Generic Report 2023

TECHNICAL MODULE IAPMD QAP 2023

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Technical Module IAPMD QAP Organiser

TM23-1 : Processing, Sectioning and H&E Staining

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TM23-2 : Periodic Acid-Schiff (PAS) Stain

Registration and Acceptance Status of TM IAPMD QAP 2023

This is a report of Anatomic Pathology laboratories involvement in the TM IAPMD QAP 2023. In this current exercise, the registration for all 17 participants was managed by Innovz Sdn. Bhd. All laboratories participated in the TM23-1, whereas 16 participated in TM23-2. The participants included 14 from the Ministry of Health Malaysia hospitals' laboratories, one private laboratory and 2 teaching hospitals' laboratories.

This exercise consisted of two modules: TM23-1 (processing, sectioning, Haematoxylin and Eosin staining) and TM23-2 (PAS stain). As for the questionnaire, all responded to both TM23-1 and TM23-2 questionnaires. All participants submitted appropriate tissue types for TM23-1, while for TM23-2 one participant submitted unsuitable tissue for assessment.

INSTRUCTIONS TO PARTICIPANTS

CLINICAL NOTES

Code	Exercise	Clinical Notes
TM23-1	1. Processing and Sectioning 2. Haematoxylin and Eosin (H&E) staining	1. Tissue type: Colon/ Appendix tissue 2. Section size: up to 15mm to 20mm in the widest dimension
TM23-2	Periodic Acid-Schiff (PAS) stain	Colon/ Appendix tissue

ASSESSMENT CRITERIA

Processing, Sectioning and H&E Stain (TM23-1)	PAS Stain (TM23-2)
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. Coverslip placed centrally over entire section and within boundaries of slide 5. Absence of bubbles and excess mounting 6. 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 neutral and acidic mucins. 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 TM-23-1

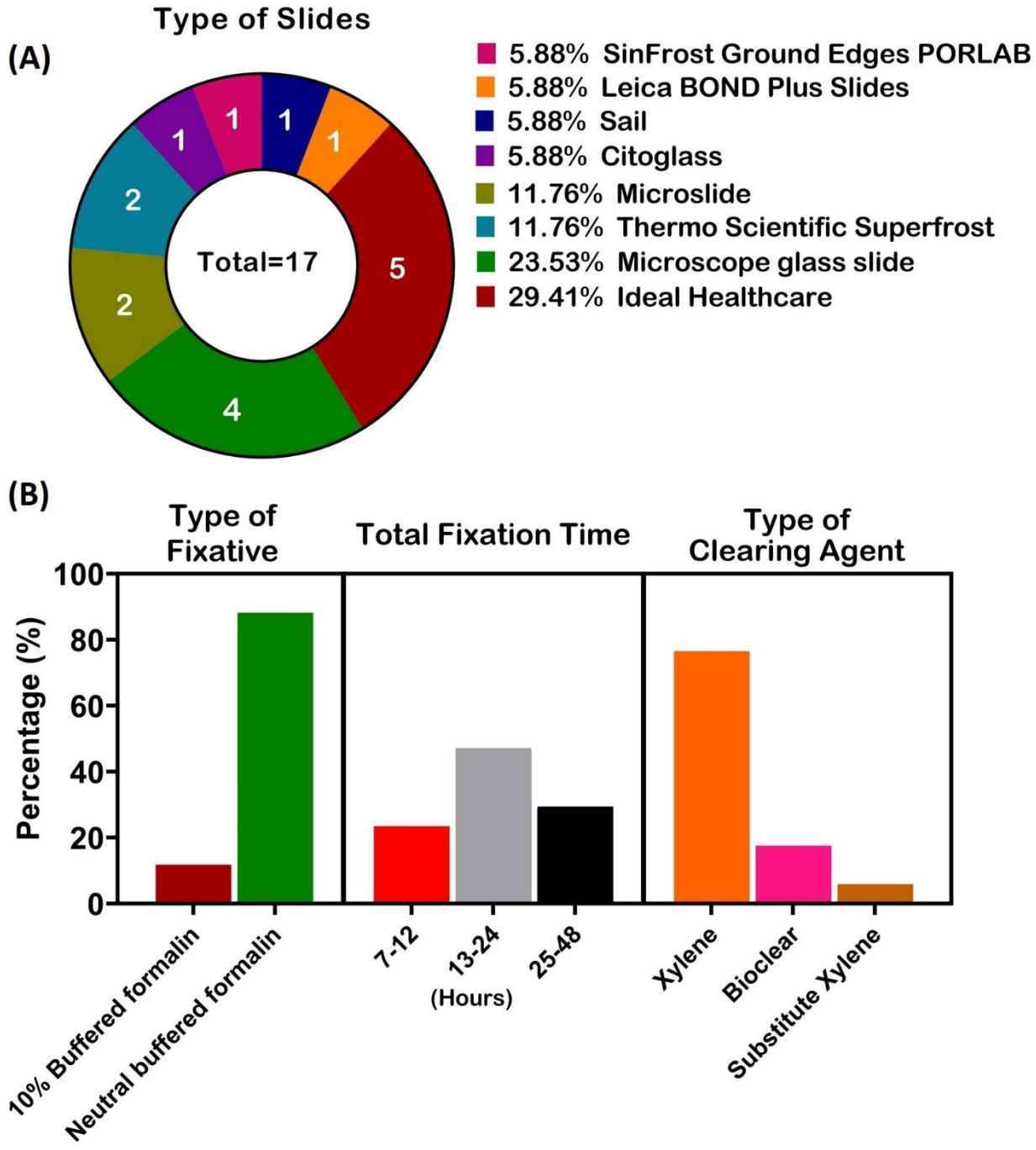

Figure 1: Responses to the questionnaires on type of slides and tissue processing

Pie and bar charts showing the survey results from 17 laboratories comprising of (A) type of slides; (B) i) type of fixative applied; ii) total of fixation time; and iii) type of clearing agent used.

Summary notes:

1. The Ideal Healthcare slide model emerged as the commonly employed choice among the surveyed laboratories, constituting 29.41% of utilisation.
2. The prevalent fixative utilised in tissue processing by the majority of laboratories was neutral buffered formalin.
3. It was observed that the fixation times for tissue processing frequently surpassed a duration of 13 hours.
4. Xylene emerged as the prevailing clearing agent employed for tissue processing.

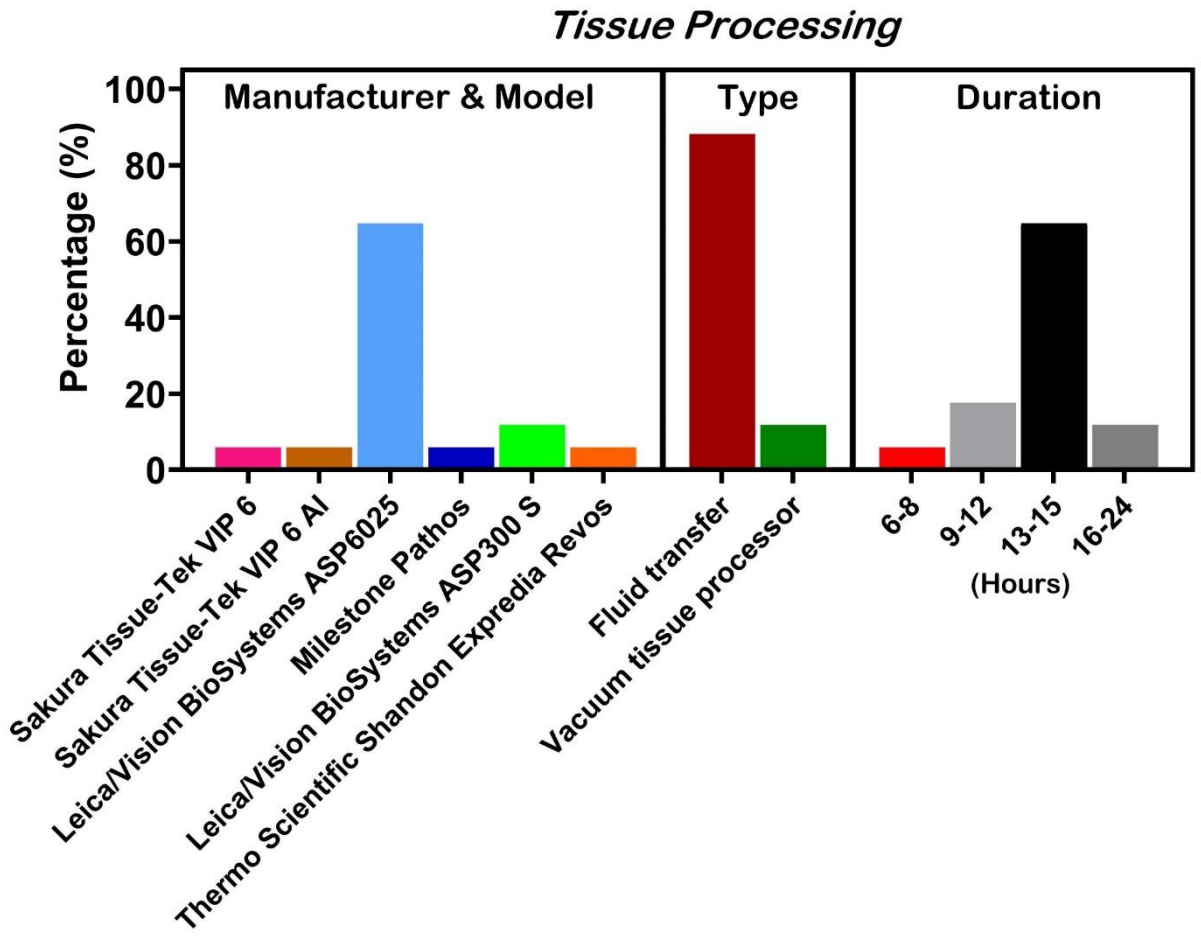

Figure 2: Responses to the questionnaires on tissue processing

Bar charts showing the survey results from 17 laboratories comprising of (i) the model of tissue processors used; (ii) type and (iii) duration of tissue processing.

Summary notes:

1. The Leica/Vision Biosystems ASP6025 tissue processor model was identified as the prevalent choice among the surveyed laboratories, accounting for 64.7% 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 and 15 hours.

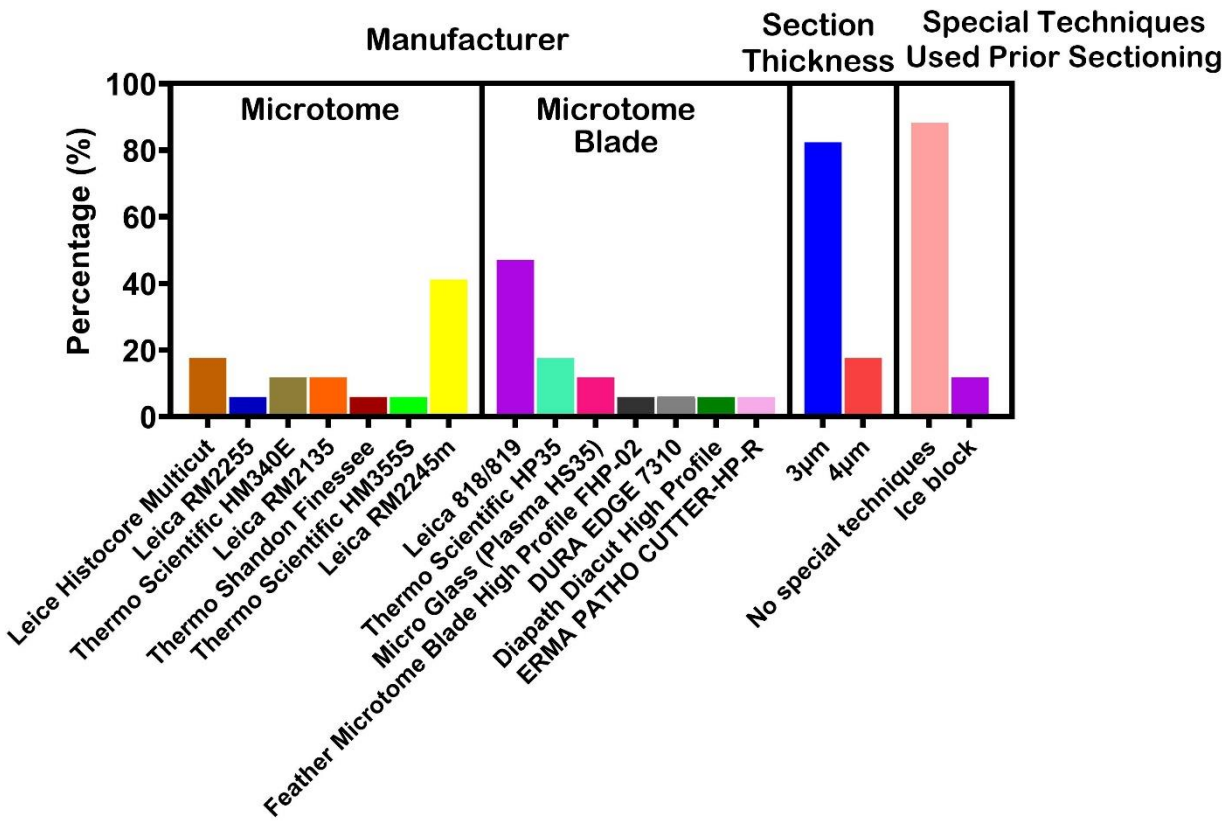

Figure 3: Responses to the questionnaires on tissue sectioning

Bar charts showing the survey results from 17 laboratories comprising of (i) type of model of microtome and microtome blade used; (ii) section thickness; and (iii) type of special techniques used before sectioning.

Summary notes:

1. The Leica RM2245m microtome (41.2%) and the Leica 818/819 microtome blade model (47.1%) were frequently employed in the survey.
2. The thickness of 3 µm for tissue sections was the preferred choice.
3. Preceding the process of tissue sectioning, the findings from the survey indicate that majority of laboratories (88.2%) abstained from employing any special techniques. Ice block treatment was found to be the least utilised method (11.8 %).

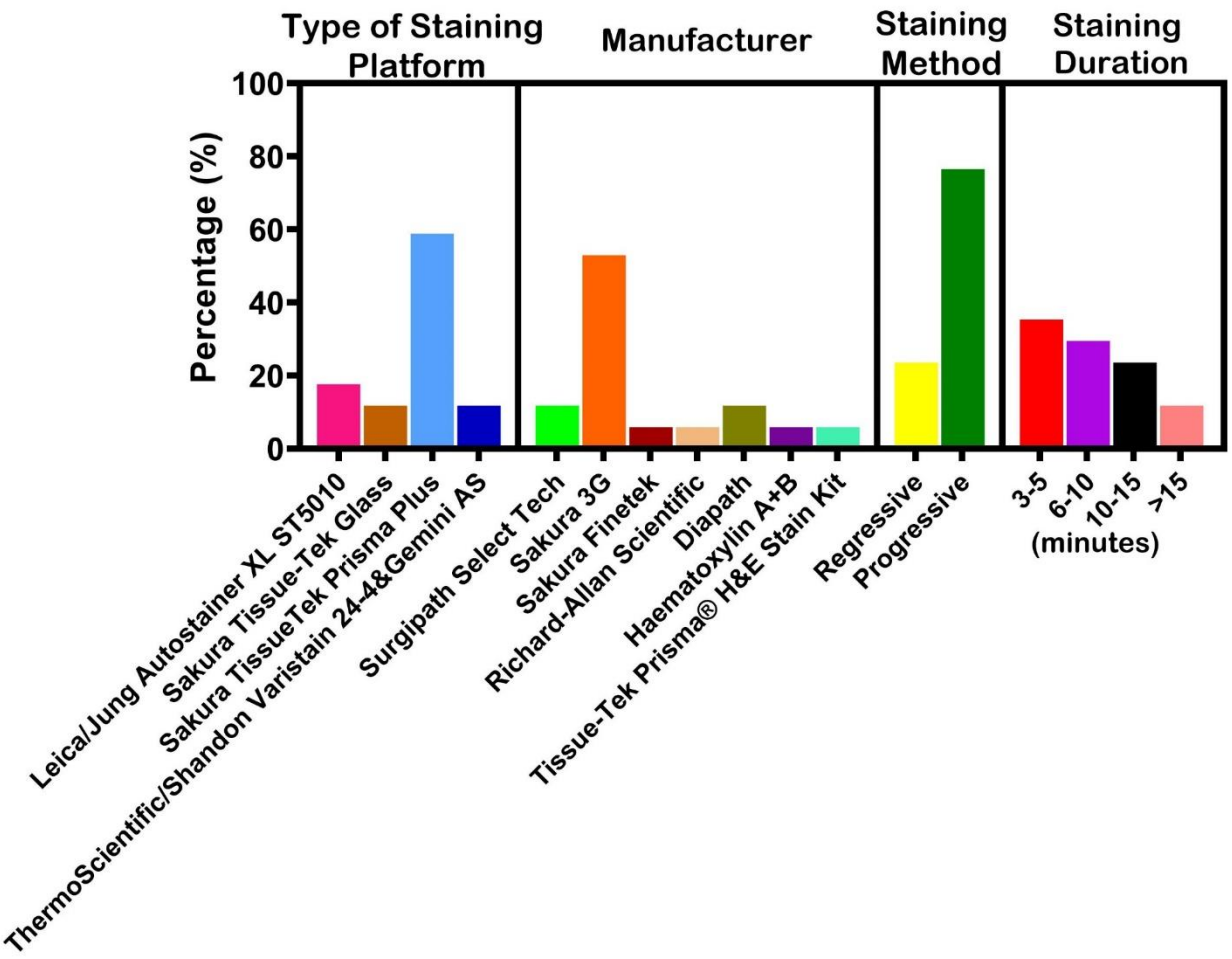

Figure 4: Responses to the questionnaires on haematoxylin in H&E staining

Bar charts showing the survey results from 17 laboratories comprising of (i) type of staining platform; (ii) manufacturer of staining solution; (iii) staining method; and (iv) staining duration.

Summary notes:

1. The Sakura Tissue Tek Prisma Plus (58.8 %) and the Leica/Jung Autostainer XL ST5010 (17.6 %) were the common tissue staining platforms utilised.
2. Sakura 3G (52.9 %), Surgipath Select Tech (11.8 %), and Diapath (11.8 %) emerged as the top three manufacturers for haematoxylin.
3. The progressive staining approach garnered the highest preference as the staining method of choice.
4. Typically, the staining duration of haematoxylin that is most favoured falls within the range of 3-5 minutes.

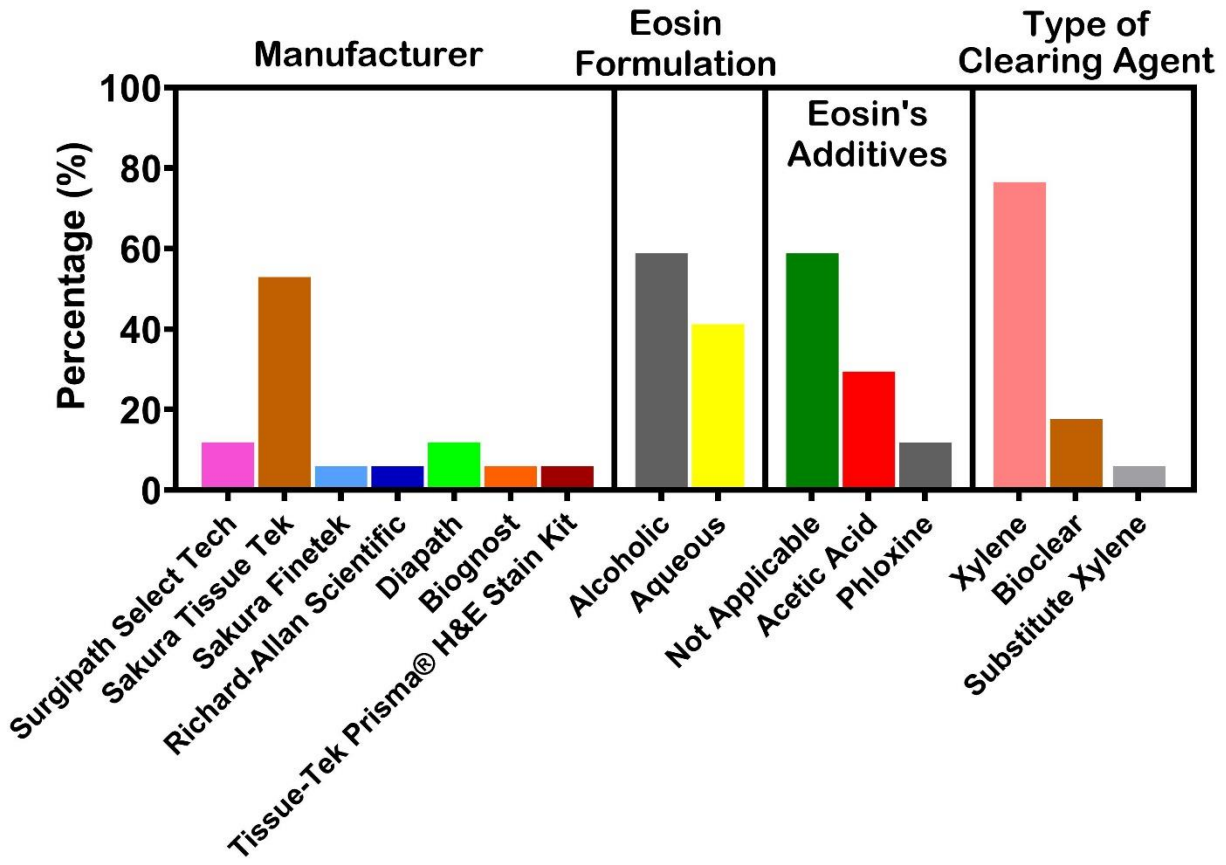

Figure 5: Responses to the questionnaires on eosin in H&E staining

Bar charts showing the survey results from 17 laboratories comprising of (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 produced by Sakura Tissue Tek received the highest favourability (52.9 %).
2. Alcoholic solvents were predominantly favoured over aqueous solvents for eosin formulation.
3. A majority of surveyed laboratories (58.8 %) refrained 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 Bioclear and substitute xylene.

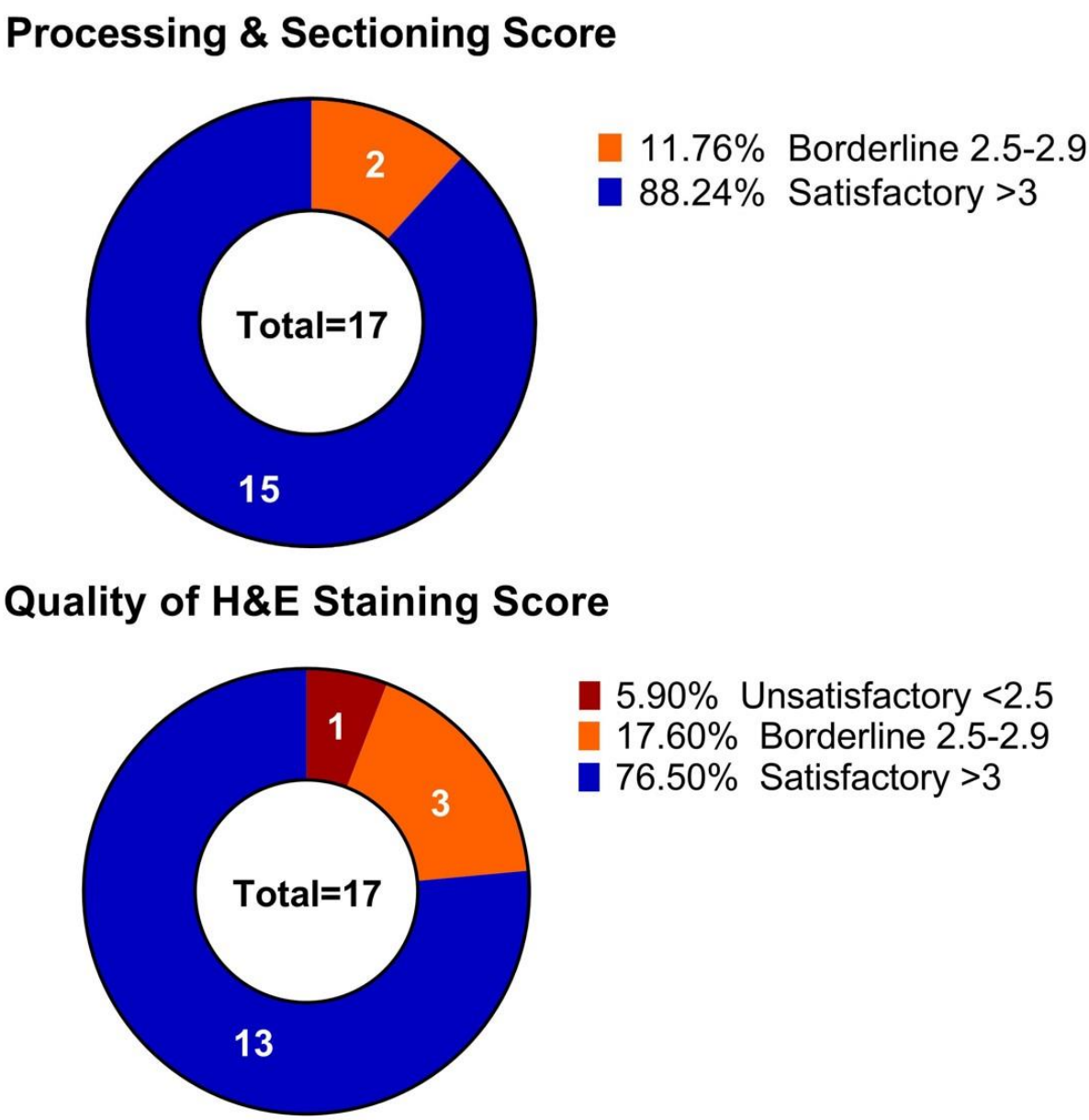

Figure 6: Technical assessment on the quality of tissue processing and H&E staining

Pie chart showing the quality of tissue processing and H&E staining analysis from 17 laboratories.

Summary notes:

1. The tissue processing and sectioning quality met the satisfactory criteria for 88.24% of the participating laboratories, while 11.76% received borderline scores. The highest and lowest scores were 4.8 and 2.8, respectively.
2. A significant majority of the participating laboratories (76.50%) achieved satisfactory scores for H&E tissue staining, while 17.60% and 5.90% of the laboratories received borderline and unsatisfactory scores each. The highest and lowest scores were 5.0 and 2.4, respectively.

Questionnaire analysis of TM-23-2

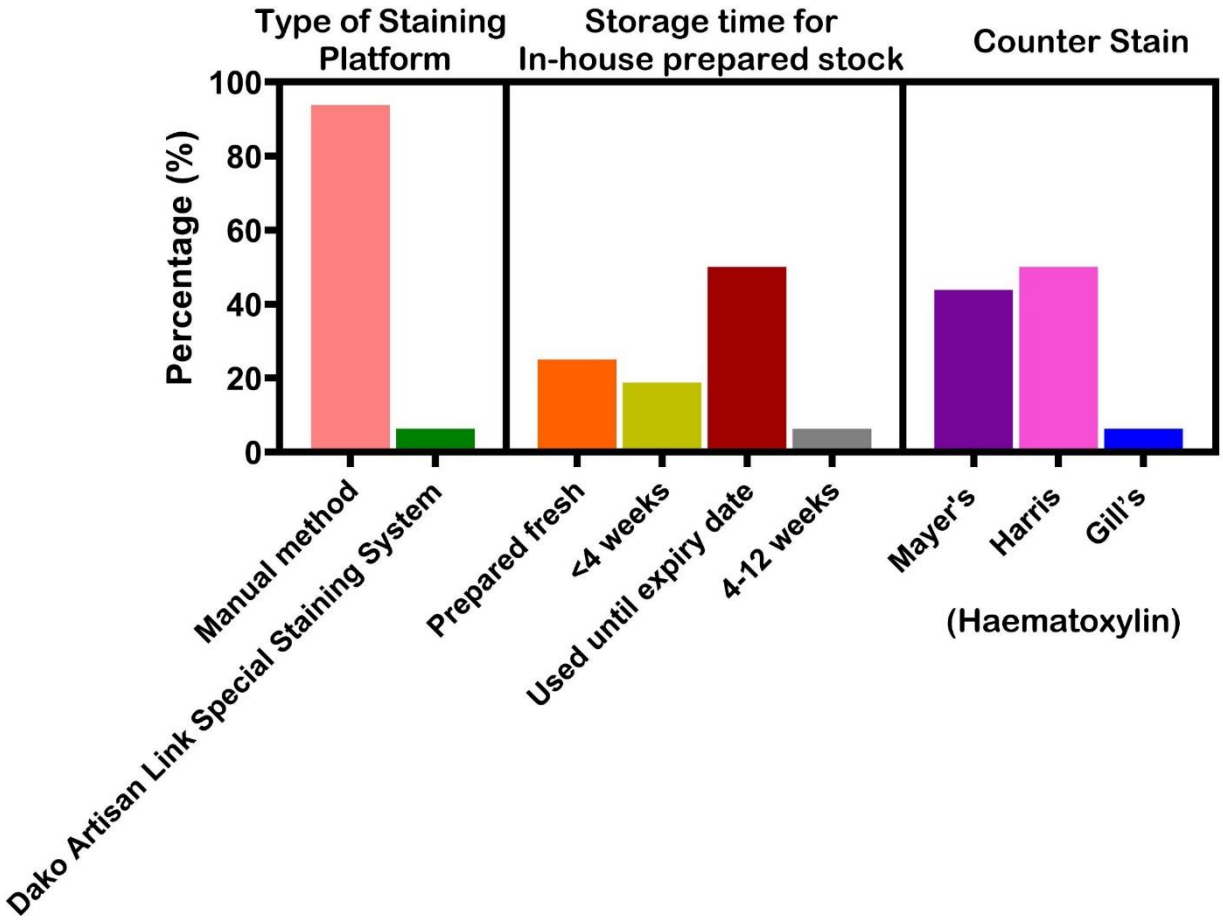

Figure 7: Responses to the questionnaires on PAS staining

Bar charts showing the survey results from 16 laboratories comprising of (i) the type of staining platform; (ii) duration of storage time for in-house prepared stock solution; and (iii) type of counter stain applied.

Summary notes:

1. The manual method accounted for the majority of PAS staining procedures (93.8%), whereas the automated staining Dako Artisan Link Special Staining System was less commonly employed.
2. In terms of stock solution usage, half of the surveyed laboratories (50%) utilized in-house prepared stock solutions until their expiry dates, while a quarter of the laboratories preferred freshly prepared stock solutions. The remaining laboratories used the stock solutions within a time frame of less than 4 weeks from the initial preparation, with only a few extended their usage to 4-12 weeks from the preparation time.
3. Harris Haematoxylin solution (50%) emerged as the most frequently used counter stain, followed by Mayer's Haematoxylin solution. Conversely, Gill's Haematoxylin solution was the least utilized among the surveyed laboratories.

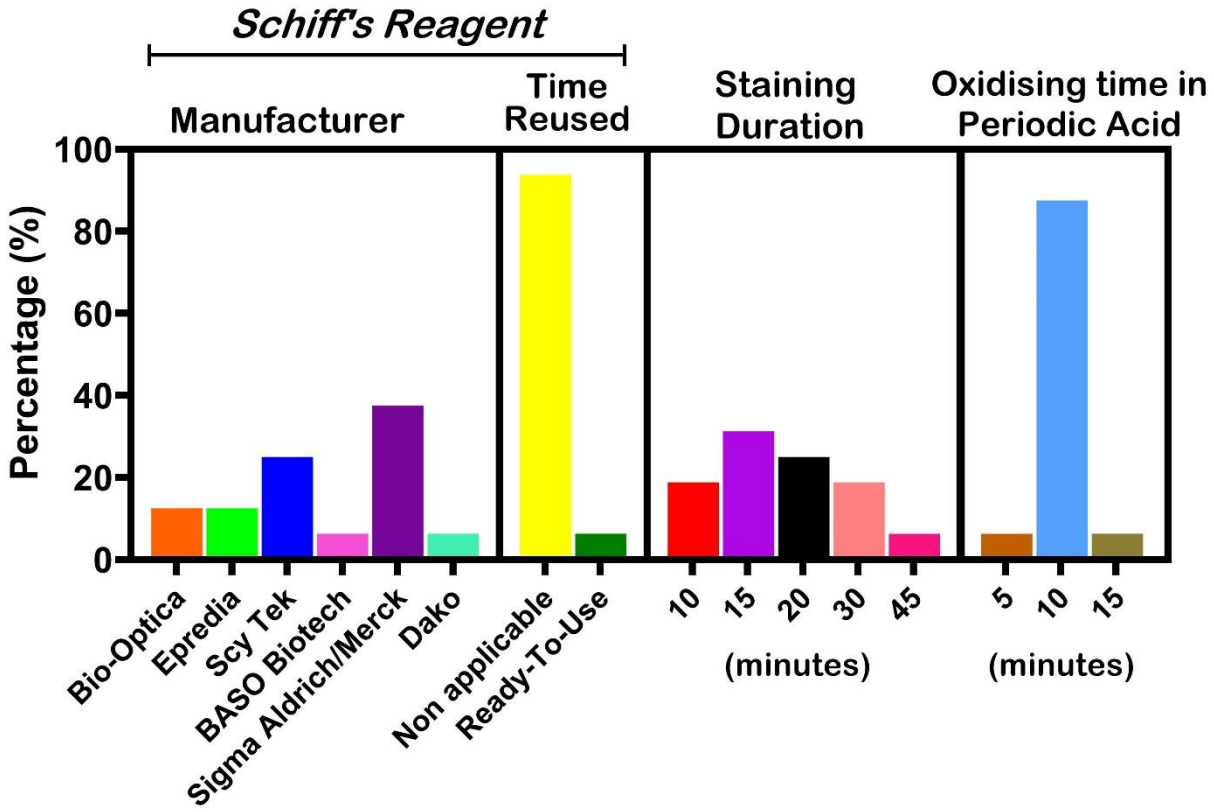

Figure 8: Responses to the questionnaires reagents used in PAS staining

Bar charts showing the survey results from 16 laboratories comprising of (i) manufacturer of Schiff's reagent staining solution; (ii) duration of Schiff's reagent reuse; (iii) staining times in Schiff's reagent solutions; and (iv) oxidising time in periodic acid solution.

Summary notes:

1. The top two manufacturers for Schiff's reagent staining solution were Sigma Aldrich/Merck (37.5%) and Scy Tek (25.0%), while Dako and BASO Biotech received the least favourable (6.3%) for their Schiff's reagent staining solution.
2. The majority of surveyed laboratories (93.8%) refrained from reusing Schiff's reagent in the PAS staining process, opted instead for ready-to-use Schiff's reagent.
3. The most frequent staining duration in Schiff's reagent solutions was 15 minutes (31.3%), while a small subset of laboratories stained for 45 minutes (6.3%) in Schiff's reagent solutions.
4. The preferred oxidising time in periodic acid solution during PAS staining was 10 minutes (87.5%). However, a few laboratories oxidised for 5 minutes (6.3%) or 15 minutes (6.3%) in periodic acid solution during PAS staining.

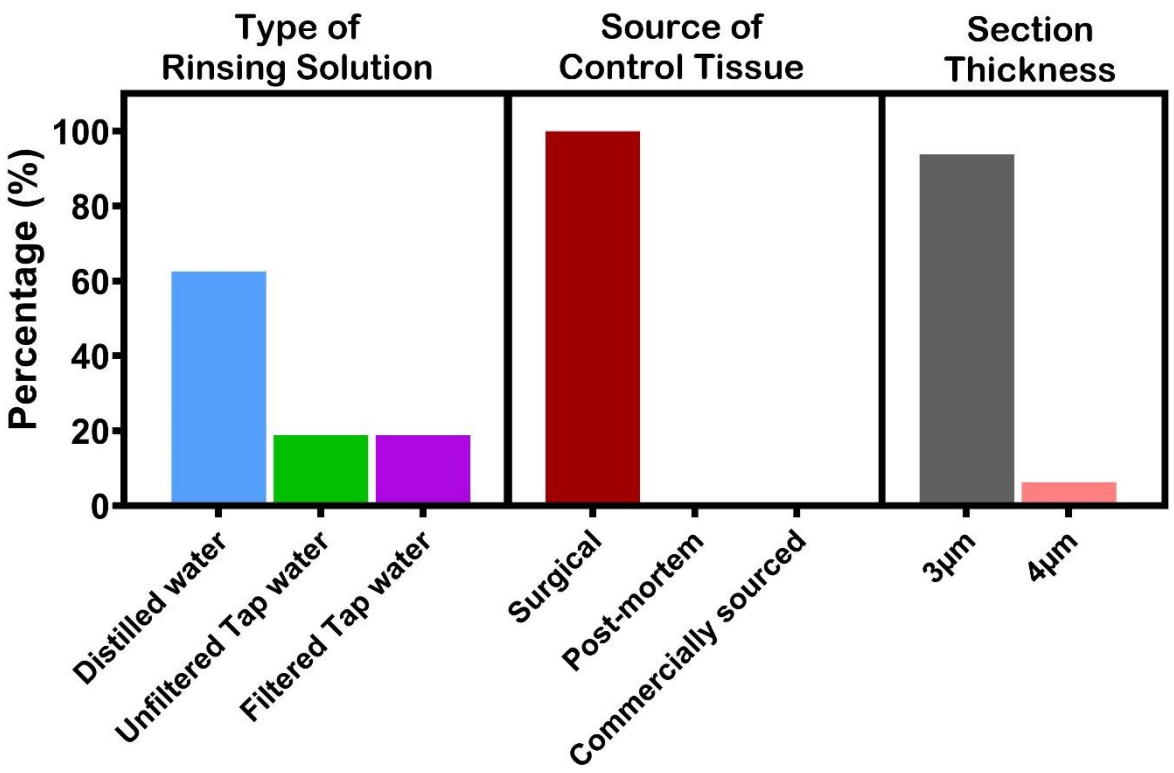

Figure 9: Responses to the questionnaires reagents used in PAS staining

Bar charts showing the survey results from 16 laboratories comprising of (i) type of rinsing solution; (ii) source of control tissue; and (iii) thickness of tissue section.

Summary notes:

1. The rinsing solution of choice for the majority of surveyed laboratories (62.5%) was distilled water, while an equal percentage of laboratories opted for either filtered or unfiltered tap water.
2. For this exercise, all control tissues utilised were sourced from surgical tissues, with none being derived from post-mortem or commercially sourced specimens.
3. The preferred thickness for tissue sections in PAS staining was 3 µm (93.8%), with only a few laboratories utilising 4 µm thickness for their tissue sections.

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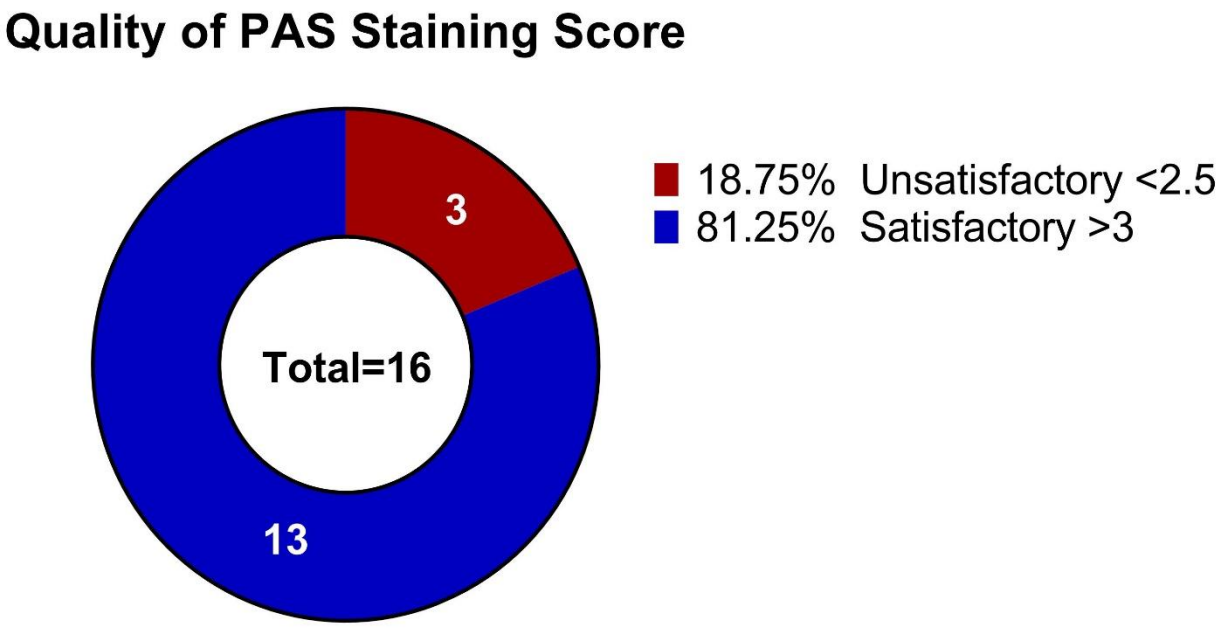

Figure 10: Technical assessment on the quality of PAS staining

Pie chart showing the quality of PAS staining analysis from 16 laboratories.

Summary notes:

1. The satisfactory scores for PAS staining were attained by a significant majority of the participating laboratories (81.25%), whereas 18.75% of the laboratories received unsatisfactory scores. The highest and lowest scores were 4.8 and 0.5, respectively.