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EQA pilot regional program for haematology in Emilia-Romagna, Italy

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Summary. - A pilot regional EQA scheme in haematology has been implemented in Emilia-Romagna. The distinctive features of this scheme are: a) inter-laboratory comparability of results on a local basis has been selected as main target of the whole scheme; b) fresh blood has been chosen as ideal control material; c) a fast and effective dispatching system of control material has been accomplished. This has been made possible by means of the hospital ambulances network and an express delivery service; d) the assessment of the analyzers calibration alignment in the whole region has been subdivided in two phases. The first one consists in aligning all analyzers of each province with the corresponding reference provincial center, the second one in aligning the analyzers of the eight reference centers in the region with each other; e) all laboratories taking part in the EQA scheme have been directly involved. Preparation of control material and comment sheet mailing to participants are carried out by eight provincial reference centers. Initial setting of the scheme targets, collegial survey of results and alteration of the scheme guidelines involve all participants in the scheme; f) a distinction between a short-term and a medium-term goal has been made. The short-term one is to improve the harmonization of results for comparability between different laboratories in the same area. The medium-term one is to set up educational programmes and consensus procedures for establishing operative protocols and for defining the allowable analytical errors, set on the basis of clinical requirements.

Key words: blood analysis, calibration, flow cytometry, haemocytometry, quality assessment.

Riassunto *(Programma pilota per la valutazione esterna di qualità per l'ematologia nella regione Emilia-Romagna).* - E' stato realizzato in Emilia-Romagna un programma sperimentale di valutazione esterna di qualità in ematologia, caratterizzato da alcune scelte qualificanti. Fra queste, le più rilevanti sono: a) di perseguire come obiettivo prioritario su ogni altro la massima confrontabilità dei dati di laboratorio prodotti nello stesso bacino d'utenza; b) di utilizzare come materiale di controllo il sangue fresco, ritenendolo ideale per questo impiego; c) di risolvere il problema della limitata quantità di sangue ottenibile per ciascun campione di controllo frazionando la regione in aree più ristrette e demandando la preparazione del materiale a otto centri provinciali. La consegna rapida tramite la rete regionale delle ambulanze risolve l'altro problema, del limitato tempo concesso per l'analisi del sangue fresco; d) di raggiungere l'allineamento delle risposte in ambito regionale perseguendo parallelamente l'armonizzazione nell'ambito di ciascuna provincia da un lato, dall'altro allineando periodicamente fra loro i centri provinciali di riferimento; e) di coinvolgere attivamente la globalità dei partecipanti, così da aumentare il loro interesse diretto al raggiungimento degli obiettivi fissati, così come all'elaborazione di obiettivi futuri; f) di considerare il miglioramento di confrontabilità dei dati per i test eseguiti a livello regionale come parte propedeutica di un programma più vasto per il miglioramento della qualità dei servizi, da perseguirsi in seconda istanza tramite l'educazione professionale continua ed il confronto periodico diretto fra tutti gli operatori coinvolti.

Parole chiave: analisi ematologiche, calibrazione, citometria a flusso, emocitometria, valutazione di qualità.

Introduction

An experimental scheme of external quality assessment (EQA) in the field of haematology has been implemented on a regional basis in Emilia-Romagna, Northern Italy.

This EQA scheme started with a first cycle of inter-laboratory collaborative studies, involving twelve public laboratories in the provinces of Ferrara and Modena in 1989 [1].

In 1990 this scheme officially came into force, after being submitted to the regional council.

The scheme has been developed in three stages:

- the first began in November 1990 and was addressed to quantitatively compare test results in the field of haemocytometry all over the region.

- the second began in January 1992 and aimed to upgrade the harmonization of results in the field of differential leukocyte count (DLC), recognition of beta thalassaemic trait and measurement of cell surface antigens of the lymphocytes.

- the third stage is focused on educational programmes. The first regional course in haematology of June 1992 dealt with the standardization of methods and implementation of the new technologies.

Participant laboratories

At present, 86 laboratories take part in the scheme. Of these, 48 are hospital laboratories, 12 public health laboratories, 2 university laboratories and 24 private ones. Fourteen hospital laboratories and five university laboratories have now jointed the side-program on cellular phenotyping by flow cytometry.

Choice of analytical goals

In setting analytical goals and in defining desirable standards of performance for our scheme, we have used the inter-laboratory data comparability as the main criterion for assessment of test results [2, 3].

This choice is motivated by reasons of clinical utility. As haematological test results are often used for monitoring purposes, and serial results from individuals are assessed for possible alterations, low inter-laboratory imprecision is required to ensure that differences are due to improvement or deterioration and not simply to the analytical variability [2-4]. Furthermore, improvement of comparability of the test results has been pointed out by a WHO expert panel as the primary goal of all regional EQA schemes [5].

Choice of materials

Commercial materials available for EQA schemes must be stable enough for use in checking reproducibility over time and/or geography. These materials usually consist of blood deprived of leukocytes and platelets, with surrogate materials added, whilst red blood cell are stabilized, usually by glutaraldehyde fixation.

Unfortunately, when blood is fixed, an alteration in morphological properties of red blood cells occurs and this affects their behaviour in the counting system, as well as the instrumental response in the system's sensing zone [4]. As a result, not all haematological instruments can guarantee fully comparable results in analyzing fresh blood samples and artificial blood controls, even if both are characterized by theoretically identical values for the same parameters [1,4]. Moreover, there is actually no commercial material available to determine the differential leukocyte count (DLC) reliably with all types of haematological analyzers.

Fresh blood has to be considered the ideal material for EQA in haematology, because it has many advantages:

- the instrumental performance obtained with the control material is the same as with patient samples;
- results obtained with any type of blood analyzers are fully comparables;
- DLC can be determined with accuracy by all types of haematological analyzers;
- the whole cycle of cellular phenotyping carried out by flow cytometry can be kept under control. By contrast, commercially available control materials allow to monitor single analysis steps only [6].

On the other hand, the use of fresh blood results in a number of additional problems [1, 7].

The chief ones are related to the limited volumes of blood which can be obtained from occasional donors. Consequently, it is not possible to despatch vials of blood from the same donor to all laboratories of the region. Other problems are linked to the need to limit the time that elapses from blood collection to its analysis to a few hours [7, 8].

Table 1. - Inter-vials variability of control material, assessed by the preparing center before despatching. Number of samples: 4. Number of vials per sample: 18, tested in duplicate

Parameter		Sample a	Sample b	Sample c	Sample d
WBC	Mean	8.16	9.40	4.58	5.80
	CV%	0.23	0.11	0.22	0.17
	Max. diff.%	2.4	3.2	4.3	3.4
RBC	Mean	3.24	3.68	3.61	2.69
	CV%	0.31	0.27	0.27	0.36
	Max. diff.%	1.8	0.8	1.9	2.0
Hb	Mean	11.2	10.1	8.7	9.4
	CV%	0.09	0.10	0.15	0.11
	Max. diff.%	0.9	1.0	2.2	1.0
MCV	Mean	107	86	78	105
	CV%	0.00	0.42	0.46	0.49
	Max. diff.%	0.0	1.1	1.2	0.9
Plt	Mean	213	682	194	391
	CV%	2.39	1.76	2.55	2.71
	Max. diff.%	7.0	4.9	7.2	9.9

WBC: 10^9 cells/l; RBC: 10^{12} cells/l; Hb: g/dl; MCV: fl; Plt: 10^9cells/l.

CV%: inter-vials variations, expressed as CV%.

Max. diff.%: maximal difference among measures carried out in duplicate on each vial series from a same blood donor.

Preparation and delivery of control blood vials

In order to overcome the above mentioned problems, we have chosen to delegate preparation of survey material to eight provincial reference centers. Each center collects from individual donors a quantity of blood not exceeding 25-30 ml, by using standard procedures [8]. This volume allows preparation of a number of vials from 10 to 25, i.e. enough for all participants of the corresponding province. Moreover, we are not confined to habitual blood donors, and occasional donors may be used as well, having previously been selected to provide a wide range of values for all parameters under control.

To comply with the need to test EDTA anticoagulated fresh blood within a few hours [8], blood samples are collected from 7.30 to 8.00 a.m., dispensed into vial sets, analyzed for validation (see below) and handed over to the ambulance staff by 9.30. Samples are received and analyzed for haemocytometry by all participants not later than 12.30, i.e. in less than 5 hours from collection.

While this procedure has been implemented for haemocytometry, due to the relatively high number of laboratories involved throughout the region and the short time available for vial sets delivery, a different system has been used for cellular phenotyping EQA scheme. In this case, only one center in the region is deputed to prepare and to despatch the survey samples because of the smaller number of participants in this side-program. Moreover, an express delivery service is used instead of ambulances network, because of the longer time allowed from blood collection to its analysis. Samples of EDTA anticoagulated blood are collected around 8.00 a.m., delivered to all participants in the region by 1.00 p.m., immediately analyzed for DLC and tested for cellular phenotype on the following morning.

Table 2. - Two EQA years in haemocytometry: 1990 and 1991

Analyte	All results	Acceptable range In no.	In %	Out no.	Out %
WBC	822	805	97.9	17	2.0
RBC	822	816	99.3	6	0.6
Hb	822	801	97.4	21	2.5
PCV	812	767	94.5	45	5.4
MCV	812	745	91.7	67	8.2
Plt	764	743	97.3	21	3.6

Table 3. - Selected criterions for exclusion of outliers

Analyte	Max allowable total bias (*) (%)	Max difference of duplicates (*) (%)
WBC	± 15.0%	± 12.0%
RBC	± 7.0%	± 3.5%
Hb	± 6.0%	± 3.0%
PCV	± 7.0%	± 3.5%
MCV	± 5.0%	± 3.5%
Plt	± 25.0%	± 15.0%

(*) Modified from [13].

Both type of samples for haemocytometry and cellular phenotyping are kept at room temperature during the whole time needed for preparation, delivery and storage, until analysis is carried out.

Beside the above-mentioned, we have had to tackle questions concerning the biological hazard control and the reliability of home made material.

As for biological hazard control, all blood donors are tested a day before blood collection, to exclude any possible contamination by HBV, HCV, HIV 1 and 2.

With reference to the quality of home made survey material, it is routinely controlled by testing in duplicate all vials before despatching. A single vial is rejected, if RBC or WBC values exceed the mean value of the corresponding series from the same donor by ± 1% or ±-2% respectively.

Table 1 shows the values recorded in a provincial reference center for 4 blood samples. Each sample has been dispensed in a series of 18 vials, and each vial has been tested in duplicate.

The maximum difference among results obtained from the same sample does not exceed 2% for RBC and haemoglobin values. This difference includes both the variability due to instrumental performance and that due to sample dispensing procedure into vials. Therefore, we assume that the maximum variability due to dispensing procedures should be approximately 1.0 - 1.5%: this would ensure that quality of the home made material meets the requirements set for the overall purposes of our EQA scheme.

With regard to platelets, the maximum variability recorded is, as a rule, somewhat above that recorded for RBC (see also Table 1). This is not likely to be caused by mishandling in the sample dispensing procedures, as RBCs show a lower variability, although they are dispensed together into the same vials. But, when analyzing the results sheets of two EQA years, this aspect appears to be negligible and the suitability of our home made material is confirmed for platelet count survey purposes too (Table 2).

Data processing and reports sent to participants

Data processing have been accomplished according to ECCLS guidelines [9], and report sheets containing the processed data are sent to all participants together with the next delivery of vial sets.

Parameters recorded in the report sheets are: individual test results, median, mean and standard deviation - the both truncated by exclusion of results which fall outside ± 3 s in a preliminary calculation - percentage bias (V), deviation index (DI) and variation index (VI). DI is a measure of how far a result differs from the mean value as multiple of the standard deviation. VI measures the extent to which the result of an investigation falls within or outside a chosen coefficient of variation (CCV). The VI is calculated dividing the percentage variation of the result from the consensus mean - i.e. (V) - by CCV [9].

The CCVs selection has accounted for a crucial step in setting up our EQA scheme [2, 3, 10-15].
For each parameter undergoing control we have decided to identify the chosen coefficient of variation (CCV) with the corresponding maximum allowable total bias (Table 3). On this basis, the reliability of individual test results is routinely determined. Poor scores are those which represent deviations more than the maximum allowable total bias; this is a very selective criterion, when compared with that adopted by others [11].

The fact that data sent by individual laboratories are processed on a provincial basis, at least for haemocytometry, could have represented a problem. But a comparison on a regional basis is possible, when considering the derived values (VI) - which reflect the percentage bias against the consensus mean of the corresponding province - instead of the absolute values recorded. On the other hand, the regional harmonization of results is also pursued by adjusting the analyzer alignment of the eight reference centers with each other when required.

Once a year, graphs showing the complete set of percentage biases for each parameter are also sent to each laboratory participant in the scheme (Fig. 1). As for haemocytometry, values are recorded as asterisks plotted on diagrams, where threshold lines indicate the maximum allowable analytical bias for the corresponding parameter (Fig. 2).

Results

Results of two years of EQA in haemocytometry are summarized in Table 2. Results outside the maximum allowable total bias formed 3.6% of the total number of 4,854. In particular, the larger part of unreliable results occurred with MCV and haematocrit. Less than 3% of 764 observation for platelet counts and 822 for haemoglobin measurements turned out to be unacceptable. As for RBC counts, 6 out of 822 fell out the allowable range. All in all, the state of the art in haemocytometry in our region has to be regarded as satisfactory.

The first survey in the field of cellular phenotyping has also provided fairly satisfactory results, as compared with other EQA schemes [6]. The coefficients of variation

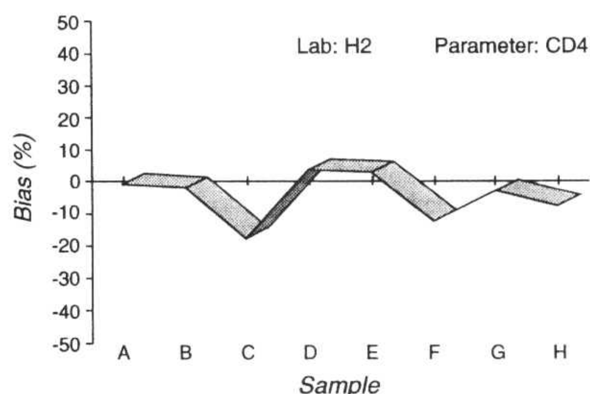

Fig. 1. - Example of a graphic report of percentage biases for CD4 test, concerning H2-labelled laboratory. Control samples: from A to H.

Table 4. - Interlaboratory survey for CD tests. First results

Sample	CD3		CD4		CD8	
	Mean value (*)	CV %	Mean value (*)	CV %	Mean value (*)	CV %
Z 1 a	308	11.0	114	19.5	134	14.7
Z 1 b	410	8.1	344	13.8	201	11.5
Z 1 c	709	11.8	479	9.9	219	2.7
Z 1 d	1035	11.0	645	8.5	365	10.2
Z 1 e	1163	9.0	494	6.4	455	16.0
Z 1 f	1437	11.9	870	10.9	551	19.5
Z 1 g	1554	8.2	1047	11.0	810	14.0
Z 1 h	1690	15.6	1428	12.6	822	12.9

(*) without exclusion of outliers.

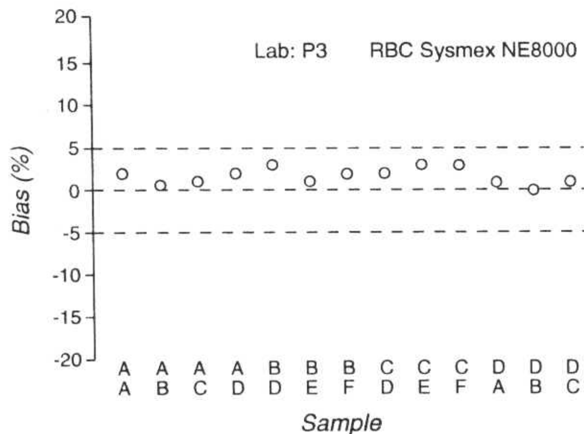

Fig. 2. - Example of threshold lines which evidentiate a percentage bias of ± 5% on a graphic report, which summarizes the percentage biases for RBC counts, carried out by P3-labelled laboratory on control samples from AA to DC.

(CVs) recorded for CD3 tests and CD4 tests were within the range of 10%, while CVs recorded for CD8 tests were somewhat higher (Table 4).

Comments

The EQA scheme we have implemented has proven to be quite effective and the scheme has allowed substantial improvement in the standard of haemocytometry. Actually, in the first six months of 1992 test results sent by the participants in the scheme exceeded the maximum allowable total bias by approximately 1%, as compared with 4% recorded in 1990.

Beside such an improvement in data comparability, the committment of all laboratory staff in pursuing goals set by the scheme and in upgrading the guidelines of the programme must be considered at least as an equally important result.

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