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

Tissue pathways for breast pathology

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Produced by	Members of the National Coordinating Committee for Breast Pathology, Professor Ian Ellis, Nottingham City Hospital (Writing Group Lead)
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The Royal College of Pathologists
2 Carlton House Terrace
London, SW1Y 5AF
Tel: 020 7451 6700
Fax: 020 7451 6701
Web: www.rcpath.org

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GENERAL INTRODUCTION

The following recommendations are regarded as a minimum acceptable practice. For a more detailed description of best practice see *Guidelines for Non-operative Diagnostic Procedures and Reporting In Breast Cancer Screening*. NHSBSP Publication No 50. June 2001[1] (these are being revised during 2009) and *Pathology of Breast Disease*. NHSBSP Publication No 58. January 2005.[2]

1. STAFFING AND WORKLOAD

There should at least be two and preferably more pathologists in a unit competent in the reporting of breast specimens, in order to provide cover for periods of leave. It is recognised that in some smaller units only one pathologist may have specialist expertise. All pathologists reporting NHS breast screening specimens should participate in the UK breast pathology EQA scheme.

A maximum workload for a full time breast pathologist should be within The Royal College of Pathologists recommended guidance.[3] An evidence based minimum workload is as yet not clearly defined. However with low breast specimen workloads pathologists must bear in mind their diagnostic experience, on-going CPD activity and EQA outcomes in assessing their ability to maintain an acceptable level of reporting expertise. Otherwise referral of the workload to a specialist centre can be considered.

2. LABORATORY FACILITIES

In addition to routine light microscopy (LM), there must be access to immunohistochemistry (IHC) and molecular (MOL) genetic testing (for example in situ hybridisation (ISH)). Molecular genetic testing facilities may be offsite.

Diagnostic LM, IHC and MOL from a single case should be coordinated by one pathologist. Reporting each in isolation may result in serious misdiagnoses or misclassification.

Additional prognostic or predictive studies such as hormone receptor or HER-2 testing may, for various reasons such as quality, skill mix or economics, be provided elsewhere. The results of these tests should be made available to the diagnostic laboratory for co-ordination with the diagnostic report, feedback and audit as required.

SECTION A: NEEDLE CORE AND VACUUM ASSISTED CORE SPECIMENS

1. SPECIMEN SUBMISSION

The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical and imaging summary (see below). It is helpful if previous histology which is pertinent to the current specimen is identified. The patient details required may vary from laboratory to laboratory but as a minimum must include full name, gender, date of birth and NHS number. The specimen container is labelled with matching patient details. When core biopsies are taken from multiple lesions/separate sites from the same patient on the same day, the clinical details must clearly indicate the source of each separate specimen.
Proper interpretation of breast core biopsies requires knowledge of details of both clinical and mammographic findings and this information must be provided on the request form. The completed request form should include clinical details, specifying the radiographic sign and the site of biopsies. Correlation is also made with previous or current fine needle aspiration cytology results.
Biopsies taken from microcalcifications are x-rayed to determine the presence of calcium. Whenever possible a radiological comment regarding the presence of representative microcalcification of the mammographic lesion in the sample is provided along with the specimen x-ray. Those cores with microcalcification can be sent in a separate pot and embedded individually to facilitate the examination of further levels if calcification is not immediately apparent on histological examination.
The specimen is received in formalin of adequate volume to ensure proper fixation. Good fixation is vital to preserve the morphological detail. Biopsies are placed in fixative solution immediately and sent promptly to the laboratory. Ideally biopsies are fixed routinely for a minimum of six hours although specimens may be fixed rapidly with the aid of microwave techniques.

2. SPECIMEN DISSECTION

Each case and samples from separate sites of the same patient are handled separately and handling instruments cleaned between samples to avoid risk of transposition or contamination. The number of core biopsy tissue samples received and their size (mm) are recorded. Needle core biopsy specimens can be placed by the radiologist or surgeon directly into cassettes appropriate for processing, which facilitates flat specimen fixation, processing and limits the need for specimen handling. Recording of number and size is not appropriate prior to processing in these circumstances.

3. SECTIONING AND STAINING

After processing, haematoxylin and eosin stained sections from one level are usually sufficient for core biopsies from mass lesions. Core biopsies taken for the investigation of microcalcification have a minimum of three levels examined. In practice most laboratories choose to examine all core biopsies from screen detected lesions at three levels initially. In problematic cases further levels and immunohistochemical studies may be helpful.

4. FURTHER INVESTIGATIONS

- Additional sections/levels at the discretion of the pathologist.
- Additional LM stains at the discretion of the pathologist.
- Special histochemical stains and immunohistochemistry are not normally required unless there are specific indications e.g. hormone receptor status for preoperative neoadjuvant therapy. Guidance for use of such stains is given.[1]
- Where local protocols require testing of samples containing tumour for therapeutic response, detailed guidance on predictor assessment including hormone receptors and HER2 status is available.[2] Laboratories providing such assays should participate in appropriate quality assurance schemes.

- With the exception of gene copy number evaluations for assessment of HER2 status, if an invasive tumour is identified, molecular investigations including in situ hybridisation are not regarded as routine at present. However there should be a route for referral to a specialist genetic or molecular pathology service for relevant cases.

5. REPORTING AND REPORT CONTENT

Reporting of both symptomatic and mammographic screening generated samples should follow the UK Guidelines.[1] Detailed guidance on reporting breast non-operative diagnostic samples is given in the above document. Key points have been replicated below for information only but all pathologists reporting breast pathology samples should be familiar with the above Guidelines in detail.

It is important to remember that histological examination of core biopsy samples is performed to fulfil the assessment process role in both symptomatic and mammographic screening settings by giving a pathology category classification (B1–5) and forms one of the components of the triple approach to diagnosis of breast cancer. This process is not designed to give a definitive diagnosis, although this is possible in the majority of cases. Thus, while most core biopsy samples can be readily categorised as normal, benign, or malignant, it must be recognised that a small proportion (typically less than 10%) of samples cannot. These categories are designed only to take account of the histological nature of the specimen and not the clinical or imaging characteristics. Similarly, it is not feasible for the pathologist to judge independently whether or not a sample is an adequate representation of a mammographic lesion. This judgement requires multidisciplinary discussion. For these reasons there is no inadequate biopsy category for core biopsy specimens. In some situations, particularly epithelial proliferation and papillary lesions, the choice of diagnostic category should be based on the features present in the sample. If a pathologist has concerns about whether or not a biopsy specimen is adequate, this should be expressed in the text of the report; this is particularly important to ensure clarity of communications for reports in the B1 and B2 categories.

REPORTING CATEGORIES

B1 (normal tissue)

This indicates a core of normal tissue whether or not breast parenchymal structures are present. Thus this category is equally appropriate for a core including normal breast ducts and lobules or mature adipose tissue or stroma only. A B1 report includes a description of the components present, and comment is made regarding the presence of breast epithelial structures. Statements can also be made to assist in mammographic clinical histology correlation, for example, a sample composed of mature adipose tissue is classified as B1 but would be consistent with an origin from a benign lipoma.

B2 (benign lesion)

A core is classified as B2 when it contains a benign abnormality. This category is appropriate for a range of benign lesions, including fibroadenomas, fibrocystic changes, sclerosing adenosis and duct ectasia, and extends to include other non-parenchymal lesions such as abscesses and fat necrosis.

B3 (lesion of uncertain malignant potential)

This category mainly consists of lesions which may provide benign histology on core biopsy but are known to show heterogeneity or to have an increased risk (albeit low) of associated malignancy.

B4 (suspicious)

Technical problems such as crushed or poorly fixed cores that contain probable carcinoma but cannot provide the definitive diagnosis are best included as B4. Similarly, apparently neoplastic cells contained within blood clot or adherent to the outer aspect of the sample are classified as B4 suspicious. Very small foci of invasive carcinoma in which there is insufficient material to allow immunocytochemical studies may also reasonably be assigned to this category.

B5 (malignant)
This category is appropriate for cases of unequivocal malignancy on core biopsy. Further categorisation into in situ and invasive malignancy is undertaken whenever possible by sub-categorisation into B5a – in situ; B5b - invasive and B5c - uncertain whether in situ or invasive. Other forms of malignancy such as malignant lymphoma are also classified as B5.

6. SNOMED CODING

Coding of both symptomatic and mammographic screening generated samples follows the UK Guidelines.[2]

7 REFERENCES

1. *Guidelines for non-operative diagnostic procedures and reporting in Breast Cancer Screening.* NHSBSP Publication No 50, June 2001. www.gov.uk/government/collections/breast-screening-professional-guidance
2. Pathology Reporting of Breast Disease. A joint document incorporating the third edition of the NHS Breast Screening Programme Guidelines for Pathology Reporting in Breast Cancer Screening and the second edition of the Royal College of Pathologists Minimum Dataset for Breast Cancer Histopathology. NHSBSP Publication No 58. January 2005. www.rcpath.org/index.asp?PageID=695 .
3. RCPath. *Guidelines on Staffing and Workload for Histopathology and Cytopathology Departments.* 2nd edition, June 2005. www.rcpath.org/resources/pdf/GuideHistoCytoWorkload0605.pdf

SECTION B: SURGICAL SPECIMENS

1. SPECIMEN SUBMISSION

After excision, it is appropriate to take a radiograph of diagnostic localisation resections. This allows confirmation of the presence of the abnormality and also its location in the specimen. The radiographs are ideally reported by the breast radiologist. The specimen radiographs must, however, be available to the pathologist so that he/she can be certain of the nature of the lesion, i.e. mass, calcification, etc. The pathologist can therefore also assess where the lesion is situated in the specimen in order to facilitate histological sampling. This may also be facilitated by guide wire localisation.
The surgeon should refrain from interfering with the specimen once it has been removed from the body i.e. no opening/slicing etc., as this may lead to distortion of the tissues during fixation. The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical summary. It is helpful if previous histology and fine needle aspiration cytology results pertinent to the current specimen are identified. The patient details required may vary from laboratory to laboratory but as a minimum must include full name, gender, date of birth and NHS number. The specimen container is labelled with matching patient details.

2. SPECIMEN PRE-DISSECTION

If the specimen is sent fresh there must be a protocol in place to ensure rapid transport to the laboratory, with refrigeration overnight if necessary. Good fixation is vital to preserve the morphological detail. This is particularly relevant for the diagnosis of some difficult intraductal epithelial proliferations. Specimens must be placed in a sufficient volume of formalin (at least twice, and preferably 5-10 times, the volume of the specimen) or other appropriate fixative inside an appropriately sized and shaped container either before or, preferably, after receipt by the laboratory. Incision of the specimen, ideally by the person who will subsequently dissect the specimen, is beneficial in achieving rapid fixation in larger specimens.

3. SPECIMEN DISSECTION

The specimen is weighed (g) and measured (mm) in three dimensions. Its external surface is painted according to local orientation protocols to demonstrate the relationship of any pathological abnormality to the surgical margins. It is then serially sliced at intervals of approximately 3–5 mm for a small diagnostic specimen. Block selection focuses on the lesional area for visible palpable abnormalities. Cases of impalpable mammographic screen detected lesions such as microcalcification, where guided block selection is required (i.e. those that are not embedded in their entirety) should undergo specimen slice x-ray examination. This enables blocks to be taken from the areas corresponding to the mammographic abnormality, as well as any other suspicious areas identified. The sites of sampling can be marked on the specimen x-ray or the x-ray of specimen slices by using a white wax (Chinagraph) pencil or other marker.
The sampling technique and the number of blocks taken are clearly dependent on the size of the specimen and the nature and size of the abnormality. If the specimen is small, it is often best to block and examine all of the tissue. Samples of approximately 30mm or less in maximum dimension are completely sliced, embedded and examined histologically.
For larger specimens, sampling should be adequate to determine accurately the size of the lesion. Sampling includes the extremes of the mammographic abnormality and adjacent tissue in order to avoid underestimation of size. This is particularly important with cases of calcification as it is recognised that mammographic size may be an underestimate of true lesion size.
If specimens are sent as more than one piece of tissue, it can be impossible to measure the absolute extent of the lesion. In these cases, it is appropriate to take a pragmatic approach and to measure the maximum size in each piece of tissue and add the dimensions to give an estimated total size. If, however, the orientation of the specimens can be determined, the true size can be ascertained more reliably. Any such estimation of lesion size is correlated with the

imaging size and if doubt exists on the true pathological size then it may be appropriate to default size assessment to the image size.

Tips on block selection

For detailed guidance on handling and dissection of breast cancer specimens see.[1]

- where there is no palpable or visible lesion submit fibrous parenchyma rather than fatty tissue
- consider submission of serial transverse slices for some specimens such as: localisation/open diagnostic biopsies, major duct excisions, cavity shave margins
- a macroscopic lesion is sampled adequately with adjacent surgical margins if considered appropriate, examples include: abscess, fat necrosis, fibroadenoma, phyllodes tumour, carcinoma
- in cases where no visible or palpable lesion is expected submit representative samples of fibrous parenchyma ± nipple/skin, examples include: gynaecomastia, prophylactic mastectomy, breast reduction, mammoplasty
- for nipple skin biopsy specimens: bisect perpendicular to the skin surface and examine histologically at 3 levels.

It is desirable to include block selection site information as part of the content of the pathology report to facilitate peer review and audit.

Additional guidance has been prepared regarding examination of cosmetic breast reduction specimens. The additional text appears in Section C.

4. SECTIONING AND STAINING

A single haematoxylin and eosin stained section is adequate for examination provided the blocks taken are fully represented on the slide.

5. FURTHER DIAGNOSTIC INVESTIGATIONS

Additional sections / levels at the discretion of the pathologist
Additional LM stains at the discretion of the pathologist.
Special histochemical stains and immunohistochemistry are not normally required unless there are specific indications. Indications for use of immunocytochemistry to support diagnosis of cases are given.[1]
Cases of invasive carcinoma require testing of samples containing tumour for therapeutic response. Detailed guidance on predictor assessment including hormone receptors and HER2 status is available.[1]
With the exception of gene copy number evaluations for assessment of HER2 status molecular investigations including in situ hybridisation are not regarded as routine at present. However there should be a route for referral to a specialist genetic or molecular pathology service for relevant cases.

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Reporting of both symptomatic and mammographic screening generated specimens follows the UK Guidelines.[1]

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8. REFERENCES

1. Pathology Reporting of Breast Disease. A joint document incorporating the third edition of the NHS Breast Screening Programme Guidelines for Pathology Reporting in Breast Cancer Screening and the second edition of the Royal College of Pathologists Minimum Dataset for Breast Cancer Histopathology. NHSBSP Publication No 58. January 2005. www.rcpath.org/index.asp?PageID=695.

SECTION C: TISSUE SAMPLING IN COSMETIC BREAST REDUCTIONS

Tissue removed from non-oncoplastic cosmetic breast procedures are generally submitted for pathology examination. It is recognised that there is a risk of identification of detection of invasive cancer, in situ carcinoma or atypical hyperplasia in such specimens, albeit at very low frequency. The risk of detection of such abnormality appears to be higher in patients over the age of 40. Pathologists requested to examine such specimens are recommended to undertake careful macroscopic examination of each specimen through visual inspection and manual palpation of the specimens and slices of the specimen at between 5 and 10mm thickness. Abnormal areas should be sampled for pathological examination. It is recommended that a minimum of two tissue blocks are taken for histological examination. Block sampling should be targeted towards white fibrous, potentially parenchymal rich and non-fatty tissue. In patients with prominent fibrous breast tissue and those over the age 40, additional block sampling can be considered.