UK NEQAS
ICC & FISH

Participants Manual
2007 – 2008

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1.0 INTRODUCTION TO IMMUNOCYTOCHEMISTRY & FISH

The External Quality Assessment Scheme for Immunocytochemistry was founded in 1985 by Mr Gerry Reynolds at Mount Vernon Hospital, Middlesex. In 1988 the scheme was recognised by the UK Department of Health and from that time it became known as the UK National External Quality Assessment Scheme for Immunocytochemistry (UK NEQAS-ICC). Currently UK NEQAS-ICC offers assessments in 7 different immunocytochemistry modules at approximately 3-monthly intervals throughout the fiscal year. These are as follows;

- General pathology
- Breast pathology (Hormonal Receptors)
- Breast pathology (HER-2)
- Lymphoid pathology
- Neuropathology
- Cytopathology
- Alimentary Tract Pathology (pilot module), includes CD117 and HNPCC (MLH1 & MSH2)

Details of each module can be found in the pages that follow. Participants are encouraged to participate in those modules that are compatible with the range of immunocytochemistry performed in their laboratory.

Fluorescent in-situ hybridisation is now becoming a major tool in cellular pathology and Dr John Bartlett leads the HER2 FISH EQA module for HER-2. Details can be found on pages 10-15.

2.0 IMMUNOCYTOCHEMISTRY

2.1 Assessment procedure

At each assessment, laboratories are sent formalin-fixed paraffin processed tissue sections (alcohol fixed cytospins for the cytology scheme) along with instruction sheets for the modules to which they have subscribed. They are requested to typically demonstrate 2 different antigens (one for the breast scheme) on the slides provided and return the best one for assessment along with their usual 'in house' control slide stained with the same marker. For most modules, one of the antigens requested is repeated from one assessment to the next for a period of 12 months and serves as a 'gold standard'. This allows participants to implement recommended changes if their quality of immuno-staining is found to be sub-standard and to test out their improved technique at the next assessment. They are also requested to complete a short questionnaire giving brief details of the antibody and method they have employed. The slides bearing each participants unique code number (to ensure anonymity) are then marked by an expert panel consisting of senior biomedical or clinical scientists and consultant histopathologists.

2.2 Interpretation of the scores achieved at assessment

Each of the 4 assessors awards marks out of 5 using the guidelines issued for each antigen. The marks are then totalled to give a score out of 20. An acceptable level of staining attracts marks greater than 12/20. A borderline mark in the 10-12/20 range indicates that whilst some information can be obtained from the slide, the staining is sub-optimal. Finally, a score of lessthan 10/20 is given for poor immunocytochemistry that has failed to clearly demonstrate the required components.

2.3 Non-UK laboratories

The scheme currently attracts members from over 30 different countries and welcomes participation from both UK and non-UK based laboratories. In some countries the number of participants is quite substantial and as a consequence there are now a few over-seas assessors assisting with assessment.

2.4 Educational remit of the scheme

A main aim of the scheme is to provide useful information on methods and reagents that allow for improved quality of immunocytochemistry. To this end, the main technical steps employed by participants at assessment are collated onto a database and subsequently tabulated to show the proportion of participants using a particular reagent that achieved acceptable staining at assessment. The results of these analyses appear in a review of each run in the scheme's Journal, of which participants each receive three copies. These reviews also contain colour prints showing optimal demonstration of the antigens assessed, examples of high scoring methods and frequency charts illustrating the distribution of participants scores for each run.

2.5 Monitoring of poor performance (applies to UK clinical laboratories only)

All UK NEQAS' are required by their accrediting body (CPA-EQA), to have in place a formal system where by the performance of all of its UK based clinical laboratories are monitored. The scheme is required to notify the National Quality Assurance Advisory Panel (NQAAP) of any cases of persistent substandard performance in participating UK clinical laboratories. They currently consist of the following procedure;

2.5.1 Warning letters (UK only)

A score of less than 10/20 for any of the antigen codes in a module on 3 successive occasions will result in a letter being sent to the laboratory concerned warning them of these 3 low scores and requesting them to contact the schemes organiser to discuss the situation. The organiser will then provide advice and assistance on how the laboratory concerned might improve their results.

2.5.2 Poor performance (UK only)

Poor performance is defined as score of less than 10/20 for any of the antigen codes in a module on 4 successive occasions. This will result in a poor performance letter being sent to the laboratory concerned informing them of the 4 low scores and requesting them to contact the schemes organiser to discuss the situation. The organiser will then provide advice and assistance on how the laboratory might improve. Failure to improve at subsequent assessment runs will necessitate in the scheme organiser taking further action. This may result in the organiser notifying the chairman of the NQAAP.

NB: Please note that whilst these criteria currently apply to UK laboratories participating in all modules, the performance monitoring guidelines for the breast hormonal receptor and HER-2 modules are outlined below in section *2.5.3* below.

2.5.3 Breast hormonal receptor and HER-2 modules

Because of the direct impact that the results of assays for hormonal receptors and HER-2 have on patient management, more stringent performance monitoring mechanisms have recently been proposed and are as follows.

2.5.3.1 Breast hormonal receptors

In order to identify and remedy sub-optimal performance for IHC receptor assays by UK laboratories within an acceptable time frame, the following procedure will be adopted. UK Laboratories achieving scores of <10 on 'in house' sections will be issued a warning letter and offered technical advise for improvement to include attendance at the UK NEQAS organisers laboratory by the poor performing laboratory's biomedical scientist. A score of <10 on 'in house' sections on a second occasion within the same fiscal year will result in the laboratory concerned being reported to the chairman of NQAAP. In addition, the UK NEQAS-ICC will approach all UK laboratories achieving a score <13 on UK NEQAS or 'in house' sections and provide advice for improvement. Any of these participants subsequently achieving a score <13 at the next two subsequent assessment runs on UK NEQAS or 'in house' sections will be issued a warning letter. With this UK NEQAS will provide further technical advice and support to include attendance at the UK NEQAS organisers laboratory by the poor performing laboratory's biomedical scientist. All attempts will be made to assist the laboratory to

improve. Failure to do so however (i.e. laboratory accruing a total of 4 successive scores <13 on the UK NEQAS or 'in house' sections) will result in the laboratory concerned being reported to NQAAP.

The overall approach will aim to ensure that very poor performing laboratories (i.e. those scoring <10 on 'in house' material) are identified immediately and given urgent warning and help to improve their performance within a 3-month period. Laboratories producing borderline performance (scores of <13 on UK NEQAS or 'in house' material) will be given no more than 12 months to show a consistent improvement in their performance to an acceptable standard. Failure to improve on either account within the designated period will result in the laboratory being reported to NQAAP. This may ultimately affect the CPA status of the laboratory concerned, with respect to offering hormonal receptor assays as predictive tests. However the laboratory will be permitted to continue participating in EQA for hormonal receptors (if it so wishes) and the chairman of NQAAP notified if it is able to show significant improvement by subsequently accruing acceptable results at all of four successive assessment runs.

2.5.3.2 HER-2 Immunocytochemistry

UK NEQAS-ICC on a quarterly basis circulates to over 200 laboratories unstained sections from a formalin fixed and paraffin processed block comprising the human breast carcinoma cell lines:

- SK-BR-3 (3+)
- MDA-MB-453 (2+)
- MDA-MB-175 (1+)
- MDA-MB-231 (0)

Participating laboratories are requested to test the UK NEQAS sections and their own 'in house' control for HER-2 and to return them to the organising centre for evaluation by a panel of 4 expert assessors using the method of evaluation initially devised for the Clinical Trials Assay (1-3), with the median value from the 4 assessors used to calculate either an 'acceptable' or 'not acceptable' level of staining. An "acceptable" level of performance would be where all 4 cell have been assessed to show the expected staining levels and intensity. An acceptable: but some disagreement between assessors' result is given when 2 of the 4 assessors have scored the cell lines as having the appropriate stain. Finally where 3-4 assessors have assessed the cell lines as 'unacceptable', the participating laboratory would be scored as showing an 'inappropriate' level of staining.

In order to identify and rectify sub-optimal performance for HER-2 assays by UK laboratories within an acceptable time frame, UK NEQAS-ICC will approach all UK laboratories achieving an inappropriate result on the UK NEQAS sections and provide advice for improvement. Any of these participants subsequently achieving an inappropriate result at two subsequent assessment runs on the UK NEQAS sections will be issued a warning letter. With the issue of this warning letter, UK NEQAS will provide further technical advise and support to include attendance at the UK NEQAS organisers laboratory by the poor performing laboratories biomedical scientist. All attempts will be made to assist the laboratory improve. Failure to do so however, with the laboratory accruing a total of 4 successive inappropriate scores on the UK NEQAS sections despite intensive advise and assistance will result in the laboratory concerned being reported to the chairman of the NQAAP. This may ultimately affect the CPA status of the laboratory concerned, with respect to offering HER-2 as a predictive test. However the laboratory will be permitted to continue participating in EQA for HER-2 (if it so wishes) and the chairman of NQAAP notified if it is able to show significant improvement by subsequently accruing acceptable results at all of four successive assessment runs.

This approach will ensure that poor performing laboratories are identified promptly and the situation rectified by appropriate action within a 12-month period.

References

1. Rhodes A, Jasani B, Anderson E et al. Evaluation of HER-2/neu immunohistochemical assay sensitivity and scoring on formalin fixed and paraffin processed cell lines and breast carcinomas: A comparative study involving results from laboratories in 21 countries. *Am J Clin Pathol* 2002; 118: 408-417.

2. Rhodes A, Jasani B, Couturier J, et al. A formalin fixed and paraffin processed cell line standard for quality control of immunohistochemical assay of HER-2/neu expression in breast cancer. *Am J Clin Pathol* 2002; 117: 81-89.
3. I O Ellis, J Bartlett, M Dowsett, S Humphreys, B Jasani, K Miller, S E Pinder, A Rhodes, and R Walker. Best practice No 176: Updated recommendations for HER-2 testing in the UK. *J Clin. Pathol.*, Mar 2004; 57: 233 - 237.
4. Mass R. The role of HER-2 expression in predicting response to therapy in breast cancer. Seminars in Oncology 2000; 27: 46-52.

2.6 End of year performance record
After the end of each fiscal year the scheme provides all participants with a summary of the results they achieved over the preceding year.

2.7 Participants help line and key personnel
Participants experiencing technical difficulties or requiring information about a particular antibody or reagent are encouraged to contact either the schemes organiser or manager for assistance. Replacement slides, following breakage, may be obtained by contacting the UK NEQAS-ICC office. Ideally all laboratories experiencing difficulties should contact the scheme for advise well before poor performance monitoring mechanisms come into effect. The schemes organiser and manager always welcome such calls. The schemes contact details are as follows;

Scheme Organiser: **Mr. Keith Miller, Tel: 020 7679 6048, email: k.miller@ucl.ac.uk**
Scheme Manager: **Dr. Merdol Ibrahim, Tel: 020 7679 8678, email: merdol.Ibrahim@ucl.ac.uk**
Office Manager: **Mrs. Ai Lin Rhodes, Tel 020 7554 8679,email: rmkdalr@ucl.ac.uk**
Office Staff: **Mrs. Maricarmen Perez, 020 7554 8677**

They may also be contacted by writing to the address on the front of this booklet.

2.8 Appeals procedure
Participants who are not satisfied with the scores received at a particular assessment run are invited to re-submit the slides for re-assessment. This re-assessment takes place at the first assessors meeting after receipt of the request for re-assessment. If the re-assessment scores are different than the original ones, the score sheets and database are amended accordingly and the participant sent amended scores and a letter of explanation.

2.9 Complaints procedure
Complaints about the service offered by UK NEQAS-ICC should be addressed to the schemes organiser, Mr. Keith Miller, Dept of Histopathology, UCL Medical School, Rockefeller Building, University St, London WC1E 6JJ

2.10 Scientific meetings and seminars
UK NEQAS-ICC holds scientific meetings and seminars when appropriate. In addition to the scientific programme these allow participants to discuss immunocytochemistry and EQA related topics with other participants, the schemes assessors and UK NEQAS-ICC personal.

2.11 Practical workshops
Practical workshops in conjunction with the University of Westminster, London, UK and their Biomedical Sciences Short Courses Unit are offered every year. As reagents and equipment for this exercise are costly, the University levies a charge for this service. Further information can be obtained by contacting Dr. Tony Madgwick at the University of Westminster on **+44 (0)20 7911 5000 x3864, email: madgwia@wmin.ac.uk**

2.12 Summary of benefits of UK NEQAS-ICC & FISH membership
In summary the main benefits for laboratories participating in UK NEQAS-ICC are;

- Four assessment runs per year.

- Specific modules catering for the specialised needs of the participant (please see pages to follow for details of specific modules).
- Assessment of two antigens per run (one for the breast scheme) on UK NEQAS and 'in house' slides.
- Score sheets following each assessment and when the staining is sub-optimal, constructive comments from the assessors.
- An end of year performance record.
- Participants 'Help-line'.
- Scientific meetings and seminars when appropriate.
- Three copies of the schemes newsletter, containing reviews of the assessment runs which include;
    - frequency charts illustrating the distribution of participant scores for each run
    - colour prints showing optimal demonstration of the antigens assessed
    - examples of high scoring methods
    - tables of the main antibodies and immunocytochemical reagents used by participants

2.13 Subscription fees

UK NEQAS for Immunocytochemistry receives no financial support other than that generated from participants subscription fees. These are set to cover the costs of running the scheme on a strict non-profit-making basis. The annual subscription fees are listed in the accompanying enrolment forms.

2.14 Registering to participate in UK NEQAS-ICC

Laboratories wishing to participate in UK NEQAS-ICC are recommended to read the detailed the descriptions of each of the modules and elect to participate in those modules that cover the range of markers used routinely in their laboratory. Further information and guidance, if required, can be obtained by contacting the scheme manager. The enrolment form accompanying this booklet should then be completed and returned either by mail to; UK NEQAS-ICC, Room 3/22, Hamilton House, Mabledon Place, London WC1H 9BB, United Kingdom, or by fax: 020 7554 8501.

3.0 IMMUNOCYTOCHEMISTRY MODULES

Currently laboratories are able to participate in up to 6 different immunocytochemistry modules (plus one pilot module), depending on their service commitments and specialised areas of interest. These modules are as follows;

MODULE 1: General Pathology

This module offers FOUR assessment runs per year with the assessment of TWO antigens on each run. One of these antigens is requested at every assessment (gold standard), whilst the other is different.

The 'gold standard' antigen for the year ahead will be S100.

Assessment will be performed on both UK NEQAS and 'in house' sections. The antigens to be assessed, apart from S100, will be chosen from the list below. Every effort will be made to ensure that only markers used by the majority of participants are selected for assessment.

Epithelial Markers
Cytokeratin,
Epithelial Membrane Antigen (EMA),

Endothelial Markers
Participants may use any of the following;
Von Willebrand Factor (FVIII related antigen),
CD31, CD34

Muscle Markers
Smooth Muscle Actin
Desmin

Urological & Prostatic Markers
Prostate specific antigen
Prostate specific acid phosphatase
LP34 or *Beta* 34E12
Cytokeratin 7
Cytokeratin 20
Cytokeratin 5 or Cytokeratin 5/6

Neuroendocrine Markers
Chromogranin
Neuron Specific Enolase (NSE)
Synaptophysin

Mesothelial Markers
Participants may used any of the following;
Carcinoembryonic Antigen (CEA), AUA-1,
Ber-EP4, HBME-1, Cytokeratin 5/6, Calretinin
Thrombomodulin

Melanoma Markers (apart from S-100)
HMB45
Melan A

Lymphoid Markers
T-Cell markers e.g.CD3
B-Cell markers e.g. CD20, CD79a
Ig light chains (occasional request only)
Leucocyte Common Antigen (CD45)
CD68

Miscellaneous
Thyroglobulin
Ki-67 or MIB1
Human Chorionic Gonadotrophin (HCG)
Calcitonin
CD56
TTF1

MODULE 2: Breast Pathology (Hormonal Receptors)

This module offers FOUR assessment runs per year with the assessment of one antigen at each run, on both UK NEQAS prepared slides and 'in house' slides. Either Progesterone (PR) or Oestrogen receptors (ER) will be chosen alternately.

MODULE 3: Breast Pathology (HER-2 Immunocytochemistry)

This module offers FOUR assessment runs per year with the assessment of HER-2 at each run, on both UK NEQAS prepared slides (formalin fixed and paraffin processed cell lines) and 'in house' slides.

****For HER-2 FISH please see section 4.0***

MODULE 4: Lymphoid Pathology

This module offers FOUR assessment runs per year with the assessment of TWO antigens at each run. **The 'gold standard' antigen for the year ahead will be Cyclin D1 (Mantle Cell Lymphoma).** Four different antibodies will be selected, along with the gold standard for the year. This 'gold standard' antigen will change annually. Assessment will be performed on both UK NEQAS prepared sections and 'in house' sections. Every effort will be made to ensure that only markers used by the majority of participants are selected for assessment. The markers to be assessed in the year ahead, apart from IgM, are likely to be chosen from the list below;

Alk-1
bcl-2
Bcl-6
CD1a
CD2
CD4
CD5
CD8
CD10
CD15
CD20
CD21
CD23
CD30
CD35
CD43
CD56
CD68
CD79a
CD138
Cyclin D1
IgD
IgM
Ig light chains
Ki-67/MIB1
Mast Cell Tryptase
Terminal deoxynucleotidyle transferase (Tdt)

MODULE 5: Neuropathology

This module will offer FOUR assessment runs per year with the assessment of TWO antigens at each run.
The 'gold standard' antigen for the year ahead will be GFAP.
The gold standard will be requested at every assessment run. Assessment will be performed on both UK NEQAS and 'in house' sections. The other antigens to be assessed in the year ahead, apart from synaptophysin, will be chosen from the list below. Every effort will be made to ensure that only markers used by the majority of participants are selected for assessment.

Neurological & Neuroendocrine Markers
Neurofilament Protein (NFP)
Glial Fibrillary Acidic Protein (GFAP)
Neuron Specific Enolase (NSE)
Chromogranin
S-100 Protein
Growth Hormone (GH)
Adrenocorticotrophic Hormone (ACTH)
Follicle Stimulating Hormone (FSH)
Luteinizing Hormone (LH)
Thyroid Stimulating Hormone (TSH)
Prolactin
Beta-amyloid
Ubiquitin
Tau-protein
Ki-67 / MIB1

Markers for metastatic Disease
Cytokeratin
Leucocyte Common Antigen (LCA)
Prostate Specific Antigen/Prostate Specific Acid Phosphatase
Desmin

MODULE 6: Cytopathology

This module will offer FOUR assessment runs per year with the assessment of TWO antigens at each run on cytospin preparations distributed by the scheme and on participants 'in house' controls, which should preferably consist of cytological preparations.
The 'gold standard' antigen for the year ahead will be CD45/LCA. The second marker to be assessed at each run will be chosen from the following:

Lymphoma Markers
CD3
CD20

Mesothelioma Markers
HBME-1
Calretinin
Thrombomodulin
Cytokeratin 5/6

Adenocarcinoma
EMA/HMFG-2
Cytokeratins

Melanoma Markers
S-100
HMB45
Melan A

MODULE 7: Alimentary Tract Pathology (Pilot Module)

Due to the ever increasing demand for specialist antibodies for gastro intestinal stromal cell tumours (GISTS) and hereditary non polyposis colorectal cancer (HNPCC) antibodies, the UK NEQAS now runs two separate assessments, one for GISTs (CD117) and the other HNPCC (MLH1 & MSH2). There will be two alternating assessments for each of the modules, 7A and 7B (see below).
SEPARATE SUBSCRIPTIONS ARE REQUIRED IF YOU WOULD LIKE TO SUBSCRIBE TO EITHER OF THE MODULES.

Module 7A: Gastrointestinal stromal tumours (GIST)

- CD117

Module 7B: Hereditary non polyposis colorectal cancer (HNPCC)

- MLH1
- MSH2
- MSH6

4.0 FLUORESCENT IN-SITU HYBRIDISATION

4.1 Overview

The UK National External Quality Assessment Module for Fluorescence in situ Hybridisation (UK NEQAS-FISH) is a novel Module organised by Dr John Bartlett, at Edinburgh Cancer Research Centre and Keith Miller of the UK National External Quality Assessment Scheme-ICC (UK NEQAS-ICC). Currently UK NEQAS-FISH will offer assessments for HER2 FISH at approximately 4-monthly intervals throughout the fiscal year. Other schemes are under consideration, including;

- Leukemia/Lymphoma (BCR-ABL)
- Neuroblastoma (N-myc)
- GIST (C-kit)

Details of the HER2 module can be found in the pages that follow. Participants are encouraged to participate if HER2 FISH is currently performed in their laboratory.

4.2 Assessment procedure

At each assessment, laboratories are sent formalin-fixed paraffin processed cell lines from a panel with known HER2 status. They are requested to demonstrate HER2 gene amplification by determination of either HER2/Chromosome 17 ratio or by HER2 copy number (in accordance with UK HER2 FISH guidelines; (1-3) on the slides provided and complete and return scores for each sample using the results scoring sheet provided. In this module, a different panel of breast cancer specimens will be sent at each assessment to ensure coverage of the critical diagnostic ranges. Within a 12 month period, at least one pellet will be repeated to serve as a 'gold standard'. This will allow participants to implement recommended changes if their quality of FISH analysis is found to be sub-standard and to test out their improved technique at the next assessment. They are also requested to complete a short questionnaire giving brief details of the probe and method they have employed. The sheets bearing each participants unique code number (to ensure anonymity) are then assessed by an expert panel consisting of senior biomedical or clinical scientists and consultant histopathologists.

4.3 Interpretation of the scores achieved at assessment

Results obtained by each laboratory will be compared with results obtained, using the same specimens, by a panel of UK reference laboratories and the known HER2 status of the cell lines being used. Results will be scored for each of the 4 pellets relating to the accuracy with which the laboratories results reflect those obtained by the reference laboratories and taking account of acceptable inter-observer variation measurements. Each of the assessors will review the results from reference laboratories and determine the ranges to be described as "appropriate" (within acceptable technical limits), "acceptable" (outwith normal limits but marginal), "inappropriate" (results outwith acceptable limits of variation) and "misdiagnosis" (results outwith acceptable limits resulting in misdiagnosis of HER2 status). Results from individual laboratories will then be scored as "appropriate" (within acceptable limits, 3 points), "acceptable" (outwith normal limits but marginal, 2 points), "inappropriate" (results outwith acceptable limits of variation, 1 point) and "misdiagnosis" (results outwith acceptable limits resulting in misdiagnosis of HER2 status, 0 points).

Scoring summary:

i) An "Appropriate" level of performance: where all 4 results are scored within the range provided by the reference laboratories. Indicated by a score of 12/12

ii) An "Acceptable" level of performance: Where performance was within a 10% (+/-) band when scored against the reference laboratories. In this case 1 or 2 samples have been scored as "Acceptable" or 1 sample was "Inappropriate". Indicated by a score of 9-11/12

iii) An "Inappropriate" level of performance. Where the participant scored >10% (+/-) band achieved by reference laboratories results. In this case 2 or more cell lines were "Inappropriate " or 3-4 samples were "Acceptable". Indicated by a score of $\leq$8/12

iv) An "Inappropriate" level of performance will also be given if the participant mis-diagnosis on one of the samples

4.4 Non-UK laboratories

The NEQAS ICC scheme currently attracts members from over 30 different countries and similarly the NEQAS FISH scheme welcomes participation from both UK and non-UK based laboratories.

4.5 Educational remit of the module

A main aim of the module is to provide useful information on methods and reagents that allow for improved quality of Fluorescence in situ Hybridisation. To this end, the main technical steps employed by participants at assessment are collated onto a database and subsequently tabulated to show the proportion of participants using a particular reagent that achieved acceptable staining at assessment. The results of these analyses appear in a review of each run in the scheme's journal, of which participants each receive three copies. These reviews will contain examples of accurate methods and frequency charts illustrating the distribution of participants scores for each run and will also, periodically, contain colour prints showing optimal demonstration of the genes assessed.

4.6 Monitoring of poor performance (UK laboratories)

Because of the direct impact that the results of assays for HER-2 have on patient management, more stringent performance monitoring mechanisms have recently been proposed for NEQAS ICC and NEQAS FISH will reflect a similar approach. There is now extensive evidence as to the value of FISH in determination of HER2 gene amplification in breast cancer, and a number of studies point to the value of quality control in the use of these tests (1-5). These new performance guidelines have still to be approved by the National Quality Assurance Advisory Panel (NQAAP) and the UK NEQAS Technology in Cellular Pathology Steering Committee and will not be implemented until this approval has been given.

4.7 HER2 FISH Assessment

In order to identify and remedy sub-optimal performance for HER2 FISH assays by UK laboratories within an acceptable time frame, the following procedure will be adopted. UK Laboratories achieving scores of <8 on UK NEQAS samples sections will be issued a warning letter and offered technical advice for improvement to include attendance at the UK NEQAS organisers laboratory by the poor performing laboratory's biomedical scientist, if appropriate. A score of <8 on UK NEQAS sections on a second occasion within the same fiscal year will result in the laboratory concerned being reported to the chairman of NQAAP. In addition, the UK NEQAS-ICC will approach all UK laboratories achieving a score <10 on UK NEQAS sections and provide advice for improvement. Any of these participants subsequently achieving a score <10 at the next two subsequent assessment runs on UK NEQAS sections will be issued a warning letter. With this UK NEQAS will provide further technical advice and support to include attendance at the UK NEQAS organisers laboratory by the poor performing laboratory's biomedical scientist. All attempts will be made to assist the laboratory to improve. Failure to do so however (i.e. laboratory accruing a total of 3 successive scores <10 on the UK NEQAS or 'in house' sections) will result in the laboratory concerned being reported to NQAAP.

The overall approach will aim to ensure that very poor performing laboratories (i.e. those scoring <8 on UK NEQAS material) are identified immediately and given urgent warning and help to improve their performance within a 3-month period. Laboratories producing borderline performance (scores of <10 on UK NEQAS material) will be given no more than 12 months to show a consistent improvement in their performance to an acceptable standard. Failure to improve on either account within the designated period will result in the laboratory being reported to NQAAP. This may ultimately affect the CPA status of the laboratory concerned, with respect to offering HER2 FISH assays as predictive tests. However the laboratory will be permitted to continue participating in EQA for HER2 FISH (if it so wishes) and the chairman of NQAAP notified if it is able to show significant improvement by subsequently accruing acceptable results at each of three successive assessment runs.

This approach will ensure that poor performing laboratories are identified promptly and the situation rectified by appropriate action within a 12-month period.

1. *Ellis IO, Bartlett J, Dowsett M, Humphreys S, Jasani, B, Miller K, Pinder S.E.Rhodes A & Walker R. Updated recommendations for HER2 testing in the UK. Journal Of Clinical Pathology Mar* 2004; **57**:233-237
2. Mass R. The role of HER-2 expression in predicting response to therapy in breast cancer. Seminars in Oncology 2000; 27: 46-52.
3. *Bartlett, JMS, Going JJ, Mallon EA, Watters AD, Reeves JR, Stanton P, Richmond J, Donald D, Ferrier R, & Cooke TG Evaluating HER2 amplification and overexpression in breast cancer. Journal of Pathology (2001) 195:422-428. ISSN 2001226661.*
4. *Bartlett, J.M.S. Mallon, E.A. & Cooke, T.G. The Clinical Evaluation of HER2 status, which test to use? J. Pathology, 2003, 199:411-417.*
5. *Dowsett, M. Bartlett, J., Ellis, I.O., Salter, J., Hills, M., Mallon, E., Watters, A.D., Cooke, T., Paish, C., Wencyk, P.M. & Pinder, S.E. Correlation between immunohistochemistry (HercepTest) and Fluorescence in situ hybridization (FISH) for HER2 in 426 breast cancers from 37 centres. J. Pathology, 2003, 199:418-423.*

All UK NEQAS's are required by their accrediting body (CPA-EQA), to have in place a formal system whereby the performance of all of its UK based clinical laboratories are monitored. The scheme is required to notify the National Quality Assurance Advisory Panel (NQAAP) of any cases of persistent substandard performance in participating UK clinical laboratories.

4.8 End of year performance record

After the end of each fiscal year the scheme provides all participants with a summary of the results they achieved over the preceding year.

4.9 Participants help line and key personnel

Participants experiencing technical difficulties or requiring information about a particular probe or reagent are encouraged to contact either the schemes organiser or manager for assistance. Replacement slides, following breakage, may be obtained by contacting the UK NEQAS-FISH office. Ideally all laboratories experiencing difficulties should contact the scheme for advice well before poor performance monitoring mechanisms come into effect. The schemes organiser and manager always welcome such calls. The schemes contact details are as follows;

Module Leader & Organiser:
Scheme Organiser: **Dr. John Bartlett, Tel: 0131 777 3584 email: jbartlett@staffmail.ed.ac.uk**
Scheme Organiser: **Mr. Keith Miller, Tel: 020 7679 6048, email: k.miller@ucl.ac.uk**
Scheme Manager: **Dr. Merdol Ibrahim, Tel: 020 7679 8678, email: merdol.Ibrahim@ucl.ac.uk**
Office Manager: **Mrs. Ai Lin Rhodes, Tel 020 7554 8679,email: rmkdalr@ucl.ac.uk**
Office Staff: **Mrs. Maricarmen Perez, 020 7554 8677**

They may also be contacted by writing to the address on the front of this booklet.

4.10 Appeals procedure

Participants who are not satisfied with the scores received at a particular assessment run are invited to approach the UK-NEQAS FISH office to discuss results and to apply to re-test samples and re-submit results. Participants are however, notified that such results will contribute to their overall performance scores and may form one of 3 consecutive unsatisfactory assessments which would lead to NQAAP being advised of poor performance. If the re-assessment scores are different than the original ones, the score sheets and database are amended accordingly and the participant sent amended scores and a letter of explanation.

4.11 Complaints procedure

Complaints about the service offered by UK NEQAS-FISH should be addressed to the schemes organiser, Mr. Keith Miller, Dept of Histopathology, UCL Medical School, Rockefeller Building, University St, London WC1E 6JJ

4.12 Scientific meetings and seminars

UK NEQAS-ICC holds scientific meetings and seminars when appropriate, it is anticipated that sessions may included in such meetings to cover the UK NEQAS-FISH until such time as the remit of this scheme expands sufficiently to warrant independent meetings. In addition to the scientific programme these allow participants to discuss methodological and EQA related topics with other participants, the schemes assessors and UK NEQAS-FISH personal.

4.13 Practical workshops

Practical workshops in conjunction with the University of Glasgow, Glasgow are offered periodically. As reagents and equipment for this exercise are costly, the University levies a charge for this service. Further information can be obtained by contacting Dr John Bartlett.

4.14 Summary of benefits of UK NEQAS-FISH membership

As for Immunocytochemistry. Please see page 6.

4.15 Subscription fees

UK NEQAS for Fluorescence in situ Hybridisation receives no financial support other than that generated from participants subscription fees. These are set to cover the costs of running the scheme on a strict non-profit-making basis. The annual subscription fees are listed in the accompanying enrolment forms.

4.16 Registering to participate in UK NEQAS-FISH

Laboratories wishing to participate in UK NEQAS-FISH are recommended to read the description above. Further information and guidance, if required, can be obtained by contacting the Module Leader and Organiser. The enrolment form accompanying this booklet should then be completed and

returned either by mail to; UK NEQAS-FISH, Room 3/22, Hamilton House, Mabledon Place, London WC1H 9BB, United Kingdom, or by fax: 020 7554 8501.

MODULE 1: Breast Pathology (HER-2 FISH)

This module offers three assessment runs per year with the assessment of HER-2 at each run, on UK NEQAS prepared slides (formalin fixed and paraffin processed – cell line xenografts and tissue arrays of breast cancers)

Assessment Panel

The assessment panel will include: Dr John Bartlett (Edinburgh), Professor Ian Ellis(Nottingham), Professor Bharat Jasani(Cardiff), Professor Elaine Kay(Dublin) & Dr Fraser Lewis(Leeds).

5.0 Membership of Steering Committee for Technical Schemes in Cellular Pathology

Chairman
Mrs Barbara Totty, Laboratory Manager, Histopathology, Addenbrooke's Hospital, Cambridge
e-mail: barbara.totty@addenbrookes.nhs.uk

Secretary
Mrs Susan Slaymark [University College London]
e-mail: sue-slaymark@betadial.co.uk

Committee Members
Dr Alan Ramsay, Royal College of Pathologists representative
e-mail: a.ramsay@ich.ucl.ac.uk

Mr David Evans, Organiser of the UK NEQAS for Cellular Pathology Technique
e-mail: david.evans@nuth.northy.nhs.uk

Mr Alan Brown, Advisory Panel
e-mail: Allan.Brown@kingstonhospital.nhs.uk

Mr Peter Ruddy, IBMS representative
e-mail: peter.ruddy@uh.n-i.nhs.uk

Mr Keith Miller, Organiser of the UK NEQAS for Immunocytochemistry & FISH
e-mail: k.miller@ucl.ac.uk