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Digital Immunohistochemistry Implementation, Training and Validation : Experience and Technical Notes from a Large Clinical Laboratory

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Abstract

Aims

To consider the value proposition of digitisation of clinical immunohistochemistry services, and to develop an approach to digital immunohistochemistry implementation and validation in a large clinical laboratory.

Methods

A methodology for slide scanning in the laboratory was developed, in addition to a novel validation exercise to allow pathologists to identify the strengths and weaknesses of digital immunohistochemistry reporting, and train in digital immunohistochemistry slide assessment.

Results

A total of 1480 digital immunohistochemistry slides were assessed by 24 consultant pathologists, with complete clinical concordance between the digital and the glass slide assessment observed. Certain stains were identified as being difficult/time consuming to assess using 20x digital slides. These stains were rescanned at 40x, which improved the confidence of the pathologists to make a digital assessment. Full digitisation of immunohistochemistry slides was achieved, introducing 6 new steps into the pre-existing laboratory workflow.

Conclusions

Whilst initially encountering challenges in terms of workflow, our experience showed that a well designed, adequately resourced and well-managed scanning process can minimise the delay in slides being made available for review. Our approach to validation highlighted the need for careful assessment of a digital pathology system and scanning protocols before pathologists are expected to transfer from the light microscope to the digital microscope for routine IHC assessment.

Introduction

The production and use of digital Whole Slide Images (WSI) for the routine assessment of histology slides, including standard haematoxylin and eosin (H&E) and immunohistochemistry (IHC) is becoming widespread, with sites across the world incorporating slide scanning in to standard operating procedures. The expanding role of the pathologist in requesting, assessing and reporting immunohistochemical biomarkers to inform drug selection for patients (eg. Her2, PDL-1) makes the accurate and efficient turnaround of these slides and reports increasingly important. The specialist expertise required to produce IHC stains means that pathologists working in remote sites often rely on external laboratories to produce such stains, so the ability to use WSI offers significant cost and time saving compared to the shipping of glass slides between sites. Whilst some IHC slides, particularly those involved in large panels for tumour identification require relatively simple,

"binary" assessment (positive or negative), others require more complex assessment of quantity or intensity of staining, and their accurate scoring forms an important part of subsequent treatment decisions (eg. ER, PR, Her2, Ki-67)). Successful implementation of a digital immunohistochemistry service requires the development and integration of new laboratory protocols and processes, and validation of the clinical usefulness of the whole slide images produced.

This paper considers the value proposition of digitising IHC slide output, and outlines an approach to digital immunohistochemistry implementation and validation in a large clinical laboratory. We will summarize the lessons we have learned from this experience, including advice and observations regarding IHC slides that our pathologists found challenging to read on the digital microscope, and provide tips for the training and validation of pathologists. We hope this paper will prove a useful starting point for any laboratory considering their own immunohistochemistry implementation.

1. Background

Leeds Teaching Hospitals NHS Trust has a single site laboratory, processing in the region of 290,000 H&E stained slides and 50000 immunohistochemistry slides per annum. We are a fully subspecialised diagnostic department with more than 40 whole time equivalent specialist pathologists, covering all major surgical pathological specialties. As of September 2018, the department has successfully implemented routine scanning of 100% of surgical pathology slides, including standard slides, large slides, H&E, IHC and special stains. The scanning deployment was implemented in phases, with a stand-alone immunohistochemistry service one of the earliest digitisations. (Figure 1)

The rationale for early introduction of digital IHC slides as opposed to a phased specialty-by-specialty approach was that this would provide *all* pathologists with the opportunity to familiarise themselves with digital slide viewing and reporting before the introduction of routine scanning of all histology slides. Secondly, at this stage in our implementation, the majority of our pathologists only had access to standard desktop computer screens, and we reasoned that IHC assessment might be less limited than H&E diagnosis on this hardware. Additionally, the nature of IHC work is that it is often required urgently to make treatment decisions at the multidisciplinary team meeting (MDT) or tumour board, and digital slides might enable better access to IHC slides prior to (and even during!) these meetings.

The IHC sample also allows the project to test the system and infrastructure with a substantial, discrete subset of slides and cases (around 20% of the final workload). This would enable the laboratory to test and embed new processes prior to full scale digital implementation, and allow users to test and provide feedback on the IT infrastructure. In addition this phased approach provided a gateway to allow Go/No-Go decisions on project progress.

2. Laboratory Implementation

2.1 Challenges to Workflow

There are challenges with scanning IHC slides. First of all, scanning involves additional steps in the laboratory process – at the point at which glass slides would normally be sent to a Pathologist for diagnosis, they are diverted to scanning and go through additional steps (See figure 2). These steps are necessary for any scanning, but will vary in their detail depending on the software, policies and processes of a site.

The steps involved in scanning require staffing resource and also increase the turnaround time (TAT) for a pathologist to receive slides for review. In the scanning workflow, the model of scanners used takes an initial photograph or "snapshot" of a slide, using tissue detection algorithms to determine what area of the slide will be scanned in detail. These snapshots are then manually checked and adjusted by operators to ensure that all necessary tissue on the slide is scanned, and that there are sufficient focus points on the slide to capture a high quality image. It is only after these snapshots are checked that full scanning is initiated by the operator. There are additional steps involving the creation of cases on the slide database and the assignment of slides to cases, which if not automated, further increase the resource required to scan slides. When IHC slide scanning began, automated case creation had been implemented, and automated assignment of slides to a case was under development.

2.2 Scanning IHC Slides

At the point at which IHC slide scanning was implemented, slides for two specialties (Breast and Neuropathology) were already being scanned as part of the pilot phase, amounting to c. 3000 slides per month. This meant that the laboratory had some familiarity with the scanning process, although given the small volumes, this was limited to a small proportion of the staff as only one member of staff was required to operate the scanners. The challenge for the laboratory was to cope with an increased volume of slides (c. 7000 slides per month total) being scanned whilst minimising the increase in Turnaround Time (TAT).

Prior to the introduction of IHC scanning, two Leica Aperio AT2 scanners and one Leica Aperio CS2 scannerswere used. The AT2 scanners are high capacity (capable of holding up to 400 slides in 10 racks of up to 40 slides) and the CS2 scanners are lower capacity but capable of handling large slides (1 rack of 5 slides, or 2 large slides). Slides were scanned at 40x magnification. To increase the scanning capacity, an additional 4 AT2 scanners and 2 more CS2 scanners were deployed. The total number of scanners deployed was based on the requirement for 100% scanning of surgical slides, but the increased volumes from IHC scanning meant that the scanners could be tested and that the lab could try different processing models. The decision was taken initially to scan IHC slides at 20x magnification – this was deemed sufficient given the nature of the pathologist review of such cases, and also meant the scan time was significantly reduced (in most cases, decreased by at least 60%). Additional staff were deployed on scanners to process the additional workload.

Some adjustments were made to the slide production process to streamline the scanning process; instead of slides being laid out in trays after being produced, they were put straight into scanning racks, and some checking steps were removed as they were viewed as adequately covered by the final check of scanned images.

2.3 Effects of Scanning IHC slides

As described above, the scanning of IHC slides adds 6 steps to the end-to-end process, but these steps need not be time consuming. Given that the time taken to scan a single IHC slide at 20x magnification is 2 minutes or less, a rack can be loaded, scanned and quality checked in under 15 minutes. Considerable time was spent by the project team evaluating the need to optimize scanning capacity and efficiency in terms of resource versus optimizing the speed, and the 15 minutes is an example of what can be achieved when speed is prioritised over scanner and operator capacity/effort. When prioritising capacity, racks will be filled to capacity and loaded when full i.e. routine cases on an overnight run. Urgent cases during the day are loaded onto a rack and that rack is loaded with only that case on, snapshotted, scanned, and quality checked in rapid succession. In short, the smaller the batch of cases/slides loaded on at once, the faster that those cases can be turned around in the process.

From the pathologist's perspective, although it is early days, there are clear areas where digital images offer opportunities for time savings. For pathologists that do not review H&E slides again (for instance relying on first-pass reports), the scanned images remove the need to move from the computer to the microscope and back again, allowing all of the supplementary report to be produced using just a computer. This can save a few minutes per case (anything up to a minute per slide, depending on office layout). There is also no need to "mark up" slides prior to viewing, again saving several seconds per slide. Annotations and measurements can be done on screen, and the macro view offered by digital images has also proven useful. These small affordances, depending on a pathologist's working style, can add up to a few minutes per case. Such gains would be increased substantially once routine H & E slides were scanned, allowing pathologists stay in one place (at the computer) to do all of the work associated with a case, rather than wearing a track in the office carpet between microscope and computer.

Further productivity gains will be obtained through the elimination of "admin" that goes with glass slides. For IHC cases, there is already a benefit from reduced need to "match up" slides, or organise the slides picked up from pigeonholes. This benefit is greatly increased when all cases are digital, potentially eliminating the need retrieve glass slides from pigeonholes at all, almost eliminating time spent walking to and from pigeonholes to collect cases. This again will result in several minutes per day being saved. Overall, we estimate that, depending on an individual pathologist's working style, and their work content, anything up to half an hour per day could be saved through the reduction of admin and need to transport glass slides, even before allowing for potential differences in time to review digital and glass slides.

3. Training and Validation

3.1 IHC training and validation Protocol

The assessment and interpretation of IHC slides sometimes requires the pathologist to make a simple distinction between a positive and a negative result, but can be complex, requiring detailed localisation of the staining and correlation with the H&E stained slide or grading of the proportion of stained cells, or the intensity of the staining. In light of this we wanted to ensure our pathologists had sufficient training and familiarity with digital IHC slide use before they started using digital IHC slides in routine practice. We developed a digital IHC training and validation protocol which is a simplified and streamlined version of the digital primary diagnostic training and validation protocol

recommended by the Royal College of Pathologists in their best practice guidance [1]. See table 1for an overview of this protocol.

Table 1. . Overview of digital IHC training and validation

Phase	Aim	Description
1. Basic Skills	Pathologist familiarisation with digital pathology software	30-60 minute session Observed practice with feedback
2. Validation and Training Cases	Pathologist familiarisation with digital IHC images Identification of challenging cases Identification of IHC types that require routine 40x scanning	Pathologist views set of approx. 10 relevant training cases, covering a range of IHC stains and scenarios Discussion and feedback
3. Ongoing surveillance	Clinical governance of digital reporting Assessment of scanning requirements for new stains/scenarios	Adhere to local/national clinical governance guidelines Consider yearly audit of proportion of digital IHC assessments

3.2 Phase 1 Basic skills training

The aim of this stage is to train each pathologist in the use of the digital pathology system. This stage can be truncated or omitted for pathologists who are already experienced in using the digital pathology system.
It consists of a short (30 mins-1 hour) training session in which the pathologist learns from an experienced user of the system (a trainer). Access to a help manual, and training slides is required. The pathologist is taught:

- The basic digital pathology workflow and layout of the software
- How to use the system to open a case/ slide and pan and zoom
- How to use the system to annotate a case and other advanced functions as necessary
- How to access the documentation for the system
- How to identify gross scanning artefacts

The trainer observes the pathologist open and read a small number of training cases and provides feedback.

3.3 Phase 2 Validation and Training Cases

The aim of this stage is to train the pathologist on the appearance of digital IHC slides. It includes exposure to cases anticipated to be challenging to diagnose digitally, and encompasses a variety of case types and stains as defined in the validation scope. Discussion with pathologists prior to validation can be used to identify stains and scenarios that are potentially difficult to diagnose on the digital platform, or those that have important therapeutic implications for patients.

A set of slides was prepared for each subspecialty, comprising a set number of IHC cases for each specialty(this varied from 6 to 15 cases, and individual case size varied from 1 to 15 immunostains)). Glass slides, digital slides and clinical information are made available to the pathologist. The cases included slides from a variety of relevant tissue types, covering a range of IHC stains and diagnostic scenarios. The cases are selected to allow the pathologist to explore specific aspects of digital immunohistochemistry, which are relevant to that individual pathologist's practise and have experience of viewing a range of features on the digital microscope. As the scope of the validation protocol is to train and validate the pathologist's use of digital for immunohistochemistry assessment only, and does not extend to primary diagnosis, it was felt that a relatively small validation set of cases should be prepared for each specialty, in contrast with the Royal College of Pathologists' Guidance on primary diagnostic validation case numbers[1] and the college of American Pathologist's guidelines[2] (approximately 2 months whole time equivalent caseload and a minimum of 60 cases, and respectively). A typical “case” for these purposes, can consist of a few representative slides, and does not have to include all material from a complete clinical case. See table 2 for examples of IHC case types included by specialty. The pathologist reviews the training set, in their own time, over a short period of time (e.g. up to 2 weeks). For each case they make notes on their digital slide diagnosis. Then they immediately review the glass slides for the same case, and note their diagnosis. They make comments on the case on a proforma (see Appendix A), including their diagnostic confidence using both the digital, and the glass slides for the case, expressed on a numerical Likert scale from 1-7 (where 1 = not confident at all and 7 = very confident.) This allows the pathologist and trainer to distinguish between slides that the pathologist finds difficult to assess on any diagnostic medium, and slides that are particularly difficult to assess confidently on the WSI.

At the end of the training set, the results are discussed at a small group training meeting. This includes discussion of the pitfalls noted in the test set, and explicit identification of the cases/ features known to be difficult. If any particular type of stain or scenario is found to be problematic on digital slides, and this is not resolved following review of digital slides and discussion within the training group, the trainer will provide more examples for training, and may offer to rescan cases at 40x equivalent magnification.

Once pathologist and trainer are both satisfied that the pathologist is familiar with the operation of the system and its use in the training cases, the pathologist can view and assess their immunohistochemistry using digital slides as default. If any areas of diagnostic difficulty have been

identified, certain glass slides may be protocolled for scanning at higher magnification, or a mandatory glass check prior to case sign out may be mandated.

3.4 Phase 3 Ongoing Surveillance

Once a pathologist has completed their training for digital IHC reporting in a particular specialty, ongoing quality assurance procedures should be followed as part of normal departmental clinical governance procedure. Local incident reporting procedures should be adhered to, as they would for conventional microscopic practise. Cases should be peer reviewed for multidisciplinary team meetings, and difficult/challenging cases should be shared for second opinion, or discussed at existing intradepartmental meetings, in settings where both glass and digital images can be studied. The department should consider introducing audit protocols to allow a random review of a proportion of an individual pathologist's digital cases on a rolling basis.

4. Outcomes

4.1 Laboratory outcomes

The increase in volumes had the desired effect of rigorously testing the laboratory process and supporting IT infrastructure. Turnaround times were perceived as longer (and in some cases were), causing considerable consternation among pathologists. These concerns were exacerbated by the fact that most pathologists were not viewing the IHC slides. It is worth noting that much of the delay was due to the need to also dispatch glass slides simultaneously, which impacted the resource required to operate the scanners. The impact of not automating slide assignment was marked – the amount of resource required to operate the scanners was much higher than anticipated. For smaller cases, the extra time is a matter of a few seconds (20 or so), but for larger cases it can be over a minute – on busy days, with multiple slides coming in for new and old cases, this can amount to a several hours of additional work (c. 0.5 FTE as a conservative estimate) . With limited resource available, the workload on scanner operators lead to some errors, with slides being allocated to the wrong cases. Although these errors were easy to correct, such errors show that operating scanners is not a trivial task, and does require operators to have knowledge of the overall process rather than just being able to load and unload a machine.

The scanning of IHC slides also highlighted the importance of the scanning process being fully integrated into the end to end laboratory process. Upstream processes, such as staining of IHC slides, were tuned to signing out of cases at the end of the working day, meaning that slides often arrived at the scanner too late to be scanned on the same day. The lack of flexibility around staff working hours meant that there was limited capacity to accommodate the extra process steps, which inevitably led to delays in scanning cases and releasing them to pathologists

Our key recommendations for workflow and scanning:

- Automate the scanning process as fully as possible to reduce resource requirements and potential for error

- Integrate scanning into the overall laboratory process management, tuning the timing of upstream processes, and resourcing accordingly. "Smoothing" the process flow in this way would enable more controlled and timely release of work.
- Additional staff are necessary to run the scanners as there is additional work – attempting to absorb the extra work involved in scanning with the same level of resourcing as without scanning without reducing the overall workload will almost inevitably lead to increased TAT.
- Consideration should be given to extending the working day to take account of the longer process.

4.2 Validation and training outcomes

A total of 24 pathologists completed the digital IHC training and validation exercise, representing 11 histopathology reporting subspecialties. The number of immunohistochemistry cases viewed per specialty specialty varied from 6 to 15 cases, and individual case size varied from 1 to 15 immunostains. A total of 1480 slides were viewed and assessed in the course of the validation by all participants. The mean satisfaction score with digital IHC slides, expressed on a Likert scale of 1-7, where 1 = not at all satisfied, and 7 = very satisfied was 5.91. The range of observed responses was 2-7. (See figure 3).

There was complete IHC assessment concordance for all cases and all observers across the validation study, with no clinically significant difference in IHC interpretation observed.

Across the validation, the average confidence score for digital slide IHC assessment was 6.1 (range 2-7), compared with 6.9 (range 6-7) for glass slides. Cases scoring low confidence values on digital slide assessment contained particular IHC stains, which pathologists almost universally reported as being difficult to assess on digital in free text comments.

Free text comments

Pathologists were encouraged to support their scoring for satisfaction with digital slides, and confidence in diagnosis on digital versus glass slides with free text commentary.

Cases scoring high confidence marks on digital slides (6 or 7), and pathologists rating their satisfaction with digital slides as high (6 or 7) gave the following feedback:

- Found digital as quick and as easy as the glass slide.
- Found it easier to spot areas of concern at low power on the digital slides than on glass.
- Positive results are spotted more quickly on the digital slide
- I find it easier to assess a multi-slide case digitally. I can see all the IHC requested at one glance, then quickly zoom in to check staining pattern.
- Easy to use and interpret.
- Quicker looking at digital images.
- Digital IHC seems more crisp.

Cases scoring low confidence marks on the digital slides (anything below 6) and pathologists rating their satisfaction with digital slides as low (anything below 6) provided the following feedback:

- Screening large volumes of tissue for rare positive cells gave me a headache.
- It took me longer to scroll through all the tissue at high power than on my light microscope.
- Need higher magnification scanning for some stains.
- H pylori blurry and difficult to spot.

In addition, our pathologists identified a number of immunostains that they found difficult to interpret with confidence using standard images captured at 20x equivalent magnification. These immunostains belong to a category of stains that either require some form of advanced assessment (eg. quantification, complex location) and/or would have direct therapeutic implications for the patient (eg. decision to offer or not offer a drug therapy). Scanning this selection of immunostained slides at 40x equivalent magnification improved the ability of our pathologists to make a confident diagnosis, and direct comparison of 20x and 40x captured images demonstrated appreciable difference in the appearance of the slides. As a result of this, these slides are now mandated for 40x equivalent scanning, whilst the remainder of the IHC workload is scanned at 20x. Pathologists can request repeat scanning at 40x of any immunostained slide which they are not confident to assess at 20x, following initial 20x assessment. (See text box for stains now routinely scanned at 40x).

IHC stains now routinely scanned at 40x equivalent magnification:

Her2

ER PR

Helicobacter pylori

Ki67

Sv40

CMV

Conclusions

In our study, we demonstrated complete concordance of WSI and glass slide assessment of IHC using digital images capture at 20x equivalent magnification. Whilst this is reassuring, it is important to consider the pathologist's confidence in their WSI assessment, and the efficiency and ease with which the diagnosis is rendered too. The majority of pathologists were satisfied and confident to use digital IHC slides rather than glass slides to report live cases, but they did highlight individual immunostains and diagnostic scenarios that were difficult to assess on standard 20x captured WSI. Our approach highlighted the need for careful assessment of a digital pathology system and scanning protocols before pathologists are expected to transfer from the light microscope to the digital microscope for routine IHC assessment.

A small number of Immunostains requiring more sophisticated assessment in terms of localisation and quantification of staining were problematic for our pathologists, who were unable to reach a confident diagnosis. For these cases, routine scanning at 40x was beneficial.

Whilst initially encountering challenges in terms of workflow, our experience showed that a well designed, adequately resourced and well-managed scanning process can minimise the delay in slides being made available for review. Furthermore, as the need for glass slides diminishes, the impact on turnaround time is even lower, as resource can be focused on making the digital images available for review rather than making the glass slides available post-scanning.

The assessment of IHC is becoming an increasingly complex and time consuming process, as more diagnostically and therapeutically useful antigens are identified and incorporated into the workload of the clinical pathologist. Pathology services are under increasing pressure to provide detailed, accurate IHC assessments within short turnaround times, at a time when many institutions are suffering from a shortage of pathologists. The judicious development and use of artificial or augmented intelligence (AI) to read and interpret IHC stained slides could provide diagnostic support to the 21st century pathologist, allowing them to concentrate on the morphology, whilst algorithms locate and quantify immunopositive regions of IHC slides.

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Figures

Figure 1. Phased implementation of 100% surgical slide scanning at Leeds Teaching Hospitals NHS Trust

Figure 2. Digital Pathology Workflow

Figure 3. Pathologist reported satisfaction with digital IHC training slides.

Appendices

Appendix A – Sample validation case proforma

Take Home Messages

Immunohistochemistry is section of laboratory work which can be digitised as a stand-alone digital pathology use case.

A well designed, adequately resourced and well managed scanning process can minimise delays in slides being available for review.

Pathologists should be offered time and support to evaluate and validate new areas of digital practice

Default scanning of problematic immunohistochemistry stain types at 40x rather than 20x equivalent magnification aids confident interpretation.

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