Specimen handling in the laboratory

- Pathologists may be asked to provide tissue samples from fresh specimens for tissue banking or research purposes. The decision to provide tissue should only be made when the pathologist is sure that the diagnostic process including the measurement of maximum depth of invasion and other important parameters that influence patient prognosis and management will not be compromised. As a safeguard, research use of the specimen may be put on hold until the diagnostic process is complete so that the specimen can be retrieved.

- The specimen must be handled in a systematic and thorough fashion to ensure completeness and accuracy of pathological data.

- The type of specimen must be recorded.
  - State whether the specimen is a cone, radical trachelectomy, hysterectomy, radical hysterectomy, +/- lymph nodes, and record any other accompanying specimens.

Cone Biopsy

- Orientate the specimen.
  - It is customary for a cone biopsy to be orientated with a suture denoting 12 o’clock.

  - Differentially ink anterior and posterior surfaces of the cone biopsy (single ink will suffice if not orientated).

- The specimen may be received fresh, or formalin-fixed.
  - If received fixed, it is strongly advised to serially section the cone in a sagittal plane, at 2-3mm intervals, with each slice placed in a separate block and clearly designated (see figure 1a below).

  - If received fresh, the specimen may be formalin-fixed immediately, and then handled as above. Alternatively, if preferred, a fresh cone biopsy may be orientated, inked, and opened longitudinally (in the lateral position, usually at 3 o’clock), and pinned on a specimen board (mucosal side up) and fixed in formalin before cutting the entire cervix by making parallel sections, 2-3mm apart, along the plane of the endocervical canal. Sections should be taken in a fashion that the epithelium is present in each section (see figure 1b below).

- It is not advisable to longitudinally open a cervical cone that has already been formalin fixed.

- Three hours minimum fixation time is recommended prior to cutting.\textsuperscript{15-16}

- Slices can typically be submitted each in an individual cassette, however, if the cone biopsy is unusually high (long), the superior and inferior portions may need to be submitted in separate cassettes (ie – composite sections may be used).

  Whichever method is used, all tissue must be submitted for histology, in as many tissue blocks as is required.\textsuperscript{15-16}
A suggested method for sectioning formalin-fixed cone biopsy specimens

Note: In the figure above the slices are labelled left to right (9 o'clock to 3 o'clock). As the cone has been orientated with a suture, it may be differentially inked for orientation of margins. Each slice is blocked separately, with composite blocks used (anterior and posterior) when slices are too large for one cassette (eg central cone, as demonstrated in composite blocks D/E, F/G).
Figure 1b  A method for sectioning fresh cone biopsy specimens
Radical trachelectomy is a form of radical surgery for the treatment of early cervical cancer, offered to a selected subset of young patients with favourable cervical tumours and a desire to maintain fertility.\textsuperscript{17,18}

The procedure may be performed vaginally or abdominally, and a pelvic lymphadenectomy is performed, as a part of the surgical procedure.\textsuperscript{1,18}

Irrespective of the technique employed, the surgical specimen comprises upper vaginal cuff, cervix, parametrium and lymph nodes.\textsuperscript{17-18}

- The specimen must be widely sampled. All tissue from the endocervical margin, parametrial and nodal tissue is submitted for histology.

  - The specimen may be handled in a variety of ways, as long as all prognostic information is obtainable by the method used. Typically the entire specimen is submitted.

- The suggested method of handling for a formalin-fixed specimen is as follows (also see Figures 2a and b below): Orientate and differentially ink the specimen, including the broad endocervical margin. The peripheral portion of the parametria are removed, leaving a small amount proximally attached to the main specimen and step sectioned, with the end-pieces placed in a separate cassette. The central portion of the main specimen is handled identically to the formalin-fixed cone, with the entire cervical canal being sagittally sectioned, including 12 and 6 o'clock margins. Tissue from the 3 and 9 o'clock poles of the specimen are transected, and serially sectioned in a coronal plane, along with a small amount of proximal parametrium to ensure detailed examination of these margins (Figures 2a and b below). Usually slices will need to be submitted as superior and inferior portions in separate casettes (ie – composite sections are often used, due to the specimen length / height, and presence of additional vaginal cuff tissue).

- Alternatively, if received fresh the specimen could be opened longitudinally at 3 o’clock after orientation and differential inking of margins. The specimen is then pinned to a board (mucosal side facing up) and fixed (as per protocol for unfixed cone biopsy).\textsuperscript{19} Once fixed, the parametria are removed and step sectioned, with the end-pieces placed in a separate cassette. The main specimen is handled identically to an opened cone.
Figure 2a  Radical trachelectomy – anterior view

(a) Remove peripheral portion of the parametrium leaving a small amount attached to the main specimen proximally.

(b) Vaginal cuff shaded

Figure 2b  Radical trachelectomy from above – suggested sectioning for fixed radical trachelectomy

Right parametrium removed and sectioned.  
Left parametrium removed and sectioned.
Simple hysterectomy

- A simple hysterectomy specimen may be opened laterally and pinned out into anterior and posterior halves as per radical hysterectomy specimen (see below). However if a simple hysterectomy has been performed following a LLETZ or cone biopsy, it may be preferable due to possible cervical stenosis, to amputate the cervix from the specimen through the upper endocervical canal, and handle the amputated cervix according to the protocol of a fixed cone biopsy (figure 1a). In this case, the entire cervix should be submitted to assess for residual carcinoma.

- If carcinoma has been found incidentally in a simple hysterectomy, in the routinely taken sections, it is advisable to return to the specimen, attempt to reconstruct it, and process as required to provide maximal pathological information.

Radical hysterectomy

- The tumour and margins must be adequately and appropriately sampled such that all required prognostic information may be obtained from the description, and histologic examination of the selected blocks.
  - There are a variety of acceptable methods for the macroscopic handling of these specimens, and handling is often guided by:
    - Whether the specimen has been received fresh or fixed
    - Whether the tumour is macroscopically visible
    - Size of macroscopic tumour
    - Single or multiple tumours previously identified
    - Macroscopic relationship of tumour to margins

- The specimen may be weighed.

- Orientate the specimen.

- Ink the resection surfaces of the specimen from the vaginal cuff up to the peritoneal reflections.
  - Inking of the parametrium is sometimes useful if definition of the true surgical margins is necessary.15

- The specimen may be received fresh or formalin fixed.

- If the specimen is received fresh or even partially fixed, it is preferable to open and pin out the entire specimen as anterior and posterior halves, leaving the cervix attached and carefully pinned (mucosal side up) as well. Also pin out any parametrial attachments.
  - This allows thorough macroscopic assessment of the tumour.

- If there is a preference to amputate the cervix (for example if the state of the specimen is such that opening may cause fragmentation, such as if there is distortion by previous LLETZ or cone biopsy) then the vaginal cuff and parametrium should be left intact, and pinned out without further slicing. Once fixed the amputated cervix will be sectioned in the same manner as a fixed radical trachelectomy (figures 2b and 3c ). Open the uterine corpus into anterior and posterior halves and pin these out as well, with the mucosal surface facing upwards.

- For any form of radical specimen, please note that if the vaginal cuff is small, this is best submitted as part of the cervical sections. If the
vaginal margin is large, separate representative radial sections should be submitted.

- Parametrial tissues would usually be present in radical specimens and should be entirely processed for histologic examination. If minimal parametrial tissue is present (for example in simple hysterectomy), then a shave of the lateral myometrial margins should be submitted, to ensure that the vascular margin is sampled.

- Sections from the uterine corpus will usually be similar to those for a routine benign uterus, as long as there is no endometrial tumour. Blocks should be directed at defining the upper extent of the cervical tumour and additional sampling of the isthmic region may be required to assess this.\(^9,15-16,20\)

- If the tumour is macroscopically small then the entire tumour should be submitted for histological examination (figure 3a).

- If the tumour is not macroscopically visible, then the entire cervix typically requires submission for histologic examination.

- If the tumour is macroscopically large, representative sections should be taken including:
  - Full-face of tumour
  - Longitudinal extent of tumour
  - Deepest point of invasion
  - All quadrants
  - Relationship of tumour to margins in sagittal and coronal plane.

- **All lymph nodes are to be processed for histologic examination.**

- Any remaining fat accompanying the specimen should also be processed.
  - There may be small lymph nodes within the fat which are not macroscopically identified.\(^{21-22}\)
  - Identifying all lymph nodes present in the specimen is important, both to allow their histological evaluation for metastases, and also as a measurement of adequacy of nodal dissection.

- Larger lymph nodes should be sliced at 2-3mm intervals. If sliced, do not process multiple nodes in the same cassette, unless inked different colours, so they can be individually identified for accurate nodal count.
  - Handling of lymph nodes is the same, irrespective of the accompanying type of cervical specimen.

- Sentinel lymph node biopsy is an emerging procedure in the management of women with early-stage cervical cancer (in patients with a low risk of lymph node metastases), but is not universally practiced.\(^9,23-24\)
  - Intra-operative frozen section may be requested on pelvic lymph nodes, especially for patients undergoing radical trachelectomy. This is particularly the case in centres where PET scanning is not readily available.\(^{17}\)
Figure 3a  Radical hysterectomy – received fresh, opened, divided and pinned out. Tumour in anterior half.

Blocks of tumour and vaginal cuff. These may be composite sections.
Blocks must incorporate the deepest point of invasion and the thickness of the cervical wall in the maximal area of invasion. $a =$ radial stromal margin measurement.

For blocking fixed amputated cervix, refer to protocol for fixed radical trachelectomy (Figure 2b).