

## EXTERNAL QUALITY ASSURANCE PROGRAMME IN HAEMATOLOGY

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### INTRODUCTION

The Haematology Laboratory encompasses a ~~mix~~ of activity not usually found in other disciplines of Pathology. The haematologist must be responsible for laboratory performance including cell counting, cell sizing, measurement of analytes both intracellularly and in the serum as well as morphology, the serology of blood banking and the preparation of blood products for transfusion.

Even if not directly responsible for patient care medical haematologists are trained and expected to provide a rapid consultation service in close liaison with clinical staff for acute and chronic haematological problems.

The design of an external Quality Assurance Programme (QAP) in Haematology must then contain challenges for a wide range of laboratory and clinical activities. It must also be acceptable in its design to participants who have a variety of backgrounds in training. Because of the mix of morphology, serology and measurement such a programme is difficult to design, organise and manage. Analysis of results is complicated because of the absence of reference material for cell sizing, cell counting and coagulation activity. Morphology is always subjective and the wide range of training of staff expected to perform morphological exercise in the laboratory causes great complications in the assessment of their performance.

### CONTENT

The actual choice and preparation of materials and the range of tests to be incorporated must be closely related to laboratory factors such as location, training of staff and equipment. There is little point in presenting a complicated morphological or specialised coagulation analysis to a laboratory staffed by a laboratory aide of one year's training in a remote locality. In other words the laboratory structure in terms of staff **training** and internal organisation and to a lesser extent equipment are the most important factors for adequate quality of results. An external QAP cannot produce improvement in a laboratory where the structure of that laboratory will not allow

it. It may simply show that such a laboratory should not exist.

Many haematology analytes are very unstable; even stabilised cell suspensions have an expiry date which can be shortened by exposure to **high** temperatures e.g. in the postal service. Stabilisation is achieved using partial **fixation**, addition of antibiotics, lyophilization etc.

### RANGE OF TESTS AND PREPARATION OF MATERIALS

#### 1. *Full blood count.*

It is generally recognised by haematologists that should all other techniques be unavailable, a spun haematocrit and the examination of a well made stained peripheral blood film gives the greatest amount of useful information in the diagnosis of haematological disorders. However the widespread use of modern equipment, different brands and techniques of cell counting and measurement mean that a compromise must be reached in sample preparation.

The Australian and most other surveys aim to provide a partially fixed wet sample which **is** suitable for most technologies including manual counting. However such samples are unsuitable to be spread and stained as blood **films**. The latter must be provided separately. Table 1 provides the technical details for **sample preparation**. Even using this carefully tried preparation **technical problems** are encountered.

- a) These red cells, particularly at low temperatures, tend to adhere to each other following this treatment and adequate (at least thirty minutes) resuspension at room temperature is required for uniform sampling.
- b) This material is not suitable for differential count and gives a total white cell count only.
- c) There are difficulties in manual counting of white cells as avian red cells are used as a substitute and some participants often are not experienced in recognising such cells as substitute white cells.

- d) These red cells swell at different rates when the mean cell volume is attempted using different technologies and thus variable answers may be obtained for this index.
- e) The formalin used to partially fix the platelets may sometimes over-fix red cells. In some machines red cells are not lysed as freely as others and over-fixed red cells may be counted under these circumstances as leucocytes.

It may be appropriate in many instances where there are small laboratories and where there has been no previous external QAP to aim at providing a test for haemoglobin estimation only. The measurement of haemoglobin is surprisingly poorly done as most laboratory methodology is aimed at less concentrated substances; thus a large dilution of the haemoglobin in its reagent must be made. The additional problem of mixing of red cells adequately also affects the other parameters referred to in the previous paragraph. A simple method of performing haemoglobin quality assurance is to provide a haemolysate. This does not necessarily need to be stabilised although high temperatures of course will cause methaemoglobin conversion which will not be measured by the oxy-haemoglobin

**TABLE 1**  
**TECHNICAL DETAILS FOR SAMPLE**  
**PREPARATION**

*Principle*

Fresh donor blood is used to prepare and stabilise whole blood specimen suitable for postal distribution. It includes a fixed platelet component and a substitution of fixed avian red blood cells for human white cells.

Both the red cells and PRP are treated with a stabilising solution. The platelets are partially fixed with a weak formalin wash. The human WBCs are removed from the RBC component and fixed avian RBCs added.

*Method*

Fresh donor blood is collected into EDTA. The pack is spun. The platelet rich plasma (PRP) separated.

The stabilising reagent contains glucose, adenine and inosine. Chloramphenicol is added as an antibacterial agent and cyclohexamide as an antifungal. The sample is stable for 6 weeks.

method used in many small laboratories. However, provided it is reasonably fresh the results seem to be encouraging. The haemolysate is simply prepared by freezing and thawing an appropriate specimen of EDTA blood, making sure that the resultant solution is homogenous by mixing well and then sucking the solution into a length of plastic tubing such as is used in drip sets or attached to plastic blood bags. The resulting long tube can be segmented using a heat sealer or stapler as is used in many transfusion centres. Such specimens are then completely sealed and can be easily sent through the post even in ordinary envelopes. There is at present a small programme using this methodology in operation in Indonesia.

*2. Coagulation.*

Even in experienced laboratories there is a little agreement still on standardisation in coagulant activity estimation. The greatest success has been in the introduction of the International Normalised Ratio (INR) with ISI conversion of different thromboplastins but this calculation is still not in uniform use throughout the world. There is still disagreement on the source of thromboplastin, as to whether it should be of animal or human origin and the degree of anticoagulation for patients on anticoagulant therapy. To complicate matters plasmas for testing must be fresh and rapidly freeze dried and even under optimal conditions normal values for a prothrombin time are difficult to obtain following such treatment. However, as far as the prothrombin time is concerned even in the presence of an extended control adjustments can be made and an appropriate International Normalised Ratio calculated. In practice the preparation of such samples in non-air conditioned premises and less than ideal circumstances is almost not worth the effort as there is a resultant variability in the performance of the different samples. Issuing of wet plasma is of no use at all. Freeze drying equipment to prepare bulk samples and ampoules is expensive and difficult to maintain.

The problems met with the specimens in prothrombin time estimation are even more greatly exaggerated with respect to Activated Partial Thromboplastin Times and special factor assays. These tests are of course even more sensitive to small factors deficiencies. To make matters worse there is no standardised method of reporting and there is a huge variety

of reagents and methods. In addition, the precision of the manual technique is extremely poor. Thus, unless there are large numbers of participants and optimal conditions of preparation there is virtually no reward in sending this determination in Quality Assurance Programmes.

Fibrinogen, however, is reasonably stable but the results of the determination are usually very poor as most methods are inadequate. The easiest methods such as heat precipitation have very poor precision and accuracy but are probably of sufficient clinical use to detect the complete absence or very low levels of fibrinogen.

### 3. Vitamin Assay

The rules that apply to the Quality Assurance of most biochemical assays would apply to the determination of B12, folate and red cell folate as well as ferritin. However, there are problems with the multiple analogues of B12 and folate that are present in serum, the different methodologies including the continued use of microbiological assays and the resultant different normal ranges. Vitamin B12 assay itself is intrinsically difficult to assay as it is widespread in nature, is in the water supply and appears to adhere to glass and plastic. B12 free reagents and glassware are difficult to prepare. The preparation of the serum samples is straight forward although obtaining sufficient serum to test for low vitamin B12 levels is for obvious reasons quite difficult. There seems to be no satisfactory artificial method of depleting serum of B12. In the Australian survey, red cell folate have been successfully determined for some time now using a freeze dried haemolysate but even so the overall results show wide variations and a totally unacceptable coefficient of variation.

### 4. Morphology

Testing of morphology is by far the most painstaking and labour intensive area. Providing over 100 blood or bone marrow smears of adequate stain quality is well nigh impossible unless films are prepared in a dry atmosphere and rapidly fixed completely in absolute anhydrous methanol prior to staining. Finding a source of sufficient quantity of high class glass slides without grease on the surface is also a problem. The selection of subjects of morphology is also difficult as, no matter how stringent the quality in the preparation area, slides seem to deteriorate in weeks or months. Thus patients must be used that are imme-

diately available and the selection of subjects for morphology is somewhat dependent on this source. The problem is overcome in the College of American Pathologists Survey by the use of 35mm colour slides but most authorities consider that this is not a real test. However it is a solution where the large number of participants or sub-optimal conditions make the preparation of real films impractical. Although a somewhat daunting task it is possible to make five or six hundred slides from a marrow aspirate provided that it is reasonably cellular and well suspended in EDTA. The EDTA diluted marrow should not be held for more than thirty minutes otherwise artifact changes rapidly ensue.

As the morphology survey will be testing quite junior laboratory staff it should be aimed to be within the capabilities of this level of staff as well as sufficiently interesting and varied to challenge senior and experienced specialists. This is achieved, if with some difficulty by regularly sending simple exercises such as normal films, simple iron deficiency anaemia, blood film artifacts etc. as well as unusual and difficult disorders such as the classification of leukaemias, storage disorders etc. Repetitive testing with similar material gives the opportunity of comparison to see if the Quality Assurance Survey is achieving any recognisable improvement. Telephone or personal comparison of results between laboratories is more common in morphology than in any other sector of an external QAP and thus mixed samples carrying the same label should be sent out at regular intervals to test the honesty of participants.

### 5. Other

There are other areas of haematology laboratory testing which could be included in a Quality Assurance Programme but are generally omitted. *Reticulocyte counting* is an extraordinarily imprecise measurement in the laboratory, the error not being confined to the microscope count but also in the preparation. It is thus difficult to provide material to cover all these aspects as reticulocytes mature quite quickly *in vitro*. Even the stained slides appear to lose some of their RNA staining over two weeks or so. *Red cell enzyme screening* tests especially Glucose 6-Phosphate Dehydrogenase deficiency could easily be tested but to my knowledge no survey includes this aspect. The immunofluorescent phenotype of circulating haematological cells is becoming of greater importance in the classification of

leukaemias and lymphomas and no doubt some means of testing these parameters will have to be designed in the future. It may be possible to partially fix cells such that the surface antigens involved in these markers are preserved.

#### 6. General

Although not specific for haematology, *laboratory safety* in haematology laboratories is often neglected as known infectious material is rarely handled. However the increasing awareness of blood contamination with hepatitis or AIDS infecting particles makes this an important aspect of Quality Assurance. Safety has been attempted in the Australian Survey using questionnaires and the results have been surprising if not somewhat alarming.

The *identification of samples* is another area frequently neglected by External Quality Assurance Programmes although a frequent error in laboratory practice. The occasional issue of deliberately mislabeled specimens is a useful exercise.

#### FREQUENCY OF SURVEYS

Chemical pathology surveys generally despatch specimens every fortnight for laboratory analysis. However this has been found rather impractical in the haematology environment and although this varies from country to country the generally accepted frequency is between once a month and once every two months for blood counts and other simple determinations. However the labour involved in the preparation of coagulation, samples for haemoglobin electrophoresis, morphology etc. limit the frequency to once every three months in the hands of the Australian survey. In other countries it tends to be even less frequent although this is naturally budget dependent to an extent. One of the major problems is statistical in that if there are too few participants and too numerous methodologies, no statistically valid comparisons can be made on cell enumeration and other assays. It is not possible to send duplicate blood count samples out at different months as the blood count samples would have exceeded their expiry. However, it is possible to use the system with freeze dried specimens and does assist to make statistics more valid. In the Australian surveys, survey participants are presented with blood count and simple slide morphology exercises once a month and a full survey including morphology, coagulation and special tests is sent once every three months.

#### ASSESSMENT OF RETURNS

There is no doubt that the major factors operating against a useful and interpretable result format for participants in the Haematology Quality Assurance Programme are:

1. A large number of different methodologies amongst a small number of participants, and
2. Subjective methods needed to assess morphological returns.

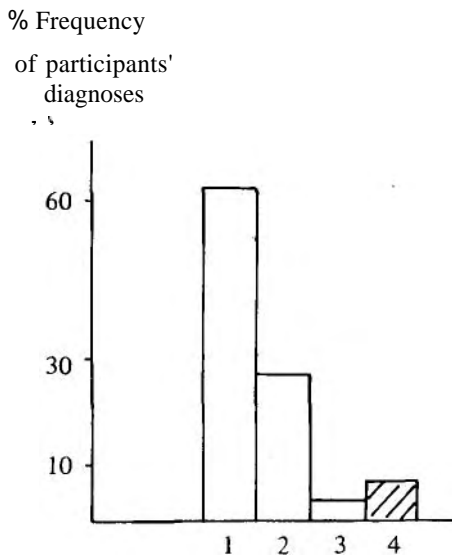
There is no easy way of overcoming these problems and in some cases it is impossible. Reference material suitable for all assay methods is unavailable and thus the setting of target values is impossible except for haemoglobin and even then such material must be in the form of haemolysate and is therefore unsuitable for other determinations. Haematology Quality Assurance Programmes throughout the world have settled on "Consensus Means" and in order for these to be relevant then there must be sufficient partakers in each group. One way around this problem is to group as many methodologies of similar type together; this technique is rather arbitrary and based on a rather anecdotal perusal of the figures but nevertheless seems to be useful in practice. Where there are less than ten participants in each group the only solutions are to issue no statistics at all or to group them together in an "All Method Mean" for a comparison which may not be entirely valid.

Morphology results may be analysed on the basis of "correct" or "incorrect" return as judged by a panel of reference laboratories or haematologists. This can be felt by participants to be somewhat arbitrary and in some cases a great deal of bad feeling can be generated because of perceived lack of fairness on the part of the survey organisers. For these reasons the Australian survey has elected to analyse participants on the basis of a relative analysis of responses from other participants (Fig. 1). Thus participants can gauge the adequacy or inadequacy of their reporting by comparing the performance of their peers. Nevertheless even here grossly inadequate results which would be considered unacceptable under all circumstances are flagged.

#### Presentation of Reports

The most confusing area for participants is the understanding of their reports. Complexities should therefore be kept to a minimum. any statements should be fully justified and the

FIG. 1: RELATIVE ANALYSIS OF PARTICIPANTS' RESPONSES



1. Thrombotic Thrombocytopenic Purpura/ Haemolytic Uremic Syndrome.
2. Microangiopathic/DIC.
3. Sickle Cell Disease
4. Others.

survey organisers should indicate their willingness to negotiate on any questions. Clarity of results presented to participants is enormously assisted if the participants' result is reprinted and sent back to them with the comparative results of other participants. Laboratories are often rather disorganised and cannot find their own results. The Australian survey also issues an Action Sheet (Fig. 2) which highlights areas of either possible deficiency in performance or total non performance. Non performers prove to be the biggest problem area as poor performers generally respond to the awareness of their deficiencies. However, most surveys in the world continue to see a significant core of laboratories who although fully enrolled in the Programme, never submit any results.

#### Poor and Non Performers

In the voluntary programme it is obviously difficult to undertake any executive action to change laboratory practices. However, with the recently enacted Registration of Laboratories in this country lack of compliance with quality control standards can result in a

laboratory's deregistration and inability to charge fees. Should the latter take place a rapid reaction usually ensues. On the other hand in a purely voluntary programme the organisers must limit themselves to negotiation, educational exercises such as educational supplements and diplomatic personal contacts. In the Haematology Survey, educational subjects become the topic of so called "Educational Supplement" once every three months and there is a continued active correspondence between participants and the College organisers of the survey.

#### CONCLUSION

The key question for Quality Assurance Programmes is the effectiveness that they have in the role of laboratory management. Certainly with regard to tests in the laboratory involving true measurements deficiencies can reasonably easily be documented and improvement in such laboratories expected. The most difficult area is morphology. Very few exercises in morphology are exactly parallel and it is thus very difficult to document improvement or deterioration. The labour intensive nature of morphology exercises both at the preparation and participant end makes statistically valid results also very difficult to obtain. The acquisition by a laboratory is of an increasing number of automated machines to take over the role of the morphology or to give assistance in terms of cell parameters seem to be resulting in a deterioration in the standard of morphology both at the Technologist and Specialist level. The Royal Australasian College of Pathologists believe that the Quality Assurance Programmes are and will play a key role of in defining these deficiencies and hopefully in their correction.'

#### REFERENCES

1. Jackson MJ, Hughes W. External quality assurance in haematology. The programme of the Royal College of Pathologists of Australasia. *Pathology* 1985; 17: 573-8.

FIG. 2. Action Sheet for RCPA Haematology QAP

Haematology QAP-RCPA Copyright

Date: 3/4/87 Page

ACTION SHEET for Participant Nu. 250 Survey No. 8702

PROF E S FINCKH 250  
INSTITUTE OF CLIN PATH  
& MEDICAL RESEARCH  
P O BOX 60  
WENTWORTHVILLE NSW 2145

TESTS OUTSIDE ACCEPTABLE LIMITS:-

TESTS NORMALLY PERFORMED BUT NO ANSWER THIS SURVEY:

Q.A.P. CHAIRMAN'S COMMENTS:-

ACTION TAKEN

LABORATORY DIRECTOR

.....  
.....  
.....  
.....

TECHNOLOGIST/SCIENTIST

.....  
.....  
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DO NOT RETURN - KEEP IN LABORATORY FOR ACCREDITATION INSPECTION