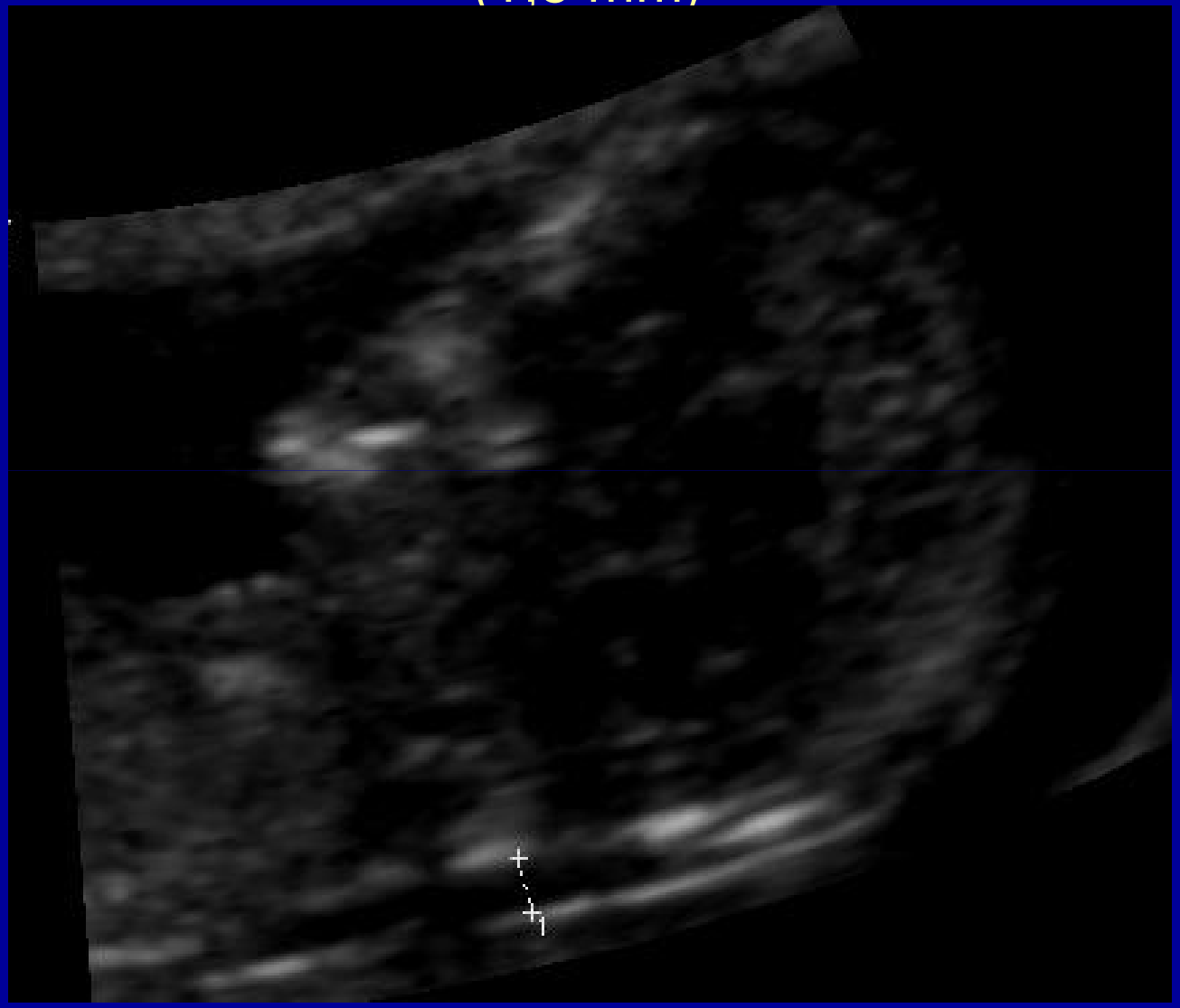
- PAPP-A - Pregnancy associated Plasma Protein-A. Placental protein which continues to increase during the term of the pregnancy
- Homotetrameric glycoprotein synthetized in chorionic villi.
- Specific and potent inhibitor of granulocyte elastase.
- Serum levels lower in Down's pregnancies in 1st trimester screening period

Nuchal Translucency (NT)

- Ideally performed between 11 & 13 weeks (10^{+2}-14^{+6} FMF).
- NT thickness is a measure of the amount of fluid at the back of the foetal neck
- 3 measurements to the nearest 0.1 mm are advised.
- The thickness is higher in Down's pregnancies during screening period.

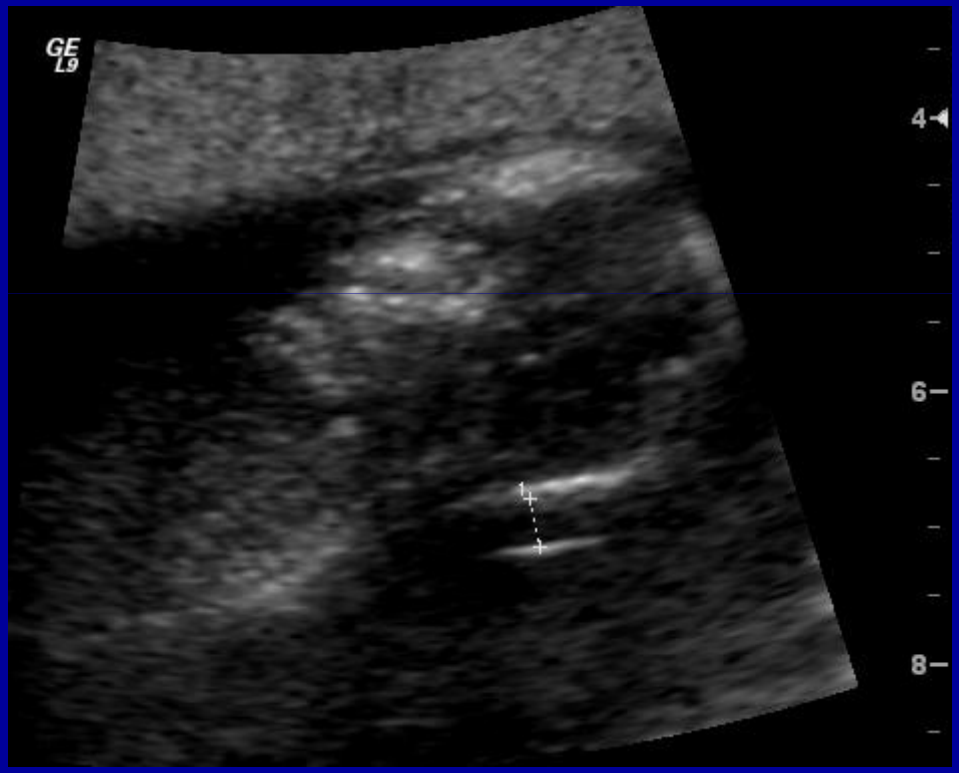
Measuring of NT

(1,5 mm)

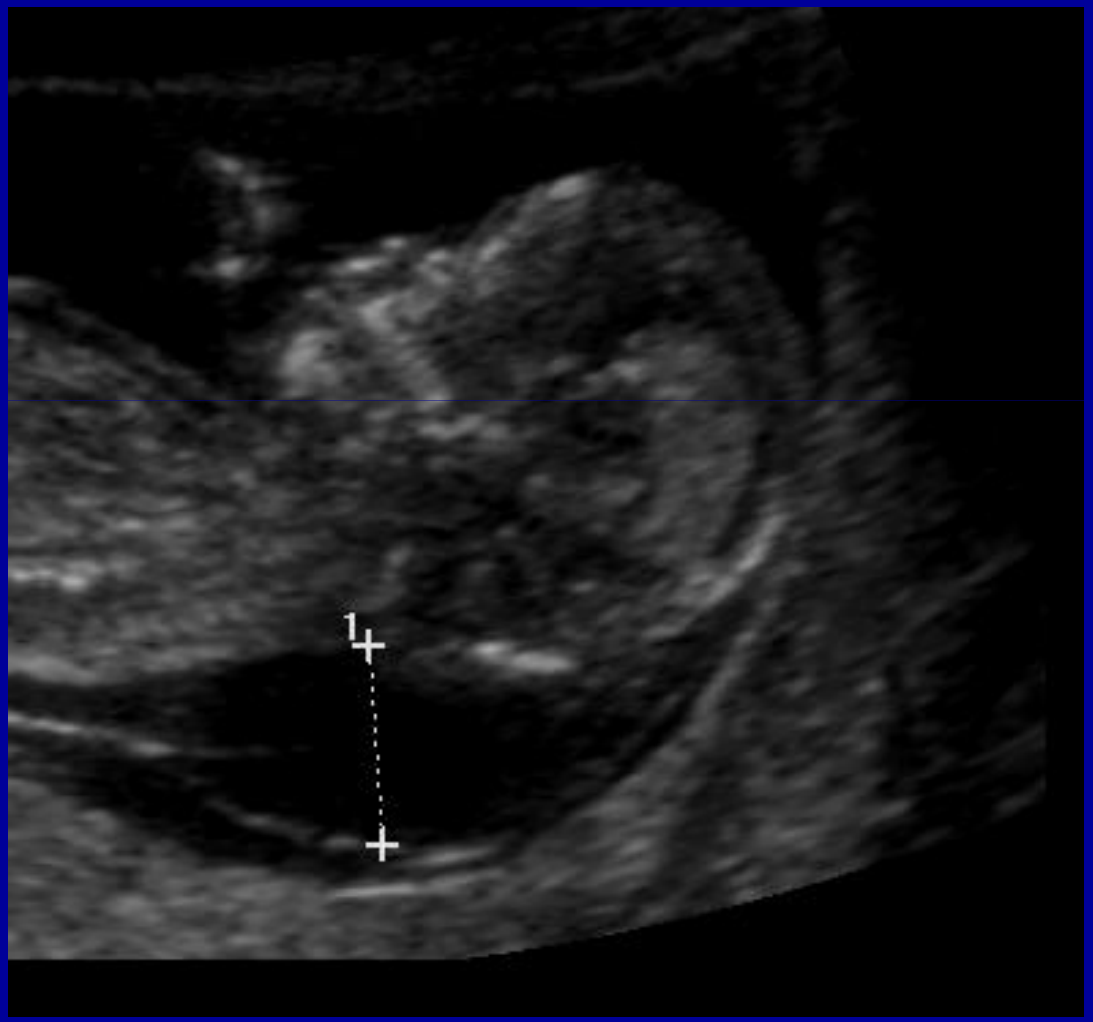


Foethus with DS

NT (3,4 mm)



Foethus with Turner syndrom
NT (10 mm)



NT – influence on result of screening

Age	30 let
NT	1,0 MoM
PAPP-A	0,65 MoM
MS-AFP	0,72 MoM
uE3	0,68 MoM
HCG	1,52 MoM
Inhibin-A	1,49 MoM

1:930 Negative

Age	30 let
NT	1,7 MoM
PAPP-A	0,65 MoM
MS-AFP	0,72 MoM
uE3	0,68 MoM
HCG	1,52 MoM
Inhibin-A	1,49 MoM

1:65 Positive

Risk calculation

- Nuchal Translucency (NT) measurement.
- Maternal age + NT account for 80% overall risk
- Maternal age + NT + Biochemistry 88-90%.

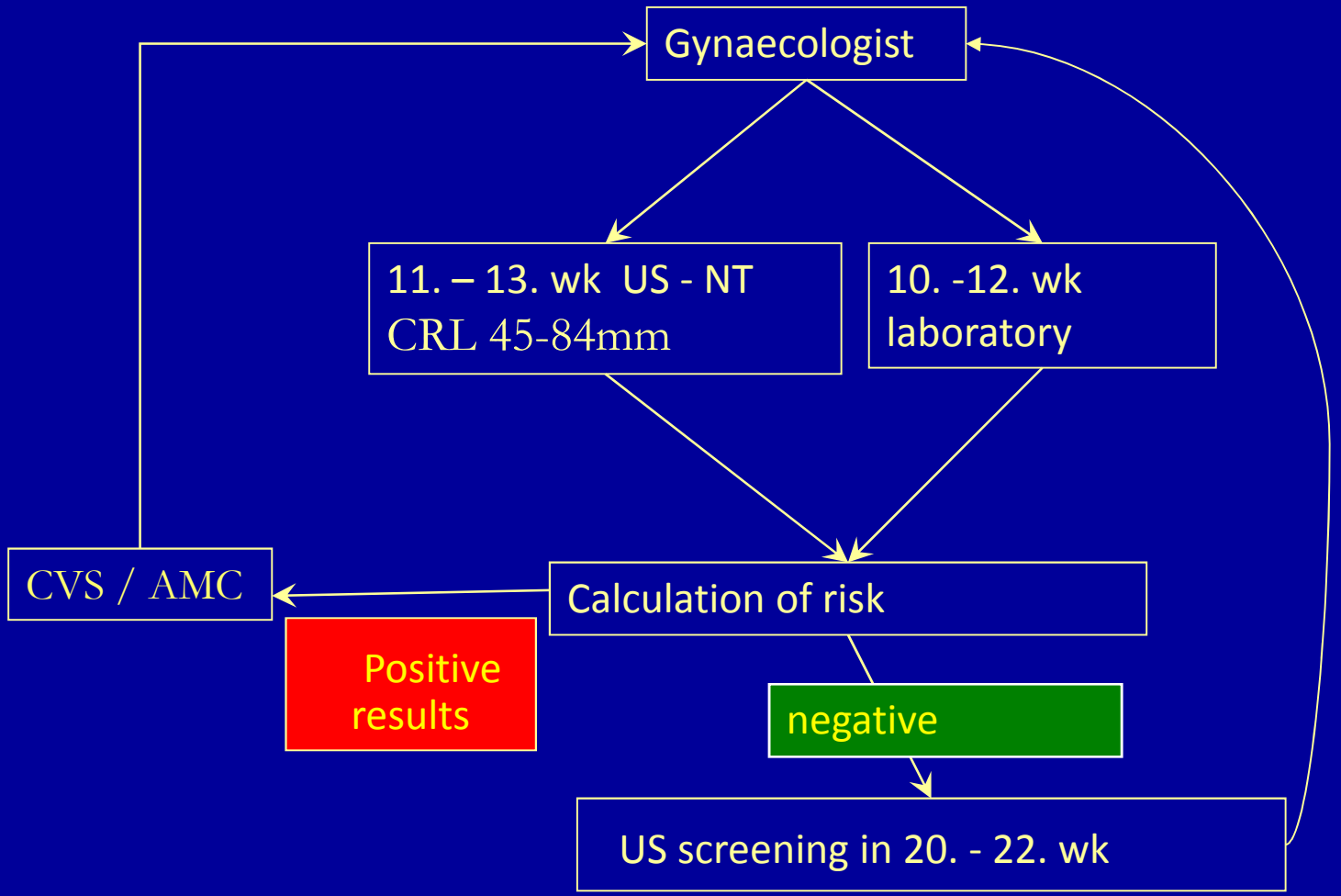
RISK ASSESMENT

- MoMs that typically yield a high risk of Down's are those where, in combination, the Free β HCG MoM is > 2.5 and the PAPP-A is < 0.4
- HIGH RISK- Woman > 35 years of age, with a NT of > 2.0 mm, and Free β HCG MoM >2.5 and PAPP-A MoM < 0.4
- LOW RISK - Women < 35 years of age, with a NT of < 2.0 mm, combined with a Free β HCG and PAPP-A MoM of 1.0.
- Further tests required at an overall risk of 1:150

Advantages of 1st Trimester Screening

- Information earlier, more options
- Reduce number of invasive procedures
- May identify other severe anomalies (or risk for) at time of scan and increased risk of adverse pregnancy outcome—referral for 2nd trimester
- Good time to date pregnancy accurately
- NT good for multiple gestation

Scheme of 1st trimester screening



Diagnostic Tests

- **Amniocentesis**
  - **Usually performed between 15 & 18 weeks.**
  - **Amniotic fluid removed by needle inserted into uterus transabdominal (located by US).**
  - **foetal cells cultured (cytogenetics lab).**
  - **Molecular biology techniques instead of full karyotyping.**
  - **Enables detection of other chromosomal abn.**
  - **Risk of miscarriage approx. 1 in 100.**

Diagnostic Tests

- **Chorionic Villus Sampling (CVS)**
  - **Performed around 11 to 13 weeks.**
  - **Chorionic villi sample removed from developing placenta (trans abdominally or trans vaginally under US control).**
  - **Slightly higher miscarriage rate than amnio.**

Screening of congenial development defects

- Currently perform:
  - screening of Down syndrom and NTD in the II.trimester of pregnacy
    - hCG
    - AFP
  - screening of DS in the I.trimester
    - Free β hCG
    - PAPP-A
    - Nuchal translucency – NT
    - Present of nose bone
  - integrated test

Integrated test

1st trimester

- determination of PAPP-A, optionally free βhCG
- determination of GA by US
- measuring of NT
- first evaluation by physician

2nd trimester

- determination of AFP and total hCG
- common evaluation with I. trimester results

AMNIO-PCR

- Only a small amount of amniotic fluid is required
- Applicable to a wide range of pregnancies (12 to 34 weeks)
- Definitive results within 24 h
- 100% accurate in the detection of major autosomal trisomies
  - Trisomy 21 (Down syndrom)
  - Trisomy 18 (Edwards syndrom)
  - Trisomy 13 (Patau syndrom)
  - Triploidy
  - Sex chromosome aneuploidy

Ethical considerations

- Who is the patient?
- Who benefits?
- Tests far from perfect - 65% detection
- Highly stressful - patients and staff
- Stigmatisation of surviving Down's patients
- Can appropriate counselling be provided?

Screening
Ethics
Religion

- What will be after diagnosis?
- Pregnant women and her family?
- Gynaecologists and midwifes – agree with abortion?
  - The Hippocratic Oath (“primum non nocere”)
  - Religion
- Czech Republic – the atheistic country

Conclusions

- Down's Syndrome screening feasible
- Technically imperfect
- New approaches may help – US, DNA technology
- Current aim is for first trimester screening
- Ethical issues cannot be ignored
- Informed decision making essential

Information for pregnant

Consultation before performation of screening

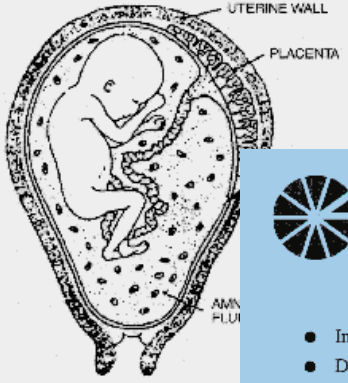
Posibility of consultation during all pregnancy

www pages

Laboratory communication with gyneacologists and geneticts

Serum Screening for Down's Syndrome and NTDs

GIVING INFORMATION ABOUT AMNIOCENTESIS



Points to discuss:

- nature of the procedure
- accuracy of the results
- risk of miscarriage
- limitations of the test
- possibility
- possibility
- care after
- length of
- in the few
  diagnosed

Serum Screening for Down's Syndrom

REPORTING SCREEN-POSITIVE RESUL
(Increased risk of Down's syndrome)

- Confirm the woman's identity.
- Remind her about the screening test for Down's syndrome and that the test divides women into tw groups, a higher risk group (screen-positive result sult).

e result and e

e a screen-po

Serum Screening for Down's Syndrome and NTDs

PRE TEST INFORMATION

- Invite an explicit decision on whether to be scree
- Determine the woman's knowledge of Down's sy and neural tube defects and give information as a
- Determine the woman's knowledge of the screen give information as appropriate.

Points to include:

"yes or no" answer – it d
groups (screen-positive r
creen-negative results).
5 women screened will
d be offered an amniocen
ill not have affected preg
screen-positive result inc
woman, knowing this in a
on hearing that she has a

ll pregnancies with Dow
e defect.
will be followed by the
-on-amniocentesis with
riage).
n which Down's syndron
s diagnosed, the woman
pregnancy.

Serum Screening for Down's Syndrome and NTDs

FACTS ABOUT SERUM SCREENING

- Serum screening identifies women with an increased risk of having a pregnancy with Down's syndrome or an open NTD so that they can be offered a diagnostic test.
- The serum markers used are alpha-fetoprotein (AFP), human chorionic gonadotrophin (total hCG or free ß-hCG) and/or unconjugated oestriol (uE_3) and/or inhibin-A.
- The test can be carried out on blood samples taken between 15 and 22 weeks – 16 weeks is ideal.

ncy with Down's
vel (usually 1 in
About 1 in 20
up, and about 1 in
nancy.

er there is an
with an open NTD.
positive (raised
ave an affected

4 women who have

men who have
d nearly all women
ly.

nstitute of Preventive Medicine

Serum Screening for Down's Syndrome and NTDs

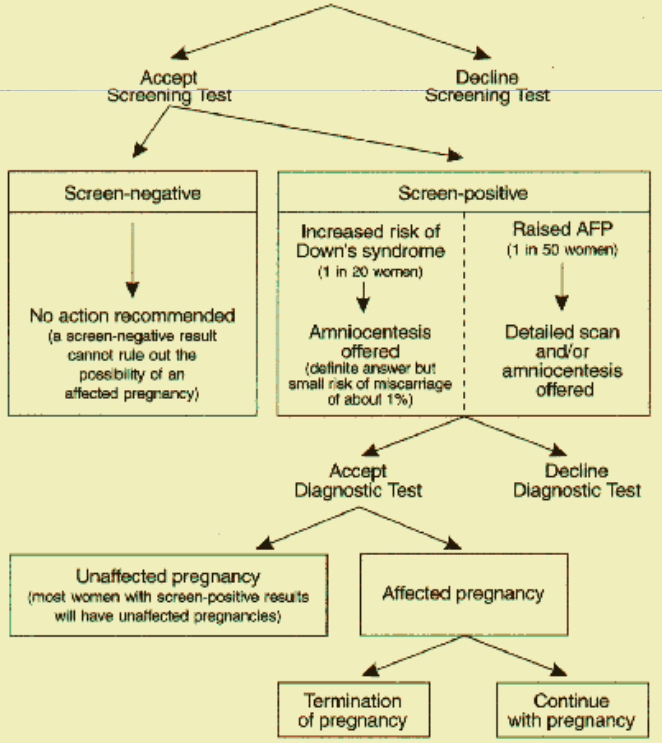
SERUM SCREENING FOR DOWN'S SYNDROME:
Probability of a screen-positive result by maternal age*

Maternal age group (years)	Probability of a screen-positive result	Proportion of Down's syndrome pregnancies detected (%)
under 25	1 in 40	60%
25-29	1 in 30	65%
30-34	1 in 15	70%
35-39	1 in 5	85%
40-44	1 in 3	95%
45 and over	> 1 in 2	>99%
All	1 in 20	75%

* Quadruple test (AFP, uE_3, inhibin-A and free β-hCG): risk cut-off level 1 in 300
Triple test (AFP, uE_3 and hCG [total or free β]): risk cut-off level 1 in 250

Serum Screening for Down's Syndrome and NTDs

DISCUSS SCREENING TEST




Antenatal Screening
Antenatal Screening for Down's Syndrome and Open Neural Tube Defects
THE INTEGRATED TEST
Information for Health Professionals
The Wolfson Institute of Preventive Medicine
St Bartholomew's & the Royal London School of Medicine and Dentistry
and
The Fetal Medicine Unit, University College Hospital
Antenatal Screening
MATERNAL SERUM SCREENING FOR DOWN'S SYNDROME AND OPEN NEURAL TUBE DEFECTS
Questions and Answers
Antenatal Screening Service
St Bartholomew's and the Royal London School of Medicine and Dentistry
Antenata
Antenatal Screening for Down's Syndrome and Open Neural Tube Defects
THE INTEGRATED TEST
Questions and Answers for women considering the test
The Wolfson Institute of Preventive Medicine
St Bartholomew's & the Royal London School of Medicine and Dentistry
and
The Fetal Medicine Unit, University College Hospital London
tenatal Screening
MATERNAL SERUM SCREENING FOR DOWN'S SYNDROME AND OPEN NEURAL TUBE DEFECTS
General Information
Antenatal Screening Service
St Bartholomew's and the Royal London School of Medicine and Dentistry

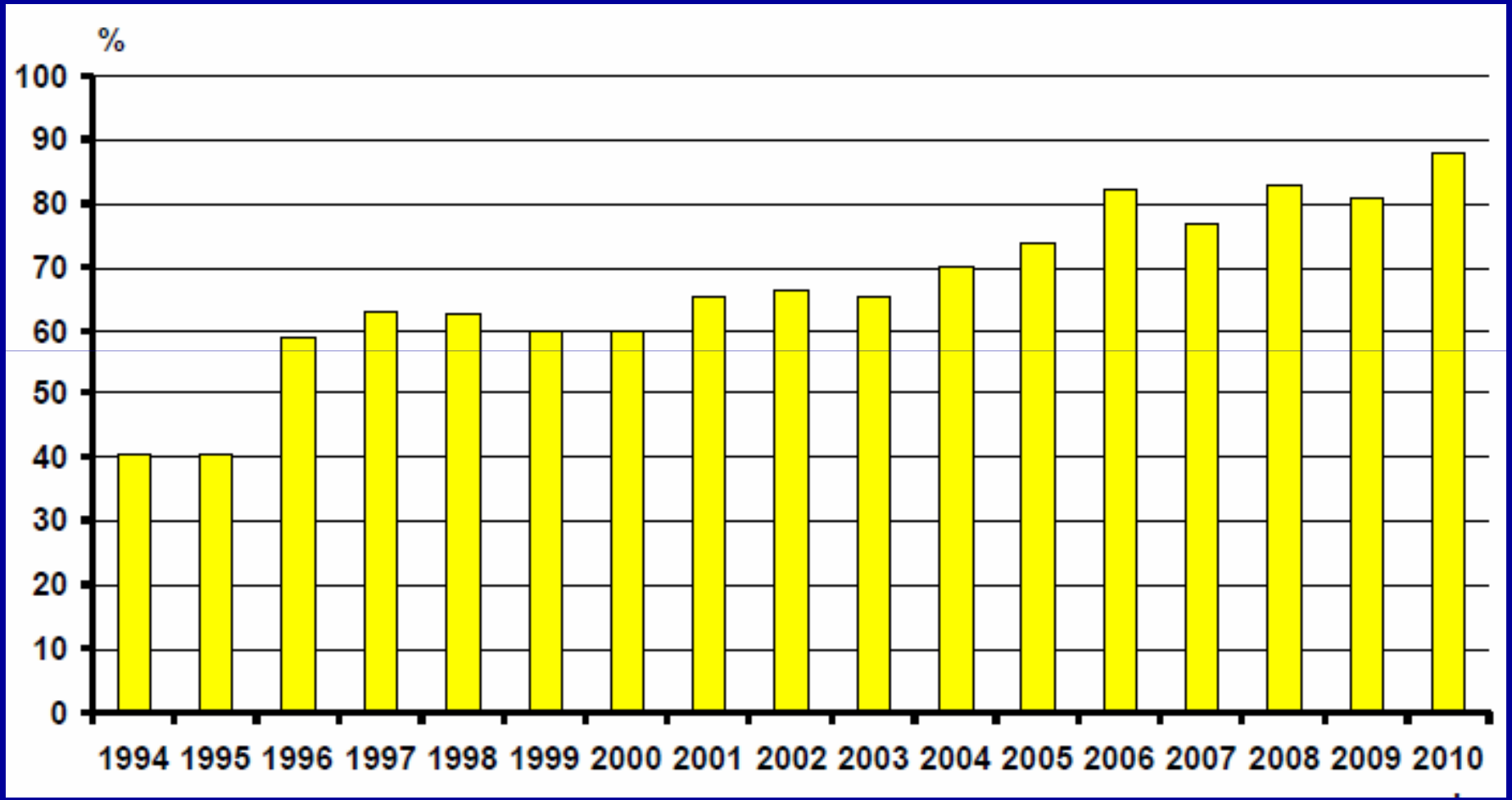
Screening of DS

	1st trimester	2nd trimester	Integrated
PAPP-A			
free β hCG			
Nuchal translucency **(NT)**			
AFP			
hCG			
uE3			

Detection rate

	FP for 85% DR	DR for 5% FP
I.trimester combined test	3,8 - 6,8%	85%
II.trimester	9,3 - 14 %	69%
integrated test	0,8 - 1,2%	94%
serum integrated test	2,7 - 5,2%	85%

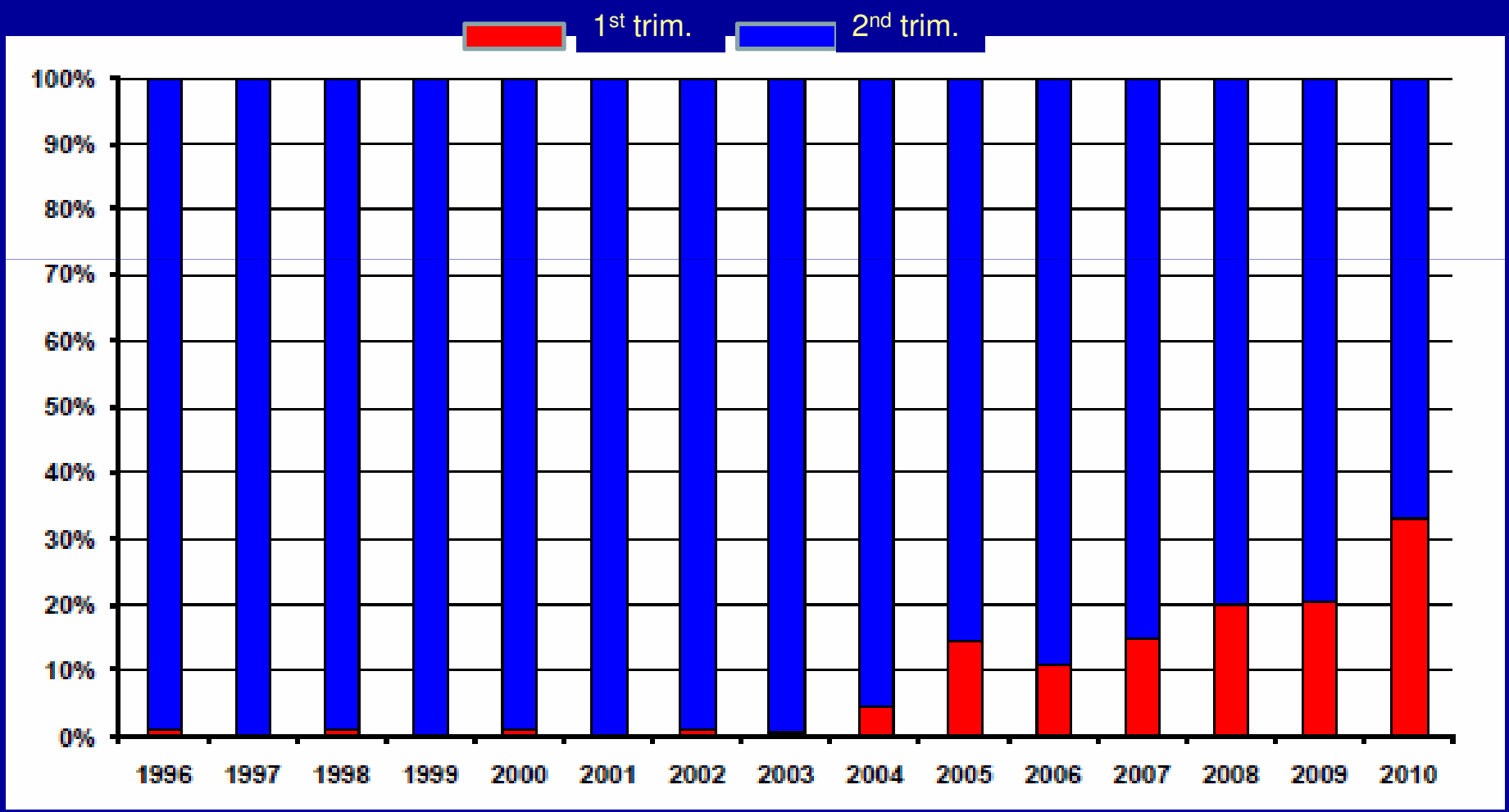
Prenatal diagnoses of DS



V.Gregor, Genetic, Thomayer Hospital, Prague , Czech Republic

Ratio DS diagnoses in the first and second trimester

V.Gregor, Genetic, Thomayer Hospital, Prague , Czech Republic

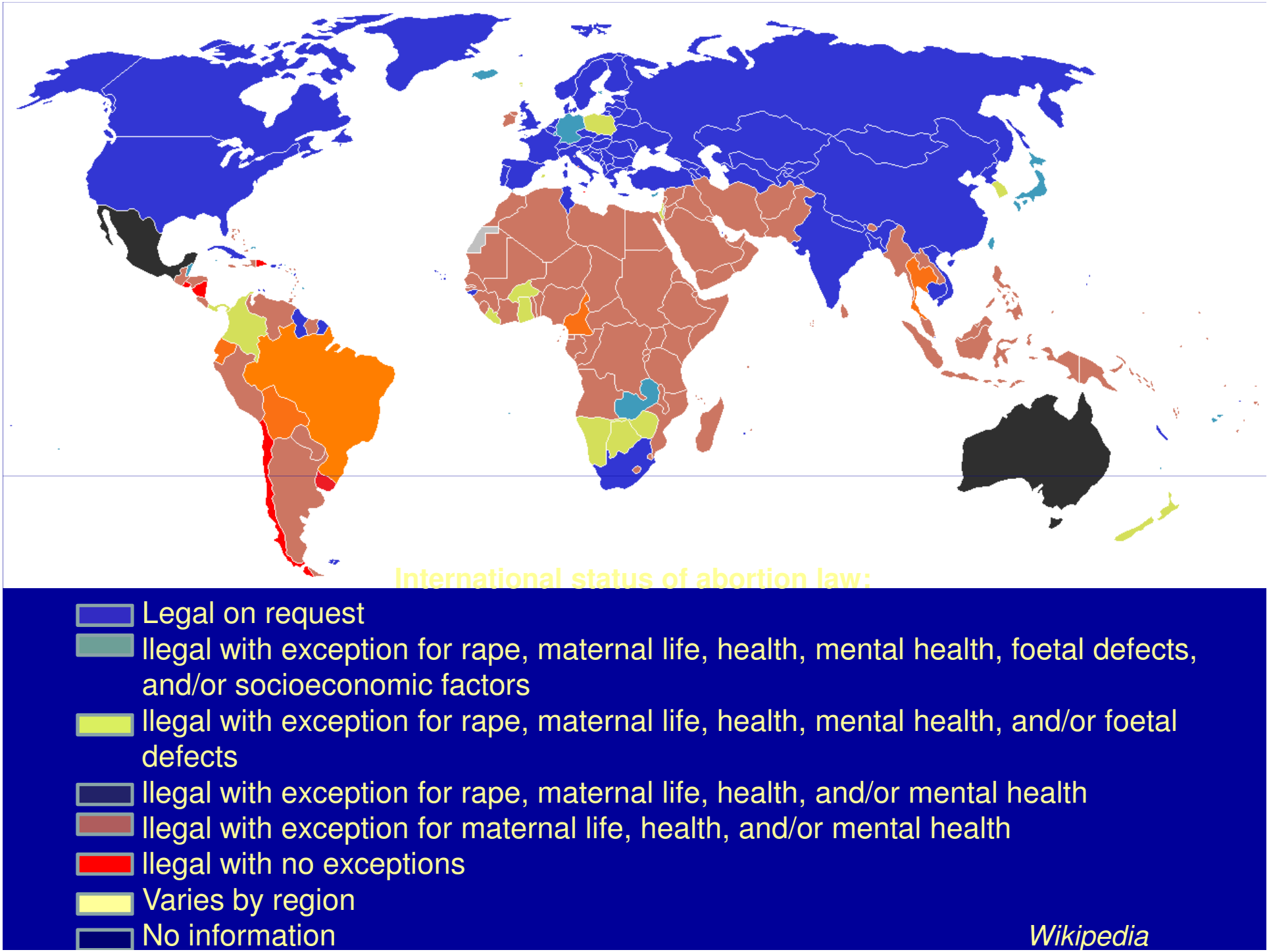


QUALITY CONTROL

- **Quality Control specimens are run daily at all levels of the working range for all assays (precision).**
- **External Quality Control for 2nd trimester is organized by SEKK or DGKC, for 1st trimester by UKNEQAS (accuracy). This allows for comparisons between different methodologies in different laboratories to be made.**

Europe

- In some countries is screening defined by state health care
- Majority of countries perform AMC after 35.
  - France > 38
  - Finland > 40

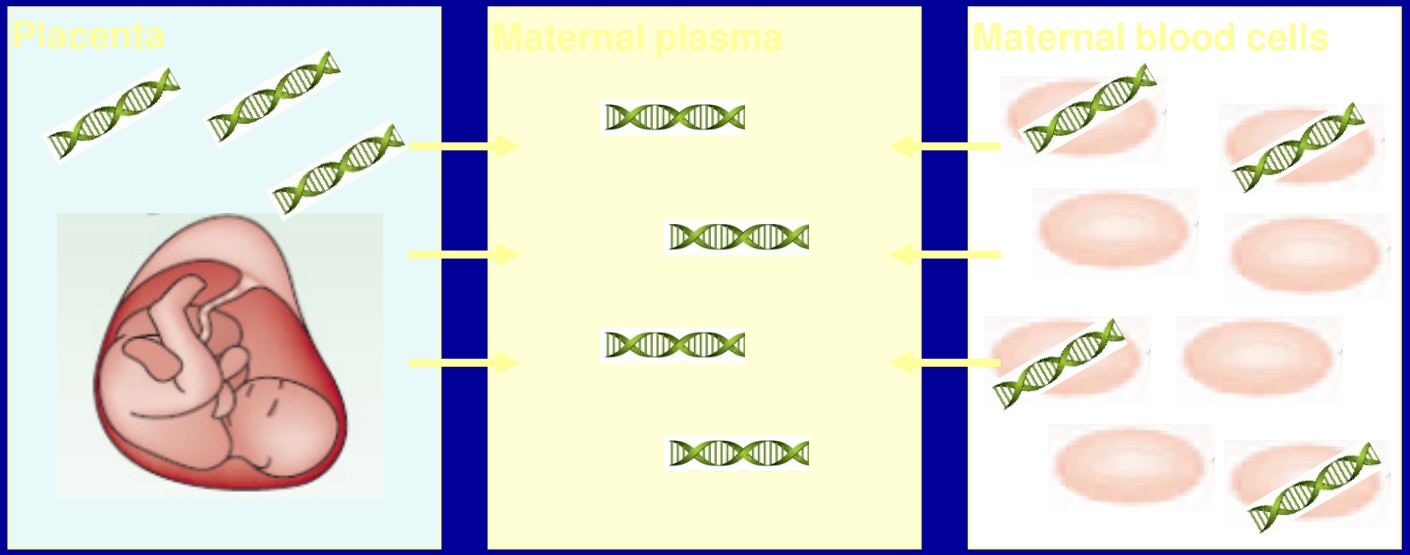

International status of abortion law:
Legal on request
Illegal with exception for rape, maternal life, health, mental health, foetal defects, and/or socioeconomic factors
Illegal with exception for rape, maternal life, health, mental health, and/or foetal defects
Illegal with exception for rape, maternal life, health, and/or mental health
Illegal with exception for maternal life, health, and/or mental health
Illegal with no exceptions
Varies by region
No information
Wikipedia

Summary

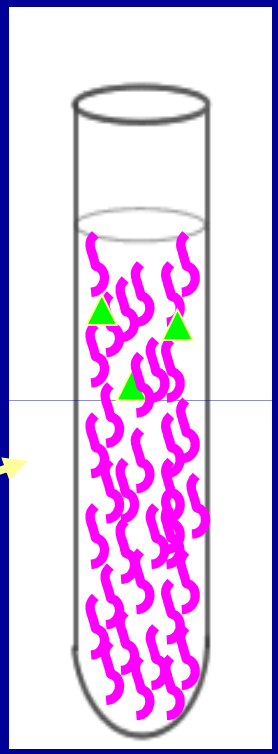
- Despite of increasing participation of US, the biochemical testing has still major role in Down's screening
- Current aim is for first trimester screening but many practical problems are associated - certification, high level of cooperation (one-day service)
- Increased standardization for all participants
- Biochemical testing for NTD is being superseded by US
- Ethical issues cannot be ignored
- Informed decision making essential

Future in prenatal testing

- 1997 First report of free foetal DNA in maternal circulation. (Lo YMD *et al*. *Lancet* 1997;350:485-7)
- Investigations focused on the role of cell-free foetal DNA and foetal messenger RNA



Extraction of cell free foetal nucleic acids



11 – 17 weeks

▲ Cell free foetal DNA (3.4%)

ʃ Cell free maternal DNA (96.6%)

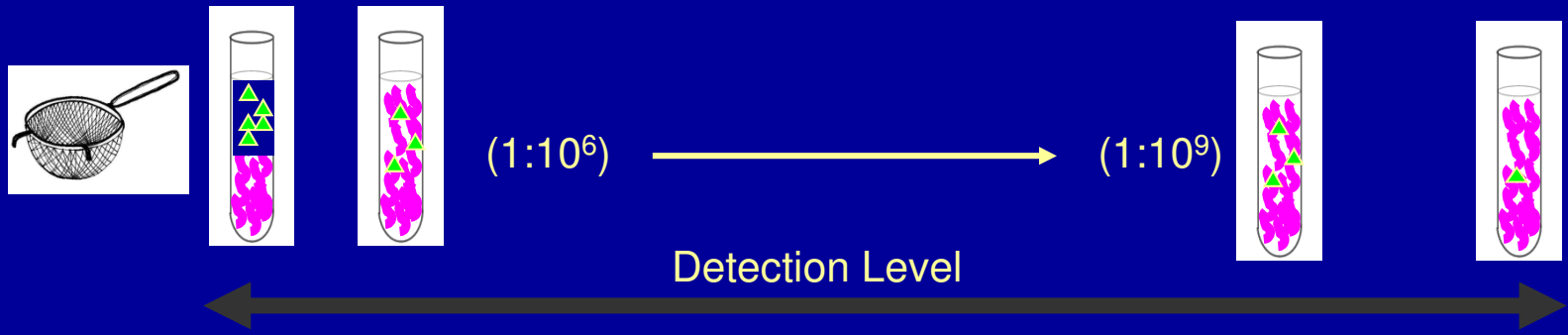
- Population variation
- Low copy number of cf foetal DNA
- Background ‘contamination’ with cf maternal DNA (94 – 97%)

Helen White, PhD. National Genetics, Reference Lab (Wessex)

New advances

Development of techniques with improved detection levels

e.g.Pyrophosphorolysis Activated Polymerisation (PAP)



- Theoretical detection level of 10^{-9} (improvement on RQ-PCR)
- foetal sexing n=54 (Boon et al., Prenatal Diagnosis 2007)
- Sensitivity 100 % (95% CI: <5.56% chance of false negative)
- Specificity 98.1 % (95% CI: 91.5% - 99.9%) [1 false positive]
- Potential for detection of paternal mutations
- Requires high purity blocked oligonucleotides – risk of false positives

Helen White, PhD. National Genetics, Reference Lab (Wessex)

Detection of aneuploidy

The ability to use NIPD to detect foetal aneuploidies, particularly trisomy 21, represents a major breakthrough in prenatal diagnosis

- Major technical challenge

Background of cf maternal DNA mean direct quantification of foetal chromosome copy number is not yet feasible

Need targets that are free from maternal background interference

- Recent major breakthroughs

Quantitative analysis of SNPs in foetal specific mRNA transcripts
(Lo et al., PNAS 2007; Lo et al., Nature Medicine 2007; Maron et al, 2007)

Epigenetic analysis (Tong et al., 2006; Old et al, 2007)

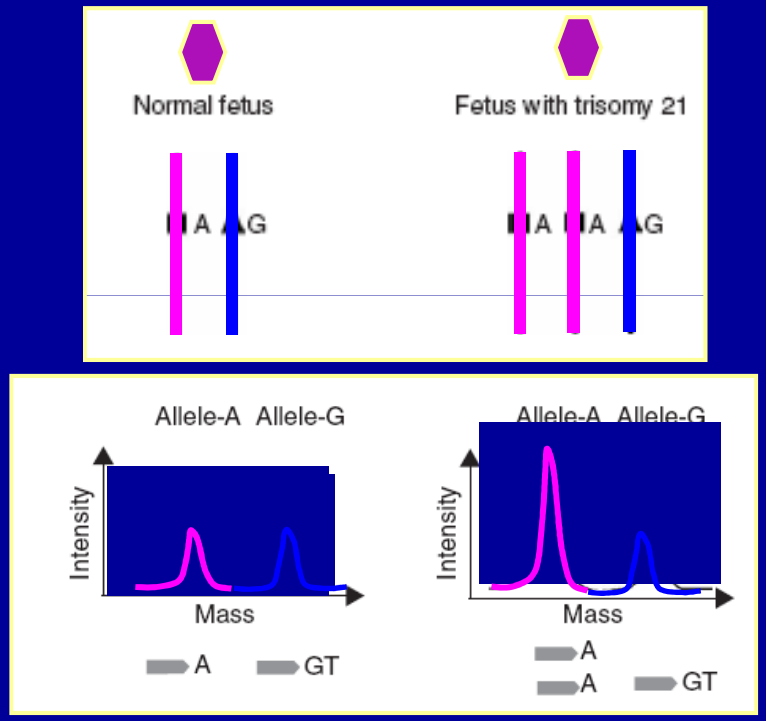
Proteomic analysis (e.g. Nagalla et al., 2007) Identification of novel protein biomarkers in maternal plasma associated with trisomy 21 pregnancies

Helen White, PhD. National Genetics, Reference Lab (Wessex)

Quantitative analysis of SNPs in foetal specific mRNA

- PLAC4 mRNA (⬢) is derived exclusively from foetal chromosome 21
- PLAC4 mRNA expressed in the placenta and is found in the plasma of pregnant women

Normal fetus



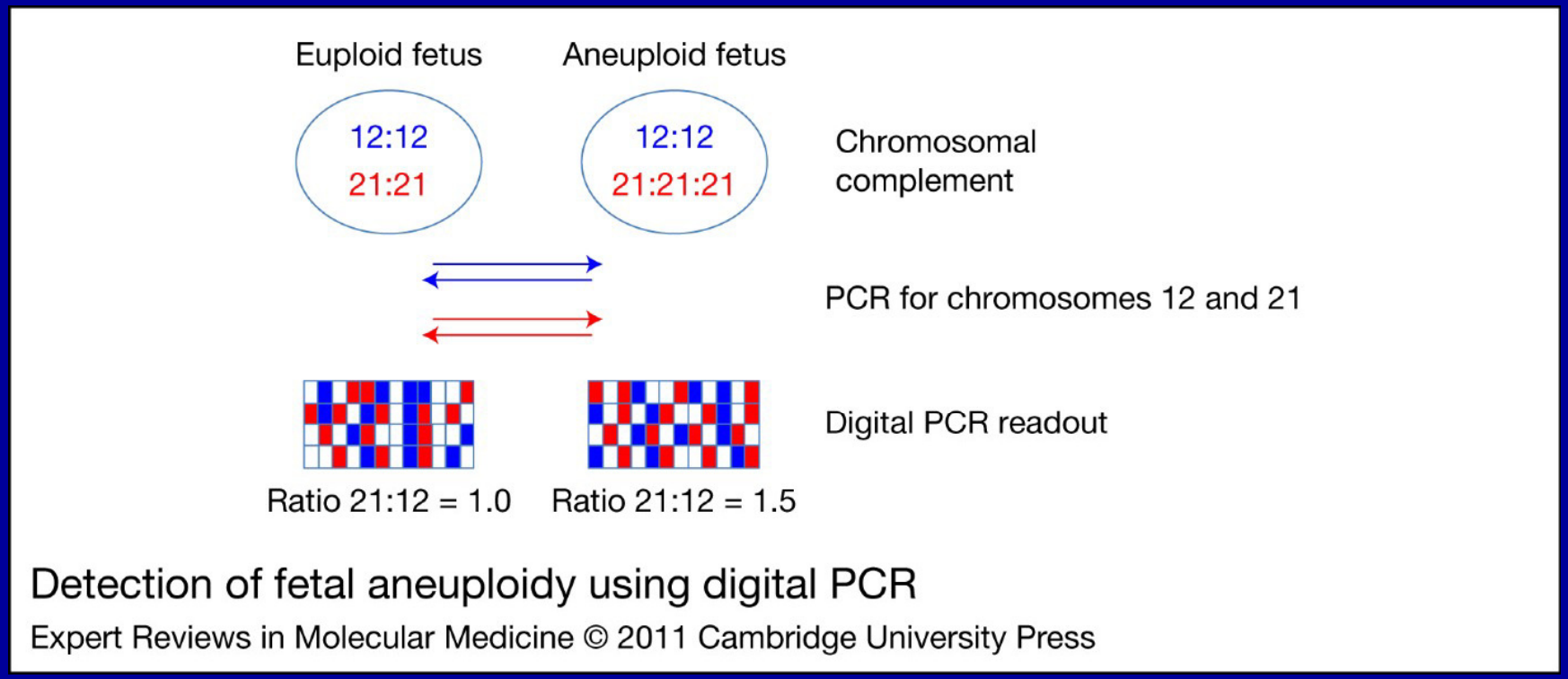
Trisomy 21 fetus

1 : 1

2 : 1

- Correctly diagnosed foetal trisomy 21 in 90% of +21 cases (n=10)
- Excluded diagnosis of trisomy 21 in 96.5% of chromosomally normal controls (n=57)
- Foetus has to be informative for SNP analysed

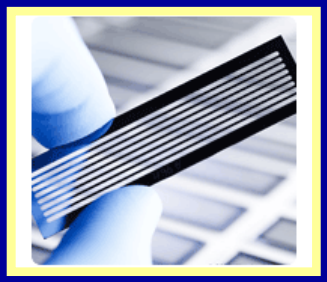
Lo et al., Nature Med & PNAS, 2007



Detection of fetal aneuploidy using digital PCR

Hahn S, Lapaire O, Tercanli S, Kolla V, Hösli I. Expert Rev Mol Med 2011:13:e16

MPS – Implementation on Illumina platform



- Sample preparation (cca. 270 USD)
- Cluster preparation (cca. 600 USD)
- sequenator (1500 USD for 1 cell, 7 pac.+QC)
- Data analysis, evaluation
- 3 working days
- Price of device – 600.000 USD

Summary

DNA Extraction

- Enrichment of cf foetal DNA – will improve reliability of testing
- Any technique that can separate cf maternal and cf foetal DNA would revolutionise NIPD

Foetal sexing and single gene disorders

- Already in diagnostic use although development of more foetal specific markers required
- New techniques with increased levels of detection are being reported in research settings. Requires diagnostic validation

Aneuploidy

- Use of mRNA SNP allele ratio for testing for DS testing has been successfully applied in research setting. Requires diagnostic validation
- Epigenetic studies ongoing and proteomics studies are promising

Lack of quality control material and method standardisation

Helen White, PhD. National Genetics, Reference Lab (Wessex)

PGD

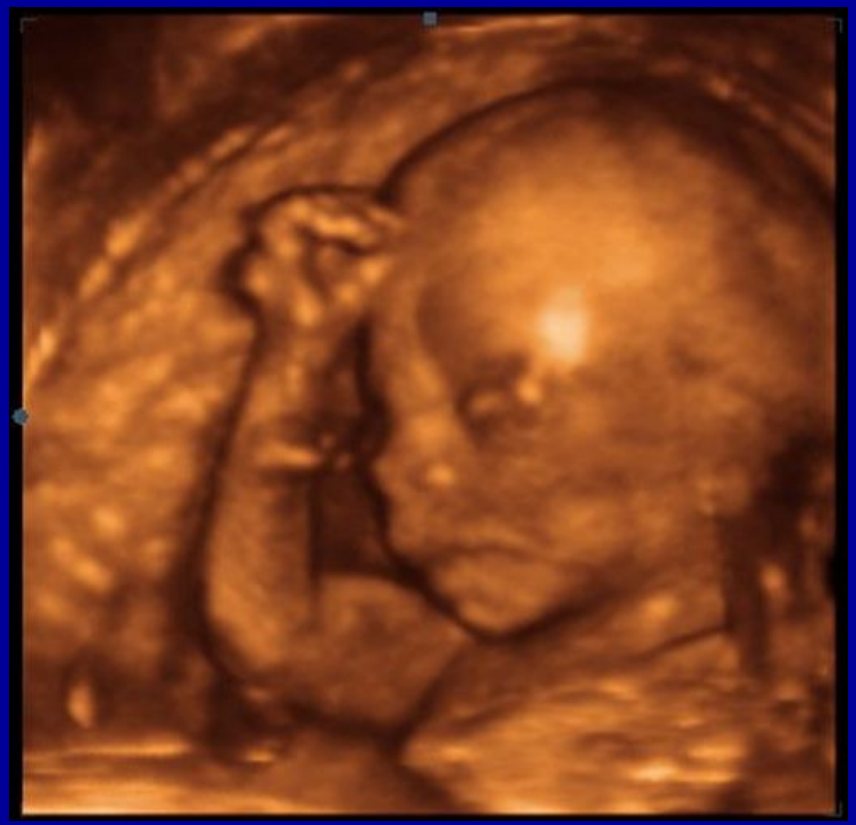
- PGD – Preimplantation genetic diagnosis
- Combines protocols of IVF and genetic testing
- Handyside and Verlinsky – 1990
- Biopsy of polar bodies of oocytes (less frequenlty)
- Biopsy of embryonal blastomeres (more frequenlty)
- Sampling – 8 cells embryos i.e. 3. days after egg fertilization
- Egg must be fertilized by one sperm (ICSI)

Methods of testing

- Fluorescence In Situ Hybridization (FISH)
- PCR
- Microarray

Diseases that can be diagnosed

- Aneuploidy (sensitivity 90%)
- Abortion of foetus when one of the parents is known as a carrier of translocations
- Monogennous diseases (sickle cell anemia, cystic fibrosis, Huntigton's disease, Tay-Sachs disease
- Carrier translocations
- Maternal Gonosomal mosaicism
-
- ***Each test is always disease specific***



Děkuji za pozornost

Literature:

- Pandya, P.P. et al (1995) Br J Obstet Gynacol 102: 957-962
- Spencer, K. et al (1999) Ultrasound Obstet Gynacol 13: 231-237
- Tasevski V, Ward P, Koe L. and Morris J. Feto-Maternal Medicine Laboratory Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, NSW AUSTRALIA
- Dr Rick Jones, Division of Clinical Sciences, University of Leeds, Leeds, UK
- Allan Thompson: NTD and Down's Screening, Bayer Diagnostics
- *Stevenson, Leslie and Sheridan, Ann.Clin.Biochem 30, 99-100 (1993)*