Generic Report 2022

TRIAL RUN

Prepared by

Technical Module IAPMD QAP Organiser

TM22-1 (Reticulin Stain)

&

TM22-2 (Processing, Sectioning and H&E Staining)

Registration and Acceptance Status of TM IAPMD-QAP (Trial Run) 2022

This is a report of Anatomic Pathology laboratories involvement in the trial run of TM IAPMD-QAP exercise. Initially, there were 37 laboratories registered for the exercise. Two laboratories withdrew from participating by informing us via email. In this current exercise, a total of 19 laboratories participated in the TM22-1, whereas 27 laboratories contributed in the TM22-2. The participants included 14 from the Ministry of Health Malaysia hospitals' laboratories, 9 private laboratories and 4 teaching hospitals' laboratories.

This exercise consisted of two modules: TM22-1 (Reticulin staining) and TM22-2 (Processing, Sectioning, Haematoxylin and Eosin staining). All participants had responded to TM22-1 and TM22-2 questionnaires. A total of 3 laboratories submitted non-tonsil tissues for TM22-2 (fibroid, lymph node and skin). Meanwhile, 8 laboratories did not submit the TM22-1 because inavailability of the reticulin staining in their centre.

INSTRUCTIONS TO PARTICIPANTS

CLINICAL NOTES

Code	Exercise	Clinical Notes
TM22-1	Reticulin stain	Liver tissue
TM22-2	Processing and Sectioning, Haematoxylin and Eosin (H&E) staining	Tissue type: tonsil Section size: 10 x 10mm to 15 x 20mm in dimension

ASSESSMENT CRITERIA

Reticulin stain (TM22-1)	Processing, Sectioning and H&E Stain (TM22-2)
1. Clear demonstration of reticular fibres and basement membrane material in the tissue. 2. Counterstain quality - complementary not obscuring (where used). 3. Staining result suitable for diagnostic reporting. 4. Uniformity of staining across the slide. 5. Absence of contaminants. 6. Absence of artefact from dehydration, clearing and mounting	**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

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

- For this technical module (trial run), slides will not be returned to the participants.

Questionnaire analysis of TM22-1

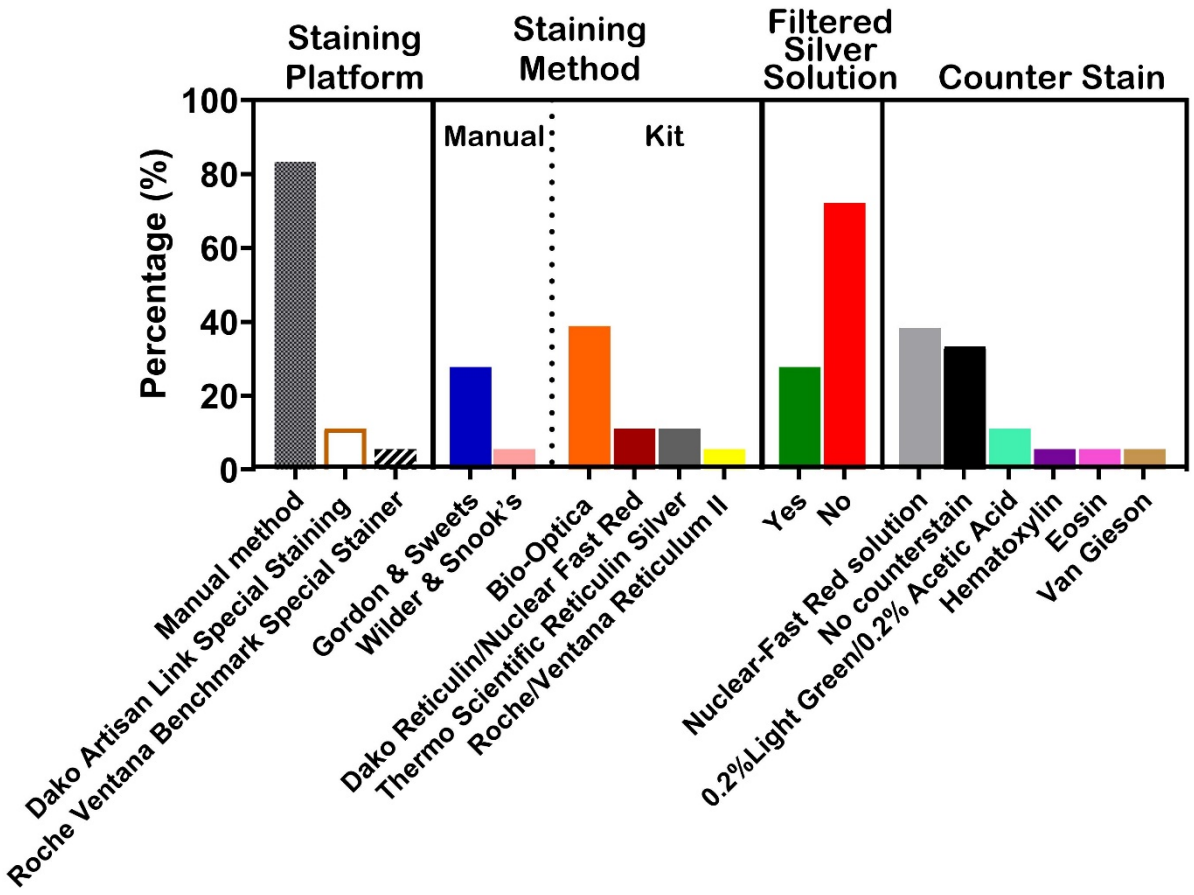

Figure 1: Responses to the questionnaires

Bar charts showing the survey results from 19 laboratories comprising of (i) the type of staining platform; (ii) type of staining methods; (iii) usage of silver solution filtration; and (iv) type of counter stain applied.

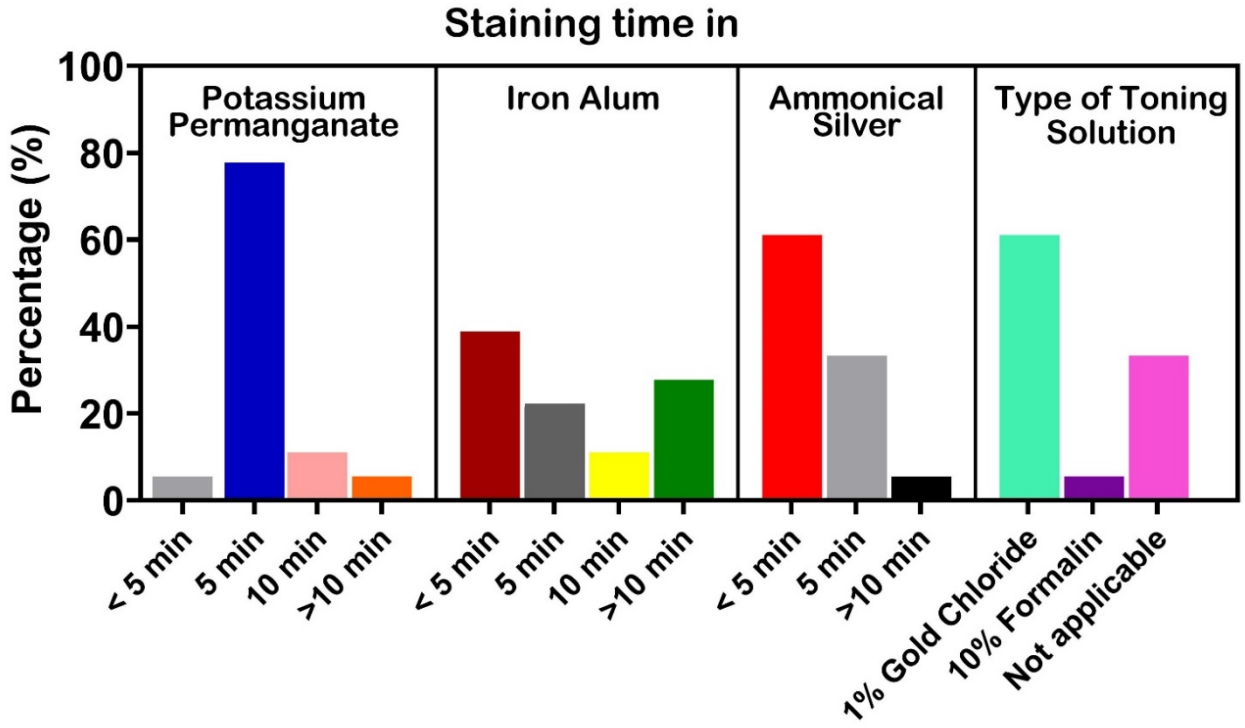

Figure 2: Responses to the questionnaires

Bar charts showing the survey results from 19 laboratories comprising of (i) staining times in potassium permanganate, iron alum, and ammonical silver solutions; (ii) type of toning solution

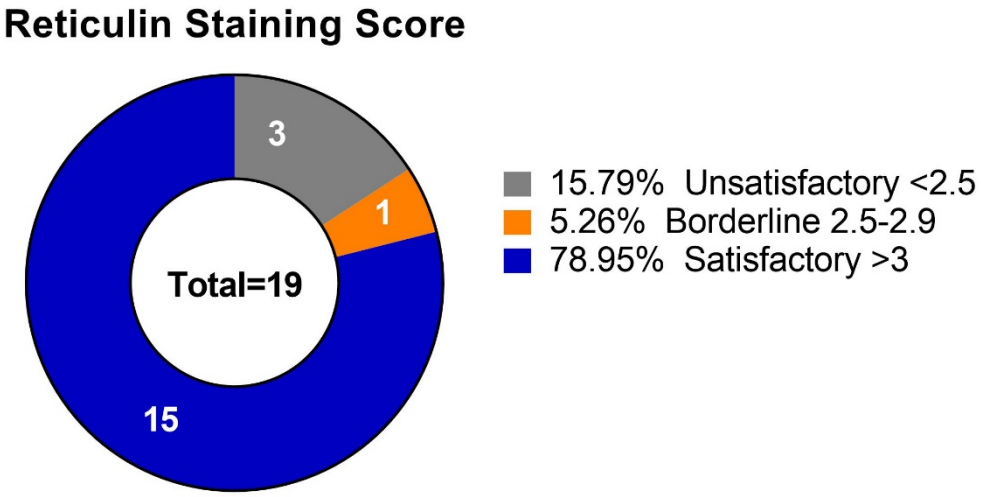

Figure 3: Technical assessment on the quality of reticulin staining

Pie chart showing the quality of reticulin staining analysis from 19 laboratories.

Summary notes:

1. Majority of the reticulin staining was performed manually (83.33%) as compared to the automated staining i.e. DAKO Special Staining System and Roche Ventana Benchmark Special Stainer (Figure 1).
2. Bio-Optica Reticulin and Gordon& Sweets were the top two common staining methods (Figure 1).
3. Bio-Optica Reticulin was the most common preferred manufacturer, followed by the in-house staining (Figure 1).
4. Nuclear fast red solution was the frequently used counter stain (Figure 1).
5. Incubation times for all staining solutions of potassium permanganate and iron alum were mostly performed more than 5 minutes except for the ammonical silver (Figure 2).
6. 1% gold chloride solution was the preferred toning solution (Figure 2).
7. The highest reticulin staining score of 4.2 in this exercise demonstrated fine and uniformity reticulin fibres. This staining used Bio Optica kit method toning with 0.2 % Gold Chloride for 2 minutes and counterstained with nuclear fast red.

Questionnaire analysis of TM22-2

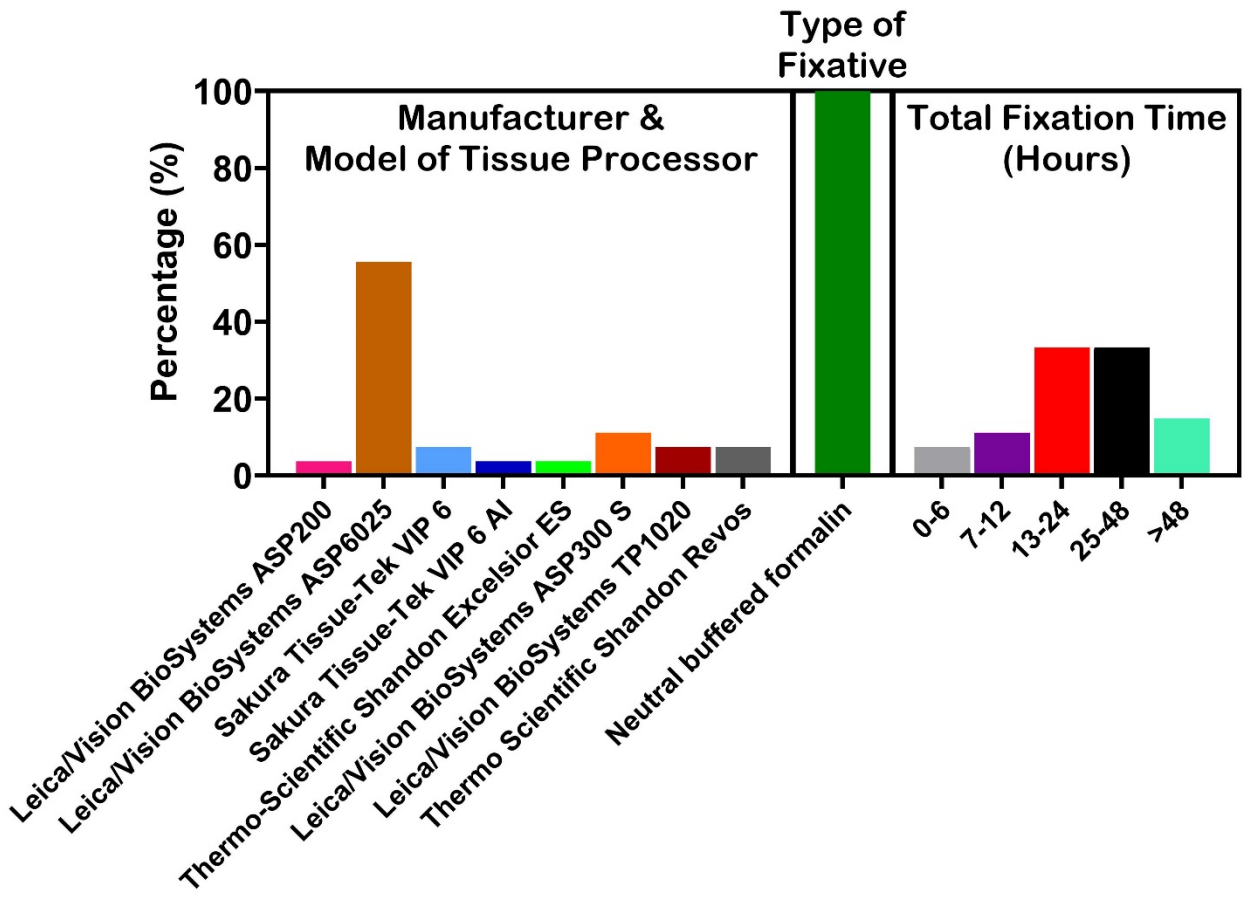

Figure 1: Responses to the questionnaires on tissue processing

Bar charts showing the survey results from 27 laboratories comprising of (i) the model of tissue processors used; (ii) type of fixative applied; and (iii) total of fixation time.

Summary notes:

1. The commonly used tissue processor model was the Leica/Vision Biosystems ASP6025 (55.6%).
2. All laboratories used neutral buffered formalin as a fixative in tissue processing.
3. Normal fixation times for tissue processing often exceeded 13 hours.

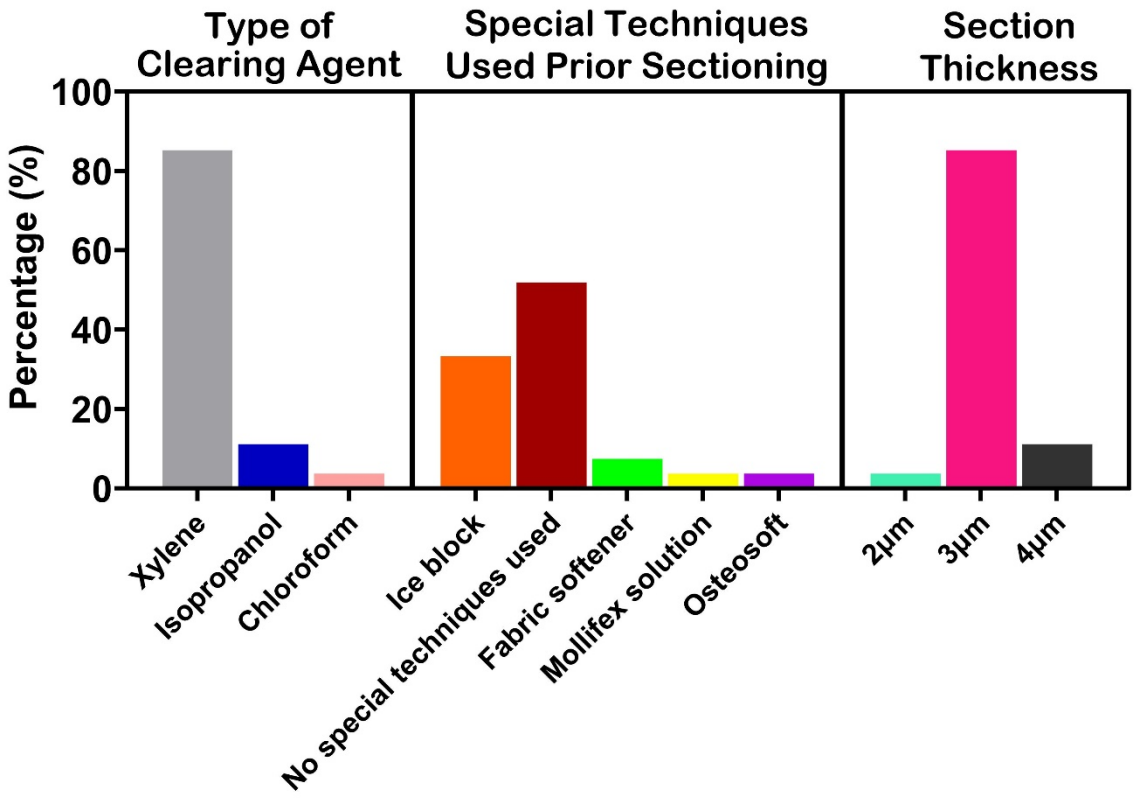

Figure 2: Responses to the questionnaires on tissue processing

Bar charts showing the survey results from 27 laboratories comprising of (i) type of clearing agent; (ii) type of special techniques used before sectioning; and (iii) section thickness.

Summary notes:

1. The most frequent clearing agent for tissue processing was xylene.
2. Prior to tissue sectioning, ice block, fabric softener, mollifex solution, and osteosoft were among the particular treatments employed. Ice block was the most often used method. The majority of laboratories (51.9%) did not perform any of the special technique.
3. Tissue sections with a thickness of 3 µm were the most preferred.
4. The highest tissue processing and sectioning score of 4.7 in this exercise demonstrated good preservation of tissue architecture with overall uniform section, absence of artefacts and no special technique was applied prior to sectioning. The laboratory used Leica ASP6025 autoprocessor.

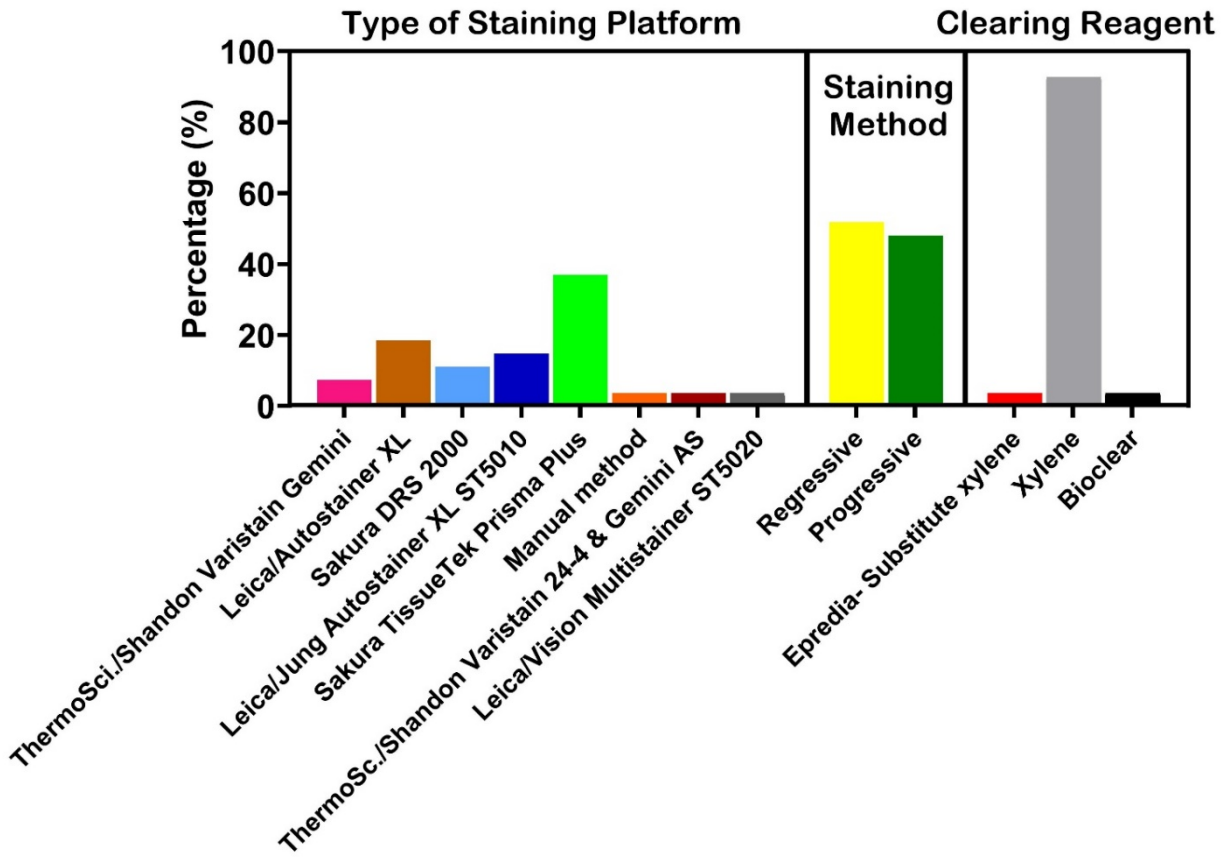

Figure 3: Responses to the questionnaires on tissue staining

Bar charts showing the survey results from 27 laboratories comprising of (i) type of staining platform; (ii) staining method; and (iii) type of clearing reagent.

Summary notes:

1. Common tissue staining platforms included the Sakura Tissue Tek Prisma Plus (37%) and the Leica/Autostainer XL(18.5%).
2. The regressive staining approach showed almost similar preference to the progressive staining method.
3. The most frequently used cleaning agent for tissue staining was xylene.

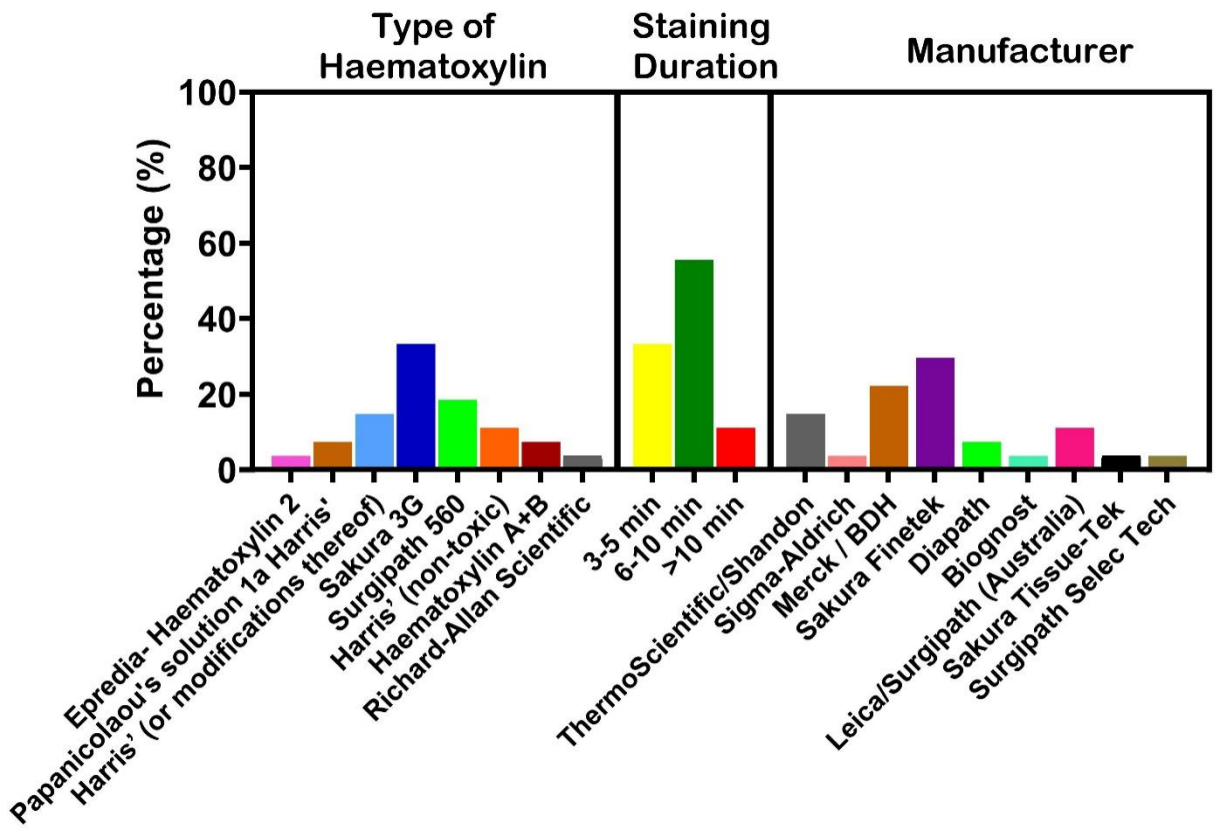

Figure 4: Responses to the questionnaires on haematoxylin in H&E staining

Bar charts showing the survey results from 27 laboratories comprising of (i) type of haematoxylin; (ii) staining duration; and (iii) manufacturer of staining solution.

Summary notes:

1. Sakura 3G was the most common type of haematoxylin (33.3%) in H&E staining.
2. The preferred staining duration in haematoxylin was around 6-10 minutes.
3. The top three manufacturers for haematoxylin were Sakura Finetek (29.6%), Merck/BDH (22.2%) and Thermo Scientific/Shandon (14.8%).

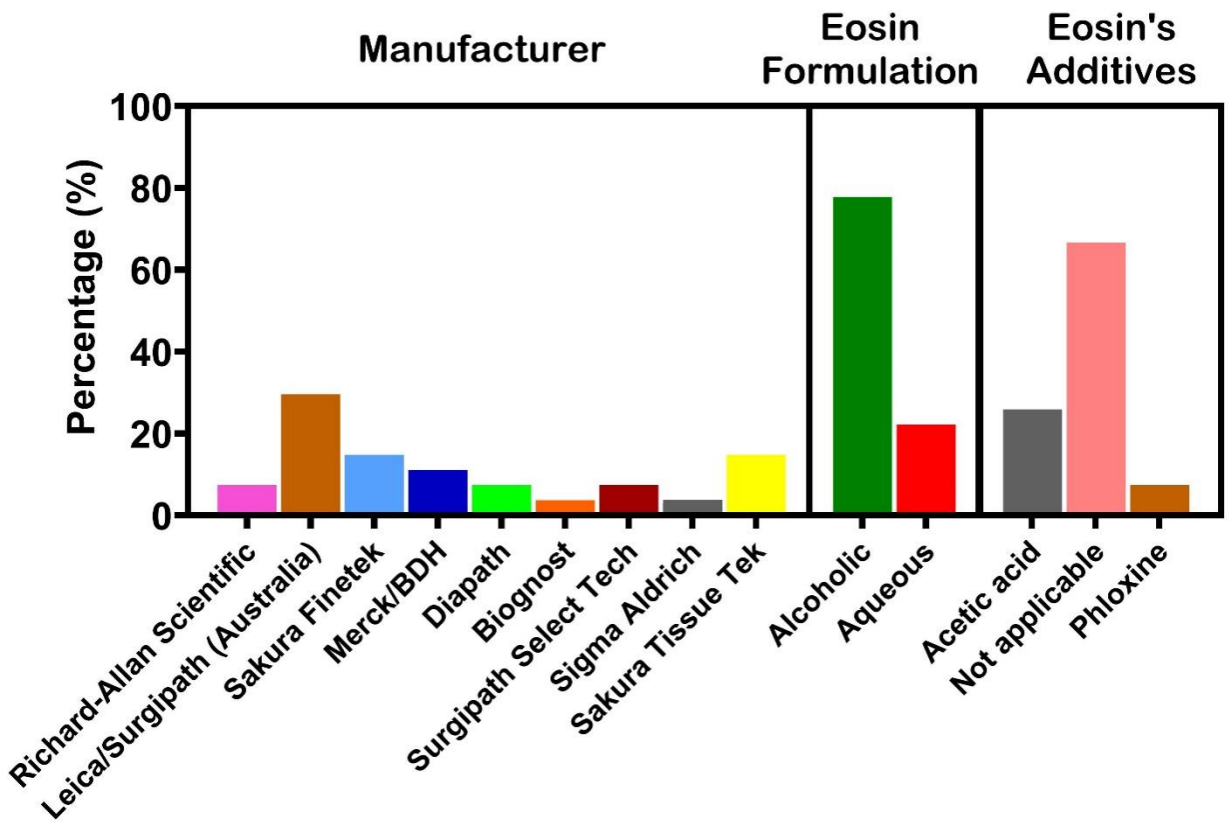

Figure 5: Responses to the questionnaires on eosin in H&E staining

Bar charts showing the survey results from 27 laboratories comprising of (i) manufacturer of staining solution; (ii) eosin formulation; and (iii) eosin's additive.

Summary notes:

1. The most favoured eosin staining solution was manufactured by Leica/Surgipath (Australia) (29.6%).
2. The most preferred solvent for eosin formulation was alcohol.
3. The majority of laboratories (66.7%) omitted the additional solution for eosin formation. However, some laboratories used acetic acid and phloxine.
4. The highest H&E staining score of 5.5 in this exercise demonstrated excellent demonstration of resting and activated lymphocytes, crisp nuclear chromatin, non-nuclear material were well stained, and adequate contrast between haematoxylin and eosin staining. The staining was performed using Sakura Tissue Tek Prisma Plus system.

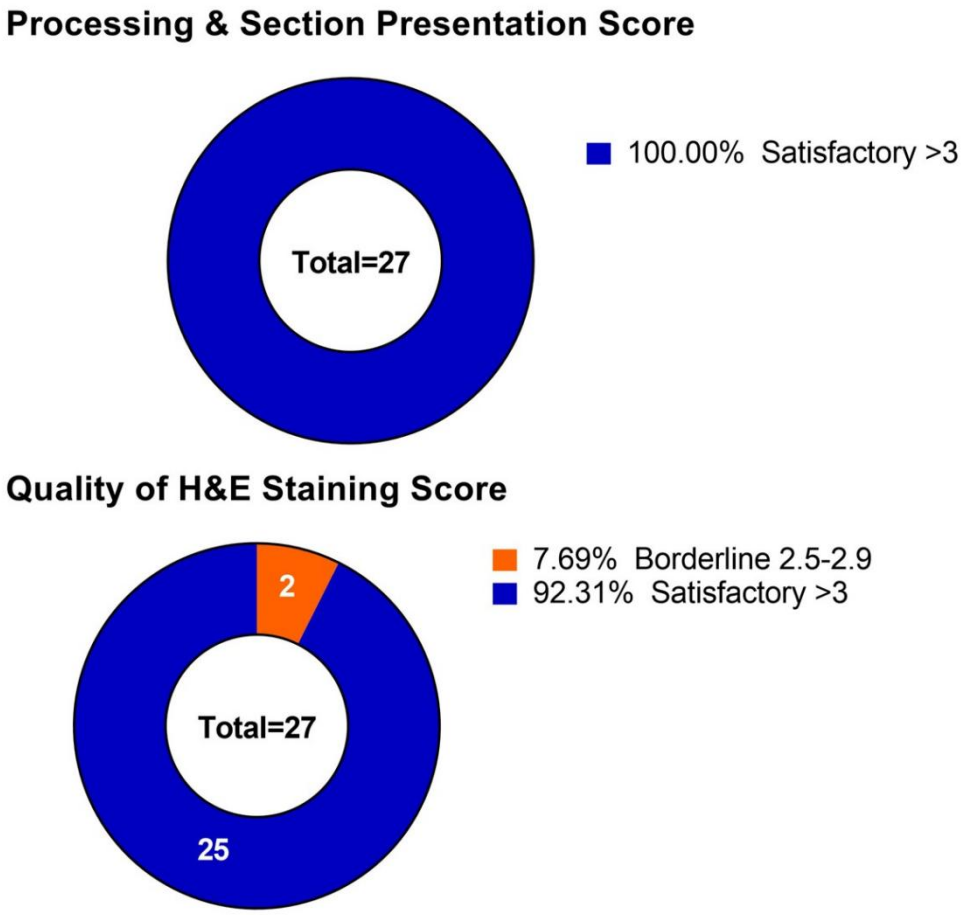

Figure 6: Technical assessment on the quality of tissue processing and H&E staining

Pie chart showing the quality of tissue processing and H&E staining analysis from 27 laboratories.

Summary notes:

1. The quality of tissue processing and sectioning were satisfactory for all participating laboratories.
2. Twenty five of the participating laboratories were given satisfactory score for H&E tissue staining, whilst two laboratories had borderline scores.