PIPADOR REVISION

MRCOG Part 1 Revision Notes

ELISA and Immunoassays

MOLECULAR BIOLOGY • LECTURE 95

VISUAL EXAMINATION REVISION NOTES

2026 Edition

MRCOG Part 1 Revision Notes

Lecture 95: Enzyme-Linked Immunosorbent Assay (ELISA)

Molecular biology • Visual revision edition

COLOUR CODE USED THROUGHOUT THESE NOTES

ANTIGEN	CAPTURE / PRIMARY Ab	DETECTION / SECONDARY Ab	SANDWICH / NORMAL	PITFALL / COMPETITIVE

LEARNING OBJECTIVES — tick off as you go

- ☐ Explain the ELISA principle and standard curve.
- ☐ Compare direct, indirect, sandwich and competitive formats.
- ☐ Describe detection systems and lateral flow tests.
- ☐ Recognise assay pitfalls: hook effect, heterophile antibodies, biotin, cross-reactivity.
- ☐ Apply to hCG, antenatal biochemistry, serology, endocrinology and IHC.

1 Principle and Formats

IN ONE LINE: *Antibody meets antigen on a plastic well; an enzyme on the antibody turns a substrate into colour or light; the signal, read against a standard curve, gives the concentration.*

ELISA formats (schematic)

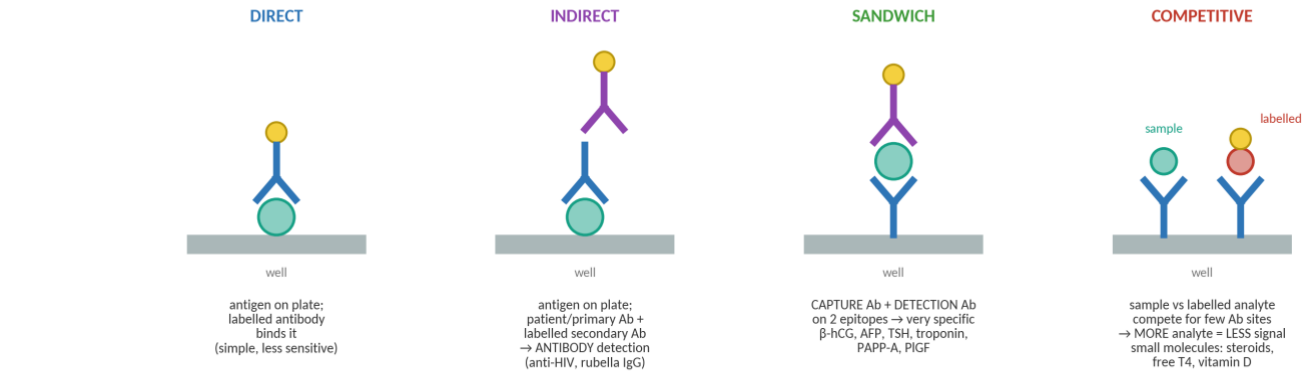


Figure 1. Direct, indirect, sandwich, competitive.

MNEMONIC / MEMORY HOOK

'Big molecules get a Sandwich; small ones Compete.' Proteins with two epitopes (hCG, TSH, PAPP-A) = sandwich; steroids, free T4, vitamin D = competitive.

Detection	Key points
Colour (classic ELISA)	HRP + TMB (blue → yellow with acid stop); alkaline phosphatase + pNPP (yellow)
Chemiluminescence (CLIA)	Enzyme or label makes light — most automated hospital analysers

Electrochemiluminescence	Very sensitive, wide range (e.g. Roche platforms — many use streptavidin–biotin)
Radioimmunoassay (RIA)	Historic ^{125}I — sensitive but radioactive
Turbidimetry / nephelometry	Immune complexes scatter light — CRP, immunoglobulins, C3/C4

2 Standard Curves and the Hook Effect

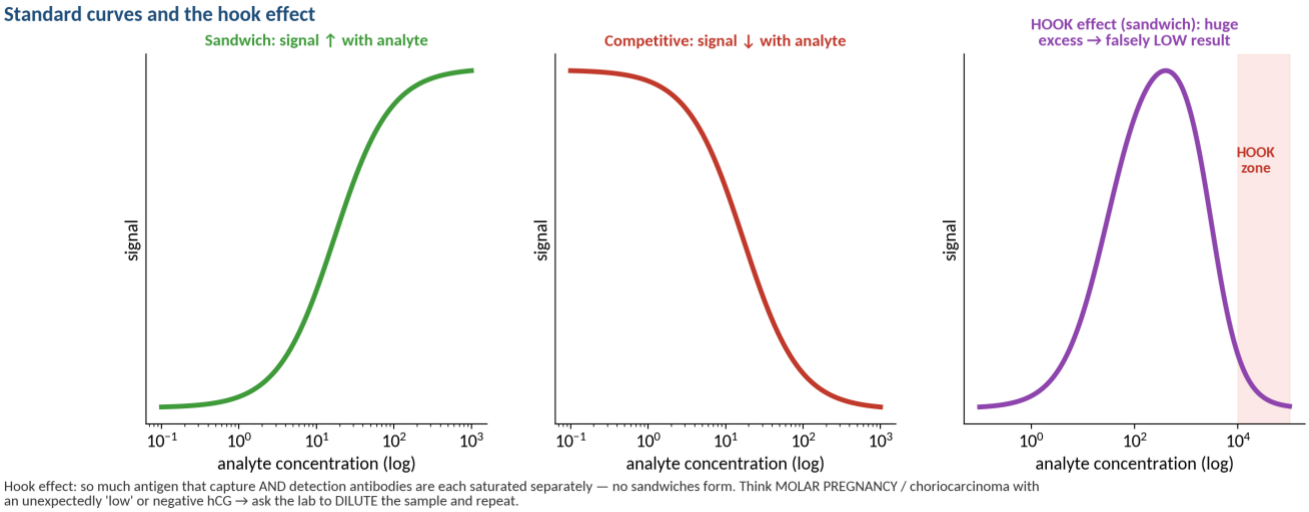


Figure 2. Curves and the hook effect.

3 Lateral Flow Tests

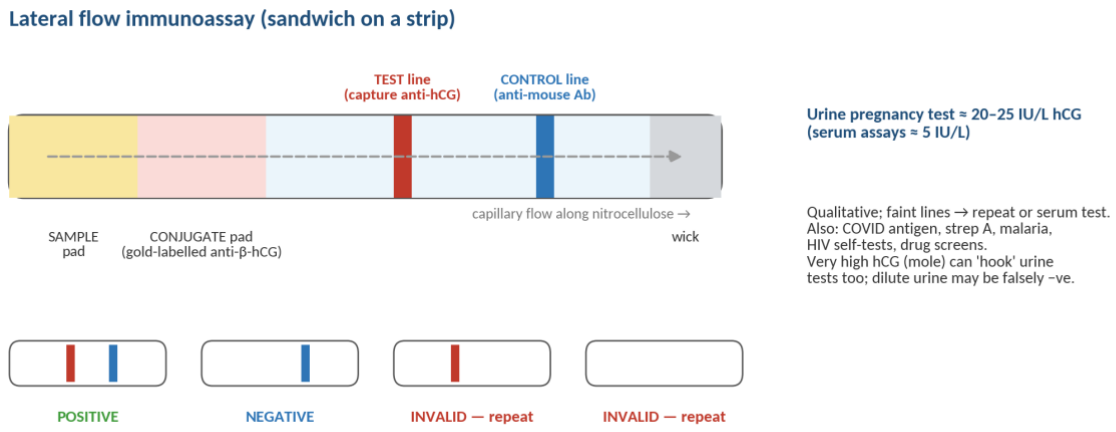


Figure 3. Lateral flow immunoassay.

4 hCG in Practice

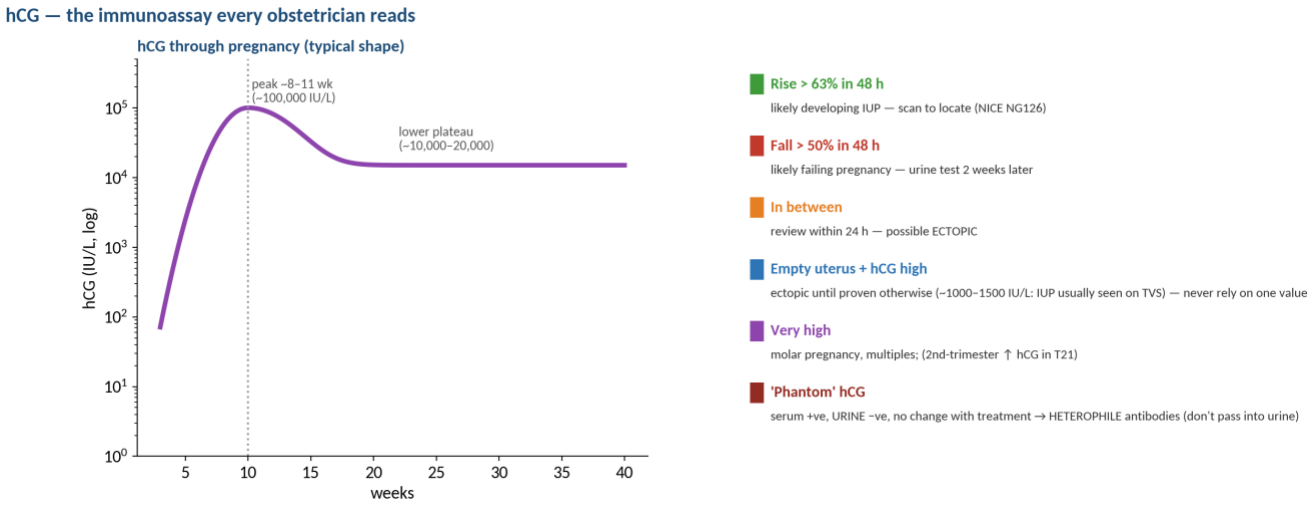


Figure 4. hCG patterns.

- hCG is also a tumour marker (**choriocarcinoma, GTN**, germ cell tumours) — follow-up after molar pregnancy uses serial hCG (UK registration with a GTD centre: Sheffield, Charing Cross, Dundee).

5 Pitfalls

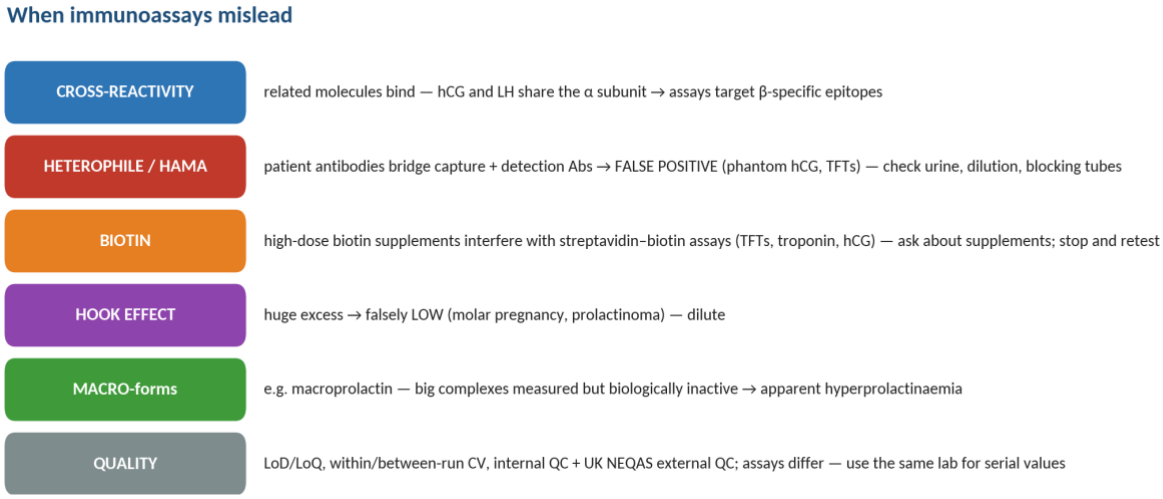


Figure 5. Interference and error.

Measure	Meaning
Sensitivity / specificity	True positives found / true negatives correctly excluded
LoD / LoQ	Lowest level distinguishable from zero / measured with acceptable precision
CV (precision)	Within- and between-run variation; internal QC + external schemes (UK NEQAS)

6 Applications in O&G

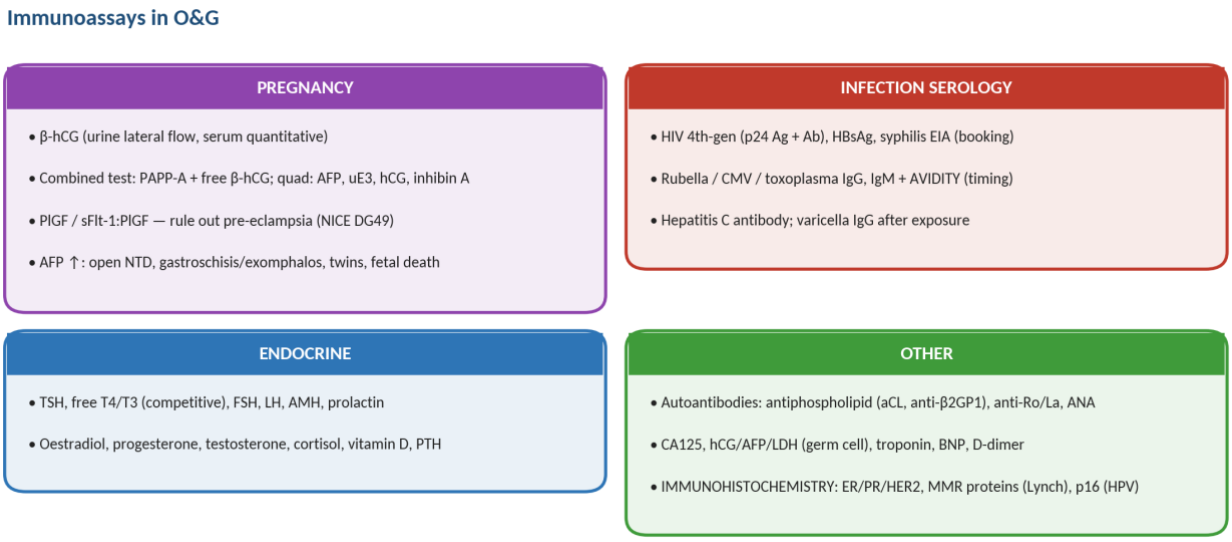


Figure 6. Immunoassays in O&G.

- **Immunohistochemistry** uses the same antibody chemistry on tissue: ER/PR/HER2 (breast), **MMR protein loss** (Lynch screening of endometrial cancer), p16 (HPV-related lesions), p53 (endometrial/ovarian subtyping), WT1/PAX8 (ovarian).

> ★ **PEARL**
>
> **Very high hCG + falsely low result = HOOK effect → dilute. Serum hCG positive but urine negative = HETEROPHILE ('phantom') hCG.**
>
> **hCG: rise > 63% in 48 h suggests IUP; fall > 50% suggests failure; in between = possible ectopic (NICE NG126).**

7 MRCOG Exam Pearls — Last-Minute Page

Numbers to know

~5 IU/L Serum hCG detection	**~20–25 IU/L** Urine test detection	**> 63%** 48-h rise (IUP likely)	**> 50%** 48-h fall (failing)
~1000–1500 hCG: IUP usually seen on TVS	**8–11 wk** hCG peak	**~100,000** Peak hCG (IU/L)	**4** ELISA formats

HIGH-YIELD CHECKLIST

- ☐ ELISA: antibody–antigen + enzyme–substrate → signal → standard curve.
- ☐ Direct, indirect (antibody detection), sandwich (proteins), competitive (small molecules).
- ☐ Competitive: more analyte = less signal.
- ☐ Lateral flow: sample → gold conjugate → test line + control line.
- ☐ Hook effect → falsely low (molar pregnancy) → dilute.
- ☐ Heterophile antibodies → false +ve; urine negative.
- ☐ Biotin supplements interfere; macroprolactin.
- ☐ hCG: 63% rise / 50% fall; never rely on one value; peak 8–11 wk.
- ☐ Antenatal biochemistry, PlGF, serology (incl. IgG avidity) are immunoassays.
- ☐ IHC: ER/PR/HER2, MMR proteins, p16.

Self-assessment — cover the right-hand column

Question	Answer
Format used for β-hCG?	Sandwich
Format used for steroid hormones?	Competitive
In a competitive assay, high analyte gives…	Low signal
Format for detecting patient antibodies (e.g. rubella IgG)?	Indirect
Mechanism of the hook effect?	Excess antigen saturates both antibodies separately — no sandwich
Clinical setting for the hook effect?	Molar pregnancy (very high hCG)
Serum hCG positive, urine negative, no trend?	Heterophile antibody interference
What does the control line show?	The strip worked (valid test)
NICE: 48-h hCG rise suggesting IUP?	> 63%
Why do hCG assays target the β subunit?	α is shared with LH, FSH and TSH

8 References

- NICE NG126. Ectopic pregnancy and miscarriage. 2019 (updated 2023).
- RCOG Green-top Guideline No. 38. Management of Gestational Trophoblastic Disease. 2020.
- NICE DG49. PlGF-based testing to help diagnose suspected pre-eclampsia. 2022.