

FRCPATH Immunology Part 2 practical examination

Station 1 – Autoimmunity

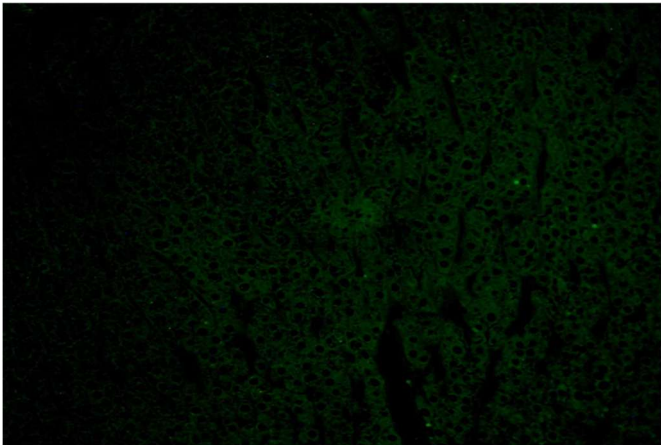
Two example questions are given, including the images that would have been provided on a laptop as Supplementary Material, followed by the answers.

Example question 1

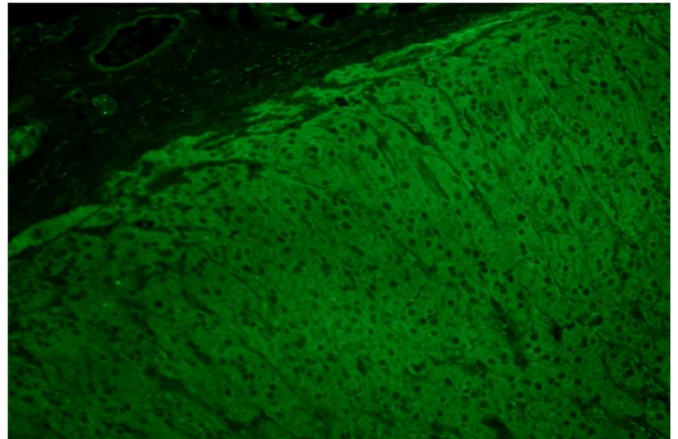
A 15 year old female with a diagnosis of autoimmune hypoparathyroidism developed new onset fatigue, dizziness and postural hypotension. Please review the results shown in Figure 1 and then answer the questions below.

Figure 1: Indirect immunofluorescence on non-human primate adrenal gland

(i) Negative control



(ii) Patient sample



- a) What pattern of staining is observed? (1 mark)
- b) What is the target antigen of the observed antibody staining? (1 mark)
- c) What other organs may be affected by this autoantibody and why? (2 marks)
- d) What primary immune disorder/diagnosis could explain the patient's symptoms and pathology? (1 mark)
- e) State the gene that is mutated in this disorder (1 mark)
- f) Which additional autoantibodies would you suggest testing? (5 marks)
- g) Name one possible infectious complication that may be seen in this primary immune disorder (1 mark)

Example Question 2

A GP has requested a connective tissue disease screen for a 35 yr old with fatigue.

Please review the results shown in Figure 2 (A-C) of the Supplementary Material and then answer the questions below.

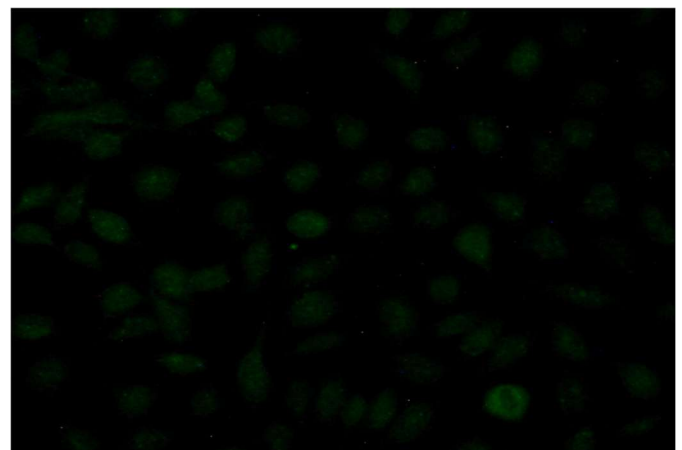
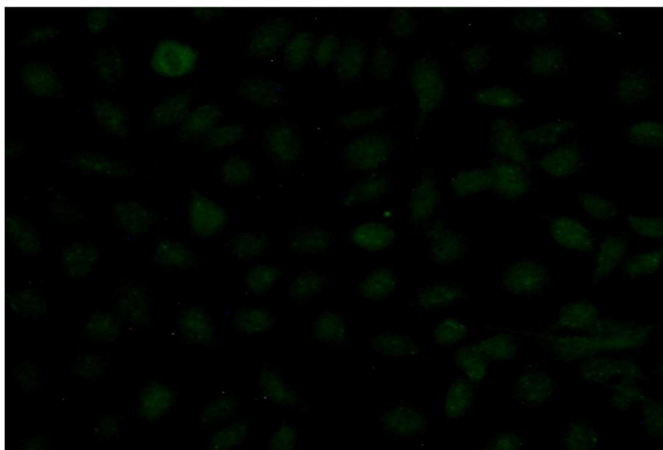
Figure 2A: Enzyme immunoassay results

Test name	Antigens included	Result	Reference range	Upper limit of assay
CTD screen	dsDNA, Ro60(SSA), Ro52, La(SSB), Sm, U1-RNP, Jo1, SCL70, Mi-2	1.3	<1.0 U/ml	30 U/ml

Figure 2B: Follow on testing by indirect immunofluorescence on HEp2 cells

(i) Negative control

(ii) Patient sample



a) Review the HEp2 results shown in Figure 2B and state the result for the patient sample (1 mark)

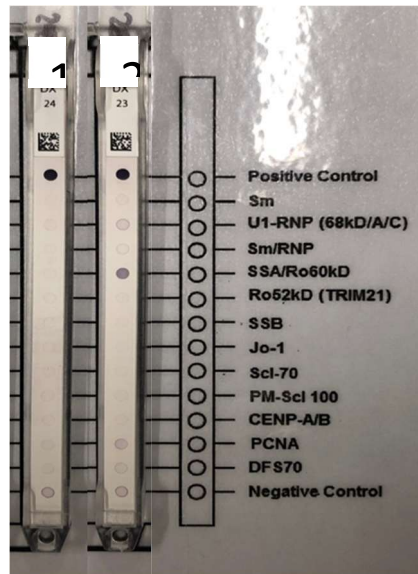
b) Explain this result in the context of the immunoassay results shown in Figure 2A (3 marks)

This sample was investigated further with immunoblotting for extractable nuclear antigens (ENA). Review the results shown in Figure 2C.

Figure 2C: Immunoblotting results for antibodies to extractable nuclear antigens

Results are shown for a healthy control (1) and a patient sample (2)

Please note, the Negative control on each strip demonstrates the maximal intensity of a negative test.



c) Please write a report for the patient sample ENA immunoblot, taking the other lab results into account, and provide an appropriate interpretative comment (3 marks)

d) Explain the ENA blot result in the context of the HEp2 results (2 marks)

ANSWERS:

Question 1

- a) Adrenal cortex (1 mark)
- b) Cytochrome P450/CYP450 (1 mark)
- c) Gonadal cells / ovaries (note patient is female) (1 mark)
The antibodies target cells that synthesise steroid hormones (1 mark)
- d) APECED/Autoimmune Polyendocrinopathy with Ectodermal Dysplasia (1 mark)
- e) AIRE/Autoimmune Regulator (1 mark)
- f) GAD antibodies (1 mark)
IA2 antibodies (1 mark)
Zinc transporter 8 (ZnT8) antibodies (1 mark)
Testes/ovarian antibodies (1 mark)
Thyroid antibodies / TPO antibodies (1 mark)
Note, only 1 mark if state islet cell antibodies
- g) Chronic mucocutaneous candidiasis/CMC (1 mark)

Question 2

- a) Negative (1 mark)
- b) Three of:
 - Weakly positive/equivocal CTD screen could be non-specific (1 mark)
 - CTD screen is a very sensitive assay / contains a mixture of antigens (1 mark)
 - CTD screen can give weak positive / equivocal results due to low level antibodies to a combination of antigens that are negative on further testing for the individual antigens (1 mark)
 - Antigen conformation/format is different in CTD screen compared to HEp2 cells (1 mark)
- c) Weak positive Ro60 (SSA) antibody (1 mark)
Ro60 antibodies can be associated with connective tissue disease, particularly Sjogren's syndrome (1 mark)

However weak positive results are not always clinically significant/interpret with caution or in clinical context (1 mark)

- d) Ro60 pos on immunoblot but not detected on HEp2 cells (or similar comment demonstrating have observed discrepancy) (1 mark - can give mark if referred to in part (c)).

Ro60 is weakly expressed in most Hep2 cell lines / weak Ro antibodies may not be detected by Hep2 (1 mark)