

Technical EQA Detailed Results

Run 2

Assessment of the grids submitted to this round of the EQA scheme along with the supporting information asked for has provided a substantial amount of information about the variation in techniques we all employ in producing our end product. This information forms a baseline of knowledge for the EQA scheme as it starts and represents our techniques and quality standards at this point in time. As the EQA scheme progresses we should hopefully see EQA scores rise as participants consider the techniques they are using and question themselves “is there a different and maybe better method of achieving their desired end product?”

On this round of EQA participants were asked to submit a stained grid of ultrathin sections for assessment.

The grids 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 = 5**
 - Is section scored/chattered
 - Is there dirt/debris on sections?
 - Are the sections folded?

- **Staining Quality** **Max mark = 5**
 - Is there sufficient contrast
 - Is there staining precipitate present?
 - Can all tissue elements be visualised?

- **Suitability of Section** **Max mark = 5**
 - Area of interest not present
 - Fixation / preparation

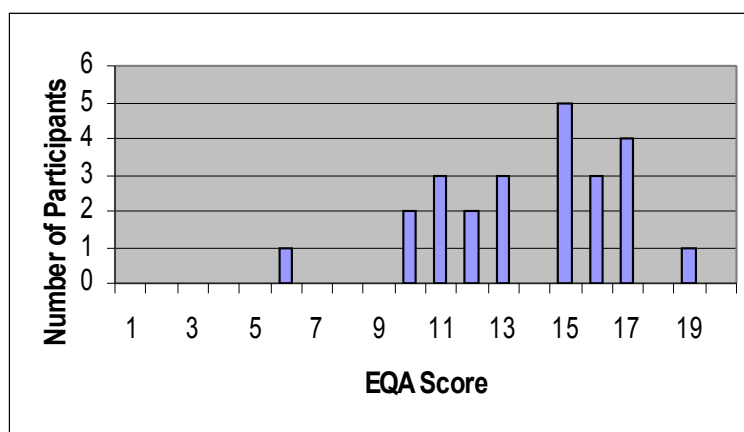
- **Overall Appearance** **Max mark = 5**
 - Grid selection

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 above represents sections of diagnostic quality. Anyone scoring under 12 should consider reviewing their section production procedures.

The scores for this round of assessment are as follows;

<u>Participant number</u>	<u>Score Awarded</u>	<u>Comments</u>
09	13.00	Poor contrast, overlapping sections scores and creases present
10	15.00	Good contrast but poor selection of grid type
13	6.25	Heavily overlaid with lead precipitate obscuring detail
02	15.00	Good contrast, Some scores present
06	17.50	Good contrast, Good sections
15	15.75	Good sections, slightly pale staining, grid mesh size good
21	17.00	Good contrast, Good sections
18	16.50	Good sections ?slightly thick
23	11.50	Precipitate over section obscuring detail
24	13.33	Some scores present and thick grid bars selected
		Some scores present and staining pale
26	12.67	Hexagonal grid with thick bars selected
05	16.00	Good contrast, Good sections
01	10.33	Spot grid selected limiting available tissue to examine
11	12.33	Good sections, staining lacks contrast
27	10.33	Sections scored and precipitate present
20	17.00	Good contrast, Good sections
04	13.00	Holes in section and some dirt present
12	17.00	Good contrast, Good sections
22	19.00	Good contrast, Good sections, plenty of tissue to examine
14	15.00	Good contrast, Good sections but grids with thick bars selected
16	11.75	Poor section quality with dirt present on sections
25	11.50	Poor section quality
19	16.50	Good contrast, Good sections
08	15.67	Formvar grids used. Good contrast & sections

Of the 24 participants who submitted grids to this round of the EQA scheme only 4 failed to reach the desired mark of 12 out of 20. In contrast 8 participants achieved a score of 16 or over out of 20 representing work of a very high standard.



Section Quality

Generally the quality of sections provided was of a good standard. However there were examples of scores and holes in excess of what might be reasonably expected (fig 1) The holes are thought to be due to placing wet grids and sections onto a surface that has adhered to the tissue, ripping holes in the sections when the grid is lifted.

Precipitate on a section is not desirable and in most cases is entirely avoidable (fig 2). Attention to cleanliness of sectioning and adherence to good staining regimes will in most cases prevent precipitate obscuring sections.

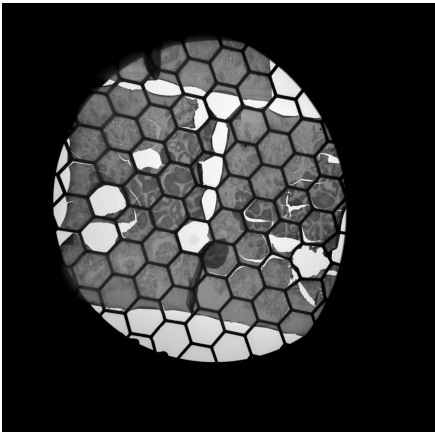


Fig.1

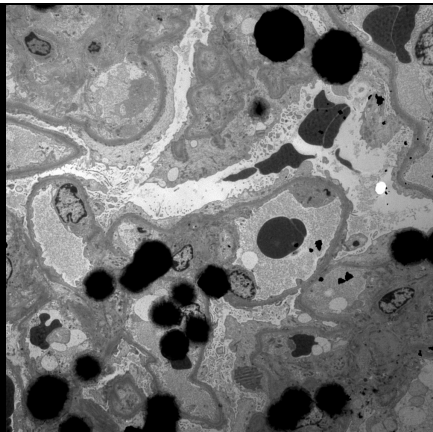


Fig.2

Fixative Used

<u>Primary Fixative Used</u>	<u>Number of Participants</u>
2.5% gluteraldehyde in cacodylate buffer	3
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	2
4% gluteraldehyde in Pipes buffer	1
2% gluteraldehyde /4% paraformaldehyde in phosphate buffer	1
1.5% gluteraldehyde/1% paraformaldehyde in phosphate buffer with sucrose	1
Buffered formalin/5% gluteraldehyde in cacodylate buffer	1
<u>Secondary Fixative</u>	<u>Number of Participants</u>
1% aqueous osmium tetroxide	8
2% aqueous osmium tetroxide	1
1% osmium tetroxide in cacodylate buffer	4
2% osmium tetroxide in cacodylate buffer	1
Osmium tetroxide and potassium ferricyanide	1

Resins Used

<u>Resin</u>	<u>Number of Participants</u>
Araldite CY212	9
TAAB EMIX Medium grade	4
TAAB Premix Medium grade	2
TAAB Spurr Hard grade	1
TAAB Epoxy 812	1
Durcopan	1
Modified Spurr	1
Lemix	1
Agar Low Viscosity	1

Staining Quality

A good staining regime imparts contrast and clarity to a tissue allowing all tissue elements and pathology to be visualised clearly and accurately. If good quality staining is not achieved then can we be absolutely sure that important features of the tissue we are examining are not being missed?

In fig 3 and fig 5 there was a distinct lack of contrast to the tissue. Other than the nuclei that stood out well it was very difficult to distinguish basement membrane/sarcomere and other cytoplasmic elements. This is in contrast to fig 4 and 6 where all tissue elements and pathology could be easily identified.

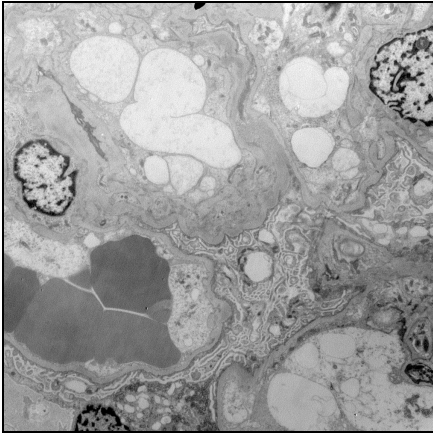


Fig. 3

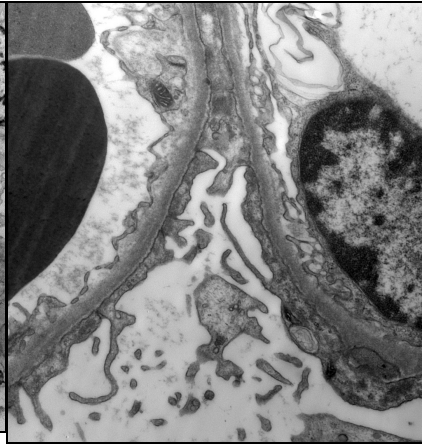


Fig. 4

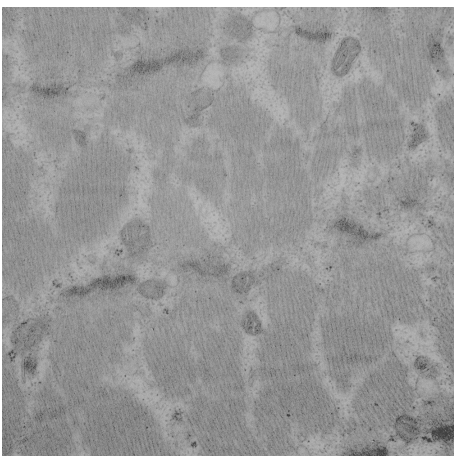


Fig.5

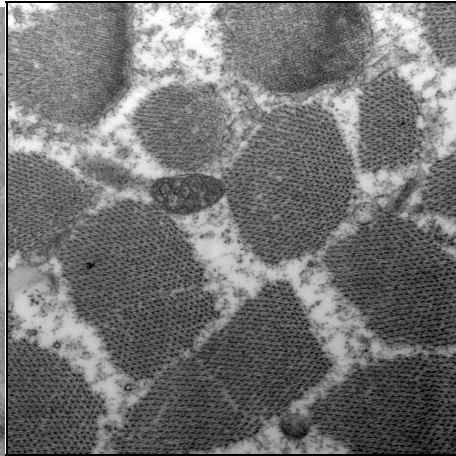


Fig. 6

Stains Used

<u>Uranyl Acetate</u>	<u>Number of participants</u>
Alcoholic uranyl acetate	1
3.3% methanolic uranyl acetate	1
4% uranyl acetate in methanol	1
5% uranyl acetate in 50% alcohol	1
Saturated alcoholic uranyl acetate	15
Aqueous uranyl acetate	1
1% aqueous uranyl acetate	1
2% aqueous uranyl acetate	1

<u>Lead Citrate</u>	<u>Number of Participants</u>
Lead citrate	9
Reynolds lead citrate	11
0.25% lead citrate	1
0.4% lead citrate in 0.1N sodium hydroxide	1

Staining Methods Producing Highest Marks

<u>Score Achieved</u>	<u>Tissue</u>	<u>Fixative Used</u>	<u>Resin Used</u>	<u>Knife Used</u>	<u>Section Thickness</u>	<u>Stain</u>
17/20	Renal	1.5% Glut. / 1% paraformaldehyde in PBS and sucrose osmium tetroxide with potassium ferricyanide	Lemix	Diatome	80nm	Saturated alcoholic Uranyl Acetate Reynolds Lead Citrate
17/20	Renal	3% Gluteraldehyde in phosphate buffer 1% Osmium Tetroxide	TAAB resin	Glass	Gold	Saturated alcoholic Uranyl Acetate Lead Citrate
17/20	Muscle	4% gluteraldehyde in pipes 1% osmium tetroxide	TAAB spur resin hard	Diamond	100nm	Saturated alcoholic Uranyl Acetate Reynolds Lead Citrate
17/20	Renal	10% formalin then gluteraldehyde / paraformaldehyde in cacodylate buffer 2% osmium tetroxide in cacodylate buffer	Modified Spurr	Glass	Silver /pale gold	Aqueous uranyl acetate (block stain) Saturated alcoholic Uranyl Acetate Reynolds Lead Citrate
19/20	Semen	3% Gluteraldehyde in 0.1M sod cacodylate 1% Osmium Tetroxide in Cacodylate buffer	Araldite	Diatome	60-80nm	Saturated alcoholic Uranyl Acetate Reynolds Lead Citrate

Grid Selection

The choice of what grid to use for ultrathin sections is dependant on many factors. Principally however grid selection is based on a balance between offering the sections sufficient support to withstand the electron beam and having sufficient tissue available between grid bars for examination. It seems excessive for a routine renal biopsy to be mounted onto a high mesh size grid with thick grid bars (fig. 7) when so much more tissue can be visualised with a grid that has thin grid bars (fig. 8). In this example up to 40% more of the glomeruli can be seen in fig. 8 than fig.7 and both sections were very stable in the electron beam

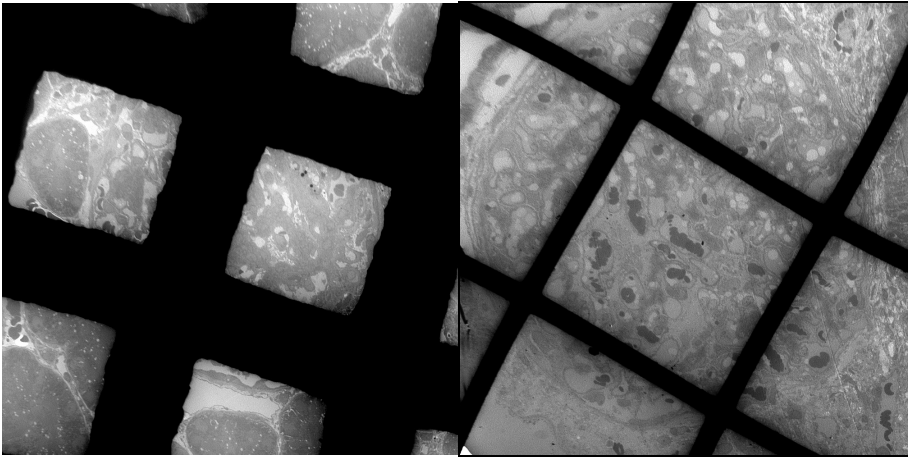


Fig. 7

Fig. 8

Grids Used

<u>Grids</u>	<u>Number of Participants</u>
TAAB Copper 200 mesh Hexagonal	1
Copper 200HS mesh – thin bar	1
Athene copper new 200 mesh	1
2x1mm formvar coated copper slot grid	1
Copper single hole grid	1
Copper 200 mesh	4
Copper 200 mesh hexagonal thin bar	1
Copper 200 mesh hexagonal coated with Coat quick G adhesive	1
Copper 200 mesh coated with pioloform	1
Copper100 mesh hexagonal	1
Copper 300HS mesh	1
Copper 400 mesh thin bar	1
Copper G202 old 200 mesh	1
Copper 200 mesh veco grid	1
Copper 150 mesh hexagonal grid	1
Copper/rhodium	1
Copper/rhodium Agar maxtaform HR24	1
Nickel 400 mesh	1