INSTRUCTIONS TO CANDIDATE

Time allowed is three (3) hours

Answers should be written in the answer book provided, writing on the right hand page only leaving the margin blank. The facing page may be used for rough work if desired.

The examination consists of:
- 2 essay style questions; each question is worth 35 marks.
  (Allow approximately 30 mins each)
- 20 short answer questions; each question is worth 5 marks.

Time allowed for writing is three (3) hours. There is an additional initial reading time of 15 minutes during which notes only may be written on the examination paper but no writing in the examination answer books is permitted at this time.
Candidates may attempt either the essay questions or the short answer questions first.

No papers or books of any kind may be taken into the examination room. No electronic devices of any type* are to be taken in to or accessed in the examination room. A non-programmable calculator only is permitted.

*This includes, but is not restricted to: phones, iPads, iPods, eBook readers, MP3 players, memory sticks (flash drives) and WiFi enabled devices of all types.

The examination paper may not be removed from the examination room.
ESSAY QUESTIONS
Both questions must be answered. Each question is worth 35 marks. Suggested time allocation is 30 minutes per question.

A. Describe in detail a method for the chemical processing and subsequent preparation (for TEM) of a renal needle biopsy that is received unfixed. Describe the processing steps you would carry out from the reception of the tissue to the point where you have a grid with sections ready to screen in the TEM. In your answer describe how you come to a decision about what tissue to ultrathin section and screen. Name the chemicals used in processing and their overall concentration and pH but do not give complete formulae, weights and volumes.

B. Describe in detail a strategy for screening, recording and photographing the diagnostic features of a renal biopsy by TEM when you have no light microscopy or immunofluorescence results or supporting clinical information.

SHORT ANSWER QUESTIONS
All questions must be answered. Each question is worth 5 marks. Suggested time allocation is 5 minutes per question.

Note that all questions refer to transmission electron microscopy (TEM) except for Q9 & Q10 which refer to scanning electron microscopy.

1. You are given a wax block containing tissue that has been sectioned for light microscopy (sections stained with H&E). An area of cells within the tissue require screening at the ultrastructural level – briefly describe how would you sample and process this tissue for TEM.

2. Why is it important to fix tissue immediately after it is collected? What artefacts will be visible in the tissue if it is not fixed promptly?

3. What is en-bloc staining? Describe an en-bloc staining procedure for TEM.

4. What are the two principal types of embedding resin that are available for TEM? Describe what each type of resin is used for.

5. In TEM – what is a semi-thin section? What are semithin sections used for? Name the common stain that is usually used to stain such sections.
6. Briefly: (a) what is the cause of the ‘Venetian blind’ effect in the micrograph below (B997: TEM image, detail of mesangium in a renal glomerulus) and; (b) how would you prevent such artefacts?

![Image](B997)

7. Ultrathin sections for TEM are usually flattened before they are viewed. In general, at what point is it best to flatten ultrathin sections and how can it be done?

8. What are the principal causes of poor image contrast when tissue is viewed in a TEM; how can the contrast be improved?

9. What is the purpose of critical point drying in the preparation of a specimen for scanning electron microscopy (SEM)?

10. What is the purpose of metal-coating a specimen for viewing with a scanning electron microscope?

11. What are the critical factors to take into account when cutting up and embedding tissue for TEM.

12. In respect to ‘uncertainty of measurement’, what are the main factors that must be taken into account when measuring the thickness of the glomerular basement membrane for diagnostic purposes?
13. What are the cell inclusions (indicated by arrows) in the TEM micrograph of a muscle biopsy (Q13) below. What is their significance?

14. Briefly describe the glomerular basement membrane changes seen in each stage of membranous glomerulonephropathy (TEM).

15. In the renal glomerulus, what are the principal ultrastructural differences that distinguish amyloid from immunotactoid? (TEM)

16. Briefly discuss the significance of tubuloreticular bodies in glomerular endothelial cells in renal disease (TEM).

17. Briefly describe the principal diagnostic ultrastructural features that are associated with glomerular capillary loops in post infectious glomerulonephritis (TEM).

18. Briefly describe the principal diagnostic ultrastructural features seen in glomerular capillary basement membranes in Alport’s syndrome (TEM).
19. Briefly describe the significant diagnostic ultrastructural features that are found in Whipple’s disease (TEM).

20. What is the object seen in cross section in the image (Q20) below? Is it normal or abnormal? (TEM)