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Precision of plasma urea and electrolytes estimated by the AutoAnalyzer

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SYNOPSIS The precision of sodium, potassium, chloride, and bicarbonate analyses carried out by the AutoAnalyzer has been studied under routine laboratory conditions. From the results it is concluded that a further increase in precision is desirable.

Electrolyte determinations now constitute one of the most frequently requested groups of analyses performed by clinical laboratories. They have a place in specific diagnosis, in the judgment of therapeutic measures, and as a general screening procedure. For such widely requested tests it is very important that both clinician and laboratory analyst should be aware of the normal range of values to be expected and the significance of variations in results. With the introduction of several new methods we have taken the opportunity to reassess the laboratory reproducibility. The paper describes the results with respect to sodium, potassium, chloride, bicarbonate, and urea analyses.

METHODS

SAMPLES The majority of analyses were carried out on plasma (lithium heparin anticoagulant) and a small number on serum. Plasma and serum results have not been differentiated.

ANALYTICAL TECHNIQUES All analyses were carried out with Technicon AutoAnalyzers. Sodium, potassium, chloride, and bicarbonate were analysed by standard Technicon methods (AutoAnalyzer Method Sheets N-20A, N-5A, and N-8A). Sodium, potassium, and chloride readings were corrected for instrumental drift on the basis of a standard after every nine specimens. Urea was determined by a modification of the diacetyl monoxime method very similar to that recently published by Marsh, Fingerhut, and Miller (1965). The urea sample line incorporated a wash-out device of the type described by Weller, Linder, Macaulay, Ferrari, and Kessler (1960). All AutoAnalyzer peaks were read twice, by different observers, to minimize reading errors.

METHODS OF ASSESSMENT Several methods were used to assess the results.

1 *Pooled sera* Routine specimens were pooled, filtered in lots of 500 ml., dispensed into 10 ml. or 5 ml. tubes and stored at −20°C. Individual tubes were thawed on the day of analysis.

2 *Repeat analyses* Routine specimens from patients were analysed on the day of receipt, stored at 4°C. overnight, and reanalysed the following day.

3 *Commercial sera* Commercial quality control sera from two manufacturers were prepared fresh daily, placed in laboratory specimen tubes and analysed.

4 *Recovery experiments* Many of the pooled sera had known amounts of NaCl, Na_2CO_3, KCl, and urea added to assess recovery of the constituents.

All the analyses reported were done amongst the routine samples during the last 12 months. The samples were all numbered in the usual way and were handled by the technicians as routine samples.

RESULTS

1 POOLED SERA Results for sodium, potassium, chloride, and bicarbonate are in Table I. For all the pools considered together the standard deviation for sodium was 1·5 mEq./l. based upon 563 determinations, for potassium 0·10 mEq./l. from 327 determinations, for chloride 1·5 mEq./l. from 317 determinations, and for bicarbonate 1·2 mEq./l. from 305 determinations.

Table II shows the means, standard deviation, and coefficient of variation for the urea pools. The mean coefficient of variation for 889 analyses was 2·9%. The coefficient of variation for urea is dependent upon the urea value, decreasing as the urea value increases. For this reason we use a coefficient of variation of 4% which underestimates the precision for high ureas but covers those falling within the normal range.

2 REPEAT ANALYSES The mean differences for duplicate analyses and the standard deviation for single analyses are in Table III. Standard deviations

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TABLE I

RESULTS FROM POOLED SERA

Sodium (mEq./l.)			*Potassium (mEq./l.)*			*Chloride (mEq./l.)*			*Bicarbonate (mEq./l.)*		
No. of Analyses	*Mean*	*S.D.*	*No. of Analyses*	*Mean*	*S.D.*	*No. of Analyses*	*Mean*	*S.D.*	*No. of Analyses*	*Mean*	*S.D.*
106	125	1·7	64	3·7	0·08	60	85	2·0	64	11·9	1·3
64	137	1·2	58	4·4	0·10	61	98	1·5	62	18·8	1·3
130	134	1·6	71	4·9	0·08	71	100	1·4	42	21·0	0·8
71	135	1·6	70	5·3	0·10	68	100	1·3	73	25·0	1·1
58	136	1·6	64	6·1	0·13	57	100	1·6	64	28·0	1·5
70	145	1·5									
64	148	1·5									

TABLE II

RESULTS FOR UREA (MG./100 ML.) FROM POOLED SERA

Number of Analyses	*Mean*	*Standard Deviation*	*Coefficient of Variation*
26	18	1·0	5·9
30	44	1·6	3·7
81	53	2·2	4·2
42	55	1·6	2·9
101	72	2·5	3·5
92	73	2·5	3·4
85	95	2·2	2·3
24	121	2·3	1·9
134	150	4·1	2·7
32	165	3·3	2·2
118	179	3·6	2·0
24	219	3·3	1·5
40	256	5·0	1·9
32	257	4·8	1·9
28	281	6·5	2·3

$$\text{Coefficient of variation} = \left(\frac{\text{S.D.}}{\text{Mean}} \times 100\right) \%$$

TABLE III

REPEAT ANALYSES ON SPECIMEN FROM PATIENTS

Analysis	*Number of Duplicates*	*Average Difference between Duplicates*	*Standard Deviation*
Sodium (mEq./l.)	166	1·4	1·2
Potassium (mEq./l.)	166	0·09	0·08
Chloride (mEq./l.)	153	1·6	1·4
Bicarbonate (mEq./l.)	231	1·7	1·5
Urea (mg./100 ml.)	175	2·7	2·4

were calculated from the mean differences between duplicates (Moroney, 1962).

3 COMMERCIAL SERA The values stated by the manufacturer, our mean results, and standard deviations are in Table IV. Each serum was analysed 40 times over a period of two months.

4 RECOVERY EXPERIMENTS The recovery results for sodium, potassium, chloride, and bicarbonate are set out in Table V. The urea recovery figures (Table VI) are all based upon pools that were analysed at least 30 times.

DISCUSSION

The introduction of semi-automation to clinical chemistry has led to improved reproducibility of results and largely removed the sources of gross errors. It has also allowed easier assessment of the precision and accuracy of routine procedures as the added work load is less noticed by the staff.

The variation in values obtained for biochemical tests may be of at least two kinds: there may be physiological fluctuations occurring from day to day or hour to hour and there is always an analytical error. Analytical errors may be divided into those

TABLE IV

RESULTS WITH COMMERCIAL SERA ANALYSED 40 TIMES

Sodium (mEq./l.)		*Potassium (mEq./l.)*		*Chloride (mEq./l.)*		*Urea (mg./100 ml.)*	
Stated	*Found*	*Stated*	*Found*	*Stated*	*Found*	*Stated*	*Found*
	S.D.		*S.D.*		*S.D.*		*S.D.*
135	136 1·4	3·6	3·8 0·08	108	107 1·3	40	39 2·7
135	139 1·5	4·9	4·9 0·11	102	101 1·4	30	27 1·2
123	125 1·8	7·2	7·1 0·13	91	91 1·2	65	67 1·7
153	150 2·2	3·1	3·2 0·08	111	109 1·7	129	130 1·8

TABLE V

RECOVERY OF CONSTITUENTS ADDED TO POOLED SERA

Sodium (mEq./l.)		*Potassium (mEq./l.)*		*Chloride (mEq./l.)*		*Bicarbonate (mEq./l.)*	
Theoretical	*Found*	*Theoretical*	*Found*	*Theoretical*	*Found*	*Theoretical*	*Found*
130	131	3·7	3·7	83	85	9	8
145	145	5·3	5·3	98	98	13	12
149	148	6·1	6·1	99	100	21	21
						24	25
						27	28

TABLE VI

UREA RECOVERY FROM POOLED SERA

Urea Added (mg./100 ml.)	*Percentage Recovery*
19	108
41	101
81	97
102	101
108	97
150	101
165	97
200	101
208	96
236	100

causing variation within one laboratory and those causing variation between different laboratories. The analytical variation to be expected from his own laboratory is what each clinician must have in mind when he appraises laboratory results from his patients.

Precision, the ability to produce closely grouped results for the same sample when analysed many times, has been divided into two factors (Hughes, 1952), repeatability and reproducibility. When figures are quoted for laboratory precision they usually cover repeatability, results obtained by one person repeating an analysis several times within the same run. As a test is performed by different staff members, reagents and standards changed, greater variation enters the results and the term 'reproducibility' becomes applicable. It is reproducibility that matters to the clinician as he must compare results from day to day in a given patient rather than results obtained within one set of analysis. Figures for reproducibility can only be obtained by studying results over a considerable period of time.

Interlaboratory variation is common, often considerable, and has been shown in several surveys (Wootton and King, 1953; Wootton, 1956; Hendry, 1963; Tonks, 1963). Whether the interlaboratory differences represent constant factors or whether they are largely due to the coincidence of error within the individual laboratories has not been established. Clearly it is desirable to have minimal variation within laboratories and concordance between laboratories. This is becoming more important as epidemiological surveys comparing results from different countries become more common.

Here we have described the reproducibility obtained within one laboratory for routine electrolyte analyses. On the basis of the results with commercial quality control sera and the recovery results we consider the values to be accurate, *i.e.*, they should not vary from those reported by other laboratories.

To the clinician a useful way to look at our results is in terms of confidence limits. The 95% confidence limits (2 S.D.s) for the true value based upon a single analysis become, for sodium ± 3·0 mEq./l., potassium ± 0·20 mEq./l., chloride ± 3·0 mEq./l., and bicarbonate ± 3·0 mEq./l. In the case of urea the expected range is so large that standard deviations must be converted to coefficients of variation; the 95% confidence limits then become ± 8% of the value found by a single analysis. For urea levels between 100 and 300 mg./100 ml. this range can be narrowed to ± 4%. It must be remembered that these figures take account of the analytical variation only from the stage the plasma or serum is separated from the cells.

In a similar study Thiers and Oglesby (1964) obtained reproducibility figures (expressed as 1 S.D.) for sodium 2·3 mEq./l., potassium 0·15 mEq./l., and chloride 2·0 mEq./l. Our comparable figures are: sodium 1·5 mEq./l., potassium 0·10 mEq./l., and chloride 1·5 mEq./l. Thiers and Oglesby (1964) do not have comparable figures for bicarbonate and urea. Our S.D. for bicarbonate is 1·5 mEq./l. and the coefficient of variation for urea 4%. These figures represent the degree of precision obtained in this laboratory under routine conditions, working with the AutoAnalyzer, an instrument now widely used in clinical laboratories.

What degree of precision is required in clinical chemistry is uncertain. Tonks (1963) used the following formula:

$$\text{Allowable limit of error (in \%)} = \frac{\frac{1}{4}\text{ normal range}}{\text{mean of normal range}} \times 100\%$$

He said that no error should be greater than 10% of the stated result. In a survey of 170 Canadian laboratories he found almost 50% of results in the

case of sodium, chloride, and urea to have errors exceeding these criteria. Zwart Voorspuij and van der Slik (1964) have suggested a ratio of physiological to analytical standard deviation of at least 3–5 to 1. With their technique for sodium analysis to achieve a ratio of 5 to 1 would have required taking the mean of seven analyses on each sample. In most cases figures for precision in clinical chemistry do not represent ideals, but merely what has been at present achieved.

Taking sodium as an example, the 95% confidence limits for a result of 140 mEq./l. are 137-143 mEq./l. When this range is compared with some of the quoted normal values for sodium, *e.g.*, those of Massachusetts General Hospital, 136-145 mEq./l. (Zervas, Holmes, Rieder, King, Beck, and Goultan, 1963), England, 133-146 mEq./l. (Varley, 1962) and 136-149 mEq./l. (Wootton, 1964), and New Zealand, 135-147 mEq./l. (Allen, 1964) it becomes clear that greater analytical precision must be achieved if further information is to be obtained from routine analyses. The same comment applies to the other constituents mentioned in this paper with the exception of potassium.

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