

QUALITY CONTROL: GOOD LABORATORY PRACTICE - AN OVERVIEW OF QUALITY ASSURANCE

S.M. Lewis

The laboratory is pivotal in medical practice as test results have a major influence on clinical diagnosis and patient management. The laboratory has an ethical obligation to produce reliable and reproducible analytic measurements and observations that are timely and cost effective. It should provide clinicians with unambiguous and meaningful reports that are relevant to the clinical problem. There is also a moral obligation to train and advance the careers of the professional and other laboratory personnel, to ensure their health and safety in the course of their work, and to advance the speciality by research and technological innovations. These objectives represent *good laboratory practice*; the mechanism for achieving this is encompassed in *total quality management* (Fig. 1). With modern instruments the analytic process and its control are becoming increasingly reliable; errors are more likely to occur during the pre- and post-analytic phases.

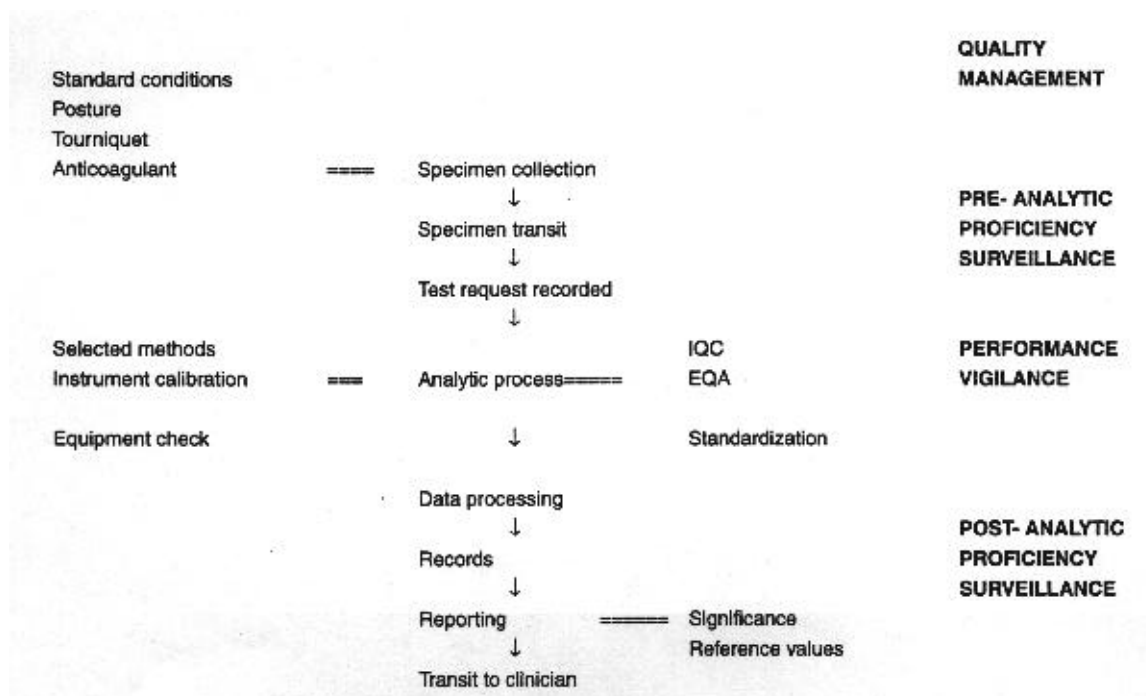


Figure 1. Flow diagram of quality management in the laboratory.

The pre-analytic phase begins with blood collection, which should be standardized to avoid subsequent measurement artefact.⁽¹⁾ Prolonged use of a tourniquet results in haemoconcentration; the patient's posture (standing, sitting or lying) and even the position of the arm during venous sampling will cause fluctuations of 5-10% in the blood count. Many blood constituents fluctuate significantly in a circadian rhythm during the course of the day. When a patient arrives at the clinic late for the appointment, flustered and breathless after having failed to find a convenient parking space, it should be

remembered that stress and exercise also lead to increases in cell concentrations and coagulation factors, notably FVIII and also tissue plasminogen activator (t-PA) with increased fibrinolytic activity.⁽²⁻⁴⁾

When blood is added to an anticoagulant such as EDTA, blood cell morphology will be affected. These effects increase if the EDTA is present in excessive concentrations, and when there is any delay between specimen collection and analysis. K3EDTA causes significant shrinking of the red cells with a decrease of 1-2% in the MCV. The International Council for Standardization in Haematology (ICSH) has recommended K2EDTA in a concentration of 1.5-2.2 mg/ml (4.55 ± 0.8 mmol/ml) as this causes less cellular change.⁽⁵⁾ However, both forms of EDTA continue to be used, thus hindering comparability of results between different laboratories. The error is compounded by the fact that in some instances less than perfect care is taken to ensure the correct ratio of anticoagulant to blood.

A serious, and potentially fatal, cause of mishap is collection from the wrong patient or subsequent specimen mix-up or transcription error. These can occur at any stage, despite, or perhaps because of, computerised systems with bar coding for patient and specimen identification, and it is essential to have a cross-check procedure.

Quality Management of the Analytic Phase

To ensure reliability in the analytic phase, procedures are required for internal quality control, external quality assessment and standardisation.⁽⁶⁾

Internal Quality Control

Internal quality control (IQC) is primarily a demonstration of precision. It ensures continual check that the established reliability of the laboratory's work does not fluctuate and that reports are validated before they are released. It is based on monitoring the procedures which are actually used for the tests in the laboratory. It includes:

- Control charts with tests on control materials
- Duplicate tests on all specimens or on a proportion of the specimens
- Delta check, comparing current test results with previous results
- Consistency of mean values of patient data;
- Correlation check (e.g., blood film features or sensibility of interrelated parameters)

Aspects of these procedures are discussed in subsequent papers in this symposium. It is worthwhile emphasising the importance of the blood film for quality control, especially so since it appears to be considered, by some, to be obsolete in competition with the increasingly automated blood count systems.

Any obvious discrepancy between the count obtained by the analyzer and subjective impression of morphology should always be checked. A glance at a blood film will confirm or refute an abnormally high or low leukocyte or platelet count. An important cause of artefactual leukopenia and thrombocytopenia is partial clotting of the specimen - this may be revealed by the presence, in the film, of fibrin strands with a mass of aggregated platelets. The film will also identify anomalies due to the presence of cold agglutinins, a high leucocyte count due to incomplete lysis of red cells in

haemoglobinopathies, etc.⁽¹⁰⁾ Counters which discriminate different types of cells by their sizes may fail to distinguish markedly microcytic red cells from platelets and, conversely, large platelets (megathrombocytes) from red cells.

Whilst it is neither practical nor necessary to stain and examine blood films from every specimen on which a blood count is carried out on an automated analyzer, it is important to recognize when a film is necessary to pick up a diagnostically informative feature. It is essential to provide staff with adequate training in morphology and the use of the microscope.

External Quality Assessment

As originally defined by WHO,⁽¹¹⁾ external quality assessment (EQA) is the objective evaluation by an outside agency of the performance by a number of laboratories on material which is supplied specially for the purpose. It is usually organized as a proficiency testing programme on a national or regional basis, whilst WHO sponsors an international scheme whose participants are intended to be reference laboratories in their respective countries.^(12,13) Analysis of performance is retrospective, the object being to achieve interlaboratory comparability or harmonization, but not necessarily accuracy unless the specimens have been assayed by a reference laboratory alongside a standard reference.

In recent years, there have been a number of publications on quality management that emphasise the importance of EQA. These include the International Organization for Standardization ISO9000 series of standards⁽¹⁴⁾ and ISO Guides 25 and 58,^(15,16) the European Committee for Clinical Laboratory Standards (ECCLS) on quality assurance,⁽¹⁷⁾ and reports of conferences of the College of American Pathologists.⁽¹⁸⁾

There is still debate on how to use the data from EQA surveys for assessing performance by participants. This problem was well demonstrated when results obtained in a survey of haemoglobin and PCV from 500 participants in one national EQA scheme were analysed by the organizers of other similar EQA schemes in the European Union. The original NEQAS assessment had identified 15 poor performers for Hb and 9 for PCV, whereas in the other schemes assessment of the same data identified 13 to 103 poor performers for Hb and 1 to 155 poor performers for PCV. Clearly, this is not just a technical problem for the organizers of EQA schemes, as it is of vital concern to the participants who may be required to show their standard of performance when contracting their services and for licencing. The discrepancies are due to the use of different methods for assigning values to the survey specimens as well as different methods for data processing.

To analyse results on EQA specimens it is first necessary to establish the target values. These might be "truth" as determined by one or more reference centres or "consensus" from the results of the participants. Referees should use reference methods which are traceable to a primary reference standard, whereas consensus is based on the use of routine methods. As consensus may be biased by the most commonly used method or instrument, it might be necessary to establish a different target value for each method. These are more convenient and practical to use than referees. Moreover, because of the absence of absolute metrological standards for most quantitative tests in haematology (haemoglobin is the outstanding exception), the consensus mean or median is, in general,

more likely to give a closer approximation of the true value. For qualitative tests, the correct result may be assumed as either that obtained as a consensus by 80% of the participants or by a consensus from 5-10 consultants who are recognized as experts in the particular field and who may be provided with additional clinical information.

Assessment of Performance

The deviation index (DI; also referred to as z-score) is used by many EQA schemes for assessing performance in quantitative tests.^(8,10,17) This is the amount of deviation from the mean (\bar{x}) relative to a unit of 1 standard deviation SD' (see below). In most cases, where results tend to be either closely bunched or widely scattered without defining a Gaussian distribution, it is better to take the median and use non-parametric statistics to estimate the central 50% spread of data (between the 25th and 75th percentiles) from which the SD is calculated as 50% spread, 1.349.⁽¹⁹⁾

SD' is the "trimmed" standard deviation, i.e., the SD which has been adjusted by excluding "blunders" and results > 3 SD in an initial calculation.

Alternative methods for adjusting the SD include:

- a) SD of best performance by referee laboratories
- b) SD of performance by a selected set of participants
- c) Calculated from a predetermined constant CV which takes account of the required level of technical competence, and the clinical utility of the test and its discriminatory function. Thus, taking as an example Hb, a CV of 1% might be required for scientific metrological studies but 5-10% will suffice for clinical purposes, when account is taken of the imprecision of routine methods as well as physiological and diurnal variations.

Performance Criteria

The DI provides a simple method for judging performance in a survey, and it also indicates whether there has been any improvement or deterioration in sequential surveys, thus distinguishing between casual errors and persistent unsatisfactory performance.

A limitation of DI is that it is purely statistical. As the state of art improves some blood count parameters will have a CV of only 1-2%. Thus, the DI will indicate poor performance with unrealistically small deviations from the median. It may be better to use clinical relevance when determining the acceptable limits of percentage deviation from the target value. There are some differences in these limits as established for CLIA 88 requirements in the USA and those proposed by some European EQA schemes⁽²⁰⁾ (Table 1).

Table 1. EQA performance assessment: allowable limits of deviation (positive or negative) as percentage of mean or median.

| CLIA 88 (1992) European schemes | | |
|---------------------------------|---|---|
| | % | % |
| Hb | 7 | 3 |

| | | |
|-------------|----|----|
| RBC | 6 | 3 |
| PCV/MCV/MCH | 6 | 4 |
| WBC | 15 | 8 |
| PLATELETS | 25 | 10 |
| PT/INR | 15 | 15 |

The more stringent limits have been proposed by the European group to provide greater sensitivity in diagnostic discrimination, but they may be impractical, resulting in an overestimate of the numbers of poor performers.

EQA schemes should assess not only technical reliability but also professional competence in interpretation of the measurements. Thus, participants should be required to report on the technical significance of their results (i.e., whether within normal reference values for the specified method) and also on the clinical significance, taking account of any clinical information provided. Incorrect interpretation of a correct quantitative result is often due to lack of understanding of the concept of reference values as described by IFCC and ICSH^(21,22) and to the use of inappropriate reference ranges. It is important in the post-analytic phase of quality management (Fig.1) for each laboratory to establish its own reference values for normals and for specific groups (e.g., smokers, pregnancy).

With qualitative tests, too, account must be taken of the clinical significance of the answer. For each test it is necessary to decide if it is as serious to err by reporting a feature which does not exist as to miss an abnormality which is present, and to weight the score accordingly. Scoring may be either as a credit for correct answers or as a penalty for an incorrect answer. Thus, using the penalty method in G6PD screening, a correct answer scores zero, whilst an error will be graded depending on the implications of misclassification for the subsequent management of the patient: it is more serious to report a low level as normal (score 5) than a normal value as low (score 3), and when the true value is intermediate, it is more serious to report it as normal in a female (score 3) than in a male (score 1).

Blood Film

Blood cell morphology is an important component of EQA. A group of expert assessors reach a consensus on the important diagnostic features of a blood film and give a weighting which will depend on the diagnostic problem in the particular case. Then, with the penalty method, a correct result scores zero, whilst every error, positive or negative, is penalised by the allocated weighting to obtain a total positive score as follows:

- Essential diagnostic feature: score 5
- Influences diagnostic decision: score 2-3
- *Might* be helpful (but not essential) in making diagnosis: score 1
- Feature providing no useful information, whether positive or negative: score 0

Summary of EQA Functions

EQA is primarily intended to check the technical competence of individual laboratories, but it also provides an overview for assessing the state of the art and for identifying problems that have occurred in instruments, reagents or kits that may be affecting an entire group of users through no fault of their own. This provides a means for verification of manufacturers' conformity to their claimed specifications and for monitoring product performance in the laboratory, as required in Europe in the context of *the Directive on In-Vitro Diagnostic Medical Devices*.⁽²³⁾

Education should be a fundamental *raison d'être* for the existence of EQA. Experience with G6PD in the UK NEQAS illustrates this. Previously, there were approximately equal numbers of laboratories using fluorescent spot test and dye decolorization. Results clearly showed the former to be more efficient, and this has persuaded users of the latter method to change, so that now over 84% of participants use the fluorescent spot test.

Another example occurred with Hb A₂ surveys. Earlier results showed such large random errors as to make the test hardly worth doing. The national professional societies collaborated with NEQAS in organizing a series of workshops, which resulted in significant improvement and the disappearance of most of the random errors. Furthermore, stimulated by the problems, ICSH developed an international reference standard which has been adopted by WHO. A similar study of the results for haematinic assays in NEQAS surveys in the UK led to ICSH/WHO international reference standards for serum vitamin B₁₂ and serum folate. More information of this type would be useful for determining the priorities for national or international agencies engaged in developing standards, and also for the International Society of Hematology when organizing the educational programmes of their Congresses.

Standardization of EQA Schemes

It is essential that participants in EQA schemes should have confidence in their efficiency and their effectiveness. ICSH has prepared guidelines for the organization and management of EQA schemes using proficiency testing. These guidelines are intended to help maintain a meaningful standard in the organization of EQA schemes and to harmonize the way in which they function. They include the following important principles and technical criteria:

- Surveys should be sufficiently frequent to make sequential performance records meaningful and to identify participants who are persistently unsatisfactory as soon as possible. Blood counts should be distributed at least monthly, other tests quarterly or more frequently depending on their clinical importance and reliability of analytic methods.
- There should be at least two specimens for every test, with values at diagnostically critical levels.
- To ensure that EQA relates to practice, survey samples should simulate natural specimens as closely as feasible, and participants should be obligated to handle them the same way they handle routine specimens.

- The material used in surveys should be stable, at least until the closing date of the survey.
- The survey specimens must test negative for HIV antibody and Hepatitis B and C antigens, and must be labelled in accordance with national regulations for packaging and transport of biological material.
- Data processing must be as rapid as possible, with prompt reports to participants.
- Organizer/participant confidentiality must be maintained. Any information on an individual's results to a third party (e.g., a licencing authority) would be provided by the participant and must *not* be the responsibility or duty of the NEQAS organizer.
- EQAS must be professionally led and should function independently of government health authorities.
- Industry may provide a useful service by organizing a scheme for users of their apparatus, but a national scheme must always be independent of industry.
- Above all, EQAS itself must not be a licensing authority nor a policing body - its primary major function is educational.

References

1. International Committee for Standardization in Haematology. Standardization of blood specimen collection procedure for reference values. Clin Lab Haematol 4:83-86, 1982.
2. van Assendelft OW, Simmons A. Specimen collection, handling, storage and variability, in Lewis SM, Koepke JA (eds): Hematology Laboratory Management and Practice. Oxford, Butterworth Heinemann, 1995, p 109-127.
3. Dacie JV, Lewis SM. Practical Haematology, 8th ed. Edinburgh, Churchill Livingstone, 1995, p 9-19.
4. Bachmann F. Molecular aspects of plasminogen, plasminogen activators and plasmin, in Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD (eds): Haemostasis and Thrombosis. Edinburgh, Churchill Livingstone, 1994, p 575-613.
5. International Council for Standardization in Haematology. Recommendations for ethylenediaminetetraacetic acid anticoagulation of blood for blood cell counting and sizing. Amer J Clin Path 100: 371-372, 1993.
6. Lewis SM, Koepke JA (eds). Hematology Laboratory Management and Practice. Oxford, Butterworth Heinemann, 1995.
7. Cavill I (ed). Methods in Hematology Vol 22: Quality Control. Edinburgh, Churchill Livingstone, 1990.
8. Lewis SM. Quality assurance in haematology. WHO document LBS 92.4. Geneva, World Health Organization, 1992.
9. Cembrowski GS, Carey RN. Laboratory Quality Management. Chicago, ASCP Press, No.189: 1989 .
10. Lewis SM. Quality assurance in laboratory haematology. Proceedings of Royal Society of Edinburgh 101B: 283-310, 1993.
11. World Health Organization. External quality assessment of health laboratories: EURO reports and studies 36. Copenhagen, WHO Regional Office, 1981.

12. Gibbs WN. The World Health Organization's international external quality assessment schemes: progress and problems. 7th International Symposium on Quality Control, Tokyo, p 257-272, 1991.
13. Lewis SM. The WHO international external quality assessment scheme for haematology. Bulletin of the World Health Organization 66: 283-290, 1988.
14. International Organization for Standardization. Quality management and quality assurance standards: ISO 9000 Parts 1-4. Geneva, ISO, 1987-1993.
15. International Organization for Standardization. General requirements for the competence of calibration and testing laboratories: ISO/IEC Guide 25. Geneva, ISO, 1990
16. International Organization for Standardization. Calibration and testing laboratory accreditation systems - general requirements for operation and recognition: ISO/IEC Guide 58. Geneva, ISO, 1993.
17. European Committee for Clinical Laboratory Standards. Standard for quality assurance, Part V: External quality assessment in haematology. ECCLS Document 3(1). Berlin, Beuth Verlag, 1986.
18. Hartmann AE, Ross JW. CAP Conference XIII on the evaluation of proficiency testing results for quantitative methods in relation to clinical usefulness. Archives of Pathology and Laboratory Medicine 112: 327-474, 1988.
19. Tukey JW. Exploratory data analysis. Boston, Addison-Wesley, 1977.
20. Lewis SM. External quality assessment, in Lewis SM, Koepke JA. Hematology Laboratory Management and Practice. Oxford, Butterworth Heinemann, 1995, p 199-217.
21. International Committee for Standardization in Haematology. The theory of reference values. Clin Lab Haematol 3: 369-373, 1981.
22. International Federation of Clinical Chemistry and International Committee for Standardization in Haematology. Approved recommendation on the theory of reference values part V: Statistical treatment of collected reference values; determination of reference limits. J Clin Chem Clin Biochem 25: 645-656, 1987.
23. Council of European Communities. Proposal for a European parliament and Council directive on in vitro diagnostic medical devices. Official Journal of the European Communities C172/02: 21-44, 1995.