UK National External Quality Assessment Scheme for Immunocytochemistry & In-Situ Hybridisation

Participants' Manual

2026-2027

External Quality Assessment Services for Cancer Diagnostics Community Interest Company
(Registration No. 10585826)

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The Scheme's Website

Visit our website at: www.ukneqasiccish.org

Download Link for This Participants' Manual

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

This document can be downloaded from our website at: www.ukneqasiccish.org

1. Introduction

History

The origins of the **United Kingdom National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation** (UK NEQAS ICC & ISH) lie in a slide exchange exercise started in 1985 by Gerry Reynolds, at that time Gerry was a medical laboratory scientist working as the laboratory lead in the Histopathology Department of Mount Vernon Hospital in London.

The slide exchange exercise quickly grew as the new science of immunocytochemistry began to be more widely used in diagnostic laboratories, and in 1988 the UK Department of Health recognised it as a 'Scheme'. From that time, it was known as the UK National External Quality Assessment Scheme for Immunocytochemistry (UK NEQAS ICC); subsequently, when *in-situ* hybridisation methodologies began to appear they were incorporated and the scheme was renamed to UK NEQAS ICC & ISH.

Aims

- To provide a scientifically-led professional External Quality Assessment (EQA) service with the primary objective of helping laboratories to evaluate their performance and to identify and implement any necessary changes for improvement.
- To achieve this by the frequent assessment of distributed samples to allow tailored feedback on performance in a timely manner.
- To distribute EQA material which closely reproduces the characteristics of clinical samples and where appropriate to supplement these with analyte controls to allow reproducible quantitative measurements to be made.
- To help ensure clinical test results are accurate and reliable and so improve patient-care.

2. UK NEQAS Code of Practice

The Scheme is a Member of the UK NEQAS Charity (https://ukneqas.org.uk/), which oversees the governance and structure of the Scheme's EQA activities. More details about the rules that govern UK NEQAS ICC & ISH can be found in the charity's Code of Practice, a copy of which can be requested from the Charity.

3. General Structure

UK NEQAS ICC & ISH offers assessments of immunocytochemistry and in-situ hybridisation techniques. These assessments are carried out at evenly spaced intervals, approximately every four months throughout the EQA year, which runs from 1st April to 31st March.

The Scheme has a modular structure to allow users to select those areas and tests which

are applicable to their own testing repertoire. Details of each Module can be found in the pages that follow. Participants are encouraged to participate in those Modules that cover the full range of immunocytochemistry and in-situ hybridisation tests performed in their laboratory.

UK NEQAS ICC & ISH is run on a strictly not-for-profit basis. All income derived from participants' subscription fees if used to run and deliver the EQA activities of the Scheme.

Scheme's Legal Entity

Hosting is provided by External Quality Assessment Services for Cancer Diagnostics (EQAS-CD), which is a not-for-profit Community Interest Company.

An ISO Accredited EQA Scheme

UK NEQAS ICC & ISH is a UKAS accredited proficiency testing provider No. 7833. As an organisation overall and in the operation of its individual assessment modules, the Scheme operates to the internationally recognised standard: ISO 17043:2023 Conformity assessment - General requirements for proficiency testing.

[Note: Pilot modules under development are not accredited. Accreditation of these is obtained prior to introducing them as full modules].

ILAC Accreditation

The Scheme is also accredited through the mutual recognition agreement with the International Laboratory Accreditation Cooperative (ILAC), which is the international organisation for accreditation bodies operating in the sphere of conformity assessment.

4. Benefits of Participation

The Scheme's remit extends beyond the assessment of technical quality of the preparations submitted by its participants. A key goal of the Scheme is education to improve quality. Therefore, the list of benefits it provides is extensive:

- Compliance with ISO/IEC 15189:2022 regarding participation in an EQA scheme.
- Three assessment runs are carried out per year.
- Specific modules cater for the specialised areas of pathology.
- Two antigens are assessed per assessment run in diagnostic biomarker modules.
- Assessment of UK NEQAS distributed material and participants' in-house samples.
- Web data entry and access to individual confidential reports.
- Constructive assessor feedback.
- Individual benchmarking graphs to track performance over time.
- Frequency charts illustrating the distribution of participant scores for

each run.
- Colour images showing optimal and sub-optimal demonstration of the antigens.
- Tables of the main antibodies and immunocytochemical reagents used by participants.
- Examples of 'Best Methods' and interactive searchable web 'Best Methods' database.
- An end of year certificate of participation (for those participants submitting materials to two runs or more).
- Annual report.
- Other articles and reviews from the scheme.
- Module reviews and articles.
- Participants 'Help-line' and details on obtaining advice.
- Referral for opinion service.
- Participant user group scientific meetings and workshops.

An International EQA Scheme

The Scheme welcomes both UK and non-UK based laboratories. It currently has participants drawn from over 50 countries.

All submissions, irrespective of the participant's country of origin, are assessed in exactly the same manner at the same assessment sessions. Assessment of slides is carried out anonymously and assessors are blinded to all identifying features for all participant centres.

Educational Remit of the Scheme

One of the main aims of the service 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. The results of these analyses are subsequently provided as feedback to laboratories in the form of tabulated data showing information on pass rates, reagents, automation and detection system employed. Best methods are also provided along with images of good and poor examples of IHC and ISH staining.

5. Subcontracted Services

UK NEQAS ICC & ISH uses external suppliers including commercial and public-sector organisations from both the UK and overseas to:

- Provide EQA material, including formalin-fixed paraffin-embedded tissues and cell lines, and cytology preparations.
- Provide section cutting services.
- Provide stained samples for validation purposes and "standard"

references.

Regardless of this, UK NEQAS ICC & ISH assesses the competency of suppliers to provide the contracted service(s) prior to engaging them.

All EQA material is checked and validated by UK NEQAS ICC & ISH prior to dispatch to participants and the Scheme assumes responsibility to its participants for all subcontracted work and services [Note 1].

[Note 1. Certain overseas participants will receive the EQA material through an authorised third-party distributor who receives the material directly from UK NEQAS ICC & ISH].

[Note 2. External service providers do not undertake the design or planning of modules, or any other operations of the scheme].

6. Modules

Available Modules are shown in Table 1.

EQA Module Description
No specific group
General Pathology
Lymphoid Pathology
Neuropathology
Cytopathology
Mis-Match Repair Proteins (MLH1, MSH2, MSH6 and PMS2)
Melanoma (Pilot)
Sarcoma (Pilot)
Breast cancer
Oestrogen Receptor (ER)
Oestrogen and Progesterone Receptor (ER and PgR)
HER2 protein over-expression by immunohistochemistry
HER2-low protein over-expression by immunohistochemistry (Pilot)
HER2 gene amplification by *in-situ* hybridisation - Technical and Interpretive

PD-L1 protein expression in Triple Negative Breast Cancer (TNBC) (Pilot)
Ki-67 (Pilot)
Non-small cell lung cancer (NSCLC)
ALK protein over-expression by immunocytochemistry
PD-L1 protein expression (Pilot)
ROS1 protein over-expression by immunocytochemistry (Pilot)
ALK gene translocation by *in-situ* hybridisation (Pilot)
ROS1 gene translocation by *in-situ* hybridisation (Pilot)
Both ALK and ROS1 gene translocation by *in-situ* hybridisation (Pilot)
Gastrointestinal tract cancers
CD117 and associated GIST markers
HER2 protein over-expression in gastric cancer
Claudin 18.2 protein over-expression in gastric cancer (Pilot)
Head and neck squamous cell carcinoma (HNSCC)
p16 protein over-expression (Pilot)
High-Risk Human Papilloma Virus (HPV) protein or RNA expression (Pilot)
Both p16 and High-Risk HPV (Pilot)
PD-L1 protein expression (Pilot)

TABLE 1. SCHEME MODULES

7. Registration and Subscription

Laboratories wishing to participate with UK NEQAS ICC & ISH are recommended to read the detailed descriptions of each of the available Modules and select those which cover the range of markers used routinely in their laboratory.

UK NEQAS ICC & ISH receives no financial support for the running of its EQA 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 are expected to join at the beginning of the EQA year.
- Participation at all (usually three) Assessment Runs during the year is expected.

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.

Alternatively, e-mail: info@ukneqasiccish.org

8. Guidelines and Procedures

Slide Distribution and Placement of Sections

Prior to each assessment run, participants receive:

- One or two duplicate microscope slides, dependent upon the Module. These bear 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).

For all Modules **except the Cytology Module** in cases where cytospin preparations are requested:

- the area towards the label end of the slide contains UK NEQAS ICC & ISH provided EQA 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, participants should carry out routine staining.

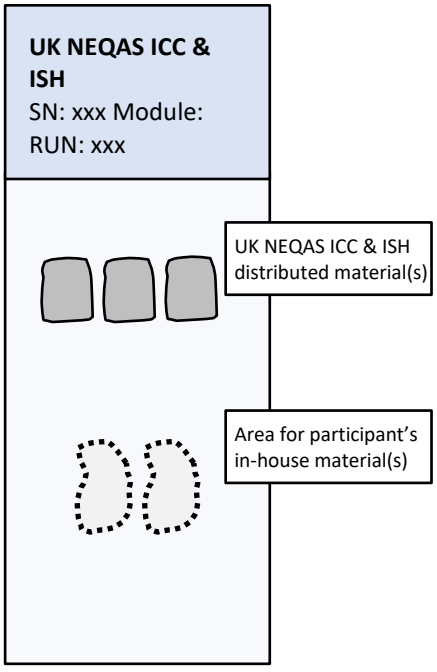


Figure 1. Distribution of samples on slide.

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

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, you MUST contact the UK NEQAS ICC & ISH offices to agree a suitable alternative.

Note that, prior to this been agreed, the UK NEQAS ICC & ISH team may refer to that year's antibody repertoire declaration made by the participant to confirm non-access to the antibody.

The data that UK NEQAS ICC & ISH collects annually via the antibody survey helps to determine which antigens will be chosen for the EQA year: The scheme tries to include mostly those antigens against which suitable antibodies are stocked by at least 95% of laboratories. Given this, it is expected that most laboratories will stock antibodies against most of the antigens listed. However, UK NEQAS does appreciate that there are several specialist centres, which may only stock and use markers within a limited area of expertise.

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. Or do not submit as unauthorised non-submissions are treated as fails.

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.

Assessment Procedure

Typically, in the diagnostic Modules, participants are asked to demonstrate two different antigens at each assessment run (in the Predictive Biomarker Modules one antigen/gene is examined at each run).

Participants are asked to stain the UK NEQAS sections using their routine method and return for assessment, along with their usual in-house control slide placed on the same

slide as the UK NEQAS material(s).

For some Modules, we may request an antigens from one assessment to the next over a number of runs. This would usually be implemented where a large number of participants had received sub-optimal scores and it allows participants to implement recommended changes to improve the quality of staining.

Participants are also required to complete details of the antibody and method they have employed on the web-based data collection forms.

Returned slides are assessed for technical quality by a panel of expert assessors comprising a mixture of senior biomedical scientists, clinical scientists, consultant histopathologists and cytopathologists. All assessors are evaluated, approved, and appropriately trained by the Scheme prior to assessing participants EQA submissions.

9. Assessment Scoring and Interpretation

This section details the guidelines assessors use when scoring participants submissions.

General assessment guide

1. Each one of the four assessors independently award a mark out of '5' using the guidelines shown in Table 2.
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.

Semi-quantitative assessments i.e., ones which have both tissues and cell lines as part of their assessed materials.

In general, a submission in which **one sample** (which can be either a tissue or a cell line) shows staining which is interpreted as being outside the expected range will receive a borderline acceptable score (12/20). If, however, **two or more samples** show out of range staining the submission will receive a score indicative of a fail (8/20).

Individual assessor scoring guide

Table 2 shows in summary the criteria our assessors use when allocating their marks.

Note that, where marks have been deducted the reason will usually be shown on individual participant reports. And, where scores of '3' or less are allocated, assessors are mandated to provide feed-back comments to explain the reason and to provide advice for corrective actions. In the case of in-house controls, marks may be deducted for the

use of inappropriate and/or inadequate control materials.

Inter-Assessor Agreement

A variance of 1 mark is allowed between assessors when assessing any given submission e.g. a mix of 4's and '5's is acceptable. This permits more 'granularity' in the final score achieved and reflects the fact that to some extent the score given by any one assessor has inevitable element of subjective variability attached to it.

Assessor's Score	Interpretation
0	**No submission**
1	**Unreadable** Clinically uninterpretable. Staining has no utility. Improvement essential. No significant demonstration of requested antigen. Excessive non-specific and/or inappropriate staining. Significant morphological damage caused by excessive pretreatment. Very poor tissue or section quality. Excessive haematoxylin counterstain completely obscuring specific ICC staining.
2	**Sub-optimal preparation that is clinically unsafe** Clinically uninterpretable. Staining has no utility. Improvement essential. Very weak demonstration of requested antigen, significantly below the expected level. For quantitative biomarkers: staining that is stronger than the expected level. Excessive non-specific and/or inappropriate staining. Significant morphological damage caused by excessive pretreatment. Very poor tissue/section quality. Excessive or very weak/absent haematoxylin counterstain.
3	**Sub-optimal preparation that is clinically readable** Although clinically interpretable with immunostaining considered to be appropriate for the target in question, the staining quality is sub-optimal, and improvement is essential. Weak demonstration of antigen, below the expected level. Non-specific and/or inappropriate staining is present but does not make the staining uninterpretable. Some morphological damage caused by excessive pretreatment. Poor tissue/section quality. Excessive or very weak haematoxylin counterstain.
4	**Good preparation that is clinically readable** Clinically interpretable with immunostaining appropriate for the target in question and of good quality. Minor improvements are possible. Demonstration of requested antigen, at the expected level of sensitivity. No non-specific and/or inappropriate staining. Good tissue and morphological preservation. Correct level of haematoxylin counterstain. Some minor aspect(s) of the preparation are not optimal.
5	**Excellent preparation that is clinically readable** Clinically interpretable with immunostaining appropriate for the target in question and of excellent quality. No improvements are required. Demonstration of requested antigen, at the expected level of sensitivity. No non-specific and/or inappropriate staining. Good tissue and morphological preservation. Correct level of haematoxylin counterstain.

Table 2. Individual assessor scores and their interpretation.

Scores between any two assessors which vary by >1 mark are not deemed to be sufficiently closely aligned e.g., a score of 3 and a score of 5. They are automatically 'flagged' by the assessment software in real-time. And, in those situations, assessors are required to agree on amended more closely aligned scores by a process of consensus review.

Distinction Between Individual Assessor Scores of '3' and '2'

An exception to the procedure of allowing a variance of 1 mark occurs when assessors are making the distinction between staining which is substantially sub-optimal, but still

clinically readable (score = 3), and staining which is sub-optimal to the degree of being of no clinical value (score = 2). These two score categories are mutually exclusive, and we therefore require unanimous consensus amongst our assessors on one or other of them.

Consequently, combined assessment scores of '9', '10' and '11' are not allocated to participants submissions by the Scheme.

Combined Assessment Scores

Participants receive a combined assessment score as a final indication of staining quality. Table 3 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 3. Interpretation of final score, produced from the 4 assessor's combined scores.

Breast HER2 IHC assessment guide

The following procedures and criteria are used in this assessment:

- Assessors evaluate each of the UK NEQAS distributed samples and provide an interpretation on the membrane staining.
- Each of the four assessors score independently using an adapted method initially devised by the Clinical Trials Assay where percentage positivity and membrane intensity are both considered.
- Assessors provide an overall score out of '5', with the four assessors' marks being added together to give a score out of '20'.
- Cell line samples are usually distributed for the Breast HER2 IHC module.
- Cell-lines show considerably less variation in their staining than do tumour tissues, but they are biological materials and they can show variability mainly due to where they are in the cell-cycle. Therefore, the overall percentage staining criteria cannot be absolutely applied, and for this reason, reference sections are

prepared by staining every 50th cut section using HER2 IHC standardised kits/assays (Agilent Dako HercepTest, Leica Oracle and Ventana Pathway 4B5). This provides a reference point to gauge the expected level of staining of participants submitted slides.

Assessors examine each sample, looking for the presence of expected cell membrane staining patterns. Assessors will mark down or fail a participant stain for the following reasons: UK NEQAS samples: Insufficient or excessive membrane staining; false positive/negative membrane staining. UK NEQAS and In-House samples: Excessive cytoplasmic/background staining; excessive/insufficient haematoxylin staining; morphological damage; poor quality of in-house control tissue, poor/inadequate choice of control tissue, poor/inadequate fixation of in-house material.

UK NEQAS Cell Line	Expected Staining	Descriptive
A: SK-BR-3	3+	Cells show strong complete membrane staining.
B: MDA-MB-453	2+	Complete membrane staining in most cells, of weak to moderate intensity
C: MDA-MB-175	1+	Cells show only partial membranous staining
D: MDA-MB-231	0	Cells are not stained

Table 4. Expected staining patterns of the UK NEQAS cell lines.

'U' Scores: assessors may also give a score of 'U', which indicates that the staining is 'uninterpretable'.

Once the membrane staining has been interpreted for each of the UK NEQAS samples, assessors then provide an overall score out of '5', based on the interpretability of the membrane staining and technical quality. The four assessor's scores are then combined to give a possible score out of '20' marks:

Final Score	Interpretation
0	**No submission.**
4 - 8	**UNACCEPTABLE** Unsuitable quality for clinical interpretation and technical improvements must be made. Marks may have been deducted due to: • Weaker/stronger than the expected level of membrane staining; • False positive/negative membrane staining; • Excessive cytoplasmic staining; • Excessive morphological damage; • Excessive staining of normal glands.
12	**BORDERLINE ACCEPTABLE** Overall, the samples are borderline interpretable. Indicating that while still being clinically relevant, technical improvements need to be made. Marks may have

	been deducted due to: • Weaker/stronger than expected membrane staining; • Some cytoplasmic staining; • Morphological damage.
13 - 15	**ACCEPTABLE** Some slight technical issues noted by some of the assessors, but overall, the staining is suitable for interpretation.
16 - 20	**GOOD to EXCELLENT** All assessors agree that, overall, for the samples distributed, the staining is at the expected level for each of the distributed samples.

Table 5. Interpretation of final score, produced from the four assessor's combined scores.

GASTRIC HER2 IHC ASSESSMENT GUIDE

UK NEQAS ICC & ISH uses an EQA specific scoring criteria when scoring the tissue sections, so as to provide participants with additional technical feedback (see Table 6).

- The Gastric HER2 scoring system is based on the original guidelines set out by Hoffman and Ruschcoff for surgical resections. The updated guidelines (Bartley et al. 2017) made no changes to the assessment of HER2 in gastric carcinoma.
- Prior to 0dispatch, and due to the heterogeneity of gastric tissue, reference sections are prepared and stained at approximately every 25th - 28th serial section using the currently available commercial kits. Samples are further validated by ISH.
- The UK NEQAS distributed Gastric HER2 slides include formalin-fixed paraffin-embedded gastric carcinoma samples with a varying range of HER2 protein expression levels. The samples do not necessarily always include (and do not necessarily run in the order of) a 3+, 2+, 1+ and 0 at each assessment run.
- During the assessment, samples are assessed independently around a multi-header microscope, with each of the 4 assessors providing their interpretation on the membrane staining.

Expected Staining	Assessment Criteria
3+	• 3+: staining is expected. • 3+/2+: 3+ membrane staining is present but also showing 2+ staining.
2+	• 2+: staining is expected. • 2+/1+: 2+ membrane staining is present but also showing 1+ staining. • 2+/3+: 2+ membrane staining is present but also showing 3+ staining.
1+	• 1+: staining is expected. • 1+/0: staining is more towards the weaker end of 1+ staining but still

	acceptable.
0	• 0: staining is expected. • 0/1+: cells are starting to show very weak membrane staining.

Table 6. Expected staining patterns of the gastric control samples.

'U' Scores: assessors may also give a score of 'U', indicating the sample is uninterpretable and substantial improvements are required. Any membrane score outside the range for each of the expected scores as indicated in Table 6 is deemed to be unacceptable. When membrane interpretation for each of the samples is complete, an individual score out of 5 is awarded, based on the interpretability of the membrane staining and the technical feedback. An overall mark is awarded by combining the four assessor's scores to give a score out of 20 (Table 7).

Final Score	Interpretation
0	**No submission.**
4 – 8	**UNACCEPTABLE** Unsuitable quality for clinical interpretation and technical improvements must be made. Marks may have been deducted due to: • Weaker/stronger than the expected level of membrane staining; • False positive/negative staining; • Excessive non-specific staining; • Excessive morphological damage.
12	**BORDERLINE ACCEPTABLE** Overall, the samples are borderline interpretable. Indicating, that while still being clinically relevant, technical improvements need to be made. Marks may have been deducted due to: • Weaker/stronger than expected membrane staining; • Excessive non-specific/background staining; • Morphological damage.
13 – 15	**ACCEPTABLE** Some slight technical issues noted by some of the assessors, but overall, the staining is suitable for interpretation.
16 – 20	**GOOD to EXCELLENT** All assessors agree that, overall, for the samples distributed, the staining is at the expected level for each of the distributed samples.

Table 7. Interpretation of final score, produced from the four assessor's combined scores.

NSCLC ALK IHC Assessment Guide

The UK NEQAS distributed material may contain up to six samples at any given Assessment Run. It will usually include a mixture of cell lines, non-small cell lung cancer (NSCLC) tissue samples of known ALK IHC expression and appendix. Reference sections are prepared by staining every 25th-28th cut sections using the Ventana ALK D5F3 companion diagnostic (CDx) assay. This provides a reference point to gauge the expected level of staining of participants submitted slides.

Assessments are carried out by four assessors scoring independently. Each assesses the UK NEQAS distributed samples and provide an interpretation on the staining intensity (scoring as 3+, 2+, 1+ or 0).

'U'/Uninterpretable Scores: Assessors may also give a score of 'U', which indicates that the cell lines / tissue sections are 'uninterpretable'.

Assessors will then also provide an overall score out of '5' with the four assessors' marks added together to give a possible score out of 20 as shown in Tables 6 and 7 above (same criteria as those used for the Gastric HER2 Module).

NSCLC PD-L1 IHC (pilot) Assessment Guide

The UK NEQAS distributed material may contain up to eight samples at any given Assessment Run It will usually include a mixture of cell lines, NSCLC tissue samples of known PD-L1 IHC expression and tonsil tissue. Reference sections are prepared by staining every 25th -28th cut sections using the Ventana/Roche and Dako/Agilent PD-L1 NSCLC IHC assays. This provides a reference point to gauge the expected level of staining of participants submitted slides.

- Assessments are carried out by assessors scoring independently out of '5', and then the average of the four assessors marks are provided as a total score out of 20. Each assesses the UK NEQAS distributed samples and provide an interpretation. The tonsil section is scored as Acceptable or Unacceptable, and the cell lines and lung tumour samples are interpreted on the percentage of tumour cells staining as 0 or <1% (negative), 1-4%, 5-9%, 10-24%, 25-49%, 50-79% and 80-100%.
- 'U'/Uninterpretable Scores: assessors may also give a score of 'U' which indicates that the cell lines / tissue sections are 'uninterpretable'.

Breast HER2 ISH Interpretive assessment guide

- At each assessment, laboratories are sent FFPE processed samples of known HER2 ISH status.
- Participants should assess the materials for HER2 gene amplification in accordance with current HER2 ISH guidelines using either:
- a dual probe assay (HER2/Cep17: ratio method)
- OR
- a single probe assay (HER2 copy).

- Participants are required to complete and return scores for each sample using the online data entry system. They are also requested to input their methodology data to provide brief details of the probe and method they have employed.
- In this module, a different panel of breast cancer specimens will be sent at each assessment to ensure coverage of the critical diagnostic ranges.

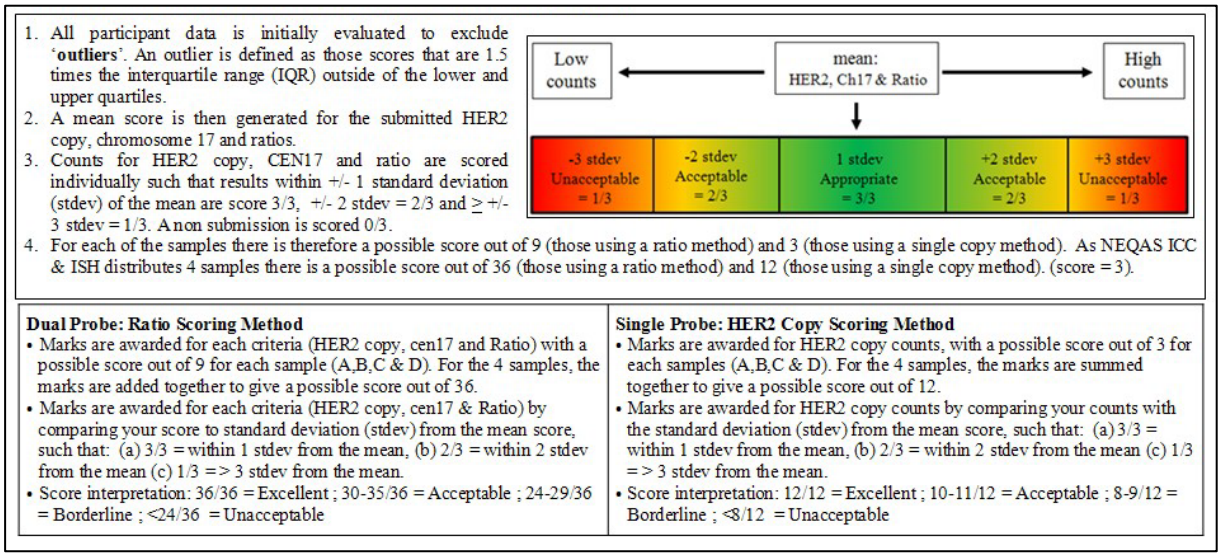


Figure 2. Statistical approach used in the ISH interpretive scoring system.

Assessment of slides utilises a statistical method in order to provide concise information with regards to the inter-observer variability in enumerating *HER2* copy, chromosome 17 and overall ratios (see Figure 2 above).

Dual Probe		**Single Probe**	
Score	**Performance Descriptor**	**Score**	**Performance Descriptor**
36/36	Excellent	12/12	Excellent
30-35/36	Acceptable	10-11/12	Acceptable
24-29/36	Borderline	8-9/12	Borderline
<24/36	Unacceptable	<8/12	Unacceptable

Table 8. Interpretation of final score.

Breast HER2 ISH Technical Assessment Guide

Chromogenic *in-situ* hybridisation (CISH) slides are technically assessed around a multi-header microscope with each slide being assessed by four independent assessors. Each assessor provides a score out of '5', and then scores are added together to give a final score out of 20.

Fluorescent *in-situ* hybridisation (FISH) slides are technically assessed by a team of assessors at the same time, by incorporating a live-feed video from the fluorescence microscope with the image viewed on a large high-definition monitor, allowing up to eight

assessors to view and score the FISH slides at the same time, and then the consensus of the assessors' marks is provided as a total score out of 20.

Assessors examine the quality of the ISH staining but DO NOT carry out probe enumeration. This is evaluated during the HER2 ISH interpretive assessment. Technical evaluation scoring procedure and criteria for interpretation are shown in the guidelines given in Table 9.

Individual Assessor Scores (see Note 1)	**Overall Scores** (see Note 2)	**Score Interpretation**
0	0	**No submission**
1 and 2	4 - 8	**UNACCEPTABLE** The UK NEQAS distributed and/or in-house samples are uninterpretable. Potential features: • Excessive or very weak/absent nuclear (DAPI) staining; • Poor probe hybridisation; • Missing HER2 or CEP17 signals, leading to incorrect copy number evaluation; • Excessive background staining.
3	12	**BORDERLINE ACCEPTABLE** The UK NEQAS distributed and/or in-house samples are interpretable, but substantial improvements in quality of staining must be made. Potential features: • Weak nuclear counter-staining; • Weak HER2 and/or CEP17 signals; • Background staining.
3 and 4	13 - 15	**ACCEPTABLE** The UK NEQAS distributed and/or in-house samples show a good standard of staining and are suitable for interpretation. Minor non-critical defects are present.
4 and 5	16 - 20	**GOOD to EXCELLENT** The UK NEQAS distributed and/or in-house samples show a very good standard of staining and are optimal for interpretation.

Table 9. Individual and combined assessment scores and their interpretation.

Note 1: individual assessor's scores are applicable to the CISH assessment only, where each assessor awards a mark between 0 – 5. Note 2: combined assessment scores are produced for both the CISH and FISH assessments, with the range being 0 – 20.

PD-L1 IN TRIPLE NEGATIVE BREAST CANCER (TNBC) ASSESSMENT GUIDE

The UK NEQAS distributed material usually contain seven samples at any given Assessment Run It will usually include a set of four cell lines, two TNBC tissue samples

of known PD-L1 IHC expression (negative and positive respectively) and tonsil tissue. Reference sections are prepared by staining every 25th -28th cut sections using the Ventana/Roche and Dako/Agilent PD-L1 assays (SP142 and 22C3). These provides reference points to gauge the expected level of staining of participants submitted slides.

- Assessments are carried out by assessors scoring independently out of '5', and then the average of the four assessors marks is provided as a total score out of 20. Each assesses the UK NEQAS distributed samples and provide an interpretation. The tonsil section is scored as Acceptable or Unacceptable, and the cell lines and lung tumour samples are interpreted on the percentage of tumour cells staining as 0 or <1% (negative), 1-4%, 5-9%, 10-24%, 25-49%, 50-79% and 80-100%.

- 'U'/Uninterpretable Scores: assessors may also give a score of 'U' which indicates that the cell lines / tissue sections are 'uninterpretable'.

Troubleshooting Interpretive and Technical module Results

Combining the results from the 'Interpretive' and 'Technical' HER2 ISH modules, allows laboratories to further troubleshoot their techniques as shown in Table 10 on the next page.

Technical Assessment Result	Interpretive Assessment Result	Interpretation and Recommended Actions
Acceptable	Appropriate or Acceptable	The UK NEQAS distributed samples show a good standard of staining and have been interpreted correctly. **No corrective action is required.**
Acceptable	Unacceptable	The UK NEQAS distributed samples show a good standard of staining BUT there is an issue with interpretation i.e., HER2 copy number and/or CEP17 incorrectly assessed. **Recommend that scoring/counting criteria are reviewed.**
Unacceptable	Appropriate or Acceptable	The technical staining quality of the UK NEQAS distributed samples is poor and therefore not suitable for interpretation. Although interpretation of these samples by the participant is correct their staining quality if present in clinical cases may lead to misinterpretation. **Recommend that technical method is optimised (or that a standardised kit/assay is used as per manufacturer's instructions).**
Unacceptable	Unacceptable	Overall, the NEQAS samples are unacceptable for technical staining and interpretation. Reporting from such cases is very likely to lead to incorrect interpretation of clinical cases. **If there is persistent underperformance:** • Seek assistance from kit/assay manufacturer.

		• Seek assistance from UK NEQAS or colleagues. • Re-validate protocol (retrospectively and prospectively). • Review scoring criteria. • Consider sending out clinical cases to a referral Centre to verify in-house results.

Table 10. Troubleshooting guidelines

10. In-house Control Tissues: Requirements and Recommendations

- In-house samples should be placed onto UK NEQAS distributed slides as shown in Figure 1 in 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.

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.

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.
NSCLC ROS1 IHC	ROS1-positive and ROS1-negative NSCLC
Breast HER2 ISH	A single sample consisting of an invasive breast tumour.
Breast Hormonal Receptors (ER	Participants in-house control tissue MUST consist of composite breast tissue (see also Note 1 about use of cell lines):

and PR)	• >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 provided the participant indicates the areas and expected levels of staining.
Breast HER2 Low IHC	In-house control material MUST include samples from 2+, 1+ and 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., 2+ and 1+ membrane expression provided 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 ROS-1 -negative NSCLC
PD-L1 in TNBC (Pilot)	Tonsil together with a positive and a negative TNBC sample
Ki-67 in Breast Cancer (Pilot)	Tonsil together with a breast cancer sample showing low proliferation (5% or less) and one showing high proliferation (20% or more)
p16 in Head &	A tonsil together with a head & neck carcinoma showing no staining for p16

Neck (Pilot)	and one showing high expression of p16
High-risk HPV in Head and Neck (Pilot)	A tonsil together with a head & neck carcinoma showing no staining for high-risk HPV and one showing expression of high-risk HPV.
PD-L1 in Head & Neck (Pilot)	PD-L1-positive and PD-L1-negative HNSCC together with tonsil
Claudin 18.2 in Gastric cancer (Pilot)	Claudin-positive and Claudin-negative gastric/gastro-oesophageal tumour together with normal gastric mucosa
Sarcoma (Pilot)	Positive sarcoma of a type appropriate for the antigen examined.
Melanoma	Positive melanoma of a type appropriate for the antigen examined.

Table 11. In-House 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.

11. 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 EQA-Manager portal.

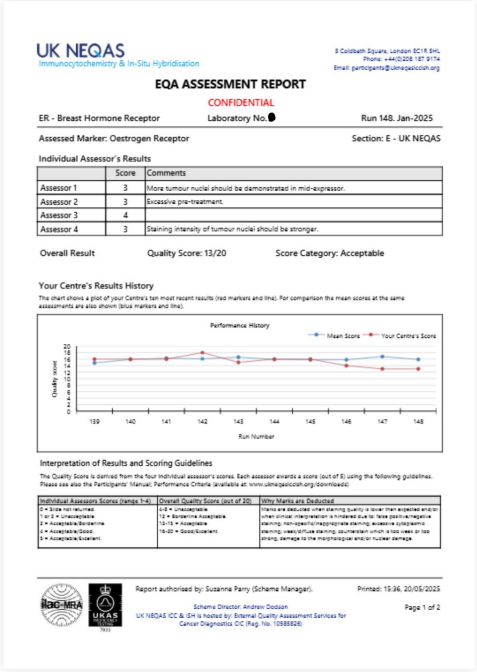
Participants also have access to graphs, technical tables showing antibodies used, automation systems and retrieval methods, along with images showing optimal and poor examples of staining. Furthermore, 'Best Methods' are also generated from anonymised

participant technical data.

Individual Participant Reports

All individual reports consist of:

- The individual assessors' scores out of 5 and total score out of 20;.
- Assessor feedback when appropriate.
- A bench-marking graphical panel showing the results for the participant over the course of 10 assessments compared to the group average.



UK NEQAS
Immunocytochemistry & In-Situ Hybridisation

EQA ASSESSMENT REPORT

CONFIDENTIAL

ER - Breast Hormone Receptor Laboratory No.● Run 148, Jan-2025

Assessed Marker: Oestrogen Receptor Section: E - UK NEQAS

Individual Assessor's Results

	Score	Comments
Assessor 1	3	More tumour nuclei should be demonstrated in mid-expressor.
Assessor 2	3	Excessive pre-treatment.
Assessor 3	4	
Assessor 4	3	Staining intensity of tumour nuclei should be stronger.

Overall Result Quality Score: 13/20 Score Category: Acceptable

Your Centre's Results History

Performance History

Mean Score Your Centre's Score

Quality score

Run Number

Interpretation of Results and Scoring Guidelines

Individual Assessors Scores (range 1-4)	Overall Quality Score (out of 20)	Why Marks are Deducted
0 = Slide not returned. 1 or 2 = Unacceptable. 3 = Acceptable/Borderline. 4 = Acceptable/Good. 5 = Acceptable/Excellent.	0-8 = Unacceptable. 12 = Borderline Acceptable. 13-15 = Acceptable. 16-20 = Good/Excellent.	Marks are deducted when staining quality is lower than expected and/or when clinical interpretation is hindered due to: false positive/negative staining; non-specific/inappropriate staining; excessive cytoplasmic staining; weak/diffuse staining; counterstain which is too weak or too strong; damage to the morphological and/or nuclear detail.

Report authorised by: Suzanne Parry (Scheme Manager). Printed: 15:36, 20/05/2025

Scheme Director: Andrew Dodson

UK NEQAS ICC & ISH is hosted by: External Quality Assessment Services for Cancer Diagnostics CIC (Reg. No. 10888626)

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Figure 3. Participant report example from the breast hormonal receptor module.

Graphical Data

Graphs are provided showing the distribution of pass rates for a particular run on both the UK NEQAS ICC and in-house samples.

This allows individual participants to gauge their performance against the rest of the participants. An example is shown below.

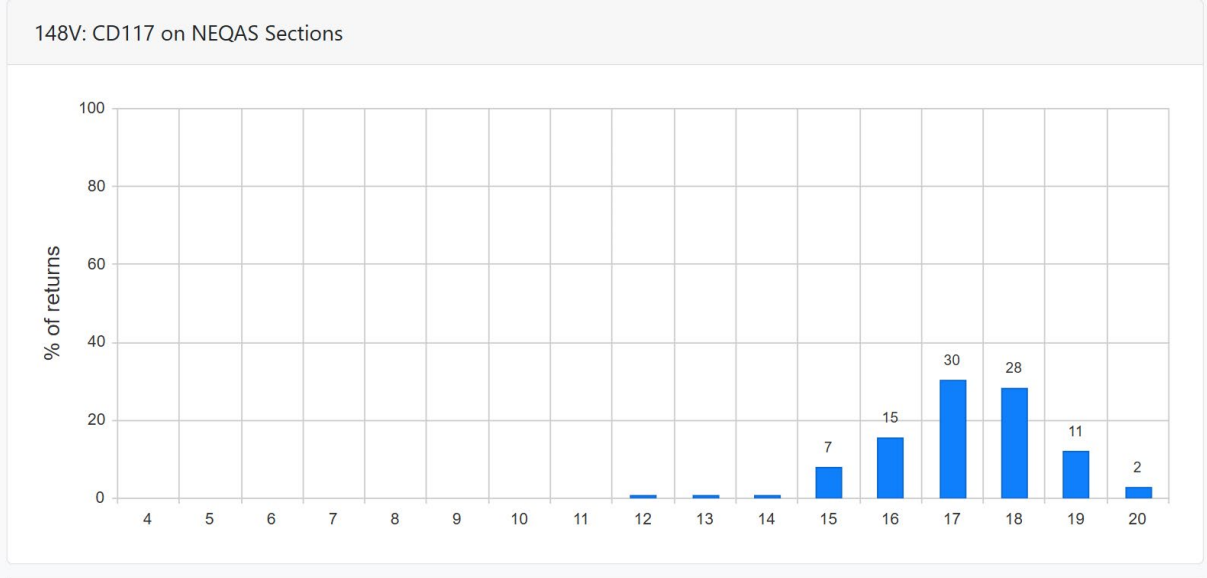

Figure 4. Example of the graphical reports from the Alimentary Tract module.

TECHNICAL DATA

Technical tables, showing participant choice of antibodies, automation systems, and retrieval methods are also provided. The data show the number of participants using a particular method (N) along with the percentage (%) that have achieved an acceptable score using the selected parameters (score≥12/20 in the case of most modules).

Antibody Summary for Tau Protein

Value	Count	Percentage
Dako / Agilent Rb Polyclonal (Concentrate) A0024	2	50
Other (please specify) Specify antibody clone, product code and supplier	3	66.67
Sigma Aldrich AT8 (Rb Polyclonal, concentrate) T6402	1	0

Figure 5. Example of the technical reports from the Neuropathology module

Selected 'Best Methods' in Reports

Best methods can be selected from any of the Modules. They can be modified to select the participants own requirements

12. Poor Performance Monitoring (UK Clinical Laboratories Only)

All UK NEQAS schemes are required by their accrediting body, UKAS (ISO/IEC 17043:2023), to have in place a formal system whereby performance of their UK clinical laboratory-based participants is monitored.

To aid laboratories in the interpretation of their performance status UK NEQAS ICC & ISH uses a 'traffic light' system.

Colour Code	Descriptor
GREEN	Participant does not have any issues with poor performance.
AMBER	Issues with poor performance, managed locally between the Scheme and the participant.
RED	Poor performance issues remain unresolved; participant is designated as a persistent poor performer.

Table 12. 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.

Each Module is treated as a separate entity; low scores from one Module are not combined with low scores from another to produce a poor performance.

Failure to resolve a RED status in a timely fashion will result in referral to the NQAAP for Cellular Pathology

It is important that a laboratory which has underperformed continues to participate at subsequent Assessment Runs in order that their continuing performance can be correctly judged (please note that un-sanctioned non-submission counts towards poor performance).

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

When a participant has received one score (in Predictive Biomarker Modules) or two scores (in Diagnostic Biomarker Modules) indicative of underperformance(s), the scheme will contact the participant with 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. Performance status remains **GREEN** at this stage.

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.

If a laboratory has not submitted for a run, then the EQA provider (UK NEQAS ICC & ISH) should be provided with 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 accepted.

This includes submission to all non-Pilot.

Action	Trigger Point	Monitoring Procedure
Offer of Assistance Letter	**Two** unacceptable scores (≤8/20) over 5 runs on UK NEQAS Gold or second antibody assessments.	Participant nominated contact is notified of repeated underperformance. Participant will be offered assistance to improve.
AMBER STATUS	**Three** unacceptable scores (≤8/20) over 5 runs on UK NEQAS Gold or second antibody assessments.	Participant nominated contact and Head of Department are notified of repeated underperformance. A 'Warning letter' is issued indicating that they are close to being deemed a poor performer.
RED STATUS	**Four** unacceptable scores (≤8/20) over 5 runs on UK NEQAS Gold or second antibody assessments.	Participant nominated contact and Head of Department are notified of repeated underperformance. A 'Red letter' is issued indicating that they are deemed to be a poor performer and are required to contact the Scheme Director.

Table 13. Sub-optimal performance action for Modules assessing diagnostic biomarkers.

Although in-house sections are not part of poor performance monitoring, they may be used to gauge overall performance in cases of poor performance. Participants should make every effort to submit appropriate control material for the antigen requested.

Predictive Biomarker Modules

Because of the direct impact that the results of assays for predictive biomarkers have on patient management, more stringent performance monitoring mechanisms are employed.

Modules designated as assessing biomarker include:

- Breast Pathology Hormone Receptors (ER and PR)
- Breast Pathology HER2 IHC
- Breast Pathology HER2 ISH
- Gastric Pathology HER2 IHC
- NSCLC ALK IHC

Note that predictive biomarker Modules currently in Pilot are not performance assessed.

Action	Trigger Point	Monitoring Procedure
Offer of Assistance Letter	**One** unacceptable score, (≤8/20) over 5 runs on UK NEQAS Gold or second antibody assessments.	Participant nominated contact is notified of repeated underperformance. Participant will be offered assistance to improve.
AMBER STATUS	**Two** unacceptable scores, (≤8/20) over 5 runs on UK NEQAS Gold or second antibody assessments.	Participant nominated contact and Head of Department are notified of repeated underperformance. A 'Warning letter' is issued indicating that they are close to being deemed a poor performer.
RED STATUS	**Three** unacceptable scores, (≤8/20) over 5 runs on UK NEQAS Gold or second antibody assessments.	Participant nominated contact and Head of Department are notified of repeated underperformance. A 'Red letter' is issued indicating that they are deemed to be a poor performer and are required to contact the Scheme Director.

Table 14. Sub-optimal performance action for Modules assessing predictive biomarkers.

Although in-house sections are not part of the poor performance monitoring system, they may also be used to gauge overall performance status in cases of poor performance. Participants should make every effort to submit appropriate control material for the antigen requested.

Poor performance monitoring is carried out over a rolling five-assessment period 5. Participants may receive a letter to confirm their current status or continuing (e.g., Amber or Red) even if this may have been triggered at a previous Assessment Run.

If a laboratory's status changes following an appeal (reassessment), a revised letter will be sent to confirm the new status.

13. 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.

14. 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.

15. Annual Report

Participants receive a summary of the results they achieved over the preceding year (annual report) for each Module they have participated in.

16. Meetings and Practical Workshops

Participant and scientific meetings, and practical workshops are organised, details of which are distributed to all UK NEQAS ICC & ISH subscribers.

These meetings provide opportunity for participants to discuss ICC and ISH techniques and applications and EQA related matters with the Scheme's assessors and UK NEQAS ICC & ISH personnel.

17. 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 listing on the website of the Scheme's accrediting body, UKAS (https://www.ukas.com/download-schedule/7833/ProficiencyTesting/).

18. 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 each Assessment Run during the EQA year is required.

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 beyond its control requires it to be done.

More details about the antigens and genes assessed can be found by referring to our published scope as listed on the UKAS website here: https://www.ukas.com/download-schedule/7833/ProficiencyTesting/

General Pathology

Antigens will be chosen from those commonly used in the diagnostic work-up of tumours.

Breast Pathology Hormonal Receptors (ER and PR)

Oestrogen Receptor (ER) and Progesterone Receptor (PR)

Breast Pathology HER2 IHC

Formalin fixed and paraffin processed cell lines showing the full range of HER2 IHC expression (3+, 2+, 1+ and 0).

Lymphoid Pathology

Antigens used for the general and specialised diagnostic, prognostic and predictive work-up of solid lymphoid proliferations to include lymphomas and bone marrows.

Neuropathology

Antigens used for the general and specialised diagnostic, prognostic and predictive work-up of tumours presenting in the central nervous system.

Cytopathology

Antigens used for the general and specialised diagnostic, prognostic and predictive work-up of tumours presenting diagnostic cytopathology.

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.

CD 117 and Associated Markers (GIST)

The primary antigen which will be requested at each Run: CD117 (c-KIT)

Second antibody/antigens, one of these will be requested on a rotational basis at each Run: DOG-1, Desmin, CD34, S100 and CDX2

Gastric HER2 IHC

Formalin-fixed paraffin-embedded gastric cancer tissue from excision samples showing varying levels of HER2 membrane protein expression.

Breast HER2 ISH (Technical and Interpretive)

Formalin-fixed paraffin-embedded breast tumour samples.

NSCLC ALK IHC

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.

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.

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 gene status.

Mis-Match Repair Proteins

- MLH1 and PMS2
- MSH2 and MSH6

The antigen pairs will be requested at alternate Assessment Runs.

NSCLC ROS1 IHC (PILOT)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded cell lines and/or lung tumour samples of known gene status and a sample of normal lung.

TNBC PD-L1 (Pilot)

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

Ki-67 in Breast Cancer (Pilot)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded breast tumour tissue from excision samples with varying levels of Ki-67 IHC expression. UK NEQAS samples will also include a piece of tonsil (reactive).

Head & Neck Pathology – p16 (Pilot)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded head & neck tumour tissues from excision samples with varying levels of p16 IHC expression (usually negative and high-level expression). UK NEQAS samples will also include a tonsil sample.

Head & Neck Pathology – high-risk HPV (Pilot)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded head & neck tumour tissues from excision samples with varying levels of high-risk HPV expression by ISH (including negative and strongly positive). UK NEQAS samples will also include a tonsil sample.

Head & Neck Pathology – PD-L1 (Pilot)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded head & neck tumour tissues from excision samples positive and negative for PD-L1 expression and a tonsil sample. Cell lines showing varying degrees of staining for PD-L1 negative to strong-positive (approximately 80% positive expression) will also be included.

Low HER2 in Breast Cancer (Pilot)

UK NEQAS distributed samples will consist of formalin-fixed paraffin-embedded breast cancer tumour tissues from excision samples with varying levels of HER2 expression by ICC (including negative (0), 1+ and 2+).

Melanoma

Antigens used for the general and specialised diagnostic, prognostic and predictive work-up of cutaneous melanoma are assessed. The material distributed will comprise tissue samples expressing the antigen under examination.

Sarcoma

Antigens used for the general and specialised diagnostic, prognostic and predictive work-up of sarcoma. The material distributed will comprise tissue samples expressing the antigen under examination.

Claudin in Gastric Cancer

Claudin 18.2 over-expression in gastric and gastro-oesophageal cancer (G/GEJ). The material distributed will comprise G/GEJ tumour samples negative for Claudin and ones over expressing the antigen.

19. UK NEQAS ICC & ISH Contact Details

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
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
Kally Sidhu	Quality Manager & Medical Laboratory Technical Officer	ksidhu@ ukneqasiccish.org
Marie Stoddart	Senior Administrator	mstoddart@ukneqasiccish.org
David Evans	Medical Laboratory Assistant	devans@ ukneqasiccish.org

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

20. UK NEQAS ICC & ISH Assessors

UK NEQAS ICC & ISH assessments are a team effort, our assessors are a key part of that team. We rely very heavily on their expert help and advice and are very grateful to them.

21. Replacement slides

Replacement slides are available upon request. Please indicate your reason for requesting a replacement in your email e.g., slide broken upon receipt, slide broken in laboratory, quality of the result is sub-optimal and requires repeat staining. Please also include your Participant Code.

Contact the Admin team. Email: info@ukneqasiccish.org; Telephone: +44(0)208 187 9174

22. 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.

23. 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).

24. 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.

25. Conflict of Interest and Impartiality Declaration

All UK NEQAS ICC & ISH employees and staff members, members of scientific advisory panels (including all active Assessors) complete an annual Conflict of Interest and Impartiality declaration. These are reviewed to ensure there is no potential for Participants' results to be subject to biased assessment.

Additionally, all staff members sign declarations as part of their Induction procedures when they join UK NEQAS ICC & ISH.

26. Discriminatory Action

The Scheme takes steps to avoid the possibility of discriminatory action resulting from Participant appeals or complaints.

In regard to appeals, these are dealt with in an anonymised way such that:

- the Participant Number is not known to the Assessor's making the reassessment.
- nor is it revealed or discussed with the assessors who performed the original assessment.

In regard to complaints:

- these are dealt with by the Scheme Organiser who maintains the Participants Identification Number confidential.
- In a case where the complaint requires discussion with one or more Assessor (either staff-based or external), the Participant Number is never revealed.

27. Associated Schemes

Cellular Pathology Techniques

Participants are assessed for the quality of their staining preparations in both Haematoxylin and Eosin-stained sections and special staining methods. For further information please contact the Scheme's using its general contact email address: cpt@ukneqas.org.uk

Molecular Pathology

GenQA provides an EQA service for a variety of molecular tests on a range of diseases. Test are carried out on the patient tumour samples providing an EQA service for a variety of molecular tests, including, Non-small cell lung cancer, Colorectal cancer, Melanoma, and Gastrointestinal Stromal Cancer. For further information please contact Dr Sandi Deans (Scheme Director); sandi.deans@ed.ac.uk

28. Steering Committee for Technical EQA Schemes in Cellular Pathology

Chairperson: Guy Orchard (guy.orchard@synnovis.co.uk).

29. General Terms and Conditions

An active participant (laboratory, organisation or individual) subscribed to our services, agrees to, and acknowledges the following:

- Inform UK NEQAS ICC & ISH of any change of personnel or contact details.
- Quote your unique participants' code whenever contacting UK NEQAS ICC & ISH.
- Ensure slides are securely packaged to prevent breakages and possible non-assessment and returned in the correct labelled slide boxes to aid sorting.
- Ensure slides are clearly labelled and concealing your site's identity.
- Adhere to submission deadlines – late submissions will be logged by the scheme.
- Prompt payment of subscription fees, your account may be suspended if payment is not received.
- Antibody repertoires, non-declaration of this may lead to a non-submission (0 score) and possible poor performance issues.
- Follow specific staining requirements for each of the subscribed modules.
- Complete entry of methodology protocols.
- Declares that the methodology submitted is the same method used in the

routine setting of the laboratory.
- Producing local procedures for EQA, including handling and interpretation of results.
- Respect the anonymity and confidentiality aspect of EQA when corresponding with other laboratories.
- Suspected collusion and/or falsification of results, data or manipulation of EQA slides will result in the participant/s being suspended from UK NEQAS ICC & ISH.
- UK NEQAS ICC & ISH requests as wide a range of markers for each module as possible but cannot cover all antigens or tissue types. Participants should have their own alternative performance assessment activities to cover their repertoire.

Provided assessment results, although confidential to each participant, may be used by the participant as they see fit (e.g., printed, placed on website etc). However, under no circumstances

If individual reports are used in any form, then the accompanying statement should be included:

"Participation in UK NEQAS ICC & ISH is not an indication of the overall performance of the participant (laboratory, organisation or individual), and as such is not an endorsement of the overall quality of staining produced".

30. Selected References

Recent Publications from the Scheme

- Dodson A, Parry S, Ibrahim M, et al. (2018). Breast cancer biomarkers in clinical testing: analysis of a UK national external quality assessment scheme for immunocytochemistry and in situ hybridisation database containing results from 199 300 patients. J Pathol Clin Res. 2018 Oct;4(4):262-273.
- Dodson A, Parry S, Lissenberg-Witte B, et al. (2019). External quality assessment demonstrates that PD-L1 22C3 and SP263 assays are systematically different [published online ahead of print, 2019 Dec 17]. J Pathol Clin Res. 2019;10.1002/cjp2.153. doi:10.1002/cjp2.153.
- Acs B, Leung SCY, Kidwell KM, Arun I, Augulis R, Badve SS, Bai Y, Bane AL, Bartlett JMS, Bayani J, Bigras G, Blank A, Buikema H, Chang MC, Dietz RL, Dodson A, Fineberg S, Focke CM, Gao D, Gown AM, Gutierrez C, Hartman J, Kos Z, Lænkholm AV, Laurinavicius A, Levenson RM, Mahboubi-Ardakani R, Mastropasqua MG, Nofech-Mozes S, Osborne CK, Penault-Llorca FM, Piper T, Quintayo MA, Rau TT, Reinhard S, Robertson S, Salgado R, Sugie T, van der Vegt B, Viale G, Zabaglo LA, Hayes DF, Dowsett M, Nielsen TO, Rimm DL; International Ki67 in Breast Cancer Working Group of the Breast International Group and North American Breast Cancer Group (BIG-NABCG). Systematically higher Ki67 scores on core biopsy samples compared to corresponding resection specimen in breast cancer: a multi-operator and multi-institutional study. Mod Pathol. 2022 Oct;35(10):1362-1369. doi: 10.1038/s41379-022-01104-9. Epub 2022 Jun 21. PMID: 35729220; PMCID: PMC9514990.
- Parry S, Dowsett M, Dodson A. (2021) UK NEQAS ICC & ISH Ki-67 Data reveal differences in

performance of primary antibody clones. Appl Immunohistochem Mol Morphol. 2021 Feb 1;29(2):86-94.

- Dowsett M, Kilburn L, Rimawi MF, Osborne CK, Pogue-Geile K, Liu Y, Jacobs SA, Finnigan M, Puhalla S, Dodson A, Martins V, Cheang M, Perry S, Holcombe C, Turner N, Swift C, Bliss JM, Johnston S; PALLET trialists. Biomarkers of Response and Resistance to Palbociclib Plus Letrozole in Patients With ER+ve, HER2-ve Breast Cancer. Clin Cancer Res. 2022 Jan 1;28(1):163-174. doi: 10.1158/1078-0432.CCR-21-1628. Epub 2021 Oct 13. PMID: 34645649; PMCID: PMC9632606.
- Hurwitz JT, Vaffis S, Grizzle AJ, Nielsen S, Dodson A, Parry S. Cost-Effectiveness of PD-L1 Testing in Non-Small Cell Lung Cancer (NSCLC) Using In Vitro Diagnostic (IVD) Versus Laboratory-Developed Test (LDT). Oncol Ther. 2022 Dec;10(2):391-409.
- Bliss JM, Tovey H, Evans A, Holcombe C, Horgan K, Mallon E, Vidya R, Skene A, Dodson A, Hills M, Detre S, Zabaglo L, Banerji J, Kilburn L, Morden JP, Robertson JFR, Smith I, Dowsett M; POETIC Trialists. Clinico-pathologic relationships with Ki67 and its change with short-term aromatase inhibitor treatment in primary ER + breast cancer: further results from the POETIC trial (CRUK/07/015). Breast Cancer Res. 2023 Apr 12;25(1):39. doi: 10.1186/s13058-023-01626-3. PMID: 37046348; PMCID: PMC10099675.
- Jasani B, Taniere P, Schildhaus HU, Blighe K, Parry S, Wilkinson D, Atkey N, Clare-Antony S, McCabe C, Quinn C; CLDN Study Group; Dodson A. Global Ring Study to Investigate the Comparability of Total Assay Performance of Commercial Claudin 18 Antibodies for Evaluation in Gastric Cancer. Lab Invest. 2024 Jan;104(1):100284. doi: 10.1016/j.labinv.2023.100284. Epub 2023 Nov 8. PMID: 37949357.
- Dodson A, Parry S. The Chemistry in Immunohistochemistry: A Reply From UK NEQAS. Arch Pathol Lab Med. 2024 Mar 1;148(3):265-266. doi: 10.5858/arpa.2023-0389-LE. PMID: 38408029.
- Torlakovic EE, Al Dieri R, Badrick T, Chen ZE, Cheung CC, Deans Z, Dodson A, Fenizia F, Kijima H, Maas J, Martinez A, Nielsen S, Patton S, Rouleau E, Schirmacher P, Shet T, Stockley T, Normanno N. Indirect clinical validation for predictive biomarkers in oncology: International Quality Network for Pathology (IQN Path) Position Paper. Virchows Arch. 2025 Sep;487(3):565-572. doi: 10.1007/s00428-025-04169-4. Epub 2025 Jul 17. PMID: 40670724; PMCID: PMC12488805.
- Parry S, Zabaglo L, Shaaban AM, Dodson A. Inter-rater agreement of HER2-low scores between expert breast pathologists and the Visiopharm digital image analysis application (HER2 APP, CE2797). J Pathol Clin Res. 2025 Nov;11(6):e70051. doi: 10.1002/2056-4538.70051. PMID: 41103138; PMCID: PMC12531420.

31. Referral for Feedback and Opinion Service

UK NEQAS ICC & ISH offers a referral service which allows registered participants to submit any marker for feedback and opinion outside the standard scheme schedule.

The service comprises of two types of request:

Quality Improvement Following Assessment (QIFA). This provides feedback on staining following re-optimisation of protocols due to low scores in a previous UK NEQAS assessment. This service is provided free of charge, and although the turnaround times will vary, the scheme aims to provide a report within 14 working days.

Feedback or Opinion of Staining. This provides feedback or opinion on markers for all other instances. It encompasses those not requested as part of the routine EQA assessments, those that fall outside the UK NEQAS ICC and ISH scope, and any that have been requested for accreditation purposes. This service has a fee, which covers the

administration and running costs incurred. The turnaround time will be in the region of three months.

Participants are not limited to the number of markers they may submit but are asked to contact the Scheme prior to sending slides so that we can address each laboratory's requirements and advise accordingly. The contact e-mail address is referrals@ukneqasiccish.org, and can also be found on the relevant forms on our website at: https://ukneqasiccish.org/participants-area/forms/

Slides may be reviewed by our own scientific staff, assessors, or external specialists, and assessment scoring and interpretation is conducted as for the routine Assessment Runs. Following review, electronic and hard copy reports will be sent out and slides returned.

This is a non-accredited activity.

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