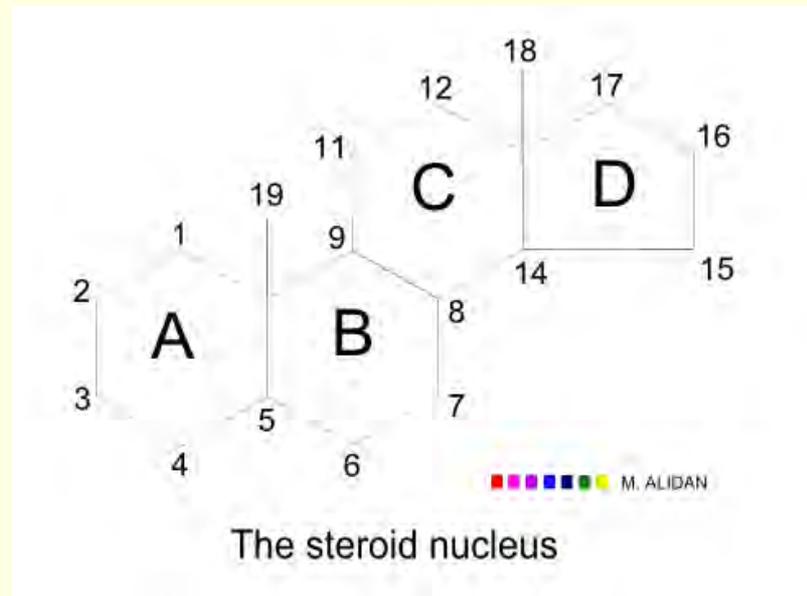


Steroids



Ronda Greaves

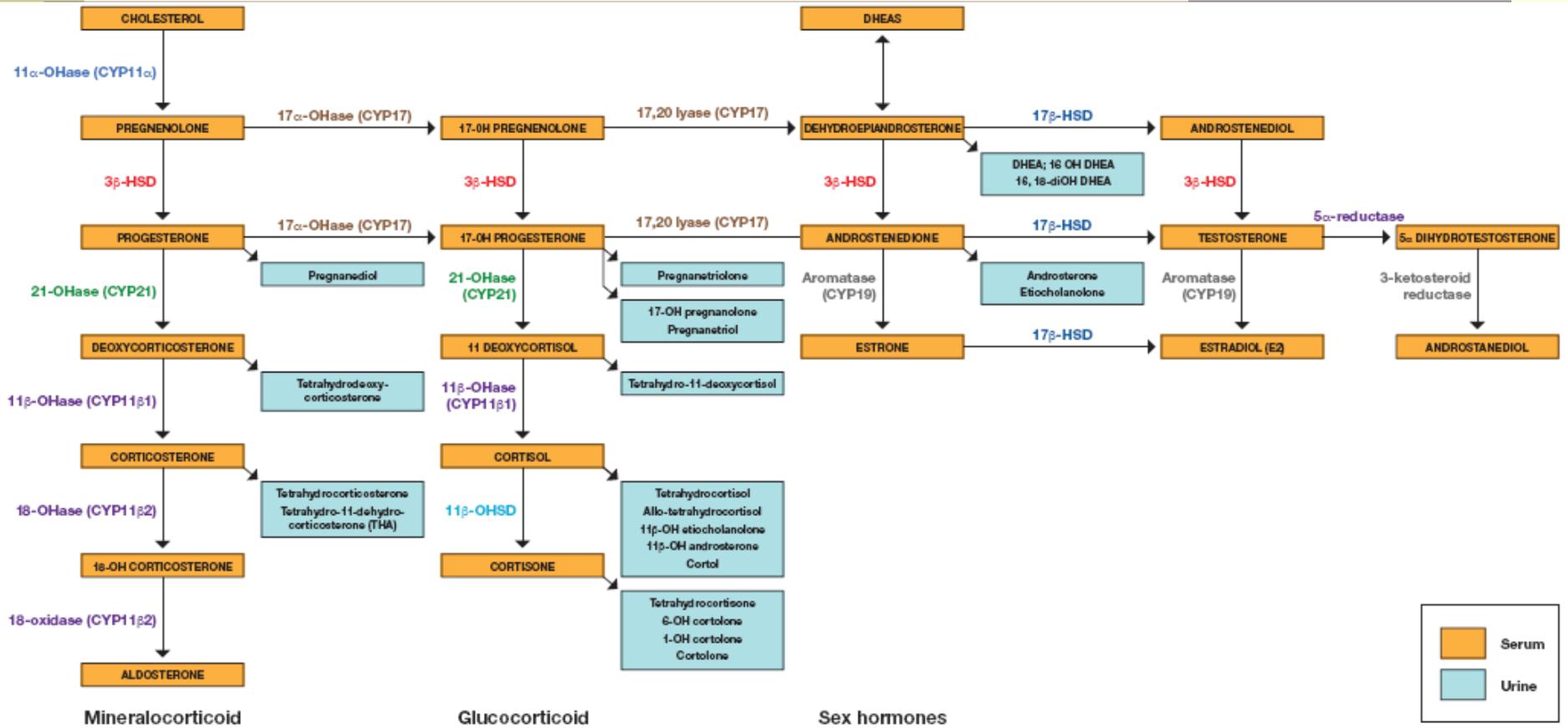
Overview

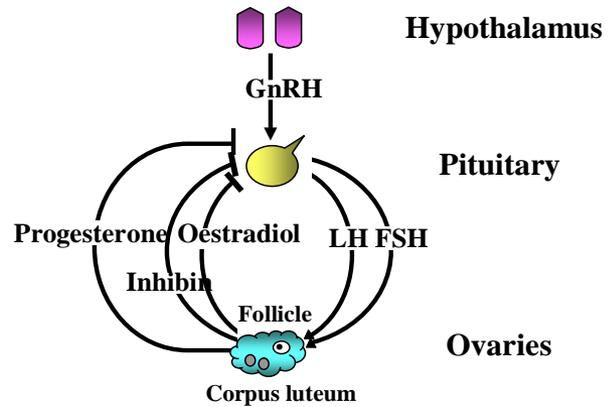
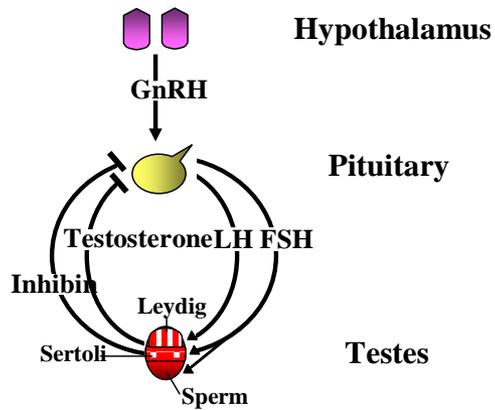
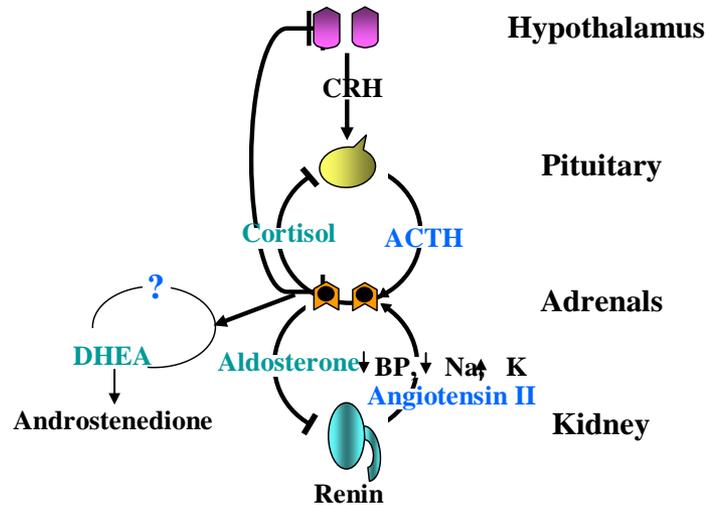


Cyclopentanophenanthrene ring

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

Steroid Pathway

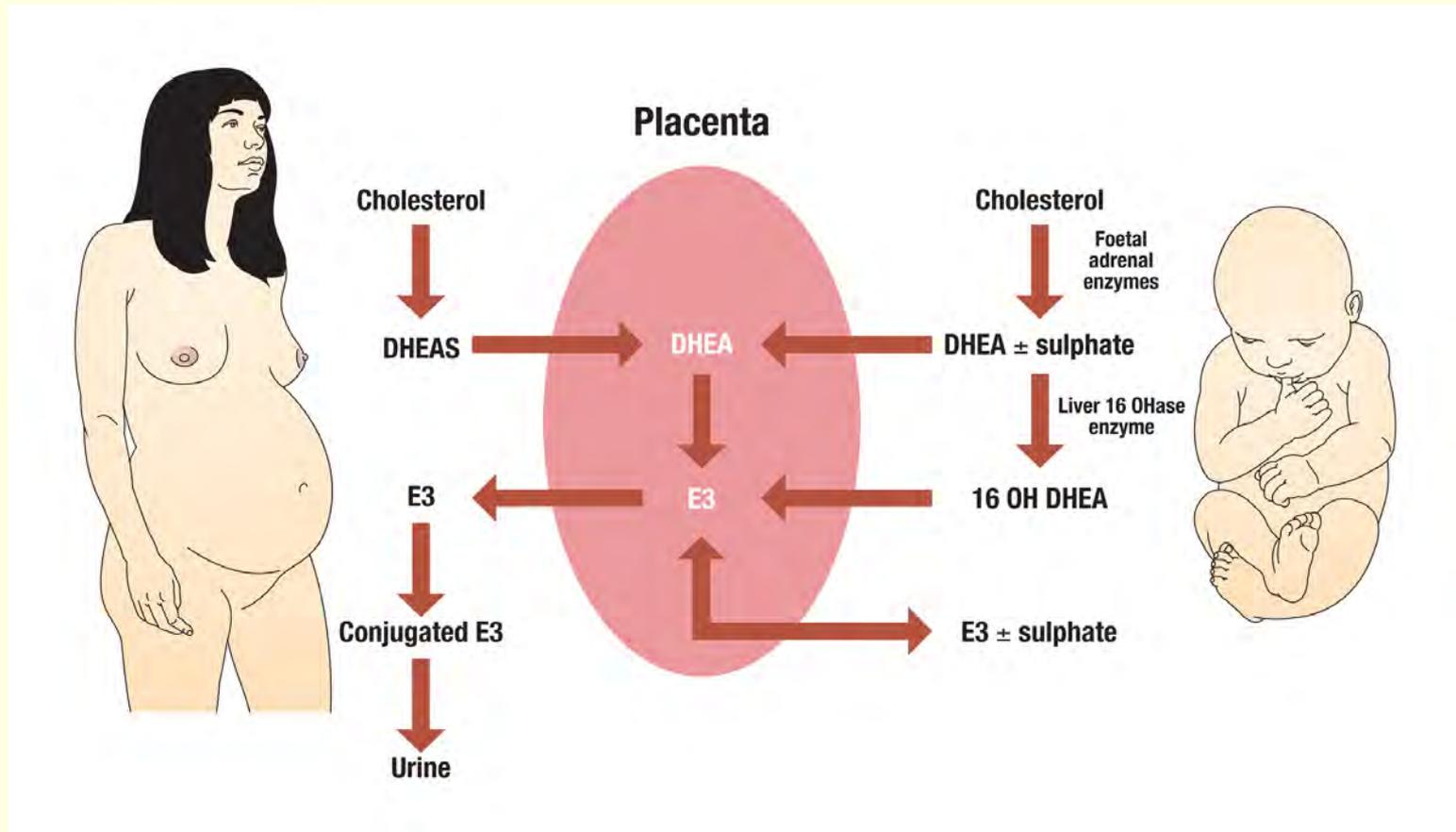






Foetal Adrenal Zone & Premature infants

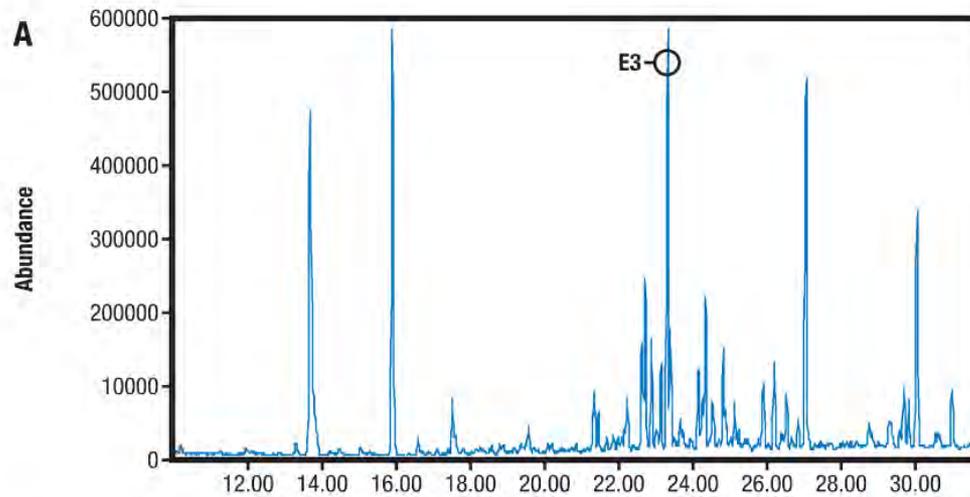
Maternal – Foetal interaction



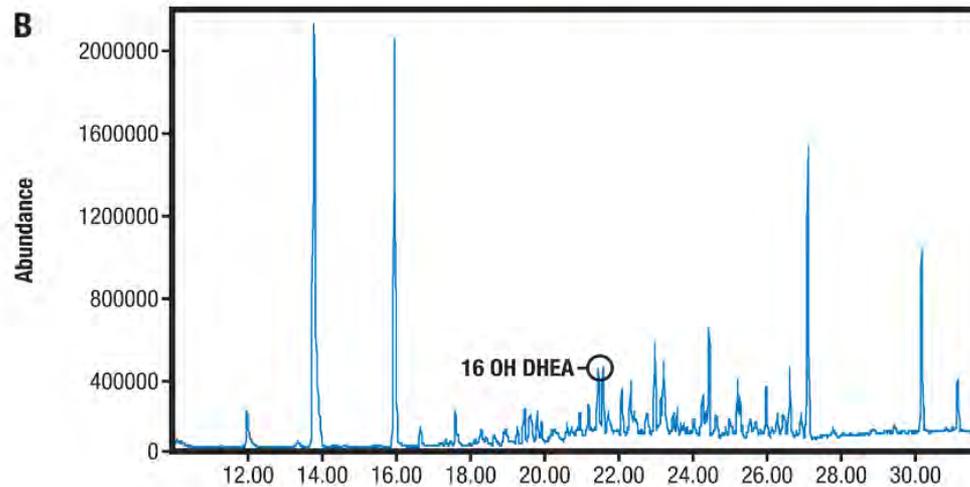
Steroid hormone concentrations in cord blood (umol/L)

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

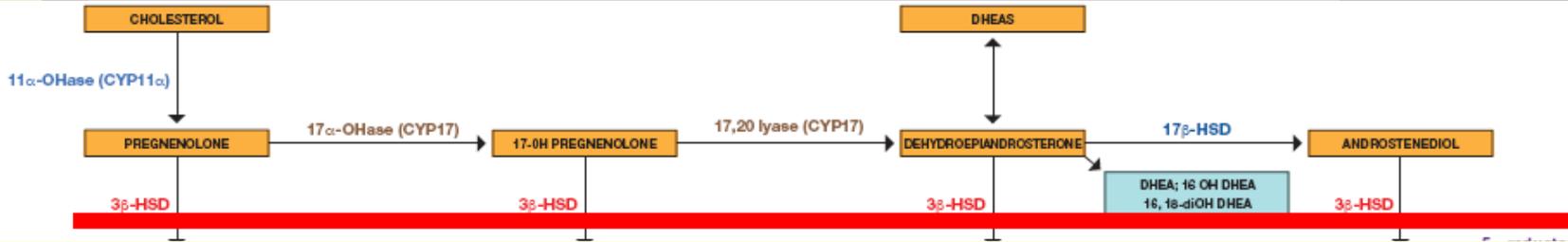


Full term neonate
4 days of age



Preterm neonate
6 weeks of age

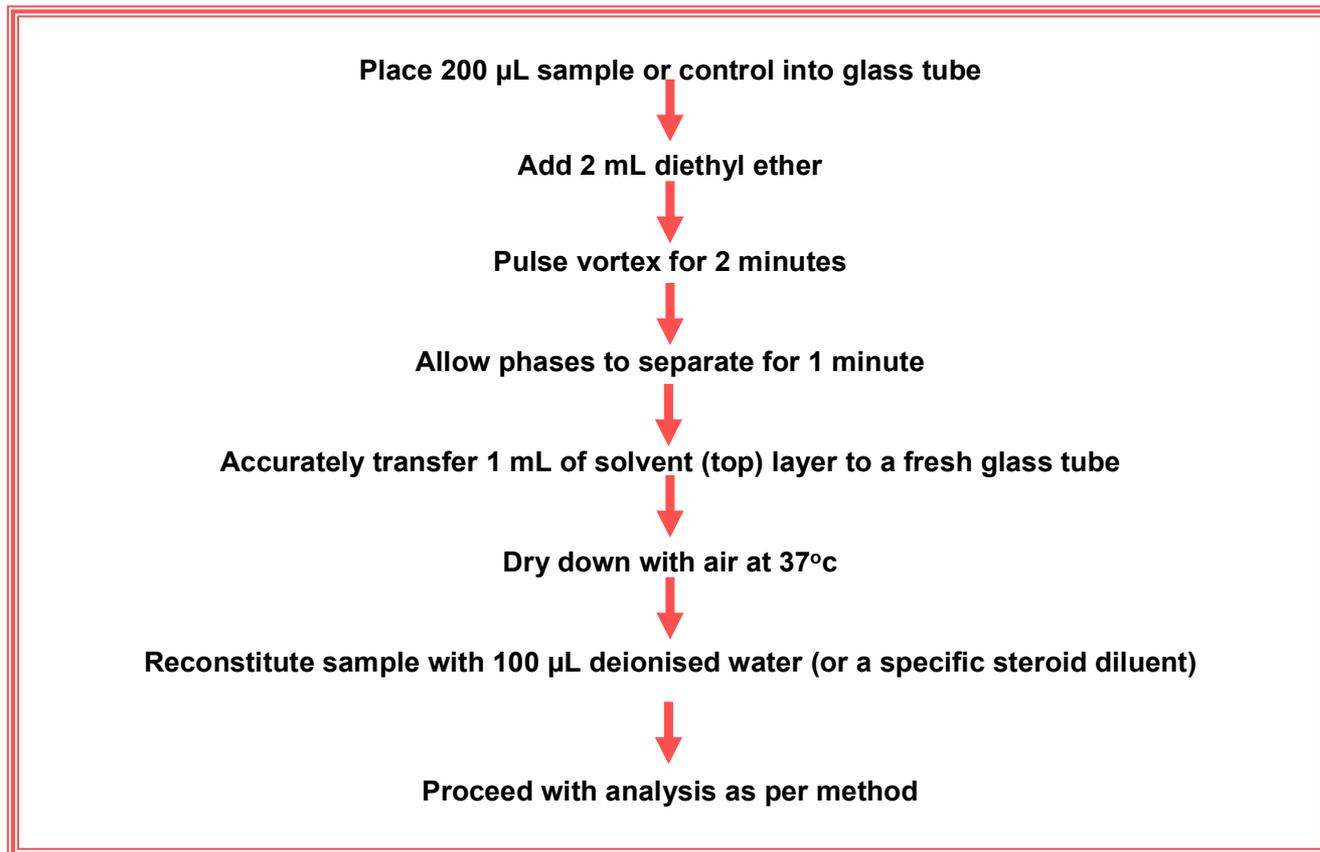
Steroid Pathway in the foetus



Persistence of the foetal adrenal zone

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

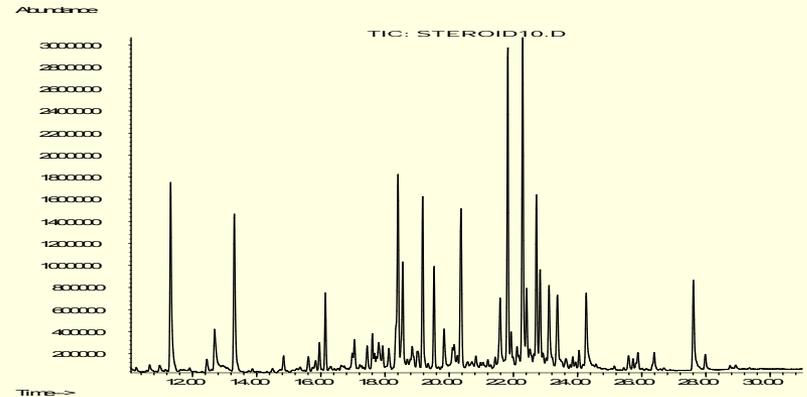
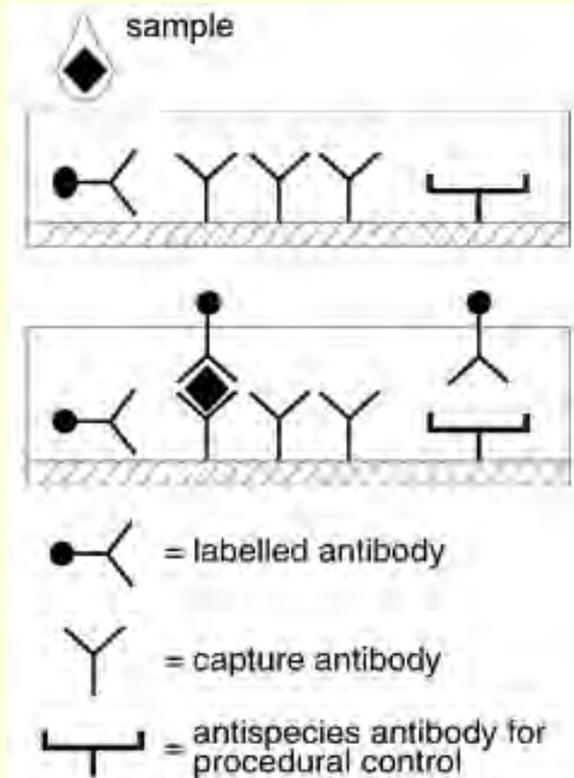
Simple liquid extraction protocol for steroids prior to immunoassay





Steroid Analysis

Immunoassay vs Chromatography

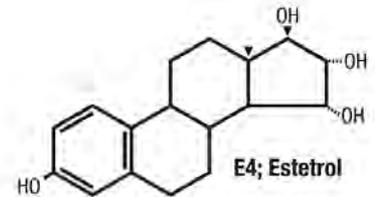
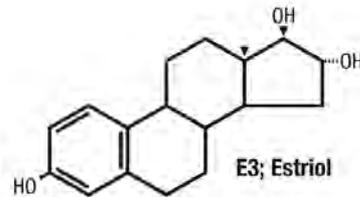
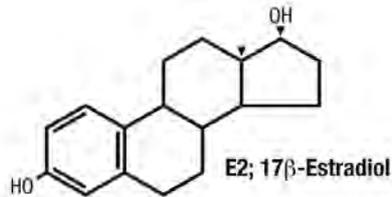
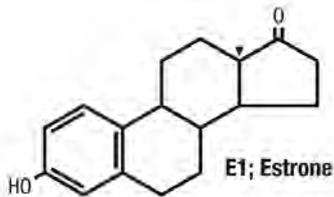
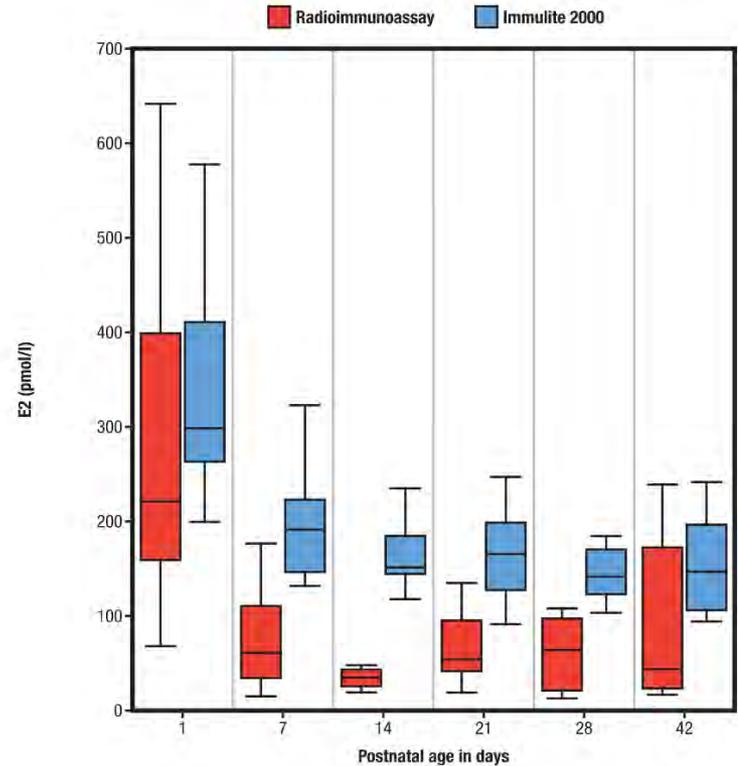
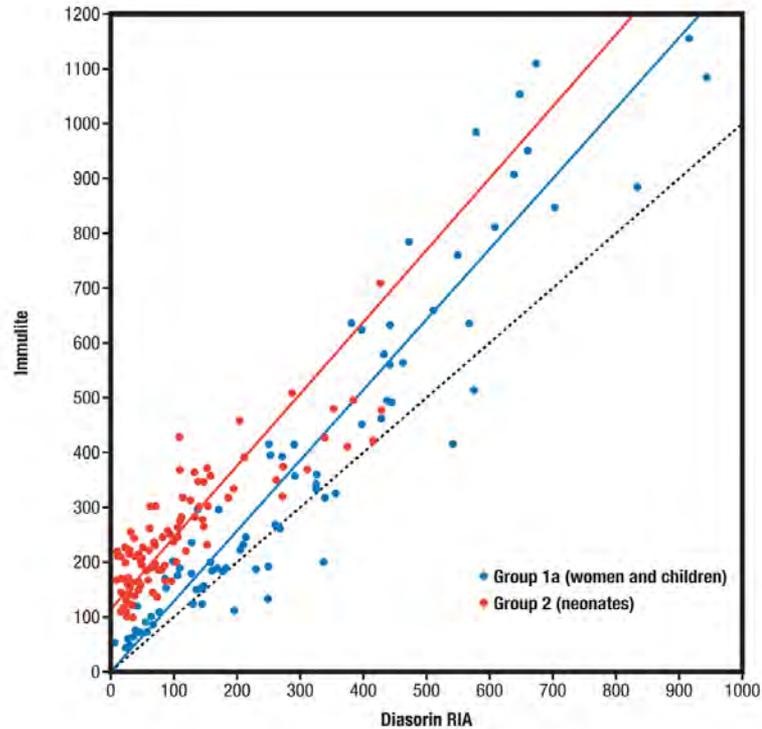


Dependent on separation of peaks

Addition of MS or TMS detection, enables specificity

Dependent on specificity of antibody

Comparison of two E2 assays in preterm neonates

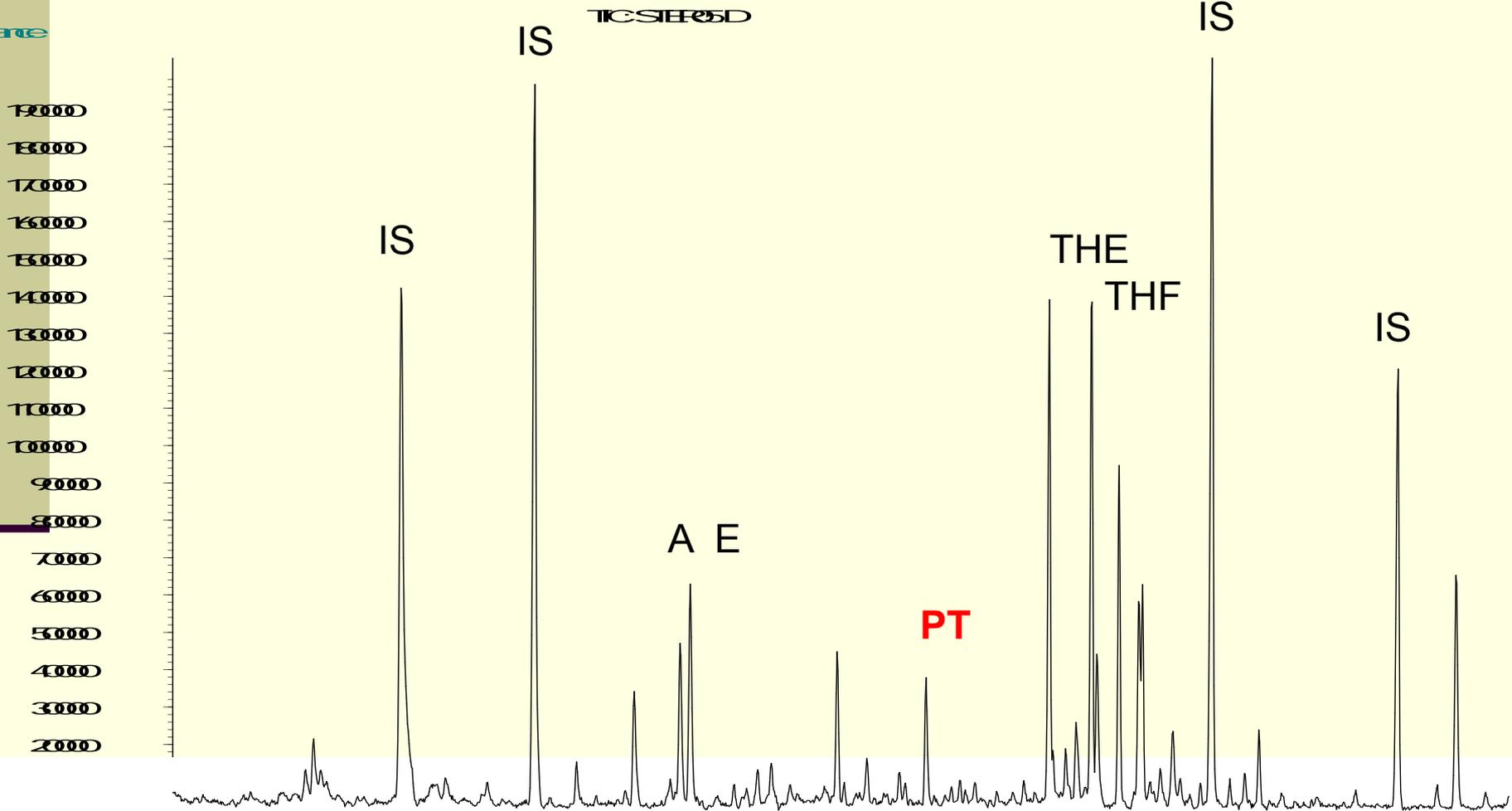


Solid phase extraction for urine steroids prior to GCMS analysis

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



Normal USP



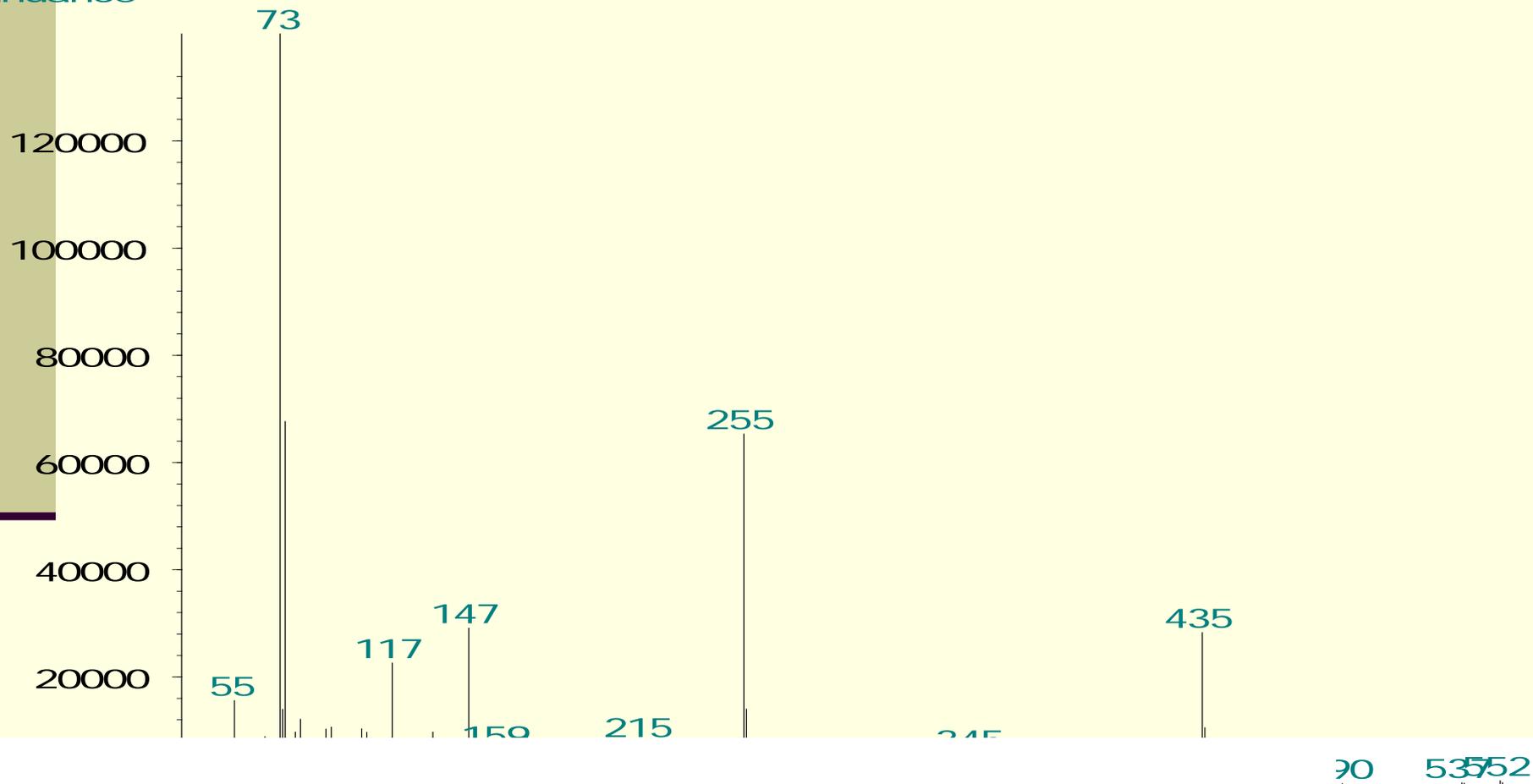
MS fragmentation

	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

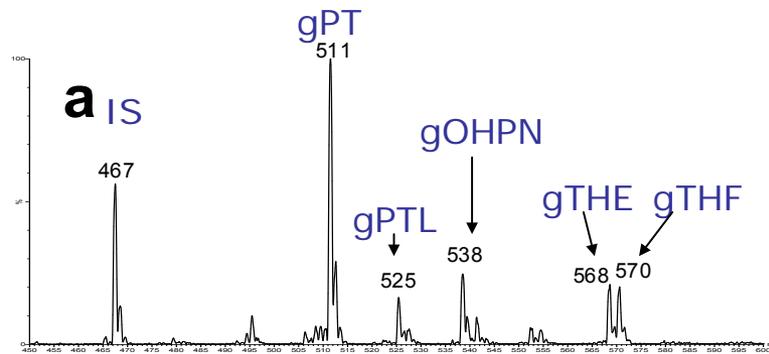
PT m/z

Scan 947 (21.935 min): STER07.D

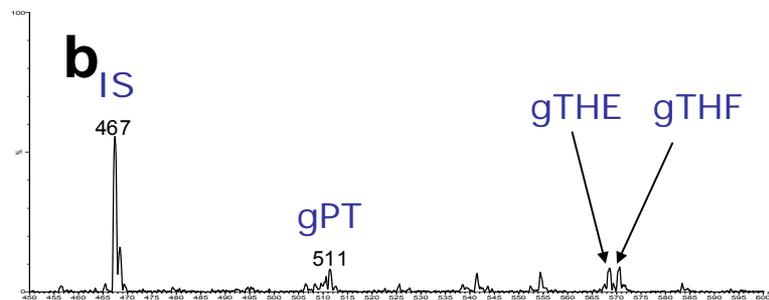
Abundance



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

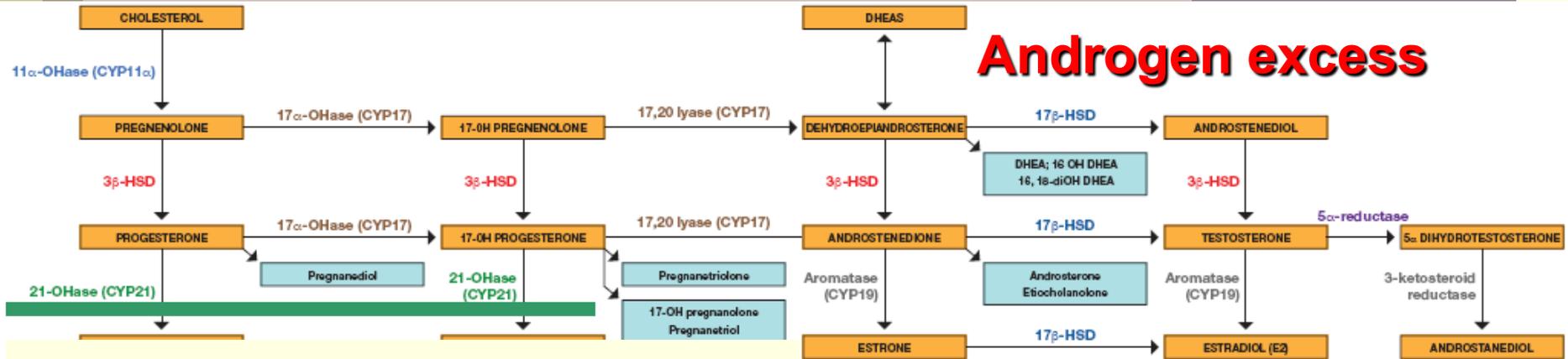


Enzyme Blocks

Congenital Adrenal Hyperplasia

Enzyme defect	CYP21	CYP11 β 1	CYP17	HSD3 β	CYP11A
Alternative name	21 OH deficiency	11 β -OH deficiency	17 α - OH deficiency	3 β -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 \approx 90% of cases	\approx 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	\uparrow 17 OHP	\uparrow 11 deoxy cortisol (S) \uparrow DOC	\uparrow preg-nenolone \uparrow DOC \uparrow cortico-sterone (B)	\uparrow DHEAS \downarrow Andro-steredione \uparrow ratio of substrate - to product \uparrow 17 OH pregnenolone \uparrow Progestrone	\downarrow Serum adrenal steroids - as the block is at the beginning of the pathway
Diagnostic urine steroids	\uparrow 17 β hydroxy pregnanolone \uparrow PT \uparrow PTL	\uparrow PT \uparrow THS \uparrow THDOC	\uparrow THDOC \uparrow THB \uparrow PD	Adrenal foetal steroids persist \uparrow P'T beyond the corrected neonatal period is diagnostic.	\downarrow Urine adrenal steroid metabolite output - as the block is at the beginning of the pathway

CAH – 21 OHase deficiency

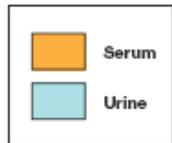


Salt loss Inadequate stress response

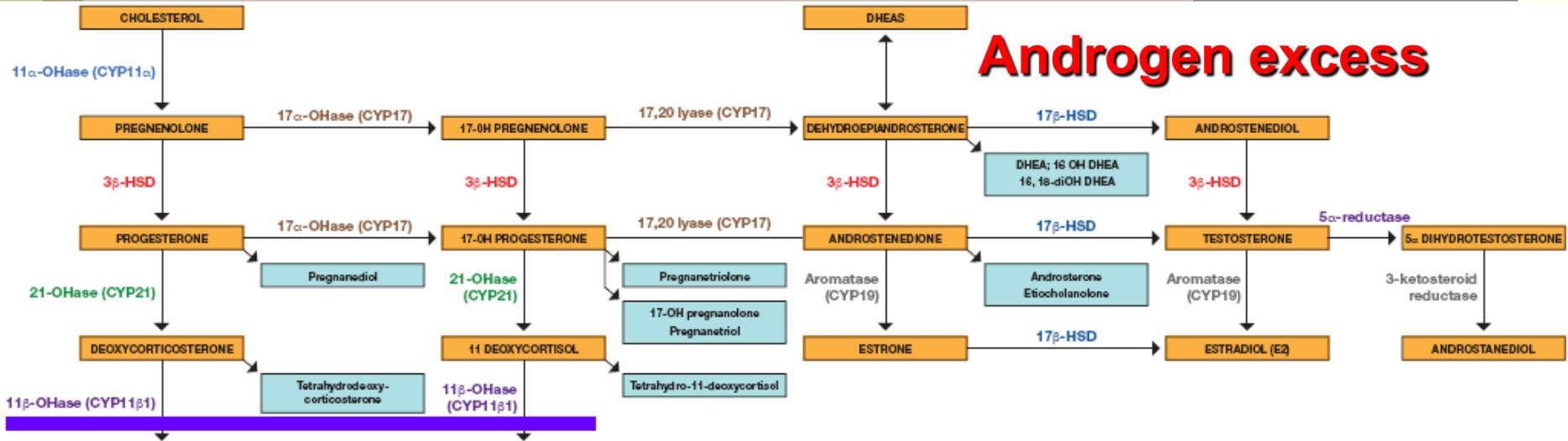
Mineralocorticoid

Glucocorticoid

Sex hormones



CAH – 11 β OHase deficiency

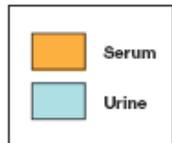


Hypertension Inadequate stress response

Mineralocorticoid

Glucocorticoid

Sex hormones

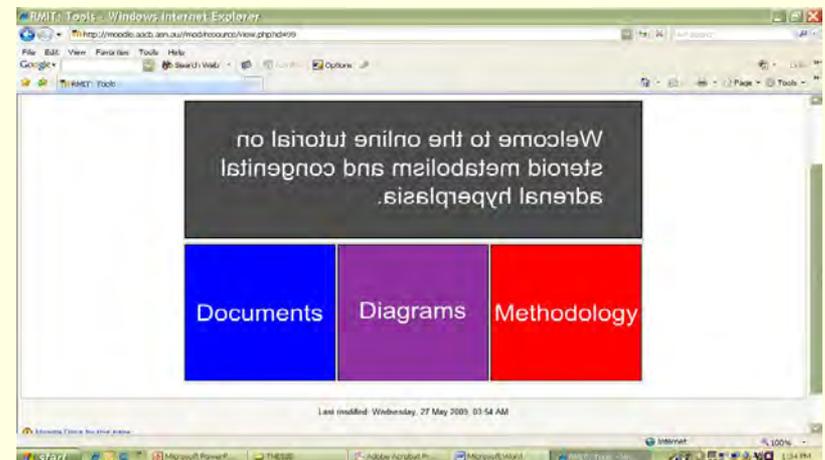
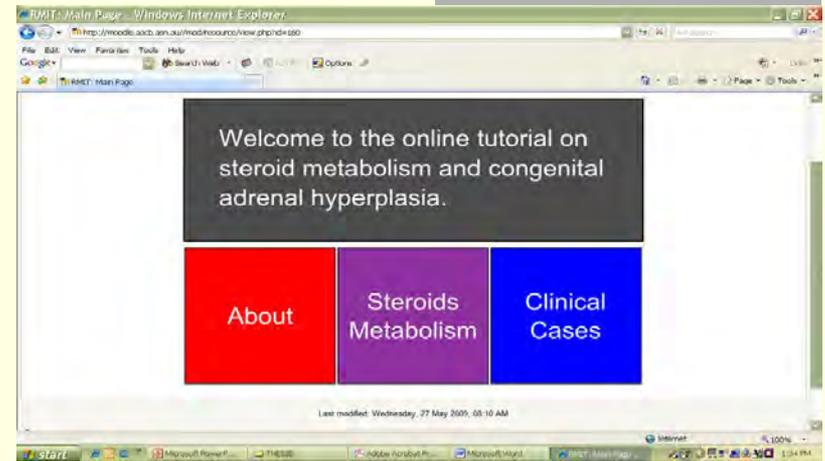




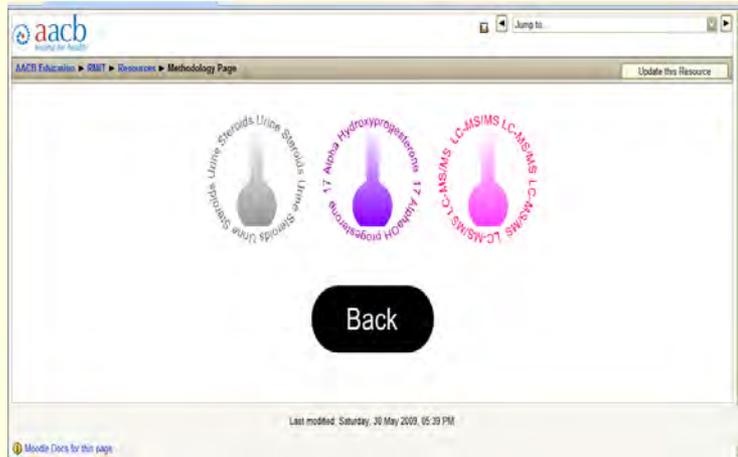
Moodle

Moodle

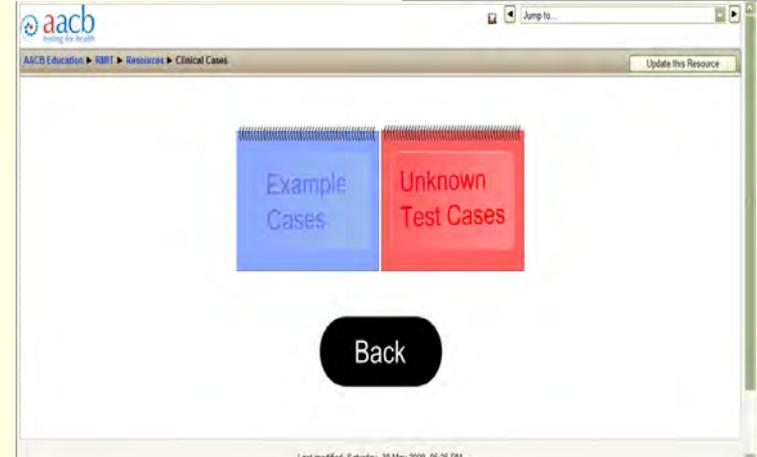
- n Current project
- n Education tool
- n Free to all
- n Just register
- n www.moodle.aacb.asn.au
- n Choose steroid course
- n Expected availability August 2009



Moodle main sections



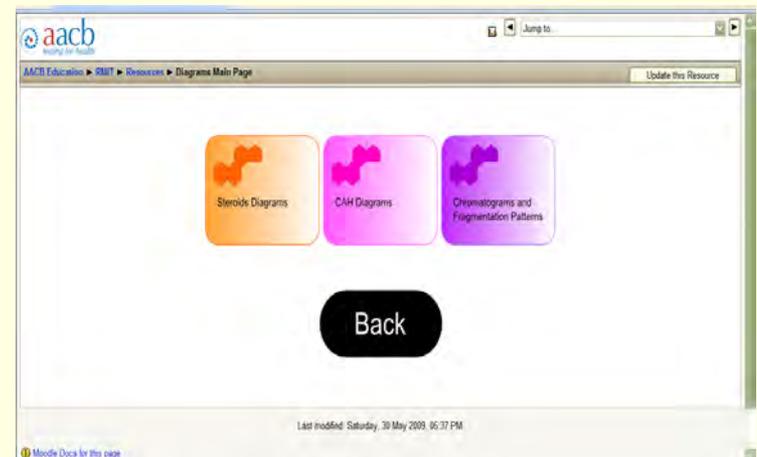
Screenshot of a Moodle page titled "Methodology Page". The page features three circular icons representing different analytical methods: "Steroids Urine Steroids Urine Steroids Urine", "Alpha Hydroxyprogesterone 17", and "LC-MS/MS LC-MS/MS LC-MS/MS". A large black "Back" button is centered below the icons. The page footer indicates it was last modified on Saturday, 30 May 2009, at 05:39 PM.



Screenshot of a Moodle page titled "Clinical Cases". The page features two rectangular icons representing "Example Cases" (blue) and "Unknown Test Cases" (red). A large black "Back" button is centered below the icons. The page footer indicates it was last modified on Saturday, 30 May 2009, at 05:39 PM.



Screenshot of a Moodle page titled "Documents". The page features three rectangular icons representing "Key Words and Abbreviations", "References", and "Quizzes". A large black "Back" button is centered below the icons. The page footer indicates it was last modified on Saturday, 30 May 2009, at 05:36 PM.



Screenshot of a Moodle page titled "Diagrams Main Page". The page features three rectangular icons representing "Steroids Diagrams", "CAH Diagrams", and "Chromatograms and Fragmentation Patterns". A large black "Back" button is centered below the icons. The page footer indicates it was last modified on Saturday, 30 May 2009, at 05:37 PM.

Moodle



Example cases



CAH

Adult Patient with 21-Hydroxylase Deficiency - Not Suppressed

11 β -Hydroxylase Deficiency Masked by Alternative Medicine

17 α -Hydroxylase Deficiency

3 β -Hydroxysteroid Dehydrogenase Deficiency

11 α -Hydroxylase Deficiency

Moodle case



The screenshot shows a Moodle course page. At the top left is the AACB logo with the tagline 'testing for health'. To the right is a search bar labeled 'Jump to...'. Below the logo is a breadcrumb trail: 'AACB Education > RMIT > Resources > A Patient with 17 Alpha Hydroxylase Deficiency'. On the far right of this bar is a button labeled 'Update this Resource'. Below the breadcrumb trail is a row of eight circular icons: a red left-pointing arrow, a purple tablet, a blue house, a yellow house, an orange gear, a grey flask, a cyan molecular structure, and a green bar chart labeled 'Diagrams'. Below the icons is the text 'History of presenting complaints:' followed by a paragraph: 'A 17 years old female was referred to endocrinologist due to sharp headache which is relieved by rest and delayed puberty.'

Past Medical History:

- n Born at term
- n Her older half sister - normal feminization and puberty
- n No family history of migraine
- n No regular medication
- n 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:

- n Ultrasound CT scans and MRI indicate absence of gonads & uterus
- n 46XY
- n Lack of adrenarche, pubertal failure, and hypertension indicate steroid synthesis defect
- n 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.

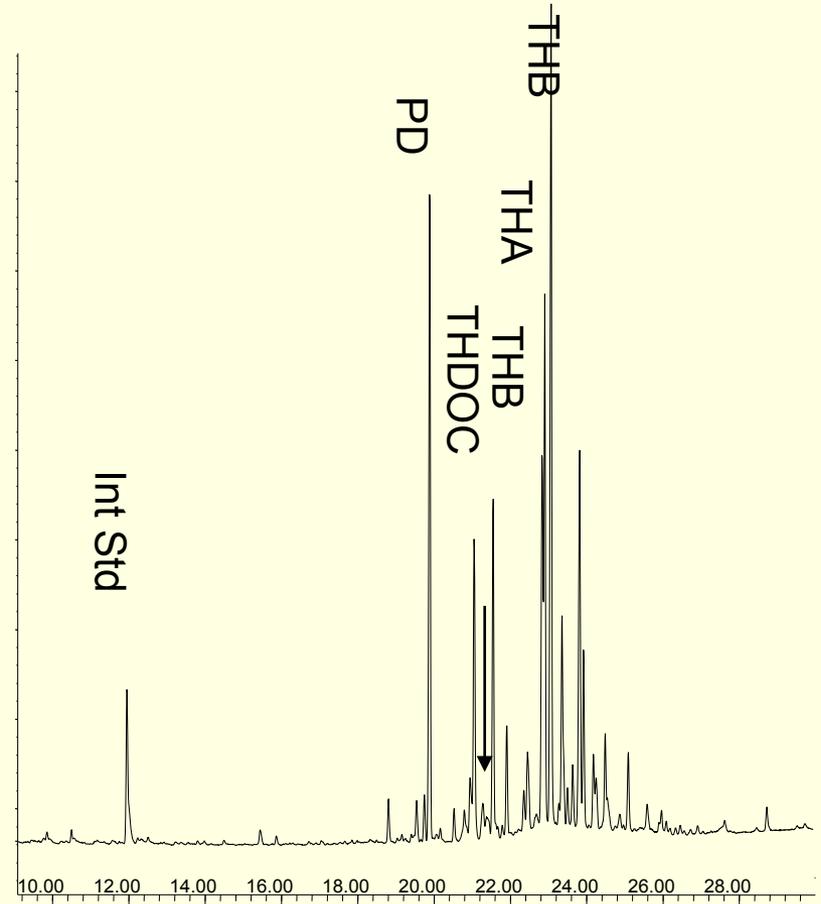
2. Hypertension due to excess mineralocorticoids level.

Treatment:

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

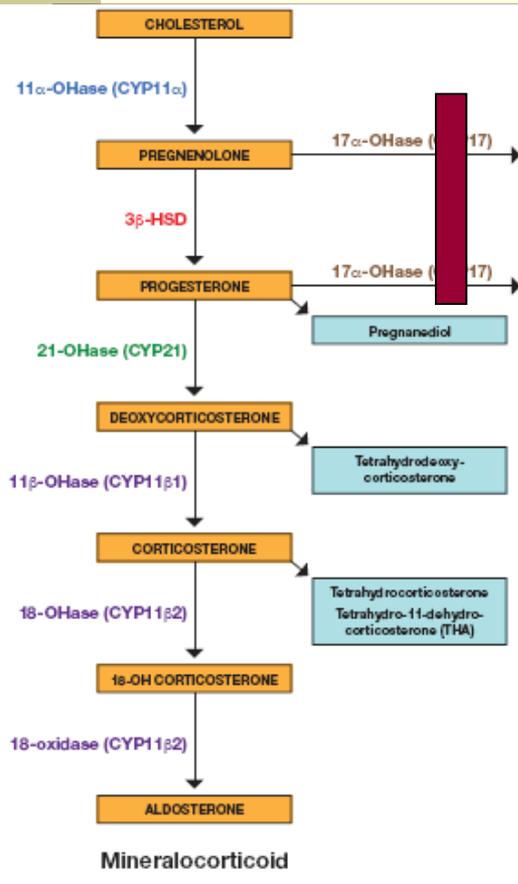
Monitoring of hypertension

Daily transdermal oestrogen patches



Urine steroid profile

CAH – 17 α OHase deficiency



**Increase in mineralocorticoids
cause of hypertension and headaches**

