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Standardisation in Histopathology – Impossible Dream or Plausible Reality?

A Diagnostic and Commercial Perspective

Standardisation in Histopathology – Impossible Dream or Plausible Reality?

A Diagnostic and Commercial Perspective

Peter Jackson[a], Michael Gandy[b], Phillip Thompson[a], Craig Barker[b]

"Standardisation" – the current buzz word in diagnostic histopathology! What does it really mean? How would it affect our day-to-day work? How can it be implemented? These are just some examples of the many questions that are raised when the topic of standardisation is discussed within the histopathology community.

The aims of this article are to highlight the role that commercial companies can play in conjunction with clinical partners in supporting the diagnostic histopathology laboratory through its continuous search for standardisation in line with the ever-increasing portfolio of diagnostic tests and accompanying guidelines available to us today[1, 2, 3].

As the pipeline of commercially available predictive and prognostic assays (e.g. HER2 protein profiling for Herceptin® immunotherapy) continues to be developed in parallel with new pharmaceutical drug discovery, continued standardisation within diagnostic histopathology will no longer be an ideal but an inevitable requirement, no matter how difficult it may seem in the short to medium term.

Complete automation within the histopathology laboratory has slowly increased with tissue processing, embedding, tinctorial stains, immunohistochemistry (IHC), *in situ* hybridisation (ISH) and image analysis (IA) becoming commonplace. However it is surprising that two of the most crucial stages in the entire histology process, specimen handling and tissue fixation, are almost always ignored.

Successful immunohistochemistry may be seen as the correct integration of several technical parameters. The aim of immunohistochemistry is to achieve reproducible and consistent demonstration of antigens with the minimum of background staining whilst preserving the integrity of tissue architecture. Specimen handling, fixation and subsequent paraffin processing are essential considerations.

Using HER2 protein profiling for Herceptin® immunotherapy as an illustrated example this article attempts to consider the pre-analytical steps associated with immunohistochemical analysis and the role which standardisation plays in producing consistent and reliable results at the pre-analytical stage.

Many of the activities discussed within this article may appear commonplace in our routine working environments. It is through the continued understanding and education coupled with the streamlined linking of these activities together that we hope will try to put the term standardisation into a relevant and practical context at the pre-analytical stage (refer to Fig. 1).

[a] Department of Histopathology & Molecular Pathology, Leeds Teaching Hospitals NHS Trust, Leeds, UK
[b] Leica Microsystems, Biosystems Division, Newcastle, UK

Building on the premise that control of good histological practice starts in theatre/surgery at point of care, we will discuss some of the routine activities that we consider have an impact not only on high profile assays such as HER2 but on all diagnostic histopathology testing ranging from initial broad spectrum H&E evaluation through to the most complex molecular characterisations.

Pre-analytic

Standardisation within histopathology starts outside of the current laboratory environment. The term "pre-analytic" in essence refers to any process that has an impact on human tissue from the instant it leaves the body until a histological staining procedure is initiated.

The pre-analytical stages of histopathology very much depend on the type of specimen in question and the subsequent analysis required. Highlighted below are the key pre-analytical stages that have a direct influence on standardisation in histopathology (refer to Fig. 2).

Open communication between laboratories and surgical services is essential to ensure that theatres, specimen transport and laboratory personnel are aware of the critical roles they play in histological standardisation.

With HER2 testing as an example and using needle core biopsy and large resection specimens to illustrate the differences between small and large specimen collection, the key aspects of the pre-analytical phases of histopathology which impact on both patient diagnosis and resultant patient care are considered.

Specimen handling – needle core biopsy

As minimal tumour is often present in needle core biopsies, appropriate handling is essential to present the greatest percentage of artefact free viable tumour for diagnostic assessment.

Whether breast needle core biopsy resections are performed using standard, ultrasound guided or mammotome-based systems, often the resultant, resected tumour is exposed at the cored surface. Therefore subsequent core handling should be kept to a minimum, using flat edged forceps to handle specimens only if required, minimising both grip and crush artefact (with optimal direct extrusion into an appropriate fixative at 20:1 fixative to specimen volume ratio).

Immediate transfer of specimen into fixative of choice ensures prompt fixation of tissue morphology. Handling of the biopsy in the laboratory should be minimised since excessive laboratory handling is considered to be a key contributor to crush artefact. Often breast needle core biopsies are larger than other needle cores such as those of prostatic, renal or hepatic origin. Personnel should be aware of potential grid-like artefacts introduced through mesh based biopsy capsules when handling larger biopsy material.

If needle core biopsies are to be x-rayed to aid in the identification of specific disease states (e.g. micro calcifications within *in situ* disease), ideally this should be subject to stringent time constraints. If a delay is likely to occur the specimen should not be allowed to dry out or placed on materials that accelerate this process (e.g. absorbent paper or gauze).

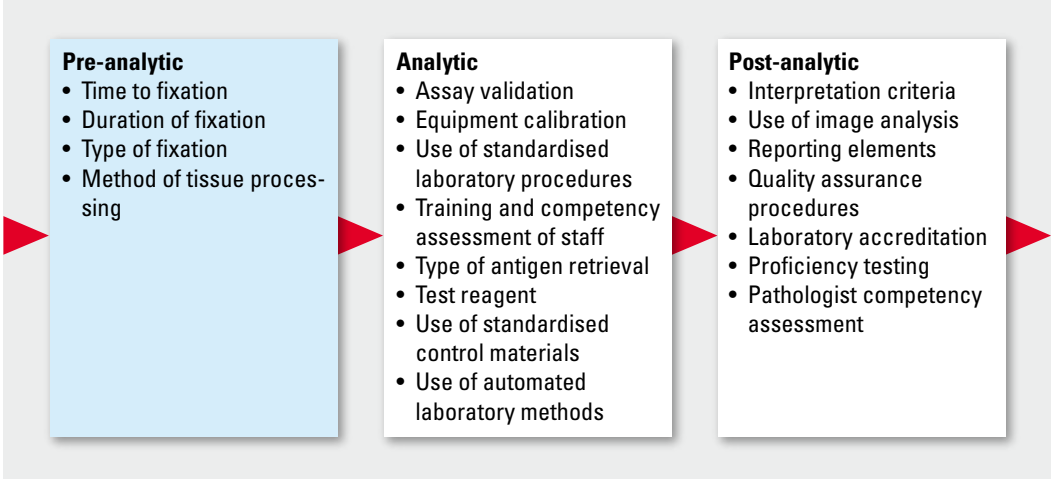

Fig. 1: Highlighting the pre-analytical, analytical and post-analytical phases of diagnostic histopathology, with key focus on the pre-analytical stage.

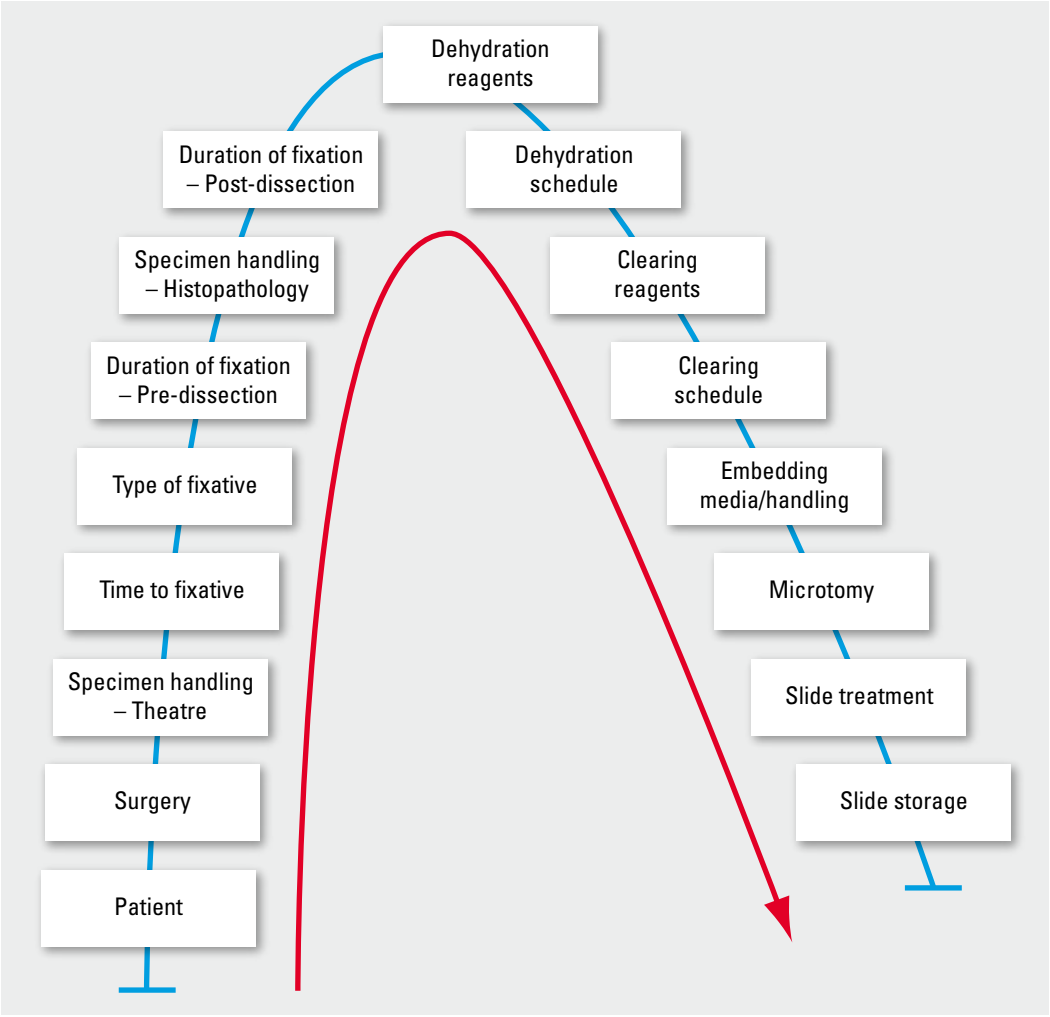

Fig. 2: Demonstrating the key phases of pre-analytical histopathology.

Further laboratory procedures may involve dying needle core biopsies to aid in visualisation at the embedding stage. Dyes that exhibit fluorescence properties may have an impact downstream in fluorescence based testing, for example when analysing breast tumours with CEP17/HER2 dual probe FISH under fluorescent microscopy, illustrating the requirement of understanding the nature and chemical properties of the substances we utilise on a routine basis.

Specimen handling – large resection

In larger surgical resections the tumour is often encapsulated within a surrounding body of adipose tissue or overlying skin. However, careful specimen handling is equally important, particularly in cases that may have key pathological features present at the surface of the specimen, to ensure that features are presented for assessment (e.g. crusting, ulceration and bleeding at the skin surface in cases of Paget's disease of the breast or dimpling/ridging of the breast, or a change in areola pigmentation in cases of inflammatory breast disease).

Furthermore surgical sutures and/or guide wires may be present and specifically attached to the specimen. It is required that these key surgical markers remain *in situ* to enable the pathologist to appropriately orientate and dissect the resected specimen.

Again, prompt fixation in a suitable volume of fixative (20:1 fixative to specimen volume ratio) is crucial to preserving diagnostic tissue structures. However, due to relatively slow penetrative effects of fixatives, larger pathology specimens should undergo appropriate specimen slicing to facilitate fixation of centrally located tumours. This is required to preserve tumour morphology and epitope antigenicity, crucial factors in end diagnosis, the aim being to enable pathologists to assign areas of tumour necrosis and amorphous cellular growth as a result

of hypoxic tumour progression and specific cellular differentiation, not as a result of inadequate fixation and sub-optimal processing.

Often larger specimens have approval for research that allows collection for tissue/tumour banking or require a fresh sample of tumour for cytogenetic evaluation (e.g. determination of ploidy status). In either scenario the specimen may be required to remain fresh (unfixed) until subsequent examination/dissection and appropriate samples are taken. In order to prevent tissue degradation, specimens should ideally be held at 4 °C.

The use of dyes has long been commonplace in pathology dissection, utilised as a tool for aiding microscopic orientation and distinguishing surgical margins. Certain dyes may exhibit radio-opaque and/or fluorescent properties. If we consider the inking of gross specimens pre and post dissection, subsequent tissue slices are often subjected to x-ray to aid in determination of specific disease states (e.g. micro calcifications within *in situ* disease), we should consider the potential nature of radio-opaque dyes and the effects they can have on radiographical interpretation. As indicated for needle core biopsies, these dyes, although utilised for a different reason may also exhibit auto-fluorescence, and may have an impact downstream in fluorescence based testing.

The assessment of sentinel lymph node status has become a routine technique for management of breast cancers. Surgical and oncology teams should be aware that the dye used should be carefully selected.

In vitro studies have shown methylene blue based dyes to adversely affect the breast hormone oestrogen receptor in ligand binding assays and in immunohistochemical demonstration for oestrogen receptor alpha (ERα) and progesterone receptor[4]. Although not a lot is known about the effects of sentinel lymph node dyes on HER2 status, the reducing nature of such dyes as methylene blue and the protein composition and location of the HER2 molecule suggest that interaction is possible, although the outcome/effects to date are unclear.

The common observation that HER2 IHC profile can change between needle core biopsy and large resection is also apparent. Change may be due to an alteration in the biology of the tumour from time of initial biopsy assessment through to full resection, especially in cases of tumours with high cellular turnover and aberrant genetic clonal/profiles. Also, a change in HER2 IHC profile may be seen simply due to the fact that the needle core biopsy may not be representative of the whole tumour and we are only observing a snapshot through a much larger 3-dimensional structure, whereby with larger resections it is easier to understand the IHC profile in the context of a larger resected area of tumour with surrounding associated tissue morphology.

However, fixation can also play a critical role. Variation in specimen to volume ratio and duration of fixation can prevent standardised results if fixation protocols for small biopsies and large resections are not calibrated.

One of the less understood potential reasons for this variability is the use of pre-operative chemo/radio therapy prior to large resection. If the aims of pre-operative therapies are to decrease and/or suppress tumour growth prior to surgery, it stands to reason that they may have an impact of HER2 receptor functionality. A study by Zhu *et al.* 2004[5] suggested that both HER2 IHC and FISH status was decreased between needle core biopsy and large resection following treatment with the Aromatase inhibitor Celecoxib Antiaromatase.

Type of fixative

In alternate diagnostic testing environments, specific tests require samples to be treated in a particular manner (e.g. EDTA based for general haematological studies, sodium citrate or sodium oxalate for more specific anti-coagulation studies). Should the histopathology laboratory consider itself any different? In fact, we do not. We have long understood the requirement for specific fixatives when specific disease states are being investigated, the utilisation of fixatives containing solutions of potassium dichromate (e.g. Zenkers solution) to demonstrate neuroendocrine cells when analysing the pheochromocytoma is an example of just one. However, the way our discipline differs is in the nature of the specimen. Although argued by our colleagues in haematology in good humour, a 1 mL vial of blood is essentially the same whoever it is taken from, whereas tissue type and size of the specimen in the histopathological environment are essentially never the same. This results in standardisation of fixation always being a compromise from specimen to specimen.

Adequate and appropriate fixation is the cornerstone of all histological and immunohistochemical preparations. Ideal fixation is a balance between good morphology and good antigenicity. Poor fixation or delay in fixation results in loss of antigenicity or diffusion of antigens into surrounding tissue (through tissue degradation and cellular lysis). Poorly fixed blocks do not process to paraffin adequately

and often have to be reprocessed or re-embedded after prolonged re-infiltration in wax.

Formalin has been used as a fixative from the end of the 19th century. It was by good fortune that Dr F. Blum used a solution of 40% formaldehyde (w/v) that he had been given to assess as an antiseptic. Diluted to 1:10 to produce a 4% solution, he reported that the solution was a good but slow acting germicide. He also noted that when the solution remained on his fingers for a short period of time, they became stiff. This stiffness was the same type of effect that he had experienced when using other fixatives such as alcohol. Based on Blum's observations this 4% solution has remained the percentage recommended for formalin fixation to date and has not been challenged despite the well documented detrimental effects formalin fixation has on tissue immunoreactivity. Despite this, formalin is the mostly widely used fixative for histological investigations. However, the formulation of the fixative used e.g. Neutral Buffered Formalin (NBF), Formal Saline, or other solutions of formalin such as Formal Calcium, has traditionally been left to each individual laboratory.

The same is true of the choice of tissue processing reagents and duration of tissue processing. This lack of standardisation of the fundamental processes of histology culminates in the production of a unique preparation – a tissue block that is unique and characteristic of the laboratory that produced it. Subsequently, sections cut from such a block will provide substrates for immunohistochemical investigations that may be vastly dissimilar to sections cut from material fixed, processed and sectioned elsewhere. A truly ubiquitous paraffin block does not therefore exist.

The main target of immunohistochemists is to devise protocols that give the greatest sensitivity without compromising specificity. The quality of the primary antibody has an enormous effect on specificity, but tissue fixation has the greatest impact on sensitivity of immunohistochemical methods. Fixation alone does not characteristically cause a loss of immunorecognition of tissue antigens. Immunorecognition for certain antigens is lost after specific combinations of fixation, tissue processing and paraffin embedding.

The Association of Clinical Oncologists/College of American Pathologists (ASCO/CAP) has addressed standardisation of fixation in the USA for general/routine immunohistochemistry. Their recommendations state that for optimal results tissue specimens with maximum dimensions of 1.0 × 1.0 × 0.4 cm should be fixed for a minimum of 3.5 hours and for a maximum of 18 hours in fresh 10% NBF. The NBF must be less than one month old. Fixed tissues that cannot be processed immediately to paraffin should be stored in 70% alcohol until processed. For general/routine immunohistochemistry in the EU/UK no official recommendations have been made regarding the type of fixative or duration of fixation. For purposes of HER2 testing ASCO/CAP specifically recommend a 6–48 hour fixation period in NBF, dependant on specimen type and size ranging from needle core biopsy to large resection[1].

The use of commercially available pre-filled pots has enabled fixative volumes to become more standardised for smaller specimens. However it is often still an arbitrary volume when it comes down to fixation of larger specimens, dependent on volume of fixative available, size of vessel and skill of the technician.

In the case of immunohistochemical based HER2 testing the performance of commercially available HER2 assays (e.g. Leica Oracle™ HER2 Bond™ IHC System, Dako HercepTest™ or Ventana Pathway™ HER2) relies on and is validated for use with standard diagnostic formalin fixation and processing schedules (refer to Fig. 3).

Time to fixative

A record of time of specimen to fixative may provide useful in determining reasons for suboptimal tissue morphology, and should be encouraged as we strive to standardise our histological processes, however this is not always available. A useful histological indicator of time to fixative is the assessment of red blood cell (RBC) morphology. A delay in time to fixative may be illustrated by poor RBC morphology and potential lysis, whereby prompt time into fixative may be illustrated by good RBC preservation and morphology.

Duration of fixation

As with the type of fixative utilised, appropriate duration of fixation has a major influence on standardisation and consistent assay performance. Specimens that are under-fixed (i.e. specimens which have limited protein or nucleic acid preservation through the suboptimal formation of aldehyde cross linkage by formalin based fixatives) will yield substandard histological morphology, nuclear detail and antigen/nucleic acid preservation, potentially compromising diagnostic evaluation.

Alternatively, prolonged fixation may result in over-fixation. Issues associated with over-fixation

Leica Oracle™ HER2 Bond™ IHC System	All specimens must be prepared to preserve the tissue for immunohistochemical staining. Standard methods of tissue processing should be used for all specimens. It is recommended that tissues are prepared in formalin-based fixatives and are routinely processed and paraffin-embedded. For example, specimens should be blocked into a thickness of 3–4 mm and fixed for 18–24 hours in 10% neutral-buffered formalin. The tissues should then be dehydrated in a series of alcohols and cleared through xylene, followed by impregnation with molten paraffin wax, held at no more than 60 °C. Tissue specimens should be sectioned between 3–5 µm. The slides required for HER2 oncoprotein evaluation and tumour verification should be prepared at the same time. To preserve antigenicity, tissue sections mounted on slides (Leica Microsystems Plus Slides – product code S21.2113) should be stained within 4–6 weeks of sectioning when held at room temperature (20–25 °C). Following sectioning, it is recommended that slides are incubated for 12–18 hours at 37 °C. Sections which require further adherence may be incubated at 60 °C for a further hour.
Dako HercepTest™	Tissues preserved in neutral buffered formalin or Bouin's fixative for routine processing and paraffin embedding are suitable for use. For example, specimens from the biopsy should be blocked into a thickness of 3 or 4 mm and fixed for 18–24 hours in neutral buffered formalin. The tissues are then dehydrated in a series of alcohols and xylene, followed by infiltration by melted paraffin held at no more than 60 °C. Properly fixed and embedded tissues expressing the HER2 protein will keep indefinitely prior to sectioning and slide mounting if stored in a cool place (15–25 °C). In the USA, the Clinical Laboratory Improvement Act of 1988 requires in 42 CFR 493.1259(b) that "The laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination". Tissue specimens should be cut into sections of 4–5 µm. The slides required for HER2 protein evaluation and verification of tumour presence should be prepared at the same time. To preserve antigenicity, tissue sections mounted on slides (SuperFrost Plus, poly-L-lysine or silanized slides) should be stained within 4–6 weeks of sectioning when held at room temperature (20–25 °C). The use of HercepTest™ on decalcified tissues has not been validated and is not recommended.
Ventana Pathway™ (4B5)	The recommended fixative is 10% neutral buffered formalin. The amount used is 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24 hour period. A 3 mm or smaller section of tissue should be fixed no less than 4 hours and no more than 8 hours. Fixation can be performed at room temperature (15–25 °C). Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades. Properly fixed and embedded tissues expressing the antigen will remain stable for at least 2 years if stored in a cool location (15–25 °C). The Clinical Laboratory Improvement Act (CLIA) of 1988, 42CFR493.1259(b) requires that "The laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination". Approximately 5 µm thick sections should be cut and picked up on glass slides. The slides should be Superfrost Plus or equivalent. Tissue should be air dried by placing the slides at ambient temperature overnight. Studies at Ventana indicate that air-dried cut tissue and cell line sections stored at 2–8 °C are stable for at least 6 months.

* Data obtained directly from individual system Instructions For Use (datasheets).

Fig. 3: Detailing the prescriptive tissue handling methodologies associated with the commercially available HER2 IHC detection system.

include formation of tissue-based pigments. However, over the years histologists have been able to read around or remove these fixative induced artefacts (e.g. the removal of formalin pigment from tissue section with the use of picric acid).

The more current issue associated with over-fixation is the effect on tissue antigens. As tissue fixation is a progressive process which can take anything up to 10–14 days to come to completion in a 1 cm^3 piece of "standard" tissue, it should come as no surprise that generally what we deal with on a routine basis in the diagnostic laboratory is essentially under-fixed material. However, when pharmaceutical clinical trials are undertaken, samples are often subject to what we term routine tissue processing as the histology is often performed in the diagnostic laboratory (in fact clinical trial specimens are most likely to be under-fixed in the same manner as our diagnostic material). This characteristic of progressive tissue fixation and its profound impact can be specifically illustrated with regard to HER2 antigen preservation.

All commercially available HER2 IHC detection systems recommend similar (not identical) stringent protocols for tissue and section handling. It is important to be aware of the subtle variations associated with each test (refer to Fig. 3).

Commercial fixation and processing recommendations are often linked to the protocols that were employed in pharmaceutical/clinical validation trials, enabling a standardised link between pharmaceutical clinical trials, commercial system validation and ultimately diagnostic testing. Pharmaceutical clinical trial assays and subsequent commercially available companion diagnostics often have a fixed Epitope Retrieval, Primary Antibody and Detection Protocols optimised specifically to complement both specimen handling, fixation and processing in both initial clinical trials and subsequent diagnostic practice.

Over-fixation from recommended standard diagnostic protocols will result in suboptimal results generally due to inadequate epitope retrieval of the HER2 epitope. An example of this is where a tumour is assessed in the routine diagnostic setting and given an appropriate HER2 IHC score. The laboratory may then obtain approval to utilise the remainder of this tumour as laboratory control material. The specimen may then be revisited at a later date, where it has been stored in fixative for a prolonged period

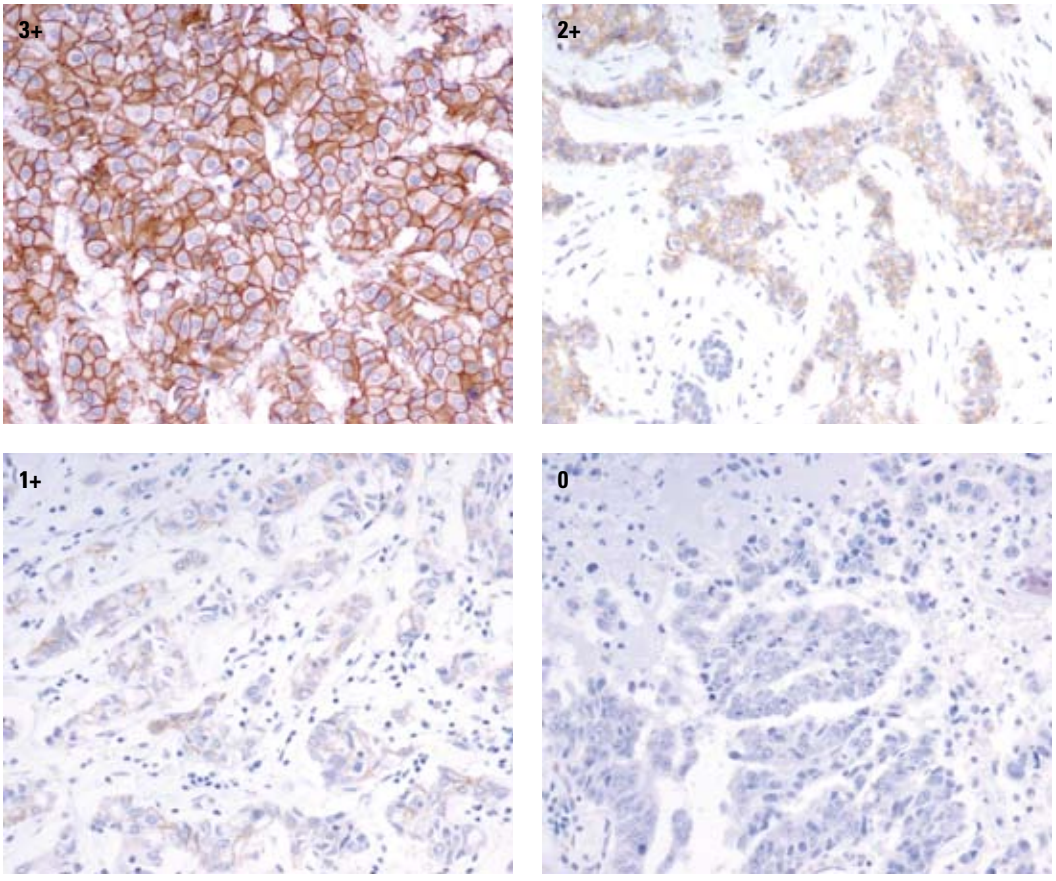

Fig. 4: Illustrating four differing invasive breast tumour samples optimally stained with the Oracle™ HER2 Bond™ IHC System (sectioned at 4 µm) demonstrating immunohistochemical staining at 0, 1+, 2+ and 3+ HER2 expression levels.

(and has been continuously undergoing fixation for the storage period). The sister block of tissue is then processed and assessed using the same HER2 assay. Taking into consideration potential tumour heterogeneity in a percentage of cases, considerable variation in IHC profile is often seen due to the effects of long term storage in fixative together with a fixed epitope retrieval protocol not designed to retrieve the HER2 antigen after an extended period of fixation.

A record of duration of fixation again may provide useful in determining reasons for suboptimal tissue, however if time to fixative is not known, duration of fixation is often an estimation based on time of sample taken and receipt in laboratory. A useful histological indicator of duration of fixation is the assessment of nuclear chromatin patterns. Detrimentally short duration of fixation may be illustrated by poor nuclear chromatin preservation and detail, whereby appropriate duration of fixation may be illustrated by good nuclear chromatin preservation and detail.

Tissue processing

Together with tissue fixation, good tissue processing is essential to producing a good end preparation. The procedure of dehydration and clearing is made easier when material is appropriately fixed in the first instance. However, careful consideration should be given to choice of dehydrant, clearing agent and regime employed, to allow for a preparation which can be fully infiltrated with molten wax which can then be appropriately sectioned and stained (refer to Fig. 3). The use of high temperature and/or microwave based tissue processors should be carefully validated by the laboratory, ensuring production of viable high quality diagnostic material suitable for all subsequent clinical evaluations. Here we illustrate four invasive breast tumours optimally fixed and processed demonstrating immunohistochemically the HER2 protein at 0, 1+, 2+ and 3+ expression levels, stained using Leica Microsystems Oracle™ HER2 Bond™ IHC System. Although we consider the use of in-house tissue controls, fixed and processed in the same manner as test tissue as gold standard laboratory control material. The use of commercial system control cell lines as a complementary tool for monitoring reagent and assay performance are invaluable. This can be demonstrated with the Oracle™ HER2 Control Slide containing formalin (NBF) fixed paraffin embedded MDA-MB-231, MDA-MB-175, MDA-MB-453 and SK-BR-3 human breast cancer cell lines demonstrating the HER2 protein at 0, 1+, 2+ and 3+ expression levels, again optimally stained using Leica Microsystems Oracle™ HER2 Bond™ IHC System (refer to Fig.5).

Section preparation

The importance of the procedure for the preparation of sections for immunohistochemical staining is often overlooked due to the routine nature of this process within the routine setting.

To produce the end product, a slide containing a correct, high quality sample that is orientated correctly and adhered sufficiently, is a multi-step technique that varies greatly between laboratories. Of these steps, section thickness, water-bath temperatures/time on water-bath and section drying/storage can account for variations in the immunohistochemical staining profile of the preparation.

Section thickness

The technique of cutting tissue sections using a microtome has changed little since it was first developed. Ergonomic enhancements have changed the microtomes appearance with motorisation and electronic components, together with the introduction of disposable microtome blades, assisting users in an attempt to produce consistent high quality, reproducible sections.

Despite the advances in microtome design, standardisation of section thickness has not been achieved, with histopathology laboratories typically sectioning at thicknesses of between 2 µm and 5 µm. Evaluation of section thickness and its consequences in immunohistochemical staining profiles has not been extensively published, but with the complexity of modern day diagnosis no longer resting on a positive or negative result, standardisation in section thickness may have to be addressed. Again using HER2 as the example model of a semi-quantitative immunohistochemical assay, the recommendations for tissue section thickness from commercially available systems range from 3 µm to 5 µm (refer to Fig. 3). Highlighting the prescriptive requirements of this essential stage of the pre-analytical process.

Water-bath

The use of a thermostatically controlled clean water-bath is an essential part of the sectioning process. Overheated water will result in the wax supporting the tissue or human cell lines to melt, resulting in damage to the tissue. Standardisation

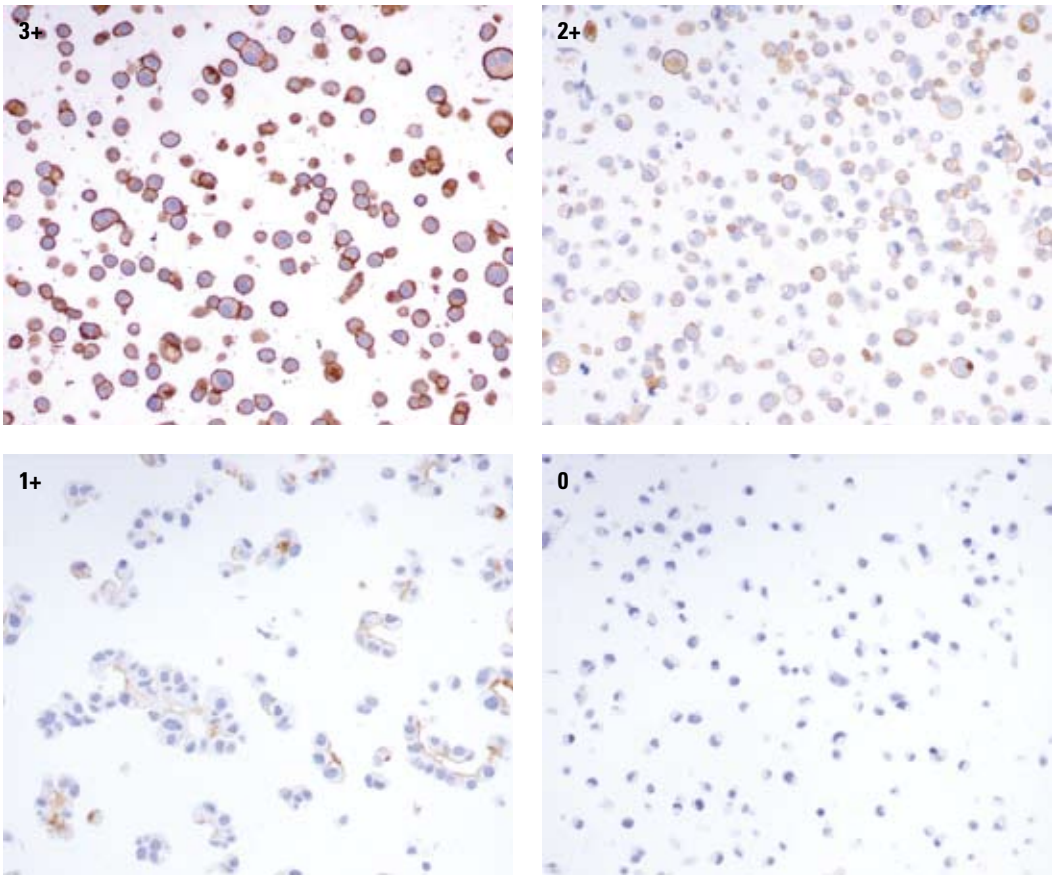

Fig. 5: Illustrating the Oracle HER2 Control Slide optimally stained with the Oracle™ HER2 Bond™ IHC System demonstrating immunohistochemical staining at 0, 1+, 2+ and 3+ HER2 expression levels.

within an individual histopathology laboratory is essential and the exact temperature of water required will vary depending on the melting point of the wax used.

The time a section is allowed to float on a water-bath should be kept to a minimum as extended times will again result in morphological damage. Tissues that are sub-optimally fixed and processed will become more evident when floated on a water-bath, as areas of tissue not fully impregnated with wax will readily interact with water and expand, destroying tissue morphology and rendering immunohistochemical analysis difficult. It is important to utilise clean microbial/contaminant free water when sectioning for immunohistochemical preparations. Bacterial contamination at all the pre-analytical stages may result in suboptimal immunohistochemical preparations, illustrated by the presence of suboptimal localisation of staining with increased non-specific background.

Section drying and storage

Standardisation in section drying should be adopted within the histopathology laboratory in an attempt to preserve antigenicity within the tissue section. Prolonged drying at high temperatures, especially on direct heat hotplates, may reduce the antigenicity within a tissue section.

Commercial recommendations for section drying range from allowing sections to dry at room temperature overnight to overnight incubation at 37 °C followed by up to 1 hour incubation at 60 °C. These methods all avoid direct heat and prolonged high temperatures gently allowing sections to adhere to slides facilitating good immunohistochemical preparations.

Section storage following slide drying plays a crucial role when archived sections are required for immunohistochemical analysis. Deterioration in antigenicity can occur with the long-term storage of cut sections[6] and should be stained within 4–6 weeks of sectioning when held at room temperature (20–25 °C).

Prolonged storage of HER2 cell lines at 2–8 °C for between 6 to 12 months has been commercially demonstrated. Preservation of the antigenicity of cell line sections is achieved through regulated fixation of the cell line epitope in question.

Summary

It is crucial that as cancer therapeutics becomes ever more patient specific and the portfolio of tissue based diagnostic assays develops, standardisation in histopathology has to become a requirement rather than a consequence of targeted testing. We hope that we have illustrated some of the profound effects the pre-analytical stages in histopathology have on the end preparation, only through the standardisation of these pre-analytical steps will be able to effectively address the requirements of the next generation of companion diagnostics.

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www.leica-microsystems.com

Editors in Chief
Anja Schué,
Corporate Communications
Anja.Schue@
leica-microsystems.com

Daniele Morpurgo,
European Marketing
Daniele.Morpurgo@
leica-microsystems.com

Layout
Heinz Flick

Cover Picture
Leica Microsystems

Production
Uwe Neumann,
Corporate Marketing & Identity

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Peter Jackson,
Department of Histopathology & Molecular Pathology, Leeds Teaching Hospitals NHS Trust, Leeds, UK,
Pete.Jackson@leedsth.nhs.uk

Michael Gandy,
Theranostics Project Manager,
Leica Biosystems Newcastle,
Michael.Gandy@leica-microsystems.com

Phillip Thompson,
Department of Histopathology & Molecular Pathology, Leeds Teaching Hospitals NHS Trust, Leeds, UK
Phillip.Thompson@leedsth.nhs.uk

Craig Barker,
Theranostics Project Manager,
Leica Biosystems Newcastle,
Craig.Barker@leica-microsystems.com

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