



REPORT OF VIRTUAL MICROSCOPY PILOT SURVEY RESULTS
2005

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Aim

The aim of this pilot survey was to determine if virtual microscope images were suitable for use as survey material for the external quality assurance program in Anatomical Pathology.

Method

Five anatomical pathology cases from past General module surveys offered by the Anatomical Pathology QAP were selected covering a variety of tissue types and lesions. One of the original H&E stained slides from a previous survey was scanned using the Aperio T2 ScanScope® and the resultant digital images were distributed on two DVD-ROMs to 91 participants who had volunteered to take part in this survey. Viewing software ImageScope® was included on the disc.

Case notes with identifying information, specimen type, age, sex, clinical notes and macroscopic description for each case were provided with the disc. Instructions for installing the viewing software, opening the images and submitting responses were provided with the case notes.

Participants were requested to submit a free text preferred diagnosis in a manner similar to that currently in use and, if required, additional comments.

The case notes were identical to those provided in the original general module surveys in which the participants had been provided with a glass slide of a H&E stained section. However, in all of the previous surveys from which these cases were chosen a preferred diagnosis was selected from a multiple choice tick box proforma. For some cases when these proformas were utilised, the multiple choice was restricted to either a neoplastic or a non-neoplastic diagnosis. In this present survey, participants were required to provide a preferred diagnosis without this assistance.

A microscopic description and target diagnosis from the contributing pathologist was withheld until after the closing date for the survey and then reported in the results. A comparison of the responses from this virtual microscope survey was made with the responses submitted for the same case in the original survey.

Results

Response rate

Of the 91 participants to whom material was sent, 42 responded, a participation rate of 46%.

However, blank submissions were received for some cases from some participants resulting in a median of 39 diagnostic responses.

Technical issues

These were raised in 22 comments:

“Negative” comments:

- Difficulty with resolution of fine detail on high power - 7 comments
- Difficulty in making a diagnosis from a computer screen compared with a microscope - 5 comments
- Uncertainty in measuring fields for mitotic figure counts - 2 comments
- Difficulty with scanning across the sections - 2 comments
- Damaged disc which could not be viewed- 1 comment
- Multiple difficulties due to the size of the viewing computer, the large file comments sizes and the autorun software installation feature and was unable to submit any diagnoses- 1 comments
- Frustration caused by installation of the autorun feature - 1 comment

“Neutral” comments were:

- While the virtual images were good, it was suboptimal for one case -1 comment (VM05-02) (Lung biopsy _Wegner’s granulomatosis – see below for further details)

“Positive” comments were:

- The images were excellent - 2 comments

Other comments from participants

62 comments related to the preferred diagnosis such as prognostic information or further tests that would be required for the diagnosis.

Responses to individual cases

Submitted preferred diagnoses have been assessed using the criteria currently used in diagnostic modules of the program as concordant, minor discordant or discordant.



Case number	VM05-01	Specimen	Large bowel wall
QAP identifier	EX00-042	Age & sex	75 year old male

Clinical notes

75 year old male of South East Asian origin

Macroscopy

The specimen consists of a 240 mm length of large bowel including the caecum and ascending colon. The appendix, up to 55 mm, is attached. The terminal 30 mm of ileum is attached approximately 60 mm from the distal cut margin of the specimen. There is a large tumour involving the entire circumference of the bowel wall over 55 mm of its length. The mucosa distant from the tumour shows slight irregularity. The section provided is from non-tumour bearing colon.

Microscopy

The background bowel wall shows schistosomiasis. This is old and calcified. It probably became inactive many years ago.

Diagnosis

INTESTINAL SCHISTOSOMIASIS

Virtual microscope survey responses	Count	Proportion
Concordant		
Schistosomiasis	28	73.7%
Parasitic infection	2	5.3%
Discordant		
Diverticulosis	3	7.9%
Normal bowel	2	5.3%
Intussusception	1	2.6%
Intestinal spirochaetosis	1	2.6%
Angiodysplasia	1	2.6%
Total	38	100.0%

Original survey responses	Count	Proportion
Concordant		
Parasitic infection, likely Schistosomiasis	169	89.4%
Parasitic infection, other	11	5.8%
Minor discordant		
Calcification	1	0.5%
Discordant		
Diverticular disease	2	1.1%
Angiodysplasia	2	1.1%
Amoebic colitis	1	0.5%
Pneumatosis intestinalis	1	0.5%
Hamartomatous polyp	1	0.5%
Ulcerative colitis & dysplasia	1	0.5%
Total	189	100.0%

Comment

In the virtual microscope survey 28 participants submitted the target diagnosis and a further 2 identified a parasitic infestation. If the response rates had been the same as the original survey the numbers would respectively have been 34 and 2. Of the discordant diagnoses interestingly only diverticulosis/diverticular disease was common to both series. If all "non-target" responses are aggregated there were 8 in the current survey and 9 originally. If the response rate had been the same as the original survey the numbers of "non-target" responses would have been only 2.

It is uncertain how far the difference between the two sets of results is due to the difference in the way in which the survey material was presented with the virtual microscope compared with a paraffin section and how far it can be attributed to the preferred diagnosis being made by free choice from an open ended list of possibilities compared with a multiple choice selection from a list with pre-determined options. Nevertheless in this case over 70% of participants achieved the target with the digital virtual microscope image.



Case number	VM05-02	Specimen	Right lung biopsy
QAP identifier	EX01-197	Age & sex	57 year old male

Clinical notes

Multiple bilateral lung opacities.

Macroscopy

The specimen is labelled "right lower lobe histology" and consists of a wedge of lung which when inflated has dimensions 110 x 40 x 30 mm. On serially sectioning through the specimen, multiple similar lesions are identified. The largest is up to 25 mm in maximum diameter. All lesions are moderately well circumscribed and are composed of white to tan reasonably solid tissue. Obvious caseous necrosis is not identified. A section from one of the larger of these nodules is provided.

Microscopy

Sections of lung tissue show an extensive but patchy process. The inflammatory process includes geographic zones of necrosis, acute necrotising vasculitis and an associated histiocytic and giant cell response. The areas of necrosis have a blue discolouration and contain numerous degenerating neutrophils. With the elastin stain, at least some of these areas are centred on blood vessels. Scattered giant cells are present about the edge of the necrosis. Small microabscesses are also present. The inflamed blood vessels are predominantly infiltrated by neutrophils but there are also accompanying histiocytes, lymphocytes, plasma cells and small numbers of eosinophils. The vasculitis involves medium and small size arteries as well as capillaries (capillaritis) and vessels within the pleura. The necrosis and inflammatory changes extend to involve bronchi and there is also a BOOP-like reaction surrounding the inflammatory foci. Within the background of the nodules, there is fibrosis. Some fibrin is present in the adjacent airspaces and there is fibrinous exudate on the pleural surface. No micro-organisms are identified with special stains (Gram, Grocott, PAS and Ziehl-Neelsen). No sarcoidal granulomata are seen. There is no evidence of malignancy. Within the uninvolved lung there is mild centriacinar emphysema. Correlation with clinical history and ANCA serology is essential.

Diagnosis

WEGENER'S GRANULOMATOSIS

Virtual microscope survey responses

	Count	Proportion
Concordant		
Wegener's granulomatosis	4	10.3%
Minor discordant		
Inflammatory myofibroblastic tumour/inflammatory pseudotumour	7	17.9%
Plasma cell granuloma	2	5.1%
Bronchiocentric granulomatosis	1	2.6%
Granulomatous inflammation NOS	1	2.6%
Inflammatory lesion with atypical granulomatous features	1	2.6%
Nodular sarcoidosis	1	2.6%
Discordant		
Metastatic melanoma	4	10.3%
Lymphomatoid granulomatosis	3	7.7%
Malignant lymphoma of lymphomatoid granulomatous type/Non Hodgkins	3	7.7%
Undifferentiated malignant neoplasm ? type	3	7.7%
Lympho-epithelioma	1	2.6%
Metastatic sarcomatoid malignant neoplasm	1	2.6%
Metastatic spindle cell lesion ?sarcoma ?sarcomatoid carcinoma ?melanoma	1	2.6%
Metastatic tumour, probably sarcoma, consistent with malignant fibrous	1	2.6%
Myofibroblastic tumour, malignant	1	2.6%
Poorly differentiated malignant neoplasm- possibly follicular dendritic cell sarcoma	1	2.6%
Pulmonary cryptococcosis	1	2.6%
Silicotic nodule	1	2.6%
Suspicious of a malignant tumour – adenocarcinoma, require further tests	1	2.6%
Total	39	100.0%



Original survey responses	Count	Proportion
Concordant		
Wegener's granulomatosis	174	78.7%
Minor discordant		
Bronchiolitis obliterans/ organising pneumonia (BOOP)	15	6.8%
Bronchocentric granulomatosis	7	3.2%
Churg-Strauss syndrome	4	1.8%
Usual interstitial pneumonitis/pneumonia	4	1.8%
Necrotising sarcoid granulomatosis	3	1.4%
Diffuse interstitial pulmonary fibrosis	2	0.9%
Complex inflammatory and fibrotic process	1	0.5%
Discordant		
Tuberculosis	2	0.9%
Polyarteritis nodosa	2	0.9%
Malignant lymphoma	2	0.9%
Hypersensitivity pneumonitis	1	0.5%
Asbestosis	1	0.5%
Eosinophilic granuloma	1	0.5%
Chronic eosinophilic pneumonia	1	0.5%
Fungal infection	1	0.5%
Total	221	100.0%

Comment

In this case there was a dramatic difference between the results of the 2 surveys. In the virtual microscope survey only 4 responses (11%) were concordant with the target while originally 174 (78.7%) were concordant. Discordances and minor discordances were correspondingly higher at 22 (56.4%) and 13 (33.3%) respectively. Also the range of discordant and minor discordant diagnoses was greater and, of particular interest, was the much greater prevalence of neoplastic diagnoses. Most of these differences were generated by free choice from an unlimited list of possibilities that were not provided as options in the original survey. (The original survey provided a multiple choice list of diagnoses limited to non-neoplastic lesions).



Case number	VM05-03	Specimen	Right breast lump
QAP identifier	EX01-137	Age & sex	54 year old male

Clinical notes

R. breast lump with nipple retraction. Lumpectomy.

Macroscopy

A roughly oval-shaped pale tan coloured tissue fragment with portions of fat adherent to one side dimensions 30 x 20 x 20mm. A white nodular area approximately 20 x 20mm is seen within the specimen. Sections of lesion and non lesional tissue provided.

Microscopy

Sections from the submitted specimen show invasive ductal carcinoma (infiltrating carcinoma NOS) with a small component of ductal carcinoma in-situ incorporated in the tumour as well as in the periphery. There is focal calcification seen associated with the invasive carcinoma. The invasive component is predominantly composed of groups and islands of pleomorphic cells with vesicular enlarged nuclei and nucleoli set in a finely granular eosinophilic cytoplasm. Tubular differentiation is seen in approximately 20 to 30% of the tumour. The outline of the infiltrating carcinoma has a roughly stellate appearance. measurements made on a single section of the tumour measures 13.7mm x 11.8mm. In the gross the nodule measured approximately 20mm in maximum diameter. The adjacent tissue is composed of dense hyalinized stroma with a few scattered ducts exhibiting some features of gynecomastia. The tumour nodule appears to abut onto the hyalinized sclerosed stroma. Groups of tumour cells are seen surrounding thick walled blood vessels and around large nerve bundles but unequivocal lymphovascular permeation is not apparent. Mitotic count amounts to 5 per 10 HPF with moderate amounts of tubule formation within the tumour and moderate nuclear pleomorphism (total score of 5) which makes this tumour grade I in the modified Bloom and Richardson histological grading system. The invasive carcinoma appears to extend to the apparent resection margin in more than one area. The DCIS component is very small and consists probably of up to 3 foci of solid type with intermediate grade cytology. Within the invasive component scattered dilated ducts containing granular eosinophilic material are present. There is a rim of fatty tissue around the tumour and groups of tumour extend into the fatty tissue and in at least two of the blocks extend up to the resection margins.

Diagnosis

INFILTRATING CARCINOMA, NO SPECIAL TYPE OR DUCTAL CARCINOMA GRADE 2

Virtual microscope survey responses**Count Proportion****Concordant**

Infiltrating ductal carcinoma	22	56.4%
Ductal carcinoma/adenocarcinoma	4	10.3%
Invasive carcinoma of no special type	2	5.1%

Minor discordant

Invasive lobular carcinoma	4	10.3%
Infiltrating breast carcinoma with ductal and lobular features	3	7.7%
Lobular cancer pleomorphic type	1	2.6%
Metaplastic carcinoma	1	2.6%

Discordant

Complex sclerosing lesion	1	2.6%
Sclerosing adenosis	1	2.6%

Total

39 100.0%

Original survey responses**Count Proportion****Concordant**

Ductal/NST invasive carcinoma	197	84.2%
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Minor discordant

Lobular carcinoma	29	12.4%
Pleomorphic invasive lobular carcinoma	1	0.4%



Anatomical Pathology

Mixed ductal-lobular carcinoma	1	0.4%
Features of invasive lobular carcinoma but probably ductal	1	0.4%
Invasive mucinous carcinoma & borderline phyllodes	1	0.4%
Medullary carcinoma	1	0.4%
Invasive carcinoma	1	0.4%
Carcinoma	1	0.4%

Discordant

Ductal hyperplasia without atypia	1	0.4%
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Total	234	100.0%
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Comment

In this case there was a better agreement between the proportions with concordant diagnoses although in the virtual microscope survey the terminology was more diverse as might be expected because of the different methods used to record the preferred diagnosis. The difference in proportions of minor discordance is unlikely to be significant. There was a higher proportion of discordances although the absolute numbers are small so this is of questionable significance. Interestingly with this case the diversity of "non concordant" diagnoses was slightly smaller in the virtual microscopy results.



Case number	VM05-04	Specimen	Lesion, right thigh
QAP identifier	EX01-122	Age & sex	28 year old male

Clinical notes

Hard tender lesion under skin of right thigh. ? Histiocytoma

Macroscopy

Tissues 15mm deep under incised pale skin 31 x 15mm.

Microscopy

Sections of skin show an unencapsulated lesion, lying within the dermis and showing limited extension into subcutaneous fat. It is separated from the overlying epidermis by a thin band of uninvolved dermis. The tumour consists of nests and fascicles of polygonal cells with finely granular, eosinophilic cytoplasm and central, round to ovoid nuclei. Occasional intranuclear inclusions are found and some nuclei contain small nucleoli. There is no evidence necrosis or significant mitotic activity. The tumour cell nests in many areas are separated by bundles of mature collagen.

Diagnosis

GRANULAR CELL TUMOUR

Virtual microscope survey responses**Count Proportion****Concordant**

Granular cell tumour	35	87.5%
Granular cell myoblastoma	1	2.5%

Minor discordant

Fibrous histiocytoma/Dermatofibroma	3	7.5%
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Differential diagnosis only

Differential 1. Alveolar soft part sarcoma. 2. Granular cell tumour.	1	2.5%
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Total

40 100.0%

Original survey responses**Count Proportion****Concordant**

Granular cell tumour	225	97.0%
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Minor discordant

Juvenile xanthogranuloma	2	0.9%
Granular cell tumour & schwannoma	1	0.4%
Xanthoma	1	0.4%
Benign fibrous histiocytoma	1	0.4%

Discordant

Actinic keratosis	1	0.4%
Atypical fibroxanthoma	1	0.4%

Total

232 100.0%

Comment

The results with this specimen are generally similar. The higher percentage of concordant responses in the original survey is probably mainly a reflection of the greater absolute number of participants.

Interestingly the proportion of minor discordances and discordances is also greater but again this is probably due to the greater number of participants and not too much should be read into this. This case is of some relevance because it is representative of the type of case that may be provided by virtual microscopy, because it is a specimen where the quantity of tissue is often insufficient to cut large numbers of paraffin sections.

Case number	VM05-05	Specimen	Right supraclavicular lymph node
QAP identifier	EX03-013	Age & sex	32 year old male

Clinical notes

Right supraclavicular lymph node ?lymphoma

Macroscopy

The specimen consists of a large nodule of tissue measuring approximately 50 x 40 x 20 mm. Sectioning reveals a nodular cut surface showing nodules of tan tissue separated by cream fibrous tissue. The nodules range in size from 2-5 mm in diameter.

Microscopy

Sections show effacement of the nodal architecture by nodules of cells separated by broad fibrous bands. The lymph node capsule is also thickened by fibrosis. The nodules are composed of a mixed population of cells including numerous Reed Sternberg cells and their variants. Lacunar variants of Reed Sternberg cells are prominent in some areas. Others cells within the nodules include lymphocytes, histiocytes and eosinophils. Occasional mitotic figures are seen. There are no sheets of Reed Sternberg cells. The Reed Sternberg cells stain positively with CD30 and CD15 but are negative for B cell (CD20, CD79 a) and T cell markers (CD3, CD45RO). The B cell and T cell markers demonstrate a mixed population of B and T cells.

Diagnosis

HODGKIN'S LYMPHOMA, NODULAR SCLEROSIS SUBTYPE

Virtual microscope survey responses

Count Proportion

Concordant

Nodular sclerosing Hodgkin's lymphoma	32	82.1%
Lymphoma, possible Hodgkin's. Require further tests.	2	5.1%
Hodgkin's lymphoma	1	2.6%

Minor discordant

Follicular lymphoma	2	5.1%
Differential diagnosis only		
Possible anaplastic lymphoma, possible infectious mononucleosis	1	2.6%
Hodgkin's lymphoma versus reactive change	1	2.6%

Total

39 100.0%

Original survey responses

Count Proportion

Concordant

Hodgkin's lymphoma, nodular sclerosing or classical	266	98.5%
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Discordant

Castleman's disease	1	0.4%
Castleman's disease & hyaline vascular follicular pattern	1	0.4%
Angioimmunoblastic lymphadenopathy	1	0.4%
Hyaline vascular follicular pattern & reactive follicular pattern	1	0.4%

Total

270 100.0%

Comments

The agreement in the two surveys in this case is good and the differences in proportions of both concordance and discordance is probably a reflection of the greater number of participants in the original survey. While low power appearances are important, to make this diagnosis with certainty would require recognition of some cellular detail with high magnification.

Discussion

While a few participants encountered some technical difficulty the number was not large, given their unfamiliarity with the product. Some problems might require the issue of a replacement disc. The problem with the autorun installation should be examined. Large file sizes may also contribute to difficulty.



Anatomical Pathology

It is evident that participants were more uncomfortable with critical issues: resolution of fine detail, mitotic counts and the one particularly difficult case (VM05-02).

While the intention of this survey was to compare virtual microscope images with glass slides, it would appear that the effect of the different methods of selecting a preferred diagnosis has also been compared.

Therefore, as far as diagnostic performance is concerned, analysis is confounded to some extent by the use of the "tick box" method of response in the initial survey compared with the open-ended "free choice" method now in use. That this may have been a factor is suggested by the divergence in the minor discordances and discordant results in the two surveys.

Consistently the virtual microscope survey produced fewer responses concordant with the "target"

However in all 5 cases some participants provided a preferred diagnosis that was concordant with the target and, except for VM05-02, the disparity was not great. Some of this may be due to the absence of a "prompt" and in those situations where there was a high frequency of concordant responses in both surveys the greater absolute number of participants in the original survey may have led to a higher proportion of concordant responses.

VM05-02 pulmonary involvement in Wegner's granulomatosis was the major exception. It was chosen deliberately to see how participants handled a difficult case of necrotising granulomatous inflammation of the lung. Given the low prevalence of this condition and the fact that lung pathology, particularly non-neoplastic lung disease, is not seen in many centres, personal experience of this is likely to be limited. It might therefore be argued that the proportion with the concordant diagnosis in the original survey was surprisingly high.

Various explanations are possible for the disparity:

- Inherent difficulty of the disease diagnosis
- Multiple choice list of diagnoses in original survey
- Small sample of pathologists in this survey
- Poor high power appearance of identifying feature on the virtual image

It is of interest, however, that the latter would also apply to some extent in VM05-5 where however participant performance was good.

Conclusion

The results of this pilot survey give qualified support for the use of virtual microscope images in future quality assurance surveys. This should however be restricted to material of a type that cannot be included in surveys at all at present because multiple paraffin sections cannot be produced. These images therefore after validation will be used in the general module for conditions that are encountered relatively frequently in small biopsies or as local lesions contained within a small area of one tissue block. They may however be used in more diagnostically demanding situations in special modules including those currently existing such as needle biopsies in the breast module or in new modules e.g. renal or liver biopsy modules. For uncommon disorders conventional paraffin sections should be retained for proficiency testing but virtual microscope images may be useful for education.

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