Immunohistochemistry Quality Assurance Program in Australasia: An Update.

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IHC QAP

- Pathologist
  - Private and public
- Member RCPA General Pathology Advisory Committee
- RCPA General Pathology Examiner
- Member RCPA Genetic Pathology Advisory Committee
- Member
  - AMP
  - ESMO
  - ASCO
- MSAC Expert standing committee
- Advisory Boards
  - Roche
  - Astra Zeneca
  - Merck Serano
  - Pfizer
IHC QAP

- Immunohistochemistry QAP
  - IHC Diagnostic
  - IHC Technical
  - IHC Breast markers (Introduced in 2003)
    - Audit (Introduced 2005)
  - HER2 BRISH (Breast introduced in 2007)
    - Gastric 2011
  - IHC Lymphoma (Introduced in 2007)
  - Molecular (Introduced 2011)
    - HPV ISH (On hold)
    - EBV ISH (on Hold)
    - KRAS (Molecular)
    - BRAF (Molecular)
    - EGFR (Molecular) – IHC/ISH 2012 on hold
    - NRAS (Molecular) – Pilot 2014
  - Neuropathology (Introduced 2012)
IHC QAP

• Homogeneity and stability testing are performed on the sections.
  – Every 20th section is stained to ensure representative tissue.
  – IHC was performed on slides over a period of 4 months to ensure antigenic site stability.
IHC QAP

• Evaluation from 0 – 5
  • The assessment criteria used are:
    – Intensity of true positivity is of reasonable strength
    – Absence of background staining (good signal to noise ratio)
    – Sensitivity – all target tissues labelled
    – Localisation – only target antigenic sites labelled
    – Chromogen character – crisp and distinct
    – Counterstain quality – complementary not obscuring
    – Absence of artefacts
  • Score
    • <2.5 is considered unsatisfactory
    • ≥2.5 and <3.0, borderline
    • ≥3.0 is satisfactory.
• Control slides are no longer assessed
  – Not stained at the same time as test
  – Control material results unknown
  – Majority satisfactory (strong positive) but do not reflect test samples
IHC QAP

• IHC Diagnostic
  – Four cases with relevant clinical details, a macroscopic description of the tissue and other relevant patient information are provided.
  – Multiple unstained slides are provided for staining with immunohistochemical markers to enable the pathologist to make a diagnosis on the case.
  – One case is a laboratory practice questionnaire common to all the immunohistochemistry modules.

  – Practical issues
    • A minimum of 10 blocks are required for each case.
      – Limitations in availability of cases and material.
IHC QAP

Responses IHD14-01
129 responses were assessed.

Responses IHD13-02
128 responses were assessed.

Diagnosis: Metastatic serous papillary carcinoma (female genital tract origin)

Diagnosis: Malignant mesothelioma
IHC QAP

Responses IHD14-03
129 responses were assessed.

Diagnosis: Metastatic epithelioid GIST

Responses IHD14-04
129 responses were assessed.

Diagnosis: Strumal carcinoid/Struma ovarii
IHC QAP

Responses IHD14-04
129 responses were assessed.

Diagnosis: Strumal carcinoid/Struma ovarii.
IHC QAP

- IHC Technical
  - Two cases per year in the area of immunohistochemistry staining to assess technical proficiency.
  - One case is a laboratory practice questionnaire common to all the immunohistochemistry modules.
IHC QAP IT14-1 SMA

SMA
There were 148 participants enrolled for this survey and 126 slides were received in time for assessment of SMA staining. 10 participants indicated that this exercise was not relevant to their laboratory.

The average assessment was 3.2.
85% were satisfactory with 9% borderline and the remaining 6% were unsatisfactory.

IT14-1 : alpha smooth muscle actin

Average Mark
IHC QAP IT14-1 SMA

Satisfactory

Borderline

Unsatisfactory

Unsatisfactory

Fig. 7

Fig. 8

Fig. 9

Fig. 10
IHC QAP IT14-1 TTF1

There were 148 participants enrolled for this survey and 118 slides were received in time for assessment of TTF1 staining. 13 participants did not submit any slides and 17 participants indicated that this exercise was not relevant to their laboratory.

The average assessment was 3.3. 80% were satisfactory with 11% borderline and the remaining 9% were unsatisfactory.
IHC QAP IT14-1 TTF1

Fig. 17: Satisfactory

Fig. 18: Satisfactory

Fig. 19: Unsatisfactory

Fig. 20: Unsatisfactory
IHC QAP IT14-2

The average assessment score was 3.3. 84% were satisfactory, 7% were marked borderline and the remaining 9% were unsatisfactory.

The average assessment was 3.4, 91% were satisfactory, 6% borderline and the remaining 3% were unsatisfactory.
IHC QAP IT11-1

**AE1/AE3**
There were 148 participants enrolled for this survey and 134 slides were received in time for assessment of AE1/AE3 staining. 10 participants indicated that this exercise was not relevant to their laboratory.

The average assessment was 2.5. 35% were satisfactory with 6% borderline and the remaining 59% were unsatisfactory.
IHC QAP IT11-1

AE1/AE3
Unsatisfactory
- Lack of staining of the basal layer.
IHC QAP

- IHC Lymphoma Markers
  – Staining to assess technical proficiency.
IHC QAP IL14-01

Results
There were 81 participants enrolled for this survey and 75 slides (93% participation) were received in time for assessment. 3 participants indicated this exercise was not relevant to their laboratory. The average assessment was 3.4. 84% of the participants were scored as satisfactory, 3% of the participants were marked borderline and the remaining participants 13% were scored unsatisfactory.

There was a marked overall improvement in the repeat CD15 exercise, 3.4/5.0 compared with 2.6 (IHL13-01). The clones MMCarb-2 and MMA LeuM1 performed well.
IHC QAP IL14-01 CD15

Fig.1: MMCarb-3 clone. Top score of 4.6. (X10)

Fig.2: LeuM1 clone. Low score of 1.4/5.0. No staining of T-cells (X20)

Fig.3: Over-retrieved. (X10)

Fig.4: Contaminant. (X20)
IHC QAP IL14-01

CD20

Results

There were 81 participants enrolled for this survey and 75 slides (93% participation) were received in time for assessment.

The average assessment was 3.6. 93% of the participants were scored satisfactory. 1% of participants had a borderline score and the remainder were marked unsatisfactory.
IHC QAP IL14-01 CD20

Fig. 5: Clone MJ1. Score = 4.2/5.0. (X20)

Fig. 6: Clone L26. Score = 3.2/5.0. (X10)

Fig. 7: Clone L26. Weak positivity. Score = 2.4/5.0. (X10)

Fig. 8: Clone L26. Follicular Lymphoma unstained. Score = 2.0/5.0. (X10)
IHC QAP IL14-02

CD30

Results

There were 82 participants enrolled in this survey and 81 slides (99% participation) were received in time for assessment. The average assessment score was 3.0. 75% of the participants were scored as satisfactory, 10% were marked borderline and the remaining 15% were assessed as unsatisfactory.

Participants in the previous survey (IHL14-1) obtained an average score of 2.8. 63% of the participants were scored as satisfactory, 14% of the participants were marked borderline and the remaining 23% participants were assessed as unsatisfactory.
IHC QAP IL14-01 CD30

Fig.9: Clone JCM182. Score = 4.0/5.0. (X10)

Fig.12: Clone BerH2. Score = 1.8/5.0. Background stain. False negativity. (X10)
IHC QAP IL14-02

CD43

Results

There were 82 participants enrolled for this survey and 65 slides (79% participation) were received in time for assessment.

The average assessment was 3.6. 80% of the participants were scored satisfactory, 17% had a borderline score and the remainder 3% were marked unsatisfactory.
IHC Neuropathology QAP

**Tau-AT8**

There were 13 participants enrolled for this survey and 8 slides were received in time for assessment of Tau-AT8 staining. Three participants indicated that this exercise was not relevant to their laboratory.

The average assessment was 2.3. Two laboratories were assessed as satisfactory, two were borderline and the remaining four laboratories were assessed as unsatisfactory.
IHC QAP IN14-01 (Neuropathology)

IDH1 – R132H

The survey was distributed to 15 participants and 12 slides were submitted to the RCPAQAP in time for assessment.

Of the submissions, 92% were assessed as satisfactory and 8% (one) was unsatisfactory. The average mark was 3.3 out of 5.0.
IHC QAP IN14-01 (Neuropathology)

Satisfactory (X20))

Unsatisfactory (X20).
**IHC QAP IHB**

- IHC QAP breast module
  - Introduced in 2003
    - Two technical exercises per year
    - Assessment of oestrogen receptor, progesterone receptor and HER2 IHC and HER2 BRISH
    - Audit introduced 2005
IHC QAP IHB14-02 ER

– 110 participants submitted a slide for assessment in IHB14-1 and 106 for IHB14-2.
– The average mark for the first 2003 survey was 2.5.
– The average mark for the IHB14-1 survey was 3.5 and 3.7 for IHB14-2.
IHC QAP IHB14-02 ER

ER
Results

The average assessment score for this exercise was 3.7. 93% of the participants had satisfactory results, 4% were borderline and the remaining 3% were unsatisfactory for assessment.

In comparison, in the previous survey (IHB14-1), the average assessment score was 3.5. 91% of the participants had satisfactory results, 7% were borderline and the remaining 2% were unsatisfactory for assessment.
IHC QAP IHB ER

ER high score 6F11

ER overretrieved

ER overstained
IHC QAP IHB14-02 ER

Antigen retrieval methods, average mark: % participants

- Roche/Ventana Benchmark Ultra (3.6)
- Roche/Vent. Benchmark XT (4.0)
- Leica/Vision Biosystems (3.7)
- PT Link (3.6)
- LEICA BOND III (3.2)
- Pressure cooker (2.8)
- DAKO OMNIS (4.2)
- Microwave (4.2)
IHC QAP IHB14-02 PR

– 106 participants submitted a slide for assessment in 2014.
– The average mark for the first 2003 survey was 3.4.
– The average mark for the 2014 IHB14-01 survey was 3.2 and the IHB14-02 survey was 3.5.
IHC QAP IHB14-02 PR

Results
The average assessment score for this exercise was 3.5. 88% of the participants had satisfactory scores, 2% were borderline and the remaining 10% were unsatisfactory in this exercise.

There was a decrease in performance compared to the previous survey (IHB14-1) where the average score was 3.2, 93% of the participants had satisfactory scores, 5% were borderline and the remaining 2% were unsatisfactory.
IHC QAP IHB PR

PR PGR636

PR SAN27

PR over retrieved
IHC QAP IHB PR

PR non specific staining

PR
IHC QAP IHB PR

IHBR Trend of Satisfactory Results - PR 2004-2014
**Staining methods, average mark: % Participants**

<table>
<thead>
<tr>
<th>Staining Method</th>
<th>Average Mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche/Ventana BenchMarkUltra</td>
<td>3.6</td>
</tr>
<tr>
<td>Roche/Ventana BenchMarkXT</td>
<td>3.4</td>
</tr>
<tr>
<td>Leica BondMax</td>
<td>3.7</td>
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<tr>
<td>Leica Bond III</td>
<td>3.7</td>
</tr>
<tr>
<td>Dako AutostainerLink</td>
<td>3.3</td>
</tr>
<tr>
<td>Manual/in house</td>
<td>3.1</td>
</tr>
<tr>
<td>Dako Autostainer</td>
<td>3.5</td>
</tr>
<tr>
<td>Roche/Ventana BenchMarkGX</td>
<td>3.0</td>
</tr>
<tr>
<td>DAKO OMNIS</td>
<td>3.6</td>
</tr>
<tr>
<td>Roche/Ventana ES</td>
<td>3.6</td>
</tr>
<tr>
<td>Sequenza</td>
<td>3.4</td>
</tr>
</tbody>
</table>

**Participation by Staining Method (%)**

- Roche/Ventana BenchMarkUltra: 37%
- Roche/Ventana BenchMarkXT: 23%
- Leica BondMax: 13%
- Leica Bond III: 9%
- Dako AutostainerLink: 7%
- Manual/in house: 5%
- Dako Autostainer: 3%
- Roche/Ventana BenchMarkGX: 1%
- DAKO OMNIS: 1%
- Roche/Ventana ES: 1%
- Sequenza: 1%
IHC QAP IHB14 HER2

– 43 participants submitted a slide for assessment in the first survey in 2003.
– 88 participants returned slides for evaluation for HER2 for the IHBR14-02 module.
– The average mark in 2003 was 4.
– The average mark for IHB14-1 HER2 and IHB14-2 HER2 was 3.4.
IHC QAP IHB14-02 HER2

HER2 Results
The average assessment score for this exercise was 3.4. 74% of the participants had satisfactory scores, 9% were borderline and the remaining 17% were unsatisfactory.

A significant decrease in performance from the previous survey (IHB14-1) was seen where the average score achieved was 3.4 with 94% of the participants achieving satisfactory scores, 2% were borderline and the remaining 4% were unsatisfactory.
**HER2 Immunohistochemistry**

Fig. 31: HER2 IHC – Satisfactory stain; clone 4B5; 3+ positive case. X20

Fig. 32: HER2 IHC – Unsatisfactory stain; False negative, clone 4B5; 3+ positive case. X20
IHC QAP IHB14-02 HER2 BRISH

HER2 BRISH

Results

35 laboratories submitted slides for assessment. The average assessment score in this exercise was 3.3, similar to the previous survey, where the average score was 3.4. 82% of the participants had satisfactory scores, 5% were borderline and the remaining 13% were unsatisfactory.

In comparison, the previous assessment showed 77% of the participants with satisfactory scores, 10% with borderline and the remaining 13% with unsatisfactory scores.
IHC QAP IHB14 HER2 BRISH

IHB Trend of Satisfactory results - HER2 BRISH 2011-2014

Survey Number

% participants

0% 10% 20% 30% 40% 50% 60% 70% 80% 90%

IH11-2 IH11-3 IH12-1 IH12-3 IH13-1 IH13-2 IH14-1 IH14-2
IHC QAP IHB14 HER2 BRISH

Fig. 37

HER2 BRISH – Unsatisfactory stain; Silver deposit; Single probe; Low Amplification. X40
IHC QAP IHB12 HER2 BRISH

HER2 BRISH Assessment of participants’ overall concordance.
RCPA QAP-06 Audit

# RCPA QAP-14 Audit

<table>
<thead>
<tr>
<th>Test result</th>
<th>Total (7739)</th>
<th>Proportion</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>6263</td>
<td>81%</td>
<td>75-80%</td>
</tr>
<tr>
<td>PR+</td>
<td>5462</td>
<td>71%</td>
<td>55-65%</td>
</tr>
<tr>
<td>ER- PR+</td>
<td>123</td>
<td>2%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>ER- PR -</td>
<td>1356</td>
<td>18%</td>
<td>15-25%</td>
</tr>
<tr>
<td>HER2 IHC+</td>
<td>957</td>
<td>15%</td>
<td>11-20%</td>
</tr>
<tr>
<td>ER+ PR+</td>
<td>5336</td>
<td>69%</td>
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<tr>
<td>ER+ PR-</td>
<td>924</td>
<td>12%</td>
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</table>
IHBR14-Audit

<table>
<thead>
<tr>
<th>Test result</th>
<th>HER2 IHC positive</th>
<th>HER2 IHC equivocal</th>
<th>HER2 IHC negative</th>
<th>Total ERPR and HER2 IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ PR+</td>
<td>370</td>
<td>847</td>
<td>3207</td>
<td>4424</td>
</tr>
<tr>
<td>ER+ PR-</td>
<td>170</td>
<td>162</td>
<td>428</td>
<td>760</td>
</tr>
<tr>
<td>ER- PR+</td>
<td>27</td>
<td>18</td>
<td>48</td>
<td>93</td>
</tr>
<tr>
<td>ER- PR-</td>
<td>388</td>
<td>148</td>
<td>609</td>
<td>1145</td>
</tr>
<tr>
<td>Total</td>
<td>955</td>
<td>1175</td>
<td>4292</td>
<td>6422</td>
</tr>
</tbody>
</table>
RCPA QAP-14 Audit

Proportion of ER positive results by number of tests per laboratory (n=7739)

There were submissions of sufficient sample size (more than 50 ER results) from 81 laboratories. Of these, 25 (31%) were significantly outside the expected range of ER positive results (i.e. <70% or >86%) and received the comment "Review required". One submission received the comment "Review recommended". A further 37 (46%) participants were marginally outside the same range of ER positive results.
RCPA QAP-14 Audit

Proportion of PR positive results by number of tests per laboratory (n=7736)
There were submissions of sufficient sample size (more than 50 PR results) from 81 laboratories. Of these, 5 laboratories fell outside the expected range of PR positive results (i.e. 55 – 65%).
There were submissions of sufficient sample size for evaluation (more than 50 ER results) from 81 laboratories. Of these, 2 laboratories were significantly outside the expected range of ER-PR+ results and received the comment “Review required.”
Proportion of HER2 IHC+ results by number of tests per laboratory n=6437
There were submissions of sufficient sample size for evaluation (more than 50 HER2 IHC results) from 81 laboratories. Of these, 29 laboratories (36%) were significantly outside the expected range of HER2 positive results and received the comment “Review required”. A further 5 laboratories (6%) were marginally outside the range of expected HER2 positive results and received the comment “Review recommended.”
RCPA QAP-14 Audit

Distribution of ER +ve results

Distribution of ER positive results (more than 50 responses) (Expected range = 75-80%)
RCPA QAP-14 Audit

Distribution of PR positive results (more than 50 responses) (Expected range 55-65%)
RCPA QAP-14 Audit

Distribution of ER-ve PR+ve results

Distribution of ER-PR+ results (more than 50 responses) (Expected range <5%)
RCPA QAP-14 Audit

Distribution of HER2 positive results (more than 50 responses) (Expected range 11-20%)
RCPA QAP-14 Audit

Correlation of HER2 IHC with HER2 CISH results

Correlation of HER2 IHC with HER2 CISH results (n=242).
RCPA QAP-14 Audit

Correlation of HER IHC with HER2 SISH results

Correlation HER2 IHC with HER2 SISH results (n=3742).
RCPA QAP-14 Audit

IH14-2 Breast Marker Audit >50 tests

Count of Participants

Concordant  Minor discordance  Assessment  Discordant

IH14-2 Breast Marker Audit survey performance
RCPA QAP-14 Audit

Breast Marker Audit results (2013 - 2014)

Assessment of concordance 2013 to 2014
RCPA QAP-14 Audit

Distribution of ER positive results 2009 - 2014

Distribution of ER+ results 2009-2014.
Conclusion

• Generally the IHC modules are performed reasonable well
  – However wide variation exists in results between laboratories and this is reflected in clinical practice.

• Breast module
  – All exercises show variation in the results for oestrogen receptor, progesterone receptor and HER2.
  – Oestrogen receptor is relatively poorly performed.
Conclusion

• Breast module
  – The IHBR-audit exercise was introduced due to concerns from participants about the supplied tissue
  – Overall results are good, but there is significant variation in individual laboratories’ results which has the potential to impact on patient treatment.
  – It is not possible to identify any definitive factor in those with unsatisfactory results.
  – Optimisation of retrieval is considered to be critical in achieving satisfactory results.
  – Validation of testing methods is essential.
New Modules

• Gastric HER2 BRISH
  – Two exercises per year
  – TMA construct
    • 30 cores per slide
  – Limited number of reporting laboratories
    • Interpretation is different to breast HER2 (cut offs different)
    • Tumour heterogeneity
    • Some Abs cross react with HER3
BRISH QAP HG12

HER2 HG12-1 Case performance.
BRISH QAP HG12

HER2 HG12-1 Assessment of participants’ overall HER2 concordance
New Modules

• Molecular AP
  – EBV and HPV ISH
    • Two exercises per year
    • TMA construct
      – ~20 cores per slide
There were 19 participants enrolled for this survey and 18 EBV ISH submissions
There were 5 participants enrolled for this survey and 3 HBV ISH submissions.
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