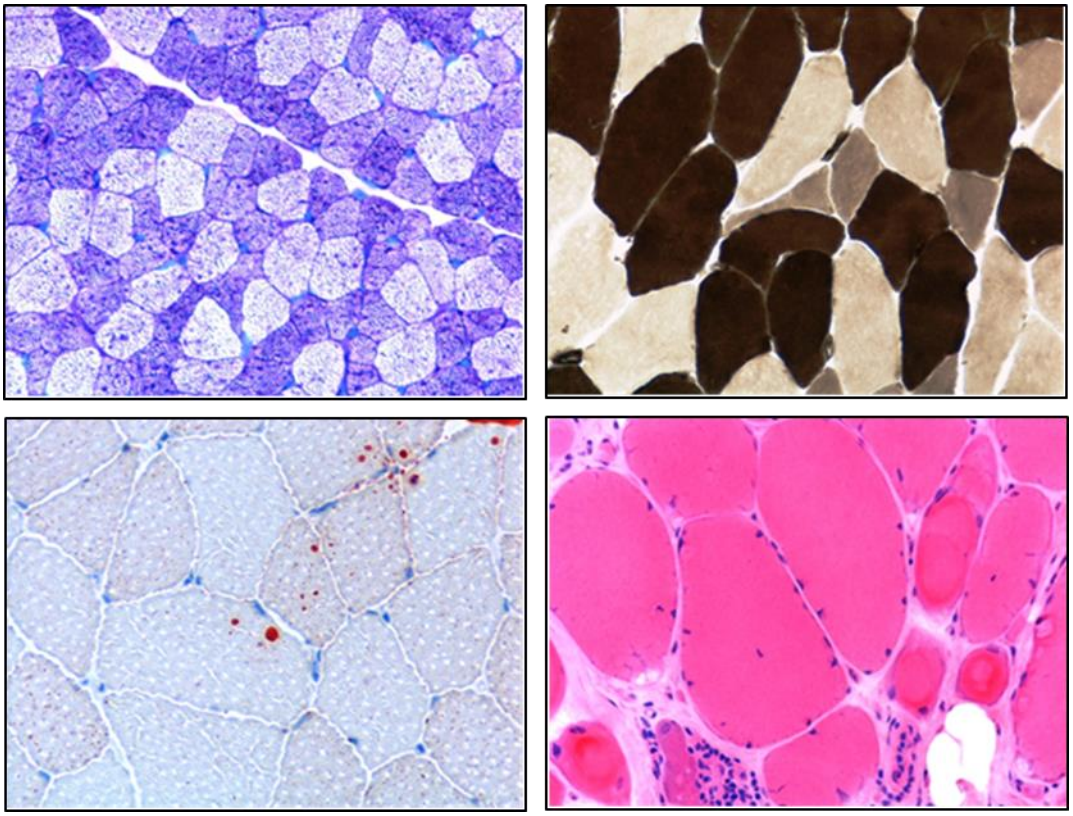
UK NEQAS
Cellular Pathology Technique

Staining Criteria Handbook

Muscle Histochemistry



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UK NEQAS Cellular Pathology Technique

Providing worldwide external quality assessment and proficiency testing for all aspects of tissue diagnostics

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Haematoxylin and Eosin Assessment Criteria

Muscle Histochemistry Haematoxylin and Eosin Assessment Criteria

Pre Cryotomy	Insufficient cellular features for assessment Drying artefact Freezing artefact Crush artefacts Cracking Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Staining	Haematoxylin Intensity too strong Haematoxylin Intensity too weak Haematoxylin colour not purple blue Haematoxylin background staining Eosin Intensity too strong Eosin Intensity too weak Eosin not selective Lack of fibre detail Uneven staining Stain deposit present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue damage Tissue exposed Water present

Description of Staining Results

The Haematoxylin and Eosin (H&E) shows muscle architecture and fibre morphology.

Haematoxylin should stain nuclei a crisp blue and fibres slightly basophilic (to show up mitochondria), eosin stains connective tissue pink.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Section quality and presentation must not impair the results.

Special Stains
Assessment Criteria

Muscle Histochemistry Acid Phosphatase Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast Not selective Background Uneven Staining Deposit / precipitate present
Counterstain	Intensity too strong Intensity too weak Stain colour Not selective Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

Acid phosphatase is an enzyme that can be found in lysosomes, with notable activity seen in necrotic fibres & macrophages plus lipofuschin in adult cases.

Acid phosphatase can be demonstrated by hydrolysis, often by azo simultaneous coupling methods.

Staining conditions must be carefully controlled to ensure specific acid phosphatase demonstration, with an acidic pH found to be optimal.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Counterstain should provide good colour contrast and assist location.

Section quality and presentation must not impair the results.

Muscle Histochemistry Cytochrome Oxidase (COx) Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast Indistinct fibre type Not selective Background Uneven Staining Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

Cytochrome oxidase (COx) is a mitochondral enzyme which can be assayed using enzyme histochemistry and used in the differential diagnosis of mitochondrial myopathies.

In normal adult muscle, type 1 fibres stain more intensely in shades of orange to brown, than type 2 fibres.

Muscle fibres without COx activity indicate an mtDNA defect.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Section quality and presentation must not impair the results.

Muscle Histochemistry Gomori Trichrome Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast Not selective Background Uneven Staining Deposit / precipitate present
Counterstain	Intensity too strong Intensity too weak Not selective Stain colour Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

The sarcoplasm of the muscle fibres should be stained green-blue, and the mitochondria within the sarcoplasm of muscle fibres should be stained red.

The number of mitochondria increases towards the periphery of the fibre where subsarcolemmal accumulations can be seen (mostly in type 1 fibres). The collagen in the endomysium and perimysium should be stained green.

If the haematoxylin counterstain is used, the nuclei should be stained grey-blue, otherwise they should also appear red.

The staining should be even, neither too dark nor too light, and the colour balance should not be shifted towards too blue /green, or too red tones.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Section quality and presentation must not impair the results.

Muscle Histochemistry Lipid Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast Not selective Background Uneven Staining Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

Lipids, compromised of fatty acids that can be present as esters or associated with carbohydrates and proteins, are a large group of organic compounds.

Lipids are the main energy source for muscle at rest and low intensity exercise.

Intra-fibre droplets maybe noted, but these should not be confused with displaced interstitial lipid from adipose tissue.

In some metabolic diseases lipid can accumulate as it cannot be processed and is stored inside the muscle.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Counterstain should provide good colour contrast and assist location.

Section quality and presentation must not impair the results.

Muscle Histochemistry NADH Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast Indistinct fibre type Not selective Background Uneven Staining Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

In normal muscle the three intensities of staining should be clearly visible corresponding to the main fibre types. Type 1 darkest, 2A intermediate, 2B lightest. Staining should be even across the section.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Section quality and presentation must not impair the results.

Muscle Histochemistry PAS Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast PAS Intensity too strong PAS Intensity too weak PAS Coloration Residual Glycogen Not selective Background Uneven Staining Deposit / precipitate present
Counterstain	Intensity too strong Intensity too weak Stain colour Not selective Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

The Schiff reaction is a histochemical method for aldehyde groups. Periodic acid oxidation creates these groups on a variety of tissue structures notably glycogen, mucins and basement membranes.

There should be complete demonstration of basement membranes of non-dystrophic muscle fibres and in blood vessels. Abnormal, disease associated, diastase resistant material should not be confused as glycogen.

Background should be minimal.

Counterstain should provide good colour contrast and assist location.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Section quality and presentation must not impair the results.

Muscle Histochemistry Primary Fibre Typing Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast Indistinct fibre type Not selective Background Uneven Staining Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

Muscle fibres can be grouped into either Type 1 or Type 2. The composition of the muscle group of the fibre type is determined by function.

Type 1 muscle fibres can be referred to as slow twitch and utilise oxidative energy pathways and sparse in ATPase.

Type 2 muscle fibres are referred to as fast twitch and further classified as 2a (oxidative, glycolytic, fatigue resistant), and 2b (glycolytic, fatigue sensitive). Both contain much more ATPase than type 1 fibres owing to the energy pathways utilised.

Fibre typing can be determined histochemically, e.g. by myosin ATPase demonstration by altering pre-incubation conditions, or immunohistochemically by myosin heavy chain isoforms.

Identifying the fibre type distribution allows assessment of composition, grouping, size & shape of fibres.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Section quality and presentation must not impair the results.

Muscle Histochemistry Succinate Dehydrogenase (SDH) Assessment Criteria

Pre Cryotomy	Drying artefact Freezing artefact Incorrect orientation
Cryotomy	Air bubbles Chatter / vibration Compressed section Folds / creases Knife back debris Knife / guide plate marks Lifting Position on slide Section overlap Section too thick Section too thin Section thickness variable Smearing Squames / floaters / fibres Waffling effect
Primary Stain	Intensity too strong Intensity too weak Stain Colour Contrast Indistinct fibre type Not selective Background Uneven Staining Deposit / precipitate present
Post staining	Air bubbles Air drying artefact Excessive mountant Mountant shrinkage Section wiped / section off slide Tissue exposed Water present

Description of Staining Results

Succinate Dehydrogenase (SDH) is a mitochondrial enzyme. Demonstration of its activity by enzyme histochemistry aids the diagnosis of mitochondrial myopathies.

SDH activity in normal adult muscle appears as blue specks, with type1 fibres producing a deeper shade than type 2 fibres.

All tissue of the section needs to be firmly adherent to the (clean) slide, evenly cut, completely dried, and properly differentiated in order to ensure even and correct (genuine) staining. No stain deposits should be identified.

The section must be flat with no folds, with no knife marks, and must not contain freezing artefact.

Section quality and presentation must not impair the results.

Assessment Criteria Definitions

- **Muscle Haematoxylin and Eosin**

- **Muscle Special Stains**

Muscle Haematoxylin and Eosin Criteria Definitions

Pre Cryotomy

The material supplied must have sufficient muscle fibres to allow assessment.

Insufficient cellular features for assessment - The material supplied does not have sufficient muscle fibres present to allow assessment

Artefacts, such as crush effects should not be introduced during the laboratory handling and preparation stages.

Crush artefacts - distortion of the fibrilar structures, particularly at one or some of the edges of the biopsy, caused by rough handling, previous to freezing

Drying artefact - Shrinking of the muscle fibres, normally producing some irregular shapes, with increased inter-fibrilar spaces. The above is usually accompanied by irregular cracks within some of the fibres

Freezing artefact - disruption of the sarcoplasm, due to multiple empty spaces, caused by the formation of ice crystals, producing a widespread “moth eaten” appearance. In severe cases, the ice crystal “hole” can be of similar diameter to the muscle fibre

Cracking - tissue breaking, perpendicular to the cutting line. These are generally caused by too low temperature of specimen during cryotomy

The orientation of the biopsy should be such that the majority of the muscle fibres have been cut transversally. It is understood that with some biopsies, particularly those obtained by needle, it may be impossible to get all the fibres transversally.

Incorrect orientation - a large proportion of the muscle fibres have been sectioned longitudinally or obliquely

Cryotomy

The section must be of appropriate thickness. If the section is too thick, it will obscure smaller changes, such as inclusion bodies; if it is too thin, it will compromise the staining intensity. It must be free from variations in thickness, creases, folds, scores and contaminants. No accumulation or fragments of tissue should be seen over or between sections. The section should be flat, with no lifted areas. There should be no holes, tearing or damage either as a result of trimming or sectioning or cover-slipping. The section must be positioned on the slide so that microscopy is not compromised.

Air bubbles - circular raised areas of tissue, well demarcated, caused by getting small air bubbles between the section and the glass, when picking the section from the cryostat knife

Chatter / vibration - very closely placed variation in thickness causing stripes of alternate staining intensity at right angles to direction of sectioning

Compressed section - this is an artefact caused when picking the section from the knife onto the slide, the section is "squashed" between the knife and the slide, causing compression and or fracture of the section.

Folds / creases - creases and folds in the section giving doubles layers that lift from the slide and stain intensely

Knife back debris - accumulation of cells that are smeared on the edge of the knife and deposited between alternate sections

Knife / guide plate marks - scores and scratches at parallel to the direction of sectioning

Lifting - areas of section that lift and do not lie flat

Position on slide - section positioned appropriately on slide for full visualisation

Section too thick - unable to focus on a single cell layer

Section too thin - loss of stain intensity and contrast because of thinness of section

Section thickness variable - varying thickness in different areas of the section, recognised by varying dye intensity

Smearing - the appearance is like an edge of the section has been dragged and stretched onto the glass. This is an artefact caused when picking the section from the knife onto the slide, due to a sideways movement of the slide, or alternatively by the section "jumping" onto the slide due to static forces

Squames / floaters / fibres - contamination by material that is not in the block, usually above the section

Waffling effect - multiple small creases, mainly within each muscle fibre

Staining

Nuclei must be stained purple/ blue with haematoxylin. Ideally, the haematoxylin should also stain many of the mitochondria, thus showing an intrafibrilar pattern, but it should not be too strong, to stain or obscure the appearance of other structures, or produce a blue tinge or colour of the myofibrils or other eosinophilic structures. This background if present must not reduce the effectiveness of the nuclear demonstration or affect the colour and selectiveness of the eosin.

Haematoxylin Intensity too strong - a loss of the chromatin granularity or excessive cytoplasmic or connective tissue staining

Haematoxylin Intensity too weak - intensity must be strong enough to allow clear demonstration of nuclear detail at a medium power

Haematoxylin colour not purple/blue - nuclei must be stained purple/ blue with haematoxylin

Haematoxylin background staining - where the haematoxylin has been differentiated out, minimal cytoplasmic or connective tissue background staining with haematoxylin must remain. If present must not reduce the effectiveness of the nuclear demonstration or affect the colour and selectiveness of the eosin

The eosin should be selective enough to demonstrate different cellular components such as collagen, cytoplasm and other eosinophilic structures. The intensity must be appropriate to the section thickness and the haematoxylin intensity. Where the intensity is too weak it will fail to allow selective demonstration of different components at low power. If the intensity is too strong it will obscure normal and some pathological structures, such as eosinophilic inclusions.

Eosin Intensity too strong - the intensity must be appropriate to the section thickness and the haematoxylin intensity. If the eosin intensity is too strong the colour and detail of the nuclear stain will be obscured and selectivity will be reduced

Eosin Intensity too weak - the intensity must be appropriate to the section thickness and the haematoxylin intensity. Where the eosin is too weak it will fail to allow selective demonstration of different components at low power

Eosin not selective - the eosin should be selective enough to demonstrate different cellular components such as collagen, cytoplasm, red blood cells, cellular granules, amyloid etc

All staining should be even across the section and there should be no dye deposits or precipitate. there should be distinct detail within the fibres being demonstrated

Uneven staining - all staining should be even across the section

Stain deposit present - dye deposits or precipitate

Lack of fibre detail - muscle architecture and fibre morphology must be clearly demonstrated

Post Staining

Coverslipping must be free from air bubbles, air drying artefact or drying back. There should be no excessive mountant, the sections must be totally covered and there must be no evidence of incomplete dewaxing or dehydration. The difficulties of processing and sectioning bony tissue will be taken into account when assessing.

Air bubbles - air bubbles within the mountant

Air drying artefact - retractile areas and tissue damage

Excessive mountant - mountant outside the coverslip

Mountant shrinkage - areas of mountant have dried back from the coverslip edges

Section wiped / section off slide - section scratched by hand or off edge of slide

Tissue damage - section scratched partially wiped or otherwise damaged by handling, after cutting had been completed

Tissue exposed - the section is not covered by the coverslip

Water present - droplets of water under the coverslip

Special Stains Criteria Definitions

Pre Cryotomy

Insufficient cellular features for assessment - The material supplied does not have sufficient muscle fibres present to allow assessment

Crush artefacts - distortion of the fibrilar structures, particularly at one or some of the edges of the biopsy, caused by rough handling, previous to freezing

Drying artefact - Shrinking of the muscle fibres, normally producing some irregular shapes, with increased inter-fibrilar spaces. The above is usually accompanied by irregular cracks within some of the fibres

Freezing artefact - disruption of the sarcoplasm, due to multiple empty spaces, caused by the formation of ice crystals, producing a widespread “moth eaten” appearance. In severe cases, the ice crystal “hole” can be of similar diameter to the muscle fibre

Cracking - tissue breaking, perpendicular to the cutting line. These are generally caused by too low temperature of specimen during cryotomy

Incorrect orientation - a large proportion of the muscle fibres have been sectioned longitudinally or obliquely

Cryotomy

Air bubbles - circular raised areas of tissue, well demarcated, caused by getting small air bubbles between the section and the glass, when picking the section from the cryostat knife

Chatter / vibration - very closely placed variation in thickness causing stripes of alternate staining intensity at right angles to direction of sectioning

Compressed section - this is an artefact caused when picking the section from the knife onto the slide, the section is “squashed” between the knife and the slide, causing compression and or fracture of the section.

Folds / creases - creases and folds in the section giving doubles layers that lift from the slide and stain intensely

Knife back debris - accumulation of cells that are smeared on the edge of the knife and deposited between alternate sections

Knife / guide plate marks - scores and scratches at parallel to the direction of sectioning

Lifting - areas of section that lift and do not lie flat

Cryotomy continued

Position on slide - section positioned appropriately on slide for full visualisation

Section too thick - unable to focus on a single cell layer

Section too thin - loss of stain intensity and contrast because of thinness of section

Section thickness variable - varying thickness in different areas of the section, recognised by varying dye intensity

Smearing - the appearance is like an edge of the section has been dragged and stretched onto the glass. This is an artefact caused when picking the section from the knife onto the slide, due to a sideways movement of the slide, or alternatively by the section "jumping" onto the slide due to static forces

Squames / floaters / fibres - contamination by material that is not in the block, usually above the section

Waffling effect - multiple small creases, mainly within each muscle fibre

Primary Stain

Background - background staining present, reducing contrast of the nuclei or obscuring detail or affecting colour of another feature. Caused by staining too long, not differentiating enough or non-selective breakdown products in old stains

Deposit / Precipitate present - random irregular or crystalline deposits above and around the cells. Old or unfiltered dyes

Indistinct fibre type - type I muscle fibres stain stronger (COx, NADH and SDH) than type II**.** The staining strength differential should be maximized. Paediatric muscles do have reduced fibre type differentiation, and it may be difficult. Also there are muscles which have a given fibre type predominance, and therefore show very few if any, darker or lighter fibres. It is however essential with COx staining that the strength of staining is such, that type II fibres are stained, to differentiate them from COx negative fibres indicative of a mitochondrial myopathy

Indistinct fibre type *(Primary Fibre Typing)* - with the acidic or alkaline pH incubation for myosin ATPase, and myosin isoforms by immuno-histochemistry, there would be distinct differentiation between type I and II fibre types. If intermediate pH for myosin ATPase is submitted for assessment, it expected that the type II fibres can be further differentiated to type IIa and IIb.

Primary Stain continued

Intensity too strong - intensity is so strong that it is hindering the fibre typing, all appear as type I. Other detail maybe missing, such as “moth eaten” fibres

Intensity too weak - intensity so low, it fails to show areas with enzyme activity. Particularly important with the COx, as it negates the difference between some type II fibres and true COx negative fibres

No contrast – colouration impairs clear visualisation / demonstration of target cells, tissue component or organism.

Not selective - Not selective of different cell types or tissue components. Old dyes. Mixed incorrectly. Not evenly applied to slide. Uneven washing. Dehydrated incorrectly

PAS Coloration - the neutral mucins are not bright magenta, and the mixed mucins are not purple. Old dyes. Mixed incorrectly. Not evenly applied to slide. Uneven washing. Dehydrated incorrectly

PAS Intensity too strong (muscle) - stain intensity is so strong that it is obscuring/ interfering with the nuclear stain or clear identification of basement membranes.

PAS Intensity too weak - where the stain is too weak it will fail to allow the clear, selective demonstration of basement membranes of muscle fibres and in vessels.

Residual Glycogen - residual glycogen present in tissue components after digestion, where glycogen should no longer be demonstrated.

Stain Colour - is not stained the appropriate colour based on the method employed and the expected staining results . Old dyes. Mixed incorrectly. Not evenly applied to slide. Uneven washing. Dehydrated incorrectly.

Uneven staining - varying intensity of staining in different parts of the slide preparation. Dyes applied but not spread evenly. Uneven dehydration.

Counterstain

Deposit / Precipitate present - Deposit / precipitate present - random irregular or crystalline deposits above and around the cells. Old or unfiltered dyes.

Intensity too strong - stain intensity is so strong that it is obscuring/ interfering with the nuclear stain or staining areas that should be another colour. Other detail may be missing

Intensity too weak - stain intensity is so weak that it is failing to demonstrate tissue components clearly

Not selective - Not selective of different cell types or tissue components. Old dyes. Mixed incorrectly. Not evenly applied to slide. Uneven washing. Dehydrated incorrectly

Stain Colour - is not stained the appropriate colour based on the method employed and the expected staining results . Old dyes. Mixed incorrectly. Not evenly applied to slide. Uneven washing. Dehydrated incorrectly.

Post Staining

Air bubbles - air bubbles within the mountant

Air drying artefact - retractile areas and tissue damage

Excessive mountant - mountant outside the coverslip

Mountant shrinkage - areas of mountant have dried back from the coverslip edges

Section wiped / section off slide - section scratched by hand or off edge of slide

Tissue damaged - section scratched partially wiped or otherwise damaged by handling, after cutting had been completed

Tissue exposed - the section is not covered by the coverslip

Water present - droplets of water under the coverslip

Appendix 1

- **Haematoxylin and Eosin Model Description**
- **Scoring guidelines**
- **Scoring based on criteria**
- **UK NEQAS CPT Stain Repertoire**

Haematoxylin and Eosin Model Description

The material supplied must be paraffin wax processed and have sufficient variety of cellular features, in particular nuclei to allow assessment. Artefacts, such as crush effects and foam insert impressions should not be introduced during the laboratory handling and preparation stages.

The tissue must show no evidence of being inadequately fixed or having had delayed fixation and there must be a clear demonstration of the chromatin detail within the nuclei at medium and low power and little red cell lysis.

There must be no evidence of poor paraffin processing. Epithelial cells groups and connective tissue components must not appear to be separated by cracking. There should be no disruption of the nuclear membrane resulting in pale, fused nuclear staining (nuclear meltdown). The appearance of bubble like artefacts over the nuclei will be noted but no marks will be deducted.

If the biopsy is suspected of being orientated in a way that fails to demonstrate the cell types or layers appropriately this will be noted but no deduction will be made from the score. Similarly if it is felt that the tissue has not been trimmed to full face, or has been trimmed past full face this will be noted but no deduction will be made.

The section must be of a thickness appropriate to the tissue type. The section is too thick if it is difficult to focus on a single layer of nuclei, or too thin if staining intensity is compromised. It must be free from variations in thickness, creases, folds, scores and contaminants. No accumulation or fragments of tissue should be seen over or between sections. The section should be flat, with no lifted areas. There should be no holes, tearing or damage either as a result of trimming or sectioning or coverslipping. The section must be positioned on the slide so that microscopy is not compromised.

Nuclei must be stained purple blue with haematoxylin. The intensity must be strong enough allow clear demonstration of nuclear detail at a medium power, but not too strong to cause a loss of the chromatin granularity or excessive cytoplasmic or connective tissue staining. Where the haematoxylin has been differentiated out, minimal cytoplasmic or connective tissue background staining with haematoxylin must remain. This background if present must not reduce the effectiveness of the nuclear demonstration or affect the colour and selectiveness of the eosin.

The eosin should be selective enough to demonstrate different cellular components such as collagen, cytoplasm, red blood cells, cellular granules, amyloid etc. The intensity must be appropriate to the section thickness and the haematoxylin intensity. Where the eosin is too weak it will fail to allow selective demonstration of different components at low power. If the eosin intensity is too strong the colour and detail of the nuclear stain will be obscured and selectivity will be reduced.

All staining should be even across the section and there should be no dye deposits or precipitate.

Coverslipping must be free from air bubbles, air drying artefact or drying back. There should be no excessive mountant, the sections must be totally covered and there must be no evidence of incomplete dewaxing or dehydration. The difficulties of processing and sectioning bony tissue will be taken into account when assessing.

Scoring Guidelines

Each **pair** of assessors complete an assessment form and data from these forms are converted into a mark out of 5, **from each assessor**. The **mark out of 5 from each assessor** is based on the criteria for a given method.

Guide lines for individual assessors mark (out of 5)

Where 0 – non submission, 1- Fail, 2 - Borderline Fail, 3 - Pass, 4 – Good, 5 - Excellent

0 – non submission

1 – Fail - No staining demonstrated based on the method employed and the expected staining results

2 – Borderline Fail - unsatisfactory demonstration based on the method employed, with expected staining results being inappropriate

3 – Pass - appropriate demonstration based on the method employed and the expected staining results, although improvements need to be made in the staining.

4 – Good – good appropriate demonstration based on the method employed and the expected staining results

5 – Excellent – excellent demonstration based on the method employed and the expected staining results

Each assessor submits their mark out of 5 based on the criteria for a given method, giving a **total score** for the submitted slide **out of 10**.

Guide lines for total score (out of 10)

Score <5 - A score of less than 5 / 10 is given for poor staining, where the participant has failed to clearly demonstrate the expected results.

Score 5/6 – a score of 5 or 6 / 10 is a pass. Whilst the staining appropriately demonstrates the expected staining results, staining is suboptimal and improvements are still required overall.

Score 7/8 – a score of 7 or 8 / 10 shows good appropriate demonstration of the expected results, and an acceptable level of quality.

Score 9/10 – a score of 9 or 10 / 10 shows excellent appropriate demonstration of the expected results, and a high level of quality.

NB. All slides which score a total mark of 4 or below must be passed to a secondary pair of assessors for further assessment before a final score is issued. If there is a discrepancy of 2 or more between the initial assessing pair e.g. 3 & 5, the slide must be passed for secondary assessing.
If there is a discrepancy of pass / fail between the assessing pair, the slide will be passed for secondary assessing.

Scoring based on criteria

Below is a ***generic ideal score*** for haematoxylin & eosin, and special stains.

It is intended purely as a guide for laboratories and assessors. Slides may not achieve all of the points listed and may have elements which span several score boundaries.

Score 5 - Excellent **Excellent nuclear and** tissue constituent staining **in a suitable preparation allowing full visualisation of nuclear and component features within the tissue.**

- Nuclear Staining intensity which demonstrates the chromatin detail clearly.
- Staining colour, intensity and balance allows clear distinction between nuclear detail and other non-nuclear features.
- Primary stain and counterstain demonstrates the appropriate tissue constituents depending on the method being employed.
- The cytoplasm must show appropriate colour spectrum.
- Counterstain intensity stain intensity does not obscure / interfere with the nuclear stain or staining areas that should be another colour. Intensity is such that it is allows clear demonstration of tissue components.
- Demonstration is appropriate based on the method employed and the expected staining results.
- Even staining across the tissue section, with minimal background staining and no dye deposits or precipitate.
- Suitable preparation to allow clear observation of the appropriate tissue constituents depending on the method being employed.
- There are no microtomy or processing irregularities.
- Dehydration, covering and labelling does not impair the visualisation of the tissue and its components.
- The overall preparation and staining is excellent and allows full visualisation of the tissue and its components.

Score 4 - Good **Good nuclear and tissue constituent staining in a suitable preparation to allow visualisation of nuclear and component features within the tissue.**

- Nuclear Staining intensity which demonstrates the chromatin detail clearly.
- Staining colour, intensity and balance may not be consistent across the preparation but allows clear distinction between nuclear detail and other non-nuclear features.

- The tissue constituents may lack appropriate colour spectrum in some areas.
- Primary and counterstain intensity may be weak or intense but does not obscure detail.
- Demonstration is appropriate based on the method employed and the expected staining results.
- There may be some uneven staining or background staining but detail is visible in the majority of the tissue.
- Suitable preparation to allow clear observation of the appropriate tissue constituents depending on the method being employed.
- There may be some microtomy or processing irregularities, but the material is adequate to assess.
- Dehydration, covering and labelling does not impair the visualisation of the tissue and its components.
- The overall preparation and staining is good and allows full visualisation of the tissue and its components. Loss of staining or preparation quality is not detrimental to the identification of tissue constituent details.

Score 3 - Pass **Adequate nuclear and tissue constituent staining in a suitable preparation to allow visualisation of nuclear and component features within the tissue.**

- There may be alteration to nuclear and non-nuclear intensity and colour, but most nuclear detail is visible.
- The tissue constituents do not show full colour spectrum. Nuclear staining may be under or over stained.
- Primary and counterstain may be weak or intense but detail is still visible. Some background staining.
- Demonstration is appropriate based on the method employed and the expected staining results.
- Staining may not be even across the preparation.
- Suitable preparation to allow clear observation of the appropriate tissue constituents depending on the method being employed.
- There may be some microtomy or processing irregularities.
- The dehydration, covering and labelling may obscure visualisation of the tissue and its components but there is sufficient visible to assess.

Score 2 - Borderline Fail **Suboptimal nuclear and or tissue constituent staining which does not allow full visualisation of nuclear and or component features. The preparation quality or method does not allow full observation within the tissue.**

- There may be alteration to nuclear and non-nuclear staining intensity and colour, but some nuclear detail is visible.
- The tissue constituents do not show full colour spectrum. Nuclear and / or tissue component staining may be under or over stained.
- Primary and counterstain may be intense and / or background staining may obscure detail.
- Demonstration is inappropriate based on the method employed and the expected staining results.
- Staining may not be even across the preparation.
- The preparation quality may be suboptimal obscuring some tissue detail. There may be areas of microtomy or processing irregularities.
- The dehydration, covering and labelling may hinder tissue visualisation.

Score 1- Fail **The nuclear and or tissue constituent staining does not allow visualisation of nuclear and / or component features or the preparation quality does not allow clear observation within the tissue.**

- Alteration to nuclear and non-nuclear staining intensity and colour which obscures nuclear detail.
- Background staining which obscures nuclear and tissue constituent detail.
- Demonstration is inappropriate based on the method employed and the expected staining results.
- Uneven or patchy staining amounting to loss of nuclear and tissue constituent detail in the majority of the tissue.
- Preparation is not suitable to allow clear observation of tissue components.
- The preparation quality may be suboptimal obscuring some tissue detail.
- The dehydration, covering and labelling obscures tissue visualisation.

Score 0 - Non submission **No slides submitted for assessment or slides returned late without contacting UK NEQAS CPT.**

UK NEQAS CPT Stain Repertoire

General Pathology (Routine Histopathology)

Stains assessed:
Selected / In-house Material
Haematoxylin and Eosin (H&E) stained sections (all 6 runs)

Distributed Material

Special A
Diastase / PAS
Elastin / van Gieson
Gram
Perls' Prussian blue
Reticulin (silver method for)
Ziehl-Neelsen

Special B
Alcian blue / PAS
Amyloid (method for)
Grocott
Haematoxylin / van Gieson
Masson-Fontana
Martius-scarlet-blue (MSB)
Copper Associated Protein (method for)

Neuropathology

Stains assessed:
Selected/In-house Material
Haematoxylin and Eosin (H&E) stained sections (all 6 runs)

Distributed Material

Special A
Diastase/PAS
Elastin/van Gieson
Gram
Perls' Prussian blue
Reticulin (silver method for)
Ziehl-Neelsen

Special B
Axonal swelling (method for)
Glial fibres (method for)
Myelin (method for)
Neurofibrillary tangles
Nissl substance
Senile plaques (method for)

For neuropathology, the methods in list B may include immunocytochemical techniques where that is the department's method of choice.

Renal Biopsy Pathology

Stains assessed:
Selected/In-house Material (all 6 runs)
Haematoxylin and Eosin (H&E) stained sections
Methenamine silver
Periodic acid-Schiff
Elastin/van Gieson

Muscle Histochemistry

Stains assessed:
Selected/In-house Material (all 6 runs)
Haematoxylin and Eosin (H&E) stained sections
Gomori Trichrome
NADH
Cytochrome Oxidase (COx)

One of the following stains will also be requested, in addition to H&E, Gomori, NADH and COx*;
Acid Phosphatase
Lipid
PAS
Primary fibre typing
Succinate Dehydrogenase (SDH)

For muscle histochemistry, the methods in list bold are requested on a rotational basis and may include immunocytochemical techniques where that is the department's method of choice.

Diagnostic Non Gynaecological Cytology

Selected/In-house Material (all 6 runs)

Stains assessed:
Papanicolaou
Romanowsky

Specimen Types:
Serous Fluid
Head and Neck
Respiratory
Urine

Bone Marrow Trephine Biopsy

Selected / In-house Material (all runs)
Stains assessed:
Haematoxylin and Eosin (H&E) stained sections
Reticulin (silver method for)

Mohs' Procedure

Selected / In-house Material (all runs)

Stains assessed:
Haematoxylin and Eosin (H&E) stained sections
Toluidine Blue

Specific Site/Tissue Composition*:
Cutaneous
Mucosal
Hair Bearing
Cartilaginous