Quality Standards for Diagnostic Electron Microscopy

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Introduction

Transmission electron microscopy (TEM) contributes significantly to diagnostics. (Stirling & Woods 2002) However, TEM laboratories are closing and the scientific and technical staff responsible for diagnostic TEM are increasingly likely to be multi-skilled rather than TEM specialists. In such an environment, quality control procedures and well-defined 'quality standards' that support laboratory operations are essential, it is also advisable to participate in an external quality assurance program (EQAP) that audits laboratory processes and outputs (such as the RCPA Quality Assurance Programs Pty Ltd internationally accredited [ILAC G13] diagnostic TEM program).

Quality control in the histopathology laboratory has been described (Furness 2002) and activities that support quality TEM practice are in place. Indeed, controlled standard operating procedures (SOPs), records of processing details and the documentation of staff training for auditing purposes are mandatory for laboratory accreditation in many countries. In respect to quality standards, while a laboratory might have no mandatory or published guidelines, most employer electron microscopists have strong personal opinions on the topic although these opinions are subjective and individuals may vary in their emphasis on specifics.

Well-defined quality standards are essential for internal and external auditing and as 'benchmarks' for staff outputs and staff training. Quality standards must recognize, but not be dominated by economic considerations, they must also go beyond 'aesthetics'; they must be realistic and focus on the concept of 'fitness for purpose'. Indicators of poor practice in all areas of tissue preparation (processing, sectioning, screening and image recording) that can impact on screening should be included. In other words, the focus should be on processes that facilitate the accurate reporting of specimens within a time frame relevant to patient care.

Quality Standards - Guidelines

The following quality standards are proposed as general guidelines.

Tissue Processing and Embedding

Principles
Tissues should be processed in a timely manner to avoid fixation and processing artefacts. Processing should also avoid delays that will impact on diagnostic evaluation and patient care.

Specifics
1. Material should normally be fully processed within 3 days of receipt and preferably not more than 5 days after receipt
2. Tissues should be processed using well-described controlled standard operating procedures
3. Tissue handling and preparation should avoid tissue damage and processing artefacts, particularly tissue extraction, precipitates and osmotic damage
4. Tissues should be dissected and embedded appropriately so that the orientation of the tissue in the resin block facilitates sectioning and the subsequent observation of all the relevant diagnostic features.

Blocks and Semi-Thin Sections

Principles
Blocks should be orientated and trimmed to minimise tissue loss and to facilitate subsequent semi-thin and thin sectioning. Semi-thin sections should be prepared so that all tissue features are clearly visible and the selection of material for thin sectioning is facilitated.

Specifics
1. The tissue should not be sectioned through unless the required features are absent and multiple levels must be cut
2. Enough blocks should be sectioned to ensure all the relevant diagnostic features have been included
3. Blocks should be trimmed and cut so that the detection of diagnostic features is maximized and no features are obscured
4. Sections should be organized on the slide so that they can be identified and located easily by light microscopy
5. Staining should be even and adequate so that all tissue components are clearly visible
6. Sections should be free of chatter and score marks, they should also be flat with no folds, wrinkles or holes
7. Sections should be clean with no dirt or particulate deposits.

Thin Sections

Principles
Thin sections should facilitate rapid and accurate screening.

Specifics
1. Thin sections should contain all the relevant features represented in the semi-thin sections and related diagnostic tests
2. Enough blocks should be sectioned to ensure all the relevant diagnostic features have been included
3. Thin sections should be located on the grid so that they can be found easily and viewed 'in toto'
4. Staining should be even and adequate so that all cell and tissue components are clearly visible
5. Sections should be free of chatter and flat with no folds, wrinkles or holes; score marks should not obscure critical diagnostic features
6. Sections should be clean with no dirt or particulate deposits.

Specimen Evaluation

Principles
Specimens should be systematically screened in a careful, thorough and timely manner. Critically, the screener must be fully competent in respect to the appropriate specimen type and complexity and if not, the specimen must be reviewed to ensure diagnostic features have not been overlooked. Badly damaged or contaminated sections should be rejected if the diagnostic features are obscured; an alternative section should be screened or the block re-cut.

Specifics
1. Grids should be screened systematically by a competent individual in a manner designed to ensure that all the relevant diagnostic features have been located
2. All relevant diagnostic features should be photographed. Images should facilitate diagnostic evaluation by the reporting pathologist and allow for a review of the specimen by a third party. Electron micrographs should include, as appropriate: A. General low magnification images that show the extent and distribution of the diagnostic features B. Specific high magnification images that show the ultrastructural details of the diagnostic features or facilitate measurements.

Micrographs

Principles
Micrographs should be clear and without imaging artefacts in order to facilitate rapid and accurate diagnosis and case review.

Specifics
1. Diagnostic features should be clearly visible and not obscured by artefacts of any type
2. Images should be in focus and without specimen drift or other types of image distortion
3. Images should have adequate contrast and brightness (especially the ‘mid-range’ grey levels) so that all the diagnostic features are clearly visible
4. Hard copy images should be of a size, contrast and density so that all the diagnostic features are clearly visible
5. Digital images should be of a size and resolution (300dpi minimum, 600 dpi recommended) so that all the diagnostic features are clearly visible.

References


Acknowledgement

I wish to thank Dr Alan Curry, Head of Electron Microscopy, Manchester Royal Infirmary, UK, for his helpful discussions and input into the content of this poster.