Characteristics and performance of the external quality assessment scheme (EQAS) for haematology in Spain. Ten years of experience

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Summary. The external quality assessment scheme for haematology (EQAS-H) in Spain started in 1984 with 56 laboratories, being 473 in 1994. Participants come from public health services (70%) and from private laboratories (30%). Surveys are performed monthly or quarterly depending on the tests and on each occasion the following samples are prepared and sent by the professional organizing team: human (HIV/HBsAg free) or equine whole blood for cell counts (erythrocytes and leucocytes), platelet suspensions for platelet counts, lyophilized plasmas for prothrombin time (PT), partial thromboplastin time (APTT), fibrinogen (F) and antithrombin III (ATIII), and blood films for cell morphology and reticuloocyte counts. In 1992 a new scheme on oral anticoagulant treatment control (OATC) has been established jointly by the Spanish haematology association (AEHH) and the Spanish society of thrombosis and haemostasis (SETH). After preparation, the control material is sent to participants in the scheme where the requested tests are performed and the results reported back to the organizer (Haematology Laboratory Department of Hospital Clinic i Provincial) for statistical analysis. For evaluating the results, laboratories are divided into four to eight groups depending on the methodologies used. Individual results are assessed against a consensus value (mean) and a deviation index (DI) from the mean, and the coefficient of variation (CV). Youden plots and other statistical information are provided for all results and groups of each parameter. More than 80% of laboratories responded regularly (up to 6 trials) for blood counts and haemoglobin and compared to the previous year (1984), the values of CV (%) improved significantly for RBC count (from 3.3 to 2.2%), haemoglobin (from 2.7 to 2.1%) and platelet count (from 22.6 to 16.3%). For coagulation tests, CV were high indicating the necessity of an improvement of the standardization in both the reagents used for the tests and the preparation of control material.

Key words: haemocitometry, coagulation tests, external quality assessment.

Riassunto (Caratteristiche e andamento dello schema di valutazione esterna di qualità per l'emotologia in Spagna. L'esperienza di dieci anni). Lo schema di valutazione esterna di qualità per l'emotologia in Spagna è iniziato nel 1984 con la partecipazione di 56 laboratori che nel 1991 erano già aumentati a 436. I partecipanti sono inseriti in servizi sanitari sia pubblici (70%) che privati (30%). Le indagini si svolgono mensilmente o una volta ogni 4 mesi, secondo il tipo di test sottoposto a controllo. Per ogni indagine, il gruppo professionale di esperti, delegato all'organizzazione dello schema, provvede alla preparazione e alla distribuzione dei seguenti materiali: sangue intero umano (non contaminato da HIV/HBsAg) o equino per i conteggi cellulari (eritrociti e leucociti); sospensioni di piastrine per i conteggi piastrinici; plasmi liofilizzati per il tempo di protrombina (PT), il tempo di tromboplastina parziale (APTT), il fibrinogeno (F) e l'antitrombina III (ATIII); strisci di sangue per la morfologia cellulare ed i conteggi di reticolociti. Nel 1992 è stato introdotto un nuovo schema per il controllo della terapia con anticoagulanti orali (OATC) organizzato congiuntamente dall'associazione spagnola per l'emotologia (AEHH) e dalla società spagnola di trombosi ed emostasi (SETH). Dopo la preparazione i campioni di materiale di controllo vengono spediti ai laboratori partecipanti che effettuano le analisi e trasmettono i risultati al laboratorio organizzatore (Haematology Laboratory Department of Hospital Clinic i Provincial) che provvede, a sua volta, all'analisi statistica. Per la valutazione dei risultati i laboratori vengono suddivisi in gruppi, da quattro a otto, secondo i metodi di analisi impiegati. I risultati individuali sono valutati a confronto con un valore di consenso (media) in base al coefficiente di variazione e all'indice di deviazione dalla media. Per tutti i risultati e relativi gruppi, per ogni parametro, vengono forniti ai partecipanti anche i diagrammi di Youden e altre informazioni statistiche. Negli ultimi anni più dell'80% dei laboratori ha risposto regolarmente (fino a 6 esercizi consecutivi) per i conteggi cellulari e emoglobina. Facendo un confronto con i primi anni di attività (fino al 1984) si è avuto un significativo miglioramento dei CV (%) per la conta degli eritrociti (da 3.3 a 2.2%), l'emoglobina (da 2.7 a 2.1%) e la conta delle piastrine (da 22.6 a 16.3%). Per i test di coagulazione i CV sono elevati ed indicano la necessità di una migliore standardizzazione dei reagenti che vengono utilizzati sia per i test che per la preparazione del materiale di controllo.

Parole chiave: emocitometria, test di coagulazione, valutazione esterna di qualità.
Introduction

Ensuring reliable results of the tests which are performed in large haematology laboratories is essential because of the problems resulting from the use of complex automated instruments and the ever increasing workload. The function of quality assessment in haematology is the establishment and maintenance of an acceptable level of comparative accuracy and precision in the data provided by the laboratory. External Quality Assessment (EQA) is an important component for quality assurance since it allows each individual laboratory to judge the comparative accuracy of its performance even if it has well established internal quality control systems [1-5]. Accordingly, the World Health Organization (WHO) has recommended that in all laboratories there should be protocols for quality assurance and national EQA schemes should be established in all countries. Up to now, different EQA schemes have been established at national level in many countries from Europe [6-9] and most of them have been officially established by government legislation.

In Spain, a voluntary national EQA Scheme (NEQAS) for general haematology was first introduced in 1982 by the Spanish Committee for Standardization in Haematology. In 1984, this scheme, (largely based on the UK NEQAS) attracted the support of the government of Catalonia (Generalitat de Catalunya) who sponsored it through its Department of Health [9]. Later, in 1985 a research grant from the Spanish Ministry of Health (FIS) allowed it to be extended to major public hospitals throughout Spain [10]. At the present, the NEQAS for haematology is run by the Spanish scientific and professional organization "Asociación Española de Hematología y Hemoterapia" (AEHH) [11]. In order to provide a better service for general laboratory practice the NEQAS for haematology works in close cooperation with the NEQAS for clinical chemistry and for microbiology which are run by their own specific professional bodies. The scheme, however, remained mainly a Catalonian one, in view of the large number of clinical laboratories and the obligatory participation for public hospitals in this region. Outside Catalonia the participation in the scheme is voluntary, although it is intended to become obligatory in some autonomous regions with transferred public health services. Laboratories in the private sector may also take part in the scheme by payment of a fee to cover the pro rata cost.

In general, the scheme follows the guidelines established by the International Council for Standardization in Haematology (ICSH) in 1986 [12] and is based on the confidentiality between the organizer and individual participants. Participation is anonymous and there are no sanctions for poor performance.

Specimens for analysis are sent to the participants monthly and nearly all arrive within two days. These include whole blood (for full blood count), platelet suspensions (for platelet count), lyophilized plasma (for prothrombin time, partial thromboplastin time, fibrinogen and antithrombin III), and stained blood films (for blood morphology and reticulocyte count). More than 80% of the results are sent back to the scheme organizer within two week limit. In 1992 a new scheme for oral anticoagulant treatment control (OATC) has been established jointly by the Spanish Associations of Haematology (AEHH) and the Spanish Society of Thrombosis and Haemostasis (SETH). OATC consists in sending two known lyophilized plasmas and reference thromboplastin. Combining the results and the data provided by the scheme, the participant is requested to modify the patient's oral anticoagulant dosification. Finally, a new scheme for the assessment of abnormal haemoglobinins (HbA2, Hbf and haemoglobinopathies) is being prepared and expected for inclusion in the Spanish NEQAS during 1995.

Materials and methods

General instructions

At the beginning of each year all applicants for participation in the scheme receive a copy of the general registration documentation which, under separate sheets, includes: 1) directions for participating in the scheme including a short description of the material and instructions for handling of the samples, and on how the results report form is to be completed, 2) codification sheet of materials used for the measurements (instruments, reagents and calibrators), 3) form sheets for communicating possible changes or modifications in the information on the registration document and 4) results report form, allowing the following data: participant reference number, date of arrival of the samples, condition of samples on arrival, and values obtained. Completed documentation should be returned to the organizer before initiating participation in the NEQAS and the results report form should be sent back before a closing date.

Preparation of EQA material

Preserved blood. - Human blood, collected in CPD anticoagulant is obtained from the Hospital Blood Banking Service from HBsAg and HIV antigens negative donors. Occasionally, equine blood is used, and is then collected in ACD-bags of up to 800 ml capacity from horses or donkeys from an abattoir.

In order to obtain the desired packed cell volume (PCV), whole blood samples are prepared by adding the required volume of plasma of the same ABO group to human erythrocyte concentrates, free from leucocytes and platelets by previous filtration through PALL® RC 50 E
filters. To simulate leucocytes, fixed avian erythrocytes are added in appropriate amounts. Finally, the antibiotics Streptomycin 100,000 U/I and penicillin 0.1 (g/l) are also added. Mixing and bottling processes are performed in a flow cabinet. Homogeneity of the samples is ensured by using a round-bottom mixing flask and a continuous mixing unit [13]. A fixed volume of mixed sample is dispensed by means of a peristaltic pump, into convenient sterile containers. Closed tubes should be kept refrigerated until packed. Homogeneity is confirmed by counts (automatically by Technicon® H*2 system) of at least 5% of tubes randomly selected. The CV obtained for each parameter must be less than 2%.

**Fixed platelets.** - They are prepared from recently obtained platelet-rich plasmas, kindly provided by the Hospital Blood Banking Service. Occasionally, equine whole blood is also used. Preparation of the fixed platelet suspensions includes a filtration by means of Pall® SQ 405 filters, a pre-incubation at 37°C with EDTA to allow platelet disaggregation, a fixation process with 9.8% formaldehyde in phosphate buffered saline (PBS) for about 48 h and the following wash procedure with PBS. After the third wash, sedimented platelets are resuspended in PBS and kept refrigerated until bottled.

Mixing and dispensing procedures are similar to those used for whole blood, including the addition of antibiotics and the homogeneity test. Both type of samples, either whole blood or fixed platelet suspensions, are finally labelled for their identification, packed in convenient envelopes and kept refrigerated until despatched by post delivery.

**Lyophilized plasma.** - This is used for the general coagulation tests, antithrombin III (ATIII) and the oral anticoagulant treatment scheme (OATS). It is obtained from daily plasma excedent of routine analysis and HIV and HBsAg testing is carried out on each sample. The fractions are analyzed and adjusted to prefixed values, divided into aliquots and lyophilized by a local commercial manufacturer. Prior to any manipulation, bulk plasma and human whole blood are analyzed again for HBsAg and HIV.

**Blood films.** - For blood morphology and reticulocyte count assessment routine selected cases are conveniently prepared. The peripheral blood smears are stained with May-Grünwald Giemsa (MGG) and for reticulocyte count, supravital new methylene blue (NMB) staining procedure is used. For blood film evaluation participants are provided with limited information on the patients from whom the films are obtained: age, sex, Hb and total WBC count are given in the instructions sheet. Participants are asked to select up to 5 significant morphology comments but also indicate the relative importance of the chosen comments. Blood films and reticulocyte count assessments are performed quarterly and in each trial only one sample is included.

**General organization**

At monthly intervals each participant receives two different specimens for blood counts, two levels of platelet suspensions and two lyophilized plasmas. For the routine blood count, participants return results for the usual parameters Hb, RBC count, packed cell volume (PCV), RBC indexes (MCV, MCH and MCHC), leucocyte count (WBC) and platelet count (PLT). For blood coagulation tests, participants return results for prothrombin time (PT) (in ratio and in international normalized ratio for OATC), partial thromboplastin time (APTT) and fibrinogen (F). Smears for peripheral blood morphology and reticulocyte count, and plasma for antithrombin III (ATIII) and OATC scheme are sent quarterly. In each trial, two different samples are sent for ATIII and OATC scheme and one sample for peripheral blood morphology and reticulocyte count. After a predetermined time, not long after the deadline set for the return of answers, the results received are processed by statistical analysis using a personal compatible computer.

For each parameter analysed, different subsets are established, depending on the instrument used by participant. The minimum is 20 participants for each subset. The number of subsets may vary from 9 for total blood cell counting to 3 for coagulation tests. In 1989 subsets for manual RBC and leucocyte counting were modified because only three participants used these methods. Accordingly, this subset has been integrated in other semiautomatics and new subsets users have been added.

For coagulation tests, the subset classification has also been modified for PT in accordance with the ISI of thromboplastins and the performance of the instrument used for the analysis.

**Processing of the data**

The data are calculated on the basis of the so-called "consensus mean" which are derived from the mean (X) and standard deviation (SD) recalculated from all the data for each parameter or method and for each result arriving at the organizer before the closing date after excluding any earlier result falling outside ± 3 SD. This allows the rapid detection of blunders in the initial stage of statistical analysis and an individual assessment of the performance by one of the following procedures: 1) deviation index (DI) which is a measure of how a test result differs from the mean value and 2) Youden plot which provides a visual indication of random or systematic errors which have been made. The deviation of each laboratory from the overall or method group
mean is expressed in percentage terms (%) and as deviation index (DI) which is expressed as follows:

<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 - 0.5</td>
<td>excellent</td>
</tr>
<tr>
<td>0.5 - 1.0</td>
<td>good</td>
</tr>
<tr>
<td>1.0 - 2.0</td>
<td>satisfactory</td>
</tr>
<tr>
<td>&gt; 2.0</td>
<td>poor (requires attention)</td>
</tr>
</tbody>
</table>

Participants receive a monthly report containing histograms, Youden plots and several statistical data including the mean (X), standard deviation (SD) coefficient of variation (CV) for: a) all participants and b) by method group and the deviation index (DI). Furthermore, they also receive a six-monthly cumulative and an annual report in which the general performance is appraised. Finally at the end of each year a comprehensive study is published in the official journal of the Spanish Association of Haematology (AEHH).

The results of the reticulocyte count are evaluated by means of the consensus mean (X) and blood films according to the results provided by five reference laboratories designated by the "Spanish Club of Cytology". For this, two levels of performance have been established: "acceptable" or "excellent" depending on the diagnostic implication of the results provided by the participant.

**Results**

As a rule, 95% of the specimens reach the participants by 2 days after posting, and more than 80% arrive back before 15 days, which is the closing date for arrival. The change in the number of participants between 1984 and 1994 and the kind of participant laboratories are represented in Fig. 1 and 2 respectively. Regarding the variation in participant response, in 1984, with 56 participants, the percentage (%) of responses was 67.9%. Although it dropped to 58.3% in 1986 (144 participants in 1985 and 230 in 1986), during 1987 and 1988 a gradual increase in both the percentage of responses (68.5% and 74.5%, respectively) and in the number of participants (262 and 280, respectively) was observed. In 1989 and 1990, with 336 and 346 participants the responses were of 69.8% and 67.9% respectively. Finally, in 1994 with 473 participants these were of 81.1%.

Figs 3 and 4 illustrate the changes in the precision (%CV) for general haematology and coagulation parameters, over the same period. For WBC count, the coefficient of variation (CV) of 17% found in 1984 dropped to 6.2% in 1994. For RBC counts, Hb, PCV, MCH and MCHC, no significant changes were observed in the values of CV during this period. Interestingly, for MCV there was a gradual decrease in CV from 5.6% in 1984 to 4.1% in 1989 and 3.3% in 1994. The platelet count documented an improvement in performance between 1984 (34.4%) and 1994 (16.3%), but the precision of this test is still poor. For coagulation tests (PT, APTT and F) an excessively wide variation between laboratories has been observed (Fig. 4). The CV for PT increased from 1984 (15.7%) to 1991 (18.2%) when the results were given in percentages and from 1989 (11.8%) to 1991 (23.2%) when given as ratio. For APTT and F an increase between 1984 (APTT: 18% and F: 18%) and 1991 (APTT: 24% and F: 32%) was observed.

**Discussion**

The essential purpose of an External Quality Assessment Scheme (EQAS) is to have an objective, independent check of laboratory results in order to establish between-laboratory comparability [1-5]. This target is reached by diminishing the dispersion of the results and by improving the reliability of the tests. The way by which an EQAS is organized depends on a number of factors. In addition to the commercial schemes, sometimes organized by instrument manufacturers for users of their products, several countries have established EQA schemes at different levels, mainly national (NEQAS) which are runned from an Academic or a public health institution. In these schemes participation is either voluntary or obligatory and they should be independent of any undue pressures [6-8, 15].
The Mediterranean EQAS for haematology started in 1984 with 56 laboratories, and rose to 473 in 1994; participants come from national health service hospitals or institutions (33%), public hospitals and laboratories (37%) and private hospitals and laboratories (30%). Surveys are performed monthly and on each occasion the control material is prepared and sent by the Haematology Laboratory Department (Hospital Clinic i Provincial of the University of Barcelona). This EQAS follows the guidelines established by the International Council for Standardization in Haematology (ICSH) [12]. Participation in the scheme is voluntary except for public hospitals and national non-hospital health services in Catalonia [9]. In Europe legislation governing the quality control of clinical laboratories has been established only in some countries, including

![Graphs showing changes in precision for general haematology parameters from 1984 to 1994.](image)

\[ Graph \text{ a:} \text{ Part 1; b: Part 2.} \]
requirements for performing internal quality control and participating in NEQAS. Although the EQA programme has many uses, one of the most important is for establishing between-laboratory comparability. Therefore, the usefulness of our scheme may be judged from the values of CV obtained for the different parameters included in the annual trials. In our EQAS, the CV values for almost all the parameters, with the exception of the coagulation tests, decreased from 1984 to 1994. This is best exemplified by the evident improvement over the years in the performance of haemoglobin (from 3.4% in 1984 to 2.5% in 1991), and WBC count (from 17.0% in 1984 to 6.2% in 1994). Results obtained for platelet count and the coagulation tests were less satisfactory. For platelet count, CV values ranged from 34.3% in 1984 to 16.3% in 1994, with CV values being 31.8% when using the manual method. Although automatic instruments allowed the CV values to drop to around 20%, this is still too high for the required performance of these instruments. Probably, one of the main reasons for the poor performance in the platelet count is the inherent technical difficulties existing for obtaining human platelet suspensions with suitable stability. Platelets are highly unstable and clumping is a frequent event in preparation conditions. Further research is needed in the future to improve this control material. Regarding the coagulation tests the extreme variability of reagents and instruments is probably the main explanation for the wide dispersion observed in the results. Changes introduced during 1989 in the subset classification of PT according to the ISI value [15] or the different thromboplasins used by the participants, have produced a better clarification of the factors involved in the accuracy of these tests.

In summary, the experience accumulated by the Spanish EQAS for haematology is satisfactory and its usefulness is supported by its current role in many laboratories from Spain. Furthermore, the increasing participation in the scheme observed since 1984 is the best proof that it has become established as a successful and valuable contribution towards good laboratory practice. In this aspect, the Catalan Government (Generalitat de Catalunya) deserves special praise for their far-sightedness in sponsoring the scheme as an official activity of its Department of Health.

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