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External quality assessment scheme for haematology in Germany

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Summary. - Quality control in haematology is performed in Germany for 20 years. Both cell count, haemoglobin measurement and differential count on smear with morphology exercise and probably diagnosis will be demanded by the participants. Until now this quality control is not mandatory, even efforts are done to change this circumstance, given by the main input of diagnostic value due to haematology results. So this regulation will be changed very soon, in order to submit haematological laboratories to governmental control, effected by the BÄK (Bundesärztekammer), as already is done in clinical chemistry. For this EQA the participants cannot expected any financial support by any organization, nor public health, nor private assurance. The role of referee laboratories and reference values as well as difficulties for the adequate reference material are discussed. For the differential count other limits have to be established: recognition of pathological blood films is one of the most important point (in sense of morphological exercise) to ensure broad knowledge of "flag interpretation". Since the last year quality control for reticulocyte count and flow cytometry for immune status and leukemia-differentiation has been established.

Key words: quality control in haematology, blood cell-counting, differential leucocyte count, external quality assessment in haematology.

Riassunto *(Schema di valutazione esterna di qualità per l'ematologia in Germania).* - In Germania la valutazione esterna di qualità in ematologia viene effettuata da 20 anni. I laboratori che partecipano al programma di valutazione debbono eseguire i conteggi cellulari, il dosaggio dell'emoglobina e l'esame dello striscio di sangue che comprende la conta differenziale dei leucociti, l'analisi morfologica e una probabile diagnosi. Fino ad oggi questo programma di controllo non è obbligatorio anche se sono stati compiuti notevoli sforzi per modificare la situazione, considerato che i dati ematologici hanno un valore diagnostico molto elevato. La legislazione vigente sarà modificata, a breve termine, in modo che i laboratori di ematologia vengano sottoposti ad un controllo governativo, effettuato dall'ordine federale dei medici (BÄK), come già avviene per i laboratori di chimica clinica. I laboratori che partecipano al programma di valutazione esterna di qualità non ricevono alcun supporto finanziario né da parte delle istituzioni pubbliche né dalle assicurazioni private. Nella discussione viene dedicata particolare attenzione alle difficoltà nella preparazione di adeguati materiali di riferimento e di controllo. Per l'analisi degli strisci di sangue debbono essere utilizzati criteri di valutazione del tutto peculiari: il riconoscimento dei preparati patologici, in base all'esame morfologico, è uno dei punti essenziali per garantire una ampia competenza nel riconoscimento dei segnali di allarme. Dall'anno scorso è stato attivato anche uno schema di controllo per la conta dei reticulociti e per la citofluorimetria sia per le immunodeficienze che per la diagnosi differenziale di leucemie.

Parole chiave: controllo di qualità in ematologia, conteggi delle cellule del sangue, conta differenziale dei leucociti, valutazione esterna di qualità in ematologia.

Introduction

External quality assessment scheme (EQAS) for haematology has been established in Germany since 20 years. Contrary to the situation in clinical chemistry, in haematology the participation in EQAS is not mandatory, although efforts are being made to establish adequate control for this primary tool of diagnosis and therapy.

Only 20% of approximately 5000 laboratories working in haematology participate in EQAS.

In Germany the Bundesärztekammer (BÄK, Federal Board of Medicine) authorized two scientific societies to organize official EQAS: the Institute for Standardization and Documentation in Medical Laboratories (INSTAND), and the German Society for Clinical Chemistry (DGKC). They therefore relate only indirectly to the Federal Minister of Health.

Both organisations are professional, non-profit bodies with a central bureau for mailing, clerical work, computerized evaluation of participants, results, etc.; experts and survey supervisors are chosen from universities, hospitals or professionals, e.g. INSTAND closely cooperates with the German Society for Haematology (DGHO).

Participants in Germany have to pay all fees by themselves; there is no support from health authorities or the insurance system.

This review concerns EQAS for haematology which is restricted to cell counting, differential leucocyte count and medical interpretation (i.e. primary diagnosis). Haemostaseology and bone marrow cytology are not included although EQAS is available, in Germany also in these fields. The examples of experimental protocols and of survey results are taken from the scheme organized by INSTAND.

Programme

Control material and dispatch

Four times a year two samples are sent out:

a) control-blood for measurement of haemoglobin, haematocrit, packed cell volume, as well as for counting erythrocytes, leukocytes and platelets;

b) smears for differential leucocyte count.

Two different procedures are used with control-blood:

a) in the DGKC scheme self- prepared control material is sent out (fresh red cells from a blood bank with supplement of stabilized leucocytes and platelets);

b) in the INSTAND scheme commercially available control blood is bought and used for EQAS purposes.

The second procedure has the advantage that this also provides participants with internal quality control, which is the most important part of good laboratory practice (GLP), according to the philosophy of INSTAND.

Target values and ranges

Target values for full blood count are obtained by reference laboratories, chosen for their range of equipment to ensure a balance between counting systems based on impedance and optical measurement, and with good reputation.

Nine laboratories/survey are involved. They have to achieve multiple measurements as shown in Fig.1.

Two technicians make duplicate measurements on two consecutive days with two different instruments. Additional visual counts (counting chamber) are required.

The target values for cell counts are found empirically; results by reference methods, as described by ICSH for haemoglobin and haematocrit [1-4] are carried out by national institutions and are also taken into consideration.

Reference methods for cell-counting are being developed. These will be a combination of impedance measurement (with dilution steps) and immuno-phenotyping.

Target values for leucocyte differential count on the smears are given by reputable haematology centers (also nine laboratories/survey).

To satisfy statistical requirements four smears are examined twice in repetition by two technicians, so that at least 800 leucocytes are evaluated, in accordance with the NCCLS-protocol [5].

Morphological comment and probability of diagnosis are also required (Fig. 2).

Target ranges are set up as shown in Table 1. The range of acceptable results (target range) is calculated using the target value and adding/substracting an amount which was declared by the BÄK, according to clinical requirements. If necessary, a method- or instrument-dependent evaluation is carried out.

Certificate

As mentioned above, control blood samples and smears are distributed four times a year. Every participant receives a report on his individual results, as shown in Fig. 3, where the good, acceptable or unsatisfactory performance may be seen at a glance.

All participants with good or acceptable results in both samples furthermore receive a certificate, valid for twelve months.

Table 1. - Analytical goals according to the recommendations of BÄK

no.	System	Analyte	Unit of measurement	Target value (method)	Maximal random analytical variability (precision) (%)	Maximal analytical bias (% of target value) accuracy (%)
1	EDTA-blood	haemoglobin	mass-concentration(g/dl)	reference value	1.7	5
2	EDTA-blood	erythrocytes	cell-concentration (no./pl)	selected method	3	9
3	EDTA-blood	leucocytes	cell-concentration (no./nl)	selected method	5	15
4	EDTA-blood	platelets	cell-concentration (no./nl)	selected method	7	21
5	EDTA-blood	haematocrit	volume fraction (1/1)	reference value	3	9
6	EDTA-blood	MCV	cell volume (fl)	selected method	2.7	8

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S O L L W E R T E R M I T T L U N G

zum Ringversuch Hämatologie - Nr. 211

Name: ..... **Rücksendetermin:** ..... **Labor-Nr.:** ..... **Probe:** .....

Bestandteile			Bemerkungen	Methode	Gerät	Tag	1. Untersucher 1. Wert	1. Untersucher 2. Wert	2. Untersucher 1. Wert	2. Untersucher 2. Wert
Hämoglobin	g/dl	HAEM				1				
						2				
Erythrozytenzahl	Ery/pl	ERYZ				1				
						2				
MCV		fl				1				
						2				
Zellp. Volumen zentrifugiert	l/l	ZPVZ				1				
						2				
Hämatokrit elektr./optisch	l/l					1				
						2				
Leukozytenzahl	/nL	LEUZ				1				
						2				
Leukozytenzahl (Kammerzählung)	/nL	LEUZ				1				
						2				
Thrombozyten Plättchenzahl	/nL	P.LZA				1				
						2				
Thrombozyten (Kammerzählung)	/nL	P.LZA				1				
						2				

Datum: ..... Unterschrift: .....

Fig. 1. - Protocol-sheet for reference-laboratories in haematology; haemoglobinometry and cell count, comprising leucocyte and platelet-count in a counting chamber (kammerzählung). Haematocrit is demanded twofold as a machine result (Hämatokrit elektr./optisch., electronic/optical) and as a centrifugation result (packed cell volume - ZPVZ). Eight measurements are required from 2 technicians on 2 instruments as a duplicate on 2 days.

S O L L W E R T E R M I T T L U N G zum Ringversuch Differentialblutbild-Nr. 212

Rücksendeanschrift: **Name:** _____

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Postfach 250211
40093 Düsseldorf
Tel.: 0211 - 33 00 33

Labor-Nr.: _____

Rücksendetermin: 06.05.95

	1.ObjTr 1.U	1.ObjTr 2.U	2.ObjTr 1.U	2.ObjTr 2.U	3.ObjTr 1.U	3.ObjTr 2.U	4.ObjTr 1.U	4.ObjTr 2.U
Myeloblasten								
Promyelozyten								
Myelozyten								
Neutrophile Metamyelozyten								
stabkernige								
polymorphkernige								
Eosinophile								
Basophile								
Monozyten atypische								
reife								
Lymphozyten atypische								
Reizformen								
typische								
Plasmazellen								
Nicht klassifizierbare Blasten								
Nicht klassifizierbare Zellen								
z u s a m m e n (=100%)								
Erythroblasten								
Sonst.Zellen auf 100 Leukozyten								

Verdachtsdiagnose: (verschlüsselt beantworten)

1. _____
2. _____
3. _____
4. _____

Weitere Fragen

Hätten Sie diesen Patienten zur Diagnostik überwiesen?

Haben Sie das Präparat neu gefärbt? (wenn ja, ankreuzen)

Wieviele Personen differenzierten das Präparat?

Wieviele Leukozyten wurden insgesamt differenziert?

Der/Die Unterzeichnende versichert, die Differenzierung selbst und/oder durch ihm/ihr unterstellte Personen in dem von ihm/ihr geleiteten Laboratorium oder in der eigenen Praxis durchgeführt zu haben.

Datum _____ Stempel/Unterschrift _____

Klinischer Befund **Proben-Nr.: 31**

79jähriger Patient, der mit Fieber und Verdacht auf Pneumonie zur Aufnahme kam.

Hb: 9.6 g/dl; Ery: 2.8 /pl; MCV 106.5 fl;
Leuko: 19.2 /nl; Thrombozyten 94 /nl.

Erythrozytenmorphologie (+,++,+++)

01		unauffällig	Veränderungen des Erythrozytendurchmessers
02		Mikrozyten	
03		Makrozyten	
04		Megalozyten	
05		Anisozyten	
06		Poikilozyten, teardrop cells	Veränderungen der Erythrozytenform
07		Ovalozyten	
08		Elliptozyten	
09		Akanthozyten, Klettenzellen	
10		Schizozyten/Fragmentozyten	
11		Drepanzozyten, Sichelzelien	
12		Echinozyten, Stechapfelform	
13		Targetzellen, Kokardenzellen	
14		Stomatozyten	
15		Anulozyten	Veränderungen der Erythrozytendicke
16		Leptozyten, Hypochromasie	
17		Hyperchrome Erythrozyten	
18		Sphärozyten, Kugelzellen	
19		Polychromatische Erythrozyten	Veränderungen des Erythrozyteninhalts
20		Basophile Tüpfelung	
21		Howell-Jolly Körperchen, Kernreste	
22		Cabot'sche Ringe	
23		Parasitenbefall	
24		Geldrollenbildung od. Veränd. d. Erythrozytenlagerung	

Leukozytenmorphologie (+,++,+++)

25		unauffällig	
26		Auer-Stäbchen	
27		Toxische Granulation	
28		Riesenstäbe	
29		Übersegmentierung, Rechtsverschiebung	
30		Döhle Körperchen	
31		Pelger/Pseudopelger	
32		Haarzellen	atypische Lymphozyten
33		Lympho/plasmozytoide Zellen	
34		Zentrozyten	
35		Atypische Plasmazellen	
36		Gumprecht Schollen, beschädigte Leukozyten	
37		Vakuolen	
38		Nekrozyten, Abbauzellen	
39		Verminderte Leukozytenzahl	
40		Vermehrte Leukozytenzahl	

Plättchenmorphologie (+,++,+++)

41		unauffällig
42		Plättchenaggregate
43		Riesenplättchen
44		Verminderte Plättchenzahl
45		Vermehrte Plättchenzahl

Fig. 2. - Protocol-sheet for reference-laboratories for differential count. 1. Obj. tr., 2 Obj. tr. = first, second smear; 1 U, 2 U = first, second technician; Klinischer Befund = legend (in general very short); Verdachtsdiagnose = most probable diagnosis/disorder. It is very important to mark the morphological abnormalities, too.

Results and discussion

An example of results obtained in one survey for full blood count is shown in Fig. 4. The coefficients of variation (CV) range between 5.4% and 5.9% for erythrocyte parameters, with the exception of centrifuged packed cell volume reaching 6.8%. The CVs of leucocyte and platelet counts are near 8% and 10% respectively, but CV increases up to 18% when leucocyte count is performed by microscope. In the same figure the participant results are compared with target values determined by referees and a satisfactory agreement is pointed out for all parameters.

As regards control material, there are some difficulties to be overcome. Thus, e.g. haematocrit/centrifuged packed cell volume are not comparable due to the stabilization of erythrocytes.

Also the matrix-effect may produce some other difficulties. For example, laboratories using the chamber-method to count leucocytes do not easily recognize the form and shape of stabilized leucocytes, and a tendency to aggregation is sometimes observed. This means that there is a large range of results for this method with random errors (Fig. 5a).

Results achieved with all methods are comparable, but the electronic cell counters show a significantly smaller scatter than visual counting (Fig. 5b).

Matrix effects were also relevant in cell counting by instruments when a new generation of electronic counters with enhanced software became available. Suddenly apparently suitable control material was no longer accepted by these technologically advanced instruments, they refused to recognize stabilized leucocyte as "real leucocyte".

This difficulty has been overcome by finding, in a pilot study, another control blood, which seems to be suitable for every instrument and method [4].

When the control material was changed as described above, we encountered another problem with platelet counts; such material, in fact, gave method dependent mean value clearly showing two maximum frequency values for optical and impedance measurements. Since this is not desirable, efforts were made to find a better control material, which seems to solve the problem [6].

It is emphasized that is of great importance not only to compare groups of instruments individually, but to ensure comparability between the systems, to avoid many difficulties for the laboratories in the routine work. We all know the problems in hospitals where different equipment is used, e.g. in emergency unit or routine, and where the technicians of the different manufacturers carry out their quarrels to the disadvantage of laboratory staff and patients. These problems need to be solved, preferably by the use of reference methods.

Finally, we should be aware of the importance of blood film EQA, which require a comment on morphological abnormalities, diagnostic-specific signs and haematological contexts.

As seen in many surveys, greater errors occur in this field than in other aspects of EQA. Participants gratefully acknowledge the value of these surveys for training and educational purpose. No machine can be better than the

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Seite 1 –E
19.6.1992

Kleines Blutbild (211)			Probe	Ihr Wert	Zielwert	Bewertungsbereich	Abw. (%)	
Haemoglobin g/dL alle Methoden M.0-99		CU26	31	18.5	18.2	17.2 - 19.2	2	+
			32	14.0	13.6	12.9 - 14.3	3	
Erythrozyten Ery/pL alle Methoden M.0-99		CU26	31	5.50	5.56	5.14 - 5.98	-1	+
			32	4.60	4.57	4.22 - 4.92	1	
MCV fl alle Methoden M.0-99		CU26	31	95.4	97.4	89.5 - 106	-2	+
			32	87.0	88.6	81.4 - 95.8	-2	
Zellpackungsvol. zentrifugiert L/L alle Methoden M.0-99			31	.470	.510	.460 - .550	-8	+
			32	.350	.380	.340 - .410	-8	
Zellpackungsvol. el./opt. alle Methoden M.0-99		CU26	31	.540	.550	.500 - .600	-2	+
			32	.400	.410	.370 - .440	-2	
Leukozyten Leuk/nL M.0-2,4-99		CU26	31	20.9	20.3	17.2 - 23.4	3	+
			32	8.30	8.20	6.96 - 9.43	1	
Plaettchen Pl/nL M.0-99		CU26	31	657	632	499 - 765	4	+
			32	240	236	186 - 286	2	

Fig. 3. - Individual survey report. Besides the general report, every participant receives his own results, showing the number of the sample, the participants values (Ihr Wert), the target values (Zielwert), the allowed range (Bewertungsbereich), deviation in % (Abweichung %) on a graphic orientation.

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Kleines Blutbild (211)	Probe	Zielwert	Bewertungsbereich	Teilnehmerkollektiv MW	Vk	Anz.	Bestehensquoten (%) Probe	Zertifikat
1. Haemoglobin g/dL								
alle Methoden	31	18.2	17.2 - 19.2	18.2	5.44	792	88.1	84.9
	32	13.6	12.9 - 14.3	13.6	6.30		90.6	
2. Erythrozyten Ery/pL								
alle Methoden	31	5.56	5.14 - 5.98	5.54	4.75	763	92.1	88.9
	32	4.57	4.22 - 4.92	4.54	4.69		93.1	
3. MCV fl								
alle Methoden	31	97.4	89.5 - 106	96.6	5.25	596	88.1	82.4
	32	88.6	81.4 - 95.8	88.3	5.74		84.4	
4. Zellpackungsvol. zentrifugiert L/L								
alle Methoden	31	.510	.460 - .550	.500	6.89	190	76.4	73.2
	32	.380	.340 - .410	.370	6.35		79.5	
5. Zellpackungsvol. el./opt.								
alle Methoden	31	.550	.500 - .600	.530	5.77	598	88.0	85.2
	32	.410	.370 - .440	.400	5.96		87.5	
6. Leukozyten Leuk/nL								
Kammerzaehlung, M.3	31	16.1	13.6 - 18.6	16.3	18.1	105	51.0	38.1
	32	6.73	5.72 - 7.74	6.84	16.8		55.3	
M.0-2,4-99	31	20.3	17.2 - 23.4	20.4	8.62	675	92.6	91.2
	32	8.20	6.96 - 9.43	8.18	6.85		96.6	
7. Plaettchen Pl/nL								
	31	632	499 - 765	630	10.5	650	91.6	89.7
	32	236	186 - 286	235	10.6		92.7	

Fig. 4. - General survey report showing number of participants, target value, target range, mean value of participants, per cent of good and acceptable performers.
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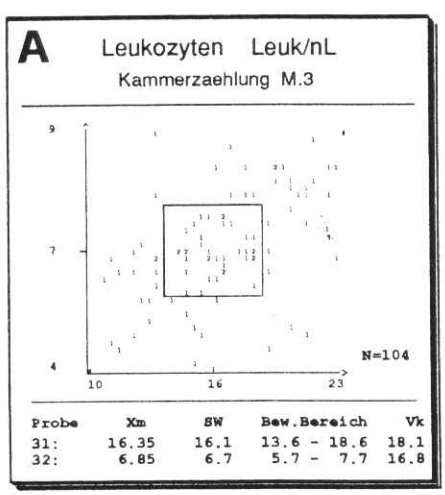
Leukozyten Leuk/nL (211-6)



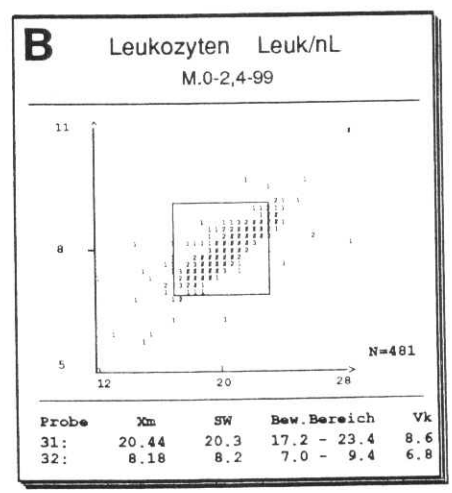


Fig. 5a, b - Comparison of results for leucocyte-counting, using: a) counting chamber and microscope; b) an instrumental method. The CV of instrumental results being lower, as seen at a glance.
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laboratory staff evaluating the flags! And as long as blasts are misinterpreted as lymphocytes all our efforts should continue to ensure better knowledge in this basic field of haematology. As regards future activities, the next item of importance is establishing EQA for immunophenotyping (flowcytometry) and reticulocytes counting. Work is in progress in this field.

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