



*Prenatal Screening for Down
Syndrome: New Developments for
Biochemical and Ultrasound
Markers*

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**Developed in collaboration with the
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Down Syndrome

- **Most common genetic cause of mental retardation**
- **Associated with typical clinical presentation such as the following dysmorphic features:**
 - **Loose skin on nape of neck**
 - **Narrow palate**
 - **Flat nasal bridge**
 - **Epicanthic folds**
 - **Gap between first and second toes**

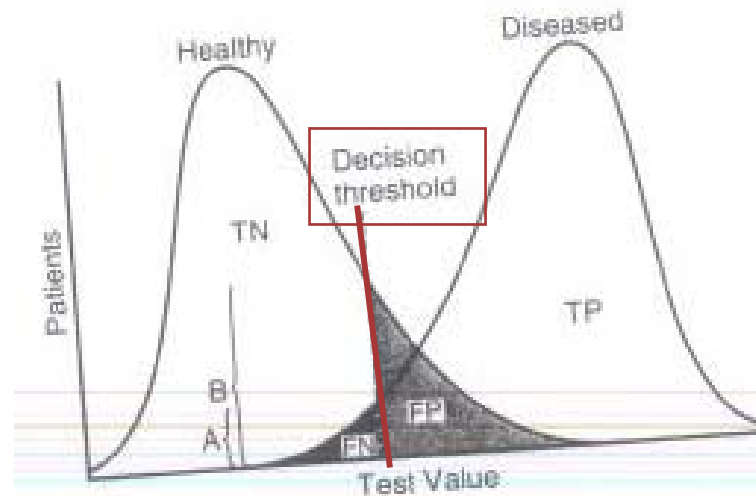


Role of screening tests

Ability to distinguish affected from non affected individuals

- Determination of detection rate (proportion of affected individuals with a screen positive test)
- Determination of false positive rate (proportion of unaffected individuals with a screen positive test)
- Performance will depend on the cut-off
- Screening test differs from diagnostic test: there will be false negative and false positive results in a screening program





Simulated distribution of healthy and diseased populations.

A/B: ratio of diseased patients to healthy patients is less than 1.

TP: true positive

TN: true negative

FP: false positive

A/B and FP will vary based on cut-off (decision threshold)

History of prenatal screening for Down syndrome

- **Early 1980s: screening based on maternal age only**
- **1984: AFP added (low level, increased risk)**
- **1988: triple test was created (AFP, hCG and uE3)**
- **Canada, US, most European countries: triple screening was used (AFP, uE3, hCG)**
- **UK: double screening was mostly used (AFP, hCG)**
- **Time window: 15-20 weeks**



History of prenatal screening for Down syndrome

- **Early 1990s: ultrasound marker nuchal translucency (NT) and biochemical markers PAPP-A and free beta hCG were reported as added value.**
- **1995: combined test was proposed**
- **Combined test at 10-13 weeks**



History of prenatal screening for Down syndrome

- 1996: dimeric inhibin A was reported as added value in second trimester screening
- MSS quad test was proposed
- Time window 15-20 weeks



History of prenatal screening for Down syndrome

- 1999: description of the mathematical model for the integrated screening test
- Integrated prenatal screening test was proposed

- Time window:
- First trimester markers (biochemical and US): 10-13 weeks
- Second trimester markers: 15-20 weeks



Evaluation of screening performance

- In view of various options, two major prospective research trials were launched:
- SURUSS
- FASTER

- Goal was to evaluate the screening performance of all options and to propose the best screening strategy



SURUSS study

- Serum URine and Ultrasound Screening Study
- Prospective UK study looking at performance of various combinations of screening markers in the first and second trimesters.



FASTER study

- First And Second Trimester Evaluation of Risks trial
- Prospective USA study looking at performance of various combinations of screening markers in the first and second trimesters



Variants screening protocols

- Beside combined screening (first trimester only); integrated screening (first and second trimester); and second trimester (triple or quad screening), various other combinations of screening markers in the first and second trimesters have been proposed, such as:
 - Sequential screening
 - Contingency screening
 - Repeated measures screening



Sequential screening

- Interim results provided when available
- Protocol associated with increased false positive rates but women with greatly increased DS risk in 1st trimester might elect early diagnostic testing (CVS) instead of waiting for 2nd trimester blood collection and results



Contingency screening

- Interim results (from first trimester) provided when risks cannot be modified with second trimester results
- Protocol associated with increased false positive rates but women with greatly increased DS risk in 1st trimester might elect CVS instead of waiting for 2nd trimester blood collection and results, while women with greatly lowered DS risk will have decreased anxiety



Repeated measures screening

First trimester (10 weeks)	Second trimester
Ultrasound marker NT	
Biochemical markers: PAPP-A and uE3	Biochemical markers: PAPP-A and uE3

Detection rate: 85%

False positive rate: 0.3%

Not tested yet on prospective studies



Example of pattern of common markers in DS cases

First Trimester
10-13 weeks

Free beta hCG	1.62 MoM 10 weeks 2.48 MoM 13 weeks
PAPP-A	0.34 MoM 10 weeks 0.58 MoM 13 weeks
Nuchal translucency (NT)	2.42 MoM 10 weeks 1.77 MoM 13 weeks

Second trimester
15-20 weeks

AFP	0.74 MoM
hCG	2.05 MoM
Unconjugated estriol	0.70 MoM
Inhibin A	1.91 MoM

Program performance- Nicolaidis

	False positive rate	Detection rate
NT	5%	77%
Free beta hCG,PAPP-A,NT	5%	87%
NT,AFP,hCG,uE3	6.5%	94%
Free beta hCG, PAPP-A, NT, AFP, hCG, uE3	Not reported	
NT, absent fetal nasal bone,free beta CG,PAPP-A	5%	97%

Program performance- SURUSS publication (2004)

(1st trimester markers at 11 weeks)	False positive rate	Detection rate
NT	15%	85%
Free beta hCG,PAPP-A,NT	4.3%	85%
PAPP-A,NT,AFP,hCG,uE3,	2.1%	85%
PAPP-A,NT,AFP,hCG,uE3, DIA	0.9% or 4.9%	85% or 94%
PAPP-A,AFP,hCG,uE3,DIA	3.9%	85%
AFP,hCG,uE3	9.3%	85%
AFP,hCG,uE3,DIA	6.2%	85%
Sequential screening	9%	94%

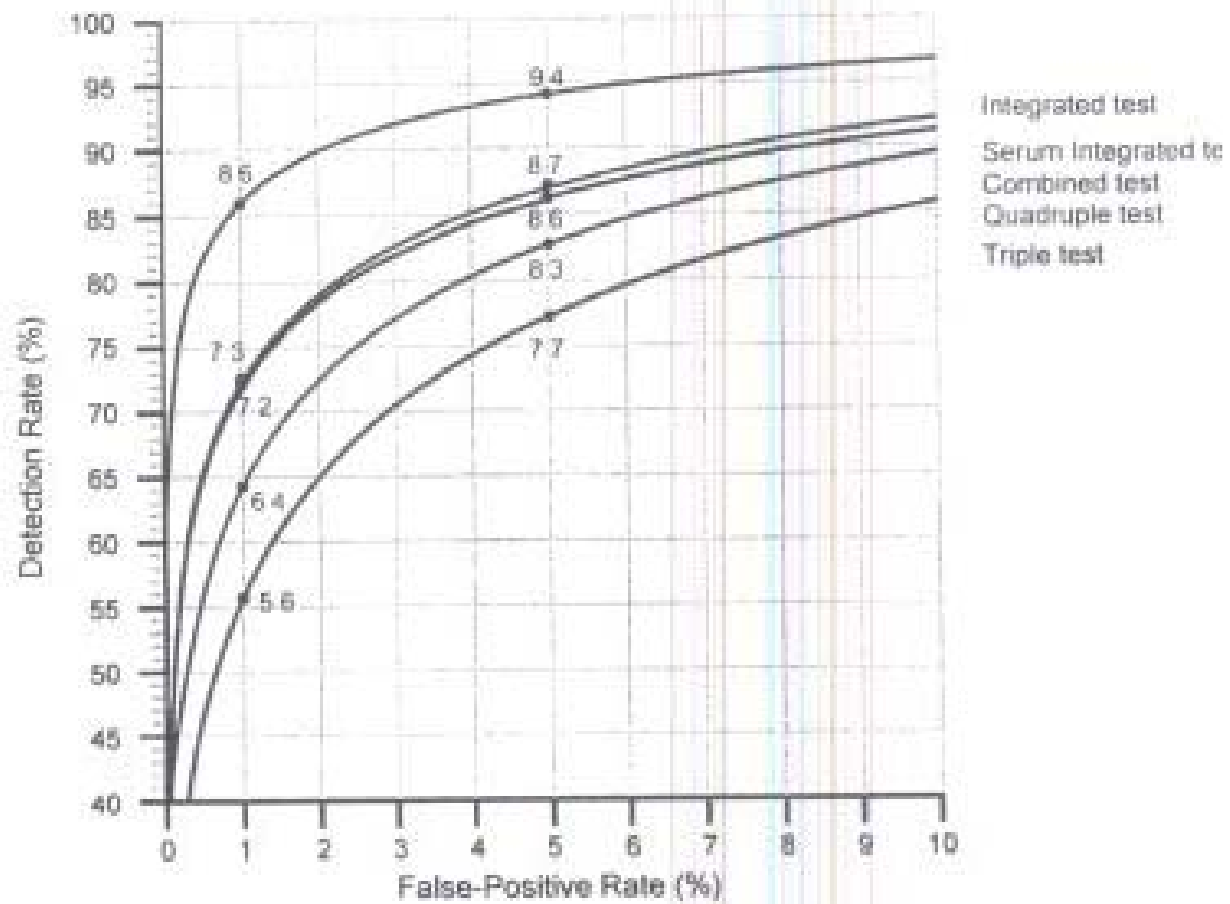


Fig. 4. Down's Syndrome detection rates and false-positive rates for specified screening tests.

Program performance- FASTER

(1st trimester markers at 12 weeks)	False positive rate	Detection rate
NT	23%	85%
Free beta hCG,PAPP-A,NT	4.8%	85%
PAPP-A,NT,AFP,hCG,uE3, DIA	0.8% or 5.0%	85% or 95%
PAPP-A,AFP,hCG,uE3,DIA	4.4%	85%
AFP,hCG,uE3	14%	85%
AFP,hCG,uE3,DIA	7.3%	85%
Sequential screening	11%	94%

DEBATE!!!

Should we screen:

- **in the first trimester of pregnancy?**
- or
- **in the second trimester of pregnancy?**
- or
- **in the first and the second trimester of pregnancy?**





**ISSUES AND
ARGUMENTS...**



Proponents of first trimester screening

- Pregnant ladies prefer EARLY prenatal diagnosis (first trimester Vs second trimester) and earlier termination of pregnancy
- Reassurance of screen negative results early in the pregnancy



Against first trimester screening

- Screening performance is lower in first trimester screening with increased patient anxiety
- Some affected pregnancies will be identified that would have spontaneously terminated a few weeks later



Proponents of second trimester screening

- 2nd trimester screening is well established and accepted
- AFP measurement allows for biochemical screening for open neural tube defects (not possible in first trimester)



Proponents of integrated screening

- Minimal benefit in early results of screening since diagnostic tools are more widely available in the second trimester (amniocentesis Vs chorionic-villus sampling)
- Debate over pros and cons of first and second trimester screening is unnecessary. With IPS, screening performance is better than all other screening modalities
- Less confusion to the patient, since she only receives one risk estimate



Against second trimester or integrated screening

- Karyotype results available approx. 2-3 weeks after amniocentesis (17-18 weeks at earliest)
- Removing 1st trimester diagnostic option if waiting for screen results of part 2 in second trimester
- Logistical difficulties and complexity in implementing IPS





**MORE ON FIRST TRIMESTER
SONOGRAPHIC MARKERS:**



Proponent of US markers

- 75% of trisomy 21 fetuses have increased nuchal translucency (NT)
- Increased NT associated with increased adverse fetal and neonatal outcome (chromosomal abnormalities, mortality, others)
- 65- 70% of trisomy 21 fetuses have absent or short nasal bone



Measurement of NT

- Needs for training, standard technique, quality assessment program
- NT should be measured before 14 weeks 0 day of gestation
- Fetal NT increases with gestational age and CRL



Measurement of nasal bone

- Needs for training (40-120 scans), standard technique, quality assessment program
- Measurement: 11 weeks 0 day to 13 weeks 6 days of gestation.
- Performance:
- False positive rate <1% (Caucasian)
- False positive rate 10% (Black)
- Detection rate (DS): 69%
- If no training by FMF, the performance decreases. Detection rate (for DS):0%

1st trimester US markers

- Other proposed markers for trisomy 21
- Abnormal flow velocity patterns in the ductus venosus, maxillary hypoplasia.
- No prospective studies with large number of patients



2nd trimester US markers

- Other proposed markers for trisomy 21
- Nuchal fold thickness, short humerus, short femur, echogenic bowel, etc.
- No prospective studies with large number of patients



Issues: NT

- Training of sonographers
- Availability outside of major cities
- Reproducibility of measurement
- Rate of unsuccessful measurements
- Unknown if NT-detected Down syndrome cases would be spontaneously lost early in pregnancy
- Divergence in the gestational age (11-14 weeks in UK Vs 10-14 weeks in USA)
- Need for high-resolution equipment
- Need for quality assessment

Examples of complexity of screening programs

Options that are offered in
Ontario, Canada

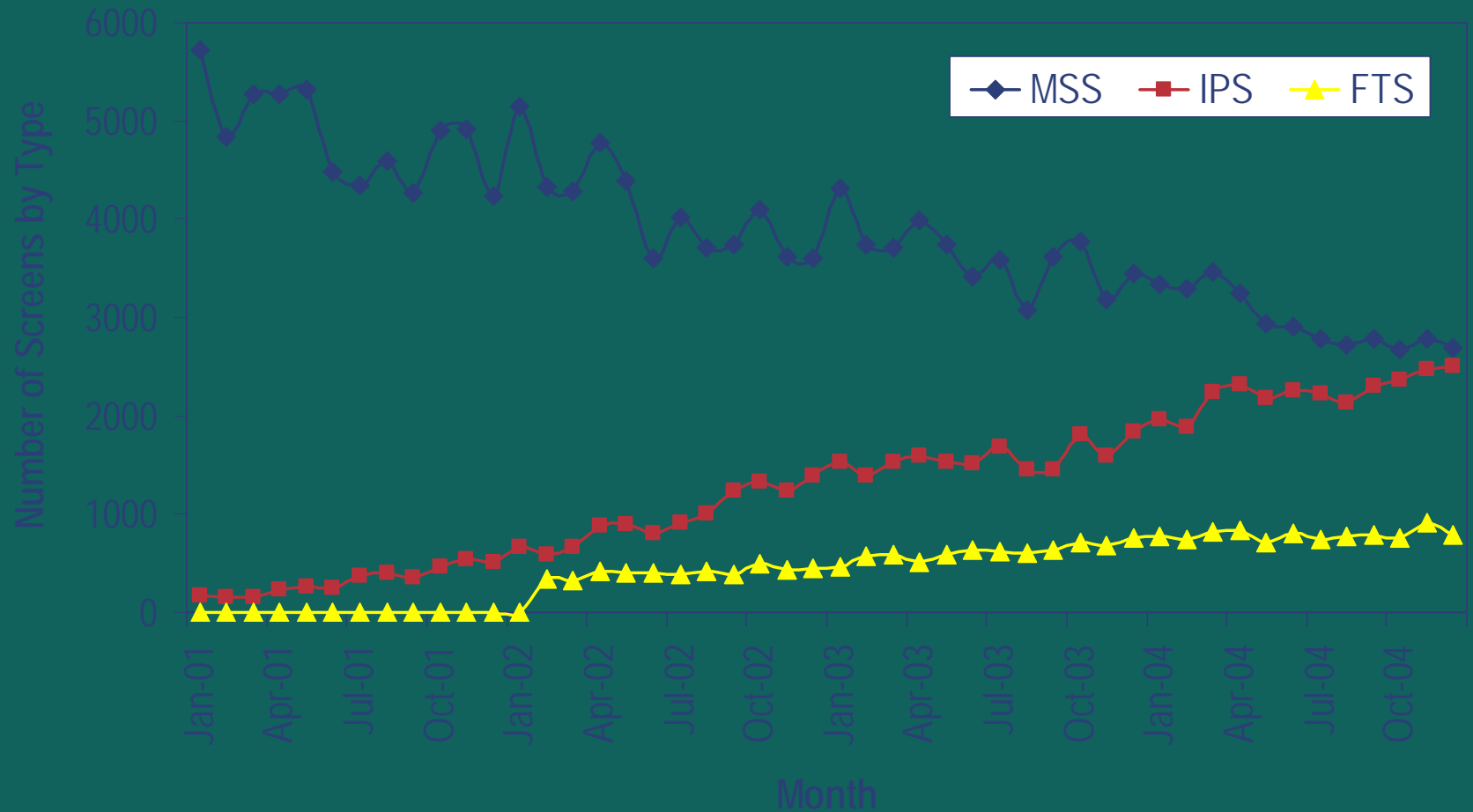


Choices in Ontario, 2006

First trimester	Free beta hCG + PAPP-A + NT
First trimester (when IPS patients do not show up for second trimester)	PAPP-A + NT
Integrated: First and second trimester	PAPP-A + NT (1 st) AFP + hCG + uE3 (2 nd)
Integrated: First and second trimester	PAPP-A (1 st) AFP + hCG + uE3 + DIA (2 nd)
Second trimester	AFP + hCG + uE3 + DIA



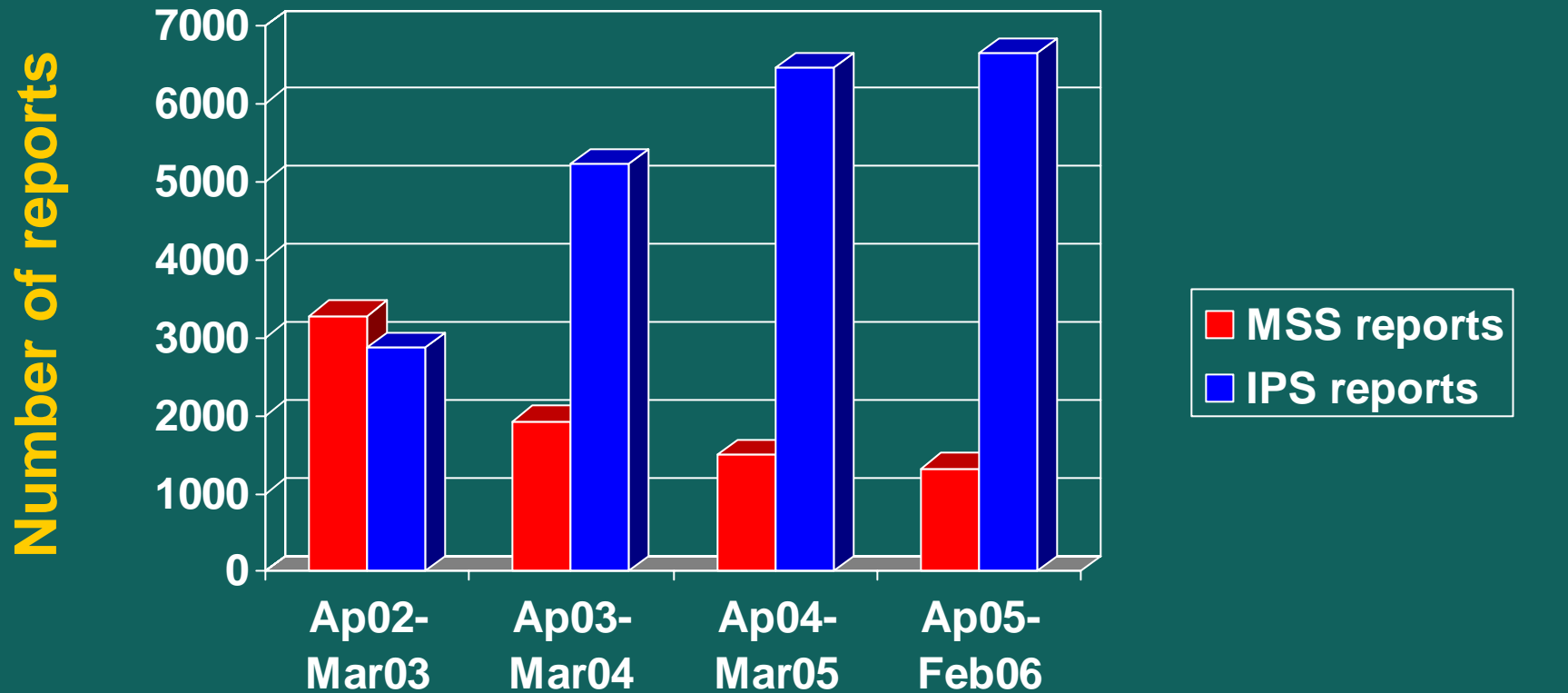
Changes in Enhanced Screening Practice in Ontario.



Slide provided by Joanne Miyazaki,
Quality Management program- Laboratory services



Statistics from Ottawa, Canada



Time period

(IPS started in July 02; MSS quad started in Dec 05)



SCREENING STATISTICS- Ottawa, Canada

- DETECTION RATE:

- IPS= 87.5%


- MSS triple= 72%

- INITIAL POSITIVE RATE:

- IPS= 5.2%

- MSS triple= 11%





Are there other usages of
first and second trimester
biochemical and sonographic
markers?



Use of prenatal screening biochemical markers for the prediction of fetal and neonatal complications

- Increased NT has been observed with aneuploidies other than trisomy 21, heart defects, genetic syndromes.
- Biochemical markers are used by some physicians to predict fetal and neonatal morbidity and mortality
- Will biochemical and US markers be used in a broader role in pregnancy monitoring, to screen for fetal and neonatal morbidity and mortality?





**Extreme levels of first
trimester Biochemical
markers**



1st trimester – Summary of publications (1)

Low PAPP-A	Intrauterine death/ miscarriage
Low PAPP-A	IUGR / low Birth weight
Low PAPP-A	Preeclampsia/ Gestational hypertension
Low PAPP-A	Fetal chromo + structural abnormalities
Low PAPP-A	Preterm delivery



1st trimester – Summary of publications (2)

Low free beta hCG	Miscarriage
Low free beta hCG	IUGR / low Birth weight
Low free beta hCG	Fetal chromo + structural abnormalities
Low free beta hCG	No Preeclampsia, No Gestational hypertension, No preterm delivery





**Extreme levels of second
trimester biochemical
markers**



Summary of 2nd trimester (high AFP, hCG, DS risk)

Adverse obstetric outcomes (Fetal death, Preeclampsia, SGA)

Fetal chromo abnormalities

Adverse neonatal outcomes (death, low birth weight)

Contradicting results:

Predictor of Fetal structural abnormalities?



Use of prenatal screening biochemical markers for the prediction of fetal and neonatal complications

Still under evaluation

No consensus on the MoM levels of biochemical markers that should be brought to attention of requesting health care providers

No prospective studies with large number of patients



Quality Management of Multiple Marker Prenatal Screening Ontario

- Comprehensive program for monitoring Biochemical markers and NT for Ontario is under development



Ontario prenatal Database

- Since 1993, Ontario repository of prenatal screening data
- Dependent of input from multiple sources
- Includes information on:
 - Patient demographics, Pregnancy details, Test and interpretation results, Counselling, cytogenetics and ultrasound, Birth outcomes



*Slide provided by Joanne Miyazaki,
Quality Management program- Laboratory services*

Current quality assurance

- Biochemical markers: external quality assessment via worldwide organizations
- Biochemical markers and initial screen positive rate: Ontario MSS database
- NT: local monitoring of sonographers and centers. This program could become an Ontario-wide monitoring program with involvement from several professional corporations




Ontario MSS Database QA

Since 1997, monthly review of:

- Analytical performance (AFP, uE₃ and hCG)
- Initial Positive rates (Down Syndrome, ONTD and Trisomy 18)
- Outcome driven



*Slide provided by Joanne Miyazaki,
Quality Management program- Laboratory services*



**Continued search
for
improved prenatal screening test
for Down syndrome**



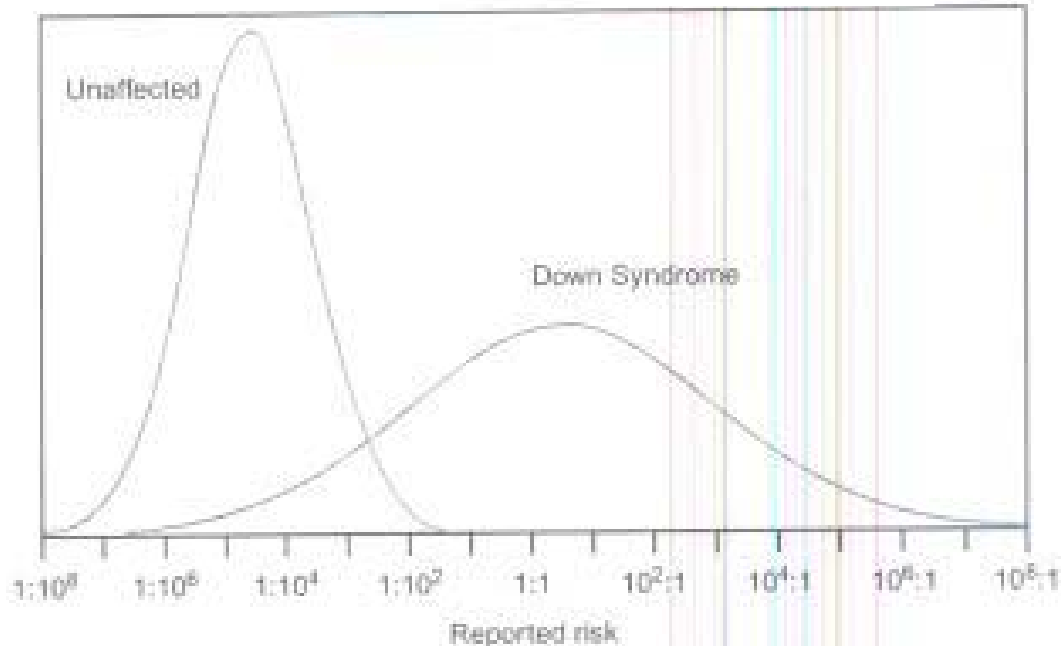


Figure 3. The distribution of the reported risk of having a Down Syndrome birth using the integrated test (first trimester nuchal translucency thickness and PAPP-A and second trimester AFP, uE₃, total hCG and inhibin-A) in affected and unaffected term pregnancies. Produced using statistical parameters cited by Wald et al.¹¹

Goal: to find combination of markers to reach two separate curves
To entirely distinguish unaffected Vs Down syndrome pregnancies



Potential new biochemical markers

or

**actual biochemical markers with different
measuring time window**



Varying time windows

1. Measurement of 1st trimester markers at two time intervals:

a) PAPP-A and NT

Time window: 10-11 weeks

b) Free beta hCG

Time window: 13 weeks

2. Dimeric Inhibin A

Time window: 7-11 weeks



Screening with new markers

1. Proform of eosinophil major basic protein (ProMBP)

Time window: 7-9 weeks; 15-19 weeks

2. Pregnancy-specific beta 1-glycoprotein (SP1)

Time window: 7-9 weeks; 10-12 weeks

3. Invasive trophoblastic antigen (ITA)

Time window: 15-20 weeks



Described in literature, still under development

Non-invasive prenatal diagnosis with fetal cells from the maternal circulation

Technical obstacles with maternal blood:

- **Maternal blood: 1-2 fetal cells per 10 million maternal cells**
- **Needs to isolate fetal cells, then to count the number of chromosomes 21 (molecular cytogenetics or PCR)**




Conclusion

- Based on laboratory performance, the best screening test for pregnant patients is:

Integrated prenatal screening

- Based on individual physician perspective, the best screening test for pregnant patients could be:

First trimester screening... integrated prenatal screening... sequential screening... integrated serum screening...



There are several remaining questions on best strategies: there are several screening options with varying screening protocols, no consensus.



Remaining questions (1)

1.- Screening protocol(s) for Down syndrome in:

- Twin pregnancies
- False positive patients in last pregnancy



Remaining questions (2)

2.- Screening protocol(s) for Down syndrome to include:

- Ultrasound markers 1st and/or 2nd trimester

3.- Screening protocol(s) for trisomy 18 in:

- Twin pregnancies
- First trimester only



REFERENCES

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- **Wald NJ et al. SURUSS in perspective. BJOG: 521-531, 2004.**
- **Note: this is a limited list of references; selection was made based on number of cases and controls included in the publications.**

