



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

TISSUE PATHWAYS FOR HEAD AND NECK PATHOLOGY

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GENERAL INTRODUCTION

1. Staffing and workload

Preferably at least two or more pathologists in a unit should be competent in the reporting of specimens from the head and neck. If one of these is not an Oral and Maxillofacial Pathologist with expertise in oral mucosal biopsies and of the special tooth-related and odontogenic pathology of the jaws, then access to this expertise should be ensured.

Pathologists reporting head and neck specimens should participate in an appropriate EQA scheme. Lead pathologists should participate in a specialist Head and Neck EQA scheme.

There are no clear maximum workloads for a full time head and neck pathologist. The Royal College of Pathologists Guidelines for Staffing and Workload in Histopathology and Cytopathology Departments¹ are a guide. Workload may vary considerably according to the nature of the specimens received. Pathologists undertaking a significant amount of oncology work will be able to report fewer requests per year than a pathologist dealing primarily with non-neoplastic specimens.

2. Laboratory facilities

The full range of routine laboratory facilities is needed, including access to immunocytochemistry and electron microscopy, which may be off site. Facilities for sectioning of hard tissue are required, including an appropriate saw (band saw or diamond-coated saw) for dissection of bone resections of the jaws, expertise in decalcification and preparation of specimens of bones and teeth. Fixation in formalin for 24-48 hours after slicing bone, and before decalcification, may improve morphology. Facilities and expertise for the preparation of ground sections of teeth are also sometimes necessary (or should be available off site).

Detailed protocols for decalcification are beyond the scope of this document (see Bancroft and Gamble⁵) and an appropriate balance needs to be reached between slower decalcification for optimal morphology and more rapid decalcification to facilitate patient management. Some decalcifying protocols may interfere with immunocytochemistry and excessive

decalcification affects the morphology. In general, strong acids e.g. nitric acid, are best avoided as decalcification is rapid and difficult to control. For most purposes, 5% formic acid is an appropriate decalcifying agent, with the end point confirmed by palpation and/or ammonium hydroxide⁵. Unless the tissue is likely to fragment or otherwise be distorted, it is recommended that bone is trimmed to block size (approximately) before decalcification. This should allow decalcification to be completed in 1-10 days, although very dense bone may take longer.

3. Specimen submission and dissection

Most specimens are received in the laboratory in formalin as routine diagnostic or therapeutic specimens according to standard procedures. For most specimens no special facilities are required for specimen dissection and preparation apart from bone and teeth as mentioned above. It is good practice to photograph large specimens so that a permanent record of the macroscopic appearance and location of blocks can be recorded and filed in the patient records. Specimen dimensions are measured in mm.

Fresh tissue specimens are occasionally required primarily for the diagnosis of vesiculobullous lesions using direct immunofluorescence. In these cases a mucosal biopsy is submitted fresh to the laboratory, either immediately wrapped in damp gauze, or in a suitable transport medium.

The guidelines for the handling of head and neck specimens may vary according to the type of specimen².

SECTION A. TISSUE PATHWAY: MUCOSAL BIOPSIES

1. Specimen dissection

Most of these specimens are small. The specimen is measured in three dimensions. Identify and describe the colour and texture of the mucosa as well as any identifiable lesion e.g. polyp, ulceration. Incisional biopsies of sufficient size are bisected through the long axis, and may be inked to indicate orientation for embedding purposes. For excision specimens the closest excision margins are often best sampled by sectioning across the short axis (transversely).

2. Sectioning

Routinely a single section is usually sufficient for diagnostic purposes. Lesions where dysplasia is suspected or needs to be excluded, or from high risk sites (e.g. floor of mouth, non-homogeneous leukoplakia) should have three levels cut at 100µm intervals.

3. Staining

Haematoxylin and eosin (H&E) stained sections are required for all cases. White lesions and dysplastic lesions, which are often infected with Candida species, may be stained using PAS with prior diastase digestion.

4. Further investigations

These are occasionally needed to confirm a diagnosis and are requested as necessary. Examples include stains for amyloid, immunocytochemistry for suspect lymphomas or melanomas.

Immunofluorescence

Fresh samples submitted for vesiculobullous disorders are stained for IgG, IgA, IgM and C3.

5. Report content

The report specifically refers to the overlying epithelium, lamina propria and other identified tissues including an indication of the depth of the biopsy (e.g. by reference to muscle on the

deep aspect). Any infective agents or dysplastic features (graded according to the WHO guidelines) are highlighted within the report. For excision specimens of oral leukoplakia, the presence and degree of dysplasia at surgical margins is noted (this is not relevant for small, diagnostic mucosal biopsies).

SECTION B. TISSUE PATHWAY: TEETH

1. Specimen dissection

Teeth are received in formalin usually with odontogenic cysts or as part of a resection specimen and may require histological examination to determine the vitality of the pulp. This can inform the pathogenesis of a periapical lesion.

Occasionally a clinical diagnosis of a tooth disorder requires histological confirmation - for example for idiopathic resorption, or developmental disorders.

Tooth notation, site, morphology, presence of caries, and filling material are recorded. Assess enamel and dentine structure including colour, transparency, banding, erosion, abrasion and relative hardness. Root number, morphology and presence of resorption are identified.

Teeth are usually decalcified before dissection and sectioning. However for diagnosis of enamel defects a ground section is required. In this case the tooth is bisected in a band saw and a ground section taken from one half and the other half submitted to decalcification.

2. Sectioning

Incisors, canines and premolar teeth are sectioned in the bucco-lingual plane. Molar teeth are sectioned mesio-distally.

3. Staining

Ground sections are viewed using Canada Balsalm as an embedding agent as this has a similar refractive index to normal enamel.

Decalcified sections are stained using H&E.

4. Further investigations

Accurate clinical information including family history, extent of teeth affected, presence of metabolic bone disorders and examination of radiographs is required for accurate diagnosis of developmental disorders.

Disorders of tooth structure require the availability of polarised light microscopy.

5. Report content

The report refers to the enamel including thickness, structure, presence of enamel matrix and appearance of amelodentinal junction, and to the dentine including the appearance and presence of mantle zone, pre-dentine, primary, secondary, tertiary and inter-globular dentine. Specifically the appearance and presence of dentine tubules including relative width and orientation as well as the location of dysplastic dentine.

Pulp examination includes assessment of the root apex, vitality, inflammation, relative size and location.

SECTION C. TISSUE PATHWAY: CYSTS – ODONTOGENIC AND NON-ODONTOGENIC

1. Specimen dissection

Most of these specimens are small, soft and fragmentary in nature. Record the number of pieces and dimensions of the largest piece. Small hard tissue fragments are common and decalcification overnight is often sufficient. Large fragments of bone and identifiable teeth or tooth fragments are described, decalcified and blocked separately. The relationship to the tooth such as attachment to the cement-enamel junction or root apex is recorded. Tooth notation, caries status and the presence of restorations are documented.

Large cysts require a description of the wall and the presence of mural thickening or nodules. Examination of the cyst lumen and its contents may reveal the presence of keratin squames, cholesterol or intraluminal nodules.

Small fragmented lesions are embedded in their entirety. Small intact cysts can be bisected. For large cysts, representative transverse slices are best. Care is taken to sample any nodules or mural thickenings.

2. Sectioning

A single section is usually sufficient for diagnostic purposes.

3. Staining

H&E stains are required for all cases.

4. Further investigations

Clinical information and preferably examination of radiographs is required for accurate diagnosis since it is often important to know the relationship to the teeth.

Unusual findings require three levels at 100µm as well as further representative samples.

Stains for PAS, alcian blue or mucicarmine may be useful in the diagnosis of glandular odontogenic cysts.

5. Report content

The report specifically describes the cyst lining and the type and nature of the epithelium e.g. the presence of keratinisation or basal palisading, mucous metaplasia, hyaline (Rushton) bodies, or atypical features. The capsule is described, particularly the presence or absence of inflammation and features such as daughter cysts, calcifications, odontogenic rests or foreign material.

SECTION D. TISSUE PATHWAY: MINOR SALIVARY GLANDS

1. Specimen dissection

Most of these specimens are relatively small and less than 15mm.

Mucoceles are usually fluctuant and may be covered by mucosa. The presence of minor salivary gland tissue is identified.

The specimen is measured in three dimensions and may be bisected in the longitudinal plane. For excision specimens, dissection is in planes appropriate to sample the closest excision margins. If multiple lobules of minor salivary gland tissue are received then the collective area is measured. Certain sites such as the upper lip are at an increased risk of tumour development even though these may clinically and macroscopically appear to be mucoceles.

Resection specimens are orientated as indicated by the surgeon on the request form. Deep and peripheral excision margins are inked. Care is taken to examine the capsule and record any areas where it is incomplete or ruptured. Where a tumour is suspected, describe its location, consistency (i.e. solid, cystic, gelatinous), capsule and circumscription.

Blocks required include:

- One block per 10mm diameter of tumour for larger specimens; most specimens will be blocked in their entirety.
- Sufficient sampling to determine adequacy of surgical margins
- Adjacent mucosa and normal salivary glands.

2. Sectioning

A single section from each block is usually sufficient for diagnostic purposes in cystic and inflammatory conditions.

3. Staining

H&E stains are required for all cases.

4. Further investigations

Stains for PAS, alcian blue or mucicarmine are useful for identifying subtle extravasation of mucin and in the diagnosis of benign salivary gland tumours.

Immunohistochemistry is occasionally useful for the diagnosis of salivary gland tumours.

5. Report content

Cysts

- Nature of cyst and lining i.e. epithelium or connective tissue
- Type of inflammatory infiltrate. Presence of atrophy, mucous extravasation, ductal ectasia and minor salivary gland tissue.

Benign tumours

- Type of tumour as based on WHO guidelines³
- Distance of tumour from the nearest peripheral margin
- Distance of tumour from the deep margin
- Presence of a capsule and any breach.

Any unsuspected malignancy is reported according to the RCPATH Head and Neck Cancer Dataset⁴.

SECTION E. TISSUE PATHWAY: MAJOR SALIVARY GLANDS

1. Specimen dissection

Submandibular and sublingual glands are usually removed entirely as a result of sialolithiasis. Radiographs may be used to identify a sialolith.

Parotid gland specimens most often comprise a superficial parotidectomy of the lower pole of the superficial lobe. Total parotidectomies are rare for benign disease but may be performed for deep lobe tumours. The superficial and deep lobes may be provided separately. Specimens should be orientated by the surgeon and in cases of doubt the surgeon must be consulted.

Superficial parotidectomy specimens resemble a triangle, with the smooth surface representing the superficial surface and the shortest profile the superior margin.

Deep lobes of the parotid and sublingual glands are difficult to orientate and are best done surgically by the operating clinician.

The submandibular gland can be orientated by the indentation produced by mylohyoid on the deep margin and by the duct at the anterior aspect.

Required measurements include:

- Dimensions and weight (g) of the specimen
- Dimension and number of cysts
- Dimensions of any identifiable tumour
- Distance to the nearest margins
- Presence of a capsule and whether or not this is intact.

Describe the location of any tumour, its consistency (i.e. solid, cystic, gelatinous), capsule and circumscription.

Blocks required include:

- One block per cm diameter of tumour
- Sufficient sampling to determine the adequacy of surgical margins
- Adjacent mucosa and normal salivary glands
- Proximal and distal aspect of nerves if identifiable
- Any intra-glandular or adjacent lymph nodes.

2. Sectioning

Routinely a single section of each block is sufficient for diagnostic purposes in cystic, infective and inflammatory conditions.

3. Staining

H&E stains are required for all cases.

4. Further investigations

Mucin stains, e.g. PAS, alcian blue or mucicarmine are helpful in the diagnosis of benign salivary gland tumours.

Immunohistochemistry is occasionally useful for the diagnosis of salivary gland tumours and for the differential diagnosis of benign lymphoepithelial lesions from extranodal marginal zone (MALT) lymphoma. This can be supplemented by molecular analysis for light and heavy chain restriction.

5. Report content

Cysts

- Nature of cyst and lining i.e. epithelium or connective tissue
- Type of inflammatory infiltrate
- Presence of atrophy, mucous extravasation and ductal ectasia
- Presence of salivary gland tissue.

Benign tumours

- Type of tumour as based on WHO guidelines³
- Distance of tumour from the nearest peripheral margin
- Distance of tumour from the deep margin

- Presence of a capsule and any breach.

Any malignancy is reported accordingly⁴.

SECTION F. TISSUE PATHWAY: JAW LESIONS

1. Specimen dissection

This category includes a number of benign lesions which necessitate major resective surgery. This includes ameloblastomas and fibro-osseous lesions among other conditions.

The presentation of jaw specimens is variable and includes enucleated specimens composed of fragmented pieces of soft tissue or bone as well as bone resections.

If multiple fragments are included the number of pieces, total dimensions and dimensions of the largest piece are recorded. It is important to determine the relationship between resection specimens and separate fragments, especially with regard to excision margins. This is particularly relevant to maxillectomy specimens which may become fragmented during removal.

If small, all samples are processed, otherwise representative sections are usually sufficient.

Some odontogenic tumours and hamartomas are cystic in nature. If associated with teeth, the relationship is documented. In addition, examination of the cyst lumen can reveal the presence of mural or luminal nodules in unicystic ameloblastomas.

For larger specimens identification of operation type and orientation is required. Photographs are used and carefully labelled to indicate orientation and the origin of blocks. Radiographs are useful in assessing the extent of the lesion, tooth resorption and the presence of calcification.

Required measurements include:

- Antero-posterior diameter along the alveolar ridge
- Maximum bone height i.e. ramus
- Dimensions of tumour
- Distance and location of the nearest margin.

Surgical margins e.g. mucosal, deep, superior limit of ramus etc. may be inked.

Small specimens can be decalcified in their entirety before sampling or blocking out. For large resections, especially of the mandible, it is often helpful to take slices of 5-8mm on a band saw. It may also be possible to slice maxillary specimens. Sometimes these are very fragile and decalcification of the entire specimen helps sampling and to preserve orientation.

As most lesions are intraosseous, dissection of soft tissue from bone is usually not necessary. However, evidence of cortical perforation requires close soft tissue examination and handling as for those resections in malignant disease⁴.

2. Sectioning

A single section of each block is usually sufficient for diagnostic purposes.

3. Staining

H&E stains are required for all cases.

4. Further investigations

Accurate clinical information is required for accurate diagnosis. In general, hard tissue lesions are not reported without examination of radiographs and/or CT images.

Congo red, alizarin red or thioflavine T are useful for the detection of amyloid proteins in adenomatoid and calcifying epithelial odontogenic tumours.

Van Gieson stains are useful in identifying dentinoid material e.g. in calcifying cystic odontogenic tumours.

Immunohistochemistry is rarely required.

5. Report content

An accurate description of any epithelium, including any reference to the formation of duct-like structures as well as the presence of atypical features such as mitotic figures. Atypical features such as pleomorphism are common in some odontogenic tumours including the calcifying epithelial odontogenic tumour. If no odontogenic epithelium is identified this is stated.

The appearance of the stroma is described including the presence of enamel, dentine, bone or other calcified material.

The presence of amyloid is confirmed with special stains.

Comment is made upon the relationship to normal structures, e.g. teeth, bone. The presence of a capsule and nature of the surgical margins are recorded.

Any malignant tumour is reported accordingly⁴.

SECTION G. TISSUE PATHWAY: NASAL CAVITY AND PARANASAL SINUSES

1. Specimen dissection

Most of these specimens are small and fragmentary in nature. Specimens should be measured in three dimensions. Identify and describe the colour and texture of the mucosa as well as any identifiable lesion e.g. polyp, ulceration. Small specimens are measured and usually embedded whole. Specimens of sufficient size are best bisected through the long axis. Larger samples and sinonasal polyps should have representative samples taken. Unilateral nasal polyps are usually blocked in their entirety because unilateral lesions have a slightly higher risk of being neoplastic than bilateral lesions. Small hard tissue fragments are common and decalcification overnight is often sufficient. Large fragments of bone should be described, decalcified and blocked separately

2. Sectioning

A single section of each block is usually sufficient for diagnostic purposes. Three levels at 100µm intervals may be indicated for more detailed examination of papillomas where dysplasia or invasive malignancy is suspected.

3. Staining

H&E stains are required for all cases.

4. Further investigations

PAS, Grocott's and Gram stains are useful for detecting fungal and bacterial infections. Mycobacterial stains are required in granulomatous conditions. If Wegener's granulomatosis is suspected, an elastic van Gieson stain may be helpful in identifying damaged vessels and further clinical information on the presence of positive c-ANCA tests and ESR is useful. Immunohistochemistry is usually not necessary in the diagnosis of benign nasal lesions. However, rare soft tissue tumours such as extrapleural solitary fibrous tumour, pituitary gland neoplasm and meningioma may occur.

Minor salivary gland tumours and fibro-osseous lesions are reported as indicated in the relevant sections.

5. Report content

The report may refer to the overlying epithelium, lamina propria and other identified tissues. Note the type of inflammatory infiltrate. Any infective agents or dysplastic features (graded according to the WHO guidelines) are highlighted. The presence and degree of dysplasia at surgical margins is noted for excision specimens, but this is usually not possible in fragmented samples..

Specific diagnoses are provided for any polypoid lesion i.e. allergic / inflammatory type, inverted papilloma, in view of the potential risk of malignant transformation in the latter.

SECTION H. TISSUE PATHWAY: LARYNX, PHARYNX AND TONSIL

1. Specimen dissection

Most of these specimens are small or fragmentary in nature. Identify and describe the colour and texture of the mucosa as well as any identifiable lesion, e.g. polyp, ulcer. Measure in three dimensions. Specimens of sufficient size are bisected through the long axis or sliced serially. Piecemeal resection specimens of the pharynx are embedded in their entirety.

Resection specimens are orientated as indicated by the surgeon on the request form. Deep and peripheral excision margins are inked. Care is taken to examine the capsule and record any areas where it is incomplete or ruptured. Describe the location of the tumour, consistency (i.e. solid, cystic, gelatinous), capsule and circumscription. Dissection should be in planes appropriate to sample the closest excision margins

Laryngectomy specimens and major resections for benign disease are treated as for laryngeal malignancy⁴.

Tonsillectomy specimens :

- Are orientated (if possible) and the deep margins inked
- Are measured in three dimensions and may be weighed.
- Are examined grossly and cut into 4-5mm transverse slices
- Should have any abnormality measured and described
- Should have representative blocks taken. If there is no macroscopic abnormality, then 2 blocks are sufficient.

Note: the ipsilateral tonsil is often the source of metastatic squamous cell carcinoma in the neck, particularly cystic metastases that can mimic branchial cysts. The primary lesion may be microscopic. In cases of tonsillectomy for patients who have proven or suspected metastasis in neck nodes and where tumour is not clearly identified within the tonsil at macroscopic examination, the tonsillectomy specimen is blocked serially and submitted in total to exclude a microscopic primary in the tonsil itself.

2. Sectioning

A single section from each block is usually sufficient for diagnostic purposes.

Lesions from high risk sites i.e. non-homogeneous leukoplakia from the vocal cords have three levels at 100µm intervals.

Retention of unstained sections is preferable, if resources permit.

3. Staining

H&E stains are required for all cases. Lesions suspicious of Candida infection are stained with PAS and diastase pre-treatment .

4. Further investigations

Immunohistochemistry is usually not required, although this may be beneficial for some rare diagnoses.

5. Report content

The report specifically refers to the overlying epithelium, lamina propria and other identified tissues.

Any infective agents or dysplastic features (graded according to the WHO guidelines) are highlighted within the report. The presence and degree of dysplasia at surgical margins is noted.

Salivary and other benign tumours are reported as discussed in the relevant sections.

SECTION I. TISSUE PATHWAY: NECK LESIONS

1. Specimen dissection

Swellings of the neck can be associated with any closely related structures such as lymph nodes, thyroid, salivary glands, blood vessels, nerves or fat. A wide variety of disease may present but most relate to cervical lymph node enlargement.

For benign disease, most neck specimens are small excisional biopsies. Neck dissection is handled as for malignant disease⁴.

Lymph nodes – small nodes (up to 4 mm in maximum dimension) are embedded whole. Nodes up to 10 mm around the equator (around the girth) are bisected longitudinally through the hilum (or “bivalved”) and embedded in total. Nodes larger than 10 mm in the equatorial plane are sliced serially at approximately 4mm intervals and have 2 or 3 representative slices embedded.

Cysts - measure in three dimensions. Thyroglossal cysts usually present as a strip of fibrous tissue surrounded by fat and muscle.

Branchial cysts are typically submitted intact. Sufficient sampling of branchial cysts is required to rule out the possibility of a cystic metastatic carcinoma. Examine the cyst lumen for nodules and record the nature of contents and thickness of the cyst wall. Small specimens are bisected or embedded intact, while larger specimens are serially sliced and representative blocks (2-4) taken.

Soft tissue tumours and carotid body paragangliomas - these are not usually orientated and may be fragmented. Ink the external surfaces and measure:

- Dimensions of the specimen
- Dimensions of the tumour (if different from those of the specimen)
- Distance from tumour to the nearest surgical margin or to marked vessels and nerves.

Describe the tumour including the colour, whether encapsulated or infiltrative, and the presence of haemorrhage and necrosis. Serially slice the tumours into 4-5mm sections. Representative blocks include one block per cm of tumour. Record the presence of necrotic and haemorrhagic areas.

2. Sectioning

A single section per block is usually sufficient for diagnostic purposes.

Lymph nodes – usually one section per block (see also lymph node Tissue Pathway)..

3. Staining

H&E stains are required for all cases.

4. Further investigations

Immunohistochemistry may be useful for a range of neck lesions including for micro-metastases, to exclude lymphomas, and in the diagnosis of soft tissue tumours.

5. Report content

For cysts the report should record:

- Cyst lining i.e. keratinisation, presence of atypical features and mitotic figures
- nature of the capsule such as fibrous, fibro-myxoid and the degree of inflammation
- Presence of foreign body reaction to ruptured cysts.

For tumours the report should record:

- Type
- Tumour – tissue interface i.e. infiltrative or encapsulated
- Relationship to major vessels and nerves
- Nearest surgical margin.

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