REVIEW ARTICLE
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Optimizing pathological assessment of breast cancer in Brazil: recommendations from a multidisciplinary working group on the tumor-tissue journey

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ABSTRACT

Timely and correct assessment of histopathological, immunohistochemical and molecular features of biopsy and surgical specimens is of paramount importance in the provision of care to patients with breast cancer, particularly in the current era of precision oncology. In order to ensure that tissue samples are obtained, processed, analyzed and reported in an optimal way, a concerted effort is required by institutions and individuals, taking into account state-of-the-art scientific and technical knowledge and circumventing logistic and operational constraints. This may be particularly challenging in some settings due to several sources of economic, structural, organizational and communication inefficiencies. In the current article, we present a brief review of breast cancer epidemiology and challenges in the disease diagnosis, especially in Brazil, and report the results of a multidisciplinary working group convened in May 2020 in an expert panel to identify and discuss the barriers and challenges related to the journey of breast cancer samples in Brazil. Following the identification of the issues, the working group also discussed and proposed recommendations for improving the journey and quality of breast cancer samples based on their professional experience and the current scientific literature, including guidelines of national and international health organizations (e.g. World Health Organization), consensus of medical societies and other published literature on the topic. We outline the most salient issues related to that journey in Brazilian public and private medical institutions, based on the experts' clinical experience, since all of them are actively working at both sectors, and discuss current recommendations to address these issues aiming at mitigating and preventing preanalytical and analytical issues affecting diagnostic and therapeutic decisions. Such issues are grouped under four headings pertaining to education, communication, procedures in the operating room and sample transportation, and procedures in the pathology laboratory. Selected recommendations based on the current literature and discussed by the group of Brazilian experts are reviewed, which may mitigate the issues identified and optimize diagnostic and therapeutic decisions for patients with breast cancer, currently the most frequent malignant tumor worldwide and in Brazil. This paper has been submitted and published jointly, upon invitation and consent, in both the *Surgical and Experimental Pathology* and the *Mastology* journals.

KEYWORDS: breast neoplasms; specimen handling; pathology; interdisciplinary communication; treatment outcome; precision medicine.

INTRODUCTION

With an estimated 2.3 million new cases every year, breast cancer is currently the most frequent non-cutaneous malignant tumor worldwide[1]. Breast cancer currently accounts for one in four new cancer cases and one in six cancer deaths among women worldwide[1], and one in eight women born in developed countries are expected to develop the disease in their lifetime[2]. The burden of breast cancer continues to increase worldwide, particularly in developing countries, notwithstanding the great achievements of the past decades in terms of mammographic screening, increased understanding of genetic and environmental risk factors, and treatment[1,3,4]. Like many countries, Brazil

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faces an increasing challenge in providing health care to cancer patients; in this country, breast cancer is now the most frequent non-cutaneous malignant tumor in both sexes combined[5], but several barriers need to be overcome in the attempt to provide comprehensive diagnosis and treatment for our patients at the national level[6-8]. Moreover, Brazil has a dual health-care system, whereby nearly 75% of the population relies on medical care provided by a government-funded public system, and the remaining 25% has access to private health insurance[9]. Despite the attempts of the public system to provide full and comprehensive care to all citizens, access to health care in Brazil is very heterogeneous.

One of the greatest recent changes in our understanding of breast cancer has been the creation of a molecular taxonomy with diagnostic and therapeutic implications[4,10,11]. As a result, systemic treatment for molecularly defined subtypes of breast cancer has led to an increasingly complex decision tree for the management of patients with early-stage, locally advanced and metastatic disease[12-18]. This approach to treatment has paved the way to precision oncology, marked by the development of monoclonal antibodies and signal-transduction inhibitors of several relevant pathogenic alterations found in breast cancer and other tumor types. Thus, therapeutic decisions are now guided by comprehensive analysis of such alterations, and the molecular profile of each patient's tumor now routinely accompanies histopathological assessment[19,20]. Moreover, biological features (tumor grade, estrogen and progesterone receptors [ER and PR] and HER2 expression) and gene expression-based assays with prognostic relevance are now included in the 8th edition of the American Joint Committee on Cancer staging manual for breast cancer[21]. Finally, reliance on genotypic and molecular phenotypic features is only likely to increase in the future, as a result of the increasing role played by precision oncology in the treatment of patients with breast cancer[22-25].

For all these reasons, timely and correct assessment of histopathological, immunohistochemical (IHC) and molecular features of biopsy and surgical specimens is of paramount importance in the provision of care to patients with breast cancer. As a result, a concerted effort needs to be continuously undertaken by institutions and individuals in order to ensure that tissue samples are obtained, processed, analyzed and reported in an optimal way that takes into account state-of-the-art scientific and technical knowledge and circumvents logistic and operational constraints. This may be particularly challenging in some settings due to several sources of inefficiency in terms of economic, structural, organizational and communication features that preclude optimal pathological assessment of tumor specimens. In the current article, we present the issues related to the journey of breast cancer samples in Brazil that were identified and discussed by a working group convened in an expert panel and review important recommendations selected by the group based on the current literature and guidelines and also on their professional experience to address these issues. This paper is part of a larger initiative that aims to improve the health-care journey of breast cancer patients in Brazil[6]. The article was developed through a collaboration between members of the Brazilian Society of Pathology, Brazilian Society of Mastology, Brazilian Society of Histotechnology, and Brazilian Society of Operating-Room Nurses, and has been published jointly by invitation and consent in both, the *Surgical and Experimental Pathology* and *Mastology* journals

METHODS

Composition, objectives and funding of the working group

The multidisciplinary working group was composed of two pathologists (HG and FMC), one breast surgeon (RMSR), one oncology nurse (MIK), and one histotechnologist (DLP) from Brazil with experience or professional focus on breast cancer. The five members work in large hospitals/services located in four states of two different regions of the country. The working group convened in May 2021 in an expert panel upon invitation from Roche Produtos Químicos e Farmacêuticos, Brazil, who also had representatives attending the meeting with the aim of organizing it. The working group attempted to identify the most salient issues related to the breast cancer tumor-tissue journey in Brazilian public and private medical institutions, based on their experience, since all of them actively work at both sectors, and discussed the current scientific literature, with the main objective of selecting and reviewing recommendations that may mitigate and prevent preanalytical, analytical, and post-analytical issues that may affect diagnostic and therapeutic decisions. The financial sponsor had no influence on the discussions during the expert panel. Hence all the recommendations reviewed here and the writing of this article rest under the entire responsibility of the authors.

Issues identified and discussed by the working group

The preanalytical, analytical, and post-analytical issues discussed by the working group members were grouped under the four headings presented below and summarized in Table 1.

Professional education and awareness

Adequate knowledge on the part of the various individuals impacted by the tumor-tissue journey is a prerequisite for all the procedural steps required in this process. Each individual needs to understand the process as a whole and in its different steps, their own role, and the roles of others. Table 1 displays the specific issues identified by the experts based on their professional experience; the prevention or resolution of these issues

Table 1. Categories and issues identified as critical for optimizing the tumor-tissue journey.

Categories of issues	Specific issues
Education	• Lack of awareness of the problem • Insufficient knowledge of the various steps of the process • Lack of attribution of clear roles for each team member • Lack of standardization of procedures • Insufficient training
Communication	• Lack of communication among team members • Lack of communication among institutional sectors or departments • Lack of attribution of clear roles for each sector or department • Insufficient provision of information to, or lack of access to, the pathologist • Insufficient provision of feedback by the pathologist
Operating room and transport	• Unduly long time before the sample reaches the laboratory • Distance between laboratory and hospital • Insufficient basic infrastructure, leading to the use of improper containers for sample conditioning and inadequate fixation procedures • Insufficient technological infrastructure, e.g., for digitalizing information • Individual dynamics of operating rooms, e.g., with regard to time-out • Logistic bottlenecks in some institutions • Heterogeneity in organization systems • Incorrect or incomplete labeling of the specimen • Incorrect or incomplete forms accompanying the sample • Poorly designed forms • Lack of standardized identification packaging containing the specimen • Incorrect packaging of the specimen, including omission of buffered formalin • Unduly long-time outside formalin, and use of non-buffered formalin • Inadequate fixation or amount of formalin given sample dimensions • Delayed transportation of the sample to the laboratory
Pathology laboratory	• Insufficient information upon receipt of sample • Incomplete or unclear specification of procedures • Incomplete information regarding time of tissue collection and immersion in formalin • Delay in gross examination and sampling before fixation • Frequent change in provider in public hospitals outsourcing pathology services

can be accomplished with continued education, the creation of standardized operating procedures, and participation in external quality assurance programs. Moreover, institutional buy-in is paramount, because the process cannot rely simply on the goodwill of a few key persons. Institutions need to recognize their role in fostering professional education and awareness, as well as enforcing operating procedures.

Communication and integration within teams

In addition to awareness of their roles in the process, individuals must establish adequate communication with other team members; likewise, adequate communication among institutional sectors or departments is vital, and managers should work to ensure the necessary procedures and infrastructure. This may be particularly critical in publicly funded institutions, where the organization of roles and structures may depend on several layers of administration. Importantly, there must be a two-way communication between the pathologist and the rest of the team, in the sense that the relevant medical and practical information needs to be provided to the pathologist, who in turn must provide feedback to the team about sample quality and issues that may arise. There is often insufficient provision of relevant details, even on the part of surgeons, and this may preclude optimal interpretation of findings. Table 1 summarizes the communication issues identified by the task force members.

Procedures in the operating room and sample transportation

Table 1 also summarizes the key issues identified by the working group members regarding the procedures required in the operating room with the aim of optimizing the quality of the sample. A key issue in some institutions is the unduly long time taken before the sample reaches the laboratory, sometimes due to internal organization of the operating room or due to the physical distance between the hospital and the laboratory where samples will be processed and analyzed. In some cases, insufficient technology, e.g., lack of electronic medical records and barcode system for digitizing information, may increase that time. Other issues may also contribute to that increase, including individual institutional features that may create additional bottlenecks. Once again, institutional will is of paramount importance toward ensuring adequate and streamlined procedures that may ensure the minimum possible time between sample collection and delivery to the laboratory, and the best possible handling of the sample during that journey.

Issues related to sample identification, labeling, conditioning and transportation may occur from sample removal to its

delivery to the pathology laboratory (Table 1). Incorrect or incomplete labeling of the specimen or filling of forms accompanying the sample are unfortunately frequent occurrences. Individuals and the institution play an important role in devoting attention to the design of the forms and the choice of packaging and labeling materials. Of particular concern is the frequent lack of awareness about the importance of buffered formalin and of swift transportation of the sample to the pathology laboratory.

Procedures in the pathology laboratory

The pathology laboratory plays a central role in minimizing issues that may compromise correct and timely information required for diagnostic and therapeutic decisions (Table 1). In addition to standardization and proper implementation of techniques related to sample processing, including those involving conditioning, specimen cleavage and fixation, laboratory personnel must ensure that sufficient information has been provided upon receipt of samples. Very often, forms accompanying samples are incompletely filled. In publicly funded institutions, the practice of outsourcing pathology services is not uncommon, and frequent change in the providers of such services may represent an important hurdle for adequate patient management.

RESULTS

Recommendations to mitigate the identified issues and optimize pathological assessment of tumor specimens

Breast specimens obtained from outpatient procedures or from procedures performed in the operating room for the diagnosis of breast cancer require attention from collection to reporting of histological results. In this journey, several factors may interfere with the quality of the final diagnosis in terms of the disease definition, type, characteristics of greater or lesser biological aggressiveness, presence of hormone receptors, and HER2 expression. These factors guide the selection of the best therapeutic option for each case and, when incorrectly evaluated, may negatively affect patient prognosis.

The tumor-tissue journey of breast specimens involves the participation of physicians, nursing team members, biomedical professionals, biologists, lab technicians, and administrative personnel. As part of the task and based on the current guidelines and the published literature, the experts discussed the steps involved in each of the three phases of the tissue processing journey to review important recommendations. Figure 1 summarizes the steps comprising the pre-analytical, analytical, and post-analytical phases of the tissue journey, although variation may exist in how the steps are grouped[26].

Based on the issues identified (Table 1), the working group selected and discussed recommendations to address each aspect. The recommendations reviewed here were based on the current guidelines and orientations published by international organizations, such as World Health Organization (WHO)[26] and the College of American Pathologists (CAP)[27], and Brazilian Society of Pathology (SBP)[28], among other documents[29,30], as well as on the professional knowledge and experience of the multidisciplinary members of the working group, especially considering the local scenario.

Recommendations are summarized in Tables 2–4 and discussed below, according to the three phases, following the criteria adopted by the WHO guidelines[26].

General recommendations

In all the steps, samples must be identified with the name of the responsible person, the date and time, to ensure traceability. The experts recommend that the sample be accompanied throughout its journey, not only by the medical request form, but also by a document listing all the steps, with the name of the person responsible for each step, date and time, either on paper or electronically. Important information includes:

- Time of sample collection
- Time of sample placing in the fixative
- Cold ischemia time
- Time of sample delivery to the person responsible for transferring it to the pathology laboratory (intra- and inter hospital transport)
- Time of entry at the pathology laboratory
- Time of macroscopic evaluation

Pre-analytical phase

Table 2 displays actions and recommendations for the different steps of the pre-analytical phase[13,16,26,27,31-34].

Sample collection and conditioning

Sample collection is under the responsibility of the physician, surgeon, or radiologist, who is also responsible for filling in the exam request form with clinical information. Information about the time of specimen collection and the time of cold ischemia (defined as the time between removal of the tissue from patient until placement into the fixative) are under the responsibility of the nursing team (operating room) or the radiology assistant (radiology services). The cold ischemia time is an important variable to be emphasized as it can alter the gene expression and protein characteristics, thus interfering with the results of IHC and molecular tests[27]. Regarding this, a cold ischemia time of less than 1 hour is recommended.

The excised material must be clearly detailed in the request form and should be checked by the nursing team before placement in the containers with fixative. Regarding the handling of the specimens before placement in the fixative, there are specific recommendations for outpatient procedures and for surgical

Table 2. Summary of actions and recommendations for the pre-analytical phase.

	Recommendations (13, 16, 26, 27, 31-34)
Sample collection and conditioning	1) Personnel responsible for specimen collection and for completing the request form with clinical information: physician, surgeon, or radiologist 2) Personnel responsible for registering information regarding the time of specimen collection and the time of cold ischemia (defined as the time between tissue removal from patient until placement into the fixative): nursing team (operating room) or the radiology assistant/ technician (radiology services). 3) The excised material must be clearly specified in the request form and checked by the nursing team before placement in the containers with fixative. 4) Handling of specimens before fixation: • Outpatient procedures: keep in saline solution if fixation will not be performed immediately (for example, in cases that require radiography or photographic documentation of the specimen) • Surgical specimens: ◦ Small samples (nodulectomies, lymph nodes, lumpectomy), measuring less than 5.0 cm or at physician discretion, can be immediately placed in the fixative, fully submerged ◦ Larger samples, such as mastectomies and wide local excisions, should be sliced in case they are not immediately sent to the pathology laboratory (see below for details) ◦ Samples that had undergone an intraoperative frozen section should be sent fresh to the pathologist, who will be responsible for the specimen manipulation until the intraoperative diagnosis. After the test, the specimen will follow the same workflow described for samples that are not submitted to intraoperative procedures. 5) Preparation of larger specimens • Specimens with larger volume need to be properly prepared for adequate fixation. Although formalin is a good fixative, its action is slow, as it penetrates the tissue with a speed of 1 mm/hour at room temperature. This information can be used to support the choice of the thickness of the fragments (thinner thickness, in case delays in the specimen dispatch to the laboratory, for example, during the weekend or holidays). It is recommended that surgical specimens be cut in parallel slices performed from the deep fascia towards the skin, without transfixing the surgical piece so it can be recomposed in the laboratory. This procedure needs to be agreed between the pathology laboratory and the surgical team. • The pathologist is responsible for training the personnel involved in the procedure after the specimen excision, such as the surgical team members, technicians, paramedics etc., depending on the local conditions. • Ideally, before slicing, the resection margins should be identified and inked. In this case, it is necessary to dry the specimen using paper towel, apply the ink followed by acetic acid or vinegar so the ink can fix properly without dissolving in formalin and during the processing, thus allowing the proper assessment of the surgical margins. • Inadequate fixation impairs the histopathological diagnosis (differential diagnosis between benign and malignant, histological tumor typing and grading, and the immunoreactivity of target molecules. 6) Specimen labeling and identification (nursing team) • Labels for container or slide identification should be printed using computers or written in pencil in adhesive tape, and contain patient's name and information about the specimen • Ideal scenario: Bar-code or QR code • The label should be placed on the primary container, not in the lid. • Certify that the received specimen matches the description provided in the medical request 7) Placement in the containers • Containers should preferably be rigid, impermeable, break-resistant, and non-reactive to fixatives • Previously identified by the nursing team 8) Fixation • Register the time the specimen was placed in the fixative • Recommended cold ischemia time: less than 1 hour • Recommended type of fixative: 10% neutral phosphate buffered formalin (40% formaldehyde diluted to 10% - elevation of pH to ~7) • Fixative volume: 10 to 20 times the size of the specimen • Fixation time of tumor samples recommended for hormone receptors and HER2: 6-72 hours
Pathological exam request	• Responsibility of the medical team • The request form must accompany the specimen during the complete journey, from collection to the end of pathological exam. • Should specify: ◦ Laboratory of destination ◦ Patient identification ◦ Clinical diagnosis/diagnostic hypothesis ◦ Summary of the clinical history ◦ Procedure performed ◦ Date of procedure • The specimens should be preferably numerate and properly described regarding its type, laterality, and topography • Type of test to be performed (e.g., immunohistochemistry, molecular tests)

Continue...

Table 2. Continuation.

	Recommendations (13, 16, 26, 27, 31-34)

Transportation to the pathology laboratory	• Forms of sending the specimen/material
	  • Intra-hospital transfer (the pathology laboratory is located in the hospital or clinic itself)
	  • Laboratory outside the hospital (transportation using messenger service or mail):
	  ○ Adequate conditioning: primary container (container with the specimen properly identified), secondary (leak-proof) and tertiary (rigid, accompanied by the identification of the sender and the recipient, identification of the biological material, and phone number contact in case of accident).

Table 3. Summary of actions and recommendations for the analytical phase.

	Recommendations[26, 28-30,35,36]

Sample reception at the pathology laboratory	1) Responsible personnel: administrative or technical employee
	2) Verify the list of dates/times registered for the steps/procedures previously performed
	3) Register date and time of sample receipt
	4) Confirm the type of tissue (fresh or fixed) and the type of fixative, and register the date of entry at the laboratory
	5) The criteria for sample acceptance and rejection and the recommendations for exams to be performed in samples with restriction must be clearly specified in written instructions
	6) Reasons for samples rejection:
	  • Samples lacking patient identification or with doubtful or incorrect data
	  • Inconsistency between the type of sample mentioned in the exam request form and the type of material received
	  • Samples without a medical request form
	7) Factors that limit sample condition (notified at the registry of exam entry)
	  • Fixative is inadequate or absent
	  • Broken or cracked containers/slides with possible partial leakage of material
	  • Information about the dates/times of the previous steps is unavailable
	  • Inadequate proportion of fixative to specimen
	  • Large specimen not previously sectioned
	  • Inadequate containers
	  • Exam request form incomplete
	8) Specimen registration and transfer to macroscopy

Specimen registration in the laboratory	• Verify if specimens retrieved from the container used for transportation match the information provided in the labels and in the request form • If specimen and identification data match, a unique identification number is attributed for the sample to allow tracking during the process • When possible, use barcode labels to improve traceability of all materials of a single case (sample fragments, paraffin blocks, histological slides, routine and special staining, etc)
Macroscopic examination	• Manually performed by pathologist or laboratory technician • Verify the correspondence between the specimen/sample identification on the label and the request form, confirming the laterality and tumor location in breast quadrants • Follow the test and sampling protocols recommended by scientific societies of pathology and international institutions • Verify if fixation was properly performed • Measure the size and weight of the tissue surgical piece • Ink the surgical margins with different ink colors • Cut the specimen into thin, parallel, and cross-sectional slices, avoiding damaging or clamping the tissue • Describe the observed alterations in relation to the color, texture, consistency, delimitation of the adjacent tissue • Measure the lesions found in the macroscopic examination • Use clean cut surfaces and instruments to avoid cross-contamination with other samples • Special care is required for fine-needle biopsies to assure the inclusion of all fragments • Choose appropriate and labeled cassettes for each type of material, avoiding placing excess material • Describe and measure the lesions visualized in the macroscopic examination, registering information regarding the topography in relation to the anatomic position and distance from the nipple (when present) and surgical margins
Histological processing	• Performed by laboratory technicians using tissue processors • Use of adequate time of tissue processing for each type of specimen • Needle biopsies require shorter time in each reagent during processing than specimens from surgical resections

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Table 3. Continuation.

	Recommendations[26, 28-30,35,36]
Paraffin embedding technique	• Performed by laboratory technician • Manually (handling-processing) or with the use of a paraffin embedding machine • Avoid excessive heating of paraffin • Check the paraffin temperature regularly • Avoid overfilling of each mold/block • Samples should be carefully oriented, handled and positioned in the inclusion blocks
Microtomy	• Performed by a laboratory technician • Use high quality blades • Optimize the knife angle of inclination in the microtome • Slice the paraffin embedded tissue blocks carefully • Avoid freezing damages • Slice blocks in thin sections (3 to 5 micrometers), gently and slowly
Tissue floatation in water bath and placement of the paraffin embedded tissue sections on slides	• Use clean water • Certify that blades/knives are clean to avoid cross-contamination • Avoid simultaneous floating of various cuts in the water bath chamber • Check water bath temperature • Avoid excessive expansion and damage of tissue sections • Carefully choose tissue section with no folding or extensive distension • Avoid the formation of bubbles under the tissue sections that could lead to the detachment of the sections during histological staining
Dehydration of histological sections	• Dry the histological section before placing it in the histological incubator to dehydrate • Incubator temperature and dehydration time should be monitored
Routine staining	• Staining with hematoxylin and eosin are routinely performed manually by the histotechnician or using specific equipment (autostainer) • Histological sections must be completely deparaffinized before staining • Reagent should be regularly renewed • Use standardized conditions and protocols for staining, adopting precise times and quality constant monitoring
Coverage of tissue sections with coverslip	• Histological sections should completely dehydrate before mounting • Place the mounting medium and cover with cover slip • Avoid excessive drying, formation of crystals or bubbles.

Table 4. Summary of actions and recommendations for the post-analytical phase.

	Recommendations[13,16,26]
Slide reception by the pathologist	• Verify the clinical data provided in the pathological exam request form (age, clinical diagnosis, clinical information, imaging findings, neoadjuvant treatment, procedures performed) • Check the identification of the slides (name, number) • Review data from the macroscopic examination (type of specimen received, sampling, lesion features of the lesion(s), specimen dimension and localization)
Slide interpretation	• Follow the recommendations of standardized manuals and guidelines: o Manual for Standardization of Histopathological Reports of the Brazilian Society of Pathology: http://www.sbp.org.br/manual-de-laudos-histopatologicos/ o Protocols for Cancer and Biomarker reporting released by the College of American Pathologists (CAP): https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates o Guidelines on TIL-assessment developed by the International Immuno-Oncology Biomarker Working Group on Breast Cancer: https://www.tilsinbreastcancer.org/ o Residual Cancer Burden Calculator after neoadjuvant treatment http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3 o AJCC/TNM for anatomopathological staging and prognosis: https://cancerstaging.org/references-tools/deskreferences/Documents/AJCC%20Breast%20Cancer%20Staging%20System.pdf • Use standardized synoptic reports specifically designed for each type of specimen • Include in the report information regarding the sample quality (see description below) o adequate: no impact on histological, immuno-histochemical and molecular assessments o limited: can possibly impact on histological, immuno-histochemical and molecular assessments o inadequate: impairment of the histological, immuno-histochemical and molecular assessments

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Table 4. Continuation.

Sample quality	• Sample quality must be assessed • If sample quality is limited or inadequate, specify the causes: ( ) Cold ischemia time: o 1h-8h o 8h-12h o 12h-24h o >24h ( ) Fixative: o Non-buffered formalin o alcohol o no fixative o other: _____ ( ) Fixative volume is inadequate ( ) Fixation time: o <6h o 6-72h o 72-96h o >96h ( ) Histological sections with technical artifacts o thick sections o signs of excessive heat in paraffin o signs of excessive heat in water bath o excess of folding o clamping artifacts o thermal artifacts o loss of material during microtomy o inadequate staining (weak or strong) ( ) Immuno-histochemistry reaction o no internal control o no external control o presence of artifacts in the histological sections o abnormal staining
Suspected inconsistencies	• Notify if clinical, imaging, histological and immunohistochemical findings are consistent. • Examples of inconsistencies: • Radiologic image with extensive microcalcifications, invasive neoplasm with apocrine pattern, but HER2-negative • Low grade carcinoma, with low proliferative activity, but hormone receptor-negative or hormone receptor-low • HER2-positive carcinoma, but with low grade, low proliferative activity • High-grade carcinoma, high proliferative activity, but hormone receptor-positive/HER2-negative

specimens, as detailed in Table 2. Large tumor specimens require preparation for adequate fixation. Recommendations regarding sectioning before fixation, including the thickness of the sections, type of fixative and fixation time are provided in Table 2. This is an important topic, as inadequate fixation impairs the histological diagnosis (differential diagnosis between benign and malignant, histological typing and grading, and the immunoreactivity of target molecules, especially those of cytoplasm or membrane localization, such as programmed death 1 ligand [PD-L1], HER2, etc)[31-33].

Recommendations regarding sample identification, which is an attribution of the nursing team, characteristics and labeling of containers, fixation registry, duration, and fixative solutions are also detailed in Table 2. 10% neutral buffered formalin is the fixative solution most frequently preferred for routine histological preparations of surgical specimens. Monitoring the fixation time is critical. For hormone receptors and HER2, a fixation time of 6-72 hours is recommended[13,16].

Exam request

As previously mentioned, the medical team is responsible for completing the request form with clinical data and specimen information. The precise and complete filling of this form is of crucial importance to the tissue journey.

Transportation

The last step of the pre-analytical phase is the transportation of the sample to the pathology laboratory, which may be located at the same hospital/service involved in the specimen resection or may be in a different, distant location. Special care must be taken when transporting surgical specimens from the operating room to outside pathology laboratories. Specimens must be transported in rigid containers, with an adequate volume of buffered formalin[35]. Information regarding current recommendations in guidelines for specimen transportation is also detailed in Table 2.

Analytical phase

The analytical phase comprises the sample/specimen reception at the pathology laboratory, sample/specimen macroscopic examination, tissue processing, paraffin embedding, sectioning/microtomy of the paraffin blocks, routine staining, special staining, IHC, and other molecular techniques such as ***in situ*** hybridization (Figure 1)[26]. To be performed with safety and quality, this phase requires the establishment of standardized procedures and efficient channels of communication between the pathology laboratory and the clinical-surgical and imaging services where the samples were obtained. In the analytical phase, only a few steps are automated, with several steps in the process being manual, relying on the care and skill of the pathologist (gross examination, specimen cleavage and selection of samples for microscopy) and the laboratory technicians (inclusion and microtomy)[28].

Factors that are determinant to the analytical phase include the criteria adopted for sample acceptance or rejection, the thickness of tissue section into cassette, tissue processor fluid maintenance, paraffin type and temperature, and validity tests and controls[26].

A summary of the actions and recommendations for the main steps of the analytical phase is presented in Table 3 and briefly described below[26,28-30,35,36].

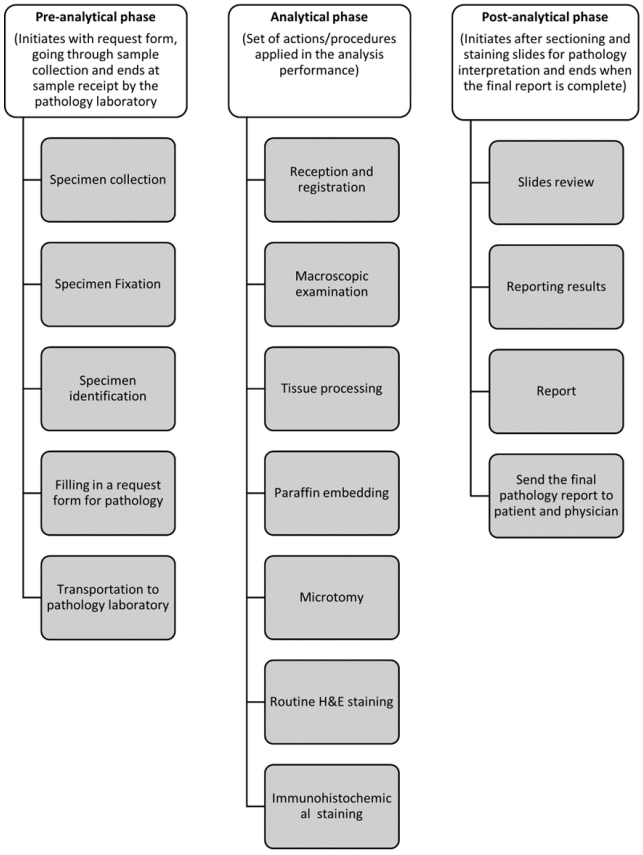

Figure 1. Flowchart of the main steps of the preanalytical, analytical, and post-analytical phases of the tissue-journey, adapted from the WHO document.

Sample reception

The reception of the pathology laboratory is where the samples are received. Upon receipt, it must be guaranteed that each specimen received is accurately labeled with the patient identification and accompanied by the examination request containing clinical information and previous laboratory tests, date and time of collection. The date and time of receipt of the material must be registered in the laboratory, confirming whether the tissue was received fresh or fixed and the type of fixative used. Predetermined rules previously established by the pathology laboratory receiving the samples should be followed for rejecting inadequate specimens whenever needed. These rules must be communicated to all physicians and healthcare professionals who send the materials. Situations in which specimens must be rejected include: unlabeled sample with no information regarding patient name and material identification; insufficient patient information; and information provided in the sample label not matching the patient name on the pathology request form[26,37]. Additionally, there are situations that do not imply rejection of material, but can interfere with the quality of the specimen, exam and results, including: damaged or leaking tube/container; inadequate volume of fixative for the amount of material; material partially dried up due to inadequate volume of fixative; and extended transportation time or other improper handling during transportation[26,28].

It is important that the laboratory communicates to the physician who requested the pathology exam any problem related to the rejection of the sample or the identification of situations that interfere with the quality of the exam.

Sample registration

Upon receipt, one important step is checking if the received specimens match the information and description provided for the case in the container labels and in the request form. Once the correspondence is confirmed, sample registration proceeds with the attribution of a unique identification number to facilitate sample tracking during the process. To improve traceability of materials (sample fragments, paraffin blocks, histological slides, routine and special staining, etc), the use of barcode labels is recommended wherever possible.

Macroscopic examination of specimens

Gross examination is performed by the pathologist or laboratory technicians. This step involves the description of the specimen in terms of shape, color, texture, consistency, and delimitation of the adjacent tissue, the measurement (size and weight) of the specimen, and its dissection. Lesions should be described and measured with information about their topography. More detailed recommendations are provided in Table 3. It is highly recommended to follow protocols and guidelines for testing and sampling established by pathology scientific societies and international institutions[29,30,36].

Histological processing

Tissue processing is performed using an automated tissue processor prior to microtomy. This equipment is maintained by lab technicians for the control of reagents used (formaldehyde, alcohols, xylene, paraffin). The time of tissue processing should be adequate to each type of specimen (Table 3).

Paraffin embedding

After processing, the tissue samples are embedded in paraffin wax. Monitoring paraffin temperature is crucial to avoid excessive heat. Samples should be carefully oriented, handled and positioned in the inclusion blocks. Specific recommendations selected by the working group based on the current guidelines and literature are listed in Table 3.

Microtomy

Sectioning the tissue block with the use of a microtome is the following step. Specific recommendations on sections thickness, quality and positioning of blades were reviewed and are provided (Table 3).

Tissue floatation in warm water bath, placement of the paraffin embedded tissue sections on slides, and dehydration of sections

As part of the process, the tissue slices are placed in a warm water bath. Precautions need to be taken to avoid cross contamination and damage of sections (Table 3). Tissue sections should be carefully selected and placed on slides. Before proceeding to staining, histological sections should be dehydrated. More detailed recommendations are displayed in Table 3 and in the original publication of the cited guidelines.

Routine and complementary stainings

Hematoxylin and eosin (H&E) are the stains routinely used in histopathology. Table 3 displays recommendations for this step. Special stainings (histochemistry) or, more often, IHC stainings, can be used to provide complementary information for diagnosis or for predictive tests for therapeutic response.

Immunohistochemical stain

It can be performed on specific equipments (autostainers) or manually using standardized procedures and specific reagents. Positive-charged or silane coated glass slides are recommended to ensure adherence of the histological sections and avoid loss of material during the different stages of the IHC technique. The choice of reagents (primary and secondary antibodies, detection system, and counterstaining) is of paramount importance and determines the quality of the reactions together with the standardization of procedures. The equipment used must be routinely calibrated. Antibodies should be chosen with care and used following the manufacturers' technical specifications, using antigen retrieval in the appropriate medium when necessary. Use an appropriate detection system, standardize washing steps and optimize counterstaining. An appropriate positive tissue external control should be included on all reactions. The WHO, the College of American Pathologists and the American Society of Clinical Oncology recommend that all primary breast tumors should be tested for hormone receptors (ER and PR) and HER2[13,16,38].

In situ hybridization

In situ hybridization should follow the same precautions recommended for the IHC method using properly fixed tissue and silane coated or positive-charged slides to avoid detachment problems and loss of material. Specific and standardized reaction protocols should be followed. Probes must be carefully chosen for each diagnostic indication, and appropriate controls used for all reactions.

Post-analytical phase

The post-analytical phase involves the interpretation of the slides and the preparation of pathology reports to describe the results. The use of synoptic reports is highly recommended to improve data reporting, as they provide a structured and standardized documentation[26].

As emphasized in the guide published by WHO in 2019, the post-analytical phase also includes the retention and disposal of all the materials containing patient tissues/samples (paraffin blocks and glass slides) and data archiving, with specific recommendations being attributed to these steps[26].

The quality of the sample must be assessed and the reasons for a sample to be considered of limited or inadequate quality must be notified, as described in the recommendations listed in Table 4[13,16,26]. Parameters used to attest the quality of a sample include the cold ischemia time, type and volume of fixative, fixation time, presence of technical artifacts, and factors affecting the IHC reaction/interpretation (e.g., the use of internal and external controls).

Recently, new categories of tumors, based on low expression of the traditional biomarkers ER and HER, have shown important prognostic and predictive differences[39]. HER2-negative 2018 ASCO/CAP group includes tumors with no staining (score 0), incomplete and faint/barely perceptible staining in up to 10% of tumor cells (score 0), incomplete and faint/barely perceptible staining in >10% of cells (score 1+), and those with weak/moderate complete membrane staining in more than 10% of cells (score 2+) with no amplification by in situ hybridization[16,40]. Breast cancer with low HER2 expression, particularly the group denominated HER2-low (score 1+ or 2+ without gene amplification), has shown response to new generation of antibody-drug conjugates, capable of delivering drug to tissues by binding to target cells[41]. However, reproducibility of the correct

classification among pathologists is suboptimal, with discordance of 35% of the cases, in part because of influence of preanalytical artifacts[42]. Pathologists should follow the specimen fixation, processing, and interpretation guidelines proposed by the 2018 ASCO/CAP HER2 test recommendations to ensure the reliability and reproducibility of classifying tumors into different expression categories of this biomarker.

DISCUSSION

The importance of pathological preanalytical and analytical issues to the adequate provision of contemporary cancer care cannot be overemphasized[6,13,16,43,44]. Most issues affecting timely and correct assessment of specimens occur in the preanalytical phase of processing[20,43,44]. Studies suggest that about 60–70% of laboratory errors are due to preanalytical factors[27]. Adequate handling of surgically removed specimens involves labeling, packaging, transportation, fixation and storage, as well as the collection and reporting of administrative, demographic and medical information. Attention to specimens at all these steps may mitigate errors and optimize histopathological, immunohistochemical, and molecular testing in breast cancer.

The relevance of the issues outlined here is only likely to increase, as a result of the increasing role played by precision oncology in the treatment of patients with breast cancer. The time from tissue removal to formalin fixation (cold ischemic time) and temperatures during fixation are crucial[13,16,45]. These parameters are particularly critical for the analysis of ER, PR, and HER2 expression[45]. Among other problems, antigen loss in formalin-fixed tissue sections is sufficient to preclude optimal diagnostic histopathology and IHC studies[44]. Even though we focus our attention on handling of samples for histopathological and IHC assessment, the problem is broader when one considers the increasing role of newer molecular-biology technologies that rely on the quality of tissue RNA in the assessment of gene expression[46]. Prognostic gene expression-based assays play an increasing role and have been increasingly used for decision-making regarding the indication of chemotherapy[47].

If the preanalytical phase is optimized, errors in the analysis or interpretation of results by the pathologist are minimized. Nevertheless, attention is needed to the frequent communication issues identified in Table 1, particularly with regard to insufficient provision of the relevant clinical information to the pathologist. Unfortunately, the pathology laboratory is also place for some of the preanalytical issues that can compromise correct and timely acquisition of information required for diagnostic and therapeutic decisions in oncology[19]. In Brazil, many hospitals do not have their own pathology laboratory, but rather outsource this service, which creates an additional layer of complexity in the attempt to minimize errors. Of note, there is frequent concern about the quality of the services provided by some of these laboratories, which are usually contracted on the basis of public procurement.

CONCLUSIONS

Ideally, patients with breast cancer should be under the care of a multidisciplinary team involving the various specialized professionals required for optimal results[6,12,19]. Although there is overlap between the function of individuals, departments and institutions in terms of their contribution to a seamless tumor-tissue journey, each participant in the process needs to be aware of their contribution and of the overall process. Education, communication, standardization of procedures, and creation of adequate infrastructure are the keys to success, and are ideally achieved in institutions motivated and with the required administrative will. These institutions are further embedded in larger publicly funded or private systems, which must recognize the importance and foster implementation of the issues highlighted here. We hope the recommendations reviewed here can play a role in that goal, and potentially inform public policy related to these issues.

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AUTHORS' CONTRIBUTIONS

HG: Conceptualization, Writing – original draft, Writing – review & editing. FMC: Conceptualization, Writing – original draft, Writing – review & editing. RMSR: Conceptualization, Writing – original draft, Writing – review & editing. MIK: Conceptualization, Writing – original draft, Writing – review & editing. DLP: Conceptualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30. https://doi.org/10.3322/caac.21590
3. Heer E, Harper A, Escandor N, Sung H, McCormack V, Fidler-Benaoudia MM. Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. Lancet Glob Health. 2020;8(8):e1027-37. https://doi.org/10.1016/S2214-109X(20)30215-1
4. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. Breast cancer. Nat Rev Dis Primers. 2019;5(1):66. https://doi.org/10.1038/s41572-019-0111-2
5. Brasil. Instituto Nacional de Câncer José Alencar Gomes da Silva. Estimativa 2020: incidência de câncer no Brasil. Rio de Janeiro: INCA; 2019 [cited on Feb 9, 2022]. Available from: https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2020-incidencia-de-cancer-no-brasil.pdf
6. Buzaid AC, Achatz MI, Amorim GLS, Barrios CH, Carvalho FM, Cavalcante FP, et al. Challenges in the journey of breast cancer patients in Brazil. Braz J Oncol. 2020;16:e-20200021. https://doi.org/10.5935/2526-8732.20200021
7. Lee BL, Liedke PE, Barrios CH, Simon SD, Finkelstein DM, Goss PE. Breast cancer in Brazil: present status and future goals. Lancet Oncol. 2012;13(3):e95-102. https://doi.org/10.1016/S1470-2045(11)70323-0
8. Cunha IW, Coudry RA, Macedo MP, Assis EACP, Stefani S, Soares FA. A call to action: molecular pathology in Brazil. Surg Exp Pathol. 2021;4(1):15. https://doi.org/10.1186/s42047-021-00096-1
9. Brasil. Agência Nacional de Saúde Suplementar. Dados Gerais. Beneficiários de planos privados de saúde, por cobertura assistencial (Brasil, 2011-2021). 2022 [cited on Feb 9, 2022]. Available from: https://www.gov.br/ans/pt-br/acesso-a-informacao/perfil-do-setor/dados-gerais
10. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747-52. https://doi.org/10.1038/35021093
11. Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. N Engl J Med. 2009;360(8):790-800. https://doi.org/10.1056/NEJMra0801289
12. National Comprehensive Cancer Network. NCCN practice guidelines in oncology. Breast Cancer – v.7.2021. 2022 [cited on Feb 9, 2022]. Available from: http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf
13. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP Guideline Update. J Clin Oncol. 2020;38(12):1346-66. https://doi.org/10.1200/JCO.19.02309
14. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early breast cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2019;30(8):1194-220. https://doi.org/10.1093/annonc/mdz173
15. Denduluri N, Somerfield MR, Chavez-MacGregor M, Comander AH, Dayao Z, Eisen A, et al. Selection of Optimal Adjuvant Chemotherapy and Targeted Therapy for Early Breast Cancer: ASCO Guideline Update. J Clin Oncol. 2021;39(6):685-93. https://doi.org/10.1200/JCO.20.02510
16. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol. 2018;36(20):2105-22. https://doi.org/10.1200/JCO.2018.77.8738
17. Burstein HJ, Curigliano G, Thurlimann B, Weber WP, Poortmans P, Regan MM, et al. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol. 2021;32(10):1216-35. https://doi.org/10.1016/j.annonc.2021.06.023
18. Cardoso F, Paluch-Shimon S, Senkus E, Curigliano G, Aapro MS, Andre F, et al. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Ann Oncol. 2020;31(12):1623-49. https://doi.org/10.1016/j.annonc.2020.09.010
19. De Las Casas LE, Hicks DG. Pathologists at the leading edge of optimizing the tumor tissue journey for diagnostic accuracy and molecular testing. Am J Clin Pathol. 2021;155(6):781-92. https://doi.org/10.1093/ajcp/aqaa212
20. Hewitt SM, Badve SS, True LD. Impact of preanalytic factors on the design and application of integral biomarkers for directing patient therapy. Clin Cancer Res. 2012;18(6):1524-30. https://doi.org/10.1158/1078-0432.CCR-11-2204
21. Hortobagyi GN, Connolly JL, D'Orsi CJ, Edge SB, Mittendorf EA, et al. Breast. In: Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. American Joint Committee on Cancer. AJCC cancer staging manual. 8th ed. New York, NY: Springer; 2017.
22. Cardoso F, van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med. 2016;375(8):717-29. https://doi.org/10.1056/NEJMoa1602253
23. Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. N Engl J Med. 2018;379(2):111-21. https://doi.org/10.1056/NEJMoa1804710
24. Early Breast Cancer Trialists' Collaborative group (EBCTCG). Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13 864 women in seven randomised trials. Lancet Oncol. 2021;22(8):1139-50. https://doi.org/10.1016/S1470-2045(21)00288-6
25. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. Adjuvant olaparib for patients with BRCA1- or BRCA2-Mutated Breast Cancer. N Engl J Med. 2021;384(25):2394-405. https://doi.org/10.1056/NEJMoa2105215
26. Guide for establishing a pathology laboratory in the context of cancer control. Geneva: World Health Organization; 2019

[cited on Feb 9, 2022]. Available from: https://apps.who.int/iris/handle/10665/330664

27. Compton CC, Robb JA, Anderson MW, Berry AB, Birdsong GG, Bloom KJ, et al. Preanalytics and precision pathology: pathology practices to ensure molecular integrity of cancer patient biospecimens for precision medicine. Arch Pathol Lab Med. 2019;143(11):1346-63. https://doi.org/10.5858/arpa.2019-0009-SA

28. Assis E. Manual de boas práticas em patologia. São Paulo: Sociedade Brasileira de Patologia, 2020 [cited on Feb 9, 2022]. Available from: http://www.sbp.org.br/publicacoes/manual-de-boas-praticas-em-patologia/

29. Ellis I, Allison KH, Dang C, Gobbi H, et al . Invasive carcinoma of the breast histopathology reporting guide. international collaboration on cancer reporting; Sydney, Australia. 2021[cited on Feb 9, 2022]. Available from: http://www.iccr-cancer.org/datasets/published-datasets/breast/invasive-carcinoma-of-the-breast

30. Royal College of Pathologists. Cancer datasets and tissue pathways. 2021 [cited on Feb 9, 2022]. Available from: https://www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html

31. Start RD, Cross SS, Smith JH. Assessment of specimen fixation in a surgical pathology service. J Clin Pathol. 1992;45(6):546-7. https://doi.org/10.1136/jcp.45.6.546

32. Williams JH, Mepham BL, Wright DH. Tissue preparation for immunocytochemistry. J Clin Pathol. 1997;50(5):422-8. https://doi.org/10.1136/jcp.50.5.422

33. MacGrogan G, Mathieu MC, Poulet B, Penault-Llorca F, Vincent-Salomon A, Roger P, et al. Pre-analytical stage for biomarker assessment in breast cancer: 2014 update of the GEFPICS' guidelines in France. Ann Pathol. 2014;34(5):366-72. https://doi.org/10.1016/j.annpat.2014.08.017

34. Santana M, Ferreira L. Diagnostic errors in surgical pathology. J Bras Patol Med Lab. 2017;53(2):6. https://doi.org/10.5935/1676-2444.20170021

35. D'Angelo R, Mejabi O. Getting it right for patient safety: specimen collection process improvement from operating room to pathology. Am J Clin Pathol. 2016;146(1):8-17. https://doi.org/10.1093/ajcp/aqw057

36. Fitzgibbons PL., Connolly JL, Bose S. et al. Protocol for the examination of resection specimens from patients with invasive carcinoma of the breast. College of American Pathologists, 2020 [cited on Feb 9, 2022]. Available from: https://documents.cap.org/protocols/cp-breast-invasive-resection-20-4400.pdf

37. Laboratory quality management system: handbook. Geneva: World Health Organization; 2005.

38. WHO Classification of Tumours Editorial Board. Breast Tumours. WHO Classification of Tumours. 5th ed. Lyon: IARC Press; 2019.

39. Najjar S, Allison KH. Updates on breast biomarkers. Virchows Arch. 2022;480(1):163-76. https://doi.org/10.1007/s00428-022-03267-x

40. Venetis K, Crimini E, Sajjadi E, Corti C, Guerini-Rocco E, Viale G, et al. HER2 low, ultra-low, and novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast cancer. Front Mol Biosci. 2022;9:834651. https://doi.org/10.3389/fmolb.2022.834651

41. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med. 2022;387(1):9-20. https://doi.org/10.1056/NEJMoa2203690

42. Schettini F, Chic N, Brasó-Maristany F, Paré L, Pascual T, Conte B, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ Breast Cancer. 2021;7(1):1. https://doi.org/10.1038/s41523-020-00208-2

43. Khoury T. Delay to formalin fixation (Cold Ischemia Time) effect on breast cancer molecules. Am J Clin Pathol. 2018;149(4):275-92. https://doi.org/10.1093/ajcp/aqx164

44. Xie R, Chung JY, Ylaya K, Williams RL, Guerrero N, Nakatsuka N, et al. Factors influencing the degradation of archival formalin-fixed paraffin-embedded tissue sections. J Histochem Cytochem. 2011;59(4):356-65. https://doi.org/10.1369/0022155411398488

45. Gundisch S, Annaratone L, Beese C, Drecol E, Marchio C, Quaglino E, et al. Critical roles of specimen type and temperature before and during fixation in the detection of phosphoproteins in breast cancer tissues. Lab Invest. 2015;95(5):561-71. https://doi.org/10.1038/labinvest.2015.37

46. Hatzis C, Sun H, Yao H, Hubbard RE, Meric-Bernstam F, Babiera GV, et al. Effects of tissue handling on RNA integrity and microarray measurements from resected breast cancers. J Natl Cancer Inst. 2011;103(24):1871-83. https://doi.org/10.1093/jnci/djr438

47. Loi S. The ESMO clinical practise guidelines for early breast cancer: diagnosis, treatment and follow-up: on the winding road to personalized medicine. Ann Oncol. 2019;30(8):1183-4. https://doi.org/10.1093/annonc/mdz201