Generic Report 2025

TECHNICAL MODULE IAPMD QAP 2025

Prepared by

Technical Module IAPMD QAP Organiser

TM25-1 : Processing, Sectioning and H&E Staining

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TM25-2 : Trichrome Stain

Registration and Acceptance Status of TM IAPMD QAP 2025

This is a report of Anatomic Pathology laboratories involvement in the TM IAPMD QAP 2025. In this current exercise, the registration for all 19 participants was managed by Innovz Sdn. Bhd. All laboratories participated in the TM25-1, whereas 15 participated in TM25-2. The participants included 13 from the Ministry of Health Malaysia hospitals' laboratories, one from the Ministry of Defence Hospital, four teaching hospitals' laboratories and one from a private laboratory.

This exercise consisted of two modules: TM25-1 (processing, sectioning, Haematoxylin and Eosin staining) and TM25-2 (Trichrome stain). As for the questionnaire, all responded to both TM25-1 and TM25-2 questionnaires. All participants submitted appropriate tissue types for TM25-1, and TM25-2 for assessment. However, upon arrival, one of the glass slides was found broken, and two participating laboratories had mislabelled their participation IDs.

INSTRUCTIONS TO PARTICIPANTS

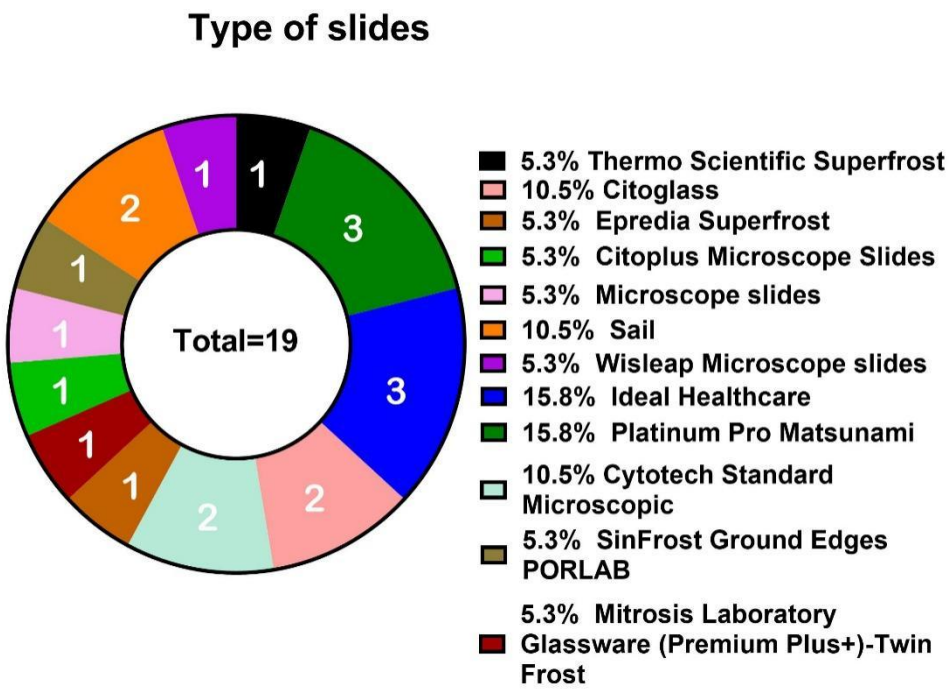
CLINICAL NOTES

Code	Exercise	Clinical Notes
TM25-1	• Processing and Sectioning • Haematoxylin and Eosin (H&E) staining	• Tissue type: Colon/ Appendix/ Small intestine tissue • Appropriate section size
TM25-2	• Trichrome stain	• Tissue type: Colon/ Appendix/ Liver/ Small intestine tissue

ASSESSMENT CRITERIA

Processing, Sectioning and H&E Stain (TM25-1)	Trichrome Stain (TM25-2)
Processing and Sectioning 1. Preservation of the general tissue architecture e.g., nuclear and cytoplasmic details. 2. Appropriate thickness of tissue section. 3. Absence of knife lines, chatter, compression or over-expansion. 4. Absence of bubbles and excess mounting 5. Absence of artefacts from dehydration, clearing and mounting. **H&E Staining** 1. The effectiveness in demonstrating nuclear membranes, nucleoli, chromatin of vesicular and hyperchromatic nuclei. 2. The effectiveness in demonstrating all non-nuclear material e.g., cytoplasm, fine and dense connective tissue fibres, skeletal and smooth muscle and red blood cells (where present). 3. Adequate contrast between haematoxylin & eosin. 4. Uniformity of staining across slide. 5. Absence of contaminants.	1. Effectiveness in demonstrating the epithelial mucin. 2. Minimal background staining. 3. Counterstain quality – complementary not obscuring (where used). 4. Staining result suitable for diagnostic reporting. 5. Uniformity of staining across the slide. Absence of contaminants. 6. Absence of artefact from dehydration, clearing and mounting.

- Assessment result (Total mark out of 5):
  - Unsatisfactory <2.5
  - Borderline 2.5 - 2.9
  - Satisfactory >3



Questionnaire analysis of TM25-1

Figure 1: Responses to the questionnaires on type of slides used
Pie chart showing the survey results regarding the brand of glass slides used by the 19 laboratories that participated.

Summary notes:

1. The Ideal Healthcare and Platinum Pro Matsunami slide brand emerged as the commonly employed choice among the surveyed laboratories, constituting 15.8% of utilisation.

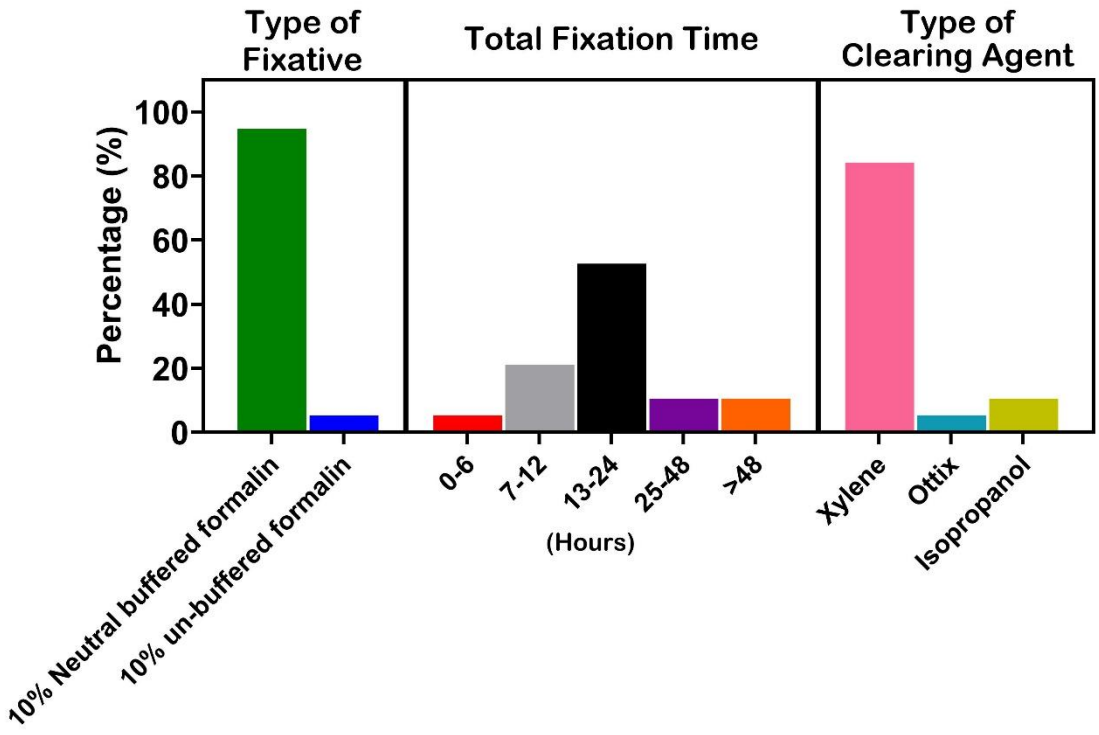


Figure 2: Responses to the questionnaires on tissue processing methods used.
Bar charts show the survey results from 19 laboratories regarding the type of fixative applied to the tissue, total fixation time and type of clearing agent used.

Summary notes:

1. Majority of the participating laboratories utilise 10% neutral buffered formalin in tissue processing as the fixative, whilst only one laboratory (5.3%) employs 10% un-buffered formalin.
2. It was observed that the fixation times for tissue processing frequently surpassed a duration of 13-24 hours.
3. The clearing agents used in tissue processing include xylene, isopropanol, and Diapath Ottix Plus, with xylene being the most commonly employed.
4. It is worth noting that fixation times exceeding 48 hours may increase the risk of tissue chattering and tearing, as well as obscure nuclear details.

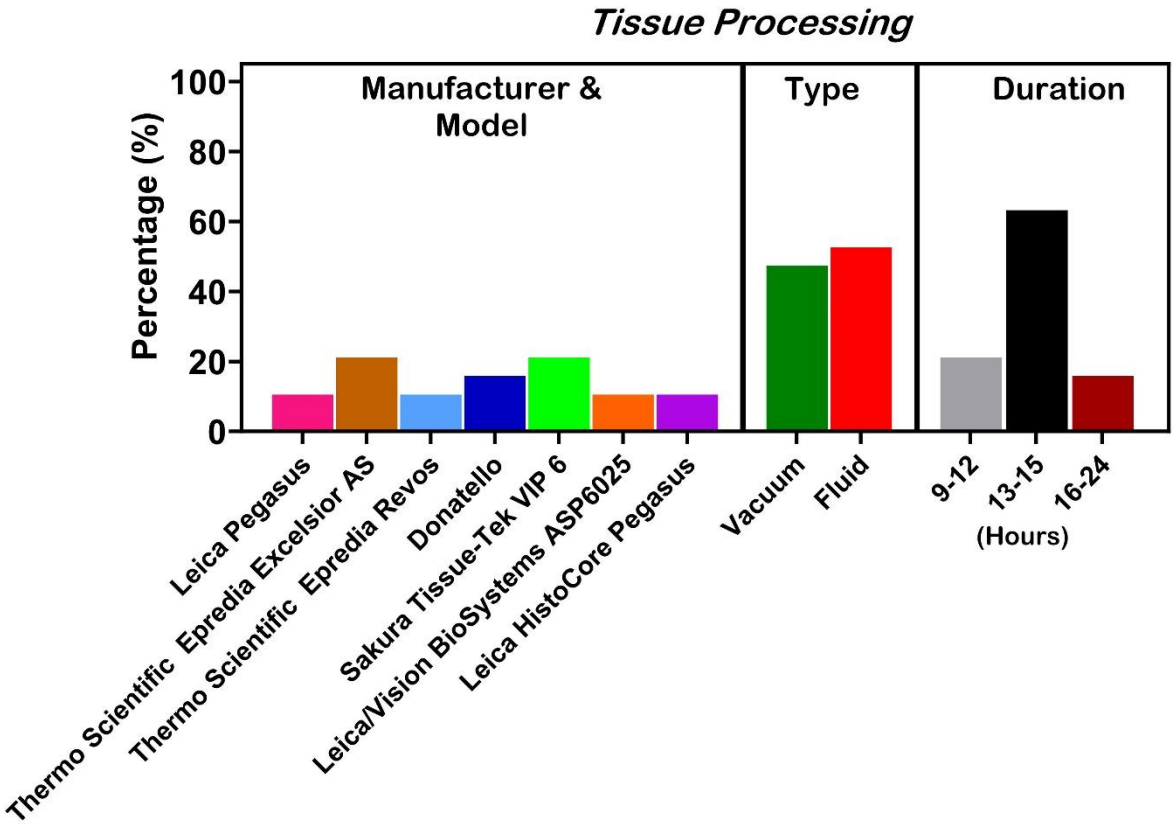


Figure 3: Responses to the questionnaires on tissue processing
Bar charts showing the survey results from 19 laboratories regarding (i) the model of tissue processors used; (ii) the type of tissue processors and (iii) duration of tissue processing.

Summary notes:

1. The Epredia Excelsior AS (Thermo Scientific) and Sakura Tissue-Tek VIP 6 tissue processors model were identified as the prevalent choice among the surveyed laboratories, accounting for 21.1% of usage.
2. The technique of fluid transfer was widely employed in tissue processing by the laboratories involved in the survey.
3. The most frequent tissue processing duration was between 13 to 15 hours.

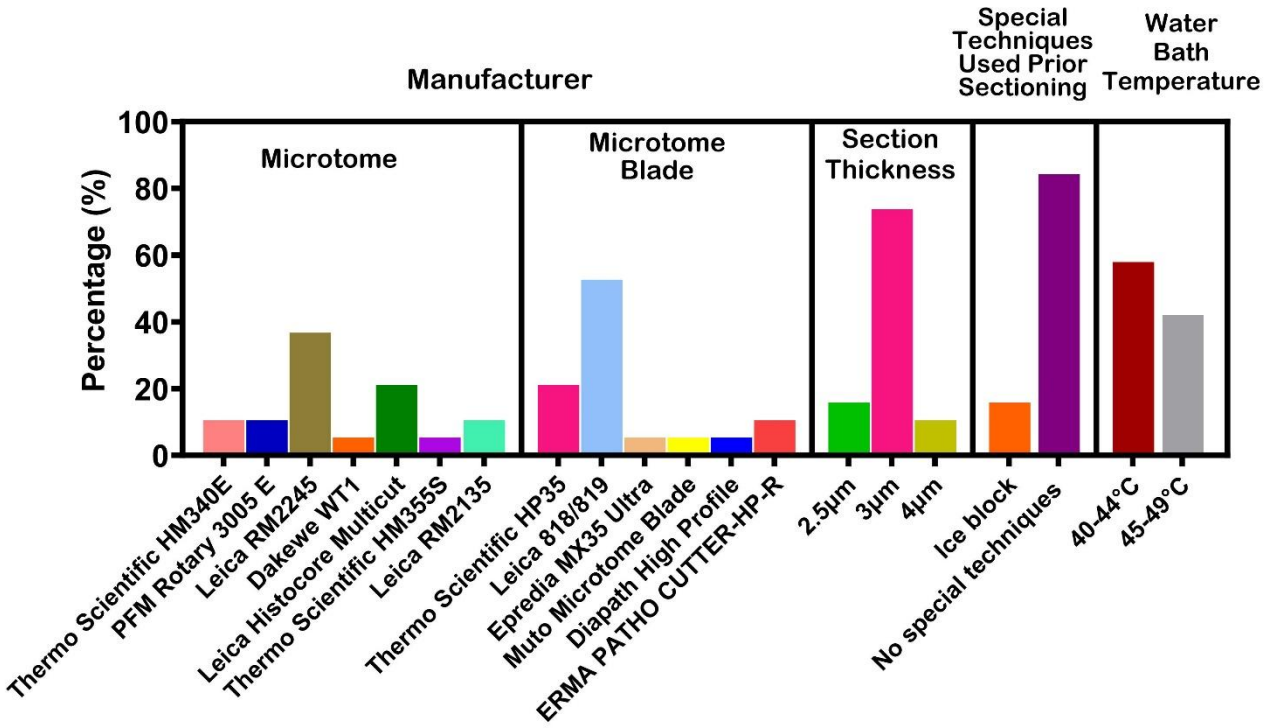


Figure 4: Responses to the questionnaires on tissue sectioning.
Bar charts showing the survey results from 19 laboratories regarding (i) the model of microtome and microtome blade used; (ii) section thickness; (iii) special techniques used before sectioning; and (iv) temperature of water bath for sectioning.

Summary notes:

1. The Leica RM2245 microtome (36.8%) and the Leica 818/819 microtome blade model (52.6%) were frequently employed by the participating laboratories.
2. Tissue thickness of 3 µm was the preferred choice.
3. The findings from the survey indicate that majority of laboratories (84.2%) did not utilise any special techniques prior to tissue sectioning. Ice block treatment was found to be the least utilised method (15.8 %).
4. Water bath set at 40-44°C during sectioning process was the most frequent technique used (57.9%).

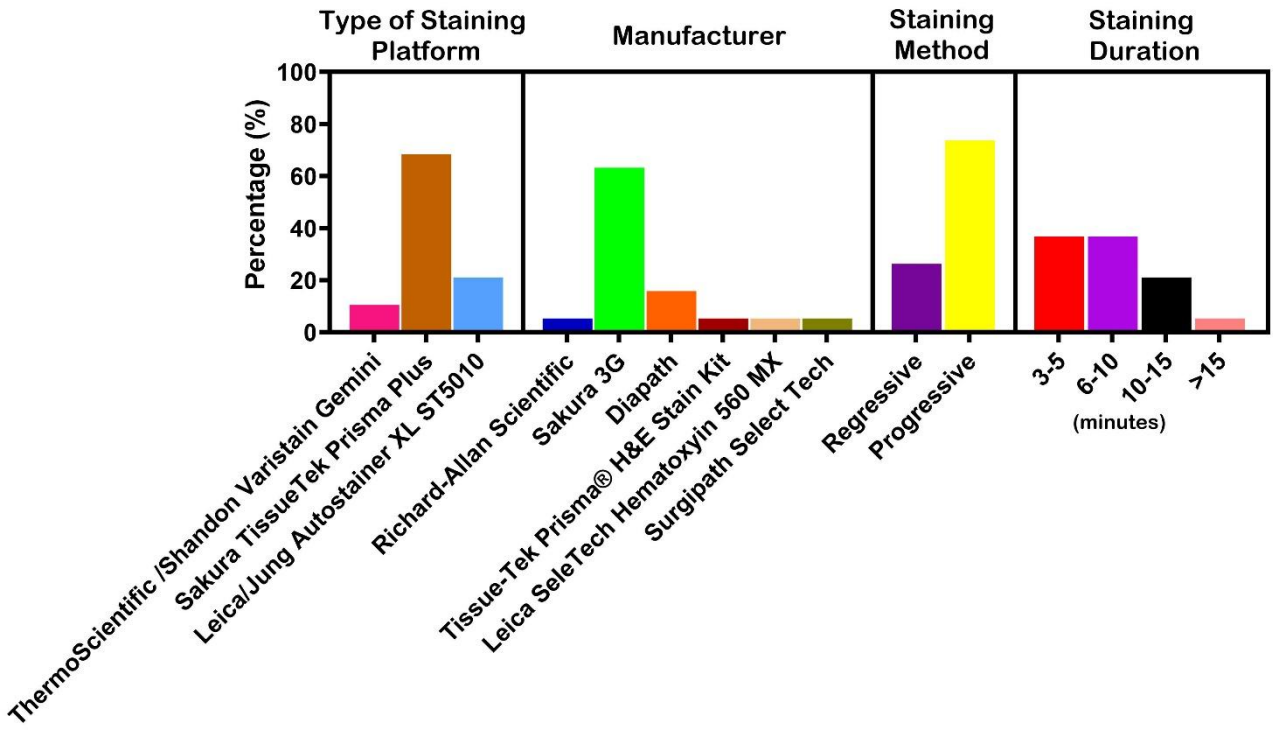


Figure 5: Responses to the questionnaires on haematoxylin in H&E staining.
Bar charts showing the survey results from 19 laboratories regarding (i) type of staining platform; (ii) manufacturer of staining solution; (iii) types of staining method; and (iv) staining duration.

Summary notes:

1. The Sakura Tissue Tek Prisma Plus (68.4 %) was the most common tissue staining platforms utilised.
2. Sakura 3G (63.2 %) and Diapath (15.8 %) emerged as the top two manufacturers for haematoxylin.
3. The progressive staining approach garnered the highest preference as the staining method of choice.
4. The staining duration of haematoxylin that is most favoured falls within the range of 3-10 minutes.

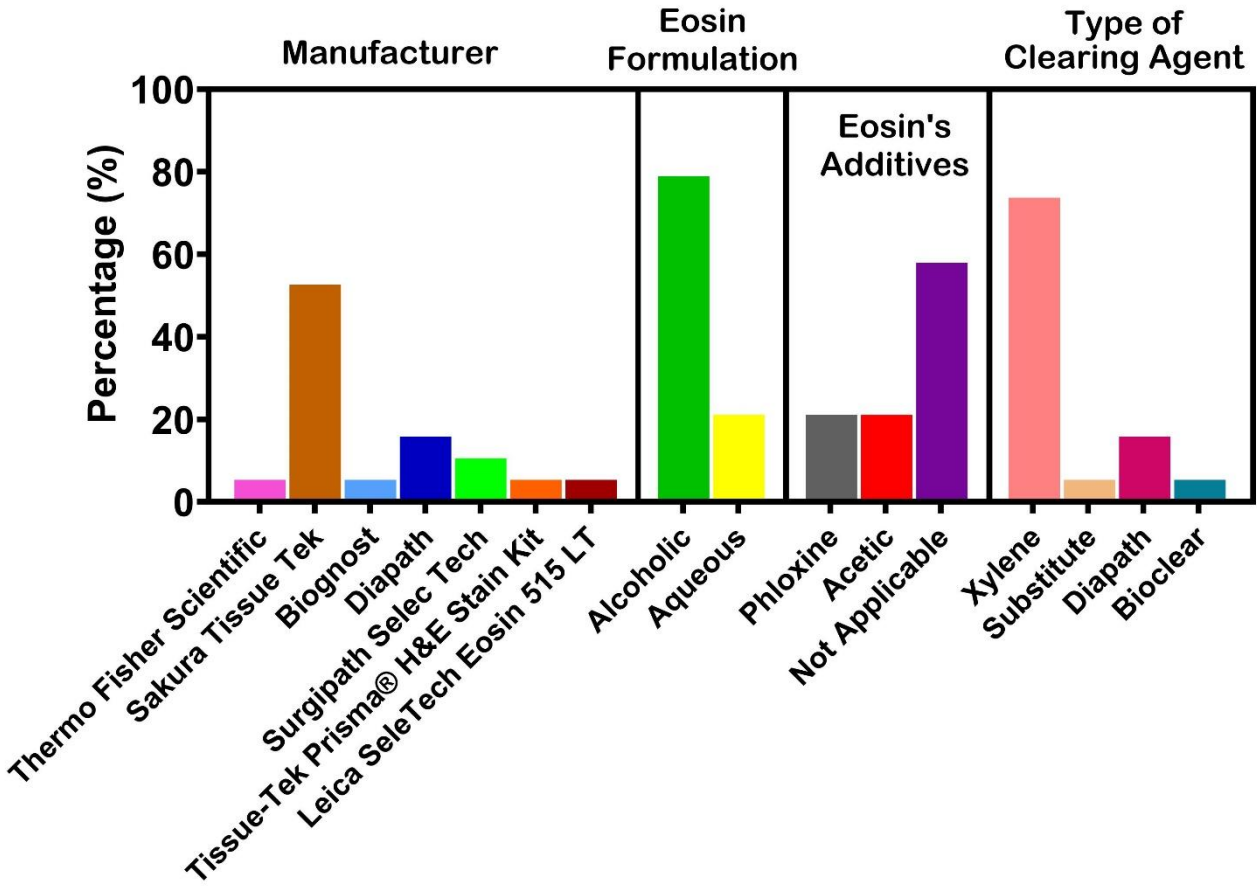


Figure 6: Responses to the questionnaires on eosin in H&E staining.
Bar charts showing the survey results from 19 laboratories regarding the (i) manufacturer of eosin staining solution; (ii) eosin formulation; (iii) eosin's additive; and (iv) type of clearing agent used.

Summary notes:

1. The eosin staining solution manufactured by Sakura Tissue-Tek was the preferred choice among participating laboratories, with a usage rate of 52.6%.
2. Alcohol-based solvent was predominantly favoured over aqueous solvent for eosin formulation.
3. Majority of the surveyed laboratories (57.9 %) opted from using additional solutions in the eosin formation process. However, a subset of laboratories utilised acetic acid and phloxine.
4. The preferred clearing agent for eosin staining in H&E was xylene, followed by Diapath.

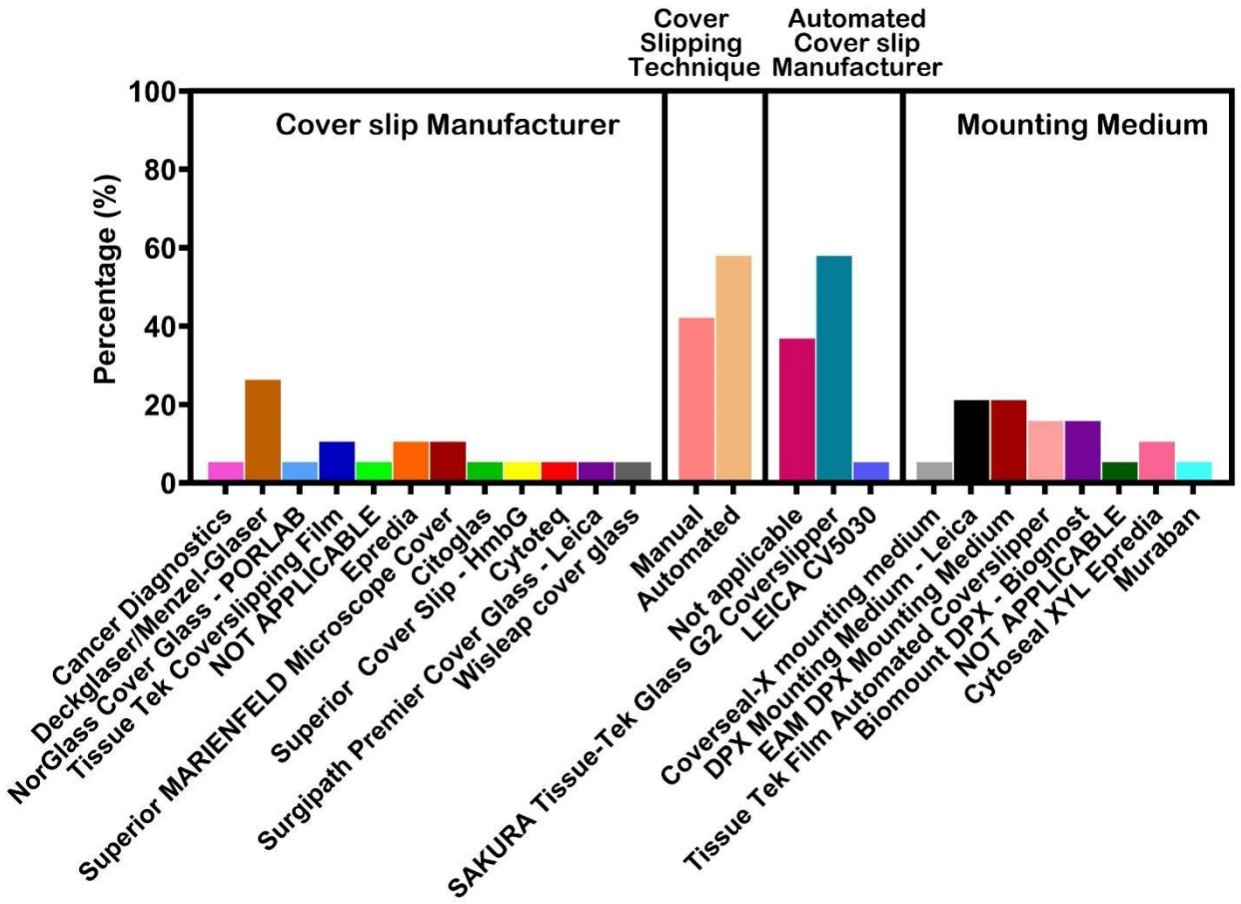


Figure 7: Responses to the questionnaires on cover slip and mounting medium used in H&E staining.

Bar charts showing the survey results from 19 laboratories regarding the (i) manufacturer of cover slip; (ii) cover slipping technique; (iii) manufacturer for automated cover slipping technique; and (iv) type of mounting medium used.

Summary notes:

1. Deckglaser/Menzel-Glaser was the predominant cover slip brand, used in 26.3% of cases.
2. Automated machines were used for the cover slipping technique in most surveyed laboratories, and SAKURA Tissue-Tek Glass G2 Coverslipper was the leading manufacturer.
3. The preferred mounting medium brand used in H&E staining were DPX Mounting Medium-Leica and EAM mounting Medium.

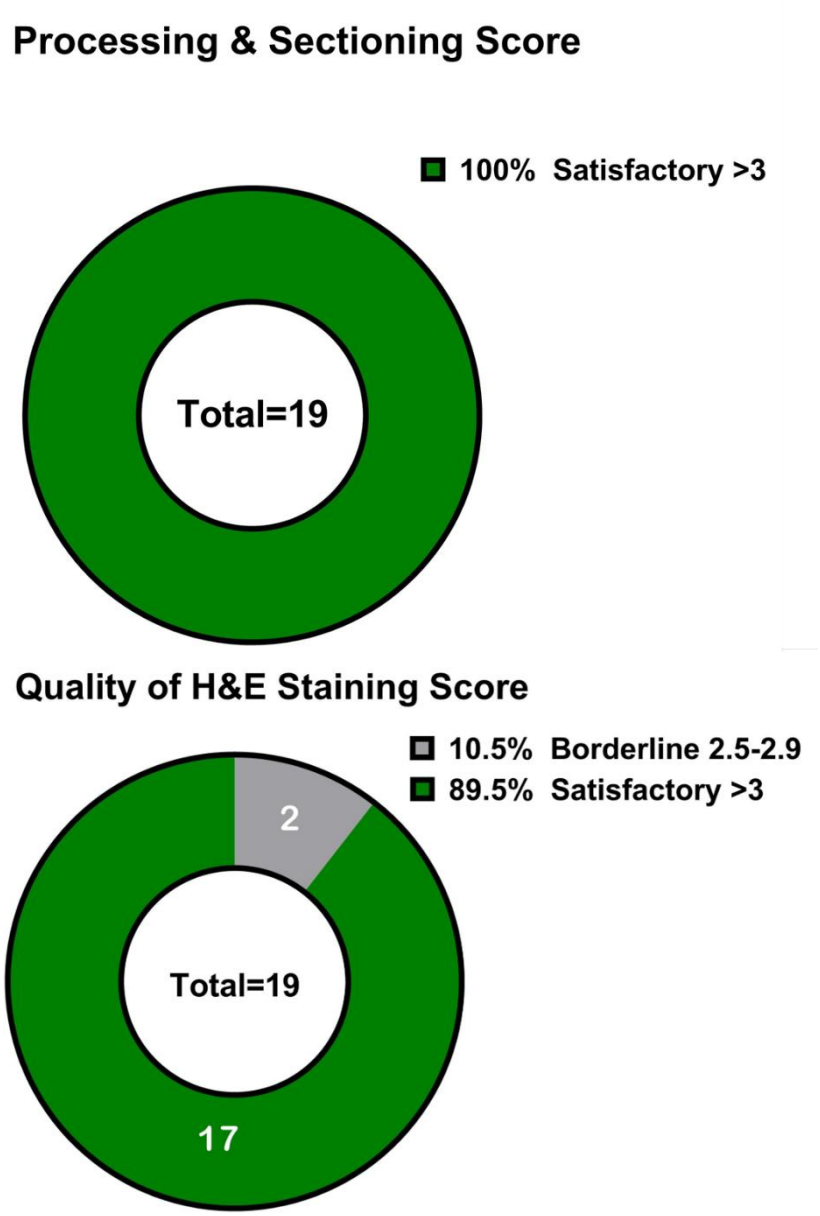
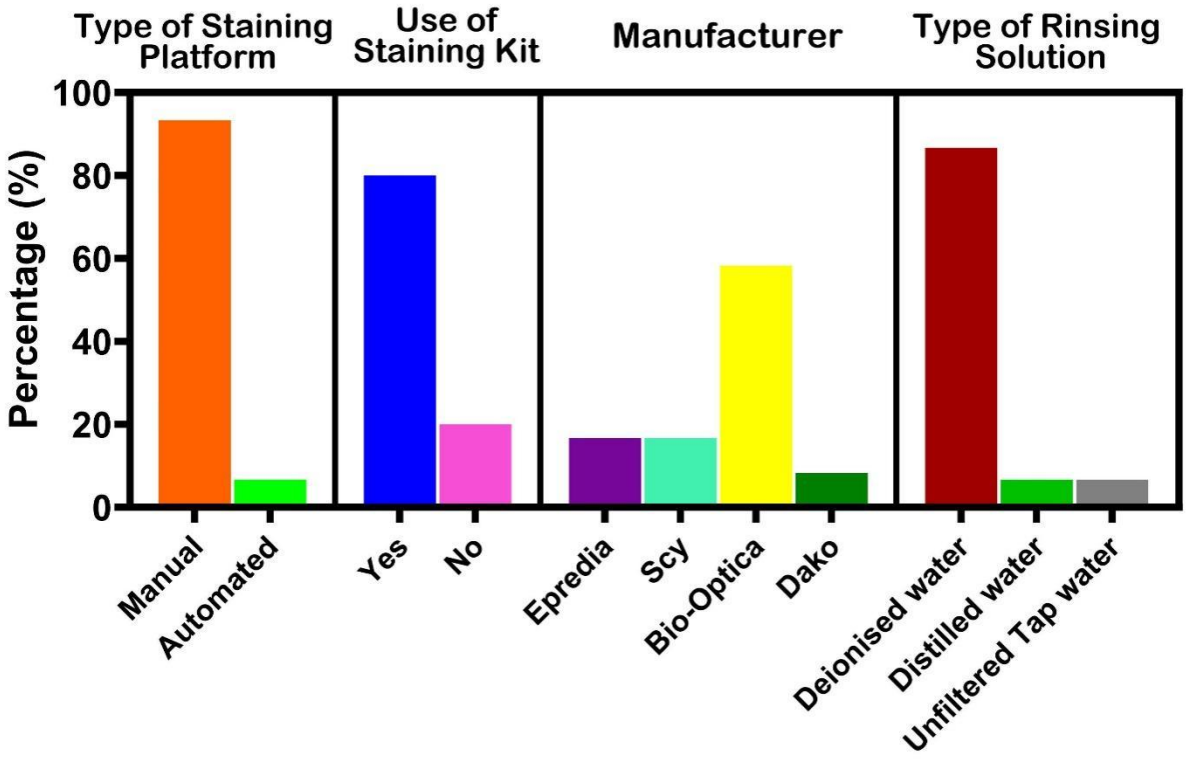


Figure 8: Technical assessment on the quality of tissue processing and H&E staining.
Pie chart showing the quality of tissue processing and H&E staining analysis from 19 laboratories.

Summary notes:

1. All laboratories showed satisfactory quality of tissue processing and sectioning. The highest and lowest scores were 4.4 and 3.0, respectively.
2. Majority of the participating laboratories achieved satisfactory scores (89.5%) for H&E tissue staining. Whilst, 10.5% of the laboratories received borderline scores. The highest and lowest scores were 4.5 and 2.8, respectively.



Questionnaire analysis of TM25-2

Figure 9: Responses to the questionnaires on Trichrome staining

Bar charts showing the survey results from 15 laboratories comprising of (i) the type of staining platform; (ii) use of staining kit; (iii) manufacturer of staining kits; and (iv) type of rinsing solution.

Summary notes:

1. The majority of participating laboratories employed the manual method.
2. Most of the surveyed laboratories used a staining kit.
3. Bio-Optica was the most preferred brand of staining kit (58.3%).
4. Deionised water was the rinsing solution of choice for 86.7% of the surveyed laboratories, while the remaining laboratories were evenly divided between using filtered and unfiltered tap water.

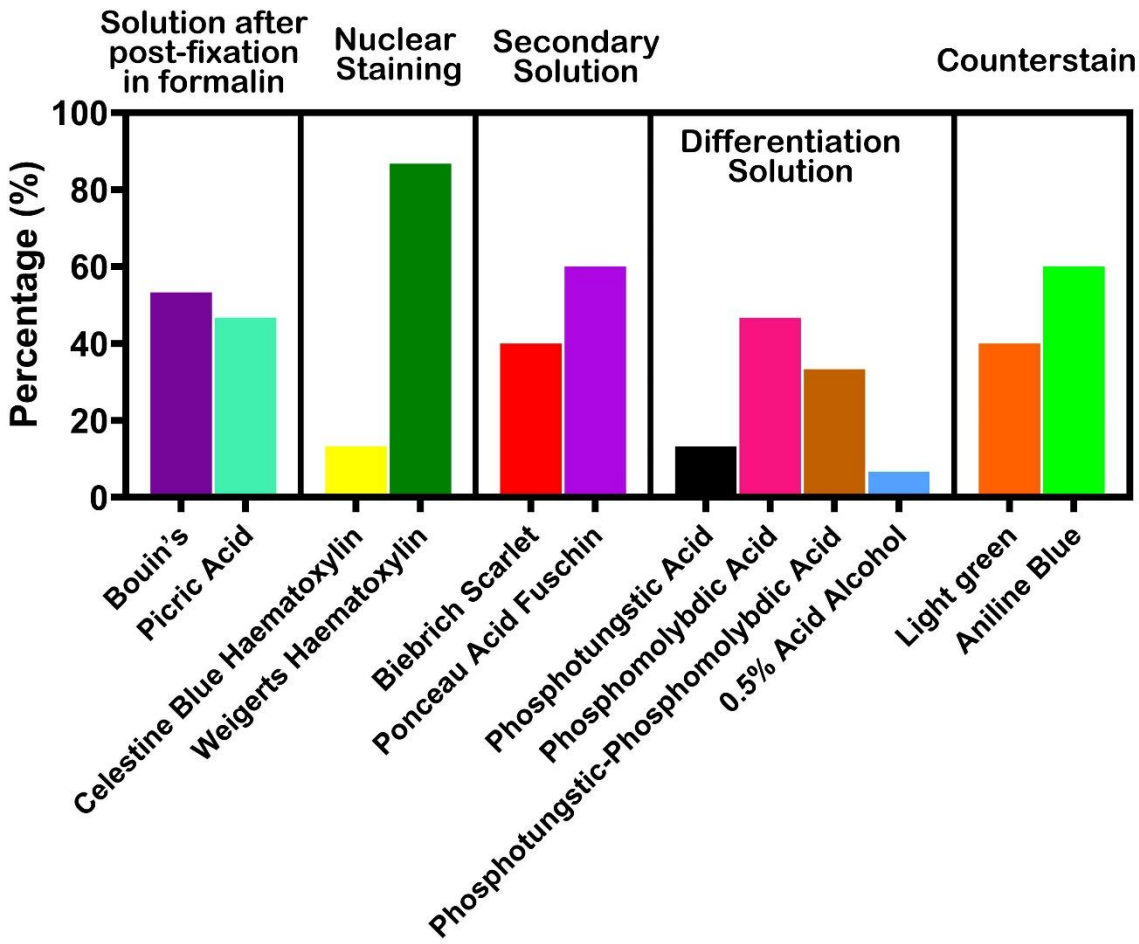


Figure 10: Responses to the questionnaires reagents used in Trichrome staining

Bar charts showing the survey results from 15 laboratories comprising of (i) type of solution used after post-fixation in formalin; (ii) type of nuclear staining; (iii) secondary solution; (iv) differentiation solution; and (v) type of counterstaining.

Summary notes:

1. Bouin's solution was the predominant post-fixation reagent used following formalin fixation, employed by 53.3% of the surveyed laboratories, while the remaining laboratories preferred picric acid solution.
2. Weigert's Haematoxylin was the nuclear stain of choice in Trichrome staining, used by 86.7% of laboratories.
3. Ponceau Acid Fuchsin was the most preferred secondary stain, surpassing the use of Biebrich Scarlet.
4. Phosphomolybdic acid was the most commonly favoured differentiation solution, selected by 46.7% of laboratories.
5. Aniline Blue received the highest preference as the counterstain of choice among the surveyed laboratories.

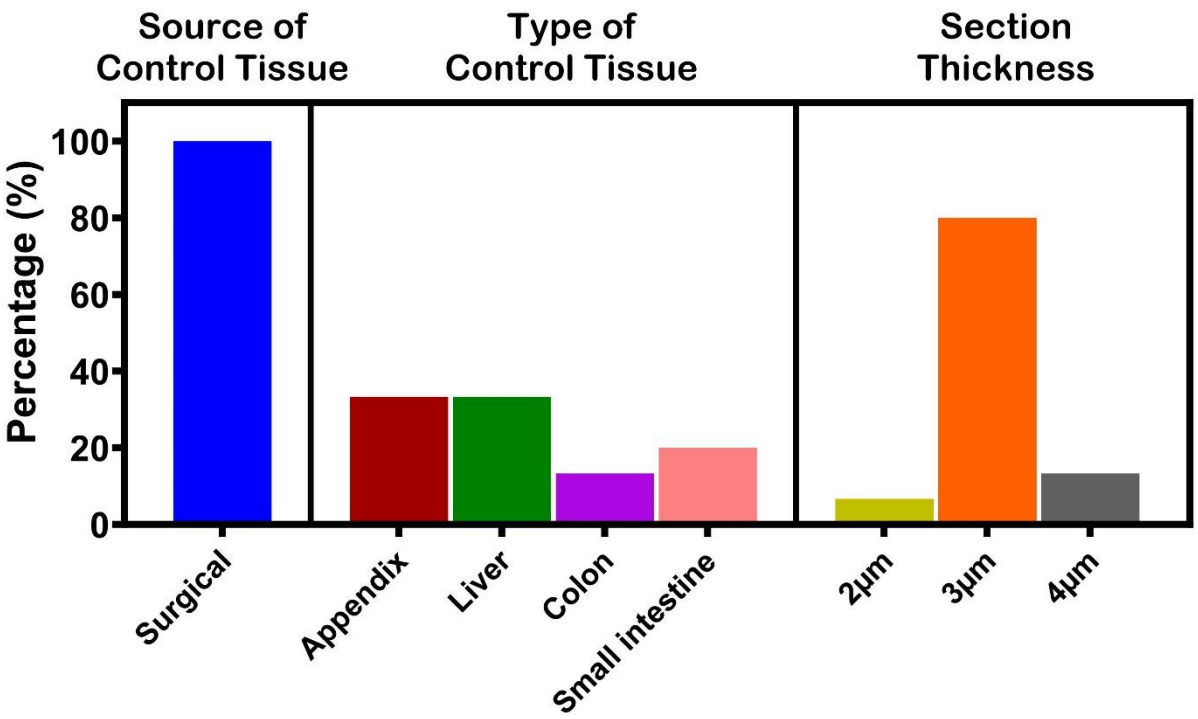


Figure 11: Responses to the questionnaires on the type of control tissue used and section thickness in Trichrome staining

Bar charts showing the survey results from 15 laboratories comprising of (i) source of control tissue; (ii) type of control tissue; and (iii) section thickness.

Summary notes:

1. In this exercise, all control tissues were obtained from surgical specimens; none were derived from post-mortem sources or commercially prepared tissues.
2. The appendix and liver were the most commonly used control tissues among the surveyed laboratories, accounting for 33.3% of usage.
3. A tissue section thickness of 3 µm was preferred for Trichrome staining by 80% of laboratories, while only a few used sections of 2 µm or 4 µm.

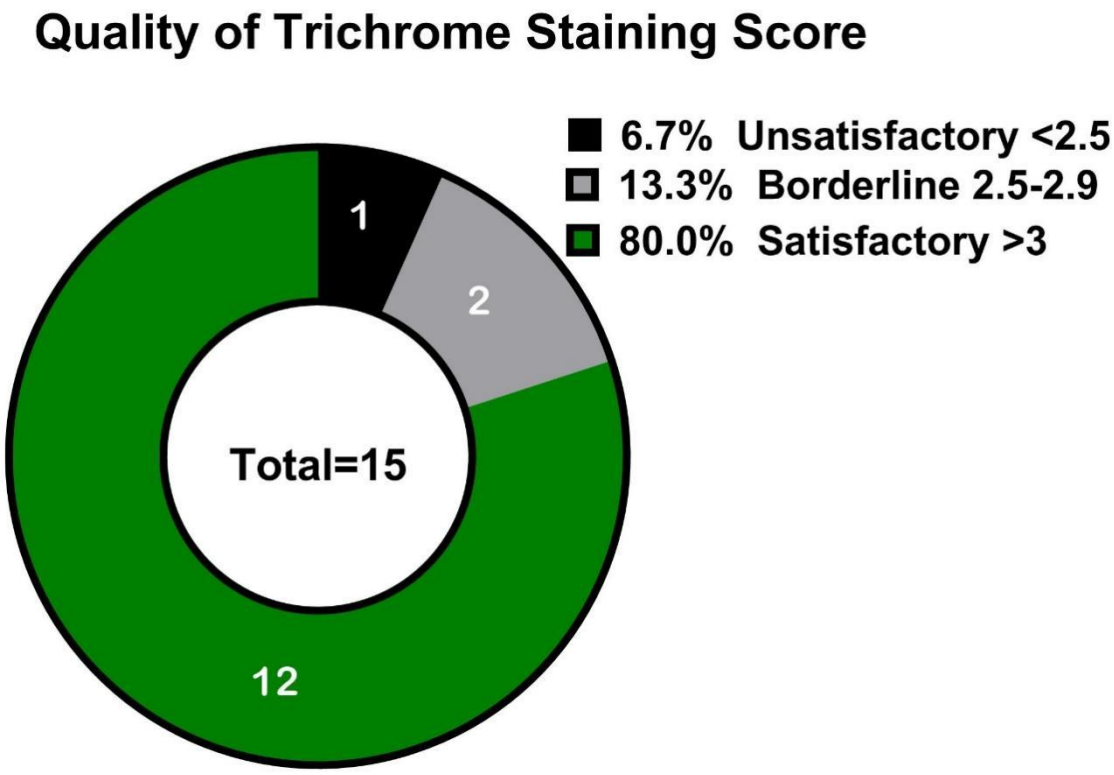


Figure 12: Technical assessment on the quality of Trichrome staining.
Pie chart showing the quality of Trichrome staining analysis from 15 laboratories.

Summary notes:

1. The satisfactory scores for Trichrome staining were attained by majority of the participating laboratories (80.0%), whereas 6.7% and 13.3% of the laboratories received unsatisfactory and borderline scores, respectively. The highest and lowest scores were 4.1 and 2.3, respectively.

TM25-1 (Processing, Sectioning and H&E Staining)

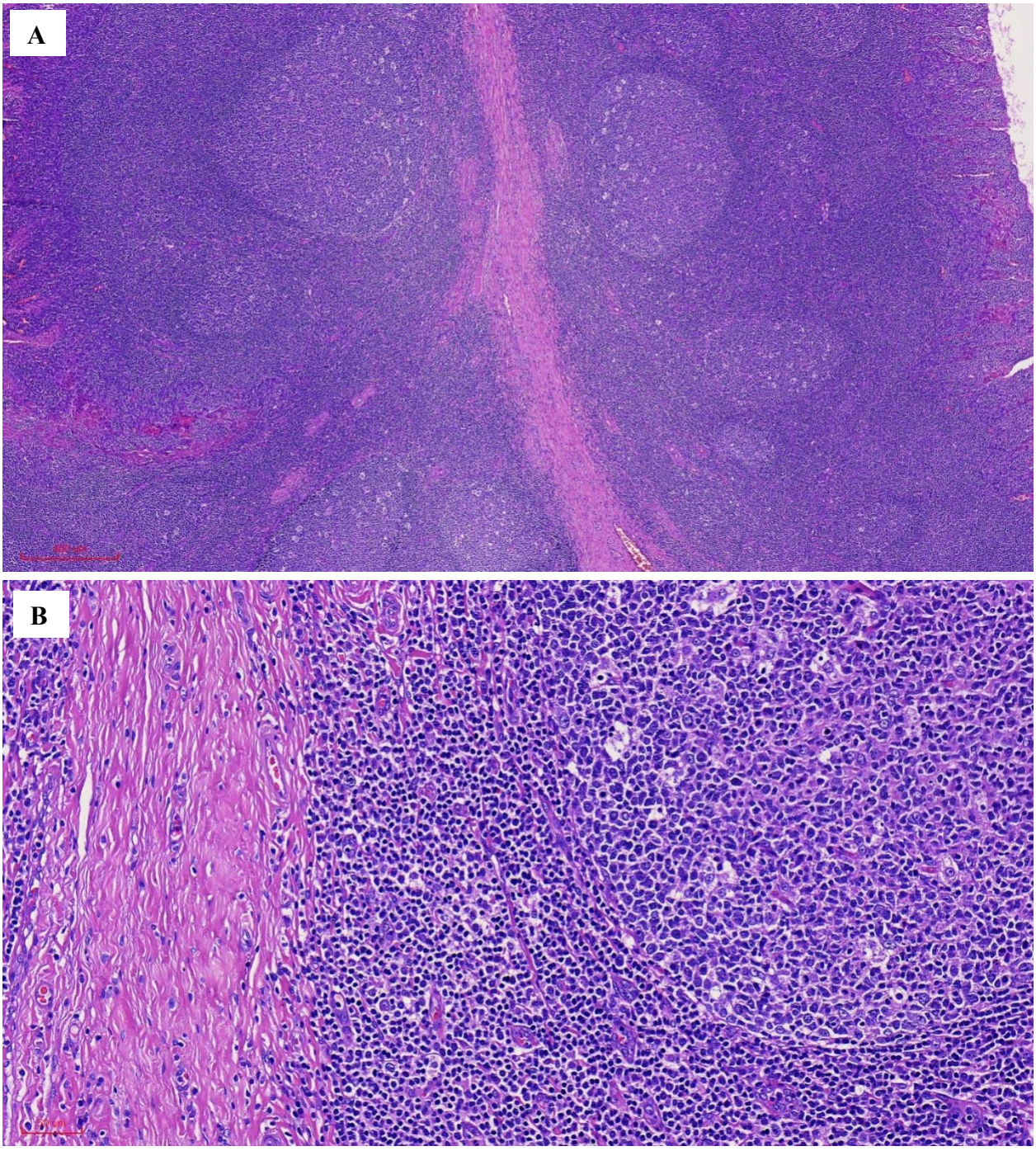


Figure 1: Highest scores demonstration of tissue processing, sectioning and staining (Tissue type: tonsil)

Processing & Sectioning*:* (A) x4, (B) x20: The tissue architecture was effectively preserved with appropriate thickness and absence of artefact. The processing was conducted using Leica PEGASUS and utilisation of ice block technique during sectioning. Score 4.4/5.0.

Staining: Excellent demonstration of staining contrast in haematoxylin and eosin, which was performed using regressive method by Thermo Shandon Varistain Gemini. Score 4.5/5.0.

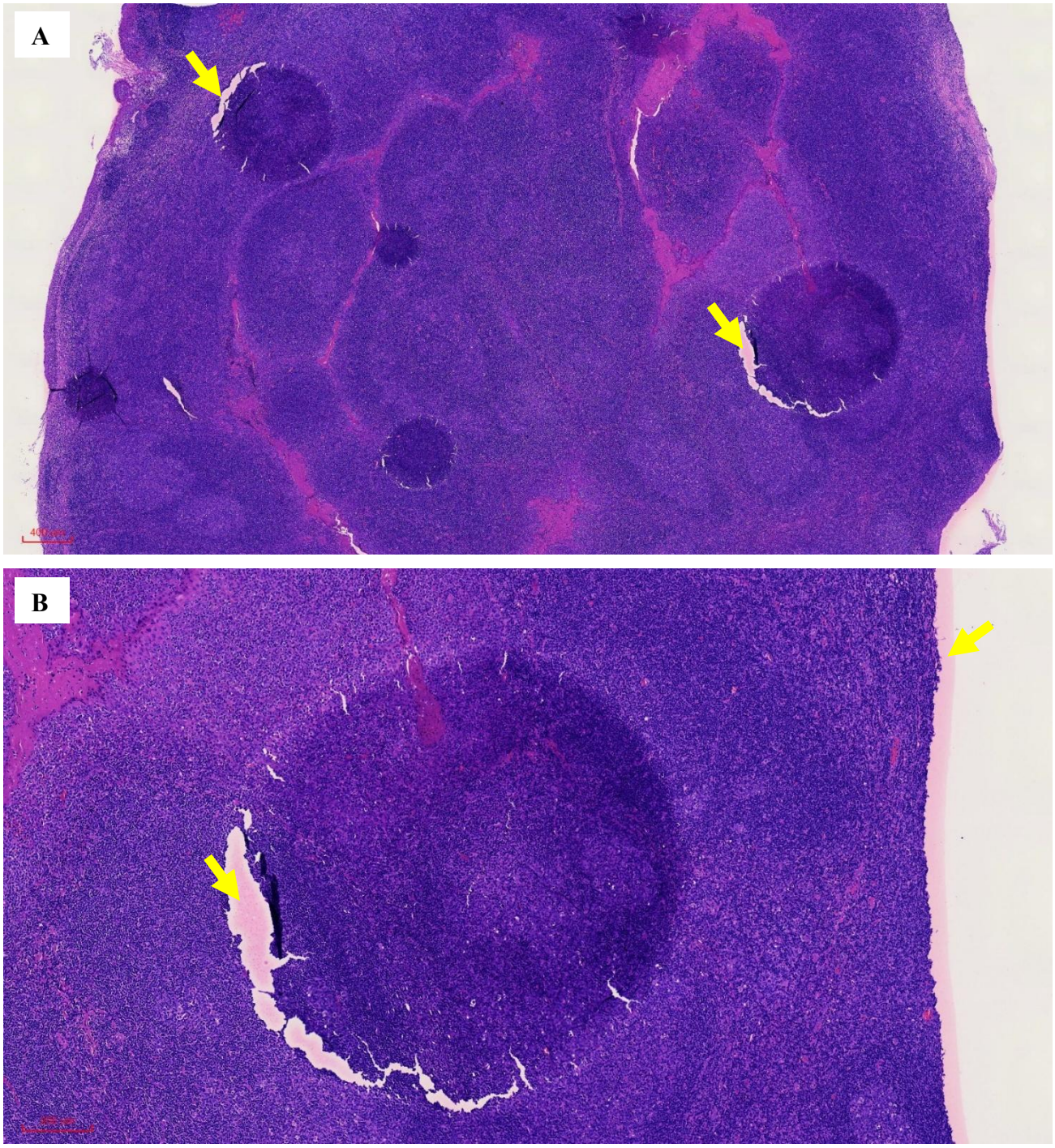


Figure 2: Demonstration of artefacts (Tissue type: Tonsil)
(A) x2, (B) x4: The sections show uneven thickness, with numerous chattering artefacts. There is presence of trapped air bubbles within the tissue. Staining residual (yellow arrows) due to inadequate rinsing are observed. Score 2.3/5.0 for specific criteria.

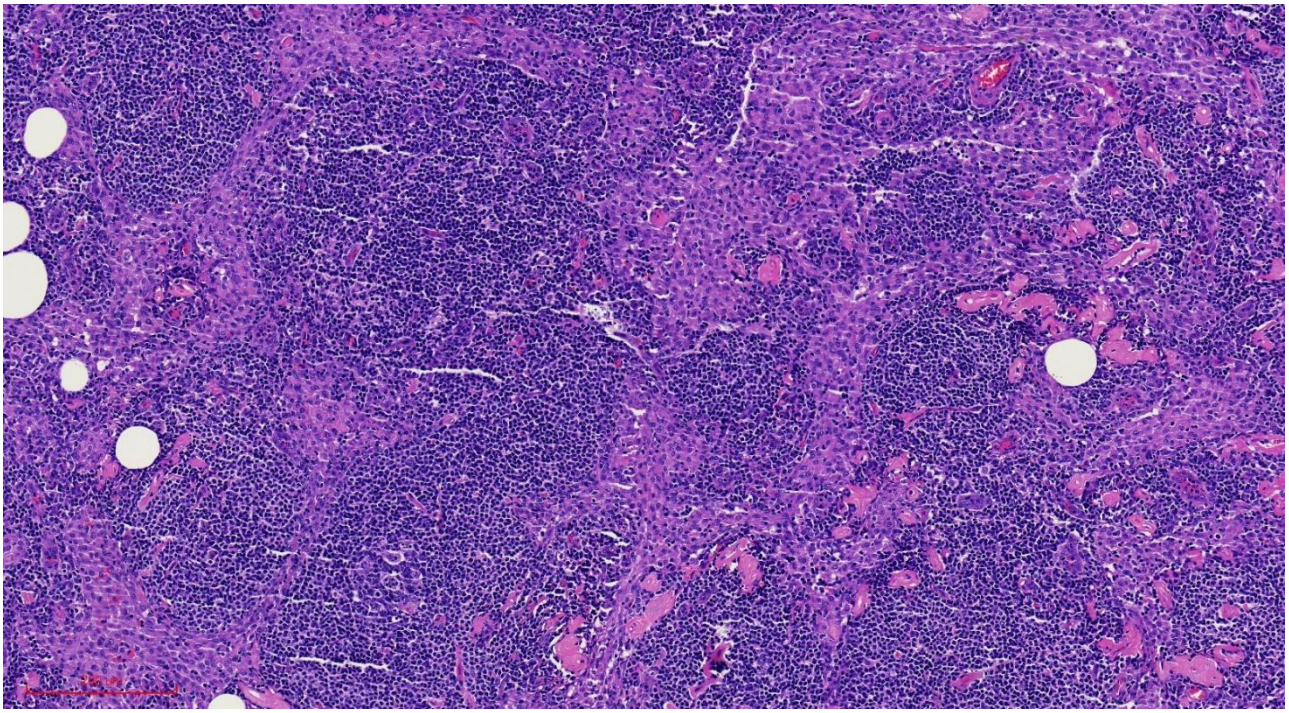


Figure 3: Demonstration of artefacts (Tissue type: Tonsil)
The section shows numerous chattering artefacts associated with over expansion. Score 3.0/5.0 for specific criteria.

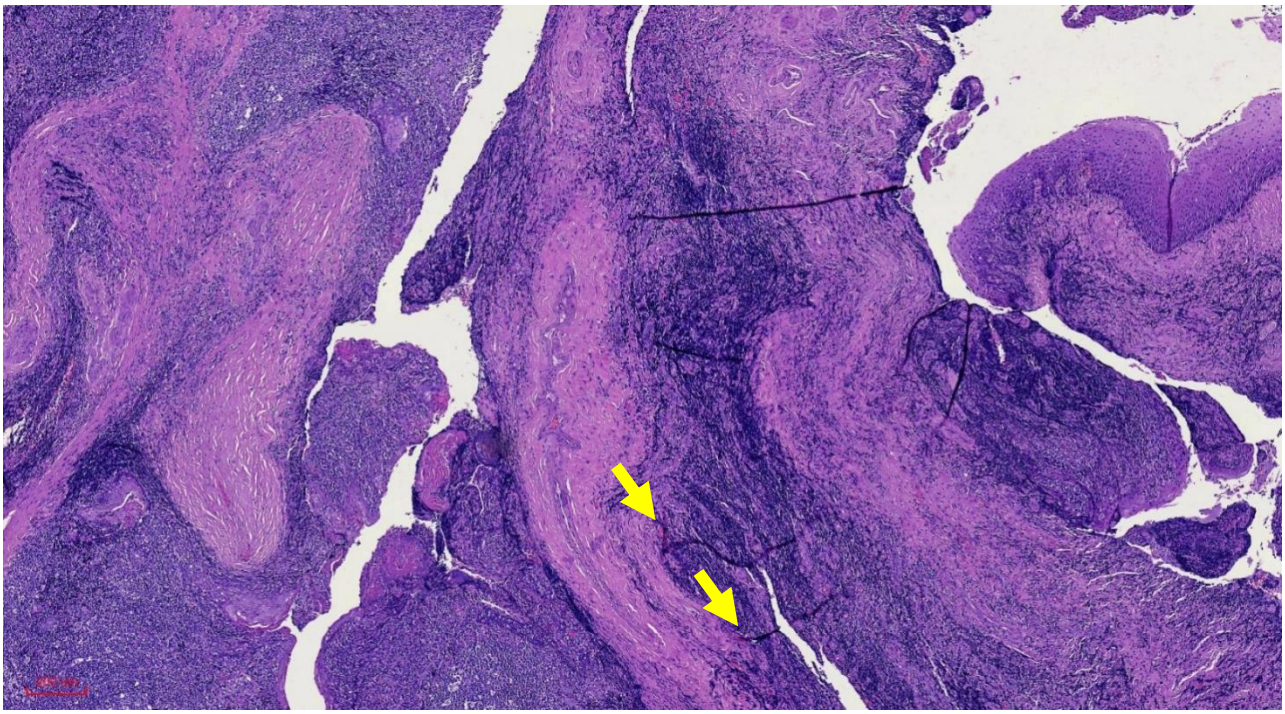


Figure 4: Demonstration of artefacts (Tissue type: Tonsil)
The section shows crush artefacts and foreign body contaminants (yellow arrows). Score 2.8/5.0 for specific criteria.

TM25-2 (Trichrome Stain)

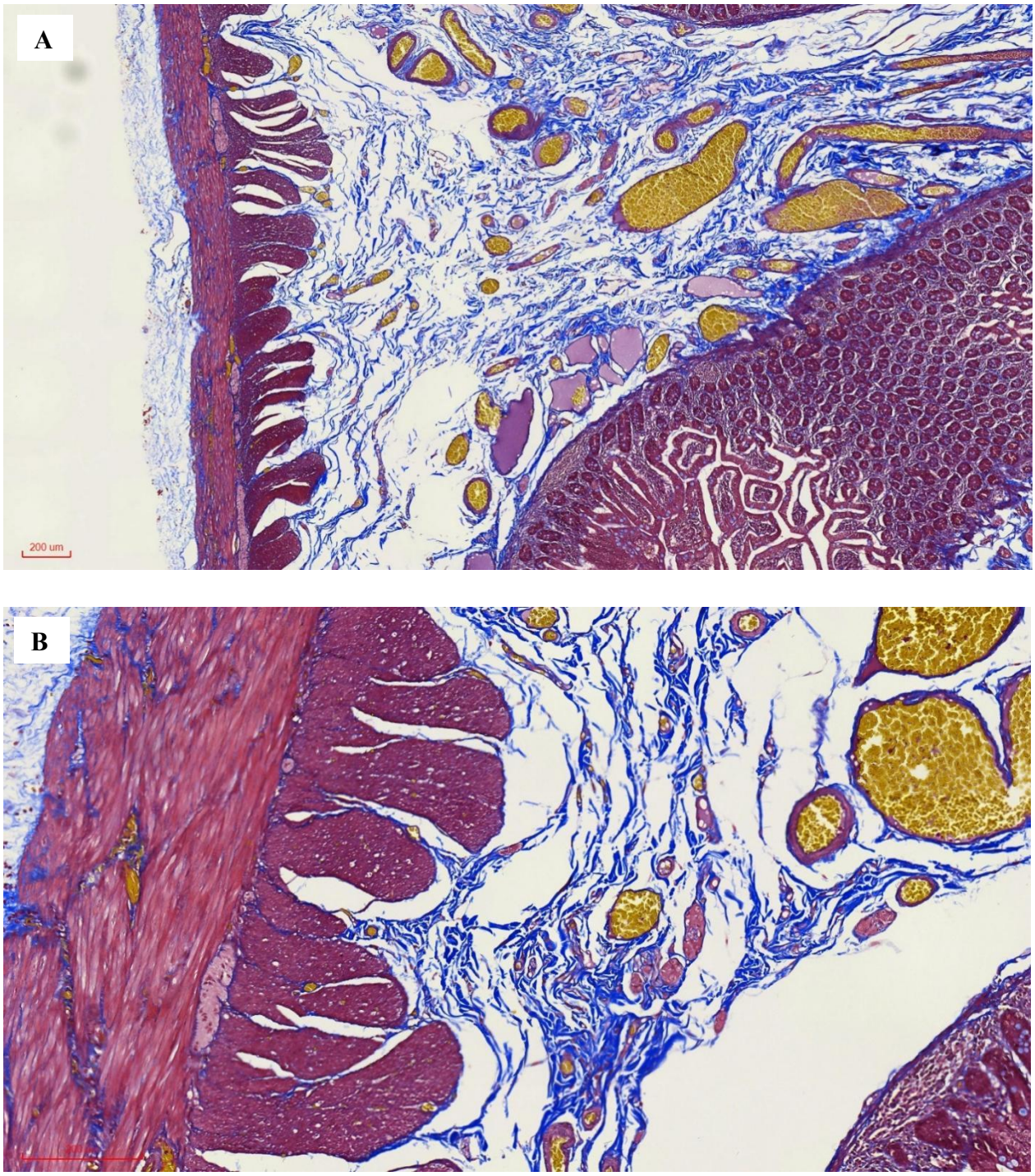


Figure 1: (A) Optimal staining of the colon tissue was achieved to highlight the muscle, collagen fibres and red blood cells (x4). (B) The staining exhibited good contrast and uniformity using manual method. The reagents are from Bio-Optica (Aniline Blue). Score 4.1/5.0 (x10).

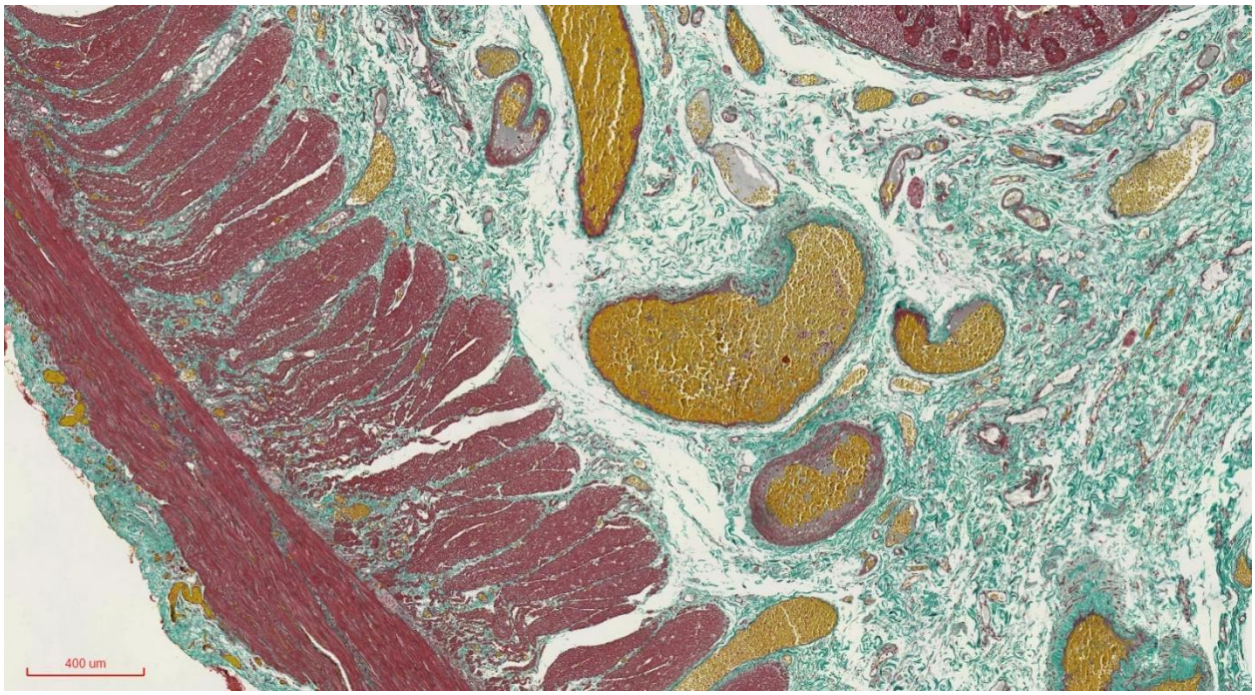
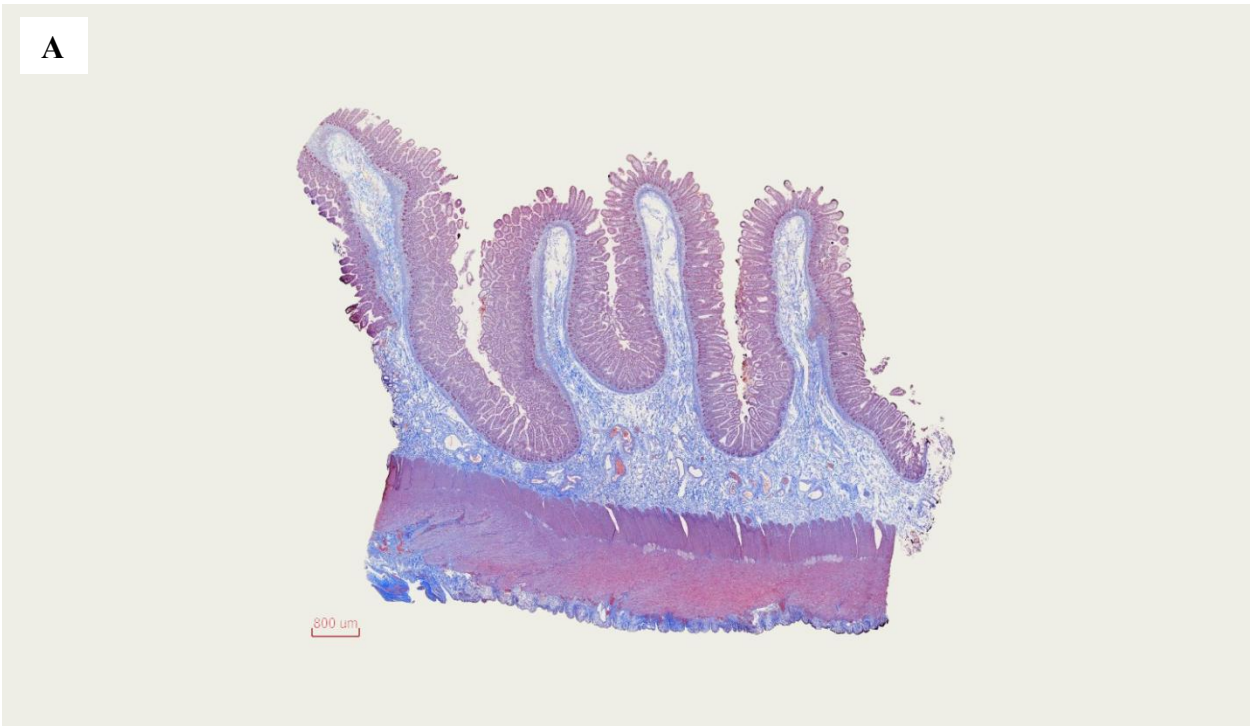


Figure 2: Optimal staining of the small intestinal tissue was achieved to highlight the muscle, collagen fibres and red blood cells. The staining exhibited good contrast and uniformity using manual method. The reagents are from Bio-Optica (Light Green). Score 4.0/5.0 (x4).



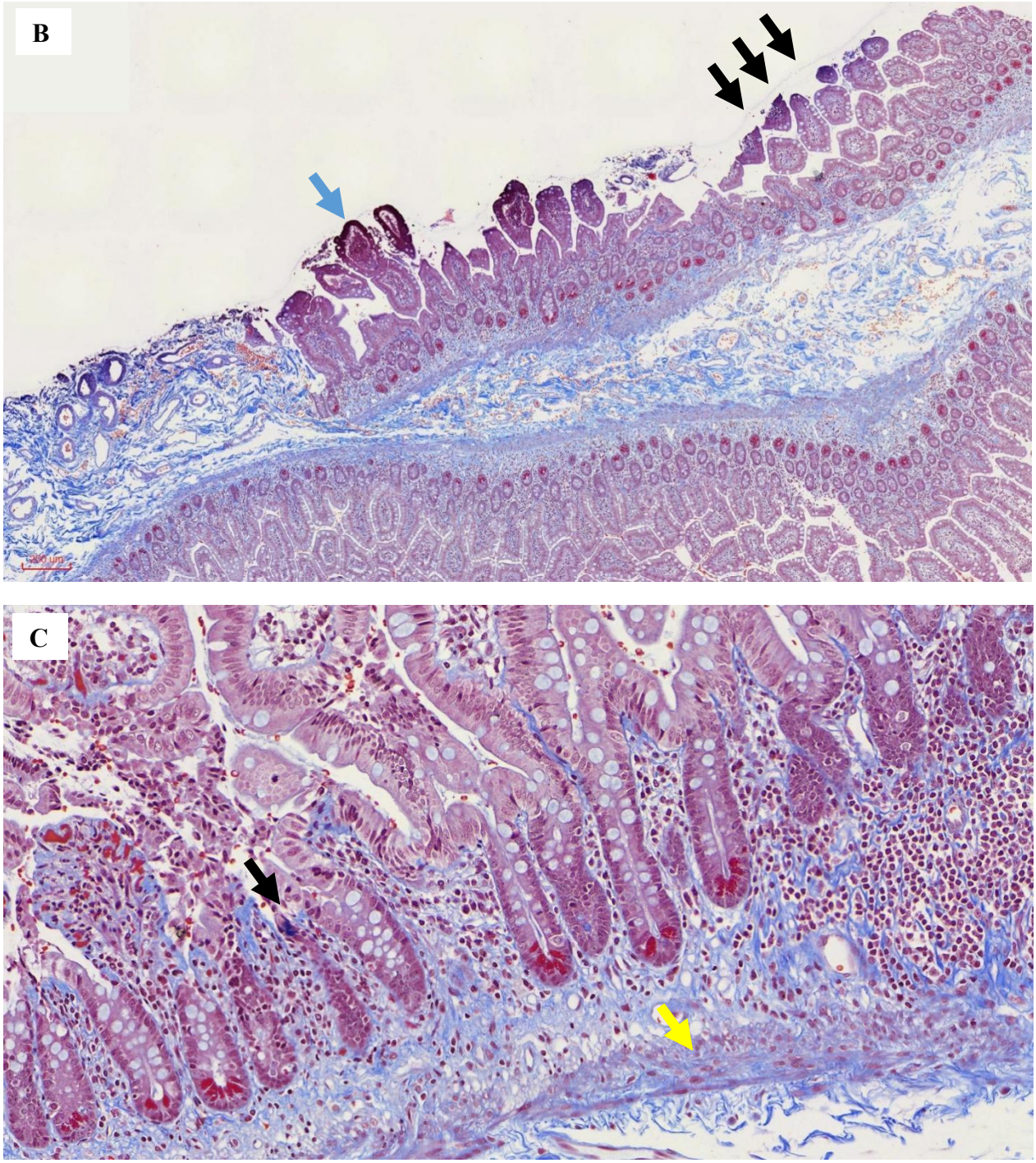


Figure 3: (A) Poor demonstration and uneven Trichrome staining of the small intestinal tissue with presence of artefacts (x0.5). (B) The higher magnification (x4) shows burnt artefacts (blue arrow) at the periphery of the tissue section, due to overheating during the staining process. Staining contaminants are present (black arrows) because of inadequate rinsing. (C) Staining precipitations are present (black arrow) with uneven and poor muscle staining (yellow arrow, x20). The procedure was carried out using the Dako Artisan Link Special Staining System. Score 2.3 /5.0.

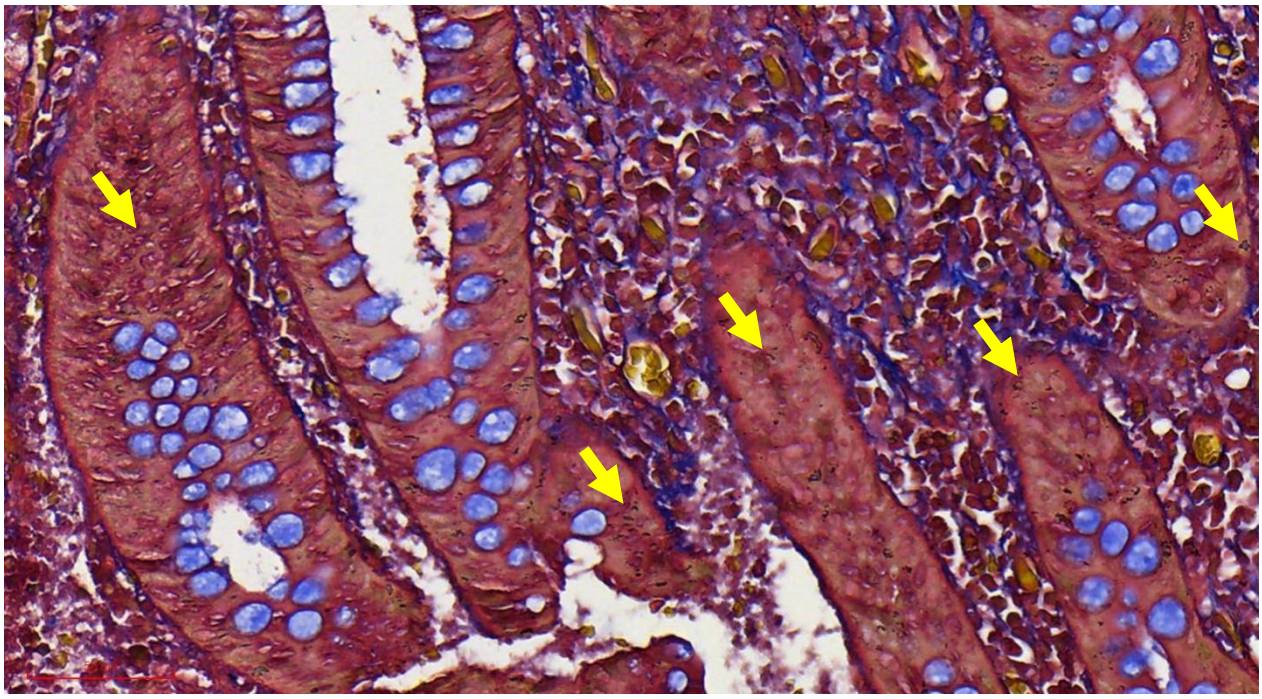


Figure 4: Staining shows dehydration artefacts (yellow arrows), poor counterstain contrast, and indistinct nuclear detail. Staining score: 2.8/5.0 for specific criteria (x10).