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Digital Pathology for the Primary Diagnosis of Breast Histopathological Specimens: An Innovative Validation and Concordance Study

Digital Pathology Validation and Training

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Abstract

Aim - To train and individually validate a group of breast pathologists in specialty specific digital primary diagnosis using a novel protocol endorsed by the Royal College of Pathologists' new guideline for digital pathology. The protocol allows early exposure to live digital reporting, in a risk mitigated environment, and focusses on patient safety and professional development.

Methods and Results - 3 specialty breast pathologist completed training in use of a digital microscopy system, and were exposed to a training set of 20 challenging cases, designed to help them identify personal digital diagnostic pitfalls. Following this, the 3 pathologists viewed a total of 694 live, entire breast cases. All primary diagnoses were made on digital slides, with immediate glass review and reconciliation before final case sign out. There was complete clinical concordance between the glass and digital impression of the case in 98.8% of cases. Only 1.2% of cases had a clinically significant difference in diagnosis/prognosis on glass and digital slide reads. All pathologists elected to continue using the digital microscope as standard for breast histopathology specimens, with deferral to glass for a limited number of clinical/histological scenarios as a safety net.

Conclusion - Individual training and validation for digital primary diagnosis allows pathologists to develop competence and confidence in their digital diagnostic skills, and aids safe and responsible transition from the light microscope to the digital microscope.

Key words

Digital pathology, validation, training

1. Introduction

1.1 Digital Pathology

Digital pathology can be defined as the use of a whole slide imaging (WSI) system to capture, transmit and store digital images of glass slides, to be viewed on a computer screen. Digital slides can be read by multiple examiners in multiple locations, facilitating remote consultations, streamlining workflows and reducing time and financial costs of transferring glass slides between locations. Instantaneous access to multiple users renders digital slide technology invaluable in a number of pathology applications including quality assurance programmes, frozen section diagnosis, multidisciplinary team meetings, clinicopathological conferences, expert panel/consensus boards and education.

1.2 Digital Pathology in Primary Diagnosis

Interest in the use of digital pathology for the primary diagnosis of histological specimens is flourishing, with a number of laboratories using digital images for primary diagnosis in at least a proportion of cases. (eg. Utrecht, Netherlands[1], Linkoping, Sweden[2], Leeds Teaching Hospitals NHS Trust and Coventry in the United Kingdom[3]). For digital pathology to be accepted and adopted on a

large scale, regulatory bodies, diagnostic departments, and individual pathologists will have to be convinced that a diagnosis made by a particular pathologist on a digital microscope is as good as a diagnosis made by the same pathologist on a conventional light microscope, and that no systematic error is introduced into the diagnostic process as a result of the technology. A recent systematic review of the diagnostic concordance of whole slide imaging and conventional light microscopy analysed data from 38 concordance studies demonstrated a mean diagnostic concordance of WSI and light microscopy (LM) of 92.4%[4]. In comparison, concordance between repeat light microscopy reads of the same case was 93.4% in those studies (n=10) that quoted it. There was a trend for increasing concordance in the more recent studies. The review found evidence to support a high level of diagnostic concordance for WSI overall. A recent systematic analysis of instances of diagnostic discordance in glass:digital comparisons of the same slides found 335 instances of diagnostic discordance, out of 8069 documented instances of a glass diagnosis being compared with a digital diagnosis (4%)[5]. The majority of these discordances would have had no clinical significance, and reflected diagnostic scenarios prone to intra- and inter-observer variation in diagnosis, regardless of the diagnostic medium used. Potential pitfalls of digital diagnosis were identified, including the detection and grading of dysplasia, and the location of small diagnostic or prognostic objects including micrometastases.

1.3 Digital pathology validation

There is little guidance available to the clinical pathologist on how to validate digital pathology for use for primary diagnosis in a real world setting. The College of American Pathologists published a guideline for digital pathology validation in 2013[6], which has formed the foundation of the majority of validation studies to date. The guidelines recommend that all departments adopting WSI for diagnosis should conduct their own validation, that at least 60 specimens should be evaluated, to assess intraobserver variation in diagnosis on digital and glass, with a washout period of at least 2 weeks between digital and glass reads of cases. Whilst this methodology provides a good baseline validation of a departmental whole slide imaging system, it may not be enough to convince the individual histopathologist that they are competent and confident to make primary diagnoses on the digital microscope.

1.3 Digital pathology in breast pathology

Digital slides are used in the undergraduate and postgraduate medical education, with breast histopathology images accessible online at sites including the online atlas for breast pathology (www2.webmicroscope.net) and the virtual microscopy website of the University of Leeds (www.virtualpathology.leeds.ac.uk). In research, digital slides allow for simplified centralised review of breast cancer material in large multicentre studies, an option explored by the Prospective Study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) cohort study, amongst many others.[7]In the LORIS trial, which aims to address the overtreatment of screen detected ductal carcinoma in situ, trial entry depends on real time review of digital slides rather than glass slides to assess eligibility. [8]

In clinical pathology, breast pathologists are under increasing pressures in terms of breast cancer case volume, case complexity, and the need for rapid evaluation and review to meet cancer diagnostic and therapeutic targets. A number of digital pathology validation studies have focused on the use of whole slide images for the diagnosis of breast biopsies. Al Janabi et al demonstrated a

93% concordance rate in a single reader study of 100 breast biopsies[9], whilst Campbell et al found intraobserver concordance between digital and glass diagnosis of 85 breast biopsies for 3 pathologists was 95.4%[10]. Both studies identified discordant diagnoses regarding a select group of diagnoses: differentiation between hyperplasia and atypical ductal hyperplasia (ADH), the differentiation of benign phyllodes tumours from fibroadenomas, and the identification of foci of microinvasion/lymphovascular invasion. In their validation study, Reyes et al found digital:glass variation in diagnosis varied between 1% and 4% for their 3 pathologists, and in all cases of discordance, the diagnostic issue was the differentiation of ductal hyperplasia from atypical hyperplasia. [11]

The majority of breast digital pathology validation studies in the literature focus on biopsy specimens, whilst in real practice, a large proportion of the pathologists time is spent viewing resection specimens, where a checklist of histological parameters of an excised tumour need to be assessed and recorded. Shaw et al published their experience reviewing both glass and digital slides of breast cancers from the POSH breast cancer cohort study[7]. 9 pathologists collected data items from digital slides of breast tumours, and then reviewed the glass slides at a later date. Diagnostic performance with the digital slides was comparable to conventional light microscopy. There was better agreement on degree of tubule formation between different reviewers using digital slides than glass slides. The authors suggest that this supports the assertion that the whole slide view provided in digital pathology permits superior assessment of the architecture of a lesion compared with light microscopy. A recent non-inferiority study compared reads of 299 breast cases by 4 pathologists, and found no significant difference in the incidence of major discordances using digital microscopy versus light microscopy.[12]

Leeds Teaching Hospitals NHS Trust made the decision to pilot digital pathology for the primary diagnosis of breast histopathology specimens, utilising a novel validation protocol which offered participant histopathologists digital microscopy training, exposure to challenging cases, and a risk mitigated early conversion to a full digital slide workload.

2. Methods

The study was performed in the histopathology department of St James University Hospital, Leeds, United Kingdom, a large academic institution with full histopathologist subspecialisation, which processes in the region of 250,000 H&E stained histology slides per annum. 3 consultant breast histopathologists with 35 years of combined practice were recruited to participate in the validation study. Scanning of all breast histopathology glass slides prior to laboratory send out was initiated in August 2016. Scanning was performed using a single Aperio AT2 scanner for standard dimension slides (Leica Aperio, Vista, US), and a single CS2 scanner (Leica Aperio, Vista, US) for large slides. Standard slides were scanned at 40x equivalent magnification, and large slides at 20x equivalent magnification. Automated scanning processes (selection of scanning area, placement of focus points) were quality checked and repeated manually by a laboratory technician where necessary. Digital images were stored in a remote digital archive, along with relevant clinical information, including a scanned copy of the original request form, and retrieved using e-Slide Manager software

(Leica Aperio, Vista, US). Images were viewed by consultant pathologists using Leeds Virtual Microscope viewing software (University of Leeds, Leeds TH NHS Trust[13]) on medical grade Coronis Fusion 6 MP, 30.40 inch screens (Barco, Kortrijk, Belgium).

The validation protocol is published as an appendix to the Royal College of Pathologists Guidelines for Digital Pathology, where it is cited as an example of best practice. The validation structure consisted of 3 phases, a training phase (T), a validation training set phase (V1), a live reporting validation phase (V2) and a summary phase (S). (See table 1 for an overview of validation procedure). Prior to the initiation of training, each participant completed a questionnaire detailing their prior experience of, and attitude towards digital pathology.

2.1 Training Phase (T1)

In T1, each participant received a one hour individual session in basic use of the digital pathology slide viewer (LVM), and the image management software (e-Slide Manager), and was issued a user manual. Participants were observed opening and evaluating cases, and given feedback regarding effective use of input modalities (mouse and keyboard shortcuts). The participant could request additional training as required.

2.2 Validation 1 – Training set (V1)

In V1, each participant received a training set of 20 cases, in glass slide and digital slide formats. The training set was designed to encompass the breadth of breast diagnosis, and confront the participant with cases which might be challenging to diagnose digitally. The cases were chosen based on clinical relevance to our department, and the challenging digital cases were selected based on a review of the literature concerning digital discordance[5]. (See table 2). Participants viewed the training set in their own time. For each case, the digital slides were viewed first, then the pathologist recorded their diagnosis, and their level of confidence in their diagnosis, on a Likert scale from 1-7, where 1 corresponded to no not at all confident, and 7 to very confident.

The pathologist then viewed the glass slides for the same case, immediately after the digital read, and recorded any alteration in their diagnosis, and their confidence in their glass slide diagnosis. When all participants had completed the training set, the results were discussed in a group with the validator, and all participants reviewed discordant cases on glass and digital slides. Pathologists identified the types of case they found problematic on digital, so that they could ensure they were vigilant for these type of error in the next phase, V2.

2.3 Validation 2 – Live cases (V2)

In V2, the totality of each participants breast pathology workload was scanned prospectively. The pathologists made their primary diagnosis on digital slides, recording it in a spreadsheet, along with their confidence in their diagnosis. All cases were then checked on glass before final reporting, and any modification to the diagnosis was recorded, along with the glass slide confidence in diagnosis, and the preferred diagnostic medium for each case. Pathologists were also asked to record any technical failures – ie. out of focus digital slides, or those with any digital artefact which might preclude confident or safe diagnosis.

All discordances were discussed at weekly to fortnightly validation meetings, were digital and glass slides were reviewed by all available participants and the validator.

When each participant had viewed 2 months whole time equivalent workload (estimated at approximately 200 cases based on departmental data), their diagnostic spreadsheets were analysed by the validator, and concordance and discordance data was summarised. This data was discussed between each participant and the validator, and the scope of that pathologists future digital pathology practice was agreed upon, with specific criteria documented for cases which require a check on glass before final sign out.

3. Results

3.1 Validation 1 – Training set (V1)

Each participant viewed the same 20 training cases on digital slides and glass, consisting of 60 slides in total. Mean diagnostic concordance for all participants was 92% (range 80% - 100%). Discordant cases concerned the following areas of diagnosis: mitotic count component of invasive tumour grading, failure to detect weddelite calcification, micrometastasis detection, and the recognition of ductal atypia.

3.2 Validation 2 – Live cases (V2)

The participants viewed a total of 694 complete breast histopathology cases, consisting of 15,000 slides. The cases were representative of the specimen type and diagnostic category mix found in the departmental breast workload. (See tables 3 and 4).

In the course of the validation, a technical failure rate of 1.0% was observed - these were cases where scanning artefact or focus issues with digital slides resulted in the pathologist rejecting the digital slides and making a diagnosis on glass. There was complete clinical concordance between the glass and digital impression of the case in 98.8% of cases. Only 1.2% of cases had a clinically significant difference in diagnosis/prognosis on glass and digital slides. (See table 5)

All discordances were reviewed on glass and digital by the validation group and trainer. Clinically significant discordances concerned the mitotic count component of invasive tumour grading, identification of weddelite calcification, identification of isolated tumour cells, assessment of a fibroepithelial lesion for cellularity, and identification of focal epithelial atypia. (See figure 1 for example images). The 2 most significant discordances both concerned the diagnosis of DCIS. In one case, a small focus of DCIS was missed on the digital read of an otherwise B3 screening case, whilst in another case, a small focus of DCIS was correctly diagnosed on the digital slide in a large, multi-slide case, but missed on the initial glass review of the case. The pathologist had to revert to the digital case to locate the corresponding glass slide, and was then able to identify the DCIS on the glass, which had been overlooked. Use of glass slides only for this case could have resulted in misclassification of a B5a case as B2. (See table 6).

3.3 Diagnostic confidence and diagnostic modality preference

Mean diagnostic confidence (on a Likert scale from 0-7) was similar for each pathologist for digital slides and for glass slides. (See table 7), although the range of diagnostic confidence scores was dramatically different for one pathologist (0-7 on digital, versus 6-7 on glass).

All of the participant pathologists identified a proportion of cases for which they preferred to use glass slides over digital slides, although digital slides were judged to be superior or equivalent to glass slides in the vast majority of cases. (See figure 2). Cases where glass slides were preferred all involved mitotic counting, weddelite detection and lymph node searches.

3.4 Beliefs about digital pathology efficiency

Prior to their validation procedure, the pathologist group predicted that viewing digital slides would be slightly slower than viewing glass slides, and that breast resections would be much slower to report on digital. After the validation procedure, the pathologists reported that they perceived their digital reads of resection cases and large/multi-level biopsies to be much faster using digital slides rather than glass slides, and resections to be either slightly faster or much faster on the digital microscope.

Prior to the validation procedure, pathologists believed the most relevant barriers to digital pathology adoption were increased time to view digital slides compared with glass slides, pathologists lack of exposure to digital pathology and pathologists' resistance to change. Following the validation procedure they identified the chief barriers to digital pathology adoption were financial cost to the department and the time taken to scan slides in the laboratory.

When asked to list the principal benefits of digital slides over glass slides, pathologists listed ease of access to previous biopsies/linked specimens, more efficient diagnosis of large cases/multi slide biopsies, diagnostic utility of the low power overview of the slide, more efficient delivery of digital slides to the pathologists desktop, enhanced opportunities to teach trainees and ergonomic benefits.

4. Discussion

Digital pathology has the potential to transform the way in which breast pathology services are delivered. Rapid transfer of images across geographical boundaries can allow for more efficient dispersal of pathology workload between linked hospitals, and make best use of pathologist manpower. Rapid access to second opinion on challenging cases, and increased collaboration between pathologists on cases could lead to significant improvements in the quality of pathology diagnosis.

Successful adoption of digital pathology for primary diagnosis in a department is dependent on individual pathologists, many with decades of experience reporting on a light microscope, engaging with a new technology, educating themselves on its limitations, and actively learning how to use software and hardware efficiently. As with the adoption of any new diagnostic procedure, patient safety should be paramount. The US Food and Drugs Administration guidance to manufacturers recommends that medical devices (including whole slide imaging systems) should be able to demonstrate established safety and effectiveness[14]. The new digital pathology guidelines published

by the Royal College of Pathologists also describe the need for individual pathologists to be validated with sufficient rigour to satisfy an internal or external observer that safety and clinical effectiveness are maintained. The document also emphasises that validation should occur in a real world context.

This study documents the first instance of use of the novel validation and training protocol for digital primary diagnosis of histological specimens recommended as an example of best practice in the Royal College of Pathologist's Guidelines for Digital Pathology (2017). The philosophy of this validation protocol differs greatly from the approach of the College of American Pathologists Guideline[6] and of other non-inferiority studies. Firstly, it is centred on the individual pathologist rather than a department as a whole, and secondly it is competence driven rather target driven. This approach takes into account the variability in IT competencies, diagnostic experience and enthusiasm for technology between pathologists, and allows all members of a department, whether enthusiasts or skeptics to develop digital pathology skills and gain confidence in their abilities. Three specialist breast pathologists viewed 694 complete "live" breast cases, including large format slides, stained with haematoxylin and eosin, immunohistochemistry and special stains. Complete clinically significant concordance was observed in 98.8% of cases, indicating excellent agreement between digital primary diagnosis and glass slide review. Our findings suggest that pathologists, given access to digital pathology training, and a risk mitigated diagnostic environment to gain real world digital reporting experience, can competently and confidently use digital pathology for primary diagnosis as standard practice.

The training and validation process allowed the participant pathologists to identify and discuss areas of digital diagnosis they found more challenging, and identify subtypes of breast case which warrant glass review of digital slides, in order to maintain patient safety and allow for further education of the pathologist and navigation of specific learning curves (eg. for confident identification of mitotic figures or navigation of lymph nodes). Identification and counting of mitotic figures was consistently highlighted as an area of difficulty for pathologists. Our pathologists perceived two causes of this difficulty in digital reporting: firstly they suggested that less contrast between chromatin and the background on digital slides made mitoses harder to identify, and secondly, they were unable to fine-focus on suspected mitotic figures on digital slides, a function they often perform on glass slides to confirm the identity of mitoses. A number of workarounds and strategies to mitigate this difficulty could be considered, including use of immunohistochemistry to highlight mitoses, the use of image analysis software to automate mitotic counts, or mandatory checks of mitotic count on glass slides prior to specimen sign out, in cases where mitotic score would affect overall grading of an invasive tumour.

Our pathologists reported perceived greater efficiency in reporting multi-slide biopsies and large resections on digital slides, which they attributed to a number of factors. This was partly because they no longer had to load and reload glass slides on the microscope stage, and could move swiftly between slides. In addition, they found the full screen low power view of individual slides enabled them to assess lesional architecture with greater ease, and they were able to make measurements using digital tools efficiently and accurately. The relative diagnostic efficiency of pathologists using digital versus glass slides deserves further attention, especially now that we have a growing cohort of pathologists with significant digital microscopy experience to compare fairly with conventional light microscopy . Others benefits of digital reporting noted by our pathologists included rapid access

to previous biopsy specimens when reviewing resections, more engaging education and training of junior colleagues, and ergonomic benefits.

As a consequence of this validation study, our validated breast pathologists now report all cases on digital slides as standard, reverting to glass following digital examination only for cases fulfilling set criteria (invasive cancers where differences in mitotic score could affect overall grade, cellular fibroepithelial lesions, cases with radiological confirmation of calcification but no calcium identified on digital, and any challenging case not encountered in the validation phase.) Next year, the laboratory at Leeds Teaching Hospitals NHS Trust will commence scanning all histopathology slides for all specialties, and all consultants will complete a validation procedure for the relevant diagnostic subspecialty. As the validation process is completed for each specialty, we will gather more data on challenging areas of digital diagnosis. It is important that individual departments share their experiences with digital pathology, and highlight areas of potential difficulty which can be prioritised in the digital training of their colleagues to ensure a safe transition from glass slide to digital slide reporting.

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BW and DT designed the study and drafted the manuscript. BW collected and analysed data. AH, RMS, AN and EV provided feedback on study design, participated in validation, and gave feedback on drafts of the manuscript.

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Figures legends

Figure 1. Examples of discordant validation cases

Clockwise from top left. Missed micrometastasis in a sentinel lymph node, Difficulty identifying mitotic figures, Missed weddelite calcification, Cellularity of stroma overcalled

Figure 2. Diagnostic preferences of individual pathologists

Table 1. Summary of validation phases

Phase	Overview
Training (T)	1:1 formalized training in digital microscope use. Observed practice with feedback.
Validation – Training Cases (V1)	Training set of 20 challenging and informative specialty specific cases. Participant views digital slides, make notes on diagnosis, immediately checks corresponding glass slides and notes any difference in opinion. Group discussion. Identify and mitigate pitfalls.
Validation – Live reporting (V2)	All cases scanned prospectively. Diagnosis made on digital slides with reconciliation with glass slides before sign out. Difficulties recorded and discussed. Library of problematic cases assembled and reviewed with group.
Summary and recommendations (S)	Validation document produced with each pathologist, documenting concordance/discordance. Recommendations made for scope of digital practice / further training.

Table 2. Validation Training Case Set

Case	Diagnosis	Domains explored
1	Benign phyllodes tumour	Diagnosis (benign fibroepithelial)
2	Fibrocystic change, weddelite calcification	Diagnosis (benign tissue), Identification of weddelite calcification
3	Fat necrosis	Diagnosis (benign/inflammatory condition)
4	Sparse residual ductal carcinoma, post chemotherapy	Diagnosis (malignant epithelial), Grading, Immunohistochemistry interpretation (sparse tumour cells)
5	Invasive ductal carcinoma, grade 2, neuroendocrine features	Diagnosis (malignant epithelial), Grading, Immunohistochemistry interpretation, Identification of neuroendocrine features.
6	High grade DCIS with small, grade 1 invasive component	Diagnosis (malignant epithelial), Grading, Identification of small invasive component
7	Atypical ductal hyperplasia, flat epithelial atypia, microcalcification, sclerosed papilloma	Diagnosis (benign and atypical epithelium, papillary lesion), Identification of microcalcification
8	Invasive ductal carcinoma, grade 3	Diagnosis (malignant epithelial), Grading, Immunohistochemistry interpretation
9	Paget's disease of nipple	Diagnosis (malignant epithelium) Immunohistochemistry/special stain interpretation
10	Fibroadenoma with ductal carcinoma in situ	Dual diagnosis (malignant epithelium and fibroepithelial lesion)
11	High grade ductal carcinoma in situ, no calcification	Diagnosis (malignant epithelium), Grading, Identification that no microcalcification is present
12	Benign sclerotic lesion	Diagnosis (benign lesion) Immunohistochemistry interpretation
13	5mm lymph node metastasis	Diagnosis (locate metastasis)
14	Organising haematoma	Diagnosis (benign/inflammatory)
15	Apocrine metaplasia with atypia	Diagnosis (borderline lesion)
16	Lymph node with micrometastasis	Diagnosis (locate micrometastasis)
17	Nipple dermatitis	Diagnosis (benign dermatosis)
18	Mucinous carcinoma, grade 1	Diagnosis (malignant epithelial), Grading, Identification of mucin
19	Pleomorphic lobular carcinoma, grade 2	Diagnosis (malignant epithelial), Grading, Identification of pleomorphic lobular component
20	Invasive lobular carcinoma, grade 2	Diagnosis (malignant epithelail), Grading, Identification of classical lobular features

Table 3. Case Mix by Specimen Type

Specimen type	Number of cases
Vacuum assisted biopsy	159
Core biopsy	397
Wide local excision	28
Mastectomy	27
Other excision	55
Immuno/special stains	28
Total	694

Table 4. Case Mix by Diagnostic Category

Diagnostic category	Number of cases
B1 (Normal tissue)	85
B2 (Benign lesion)	308
B3 (Lesion of uncertain malignant potential)	51
B4 (Suspicious)	5
B5a (Malignant – in situ)	43
B5b (Malignant- invasive)	145
LB1 (No lymphoid tissue)	1
LB2 (Benign lymphoid tissue)	22
LB5 (Malignant, metastatic carcinoma or other)	5
Other	29
Total	694

Table 5. Live reporting validation statistics.

	Pathologist 1	Pathologist 2	Pathologist 3	All pathologists
Technical failure rate	0.7%	1.4%	1.0%	1.0%
Complete concordance	95.0%	96.2%	97.4%	96.2%
Any observable difference	5.0%	3.8%	2.6%	3.8%
Complete clinical concordance	99.3%	99.1%	98.5%	98.8%
Clinically significant observable difference	0.7%	0.9%	1.5%	1.2%

Table 6. Discordant cases from the live reporting phase of validation (V2)

Specimen	Digital Diagnosis	Glass diagnosis	Preferred diagnosis
Core biopsy	Grade 2 invasive ductal carcinoma	Grade 3 invasive ductal carcinoma	Glass
Vacuum biopsy	Benign phyllodes tumour	Fibroadenoma with inflammation	Glass
Vacuum biopsy	Columnar cell change	Columnar cell change plus atypical intraductal proliferation	Glass
Vacuum biopsy	Sclerosing adenosis	Sclerosing adenosis, small focus ductal carcinoma in situ	Glass
Vacuum biopsy	Microcysts	Microcysts and weddelite calcification	Glass
Sentinel node	Benign	Isolated tumour cells	Glass
Vacuum biopsy	Columnar cell change	Columnar cell change, single focus atypical cells	Glass
Vacuum biopsy	Small focus of ductal carcinoma in situ	Benign	Digital

Table7. Diagnostic confidence using digital and glass slides (0= not at all confident, 7 = very confident)

	Digital slides		Glass slides	
	Mean confidence (0-7)	Range	Mean confidence (0-7)	Range
Pathologist 1	6.70	4-7	6.80	4-7
Pathologist 2	6.90	4-7	6.90	4-7
Pathologist 3	6.79	0-7	6.99	6-7