The Royal College of Pathologists
Pathology: the science behind the cure

FRCPath Immunology Part 2 practical examination

Station 5 – Flow cytometry

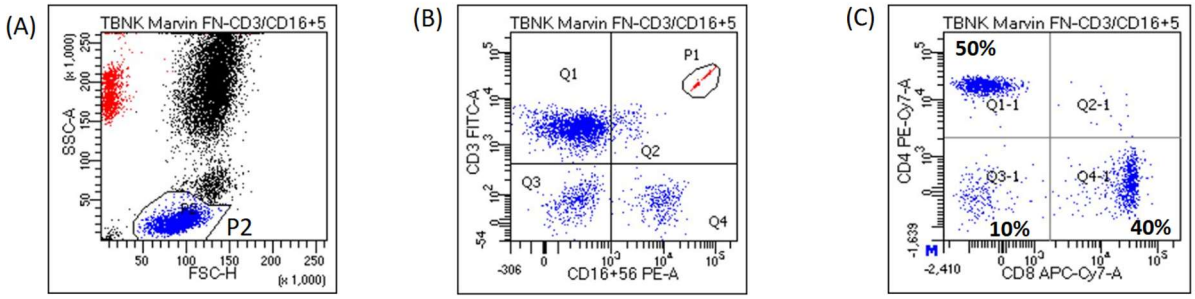
Two example questions are given, including the images that would have been provided on a laptop as Supplementary Material, followed by the answers.

Example question 1

A 2 year old male presents with cervical lymphadenopathy and splenomegaly. Biopsy from a pre-auricular lymph node shows paracortical T cell expansion and follicular hyperplasia but no evidence of malignancy and immediate cultures are negative. Since having the biopsy, he has been experiencing intermittent discharge of yellowish-red liquid from the biopsy site. His full blood count shows anaemia and thrombocytopaenia.

Review the flow cytometry results shown in Figure 1 and then answer the questions below.

Figure 1: Flow cytometric analysis



(a) Summarise the differential diagnosis for this case (3 marks)

(b) State the populations depicted by the following gates in Figure 2A and Figure 2B

(i) Gate P1 (1 mark)

(ii) Gate P2 (1 mark)

(c) Give a cell type that is found in the following quadrants shown in Figure 2B:

(i) Quadrant Q2 (1 mark)

(ii) Quadrant Q3 (1 mark)

(d) What is the name of the cell populations shown in Figure 2C in quadrant Q3-1 (1 mark)

(e) What further tests would you perform by flow cytometry to investigate this further and why? (4 marks)

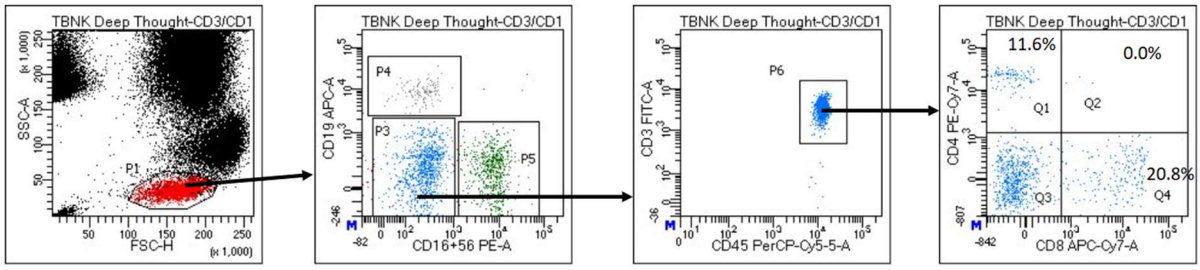
Example question 2

An 8 year old girl presents with an 18 month history of recurrent chest infections. She had normal developmental milestones and an uneventful early childhood. She has developed widespread molluscum over the past year and was diagnosed with hypothyroidism two years ago. Her lymphocyte count has been persistently low (0.2 – 0.3 X10^9/L) over the past 2 years.

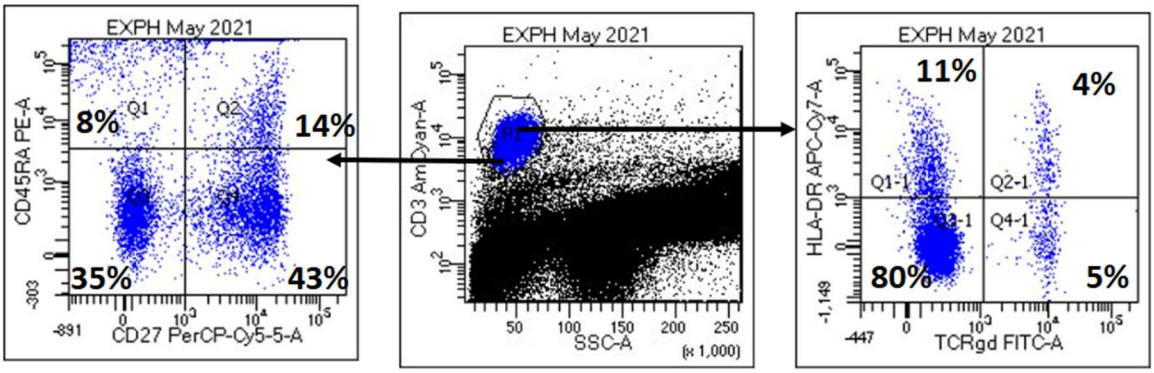
Review the flow cytometry results in Figure 2(A,B) and then answer the questions below.

Figure 2A: Flow cytometry analysis

Tube 1



Tube 2



The flow cytometry analysis is from a single platform methodology using counting beads to obtain cell counts. The gating strategy used is shown for Tube 1. The volume of sample used in the test was 50uL and total number of beads in the tube was 49100.

Figure 2B: Table of event counts

	Number of Events	Cell count (cells/µl)
P1	1693	134
P3	939	-
P6	931	**[A]**
Q1	108	9
Q2	3	0
Q3	626	50
Q4	194	15
P4	127	**[B]**
P5	601	**[C]**
Bead Events	12369	-

(a) Calculate the missing cell counts in Table 2B (expressed as cells/ul) using the available information:

(i) [A]

(ii) [B]

(iii) [C]

(b) From the results shown in Figure 2A for Tube 2, state the proportion (expressed as a percentage of T cells) of:

(i) Naïve T-cells (1 mark)

(ii) Activated T-cells (1mark)

(c) These results are brought to you because they failed technical validation. State why they failed technical validation and suggest a possible cause for this using information from Figures 2A and 2B (2 marks)

ANSWERS

Question 1

(a) Immune deficiency e.g. ALPS (1 mark)
Infections – TB, Cat scratch disease (Bartonella) (1 mark for any of these)
Autoimmune conditions e.g. autoimmune cytopenias (1 mark)

(b) (i) P1 – Counting beads (1 mark)
(ii) 2 - Lymphocytes (1 mark)

(c) (i) Q2 – NKT cells (1 mark)
(ii) Q3 – B-cells (1 mark)

(d) Double-negative T-cells (1 mark)

(e) Test: Alpha/Beta, gamma/delta (1 mark)
Why: Assessment of identity of double negative T cells/Elevated double negatives (>2.5% of CD3) meets diagnostic criteria for ALPS (1 mark for any of these or similar)
Apoptosis functional assay (1 mark)
Why: Apoptosis is impaired in ALPS. DNTs expanded because of impaired FAS/FASl and caspase dependent killing (1 mark for either of these or similar)

Question 2

(a) (i) 74 (1 mark)
(ii) 10 (1 mark)
(iii) 48 (1 mark)

(b) (i) 14 (1 mark)
(ii) 15 (1 mark)

(c) Fails T-sum (1 mark)
Likely increased proportion of double negative Alpha/Beta T-cells as cannot be explained by Gamma/Delta proportion (1 mark)