The Royal College of Pathologists
Pathology: the science behind the cure

Tissue pathways for gynaecological pathology

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Comments	This document supersedes the 2008 document of the same name. In accordance with the College's pre-publications policy, this document was on The Royal College of Pathologists' website for consultation from 1–29 December 2014. Twenty items of feedback were received. Please email publications@rcpath.org to see the responses and comments. **Dr David Bailey** **Vice-President for Communications**

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Foreword

The tissue pathways published by The Royal College of Pathologists (RCPath) are guidelines, which enable pathologists to deal with routine surgical specimens in a consistent manner and to a high standard. This ensures that accurate diagnostic and prognostic information is available to clinicians for optimal patient care and ensures appropriate management for specific clinical circumstances.

It may rarely be necessary or even desirable to depart from the guidelines in the interests of specific patients and special circumstances. The clinical risk of departing from the guidelines should be carefully considered by the reporting pathologist; just as adherence to the guidelines may not constitute defence against a claim of negligence, so a decision to deviate from them should not necessarily be deemed negligent.

The guidelines themselves constitute the tools for implementation and dissemination of good practice.

The stakeholders consulted for this document were members of British Association of Gynaecological Pathologists (BAGP) and British Gynaecological Cancer Society (BGCS).

No major organisational changes or cost implications have been identified that would hinder the implementation of the tissue pathways.

The information used to develop this tissue pathway was collected from electronic searches of the medical literature, previous recommendations of the RCPath and local guidelines in the United Kingdom. Much of the content of the tissue pathways represents custom and practice, and is based on the clinical experience of the authors. For the reporting guidance and related appendices, this includes referral practice and experience from the evaluation of responses in the UK Gynaecological EQA Scheme (evidence corresponding to 'good practice point' in Appendix B). Published evidence to support the recommendations has been identified by PubMed searches and referenced where appropriate. The evidence was evaluated using modified SIGN guidance. Consensus of evidence in the tissue pathways was achieved by expert review. Gaps in the evidence were identified by College Fellows via feedback received from consultation.

A formal revision cycle for all tissue pathways takes place on a four-yearly basis. However, each year the College will ask the authors of the tissue pathways, in conjunction with the relevant subspecialty advisor to the College, to consider whether or not the document needs to be updated or revised. A full consultation process will be undertaken if major revisions are required. If minor revisions are required, an abridged consultation process will be undertaken, whereby a short note of the proposed changes will be placed on the College website for two weeks for Fellows' attention. If Fellows do not object to the changes, the short notice of change will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the publications page of the College.

This tissue pathway has been reviewed by the Working Group on Cancer Services and was on the College website for consultation with the membership from 1–29 December 2014. All comments received from the Working Group and membership were addressed by the authors to the satisfaction of the Chair of the Working Group and Director of the Clinical Effectiveness Unit.

This pathway was developed without external funding to the writing group. The College requires the authors of tissue pathways to provide a list of potential conflicts of interest; these are monitored by the Director of the Clinical Effectiveness Unit and are available on request. The authors of this document have declared that there are no conflicts of interest.

1 Introduction

The previous guidelines, *Tissue pathways for gynaecological pathology*, were published in 2008. They have now been revised to ensure that all recommendations are up to date, that terminology complies with recommendations in the 2014 revision of the WHO classification of tumours of the female reproductive tract,[1] and that the document complies with the revised format of the tissue pathway series.

This document provides guidance on the specimen handling and reporting of tissue specimens from the vulva, vagina, cervix, endometrium, myometrium uterus and adnexa and relates primarily to those biopsies taken for the investigation of benign or pre-neoplastic conditions at these anatomical sites. The specimens described in this guideline are currently reported by most histopathology departments in the UK.

The purpose of these guidelines is to promote a uniform good practice of specimen handling and reporting in histopathology departments and to assist cellular pathologists to provide a high standard of care for patients in the reporting of benign and pre-neoplastic gynaecological specimens. The tissue pathways are important as they provide a consistent approach to managing this range of pathological specimens and highlight the use of ancillary techniques when appropriate.

There is very little literature on the handing of samples resected for benign and non-neoplastic conditions, but a good overview and clear guidance can be found in several standard gynaecological pathology reference books.[2-4] The tissue pathways should be used in conjunction with the datasets on gynaecological cancers.[5-8]

Target users of this guideline

The primary users of the tissue pathway documents are trainee and consultant cellular pathologists. The recommendations will also be of value to biomedical scientist advanced practitioners who currently deal with this range of specimens in some histopathology laboratories in the UK, histology laboratory managers, users of a gynaecological pathology service and service commissioners.

2 Generic issues relating to staffing, workload and facilities

The following recommendations should be met for a general level of acceptable practice.

i. The diagnostic laboratory should have sufficient pathologists, biomedical scientists and clerical staff to cover all of its functions. In general, staffing levels will follow the workload guidelines of The Royal College of Pathologists.

ii. Pathologists should:

- participate in audit
- participate in the College's continuing professional development (CPD) scheme
- participate in relevant external quality assessment (EQA) schemes of a general or specialist nature
- via their pathology department, have standard gynaecological pathology texts available for reference[2,3]
- have access to specialist referral opinions on a local network or national basis.

iii. The laboratory should:

- be equipped to allow the recommended technical procedures to be performed safely
- be accredited by UK Accreditation Service (UKAS) or equivalent
- participate in the UK National EQA Scheme for Cellular Pathology Technique
- participate in the UK National EQA Scheme for immunohistochemistry and *in-situ* hybridisation (if these techniques are used in the diagnostic pathway).

iv. Reports should be held on an electronic database that has facilities to search and retrieve specific data items, and that is indexed according to Systematised Nomenclature of Medicine Clinical Terms (SNOMED) T, M and P codes or SNOMED-CT. It is acknowledged that existing laboratory information systems may not meet this standard. However the ability to store data in this way should be considered when laboratory systems are replaced or upgraded.

v. The laboratory should comply with the College's key performance indicators[9] to ensure that cellular pathology turnaround times are monitored and audited, particularly where these are linked to patient pathways.

vi. Workload data should be recorded in a format that facilitates the determination of the resources involved.

3 Vulval and vaginal biopsies

3.1 Vulval and vaginal epithelial biopsies

3.1.1 Specimen submission

Most biopsies are submitted as fixed specimens to the laboratory in formalin. Biopsies will vary according to the size of the lesion and range from small punch biopsies that are up to several millimetres long and 2–4 mm thick, to larger ellipse biopsies of similar size to skin excisional biopsies. In some institutions, small biopsies may be mounted onto a card. Small lesions such as cysts or papillomas may be submitted intact or in fragments.

Careful handling of these specimens is recommended to prevent surface trauma and disruption or loss of surface epithelium.

3.1.2 Specimen dissection and block selection

It is important to examine carefully the contents of the container and the under-surface of its lid to ensure that any stray fragments of tissue are recovered.

Small biopsy fragments should be counted. Their size must be recorded (maximum dimension of largest and smallest if ≤3 fragments, or measure in aggregate if >3) as should the shape, colour and texture (mucoid, granular, friable) if appropriate. If biopsy fragments are very small, they should be wrapped in tissue or placed between layers of foam, in a mesh bag or wire basket to avert tissue loss during processing. All of the material submitted must be processed for histological examination.

Punch biopsies should be measured in three dimensions. Small punch biopsies <3 mm should be processed whole. Larger punch biopsies >3 mm may need to be bisected perpendicular to the epithelial surface and both halves processed. The bisected halves must be placed in the cassette for embedding with the epithelium clearly visible for orientation.

Larger pieces of tissue should be measured individually. If skin ellipses are narrower than 3 mm, they should be processed (and embedded) as received, but if wider than 3 mm, bisected longitudinally and both halves processed, particularly if ellipse excisions are submitted for non-neoplastic conditions such as lichen sclerosus. If a surface lesion is readily identified, it must be fully described and measured, and the macroscopic distance from the closest margin noted. Wider/larger ellipses that include a well-defined lesion should be cut in transverse section, perpendicular to the long axis of the ellipse, to include the nearest resection margins. If appropriate, the entire specimen should be processed in 2–3 mm serial transverse sections. The sections should be placed in separate cassettes. The blocks containing the end slices should be noted (these will usually be the first and last blocks in the sequence). It may be appropriate to ink the resection margins if the clinician has orientated the specimen, or to assess the margin of clearance of a well-defined lesion.

3.1.3 Embedding options

All biopsies must be properly orientated to provide vertical, full-face sections to reduce the potential of cross-cutting artefact and potential for misinterpretation of stromal invasion.[8]

The flat, cut face of any sliced specimens must be embedded downwards to ensure that this surface/face is cut by the microtome. Intact biopsies should be orientated carefully and embedded on edge, with the epithelial surface perpendicular to the face of the block to be cut by the microtome.

3.1.4 Sectioning and staining

A single haematoxylin and eosin (H&E)-stained section representing a full face of the blocked specimen is adequate for the initial microscopic examination of larger specimens but small fragmented biopsies and punch biopsies should be sectioned at three levels. Depending on the histological findings, levels may be requested at the discretion of the reporting pathologist.

3.1.5 Further investigations

If appropriate, the following histochemical stains may be required:

- PAS(D) or Grocott stains for the identification of fungal hyphae and spores
- silver stains for the identification of spirochaetes
- Giemsa stain for Donovan bodies
- elastic van Gieson (EVG) to help confirm lichen sclerosus – the zone of homogenised dermal collagen does not usually contain elastic fibres in contrast to the mid and lower dermis where there is an increase in elastic fibres.[10]

[Level of evidence – D]

Immunohistochemistry for p16 may help to confirm a high-risk HPV-associated lesion in VIN of classical type or in cases of VaIN. Ki67 and aberrant p53 expression may assist in the diagnosis of differentiated VIN.[11–13] Primary vulval Paget disease usually expresses CK7, CEA, CAM5.2, EMA and GCDFP15, but not CDX2, uroplakin, p63, oestrogen and progestogen receptors (ER and PR) or melanoma markers.[12,14] CK20 is usually negative or exhibits focal weak immunostaining.

[Level of evidence – C]

3.1.6 Report content

- Pre-neoplastic/*in-situ* squamous lesions of the vulva and vagina should be graded. The WHO recommends a two-tier grading system: low-grade squamous intraepithelial lesion (equivalent to VaIN 1 and VIN 1), and high-grade squamous intraepithelial lesion (equivalent to VaIN 2 and 3, and VIN 2 and 3 of classical type).[1] In the UK, adherence to VIN/VaIN terminology is recommended at present.
- Differentiated VIN is not graded but it may be useful to state on the report that this is a form of non-HPV related VIN which is automatically regarded as high grade.
- Resection margins must be assessed and the status of these must be documented in the pathology report.
- If an invasive component is present, pathologists should use the RCPath cancer dataset for the histopathological reporting of vulval neoplasms.[8]
- Non-neoplastic epithelial diseases of the vulva, e.g. lichen sclerosus, squamous hyperplasia, lichen planus and other vulvar dermatoses should be classified according to the ISSVD 2006 and 2011 nomenclature and classification for vulvar disease.

3.2 Vulval and vaginal soft tissue excisions

3.2.1 Specimen submission

Most specimens are submitted in a fixed state, in formalin, and are usually taken during the investigation of a vulval or vaginal soft tissue mass. Lesions may be submitted intact but large, more deeply sited soft tissue lesions may be submitted in fragments.

3.2.2 Specimen dissection and block selection

If specimens are submitted piecemeal or only very small diagnostic biopsies have been taken, it is important to examine carefully the contents of the container and the under-surface of its lid, to ensure that small, stray fragments of tissue are recovered.

The number of pieces received must be recorded, as well as their size (larger pieces should be measured individually in three dimensions; aggregated measurements are adequate if more than three pieces of tissue are received). The shape, colour and texture (fibrous, rubbery, myxoid, mucoid, granular, friable) should be documented. If biopsy fragments are very small, they should be wrapped in tissue, placed between layers of foam or in a mesh bag or wire basket to avert tissue loss during processing.

The number, size, shape and consistency of larger pieces of soft tissue should be recorded. If appropriate, excision margins can be inked. Larger fragments may need bisection or slicing so that the tissue can be accommodated in a standard cassette. Myxoid, oedematous, haemorrhagic or necrotic areas should be well sampled. Any attached, macroscopically uninvolved soft tissue must be sampled and examined carefully to identify infiltration/invasion, and where the gynaecologist has orientated a specimen, excision margins should be sampled and the cassettes containing these should be clearly labelled.

3.2.3 Embedding options

If bisected or sliced, the flat, cut face must be embedded downwards to ensure that this surface is cut by the microtome and full-face sections are obtained.

3.2.4 Sectioning and staining

A single H&E-stained section representing a full face of the blocked specimen is adequate for the initial microscopic examination of larger specimens. Small biopsies may require

further levels, and additional levels may be required on larger specimens depending on the histological findings.

3.2.5 Further investigations

Mucin stains (ABPAS-D) may be helpful to identify interstitial mucoid material in cases of superficial angiomyxoma.

Immunohistochemistry including ER, PR, SMA, CD34, desmin, h-caldesmon, S100 and HMGA2 are useful in establishing the diagnosis of vulvovaginal soft tissue neoplasms.[12,15–17] HMGA2 may also be used in the assessment of resection margins in cases of aggressive angiomyxoma.

[Level of evidence – C]

3.2.6 Report content

- Vulvovaginal soft tissue neoplasms include a wide range of differential diagnoses and some lesions may exhibit overlapping morphological and immunohistochemical features. In problematic cases an expert opinion should be sought.
- Where possible, the report should include the status of excision margins.

3.3 Vulval and vaginal cysts

3.3.1 Specimen submission

Most specimens are submitted in a fixed state, in formalin. Cysts may be submitted intact or in fragments.

3.3.2 Specimen dissection and block selection

If submitted piecemeal, the number, size, shape and colour of the fragments should be described. If submitted intact, the size of the cyst and the external appearance of the capsule should be recorded. If the clinician orientates the specimen or a neoplasm is suspected, it may be prudent to ink the external surface/cyst capsule to assess the status of the excision margins. In some cases, an ellipse of overlying surface epithelium may be attached. The cyst contents should be noted, as should the wall thickness, and the presence of solid and/or papillary areas. These should be preferentially sampled.

Very small cysts can be processed whole; small cysts may need to be bisected, and representative tissue sections of larger cysts should be processed. The number of blocks will depend on the size of the cyst and the presence of unusual features.

3.3.3 Embedding options

Optimally orientated cyst wall should be embedded with the cyst wall and lining perpendicular to the face of the block.

3.3.4 Sectioning and staining

A single H&E-stained section representing a full face of the block is adequate for initial microscopic examination. Depending on the findings, levels may be requested at the discretion of the reporting pathologist.

3.3.5 Further investigations

Neoplastic, cystic adnexal tumours or rare neoplastic lesions of Bartholin gland may require immunohistochemistry and referral for expert opinion. CD10 may be useful to identify endometrial stroma in cases of cystic endometriosis.

[Level of evidence – C]

3.3.6 Report content

In the vulva, diagnoses include a range of cystic lesions specific to the female genital tract, e.g. cysts related to Bartholin gland, cysts that arise in anogenital mammary-like glands[18–20] and cysts arising in local skin adnexal structures.

[Level of evidence – C]

The histological diagnosis should be reported in a clear and succinct manner and, if appropriate, the status of the resection margins should be documented.

In the vagina, the most common type of cyst in one study was a mucus-secreting Mullerian cyst,[21] but epidermal inclusion cysts (some arising at the site of previous episiotomy), cysts of hymenal, mesonephric and endometriotic type may also occur.

4 Cervix

4.1 Cervical biopsy (NOS), punch, loop, cone and wedge biopsy

4.1.1 Specimen submission

Cervical biopsies (including punch biopsies) are usually carried out as a diagnostic procedure, after an abnormal smear. They are colposcopically directed and may be up to several millimetres long and 2–4 mm thick. Fixation of small biopsies in eosin-tinted formalin may facilitate their identification and orientation.[22] In some institutions, such biopsies are mounted onto a card or filter paper. One study has shown that specimens that were mounted on filter paper before fixation were more likely to be optimally oriented, to have a preserved squamocolumnar junction and to have intact surface epithelium.[22]

[Level of evidence – D]

Wedge biopsies are larger than punch biopsies, but generally smaller than cone/large loop excision of the transformation zone (LLETZ) biopsies. They are carried out at the time of colposcopy for women with abnormal smears as part of a ‘see and treat’ therapeutic procedure, or as an alternative diagnostic procedure to a punch biopsy to confirm neoplasia before definitive treatment.[23]

Cervical loop and cone biopsies (including LLETZ) are carried out for women with abnormal smears as part of a ‘see and treat’ or following a positive punch biopsy, i.e. these can be either diagnostic or therapeutic procedures. Cone biopsies are performed using a scalpel ('cold knife'), but more commonly large loop diathermy methods are used to the same effect, with the advantage of reduced bleeding, better healing and preservation of cervical anatomy. Loop diathermy methods also have the advantage of being performed without a general anaesthetic, as an outpatient procedure. A disadvantage of loop diathermy is the artefact at the resection margins that results from electrothermal damage. This may impair histological diagnosis, and also the assessment of resection margins, especially in cases of glandular neoplasia.[24] For this reason, cone biopsy is a preferred procedure in some, but not all, institutions for the assessment of glandular lesions of the cervix.

Most specimens are submitted in formalin, in the fixed state. In some departments loop or cone biopsies may be submitted as fresh specimens with a marker suture in the 12 o'clock position to allow the loop to be orientated, opened and pinned out before fixation, to optimise block taking.

Careful handling of all of these specimens is recommended to prevent surface trauma and disruption or loss of surface epithelium. Pathologists should be aware that opening an intact loop/cone biopsy may result in damage to the surface epithelium. This is not advised. Similarly the os in such specimens must not be probed.

4.1.2 Specimen dissection and block selection

Cervical biopsy (NOS) and punch biopsies

- It is important to examine carefully the contents of the specimen container and the under-surface of its lid to ensure that all stray fragments of tissue are recovered.
- Record the number and size of biopsy fragments; note the maximum dimension of the largest and smallest fragments in three dimensions if there are three or fewer fragments, if there are multiple fragments (often these are mucoid) measure their aggregated size.
- The colour and texture (mucoid, granular, friable) should be documented.
- If biopsies are >5 mm in dimension, bisect these transversely, perpendicular to the mucosal surface, to produce two pieces.
- If fragments are very small, wrap them in tissue and place them between layers of foam sponge, in mesh bags or wire cages to avert tissue loss during processing.
- Process all of the biopsies, including mucoid fragments.

Cervical loop and cone biopsies (including LLETZ) are roughly conical in shape when received intact.

- In some centres, a specific position (usually 12 o'clock) is marked with a suture, or the specimen may be orientated and pinned to a corkboard.
- Record the colour, consistency and presence of any surface lesions.
- The specimen may be opened at one end (giving a U-shape) and form a flattened, curved specimen or in some instances submitted as multiple specimens/loops.
- Measure all specimen/s in three dimensions: the antero-posterior, lateral-lateral dimensions and thickness of an intact central loop/cone biopsy should measured; a flat/opened loop biopsy must also be measured in three dimensions and care taken to provide a clear statement of exactly what is being measured – the circumference of an opened, flattened loop/cone biopsy is different from that of an intact conical specimen.
- If multiple loop biopsies are submitted, note the number of pieces, measure the smallest and largest in three dimensions or measure the maximum dimension if the pieces are small.
- If specific margins have not been indicated, the entire excision margin may be painted with ink to assist with margin identification in the histological sections, although this is not usually necessary.
- Intact central loop/cone biopsies can be sectioned in one of several ways, although there are two preferred, widely used methods. Because the external os in most parous women is transverse and slit-like, loop/cone biopsies can be sliced serially at 2–3 mm

intervals,[3,23] from one edge to the other in a sagittal and parasagittal plane (beginning at the 3 or 9 o'clock edge), perpendicular to the transverse axis of the external os. This avoids the problems of interpretation that may arise when dysplastic epithelium arises on the narrow end of a wedge shaped block (if a loop/cone specimen is sectioned radially – see below), and facilitates assessment of tumour volume in small lesions or neoplasms.[25] However, this method does not easily allow direct correlation of cervical intraepithelial neoplasia (CIN) with the specific position on a clock face[2] that the radial method of sampling permits.

- The radial method involves the sampling of an intact loop radially, in wedge-shaped slices, according to the hours on a clock face. This is a useful method of sampling if accurate mapping of a lesion is desired,[2] although this is not usually necessary. The disadvantage of radial slicing is that the surface epithelium may be incomplete at the internal os because of the difficulties in sectioning the thin/narrow central ends of the tissue slices.

  In either case, submit the slices in separate, sequentially numbered blocks (corresponding to the hours on a clock face if radial sampling has been carried out, e.g. block 1 = 1 o'clock, etc.).

- Process an opened loop biopsy and individual loops if multiple specimens are submitted, in serial transverse sections in specifically designated cassettes.

- Take care to ensure that the correct cut face is placed face down in the cassette. If desired, the opposite cut face can be marked with ink, to ensure that the correct (non-inked) side is embedded downwards to be cut by the microtome.

- Each piece of tissue should be placed in a single cassette.[26] Some pathologists suggest that if the slices are small, two or three may be placed in one cassette for reasons of convenience and economy[2,4] but placing multiple slices in one cassette should be avoided because this makes it impossible to measure the horizontal size (in third dimension) of any small invasive lesion and compromises accurate staging.

- The deep radial margin must not be trimmed off.

- In all cases, all of the tissue must be submitted.

Wedge biopsies may be over 10 mm in maximum dimension, and occasionally more than one biopsy is submitted.

- Provide the size in three dimensions for each biopsy or the maximum dimension if small.

- Record the colour, consistency and presence of any surface lesions.

- Cut/slice the specimen/s perpendicular to the transformation zone (this is usually visible macroscopically) or perpendicular to the long axis to ensure that both ectocervical and endocervical edges of the specimen appear in their normal anatomical context in the sections.

- Process all specimens in their entirety, with each slice in a separate cassette (see above).

4.1.3 Embedding options

Cervical biopsies (including punch biopsies)

- If bisected, embed the flat, cut end downwards to ensure that this surface is cut by the microtome.

- Orientate intact biopsies carefully and embed these on edge, with the epithelial surface perpendicular to the face of the block to be cut by the microtome.

Cervical loop, cone biopsies (including LLETZ) and wedge biopsies

- Embed the sections cut face down to ensure that the correct surface is cut by the microtome.
- In some centres, for the purpose of expediency, the excision margins of loop biopsies are assessed by embedding the outer (curved) surface of the first and last slices of the loop face down for sectioning, instead of the cut surface. This avoids having to request additional levels to assess these margins.

4.1.4 Sectioning and staining

H&E-stained sections representing the full face of the block are adequate for initial microscopic examination. Additional levels may be necessary.

Cervical biopsies (including punch biopsies)

In general, it is recommended that levels of such biopsies are cut.[23]

[Level of evidence – D]

Although the precise number of levels is not always specified, three levels are recommended.

Step-serial sectioning is not necessary as a routine.

Cervical loop, cone biopsies (including LLETZ) and wedge biopsies

A single level from each block is likely to suffice initially.[23, 26]

[Level of evidence – D]

Some laboratories provide a ‘deeper’ as standard along with the index section.

Deeper levels will be required if sections are incomplete or if there are difficulties in identifying a lesion that might account for the abnormal cells in an antecedent smear.

One study has shown that examination of a only a single further level is adequate in those specimens where surface epithelium or squamocolumnar junction is missing, or in circumstances where there is a discrepancy between the histological findings and smear.[27]

[Level of evidence – D]

If invasive disease is suspected on the basis of the cytological, colposcopic or histological features,[28] further levels must be examined.

4.1.5 Further investigations

Mucin histochemistry (AB/PAS+/–D) can be used to identify gastric or intestinal differentiation in endocervical glandular lesions.

Immunohistochemistry (Ki-67, p16) may be helpful to differentiate between metaplastic and neoplastic changes in endocervical glands, or to distinguish atrophy, immature metaplasia or regenerative changes from CIN.[16, 29–32]

[Level of evidence – C]

In squamous lesions, strong, diffuse, 'block' immunopositivity for p16, i.e. staining of both the cytoplasm and nucleus of affected cells involving the basal layers and usually extending above this, is regarded as diagnostic of the presence of high-risk HPV.[33–36] A proportion of low-grade lesions associated with high-risk HPV also show 'block' positivity but in general, in cases of low-grade CIN, p16 immunopositivity is confined to the basal and parabasal layers. Negative or non-block-positive staining strongly supports a diagnosis of a low-risk HPV associated lesion (low-grade CIN) or a non-HPV-associated pathology.

[Level of evidence – C]

4.1.6 Report content

Cervical biopsies (including punch biopsies)

- The report should incorporate the macroscopic description of the specimen and identify the area/s of the cervix from which the biopsy has originated, i.e. ectocervix, endocervix, transformation zone.
- Where artefact or epithelial loss impairs interpretation of the biopsy, this must be stated in the report.[26]
- Pathologists must have access to the cytological report when writing the histology report to allow correlation between cytological and histological findings.
- Report all grades of squamous and/or glandular intra-epithelial neoplasia. Invasive lesions must be reported and graded according to national protocols and guidelines.[6]
- Report koilocytosis and koilocytosis-associated changes, but mention CIN first unless the CIN represents only a minor component of a predominantly koilocytic lesion. The pathologist must be mindful of the cytological report when writing the histology report, and include all pathological lesions (neoplastic and non-neoplastic) that may be associated with, or account for the reported cytological abnormalities.
- When a biopsy fails to reveal the source of the abnormal cells in a smear, it is important to differentiate between a biopsy that is technically adequate but fails to identify a lesion, and a biopsy that is technically inadequate.
- The limitations of punch biopsies in the detection of CIN[37] and particularly high-grade CIN are recognised.[38]

[Level of evidence – D]

Cervical loop, cone biopsies (including LLETZ) and wedge biopsies

- The report should incorporate the macroscopic description of the specimen, and identify the tissue components that are present, i.e. ectocervix, endocervix, transformation zone, isthmus.
- Record features that impair interpretation, e.g. opened loop, fragmentation, surface epithelial loss, surgical/operative trauma, thermal artefact.
- Pathologists must have access to the cytological report when writing the histology report to allow correlation between cytological and histological findings.
- Report all grades of squamous and/or glandular intra-epithelial neoplasia and the presence of endocervical crypt involvement. Note the position and distribution of a lesion if an orientated specimen has been submitted. Classify, grade and stage invasive lesions according to national protocols and guidelines.[6]

- Report significant inflammation or inflammation associated with specific pathological features, e.g. follicular cervicitis, herpesvirus infection. Note koilocytosis and pathological lesions (neoplastic and non-neoplastic) that may be associated with, or account for, the reported cytological abnormalities.
- The report must indicate whether or not the abnormal squamous or glandular epithelium has been completely excised. If the most lateral blocks of an intact LLETZ specimen bear abnormal squamous or glandular surface epithelium, this indicates involvement of the ectocervical resection margins. Fragmentation usually precludes an adequate assessment of the margins. Pathologists must exercise caution in assessing excision of a lesion when opened, fragmented or multiple loop biopsies have been submitted.

4.2 Manchester repair

4.2.1 Specimen submission

Manchester repair is performed for uterine prolapse, and comprises amputated cervix, usually with one or two triangular pieces of mucosa from the anterior and posterior vaginal walls attached.

4.2.2 Specimen dissection and block selection

Measure the main specimen in three dimensions and record the length of the attached vaginal mucosa.

Describe the surface of the cervix and any lesions on the cervical surface or the vaginal mucosa.

Sample the cervix according to the recommended protocols for cervical sampling in a non-malignant hysterectomy specimen, i.e. two midline blocks of cervix, one each from the anterior and posterior cervical lips.[3,25] If possible, include vaginal mucosa in continuity with the cervix.

If there is a history of current or previous abnormal smears, process the specimen as a loop/LLETZ (see section 4.1 on Cervix, above).

4.2.3 Embedding options

Ensure sections are cut at right angles to the epithelial surface.

4.2.4 Sectioning and staining

A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination. Depending on the findings, levels may be requested at the discretion of the reporting pathologist.

4.2.5 Further investigations

If CIN or cervical glandular intraepithelial neoplasia (CGIN) is found the whole of the cervix must be processed and the specimen should be managed in the same way as a loop/LLETZ as described above.

4.2.6 Report content

- The report should incorporate the macroscopic description of the specimen.

- If CIN or CGIN is identified this must be graded and reported according to the recommendations described above.
- The report must include assessment of the resection margins and indicate whether or not excision of the lesion is complete.

4.3 Endocervical polypectomy

4.3.1 Specimen submission

Most endocervical polyps are asymptomatic and identified incidentally at the time of smear taking. They are usually removed by avulsion.

Larger polyps may be removed by excision at the time of colposcopy. Most are benign, smooth surfaced and pedunculated.

4.3.2 Specimen dissection and block selection

Measure the maximum dimensions if submitted as a single polyp. If multiple fragments are sent, measure the size of the smallest and largest. If the fragments are very disrupted and friable, measure their aggregated size.

Describe the colour and texture (e.g. spongy, solid, mucoid).

If a single large polyp >5 mm is submitted, bisect the polyp longitudinally parallel to the axis of the stalk, and submit the whole polyp for processing. If the polyp is very large, the polyp halves may need to be cut into smaller pieces and sampled in more than one cassette.

If multiple fragments are sent, place all of the fragments in a mesh bag or wire/mesh basket for processing.

4.3.3 Embedding options

If the polyp has been sliced, embed the sections cut face down to ensure that the correct surface is cut by the microtome.

4.3.4 Sectioning and staining

A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination.

Depending on the appearances, levels may be requested at the discretion of the reporting pathologist.

4.3.5 Further investigations

Carcinoma, CIN, CGIN, or other lesions may require further levels for diagnosis.

4.3.6 Report content

- The report should incorporate the macroscopic description of the specimen.
- Endometrial polyps (benign or malignant) can sometimes protrude through the cervix and be mistaken for endocervical polyps; report these according to the recommendations for endometrial polyps (see section 5 below).
- Occasionally, prominent Nabothian follicles may be mistaken for endocervical polyps.

- Report the presence of significant inflammation or metaplastic changes and review cervical cytology reports when writing the histology report, to correlate any low-grade smear changes with metaplastic or reactive changes on the surface of inflamed endocervical polyps.

4.4 Endocervical curettage

4.4.1 Specimen submission

Such specimens are uncommon and are usually submitted to identify the presence of CGIN or CIN in the endocervical canal or to assess whether endometrial carcinoma has spread to involve the cervix. They are typically scanty and comprise mucus and blood admixed with small tissue fragments. Because of this, they must be handled carefully.

4.4.2 Specimen dissection and block selection

Measure the aggregated size of the sample in three dimensions after it has been filtered into a mesh bag, and record the colour and texture (mucoid, spongy, firm). The entire sample must be processed, preferably in a mesh bag or wire/mesh basket to avert the loss of tiny fragments. Avoid the use of filter paper and sponges because of the possibility of losing tissue fragments that become entrapped or adherent.

4.4.3 Embedding options

No specific issues.

4.4.5 Sectioning and staining

A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination.

Depending on the appearances, levels may be requested at the discretion of the reporting pathologist.

4.4.6 Report content

- The report should incorporate the macroscopic description of the specimen.
- Report neoplasia (either CGIN, CIN, invasive cervical or endometrial carcinoma) and classify and grade this according to national protocols and guidelines.
- The small volume of material available for examination in such specimens may impair assessment in which case further biopsy may be needed.

5 Endometrial specimens

5.1 Curettings, pipelle biopsies and TCRE (transcervical resection of the endometrium) specimens

5.1.1 Specimen submission

All specimens must be submitted in an adequate volume of fixative (usually 10% formalin) to ensure proper fixation. Bouin’s fluid is no longer used as it is carcinogenic.

The specimen must be accompanied by a fully completed request form including information about:

- the menstrual status (the date of the last menstrual period [LMP] should be provided for premenopausal and perimenopausal women)
- any relevant history of hormone treatment or use of tamoxifen, previous endometrial ablation
- endometrial thickness
- the presence of an intrauterine device (IUD) if applicable.

5.1.2 Specimen dissection and block selection

Describe the colour of the specimen. Provide a semi-quantitative estimate of the volume by measurement in three dimensions in millimetres, or give the maximum dimension if the specimen is small.

The quantity of a specimen may also be assessed by its weight:

- scanty <½ cassette or <0.5 g
- moderate >½–1 cassette or 0.5–1.0 g
- bulky >1 cassette or >1 g.

Filter or centrifuge small/invisible samples. All of the tissue submitted must be processed for histological examination.

Describe the colour and texture of polyps if these can be identified; comment on the presence of necrosis or haemorrhage. Measure polyps or submucous fibroids in millimetres in three dimensions.

It may be necessary to slice large polyps into multiple pieces. The base of the stalk should be identified if possible, and examined in a separate cassette. All of the tissue from polyps must be submitted for examination.

5.1.3 Embedding options

There are no specific issues related to specimen embedding.

5.1.4 Sectioning and staining

A single H&E-stained section representing a full face of the block is generally adequate for the initial microscopic examination.

Depending on the histological findings, levels may be requested at the discretion of the reporting pathologist although Hill *et al*[39] suggest that two levels at the outset is optimal.

5.1.5 Further investigations

Histochemical stains may be carried out as appropriate for the H&E findings, e.g. Ziehl-Neelsen stain in cases of granulomatous inflammation, Gram and silver stains for the identification of actinomyces species, and PAS or Grocott for fungi. Immunohistochemistry may occasionally be helpful to differentiate between endometrial epithelial metaplastic processes and malignancy.[40]

[Level of evidence – C]

5.1.6 Report content[40–42]

- A comment about the adequacy of the sample may be appropriate. The adequacy of a sample depends not only on the quantity of material submitted but also on the clinical setting. A scanty specimen may contain sufficient material for assessment in a post-menopausal woman with an endometrial thickness of <4 mm and therefore represent an adequate sample. A sample of similar quantity in a pre- or perimenopausal woman (or even in a postmenopausal woman) with thickened endometrium is inadequate. A diagnostic algorithm for assessing sample adequacy has been proposed by McCluggage[40] who suggested labelling a specimen as 'inadequate' in the absence of endometrial tissue or 'unassessable' where only minimal endometrial tissue was present. However, a recent study has shown that that categorising scanty endometrial specimens as 'inadequate' or 'unassessable' had no clinical implications because both of the latter categories prompted clinicians to carry out further investigations in the context of the clinical situation.[43]

- The report should indicate the phase of the menstrual cycle and correlate this with the LMP and any other clinical information provided. Accurate dating of samples from infertility patients should be attempted provided that all of the relevant clinical information has been given.

- Exogenous hormone/drug effect should be noted, and if possible, categorisation of dysfunctional bleeding should be attempted, i.e. the report should describe the ovulatory or luteal phase defect that is present.

- Report the presence of non-endometrial tissue. This is important because the identification of adipose tissue or tissue of bowel wall origin is strongly suggestive of uterine perforation. The finding of these tissues should be conveyed to the clinical team as a matter of urgency. Other rare situations where adipose tissue may be encountered in endometrial samples include tissue fragments derived from uterine lipoleiomyomas, lipomas, hamartomas or metaplastic adipose tissue within the endometrial stroma. Cervical tissue is also commonly represented and may exhibit a variety of squamous or glandular abnormalities.

- Pathologists must take care to avoid misinterpreting artefactual changes in endometrial biopsy specimens as hyperplasia. Gland telescoping and artefactual gland crowding and compression ('moulding') may be misinterpreted as hyperplasia. Tearing/clefting of the tissue around the glands may provide a clue to the diagnosis of this artefact.

- It may not be possible to comment on the endometrium in a TCRE specimen because of diathermy artefact. Recognition of tangential cutting artefact should prevent over-diagnosis of adenomyosis in TCRE specimens.

- According to the revised 2014 *WHO Classification of Gynaecological Tumours*,[1] endometrial hyperplasia should be classified as hyperplasia with or without cytological atypia. Atypical hyperplasia is characterised by crowded, cytologically atypical tubular or branching glands. The most reliable indicators of atypia are nuclear enlargement, pleomorphism, rounding, loss of polarity and nucleoli. Mitoses are present but may not be conspicuous. Cytonuclear changes that accompany endometrial metaplasia may mimic atypia.

- Proliferative activity in endometrial samples from postmenopausal women should be reported, as this finding indicates ongoing oestrogenic stimulation. The oestrogenic stimulation may be exogenous, i.e. as a result of hormone replacement therapy/ administration or due to endogenous oestrogen production, which may result from an increased body mass index (BMI) or an ovarian tumour such as a fibroma or granulosa cell tumour.

6 Uterus

6.1 Specimen submission[2, 44]

Specimens are usually submitted in the fixed state. The volume of fixative must be adequate to ensure proper fixation. Hysterectomy (with or without cervix and the adnexa) may be performed for a wide range of clinical conditions including uterovaginal prolapse, fibroids, adenomyosis, endometriosis, dysfunctional uterine bleeding, persistently abnormal cervical cytology (± previous cervical biopsy/LLETZ) and in some cases for obstetric complications. If performed for malignancy, the RCPath cancer datasets must be used.[5,45]

The reason for the hysterectomy should be provided on the clinical request form and any relevant clinical information that may affect histological interpretation should be disclosed. Such information includes prior endometrial ablation, pre-operative treatment with hormones, tamoxifen or uterine embolisation, which can significantly alter the morphology of fibroids. History of rapid growth of a fibroid, especially in a postmenopausal woman is important. Hysteroscopic/transcervical endometrial resection also changes the appearances of the endometrium and myometrium, and may be associated with uterine wall perforation. A patient's cervical screening history may be pertinent if the patient has had previous loop/LLETZ biopsies for CIN and/or if there is persisting abnormal cytology.

Laparoscopic hysterectomies may be submitted as morcellated specimens. There should be a previous endometrial sample to exclude any endometrial abnormality. This procedure should not be performed if there is a history of atypical endometrial hyperplasia or gynaecological neoplasia.

If the hysterectomy is performed for an obstetric complication, the clinician must specify at what stage of the delivery the uterus was removed; what the indication for the hysterectomy was (intractable intra-partum/post-partum haemorrhage, uterine rupture, abnormal placental implantation) and if there is a history of a previous Caesarean section. If relevant, the condition of the newborn should be disclosed.

6.2 Specimen dissection and block selection

The specimen should be orientated using the following anatomical landmarks:

- the posterior peritoneal reflection extends lower on the surface of the pouch of Douglas in comparison with the anterior peritoneal reflection
- the ovaries are normally sited posterior to the fallopian tubes.

The absence of a cervix must be noted in subtotal hysterectomy specimens as young patients who undergo this procedure will still require cervical smears as part of the National Cervical Screening Programme. The submission of both intact adnexa must be confirmed when a risk-reducing (prophylactic) hysterectomy is carried out for a family history of ovarian cancer. Describe surgical or traumatic lesions and serosal abnormalities, e.g. adhesions, endometriosis, etc. Interruptions in the fallopian tubes as a result of prior tubal ligation should be recorded and if sterilisation clips are present in the fallopian tubes these should be described.

It is standard practice to measure the uterus in millimetres in three dimensions (fundus to cervix; cornu to cornu and anterior surface of the body to the posterior surface) and describe its shape and symmetry. If a vaginal cuff is attached, this is usually asymmetrical. Describe and measure the cuff in millimetres where it is longest. Any developmental abnormalities such as arcuate, bicornuate, didelphys or unicornuate uterus should be noted. The weight of the uterus can be recorded if desired. Both the dimensions and weight of the uterus are variable and are related to age, parity, body mass index, phase of the menstrual cycle and

other associated pathological processes (fibroids, adenomyosis, etc.) and are therefore of limited clinical significance.[46–51]

[Level of evidence – C)

Note recent or past Caesarean section sites in hysterectomies performed for obstetric complications. A recent Caesarean section usually takes the form of a transverse incision through the anterior lower uterine segment. Rarely a vertical, classical surgical incision through the uterine body may be performed. If uterine rupture is identified, the site should be clearly and carefully described, as should abnormal sites of placental implantation. There may be medicolegal issues associated with obstetric hysterectomies and photographic images of these specimens are recommended.

Morcellated specimens are submitted in multiple pieces but it may be possible to identify endometrial, endocervical or serosal surfaces and also fibroid-derived fragments. The introduction of intrauterine methylene blue preoperatively is an easy, cheap, harmless, and quick method to reduce the time spent on macroscopic examination, as it allows identification of the endometrial lining and reduces the number of tissue blocks submitted for histological examination.[52]

If the hysterectomy has been done for persistent abnormal cervical cytology or if a cervical lesion is suspected, some pathologists amputate the cervix and dissect the cervix in the same way as a LLETZ or cone biopsy (see section 4.1 above)

There are several ways of opening the uterus, depending on the preference and experience of the pathologist. Some pathologists prefer to open the uterus in the sagittal plane while others open it coronally along the lateral borders and between the cornua. The method of opening may be affected if the uterus is markedly distorted, e.g. by multiple fibroids. Opening should be adjusted to optimally expose the uterine cavity. Gentle probing of the uterus may be necessary to aid the identification and orientation of the cavity. Whatever the method of opening, the uterine cavity should be exposed for inspection. The myometrium can then be examined by multiple parasagittal or horizontal incisions.

Describe the appearance of the cervix, note if there is scarring as a result of previous loop/LLETZ biopsies and if any polyps are present.

Describe the appearance of the endometrium (cystic, granular, irregular). Some pathologists measure the endometrial thickness in millimetres although there are no systematic studies that demonstrate correlation between macroscopic measurements of endometrial thickness and pathological abnormalities such as endometrial hyperplasia. If endometrial polyps are present, note the number, size, location and appearance of the polyp/s. Record the presence of an IUD and specify the location. Old Caesarean section scars (usually transmural, in the anterior uterine isthmus, below the peritoneal reflection) should be noted.

The myometrium may show adenomyosis (manifest as diffuse myometrial thickening with coarse trabecular bands and small, interspersed slit-like spaces that may contain brown fluid). When fibroids are present, note their number (an estimate is acceptable if numerous), size (usually of the largest and smallest fibroid if there are multiple), location (submucosal, intramural or subserosal), outline (discrete or poorly defined), texture (firm, soft, gritty), colour and appearance (whorled, cystic, presence of fatty, calcified or myxoid areas). It is important to note the presence of necrosis. This may impart a salmon pink or grey colour. Haemorrhage should also be recorded. This may be focal/spotty or widespread and may be associated with necrosis.

Examine and describe the serosal surface. Note the presence of a recent or past perforation/adhesions or haemorrhagic areas, which may represent endometriosis.

General guidance about block selection

- Cervix: two representative blocks of the anterior and the posterior cervical lips, to include the transformation zone. Sample the entire transformation zone if there is a recent history of CIN or CGIN or if the most recent cervical cytology sample was abnormal. Refer to the LLETZ/cone biopsy section if required (see section 4.1).

- Endometrium: two blocks, one each of the full thickness of the anterior and posterior uterine walls if possible. These blocks should include the myometrium with serosa at one surface of the block and endometrium at the other. Multiple blocks are required if there is a pre-operative diagnosis of hyperplasia. If hyperplasia is not identified at microscopy in a patient with a prior biopsy diagnosis of hyperplasia, the remaining endometrium and both of the cornu may need to be sampled. When there is a pre-operative diagnosis of atypical hyperplasia specimens should be handled in the same way as uteri resected for endometrial adenocarcinoma.

- Sample endometrial polyps thoroughly and include the base of the polyp/s that abut the contiguous background endometrium.

- Myometrium: fibroids/leiomyomas (note that the term 'fibroid' should only be used for the macroscopic description) are common and leiomyosarcomas rare, so the majority of uterine smooth muscle tumours are likely to be benign. When fibroids appear typical on gross examination, minimal sampling, usually one block of the largest fibroid and one or two others selected at random, will suffice. More extensive sampling is required when there are clinical or macroscopic variations from the norm. If clinical information is provided that suggests unusually rapid growth or abnormal radiological appearances, particularly in postmenopausal women, more extensive sampling may be indicated especially if the macroscopic appearances are abnormal. Any fibroid other than the typical circumscribed, firm, white, whorled mass that stands proud of the cut surface of the myometrium should be examined carefully. The presence of softening, haemorrhage, cystic degeneration or variation in colour should prompt sampling of up to one block per 1–2 cm of the fibroid mass. The junction with normal myometrium should be sampled.[42]

- Adnexa: see sections 7 and 8.

Block selection in specific situations

- Morcellated hysterectomy: attempts should be made to sample the cervical, endometrial and serosal surfaces and any focal lesions such as polyps or fibroids.

- Obstetric hysterectomy: several blocks of the fresh Caesarean section site should be taken, as should the edges of a traumatic rupture. Ragged, haemorrhagic tissue lining the uterine cavity may represent retained, adherent placental tissue and should be sampled thoroughly. In cases of placenta praevia, accreta and increta, the interface between the placenta and adjacent myometrium should also be sampled.

- Prophylactic hysterectomy for Lynch syndrome: the lower uterine segment should be processed in its entirety with representative anterior, posterior, right and left lateral blocks from the cervix. In the absence of a macroscopic lesion in the endometrium, the entire endometrium should be submitted for histological examination. This should be performed in a systematic way, e.g. from superior to inferior. The adnexa should be amputated and handled according to the SEE-FIM (**s**ectioning and **e**xtensively **e**xamining the **fim**briated end of the fallopian tube) protocol for risk-reducing (prophylactic) bilateral salpingo-oophorectomy specimens performed for BRCA mutations (see section 8). Any abnormality that is identified in the specimen at macroscopic examination must be submitted for histological examination.[53]

[Level of evidence – D]

6.3 Sectioning and staining

A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination. Depending on the histological findings, levels may be requested at the discretion of the reporting pathologist.

6.4 Further investigations

ABPASD is helpful in differentiating between oedema and myxoid change in leiomyomas and in the diagnosis of myxoid smooth muscle tumours.

SMA, desmin, CD10, h-caldesmon may be helpful to differentiate endometrial stromal from smooth muscle lesions, although there may be immunophenotypic overlap.

p53 and Ki67 immunostaining of stretches of cytologically atypical epithelium in the fallopian tube or endometrial cavity, especially on the surface of polyps, can be helpful to confirm serous tubal intra-epithelial carcinoma (STIC) or serous endometrial intra-epithelial carcinoma (SEIC).

[Level of evidence – C].

6.5 Report content

- Cervix: report CIN and CGIN if present and assess excision margins.
- Endometrium: see section 5.1.6 above. If the hysterectomy was performed for hyperplasia, comment on the presence of residual/persistent hyperplasia. Report on polyps if these are present and the effects of hormonal therapy.
- Myometrium: comment on presence or absence of adenomyosis. If leiomyomas are present, assess and comment on the following features if appropriate:
  - recognised variants including cellular, epithelioid, mitotically active, myxoid, dissecting/cotyledonoid, hydropic, apoplectic and lipomatous leiomyomas, also leiomyoma with bizarre nuclei
  - degenerative changes (hyaline, hydropic, including perinodular hydropic and red degeneration)
  - unusual growth patterns (dissecting or intravenous growth patterns)
  - junction with normal myometrium
  - mitotic activity (by convention mitotic counts in smooth muscle tumours are expressed as the number of mitoses/10HPF; 1HPF = 0.1734 mm^2; atypical forms must be noted)
  - necrosis (coagulative or ischaemic/infarct type)
  - nuclear atypia (severity; distribution – diffuse or localised)
  - lymphovascular involvement.
- Uterine serosa: report on endometriosis or endosalpingiosis if present.
- Obstetric hysterectomy: report on the state of any tears/rupture, the Caesarean section site, the site of placental implantation (note if this is ectopic, i.e. in the fallopian tube, cervix or on the serosa), the presence of placenta praevia, the state of the placental

bed and on infiltration of the myometrium by placenta accreta, increta or percreta. The identification of subinvolutional changes in placental bed vessels may be the cause of post-partum bleeding.

7 Myomectomy specimens

7.1 Specimen submission

Specimens are submitted to the laboratory in a fixed state, in formalin. Myomectomies may be performed laparoscopically, hysteroscopically or during the course of laparotomy. The procedure is usually performed in women with infertility thought to be related to the presence of fibroids, or in women with symptomatic fibroids who wish to retain their uterus. All relevant clinical information that may affect histological interpretation should be provided. Such information includes prior endometrial ablation, pre-operative treatment with hormones, tamoxifen or uterine embolisation, all of which can significantly alter the morphology of fibroids. The specimens are sent for histological examination to confirm the diagnosis of leiomyoma and to exclude malignancy. One or more fibroids may be resected, and are usually submitted intact if removed at laparotomy. If removed laparoscopically or hysteroscopically, the fibroids may be submitted as disrupted fragments or morcellated specimens.

7.2 Specimen dissection and block selection

Intact myomectomy specimens should be weighed and measured. The presence of a smooth serosal surface should be noted and suggests a subserosal origin. The fibroid/s should be sliced at 5–10 mm intervals and the cut surfaces examined and described. Fragmented or morcellated specimens should also be weighed and a semi-quantitative estimate of the volume should be provided by measurement in three dimensions in millimetres. Fragmented or morcellated specimens must also be sliced. The cut surface should be described as indicated in section 6.2 above. If slicing reveals any deviation from the typical macroscopic appearance of a white, whorled cut surface, this must be noted and sampling of up to one block per 1–2 cm of the maximum diameter of the fibroid should be undertaken. If the cut surface shows no gross abnormality on slicing, routine sampling of one block per leiomyoma is sufficient, with up to three blocks of representative blocks of morcellated or fragmented specimens. Where possible, the blocks should include the interface between the normal myometrium and the fibroid.

7.3 Sectioning and staining

A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination.

7.4 Further investigations

ABPASD is helpful in differentiating between oedema and myxoid change in leiomyomas and in the diagnosis of myxoid smooth muscle tumours.

7.5 Report content

Leiomyomas (and their variants) should be reported in a similar manner to leiomyomas that are identified in hysterectomy specimens (see section 6.5 above).

8 Ovary

8.1 Oophorectomy, ovarian cystectomy, ovarian biopsy

8.2 Specimen submission

Most specimens are submitted to the laboratory in a fixed state, in formalin. Non-neoplastic ovaries may be removed as part of a hysterectomy and bilateral or unilateral salpingo-oophorectomy for uterine, pelvic, ovarian or tubal disease. Ovaries, usually with the attached fallopian tube, may be removed without the uterus for pelvic pain (often due to pelvic inflammatory disease or adhesions), cysts, mass lesions, endometriosis and torsion/oedema or as a risk-reducing (prophylactic) measure in women with BRCA mutations. Wedge biopsies are nowadays uncommonly performed but may be undertaken to investigate infertility, polycystic ovarian disease or for other specific clinical indications. Cystectomy is performed for clinically and radiologically benign cysts and to preserve fertility.

8.3 Specimen dissection and block selection[54]

Avoid excessive handling of the surface to prevent abrasion of the delicate covering mesothelium. Measure the ovary in three dimensions and describe the appearance of the ovarian surface; any capsular breaches/tears, surface papillary or solid projections must be recorded and measured.

Slice small ovaries in the coronal/longitudinal plane, or serially in the parasagittal plane in risk-reducing (prophylactic) salpingo-oophorectomy specimens. Note any cysts; measure the largest dimension or preferably measure their size in three dimensions, record their internal structure (unilocular, multilocular, solid or papillary areas) and describe their contents (watery, serous, mucoid, gelatinous, blood-stained, altered blood [‘chocolate’-like material]). Multiple small subcapsular cysts may indicate polycystic ovarian syndrome. For cysts with complex internal structures, solid or papillary areas, follow the guidelines in the RCPath ovarian cancer dataset.[7] Sample thin-walled cysts by rolling up the wall to give a ‘Swiss roll’ block. Sample and describe para-ovarian cysts in the same way. Block the cyst wall, solid, soft or papillary areas in endometriotic cysts to exclude/document atypia, hyperplasia or tumour. In dermoid cysts, there may be hair or sebum in the cyst lumen, with bone and teeth in the cyst lining and wall. Take blocks from the Rokitansky tubercle, cyst wall and any grossly different areas.

One block is sufficient for a normal ovary. The block should include cortex, medulla and hilus. Several blocks may be needed for otherwise normal ovaries larger than 25 mm greatest dimension. Block papillary and solid areas to identify possible borderline tumours (see the RCPath ovarian cancer dataset)[7]. One block per centimetre of a solid or papillary area is recommended for areas under 100 mm and two per 10 mm for larger lesions, but there is no good evidence to support this recommendation.[55, 56]

[Level of evidence – D]

If the ovary contains a solid tumour or tumour component on slicing, measure the maximum dimensions of the solid component, and describe its colour and appearance (whorled, calcified, haemorrhagic). Try to identify residual ovary if possible.

Block entire wedge biopsy specimens and ensure a vertical section through the cortex, medulla and capsule. Small samples can be bisected along their long axis. Take multiple blocks from larger samples at right angles to the long axis of the specimen.

Block the whole ovary (and fallopian tube) in patients with BRCA mutations who have risk-reducing (prophylactic) salpingo-oophorectomies according to the SEE-FIM protocol to

identify any microscopic tumours or precursor lesions which are especially likely in the tubal fimbriae.[57,58,59]

[Level of evidence – C]

One study suggests that multiple deeper sections allow detection of additional premalignant or malignant lesions.[60] Downes *et al* suggest that the ovaries (and tubes) of patients with Lynch syndrome should be processed in the same way.[53]

If the fallopian tube is submitted with the ovary, this should be measured and described (see section 8 below).

8.4 Sectioning and staining

Orientate cyst wall to ensure a vertical section through the epithelial lining, wall and surface. A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination. Depending on the appearances, levels may be requested at the discretion of the reporting pathologist. A reticulin stain may be helpful to identify the structure of the ovary in infarcted/torted specimens.

8.5 Further investigations

Immunohistochemistry may be required to characterise ovarian tumours.[61]

8.6 Report content

- Oophorectomy for pelvic pain: fibrous capsular adhesions may be present as a result of endometriosis or past pelvic inflammatory disease. Note the presence of inflammation and evidence of old/recent bleeding. Two of three features (organising blood, endometrial-type stroma and endometrioid epithelium) are usually required for a diagnosis of endometriosis. Occasionally stromal endometriosis, i.e. endometriosis that consists only of endometrial-type stroma and no endometrial glands, is seen.[62]
- Oophorectomy for venous infarction/torsion: there may only be minimal viable ovarian tissue for assessment and a reticulin stain can be helpful to identify the architecture of the infarcted tissue. If the architecture of a neoplasm is identified, it may not be possible to determine its nature if the ovary is completely infarcted.
- Wedge biopsy for infertility: note the presence of primordial follicles and signs of previous ovulation such as corpora albicantes and lutea. Record the finding of dysmorphic follicles, inflammation and fibrous capsular thickening with abnormal cystic follicles in polycystic ovaries.
- Oophorectomy for an ovarian cyst/s: record the nature of functional cysts, the presence of cortical inclusion cysts or endometriosis. If there is a benign ovarian epithelial tumour, this should be classified according to the revised 2014 WHO classification of ovarian tumours.[1] If an ovarian tumour, borderline or malignant, is present, report this according to the RCPath ovarian cancer dataset.[7]

9 Fallopian tube

9.1 Specimen submission

Most specimens are submitted to the laboratory fixed in formalin. Short segments of the fallopian tube are most commonly resected as part of a sterilisation procedure, and either a segment or the entire fallopian tube may be excised for hydrosalpinx, pelvic inflammatory disease, endometriosis or ectopic pregnancy. The laterality of the tubes (or segments of

tube) should be indicated ('left' and 'right') by the clinician to enable subsequent exploration of the correct site if necessary. Fallopian tubes may be submitted with an excised ovary, or with total abdominal hysterectomy and oophorectomy specimens for a range of benign and malignant conditions. Fallopian tubes also accompany oophorectomy specimens when risk-reducing (prophylactic) surgery is performed for inherited ovarian cancer syndromes.

9.2 Specimen dissection and block selection[63]

9.2.1 Tubal segment for sterilisation

Measure the length and diameter of the tube segment in millimetres. Confirm the absence of the fimbrial end. Submit at least one transverse slice of each fallopian tube to confirm the presence of fallopian tube in full cross-sectional profile.

9.2.2 Salpingectomy for sterilisation

Measure the length and maximum diameter of the tube in millimetres. Confirm the presence of the fimbrial end. Note any serosal abnormality (adhesions, haemorrhage, adherent cysts) and evidence of previous surgery, including indications of past sterilisation such as the presence of a Filshie clip. Due to the coiling and natural tapering of the tube, it may not be possible to provide accurate dimensions for a normal tube. Take at least one block of each fallopian tube to confirm tube in full cross-sectional profile, and process both fimbrial ends in their entirety. It may be convenient to take two cross-sections from each tube to ensure a full-face section.

9.2.3 Failed sterilisation

Measure the length and maximum diameter of the tube in millimetres. If the tube is distorted it may not be possible to obtain accurate macroscopic dimensions. It may be helpful to photograph the specimen/s as the subsequent findings may have medicolegal implications. Note the presence of fimbriae and any other signs of previous surgery such as suture material or a clip. Note the position of any clip/s or suture material. Block the whole tube (levels may be necessary) to identify possible recanalisation. Where there is gross distortion it may be necessary to take longitudinal blocks. If dissection is difficult because of clips, plastic embedding and sectioning may be required.[64]

9.2.4 Salpingectomy for other reasons

Record the length and maximum diameter in millimetres of the tube if dilated. Note the presence of the fimbrial end. If macroscopically normal and excised as part of a procedure carried out for other gynaecological pathology, take one or more cross-sections through the tube and process the entire fimbrial end.

9.2.5 Salpingectomy for pelvic inflammatory disease

Record the length and maximum diameter in millimetres of the tube. Take one or more transverse sections from the dilated segment of a hydro- or pyosalpinx. Include one block of non-dilated tube (usually isthmic end), if present, to identify previous or concomitant chronic inflammation/evidence of pelvic inflammatory disease. Sample adhesions to the tube, ovary and other attached structures. Block the fimbrial end in its entirety.

9.2.6 Salpingectomy for ectopic pregnancy

See also section 10 below. Record the length and maximum diameter in millimetres of the tube. Note any areas of rupture and describe the contents of the tube. Sample the dilated portion of the tube or implantation site on the surface of the tube. Also sample the non-dilated tube, usually the isthmic end, to document pre-existing inflammation or structural

abnormality. Separately submitted blood clot in the specimen container may contain products of conception and should also sampled. Block the fimbrial end in its entirety.

9.2.7 Risk-reducing (prophylactic) salpingo-oophorectomy for inherited/familial ovarian cancer

Follow the SEE-FIM protocol, i.e. the fimbrial end should be bisected along its long axis and both halves processed, along with serial transverse sections of the entire fallopian tube.[58,65–67]

[Level of evidence – C]

9.3 Sectioning and staining

Ensure that cross-sections of tube are orientated with the cut face at right angles to the plane of section. A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination. Depending on the appearances, levels may be requested at the discretion of the reporting pathologist.

In case of pelvic inflammatory disease Gram, PAS or Grocott for fungi and Ziehl-Neelsen stains may be required to rule out specific causes of inflammation.

9.4 Further investigations

There are no specific investigations that are required for benign disease.

9.5 Report content

9.5.1 Tube segment or salpingectomy for sterilisation

Confirm that a complete cross-sectional profile of fallopian tube is seen.

9.5.2 Failed sterilisation

Confirm that a complete cross-sectional profile of fallopian tube is identified. Identify and document evidence of previous surgery, including the presence and position of a clip/s. Note if recanalisation is present; this may require additional levels.

9.5.3 Salpingectomy for pelvic inflammatory disease

Record the presence of inflammation (acute, subacute, chronic, xanthogranulomatous). If present, report on the presence of plical fibrosis, fusion, simplification and salpingitis isthmica nodosa, and note if histochemical stains identify specific microorganisms. Chlamydia infection may be associated with lymphoid follicle formation. Exclude malakoplakia and other causes of granulomatous inflammation.

9.5.4 Salpingectomy for other reasons

Document any significant incidental findings such as epithelial metaplasia, endometriosis or serous tubal intra-epithelial neoplasia (STIC) if present.

9.5.5 Salpingectomy for ectopic pregnancy

See section 10 below. The identification of a placental implantation site, villi or fetal parts confirms an ectopic gestation. Beware of over-diagnosing hydatidiform mole in early tubal pregnancies.[68] Document any pre-existing pathology that may have predisposed to the development of an ectopic pregnancy, e.g. pelvic inflammatory disease, tubal diverticulum or endometriosis.

9.5.6 Risk-reducing (prophylactic) salpingo-oophorectomy for inherited/familial ovarian cancer

Record the presence of STIC or early tubal carcinoma using the RCPath ovarian/fallopian tube cancer dataset.[7] p53 and Ki67 immunohistochemistry may be helpful in confirming a diagnosis of STIC.

[Level of evidence – C]

10 Products of conception (pregnancy remains; pregnancy loss)

10.1 Specimen submission

Most specimens are submitted to the laboratory fixed in formalin. In some situations, e.g. if the patient has a history of recurrent miscarriages, fresh specimens may be submitted because karyotyping will be required in addition to routine histopathology. The request for karyotyping should be clearly indicated on the request form and the laboratory should have standard operating procedures in place to facilitate the transfer of some of the fresh tissue to a genetics department. All specimens must be accompanied by a fully completed request form with adequate clinical information, including ultrasound appearance, gestational age, history of previous trophoblastic disease, serum hCG (human Chorionic Gonadotrophin) level and date of delivery in cases of post-partum haemorrhage.

Specimens submitted include products of conception, retained products of conception, tissue originating from medical termination of pregnancy (MTOP) and suction termination of pregnancy (STOP). Tissue from MTOP or STOP is not usually submitted for histopathological examination unless the obstetrician has observed an abnormality on the ultrasound scan or during the procedure, or there are other clinical reasons to suspect an abnormality.

Products of conception are sometimes sent in a length of stockinette or in the specimen receptacle of a suction device. If the specimen is bulky, it may be preferable to open the stockinette to ensure adequate overnight fixation prior to specimen sampling.

10.2 Specimen dissection and block selection

Weigh, estimate the volume or measure the specimen in three dimensions. Describe the colour and consistency. Comment on whether spongy placental tissue and/or a gestational sac is present. Glistening membranous tissue may include the implantation site and should be sampled. Look for vesicles suggestive of trophoblastic disease. Measure the maximum vesicle diameter in millimetres. Measure the maximum dimension of the gestational sac, if present, and describe the contents. Comment on the presence of fetal parts. If identified, measure the foot length of the fetus. Avoid sampling fetal tissue.

If adipose tissue is identified macroscopically, it may have originated from the peritoneal cavity due to uterine perforation. The specimen should be processed immediately for histological examination and if adipose tissue is confirmed the clinician must be informed immediately.

Representative material that includes placental tissue, membranes and the implantation site should be sampled. The sampled tissue may be wrapped in paper, placed between layers of foam or in mesh/wire baskets to prevent loss of material and contamination of other specimens. One tissue block is sufficient to confirm normal products of conception.[69]

[Level of evidence: D]

If vesicles are identified macroscopically or if there is clinical suspicion of molar pregnancy, up to three blocks should be sampled *ab initio* in order to prevent a delay in diagnosis. This is important in cases of partial mole as only a small percentage of villi may be abnormal.

Further blocks may be required if the initial section has failed to sample products of conception and confirmation of an intrauterine pregnancy is required, or if there is a suspicion of trophoblastic disease or malignancy.

10.3 Sectioning and staining[23]

A single H&E-stained section representing a full face of the block is adequate for the initial microscopic examination.

10.4 Further investigations

Immunohistochemical studies may be necessary depending on H&E appearances. All trophoblastic cell lineages express cytokeratins (AE1/3, CAM 5.2), inhibin and CD10. hPL (human Placental Lactogen), β-hCG and p63 can assist in the identification of particular trophoblastic populations. $p57_{KIP2}$ may help in confirming a diagnosis of complete hydatidiform mole; however it cannot be used to differentiate between partial mole and hydropic abortus.[70–72]

[Level of evidence – B]

$p57_{KIP2}$ is a cyclin-dependent kinase inhibitor that is paternally imprinted and maternally expressed. Complete moles lack maternal genes and have androgenetic diploidy; therefore $p57_{KIP2}$ expression in villous cytotrophoblast and villous mesenchymal cells is absent or minimal. Although normal syncytiotrophoblast does not usually express $p57_{KIP2}$, nuclear positivity can sometimes be seen in complete hydatidiform mole.[73] Extravillous trophoblast in complete hydatidiform mole may express $p57_{KIP2}$. In partial hydatidiform mole and hydropic abortus (both of which contain maternal genes) villous cytotrophoblast and villous mesenchymal cells are positive for $p57_{KIP2.}$ Ideally, the block selected for $p57_{KIP2}$ immunohistochemistry should contain decidua, which will act as an internal positive control.

Placental site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumour (ETT) are both rare tumours that arise from extravillous trophoblast. PSTT expresses hPL, focal β-hCG and is p63 negative, whereas ETT is positive for p63 and usually negative for hPL. ETT also expresses PLAP. Both of these tumours should be differentiated from an exaggerated placental site and a placental site nodule; the Ki67 index may helpful in this respect. Choriocarcinoma is positive for β-hCG and other trophoblastic markers.

All cases of suspected gestational trophoblastic disease must be referred to a specialist centre where flow cytometry and/or further molecular and immunohistochemical studies may be performed to confirm the diagnosis. There are three national specialist centres for the investigation and follow-up of cases of trophoblastic disease (see below).

10.5 Report content

- Comment on the presence and appearance of chorionic villi (normal, sclerotic, oedematous). If placental membranes are present, this should be reported.
- If villi are absent, the presence of extravillous trophoblast and placental implantation site confirm an intrauterine pregnancy. If gestational endometrium or decidua is present but no placental implantation site or trophoblast is identified, then an ectopic pregnancy should be suspected and the results communicated urgently to the ward and/or clinician.

- If gestational trophoblastic disease (GTD) is identified, i.e. partial or complete hydatidiform mole, choriocarcinoma, placental site or epithelioid trophoblastic tumour, the pathological features should be described and clearly reported and the information should be communicated to the ward and/or clinician urgently if the diagnosis is unexpected. A diagnosis of GTD will result in referral of the patient to one of three national centres for the investigation, treatment and follow-up of patients with trophoblastic disease. These centres are based at Ninewells Hospital (Dundee), Charing Cross Hospital (London) and Weston Park Hospital (Sheffield). At these specialist centres, flow cytometry and image ploidy analysis may be performed. A mechanism should be in place to refer material to one of the specialist centres if required for diagnostic purposes. All patients with a diagnosis of gestational trophoblastic disease must be registered with one of the three national centres and clinicians are responsible for ensuring that there are mechanisms in place to do this. Once a patient has been registered, central review of diagnostic material will take place and departments should document despatch of the material required for review according to standard laboratory operating procedures.

- The presence of mature adipose tissue should be commented upon and communicated to the ward and/or clinician, as indicated above.

- It is unusual to see smooth muscle in these specimens, but if present it should be commented upon. The presence of smooth muscle may be due to a submucosal leiomyoma but if deep curettage has been performed, there is a possibility of developing subsequent Asherman's syndrome.

10.6 Specimen disposal

A standard operating procedure must be place that details the method of sensitive disposal of specimens containing fetal tissue/parts (Human Tissue Authority, www.hta.gov.uk). The HTA advises: "Cremation and burial should be the default methods of disposal for all pregnancy loss and termination of pregnancy, regardless of whether or not there is discernible fetal tissue".

11 Criteria for audit

11.1 Staffing and workload

Annual review of numbers and types of specimens reported by each pathologist; EQA and RCPath CPD compliance.

11.2 Timeliness of report

Confirmation of compliance with RCPath key performance indicators (KPIs) (see www.rcpath.org/clinical-effectiveness/kpi/KPI) is required by providing published monthly audit reports on seven- and ten-day turnaround of specimens (KPI 6.4 for histopathology cases that are reported, confirmed and authorised within seven and ten calendar days of the biopsy being taken).

Standard: 80% of cases must be reported within seven calendar days and 90% of all cases within ten calendar days.

11.3 Monitoring delayed reports

A published report on the number and percentage cases reported after 20 days must be provided (KPI 6.5 for monitoring delayed cellular pathology reports requires there to be a documented system in place to identify, manage and report cases remaining unreported

longer than is anticipated. Exception reporting must be undertaken of all cases (including decalcified cases) remaining unreported after 20 calendar days).

Standard: 100% compliance.

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Appendix A AGREE compliance monitoring sheet

The tissue pathways of The Royal College of Pathologists comply with the AGREE standards for good quality clinical guidelines. The sections of this tissue pathway that indicate compliance with each of the AGREE standards are indicated in the table below.

AGREE standard	Section of document
SCOPE AND PURPOSE	
1. The overall objective(s) of the guideline is (are) specifically described.	Introduction
2. The clinical question(s) covered by the guidelines is (are) specifically described.	Foreword and Introduction
3. The patients to whom the guideline is meant to apply are specifically described.	Introduction
STAKEHOLDER INVOLVEMENT	
4. The guideline development group includes individuals from all the relevant professional groups.	Foreword
5. The patients' views and preferences have been sought.	n/a *
6. The target users of the guideline are clearly defined.	Introduction
7. The guideline has been piloted among target users.	Foreword
RIGOUR OF DEVELOPMENT	
8. Systematic methods were used to search for evidence.	Foreword
9. The criteria for selecting the evidence are clearly described.	Foreword
10. The methods used for formulating the recommendations are clearly described.	Introduction
11. The health benefits, side effects and risks have been considered in formulating the recommendations.	Foreword
12. There is an explicit link between the recommendations and the supporting evidence.	Throughout
13. The guideline has been externally reviewed by experts prior to its publication.	Foreword
14. A procedure for updating the guideline is provided.	Foreword
CLARITY OF PRESENTATION	
15. The recommendations are specific and unambiguous.	2–10
16. The different options for management of the condition are clearly presented.	Throughout
17. Key recommendations are easily identifiable.	Throughout
18. The guideline is supported with tools for application.	2–10
APPLICABILITY	
19. The potential organisational barriers in applying the recommendations have been discussed.	Foreword
20. The potential cost implications of applying the recommendations have been considered.	Foreword
21. The guideline presents key review criteria for monitoring and/audit purposes.	11
EDITORIAL INDEPENDENCE	
22. The guideline is editorially independent from the funding body.	Foreword
23. Conflicts of interest of guideline development members have been recorded.	Foreword

* The Lay Advisory Committee (LAC) of The Royal College of Pathologists has advised that there is no reason to consult directly with patients or the public regarding this tissue pathway because it is technical in nature and intended to guide pathologists in their practice. The authors will refer to the LAC for further advice if necessary.

Appendix B Summary table – Explanation of grades of evidence

(modified from Palmer K *et al. BMJ* 2008; 337:1832)

Grade (level) of evidence	Nature of evidence
Grade A	At least one high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target cancer type or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target cancer type.
Grade B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in A.
Grade C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high quality case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in B.
Grade D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group