UK NEQAS
International Quality Expertise

UK NEQAS for Immunocytochemistry & In-Situ Hybridisation

Participants' Quick Guide

2024-2025

Table of Contents

Download Link for This Participants' Manual and the Participants' Quick Guide

This Participants' Manual is a comprehensive reference guide to all aspects of the services and the procedures followed by UK NEQAS ICC & ISH.

We have also produced this user-friendly 'Quick Guide'.
It is a useful ready reference that contains answers to the most frequently asked questions.

Both documents can be downloaded from our website at: www.ukneqasiccish.org

1. Modules

Code	EQA Module Description
	No specific group
1	General Pathology
4	Lymphoid Pathology
5	Neuropathology
6	Cytopathology
13	Mis-Match Repair Proteins (MLH1, MSH2, MSH6 and PMS2)
	Breast cancer
2a	Oestrogen Receptor (ER)
2b	Oestrogen and Progesterone Receptor (ER and PgR)
3	HER2 protein over-expression by immunohistochemistry
24	HER2 protein LOW over-expression by immunohistochemistry (see NOTE 1)
9	HER2 gene amplification by *in-situ* hybridisation - Technical and Interpretive
15	PD-L1 protein over-expression in Triple Negative Breast Cancer (TNBC) (Pilot)
16	Ki-67 (Pilot)
	Non-small cell lung cancer (NSCLC)
10	ALK protein over-expression by immunocytochemistry
11	PD-L1 protein over-expression (Pilot)
14	ROS1 protein over-expression by immunocytochemistry (Pilot)
12a	ALK gene translocation by *in-situ* hybridisation (Pilot)
12b	ROS1 gene translocation by *in-situ* hybridisation (Pilot)
12	Both ALK and ROS1 gene translocation by *in-situ* hybridisation (Pilot)
	Gastrointestinal tract cancers
7	CD117 and associated GIST markers
8	HER2 protein over-expression in gastric cancer
	Head and neck squamous cell carcinoma (HNSCC)
17a	p16 protein over-expression (Pilot)
17b	High-Risk Human Papilloma Virus (HPV) protein or RNA expression (Pilot)
17	Both p16 and High-Risk HPV (Pilot)

Table 1. Scheme Modules

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.

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

Email: arhodes@ukneqasiccish.org; Telephone: +44(0)208 187 9174.

3. Guidelines and Procedures

Slide Distribution and Placement of UK NEQAS and In-House Controls

Prior to each assessment run, participants receive:

- One or two duplicate microscope slides, bearing appropriate UK NEQAS ICC & ISH control materials.
- An assessment run 'cover letter' providing information and instructions (a copy is also sent to the participant laboratory's contact e-mail address).
- More detailed module specific instruction sheets can be found on the UK NEQAS ICC & ISH website.

For all Modules (except Cytology Module in cases where cytospin preparations are requested):

- the area towards the label end contains UK NEQAS ICC & ISH provided sample(s).
- the area at the lower end of the slide is used by participants to mount their own in-house samples/controls.
- Slides are distributed with the mounted sections 'unbaked'.
- Upon receipt, participants should mount their in-house control material onto the same slide that contains the UK NEQAS ICC & ISH section(s).
- After mounting their own control materials, participants should heat slides in a slide-drying oven at either 37°C overnight or 55-60°C for 1 hour to ensure adequate section adhesion.
- As soon as possible after the slide drying has been completed, participants should carry out their routine staining procedure.

By convention, microscope slides distributed by the Scheme are separated into two areas (illustrated in Figure 1):

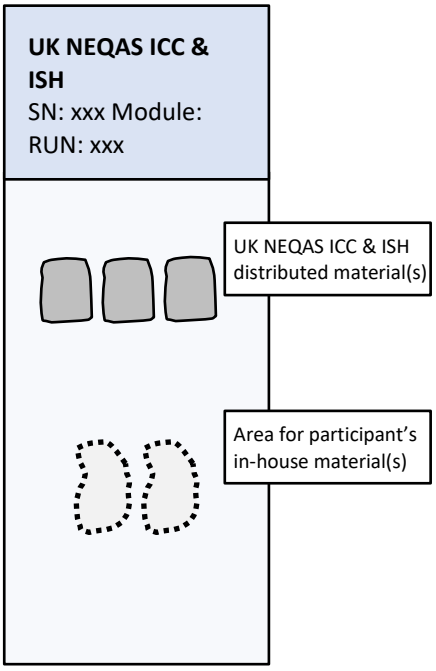

Figure 1. Distribution of samples on slide.

It is very important that participants prepare control samples which are appropriate for the antigen that is being assessed. Ideally, the control tissues chosen should fit within the designated area on the same slide that holds the UK NEQAS ICC & ISH section(s). If this is not possible, it is permissible for them to be mounted on a separate slide.

Cytology Module cytospins only: participants who request cytospin samples as their UK NEQAS distributed material are required to submit a separate slide for their in-house control sample; the in-house sample should ideally be a cytospin from a cytology preparation. And the staining method carried out should be the same for both the UK NEQAS distributed and the in-house samples. Participants who

request a cell block sample should place their in-house section on to the same slide as the UK NEQAS sample where possible.

Antibody Not Stocked

If a suitable antibody against the antigen chosen for assessment is not stocked by a participant, they MUST contact the UK NEQAS ICC & ISH offices to agree a suitable alternative.

If an alternative antibody is provided, slide(s) will be treated and marked in the same way as the original antibody and will count towards a participant's performance record. It is therefore important that you contact the UK NEQAS ICC & ISH office to ask for an alternative, and do not choose your own alternative.

Web Based Data Entry System and Accessing Online Reports

Participants have access to the UK NEQAS ICC & ISH web data entry and report system, which provides:

- Comprehensive instructions for each assessment.
- Individual participant-specific assessment reports.
- Selected assessment images showing optimal staining results and common features of sub-standard staining.
- Assessment run results presented Graphically and in Tabulated format.

4. Assessment Scoring and Interpretation

General assessment guide

1. Each one of the four assessors independently award a mark out of '5' using the pre-determined guidelines.
2. Marks are added together to give a final score out of 20.
3. An acceptable level of staining is indicated by a score of at least 13/20.
4. A borderline acceptable score of 12/20 indicates that whilst the staining may show some clinical relevance, the staining is sub-optimal, and improvements are required.
5. A score of 8/20 or less is given for a poor quality of immunocytochemistry, which is of no clinical relevance. Significant improvements are required.

Combined Assessment Scores

Participants receive a combined assessment score as a final indication of staining quality. Table gives an indication of how these scores should be interpreted and what actions, if any are required.

Final Score	Interpretation
0	**No submission.**
4 - 8	**UNACCEPTABLE** Unreadable/clinically uninterpretable. Staining has no utility. Improvement essential.
12	**BORDERLINE ACCEPTABLE** Although clinically interpretable with immunostaining considered to be appropriate for the target in question, the staining quality is sub-optimal, and improvement is essential.
13 - 15	**ACCEPTABLE** Clinically interpretable with immunostaining appropriate for the target in question and of good quality. Improvements are required.
16 - 20	**GOOD to EXCELLENT** Clinically interpretable with immunostaining appropriate for the target in question and of good to excellent quality. Minor improvements may be possible.

Table. Interpretation of final score, produced from the 4 assessor's combined scores.

5. In-house Control Tissue Requirements and Recommendations

In-house samples should be placed onto UK NEQAS distributed slides.

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 sub-optimal 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.

Required 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.

IMPORTANT NOTE: Cell lines are an acceptable substitute to tissues as in-house controls, but only when used in conjunction with a piece of the participant's own in-house tissue.

While cell-line controls can inform on the quality of immunocytochemical staining in the same way that tissues do, they have not been subjected to the participant's pre-analytical procedures. Therefore, in-house tissue is requested in addition to cell-lines to allow the assessment of the adequacy of pre-analytical processes i.e., fixation and processing, both of which have significant bearing on the outcome of any subsequent immunocytochemical testing.

In the participants' day-to-day internal quality control there is no necessity to include a piece of tissue when cell-lines are used. As the adequacy of pre-analytical processes can be assessed on the tissue undergoing testing.

Regardless of whether tissues or cell-lines, or a mixture of both are used, it is still necessary to encompass the varying expression levels that are clinically important.

Cell lines included with commercial kits or assays are an acceptable alternative internal control to those produced in-house provided they cover the critical decision-point range for the assay. And here again, a piece of the participant's own in-house tissue must also be included.

6. Participant Reports

At the end of each assessment, participants are sent notification via email that reports are available to view and download from the UK NEQAS ICC & ISH website.

7. Poor Performance Monitoring (UK Clinical Laboratories Only)

All UK NEQAS schemes are required by their accrediting body, UKAS (ISO/IEC 17043:2010), to have in place a formal system whereby performance of their UK clinical laboratory-based participants is monitored.

8. Poor Performance Monitoring of Non-UK Participants

UK NEQAS ICC & ISH does not have a mandate to report poor performance of non-UK based participants. But in order serve those participants as well as is possible, the Scheme will contact them at Amber and Red trigger points to offer help and assistance on a voluntary basis.

9. End of Year Performance Record / Certificate of Participation

At the end of each EQA year, the Scheme provides all participants with a printed 'certificate of participation', listing all modules participated in. For each module, laboratories must have submitted at least twice during the EQA year. Participants also receive a summary of the results they achieved over the preceding year (annual report).

10. The Scheme's Scope

For a full list of antigens (examined using ICC) and genes (examined using ISH) that are able to be assessed by UK NEQAS ICC & ISH (its Scope), please refer to the website of the Scheme's accrediting body: **CLICK HERE**

11. The Scheme's Modules: General Remarks

Laboratories are welcome to participate in any of the Modules, depending on their service commitments and specialised areas of interest. All modules offer three Assessment Runs per year. Participants are assessed on both the UK NEQAS distributed materials and participant's own in-house controls.

Participation in all Assessment Runs during the EQA year is expected.

The Scheme will make every effort to ensure that, where specified the stipulated requested markers and are assessed as stated but reserves the right to change them for suitable alternatives where circumstances require it to be done.

12. UK NEQAS ICC & ISH Contact Details and Personnel

Contact

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

UK NEQAS ICC & ISH, 5 Coldbath Square, London EC1R 5HL United Kingdom

Telephone: (+44) (0)208 187 9174. Email: info@ukneqasiccish.org

Alternatively, email the appropriate UK NEQAS ICC & ISH staff member using the contact details below.

Name	Position	Email
Andrew Dodson	Scheme Director	adodson@ukneqasiccish.org
Suzanne Parry	Scheme Manager & Deputy Scheme Director	sparry@ukneqasiccish.org
Ai Lin Rhodes	Office Manager	arhodes@ukneqasiccish.org
Michelle James	Quality Manager & Scientist	mjames@ukneqasiccish.org
Dawn Wilkinson	Deputy Scheme Manager & Scientist	dwilkinson@ukneqasiccish.org
Deepa Nayar	Staff Scientist	dnayar@ ukneqasiccish.org
Fitim Berisha	Staff Scientist	fberisha@ ukneqasiccish.org
Lila Zabaglo	Staff Scientist	lzabaglo@ ukneqasiccish.org
Sumera Khalid	Staff Scientist	sskhalid@ukneqasiccish.org
Kally Sidhu	Medical Laboratory Technical Officer	ksidhu@ ukneqasiccish.org
Marie Stoddart	Senior Administrator	mstoddart@ukneqasiccish.org
Wendy Fernandes	Administrative Assistant	wfernandes@ukneqasiccish.org
David Evans	Medical Laboratory Assistant	devans@ ukneqasiccish.org

Table 15: UK NEQAS ICC & ISH Personnel and their contact details.

13. Replacement slides

Replacement slides for those which arrive broken may be obtained by contacting the Admin team. Email**: info@ukneqasiccish.org; Telephone: +44(0)208 187 9174**

Please include your Participant Code and the reason why you are requesting replacements.

14. 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 recently completed run.

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

An 'Appeal Against Assessment Result' form can be found on the UK NEQAS ICC & ISH website.

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. The service we offer differentiates between:

- Those requests that relate to improvements to a protocol initiated by a poor result at assessment – this is the **Quality Improvement Following Assessment** service.
- Those requests that are initiated by the laboratory to introduce a new primary antibody or to improve an existing procedure that do not relate to their performance at assessment – **Referral Request - for Feedback or Opinion**.

Request forms for both can be downloaded from our website (IMPORTANT: **do not use the UK NEQAS ICC & ISH Appeal Against Assessment form**).

Ideally, all laboratories experiencing difficulties should contact the scheme for advice well before poor performance monitoring mechanisms are triggered.

15. 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 from our website. (Do not use this form if requesting a reassessment).

16. Confidentiality Policy

UK NEQAS ICC & ISH maintains the confidentiality of a participants' performance results at all times; except where the scheme is obliged to inform regulatory bodies (NQAAP) of UK clinical laboratories that are persistent poor performers; this is to ensure that patient safety is not endangered.

- **During assessments, and at any subsequent use of data for educational purposes, the participants' identity is never disclosed.**
- **Linkage of the unique participation code with the identity of the centres is only available for selected UK NEQAS ICC & ISH staff members.**

Where a third party or an interested party enquires about the use of an individual participants' data, this

will only be disclosed if the participant waives its right to confidentiality. UK NEQAS ICC & ISH may provide anonymised data to third parties that have a direct involvement in UK NEQAS ICC & ISH.

If UK NEQAS ICC & ISH is legally obliged to provide data, to a regulatory body or another organisation, the participants will be informed in all such instances.

The host organisation of UK National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation is:

External Quality Assessment Services for Cancer Diagnostics.

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