The Hycel-M Multichannel Analyzer—A Model for Evaluation

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We evaluated the Hycel-M Multichannel Analyzer on behalf of the British Department of Health and Social Security. The purpose of the evaluation was to determine the precision of the instrument over a prolonged period of time, to compare its results with those of well-tried laboratory procedures, and to determine how the instrument would perform under the rigors of routine analysis. In addition, experiments were designed and carried out to test specifically certain new features of the instrument. Some new approaches to the analysis of the data generated have been examined and are discussed. The findings are presented as a demonstration of an evaluation procedure.

Additional Keyphrases: instrumentation · discrete analysis · analytical error

The work reported here took place as part of an evaluation of the Hycel-M multichannel analyzer undertaken on behalf of the British Department of Health and Social Security (DHSS). The evaluation has followed the recommendations suggested by Broughton et al. (1), but in addition has complied with requirements set out by the DHSS, some of them specific for this evaluation. We believe these to be important and stringent requirements, which could usefully be adopted in the evaluation of other instruments. The purpose of this paper, therefore, is to present the data we obtained as an example of the evaluation procedure.

The Instrument

The Hycel-M multichannel micro-chemistry analyzer is a discrete selective chemical analyzer of 30 channels, which will process specimens at the rate of 120 per hour with a throughput time of 15 min. The analyses are done by conventional techniques: end-point colorimetry, ultraviolet rate-reaction determination for enzymes, and flame photometry for sodium and potassium.

Samples are placed in a continuous serum belt designed to accommodate Vacutainer-type tubes. Each tube is identified by a half bar-code label, the other identical half being attached to a request card, giving a secure sample-identification procedure. The tests required are scheduled by means of a request card. The unique feature of the Hycel-M is a serum-dispense arm, which travels across 30 reaction tubes, one of each chemistry channel, and dispenses sample into those tubes for which the analysis was requested. The reaction tubes are immersed in a 37 °C water bath, and reagents are added by the action of solenoid-operated valves. Finally, color development or rate of change of absorbance, whichever is appropriate, is measured in a "read" cell, where 32 absorbance measurements are made during 15 s, from which the analyte concentration or enzyme activity is calculated. For calibration, a predetermined sequence of calibrator materials is used.

The instrument is controlled by its own computer, in which floppy magnetic diskettes are used for storage of programs and of patient-sample and control data. For operator access, there is a 16-key keyboard, and information and instructions are imparted to the operator by appearing on the face of a cathode ray tube (CRT).

Carryover and Precision

These aspects of the instrument's performance were tested by repeated analysis of sera with high, medium, and low values for each analyte, designated Hi, Med, and Lo, respectively. These were prepared from commercial material, e.g., Autosect M and H from Wellcome Reagents Ltd., Beckenham, Kent, U.K., and Precilip EL from BCL (London) Ltd., Lewes, East Sussex, U.K.

We analyzed each serum on 16 consecutive working days, each being assayed 16 times on each day in a fixed predetermined sequence. A mean, standard deviation (SD), and coefficient of variation (CV) were determined for each of the 16 days. From these data we calculated the mean within-batch performance, and also the between-batch performance by summing all the data from 16 days. This is summarized in Table 1. In the case of two of the analyses, unsaturated iron-binding capacity and creatine kinase, some data were excluded. On two days some of the values recorded for unsaturated iron-binding capacity for all three sera were analytically incompatible with all other recorded data. No explanation was found for this, but we believed that recalculations should be made after excluding all the results for this assay from these two days. Similarly, with creatine kinase all the values for the Med control from one day were analytically incompatible with all other recorded data, and were excluded.

In addition, the trend-free standard deviation SDтро has been calculated. This is defined as a measure of the variation of consecutive values in a series of data about any existing slow drift, be it consistent in one direction or sinusous (2), and is calculated thus:

\[
SD_{тр} = \frac{1}{1.128} \times \left( \frac{\sum |x_m - x_{m+1}| - |x_1 - x_n|}{n - 1} \right)
\]

where \(x_1 = \) first datum point, \(x_n =\) last datum point, \(x_m =\) consecutive data points, and \(n =\) total number of data points.

This may be compared with the between-batch standard deviation in the form of the ratio SD/SDтро (Table 1). A significant trend is demonstrated if this ratio exceeds 1.25, this being the \(t\) value for \(p = 0.05\) for 14 degrees of freedom, the number of degrees of freedom derived from the equation for the calculation of SDтро when \(n = 16.\) Using this criterion, we found that the only analysis to show a significant trend was the Med control for creatine kinase. This ceased to be significant when data was recalculated after making the exclusions mentioned above.

Carryover was determined by ranking the data obtained for the Med control according to the preceding sample. Thus,
where the preceding sample is a Lo control, data are represented by (Lo ↔ Med), etc. If the mean value for these groups of ranked data, then negative and positive ratios may be calculated as follows: Negative interaction ratio = \( \frac{\overline{x}(\text{Med} \rightarrow \text{Med}) - \overline{x}(\text{Lo} \rightarrow \text{Med})}{\overline{x}(\text{Lo} \rightarrow \text{Med})} \times 100 \)

Positive interaction ratio = \( \frac{\overline{x}(\text{Hi} \rightarrow \text{Med}) - \overline{x}(\text{Hi} \rightarrow \text{Lo})}{\overline{x}(\text{Hi} \rightarrow \text{Lo})} \times 100 \)

Table 1 shows the results of the calculation of interaction ratios. Almost half of the carryover is not significant as compared with the precision. No data were included for alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase.

**Table 1. Precision and Carryover Data**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Low CV, %</th>
<th>Low</th>
<th>With</th>
<th>Mean</th>
<th>Be-</th>
<th>SD/SD(_T)</th>
<th>Medium CV, %</th>
<th>Medium</th>
<th>With</th>
<th>Mean</th>
<th>Be-</th>
<th>SD/SD(_T)</th>
<th>High CV, %</th>
<th>High</th>
<th>With</th>
<th>Mean</th>
<th>Be-</th>
<th>SD/SD(_T)</th>
<th>Interaction ratio</th>
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<td>Glucose</td>
<td>mmol/L</td>
<td>3.23</td>
<td>2.2</td>
<td>5.4</td>
<td>1.21</td>
<td>10.20</td>
<td>2.2</td>
<td>2.0</td>
<td>9.02</td>
<td>1.467</td>
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<td>0.98</td>
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<td>Creatinine</td>
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<td>393.5</td>
<td>2.1</td>
<td>2.7</td>
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<td>772.9</td>
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<td>1.9</td>
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<td>0.84</td>
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<td>Urea</td>
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<td>g/L</td>
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<td>Conjugate bilirubin</td>
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<td>11.6</td>
<td>19.9</td>
<td>1.15</td>
<td>8.95</td>
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<td>Total bilirubin</td>
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<td>1.03</td>
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<td>16.8</td>
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<td>4.1</td>
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<td>capacity</td>
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<td>Aspartate aminotransferase</td>
<td>U/L</td>
<td>25.5</td>
<td>10.6</td>
<td>4.0</td>
<td>1.11</td>
<td>85.9</td>
<td>2.7</td>
<td>3.5</td>
<td>1.43</td>
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<tr>
<td>Lactate dehydrogenase</td>
<td>U/L</td>
<td>68.4</td>
<td>5.2</td>
<td>2.0</td>
<td>0.88</td>
<td>216.6</td>
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<td>Alanine aminotransferase</td>
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<td>Hydroxybutyrate dehydrogenase</td>
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* Recalculated with exclusions (see text).
transferrin, because Med activity is slightly higher than HI, and therefore the calculation is not valid (see Table 1).

Comparisons of Methods
Results of analyses done with the HyCel-M were compared with those for analyses done on the same specimens by our "in-house" laboratory methods. Routine patients' samples were used, with the aim of carrying out about 200 comparisons of which at least 10% would be outside the reference interval for healthy persons.

From these data, the standard deviation of the pairs, a t-test, and a regression equation were derived (Table 2). Skewness and kurtosis of the distribution of the difference of the pairs were also determined, and re-determined after log10 transformation of the original data. However, these results did not add further useful information and therefore have not been included or recommended for future evaluation programs. Graphical representation of the data was helpful. We used two methods: the plot of data points of HyCel-M results vs "in-house" method results, including the line of the regression equation and the line \( y = x \); and secondly the plot of the mean of the pairs vs their difference. These two representations demonstrate the presence of good correlation between methods, or whether there is a bias, and whether it is concentration related. The second type of representation is particularly useful in demonstrating bias. It is not practicable to present all graphical data here, but some examples illustrating these points are shown in Figure 1.

Urgent ("Stat") Analyses
We determined the ability of the instrument to maintain its calibration over long intervals without recalibration. Calibration was carried out in the usual way and then 10 identical samples of a commercial serum were analyzed. Analysis was repeated at intervals up to 24 h, but was followed by a blank/control update sequence and a further 10 samples of the control material. This sequence allows the blank voltages to be reset if necessary, and the values obtained for control materials are used to check whether the instrument was in control or not.

With the following exceptions, results were acceptably consistent and precise, after applying the blank/control sequence correction, as compared with data in Table 1, throughout 24 h.

Glucose. Acceptable to 12 h, but with a significant change in mean value at 24 h.

Triglyceride. Acceptable to 12 h, but with a significant change in precision at 24 h.

Chloride. Some erratic results.

Calcium. Unacceptably erratic after 0 h.

Hydroxybutyrate dehydrogenase. Acceptable to 10 h, after which substrate depletion was observed.

Urine analyses for sodium, potassium, and chloride were not done, because the analytical range of the instrument was too narrow for this to be practicable.

Linearity and Accuracy
The linearity of response of each non-enzymic assay was determined, except for unsaturated iron-binding capacity. Where possible, we did this by using primary standards prepared from ANALAR-grade reagents. Where this was not practicable, commercial preparations were used. Linearity was demonstrated within the range claimed by the manufacturer (and often further), except for phosphate, where linearity extended only to 2.2 mmol/L, although claimed to 3.2 mmol/L.
Fig. 1. Examples of graphical representation of data presented in the Tables.

In figures a, c, and e the continuous line represents the regression equation of \( y \) on \( x \), and the broken line \( y = x \). In parts b, d, and f the continuous horizontal line represents the value of \( y - x = 0 \) for all values of \( (x + y)/2 \). a,b: Comparative data for cholesterol. This is an example of a good comparison with no bias. In a the regression line lies close to \( y = x \), and in b the points are evenly distributed about \( y - x = 0 \). c,d: Comparative data for total protein. This is an example of a biased correlation that is not concentration related. In c the regression line is parallel, but above the line \( y = x \), and in d the points are distributed evenly, but predominantly above \( y - x = 0 \). These points indicate that the HyCel-M statistically gives a constant higher value. e,f: Comparative data for glucose. This is an example of a concentration-related, biased correlation. In e the regression line is divergent from \( y = x \), and in f the points lie increasingly above the line \( y - x = 0 \) as \( (x + y)/2 \) increases. These points indicate that the HyCel-M gives a value that statistically is higher by a constant percentage (about 25%).
Precision of Reagent Pumps

Reagent delivery is controlled by a solenoid-driven reagent pump. Reagent pumps were selected without conscious bias, except to cover all delivery volumes (100 to 1500 μL) for a precision study. From an acidified solution of potassium dichromate of known concentration, 20 to 25 aliquots were collected from each pump, diluted to a constant fixed volume (250 mL), and their absorbances measured at 350 nm with a Pye-Unicam SP500 spectrophotometer. The precision of the spectrophotometer was determined over the same absorbance range, and by assuming that the precision of measurement of absorbance of the aliquots dispensed by a reagent pump is made up of only the precision of the pump and the precision of the spectrophotometer, and that these variables are independent, then the overall precision is the root of the sum of the precision of the pump and spectrophotometer. From this we determined the precision of the pump. By using the molar absorptivity coefficient for potassium dichromate at 350 nm it is possible to calculate the mean pump volume dispensed.

Of the pumps tested, most had a CV of 3.0% or less; the highest CV was 6.6%. Most were accurate to within 3.0% of their stated volume, and the worst was 8.8%. Precision and accuracy are independent of the volume dispensed.

Precision of Serum Dispenser

Serum is dispensed into reaction tubes from a syringe and needle system, which moves with a continuous motion over the reaction tubes, dispensing a predetermined volume of serum into appropriate reaction tubes for the analyses requested. We tested the precision of this system on all channels by the repeated (n = 20) dispensing of a pooled serum containing sodium [51Cr]dichromate. By repetition, 20 data points for each channel were obtained. Each sample was counted to 20,000 counts, giving an imprecision of counting of 0.707%, which was substracted from the CV for each set of 20 data points by the method of summing the squares as described in the previous section, thus determining the precision due solely to the serum dispenser.

Precision varied from 0.65 to 5.4% and was independent of volume dispensed, but appeared to be related to the position of the channel, those being dispensed first being the more precise.

Instrument Components

Other components of the instrument were not evaluated thoroughly but we can comment concerning their performance during the evaluation.

The instrument is fitted with two bar-code readers, one mounted near the serum dispenser and the other a hand-operated wand, which may be used for identifying blocks of samples on which identical analyses have been requested. Both worked well throughout, although the mounted reader failed to read a label if it was misaligned.

The request card reader functioned satisfactorily, although cards were difficult to feed into the reader if not lined up carefully. The card printer was less satisfactory; it was noisy and on some occasions results were printed out of phase, sometimes appearing against the wrong assay. The field size was not large enough to cope with SI units; with some analyses one column of figures was lost.

The ability of the 37 °C water bath to maintain a constant temperature within reaction tubes after reagent addition was examined with a thermistor probe. We concluded that temperature equilibrium had probably just been reached in all assays by the time that absorbance measurements were made subsequent to final reagent additions.

Conclusions

During this evaluation we investigated several methods of statistical analysis for processing the data from experiments on carryover, precision, and comparison of methods. Some of these were discarded as unsuitable—for example, tests for skewness and kurtosis of the differences in the comparisons of methods. We concluded that these did not add significantly to the analysis of the data. Similarly, log10 transformed data were omitted. Emphasis has been placed on graphical representation, because we believe this is often more helpful than statistical analysis. For this reason the plot of mean against difference has been included in the comparison of methods, because this is a useful way of demonstrating bias. We also believe that the use of a previously defined sequence of controls with low, medium, and high values is a realistic way to determine precision and carryover, more akin to the normal routine use of an instrument.

The use of instruments under specialized circumstances, or for special analyses such as stat and urine analysis, is frequently omitted from an evaluation, but we believe it is an important aspect.

Finally, it may be important to evaluate a particular part of an instrument’s function if its method of operation is conceptually new, for example, the reagent-pumping system and the serum-dispense mechanism.

During the time the instrument was in our laboratory some 40,000 data points were generated, and although some “rogue” results were obtained, we believe these were acceptably infrequent. We therefore believe that the data presented accurately represents the instrument’s performance in terms of accuracy and precision. From this, a number of salient points emerge.

- The determination of glucose is inaccurate, probably owing to misalignment of the calibrating material.
- Creatinine analysis was generally good except that DATA ERROR was recorded too frequently with urine analysis to make the method reliable for analysis of urines.
- Urea analysis was not acceptably precise. In addition, the range is relatively small (up to about 20 mmol/L), although we realize this is a common problem with enzymic methods.
- There appears to be an accuracy problem in urate de-
termination, although this is not apparent with urinalysis. This discrepancy is difficult to explain.

- The flame photometer gave much better results for potassium than for sodium, which showed large variations in accuracy over long time periods, even with a calibration update. Its usefulness in urinalysis is limited by the restrictions of the calibrating procedure, especially for sodium.
- Urine chloride determination is restricted similarly to that of sodium and potassium.
- Calcium determination is not acceptably precise. This is probably a reagent problem, because some instability of the color reagent was noted.
- The precision of enzyme activity determination was generally good, although measurements are probably being made just as constant temperature is attained. This may be affecting the precision of aminotransferase activity determination.

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References


Free-Cortisol Assay by Immunoextraction: Comparison with an Equilibrium Dialysis Procedure

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We describe a new method for directly determining the apparent free cortisol concentration in plasma samples by use of an antibody-coated test-tube RIA. Buffer-diluted plasma or standard serum is added to antibody-coated test tubes, incubated at 4 °C for 4 h, and the solutions are aspirated. ¹²⁵I-labeled cortisol is added to each tube and incubated for 3 h at 4 °C. Then the inside of the tubes are washed and their radioactivities counted. The standard curve is in terms of free cortisol, the standard serum solutions having been measured with an equilibrium dialysis procedure. Plasma samples (n = 155) from normal subjects and from various patients, measured with the new immunoextraction method and the equilibrium dialysis technique, gave results that correlated well (r = 0.847, p <0.001). Results by this direct RIA also correlate well with the clinical adrenocortical status of patients for whom data on total plasma cortisol may be misleading. This simple, easy RIA is suited to be the routine method for free cortisol in plasma.

The free (non-protein-bound) fraction of steroid hormones has a major role in determining their biological activity. Therefore, assay of free steroid appears to furnish more reliable information about the functional status of these hormones, especially in cases where the binding capacity of plasma proteins is altered.

Recently, we described a highly sensitive method for determining the apparent free cortisol concentration (AFCC) in plasma samples by means of an RIA in which an antiserum is directly coated on the test tube wall (1). The diffusible (free) cortisol in the plasma dialysate after an equilibrium dialysis (ED) is measured directly by this RIA method.

However, ED has the disadvantage of a long incubation interval with respect to other techniques that are available for the measurement of the free hormone fraction, such as gel-equilibrium, ultrafiltration, and ultracentrifugation. Additional drawbacks are the volume of plasma necessary for the assay (1 mL) and the need to determine AFCC by two steps (ED and RIA).

Therefore, we have developed a direct assay of free cortisol in plasma, in which the same antibody-coated-tube RIA is used as for the ED procedure (1). Our procedure is based on the immunoextraction of cortisol in unknown samples, which is measured from a calibration curve prepared by use of human serum standards in which the free hormone content was previously determined by the ED system (1) as the comparison method.

Here we describe the methodological characteristics of this new method and compare results with those by the previously described ED procedure.

Materials and Methods

AFCC was measured in plasma samples by the RIA plus ED technique as previously described (1, 2). The ED was performed with 1 mL of plasma at 37 °C for 20–24 h vs 5 mL of buffer. The diffusible cortisol in the dialysate was then measured by an RIA method in which an antiserum is directly coated on the test tubes and ¹²⁵I-labeled cortisol is used as the tracer [SPAC Cortisol, kindly supplied by Byk Gulden Italia S.p.A., Cormano (MI), Italy] (1).

This same RIA method was also used for the direct assay

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