Session 1: Lung – Chair: Keith Miller

1. Lung: diagnosis, ICC, ALK and PDL-1
   Professor Andrew G Nicholson
   Consultant Histopathologist, Royal Brompton And Harefield Hospitals NHS Foundation Trust

2. UK NEQAS ICC & ISH ALK IHC module – update and review.
   Dr Merdol Ibrahim
   UK NEQAS ICC & ISH Scheme Manager

3. IHC-based biomarker testing in Lung
   David Allen
   Laboratory Services Manager, Lead BMS. UCL-AD

Session 2: Diagnostic IHC – Chair: Keith Miller

4. Are there any new biomarkers in Gastrointestinal tumours?
   Dr Manuel Rodriguez-Justo
   Consultant Histopathologist, UCL Hospitals

5. Immunohistochemistry in bone and soft tissue tumours: Overview and illustrative cases.
   Prof. Chas Mangham
   Consultant Histopathologist, Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry
Session 3 – Chair: Suzanne Parry Assistant Scheme Manager

UK NEQAS ICC & ISH scheme updates

6. Part 1:
   - New Website UK NEQAS ICC & ISH Support Scientist
   - Journal

7. Part 2:
   - Slide survey Dawn Wilkinson.
   - News, future plans UK NEQAS ICC & ISH Support Scientist

8. A day in the life of a UK NEQAS ICC & ISH assessor
   Julie Williams

9. Uncertainty in Cellular Pathology
   Dr Merdol Ibrahim, UK NEQAS ICC & ISH Scheme Manager.

10. CADQAS
    Keith Miller, UK NEQAS ICC & ISH Director
Lung: Diagnosis, ICC, ALK and PD-L1

Professor Andrew G Nicholson, DM, FRCPath
Consultant Histopathologist, Royal Brompton and Harefield NHS Foundation Trust
Professor of Respiratory Pathology National Heart and Lung Division
Imperial College, London, United Kingdom

AIMS OF PRESENTATION

• Review of 2015 WHO classification system.
• Recognise the increasing importance of immunohistochemistry and genetics in the diagnosis of lung tumours.
• Current status of ALK testing
• Current status of PD-L1 testing

CLASSIFICATION LARGELY DEVELOPED WITHIN THE IASLC PATHOLOGY COMMITTEE

WHERE WAS WHO DEVELOPED?

157 Authors from 29 countries

WHAT IS A WHO CLASSIFICATION?

A pathologic and genetic classification of human tumors designed to be accepted and used worldwide.

• Provides standard criteria for
  – Pathology diagnosis
  – Clinical practice
  – Cancer registration
  – Epidemiologic studies
  – Clinical trials
  – Cancer research

THE REQUIREMENTS OF A CLASSIFICATION SYSTEM

• REPRODUCIBLE (strict and recognizable set of criteria)
• GLOBALLY APPLICABLE (…that everyone can apply)
• THOROUGH (…which can deal with atypical variants)
• DYNAMIC (…adapts to recent advances)

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SPECIFIC TERMINOLOGY AND CRITERIA FOR ADENOCARCINOMA, SQUAMOUS CELL CARCINOMA AND NON-SMALL CELL CARCINOMA-NOS IN SMALL BIOPSIES AND CYTOLOGY

2015 WHO Classification in resection specimens

Morphology/Stains New Small Biopsy/Cytology

Terminology

ADENOCARCINOMA (Predominant pattern)

Acinar
Papillary
Solid
Micropapillary

Morphologic adenocarcinoma patterns clearly present

Adenocarcinoma (describe identifiable patterns present)

Lepidic (nonmucinous)

Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)

Invasive mucinous adenocarcinoma

Colloid adenocarcinoma

Fetal adenocarcinoma

Enteric adenocarcinoma

Invasive mucinous adenocarcinoma (describe patterns present; use term mucinous adenocarcinoma with lepidic pattern if pure lepidic pattern – see text)

Adenocarcinoma with mucinous features

Adenocarcinoma with fetal features

Adenocarcinoma with enteric features

SQUAMOUS CELL CARCINOMA

Morphologic squamous cell patterns clearly present

Squamous cell carcinoma

Adapted from: Travis WD et al. IASLC/ATS/ERS classification of ADCs J Thor Oncol 2011;6:244-285

DO NOT CLASSIFY BIOPSIES AS ADENOCARCINOMA IN SITU

Adenocarcinoma with a purely lepidic pattern in this sample

Biopsy here will show lepidic only

Biopsy here will show lepidic and other “invasive patterns”

CLASSIFICATION FOR SMALL BIOPSIES/CYTOLGY COMPARING 2015 WHO TERMS WITH NEW TERMS FOR SMALL CELL CARCINOMA, LARGE CELL NEUROENDOCRINE CARCINOMA, ADENOSQUAMOUS CARCINOMA AND SARCOMATOID CARCINOMA

SMALL CELL CARCINOMA

Small cell carcinoma

LARGE CELL NEUROENDOCRINE CARCINOMA (LCNEC)

Non-small cell carcinoma with neuroendocrine (NE) morphology and positive NE markers, possible LCNEC

ADENOSQUAMOUS CARCINOMA

Morphologic squamous cell and adenocarcinoma patterns present: Non-small cell carcinoma, NOS, (comment that adenocarcinoma and squamous components are present and this could represent adenosquamous carcinoma).

No counterpart in 2015 WHO classification

Morphologic squamous cell or adenocarcinoma patterns not present but immunostains favor separate glandular and adenocarcinoma components Non-small cell carcinoma, NOS, (specify the results of the immunohistochemical stains and the interpretation)

Comment: this could represent adenosquamous carcinoma.

Pleomorphic, spindle and/or giant cell carcinoma

NSCC with spindle and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)
SPECIFIC TERMINOLOGY AND CRITERIA FOR ADENOCARCINOMA, SQUAMOUS CELL CARCINOMA AND NON-SMALL CELL CARCINOMA-NOS IN SMALL BIOPSIES AND CYTOLOGY †

2015 WHO Classification
in resection specimens

Morphology/Stains New Small Biopsy/Cytology †

Terminology

Adenocarcinoma (solid pattern may be just one component of the tumor) ‡
Morphologic adenocarcinoma patterns not present, but supported by special stains, i.e. +TTF-1

Non-small cell carcinoma, favor adenocarcinoma‡

Squamous cell carcinoma, (nonkeratinizing pattern may be just one component of the tumor) ‡
Morphologic squamous cell patterns not present, but supported by stains i.e. +p40

Non-small cell carcinoma, favor squamous cell carcinoma

LARGE CELL CARCINOMA
No clear adenocarcinoma, squamous or neuroendocrine morphology or staining pattern ‡
Non-small cell carcinoma, not otherwise specified NSCLC-NOS‡‡

†† Metastatic carcinomas should be carefully excluded with clinical and appropriate but judicious immunohistochemical examination.

‡The categories do not always correspond to solid predominant adenocarcinoma or non-keratinizing squamous cell carcinoma respectively. Poorly differentiated components in adenocarcinoma or squamous cell carcinoma may be sampled.

‡‡ NSCLC-NOS pattern can be seen not only in large cell carcinomas but also when the solid poorly differentiated component of adenocarcinomas or squamous cell carcinomas are sampled but do not express immunohistochemical markers or mucin.

Thyroid transcription factor-1 (TTF-1), WHO – World Health Organization

Adapted from: Travis WD et al. IASLC/ATS/ERS classification of ADCs J Thor Oncol 2011;6:244-285

GENERAL PRINCIPLES
• Cut tissue block as sparingly as possible
• Obtain unstained slides for molecular at time of cutting block for IHC
• Minimize stains to maximize tissue for molecular testing by using a limited panel of IHC (i.e TTF-1 and P40)
• Further molecular testing, if clinically appropriate, can be performed on remaining tissue

CYTOLOGY IS A POWERFUL TOOL FOR CLASSIFYING NSCLC


"4.2: Expert Consensus Opinion.
- Cytologic samples are suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations."

“Pseudosquamoid” solid ADC

Adapted from: Travis WD et al. IASLC/ATS/ERS classification of ADCs J Thor Oncol 2011;6:244-285
Potential targetable oncogenes by histology subtype.

Undifferentiated Adenocarcinoma

Molecularly Targeted Therapies in Non–Small-Cell Lung Cancer Annual Update 2014

Next generation sequencing

IMMUNOMODULATORY THERAPY (e.g. PD-L1)

• INVASIVE
• PREINVASIVE LESIONS

NEXT GENERATION CLASSIFICATION

Travis WD et al. JTO 2011;6:244-286

WHO Classification Of Adenocarcinoma 2004

Adenocarcinoma

Mixed subtype
Acinar
Papillary
Bronchioloalveolar carcinoma

Nonmucinous
Mucinous

Mixed mucinous and non-mucinous
Solid adenocarcinoma with mucin formation

Variants

* Need for greater clinical relevance and need to take into account rapid evolving molecular advances

Adapted from:
Travis WD et al. IASLC/ATS/ERS classification of ADCs / Thor Oncol 2011;6:244-286
**ADENOCARCINOMA-IN-SITU**

5mm or less = "microinvasion"
No necrosis
No lymphatic or pleural invasion
No spread through air-spaces (STAS)

**Minimally invasive adenocarcinoma**

**Updated ADC Classification ...is it working?**

- IO variation
  - Kappas range from moderate to good for classic cases (problem with definition of invasion)
- PREDOMINANT PATTERN
  - Several papers, higher stages different countries
- CORRELATION WITH MOLECULAR SUBTYPES
  - Predominant pattern, Signet ring, TTF-1 +ve

**AIMS OF PRESENTATION**

- Review of 2015 WHO classification system.
- Recognise the increasing importance of immunohistochemistry and genetics in the diagnosis of lung tumours.
- Current status of ALK testing
- Current status of PD-L1 testing

**SQUAMOUS CELL CARCINOMA (SQCC)**

"A malignant epithelial tumour showing keratinization and/or intercellular bridges that arises from bronchial epithelium"
Squamous cell carcinoma (WHO 2004)

- Squamous cell carcinoma; variants:
  - Papillary
  - Clear cell
  - Small cell
  - Basaloid
- Adenosquamous carcinoma
- Large cell carcinoma:
  - Basaloid carcinoma subtype
- Pre-invasive lesions:
  - Squamous cell carcinoma in situ

2015 WHO CLASSIFICATION SQUAMOUS CELL CARCINOMA

- Keratinizing
  - now need IHC – P40 positive, TTF-1 negative
- Non-keratinizing
  - Basaloid carcinoma
    - now need IHC – (+p40, -TTF-1 & NE markers)
    - r/o LCNEC & SCLC

2015 WHO CLASSIFICATION NEUROENDOCRINE TUMORS

- Small cell carcinoma
  - Combined SCLC
- Large cell neuroendocrine carcinoma
  - Combined LCNEC
- Carcinoid tumor
  - Typical carcinoid
  - Atypical carcinoid

LARGE CELL CARCINOMA

2004

- Large cell carcinoma
  - Large cell neuroendocrine carcinoma 8013/3
  - Combined large cell neuroendocrine carcinoma 8013/3
  - Basaloid carcinoma 8123/3
  - Clear cell carcinoma 8310/3
  - Large cell carcinoma with rhabdoid phenotype 8014/3

2015...

- Large cell carcinoma
  - Null phenotype on IHC
  - Unclear phenotype on IHC
  - No IHC available
  - ADC on IHC (NOW UNDER ADC, solid subtype)
  - SQCC on IHC (NOW UNDER NON-KERAT SQCC)

** Clear cell and rhabdoid (more aggressive) are cytologic features with no current prognostic/predictive significance, but may be relevant to differential diagnosis – comment as a percentage of tumor.

Subtyping of resected morphologically undifferentiated non-small cell carcinomas (formerly large cell carcinoma)

- Adenocarcinoma, solid subtype
- Non-keratinising squamous cell carcinoma
- Large cell carcinoma
- Clear cell carcinoma
- Rhabdoid carcinoma
- Undifferentiated carcinoma

Figure 105: Keratinizing squamous cell carcinoma is rare in SQCC. It is seen mostly in the periphery of SQCCs near small blood vessels or lymphatic vessels. It has a distinctive cribriform pattern. The keratinizing cells are small and monotonous, with a granular nuclear chromatin pattern. The stroma is typically edematous and rich in blood vessels. Immunohistochemistry is not required for this diagnosis.

** Clear cell and rhabdoid (more aggressive) are cytologic features with no current prognostic/predictive significance, but may be relevant to differential diagnosis – comment as a percentage of tumor.

* All IHC can only be done if sufficient tissue is available.

1 In cases where there is morphological evidence of either squamous cell carcinoma or adenocarcinoma, then immunohistochemistry is not required to assess undifferentiated areas.
SEER data on incidence of non-small cell carcinomas (Lewis DR et al. Cancer 2014;2014(18):2883-92)

**AIMS OF PRESENTATION**

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**Response to Crizotinib in Patients With NSCLC Carrying an EML4-ALK Translocation...**

- A receptor tyrosine kinase anaplastic lymphoma kinase (ALK) fuses to the echinoderm microtubule-associated protein-like 4 (EML-4)
- More than 20 EML4-ALK variants have been identified, nine of which are shown here. Three other partner proteins have been identified in NSCLC: TFG, KIF5B, and KLC1. Three different KIB5B-ALK variants have been identified (not shown).

**Comparison of the methods used for ALK fusion detection by Tuononen et al. (n=5 cases)**

<table>
<thead>
<tr>
<th>Method</th>
<th>FFPE material</th>
<th>Amount of material required</th>
<th>Sensitivity</th>
<th>Time used for analysis</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH</td>
<td>Yes</td>
<td>One tissue section (2.5 μm thick)</td>
<td>10–15%</td>
<td>2-3 days</td>
<td>Medium (~200 euros)</td>
</tr>
<tr>
<td>IHC</td>
<td>Yes</td>
<td>One tissue section (2.5 μm thick)</td>
<td>5–10%</td>
<td>Half a day</td>
<td>Low (~20 euros)</td>
</tr>
<tr>
<td>Real-time RT-PCR</td>
<td>Yes</td>
<td>0.1–0.5 μg of RNA</td>
<td>1–5%</td>
<td>1 day</td>
<td>Medium (~150 euros)</td>
</tr>
<tr>
<td>Targeted resequencing</td>
<td>Yes</td>
<td>2–3 μg of DNA</td>
<td>Not determined</td>
<td>10 days</td>
<td>High (~1000 euros)</td>
</tr>
<tr>
<td>Possibility to see large range of other gene mutations</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

From: Tuononen et al. Table 2  Biomed Research international 2013, Article ID 757490,
IHC seems to be a reliable screening tool for the ALK translocation (compared to FISH +ve)

- Boland JM et al. Hum Pathol. 2009;40(8):1152-1158 (Dako, 5A4)
- Conklin CM et al. J Thoracic Oncol. 2011;6:45-51. (D5F3 and 5A4)

High interobserver reproducibility amongst pathologists for IHC reading

**What is the most efficient way of testing for the ALK translocation?**

JULY 2011  
MARCH 2012

A comparison of FISH and Immunohistochemistry in the detection of ALK rearrangements in lung ADC

Staining intensity was the most discriminating measure overall. Proportion scoring did not improve the last, so intensity alone was used:

- No FISH-IHC cases were seen (100% specificity)
- Antibodies were of variable sensitivity, though very similar

IHC Positive-1  
IHC Negative

H&E resist Novocastra(5A4) Dako(ALK) Ventana(5D3)

**Clinical, imaging, and histological parameters for targeting ALK translocations**

- Decision to use Dako antibody, based on cost (£8.65 vs ~£30 per test excluding labour) and availability of platform (cost of a Ventana machine...)
- FISH confirmation when IHC positive, if tissue available
- Screen stage 3 and above
- Began September 2013

Audited July 2014

- 168 cases screened with 11 positive cases.
- Of these 7 of 7 confirmed by FISH (two not tested, one awaited, and one had no tissue left after IHC).
- An additional 14 patients with stage 3 or above were not screened.
- Of 10/11, 8 have been treated, 4 showing response, 1 stopped for toxicity, 1 just started, 3 still on 1st line, 1 advanced surgical case
**AIMS OF PRESENTATION**

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**Markers of immune function: cell surface molecules**

- Expression of cell surface molecules determining T-cell function may have predictive significance
- The inhibitory (checkpoint) molecule PD-L1 is associated with poor prognosis in patients with NSCLC

**PD-L1 as a target for therapy**

- Lung Cancer:
  - Interruption of the interaction of programmed death receptor-1 (PD-1) and its ligand (PDL-1) between tumour cells and immune effectors cells, using monoclonal antibodies
  - Expression of cell surface molecules

- The inhibitory function may have determining T-cell surface molecules


- Never smokers, adenocarcinomas (and young age) multi wild, signet ring component in 10%
- Combinational test for parallel and combinational platforms

**ALK testing - 2015**

- Actionable mutation (crizotinib, others...)
- Laboratories should use an ALK FISH assay using dual-labelled break-apart probes for selecting patients for ALK status-related therapy.
- ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for ALK FISH testing.
- Companion diagnostic "recommended", although others (cheaper) seem to work with similar efficacy, if properly validated.
- Support funding from pharmaceutical industry ceased in 2015.
- ALK immunohistochemistry and genetics in the diagnosis of ALK testing - 2015

- Percentage of laboratories are testing in the UK.

- The inhibitory effectors cells, using monoclonal antibodies
  - Stand alone
  - Kit based Assay
  - Methodology Kit assay Automated
  - Respond to crizotinib
Challenges in developing a PD-L1 biomarker assay

• PD-L1 expression variable and dynamic within tissues
• STILL unclear what level of PD-L1 expression is going to be the cut-off
• Relevance of co-localisation with TILs (relevance in relation to TBNAs…)
• Issues to be addressed in developing reliable and reproducible assay(s) for routine clinical in the UK:
  • Variability in tissue collection timing
  • Which cells to sample (primary tumour, metastasis, archival versus new sample)
  • IHC criteria are different for each assay
  • Training and interpretation
  • Verification and validation (number of cases, quality assurance (NEQAS), etc…)

Best Practice for Usage of Tissues – Everyone has a Role to Play!

• Pre-examination phase:
  – Identify those who you would consider for targeted therapy
  – Handle tissue appropriately (right media, timely fixation etc)
  – Put core biopsies in separate pots

• Examination phase ("judicious use of tissue"):
  – Consider separate blocks for different cores
  – Cut into the tissue carefully (take spare sections for IHC up front)
  – Selection for testing based on histology
    - ADC versus SQCC
    - Apply immunohistochemistry appropriately (ideally only once)
    - Specific antibodies (ALK, ROS)

• Verification and validation (number of cases, quality assurance (NEQAS), etc…)

• Post-examination phase:
  – Enhance tumour load by microdissection
  – Tissue result goes into the pathology report, with explanation of the result and the assay undertaken

CONCLUSION: HANDLING OF SMALL BIOPSIES AND CYTOTOLOGICAL SAMPLES

<table>
<thead>
<tr>
<th>Biopsy Type</th>
<th>Recommended Handling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Block</td>
<td>Fixation in formalin</td>
</tr>
<tr>
<td>Core Biopsy</td>
<td>Separate blocks</td>
</tr>
<tr>
<td>cytology</td>
<td>Microdissection</td>
</tr>
</tbody>
</table>

CONCLUSION

The WHO 2015 classification of lung tumours: evolution of tumour classification in lung cancer

• …is a more biologically based classification, although is still based primarily on microscopic features.
• …greater reliance on immunohistochemistry
• …is more relevant to both clinical management of patients and more closely allied to molecular classification.

• All staff dealing with lung cancer patients should use the classification, and ensure that tissue is handled as efficiently as possible to ensure optimal patient management.
  – Pre-examination phase
  – Examination phase
  – Post-examination phase
  – Research/clinical trials

• Future classification
  – Balance of morphology and molecular data needs to be maintained

PUBCAN – ONLINE WHO BOOK COMING SOON – CAN BE UPDATED

www.pubcan.org
ALK IHC in Non-Small Cell Lung Carcinoma (NSCLC): Establishing a Robust EQA

Merdol Ibrahim
UK NEQAS ICC & ISH, London
merdol.ibrahim@ucl.ac.uk

References

NSCLC ALK
- Accounts for approximately 80% of lung cancers, with a 5 year survival rate of 17%
- EML4-ALK fusion gene identified in 3-7% of patients
- Patients may be eligible candidates for more precise targeted therapies e.g. Crizotinib – ORR 60%... (Ceritinib (Novartis), Alectinib (Roche).

Normal:
- EML4 on opposite strand to ALK, and both probes on the ALK gene (orange/green) are close together
- Inversion of EML4 N-terminal with the kinase domain of ALK
- Increased distance between the orange/green probes

Inversion with Deletion:
- Inversion + deletion of the proximal part = single red orange signal.

IHC and NSCLC ALK
- Brightfield immunohistochemistry (IHC) has also enhanced the possibility of detecting ALK rearrangements.
- IHC for ALK rearrangements can be challenging due to the relatively low ALK protein concentrations
- IHC more cost effective, identify tumour better and less time consuming
- IHC vs FISH sensitivity
  - Depends on the publications you read

Survey of potential NSCLC ALK EQA participants (n=102)

Would you be interested in participating in an ALK EQA?

Result n %
YES 90 88%
NO 2 2%
Unsure 10 10%

Do you currently carry out diagnostic NSCLC ALK testing?

Result n %
YES 79 77%
NO 16 16%
Research Only 7 7%

In IHC Your Front-line Method?

Result n %
Yes 65 64%
No 37 36%

Do you currently participate in an ALK EQA?

Result n %
Yes 65 64%
No 37 36%

Setting up a NSCLC ALK EQA

Establish a rigid ALK IHC EQA module and to identify and assist laboratories who may be having technical issues

Pre-pilot advisory group meeting February 2015:

Pathologists
Dr Merdol Ibrahim
Dr Erik Thunnissen (Amsterdam)
Dr Philip Jassim (Birmingham)
Prof John Gosney (Liverpool)
Prof Keith Kerr (Scotland)

Scientists
Dr Jane Starcynski (Birmingham)
Mr Steven Forrest (Liverpool)
Dr Perry Maxwell (Belfast)
Mr David Aller (UCL)
Dr Tony O’Grady (Dublin)

UK NEQAS ICC & ISH:
Dr Merdol Ibrahim
Ms Suzanne Parry
Mrs Dawn Wilkinson
Mr Neil Bilbe
Mr Keith Miller

Pathologists
Dr Erik Thunnissen (Amsterdam)
Dr Philip Jassim (Birmingham)
Prof John Gosney (Liverpool)
Prof Keith Kerr (Scotland)

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Mr David Aller (UCL)
Dr Tony O’Grady (Dublin)
Setting up a NSCLC ALK EQA

Establish a rigid ALK IHC EQA module and to identify and assist laboratories who may be having technical issues

- Due to the various antibodies and methodologies:
  - Assessment not focused on staining intensity, but on the interpretability of the samples.
  - Interpretation for initial pilot decided as a simple ‘+ve’/’-ve’ (positive/negative)
  - Updated to provide further information on intensity
    - +ve (3+), +ve (2+) and +ve (1+)
  - Assessment panel provide their own ‘independent’ interpretation of EQA samples
  - Scoring system based on each 4 assessor scoring independently out of 5 marks, with scores summed to give a potential score out of 20

ALK IHC: Sample Distribution

**Sample Placement**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Distribution</th>
<th>FISH status (Vysis)</th>
<th>IHC status (Roche D5F3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cell line: 50% knock in, 50% adenocarcinoma</td>
<td>-ve</td>
<td>Approx. 50% +ve &amp; 50% -ve</td>
</tr>
<tr>
<td>B</td>
<td>Cell line: 50% knock in, 50% adenocarcinoma</td>
<td>-ve</td>
<td>100% +ve</td>
</tr>
<tr>
<td>C</td>
<td>Cell line: 50% knock in, 50% adenocarcinoma</td>
<td>+ve (Break apart: inversion)</td>
<td>Approx. 50% +ve &amp; 50% -ve</td>
</tr>
<tr>
<td>D</td>
<td>Cell line: 50% knock in, 50% adenocarcinoma</td>
<td>+ve (Break apart: inversion)</td>
<td>Approx. 50% +ve &amp; 50% -ve</td>
</tr>
<tr>
<td>E</td>
<td>NSCLC tumour adenocarcinoma</td>
<td>+ve (Break apart: inversion + deletion)</td>
<td>+ve</td>
</tr>
<tr>
<td>F</td>
<td>NSCLC tumour adenocarcinoma</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

**Method used can change interpretation**

1st EQA

- FISH Confirmation of UK NEQAS EQA Samples
  - A: -ve
  - B: -ve
  - C: +ve (inversion)
  - D: +ve (inversion)
  - E: +ve (inversion + deletion)
  - F: -ve

2nd EQA

- Tested as:
  - -ve tumour (E)
  - +ve tumour (F)
  - +ve cell line (C)

**IHC and NSCLC ALK**

- Three IHC protocols:
  - DSF3 + Benchmark + Optiview
  - 5A4 + Benchmark + Optiview
  - 5A4 + BondMax + Bond Polymer Refine

**Concordance**

- Retrospective cohort: 1/25 FISH +ve (4%) = IHC false -ve
- Prospective cohort:
  - 3/32 FISH-positive (9.4%) = IHC false -ve
  - 2/271 FISH-negative (0.7%) = IHC false +ve

**ALK IHC: Pass Rates NEQAS Samples**

- 1st EQA:
  - n = 26
  - Unacceptable & Borderline = 17%
  - 44% new participants

- 2nd EQA:
  - n = 52
  - Unacceptable & Borderline = 30%
  - 46% new participants
ALK IHC:
Antibody and Pass Rates

Roche (D5F3) Failures
Excessive tyramide staining

Dako (ALK1):
Only recommended for lymphoma

Does staining intensity really matter?

Not Just Detection Method

- Roche (D5F3), 53% of users, Pass Rate 80%
- Dako (ALK1), 6% BUT clone NOT recommended by Dako
- Failure not necessarily due to antibody clone alone

UK NEDAS
**Not Just Detection Method**

**Antibody clone + Dilution + Retrieval + Incubation**

<table>
<thead>
<tr>
<th>Primary Antibody Automation Instrument Detection Kit</th>
<th>Excellent</th>
<th>Acceptable</th>
<th>Borderline</th>
<th>Uncceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>LabVision Autostainer Dako Envision HRP/DAB</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Leica Bond-III Leica Bond Polymer Refine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
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<tr>
<td>Ventana Benchmark XT Ventana UltraView Kit</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Dako (ALK1)</td>
<td></td>
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<tr>
<td>Dako Autostainer Link 48</td>
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<tr>
<td>Dako EnVision FLEX+ (K8002/12)</td>
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<tr>
<td>Leica Bond Max Bond Polymer Refine Red (DS9390)</td>
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<td>Leica Bond-III Leica Bond Polymer Refine (DS9800)</td>
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<td>None (Manual) Polink-2 HRP Broad with DAB Kit</td>
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<td>Thermo (5A4)</td>
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<td>Zytomed (p80)</td>
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**Interpretive Scoring Methods Used: Run 111 (4%)**

<table>
<thead>
<tr>
<th>Antibody (clone)</th>
<th>n</th>
<th>+ve / -ve</th>
<th>3+,2+,1+, neg.</th>
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</thead>
<tbody>
<tr>
<td>Ventana/Roche (D5F3)</td>
<td>26</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Novocastra NCL-ALK (5A4)</td>
<td>2</td>
<td>100%</td>
<td>43%</td>
</tr>
<tr>
<td>Cell Signalling Tech. (DSF3)</td>
<td>2</td>
<td>-</td>
<td>100%</td>
</tr>
<tr>
<td>Dako M7193 (ALK1)</td>
<td>2</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Novocastra PA0306 (5A4)</td>
<td>1</td>
<td>100%</td>
<td>-</td>
</tr>
</tbody>
</table>

| Overall | 87% | 13% |

- UK NEQAS ALK EQA now includes intensity feedback:
  - +ve (3+) / +ve (2+) / +ve (1+)
- Intensity scoring may help labs to further troubleshoot their method

**Interpretive Scoring Methods Used**

**JCO 32 (25): 2780-2788**

**ALK IHC: Pass Rates**

**In-house samples**

Unacceptable & Borderline = 19%

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>No. of in-house controls submitted per participant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung adenocarcinoma (+ve)</td>
<td>34</td>
</tr>
<tr>
<td>Lymphoma (+ve)</td>
<td>11</td>
</tr>
<tr>
<td>Appendix (+ve)</td>
<td>69</td>
</tr>
<tr>
<td>Other Positive</td>
<td>46</td>
</tr>
<tr>
<td>Lung adenocarcinoma (-ve)</td>
<td>47</td>
</tr>
<tr>
<td>Other Negative</td>
<td>35</td>
</tr>
<tr>
<td>Small cell lung carcinoma (+ve)</td>
<td>12</td>
</tr>
<tr>
<td>Squamous cell carcinoma (-ve)</td>
<td>12</td>
</tr>
<tr>
<td>Tonsil (-ve)</td>
<td>12</td>
</tr>
</tbody>
</table>

**Submitted In-house Controls: Run 110 (3rd assessment)**

<table>
<thead>
<tr>
<th>No. of in-house controls submitted per participant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>&gt;3</td>
</tr>
</tbody>
</table>
In-house Control:
What is an acceptable control/s?

- +ve Lung adeno
- -ve Lung adeno
- Appendix

- Ideally +ve & -ve lung tumours (with appendix?)
- Lymphoma not ideal (as illustrated with Dako clone)

Pass Rates
1 Year of EQA

<table>
<thead>
<tr>
<th></th>
<th>Unsatisfactory</th>
<th>Borderline</th>
<th>Acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 (n=60)</td>
<td>14%</td>
<td>3%</td>
<td>83%</td>
</tr>
<tr>
<td>100 (n=50)</td>
<td>10%</td>
<td>10%</td>
<td>80%</td>
</tr>
<tr>
<td>110 (n=58)</td>
<td>5%</td>
<td>3%</td>
<td>92%</td>
</tr>
<tr>
<td>118 (n=42)</td>
<td>3%</td>
<td>3%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Methods Used
1 Year of EQA

- 85% of EQA users use Roche (D5F3): 89% pass Rate
- 15% Leica (5A4): 67% pass rate
- 3% (n=1) Dako (ALK1): But NOT recommended for NSCLC

Data indicates that it’s not just the clone but the detection system applied... more sensitive detection required

Please follow commercial company protocols

- EQA shows multiple methods: Highlights potential ‘pitfalls’
- ALK IHC EQA now offered 4 x per year
- ALK FISH in development

Preston Cellular Pathology Weekend

- 18th-20th March 2016
- Marriott Hotel, Preston
- Info: Christine Thomas — christine.thomas@lthtr.nhs.uk

ALK Summary

- 85% of EQA users use Roche (D5F3): 89% pass Rate
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- 3% (n=1) Dako (ALK1): But NOT recommended for NSCLC

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- ALK IHC EQA now offered 4 x per year
- ALK FISH in development

Thank You
.... & Acknowledgments

UK NEQAS ICC & ISH:
Ms Suzanne Parry
Mrs Dawn Wilkinson
Mr Neil Bilbe
Mr Keith Miller

Novartis Pharmaceuticals UK
- Provided an educational grant, used to help setup the ALK EQA module.
- Novartis are not privy to any data/results until they are publicly available.

Assessment Panel:
Dr Eric Thunnissen (Amsterdam)
Dr Philippe Taniere (Birmingham)
Dr Perry Maxwell (Belfast)
Dr Tony O’Grady (Dublin)
Dr Jane Starcynski (Birmingham)
Dr John Gosney (Liverpool)
Dr Keith Kerr (Scotland)

### Aim of an IHC NSCLC ALK EQA

<table>
<thead>
<tr>
<th>Question</th>
<th>Result</th>
<th>Yes</th>
<th>No</th>
<th>Unsure</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you currently participate in an NSCLC ALK EQA?</td>
<td>No</td>
<td>13</td>
<td>34</td>
<td>53</td>
<td>20</td>
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<tr>
<td>Would you be interested in participating in the UK NEQAS NSCLC ALK EQA?</td>
<td>Yes</td>
<td>39</td>
<td>56</td>
<td>7</td>
<td>48</td>
</tr>
</tbody>
</table>

### Survey: Which Methods are lab using?

<table>
<thead>
<tr>
<th>Method</th>
<th>Result</th>
<th>Yes</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventana/Roche D5F3</td>
<td>44</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novocastra 5A4</td>
<td>16</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (specify below)</td>
<td>8</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dako ALK1: CD246</td>
<td>7</td>
<td>93</td>
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<tr>
<td>Thermo/Neomarkers A4</td>
<td>4</td>
<td>96</td>
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### ALK IHC Pre-pilot Survey

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<tbody>
<tr>
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<td>Dako PTLink</td>
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<td>2</td>
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</table>

### Which ALK IHC staining method/s do you use?

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</table>
Slide-Based Biomarker Testing in Lung

David Allen
Laboratory Services Manager–IHC&ISH
UCL–Advanced Diagnostics

Predicative Biomarker Testing

• Era of personalised medicine
• Identify the “right treatment for the right patient at the right time”

• Organ vs biomarker based disease?
  – Over 1500 active biomarker based clinical trials in US
  – 106 FDA approved indications for targeted treatments

Current Biomarker Testing

• Breast – ER, HER2, AR, MET
• Lung – EGFR, ALK, ROS1, NTRK1, KRAS, BRAF, MET, RET, FGFR1-3, HER2, PIK3CA
• GI – KRAS, NRAS, EGFR, HER2, c-Kit, MET
• Melanoma – BRAF
• Prostate – AR
• Head & Neck – p16, HPV, EGFR
• Cervical – p16, HPV
• Haematopathology – CD20, CD52, c-Kit
• Neuropathology – IDH1, 1p/19q deletions, MET
ALK Gene Rearrangement in NSCLC

- First identified in 2007
- At least 27 known ALK gene rearrangement variants
- Rearrangement leads to kinase expression, activation and oncogene addiction
- Crizotinib approved by the FDA in 2011

ALK Fusions – Mode of Action

- Crizotinib (Xalkori, Pfizer)
  - Selective small-molecule inhibitor of anaplastic lymphoma kinase (ALK) receptor tyrosine kinase
  - Used to treat advanced or metastatic NSCLC
  - 60% response rate with average extra 7.7 months progression free survival compared with standard treatment
  - 2nd line: Ceritinib, Novartis (LDK378) approved April 2014 for Crizotinib resistant ALK + NSCLC

ALK Testing in NSCLC

- ALK gene rearrangements in NSCLC used to stratify patients for treatment with Crizotinib
- Main methods employed
  - Breakapart FISH: direct visualisation of rearrangements
  - IHC: indirect visualisation of rearrangements/direct visualisation of protein overexpression
    - Ventana XT OptiView with TSA amplification
  - RT-PCR: detection of fusion transcripts
  - NGS: Oncomine
ALK Testing in NSCLC

- IHC: Strong granular cytoplasmic staining in any percentage of tumour cells
- FISH: ≥15/50 nuclei – evidence of translocation
  - ≥5 to <15/50 - count extra 50 nuclei. Positive if ≥15/100

ALK FISH Reporting

- Manual reporting as per Abbott ALK NSCLC FISH Test Instructions for Use
- Ensure were possible H&E and any associated immunohistochemistry is available to assist in tumour assessment
- Slides assessed manually down the eyepiece (not on screen)
- DAPI, single colour (specific for Spectrum Orange & Spectrum Green) and triple filters ALL used as part of the assessment to accurately characterize ALK translocations & copy number variations

ALK Re-arrangements in NSCLC

- Normal fusion profile
- Copy number gain of fusion signals
- Copy number gain of green signals
- Copy number gain of ALK signals
- Copy number loss of green probe
- Copy number gain of ALK signals (no translocations)

Roche ALK IHC

Roche ALK IHC - False Negative

UCL-AD ALK Testing Pathway

Roche ALK IHC (D5F3)

Abbott ALK FISH

Material used from Dr. Jane Starczynski, Heartlands Hospital, Birmingham.
ALK Re-arrangement in NSCLC

ALK FISH Reporting Mechanism

1. Identify Invasive Tumour
2. Assess all tumour looking for any areas of sample heterogeneity (very, very rare)
3. Assess 50 tumour cells for gene re-arrangement patterns

- \(< 5/50 \text{cells} = \text{NEGATIVE}\)
- \(\geq 25/50 = \text{POSITIVE}\)

Equivocal (5-25/50 cells or 5-50%)

1. Assess a further 50 tumour cells for gene re-arrangement patterns
2. Collate with first 50 tumour cells to give a final result from 100 tumour cells

- \(< 15\% (<15/100) = \text{NEGATIVE}\)
- \(\geq 15\% (\geq 15/100) = \text{POSITIVE}\)

Testing Data: UCL-AD (Dec 12 – Sept 15)

- 4614 ALK requests
- 244 inadequate specimens (5.3%)
- 4373 cases reported
- 475 cases FISH analysed (9%)
- 88 positive cases (2.05%)
- 16 cases IHC positive/FISH negative
  - (rolling total positivity 2.3% if included)
- 10 cases IHC weak/FISH positive
  - (11.4% of positive cases)
We know these patients have a gene rearrangement
Do we know if those patients who show clear disease progression lack ALK protein?
IHC & ISH should be included in ALL ongoing ALK targeted therapy trial to determine best companion diagnostic assay

ROS1 Translocations in NCSLC
- 7 known gene partners
  - TPM3-ROS1, t(1;6)(q21.2;q22)
  - SDC4-ROS1, t(6;20)(q22;q12)
  - SLC34A2-ROS1, t(4;6)(q15.2;q22)
  - CD74-ROS1, t(5;6)(q32;q22)
  - LR63-ROS1, t(6;12)(q22;q14.1)
  - GOPC-ROS1, del(6)(q22q22.3)
  - EZR-ROS1, inv(6)(q22q25.3)
- Sensitive to kinase inhibitors – Crizotinib (off label/trials)
- 2-3% of NSCLC – probably much less in real life
- Independent biomarker
  - Data suggests it occurs without accompanying EGFR, KRAS, ALK, HER2 or RET alterations

ROS1
- Phase II clinical trials for ROS1 positive patients active
  - Crizotinib & Certinib
  - Very good initial results, but very difficult to recruit patients, 50 patients over 3 years!!
- FISH assay – Kreatech, Abbott, Cytocell, Zytovysion, Dako/Agilent
- IHC assay – Cell Signalling Technologies, clone D4D6
  - Early data shows very high sensitivity & specificity
  - 80 cases assessed by FISH and IHC at UCL-AD
    - 100% concordance between FISH and IHC (3 positive cases!!)
    - Verified through cell lines

NTRK1 Translocations in NSCLC
- Neurotrophic Tyrosine Kinase Receptor 1
- Has been shown to respond to kinase inhibitors
- 3% of NSCLC (very limited data)
- Zytovysion FISH probe
RET Translocations in NSCLC

- Limited expression in adult tissue
  - Developmental tyrosine kinase
  - RET translocations lead to active protein
    - Signal transduction and activation of cell signalling pathways
- Sensitive to kinase inhibitors
  - Ponatinib FDA approved for CML, Ph+ ALL
  - Cardiotoxicity – suspended Oct 2013
- 1-2% of NSCLC
- Analysis by FISH only
  - Very poor IHC

EGFR Mutation Specific IHC

- Antibodies against specific EGFR mutation/deletion
- Highly specific, but may lack sensitivity
  - Reverses the general IHC to Molecular testing paradigm
  - IHC negative cases must go for PCR specific mutation testing
    - In UCL-AG it will be mandatory for all requests to have concurrent IHC & NGS testing
    - Use will be directed towards patients who cannot wait for results from QPCR/NGS pipelines
  - EGFR Exon 19 & 21
    - Specificity 90-99%
    - Sensitivity 65-85%

EGFR Mutation Specific IHC

- Ventana/Roche
  - E746-A750 (SP111) & L858R (SP125)
    - CE-IVD
- Cell Signalling Technologies
  - E746-A750 (D6B6) & L858R (43B2)
    - RUO
- Scoring Criteria
  - 0: No expression
  - 1+: Weak membrane or cytoplasmic expression in >10%
  - 2+: Moderate membrane or cytoplasmic expression in >10%
  - 3+: Strong membrane or cytoplasmic expression in >10%

NSCLC – Research & Clinical Trials

- TRACERx (Tracking Cancer Evolution through Therapy)
- 850 patients over 9 years
- Samples taken before and after surgery and during treatment from multiple sites within the tumour
  - Will track in real time how tumours evolve, mutate and gain resistance mechanisms
Biomarker Negative NSCLC

- No identifiable target in 35-45% of cases
  - Probably no such entity
  - Currently huge research focus on these patients
Are there any new biomarkers in gastrointestinal cancers?

Manuel Rodriguez-Justo

Friday 6th November, 2015

"Old friends" with new roles
- HER2
- BRAF
- FGFR

"New kids on the block"
- HER3

"Rising stars"
- Checkpoint Inhibitors / Immune Modulators

HER Family

Family of closely related tyrosine kinases.
- HER-1 / EGFR / erbB1
- HER-2 / neu / erbB2
- HER-3 / erbB3
- HER-4 / erbB4

EGFR (HER-1) and HER-2 widely implicated in tumourigenesis.
ErbB Receptor dimerisation & downstream pathway

Pharmacological intervention - antibodies

Anti–HER Family monoclonal antibody therapies
- EGFR
  - Cetuximab
  - Panitumumab
- HER-2
  - Trastuzumab (Herceptin)
- Rather expensive therapies (≈ £2,000 per month).
- Side effects do occur.

HER2
Which antibody?
Which platform?
Scoring system?
HerceptTest antibody (Dako A/S Glostrup) manually & VENTANA 4B5 antibody on Bench Mark Ultrasystem

Amplification: FISH (PathVysion HER-2 DNA Probe Kit); SISH with VENTANA 4B5 Inform HER2 dual-color

Weak staining on both TMAs
HER3

- HER3 receptor lacks intrinsic tyrosine kinase activity
- Heterodimerisation with other HER members is a requirement to initiate signal transduction
- Receptor overexpression the most common cause of inappropriate signalling
- The most active heterodimer is the HER3/HER2 dimer

HER3 expression in patients with primary colorectal cancer and corresponding lymph node metastases related to clinical outcome:

- 236 CRC, stage II and III
- HER3 staining in primary and lymph node metastases
- FISH for HER3 in 58 cases (primary tumours)
- 70% primary tumours & 75% LN+ overexpression HER3
- Overexpression in primary tumour correlates with expression in the LN mets
- A high HER3 expression in the primary tumour was associated with worse clinical outcome
- FISH: no gene amplification in any of the cases

Patritumab is a fully human anti-HER3 monoclonal antibody that binds to the extracellular domain of HER3, promoting receptor internalization and degradation and inhibiting ligands from binding HER3

FISH: no gene amplification in any of the cases
• No consensus for the IHC HER3 staining procedure in CRC (membrane only? Cytoplasmic and membrane?)
• Is the Hercept test, Dako interpretation adequate for assessment? Is the gastric scoring suitable for CRC?
• Proposed scoring systems: Membrane 0-3, lower limit of positive expression: 10% CRC cells [F Ledel 2014]

BRAF
• Proto-oncogen (chromosome 7q34)
• BRAF is mutation in 3-15% mCRC and mutually exclusive with RAS mutations
• Most common mutation V600E (80%)
• mut-BRAF strong negative prognostic factor in mCRC regardless of treatment
• mBRAF negative predictor of response to anti-EGFR moAB? (controversial data)

Pitfalls
1. CRC mets to lung → positive staining in bronchial glands
2. Nuclear staining in benign colonocytes

VE1 staining by two different IHC methods (Leica Bond and Ventana BenchMark) in whole tissue sections

480 CRCs
• 323 BRAF wildtype,
• 142 BRAF V600E mutation
• 15 BRAF non-V600E mutation
Positivity defines as staining in ≥20% of tumour cells (sensitivity 75% and specificity 93% for BRAF V600E mutation)

<table>
<thead>
<tr>
<th></th>
<th>BOND</th>
<th>VENTANA</th>
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<tr>
<td></td>
<td>142 mBRAF</td>
<td>57 mBRAF</td>
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<tr>
<td></td>
<td>77(54%) diffuse VE1 staining</td>
<td>36(63%) diffuse VE1 staining</td>
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<tr>
<td></td>
<td>48 (33%) heterogeneous</td>
<td>6 (11%) negative (&lt;20% tumour cells)</td>
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<tr>
<td></td>
<td>17 (12%) negative</td>
<td>16 (48%) negative or weak</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 (45%) heterogeneous staining</td>
</tr>
<tr>
<td></td>
<td>323 wtBRAF</td>
<td>33 wtBRAF</td>
</tr>
<tr>
<td></td>
<td>196 (61%) negative</td>
<td>16 (48%) negative or weak</td>
</tr>
<tr>
<td></td>
<td>127 (39%) positive VE1 staining (7 diffuse)</td>
<td>15 (45%) heterogeneous staining</td>
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VE1 IHC produces suboptimal results in CRC and should not be used to guide patient management.
Poorly differentiated adenocarcinoma with prominent inflammatory response

Loss of MLH1/PMS2 expression

Screening for Lynch syndrome

BRAF V600E-specific immunohistochemistry for the exclusion of Lynch syndrome in MSI-H colorectal cancer

91 MSI-H CRC tested for Lynch syndrome.

Concordance VE1 and molecular BRAF mutation status:
- 90/91 (99.9%) MSI-H samples.
  - All 11 tumors classified as mBRAF (Sanger sequencing) were immunopositive
  - 79/80 (98.8%) wtBRAF tumors showed negative staining.

None of the tumors with MMR gene germline mutation carriers displayed positive VE1 staining.

VE1 (IHC) in the diagnostic panel of Lynch syndrome → advantage over MLH1 promoter methylation studies (time consuming and expensive)

FGFR (2)
• Located in chromosome 10q26
• Single transmembrane receptor
• Alternative splicing isoforms
  Extracellular domain: IIIb and IIIc isoforms
  Intracellular domain: C1, C2 and C3 variants
• Genetic alterations and over-expression of FGFR-2 frequently reported in GI cancers

FGFR2: How we define amplification?

- Non-amplified
- Amplified

Gene amplification
- Gastric Ca
- Breast Ca

Misense mutation
- Endometrial Ca
- Lung Ca

SNPs
- Endometrial Ca
- Breast Ca

Activating mutation
- Endometrial Ca

Overexpression C3
- Gastric Ca

Increase IIIb or IIIc
- Oesophageal Ca
- Gastric Ca
- Colorectal Ca

A Phase II, Single Arm Study of BGJ398 in Patients With Advanced Cholangiocarcinoma

Prognostic Value of FGFR Gene Amplification in Patients with Different Types of Cancer: A Systematic Review and Meta-Analysis

PLOS One 2014
Immune checkpoint inhibitors
- Targeting molecules that serve as checks and balances in the regulation of immune responses.
- By blocking inhibitory molecules or, alternatively, activating stimulatory molecules, these treatments are designed to unleash or enhance pre-existing anti-cancer immune responses.

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency
41 patients with progressive metastatic carcinoma with or without mismatch-repair deficiency
Mismatch-repair status predicted clinical benefit of immune checkpoint blockade with pembrolizumab.

- CD8+ lymphoid infiltrate associated with objective response
- CD8+ cells prominent at the invasive front of the tumour
- Membranous PD-L1 expression only in patients with MMR-deficient tumours
PD-L1 / PD1

Which antibody?

VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody with OptiView DAB IHC Detection on the Ventana BenchMark ULTRA Advanced Staining Platform

PD-L1 IHC 22C3 pharmDx test (FDA approved for NSCLC)

Scoring system?

- Cut-off values (20%? 50%?)
- Scoring in tumour cells? Immune cells?

SUMMARY

BRAF:
VE1 only detects V600E amino acid substitution
Light staining and/or heterogenous positivity
Alternative to BRAF mutation analysis?

HER2/HER3
Lack of scoring systems in CRC samples
Need to consider tumour heterogeneity for FISH assessment
**SUMMARY**

**BRAF:**
VE1 only detects V600E amino acid substitution
Light staining and/or heterogenous positivity
Alternative to BRAF mutation analysis?

**HER2/HER3**
Lack of scoring systems in CRC samples
Need to consider tumour heterogeneity for FISH assessment

**FGFR**
Need to define groups for clinical trials: high-level amplification, low-level, no amplification...

**PD-L1 / PD1**
Different antibodies associated with different drugs might require different scoring systems, cut-off values...
Need to assess immune response
Immunohistochemistry in Bone and Soft Tissue Tumours: Overview and Illustrative Cases.

Chas. Mangham
RJAH Orthopaedic Hospital, ROH Orthopaedic Hospital and Universities of Liverpool and Manchester
Bone and Soft (Somatic) Tissue Tumours

- Tumours arising in somatic tissue and/or showing mesenchymal differentiation (the latter could arise in any organ – including viscera)
- Relatively rare tumours
  - Benign:Malignant – 100:1
- Sarcoma incidence – approximately 50 per million per year (i.e. about 3000 new cases per year in UK)
- NIH, National Cancer Institute, SEER data
  - Cancer arising in bone – 0.2% of new cancer cases
  - Cancer raising in soft (somatic) tissue – 1% of new cancer cases
- All age groups affected (no paediatric/adult split)
- Total of approx. 80 malignant subtypes
- Commonest malignant bone tumours:
  - Osteosarcoma, chondrosarcoma, Ewing’s sarcoma, chordoma
- Commonest malignant soft tissue tumours:
  - Myxofibrosarcoma, well differentiated liposarcoma/ALT, leiomyosarcoma, undifferentiated pleomorphic sarcoma (“MFH”)
England: Organisation of sarcoma services

• Soft tissue sarcomas:
  – Regional centres, usually major teaching hospitals

• Bone sarcomas:
  – National centres:
    • RVI, Newcastle
    • RJAH, Oswestry (part of Greater Manchester & Oswestry Sarcoma Service - GMOSS)
    • ROH, Birmingham
    • NOC, Oxford
    • RNOH, London
WHO classification – soft tissue tumours, 2013
### WHO classification – bone tumours, 2013

#### CHONDRIOGENIC TUMOURS
- Benign: Osteoma, Osteoid osteoma, Osteosarcoma
- Intermediate (locally aggressive): Chondroblastoma, Chondrosarcoma, Osteosarcoma
- Malignant: Chondrosarcoma

#### KNOBBLY TUMOURS
- Benign: Osteoma, Osteoid osteoma
- Intermediate (locally aggressive): Chondroblastoma, Chondrosarcoma, Osteosarcoma
- Malignant: Chondrosarcoma

#### HEMATOMATOUS NEOPLASMS
- Benign: Hemangioma, Hemangiendothelioma
- Intermediate (locally aggressive): Angiomyxofibrous tumor, Angiosarcoma

#### OSTEOCLASTIC GIANT CELL RICH TUMOURS
- Benign: Giant cell lesion of the small bones
- Intermediate (locally aggressive, rarely metastasizing): Giant cell tumour of bone
- Malignant: Malignant Giant cell tumour of bone

#### OSTEIOGENIC TUMOURS
- Benign: Osteoma, Osteoid osteoma, Osteosarcoma
- Intermediate (locally aggressive): Osteosarcoma
- Malignant: Low-grade central osteosarcoma, Conventional osteosarcoma, Chondroblastic osteosarcoma, Fibroblastic osteosarcoma, Osteoblastic osteosarcoma, Telangiectatic osteosarcoma, Soft tissue osteosarcoma, Secondary osteosarcoma, Periosteal osteosarcoma, Periosteal osteosarcoma, High-grade surface osteosarcoma

#### MYEOGENIC TUMOURS
- Benign: Lymphangioma
- Intermediate (locally aggressive): Angiomyxofibrous tumor, Angiosarcoma

#### LIPIOGENIC TUMOURS
- Benign: Lipoma, Liposarcoma
- Intermediate (locally aggressive, rarely metastasizing): Liposarcoma

#### MISCELLANEOUS TUMOURS
- Intermediate (locally aggressive): Langerhans cell histiocytosis, Monoosteoclastitis
- Malignant: Erdheim-Chester disease

#### TUMOURS OF UNDEFINED NEOPLASTIC NATURE
- Benign: Bone pseudo-tumour
- Intermediate: Osteosarcoma, Chondrosarcoma, Fibro-osseous lesion, Chondroblastoma, Angiomyxofibrous tumor, Angiosarcoma
Bone and soft tissue pathology: Immunohistochemistry

• One important difference from other labs:

• Antigen retrieval:
  – Avoid microwave or pressure cooker pre-treatment – bone and cartilage sections detach
  – Overnight (16 hours), stirred at 65 degrees C in 10mM TRIS, 1mM EDTA, 0.05% Tween20 pH 9.0
## IHC Request Forms

### Immunohistochemistry Request Form

<table>
<thead>
<tr>
<th>Patient's name:</th>
<th>Laboratory no:</th>
<th>Block no:</th>
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<tbody>
<tr>
<td>Differential diagnosis:</td>
<td></td>
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<tr>
<td>Requesting Pathologist:</td>
<td>Date:</td>
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<tr>
<td>Please tick box if required:</td>
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<tr>
<th>CD1a</th>
<th>CEA</th>
<th>Desmin</th>
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<tr>
<td>CD3</td>
<td>CK MNF116</td>
<td>SMA</td>
<td>HMB45</td>
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<td>CD4</td>
<td>EMA</td>
<td>Caldesmon</td>
<td>Melan A</td>
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<tr>
<td>CD5</td>
<td>AE-1/AE-3</td>
<td>Myo-D1</td>
<td>GFAP</td>
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<tr>
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<td>CK7</td>
<td>Myo-4</td>
<td>NF</td>
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<td>CK20</td>
<td>Myogenin</td>
<td>NSE</td>
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<td>CK21</td>
<td>Myoglobin</td>
<td>Vimentin</td>
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<td>CK5</td>
<td>Chromogranin</td>
<td>Synaptophysin</td>
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<tr>
<td>CD23</td>
<td>PSA</td>
<td>Ki-67</td>
<td>WT1 (C-19)</td>
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<tr>
<td>CD30</td>
<td>PAP</td>
<td>P63</td>
<td>WT1 (Dako)</td>
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<td>CD31</td>
<td>EHR</td>
<td>WT1</td>
<td>FLI-1</td>
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<td>CD34</td>
<td>TTF-1</td>
<td>Vimentin</td>
<td>VWF</td>
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<td>Vimentin</td>
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91 primary antibodies
Bone and Soft Tissue Tumours

- Immunohistochemistry of soft tissue tumours – review with emphasis on 10 markers.
  M. Miettinen, Histopathology 2013 ARI

- The 10 markers:
  - CD31, CD34, desmin, DOG1, EMA, ERG, keratins, SMA, S100, KIT
  - 6 for practical triage: CD34, keratin cocktail (AE1/AE3), S100, SMA, desmin, EMA (all multispecific)
  - Differential diagnosis: fibroblastic, myoid, nerve sheath and perineurial, synovial & epithelioid sarcoma.
From scratch on a super tight budget

• I would recommend stocking:
  – S100
  – CK
  – SMA
  – CD34
  – CD31
  – Ki67

• These can get the pathologist out of trouble

• Straw poll of Bone & ST tumour pathologists:
  – If I could have just one immunohistochemical marker it would be: **S100**
Bone and soft tissue pathology

• Immunohistochemistry used:
  – To confirm suspected diagnosis
  – To resolve differential diagnosis
  – To highlight/rule out presence of abnormal cell population (e.g. metastatic carcinoma)
  – To exaggerate architecture of tumour (vimentin)
  – To highlight presence of structures – e.g. neurofilament for nerves, vWF or ERG for presence of vascular invasion
  – To help grade tumour – e.g. Ki67, p53
  – To help guide therapy – e.g. c-kit in GIST, ER/PR and Her2 in metastatic breast carcinoma
IHC in bone and soft tissue tumours is a diagnostic adjunct

- Hardly any marker is monospecific

- No markers that can distinguish between benign and malignant
  - Closest are p53 and ki67 (when very high >80%)

- Most useful when integrated into the evaluation of a case by an experienced histopathologist after careful evaluation of the H&E, radiology and clinical context.

- Repeated calls for IHC diagnostic algorithms
  - Fraught with danger
No completely fixed rules:

Beware of immunohistochemistry - report of a cytokeratin-, desmin- and INI-1-negative pelvic desmoplastic small round cell tumor in a 51 year old woman

GST Soon, F Petersson


2015, but true for every year since immunohistochemistry was introduced into diagnostic Histopathology.
Soft tissue leiomyosarcoma

Desmin
Soft tissue leiomyosarcoma

Caldesmon

SMA

Ki67

Calponin
Example of multispecific but highly useful antibody – CD34

- CD34
  - Endothelial cells, perineural and periadnexal fibroblasts, dermal (and other specific-site) fibroblasts.
  - Fibroblastic tumours:
    - Dermatofibrosarcoma protuberans, solitary fibrous tumour, myxofibrosarcoma, dedifferentiated liposarcoma, superficial acral angiomyxoma, vasoformative tumours, spindle and pleomorphic cell lipoma, epithelioid sarcoma, neurofibroma.

- Similar points could be made about other multispecific, but highly useful, markers:
  - S100, desmin, EMA, keratins, SMA, CD31 etc.

MOST Abs ARE MULTISPECIFIC AND USED IN H&E CONTEXT AND AS PANELS
Examples of monospecific antibodies

- HHV8 in Kaposi’s sarcoma, myoD1 and myogenin in rhabdo-tumours, brachyury in notochordal tumours (but see later!)

- Errr, that’s it!

- Most are only monospecific until we’ve had time to fully test them.
PEComa – HMB45 and myogenic markers
PEComa – HMB45 and myogenic markers
Caldesmon

Ki67

S100

TFE3

Ki67

PEComa – HMB45 and myogenic markers
Diagnosis

• PEComa – TFE3 positive subtype

• Combination of morphology and unique combination of IHC markers (melanocytic and myogenic)

• TFE3 – originally “specific” for alveolar soft part sarcoma, but now granular cell tumour and subsets of PEComa and translocation renal cell carcinomas.
Brachyury and notochordal tumours
Brachyury

Great! – a mono-specific marker for notochordal differentiation

BUT

Expression of Brachyury in Hemangioblastoma
Potential Use in Differential Diagnosis

Valeria Barresi, MD, PhD,* Enrica Vitarelli, MSc,* Giovanni Branca, MD,* Manila Antonelli, MD,† Felice Giangaspero, MD,† and Gaetano Barresi, MD*
Angiosarcoma and ERG
ERG

• ERG – endothelial cells, but now cartilaginous tumour cells and megakaryocytes.
Solitary Fibrous Tumour and STAT6
STAT6

• Originally SFT, but now, albeit very rarely, dedifferentiated liposarcoma and deep fibrous histiocytoma

• (nb. In our hands, this can be a “dirty” antibody)
Epithelioid Sarcoma and INI1
INI1

- INI1 – epithelioid sarcoma, but now epithelioid malignant peripheral nerve sheath tumour, myoepithelial carcinoma, extraskeletal myxoid chondrosarcoma, sinonasal basaloid carcinoma, collecting duct carcinoma of the kidney and rhabdoid carcinoma of the gastrointestinal tract.
Other (initially) monospecific antibodies

- KIT – GIST, but also mastocytoma, and minority of Ewing’s sarcoma, extraskeletal myxoid chondrosarcoma and synovial sarcoma
- DOG1 – GIST and rarely smooth muscle tumours, endothelial cell tumours and synovial sarcoma
Sclerosing rhabdomyosarcoma – desmin and myoD1
Sclerosing rhabdomyosarcoma – desmin and myoD1
Low grade fibromyxoid sarcoma and muc4
Intramuscular myxoma and muc4
Stick with what you know

• Widely used, established markers have the great benefit of being backed by enormous experience and data

• Novel, “best thing since sliced bread” markers
  – Scant data/experience, unknown pitfalls
  – Use them and gain experience but proceed with caution
Case examples where IHC was crucial

• Pseudomyogenic haemangioendothelioma
• Follicular dendritic cell sarcoma
• Angiomatoid fibrous histiocytoma

• IHC as a powerful adjunctive technique, alongside more recent bedfellows – FISH and other molecular pathology techniques, RT-PCR and single nucleotide mutation DNA sequencing
Pseudomyogenic haemangioendothelioma
Soft tissue follicular dendritic cell sarcoma

- Autoimmune Encephalitis Screen:
  - GABA\(_{\text{B1}}\)
  - NMDA
  - AMPAR1
  - AMPAR2
  - LGI1
  - CASPR2

- Paraneoplastic screen – monkey cerebellum:
  - Recoverin
  - Yo (PCA-1)
  - Ma (Ma1)
  - Ta (Ma2)
  - Hu (ANNA1)
  - Ri (ANNA2)
  - GAD
  - CV2/CRMP5
  - Amphiphysin
  - AGNA
  - Tr

Positive reaction with anti-Hu antibodies.
Patient’s serum antibody binds to the granular and Purkinje neurones.
Soft tissue follicular dendritic cell sarcoma
Soft tissue follicular dendritic cell sarcoma
Soft tissue follicular dendritic cell sarcoma

Immunohistochemistry:
CD21 – positive

CD21
FDC Sarcoma

- Wide age range – mainly adult. Median age 44 years
- M = F
- Relationship to Castleman’s disease
- Can be intra- or extra- nodal. Wide variety of reported sites.
- Median size – 5 cms at presentation
- Systemic symptoms are common in inflammatory pseudotumour-like variant (occurs in liver and spleen) – fever, weight loss etc. Otherwise, systemic symptoms are uncommon.
- Spindled & ovoid cells forming whorls, fascicles, storiform arrangements, sheets or nodules. Monotonous with indistinct cell borders, moderate amount of cytoplasm, granular chromatin with small nucleoli. Typical feature is a sprinkling (salt and pepper) of small lymphocytes. Less commonly, may show increased nuclear atypia, high mitotic rates and necrosis.
- Immunohistochemistry:
  - CD21, CD23, CD35, clusterin – positive
  - S100, EMA – variably positive
  - CD1a, CD34, CD3, CD79a, CD20, CD30, CD45, cytokeratin – negative
- Reactive salt & pepper lymphocytes are B and/or T cells
- Most cases have a relatively indolent course with 15% long term mortality rate. However, tumours displaying high mitotic rates, significant nuclear atypia and necrosis may prove rapidly fatal (Chan et al, 1997 Cancer 79: 294-313).
Angiomatoid fibrous histiocytoma of bone
Angiomatoid fibrous histiocytoma of bone

Immunohistochemistry:

Ki67 - ~ 5%

Desmin - ++

EMA, CK - focally +

CD31, CD34, CD45, S100, SMA, HMB45, myoD1, myoglobin, myf4, myogenin - all negative.
Angiomatoid fibrous histiocytoma of bone
Angiomatoid fibrous histiocytoma of bone

Desmin

Ki-67
FISH – “break apart” for EWSR-1
RT-PCR using newly designed primers - frozen and paraffin tissue

229 bp

114 bp
RT-PCR generated amplicon sequence

EWS exon 7 | ATF1 exon 5
Confirmed angiomatoid fibrous histiocytoma with EWSR1ex7-ATF1ex5 translocation

- As in the previously reported non-soft tissue cases, this case has an EWSR1/ATF1 translocation rather than the much commoner EWSR1/CREB1 variant seen in soft tissues.
Summary

• IHC is very important in Bone and Soft Tissue Tumour classification
• New antibodies every few months
• Stick with tried and tested antibodies and fully evaluate new markers
• Integrate with (other) molecular pathology techniques
**UK NEQAS ICC & ISH New Website**

Neil Bilbe. FIBMS. Support Scientist.

---

**THE NEED FOR CHANGE... Old Website**

- Originally created in 1990s.
- Custom built by UCL – hosted on local server.
- Static not dynamic.
- Not aesthetically pleasing.
- Not fit for the 21st Century!

---

**THE NEED FOR CHANGE**

- During a CPA visit noted that no update field.
- No real opportunity to incorporate sponsorship.

---

**THE NEW SITE**

- Started to plan it Summer 2014.
- Were given numerous templates to view – in a sort of paper patchwork quilt.
- Narrowed this down to about 3 or 4.
- Developers went away and started to build.
- Late 2014 had a good framework to fine tune.
- Website uses WordPress – originally designed for Blogs.

---

**THE NEW SITE**

- The UK National External Quality Assessment Scheme for Immunocytochemistry and In Situ Hybridisation (ICC & ISH).
- Website uses WordPress – originally designed for Blogs.

---

**THE NEW SITE**

- The new website is available at [www.mezzaninecreative.co.uk](http://www.mezzaninecreative.co.uk).

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**THE NEW SITE**

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FEATURES

• Translation function allows all labs to read

• Embed forms and important scheme documents – again this is translatable

• Easily contact us: online form or info@uknegasiccish.org

• Keep up to date with events and meeting, ........slide dates ..... calendar

• Sponsorship opportunities – helps fund site and aid development
  • Initially offered free 3 month appearance
  • Banner on every webpage
  • Click on any icon

• Directly to Sponsor’s page(s)
FEATURES
• Interactive – Best Methods Page

TRAFFIC
• How many are visiting us?

FEATURES
• Initially there was a Print problem but now fixed

TRAFFIC... Where are you going?
• How many are visiting us?
UK NEQAS ICC & ISH
Immunocytochemistry Journal

Neil Bilbe. FIBMS.
Support Scientist.

HISTORY AND PROGRESSION

• Previously produced as a bona fide Journal
• First volumes from c. 2001
• Up until 2007 (Run 77)

HISTORY AND PROGRESSION

• Expensive to produce: £Ks per run!
• Journal: vital record for scheme and participants
• Review of material, runs, submissions, results....

HISTORY AND PROGRESSION

• Expensive to produce: £Ks per run!
• Journal: vital record for scheme and participants
• Review of material, runs, submissions, results....
• .......problems, scheme write ups, articles, news
HISTORY AND PROGRESSION

• Expensive to produce: £Ks per run!
• Journal: vital record for scheme and participants
• Review of material, runs, submissions, results....
  ......problems, scheme write ups, articles, news
• So new format and approach needed

NEW JOURNAL FORMAT

• From Run 96

NEW JOURNAL FORMAT

• Summary page

NEW JOURNAL FORMAT

• Images: Excellent, Borderline, and Poor

NEW JOURNAL FORMAT

• Distribution graphs each antigen (NEQAS & In-house)
NEW JOURNAL FORMAT
• Reagents: 1° ab, detection, retrieval, automation.....

NEW JOURNAL FORMAT
• Articles, news, reviews, and updates

NEW JOURNAL FORMAT
• Available online - Menu at top of every page:

NEW JOURNAL FORMAT
• Available online - Menu at top of every page:

NEW JOURNAL FORMAT
• Available online - Menu at top of every page:
• Go to the Journal Page:
NEW JOURNAL FORMAT

• Go to the Journal Page:
• Click on the link

• Opens up Journal pdf for download

N.B. Turnaround time for Journal

YOUR VIEWS

• Do you have any interesting articles?
• Wide ranging appeal and interest
• Send them to us for consideration
• We may add a rider or disclaimer though

THANK YOU!
UK NEQAS ICC & ISH User Survey 2015
Neil Bilbe. FIBMS.
Support Scientist.

SURVEY OUTLINE
- Web-based Survey May 2015
- CurrentlyApprox. 600 labs registered start EQA year
  - 224 UK & Eire labs registered as active (42%)
  - 372 Overseas labs registered (58%)
- 117 surveys responses (20%)
  - Duplicate, incompleted, multiple replies
  - Failure to complete

Incomplete Data is also useful
- If we filter data by ‘Completeness’ then run the risk of perhaps missing a dissatisfied reply or comment!

.....lies, damned lies, and statistics!
Mark Twain. c. 1895.

REPLIES BY REGION
- Overall responses from active labs
  - UK & Eire = 21%
  - OS = 19%

REPLIES BY MODULES & PARTICIPATION
- Average no. modules subscribed = 3.7
- Approx. 25% of labs registered for a module responded to the survey (median = 26%)
**ARE YOU SATISFIED?**

How you rated the scheme!

- Very Satisfied (39) 37.5%
- Satisfied (60) 57.6%
- Neutral (5) 4.8%

No Dissatisfied responses!!

**ARE YOU SATISFIED?**

How you rated the scheme - Regional

<table>
<thead>
<tr>
<th></th>
<th>UK (44)</th>
<th>US (89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Satisfied</td>
<td>10 (22.7%)</td>
<td>21 (23.6%)</td>
</tr>
<tr>
<td>Satisfied</td>
<td>26 (59.1%)</td>
<td>34 (38.2%)</td>
</tr>
<tr>
<td>Neutral</td>
<td>1 (2.2%)</td>
<td>4 (0.7%)</td>
</tr>
<tr>
<td>Very/Dissatisfied</td>
<td>3 (6.8%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

There were some replies with no response to Q. 28.

**ARE YOU SATISFIED?**

How you rated the scheme – comparison with 2014 survey

<table>
<thead>
<tr>
<th>Responses</th>
<th>2014 (%)</th>
<th>2015 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Satisfied</td>
<td>34.1</td>
<td>37.5</td>
</tr>
<tr>
<td>Satisfied</td>
<td>57.3</td>
<td>57.5</td>
</tr>
<tr>
<td>All other responses</td>
<td>8.5</td>
<td>4.8</td>
</tr>
</tbody>
</table>

- Overall levels of satisfaction remain relatively unchanged
- Increase in the number of Very Satisfied (↑ 3.4%) responses
- Decrease in Neutral responses (↓ 3.7%)

**SCORES (OUT/10) BY MODULE**

<table>
<thead>
<tr>
<th>Module</th>
<th>2014 Score</th>
<th>2015 Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Module A</td>
<td>7.0</td>
<td>7.2</td>
</tr>
<tr>
<td>Module B</td>
<td>6.5</td>
<td>6.7</td>
</tr>
<tr>
<td>Module C</td>
<td>5.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Module D</td>
<td>5.3</td>
<td>5.5</td>
</tr>
</tbody>
</table>

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<td>6.7</td>
</tr>
<tr>
<td>Module C</td>
<td>5.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Module D</td>
<td>5.3</td>
<td>5.5</td>
</tr>
</tbody>
</table>

4 respondents to 28 module.
**SCORES (OUT/10) BY MODULE**

Average score out of 10 by Module

Av. score was 8.3 (8.2 last year), with a low of 8.1 and a high of 8.4.

How relevant is this? Perhaps we should be asking labs to score each of their modules out of 10!

**MOANS AND GROANS**

Response to individual questions (Q: 1-16) requiring a rating of between Very Satisfied (Highest) to Very Dissatisfied (Lowest) are not mandatory.

Therefore the number of participants leaving feedback varied from question to question, and topic to topic.

**MOANS AND GROANS**

Out of a maximum of 117 replies: 9/16 of the Questions were fully completed (117/117)

**MOANS AND GROANS**

.......... to a low of 100/117 replies for others

**MOANS AND GROANS**

.......... to a low of 100/117 replies for others – these were mainly for the 3 questions relating to UK NEQAS ICC & ISH Meetings

So many just replied Neutral or did not respond.
MOANS AND GROANS

- The average number of responses per question was 113
- The ‘level of dissatisfaction’ is arrived at by adding up all the very/dissatisfied replies
- Then calculating these as a % of the total number of responses

• Total number of replies = 1815
• Total number of very dissatisfied and dissatisfied replies = 63
• Therefore 3.5% of replies were ‘dissatisfied’
• Slight increase from last year’s 3.1%
• There is a 5 year average of 3%

The main areas of concern from participants

1. Turnaround times: up to 8.5% from 6%
2. Assessor comments: 14.5% up from 10%
3. Web based format of results: 6% up slightly
4. ‘Location of Meetings’: 6% down from 7% but still the 4th highest topic for dissatisfaction.

COMMENTS

169 of them!

- Problem is:

People put comments

.....but not necessarily in the right place
Q. How do you rate the quality of the NEQAS samples sent to you?

Comment: My slides always arrive late!

Q. Overall how do you rate UK NEQAS ICC & ISH out of 10?

Comment: I have 20 antibodies which UK NEQAS ICC & ISH have never requested and I have UKAS coming next week!

COMMENTS

Breakdown by section:
- Sample and results section (17 comments)
- Participant feedback and communication section (23)
- UK NEQAS ICC & ISH meetings section (10)
- Complaints about the service (6)
  - Treatment of in-house samples and UK NEQAS ICC & ISH samples (20)
  - Assessment of in-house controls (12)
  - Quality of the UK NEQAS ICC & ISH EQA material (17)
- Use of EQA results to improve in-house staining (43)
- Reassessment requests (8)
- General comments and feedback about the service (13)

COMMENTS

- More feedback on possible best methods*
- Too much information sent out on cover letters*
- Turnaround times could be better
- More detailed comments for sub-optimal results
- Fixation differences between NEQAS – In-house*
- Staining and assessment of in-house controls*
- Variable section quality
- Reassessment of slides
- Same slide EQA procedure*
- More antibodies for certain modules*
- Plus lots of complimentary comments about the scheme


WE DO LISTEN!

Venue and date of this meeting:
- Day towards the end of the week more popular
- Here you are in London on a Friday!!
WE DO LISTEN!

Important points to remember:

• Any enhancements or suggestions must benefit a large percentage of the participants

• Requires:
  ❖ Discussion with UK NEQAS ICC & ISH management
  ❖ Approval from Steering Committee
  ❖ Costed - including potential level of module fees
  ❖ Liaise with: sample suppliers, database providers, commercial sector (equipment/educational grant)

HOW HAVE WE LISTENED!

• More antigens tested. Golds: some now alternating; e.g. SMA/Desmin
• New antibodies per modules: General, Lymphoma, Neuro*, Cytology, GIST
• Fixation, treating UK NEQAS ICC & ISH & in-house differently (Dawn)
• Cell blocks – UK led request now > 90% UK labs receive CB slides
• New website (presentation to follow)
• Feedback and reports: Improved scoring sheets for assessors and therefore results and feedback. Benchmarking graphs over 10 runs
• Journal greatly improved (presentation to follow)
• Meetings – 3rd year of Participant Meetings, workshops coming!

SURVEY SUMMARY

• Response level of < 20% was disappointing
• 25% - 30% in previous few years
• Reminder to participants not sent out this year!
• Levels of satisfaction remain high at c. 95%
• Score out of 10 very consistent at > 8/10
• E-Journal feedback continues to ↑
• Quality of sample ratings also steadily rising ..........
• ..... Need more excellent ratings though
• Overall rating better from UK & Eire labs (97.8%) than OS (93.3%)
• But for individual questions or topics; e.g. TAT and web based format UK labs less satisfied than OS........
• ..........? greater pressure or emphasis on TAT in UK and higher expectations of online and IT related issues!

THANK YOU!
Single slide assessment. “STICK WITH US”

Change to “single slide” assessments

• Pilot for ER Introduced May 2014
• Rolled out to all modules in May 2015 Run 110
• Although the majority of participants are happy with the single slide approach there have been some adhesive issues.

UK NEQAS ICC TEAM

“WHY?”

• To comply with our ISO standards 17043: Ensure same protocol is applied to both samples (stained at same time, same type of slide, same batch, same run and on the same position within the machine).
• Reduce laboratory EQA reagent costs
• Decrease assessment and report TAT’s
• To manage UK NEQAS increase in slide workload
• Easier comparison with NEQAS and IN-HOUSE samples by assessing slides in parallel, increasing consistency and reproducibility by our assessors.
• Allow assessors more opportunity to provide greater feedback

SLIDE SURVEY

‘Participants’ in-house controls not adhering onto NEQAS slides
153 Responses

Modules Participating in 2015-2016
SECTION LIFTING

UK & EIRE (63% lifting)
Have you experienced any section lifting with the new slides?

Overseas (22% lifting)
Have you experienced any section lifting with the new slides?

% LIFTING BY MODULE

<table>
<thead>
<tr>
<th>Module</th>
<th>Total</th>
<th>Lifting</th>
<th>% Lift</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>125</td>
<td>21</td>
<td>17%</td>
</tr>
<tr>
<td>Receptor Hormone</td>
<td>107</td>
<td>33</td>
<td>31%</td>
</tr>
<tr>
<td>Neuro</td>
<td>26</td>
<td>3</td>
<td>12%</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>76</td>
<td>6</td>
<td>8%</td>
</tr>
<tr>
<td>HER2 IHC</td>
<td>77</td>
<td>12</td>
<td>16%</td>
</tr>
<tr>
<td>GIST</td>
<td>39</td>
<td>6</td>
<td>16%</td>
</tr>
<tr>
<td>HNPCC</td>
<td>22</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>GASTRIC</td>
<td>17</td>
<td>3</td>
<td>18%</td>
</tr>
<tr>
<td>SH</td>
<td>51</td>
<td>5</td>
<td>10%</td>
</tr>
</tbody>
</table>

% LIFTING BY SINGLE MODULES

<table>
<thead>
<tr>
<th>Module</th>
<th>Total</th>
<th>Lifting</th>
<th>% Lift</th>
</tr>
</thead>
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<tr>
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<td>26</td>
<td>3</td>
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</tr>
<tr>
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<td>4</td>
<td>3%</td>
</tr>
<tr>
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<td>77</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>GIST</td>
<td>39</td>
<td>4</td>
<td>5%</td>
</tr>
<tr>
<td>HNPCC</td>
<td>22</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>GASTRIC</td>
<td>17</td>
<td>1</td>
<td>6%</td>
</tr>
<tr>
<td>SH</td>
<td>51</td>
<td>4</td>
<td>8%</td>
</tr>
</tbody>
</table>

How long after receipt of the NEQAS slides do you handle them?

<table>
<thead>
<tr>
<th>Result</th>
<th>Responses</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate</td>
<td>20</td>
<td>40%</td>
</tr>
<tr>
<td>Within a few days</td>
<td>100</td>
<td>20%</td>
</tr>
<tr>
<td>Ask prior to closing site</td>
<td>10</td>
<td>2%</td>
</tr>
<tr>
<td>Depends on workload</td>
<td>41</td>
<td>20%</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
<td>0%</td>
</tr>
</tbody>
</table>

After placing your control section onto the NEQAS slide, how long do you drain the slide for?

<table>
<thead>
<tr>
<th>Duration</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>26</td>
</tr>
<tr>
<td>2 min</td>
<td>70</td>
</tr>
<tr>
<td>3 min</td>
<td>36</td>
</tr>
<tr>
<td>4 min</td>
<td>16</td>
</tr>
<tr>
<td>5 min</td>
<td>12</td>
</tr>
<tr>
<td>6 min</td>
<td>5</td>
</tr>
</tbody>
</table>

Is this your usual draining time?

95%
Did you sections fall off?  
149 responses

How do you dry your slides?

What temperature do you dry your slides?

How long do you dry your slides for?

Is this your usual drying method?

Is this your usual drying method  
146 responses
What slides do you currently use for IHC?

<table>
<thead>
<tr>
<th>Result</th>
<th>Response</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo-Coolfix</td>
<td>1</td>
<td>0.6%</td>
</tr>
<tr>
<td>Thermo Superfast</td>
<td></td>
<td>35.7%</td>
</tr>
<tr>
<td>Temo (Ventana)</td>
<td>3</td>
<td>1.5%</td>
</tr>
<tr>
<td>Leica Bond Plus</td>
<td>23</td>
<td>13.9%</td>
</tr>
<tr>
<td>CellPath Histolab</td>
<td>2</td>
<td>1.2%</td>
</tr>
<tr>
<td>Dako BondFix</td>
<td>1</td>
<td>0.6%</td>
</tr>
<tr>
<td>VWR Superfast</td>
<td>9</td>
<td>5.4%</td>
</tr>
<tr>
<td>Surgipath Ultra</td>
<td>17</td>
<td>10.3%</td>
</tr>
<tr>
<td>Other</td>
<td>43</td>
<td>26.6%</td>
</tr>
</tbody>
</table>

Comments from Participants

- Sometimes we may have to extend the oven drying time to ensure excess water that has been ‘trapped’ under the in-house sections, and have noticed that this may dry out the edges of the NEQAS sections. The oven drying step is at 60 degrees, would it be of any value to the NEQAS committee to record this step when submitting slides?
- We have had no problems with the slides. We like the idea of cutting our current control onto the slides.
- Fixation of NEQAS tissue differs from in-house tissue and may therefore require different antigen retrieval - this is not possible with these slides. If we receive cases referred to our lab we may use a different antibody retrieval process dependent on the fixation.
- We have not experienced any problems with the single slide submission and much prefer it to the previous method (you know there is no difference in immune-staining between control and test tissues).
- Due to differences in processing between NEQAS tissue and in-house tissue, we generally notice that the NEQAS tissue is staining weaker than our in-house tissue. Quality of NEQAS tissue not always optimal!
- Since using the single slide system our UNIQAS results have dropped fairly significantly. Are other labs having any similar problems?

Conclusion from survey

- No single common denominator
- Survey has highlighted a number of different variables within the pre-analytical process which may have to be standardised.

NEQAS Recommendations

- Drain NEQAS slide for 30 mins vertically.
- Ensure no water is under the wax, prior to baking.
- All slides sent out unbaked from NEQAS (except cytology)
- Bake the slide (60°C for 1 hour or 37°C overnight).
- Use distilled water in your water bath if having adhesion problems.
- Stain our NEQAS slides as soon as possible.

Impact of the microscope slide in IHC

- The advent of positively-charged slide has definitely helped improve tissue retention, however tissue lifting may still occur.
- Best prevention is properly fixed and processed tissue however to augment performance the slide you use is important.
- You may want to learn more about what the manufacturers have been quietly introducing!

Not all positively charged slides are the same!
Hydrophobic vs Hydrophilic

- Traditionally adhesive slides have been "Hydrophobic" manufacturers behind the scenes have been quietly introducing hydrophilic slides.
- How do you know if the slides are hydrophilic or hydrophobic?
We decided to investigate!

Slide Wettability

Fig 1: Wettability is one of the most important features of adhesion slides. The smaller contact angles indicate greater hydrophilicity and larger contact angle s indicate greater hydrophobicity. Lower contact angles encourage reagent dispersal.

Hydrophobic vs Hydrophilic

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Hydrophilic Slides</th>
<th>Hydrophobic Slides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Collection on Slide</td>
<td>Hydrophilic glass slides</td>
<td>Hydrophobic glass slides</td>
</tr>
<tr>
<td>&quot;Wettability&quot; of Section</td>
<td>Hydrophilic surfaces are slippery to the touch. Tissue section is released from the slide quickly and smoothly</td>
<td>Hydrophobic surfaces interact with water leading to stiction &amp; sections sticking to the slide</td>
</tr>
<tr>
<td>Draining Time</td>
<td>Water collects underneath the tissue sections faster and is drained out quicker</td>
<td>Water drains off the slide surface leaving the sections intact</td>
</tr>
</tbody>
</table>

Hydrophobic vs Hydrophilic

- Slides that displayed a tendency to be hydrophobic experienced tissue wrinkling and damage upon retrieval by heat-based methods due to hydrophobic slides trapping water.

Hydrophobic vs Hydrophilic

- Slides are more prone to moving on the slide.
- Pick up the sections in a more vertical manner from the water bath may help to avoid condensing excess water with hydrophobic slides as well as helping to avoid larger pools of water to collect under sections on hydrophilic slides...so when in doubt do it straight in and straight out when picking up sections on slides.
Information on slides

- **FISH**: slides made of white crown glass give highest level of transmission and no auto fluorescence.
- **Hydrophilic slides**: 3 times more +ve charge.
- **Hydrophilic slides**: some are treated with an electrical plasma beam to eradicate oils and residue- Wipe slide with alcohol?
- **Positive charge** on the slides dissipate with time.

NEQAS R&D: In-House Experiment

- Water Collection on slide/Dip test – wettability
- Movability of section
- Draining time
- Cutting in-house on to previous run slide (mimicking real time in laboratory)

NEQAS R&D

200ul water per slide

- **Wettability**
  - 1: Being hydrophilic
  - 2: Middle of spectrum
  - 3: Hydrophobic
- **Movability**
  - 1: Moves easily
  - 2: Moves
  - 3: No movement
- **Draining time**
  - 1-5 mins
  - 2-10 mins
  - 3-15 mins
  - 4-20 mins
  - +MINS

NEQAS R&D- Cut in-house onto NEQAS Control

NEQAS R&D Conclusions

- UK NEQAS slides display both hydrophobic and hydrophilic properties.
- If you are routinely using hydrophilic slides you REQUIRE to drain the NEQAS slides for a longer period of time.
- UK NEQAS is looking into alternatives.
- There may never be a one slide fits all solution.
- We always use in-date slides and we record batch numbers against each laboratory.
- The majority of laboratories have had success with the single slide approach, on NEQAS slides.

As Lady Macbeth said to her husband ‘Screw your courage to the sticking place.’

I urge you to have the courage to stick with us, and we’ll seek to overcome this together.
NEQAS Future Plans

- **ALK FISH**: In the pipeline and a pre-pilot is being planned with some expert assessors to determine scoring and feasibility.

- **PD/PD-L1**: Pre-pilot Apr/May 2016. will be a challenge, as there may be 4 companion assays with varying cut-offs.

- **Workshops in 2016:**
  - Uncertainty in cellular pathology
  - Good, Bad and Ugly in cellular pathology
  - Let us know what you would like?

- **ISO:17043 inspection**: February 2016

WE ARE MOVING!

"It appears I'm being relocated."
Day in the life of a UK NEQAS ICC & ISH assessor
Julie Williams
Lead Assessor for General Scheme
UK NEQAS ICC & ISH

Early history of the scheme
- 1985 - National IHC scheme for intraepithelial cholesterol saturated imaging of the breast (CISH). The first scheme was designed by the author. Generating tissue and Weense scheme run by Brian Hopkins. Inoculation General Hospital in London.
- Run 1 - one antibody (HCAM) with 2 processors/ 4 observers. Each slide being assessed by 4 assessors. Slides were sent between labs for assessing. 12/20 or above was the required mark. 2 runs per year.
- Run 1/2 - 4 runs per year following additional funding from NHS.
- Run 3 - feedback from assessors (31 returns).
- Run 4 - information on number of labs using which primary antibody supplier and detection method in each of 38 returns.
- Run 5 - 1993 - first attempt to submit completed data for analysis using the Mount Vernon Computer scheme.
- To assess whether use of internally supplied material was a valid measure of assessing laboratory performance in the UK (71 labs).
- Run 6 - 1999 - official recognition of scheme, adopted as full scheme in IHC. First in histopathology (80 labs).
- Run 10 - interpretation grade introduced with peer reviews (Run 20 bars charts).
- Run 23 (1993) - best methods introduced.

Participant numbers

1985 v 2015

1985

- 1 'general' module
- 17 participants
- Total pool of assessors - 5
- Total required per run - 4

1985 modules - General, Breast Hormone Receptors, Breast HER2 IHC, Gastric HER2 IHC, Lymphoma, Neuroendocrinology, Cytology, Gastrointestinal, 7 IMF modules - HER2 ISH interpretive, HER2 Technical (pilot)

- 612 participants from 50 countries
- Total pool of assessors - 80 from 6 countries
- Total required per assessment - 75
- General, Breast Hormone Receptors, Breast HER2 IHC, HER2 ISH interpretive, HER2 Technical all require 2 teams.

2015

- 9 IMF modules - General, Breast Hormone Receptors, Breast HER2 IHC, Gastric HER2 IHC, Lymphoma, Neuroendocrinology, Cytology, Gastrointestinal.

- 1 IMF module - Her 2 ISH Technical (pilot)

- 50 participants from 20 countries
- Total pool of assessors - 100 from 6 countries
- Total required per assessment - 75
- General, Breast Hormone Receptors, Breast HER2 IHC, HER2 ISH interpretive, HER2 Technical all require 2 teams.

Lead Assessors
- General Pathology - Julie Williams, Portsmouth (Team 1) supported by Perry Maxwell, Belfast (Team 2)
- Breast Hormone Receptor – Keith Miller (Team 1) & Suzanne Parry or Merdol Ibrahim (Team 2)
- Breast HER2 IHC – Keith Miller (Team 1) & Suzanne Parry or Merdol Ibrahim (Team 2)
- Gastric HER2 IHC – Herd Ibrahim
- Lymphoma – David Blythe (Leeds)
- Neuroendocrinology – Neil Billie
- Cytology – Neil Billie & Inera Srebrenik Kirbi, Ljubljana, Slovenia
- Gastrointestinal Tract – Suzanne Parry
- Gastrointestinal Tract – Lynne Sandozian, NIH/NC - Suzanne Parry or Keith Miller
- NSCLC ALK IHC (pilot) – Merdol Ibrahim
- Her 2 ISH – Interpretive – Merdol Ibrahim
- Her 2 ISH – Technical (Pilot) – Merdol Ibrahim (ISH) & Suzanne Parry (CISH)
How I became an assessor

- Worked in the immunocytochemistry lab at Southampton under Brian Mepham 1984-1997
- Joined NEQAS as an assessor in 2000 following recommendation from Brian Mepham
- Was asked to take on Lead Assessor for General Scheme in 2004 (Run 63).
- Led both teams at the beginning (16 days per year)

How to become an assessor

- Originally recommendations from other assessors

Now UKNEQAS require certain criteria with evidence i.e. via CV
- MSC, Fellowship or equivalent
- Several years experience particularly in immunocytochemistry
- BMS Band 7 equivalent or above
- Professional registration – HCPC or RCPATH
- Potential assessors invited to view an assessment – carry out mock assessment
- Sign assessor agreement form
- Need to commit to attending at least 2 assessments per year

The assessment

- 5000 slides to be divided by module and sent out.

Assessors – four assessors will mark each slide out of 5 and will also record any constructive comments if score of 3 or below.

Lead Assessor – drives the microscope while the others score and acts as the adjudicator if there are any major discrepancies in the scoring i.e. if the score between 2 assessors differs by more than one

Two assessments run on the same day one in each of the rooms

Assessors
- Lin Rhodes – Office Manager
- Clara Lynch – Office staff

The assessment (General)

- Assessors: four assessors will mark each slide out of 5 and will also record any constructive comments if score of 3 or below.
- Lead Assessor: drives the microscope while the others score and acts as the adjudicator if there are any major discrepancies in the scoring i.e. if the score between 2 assessors differs by more than one.
- Two assessments run on the same day one in each of the rooms.

Assessors
- Lin Rhodes – Office Manager
- Clara Lynch – Office staff

General Assessment – Day 1

- Lead Assessor/Assessors: before starting assessment review the gold standard stained by lab supplying sections. The gold standard is then checked again throughout the assessment (QA).
- The gold standard antibody on the NEQAS slide is assessed first (slide A), then the in-house section (slide B). Then the Micros section of the second antibody (slide C) and finally the in-house section (slide D). Note: system changing
- Assessor: The slides are examined anonymously (unless it is your own lab slides) and scored out of 5 (total score 20). The scores are entered straight on to the computer by participant number and after 10 slides the records are saved. It is at this point that the computer will flag up any discrepancies i.e. difference in some of more than 1. If two scores are more than one mark different the slide will be reassessed. The number of discrepancies will vary as it depends on both the assessors i.e. can get hard and soft assessors, and the antibody being assessed as some are more difficult to assess than others.
UK NEQAS ICC & ISH Participants Meeting 2015
Hamilton House, London

General Assessment – Day 2

- Lead/Assessors: finish off assessing slides – the time taken depends on the antibody and control tissue used especially for the in-house controls as these can be far more difficult to assess.
- Each team will access 600 or more slides over the 2 days
- Assessors: Audit of NEQAS section quality
- Lead/Assessors: audit of scoring
- Lead/Assessors: carry out any reassessments and late returns
- Lead assessor: Select slides for photography

After the assessment is finished

- Take photos for journal write up
- Prepare write up
- Prepare statistics for journal
- Update website
- Pack and send the slides back
- Trouble shooting poor performance

Carried out by small NEQAS team Merdol, Suzanne, Neil, Dawn, Lin and Clara

Suzanne Parry – Assistant Scheme Manager

General Assessment Combined scoring guide

- 16 – 20/20: Excellent
- 13 – 15/20: Acceptable
- 10 – 12/20: Borderline acceptable
- 4 – 9/20: Unacceptable

For more detail of scoring please see individual Assessment Reports or the Participants manual (available on line)

Scoring: αSMA in appendix (Gold Standard 2015-16)

Optimal staining with αSMA in section of appendix ENQAS week 40 showing strong staining of the vessels in the mucosa and submucosa and the delicates fibres which extend into the epithelial crypts of the appendix.

Score = Grade x 5 Overall score: 20/20

 Poor staining with αSMA. Note: weak or negative staining of smooth muscle cells.

Optimal staining with αSMA in section of appendix ENQAS week 40 showing strong staining of the vessels in the mucosa and submucosa and the delicates fibres which extend into the epithelial crypts of the appendix.

Score = Grade x 5 Overall score: 20/20
Scoring: αSMA - leiomyosarcoma

- Strong staining of αSMA in the tumour
- Weak staining of αSMA in the tumour

Methodology:
- Dako 1A4 RTU, Dako Omnis with 30 min on board retrieval

Score = 5+5+5+5 Overall score: 20/20

Scoring: Ki 67 in tonsil & breast (Gold standard 2013-14)

- Strong staining of Ki67 in tonsil and high expressing breast (NEQAS section)
- Adequate staining of Ki67 in tonsil but very weak staining in high expressing breast (NEQAS section)

Methodology:
- Dako MiB1, PT link, on an Autostainer

Score = 5+5+5+5 Overall score: 20/20

When marking slides:
- Deduct marks if slides are not readable.

Poor in house section quality

- Shattering of collagen and detachment of muscle from slide
- Extra material or ‘floater’ on section

Note: No marks deducted
General Scheme poor performance table

<table>
<thead>
<tr>
<th>ID</th>
<th>Score</th>
<th>Result</th>
</tr>
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</tr>
</tbody>
</table>

(www.ukneqasiccish.org)

New Developments

- Use of new slides for NEQAS controls to standardise pre-treatment.
- Assessors to assess in-house staining directly after NEQAS slide
- Waiting for upgrade to scoring software
- What do participants think?

Not all hard work!!

Chance to network and compare notes on new developments, UKAS etc.
**Uncertainty in Cellular Pathology: What’s Yours?**

Merdol Ibrahim
UK NEQAS ICC & ISH, London
merdol.ibrahim@ucl.ac.uk

**Outline**
- UKAS and Measurement of Uncertainty
- Uncertainty Workshop Data
- UK NEQAS ICC & ISH Uncertainty Examples
- References

---

**Increasing workloads & Considerations in Cellular Pathology**

**UKAS ISO Standards**

UKAS ISO Standards

- **ISO 15189**: Medical laboratories -- Requirements for quality and competence
- **ISO 17043**: UK NEQAS...General requirements for proficiency testing

**UKAS ISO Standards**

ISO 15189

- Uncertainty: ISO 15189-2012
  - The laboratory shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients’ samples.
  - The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty.

**ISO 17025:2005**

**More Practical Approach**

- If nature of the test prevents calculation of uncertainty of measurement:
  - Lab shall at least attempt to identify all the components of uncertainty
  - Make a reasonable estimation
  - Ensure that the form of reporting of the result does not give a wrong impression of the uncertainty.

- Reasonable estimation
  - Based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data

**Important that Labs clearly define processes & have SOPs**

Very relevant and referenceable

---

www.rcpath.org/publications-media/publications

Prof. Tim Helliwell, Dr Tom Giles & Ms Sharon Fensome-Rimmer

- Document produced at the request of the Specialty Advisory Committee (SAC) for Cellular Pathology
- Provide laboratories with an approach to the assessment of the uncertainty of measurement
- Discussed with UKAS assessment team...towards a shared understanding of the most appropriate ways to meet ISO 15189 standard on ‘assessment of uncertainty’
Testing for Uncertainty Workshop

Pinpoint where you may have uncertainty

Tools you will need
- Consideration guide
- Post-it notes
  - GREEN
  - AMBER
  - RED

Preanalytic
- e.g. Fixation

Analytic
- e.g. Staining
- Controls, Reporting

Post-Analytic
- e.g. TATs / IT / Audit

Consideration Matrix Board
Breakdown of cellular pathology testing phases

Post-analytic

Turn around Times (TAT)
What is your TAT?
How do you calculate your TAT?

Audit
Regular audit of HER2 rates?
Weekly Monthly?

IT
LIMS, Barcode system / Tracking, Reporting

Post-it Notes
Make comments on coloured ‘post-it’ notes

Not sure about referred sample fixation
No idea of sample fixation time!!

No uncertainty
Some uncertainty
A lot of uncertainty

Completed Matrix Board
Place in Staff cafeteria for anonymous collection!
Build up your evidence

Heat Map of Uncertainty

Stages of Uncertainty

n=53

n=159
n=212
n=158
Order of Greatest Uncertainty

- Red: Lot of Uncertainty
- Amber: Some Uncertainty
- Green: No Uncertainty

Fixation

- Sample ischemia time unknown
- Time in fixative not known / No control over fixation
- Referring site so no idea of fixation times or protocols
- Lack of forms indicating fixation times
- Weekends - samples can be over-fixed
- pH of formalin not taken
- Surgical samples not always ‘opened’: “depends on pathologist”

- Cold ischemia time audit is carried out
- Have good fixation times including min/max times
- pH taken of fixative
- Use Datix (www.datix.com) (patient safety software for healthcare risk management,) for samples fixed for >72 hours
- Commercial fixative supplied to surgery clinics

Effect of Endothelial Camellia Fixation on Estrogen and Progesterone Receptors in Breast Cancer

- Need for better collaboration with surgical teams

Antibody Validation

- How do I validate?
- Out of date antibodies?
- Verification:
  - IVD/CE marked antibodies, kits/assays e.g. ALK FISH, Her2 kits (Herceptest, Ventana IHC kit, Leica Oracle kit)
  - Less complex procedures
- Validation:
  - Lab devised, ‘home brew’ antibody assay, out of date antibodies
  - More complex procedures

Fixation: Q.V.T

- Quality
  - CE marked
  - 15-20:1 (In tissues)
- Volume
  - 6-8 hrs min for core biopsies
  - 24-48hrs (72 hrs to cover weekends!) surgical excisions

Antibody Validation

- Verification or Validation?
- Start with commercial company recommendations / data sheets
- Lab devised methodologies: Onus on yourselves
- Best Methods database: www.ukneqasiccish.org
- Data analysis /statistics
- References

- Sensitivity & specificity
- Common pitfalls
- More vendor based validation procedures
**Antibody Validation: References**

Updated UK Recommendations for HER2 assessment in breast cancer

Marketing A et al. 2015 100 IHC and ISH cases 95% concordance

- Principles of Analytic Validation of Immunohistochemical Assays
  - Guideline from the College of American Pathologists and College
    Quality review
  - 90% Concordance: Too low for UK?
  - Biopath: 40 Cases: 20 +ve & 20 -ve (full clinical range)
  - Non predictive assays: 10 +ve & 10 -ve
  - Change in protocol: dilution, vendor, incubation, retrieval
  - Change in antibody clone: full re-validation

Document all validation and verification procedure: SOPs

**Statistics: Antibody Validation**

- Arch Pathol Lab Med 2014 138: 1432-1443

Contingency Table

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<tr>
<th>New Test</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
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<tbody>
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<td>Positive</td>
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<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>25</td>
<td>40</td>
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</table>

**Statistics**

- Concordance: 35/40 = 87.5%. Does not hit 90% benchmark
- Kappa = 0.7, substantial agreement but concordance not met

**Out of Date Antibodies**

- CPA accepted ‘out of date antibodies (except biomarkers)’ BUT Validation required
- UKAS... also appears to accept out of date antibodies BUT Confusion as to what validation is exactly required

“We are starting to feel that this standard is unachievable in Cellular Pathology”

UKAS assessors

- Lab could take on the role of the ‘manufacturer’, & give an expiry date.
- Validate across all tissue/tumour types & all possible usage situations

ISO 17025:2005

- Based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data

**Out of Date Antibodies**

- Extension of Useful Reagent Shelf Life Beyond Manufacturers’ Recommendations

**Antibody Expiration in the Context of Resource Limitation**

- What is the Evidence Basis?
  - Admission of ‘sliding scale’? Satisfactory performance of primary antibodies beyond manufacturers’ recommended expiration dates
  - App. Immunohistochem. 1998 122 (12) 4 1051-1052
  - NSE, HMB45, CLA & S100

**EQA & Uncertainty in Antibody Staining**

- Same Antibody = Two Results: Whose right?

**Histopathology 2013, 63, 569-876**

“Vintage” antibodies

All Referenceable BUT... ...is time up for out of date antibodies!

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ER UK Data: Multiple Methodologies
6F11 (Concentrate) Clone on The Leica Bond Max

<table>
<thead>
<tr>
<th>Antibody dilutions</th>
<th>Retrieval Times (mins)</th>
<th>Incubation Times (mins)</th>
<th>Protocol Variations</th>
</tr>
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<tbody>
<tr>
<td>1:25</td>
<td>15</td>
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<td>22/29 (76%)</td>
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Too Many Protocols! All Validated / re-validated?

Retrieval

Antibody dilutions
<table>
<thead>
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</tbody>
</table>

Incubation

Correlation of ER (6F11) IHC & ESR1 mRNA

Correlation of ER (6F11) IHC & ESR1 mRNA

ER IHC & mRNA Good Comparative Correlation

ER IHC & ESR1 mRNA H-Scores in Distributed EQA Samples

Transport

74% some/lot of uncertainty

- Referral centre: no idea of transport time
- No special arrangement for Fridays/weekends
- Causes bottleneck...batching of work
- Inter-hospital transport problems
- Delays TATs as no control over when sample arrives

IT

77% some/lot of uncertainty

- No/Poor Lims system / outdated / ancient
- Rubbish system
- Quite paper based!
- Not adequate or fit for purpose
- No barcoding: difficult to monitor
- No specimen tracking
- Manually create PDF reports – Delays in reports

- LIMS tracks cases and reports
- Have barcode system and electronic reporting
- Tracking in places
Audits & TATs

- Done only yearly / bi-yearly / episodic
- No time / too busy / understaffed
- What should we audit?
- No audit of TATs
- Only if there is a problem
- Not regular because of IT problems

TATs: 56% some/lot of uncertainty

- Not measured: Too busy to audit
- Presume ok if no complaint
- Not done: staff shortage
- Often delayed due to poor tissue quality
- Variable depends on pathologist
- Behind due to workload issues
- Poor TATs due to batching

Importance of Clinical Audit

Web based breast HER2 audit

Control Material

53% some/lot of uncertainty

- No on-slide controls
- Kit controls, only one per run
- Problems sourcing control material
- Variability in quality of control material
- Yes, but no on-slide
- In-house control a pain!
- Commercial controls too expensive

- Use on slide control
- Use controls to monitor batch to batch variability

Control Material

Standardization of Negative Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Panel

- Lack of tissue homogeneity makes it impossible to determine the ‘true value’ of a measurement.
- Pragmatic approach is appropriate based on published literature and clinical experience.
  - Consider which reported measured values are clinically critical rather than descriptive
  - A more descriptive evaluation of uncertainty is likely to be appropriate
- Laboratory should consider relevant aspects for themselves.
  - Create a simple matrix board of uncertainty: post-it!
  - Consider which methods are best to achieve clinically reliable measurements... Verify or Validate
  - Ensure lab equipment is calibrated regularly to a traceable standard
  - Assess equipment achieves the desired objectives... Audit!
  - Well defined SOPs

 summary

<uk nedas>
Acknowledgments

• Advanced Cell Diagnostics: RNAscope: mRNA staining
• Dako: Providing ER/PR Cell lines
• Roche products, Leica & Dako: Staining of NEQAS samples using recommended protocols

Uncertainty in methods applied to EQA material

Slide EQA

Participants cut their control material alongside the NEQAS EQA samples

1. Ensures same protocol is applied to all samples... UKAS questioned
2. Reduces Laboratory EQA reagent costs
3. Faster assessments TATs

NEQAS Is Not Infallible

1) Participants sample not adhering to slide too well. Survey lab section drying time & slides used.
2) Lymphoma Cyclin D1 assessment... Very low pass rate: Audit indicated problem with EQA tissue
In Poundbury Cancer Institute, Dorset

Where is CADQAS?

- In Poundbury Cancer Institute, Dorset

CADQAS: Digital Classroom

- Educational remit:
  - Training on a range of cancer diagnostic tests on a number of different platforms

Why Dorset?

- Enthusiastic pathologists who are interested in QA
- Affordable facilities:
  - For research and development
  - For teaching/training
- Good transport links
  - Southampton airport
  - Trains to London
- Good, affordable hotels
- Beautiful part of the UK...

www.thepathologist.com
Prince of Wales getting feedback from UK NEQAS…

CADQAS

Important points:
- Community Interest Company
- Not-for-Profit
- Linked to UK NEQAS ICC&ISH
- Educational remit
- Independent

Who is involved?

Keith Miller
Director of UK NEQAS ICC&ISH & CADQAS CIC

Sarah Wedden
Director of CADQAS CIC

Corrado D’Arrigo
Founder of Poundbury Cancer Institute

Multiplex IHC using 4 antibodies:
CK5, p63 Ki67, P504S

- Multiplex staining is performed routinely & prospectively on all prostate cores @ DCH
Why is CADQAS needed?

• Many new targeted therapies in development which require many new slide based companion diagnostics
  – Quality Assessment programme also needs to be in place at product launch
  – The tests need to be working effectively, to be performed correctly, interpreted accurately and evaluated on appropriate tissue/cell lines
• There is a need to engage with Industry early