UK NEQAS
International Quality Expertise

50 Years as World Leaders in EQA 1969–2019

UK NEQAS for

Immunocytochemistry & In-Situ Hybridisation

Participant's Manual – Quick Guide

2019 - 2020

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The host organisation
of the
UK National External Quality Assessment Scheme
for Immunocytochemistry and In-Situ Hybridisation
is
External Quality Assessment Services for Cancer Diagnostics
Community Interest Company
Limited by Guarantee, Company number: 10585826

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1. UK NEQAS ICC & ISH Contact Details and Personnel

Contact

Address all correspondence to the UK NEQAS ICC & ISH office:

UK NEQAS ICC & ISH,
Office 127, Finsbury Business Centre,40
Bowling Green Lane,
London EC1 0NE UK.
Telephone: (+44) (0)207 415 7065.
Email: info@ukneqasiccish.org

Alternatively, email or call the appropriate UK NEQAS ICC & ISH staff member using the contact details given in the Table below.

Name	Position	Telephone	Email
Suzanne Parry	Scheme Manager	020 7415 7038	sparry@ukneqasiccish.org
Jamie Hughes	Deputy Manager	020 7415 7168	jhughes@ukneqasiccish.org
Ai Lin Rhodes	Office Manager	020 7415 7065	arhodes@ukneqasiccish.org
Neil Bilbe	Operations Manager	0207 415 7181	nbilbe@ukneqasiccish.org
Chris-Jude Quaye	Quality Manager	020 7415 7038	cjquaye@ukneqasiccish.org
Andrew Dodson	Scheme Director	0207 415 7065	adodson@ukneqasiccish.org

Table 1: UK NEQAS ICC & ISH Personnel and their contact details.

2. Registration and Subscription

Laboratories wishing to participate in one or more UK NEQAS ICC & ISH modules are recommended to read the detailed descriptions of each of the modules and elect to participate in those modules that cover the range of markers used routinely in their laboratory.

UK NEQAS ICC & ISH receives no financial support for the running of the Scheme, other than that generated from participants' subscription fees. These are set to cover the running costs of the scheme on a strictly non-profit basis. The annual subscription fees are provided to all currently subscribed members and can be sent out on request to prospective new participants.

- Subscription fees are payable prior to the start of the EQA financial year, which runs from April to March. They are collected by and made payable to our host organisation: External Quality Assessment Services for Cancer Diagnostics, which is a not-for-profit company;
- Fees are non-refundable;
- Participants enrolled in the current year's EQA service will automatically be sent

subscription renewal forms. Non-return of subscription forms will be taken to mean that a participant no longer wishes to continue with their subscription;

- Participants must inform UK NEQAS ICC & ISH in writing if they wish to cease participating in any of its modules;
- Participants must inform UK NEQAS ICC & ISH in writing of any changes in contact details;
- New participants can join at any time throughout the year. Subscription fees may be reduced according to the remaining EQA period at the point of joining.

Subscription forms and further information about registration can be obtained by contacting the Scheme's Office Manager, Lin Rhodes.

Email: arhodes@uknegasiccish.org Telephone: +44(0)20 7415 7065

3. Poor Performance Monitoring (UK Clinical Laboratories)

All UK EQA schemes are required by their accrediting body (UKAS ISO 17043/15189), to have in place a formal system whereby the performance of all 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.

From September 2010, NQAAP required all EQA schemes to use a 'traffic light' system for the grading of all its UK participants:

Colour Code	DESCRIPTOR
GREEN	No Issues with poor performance
AMBER	Issues with poor performance, managed locally by the Scheme
RED	Poor performance issues unresolved; persistent poor performer referred to NQAAP

Table 2. Traffic Light system used for grading sub-optimal performance

The UK NEQAS ICC & ISH Poor Performance monitoring covers the five most recent runs following the upload of reports after each assessment.

For the generic modules, each module is treated as a separate entity; low scores from one module are not combined with low scores from another module to produce a poor performance. Therefore, a laboratory that is underperforming in one module may continue to submit returns for any of the other generic modules in which they are not underperforming.

Although in-house sections are not part of the front-line poor performance monitoring procedure, the importance of good in-house staining is to be emphasised and laboratories may be contacted if their in- house controls are suboptimal, or their choice of in-house control material is not appropriate. It will not be acceptable to perform well on UK NEQAS ICC & ISH material alone. Laboratories with persistent suboptimal staining of their in-house material will be contacted, and their EQA results discussed with a view to further action being taken if the situation continues.

Offer of Assistance Letters (UK Clinical Laboratories only)

When a laboratory has under-achieved in one (Biomarker) or two (Generic) assessments, the scheme will notify the laboratory to alert them of this situation. This will be an 'Offer of Assistance' letter. Although participants are not obliged to contact UK NEQAS ICC & ISH at this point, they may still wish to do so for advice and feedback to improve on future assessment results. Please note that for the generic modules it is possible to have one failed score, then two failed scores at a subsequent run and therefore receive an Amber letter (3 failed scores over 5 runs).

Poor Performance Monitoring (Non-UK Clinical Laboratories only)

From the start of the 2019 – 2020 EQA year UK NEQAS ICC & ISH will be monitoring Non-UK Clinical laboratories (Overseas). Overseas labs will only receive **Amber** and **Red** letters and will not be sent 'Offer Of Assistance' letters.

Non-submission of Slides

This will result in a score of zero (0), and will be included in poor performance monitoring, unless the laboratory has informed UK NEQAS ICC & ISH of a valid reason for the non-submission.

NQAAP has stated that submission rates should be **100%** for all UK Clinical laboratories. If a laboratory has not submitted for a run, then the EQA provider (UK NEQAS ICC & ISH) should be given/sent a valid explanation or reason why; e.g. antibody not stocked (and an alternative could not be provided), not clinically testing or testing being outsourced.

Retrospective explanations following the production of results, and subsequent poor performance reports, may not be acceptable.

Important: The monitoring procedure shown above is liable to change depending on approval from NQAAP. UK participants will be notified of any changes in a separate communication.

4. MODULE 1: General Pathology

Requested Antigens

- Pan-cytokeratin's e.g. AE1/AE3, MNF116 etc;
- Endothelial marker e.g. CD31, CD34 etc;
- CD56;
- E-cadherin;
- Prostate Specific Antigen (PSA);
- CD3;
- Calretinin;
- CDX2.

There is no Gold Standard for this Module. The eight antigens in the list will be assessed, with two being selected in turn at each of the four Assessments during the year, i.e. each will be examined once. Every effort has been made to ensure that only markers used by the majority of participants have been selected.

5. MODULE 2: BREAST PATHOLOGY HORMONAL RECEPTORS (ER AND PR)

GOLD STANDARD ANTIGENS

- Oestrogen Receptor (ER)
- Progesterone Receptor (PR)

6. MODULE 3: BREAST PATHOLOGY HER2 IHC

Formalin fixed and paraffin processed cell lines showing the full range of HER2 IHC expression (3+, 2+, 1+ and 0) are generally used as the UK NEQAS assessment samples.

7. MODULE 4: LYMPHOID PATHOLOGY

GOLD STANDARD AND SECOND ANTIGENS

Two Gold Standard antigens will be used; each will be used for two Assessment Runs:

- CD30
- Cyclin D1

A second antigen 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.

ALK-1	CD21	CD68	Lambda light chain
BCL-2	CD23	CD79a	EBV - LMP1
BCL-6	CD3	CD8	Mast Cell Tryptase
BRAF	CD35	Cytomegalovirus	MUM-1
CD10	CD4	DB44	PAX-5
CD15	CD43	Immunoglobulin D	SOX-11
CD1a	CD5	Immunoglobulin M	Terminal deoxynucleotidyl transferase (Tdt)
CD2	CD56	Kappa light chain	
CD20	CD61	Ki67	

8. MODULE 5: NEUROPATHOLOGY

GOLD STANDARD AND SECOND ANTIGENS

Two Gold Standard antigens will be used; each will be used for two Assessment Runs:

- Glial Fibrillary Acidic Protein (GFAP)
- Neurofilament Proteins (NFP)

A second antigen 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.

Acid Phosphatase	Beta-amyloid	Cytokeratin 7 or 20
Adrenocorticotropic Hormone (ACTH)	CD34	Desmin
ATRX	CD45 (LCA)	EMA
BAF47 (INI1)	CD68	Follicle Stimulating Hormone (FSH)
	Chromogranin A	

Growth Hormone (GH)	Pan-cytokeratin	Thyroid Stimulating Hormone (TSH)
Ki-67	Prolactin	Ubiquitin
Luteinizing Hormone (LH)	Prostate Specific Antigen (PSA)	
NeuN	S-100	
Neuron-Specific Enolase (NSE)	Tau-protein	

9. MODULE 6: CYTOPATHOLOGY

GOLD STANDARD AND SECOND ANTIGENS

Two Gold Standard antigens will be used; each will be used for two Assessment Runs:

- Pan-cytokeratins e.g. AE1/AE3, MNF116 etc;
- Calretinin.

A second antigen 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.

BerEP4	EMA	MOC-31
CD20	Oestrogen Receptor (ER)	p63
CD3	HBME-1	Progesterone Receptor (PR)
CD45 (LCA)	Ki-67	Thrombomodulin
Cytokeratin 20	Melanoma Markers e.g.Melan A, HMB45, S100	TTF1
Cytokeratins 5/6		WT

Cytospin preparations or cell block sections are distributed by the Scheme dependent on the indicated participant preference.

Participants' in-house controls should preferably consist of complimentary preparations depending on the requested choice of sample for assessment, i.e. if you request a cytospin from us we will expect to see a cytospin in-house control, and similarly for cell block preparations.

10. MODULE 7: ALIMENTARY TRACT PATHOLOGY (GIST)

GOLD STANDARD AND SECOND ANTIGENS

The Gold Standard antigen will be:

- CD117 (c-KIT)

A second antibody/antigen will be chosen from the list below:

DOG-1
S100
CD34
Desmin

11. MODULE 8: GASTRIC HER2 IHC

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded gastric cancer tissue from excision samples showing varying levels of HER2 membrane protein expression.

12. MODULE 9: BREAST HER2 ISH (TECHNICAL AND INTERPRETIVE)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded breast tumour samples

13. MODULE 10: NSCLC ALK IHC

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded lung tumour tissue from excision samples, and also cell lines with varying levels of ALK IHC expression. UK NEQAS samples will also include an appendix.

14. MODULE 11: NSCLC PD-L1 (PILOT)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded lung tumour tissue from excision samples, and also cell lines with varying levels of PD-L1 IHC expression. NEQAS samples will also include a tonsil sample.

15. MODULE 12: NSCLC ALK/ROS1 FISH (PILOT)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded cell lines and/or lung tumour samples of known expression.

16. MODULE 13: MISMATCH REPAIR PROTEINS

GOLD STANDARD

The Gold Standard antigens will be:

- MLH1 and PMS2
- MSH2 and MSH6

The antigen pairs will be requested at alternate Assessment Runs.

17. IN-HOUSE CONTROL TISSUE REQUIREMENTS AND RECOMMENDATIONS

- In-house samples should be placed onto UK NEQAS distributed slides as shown in Section 3 of this Manual.
- Appropriate controls must be used as outlined in the relevant Section below.
- Quality of the submitted in-house tissue is important. Tissues must be well fixed and processed with well-preserved morphology. Poor fixation, damage caused by excessive antigen retrieval, and inappropriately weak or strong counterstain will be taken into consideration when assessing quality. As will poor section quality and the use of excessively thick or thin sections.
- Online data sheets MUST be fully completed, indicating the tissue/tumour type, and where appropriate, which component has been used to control the staining (for example, in the breast module whether the *in-situ* carcinoma is to be assessed rather than the invasive component).
- We DO NOT require submission of unstained in-house controls for any of our Modules.

Suitable In-House Control Materials

For all modules, in-house tissue must include appropriate controls for the antigen requested. Marks will be deducted for inappropriate controls.

Module	Suitable In-House Control(s)
Alimentary Tract GIST	GIST and appendix *or* GIST with included normal mucosa.
Mismatch Repair Proteins	Tumour showing loss of expression (deficient) and appendix *or* tumour showing loss of expression (deficient) together with normal epithelium
Lymphoid Pathology	Lymphoma appropriate to the antigen assessed and tonsil.
NSCLC ALK IHC	ALK-positive and ALK-negative NSCLC and appendix are required.
NSCLC PD-L1 IHC (pilot):	PD-L1-positive and PD-L1-negative NSCLC together with tonsil.
Breast HER2 ISH	A single sample consisting of an invasive breast tumour.
Breast Hormonal Receptors (ER and PR)	Participants in-house control tissue MUST consist of composite breast tissue (see also Note 1 about use of cell lines): • >80% positive tumour with high intensity (Allred/Quick score 7-8) • 30-70% positive tumour with low or moderate intensity (Allred/Quick score 4-6) • negative tumour, ideally including normal glands (Allred/Quick score 0)
Breast HER2 IHC	In-house control material MUST include samples from 3+, 2+ and 1+/0 HER2 expressing invasive breast cancer cases (see Note 1 about use of cell lines). DCIS breast tissue showing differing levels of membrane staining is an acceptable alternative. However, laboratories must indicate which component they have scored, or the invasive component, if present, will be assessed. It is also acceptable to submit a heterogeneous in-house tumour control with areas of e.g. 3+ and 2+ membrane expression as long as the participant indicates the areas and expected levels of staining.
Gastric HER2 IHC	In-house control material MUST include 3+, 2+ and 1+/0 HER2 expressing cases preferably of gastric tumour, although breast tumour is also acceptable (see also Note 1 about use of cell lines). DCIS breast tissue showing differing levels of membrane staining is an acceptable alternative. Laboratories must indicate on their datasheet which component of the tumour they have scored, otherwise the invasive component, if present, will be assessed. It is also acceptable to submit a heterogeneous in-house tumour control with areas of e.g. 3+ and 2+ membrane expression as long as the participant indicates the areas and expected levels of staining.
ALK FISH (pilot):	ALK-positive and ALK-negative NSCLC
ROS1 FISH (pilot):	ROS-1-positive and ALK-negative NSCLC

Table 3. In-House Controls

Note 1: cell lines are an acceptable substitute, but only when used alongside a piece of the participants own in-house tissue, and it is still a requirement to include the varying expression levels. However, cell lines included with commercial kit/assays are not an acceptable substitute and will not be assessed.

18. Appeals and Help

Participants who are not satisfied with their scores can appeal, and have their slides reassessed.

Reassessments take place at the first assessors meeting after receipt of the request. If the reassessment scores are different from the original ones, the score sheets and database are amended accordingly, and the participant is sent amended scores and a letter of explanation.

An appeal can only be made from the most recent completed run.

Only originally submitted slides will be reassessed. We are unable to reallocate or update marks on newly stained slides.

A Reassessment form can be found on the UK NEQAS ICC & ISH website:

https://www.ukneqasiccish.org/wp/wp-content/uploads/2018/08/NEQ-MF17-Request-for-Reassessment-of-Slides-v6.pdf

Participants experiencing technical difficulties or requiring information about a particular antibody or reagent are encouraged to contact the Scheme

UK NEQAS ICC & ISH is always ready to assist with advice and troubleshooting.

Participants are welcome to send in slides asking for feedback and advice at any time (see link to referral form below) or include an enclosed letter with your slides. Do not use the UK NEQAS ICC & ISH reassessment forms for this service.

https://www.ukneqasiccish.org/wp/wp-content/uploads/2018/12/request_for_feedback_opinion.pdf

Ideally, all laboratories experiencing difficulties should contact the scheme for advice well before poor performance monitoring mechanisms come into effect.

19. Complaints Procedure

Formal complaints about the service (**not an appeal against your score**) offered by UK NEQAS ICC & ISH must be addressed to the Scheme's Director, Mr Andrew Dodson; please use the official complaint form which also has the scheme Director's contact details. The document is available at:

https://www.ukneqasiccish.org/wp/wp-content/uploads/2019/01/NEQ-MF4-Participant-Complaint-Form_v8-Re-UPDATED.pdf

Do not use the above form if requesting a reassessment.