UNIVERSITI KUALA LUMPUR
INSTITUTE OF MEDICAL SCIENCE TECHNOLOGY

FINAL EXAMINATION

MARCH 2025 SEMESTER

COURSE CODE	:	HDB30703
COURSE TITLE	:	CLINICAL LABORATORY HISTOLOGY
PROGRAMME NAME	:	BACHELOR OF BIOMEDICAL SCIENCE (HONOURS)
DATE	:	30 JUNE 2025
TIME	:	2:00PM - 5:00PM
DURATION	:	3 HOURS

INSTRUCTIONS TO CANDIDATES

1. Please read the instructions given in the question paper CAREFULLY.
2. This question paper is printed on both sides of the paper.
3. This question paper consist of TWO sections.
4. Answer ALL questions for Section A.
5. Section B consist of four questions. Answer THREE (3) questions only.
6. Please write your answer on the answer booklet provided.
7. Please answer all questions in English only.
8. Please answer MCQ/EMQ questions using OMR sheet. ☐ *Tick if applicable*
9. Refer to the attached Formula/ Appendies. ☐ *Tick if applicable*

THERE ARE 20 PAGES OF QUESTIONS INCLUDING THIS PAGE

SECTION A (Total: 40 marks)

Answer ALL questions.

Please use the answer booklet provided.

1. Which of the following tasks can be performed by a histotechnologist but not typically by a histotechnician?

   A. Performing quality control on prepared stained slides.

   B. Staining tissue using routine hematoxylin & eosin (H&E) dyes.

   C. Performing paraffin embedding.

   D. Cutting tissue sections using a microtome.

2. Which of the following specimens is best grossed by straining and wrapping in filter paper before cassette placement?

   A. Skin excision biopsy

   B. Rectal resection

   C. Cone biopsy of a cervical specimen

   D. Endometrial curettage specimen

3. Accreditation of a histology laboratory ensures _____.

   A. more efficient use of resources and reduced operational costs

   B. faster patient turnaround times

   C. compliance with minimal safety standards

   D. recognition of technical competence and quality

4. Incorrect or missing suturing during specimen collection may result in _____.

   A. delayed transcription of the report

   B. misinterpretation of specimen margins

   C. increased fixation time

   D. excessive paraffin embedding

5. A histopathology lab receives a testis biopsy and plans to perform a trichrome stain to evaluate connective tissue. Which of the following fixatives should be used to maximize stain quality?

   A. Zenker's

   B. Carnoy's

   C. Bouin's

   D. Glutaraldehyde

6. Which of the following statements is true regarding quality systems in a histology laboratory?

   A. External Quality Assurance (EQA) is performed daily by lab staff.

   B. Internal Quality Control (QC) monitors day-to-day consistency within the lab.

   C. Internal Quality Control (QC) compares data between labs.

   D. External Quality Assurance (EQA) is based on equipment calibration.

7. If a specimen is received without a request form, the histotechnician should _____.

   A. attach a generic request form

   B. hold the specimen and seek clarification

   C. process it and inform the clinician later

   D. return it to the submitting unit

8. If a colectomy resection specimen is received, the histopathologist should begin the grossing by:

   A. Marking the entire specimen with multiple ink colours before grossing.

   B. Cutting the specimen into small cubes of similar size.

   C. Rolling the specimen in gauze to absorb excess fluid before inking.

   D. Recording the quantity and placing it in a cassette without sectioning.

9. You have just received a small biopsy specimen measuring approximately 2mm thick and 3mm² in surface area. The tissue is to be fixed in 10% neutral buffered formalin for routine H&E staining. Which of the following fixation protocols is most appropriate to ensure proper penetration and optimal histological preservation?

   A. Fix the tissue at 4°C for 12 hours.

   B. Fix the tissue at room temperature for 6 hours.

   C. Fix the tissue at 60°C for 24 hours.

   D. Fix the tissue at room temperature for 2 hours.

10. A histotechnician unintentionally used unbuffered formalin with a pH of 4.5 to fix a surgical tissue sample. Upon microscopic examination after H&E staining, which of the following artifacts is most likely to be observed in the section?

    A. Dark brown pigment deposits.

    B. Excessive eosin binding.

    C. Poor nuclear staining.

    D. Marked tissue shrinkage and distortion.

11. You are assigned to process a tissue sample from a fat-containing tumor to evaluate its lipid content under the microscope. Which of the following fixation methods would best preserve the lipids in the tissue?

    A. Fix in Zenker's solution for 4 hours.

    B. Perform cryostat sectioning without prior fixation.

    C. Fix in B5 for better nuclear contrast.

    D. Use Bouin's solution followed by paraffin embedding.

12. Which of the following combinations is most effective during the dehydration step (tissue processing) to maximize reagent penetration?

    A. High viscosity reagent and low temperature.

    B. High temperature and no vacuum.

    C. Strong vacuum and rapid agitation.

    D. Low viscosity reagent and gentle agitation.

13. After paraffin wax infiltration, a histotechnician notices that a liver resection specimen remains soft. What is the most likely cause?

    A. The specimen was over-dehydrated.

    B. The infiltration time was too long.

    C. The paraffin wax was too hot.

    D. The specimen was grossed too thick.

14. You are embedding a cross-section of a fallopian tube for routine hematoxylin and eosin (H&E) staining. How should it be oriented in the mould to show all its histological layers?

    A. Longitudinally

    B. Diagonally

    C. Rolled into a coil

    D. Transversely

15. After paraffin embedding, a histotechnician observes that the tissue specimen is not fully visible within the solidified wax block. Which of the following is the most appropriate troubleshooting step to address this issue?

A. Add more paraffin layers after the block has cooled.

B. Soak the block in cold water to improve visibility during sectioning.

C. Replace the paraffin wax with a lower melting point type.

D. Re-melt the block and orient the tissue closer to the surface.

16. Which of the following situations justifies the use of a cryotome?

A. Preparation of 70nm sections for electron microscopy.

B. Immediate surgical diagnosis of soft tissue tumor.

C. Sectioning of resin-embedded bone.

D. Routine histopathology of paraffin-embedded lung tissue.

17. Which of the following shows the correct sequence of steps before beginning tissue sectioning using a microtome?

A. Trim the block → Chill the block → Secure the block in the microtome holder → Adjust blade clearance

B. Adjust blade clearance → Trim the block → Chill the block → Secure the block in the microtome holder

C. Secure the block in the microtome holder → Chill the block → Trim the block → Adjust blade clearance

D. Chill the block → Trim the block → Secure the block in the microtome holder → Adjust blade clearance

18. Which of the following blades is most suitable for sectioning hard or dense tissues using a rotary microtome?

    A. Disposable low-profile blade

    B. Disposable high-profile blade

    C. Non-disposable tungsten carbide knife

    D. Diamond knife

19. During ribbon flotation, you observe that the tissue ribbons are wrinkling and folding, making it difficult to mount them smoothly onto slides. What is the most likely source of this error?

    A. The water bath temperature is too low, around 35-40°C.

    B. Using distilled water instead of detergent solution in the flotation bath.

    C. Using tap water instead of distilled water in the flotation bath.

    D. The water bath temperature is too high, above 55°C.

20. Which of the following staining techniques is most likely due to van der Waals interactions rather than ionic (Coulombic) bonding?

    A. Congo red

    B. Periodic acid Schiff's

    C. Hematoxylin

    D. Toluidine blue

21. What is the main function of acid alcohol in the hematoxylin and eosin (H&E) procedure?

    A. Prevents fading of the eosin stain.

    B. Removes excess hematoxylin.

    C. Intensifies cytoplasmic staining.

    D. Serves as a mordant for eosin.

22. Which of the following is the most likely cause of a hazy or milky appearance beneath the coverslip after mounting with DPX?

A. Overheating during the flotation step.

B. Excessive hematoxylin staining.

C. Incomplete dehydration before clearing.

D. Remnant of acid alcohol.

23. Which of the following best describes the function of a mordant in hematoxylin staining?

A. It is used to differentiate overstained sections.

B. It counteracts acidic substances in the cytoplasmic regions.

C. It strengthens the binding of hematoxylin to nuclear elements.

D. It helps remove excess stain from the nuclei and cytoplasm.

24. In Masson-Goldner's trichrome stain, the red-stained component represents the _____.

A. osteoid

B. hematopoietic cells

C. nuclei

D. mineralized bone matrix

25. Inadequate reduction of silver during Gordon and Sweet staining will result in:

A. Pale visualization of reticulin

B. Overstained reticular fibers

C. Elastic overstaining

D. Diffuse elastic staining

26. A pathologist requests a VVG stain for an aortic tissue sample. After VVG staining, the section of the aortic tissue sample reveals fragmented elastic fibers. What is the most likely diagnosis?

    A. Arteriosclerosis

    B. Capillary congestion

    C. Solar elastosis

    D. Pulmonary fibrosis

27. Following Alcian Blue–Periodic Acid–Schiff (AB-PAS) staining, prominent blue staining is observed in a gastric biopsy. What is the most appropriate interpretation?

    A. Increased acid mucin production.

    B. Presence of only neutral mucins.

    C. Indication of *H. pylori* colonization.

    D. Dominance of proteinaceous secretions.

28. A histotechnologist observes predominantly brown staining within the colonic crypts following Alcian Blue–High Iron Diamine (AB-HID) staining. What does this finding indicate?

    A. High levels of neutral mucins.

    B. Predominant protein secretion.

    C. Elevated sulfomucin content.

    D. Decreased mucin production.

29. Which of the following neoplasms is most effectively demonstrated by mucicarmine staining for diagnostic confirmation?

    A. Serous cystadenoma

    B. Signet-ring carcinoma

    C. Renal clear cell carcinoma

    D. Squamous cell carcinoma

30. Which pigment is most likely represented by brown-black granules observed in tissue fixed with unbuffered formalin?

A. Melanin

B. Lipofuscin

C. Acid hematin

D. Hemosiderin

31. Which of the following is identified as a beaded, golden-brown, dumbbell-shaped body in lung tissue and is commonly associated with industrial exposure?

A. Carbon granule

B. Amalgam pigment

C. Tobacco inclusion

D. Asbestos body

32. What is the characteristic shape of uric acid crystals seen under polarized light?

A. Needle-like structures

B. Cuboidal blocks

C. Round granules

D. Hexagonal plates

33. Which of the following findings is most consistent with McArdle disease?

A. A complete absence of acetylcholinesterase staining in a muscle biopsy.

B. A complete absence of phosphorylase staining in a muscle biopsy.

C. A positive acetylcholinesterase staining in a muscle biopsy.

D. A positive phosphorylase staining in a muscle biopsy.

34. In the enzyme histochemical diagnosis of osteoporosis, which enzyme activity provides insight into bone resorption?

A. Decreased alkaline phosphatase indicating suppressed osteoblasts.

B. Increased acid phosphatase indicating active osteoblasts.

C. Increased acid phosphatase indicating active osteoclast.

D. Decreased alkaline phosphatase indicating suppressed osteoclasts.

35. Which of the following findings indicates cytochrome c oxidase (COX) enzyme deficiency?

A. Presence of patchy and pale vacuoles in certain muscle fibers.

B. Absence of staining in certain muscle fibers.

C. Presence of intense and dark vacuoles in all muscle fibers.

D. Enhanced staining in all muscle fibers.

36. Which enzymatic stain helps distinguish type I from type II muscle fibers based on oxidative capacity?

A. NADH diaphorase

B. Myeloperoxidase

C. Acid phosphatase

D. Acetylcholinesterase

37. Which of the following diagnostic applications most commonly utilizes the immunofluorescence (IF) protocol?

A. Detection of amyloid deposits in brain tissues.

B. Identification of fungal infections in lung biopsies.

C. Grading of tumor cells in breast carcinoma.

D. Diagnosis of glomerulonephritis in renal tissue.

38. Which of the following challenges must be anticipated while using frozen sections for the immunofluorescence protocol?

A. Increased antibody binding

B. Overfixation of tissues

C. Tissue thickness challenges

D. Morphological problems

39. During analysis of a renal biopsy using the immunofluorescent (IF) staining technique, an orange fluorescence is observed at the glomerular basement membrane. Which fluorophore was likely used?

A. Fluorescein isothiocyanate

B. Hydroxycoumarin

C. R-phycoerythrin

D. Tetramethylrhodamine isothiocyanate

40. A histotechnician includes a "no primary control" slide in an IHC protocol. Which of the following best explains the purpose of this control?

A. To verify that the counterstain does not mask the antigen signal.

B. To ensure that the chromogen reacts only with the primary antibody.

C. To test the sensitivity of the antigen retrieval method.

D. To validate the nonspecific binding of the secondary antibody.

SECTION B (Total: 60 marks)

Answer THREE (3) questions only.

Please use the answer booklet provided.

Question 1

A histotechnician was tasked with sectioning and staining a paraffin-embedded cervix tissue block. During microtome sectioning, the tissue sections repeatedly stuck to the block face on the return stroke and frequently split in the middle. After minor adjustments, a few usable sections were obtained and processed using the standard hematoxylin and eosin (H&E) staining protocol. However, microscopic examination revealed pale nuclei, despite adequate eosin staining. The histotechnician suspected that technical errors had occurred during both the sectioning and staining steps.
Based on this scenario, answer the following questions:

(a) Predict two possible causes of the section sticking to the block face on the return stroke and suggest a solution for each cause.

(4 marks)

(b) Predict two possible causes of the section splitting in the middle and suggest a solution for each cause.

(4 marks)

(c) Predict two possible causes of the pale nuclei of the H&E-stained cervix tissue section and suggest a solution for each cause.

(4 marks)

(d) Compare the staining properties of hematoxylin and eosin in terms of target structures and chemical affinities.

(4 marks)

(e) Explain the preferred orientation of a paraffin-embedded cervix tissue block to the microtome blade.

Refer Below - Figure1 : The image illustrates the orientation of a cervix tissue block to the microtome blade. .

(1 marks)

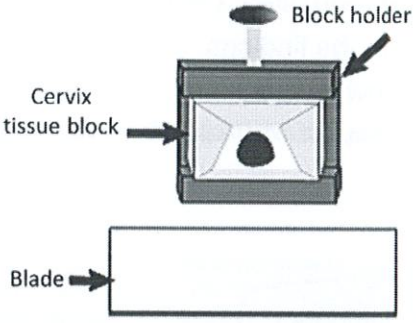

Figure 1: The image illustrates the orientation of a cervix tissue block to the microtome blade.

(f) State three acceptable criteria for mounting media.

(3 marks)

Question 2

A liver biopsy was obtained from a 58-year-old patient with a long-standing history of alcohol-related liver disease. A histotechnician was instructed to perform Van Gieson's trichrome staining technique as part of the diagnostic protocol. The microscopic observation revealed red-stained abnormal tissue (outlined by dashed lines) between clusters of hepatocytes. The histotechnologist requested a Masson's trichrome stain on a serial section of the same biopsy to cross-validate the findings.

Based on the case, answer the following questions.

Refer Below - Figure2 : The microscopic image of liver biopsy tissue stained by Van Gieson's trichrome staining technique. .

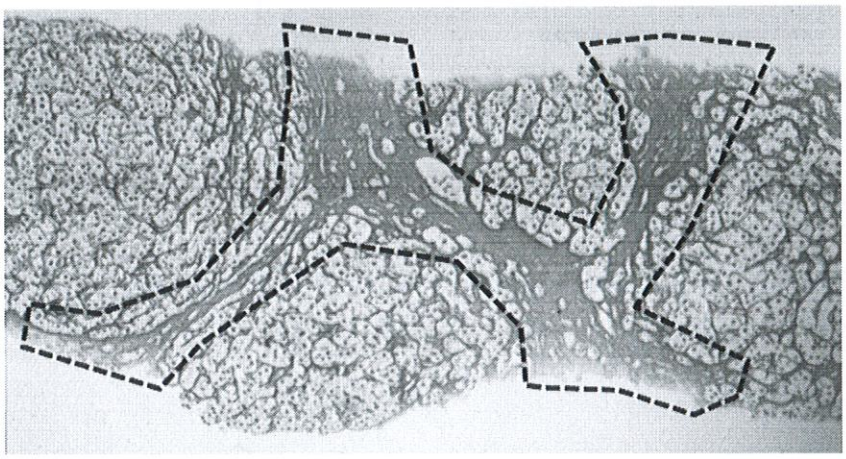

Figure 2: The microscopic image of liver biopsy tissue stained by Van Gieson's trichrome staining technique.

(a) Name the abnormal tissue (outlined by the dashed line) in the Van Gieson-stained section.

(2 marks)

(b) Based on the presence of the abnormal tissue (outlined by the dashed line) in the Van Gieson-stained liver section, predict the most likely stage or condition of the patient's liver.

(2 marks)

(c) Name the staining reagent that stained the abnormal tissue (outlined by the dashed line) red.

(2 marks)

(d) State two possible colours of the abnormal tissue (outlined by the dashed line) when the liver section is stained using Masson's trichrome technique.

(2 marks)

(e) State two staining dyes responsible for the two possible colours of the abnormal tissue (outlined by the dashed line) when the liver section is stained using Masson's trichrome technique.

(2 marks)

(f) Compare the staining reagents and staining colours of muscle fibers when using Van Gieson and Masson's trichrome staining techniques.

(4 marks)

(g) Explain another three diagnostic applications of Van Gieson's trichrome staining technique.

(6 marks)

Question 3

A 52-year-old male presents with chronic diarrhea, significant weight loss, and arthralgia. An endoscopic biopsy of the small intestine is performed and processed in the histopathology lab. A histotechnician performs periodic acid-Schiff with diastase (PAS-D) staining as part of a differential diagnostic work-up. Microscopic evaluation of the lamina propria by a histotechnologist reveals numerous macrophages containing PAS-D–resistant inclusions (arrow). These findings raise suspicion of X disease, a rare bacterial infection.

Based on the case, answer the following questions.

Refer Below - Figure3 : The microscopic image of small intestine biopsy tissue stained by periodic acid-Schiff with diastase .

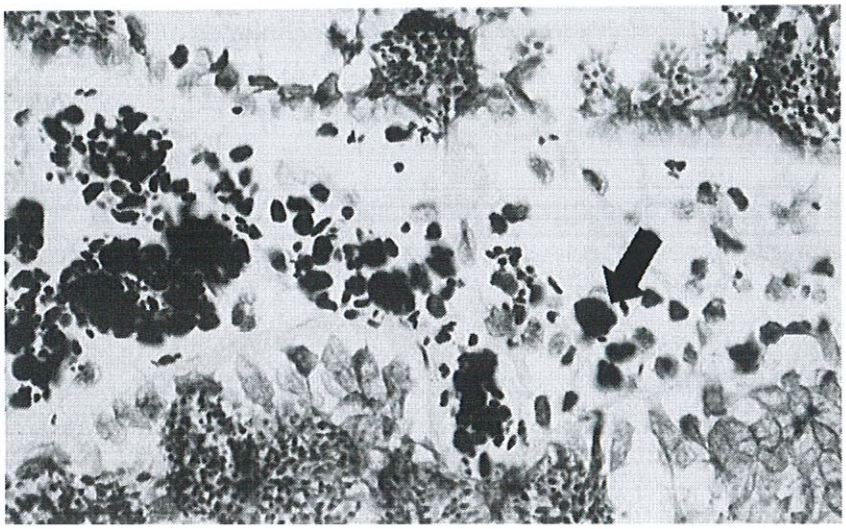

Figure 3: The microscopic image of small intestine biopsy tissue stained by periodic acid-Schiff with diastase

(a) Describe the staining principle of periodic acid and Schiff reagents.

(4 marks)

(b) Should diastase digestion be performed before or after applying the PAS stain in the PAS-D technique? Justify your answer based on the staining principle.

(2 marks)

(c) Based on the presence of numerous macrophages containing PAS-D–resistant inclusions (arrow), predict the most likely X disease.

(2 marks)

(d) Predict the potential misinterpretation that could occur if the section is stained with PAS but not treated with diastase.

(4 marks)

(e) Predict the outcome if the Schiff reagent used in the PAS-D procedure has deteriorated. How would this affect your results and diagnosis?

(4 marks)

(f) Explain another two diagnostic applications of PAS-D staining technique.

(4 marks)

Question 4

A 62-year-old woman presents with a suspicious breast lump. A core needle biopsy is performed, and the sample is processed into formalin-fixed paraffin-embedded (FFPE) tissue blocks. Immunohistochemical (IHC) staining is requested to detect the estrogen receptor (ER), which is located in the nucleus. The histotechnologist performs the IHC staining technique using primary monoclonal antibodies, followed by incubation with biotinylated secondary antibodies. The biotinylated secondary antibodies are conjugated to horseradish peroxidase (HRP), and 3,3′-Diaminobenzidine (DAB) is used as the chromogenic substrate. Microscopic analysis reveals a weak nuclear staining pattern in both the patient and positive control slides (Figures A and B). The positive control slide is expected to show a strong nuclear staining pattern (Figure C).

Based on the case, answer the following questions.

Refer Below - Figure4 : The microscopic images of the breast biopsy of tissue stained by the IHC staining technique. .

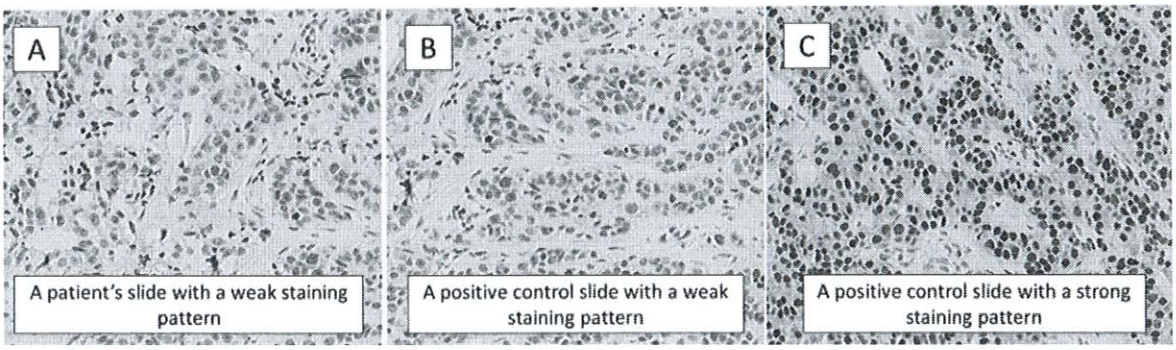

Figure 4: The microscopic images of the breast biopsy of tissue stained by the IHC staining technique.

(a) Explain two advantages of using FFPE tissue for IHC.

(2 marks)

(b) Explain the necessary step to detect estrogen receptor (ER) in the nuclei during IHC staining, and recommend two detergents for this step.

(4 marks)

(c) Explain two advantages of using monoclonal antibodies in IHC.

(2 marks)

(d) Explain the characteristics of the biotinylated secondary antibody.

(2 marks)

(e) Predict the final reaction product colour produced when horseradish peroxidase (HRP) reacts with 3,3′-Diaminobenzidine (DAB) during IHC staining.

(1 marks)

(f) Suggest three possible reasons for the weak staining observed in the positive control slide during IHC, and explain how each issue could be resolved.

(6 marks)

(g) Can the results of the IHC be accepted? Justify your answer.

(3 marks)

END OF EXAMINATION PAPER