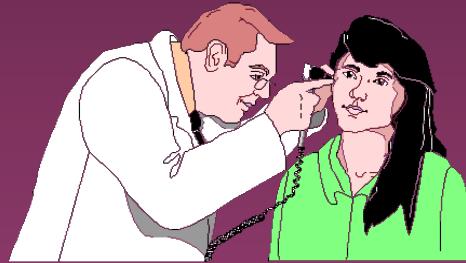**Laboratoire d'Hormonologie, Maternité**

DOWN'S SYNDROME SCREENING
Paul Bischof, PhD

DOWN'S SYNDROME SCREENING

- DETECTING DOWN'S
- CALCULATING THE RISK
- A WORD ABOUT HCG
- A WORD ABOUT PAPP-A
- COMPARISON BETWEEN TESTS
- WHAT DO WE NEED IN PRACTICE

# DETECTING DOWN'S

THE RISK AT TERM OF CARYING A TRISOMY 21 CHILD CAN BE EVALUATED INDIVIDUALLY BETWEEN 10 3/7 AND 13 6/7 AND BEWEEN 14 0/7 AND 18 6/7 WEEKS OF PREGNANCY BY COMBINING SERUM ASSAYS AND ULTRASONOGRAPHIC MEASUREMENTS

DETECTING DOWN'S

FIRST TRIMESTER 10 3/7 TO 13 6/7 WEEKS	SECOND TRIMESTER 14 0/7 TO 18 6/7 WEEKS
PAPP-A + free Beta hCG	AFP + free Beta hCG,
NT	AFP + hCG +uOestriol
CRL	BPD
Maternal Age	Maternal Age
Maternal Weight	Maternal Weight
Obstet. History	Obstet. History

A PRECISE AND REPRODUCIBLE MEASUREMENT OF NUCHAL TRANSLUCENCY
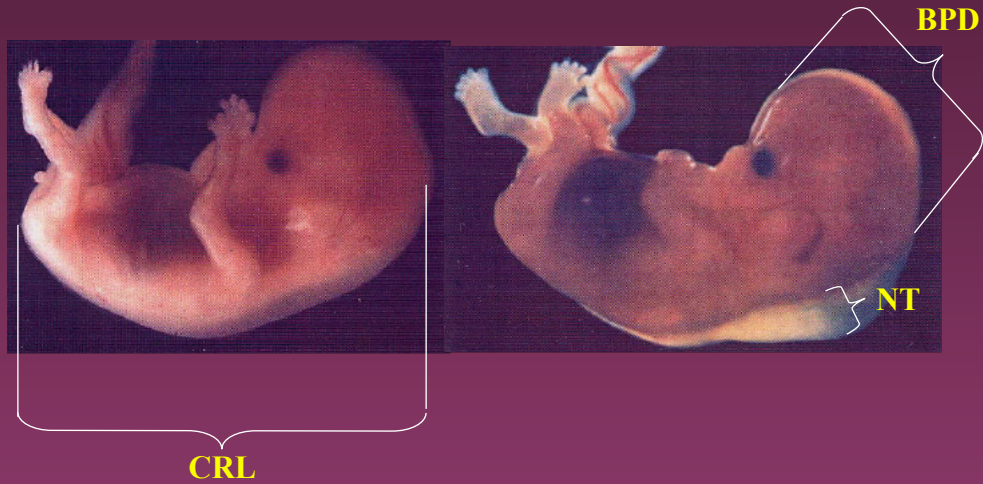
Marker	#Studies	# Down's	MoMs	CI
hCG	9	156	1.17	1.09-1.26
AFP	12	154	0.77	0.71-0.83
Free beta hCG	5	93	**1.86**	1.56-2.22
PAPP-A	5	91	**0.36**	0.27-0.49
uE3	4	64	0.66	0.54-0.80
free alpha hCG	3	51	0.97	0.75-1.26
SP1	4	54	0.71	0.47-1.06
CA125	2	23	0.87	0.26-2.90
Alk. Phos.	1	21	0.97	0.57-1.66
Inhibin	1	77	1.19	1.05-1.35

DOWN'S SYNDROME SCREENING

Calculating the risk

DOWN'S SYNDROME SCREENING

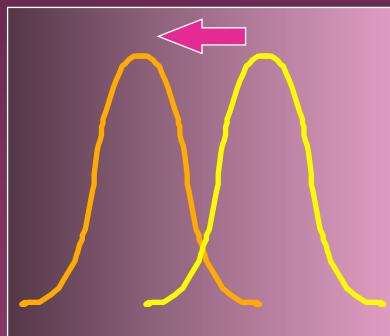| AGE	RISK
21	1507
23	1447
25	1305
27	1208
29	1018
31	797
32	683
33	575
34	474
35	380
36	308
37	243
38	190
39	147
40	113
41	87
42	66
43	50
44	38
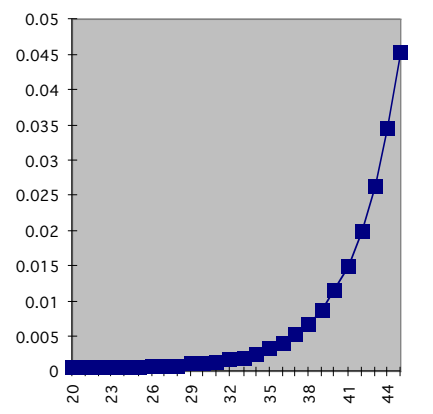
CALCULATING THE RISK
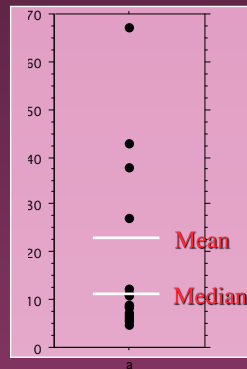
Control values are regressed by days of pregnancy and expressed in Medians per Day.

The individual values are devided by the median corresponding to the same gestational age as the control population.
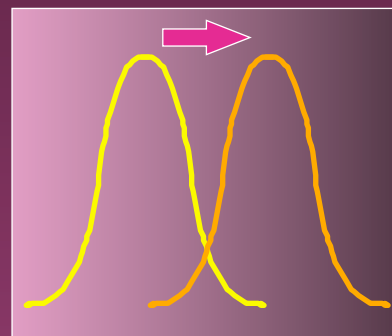
MoM

CALCULATING THE RISK

PAPP-A is dereased in Down's

Free beta hCG is increased in Down's
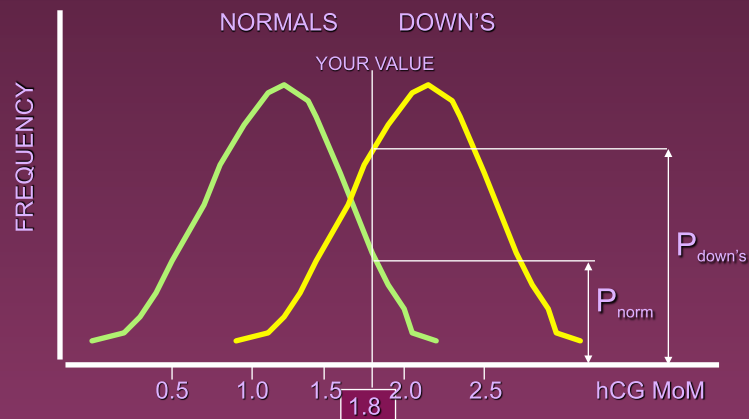
CALCULATING THE RISK

$$F_{hCG} = P_{down's} \big/ P_{norm} \qquad F_{AFP} = P_{down's} \big/ P_{norm}$$

RISK=   AGE RISK X F_{hCG} X F_{AFP}

Corrected for weight, number of foetuses and ethnic group

DOWN'S SYNDROME SCREENING

A word about hCG

Human Chorionic Gonadotropin

A WORD ABOUT hCG

N=636

(y-axis: hCG 10³ mIU/ml — values 0, 12, 25, 50, 100; x-axis: Weeks from LMP — 8 12 16 20 24 28 32 36 40)
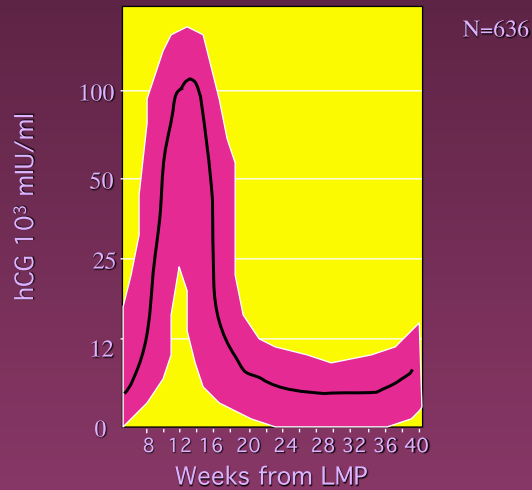
A WORD ABOUT hCG

THE STRUCTURE OF HUMAN CHORIONIC GONADOTROPIN

αSubunit
92 amino acids
5 disulfide bridges
2 oligosaccharide sites

βSubunit
145 amino acids
6 disulfide bridges
6 oligosaccharide sites

A WORD ABOUT hCG

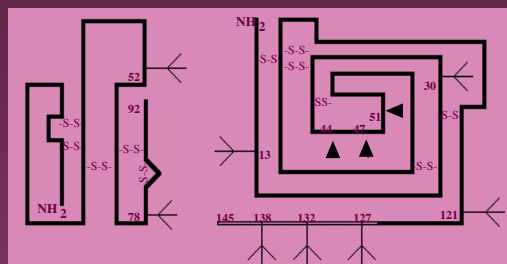| Holo hCG	Nicked hCG	Free β hCG	Nicked free β	Free α hCG
FIirst trimester pregnancy				
100	<1 - >1	<1	<1 ->1	0.3 - 4
Hydatidiform molar pregnancy				
100 ✐	?	10 - 37	8 - 24	> 0.3 - 4
Choriocarcinoma				
100 ✐	?	> 30	> 24	> 0.3 - 4
Second trimester pregnancy				
100 ↘	<1	<1	?	10 - 30
Down's syndrome				
100 ✐	>2	>2	✐	10 - 30

A WORD ABOUT hCG
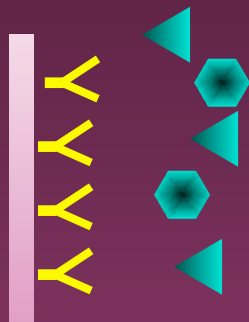
CAPTURING ANTIBODY   DETECTING ANTIBODY
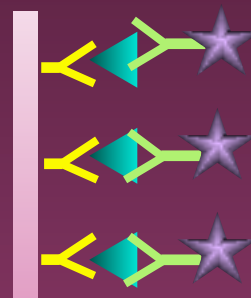
A WORD ABOUT hCG
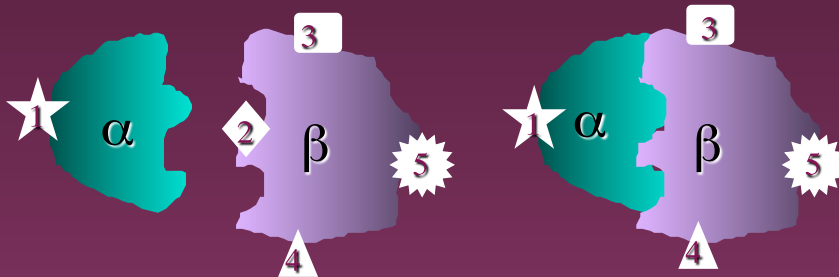
Free beta hCG Capturing epitope 2 Detecting epitope 3, 4 or 5
Total hCG   Capturing epitope 3 Detecting epitope 1,4 or 5
Holo hCG   Capturing epitope 1 Detecting epitope 3, 4 or 5

A WORD ABOUT hCG

EFFECT OF STORAGE AT ROOM TEMPERATURE

HEPARINIZED PLASMA   **HEPARINIZED BLOOD**

Free Beta hCG
(Percent from initial value)

Time from venipuncture in hours
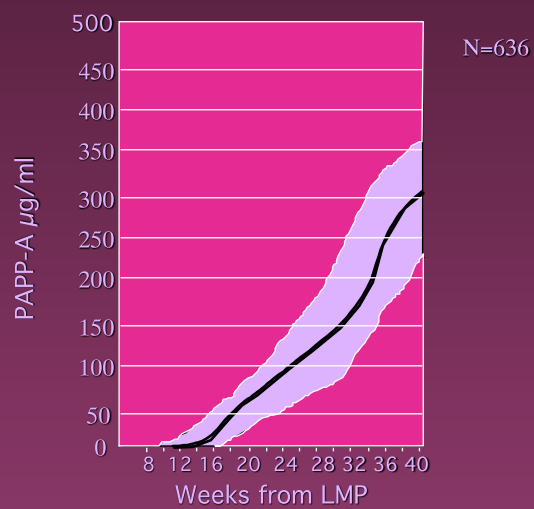
A WORD ABOUT PAPP-A

A WORD ABOUT PAPP-A

A WORD ABOUT PAPP-A

800 000

200 000   400 000

—S —   —S —

—S —   —S —
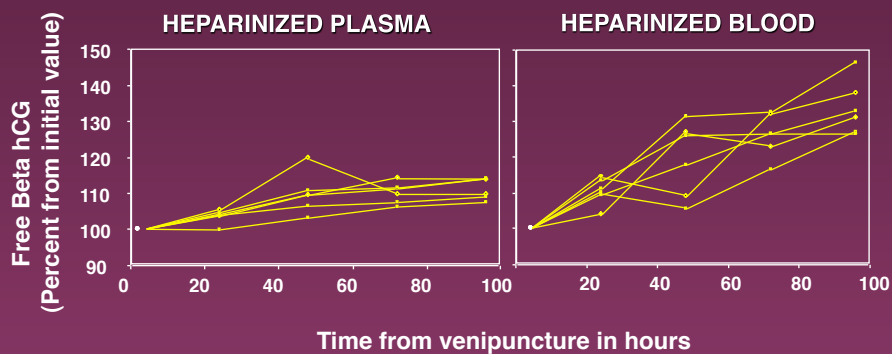
proMBP

Major Basic Protein
(Eosinophiles et placenta)
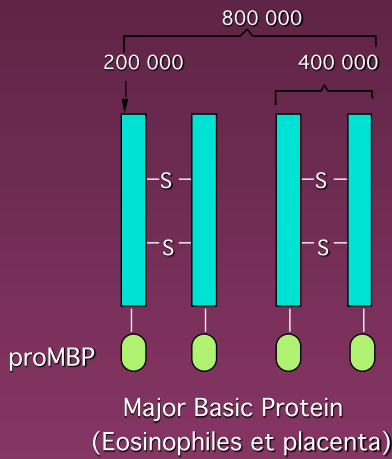
PREGNANCY ASSOCIATED
PLASMA PROTEIN-A   (PAPP-A)

Glycoprotein
Produced by the placenta
Binds Heparin
Binds proMBP

Contains Zn++

Gene on chr. 9

A WORD ABOUT PAPP-A

Available Assays to Measure PAPP-A

Polyclonal Antibodies		
Bischof et al 1979	Immunoelectrophoresis
Folkersen et al 1981	Immunoelectrophoresis
Bischof et al 1981	Radioimmunoassay
Sinosich et al 1982	Radioimmunoassay
Anthony et al 1983	Radioimmunoassay
Pinto-Furtaldo et al 1984	Radioimmunoassay
Bersinger et al 1983	Enzyme immunoassay
Pledger & Bellfield 1983	Enzyme immunoassay
Mac Donald et al 1984	Enzyme immunoassay

Monoclonal Antibodies		
Amersham 1997	Enzyme immunoassay
Johnson & Johnson 1997	Enzyme immunoassay
Cis bio International 1998	Chemie luminescence

DOWN'S SYNDROME SCREENING

PAPP-A is an IGFBP-4 protease

steroidogenesis
mitogen
invasion
aa uptake

IGF II

IGFBP-4

proMBP

PAPP-A

proMBP

IGFBP-4 IGF II

PAPP-A

IGFBP-4 IGF II + IGF II

DOWN'S SYNDROME SCREENING

IGFBP-4

IGF II

PAPP-A

IVS

Decidual Cells

Extravillous cytotrophoblast

Anchoring villous

Syncytio-trophoblast

Floating villous
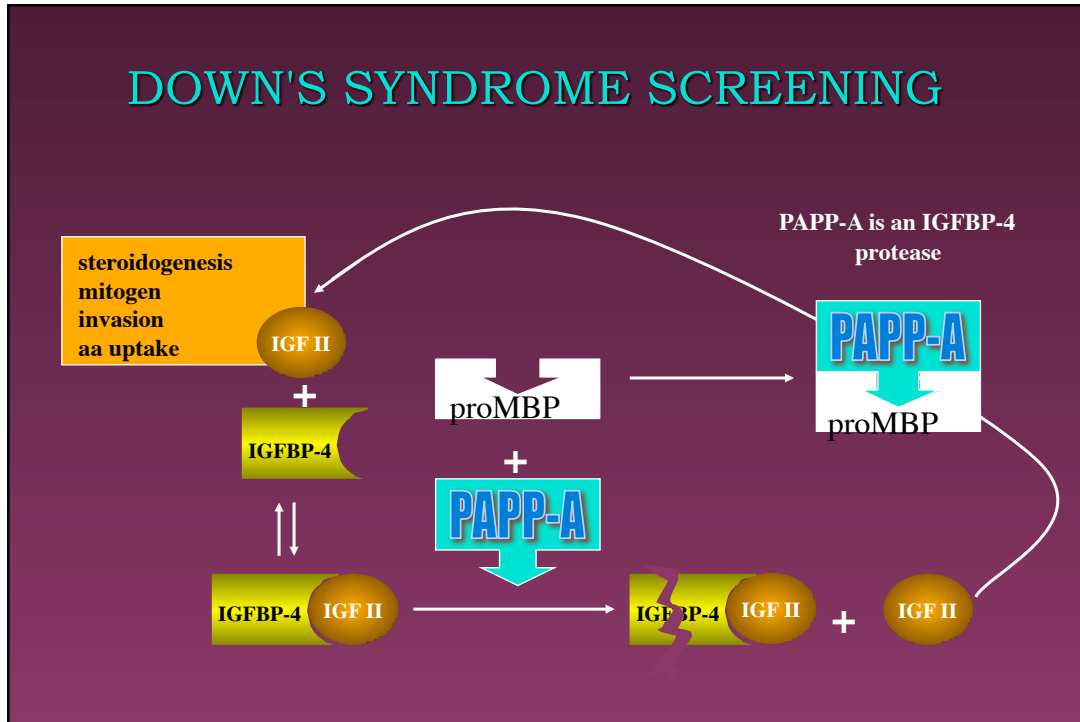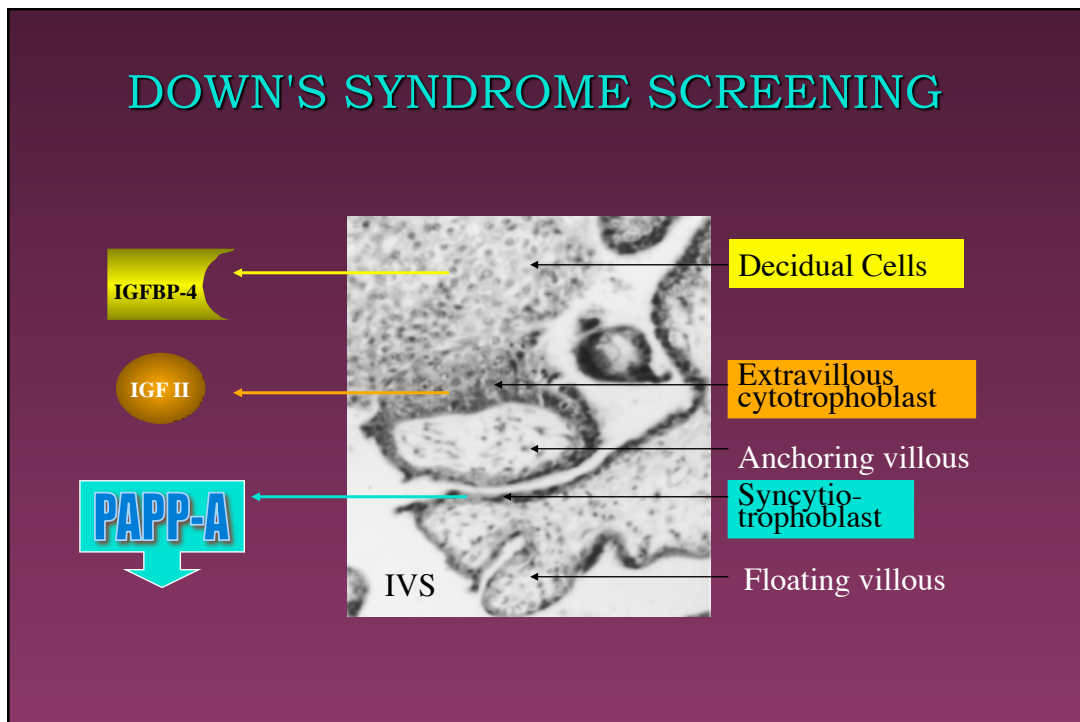
A WORD ABOUT PAPP-A

PAPP-A Measurements on Kryptor

955 Normal pregnancies between 10 and 13 Weeks and 127 Down's

Gest. Age Weeks	N	Medians mu/ml
10	37	1.48
11	304	2.20
12	427	3.13
13	187	4.71

K. Spencer

A WORD ABOUT PAPP-A

PAPP-A Measurements on Kryptor

Median MoMs in Down's

2.19 MoM for Free Beta

0.45 MoM for PAPP-A

2.40 MoM Nuchal Translucency

K. Spencer

A WORD ABOUT PAPP-A

PAPP-A Measurements on Kryptor

Detection rates

30% with free beta
39% with PAPP-A
43% with PAPP-A and age
47% with free beta and age
61% with free beta, PAPP-A and age
85% with free beta, PAPP-A, age and nuchal transl.

K. Spencer

DOWN'S SYNDROME SCREENING

Comparison
between tests

DOWN'S SYNDROME SCREENING
Retrospective Study
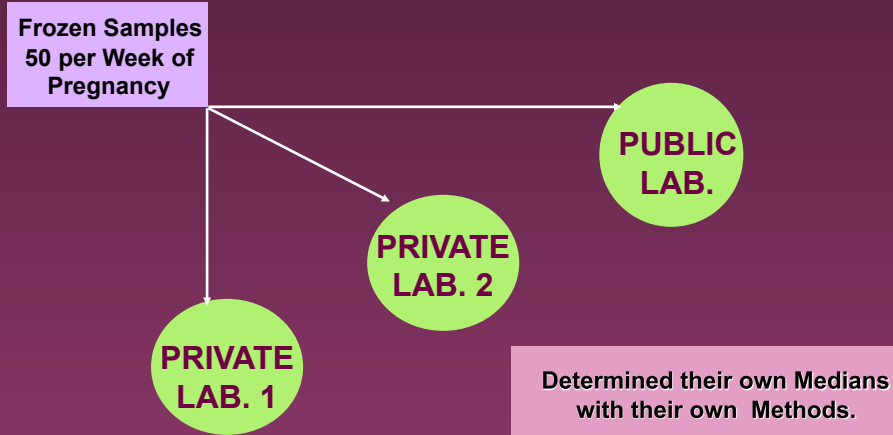
Frozen Samples
50 per Week of
Pregnancy

PUBLIC
LAB.

PRIVATE
LAB. 2

PRIVATE
LAB. 1

Determined their own Medians
with their own Methods.

DOWN'S SYNDROME SCREENING
Retrospective Study

Blood Samples

PUBLIC
LAB.

hCG, AFP
uE_3 , FBhCG

PRIVATE
LAB. 2

PRIVATE
LAB. 1

hCG, AFP

Calculate the risks
based on MoM
using What if (LMS)
and CIS bio
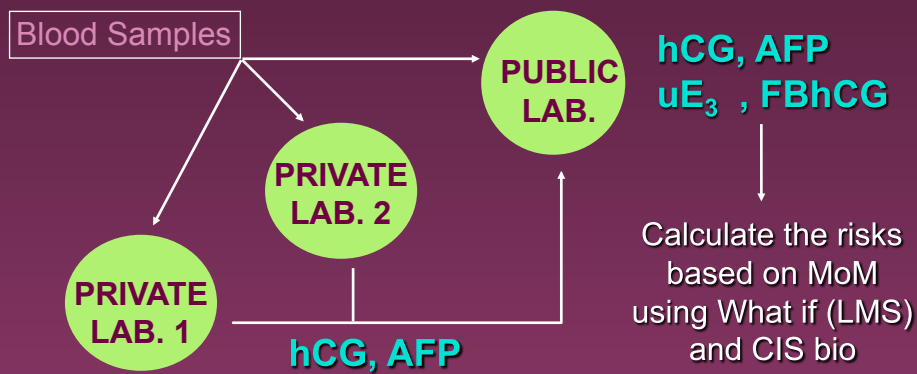
DOWN'S SYNDROME SCREENING
Retrospective Study

	AFP	hCG	FBhCG	uE$_3$
Private Lab. 1	ES 600	Stratus		
Private Lab. 2	IMX	IMX*		
Public Lab.	IMX	IMX**	CIS bio	Kodak

* holo hCG, ** total hCG

DOWN'S SYNDROME SCREENING
Retrospective Study

2516 pregnant women bled between 15 & 18 weeks
(from LMP and/or by scan)

18 stored Down's syndrome serum samples
5 prospective Down's syndrome serum samples

DOWN'S SYNDROME SCREENING
Retrospective Study

2516

5.3% > 35 years of age
Median 36.2 years.

94.7% < 35 years of age
Median: 29.1 years.

Detection rate of Down's according to serum markers (>1:380)

	TT AFP hCG uE_3		DT AFP FBhCG		TTFB AFP FBhCG uE_3	
	N	%	N	%	N	%
Stored samples (N= 18)	12	67	15	83	14	78
Per-study samples (N= 5)	3	60	2	40	1	20
TOTAL (N= 23)	15	65	17	74	15	65

DOWN'S SYNDROME SCREENING
Retrospective Study

False positives at a cut-off risk of 1:380

Weeks	N	TT		DT		TTFB	
		n	%	n	%	n	%
15	442	52	11.8	29	6.6	36	8.1
16	1368	95	6.9	90	6.6	82	6.0
17	558	39	7.0	36	6.5	34	6.1
18	148	16	10.8	12	8.1	13	8.8
	2516	202	8.0*	167	6.6*	165	6.6*

p<0.0037

p<0.013

DOWN'S SYNDROME SCREENING
Retrospective Study

CONCLUSIONS

Unconjugated oestriol does not add any significant advantage to the combined use of AFP and FBhCG in terms of detection rate or false positive rate.

FBhCG does not add any significant advantage over hCG to detect Down's syndromes.

FBhCG when used instead of hCG together with AFP or with AFP and unconjugated oestriol significantly reduces the number of false positive cases at a cut off risk of 1:380.

DOWN'S SYNDROME SCREENING
Prospective Study

	AFP	FBhCG
Private Lab. 1	ES 600	
Private Lab. 2	IMX	CIS bio
Public Lab.	IMX	CIS bio

CALCULATES ALL RISK FACTORS

DOWN'S SYNDROME SCREENING
Prospective Study

HOW GOOD WAS OUR **DOUBLE TEST** USING CIS FBETA AND IMX AFP

7027 mothers

<35 years: 6559 (93%) Risk<1/380 6107 (93.1%)
Risk>1/380   452 (6.9%)

>35 years: 468 (6.7%) Risk<1/380   339 (72.4%)
Risk>1/380   129 (27.6%)

DOWN'S SYNDROME SCREENING
Prospective Study

7027 mothers

1O DOWN'S IN WOMEN <35 YEARS, 8 DOWN'S DETECTED

2 DOWN'S IN WOMEN >35 YEARS, 1 DOWN'S DETECTED

---

12 DOWN'S   **9 DOWN'S DETECTED**

DETECTION RATE 75%

DOWN'S SYNDROME SCREENING

OTHER ANOMALIES DETECTED:

1 XO, 1 MATERNAL TRANSLOCATION, 3 INVERSIONS OF
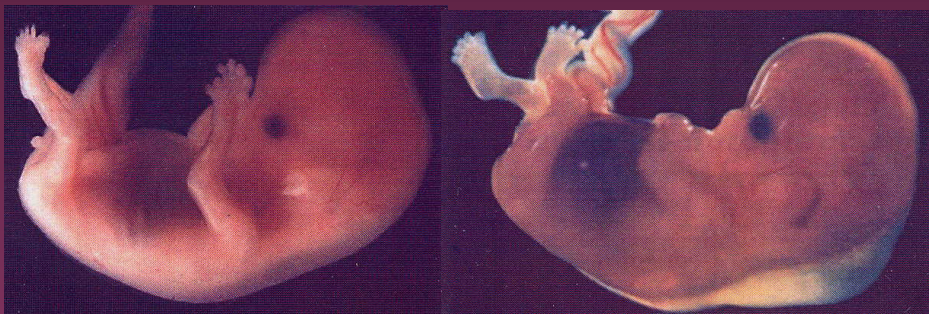PATERNAL ORIGIN, 1 XXY, 1 CRI DU CHAT

NOT DETECTED:

1 TRISOMY 18 WITH LOW FBETA

WHAT DO WE NEED IN PRACTICE

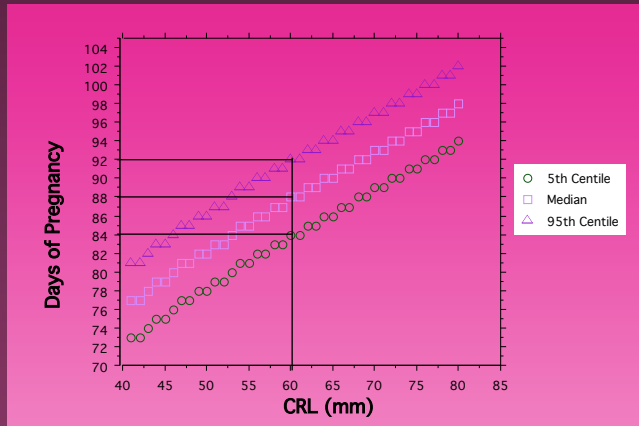# WHAT DO WE NEED IN PRACTICE

A PRECISE AND REPRODUCIBLE MEASUREMENT OF NUCHAL TRANSLUCENCY
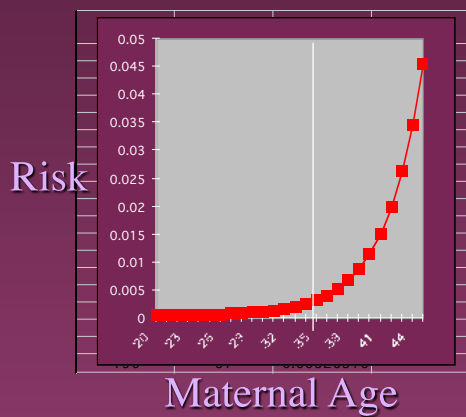
WHAT DO WE NEED IN PRACTICE

Robinson & Fleming 1975

LMP

CRL only

WHAT DO WE NEED IN PRACTICE

The a priori risk includes

Risk

Maternal Age

+

Obstetric History: Previous Down's Syndrome

WHAT DO WE NEED IN PRACTICE

**WEIGT
IS
IMPORTANT**

WHAT WE DONT WANT IN PRACTICE

**BIOCHEMISTRY ALONE IN
THE FIRST TRIMESTER**

**RISK ASSESSMENT IN FIRST
AND SECOND TRIMESTER**

SOME EXAMPLES

M o M s				
free β hCG	PAPP-A	NT	Risk	Outcome
0.71	1.91	0.89	1: 8348	N
3.33	0.45	0.61	1:128	FP
3.67	0.45	0.94	1:180	T21
3.36	0.44	4.68	1: 9	X0

Results kindly provided by *Dianalab* Geneva

When I said I, I meant We and when I said We I meant They

Top to bottom & left to right

Chantal Souverain
Nicole Jastrow
Luise Haenggeli
Melvina Araman
Elivire Henrichoz
Kim Truong
Arielle Meisser
Christine Wuillemein
Dorina Islami
Jocelyne Hediger
Claire Gruffat