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The calibration 2000 project for serum proteins

I.S. KLASEN[1], E.G.W.M. LENTJES[2], C.M. JOL-van der ZIJDE[3], E.T. BACKER[4], A.W.H.M. KUYPERS[1] and H. BAADENHUIJSEN[1]

The introduction of a new reference preparation for proteins in human serum (CRM 470, RPPHS)* has improved the between-laboratory agreement of the serum protein results in immuno-assays. CRM470, however is not intended for use as a working calibrator in the daily routine. Working calibrators, although all deduced from CRM470, are still diverse. The effects of use and the development of a universal calibrator for serum proteins in the Calibration 2000 project are described.

Key words: Calibration 2000; harmonisation; commutability; serum proteins; CRM470

The history of calibrators for serum proteins is complex (1). Before 1994, the serum protein results obtained by immuno-assays using various primary and secondary calibrators were extremely diverse. Moreover, in the past, calibrators were developed for radial immunodiffusion techniques. Their turbidity makes them unsuitable for the turbidimetric and nephelometric techniques used routinely nowadays. In 1993/1994 a new international reference preparation has been released by the BCR under the name of CRM470 and by the CAP under the name of RPPHS (1,2). CRM470 is intended for the use as a secondary reference material, from which values have to be transferred to working calibrators (tertiary calibrators) for immunoassay of serum proteins. Providers of serum protein calibrators all have produced their own working calibrators. The introduction of CRM470 has already reduced the interlaboratory variation of most serum proteins as can be observed in the reports of the national quality assessment scheme. The easy availability of a universal calibrator to control for drifting of the commercial calibrators may further improve this. The effect of use of a universal tertiary calibrator is studied here in a pilot experiment by means of the distribution of a serum sample in the national quality assessment scheme. This serum can be considered as a tertiary calibrator to which consensus values were assigned deduced from CRM470. In a more elaborate study, under auspices of the Calibration 2000 project of among others the SKZL and SKMI, a calibrator, commutable with patient sera, will be defined for a more permanent use. If CRM470 itself appears to be commutable with patient sera, CRM470 can be used as a calibrator to control for drifting. If not, the use of commutable tertiary calibrators has to be considered for this purpose. Value assignment to the tertiary calibrators, using CRM470 as a secundary calibrator, will take place. The design of the selection process for the most suitable calibrator will be presented shortly.

METHODS

Pilot experiment of tertiary reference material in the national quality assessment scheme

A pooled batch of 500 donorsera was used. After filtration at 0.22 µm this batch was snap-frozen in small aliquots in acetone/dry ice and stored at –70 °C. Six laboratories received this serum (hereafter called serum A) together with CRM470. CRM470 was reconstituted by these laboratories in aqua dest by weighing of the solvent and letting it stand overnight. Consequently, the six laboratories measured as many proteins as possible in serum A and CRM470, on three separate days, in triplicate, each time using new ampoules of calibrators. Proteins involved were: IgG, IgA, IgM, C3, C4, α_1-antitrypsin, ceruloplasmin, haptoglobin, transferrin and albumin.

In these laboratories, labs using rate nephelometry (Beckman Array, n=2), endpoint nephelometry (Behring BNA, n=2; Roche Cobas Fara or Hyland disc, n=1) and radial immunodiffusion (n=2) were represented. Of these results consensus values were calculated. The consensus values for each protein were compiled of the results of at least four laborato-

Department of Clinical Chemistry, University Hospital Nijmegen St Radboud[1], Nijmegen; Department of Clinical Chemistry, Leiden University Medical Center[2], Leiden; Department of Pediatrics, Leiden University Medical Center[3], Leiden; Department of Clinical Chemistry, Diaconessen Hospital[4], Leiden

Corresponding author: Dr. I.S. Klasen, Department of Clinical Chemistry, University Hospital St Radboud, PO Box 9101, 6500 HB Nijmegen, The Netherlands.
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E-mail: I. Klasen@ckcl.azn.nl

*: Non-standard abbreviations: BCR: Community Bureau of Reference of the Commission of the European Communities; CAP: College of American Pathologists; CRM: Certified Reference Material; RPPHS: Reference Preparation for Proteins in Human Serum; SKZL: Stichting Kwaliteitsbewaking Ziekenhuis Laboratoria (Dutch Foundation for Quality Assessment in Heath Care Laboratories); SKMI: Stichting Kwaliteitsbewaking Medische Immunologie (Dutch Foundation for Quality Assessment in Immunology).

Table 1. Mean between-laboratory CV (%) of the 12 proteins harmonised by CRM470 in the four sera in a quality survey before introduction of CRM470 (January 1995) and of a recent one (January 1999).

Serum protein	before CRM470	after CRM470
IgG	5.9	5.0
IgA	6.8	6.3
IgM	9.5	9.2
C3	13.4	6.5
C4	9.6	9.2
α_1-antitrypsin	18.1	7.7
α_1-acid glycoprotein	21.7	4.9
Ceruloplasmin	10.7	17.7
Haptoglobin	11.3	6.6
Transferrin	7.3	4.8
Prealbumin	4.9	5.9
Albumin	5.9	5.5

ries. All the available results were included, however not all the laboratories were able to determine all proteins.

In the next phase, serum A was distributed in the national quality assessment scheme together with three test samples (B,C,D). The four sera were all measured in the daily routine of the participating laboratories (depending on the protein measured by at the most 112 participants). All laboratories were at the same time questioned whether their results were considered to be traceable to the CRM470 calibrator or not. At that time (1996) about 80% of the participating laboratories reported their results based on a CRM470 related accuracy. Results of serum B, C and D were subsequently recalculated as follows:

A*/A x B (or C or D)

A* is the value of serum A as transferred from CRM470 by the expert laboratories, A, B, C, D are the values as measured by a participating laboratory.

RESULTS AND DISCUSSION

Effect of the introduction of CRM470

Since the introduction of the CRM470 calibrator a reduction of the between-laboratory CV in the SKZL/SKMI quality assessment schemes has been noticed. Table 1 shows the mean between-laboratory CV of the 12 proteins characterised in CRM470 in the four sera in a survey before introduction of CRM470 (January 1995) and of a recent one (January 1999).

The CV of ceruloplasmin increased after introduction of CRM470. It is unclear whether this is caused by usage of different calibrators deduced from CRM470, or by method differences. Introduction of CRM470 also did not improve the CV of IgM and C4 much. Use of a universal tertiary calibrator might give insight into the cause of this.

Pilot study of tertiary reference material in the national quality assessment scheme

To analyse the effect of the use of one universal calibrator on the between-laboratory CV, a designated tertiary standard material was distributed in a serum protein quality assessment survey.

Table 2 shows the between-laboratory CV of the 10 proteins involved before and after recalculation for the three additional sera (B, C, D). This was also performed separately for the initial results that were considered to be based on CRM470, and for those that were not CRM470 related. For almost all proteins, the recalculation results in an improved CV. As can be expected, the CV of the non-CRM related results improved the most. Figure 1 shows an example of the effects of recalculation of the results for one protein (α_1-antitrypsin) in one serum sample. Two effects can be seen. Firstly the results of the laboratories that do not report based on CRM470 shift into the major population. Secondly the variation of the results based on CRM 470 calibration is also reduced, as the dispersion of the results in this population is reduced.

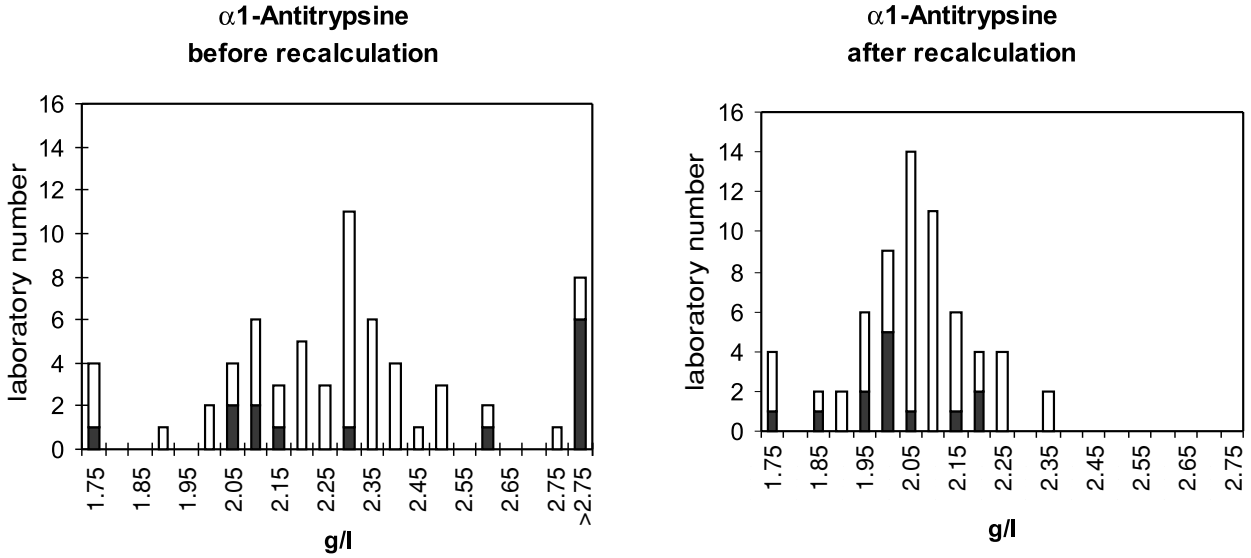

Figure 1. Frequency distribution of results for α_1-antitrypsin in a quality survey serum, before and after recalculation. Shaded bars represent results of laboratories originally not based on CRM470.

Table 2. CV (%) for 10 serum proteins in three sera of the quality survey before and after recalculation as described in the methods section. Results are presented for all laboratories, and separately for those that report based on CRM470 and those that do not.

			before recalculation			after recalculation		
Protein	calibration	n	B	C	D	B	C	D
IgG	All	112	12.2	10.8	7.8	8.8	9.6	4.4
	CRM470	87	7.1	10.4	6.2	5.1	10.2	4.6
	non-CRM470	25	19.2	12.1	10.9	15.2	6.8	3.9
IgA	All	110	12.2	11.7	9.5	9.9	9.1	6.6
	CRM470	87	7.4	10.2	8.1	5.8	9.4	6.7
	non-CRM470	23	20.1	15.3	12.5	18.1	7.7	6.5
IgM	All	110	19.6	32.0	17.5	13.5	27.7	11.1
	CRM470	87	8.7	32.6	9.4	10.9	30.2	11.9
	non-CRM470	23	29.0	27.8	26.0	20.2	14.0	7.5
C3	All	41	15.8	16.4	14.6	15.3	6.8	5.9
	CRM470	30	7.5	9.0	6.6	4.5	6.6	5.8
	non-CRM470	11	28.7	26.2	26.2	27.4	6.5	6.5
C4	All	44	18.2	11.7	10.2	16.5	13.6	10.5
	CRM470	32	7.3	10.5	9.0	9.2	13.3	9.7
	non-CRM470	12	29.6	14.2	12.4	28.3	14.3	12.7
α_1-antitrypsin	All	64	25.4	16.0	18.0	11.9	6.8	7.9
	CRM470	51	14.1	12.8	12.9	7.3	7.1	8.0
	non-CRM470	13	31.7	19.1	24.0	19.1	5.0	7.9
Ceruloplasmin	All	28	15.2	13.0	12.1	16.7	9.6	6.3
	CRM470	22	12.0	12.1	12.7	5.4	9.2	6.3
	non-CRM470	6	22.3	12.1	8.7	29.7	7.2	7.0
Haptoglobin	All	85	25.5	15.9	13.8	14.0	14.0	15.8
	CRM470	68	11.0	12.6	9.8	9.4	13.0	15.1
	non-CRM470	17	38.6	19.5	18.5	25.4	17.1	18.6
Transferrin	All	78	15.6	12.3	9.9	10.7	9.7	13.0
	CRM470	59	8.5	12.8	9.2	4.4	10.4	6.2
	non-CRM470	19	24.6	10.8	11.3	18.9	6.6	5.0
Albumin	All	72	13.0	9.3	9.2	10.0	6.9	6.8
	CRM470	47	8.1	8.1	7.8	5.8	6.2	6.1
	non-CRM470	25	19.1	12.7	11.7	15.3	8.5	8.2

Although the recalculation using the consensus values for serum A also deminishes the interlaboratory variation not caused by calibration differences, the shift of the non-CRM based results after recalculation clearly shows the effect of the usage of a universal calibrator. Especially in serum B CV values of non-CRM-based results are still higher after recalculation. Differences found here are probably method and matrix related, as in this non-CRM based group a large variety of methods are represented. In this study serum A consisted of liquid frozen serum, whereas serum B, C and D were lyophilised material. We cannot rule out that this difference has affected the results. In the pilot experiment described here, serum A was designed to be used once. In the Calibration 2000 project a tertiary standard serum will be developed that will be available for a long time as 'anchor' to CRM470. Laboratories can check for drifting of the commercial calibrators, for example each half year. The CV of the January 1999 survey in Table 1 can be considered as starting point of the calibration 2000 project for serum proteins. The design of this calibration 2000 project will be as follows:

- Collection of suitable commercial and non-commercial calibrators
- Inventarisation of the currently used methods in the quality assessment scheme
- Design of a twin study modified after (3) to assess commutability. Shortly, laboratory couples (twins) will be selected that use different methods. Each laboratory will measure the candidate calibrators (supplied to the apparatus as patient sample) together with patient sera within one run for each parameter. If financially feasible also CRM470 itself will be included in this study. The regular quality assessment scheme will be used for data collection and processing. In this way the calibrator which shows the best commutability with patient sera will be chosen. Methods that show insufficient specificity will be advised to be abandoned.
- Value assignment to the chosen calibrator. This will take place as described above for serum A. If CRM470 itself appears to be the best choice, the project will be completed in this phase.
- Distribution of the chosen serum as tertiary calibrator with defined tracability to CRM470.

We expect that use of this tertiary calibrator on a regular basis will improve interlaboratory CV.

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Automatische detectie van linksverschuiving en onrijpe granulocyten door de Sysmex SE-9000 hematologieautomaat: waarde van het IMI-kanaal bij de leukocytendifferentiatie

J.J.H. HENS en R.J. KRAAIJENHAGEN

De sensitiviteit en specificiteit van de automatische melding voor linksverschuiving en aanwezigheid van onrijpe granulocyten in de leukocytendifferentiatie werden onderzocht op de Sysmex SE-9000 hematologieautomaat. In 298 bloedmonsters afkomstig van zowel klinische als poliklinische patiënten is een leukocytendifferentiatie van het perifere bloed uitgevoerd. Als standaard methode voor leukocytendifferentiatie is gebruik gemaakt van de microscopische beoordeling van 100 leukocyten in bloeduitstrijkjes. De sensitiviteit en de specificiteit van linksverschuiving op de SE-9000 bedragen respectievelijk 100% en 80%; voor de aanwezigheid van onrijpe granulocyten respectievelijk 70% en 86%. Wij concluderen dat de automatische melding "Linksverschuiving" en "Aanwezigheid van onrijpe granulocyten" door de SE-9000 beperkt bruikbaar is bij het reduceren van het aantal uit te voeren microscopische leukocytendifferentiaties.

Trefwoorden: automatische leukocytendifferentiatie; Sysmex SE-9000; IMI-kanaal; linksverschuiving; onrijpe granulocyten

De leukocytendifferentiatie wordt voornamelijk ten behoeve van infectiediagnostiek veelvuldig gebruikt (1). Bij bacteriële infecties kunnen leukocytose met linksverschuiving, toxische korreling, vacuolisatie en lichaampjes van Döhle worden waargenomen; bij virale infecties komt vaak lymfocytose voor. Door toepassing van automatische telapparatuur is het mogelijk grote aantallen leukocyten te tellen en deze op soort en ontwikkelingsstadium te differentiëren. Hierdoor is de precisie van de huidige celtelapparatuur superieur aan die van de microscopische beoordeling van de leukocytendifferentiatie (2). Of dit ook voor de juistheid van de leukocytendifferentiatie geldt valt te bezien.

Hoewel de Sysmex SE-9000 hematologieautomaat al sinds 1994 op de markt is en een aantal bijzondere mogelijkheden biedt ten aanzien van het signaleren van onrijpe granulocyten, is het tot op heden nog steeds niet zo dat de microscopische differentiatie van leukocyten door de automatendifferentiatie te vervangen is. De in deze studie onderzochte SE-9000 genereert bij de leukocytendifferentiatie van het perifere bloed voor "Linksverschuiving" en "Aanwezigheid van onrijpe granulocyten" twee afzonderlijke resultaten, namelijk een zogenaamde Q-flag voor "L-shift?" en "Imm. gran?". De SE-9000 maakt bij de bepaling hiervan in belangrijke mate gebruik van het zogenaamd IMI-kanaal, waarbij IMI de afkorting is van *IM*mature *I*nformation. Voor deze studie hebben wij ons de vraag gesteld in hoeverre de door de Sysmex SE-9000 hematologieautomaat gedetecteerde linksverschuiving en aanwezigheid van onrijpe granulocyten klinisch te gebruiken zijn en de arbeidsintensieve microscopische differentiatie zouden kunnen vervangen (3).

MATERIAAL en METHODEN

In bloed, afgenomen in 3 ml K_3-EDTA vacutainers, werd binnen vier uur een leukocytentelling en -differentiatie uitgevoerd met een 7-kanaals Sysmex SE-9000 hematologieautomaat (Toa Medical Instruments, Kobe, Japan). Van alle monsters zijn eveneens bloeduitstrijkjes gemaakt, waarin na fixatie en kleuren volgens de methode van May-Grünwald Giemsa, 100 leukocyten microscopisch zijn gedifferentieerd (4). Elk monsterpreparaat is door één routine analist

Klinisch Chemisch Laboratorium, Ziekenhuis Eemland, Amersfoort

Correspondentie: Dr. J.J.H. Hens, Klinisch Chemisch Laboratorium, Ziekenhuis Eemland, Postbus 1502, 3800 BM Amersfoort.
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