Answers to self-check questions

Chapter 2

2.1 Serum is a heterogeneous mix of proteins, hormones, metabolites, ions etc. As we are mostly interested in the immunoglobulin fraction a stain is required which will identify these. By using a protein selective stain the molecules of interest can be visualized without excessive interference from other serum components.

2.2 As a minimum, a positive monoclonal sample should be run to demonstrate that patient samples containing significant monoclones can be identified. The monoclone level should be low enough to ensure that small but significant monoclones can be identified. However, if the control sample is also being used as a densitometric control, it needs to be at such a level that an acceptable CV can be achieved on the densitometry results. We find that for serum a single positive control with a monoclone of ~4g/L is acceptable. However for urine we use both a qualitative control with a monoclone level of ~0.01g/L and a quantitative control with a monoclone level of ~0.04g/L. A negative control can be included to show that the electrophoresis has achieved adequate separation, but this information can equally be gained from the patient samples.

2.3

Advantages:

- Stability—monoclonal OCBs are stable, allowing the production of control preparations with a long shelf life.
- Distinct—monoclonal OCBs give a strong, distinct banding pattern making it easy to determine that the sample has applied and run successfully.
- Low background—monoclonal OCB preparations contain little or no polyclonal IgG, enhancing the clarity of the banding pattern for ease of reading.

Disadvantages:

- The presence of a monoclonal OCB control banding pattern gives no guarantee that patient samples in the run have run successfully (unless they too are monoclonal).
- Unfocused gels may show a recognizable monoclonal pattern whilst obscuring weaker positive bands in patient CSF.
- Weak positive bands in patient CSF may not be recognized if there is a strong positive polyclonal background.
- IgG in patient CSF may deteriorate with time, giving a false negative result.

Chapter 3

3.1

- An induction phase in which the immune system forms IgE antibodies in response to initial exposure to the allergen.
- A reactive phase in which an allergen binds to IgE molecules, present on Fc receptors on mast cells and basophils, causing the cells to release pre-formed or rapidly synthesized mediators.

3.2

- Clinical assessment supplemented by laboratory investigations.
- Clinical history, skin tests, intradermal tests, challenge tests, physiological tests.
- Allergen-specific IgE, allergen-specific IgG, basophil activation, mast cell tryptase measurement, eosinophilic cationic protein.

3.3 Check for false positive results in relation to the clinical history and patterns suggestive of sensitization to cross-reactive allergens.

3.4 Defining a patient's reactivity at the level of individual disease-eliciting allergen components using recombinant reagents.

3.5

- To confirm that allergen-specific IgE results are not due to non-specific IgE antibody binding.
- Non-allergic conditions: parasitic infections; immunodeficiency (hyper IgE syndrome; Wiskott–Aldrich syndrome; Omenn's syndrome); vasculitis (Churg–Strauss syndrome); IgE myeloma.

3.6 Levels in the blood peak within 1–3 hours post-reaction and return to baseline within 12 hours.

3.7 Farmer's lung; allergic alveolitis and allergic bronchopulmonary alveolitis; bird fancier's lung.

Chapter 4

4.1

- Classical pathway—antibody/antigen complexes.
- Alternative pathway—stabilization of C3 convertase by bacterial cell walls.
- Lectin pathway—mannose and N-acetyl glucosamine residues in bacterial cell walls.

4.2 Any five out of the seven regulatory proteins below:

C1 esterase inhibitor (C1inh)	Binds and deactivates C1qrs complex
Factor I (fI)	Cleaves C3b and C4b
C4 binding protein (C4bp)	Accelerates decay of C4bC2b and acts as a cofactor for fI cleavage of C4b
Factor H (fH)	Accelerates decay of C3bBb and acts as a cofactor for fI cleavage of C3b
Complement receptor 1(CR1)	Accelerates decay of both C4bC2b and C3bBb. Acts as a cofactor for fI cleavage of C3b and C4b
Decay accelerating factor (DAF)	Accelerates decay of C4bC2b and C3bBb
Membrane cofactor protein (MCP)	Cofactor for fI cleavage of C3b and C4b
CD59	Prevents formation of the membrane attack complex (blocks incorporation of C9)

4.3 Poor handling results in complement consumption, leading to falsely low CH50 results.

4.4

- C3 (normal).
- C4 (usually, but not always low).
- C1 esterase inhibitor levels (low in type 1 HAE, normal in type 2 HAE).
- C1 esterase inhibitor function (low in type 1 and 2 HAE).

4.5

- C3 nephritic factor is an IgG auto-antibody that stabilizes the C3bBb convertase, promoting the continued activation of C3 and profound hypocomplementaemia. C3NeF has been found in association with membranoproliferative glomerulonephritis (MPGN) and partial lipodystrophy.
- C3 nephritic factor should be measured when the above conditions are present, and is suggested by a low C3 with normal C4.

Chapter 5

5.1 Anti-dsDNA.

5.2 Because the symptoms of this disease are varied and it may mimic other diseases.

5.3 Joint.

5.4

- Limited cutaneous systemic sclerosis—anti-centromere.
- Diffuse cutaneous systemic sclerosis—anti-Scl-70.

5.5 Sjögren's syndrome.

5.6 Anti-phospholipid syndrome.

Chapter 6

6.1 Upper airways; lung; kidney.

6.2 Anti-GBM disease.

6.3 Anti-proteinase 3 and anti-myeloperoxidase.

6.4 There is strong evidence that the degree of kidney damage at the time of onset of treatment is the biggest factor in determining long-term kidney survival, so rapid diagnosis is required.

Chapter 7

7.1 The three requirements are the appropriate genetic background (predisposition), an environmental trigger (e.g. viral infection), and bad luck (as many others fulfilling the previous two categories will not develop autoimmune disease).

7.2 The pituitary controls the function of the thyroid gland. If the pituitary is not working properly, it is unable to control the thyroid gland properly, resulting in thyroid disease.

7.3 Hyperthyroidism refers to an overactive thyroid; hypothyroidism to an underactive thyroid. The effects of hyperthyroidism are an increased metabolism leading to symptoms such as nervousness.

7.4 Thyroglobulin, thyroid peroxidase, and TSH receptors.

Chapter 8

8.1

(a) Sub-cutis, dermis, epidermis.

(b) The epidermis, including the dermal–epidermal junction.

8.2 There are many, including systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, scleroderma, anti-phospholipid syndrome, ANCA-associated vasculitis, and cryoglobulinaemia.

8.3 There are several, including scalded skin syndrome (*Staphylococcus*), shingles (herpes zoster) and herpes simplex (cold sore virus).

8.4 Bullous pemphigoid is primarily a disease of the elderly. Most patients present between the ages of 50 and 90 years, with the majority over 70 years of age.

8.5

- Topical corticosteroid (i.e. applied as a cream or ointment to the skin)
- Tacrolimus
- Oral prednisolone
- Azathioprine
- Mycophenolate mofetil
- Cyclophosphamide
- Methotrexate
- Dapsone.

8.6

- Bullous pemphigoid—BP180 (collagen XVII), BP230 (dystonin).
- Mucous membrane pemphigoid—BP180, laminin 332 (laminin-5), laminin 311, α6β4 integrin.
- Epidermolysis bullosa acquisita—collagen VII.
- Pemphigoid gestationis—BP180, sometimes also BP230.
- Linear IgA disease—BP180.

8.7 Adults aged 30–50 years.

8.8 The intercellular bridge structure, the desmosome.

8.9 Main target is demoglein-1.

8.10

1) Upper (epidermal) surface stain—bullous pemphigoid.

2) Lower (dermal) surface—epidermolysis bullosa acquisita.

3) Both upper and lower—mucous membrane pemphigoid but also 5% of bullous pemphigoid.

8.11 There is no correlation in the pemphigoid diseases. In contrast, in pemphigus antibody concentration varies according to disease activity and may be used to monitor disease response to therapy. Either titre on indirect immunofluorescence or ELISA, units of anti-desmoglein may be used.

Chapter 9

9.1 The therapy is different and application of the wrong therapy may well be fatal. The treatment for AIH is immunosuppression, and in cases of infection, especially infection by the hepatitis viruses, the closing down of the immune system would allow the virus to cause irreparable hepatic and other organ system damage.

9.2 Cholangiography is the imaging of the biliary tree and there are several forms of this technique. The histology and auto-antibody profiles of children with ASC and AIH are very similar and it is only the cholangiogram that is able to demonstrate the characteristic abnormalities of ASC. This is even truer of those cases where there is no histological evidence of biliary tree involvement.

9.3 Anti-M2 has been found in cases of urinary tract infection in females. The presence of this auto-antibody can be explained by molecular mimicry. There are epitopes present in the pyruvate dehydrogenase complex of M2 that are also found in *E. coli* (the organism of the infections described).

Chapter 10

10.1 At least three elements should be included in the definition (immunity, cancer, and neurological disorder). A fourth is the remote effects of the cancer.

10.2 A neurological deficit that is most frequently associated with the neoplasm.

10.3 IIF screen alone can be uninformative as there are many antigens which can colocalize with specific paraneoplastic targets.

Chapter 11

11.1 CD3 and CD8.

11.2 B cells: all Btk^+.

Monocytes: two populations—one Btk positive and one Btk negative.

Chapter 12

12.1 HIV infection is usually determined using an ELISA which combines the testing of p24 antigen and anti-HIV antibodies, together with other HIV-1 antigens, such as gp160, gp41. Some HIV tests differentiate between HIV-1 and HIV-2 although confirmation is usually by Western blot or line probe assays—reactivity to gp120 and gp41 is indicative of HIV-1 infection while samples positive for HIV-2 would react with gp105 and gp36, but not with gp120 and gp41. In developing countries, point of care or rapid screening tests may be offered.

12.2 Yes. As in 12.1, most ELISAs differentiate between HIV-1 and HIV-2, although confirmation of HIV-1 or HIV-2 infection is usually by Western blot.

12.3 The term seroconversion refers to the development of antibodies following infection or immunization, i.e. the change from an antibody negative to antibody positive state. In HIV-1 infection, however, individuals are said to have 'seroconverted' following a 'seroconvertion illness' which may preceed the development of antibodies and may be marked by a significant viraemia.

12.4 During seroconversion illness patients may experience flu-like symptoms, myalgia, pyrexia, and lymphadenopathy. Occasionally patients present with a mobilliform rash.

12.5 Long term non-progressors or elite controllers (those who maintain a viral loads of < 50 copies/mL) are individuals who have been infected with HIV virus but have sustained low level viraemia and normal CD4 counts in the absence of antiretroviral therapy.

12.6 Disease progression and/or therapy efficacy can be determined by monitoring CD4 counts and HIV viral load. In treatment naïve patients, approximately one third will present with a CD4 count of < 200 cells/μL—these patients, even after commencing antiretroviral therapy, will have a significantly higher risk of disease progression or death than those starting with higher CD4 counts. Patients presenting with low CD4 counts may have contracted the disease a long time ago and the CD4 count may be a reflection of the progressive damage to the immune system over time. Predictions may be made about possible disease progression from initial CD4 count and viral load.

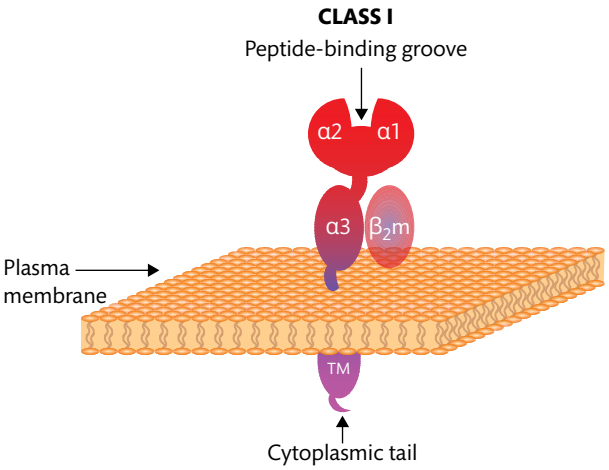
12.7 Treatment failure may occur for a number of reasons: development of resistant strains, suboptimal absorption, or maybe due to patient non-compliance.

12.8 If mutations are not detected following genotypic resistance testing, a failure in antiretroviral therapy may be due to non-compliance or poor absorption—therapeutic drug monitoring may be useful in this circumstance.

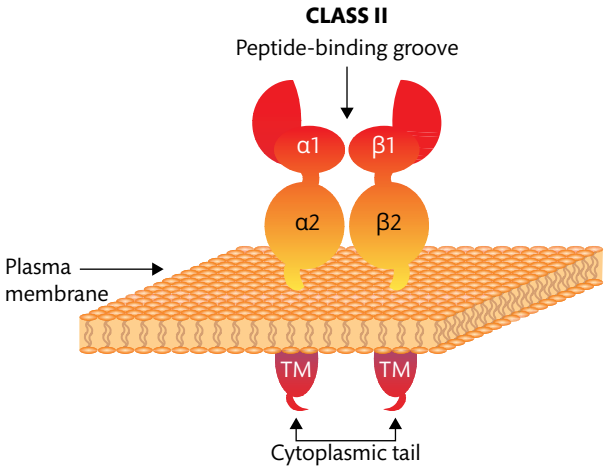
12.9 The failure to produce an effective HIV-1 vaccine is in part down to the high mutation rate of the HIV-1 virus and inability of the immune system to produce a broad spectrum of cross-neutralizing antibodies capable of recognizing a range of target envelope antigens.

Chapter 13

13.1 Diagrams of HLA 1 and 2



13.2 MHC class I and II both present peptides that interact with the TCR. Class I is a closed binding groove with more restricted peptide length than class II. MHC I uses CD8 as a co-receptor, whereas MHC II uses CD4.



13.3 HLA alleles are named as:

HLA – Gene-*-2 digit allele group :3 digit protein group:2digit synonym: 2digit non coding variance:expression digit

e.g. HLA-A*02:101:01:02N

13.4 HLA molecular typing may either be by PCR-SSP, PCR-SSOP, or sequence-based typing. Both SSP and SSOP require that the allele is already known, since sequence-specific probes are generated. With sequence-based typing new alleles can be identified and characterized, since a whole HLA sequence will be generated.

13.5 HLA antibodies may be detected by ELISA or LUMINEX technologies.

13.6 Kidneys are allocated based on need and probability of greatest benefit. Included in this are age, cold ischaemia time, and HLA match.

Tier	Patients
A	000 mismatched paediatric patients—highly sensitized* or HLA-DR homozygous
B	000 mismatched paediatric patients—others (all except those in Tier A)
C	000 mismatched adult patients—highly sensitized* or HLA-DR homozygous
D	• 000 mismatched adult patients—others (all except those in Tier C) • Favourably matched paediatric patients (100, 010, 110 mismatches)
E	All other eligible patients

Usually one kidney is retained locally and the other offered nationally unless the donor is < 5 years or > 50 years of age, in which case both are retained locally.

13.7 Cross matching may be 'real' or virtual. Virtual cross matching uses known donor specific antibody status in the recipient and compares this with the HLA antigens of the donor. Live cross matching may be by complement dependent cytotoxicity (CDC) assay, flow cross match, or solid phase assay.

13.8 The ability of the T cell adaptive immune system to see antigens is dependent on the MHC binding of a peptide and then presenting this to T cells. Certain HLA types will preferentially bind and present certain antigens. In extreme cases individuals will only develop a disorder if they have the specific HLA haplotype that allows the presentation of immune pathogenic peptides. Coeliac disease is a good example where only certain DQ2/8 heterodimers allow presentation of deamidated gliadin peptides which cause disease.