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INTERLABORATORY STUDY INTO THE PROFICIENCY OF SERUM CHOLINESTERASE ACTIVITY MEASUREMENT

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The measurement of cholinesterase activity is an important function of a clinical laboratory. Participation in appropriate quality assurance schemes is essential in ensuring a high analytical standard. Samples of human serum were distributed to thirty-five laboratories for the measurement of cholinesterase activity. Because of methodological differences between the participants, findings of each laboratory were compared either by the use of duplicate samples or by analysis of six mixtures of two samples, one having a high and one a low activity. Of 4,964 distributed samples 95% were analysed and findings reported in 596 reports. Thirty-four percent of all reports were considered very good (less than 5% within-run error) and 38% less than satisfactory (within-run error over 10%). Access to a proficiency programme such as this enables laboratories to evaluate the quality of their analytical service.

Key words:
activity measurement, butyrylcholinesterase, human serum cholinesterase, proficiency programme, quality control

Serum cholinesterase (butyrylcholinesterase, acylcholine-acylhydrolase, EC 3.1.1.8) is an enzyme with no known physiological function. However, it is a marker of organophosphate and carbamate exposure, as any reduction in its activity suggests absorption of these compounds. This enzyme is responsible for the breakdown of short-acting muscle relaxants of the succinyldicholine (Suxamethonium) type.

Individuals with low cholinesterase activity or certain genetic abnormalities hydrolyse them slowly, which puts them at risk, since the prolonged action of these relaxants paralyses the respiratory muscles. Unless artificial ventilation is continued, death will occur. Finally, since the enzyme is synthesised in the liver, a decrease in its activity occurs in circumstances of impaired liver function. For all these reasons, the accurate measurement of serum cholinesterase activity is important in clinical practice (1-3).

In order to give laboratories an opportunity to assess their ability to measure the enzyme activity reliably, a Proficiency Programme was designed by the Cholinesterase Investigation Unit of St James's University Hospital in Leeds, UK. Experience in the organisation of the scheme and analysis of results was assisted by an earlier investigation into cholinesterase phenotyping undertaken in the same unit (4,5). Thirty-five laboratories from the United Kingdom, Canada, Croatia, and New Zealand participated initially. During the four and a half years that the programme continued, five laboratories discontinued the assay either because they were unable to maintain a satisfactory standard of performance or for other reasons. Seven other laboratories joined in later.

METHODS, DISTRIBUTION OF SPECIMENS, AND REPORTING OF RESULTS

Before entering the programme all laboratories were required to complete a questionnaire providing details of their analytical procedure and the frequency of its performance. Approximately half of the participants used benzoylcholine as the substrate and the remainder acetyl-, propionyl- or butyryl-thiocholines either at 25 °C or 37 °C. Methods followed those originally published or were adapted from *Kalow* (6) and *Ellman and co-workers* (7). At the beginning of each year, a timetable was set giving the dates for the distribution of specimens and the deadlines for the return of analytical findings. It also indicated when cumulative reports could be expected. Results not received by the specified date were excluded from consideration and classified as "no report".

Serum was provided by human volunteers. Eight specimens in all but one run were distributed in volumes of approximately 0.3 ml every three months, 18 runs in all. On fourteen occasions one specimen had a zero or low activity (A) and one a high activity (B). The remaining six specimens were mixtures of A and B in proportions decided upon by the organiser. A and B were distributed randomly among the eight specimens. On four occasions the eight samples comprised four pairs of duplicates. The activity of specimens varied between zero and 1.3 μmol/min/ml with benzoylcholine as substrate. Specimen B was usually serum taken from one of the organisers (Robert T. Evans).

Since the delay in transit would have given overseas laboratories a shorter time to perform their analyses than those in the United Kingdom, all material destined for participants outside the UK was posted several days earlier. Because of inherent stability, serum cholinesterase specimens were shipped at ambient temperature.

CALCULATIONS

In view of the wide variation in methods and in order to provide a valid comparison of results between laboratories, the measured activities of specimens A and B for each participant were plotted at positions 0 and 10 on an arbitrary scale (Figure 1). The activities of the six other specimens were then inserted in positions depending on their content of A and B. For example, the position of a mixture A:B=40:60 was 6.0. If all activities are measured without error the six points fall on a straight line joining the activities of A and B (Figure 1).

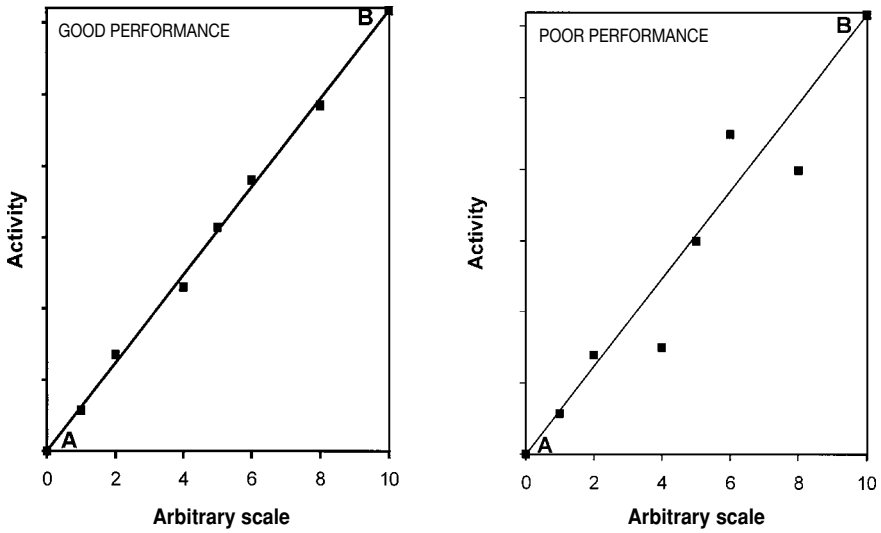

Figure 1 *Cholinesterase activity of serum samples measured with good and poor performance.* A and B were specimens with the lowest and the highest activities, and the other six specimens mixtures of the two. The abscissa is on an arbitrary scale from 0 to 10, and the positions of the six specimens reflect the proportion of B in the mixture. The scale on the ordinate varies according to the range of activities in the eight specimens.

Absolute difference between each observed activity and that expected (taken from the line of the A-B plot) was calculated and expressed as a percentage of expected activity. For every single laboratory the A-B plot was constructed using the reported values for each run, and the expected values of six other specimens were then read from the straight line on the A-B plot. The within-run imprecision of measurements was evaluated for the six mixture specimens (that is, not for A and B). The imprecision of duplicate measurements was evaluated by expressing the differences between duplicates as a percentage of the mean.

Any absolute difference of less than 2% was considered excellent. Results between 2 and 5% were regarded as very good, and between 5 and 10% as satisfactory. Differences

greater than 10% were less than satisfactory. The organiser based these performance criteria on proposed analytical goal-setting in clinical chemistry, particularly for cholinesterase activity measurements (8-11).

In average, sixteen laboratories performed their measurements using benzoylcholine as the substrate at 25 °C. We believed that the number of results sufficed for a statistical analysis. This enabled comparison between individual laboratory measurements and those of the group. Each specimen was calculated the mean, standard deviation (SD_{group}) and coefficient of variation (CV_{group}) of the reported activities for the group. Any result falling outside three standard deviations was excluded and the calculation was repeated. Each activity measurement was then reported in terms of its divergence from the mean value of the group.

Differences between duplicates were the basis for testing the imprecision of measurements undertaken using benzoylcholine or thiocholines as substrates (benzoylcholine and thiocholine laboratory groups). A Mann-Whitney nonparametric test was used to establish the difference between the medians of the two groups. Differences between distribution profiles for the two groups were tested by the Kolmogorov-Smirnov two-sample test, which is based upon distances between cumulative distribution functions.

Statistical analysis (12) was performed using the Microsoft Excel and Statgraphics Plus programme package.

RESULTS

Of the 4,964 specimens distributed, 4,737 (95%) were reported upon (Table 1). We consider this a satisfactory response. All laboratories received cumulative data tables and a figure illustrating their own results (Figure 1).

Table 1 *Summary of serum cholinesterase activity measurements reported during the first* and second half of the programme. Differences between measured and expected activities are expressed as a percentage of the expected value, and between duplicate specimens as a percentage of the mean. Of the total number of reports, 28% were with differences between 5 and 10%.

Number of reports received during the programme		With all differences less than 2%	With all differences less than 5%	With at least one difference greater than 10%
First half	292	6	69	124
Second half	304	24	105	105
Total	596 (100%)	30 (5%)	174 (29%)	229 (38%)

Of the 596 reports which contained the results of a complete series of eight specimens, 38% had at least one difference greater than 10% between the measured and expected value or between the duplicates (Table 1). An improvement in the performance of

participants was observed in the second part of the programme with the drop in the number of differences greater than 10% and increase in the number of differences of less than 5%. This improvement is partly the result of the withdrawal of a number of centres dissatisfied with their own performance. We recognise that an error in the measurement of the activity of either A or B specimens affects the interpretation of those of 1-6 even if 1-6 may have been analysed appropriately. In only 19 reports did a positive or negative shift of activities of all six specimens suggest an error in the measurement of either A or B.

The results obtained using benzoylcholine were compared with those using the three thiocholine esters by analysis of the difference between duplicates for thirty sets of duplicates which were part of the programme (Table 2). Both groups were had asymmetric distributions, the skewness being 4.9 and 7.6 for benzoylcholine and the thiocholines, respectively (Table 2). A significant difference between the two groups was found by means of the Mann-Whitney test (significance levels <0.001).

Table 2 *Average and median differences (%) between duplicate activity measurements and skewness of distribution of differences for measurements by benzoylcholine and by thiocholine substrates.*

Substrate	Number of pairs of duplicate results	Differences (%)		
		Average	Median	Skewness
Benzoylcholine	438	4.4	2.7	4.9
Thiocholine esters	470	3.0	1.5	7.6

The median of the difference between duplicates for the thiocholine group was significantly lower than that for benzoylcholine. The same significance of difference between the groups was obtained when we omitted differences greater than 30% (nine and seven results for benzoylcholine and thiocholine group, respectively) which we considered to be random errors. A higher frequency of lower values (less than 10%) was found for thiocholine than for benzoylcholine group, as shown by the Kolmogorov-Smirnov test. This is in accordance with an earlier report of a lower precision of benzoylcholine than of acetylthiocholine in measuring cholinesterase activity (13). Because the two groups of participants may have differed in the technical level and because both the averages and medians of differences for both groups were less than 5%, we do not think that thiocholine esters are sufficiently superior to benzoylcholine to recommend laboratories which use benzoylcholine to change the substrate.

CONCLUSION

Any clinical laboratory undertaking biochemical investigations must maintain a reliable standard of analytical work in order for correct conclusions to be drawn and appropriate advice offered to patients. During the period covered by this programme, participating laboratories were given the opportunity to assess the accuracy and reproducibility of their

assays. Through this experience they were able to recognise their ability or otherwise to reach acceptable analytical standards and take steps when necessary to either improve or discontinue the test. Their activity led to an improvement in performance during the programme.

It is evident from our study that it is not easy to attain the quality of cholinesterase measurement which we regard good or excellent, and that a significant proportion of results, even when issued by laboratories very committed to the achievement of high standards, are less than satisfactory. Although internal systems of quality control are essential in analytical laboratories, an external programme such as the one described in this paper provides additional confidence in the results obtained and hence the advice given.

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Sažetak

MEĐULABORATORIJSKA PROVJERA KAKVOĆE MJERENJA AKTIVNOSTI SERUMSKE KOLINESTERAZE

Mjerenje aktivnosti serumske kolinesteraze važna je zadaća kliničkih laboratorija. Smanjena aktivnost kolinesteraze upućuje na smanjenje funkcije jetre ili može biti znak i mjera stupnja izloženosti organskofosfornim spojevima i karbamatima. Serumska kolinesteraza (butirilkolinesteraza) razgrađuje kratkodjelujuće mišićne relaksanse kao što je sukcinilkolin, pa je prije njihove primjene pacijentima potrebno provjeriti aktivnost toga enzima u serumu. Kako bi se osigurao visoki analitički standard mjerenja, poželjno je da laboratorij sudjeluje u programima kontrole kakvoće mjerenja. Tijekom četiri i pol godine proveden je program provjere osposobljenosti laboratorija za mjerenje aktivnosti kolinesteraze u serumu. U programu je sudjelovalo u prosjeku trideset pet laboratorija. Svakom laboratoriju poslano je u 18 navrata po 8 uzoraka ljudskog seruma. Laboratoriji su mjerili aktivnost metodom koja je već bila uvedena u dotičnom laboratoriju. U prosjeku oko 16 laboratorija rabilo je benzoilkolin kao supstrat za mjerenje aktivnosti kolinesteraze, a ostali laboratoriji rabili su tiokolinske supstrate. Nepreciznost mjerenja svakog laboratorija provjeravana je usporedbom uzoraka u duplikatu i analizom rezultata mjerenja 6 uzoraka, koji su bili priređeni miješanjem u određenom omjeru jednog uzorka velike i jednog uzorka male ili nikakve aktivnosti: omjere miješanja znao je samo organizator. Od 4964 razaslana uzorka, 95% ih je bilo analizirano i laboratoriji su poslali svoje nalaze organizatoru. Prilikom idućeg slanja uzoraka sudionici su dobili rezultate svoje analize, kao i rezultate drugih laboratorija. Organizator je ukupno primio 596 izvještaja s nalazima mjerenja aktivnosti uzoraka. U oko 34% izvještaja rezultati su dobiveni s nepreciznošću mjerenja manjom od 5%, dok je u 38% izvještaja bilo barem jedno mjerenje određeno s nepreciznošću većom od 10%. Mjerenja obavljena s benzoilkolinom kao supstratom bila su nepreciznija nego ona s tiokolinskim supstratom, ali razlika u nepreciznosti nije bila dovoljno velika da bi se laboratorijima savjetovalo da promijene supstrat u svojim mjerenjima.
Na osnovi iscrpne analize rezultata koju su redovito dobivali, sudionici su mogli procijeniti svoju osposobljenost za mjerenje aktivnosti toga enzima i, ako je bilo potrebno, poboljšati svoja mjerenja. U drugom dijelu programa povećao se broj izvještaja s nalazima veće preciznosti.

Ključne riječi:
benzoilkolin, butirilkolinesteraza, kontrola kakvoće mjerenja, ljudska serumska kolinesteraza, nepreciznost mjerenja, priprava uzoraka za analizu, provjera stručnosti, supstrati, tiokolini

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