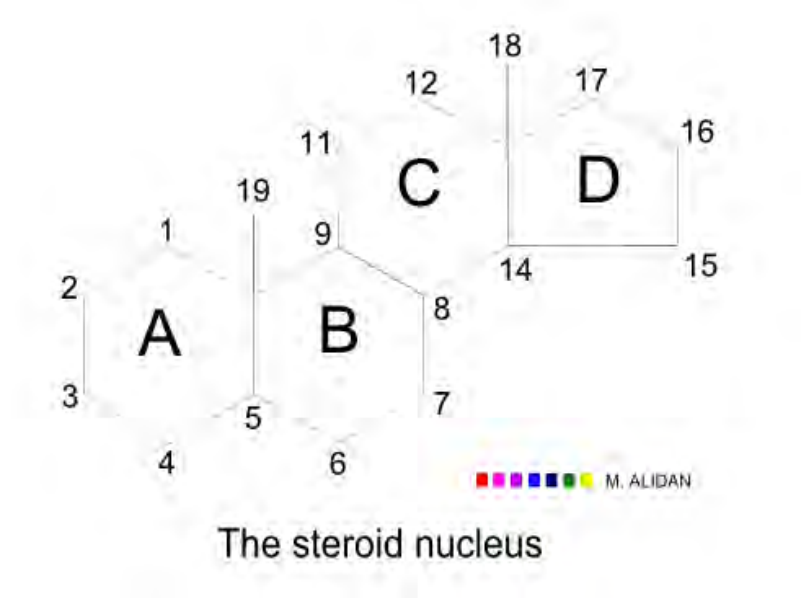
Steroids

Ronda Greaves

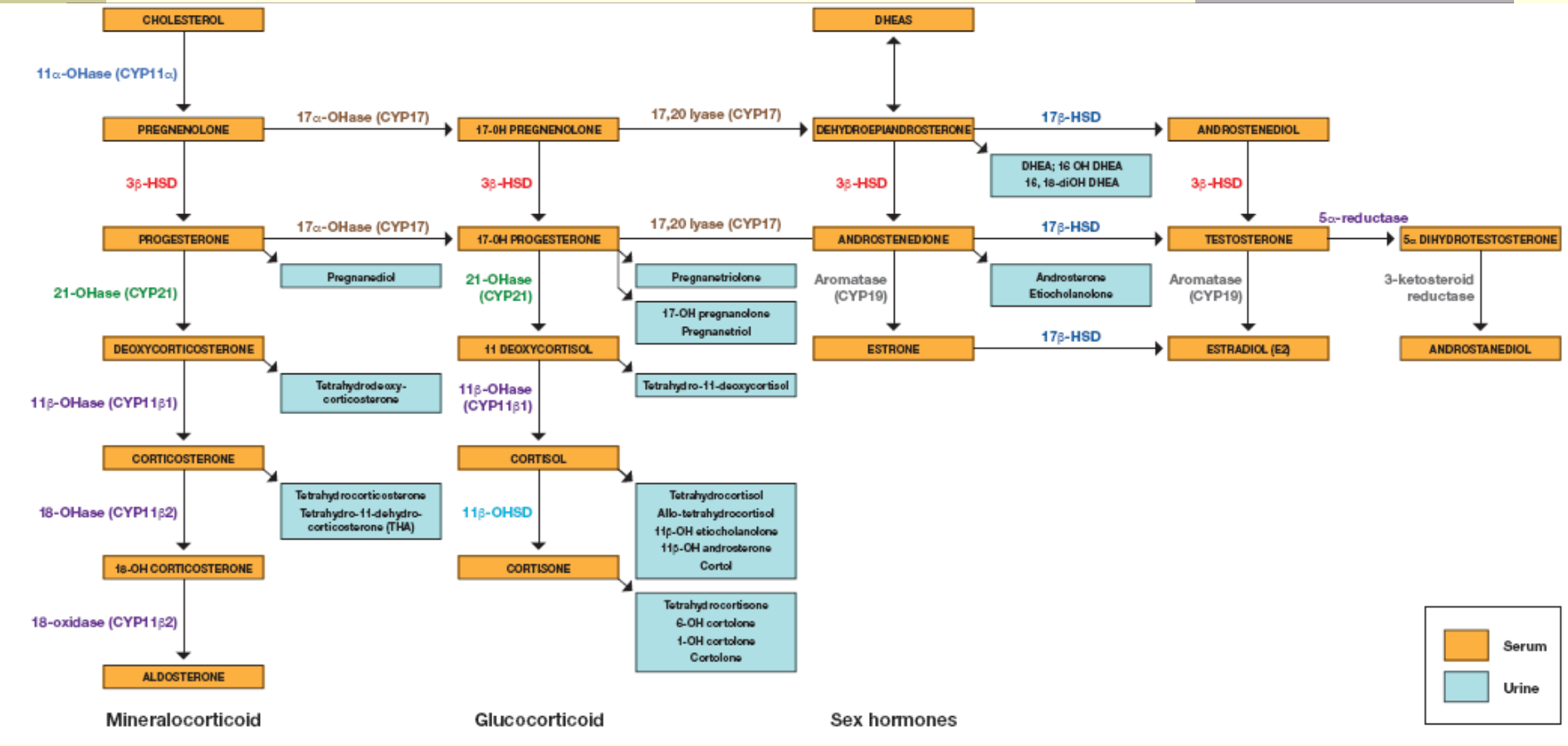
Overview

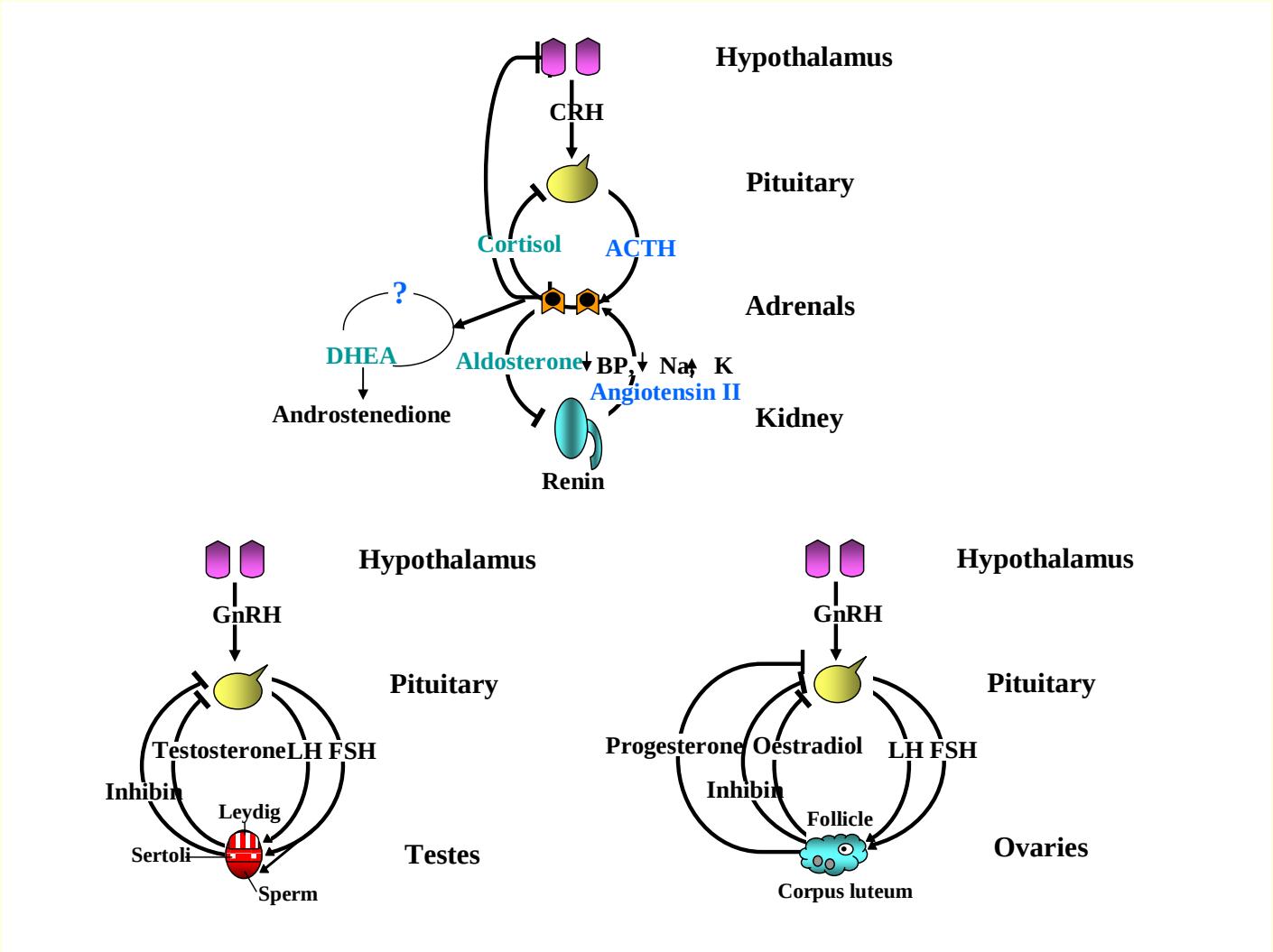


Cyclopentanophenanthrene ring

- **Structure + Pathway**
- **Foetal Adrenal Gland**
- **Analysis**
- **Enzyme Blocks**
- **Moodle**

Steroid Pathway

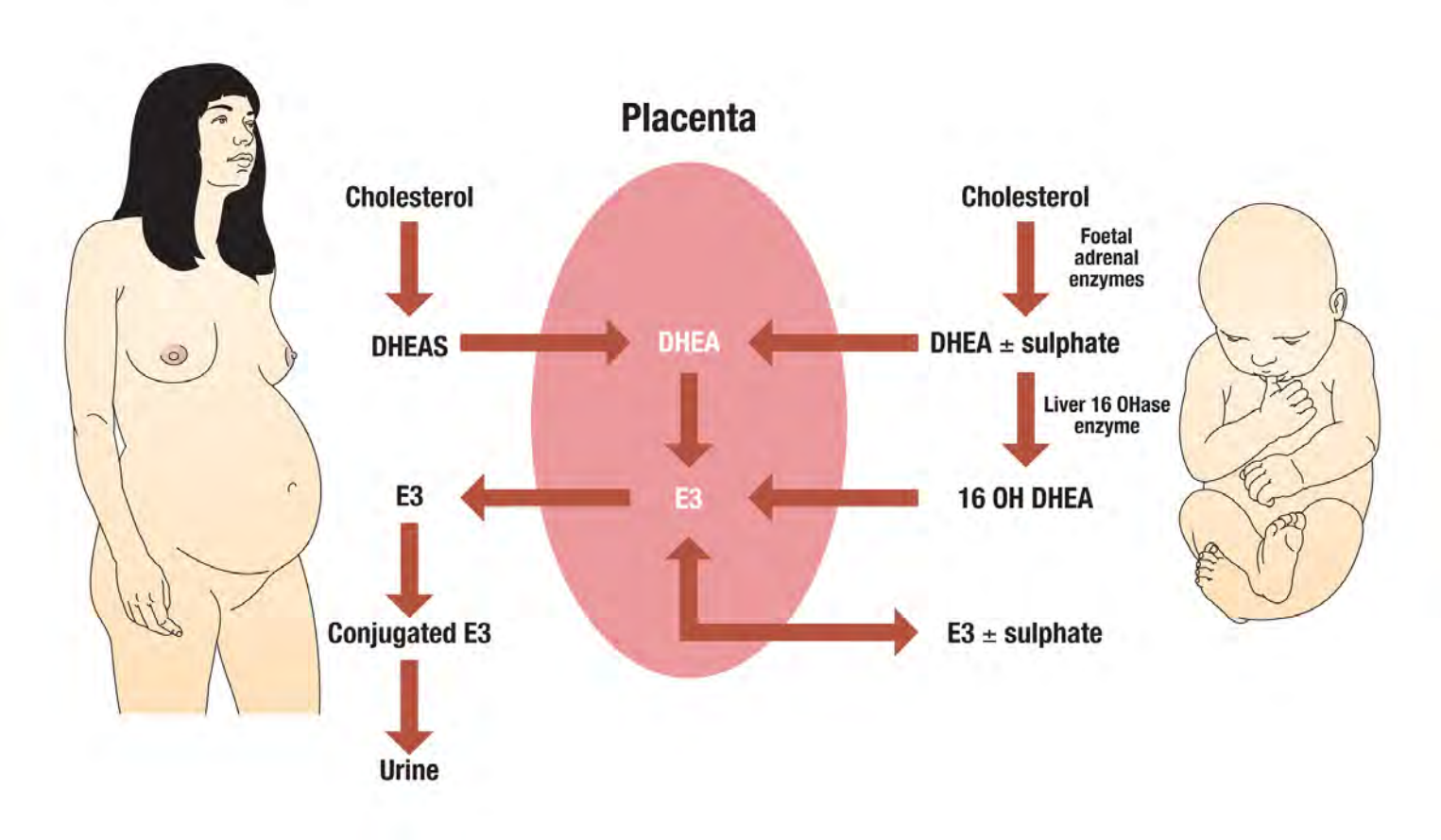




Diagrams courtesy of Dr Anna Lauber, Kinderspital Zurich

Foetal Adrenal Zone & Premature infants

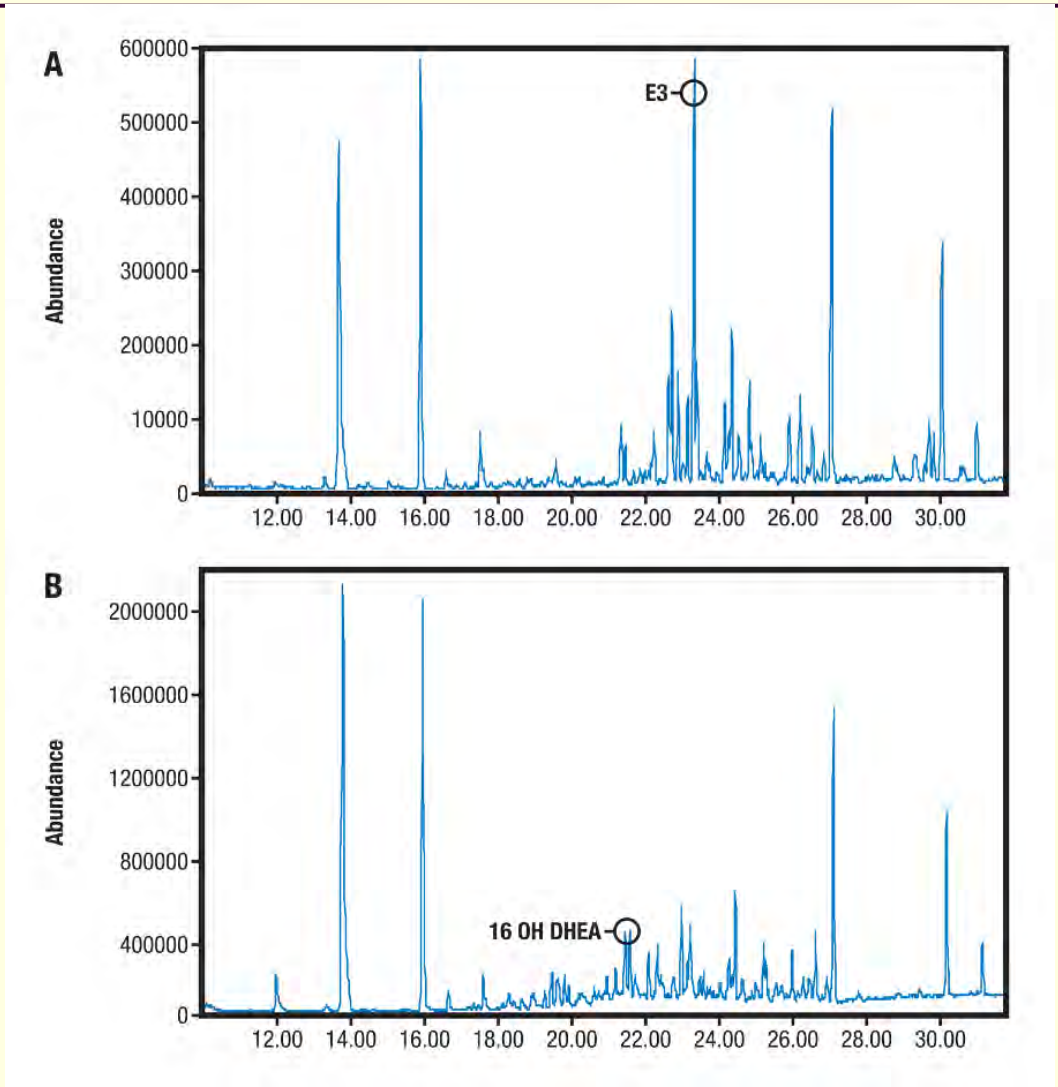
Maternal – Foetal interaction



Steroid hormone concentrations in cord blood (umol/L)

Steroid	Cord blood	Adult
DHEA	4	2-10
16a-OH DHEA	8	
16-oxo-androstenediol	2	
5-androstene-3b,16a, 17b-triol	1	
Pregnenolone	4	
16a-OH pregnenolone	3	
17OH pregnenolone	3	
5-androstene-3b, 17b-diol	8	0.2
5-androstene-3b, 17a-diol	5	1
5-pregnene-3b, 20a-diol	4	1
21 OH pregnenolone	3	
5-pregnene-3b, 20a, 21-triol	1	
5-pregnene-3b, 17a, 20a-triol	1.5	0.2

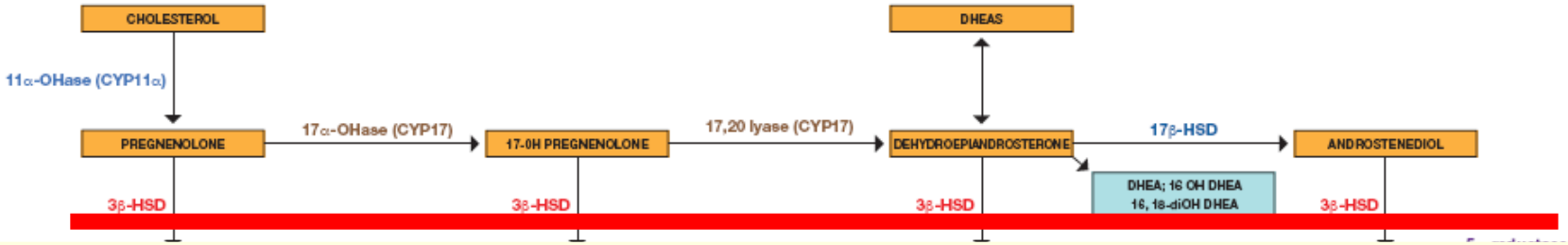
Steroid chromatograms



Full term neonate
4 days of age

Preterm neonate
6 weeks of age

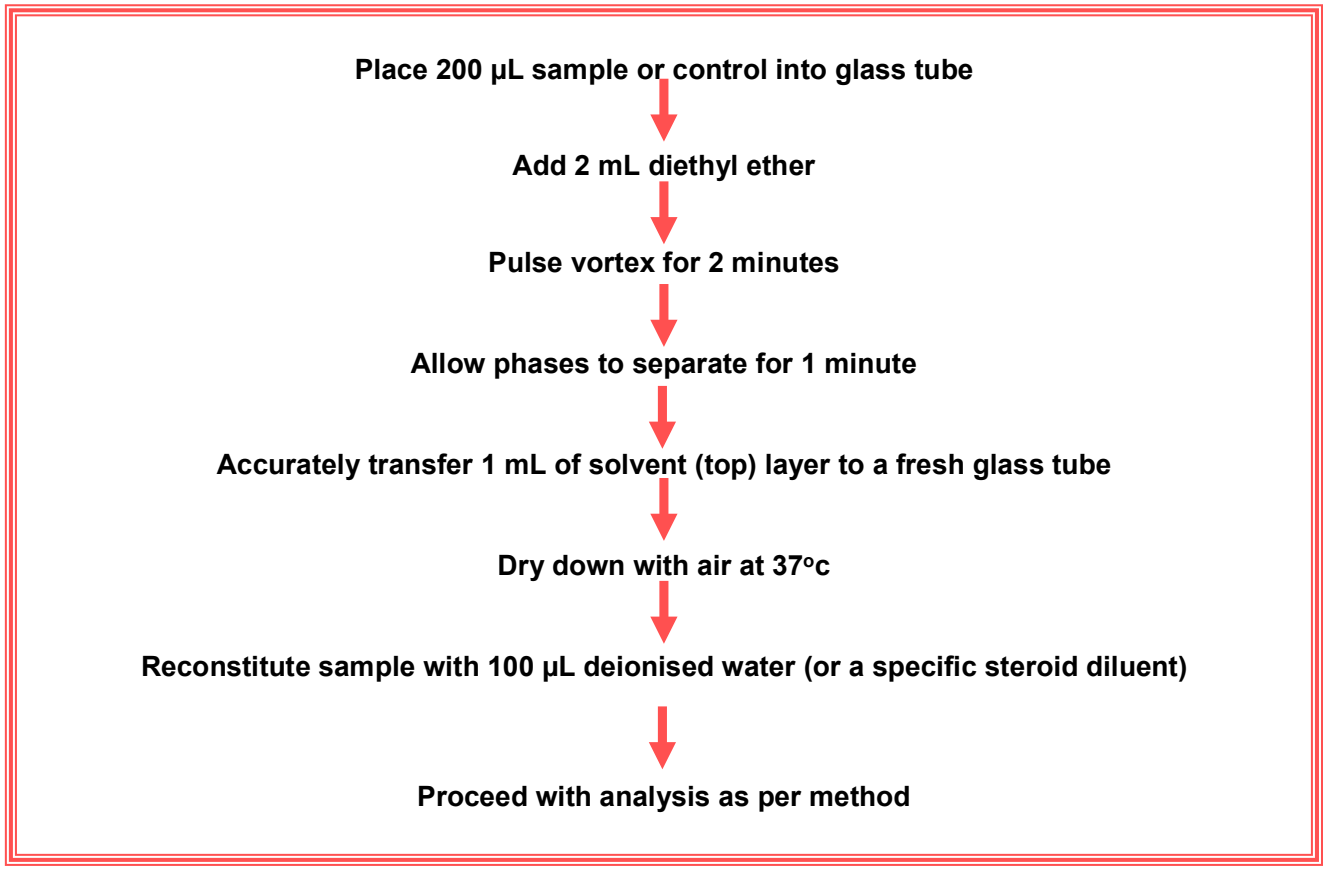
Steroid Pathway in the foetus



Persistence of the foetal adrenal zone

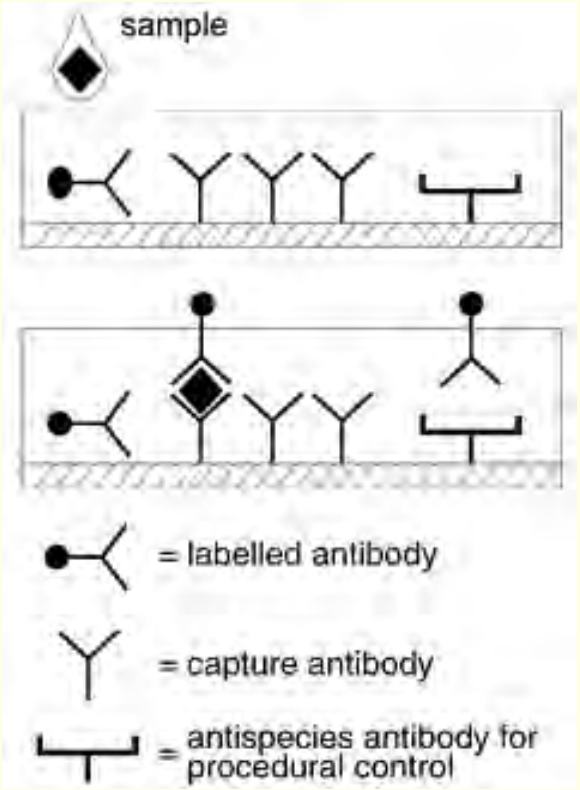
- Due to the low activity of the enzyme 3 beta HSD
- Not a cytochrome P450 enzyme
- Despite early delivery this enzyme will not switch on until at least the equivalent of term
- Persistence of foetal adrenal steroids in premature neonate
- In the laboratory we do not know the gestational age of a baby – we only have the date of birth
- Foetal adrenal steroids can interfere in some steroid immunoassays
- Perform extraction step on babies <3 months of age for e.g. testosterone, androstenedione & 17 OHP

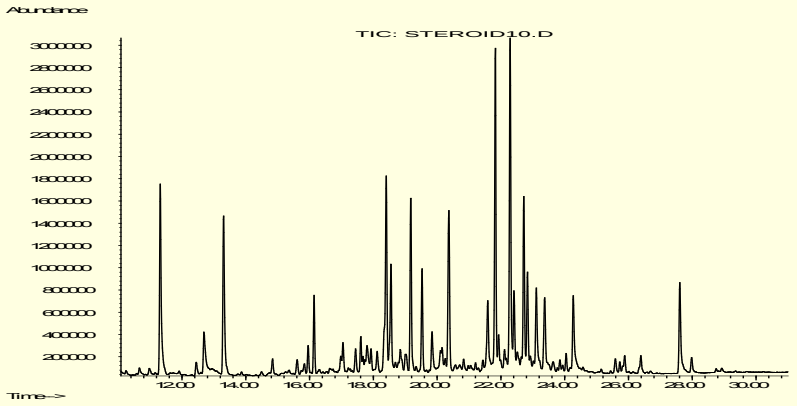
Simple liquid extraction protocol for steroids prior to immunoassay



Steroid Analysis

Immunoassay vs Chromatography



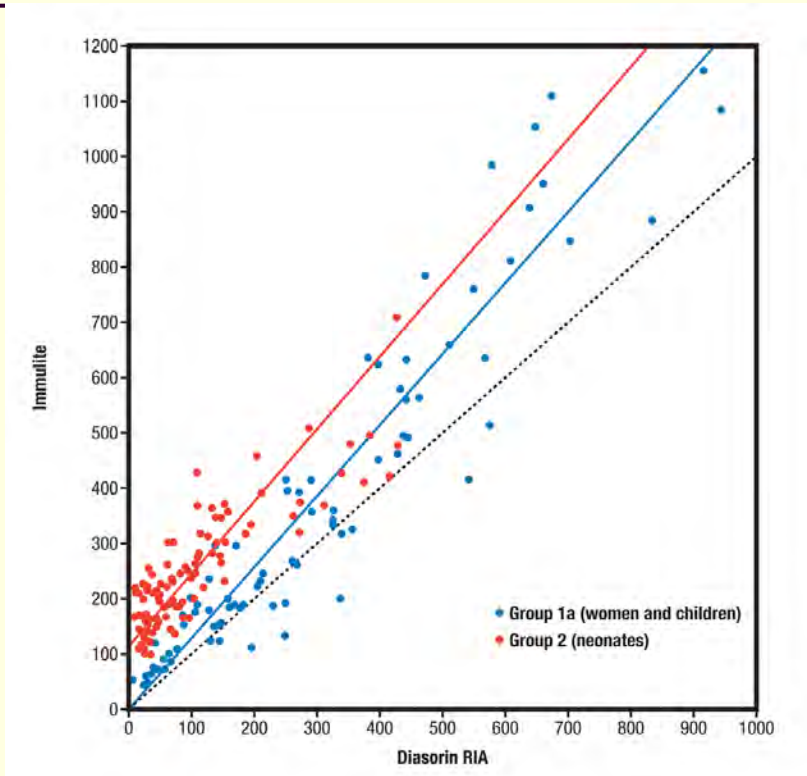


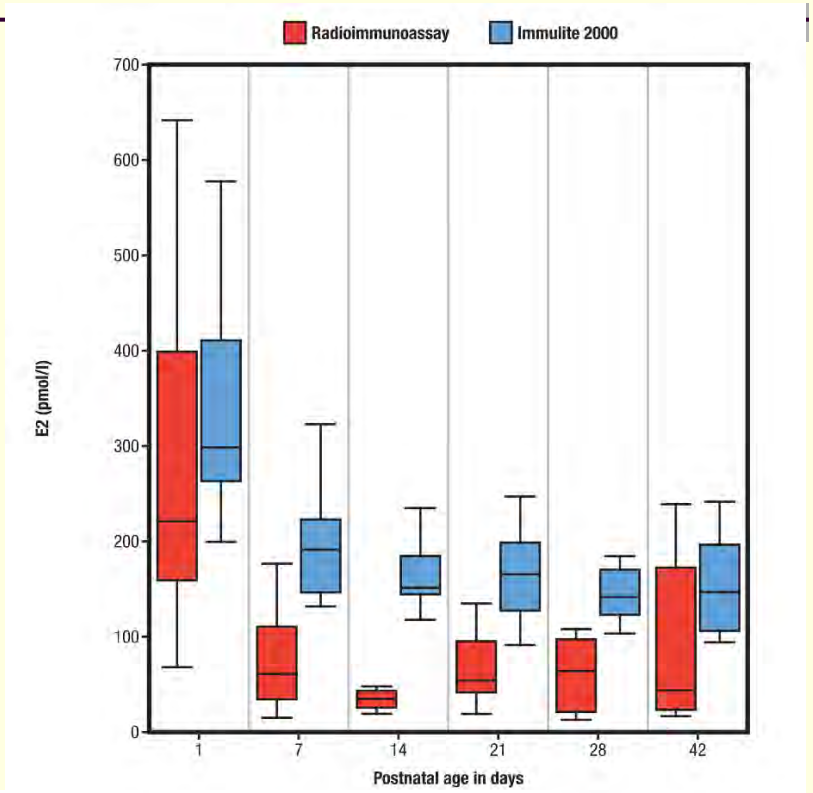
Dependent on separation of peaks

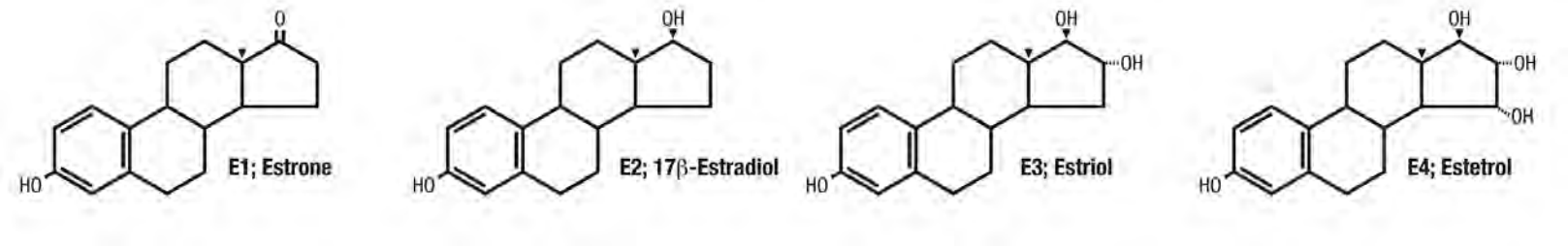
Addition of MS or TMS detection, enables specificity

Dependent on specificity of antibody

Comparison of two E2 assays in preterm neonates





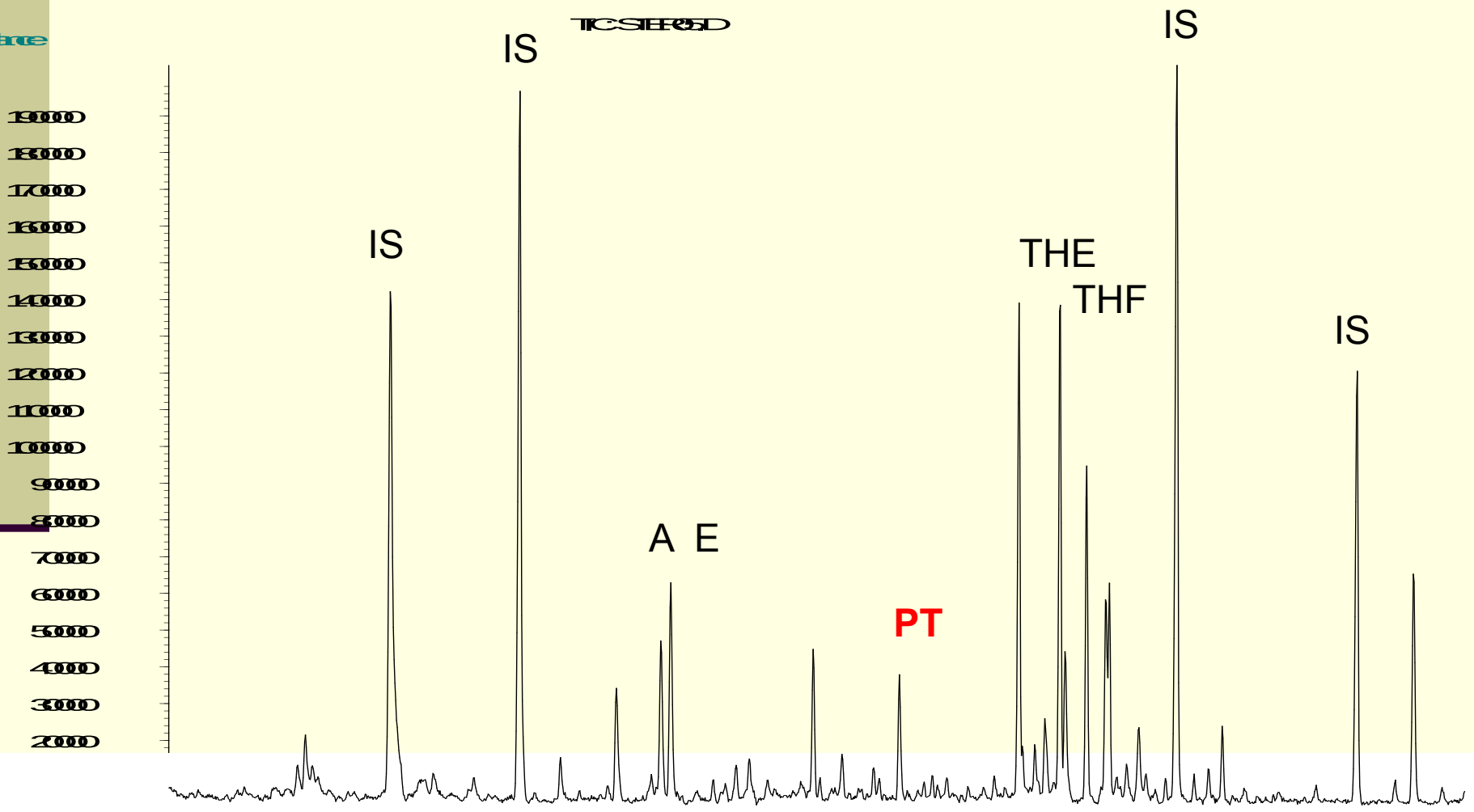


Presented at the AACB ASM 2003

Solid phase extraction for urine steroids prior to GCMS analysis

- C18 columns
- Prime with methanol → to waste
- Wash with water → to waste
- Load prepared sample → to waste
- Wash with water → to waste
- Elute with methanol → collect for next step

Normal USP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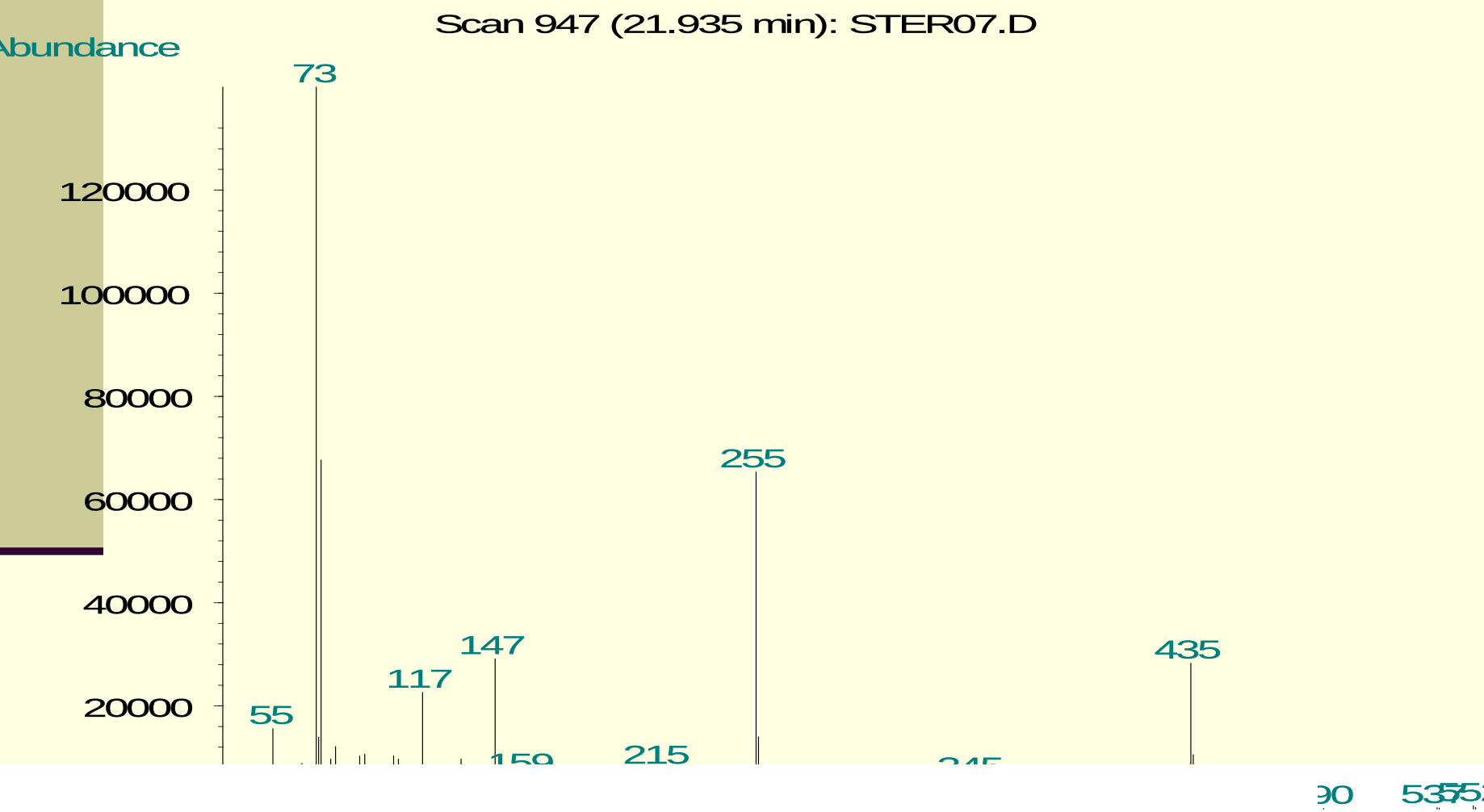
5000
4000
3000
2000

MS fragmentation

	Example
Total MW	Pregnanetriol + TMSI; **552** M+
-	-
MO: keto group fragments of 31	0 x 31
-	-
TMSI: OH group fragments of 90	3 x 90
=	=
Major ions	**255,345,435**

PT m/z


Scan 947 (21.935 min): STER07.D
Abundance
73
120000
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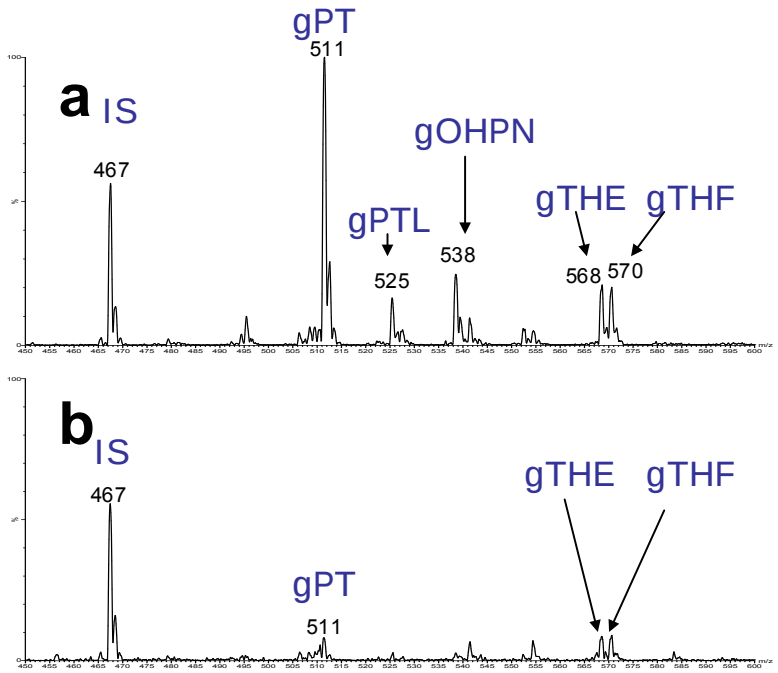
80000
60000
255
40000
147
435
117
20000
55
215
90
535
552

Tandem Mass Spec for steroids



High 17a-OHP group

Low 17a-OHP group

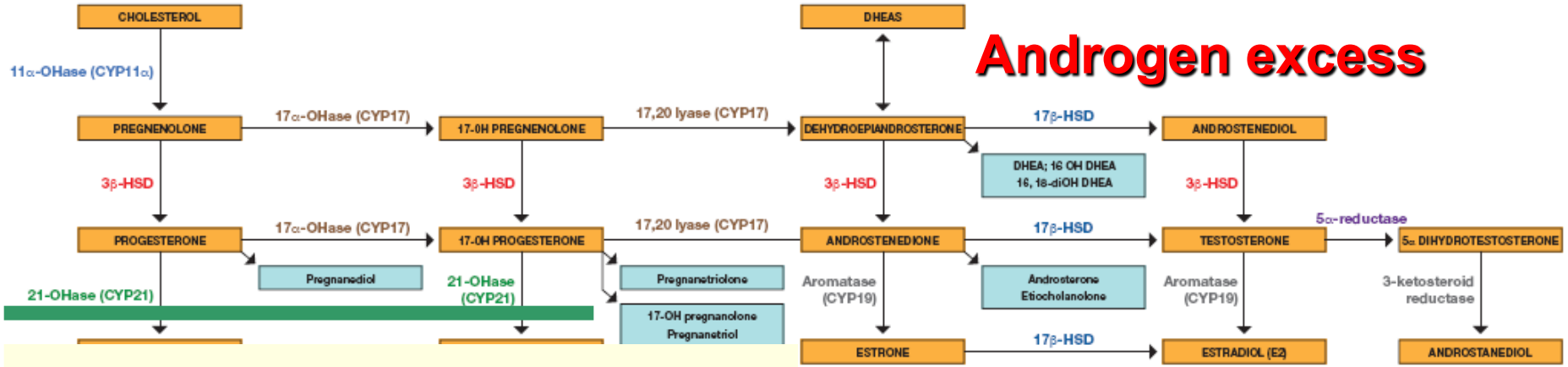
M-H: negatively charged pseudomolecular ion,
m/z: mass to charge ratio

Enzyme Blocks

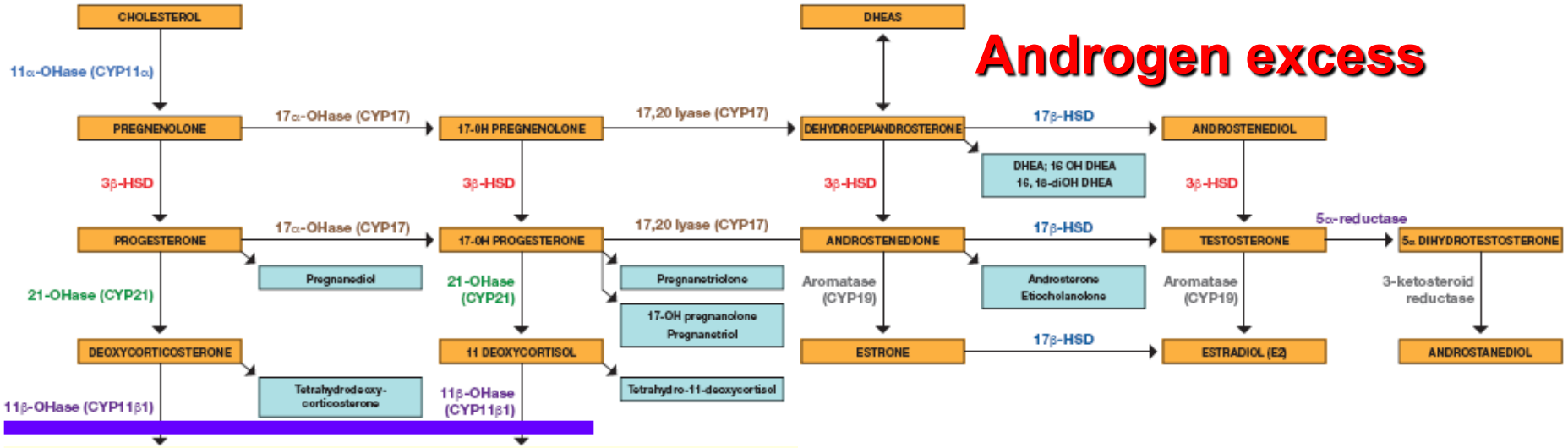
Congenital Adrenal Hyperplasia

Enzyme defect	CYP21	CYP11β1	CYP17	HSD3β	CYP11A
Alternative name	21 OH deficiency	11β-OH deficiency	17α- OH deficiency	3β-hydroxysteroid dehydrogenase deficiency	Lipoid / 20,22-desmolase deficiency 11 α OH deficiency STAR
Chromo-some	6p	8q	10q	1p	8p 15q
Frequency	1 : 11800 to 21800 ≈ 90% of cases	≈ 5% of cases	rare	rare	rare
Clinical features	Ambiguous genitalia in females	Ambiguous genitalia in females Increased blood pressure	No puberty in females Under-virilized males Increased blood pressure	Ambiguous genitalia mild in females Under-virilized males	No puberty in females Under-virilized males
Diagnostic serum steroids	↑ 17 OHP	↑ 11 deoxy cortisol (S) ↑ DOC	↑ preg-nenolone ↑ DOC ↑ cortico-sterone (B)	↑ DHEAS ↓ Andro-stenedione ↑ratio of substrate - to product ↑ 17 OH pregnenolone ↑ Progestrone	↓ Serum adrenal steroids - as the block is at the beginning of the pathway
Diagnostic urine steroids	↑ 17β hydroxy pregnanolone ↑ PT ↑ PTL	↑ PT ↑ THS ↑ THDOC	↑ THDOC ↑ THB ↑ PD	Adrenal foetal steroids persist ↑ P'T beyond the corrected neonatal period is diagnostic.	↓ Urine adrenal steroid metabolite output - as the block is at the beginning of the pathway

CAH – 21 OHase deficiency



CAH – 11 β OHase deficiency

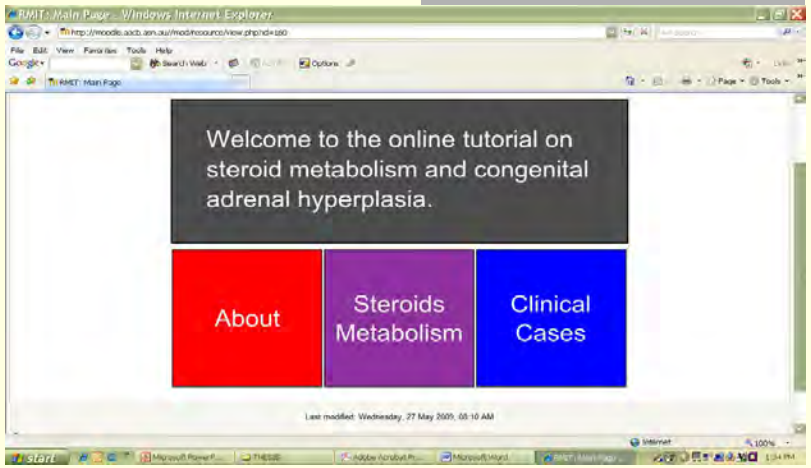


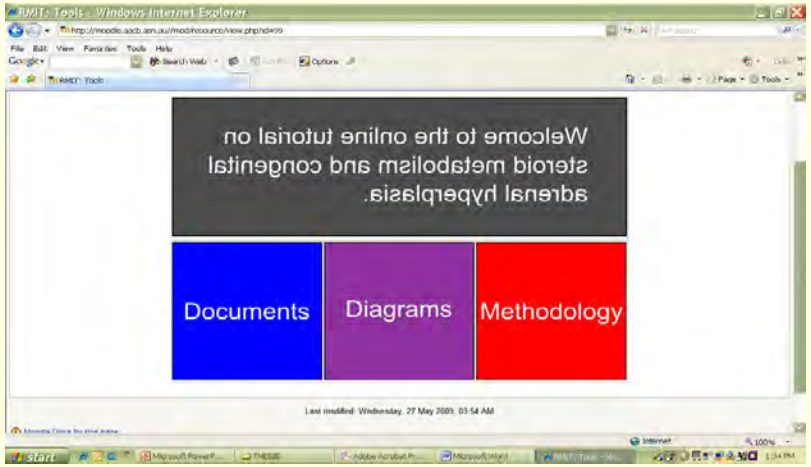
Moodle

Moodle

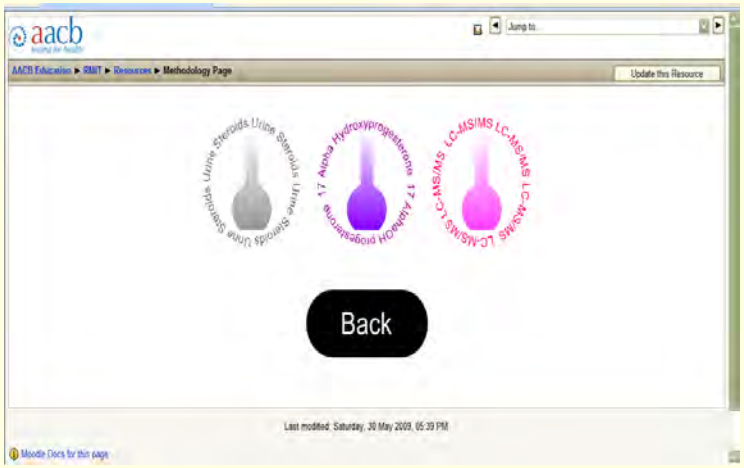
- Current project
- Education tool
- Free to all
- Just register
- www.moodle.aacb.asn.au
- Choose steroid course

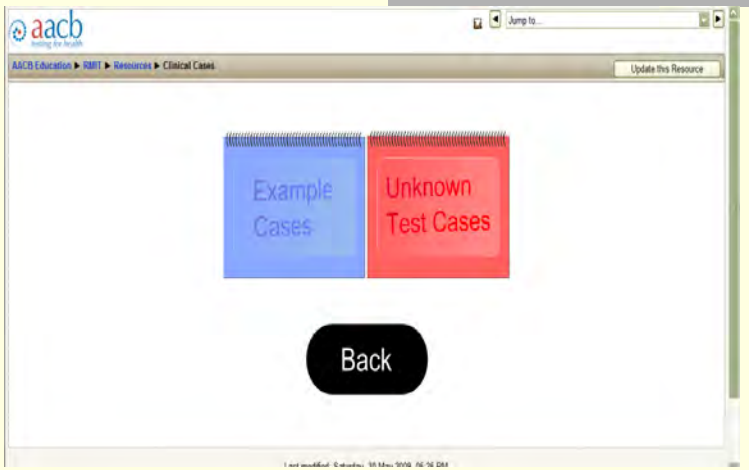
- Expected availability August 2009

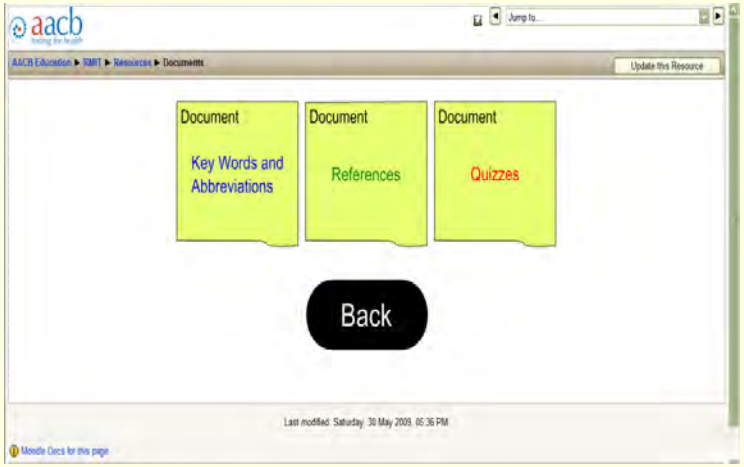


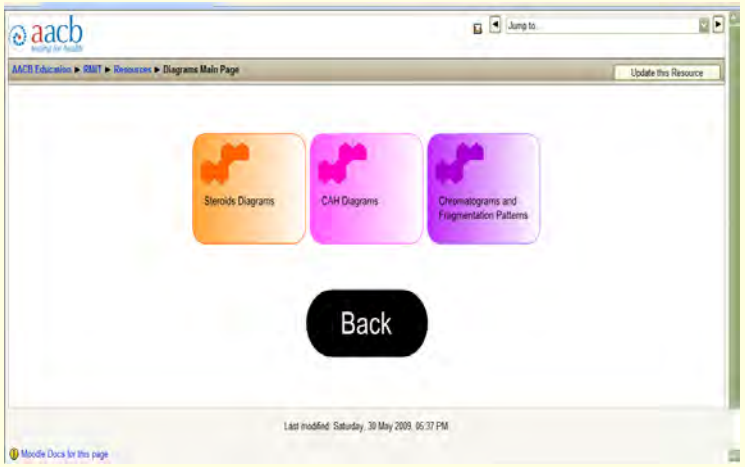


Moodle main sections

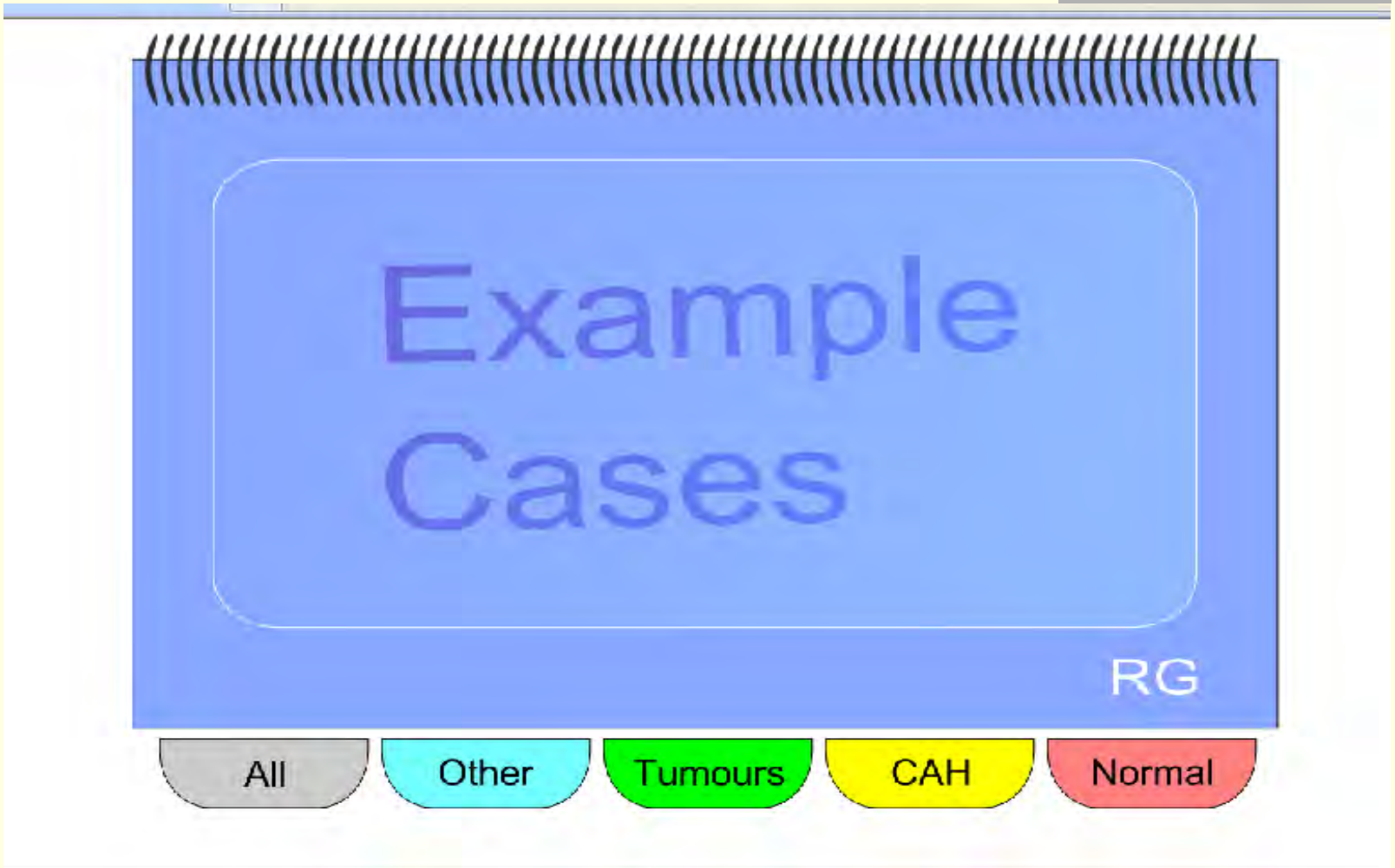




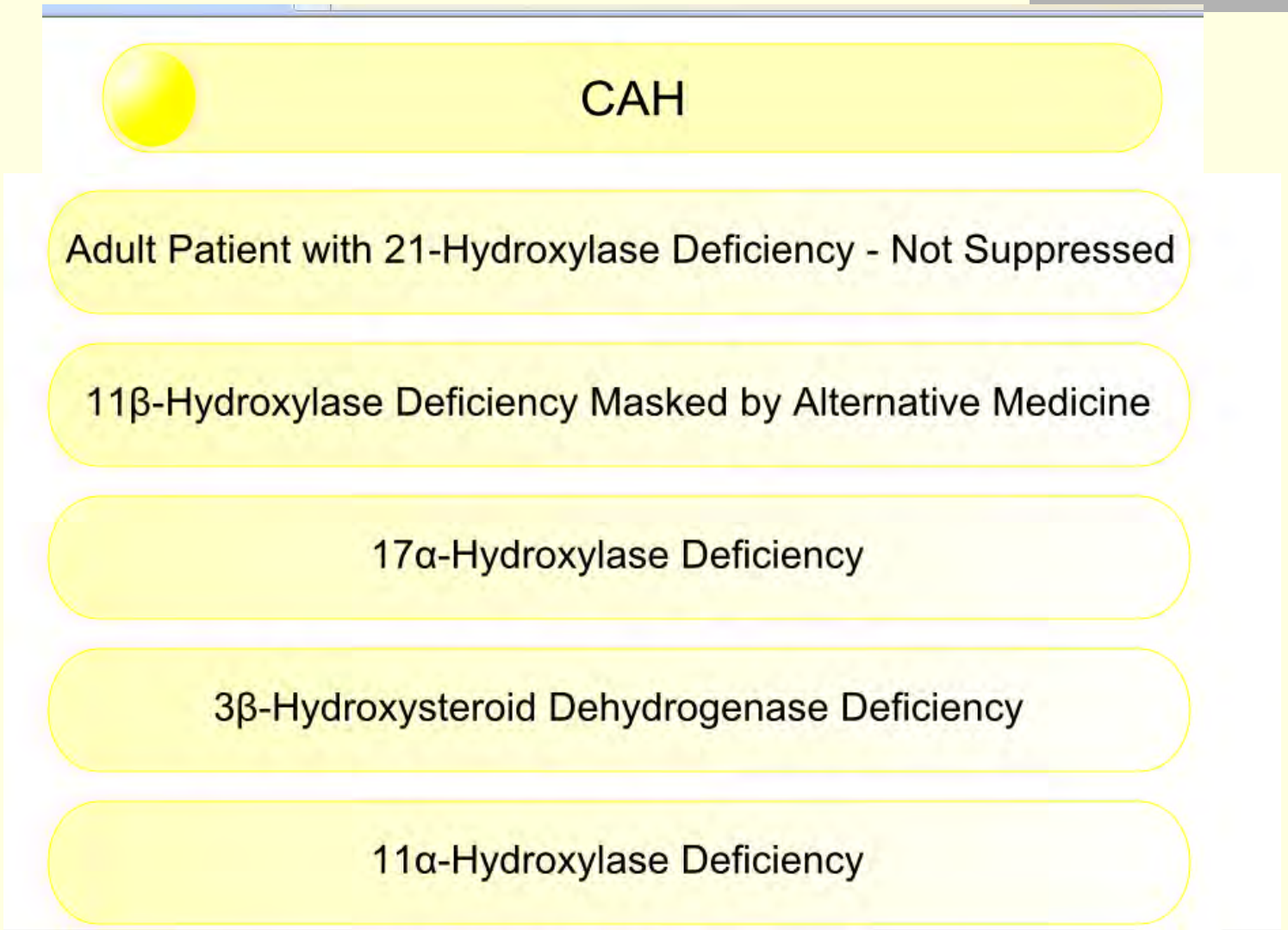




Moodle



Example cases



Moodle case

Past Medical History:

- Born at term
- Her older half sister - normal feminization and puberty
- No family history of migrain
- No regular medication
- No history of allergy

Investigations

Physical examination:

Android body.
Height: 170 cm (87 percentile)
Weight 68 Kg (86 percentile)
BMI: 23.5 (76 percentile)
Blood pressure 150/98
No evidence of adrenarche development.
Pre-pubertal with Tanner stage B1P1

Laboratory investigations:

- Ultrasound CT scans and MRI indicate absence of gonads & uterus
- 46XY
- Lack of adrenarche, pubertal failure, and hypertension indicate steroid synthesis defect
- Serum and urine steroids requested

Biochemistry

Test	Result	RI
ACTH (post synacthen)	76.8	2-10.0 pmol/L
Cortisol (pre & post synacthen)	48 to 49	nmol/L 3x pre synacthen
17 OHP	undetectable	<8.0 nmol/L
Testosterone	0.5	1.0 – 4.5 nmol/L
E2	45	90-110 pmol/L
Progesterone	23	<2.9 nmol/L
LH	26.7	0.6 – 9.8 IU/L
FSH	68.0	1.4 – 6.8 IU/L

17 α hydroxylase deficiency

Diagnosis:

1. CAH due to 17 α hydroxylase deficiency with deficiency of cortisol, oestrogen and androgens and increased mineralocorticoids.

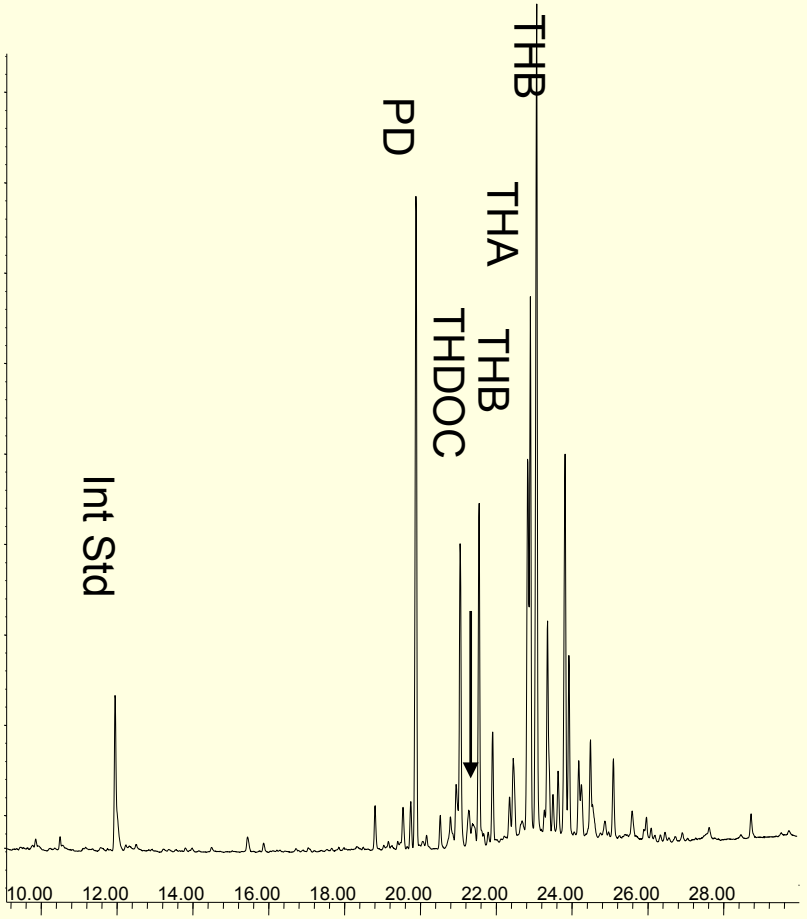
2. Hypertension due to excess mineralocorticoids level.

Treatment:

10 mg/day (15mg/m2) of hydrocortisone.

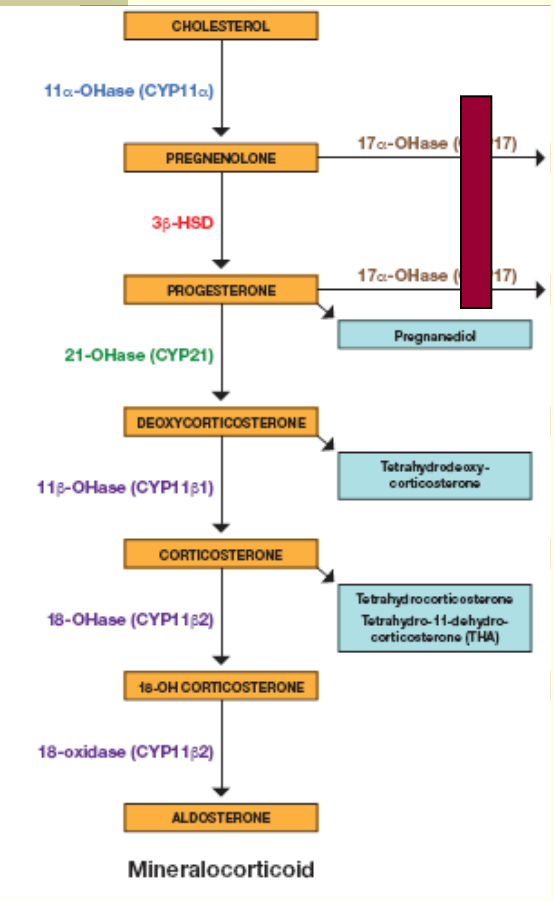
Monitoring of hypertension

Daily transdermal oestrogen patches



Urine steroid profile

CAH – 17 α OHase deficiency



Increase in mineralocorticoids
cause of hypertension and headaches


Bitte diese Katze nicht
berühren
ist sehr ängstlich
Danke!