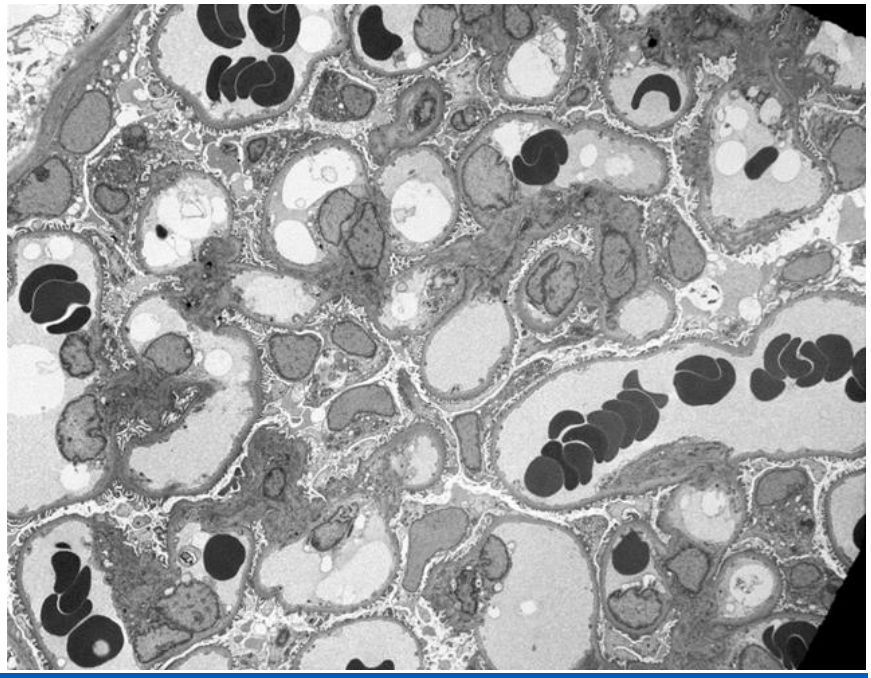
Manchester University
NHS Foundation Trust

Division of Laboratory Medicine



CELLULAR PATHOLOGY ELECTRON MICROSCOPY

USER GUIDE FOR EXTERNAL SERVICE USERS

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1. OVERVIEW OF THE SERVICE

Manchester University NHS Foundation Trust (MFT) Cellular Pathology Electron Microscopy (EM) Service is situated on the Ground Floor of Clinical Sciences Building 1 at Manchester Royal Infirmary and has formed part of the Cellular Pathology Adult Histopathology Service for over 50 years. We provide an adult and paediatric diagnostic transmission electron microscopy service for MFT and for several external Trusts nationwide.

The service is well-equipped with modern equipment that includes a JEOL JEM-1400 transmission electron microscope and other high-end, specialist equipment ultramicrotomes to enable optimum sample preparation. A close team of 2.5 WTE (whole-time equivalent) skilled & experienced Biomedical Scientist (BMS) staff and a 0.5 WTE Medical Laboratory Assistant work permanently within the Unit to provide a high quality EM service with the additional support of a rotating BMS form a pool of staff members who rotate into all areas of Adult Histopathology. Together, the team process around 500 samples per year.

We participate in a dedicated international EM external quality assurance scheme; performing well, to date, without exception and have a well-established, comprehensive quality management system in place. As part of the MFT Cellular Pathology Department, The EM unit is accredited to ISO 15189: 2012.

2. OPENING HOURS

The Unit is open and staffed from 08:00 – 17:30 Monday to Friday (except bank holidays). **Samples sent via courier/postal service should be sent to arrive within these hours to minimise the risk of undelivered or misplaced samples.**

3. REPERTOIRE

Renal Pathology is the mainstay of Electron Microscopy at MFT. More than 95% of the samples handled are renal and the Unit holds primary expertise in this area of diagnostic ultrastructural pathology. The MFT Histopathology Consultant Specialist Renal Pathologists work closely with the EM Team.

The remaining workload comprises the following specimen types:

- Muscle samples
- Duodenal samples for microvillous inclusion disease & tufting enteropathy
- Ophthalmic samples

4. PRE-EXAMINATION PROCESSES

4.1. TISSUE SAMPLING SPECIFICATIONS

Electron microscopy is sensitive to tissue handling procedures. Whilst we recognise and appreciate that non-MFT service users may prefer to determine their own laboratory procedures relating to the fixation and handling of the tissue samples that they send to us for examination, service users are reminded that fixation and handling procedures affording satisfactory light microscopy fixation, may not be suitable or satisfactory for ultrastructural studies.

Therefore, we strongly recommend that all service users meet the following specifications for all tissue samples to be processed for EM. Failure to do this is likely to result in sub-optimal tissue preservation and may place limitations on the ultrastructural interpretation of the sample. **Deviations from these recommendations by service users should be discussed and agreed with the Electron Microscopy Lead Biomedical Scientist before specimens are sent.**

a) Samples are placed into fixative immediately upon removal from the patient. 2.5% buffered glutaraldehyde is preferred but 10% Neutral Buffered Formalin is acceptable. ***The transport of samples from theatre or clinic for the application of fixative within the laboratory is not advised.***

b) If samples are fixed initially in Formalin, they are transferred to 2.5% buffered glutaraldehyde at the earliest opportunity and fixed at approximately 4 - 20°C for between approximately 24 – 72 hours, after which time the sample must be transferred to 0.1M phosphate buffer (pH 7.4) for transport (see section 4.2 for transport guidance).

c) Samples fixed in buffered glutaraldehyde are not larger than approx. 1mm^3 in size. Strips or cores of tissue up to 1mm thick/diameter are acceptable. However, lengths tissue over 3-4mm are generally to be considered excessive for EM purposes.

d) Tissue must be kept hydrated in an isotonic medium before and during transport to the EM laboratory.

Please note that we do not currently provide external service users with suppliers of fixative and/or buffer.

4.1.1. Main factors causing ultrastructural preservation artefact

Below is a summary of the common factors that negatively affect the performance of the examination and/or interpretation of EM results:

- **Delayed fixation:** Detrimental ultrastructural changes begin immediately upon removal of blood supply. The longer the delay: the more significant the ultrastructural artefact. *Note: Although the use of saline may be advantageous from the point of view of preventing the tissue drying out, it does not provide fixation and can cause cell swelling.*
- **Inadequate fixation:** This can affect a sample even if immediately placed into fixative and be caused by multiple factors including:
  - sample size too large
  - fixation time too short
  - fixative to weak
  - wrong fixative
- **Defective buffer (fixative vehicle):** A suboptimal pH of the buffer can lead to significant artefactual changes to tissue ultrastructure. The effect is time dependant.
- **Dewaxed samples:** The ultrastructure of tissue samples that are embedded in paraffin wax and subsequently dewaxed and reprocessed into resin for electron microscopy is significantly affected by the stresses afforded to it by the process involved. The ultrastructural appearances of dewaxed samples are unreliable and potentially misleading in certain diagnostic scenarios (see also section 4.1.3.)

4.1.2. Storage of fixed samples prior to transport to MFT

Fixed tissue samples must be stored in 0.1M phosphate buffer (pH 7.4) between approximately 4 - 20°C (room temperature). It is recommended that they are sent to us as soon as is feasible to the sender. Prolonged storage may affect ultrastructural detail.

4.1.3. Wax blocks

For certain clinical questions, a limited amount of useful information can sometimes be gained by de-paraffinising the tissue and reprocessing for EM. Where EM examination on tissue sent in fixative has not proved possible, the requesting pathologist may choose to send formalin fixed paraffin embedded (FFPE) tissue remaining from light microscopy procedures. However, before sending, service users should be fully aware of the limitations of examining de-paraffinised tissue ultrastructurally with respect to the clinical question. This can be advised on a case by case basis, if necessary, by contacting the Electron Microscopy Lead BMS.

Again, the quality and reliability of the ultrastructural review is dependent on the quality of initial Formalin fixation and tissue processing. FFPE tissue sent for EM should not be that which may have been frozen and used for immunofluorescence procedures prior to being fixed. Similarly, the sending of FFPE tissue which may have originally travelled from the ward/clinic to the laboratory in saline or similar solution is strongly discouraged. **See section 4.5 for further information on sending FFPE material.**

4.2. SPECIMEN PACKAGING & TRANSPORT

It is requested that samples are not sent ad hoc without notifying the Cellular Pathology Electron Microscopy Lead BMS or other, previously agreed, members of MFT electron microscopy staff (see section 10).

It is also requested that no more than approximately 5 samples are sent in any single batch. Excessive batching of samples is discouraged as this can cause demanding spikes in workload, particularly if priority samples are included. Where samples arrive in batches of 6 or more, the turnaround time cannot be guaranteed for all samples within it.

All packages should be addressed to:

Electron Microscopy
1st Floor
Clinical Sciences Building 1
Manchester Royal Infirmary
Oxford Road
Manchester
M13 9WL

4.2.1. Packaging

It is a legal requirement that all diagnostic samples carried on the public road must be packaged and transported in compliance with *The Carriage of Dangerous Goods and Use of Transportable Pressure Equipment Regulations 2011.* These regulations set into UK Law the directives of the European *ADR*[1] agreement concerning the carriage of dangerous goods by road. Under these regulations, it is the sender's responsibility to ensure that they are complied with.

Paragraph 2.2.62.1.5.8 of the *ADR* (2015) states that: *"Human or animal specimens for which there is a minimal likelihood that pathogens are present are not subject to ADR if the specimen is carried in a packaging which will prevent any leakage and which is marked with the words "Exempt human specimen" or "Exempt animal specimen."*

The MFT Cellular Pathology EM Service holds the position that this paragraph applies to all EM samples sent to it **on condition that phosphate buffer[2] is the transport medium and that all samples have been fixed in glutaraldehyde[3]** (as specified in section 4.1 of this document).

Therefore, if these two conditions are met, all packages containing samples sent to the MFT Cellular Pathology EM Service should be clearly labelled with the words: **EXEMPT HUMAN SPECIMEN.**

Paragraph 2.2.62.1.5.8 continues to clarify that the packaging is deemed to comply with the requirement of "preventing any leakage" only if it meets the following conditions:

a) *The packaging consists of three components:*

   i. *a leak-proof primary receptacle(s)*
   ii. *a leak-proof secondary packaging; and*
   iii. *an outer packaging of adequate strength for its capacity, mass and intended use and with at least one surface having minimum dimensions of 100mm x 100mm;*

b) *For liquids, absorbent material in sufficient quantity to absorb the entire contents is placed between the primary receptacle(s) and the secondary packaging so that, during carriage, any release or leak of a liquid*

[1] *"Accord européen relatif au transport international des marchandises dangereuses par route"*
[2] Since phosphate buffer is a non-hazardous substance.
[3] As this will cause any pathogens present in the sample to be inactivated.

substance will not reach the outer packaging and will not compromise the integrity of the cushioning material;

c) *When multiple fragile primary receptacles are placed in a single secondary packaging, they are either individually wrapped or separated to prevent contact between them.*

Therefore, in addition to the "Exempt human specimen" labelling, service users must ensure these 'leakage prevention' requirements are met.

4.2.2. Transport

Unless there are pre-existing local area lab-to-lab transport arrangements in place between MFT Laboratory Medicine and the sender's laboratory, the sender is responsible for specimen transport arrangements. Where lab-to-lab transport exists, arrangements to use this mode of transport should be made.

Samples should be sent to arrive within laboratory opening hours (see section 2) to minimise the risk of undelivered or misplaced samples

4.3. SPECIMEN ACCEPTANCE CRITERIA

Electron microscopy samples must arrive at the laboratory in suitable sample containers and with relevant requesting information. The specific requirements for both of these are detailed below. Additionally, it must be established (either by prior arrangement or on the requesting paperwork) from which institution the sample has been referred, the requesting pathologist, where to send the results of the examination and by what means.[4]

Our Specimen Acceptance Policy dictates that the following essential patient identifiers must be present as a minimum on <u>both the requesting paperwork and the sample container:</u>

- Family name
- First name
- Date of birth
- Sender's reference number (e.g. laboratory number)

[4] Results will only be communicated back to the requesting pathologist or Histopathology department and not directly to Ward clinicians.

The following information must be provided on the specimen container and/or the requesting paperwork before the sample is processed since specific processing and/or embedding procedures may be required accordingly:

- The nature of the tissue (i.e. specimen type)

The following information is requested to be provided on requesting paperwork (or other means). If not provided, but all other acceptance criteria are met, the sample will be processed but may not be examined ultrastructurally until the information is provided.

- Clinical details and/or the specific rationale for EM examination.[5]

The following information is also requested to be provided with requesting paperwork (or other means) but, if absent, would not preclude sample acceptance, processing or examination provided that all other acceptance criteria are met.

- Full patient address
- NHS Number (if available)
- Number of pieces of tissue sent in the specimen container

A summary of required specimen acceptance/requesting information criteria

Information required	Requesting paperwork	Specimen container	Either	Requesting paperwork or by other means (e.g. email to Lead BMS)
Family name	✓	✓		
First name	✓	✓		
Date of birth	✓	✓		
Sender's reference number (e.g. lab number)	✓	✓		
The nature of the tissue (i.e. specimen type)			✓	
Clinical details and/or the specific rationale for EM				✓
Notification marking the sample as urgent/priority				✓
Patient address†	✓			
NHS Number†	✓			
Nº tissue pieces sent in the specimen container†			✓	

† Preferable, but not mandatory

[5] This is particularly important for non-renal samples to allow a better ultrastructural examination to take place since there may be no expertise in ultrastructural pathology of the particular tissue type concerned. The examination may be delayed until this information is provided.

4.3.1. Specimen ID discrepancy procedure

In the event that any of the essential patient identifiers are missing from either the paperwork or the sample container, or there is a mismatch of information between them, the sender will be notified. Provided that three of the four essential identifiers are present and match-up, it will be sufficient for the sender to give the correct information over the telephone or via email. The sample will then be received into the laboratory and processed. However, if this condition is not met, the sample will be placed on hold and not accepted into the laboratory. The sender will be contacted for advice from the requesting pathologist as to whether the sample should be repackaged and returned for correction or whether there is a clinical need to receive into the EM laboratory and process it straight away. ***Please note that if the latter is the case then this advice must be received in an email from the requesting pathologist acknowledging the error and providing the correct information.***

For any other discrepancy with respect to the specimen acceptance policy outlined above, verbal or email clarification will be sufficient.

4.4. SPECIMEN PROCESSING & PREPARATION FOR EXAMINATION

Tissue samples are each assessed for appropriate size, dissected (if necessary) and then processed into resin blocks.

Semi-thin sections are then cut from the blocks to identify those with suitable regions of interest. Those showing regions of interest are cut to produce ultra-thin sections which are subsequently mounted onto copper grids. Grids/sections are then stained ready for electron microscopy examination.

All material is then archived in accordance with current Royal College of Pathologists guidelines as a minimum.

4.5. SENDING PREVIOUSLY PROCESSED TISSUE (WAX BLOCKS, EM BLOCKS & EM GRIDS)

These should be sent in the form in which they are created at the sending laboratory. They must be accompanied with requesting paperwork conforming to the specifications in section 4.3

External users forwarding FFPE material for EM must also provide precise information which allows localised extraction of the area of interest from the wax block. The following represents an order-of-preference list of ways in which this

information can be obtained from the sender and which, ideally, would be provided along with the material itself.

1. A light microscopy slide containing the section which best represents the tissue at the block face. The slide must be indelibly marked in a way to clearly indicate the area of interest.
2. The area of interest is clearly marked on the block itself.
3. Confirmation that the tissue remaining in the block can be dewaxed in its entirety (n.b. only be acceptable where there is not an unreasonably large amount of tissue in the block. See senior member of EM staff if necessary).

Senders sometimes prefer to determine an area of interest and extract it themselves, before sending. This is acceptable but not preferable to the above as it is often helpful to be able to assess the characteristics of the tissue macroscopically before any EM process begins.

5. EXAMINATION PROCESSES

Samples are examined ultrastructurally by a competent, experienced Advanced or Lead Electron Microscopy Biomedical Scientist.

As standard, the ultrastructural examination process takes place as described below. However, if service users have specific preferences or requirements with respect to the approach, these can be discussed as necessary.

5.1. DIGITAL ELECTRON MICROGRAPHS (IMAGES)

The electron microscope is fitted with a very high resolution side-mount digital camera controlled by digital imaging software on a PC. Biomedical Scientists are thus able to capture as many images as necessary in order to provide an accurate representation of the sample and to illustrate any ultrastructural pathology identified. There is no set number or limit to the images captured during the examination: the number varies according to the needs of each case as determined by the judgement and expertise of the Biomedical Scientist.

Images are routinely captured in 8-bit greyscale tiff format with a resolution of 8 Megapixels. Each image is annotated in order to label it with anonymous patient identifiers provided by the sender and a unique image number. Personally identifiable patient details are not used to comply with information governance.

The image capture software has calibrated functionality to enable one-dimensional measurements to be made (nanometers or micrometers) of any structure and displayed on the image.

5.2. ULTRASTRUCTURAL COMMENTARY

To complement the images provided, a text commentary is provided as a standalone text file. This provides a basic, concise summary of the ultrastructural findings. These ultrastructural comments do not constitute a histology report and are to be interpreted by the Histopathologist in the context of the clinical, light microscopic and immunohistochemical findings.

6. POST-EXAMINATION PROCESSES: COMMUNICATION OF RESULTS

Routinely, images and ultrastructural comments are saved locally, copied to cd and sent to the service user via post. Additionally, images can be emailed, upon request, ad hoc (e.g. where EM results needed urgently for MDT).

7. TURNAROUND TIMES

Electron microscopy is a highly skilled, specialised service and one which is a lengthy process intrinsically. It is highly sensitive to staffing pressures and unexpected increases in workload. Therefore, it is difficult to guarantee consistency of turnaround times (TATs).

Average routine turnaround times, in accordance with service user requirements, are detailed in individual service level agreements. No turnaround time is guaranteed when samples are received in single batches greater than 5 samples or when multiple batches totalling more than 5 samples are received in the same week from any given external service user.

However, samples that are requested by the Pathologist to be prioritised for valid clinical reasons are always expedited through the system with priority over all routine samples already in process and are examined in the order in which they are received. Digital images should be available within 2 - 6 working days of receipt/notification of priority status dependant on the number of priority samples already in the system at the time.

8. SERVICE CHARGES & BILLING

The Cellular Pathology Department currently has several standard charges for its Electron Microscopy Service, dependant on whether the sample requires ultrastructural examination only (i.e. pre-prepared grids), technical work in addition (tissue processing, semi-thin sectioning and/or ultra-thin sectioning) or whether the specimen is to be processed into resin blocks for archiving only.

The specific charge amounts will be detailed within Service Level Agreements. For ad-hoc referrals, where no formal SLA exists, the service user will be advised of the charges on enquiry.

Billing should take place monthly. The service user is invoiced for all requests completed since the previous billing cycle. Backing information is provided.

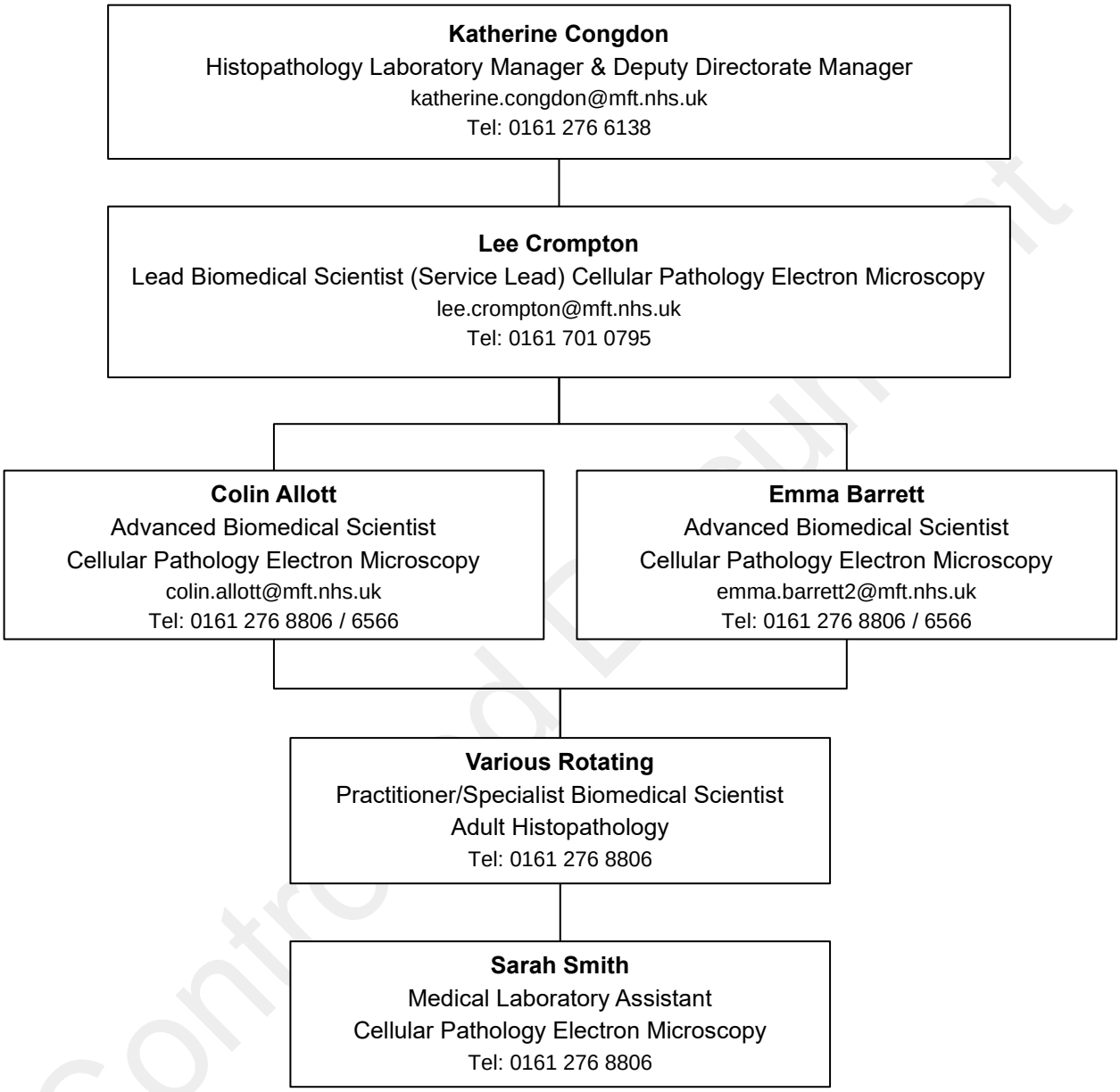
9. SERVICE LEVEL AGREEMENTS & SERVICE REVIEW

Establishment of the sustained provision of electron microscopy services will be done so under a formal SLA between MFT and the service user.

Under the SLA, MFT will seek regular interaction on a mutually agreeable basis to ensure satisfaction with performance. The MFT Electron Microscopy Service periodically issues user surveys in which the service user is kindly requested to participate.

Other service review interaction may be in the form of pre-arranged meetings, telephone conversations, email, etc. as appropriate.

10. STAFFING STRUCTURE



11. ENQUIRIES & COMPLAINTS

Enquires relating to individual samples should be made to the Electron Microscopy Lead BMS or other senior member of the EM team (see section 10 for contact details).

Enquiries and/or complaints relating to service provision should be made to either the Electron Microscopy Lead BMS or the Histopathology Laboratory Manager, as appropriate.

Lee Crompton Lead BMS: Cellular Pathology Electron Microscopy 1st Floor, Clinical Sciences Building 1 Manchester Royal Infirmary Oxford Road Manchester M13 9WL lee.crompton@mft.nhs.uk	Katherine Congdon Histopathology Laboratory Manager & Deputy Directorate Manager 1st Floor, Clinical Sciences Building 1 Manchester Royal Infirmary Oxford Road Manchester M13 9WL katherine.congdon@mft.nhs.uk