EXTERNAL QUALITY ASSESSMENT SCHEME FOR
THE EVALUATION OF PAPANICOLAOU STAINING
IN CERVICAL CYTOLOGY

Protocol and Standard Operating Procedures

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PREFACE

This document was written by a working group set up to devise a protocol and standard operating procedures for the assessment of Papanicolaou stained cervical cytology samples in the UK Cervical Screening Programme. It will be reviewed on an annual basis and may be subject to change.
MEMBERS OF THE TECHNICAL EXTERNAL QUALITY ASSESSMENT (EQA) WORKING GROUP

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ACKNOWLEDGEMENTS

This document is based largely upon the information and experiences provided from technical external quality assessment (EQA) schemes that have been introduced regionally over the last few years. We are indebted to the hard work and insight provided by previous technical EQA groups. Special thanks are also due to colleagues for their assistance in formulating the definition and categories in the scoring scheme. All have contributed substantially to the development of this publication.
External Quality Assessment Scheme for the Evaluation of Papanicolaou Staining

PROTOCOL

1. INTRODUCTION

External quality assessment (EQA) is an essential part of the wider quality assurance function. The fundamental purpose of EQA is to maintain and improve the quality of patient care by promoting a high standard of performance. This is facilitated through an independent system of checking laboratory results by an external agency. Consequently, an acceptable degree of reliability and consistency is achieved through education, advice and support to all participants.

The staining of cervical cytology samples by the Papanicolaou technique is used throughout the UK Cervical Screening Programme. The technique, as published by Papanicolaou in 1942, demonstrates the hormonal variations expressed in cervical and vaginal epithelium. Its efficacy in facilitating the accurate assessment of cervical cytology samples has subsequently become universally acknowledged.

The Papanicolaou technique provides the cytologist with the means to differentiate and evaluate both nuclear and cytoplasmic characteristics of the cell and is an integral part of the screening process. Any failure or deterioration in this staining procedure may give rise to substandard results and the potential for misinterpretation of the cervical cytology sample. Consequently, the need for quality control of this staining technique is vital.

The purpose of this document is to ensure that standards are set for routine staining in cervical cytology so that performance can be monitored and practice improved where necessary. This scheme is equally applicable to conventional smears and liquid based preparations.

This protocol and standard operating procedures (SOPs) constitute a framework for the scheme handbook manual. The handbook is designed to be read in conjunction with the scheme protocol and gives practical assistance in organising and applying the scheme. It is anticipated that the scheme will develop over time and changes will be made accordingly.

2. AIMS

The scheme aims to:

- provide an external assessment of the quality of Papanicolaou staining in cervical cytology samples
- establish minimum quality standards for staining
- maintain and improve quality by achieving consistent good practice
- identify substandard staining quality and the reasons for this and enable remedial action
- provide advice and practical help to laboratories
- promote education and training through formal feedback
- achieve recognition through the appropriate accreditation bodies.
The Joint Working Group for Quality Assurance (JWG) is recognised by the Department of Health (DH) as the independent body responsible for Pathology EQA in the United Kingdom (Figure 1). Membership of the JWG comprises representatives of the pathology professions and societies, chairpersons of the National Quality Assurance Advisory Panels (NQAAPs) and observers from national government offices and Clinical Pathology Accreditation (CPA) (UK) Limited. Its remit is to oversee all EQA in the UK, to approve and register schemes, set policies and maintain appropriate professional standards.

The JWG is responsible for the recognition of the NQAAPs and steering committees and for scheme related professional matters. Advisory panels are convened for all pathology disciplines and their remit is to monitor substandard performance.

Figure 1 Pathology EQA in the UK.
3.1 **Scheme provider**  
The scheme provider is the NHS Cervical Screening Programme (NHSCSP).

3.2 **National scheme organiser**  
An individual will be identified to undertake the role of scheme organiser at a national level. It is envisaged that this individual will be drawn from the members of the NHSCSP National Coordinating Group for Laboratory Quality Assurance.

3.3 **Steering committee**  
The NHSCSP National Coordinating Group for Laboratory Quality Assurance will act as the scheme’s steering committee. The remit of the steering committee is to review the objectives of the scheme and to advise on its scientific content.

The steering committee will hold a list of trained assessors.

3.4 **Scheme organisation**  
The scheme will be organised through the regional quality assurance framework of the NHSCSP (illustrated in Figure 2) using a similar infrastructure to that of the NHSCSP External Quality Assessment Scheme in Gynaecological Cytopathology. The scheme infrastructure is illustrated in Figure 3. Although organised locally, each region will operate to the same national protocol.

The scheme handbook describes the organisation of the scheme and lists the key personnel involved.

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**Figure 2** Quality assurance relationships for pathology in the NHSCSP.
Figure 3  Scheme infrastructure.
3.4.1 Regional scheme organiser

At a local level, the regional scheme organiser will hold overall responsibility for the scheme. It is recommended that this individual is a member of the regional pathology quality assurance coordinating group. This individual is expected to work in collaboration with the lead biomedical scientist from the regional pathology quality assurance coordinating group. Where this is not practical, the chairman of the group and the regional cervical screening quality assurance (QA) director (or equivalent in Wales, Scotland and Northern Ireland) should identify another suitable individual.

3.4.2 Technical EQA facilitator

The regional cervical screening QA director (or equivalent in Wales, Scotland and Northern Ireland) will identify an individual to undertake the day to day running of the scheme. It is recommended that this individual is able to demonstrate technical competence. The technical EQA facilitator may wish to enlist some secretarial support (see section 7.1).

3.5 Scheme secretariat

The national office of the NHSCSP will provide the secretariat for the scheme. An individual will be identified to work with the technical EQA facilitators and to act as the link between them and the steering committee.

3.6 Funding

It is expected that the scheme will be funded through the regional quality assurance framework of the NHSCSP (for England only).

3.7 Terms and conditions of participation

- The scheme is mandatory for all cytology laboratories in the NHS CSP.
- The medical head of department will be responsible for registering the laboratory as a participant in the scheme.
- All participants in the scheme will operate to the same national protocol.
- An up to date list of the participating laboratories and official contacts in England will be held by the NHSCSP national office. This service will be offered to participating laboratories in Wales, Scotland and Northern Ireland.
- A certificate of participation will be issued annually by the regional scheme organiser.

4. SCHEME DESIGN

4.1 How slides are selected

There will be four rounds of slide assessment per year. Each laboratory will be required to submit four Papanicolaou stained cervical cytology samples per round, of which two will be assessed (SOP 3) and the other two samples will be held in reserve and assessed if one or both of the original two is found to be substandard (SOP 6).

The slides selected for submission must be negative. Ideally, these should be adequately cellular and around mid-cycle from premenopausal women. Samples with a heavy bacterial component should be avoided.

The group gave careful consideration to the use of dyskaryotic cellular material. However, evidence gathered from regional schemes suggests that the inclusion of positive material may result in anomalous assessment.
Participating laboratories will also be requested to submit their staining protocols, including details of the supplier of the stains and reagents used at every assessment.

The technical EQA facilitator will determine the slides to be assessed (SOP 2).

The technical EQA facilitator will arrange for the slides to be assessed by a minimum of four assessors.

The slides will be anonymised, coded and sent with accompanying paperwork to the assessment team.

The technical EQA facilitator will be able to identify the location of any slide required for review by the originating laboratory.

After assessment, the slides will be returned to the originating laboratories.

For educational and training purposes, images of representative slides from each circulation will be taken and made available to all participating laboratories.

### 4.2 Components assessed

#### 4.2.1 Nuclear staining

Haematoxylin staining of individual nuclei should be:

- clearly visible at low power (10× objective), and
- blue to black in colour.

At high power (40× objective), nuclear chromatin should be:

- clearly demonstrated, and
- appear granular, crisp and distinct.

There should be no background staining, apart from cervical mucus, and haematoxylin should not adversely affect the colours of the counterstains.

#### 4.2.2 Counterstains

- Superficial squamous cells should stain pink; less mature cells should stain blue/green and fully keratinised cells should stain orange/yellow.
- Those colours present should be of equal intensity.
- There should be cytoplasmic translucency with a sharp contrast to the nuclear stain.

Polychromasia may be encountered within metaplastic cells in which two distinct colours are present in the cytoplasm.
Several scoring systems have been developed in the past for use on a regional basis. The underlying principles of these are much the same, and the bulk of the assessment is divided between the characteristics of nuclear staining and those of cytoplasmic staining. The scoring of the proposed scheme will be restricted to nuclear staining and cytoplasmic staining.

A criticism of existing scoring systems is that they give equal weight to nuclear and cytoplasmic staining, which conflicts with the greater importance of the nuclear characteristics. A major disadvantage of an equal weight scoring system is that a slide failing on the nuclear component of the score may nevertheless be deemed to pass overall on the basis of a high cytoplasmic score. Weighting of scores offers a refinement to the scoring system that could correct for this imbalance. However, a simple weighting of the nuclear and cytoplasmic scores itself produces anomalies; for example, even a substantial weighting of 2:1 in favour of the nuclear score leaves the possibility of seriously unbalanced slides achieving an adequate or even a good overall rating. After careful consideration, the working group felt that weighting would produce its own anomalies and would be difficult to apply and understand. An alternative approach has therefore been chosen.

This scheme uses raw scores but restricts the acceptable and good categories to slides that score above a set minimum on both nuclear and cytoplasmic assessment. By setting different minimum scores for nuclear and cytoplasmic assessment, a controlled automatic weighting of the final score is produced. The scheme also takes into account the balance of nuclear and cytoplasmic scores, rejecting slides that show well on one but poorly on the other.

It is acknowledged that even these restrictions could allow slides with certain extreme kinds of imbalance between the components of the nuclear staining score, or of the cytoplasmic staining score, to be rated as ‘good’. This can be avoided only by placing restrictions on the minimum allowable on each of the six components of the score. This was considered, but thought to be an overly complex way of dealing with what are, as far as could be established, rare occurrences for the nuclear component, although they are more likely for the cytoplasmic component.

Each slide is rated on six characteristics: three for nuclear and three for cytoplasmic staining. These are detailed below. For each characteristic, a score in the range 1–5 may be given. In common with other schemes, points are taken off the maximum of 5 for detrimental features, rather than being built up from the minimum of 1 for positive features.

A slide may score in the range 3–15 on nuclear staining (N) and 3–15 on cytoplasmic staining (C), giving a range for the total score of 6–30.
5.3 Overall slide ratings

Slides will be rated as falling into one of four categories according to the scores:

- good
- acceptable
- marginal
- substandard.

It is important to note that, because the scores are criterion-based, a score of 3 on any individual criterion cannot be interpreted as meaning that the slide is ‘average’ on that criterion. A slide that scores 3 for each characteristic, and therefore has a total score of 18, does not have an average score nor is it an acceptable slide. Setting different minimum scores for nuclear or cytoplasmic staining means that a score of 3 for any characteristic cannot be an average score.

5.3.1 Good

To be rated good, a slide must:

- score at least 25 overall, and
- score at least 12 on nuclear staining, and
- score at least 11 on cytoplasmic staining.

Note that slides scoring 25 or more will, however, not be classed as good in cases where the imbalance between the nuclear and cytoplasmic components is too great. Slides with the following component scores will be reduced to acceptable on account of imbalance:

- N15 and C10
- N11 and C14
- N11 and C15
- N10 and C15.

5.3.2 Acceptable

Similarly, to be rated acceptable, a slide must:

- score at least 20 overall, and
- score at least 10 on nuclear staining, and
- score at least 9 on cytoplasmic staining.

Slides scoring 20–24 will not be classed as acceptable where the imbalance between the nuclear and cytoplasmic components is too great. Slides with the following component scores will be reduced to marginal or substandard on account of imbalance:

- N9 and C11 or more (marginal)
- N8 or less and C12 or more (substandard)
- N12 or more and C8 or less (substandard).

5.3.3 Marginal

To be rated marginal a slide must:

- score at least 18 overall, and
- score at least 9 on nuclear staining, and
- at least 9 on cytoplasmic staining.

(See also section 10.1.)
5.3.4 **Substandard**  
Slides rated as **substandard** will be subjected to certain actions. The action points are described in section 8 of this document.

5.3.5 **Graphical representation of scores and ratings**  
The allowable scores and final ratings are represented graphically in Figure 4. The raw scores are unweighted but restricted.

![Figure 4 Graphical representation of scores and ratings](image)

### 5.4 Scoring criteria

Each assessor will mark the slides independently. The assessors’ mark sheet is attached in Appendix 1. For a slide to be given a final rating of good, at least three of the four assessors must rate it good. Similarly, for a slide to be given a final rating of acceptable (rather than marginal or substandard), at least three of the four assessors must rate it acceptable. The assessors must reconvene as a panel and produce a final consensus report.

Assessors will evaluate and mark the slides according to the criteria detailed below. The scoring scheme for both nuclear and cytoplasmic staining is given in Appendix 2.

### 5.4.1 Nuclear stain

**Differentiation of the haematoxylin**  
Adequate differentiation is characterised by clear delineation of nuclear components and lack of residual haematoxylin stain in the cytoplasm of cells.

The score allocated to this criterion indicates the intensity of nuclear staining. It is recognised that this depends upon the degree of differentiation, the time in haematoxylin solution, the type of haematoxylin and/or any combination of these factors.
A low score may result from either very dark staining affecting cytoplasmic colour or, conversely, very pale nuclear staining.

**Clarity of chromatin pattern**
Chromatin should appear crisp and distinct. It is recognised that a maximum score may only be achievable in a cervical cytology sample with optimal fixation.

**Haematoxylin colour**
Haematoxylin colour should be blue to black.

A pictorial aid to the assessment of nuclear staining is given in Appendix 3.

### 5.4.2 Cytoplasmic stain

**Colour spectrum**
This may be defined as an appropriate range of cytoplasmic colour, as demonstrated in Papanicolaou’s method.

**Intensity of cyanophilia**
This relates directly to the depth of blue/green colour present.

**Intensity of eosinophilia/orangeophilia**
This relates directly to the depth of pink/orange colour present. Eosinophilia and orangeophilia are combined because it is recognised that the orangeophilic cellular material may not always be present in test material.

### 5.5 General (non-scoring) aspects of slide assessment

There are a number of factors that may affect the interpretation of the slide. These include:

- fixation
- preparation
- presentation
- translucency.

Fixation and slide preparation often lie outside the direct control of the laboratory. Substandard fixation may result in cellular distortion, leading to an unusual staining pattern. The cytoplasm of such cells may take up excessive eosin and the nuclear staining with haematoxylin will be less than optimal.

Thick tissue fragments or multilayered aggregations of cells due to substandard spreading technique may result in improper dye penetration and colours that are not normally expected.

The elements associated with the presentation of material include uneven staining, incomplete dehydration and adequacy of mounting (eg air bubbles). The presence of excessive ‘cornflake’ artefact may deleteriously affect the presentation of material. The reason for its presence is the subject of some dispute, but it may be surmised that it often originates from substandard laboratory procedures rather than substandard fixation.
Translucency is the ability to resolve individual cellular detail within clusters or groups and is influenced by cervical cytology sample thickness and the ‘clearing’ properties of the solvents used.

Although all of these factors will be noted and commented on by the assessors, they will not influence the overall slide scores for the purpose of what is primarily a technical external quality assessment of laboratory staining.

5.6 Scheme audit

Participating laboratories should be aware that from time to time a small number of their slides may be retained for audit purposes, i.e., for quality control of subsequent assessments. These slides will form a bank held by the technical EQA facilitator for a limited period of five rounds.

Each region will also produce a bank of control slides to seed the assessments. These slides will be interchangeable between regions to assist in the audit of the assessors’ performance.

6. Assessor Training

Owing to the nature of the slide assessment process and the inherent scope for interobserver variation, the group advocates that a single national scheme is devised for assessor training to ensure a consistent approach (SOP 4).

Assessment is best and taken at venues with high quality microscopes, multiheaded discussion microscopes and photomicrography. The need for consensus agreement, which necessitates the simultaneous assessment of slides using a multiheaded microscope (SOP 5), should not be underestimated.

7. Feedback to Participants

Nationally collected data will be the property of the NHS Cervical Screening Programme.

7.1 Distribution of results

The technical EQA facilitator may wish to use secretarial assistance (termed ‘EQA secretary’ in other schemes) to ensure that the reports and correspondence generated are correctly addressed and distributed.

Secretarial assistance can also be used for any correspondence to laboratories regarding performance issues. The secretary may be kept in ignorance about the contents of the correspondence.

The secretary may only divulge the link between a laboratory’s name and a participant’s code in writing to the laboratory official who requests a reminder of the participant’s code number. This will not be divulged orally.

7.2 Reports to participating laboratories

Participating laboratories will receive a report for each of the two slides assessed.
The report will include:

- images of highest and lowest scoring staining
- details of the staining methods used by the highest scoring laboratory(ies) in that round
- a graphical representation of current scores compared with the scores for participating laboratories in the same region
- historical data accumulated during the operation of the scheme.

A comprehensive results package is described in the scheme’s handbook.

8. SUBSTANDARD PERFORMANCE

8.1 Action points

Certain actions will be activated when a slide is rated as substandard (SOP 6).

8.1.1 Action within the assessment panel

- One slide out of the two selected is rated as substandard.

The other slides from the set of four originally submitted will also be assessed.

- One slide in the set of four is rated as substandard.

No further action ensues at least until the next round.

8.1.2 Local action point

- Two (or more) slides in the set of four are rated substandard.

If the local action point is activated, the technical EQA facilitator will inform the regional scheme organiser. The regional scheme organiser will notify the clinical head of the laboratory and initiate appropriate advice. The regional organiser may wish to involve the biomedical scientist member of the regional QA team in determining the appropriate advice. The regional organiser will advise the QA director (or equivalent in Wales, Scotland and Northern Ireland) that the local action point has been triggered.

The laboratory will be expected to discuss its slide assessments with the regional scheme organiser in collaboration, if appropriate, with the biomedical scientist member of the regional QA team (or equivalent in Wales, Scotland and Northern Ireland).

The regional scheme organiser will ask the laboratory to confirm receipt of formal notification that the local action point has been triggered and an explanation sought, and will also ask for an outline plan for remedial action.

8.1.3 National action point

- At least one slide is rated as substandard in each of three out of five consecutive rounds.

If the national action point is activated, the technical EQA facilitator will inform the regional scheme organiser. The regional scheme organiser will notify the clinical head of the laboratory, the QA direct-
tor (or equivalent in Wales, Scotland and Northern Ireland) and the chairman of the NQAAP.

The clinical head of the laboratory will be expected to contact the regional scheme organiser (or equivalent in Wales, Scotland and Northern Ireland) for advice with a view to reaching a solution. The regional scheme organiser may wish to involve the biomedical scientist representative on the regional QA team. All parties are expected to agree how to manage the situation and keep the technical EQA facilitator informed.

- The laboratory does not respond to or remedy the situation.

Where remedial action is identified and is either not instituted or fails to improve laboratory performance, the technical EQA facilitator should inform the regional scheme organiser. The regional scheme organiser should then refer the matter to the QA director (or equivalent in Wales, Scotland and Northern Ireland). The QA director may then refer the matter to the screening commissioner and the chief executive of the trust.

A flow diagram to illustrate the actions following identification of substandard performance is illustrated in Figure 5.
Four slides per laboratory available at assessment

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Action within the assessment panel

One slide out of two is rated substandard

Slides 3 and 4 will also be assessed

**LOCAL ACTION POINT**

Two or more slides in the round are rated substandard

1. The technical EQA facilitator informs the regional scheme organiser. The regional scheme organiser will notify the medical head of the laboratory and initiate appropriate advice. (The regional organiser may wish to involve the biomedical scientist member of the QA team if appropriate.) The regional organiser will advise the QA director (or equivalent in Wales, Scotland and Northern Ireland) that the local action point has been triggered

2. Laboratory discusses slide assessments with regional QA director (and/or biomedical scientist member of the QA team if considered appropriate) (or equivalents in Wales, Scotland and Northern Ireland)

3. Laboratory confirms receipt of formal notification that the local action point has been triggered. Laboratory offers an explanation and outlines a plan for remedial action

**NATIONAL ACTION POINT**

At least one slide is rated as substandard in each of three out of five consecutive rounds

1. The technical EQA facilitator informs the regional scheme organiser. The regional scheme organiser notifies the medical head of the laboratory, the QA director (or equivalent in Wales, Scotland and Northern Ireland) and the chair of the NQAAP

2. Agree how the situation will be managed

3. The regional organiser may wish to involve the regional biomedical scientist member of the QA team (or equivalent in Wales, Scotland and Northern Ireland)

**PERFORMANCE MANAGEMENT ACTION POINT**

The laboratory does not respond to or remedy the situation

The QA director may discuss the matter with the screening commissioner and chief executive of the trust

*Figure 5* Substandard performance – action points.
9. CONFIDENTIALITY

9.1 Scheme confidentiality

In this protocol, the scheme is confidential under the conditions of participation in EQA schemes determined by the professional bodies through the Joint Working Group for Quality Assurance (JWG). Special arrangements will be employed when local and national action points are triggered.

9.2 Confidentiality when action points are triggered

Results for any participating laboratory are confidential between the laboratory concerned and the technical EQA facilitator.

Anonymity will be broken in the following circumstances:

• in the case of substandard performance resulting in actions requiring the involvement of the regional scheme organiser (or equivalent in Wales, Scotland and Northern Ireland) and the biomedical scientist member of the regional QA team, if appropriate
• in the case of appeals that may involve the regional scheme organiser (or equivalent in Wales, Scotland and Northern Ireland) and advice from the lead biomedical scientist member of the regional QA team, if appropriate.

10. EDUCATION AND SUPPORT

The scheme is designed to be educational and the feedback is designed to assist participating laboratories. Examples of high scoring slides and details of the method used will be provided.

Technical assistance and support will be made available if required.

10.1 Slide performance – marginal category

Laboratories in this category are encouraged to review their staining procedures and seek external advice and support.

10.2 Participants’ feedback meetings

Regular feedback meetings will be organised by the regional scheme organiser for the participating laboratories in each region. These meetings will be educational and will provide a forum for participants to review the assessments and contribute to the scheme.

10.3 Facilitators’ meetings

The technical EQA facilitators will meet once a year. These meetings will provide a forum for facilitators to contribute to the development of the scheme.

11. SCHEME DEVELOPMENT

It is recognised that the scheme will continue to develop with experience.

No amendments may be made to this protocol. Any suggestions or proposals for change must be submitted for consideration by the national steering committee.

Proposed alterations to the scheme will be managed as follows:

• any proposals for change will first be discussed at the regional participants’ meetings, and a draft revision to the relevant SOP will be produced
• the draft revision will be submitted to the regional scheme organiser for consideration and approval
• subject to local approval, the draft revision will be submitted to the national technical EQA facilitators’ group for discussion and wider agreement
• a final draft revision will then be submitted by the national office to the steering committee for consideration
• once approval has been granted, the revised SOP will be implemented nationally.

12. APPEALS

Participating laboratories may request the reassessment of any slide considered to be marked inappropriately (SOP 8).

13. COMPLAINTS

The scheme has a formal complaints procedure (SOP 9).
STANDARD OPERATING PROCEDURE 1

Terms and conditions of participation

1. The scheme is mandatory for all cytology laboratories working in the NHS Cervical Screening Programme (NHSCSP). All laboratories will receive a copy of the scheme protocol and standard operating procedures.

2. The head of department will be responsible for registering the laboratory with the regional organiser as a participant in the scheme.

3. All participating laboratories in the scheme will operate to the same protocol.

4. No amendments will be made locally to the protocol.

5. Any suggestions or proposals for change must be submitted to the national steering committee for consideration.

6. An up to date list of the participating laboratories and official contacts in England will be held by the national office of the NHSCSP. This service will be offered to participants in Wales, Scotland and Northern Ireland.

7. A certificate of participation will be issued to the laboratory on an annual basis.

Signed _________________________ (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 2

Scheme administration

The technical EQA facilitator will:

1. Establish the workload and numbering system in operation in the participating laboratory to facilitate cervical cytology sample selection by date range.

2. Request the submission of four Papanicolaou stained cervical samples on a quarterly basis, i.e., one slide from each week reported in the previous complete calendar month.

   Cervical cytology samples selected for submission must be negative. Ideally, these should be adequately cellular and around mid-cycle from premenopausal women. Cervical cytology samples with a heavy bacterial component should be avoided.

3. Request details of the staining protocols, including:
   - the automated procedure
   - the supplier of the stains
   - the reagents, and
   - the mountant used.

4. Anonymise the cervical cytology samples provided.

5. Supply anonymised, coded cervical cytology samples and appropriate paperwork, e.g., assessment forms, to the independent assessment team.

6. Identify appropriate venues for assessments and ensure that assessments are undertaken as detailed in the scheme protocol.

7. Undertake an analysis of results as detailed in the scheme protocol.

8. Return cervical cytology samples to the originating laboratory together with a breakdown of performance. The report will include:
   - images of highest and lowest scoring staining
   - details of the staining methods used by the highest scoring laboratory(ies) in that round
   - a graphical representation of current performance
   - historical data accumulated during the operation of the scheme.

The comprehensive results package is described in the user manual.
9. Provide feedback on performance to the QA team (or equivalent) highlighting any occurrence of poor performance.

10. Coordinate any laboratory queries or appeals that may arise following poor performance.

11. Identify the location of any slide if required for review.

12. Return a submitted negative slide to the clinical head of the department if the assessors think it is potentially abnormal, and advise both the clinical and scientific heads of department of the reason for its return.

Signed _________________________ (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 3

Participation in the scheme

Each participating laboratory will:

1. Supply the technical EQA facilitator with data on the workload and numbering system employed by the laboratory.

2. Supply the technical EQA facilitator with details of the current staining regime:
   • staining schedule
   • staining brands
   • reagents
   • mountant.

   This information will be kept on file for educational purposes only and will not form part of the assessment process.

3. Supply four stained cervical cytology slides on a quarterly basis as requested by the technical EQA facilitator. The slides selected for submission must be negative. Ideally, these should be adequately cellular and around mid-cycle from premenopausal women. Samples with a heavy bacterial component should be avoided.

4. Ensure that any material sent through the post is packaged safely.

5. Liaise with the technical EQA facilitator regarding the results.

6. Raise any concerns/complaints with the technical EQA facilitator.

Signed _________________________ (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 4

Selection and training of assessors

Assessors will be appointed by the regional QA teams (or equivalent in Wales, Scotland and Northern Ireland). They will undergo appropriate training with a view to forming a national network.

1. Individuals selected as assessors must have a minimum of five years’ experience in the reporting of cervical cytology samples.

2. Individuals appointed to the role will serve in this capacity for a minimum of two years to promote continuity of assessment.

3. Assessors will undergo one day of formal training in order to standardise the assessment process.

4. Members of the national technical EQA working group will be involved in the initial delivery of training. Training will consist of practical microscopy and a discussion seminar to promote a national standardised approach.

5. A minimum of six individuals per region should undergo training in order to provide the four assessors needed in the assessment process.

Signed _________________________ (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 5

Assessment process

Each slide will be assessed by a minimum of four assessors.

1. The assessment of slides will be undertaken in two distinct stages:
   - independent examination of cervical cytology samples and
   - subsequent scoring, followed by
   - multiheaded microscope group discussion and consensus agreement.

   These should take place sequentially at the assessment session.
   All assessments will be made using a colour corrector/blue filter.

2. Assessors should base their judgement on representative areas of
   the slide. However, areas of poor fixation, air-drying or obscuration
   should be avoided whenever possible.

3. Slide assessment will be based upon the six criteria as detailed in
   the scheme protocol.

4. Scoring for each criterion will be out of a maximum of 5 and marks
   will be deducted for any perceived deficiencies.

5. Each assessor will complete an assessment form.

6. The assessors will reconvene as a panel, compare individual assessments
   and produce a final ‘consensus’ report.

7. The slide bank for assessment will be seeded with three control slides
   to validate the assessors’ performance.

8. The assessment results will be returned with the slides to the technical
   EQA facilitator for analysis.

Signed _________________________ (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 6

Substandard performance

Certain actions will be activated when a slide is rated substandard.

1. Action within the assessment panel

   • One slide out of two is rated substandard.
     
     The other slides from the set of four originally submitted will also be assessed.
   
   • One slide in the round is rated substandard.
     
     No further action ensues at least until the next round.

2. Local action point

   • Two (or more) slides in the round are rated substandard.

   If the local action point is activated, the technical EQA facilitator will inform the regional scheme organiser. The regional scheme organiser will notify the clinical head of the laboratory and initiate appropriate advice. The regional organiser may wish to involve the biomedical scientist member of the regional QA team in determining the appropriate advice. The regional organiser will advise the QA director (or equivalent in Wales, Scotland and Northern Ireland) that the local action point has been triggered.

   The laboratory will be expected to discuss its slide assessments with the QA director (or equivalent in Wales, Scotland and Northern Ireland), in collaboration with the biomedical scientist member of the QA team if appropriate.

   The regional scheme organiser will ask the laboratory to confirm receipt of formal notification that the local action point has been triggered and an explanation sought, and will also ask for an outline plan for remedial action.

3. National action point

   • At least one slide is rated as substandard in each of three out of five consecutive rounds.

   If the national action point is activated, the technical EQA facilitator will inform the regional scheme organiser. The regional scheme organiser will notify the clinical head of the laboratory, the QA director (or equivalent in Wales, Scotland and Northern Ireland) and the chairman of NQAAP.
The clinical head of the laboratory will be expected to contact the regional scheme organiser (or equivalent in Wales, Scotland and Northern Ireland) for advice with a view to reaching a solution. The regional scheme organiser may wish to involve the biomedical scientist member on the regional QA team. All parties are expected to agree how to manage the situation and to keep the technical EQA facilitator informed.

4. Performance management action point

- The laboratory does not respond to or remedy the situation.

Where remedial action is identified and is either not instituted or fails to improve laboratory performance, the technical EQA facilitator should inform the regional scheme organiser. The regional scheme organiser should then refer the matter to the QA director (or equivalent in Wales, Scotland and Northern Ireland).

The QA director may discuss the matter with the screening commissioner and the chief executive of the trust.

Signed _________________________  (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 7

Confidentiality

In this protocol, the scheme is confidential under the conditions of participation in EQA schemes determined by the professional bodies through the Joint Working Group. However, if the local action point is activated, a laboratory will be expected to extend confidentiality to the regional scheme organiser, who may then extend it to the biomedical scientist member of the regional QA team (or equivalent in Wales, Scotland and Northern Ireland). The QA director (or equivalent in Wales, Scotland and Northern Ireland) will be informed at the local action point, and the NQAAP chairman will be informed at the national action point.

Results for any participating laboratory are confidential between the laboratory concerned and the technical EQA facilitator.

Anonymity will be broken in the following circumstances:

• in the case of substandard performance resulting in actions requiring the involvement of the regional scheme organiser (or equivalent in Wales, Scotland and Northern Ireland) and biomedical scientist member of the regional QA team, if appropriate
• in the case of appeals that may involve the regional scheme organiser (or equivalent in Wales, Scotland and Northern Ireland) and advice from the biomedical scientist member of the regional QA team, if appropriate

Signed _________________________ (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 8

Appeals

1. If a laboratory wishes to appeal against a particular result, it should do so by writing to the technical EQA facilitator within seven days of receiving the result.

2. Appeals will be logged, together with a summary of the communication and subsequent discussions that may involve the assessors and/or the biomedical scientist member of the regional QA team.

3. If necessary, the material will be reassessed by a different team of assessors.

4. If the matter is not resolved, it will be referred to the regional scheme organiser. If the matter becomes intractable, then it will be referred to the steering committee.

5. Local appeals should be resolved before the next assessment.

Signed _________________________ (Regional scheme organiser)

Dated _________________________
STANDARD OPERATING PROCEDURE 9

Complaints

In the first instance, complaints about the organisation and conduct of the scheme should be made to the technical EQA facilitator. A record will be kept of all complaints plus the subsequent outcome.

In the event of a complaint being handled to the dissatisfaction of the participating laboratory, the laboratory representative can complain directly to the regional scheme organiser. If the participating laboratory is not happy with the outcome, then a complaint may be made directly to the national organiser or chairman of the steering committee.

Signed _________________________ (Regional scheme organiser)

Dated _________________________
### ASSESSORS’ MARK SHEET

<table>
<thead>
<tr>
<th>Slide code number:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall impression (G = good; A = acceptable; M = marginal; S = substandard)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Non-scoring features (please tick as appropriate)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Yes [ ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uneven staining</td>
<td></td>
</tr>
<tr>
<td>Incomplete dehydration</td>
<td></td>
</tr>
<tr>
<td>Carbowax deposit</td>
<td></td>
</tr>
<tr>
<td>Air bubbles</td>
<td></td>
</tr>
<tr>
<td>Retraction of mountant</td>
<td></td>
</tr>
<tr>
<td>Translucency</td>
<td></td>
</tr>
</tbody>
</table>

### Assessment of nuclear staining

- **[A] Differentiation** Score
  - Please tick as appropriate: (?underdifferentiated) Too dark
  - (?overdifferentiated) Too pale

- **[B] Haematoxylin colour** Score

- **[C] Chromatin** Score

**Subtotal**
### Assessment of cytoplasmic staining

<table>
<thead>
<tr>
<th>[D] Intensity of cyanophilia</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Please tick as appropriate:</td>
<td></td>
</tr>
<tr>
<td>Too dark</td>
<td></td>
</tr>
<tr>
<td>Too pale</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[E] Intensity of eosinophilia/orangeophilia</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Please tick as appropriate:</td>
<td></td>
</tr>
<tr>
<td>Too dark</td>
<td></td>
</tr>
<tr>
<td>Too pale</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[F] Colour spectrum</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtotal</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total overall score</th>
</tr>
</thead>
</table>

**Final overall slide rating**
- (G = good; A = acceptable; M = marginal; S = substandard)

Assessors' signatures:  
Date:  

February 2004
## SCORE SHEET FOR THE ASSESSMENT OF NUCLEAR AND CYTOPLASMIC STAINING

<table>
<thead>
<tr>
<th>Nuclear staining</th>
<th>Marks deducted</th>
<th>Final score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[A] Differentiation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1 Optimal intensity of nuclear staining in virtually all nuclei</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>A2 Optimal intensity of nuclear staining in the majority of nuclei with acceptable staining in the remainder of nuclei</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>A3 Acceptable intensity of nuclear staining without adversely affecting cytoplasmic stains</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A4 Haematoxylin present but underrepresented</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>A5 Nuclei overstained and affecting cytoplasm</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>A6 Little or no haematoxylin present</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>A7 All nuclei heavily overstained, with haematoxylin in cytoplasm throughout</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>[B] Haematoxylin colour</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1 Blue/black colour in virtually all nuclei</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>B2 Blue/black colour in the majority of nuclei</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>B3 Purple/blue colour in the majority of nuclei</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>B4 Pink/red/green colour in more than 50% of nuclei</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>B5 Pink/red/green colour in virtually all nuclei</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>[C] Chromatin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1 Crisp and distinct pattern in virtually all nuclei</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>C2 Crisp and distinct chromatin pattern in the majority of nuclei</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>C3 Chromatin visible, but lacking definition, in the minority of nuclei</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>C4 Chromatin visible, but lacking definition, in the majority of nuclei</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>C5 Lack of chromatin definition in all of nuclei</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
### Cytoplasmic staining

<table>
<thead>
<tr>
<th>[D] Intensity of cyanophilia</th>
<th>Marks deducted</th>
<th>Final score</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1 Optimal intensity of cytoplasmic staining throughout the slide</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>D2 Good intensity of cytoplasmic staining throughout the slide</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>D3 Acceptable intensity of cytoplasmic staining throughout the slide</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>D4 Inappropriate overall intensity, ie</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• present, but too pale, cyanophilia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• present, but too dark, cyanophilia</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>D5 Overtly inappropriate intensity, eg cyanophilia virtually absent</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[E] Intensity of eosin/orangeophilia</th>
<th>Marks deducted</th>
<th>Final score</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 Optimal intensity of cytoplasmic staining throughout the slide</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>E2 Good intensity of cytoplasmic staining throughout the slide</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>E3 Acceptable intensity of cytoplasmic staining throughout the slide</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>E4 Inappropriate overall intensity, ie</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• present, but too pale eosinophilia/orangeophilia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• present, but too dark eosinophilia/orangeophilia</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>E5 Overtly inappropriate intensity, eg eosinophilia/orangeophilia virtually absent</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[F] Colour spectrum</th>
<th>Marks deducted</th>
<th>Final score</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 Colour range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• All three colours equally represented, including subtle shades of pink/orange, orange/yellow and green/blue</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>F2 Colour range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• All three colours equally represented, but lacks subtle shades</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>F3 Colour range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• All three colours present, but one or more is underrepresented in the minority of the slide</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>F4 Colour range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• One or more colours is grossly underrepresented or absent in the majority of the slide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Must take account of hormonal status, eg a cyanophilic atrophic cervical cytology sample should not be given a poor score</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>F5 Colour spectrum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• All green</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• All pink</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• All orange</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Two tone (two colours only, ie no spectrum)</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
APPENDIX 3: A PICTORIAL AID TO THE ASSESSMENT OF PAPANICOLAOU STAINING OF CERVICAL CYTOLOGY SAMPLES

Plate 1 Good nuclear staining in endocervical cells. At low power, the blue/black colour of the nuclei can be appreciated.

Plate 2 Good nuclear staining in the same group of endocervical cells. Note the crisp and distinct chromatin pattern apparent at high power.

Plate 3 An example of underdifferentiation. At low power, note how the cytoplasm of the superficial and intermediate cells retains haematoxylin, resulting in a ‘muddy’ appearance.

Plate 4 An example of poor nuclear staining. At high power, note the indistinct chromatin pattern and the presence of reddish nuclei.
Plate 5 An example of excellent staining. A good range of cytoplasmic colours reflecting the hormonal status of cells, together with crisp and distinct nuclear staining. The presence of glycogen is also clearly visible in some intermediate cells.

Plate 6 An example of good cytoplasmic staining demonstrating keratohyaline granules and translucency of cells.

Plate 7 A degree of amphophilia is acceptable in metabolically active cells. This does not detract from the crisp and distinct nuclear staining.

Plate 8 A well stained preparation demonstrating good cytoplasmic intensity, translucency and distinct blue/black nuclei. It is recognised that in a cervical cytology sample with a predominantly progestogenic pattern the colour range will be affected. This would not however be detrimental to the score.
REFERENCES

4. External Quality Assessment Scheme Accreditation. Clinical Pathology Accreditation (UK) Ltd.
6. External Quality Assessment Scheme for Gynaecological Cytopathology. NHS Cancer Screening Programmes, 2003 (NHSCSP Publication No 15)
FURTHER READING
