

Technical EQA Detailed Results

Run 3

On this round of the EQA scheme participants were asked to submit a stained survey section for assessment.

The value of survey sections could be debated with some having the view that they are of limited value and are only used for locating glomeruli or confirming the presence of muscle fibres. Some people choose not to produce survey sections at all and proceed directly to ultrathins. Others use survey sections as part of the diagnostic process and place a high value on the quality of the sections and staining.

However we choose to use survey sections we should all retain the capacity and expertise to produce survey sections of good quality that are well stained and presented. This enables us to have the maximum amount of information available to us in a well stained section to allow an objective and informed selection of tissue for ultrathin sectioning.

The survey sections were scored out of a maximum mark of **20** by each assessor. The 20 marks were subdivided into the following sections;

- **Section Quality** **Max mark = 4**
 - Is section scored/chattered
 - Is there dirt/debris on sections?
 - Are the sections folded?

- **Staining Quality** **Max mark = 14**
 - Is there sufficient contrast
 - Can all tissue elements be visualised?

- **Overall Appearance** **Max mark = 2**
 - Area of interest not present

The scores from each of the 4 assessors were then averaged to produce a single final mark for each participant. A score of 12 and over Indicates work of acceptable standard. A score of 16 and over Indicates work of very high Standard.

The marking scheme was adjusted in this round of assessment to put a heavier weighting onto the area of staining quality which was the area considered to be of greatest value.

Scores for this round of assessment are as follows;

<u>Participant No.</u>	<u>Averaged Mark</u>	<u>Comments</u>
1	15	Staining Pale and flat –lacks contrast
2	20	Excellent section and stain
4	17	Good stain –some water marks and precipitate
6	15	Whole core blocked – some section damage and folding on edge
10	14	Section too thick hence lacks clarity
11	17	Good section
12	19	Good section and well stained
13	11	Stain appears green, section thick and overall lacks detail
14	17	Tissue has been trimmed in to, Good staining contrast
15	17	Section wrinkled, good staining
16	19	Very good section and stain
19	18	Good section and stain, flat section
22	18	Large section, flat and well stained
23	13	Poor staining – uneven and lacks detail.
24	17	Good staining of tissue structures
25	17	Stain appears purple – good detail. Some wrinkling of section
26	15	Staining lacks contrast, loses detail of tissue
27	16	Pale staining with some wrinkles in section

Section Quality

The majority of the sections received for assessment were of a good standard with few section quality issues. The advisability of blocking out whole cores of renal tissue into a single block was discussed. The large size of the block leads to unavoidable folding of the sections that obscured detail. Importantly it was felt that blocking out a whole core would mean that diagnostic tissue would have to be sacrificed in preparing a trapezium of a suitable size for ultrathin sectioning. If the core was divided into smaller separate blocks then potentially less tissue would be lost. (fig. 1)

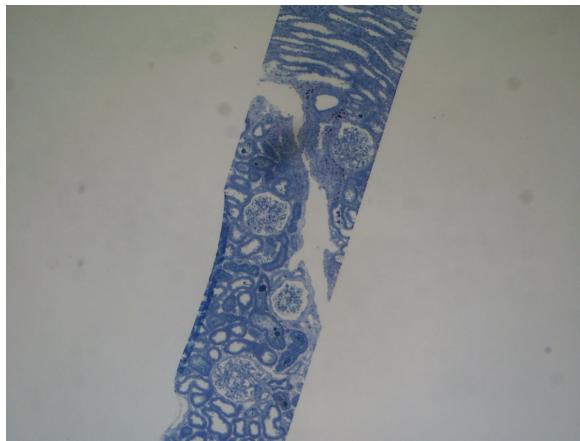


Fig. 1

Fixative Used

<u>Primary Fixative Used</u>	<u>Number of Participants</u>
2.5% gluteraldehyde in cacodylate buffer	4
3% gluteraldehyde in cacodylate buffer	1
4% gluteraldehyde in cacodylate buffer	1
5% gluteraldehyde in cacodylate buffer	1
2% gluteraldehyde in phosphate buffer	2
2.5% gluteraldehyde in phosphate buffer	3
3% gluteraldehyde in phosphate buffer	4
4% gluteraldehyde in phosphate buffer	1
2.5% gluteraldehyde in Pipes buffer	1
2% gluteraldehyde in Pipes buffer	1

Resins Used

<u>Resin</u>	<u>Number of Participants</u>
Araldite CY212	6
TAAB EMIX Medium grade	4
TAAB Premix Medium grade	1
Durcopan	1
Epon	1
Agar Low Viscosity	1
TAAB low viscosity	1
Poly/BED 812	1

Section Thickness

<u>Section Thickness</u>	<u>Number of Participants</u>
0.4um	1
0.45um	1
0.5 um	4
0.6um	1
0.75um	1
1um	6
2um	2
62um	1

Staining Quality

Considerable variation was seen in the staining quality of sections both in the stain used and also variations of apparently the same stain. Figs 2 represents 3 variations in 1% toluidine blue in 1% borax all on renal tissue.

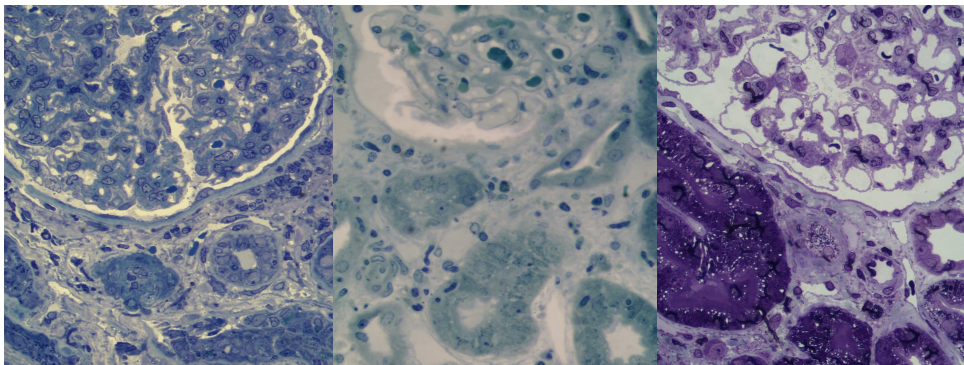


Fig. 2

We should be looking at our staining and considering if the staining is producing the expected outcome. Can we identify the tissue elements that the stain is meant to be staining and are those tissue elements the expected colour? If the answers to either of these questions are no then the staining regime or reagents used should be examined closely. Failure to produce good quality staining increases the chances of missing structures / pathology of diagnostic value. Fig. 3 compared to fig.4 highlights how much more information can be gained from a well stained section compared to one of inferior quality. What diagnostic features do we risk missing in fig 3?

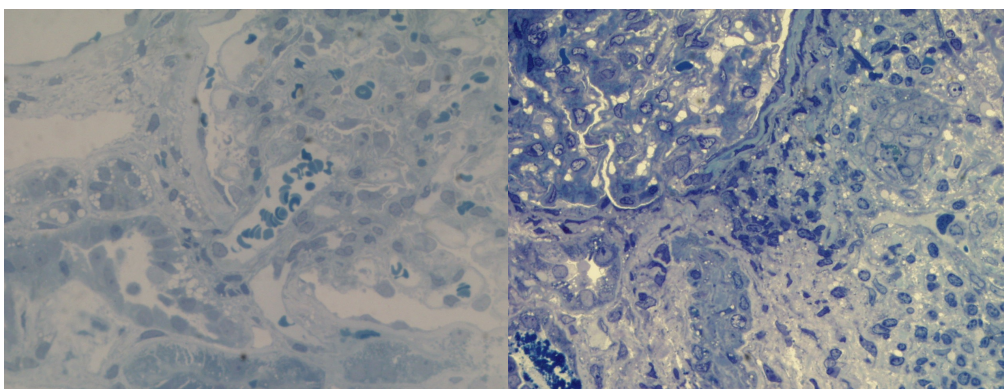


Fig. 3

Fig. 4

Is the stain used the most appropriate one for the purpose? Fig. 5 shows a muscle bx stained with paragon a toluidine blue, basic fuchsin mix. Fig. 6 is a similar muscle bx stained with toluidine blue. Although the participant must have good reasons for selecting this stain it was felt that less information could be obtained from this stain than from the alternative toluidine blue stain.

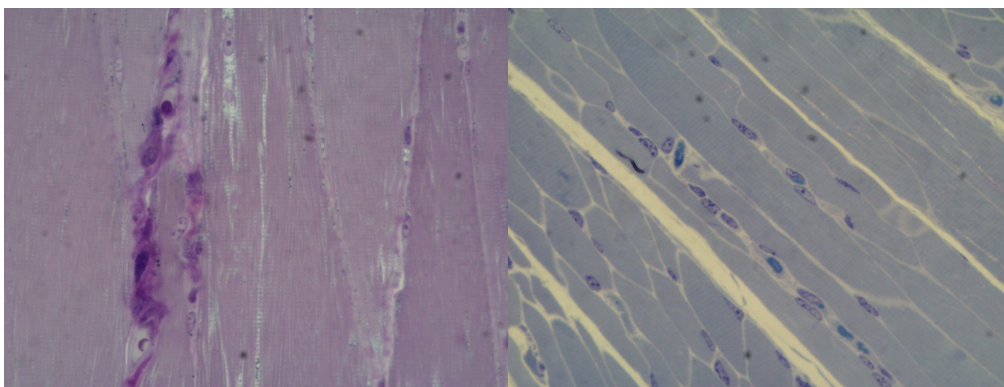


Fig. 5

Fig. 6

Stains Used

<u>Stains Used</u>	<u>Number of Participants</u>
Azur II / Methylene Blue	1
Thionine / acridine orange	2
Toluidine Blue	1
0.5% toluidine blue in 1% borax	3
1% toluidine blue in 1% borax	7
1% toluidine blue in saturated borax	1
0.2% toluidine blue in 2.5 % sodium carbonate	1
Toluidine blue and pyronin Y in borax	1
Paragon (toluidine blue/basic fuchsin)	1

Staining Methods used achieving highest marks

- **This stain achieved a score of 19/20 on a section of sural nerve.**

Acridine Orange Stain

Acridine Orange	2.5g
Distilled water	62.5 ml
0.1N NaOH	62.5ml

Add all reagents together, heat gently, stir well and filter. Keep in a refrigerator.

Thionin Stain

Thionine	0.5g
Distilled water	100ml
90% industrial methylated spirit	125ml
0.1N NaOH	25ml

Add reagents together in order given above, stir well and heat until approx 50°C: filter. Cool and store in refrigerator.

Staining Procedure

1. Place a few drops of thionin stain onto the sections and heat gently for 2-5 mins until well coloured.
2. Wash slide by flooding gently with distilled water.
3. Stain with acridine orange stain for 2-5 mins heating gently. Control colour visually.
4. Wash in distilled water.

- **This stain achieved a score of 19/20 on a section of a renal biopsy**

1% Toluidine Blue Staining Solution

Toluidine blue (Agar R1727	1g
Borax (di-sodium tetraborate 10-hydrate, BDH)	1g
Deionised water	100ml

Dissolve the borax in the water and then add the dye and stir well. Filter before use.

Staining Method

1. Dry the sections on the slide on a hot plate at approx 90°C
2. Filter 1% toluidine blue onto the slide and leave for 30 secs.
3. Rinse the slide gently in running water then replace on the hotplate to dry.