MINIMUM STANDARDS FOR HANDLING AND REPORTING BREAST PATHOLOGY SAMPLES

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Foreword

It is my pleasure to write this foreword for the first ever document designed and formatted for the purpose of standardized reporting of breast pathology samples in Nigeria.

It is paramount now to have structured documents for standardized pathological reporting in Nigeria, and also across the entire West African sub- region.

This will facilitate standardized reporting of breast pathology and allow for correlation of results and collaboration amongst Pathologists across Nigeria. It will also enable us to compare data with our counterparts in other countries and regions of the world.

It is hoped that this initiative which was as a result of a workshop on breast pathology will be replicated to cover all other organ / systems of the human body.

This way, the ease of translational research will be guaranteed across the sub -region.

I must commend the team who worked assiduously to put together this document and must also particularly appreciate Roche for sponsoring the workshop that led to the production of this document.

We will ensure at the West African Division of the International Academy of Pathology that all other organ/ systems requiring similar standardized reporting are covered in the next few years.

I hope pathologists and clinicians across Nigeria and also the West African sub-region will find this document very useful.

Dr Yawale Iliyasu

President

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Introduction

Evidences have shown that there is no standard format for breast pathology reports in Nigeria. Standardization of pathology reporting systems is the optimal way to insure that information necessary for patient management are included in pathology reports. These include the assessment of prognostic and predictive factors such as grading and staging.
In May 2010, a meeting was convened in Nigeria bringing together Pathologists and Medical Laboratory Scientists from various states with different levels of expertise and practice, to discuss the modalities for standardization of breast pathology and immunohistochemistry and to draw up guidelines for the practice of breast pathology. The subcommittee was headed by Dr Oriolowo.
This document is the outcome of the workshop.
The goal of this activity is to describe and explain proper handling of breast tissue specimens to ensure accuracy of results and subsequent treatment decisions.
The ultimate goal is to improve patient outcomes by optimizing treatment on the basis of tumour characteristics.

Learning Objectives

Outline best practices with respect to obtaining breast tissue specimens, preserving and transporting for laboratory examination and analysis and specimen triage procedure.

SUMMARY OF THE MINIMUM CLINICAL INFORMATION REQUIRED TO BE SUBMITTED ON REQUEST FORM

1. **Name of Patient:** Surname, first and other names. These should be the full names and not nicknames
2. **Age and sex of Patient:** Preferably date of birth
3. **Clinical presentation:**

a. Palpable lump, nipple discharge, impalpable screen –detected lesion, cyclical breast pain
b. Duration of symptoms:
c. If it is a recurrence all information with regards to previous recurrences, prior surgery and treatments should be provided.
d. For referrals from other centres, it is mandatory that copies of previous reports and slides are reviewed by the pathologist in the centre before any form of treatment is given to the patient for medico legal reasons.

4. **Cytological diagnosis:** C1-5

C1- Unsatisfactory, C2- benign, C3- atypical, C4- suspicious of malignancy, C5- malignant

5. **Clinical Impression and findings:**

1. **Clinical findings:** Side and site of lesion: precise location of the lesion including side and quadrant including the presence or absence of lymph nodes and metastasis
2. **Clinical diagnosis:** S1-5

S1 = normal, fatty breast tissue, S2 = benign nodularity, S3 = indeterminate, probably benign, S4 = indeterminate, probably malignant, S5 = almost certainly malignant.

6. **Radiological features and diagnosis:**

1. **Radiological features:** micro calcification, asymmetric density, architectural distortion, mass lesion
2. **Radiological diagnosis:** R1-5

R1 = normal, fatty breast tissue, R2 = benign nodularity, R3 = indeterminate, probably benign, R4 = indeterminate, probably malignant, R5 = almost certainly malignant.

7. **Specimen type:**

1. Excision, incision, core needle biopsy mastectomy simple or radical should be stated
2. Lymph nodes: clearance, sentinel, etc.
8. **History of chemotherapy or hormonal therapy:** particularly HRT
9. **Whether patient is pregnant or lactating.**
10. **Family history**
11. **Any special requirements should also be stated:**
12. **Submission:** It is advisable that all samples are submitted intact and unfixed. If from a remote hospital, sample should be carefully sliced but not completely to maintain orientation and promptly fixed in 10 times its volume of 10% neutral formalin. Cotton wool can be placed in between the slices to aid prompt fixation. Time of fixation should be noted on the form

MINIMUM INFORMATION TO BE INCLUDED IN PATHOLOGY REPORT

1. **Organ site and type of operation**
2. **Primary tumour diagnosis**

a. Size of tumour
b. Histological grade
c. Extent of tumour

3. **Resection margins**
4. **Lymph nodes**

a. Number examined
b. Size of lymph nodes
c. Number positive

5. **Sentinel lymph nodes**

a. Number examined
b. Number positive

6. **Other findings**

a. Lymphatic and or vascular invasion
b. Benign breast disease

7. **Ancillary studies**

a. Oestrogen receptor expression determined by immunohistochemistry (clone should be specified) and is positive in invasive/in-situ carcinoma
(See appendix for quantitative quick score)
b. Progesterone receptor expression determined by immunohistochemistry (clone should be specified) and is positive in invasive/in-situ carcinoma
(See appendix for quantitative quick score)
c. HER-2/neu expression is determined by immunohistochemistry (clone , test), the result is as follows:

i. 0 Membrane staining for HER-2/neu in less than 10% of tumour cells
ii. 1+Faint/barely perceptible HER-2/neu membrane staining detected in more than 10% of tumour cells. The cells are only stained in part of the membrane
iii. 2+ Weak to moderate HER-2/neu complete membrane staining detected in more than 10% of tumour cells or <30% with strong staining
iv. 3+ Strong HER-2/neu complete membrane staining detected in >30% of tumour cells

Note- 0/1 reaction- Negative, 2+ reaction- Equivocal, 3+ reaction- Positive

d. HER-2/neu gene amplification is determined by fluorescence in-situ hybridization (test), the result is as follows:
i. Aneusomy, high amplification
ii. Aneusomy, low amplification
iii. Amplification is not identified
8. **Pathological staging using the TNM staging system**

HANDLING OF SPECIFIC SPECIMEN TYPES

Specimen types
1. FINE NEEDLE ASPIRATION BIOPSY
2. LARGE CORE NEEDLE BIOPSY
3. OPEN SURGICAL BIOPSY
a. Incisional
b. Excisional
4. NEEDLE LOCALIZATION EXCISIONAL BIOPSY SPECIMENS AND CORE NEEDLE BIOPSY
5. MASTECTOMY

LARGE CORE NEEDLE BIOPSY SPECIMENS

Specimen processing:
1. Needle or incision biopsy specimens are processed for histological examination in their entirety.
2. All cores are wrapped in paper on a flat surface and aligned parallel. Three levels are ordered

Limited information about the characteristics of a lesion is obtained from such small specimens. The samples are suitable for frozen section and immunohistochemical analysis of steroid receptors

INCISION BIOPSY

Incisional biopsy are almost always taken to evaluate unresectable invasive carcinomas to confirm the clinical diagnosis and to obtain sample for hormone receptor status. *A cautery type scalpel must not be used in obtaining an incisional biopsy*.
Specimen processing:
1. The entire sample is processed.
2. Indicate the number of fragments submitted.

EXCISION BIOPSY FOR PALPABLE LUMPS.

1. Submit specimen intact, and unfixed
2. Measure the specimen before cutting and weigh (over 50 g) noting any orientating sutures.
3. Orient specimen on bench anteroposteriorly, noting superior, inferior, medial and lateral margins. Unorientated specimen maybe inked all black. Orientated specimen should be inked with coloured inks to identify specific margins. Contact surgeon if there is any ambiguity about the orientation of sample before processing.
4. Blot dry, apply India ink to surface, and blot dry again.
5. If indicated, take a radiograph of the specimen.

6. Section specimen: if specimen is 3 cm or smaller, cut 3- to 4-mm slices; record no of slices
7. If it is larger, bisect specimen transversely, fix the residual hemispheres for 1 to 2 hours, place cut surface down, and take sagittal blocks through superior and inferior portions.
8. Take a sample for hormone receptor studies/molecular studies/EM
9. Description
a. Dimensions, shape and consistency of specimen
b. Appearance of cut sections: fibrosis, cysts (size, number, content), calcification, tumour masses (size in three dimensions, colour, borders, consistency, necrosis, distance from surgical margins)
10. Sections for histology
a. Small specimens: submit in their entirety (up to five cassettes).
b. Larger specimens: thorough sampling. At least two thirds of the breast tissue (exclusive of adipose tissue) should be processed.
c. This should include any grossly visible lesions and the 6 inked surgical margins.
d. If no gross lesion is evident, submit at least 10 cassettes including the most fibrous areas rather than pure adipose tissue. If carcinoma in situ or atypical hyperplasia is found in this tissue the entire specimen is submitted for histopathological examination.
11. Margins of the tumour and surrounding breast - lymphatic emboli and in situ carcinoma outside the lesion. Take at least 2 sections from each margin. The sections may be put in the same cassette as the tumour.
12. If skin is in present submit at least 1 section,
13. Do not take tissue for special studies or research unless a definitive diagnosis of invasive carcinoma has been established.

MAMMOGRAPHICALLY DIRECTED EXCISION

Procedure

1. Specimen should not be frozen *because* of calcifications
2. Obtain radiograph of intact specimen.
3. Measure specimen before cutting.
4. Blot dry, apply India ink to surface, and blot dry again.
5. Slice specimen through the equatorial plane at 3- to 4-mm intervals.
6. Obtain radiograph of sliced specimen. Some authors have found the use of a Perspex grid useful for the subsequent localization of the lesion.
7. Label slices on the radiograph.
8. Take a sample for hormone receptor analysis only if tumour is grossly visible and of sufficient size.

Specimen radiograph

Specimen radiography: Specimen radiography confirms the presence of the calcification within the specimen. Final pathology is atypical ductal hyperplasia with associated micro calcifications and intraductal papilloma

Core needle biopsy specimen with India ink

Description

1. Dimension and consistency of specimen
2. Appearance of cut sections: fibrosis, cysts (size, number, content), calcification, tumour

masses (size in three dimensions, colour, borders, consistency, necrosis, distance from surgical margins)
Sections for histology
1. Submit in its entirety. Label cassettes as in the radiograph.
2. Multiple tissue levels of the CNB specimen are often required for histological examination.

NEEDLE LOCALIZATION EXCISION BIOPSY
1. After excision biopsy, the surgeon orients the specimen and hand delivers it to the pathology department.
2. The pathologist inks the lateral, medial, superior, inferior, superficial, and deep margins of the specimen in a colour-coded fashion.
3. A radiograph of the specimen is obtained to confirm the adequacy of the excision.
4. If the mass or micro calcifications are not noted on radiographs of the serial sections, further excision is performed.

MASTECTOMY

Procedure:

A. **First day**

1. Weigh the specimen.
2. Orient the specimen. In radical mastectomy cases, use the axillary fat as a marker for the lateral side and the surgical section of the muscle as a marker for the upper side.
3. Place the specimen on the cutting board, posterior side up, with its most inferior point toward the dissector.
4. The specimen is oriented as if the dissector were standing behind it.
5. Dissect lymph nodes groups
6. Turn the specimen around, with skin side up and the 6 o'clock position nearest the dissector
7. Evaluate features of the external appearance and measure. Palpate the specimen for masses or nodularity.
8. With a water-resistant marker, divide the breast specimen into four quadrants: upper outer, lower outer, lower inner, and upper inner.
9. With a long sharp knife, cut the entire breast longitudinally into slices about 2 cm thick. One of the cuts should be exactly through the level of the nipple.
10. Lay out the slices in order on a flat surface, maintaining orientation. Examine each slice carefully; take photographs and x-ray film, if indicated. Take a sample for hormone receptor studies,
11. Fix all the slices overnight, keeping their orientation either by laying them flat sequentially in a long pan (preferable) or by stringing them together

B. **Second day**

1. Lymph node specimens: shred the axillary tissue and dissect out all lymph nodes,
2. Nipple specimen: if following fixation the nipple is erect, if it is retracted or inverted, cut several parallel sections, about 2 to 3 mm apart, perpendicular to the skin surface through the nipple and areola.
3. Breast specimen: re-examine the slices, make additional cuts, if necessary, and take sections for histology.

Description

Side (right or left) and type of mastectomy

A. List of structures included in specimen: skin, nipple, breast, major and minor pectoralis muscles, fascia, axillary tissue, chest wall structures

B. Weight and dimensions (greatest length of skin and length perpendicular to it)

C. Features of external appearance: a. Shape and colour of skin

D. Location and extent of skin changes (scars, recent surgical incisions, erythema, oedema, flattening, retraction, ulceration)

E. Appearance of nipple and areola (erosions, ulceration, retraction, inversion)

F. Location of lesions and other features, which can be designated by stating their distance from nipple and quadrant on their direction in clock face numerals

G. Description of abnormalities on palpation, if any

H. Features of cut sections:

a. Relative amounts of fat and parenchyma

b. Cysts and dilated ducts: size, number, location, content

c. Masses: quadrant and distance from nipple, depth beneath skin, size, shape, consistency, colour; presence or absence of necrosis, haemorrhage or calcification, relation or attachment to skin, muscle, fascia, or nipple

d. Lymph nodes, if present: number of nodes in each group, size of largest node in each group, and sizes and locations of nodes containing grossly evident tumour

I. Sections for histology

a. Breast: take three sections of tumour; sample all lesions noted grossly or radiographically; take at least one section from each quadrant

b. Nipple: submit entire specimen in sections

c. Pectoralis major muscle (in radical mastectomies): take one section from any grossly abnormal area or, if none is found, from the area closest to the tumour.

d. Lymph nodes: all identified nodes should be processed for histology. Small nodes are submitted entirely; nodes over 0.5 cm in diameter are sliced.

e. If the axillary fat is grossly involved, a representative section should be taken.

Axillary lymph nodes

1. The lymph node groups should be tagged or submitted as separately identified specimens.
2. Careful manual dissection of the unfixed axillary fat is the most cost- effective method for isolating lymph nodes. Considerable time and expense required for clearing is not cost effective.
3. Avoid stating that metastases are grossly present or absent.

Specimen Radiography

1. Lesions that contain calcifications.
2. Clinical mammographic findings. negative- :
3. Alterations in parenchymal pattern, skin changes, vascular abnormalities, and ill-defined mass lesions
4. Specimen compression devices have proven useful for localizing non calcified lesions in specimen radiographs.
5. Compare specimen X-ray with mammogram
6. Perform intraoperatively.
7. Colourless calcium oxalate crystals are difficult to identify in haematoxylin and eosin

stained sections with regular light microscopy

Intraoperative Thermal (Electrocautery) Damage to Biopsy Specimens
This reduces oestrogen receptor activity (FN).Thermal damage is maximal at or limited to the surface of the specimen. The artefacts interfere with the assessment of nuclear and histological grade as well as distort distinction between normal, hyperplasic, and neoplastic tissue
Cautery effect
1. Most of the epithelium in this duct shows thermal artefacts which make it impossible to distinguish between hyperplasia and intraductal carcinoma. Parallel linear cracks are typical of thermal artefacts.
Extreme thermal damage is seen at the edge of a specimen. Coagulation of proteins in the stroma causes the marginal tissue to stain more deeply. IHC Assay Total Test Concept

TOWARDS STANDARDIZATION OF TESTING
The three standard tumour markers for the management of patients with breast cancer are oestrogen receptor (ER), progesterone receptor (PR) and Human Epidermal growth factor receptor 2 (HER2). The clinical significance of ER, PR and HER2 has rendered their assessment in primary invasive breast cancer mandatory.
The accuracy of the method used determines the appropriate application of treatment and leads to significant improvements in survival.
Standardized testing is only possible after all aspects of ER, HER2 testing—pre-analytical, analytical, and post-analytical, have been closely controlled.

Pre-analytical
Test selection
Specimen type, acquisition, transport time
Fixation: type and time
Tissue processing, type, and temperature

Analytical
Immunohistochemical procedure
Protocol, control selection
Regent validation
Technician training/certification
Laboratory certification

Post-analytical
Control evaluation
Results interpretation
Results reporting
Pathologist, experience and CME

General concepts
The warm and cold ischemic times are widely accepted as important variables in the

analysis of labile macromolecules such as proteins, RNA, and DNA from clinical tissue samples. The pathologist should effectively communicate this priority to all members of the breast care management team so processes are put in place to make sure these times are routinely recorded. The time from tumour removal to fixation should be kept to # 1 hour to comply with these recommendations.

TISSUE HANDLING

Optimal tissue handling requirements for HER2

1) It was recommended that the pathologist be informed before going for surgery, the operation list could also be sent to the lab to help the Pathologist make adequate preparation to receive samples.
2) 10% Neutral Buffered formalin should be the standard fixative. Should be supplied fresh weekly to the theatre. Surgeons to avoid using old stock."
3) Specimens should be placed IMMEDIATELY after harvesting in the appropriate amount of formalin and sent same day to the histopathology laboratory. Specimens should be processed within 24 hours of receipt in the laboratory. Care must be taken to ensure that the specimen fixes for at least 6 hours and not left in the fixative for more than 48 hours before processing begins.
4) Specimen is to be fixed immediately in appropriate amounts of formalin. If tumour > 2cm, cut should be made into the lesion serially to aid permeation of formalin.
5) The volume of fixative should be at least five times the volume of the specimen the volume could be changed if it becomes bloody after an hour.
6) Use an adequate sided container; place gauze at the bottom of the container to allow formalin permeates also from below.
7) Time and date of fixation should be noted. Axillary tissue should be properly identified, if separate from the rest of the specimen.

Optimal tissue handling requirements for ER/PR testing

Accession slip and report must include guideline-detailed elements.
Time from tissue acquisition to fixation should be as short as possible.
Samples for ER and PR testing are fixed in 10% NBF for 6 to 72 hours.
Samples should be sliced at 5-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of NBF to allow adequate tissue penetration. If tumour comes from remote location, it should be bisected through the tumour on removal and sent to the laboratory immersed in a sufficient volume of NBF. Cold ischemia time, fixative type, and time the sample was placed in NBF must be recorded. Storage of slides for more than 6 weeks before analysis is not recommended. Time tissue is removed from patient, time tissue is placed in fixative, duration of fixation, and fixative type must be recorded and noted on accession slip or in report.

FIXATION

Type of fixative

1. Only 10% Neutral buffered formalin (NBF) should be used as the fixative for breast tissue specimen

2. Higher or lower concentrations of NBF are not acceptable.
3. This recommendation is based on published literature regarding the expected or characteristic immunoreactivity for ER in breast cancer.
4. If the laboratory uses a formalin alternative for fixation, the assay must be validated against NBF fixation.

Duration of fixation

1. Breast tissue specimens must be fixed in 10% NBF for no less than 6 hours and for not more than 72 hours before processing. Complete tissue fixation usually requires 24 hours,
2. Under fixation of breast tissue may lead to false-negative ER results.
3. Over fixation is likely to be less problematic than under fixation but potentially could also lead to false-negative results.
4. Fixation of small specimens.

a. Needle core biopsies -often placed in formalin in a more timely fashion, will infiltrate more quickly because of their size, and thus may be exposed to more uniform and consistent tissue fixation.

5. If core samples are large and representative of the resection specimen,

The Panel recommends that such samples preferably be used for ER and PR analyses if they have been fixed a minimum of 6 hours in 10% NBF.

Conclusion

If these guidelines and measures developed for breast specimens handling as well as for testing of ER, PR, and HER2 status are strictly complied with within the limits of local constraints, it is expected that there will be an improvement in the performance of laboratories using these and future predictive testing methods. The ultimate goal will be an improvement of patient outcomes by optimizing treatment on the basis of tumour characteristics.

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Appendix I- WHO Classification of breast tumours (2003)

Epithelial Tumours

- Invasive ductal carcinoma, not otherwise specified (NOS) - 8500/3
  Mixed type carcinoma
  Pleomorphic carcinoma - 8022/3
  Carcinoma with osteoclastic giant cells - 8035/3
  Carcinoma with choriocarcinomatous features
  Carcinoma with melanotic features
- Invasive lobular carcinoma - 8520/3
- Tubular carcinoma - 8211/3
- Invasive cribriform carcinoma - 8201/3
- Medullary carcinoma - 8510/3
- Mucinous carcinoma and other tumours with abundant mucin
  Mucinous carcinoma - 8480/3
  Cystadenocarcinoma and columnar cell mucinous carcinoma - 8480/3
  Signet ring cell carcinoma - 8490/3
- Neuroendocrine tumours
  Solid neuroendocrine carcinoma
  Atypical carcinoid tumour - 8249/3
  Small cell / oat cell carcinoma - 8041/3
  Large cell neuroendocrine carcinoma - 8013/3
- Invasive papillary carcinoma - 8503/3
- Invasive micropapillary carcinoma - 8507/3
- Apocrine carcinoma - 8401/3
- Metaplastic carcinomas - 8575/3
  Pure epithelial metaplastic carcinomas - 8575/3
    Squamous cell carcinoma - 8070/3
    Adenocarcinoma with spindle cell metaplasia - 8572/3
    Adenosquamous carcinoma - 8560/3
    Mucoepidermoid carcinoma - 8430/3
  Mixed epithelial/mesenchymal metaplastic carcinomas - 8575/3
- Lipid-rich carcinoma - 8314/3
- Secretory carcinoma - 8502/3
- Oncocytic carcinoma - 8290/3
- Adenoid cystic carcinoma - 8200/3
- Acinic cell carcinoma - 8550/3
- Glycogen-rich clear cell carcinoma - 8315/3
- Sebaceous carcinoma - 8410/3
- Inflammatory carcinoma - 8530/3
- Lobular neoplasia
  Lobular carcinoma in situ - 8520/2
- Intraductal proliferative lesions
  Usual ductal hyperplasia

Flat epithelial atypia
Atypical ductal hyperplasia
Ductal carcinoma in situ - 8500/2
- Microinvasive carcinoma
- Intraductal papillary neoplasms

Central papilloma - 8503/0
Peripheral papilloma - 8503/0
Atypical papilloma
Intraductal papillary carcinoma - 8503/2
Intracystic papillary carcinoma - 8504/2
- Benign epithelial proliferations

Adenosis including variants: sclerosing adenosis, apocrine adenosis, blunt duct adenosis, microglandular adenosis, adenomyoepithelial adenosis
Radial scar / complex sclerosing lesion
Adenomas
Tubular adenoma - 8211/0
Lactating adenoma - 8204/0
Apocrine adenoma - 8401/0
Pleomorphic adenoma - 8940/0
Ductal adenoma - 8503/0

Myoepithelial lesions
- Myoepitheliosis
- Adenomyoepithelial adenosis
- Adenomyoepithelioma - 8983/0
- Malignant myoepithelioma - 8982/3

Mesenchymal Tumours
- Haemangioma - 9120/0
- Angiomatosis
- Haemangiopericytoma - 9150/1
- Pseudoangiomatous stromal hyperplasia
- Myofibroblastoma - 8825/0
- Fibromatosis (aggressive) - 8821/1
- Inflammatory myofibroblastic tumour - 8825/1
- Lipoma - 8850/0

Angiolipoma - 8861/0
- Granular cell tumour - 9580/0
- Neurofibroma - 9540/0
- Schwannoma - 9560/0
- Angiosarcoma - 9120/3
- Liposarcoma - 8850/3
- Rhabdomyosarcoma - 8900/3
- Osteosarcoma - 9180/3
- Leiomyoma - 8890/0
- Leiomyosarcoma - 8890/3

Fibroepithelial Tumours
- Fibroadenoma - 9010/0
- Phyllodes tumour - 9020/1
  Benign - 9020/0
  Borderline - 9020/1
  Malignant - 9020/3
- Periductal stromal sarcoma, low grade - 9020/3
- Mammary hamartoma

Tumours of the nipple

- Nipple adenoma - 8506/0
- Syringomatous adenoma - 8407/0
- Paget disease of the nipple - 8540/3

Malignant lymphoma

- Diffuse large B cell lymphoma - 9680/3
- Burkitt lymphoma - 9687/3
- Extranodal marginal-zone B-cell lymphoma of MALT type - 9699/3
- Follicular lymphoma - 9690/3

Metastatic tumours - Tumours of the male breast
- Gynaecomastia
- Carcinoma
  Invasive - 8500/3
  In situ - 8500/2

Appendix II-Tumour Grading

A. **Modified Scarff- Bloom- Richardson grading of breast carcinoma**

Feature	**score**
Tubule formation	
Majority of tumour (>75%)	1
Moderate degree (10-75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small regular uniform cells (size of normal cells uniform chromatin)	1
Moderate increase in size and variability (open vesicular nuclei with visible nucleoli)	2
Marked variation especially large bizarre nuclei (vesicular with prominent often multiple nucleoli)	3
Mitotic count	
0to 10/10HPF	1
11 to 19 /10 HPF	2
>20/10 HPF	*3*

Assess mitotic count at the periphery of the tumour. Count at least 10 fields.

Overall Tumour Grade

3 to 5 points Grade I, well differentiated

6 to 7 points grade II, moderately differentiated

8 to 9 points Grade III, poorly differentiated.

Note: *all tumours should be graded regardless of histological types.*

B. **Grading of phyllodes tumour**

FEATURE	**LOW GRADE**	**INTERMEDIATE GRADE**	**HIGH GRADE**
Cellularity	Mildly cellular	More cellular	Usually highly cellular
Cellular and nuclear pleomorphism	Minimal	More evident	May be marked
Mitotic rate	0-1/10HPF	2-5/10HPF	>5/10HPF
Stromal overgrowth	Absent or focal	Often present	Often marked (differential diagnosis of sarcoma)
Borders	Pushing	Invasion into stroma+	Invasion into stroma +++
Heterologous elements	Benign lipomatous	Benign lipomatous	Malignant sarcomatous

HPF = high power field

Appendix III- TNM staging of breast tumours
Primary tumour (T)

● **TX:** Primary tumour cannot be assessed (includes cases with tumour present at margin by **macroscopic** examination, because total extent of tumour cannot be assessed)
● **T0:** No evidence of primary tumour
● **Tis:** Carcinoma in situ
● **Tis (DCIS):** Ductal carcinoma in situ
● **Tis (LCIS):** Lobular carcinoma in situ
● **Tis (Paget's):** Paget's disease of the nipple NOT associated with invasive carcinoma or carcinoma in situ (DCIS or LCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget's disease are categorized based on the size and characteristics of the parenchymal disease, although the presence of Paget's disease should still be noted.
● **T1:** Tumour 2 cm (20 mm) or less in greatest dimension
● **T1mic:** Tumour 1 mm or less in greatest dimension (if multiple foci of microinvasion, use largest focus and add a comment, don't add sizes of individual foci)
● **T1a:** Tumour more than 1 mm, but 5 mm or less in greatest dimension
● **T1b:** Tumour more than 5 mm, but 10 mm (1 cm) or less in greatest dimension
● **T1c:** Tumour more than 1 cm, up to 2 cm in greatest dimension
● **T2:** Tumour more than 2 cm, up to 5 cm in greatest dimension
● **T3:** Tumour more than 5 cm in greatest dimension
● **T4:** Tumour of any size with direct extension to chest wall or skin as described below (invasion of dermis alone does not qualify as T4)

T4a: Extension to chest wall, not including only pectoralis muscle adherence/invasion

T4b: Ulceration or ipsilateral satellite nodules or oedema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma

T4c: Both T4a and T4b

T4d: Inflammatory carcinoma (clinical diagnosis characterized by diffuse erythema and oedema [peau d'orange] involving a third or more of the skin of the breast; skin changes are due to lymphedema caused by tumour emboli within dermal lymphatics, which may not be obvious in a small skin biopsy; however, tissue diagnosis is necessary to demonstrate an invasive carcinoma in the underlying breast parenchyma or at least in the dermal lymphatics; note that either tumour emboli in dermal lymphatics or locally advanced breast cancers directly invading the dermis or ulcerating the skin, without the clinical skin changes described above, do NOT qualify as inflammatory carcinoma. Dimpling of the skin, nipple retraction or any other skin changes except those described under T4b and T4d may occur in T1-3 without changing the classification.

Notes:
● Measure invasive component only, not DCIS
● T classification traditionally assumes there was no prior treatment; can stage after preoperative (neoadjuvant, primary) chemotherapy, but should indicate that prior treatment was received (**J Natl Cancer Inst 2005; 97:1137**)

● pT classification requires pathological examination of a primary carcinoma with no **gross** tumour at resection margins (but can classify if only microscopic tumour is present at resection margin)
● If tumour size is slightly less than or greater than a cut-off for a given T classification, it is recommended that the size be rounded to the millimetre reading that is closest to the cut-off.
● Can attempt to reconstruct original tumour size if multiple biopsies/excisions; due to difficulties in adding sizes from two resections, may want to report "at least pT_, a more accurate estimate may be based on imaging studies"
● If there are multiple simultaneous, macroscopically measurable, ipsilateral invasive tumours, use largest size, don't sum sizes (but add a comment)
● Tumour in pectoralis muscle should be measured with the breast tumour to determine the tumour size and T category
● Simultaneous bilateral breast carcinomas are staged as separate primaries in separate organs
● Gross measurement is recommended (either fresh or fixed); however if significant in situ disease is present or invasive tumour extends microscopically beyond the grossly measured mass, then microscopic measurements may be more accurate; using microscopic measurements only is discouraged, because processing artefact may cause significant tissue expansion or shrinkage (**Hum Pathol 2005;36:756**)

Regional lymph nodes (pN)
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Note: IHC means immunohistochemistry, ITC means individual tumour cells

● **pNX:** Regional lymph nodes cannot be assessed (e.g., previously removed or not removed for pathological study)
● **pN0:** No regional lymph node metastasis identified histologically
● **pN0 (i-):** No regional lymph node metastases histologically, negative IHC
● **pN0 (i+):** Malignant cells in regional lymph node(s) no greater than 0.2 mm (detected by H&E or IHC including ITC)
● **pN0 (mol-):** No regional lymph node metastases histologically, negative molecular findings (RT-PCR)
● **pN0 (mol+):** Positive molecular findings (RT-PCR), but no regional lymph node metastases detected by histology or IHC
● **pN1:** Micrometastases; or metastasis in 1-3 axillary lymph nodes; OR in internal mammary nodes with metastases detected by sentinel lymph node biopsy but not clinically detected apparent
● **pN1mi:** Micrometastases (greater than 0.2 mm or more than 200 cells, but none greater than 2.0 mm)
● **pN1a:** Metastasis in 1-3 axillary lymph nodes, at least one metastasis greater than 2.0 mm
● **pN1b:** Metastasis in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected
● **pN1c:** Metastasis in 1-3 axillary lymph nodes and in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not

clinically detected
● **pN2:** Metastasis in 4-9 axillary lymph nodes; or in clinically detected internal mammary lymph nodes in the absence of axillary lymph node metastasis
● **pN2a:** Metastasis in 4-9 axillary lymph nodes (at least one tumour deposit larger than 2.0 mm)
● **pN2b:** Metastasis in clinically detected internal mammary lymph nodes in the absence of axillary lymph node metastases
● **pN3:** Metastasis in 10 or more axillary lymph nodes; or in infraclavicular (level III axillary) lymph nodes, or in clinically detected ipsilateral internal mammary lymph nodes in the presence of one or more positive level I or II axillary lymph nodes; or in more than 3 axillary lymph nodes and in internal mammary lymph nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected; or in ipsilateral supraclavicular lymph nodes
● **pN3a:** Metastasis in 10 or more axillary lymph nodes (at least one tumour deposit greater than 2.0 mm); or metastasis to the infraclavicular (level III axillary) lymph nodes
● **pN3b:** Metastases in clinically detected ipsilateral internal mammary lymph nodes in the presence of 1 or more positive axillary lymph nodes; or in 4 or more axillary lymph nodes and in internal mammary lymph nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected
● **pN3c:** Metastasis in ipsilateral supraclavicular lymph nodes

Notes:
● "i" stands for isolated tumour cells (ITC); "i+" means small clusters of tumour cells detected by H&E or IHC that are 0.2 mm or less (**Am J Surg Pathol 2005;29:136 (author reply)** or fewer than 200 cells in a single histological cross-section); isolated tumour cells are not counted as a positive node below
● In practice, the distinction between ITC and micrometastases is often difficult and without prognostic significance (**Cancer 2008; 112:1672**)
● Classification based solely on sentinel lymph node biopsy without subsequent axillary lymph node dissection is designated (sn) for "sentinel node", for example, pN0(sn)
● "Clinically detected" is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination, and having characteristics highly suspicious for malignancy or a presumed pathological macrometastasis based on fine needle aspiration biopsy with cytological examination
● Tumour in axillary fat without evidence of residual lymph node tissue is classified as a positive axillary lymph node
● Lymph node ratio (LNR, the ratio of positive over excised lymph nodes) is suggested as an alternative to pN staging (**J Clin Oncol 2009; 27:1062**)
● requires resection and examination of at least the low axillary lymph nodes (level I)

Distant Metastasis (M)
● **M0:** No clinical or radiographic evidence of distant metastases; includes M0(i+)
● **cM0(i+):** No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumour cells in circulating blood, bone marrow or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases

● **M1:** Distant detectable metastases as determined by classic clinical and radiographic means or histologically proven larger than 0.2 mm

Notes:
● M0 is a clinical diagnosis, pM0 is an invalid designation
● Stage designation may be changed if postsurgical imaging studies reveal the presence of distant metastases, providing that the studies are carried out within 4 months of diagnosis in the absence of disease progression and provided that the patient has not received neoadjuvant therapy

APPENDIX IV- THE SEMIQUANTITATIVE QUICK SCORE METHOD OF EVALUATION (FOR ER AND PR)

With this method, the intensity of the immunohistochemical reaction as viewed under the light microscope is recorded as either:
0, negative (no staining of any nuclei even at high magnification);
1, weak (only visible at high magnification);
2, moderate (readily visible at low magnification); or
3, strong (strikingly positive even at low power magnification).
The proportion of tumour nuclei showing positive staining was also recorded as either:
zero (0),
approximately 1–25% (1),
26–50% (2),
51–75% (3), or
76–100% (4).
The score for intensity is then added to the score for proportion, giving the quick score with a range of 0–7.